

**Genetics, Sources, and Mapping of Stem Rust  
Resistance in Barley**

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# **Dedication**

I dedicate this to my family who has supported me without question.

## Abstract

Stem rust is a devastating disease of cereal crops worldwide. In barley (*Hordeum vulgare*), the disease is caused by two pathogens: *Puccinia graminis* f. sp. *secalis* (*Pgs*) and *Puccinia graminis* f. sp. *tritici* (*Pgt*). In North America, the stem rust resistance gene *Rpg1* has protected barley from losses for more 60 years; however, widely virulent *Pgt* races from Africa in the Ug99 group threaten the crop. To identify novel quantitative trait loci (QTL) for stem rust resistance, bi-parental and association mapping studies were undertaken in the Barley iCore Collection (BCC) held by the USDA National Small Grains Collection. Association mapping studies of the BCC were conducted for seedling resistance to *Pgt* race TTKSK (Ug99 group) in the greenhouse and adult plant resistance (APR) to *Pgt* TTKSK composite in Njoro, Kenya and *Pgt* race QCCJB in St. Paul, MN. A major effect QTL (*Rpg-qt1-5H-11\_11355*) for APR in all locations was identified on chromosome 5H. This QTL represents a unique locus for APR and has been confirmed in other studies. Subsequently, 290 of the most resistant BCC accessions, the BCC Selects (BCCS), were screened for resistance *Pgt* races MCCFC, QCCJB, HKHJC, TTKSK, TTKST, TKTTF, and TRTTF, and also *Pgs* isolate 92-MN-90. From this investigation, four BCCS accessions were postulated to carry *Rpg1*, 14 to carry *Rpg2*, 91 to carry *Rpg3*, four to carry *rpg4/Rpg5*, and 59 to carry potentially novel resistance genes. To map the APR genes of *Rpg2* and *Rpg3* in Hietpas-5 (CIho 7124) and GAW-79 (PI 382313), respectively, two biparental populations were developed with Hiproly (PI 60693), a stem rust susceptible accession. Both populations were phenotyped to the domestic *Pgt* races of MCCFC, QCCJB, and HKHJC in St. Paul and to *Pgt* TTKSK composite in Njoro. In the Hietpas-5/Hiproly population, a major effect QTL was identified on chromosome 2H, which is proposed as the location for *Rpg2*. In the GAW-79/Hiproly population, a major effect QTL was identified on chromosome 5H and is the proposed location for *Rpg3*. The resistance sources identified and characterized in this study enhance barley breeding programs focused on stem rust resistance.

# Table of Contents

|   |             |
|---|-------------|
| <b>Acknowledgements .....</b>                                     | <b>i</b>    |
| <b>Dedication .....</b>   | <b>iii</b>  |
| <b>Abstract.....</b>  | <b>iv</b>   |
| <b>Table of Contents .....</b>                                    | <b>v</b>    |
| <b>List of Tables .....</b>                                       | <b>x</b>    |
| <b>List of Figures.....</b>                                       | <b>xiv</b>  |
| <b>List of Online Resources.....</b>                              | <b>xxii</b> |
| <b>Chapter 1 General Introduction and Literature Review .....</b> | <b>1</b>    |
| Barley Cultivation and Uses .....                                 | 2           |
| Barley Biology.....   | 2           |
| Barley Genetics .....   | 3           |
| Barley Genetic Resources.....                                     | 4           |
| Genetics of Disease Resistance .....                              | 5           |
| Stem Rust Biology.....  | 6           |
| Races of Stem Rust.....   | 7           |
| History of Stem Rust on Barley.....                               | 8           |
| Breeding for Stem Rust Resistance in Barley.....                  | 10          |
| Resistance Gene <i>Rpg1</i> .....                                 | 12          |

|  |           |
|--|-----------|
| Resistance Gene Complex <i>rpg4/Rpg5</i> .....   | 13        |
| Other Resistance Genes .....   | 14        |
| Stem Rust QTLs .....   | 15        |
| Introduction to Genetic Mapping .....  | 17        |
| Linkage Estimation.....  | 17        |
| Genetic Markers .....  | 19        |
| Bi-parental Mapping Populations.....   | 20        |
| Genome-Wide Association Study .....  | 22        |
| <br><b>Chapter 2 Genome-Wide Association Study of Stem Rust Resistance in a World<br/>Collection of Cultivated Barley.....</b> |           |
| <b>25</b>  | <b>25</b> |
| Introduction .....   | 26        |
| Materials and Methods .....  | 32        |
| Plant Materials .....  | 32        |
| Genotyping.....  | 33        |
| Adult Plant Rust Evaluations in Kenya.....   | 33        |
| Adult Plant Rust Evaluations in the United States .....  | 35        |
| Seedling Stem Rust Evaluations to Race TTKSK in the BSL-3.....   | 37        |
| Association Mapping .....  | 39        |
| Results .....  | 41        |
| Adult Plant Rust Evaluations .....   | 41        |



|  |           |
|--|-----------|
| Seedling Stem Rust Evaluations to Race TTKSK in the BSL-3 .....  | 42        |
| Association Mapping .....  | 43        |
| Effect of Identified QTL .....   | 45        |
| Discussion.....  | 46        |
| Figures .....  | 54        |
| Tables .....   | 70        |
| Online Resource Captions .....   | 80        |
| <b>Chapter 3 Sources and Genetics of Stem Rust Resistance in a World Collection of Cultivated Barley .....</b> | <b>81</b> |
| Introduction .....   | 82        |
| Materials and Methods .....  | 87        |
| Plant Materials .....  | 87        |
| Stem Rust Isolates and Races.....  | 89        |
| Adult Plant Phenotyping .....  | 89        |
| Seedling Phenotyping.....  | 91        |
| Gene Postulation .....   | 93        |
| Genotyping for <i>Rpg1</i> .....   | 96        |
| Genotyping for <i>Rpg5</i> .....   | 96        |
| Results .....  | 98        |
| Adult Plant Resistance .....   | 98        |

|  |            |
|--|------------|
| Seedling Resistance.....   | 99         |
| Genotyping for <i>Rpg1</i> and <i>Rpg5</i> .....   | 101        |
| Discussion.....  | 102        |
| Figures .....  | 106        |
| Tables .....   | 114        |
| Online Resource Captions .....   | 186        |
| <b>Chapter 4 Mapping Adult Plant Stem Rust Resistance in Barley Accessions Hietpas-5 and GAW-79.....</b> | <b>187</b> |
| Introduction .....   | 188        |
| Materials and Methods .....  | 193        |
| Stem Rust Controls .....   | 193        |
| Mapping Population Development.....  | 193        |
| Rust Phenotyping .....   | 195        |
| Genotyping.....  | 200        |
| Linkage Map Construction.....  | 202        |
| QTL Detection .....  | 203        |
| Results .....  | 204        |
| Genotyping .....   | 204        |
| Map Construction.....  | 205        |
| Rust Resistance Phenotyping .....  | 206        |

|                                |            |
|--------------------------------|------------|
| Discussion.....                | 214        |
| Figures .....                  | 224        |
| Tables .....                   | 251        |
| Online Resource Captions ..... | 265        |
| <b>References .....</b>        | <b>267</b> |
| <b>Appendix.....</b>           | <b>280</b> |

# List of Tables

|  |           |
|--|-----------|
| <b>Chapter 2 Genome-Wide Association Study of Stem Rust Resistance in a World Collection of Cultivated Barley.....</b>   | <b>25</b> |
| <b>Table 2.1.</b> Frequency of seedling and adult plant stem rust resistance in the Barley iCore Collection (BCC), with accessions categorized into the highly resistant (Class 1) or the resistant (Class 2) based on the reaction of Q21861 and Q/SM20, respectively.....                              | 70        |
| <b>Table 2.2.</b> Markers significantly associated with seedling and adult plant stem rust reactions in the Barley iCore Collection (BCC) using datasets corrected for seasonal and spatial variability. ....  | 71        |
| <b>Table 2.3.</b> Markers significantly associated with adult plant stem rust reactions in the Barley iCore Collection (BCC) using raw adult plant severity values. ....   | 72        |
| <b>Table 2.4.</b> Quantitative trait loci (QTL), genomic positions, and associated markers detected in the Barley iCore Collection (BCC) for seedling and adult plant stem rust resistance. ....   | 74        |
| <b>Table 2.5.</b> Accessions from the Barley iCore Collection (BCC) that were classified as either highly resistant (Class 1) or resistant (Class 2) to stem rust infection at the adult stage in all locations based on the performance of Q21861 or Q/SM20, respectively.....                          | 76        |
| <b>Table 2.6.</b> Disease reduction effects of significant markers by nursery for both adult plant stem rust severity (%) to <i>Pgt</i> races QCCJB and TTKSK and seedling TTKSK coefficient of infection values, identified by genome-wide association study in the Barley iCore Collection (BCC). .... | 78        |
| <b>Chapter 3 Sources and Genetics of Stem Rust Resistance in a World Collection of Cultivated Barley .....</b>   | <b>81</b> |
| <b>Table 3.1.</b> Reaction mode of differential wheat, barley, and rye lines to specific races or isolates of the stem rust pathogen, <i>Puccinia graminis</i> . ....  | 114       |
| <b>Table 3.2.</b> Pearson correlation for adult plant disease severities (averaged within race) of   |           |

|  |     |
|--|-----|
| Barley iCore Collection Selects (BCCS) to <i>Puccinia graminis</i> f. sp. <i>tritici</i> races <i>Pgt</i> TTKSK Composite in Njoro, Kenya and QCCJB, MCCFC, and HKHJC in St. Paul, MN. ....  | 117 |
| <b>Table 3.3.</b> Threshold values used for assigning accessions of the Barley iCore Collection Selects (BCCS) into adult plant stem rust reaction classes where highly resistant (Class 1) and resistant (Class 2). ....  | 118 |
| <b>Table 3.4.</b> Linear score for conversion of the raw seedling infection type scores.....   | 120 |
| <b>Table 3.5.</b> Molecular markers used to detect functional <i>Rpg1</i> genotypes. ....  | 121 |
| <b>Table 3.6.</b> Molecular markers used to detect common functional <i>Rpg5</i> and non-functional <i>rpg5</i> genotypes.....   | 122 |
| <b>Table 3.7.</b> Molecular markers used to detect rare non-functional <i>rpg5</i> genotypes.....  | 123 |
| <b>Table 3.8.</b> Adult plant disease severity and infection responses of Barley iCore Collection Selects (BCCS) to <i>Puccinia graminis</i> f. sp. <i>tritici</i> races HKHJC, QCCJB, and MCCFC in St. Paul, MN and <i>Pgt</i> TTKSK composite in Njoro, Kenya. ....  | 125 |
| <b>Table 3.9.</b> Barley iCore Collection Selects (BCCS) exhibiting the highest levels of adult plant stem rust resistance over multiple years and races.....  | 144 |
| <b>Table 3.10.</b> Seedling reactions of Barley iCore Collection Selects (BCCS) to <i>Puccinia graminis</i> f. sp. <i>tritici</i> races TTKSK, TTKST, TKTTF, TRTTF, QCCJB, HKHJC, and MCCFC and also <i>Puccinia graminis</i> f. sp. <i>secalis</i> isolate 92-MN-90. ....   | 146 |
| <b>Table 3.11.</b> Pearson correlation coefficient among seedling infection types of Barley iCore Collection Selects (BCCS) to <i>Puccinia graminis</i> f. sp. <i>tritici</i> races TTKSK, TTKST, TKTTF, TRTTF, QCCJB, HKHJC, and MCCFC and also <i>Puccinia graminis</i> f. sp. <i>secalis</i> isolate 92-MN-90. .... | 165 |
| <b>Table 3.12.</b> Resistance genes in the Barley iCore Collection Selects (BCCS) postulated by comparison with the reaction of barley stem rust controls carrying known resistance genes as executed in the RGenePos package in R.....  | 166 |

|   |            |
|---|------------|
| <b>Table 3.13.</b> Genotype outcomes of the <i>Rpg1</i> and <i>rpg4/Rpg5</i> markers for the Barley iCore Collection Selects (BCCS) and barley stem rust controls carrying different resistance genes.<br>.....   | 167        |
| <b>Chapter 4 Mapping Adult Plant Stem Rust Resistance in Barley Accessions Hietpas-5 and GAW-79</b> .....   | <b>187</b> |
| <b>Table 4.1.</b> Summary statistics of linkage maps for the Hietpas-5/Hiproly and PI 382313/Hiproly recombinant inbred line populations.....   | 251        |
| <b>Table 4.2.</b> The mean and range of stem rust severities for barley and wheat controls infected with various races of <i>Puccinia graminis</i> f. sp. <i>tritici</i> in St. Paul, MN and Njoro, Kenya, 2015-2016. ....  | 252        |
| <b>Table 4.3.</b> Broad sense heritability ( $H^2$ ) of stem rust resistance in the Hietpas-5/Hiproly and PI 382313/Hiproly recombinant inbred line populations. ....   | 254        |
| <b>Table 4.4.</b> Chi-square test for inheritance of stem rust ( <i>Puccinia graminis</i> f. sp. <i>tritici</i> ) adult plant resistance in the Hietpas-5/Hiproly and PI 382313/Hiproly recombinant inbred line populations and leaf rust ( <i>Puccinia hordei</i> ) seedling and adult plant resistance in the Hietpas-5/Hiproly population..... | 255        |
| <b>Table 4.5.</b> Correlations between stem rust severity and agro-morphological traits in the Hietpas-5/Hiproly and PI 382313/Hiproly recombinant inbred line populations. ....  | 257        |
| <b>Table 4.6.</b> Quantitative trait loci detected for stem rust resistance and agro-morphological traits in the Hietpas-5/Hiproly recombinant inbred line population.....  | 259        |
| <b>Table 4.7.</b> Quantitative trait loci detected for stem rust resistance and agro-morphological traits in the PI 382313/Hiproly recombinant inbred line population.....  | 262        |
| <b>Appendix</b> .....   | <b>280</b> |
| <b>Appendix A1.</b> Previously identified quantitative trait loci (QTL) stem rust resistance. ....  | 280        |
| <b>Appendix A2.</b> Two-hundred and ninety accessions of the Barley iCore Collection Selects  |            |

(BCCS) chosen on the basis of their stem rust resistance from the 1,860 member Barley  
iCore Collection (BCC)..... 288

# List of Figures

## Chapter 2 Genome-Wide Association Study of Stem Rust Resistance in a World Collection of Cultivated Barley..... 25

**Figure 2.1.** Principal component analysis of all accessions of the Barley iCore Collection (BCC) (n = 1,860). Accessions are colored according to results from STRUCTURE analysis by Muñoz-Amatriaín et al. (2014)..... 54

**Figure 2.2.** Performance of barley (Hiproly, PI 532013, Chevron, and Q21861) and wheat (LMPG-6 and Red Bobs) controls in multiple stem rust screening nurseries in Njoro, Kenya and St. Paul, MN, where nurseries are abbreviated as: 2013 St. Paul (StP13), 2014 St. Paul (StP14), 2014 Kenya main-season (KEN14m), 2014 Kenya off-season (KEN14o), 2013 Kenya winter (KEN13w) and 2014 Kenya winter (KEN14w). Five statistics (bars) are represented in each box plot from bottom to top: the smallest observation, lower quartile, median, upper quartile, and largest observation, respectively. Data points positioned outside this range are extreme values and are represented as circles..... 57

**Figure 2.3.** Performance of all Barley iCore Collection (BCC) accessions in multiple stem rust screening nurseries in Njoro, Kenya and St. Paul, MN, where nurseries are abbreviated as: 2013 St. Paul (StP13), 2014 St. Paul (StP14), 2014 Kenya main-season (KEN14m), 2014 Kenya off-season (KEN14o), 2013 Kenya winter (KEN13w) and 2014 Kenya winter (KEN14w). Five statistics (bars) are represented in each box plot from bottom to top: the smallest observation, lower quartile, median, upper quartile, and largest observation, respectively. Data points positioned outside this range are extreme values and are represented as circles..... 59

**Figure 2.4.** Response of all barley controls to seedling infection by *Puccinia graminis* f. sp. *tritici* race TTKSK. Raw infection type values were converted to Coefficient of Infection (CI) values as described by Zhou et al. (2014). Five statistics (bars) are represented in each box plot from bottom to top: the smallest observation, lower quartile, median, upper quartile, and largest observation, respectively. Data points positioned outside this range are



extreme values and are represented as circles. .... 61

**Figure 2.5.** Distribution of seedling stem rust reactions to race TTKSK in the Barley iCore Collection (BCC). Raw infection type values were converted to Coefficient of Infection (CI) values as described by Zhou et al. (2014). The reactions of susceptible controls Chevron and Hiproly and resistant controls Q21861 and Q/SM20 are also shown for comparison. .... 63

**Figure 2.6.** Manhattan plots showing significant marker-trait associations for stem rust severity (0-100%) in multiple stem rust screening nurseries in Njoro, Kenya and St. Paul, MN. Nurseries are abbreviated as: 2013 St. Paul (StP13), 2014 St. Paul (StP14), 2014 Kenya main-season (KEN14m), 2014 Kenya off-season (KEN14o), 2013 Kenya winter (KEN13w), and 2014 Kenya winter (KEN14w). Blue lines indicate threshold levels for declaring significant marker-trait associations using a false discovery rate correction of,  $q$ -value  $< 0.05$ . Q-Q plots are depicted to left of each Manhattan plot. .... 66

**Figure 2.7.** Manhattan plot showing significant marker-trait associations for stem rust reaction type at the seedling stage to *Puccinia graminis* f. sp. *tritici* race TTKSK. Raw infection type values were converted to Coefficient of Infection (CI) values as described by Zhou et al. (2014). The two replicates of BSL3-1 and BSL3-2 were averaged and then  $\log_{10}$  transformed to normalize the data (TTKSK\_Seedling). Blue lines indicate threshold levels for declaring significant marker-trait associations using a false discovery rate correction of,  $q$ -value  $< 0.05$ . A Q-Q plot is depicted to the left of the Manhattan plot..... 67

**Figure 2.8.** Manhattan plots showing significant marker-trait associations for stem rust severity (0-100%) in multiple datasets corrected for field spatial and trial variability. Data sets were: Kenya Spring nurseries (Kenya\_Spring\_A), Kenya Winter nurseries (Kenya\_Winter\_A) and St. Paul Spring nurseries (StP\_Spring\_A). Blue lines indicate threshold levels for declaring significant marker-trait associations using a false discovery rate correction of,  $q$ -value  $< 0.05$ . Q-Q plots are depicted to left of each Manhattan plot.... 69

**Chapter 3 Sources and Genetics of Stem Rust Resistance in a World Collection of Cultivated Barley ..... 81**

**Figure 3.1.** Principal Component Analysis of the 1,860 accessions in the Barley iCore

Collection (BCC) showing the distribution of the 290 accessions of the Barley iCore Collection Selects (BCCS) among the population clusters. Accessions in the BCC are colored according to the STRUCTURE analysis results by Muñoz-Amatriaín et al. (2014) with BCCS accessions shown in black. .... 106

**Figure 3.2.** The range of stem rust infection types (ITs) observed on barley as assessed using the 0-4 scale of Stakman et al. (1962) in comparison to a linear score (LS) developed in this study. Barley accessions exhibiting a LS of < 8 (IT < 23-) were considered resistant, and those with a LS > 8 were considered susceptible. .... 107

**Figure 3.3.** Performance of barley (Hiproly, PI 532013, Chevron, Q21861, and Q/SM20) and wheat (LMPG-6 and Red Bobs) stem rust controls in multiple stem rust screening nurseries in Njoro, Kenya and St. Paul, MN where nurseries are abbreviated as: 2015 St. Paul *Puccinia graminis* f. sp. *tritici* (*Pgt*) race HKHJC (StP15\_HK), 2015 St. Paul *Pgt* race MCCFC (StP15\_MC), 2013 St. Paul *Pgt* race QCCJB (StP13\_QC), 2014 St. Paul *Pgt* race QCCJB (StP14\_QC), 2015 St. Paul *Pgt* race QCCJB (StP15\_QC), 2014 Kenya off-season (KEN14o), 2014 Kenya main-season (KEN14m). *Pgt* races in Kenya were *Pgt* TTKSK composite (TTKSK, TTKST, TTKTK, and TTKTT). Five statistics (bars) are represented in each box plot from bottom to top: the smallest observation, lower quartile, median, upper quartile, and largest observation, respectively. Data points positioned outside this range are extreme values and are represented as circles. .... 111

**Figure 3.4.** Examples of the diversity for adult plant infection response (IR) in barley accessions to *Puccinia graminis* f. sp. *tritici* in the field in Greytown, South Africa. IR ratings are R = Resistant, MR = Moderately Resistant, MS = Moderately Susceptible, and S = Susceptible. .... 112

**Figure 3.5.** Diversity of stem rust infection types observed on the first leaves (adaxial side) of accessions from the Barley iCore Collection Selects (BCCS). From left to right: BCC2297 (IT 0; to race TKTTF), BCC434 (0;n to TRTTF), BCC212 (0;1 to TTKST), BCC168 (0;1,2 to TRTTF), BCC1103 (10; to TTKST), BCC1578 (1n to TTKST), BCC1624 (1 to TTKST), BCC2558 (120; to TTKST), BCC1720 (12 to TKTTF), BCC1548 (21 to TRTTF), BCC654 (21n to TRTTF), BCC1917 (2n to TTKST), BCC1875 (23-1 to

|   |     |
|---|-----|
| TRTTF), BCC394 (23-c to TTKST), BCC308 (3-2 to TRTTF), BCC1872 (3 to TKTTF), BCC2856 (3-3 to TTKST), BCC2134 (33+ to TTKST), and BCC2856 (3+ to TTKST on abaxial side). ..... | 113 |
|---|-----|

**Chapter 4 Mapping Adult Plant Stem Rust Resistance in Barley Accessions Hietpas-5 and GAW-79..... 187**

|  |     |
|--|-----|
| <b>Figure 4.1.</b> Linkage map of the Hietpas-5/Hiproly recombinant inbred line population showing calculated centiMorgan (cM) lengths for the seven chromosomes of barley. The red “C” indicates the approximate centromere position..... | 224 |
|--|-----|

|  |     |
|--|-----|
| <b>Figure 4.2.</b> Linkage map of the PI 382313/Hiproly recombinant inbred line population showing calculated centiMorgan (cM) lengths for the seven chromosomes of barley. The red “C” indicates the approximate centromere position..... | 225 |
|--|-----|

|  |     |
|--|-----|
| <b>Figure 4.3.</b> Performance of barley mapping parents (Hietpas-5, PI 382313, and Hiproly) and barley (PI 532013, Chevron, Q21861, and Q/SM20) and wheat (LMPG-6 and Red Bobs) stem rust controls in multiple stem rust screening nurseries in Njoro, Kenya, Greytown, South Africa, and St. Paul, MN. Nurseries are abbreviated as: 2015 St. Paul <i>Puccinia graminis</i> f. sp. <i>tritici</i> ( <i>Pgt</i> ) race HKHJC (StP_HK15), 2015 Kenya off-season <i>Pgt</i> TTKSK composite (includes races TTKSK, TTKST, TTKTK and TTKTT) (KEN_15o), 2015 St. Paul <i>Pgt</i> race MCCFC (StP_MC15), 2016 St. Paul <i>Pgt</i> race MCCFC (StP_MC16), 2015 St. Paul <i>Pgt</i> race QCCJB (StP_QC15), 2016 St. Paul <i>Pgt</i> race QCCJB (StP_QC16), and 2016 Greytown (SA_16) <i>Pgt</i> race PTKST. Five statistics (bars) are represented in each box plot from bottom to top: the smallest observation, lower quartile, median, upper quartile, and largest observation, respectively. Data points positioned outside this range are extreme values and are represented as circles. .... | 229 |
|--|-----|

|   |     |
|---|-----|
| <b>Figure 4.4.</b> Adult plant infection responses of Hietpas-5 (left), Hiproly (center), and PI 382313 (right) to <i>Puccinia graminis</i> f. sp. <i>tritici</i> race QCCJB in St. Paul, MN in 2015. ... | 230 |
|---|-----|

|  |     |
|--|-----|
| <b>Figure 4.5.</b> Histogram of stem rust severity averaged over all locations for 200 recombinant inbred lines of the Hietpas-5/Hiproly population..... | 231 |
|--|-----|

**Figure 4.6.** Histogram of stem rust severity averaged over all locations for 200 recombinant inbred lines of the PI 382313/Hiproly population..... 232

**Figure 4.7.** Boxplots of stem rust severity for recombinant inbred lines (RILs) and parents of the Hietpas-5/Hiproly (HH) population within each trial. The trial locations and years, along with the predominant *Puccinia graminis* f. sp. *tritici* race present, are abbreviated as follows: StP\_HK15 for St. Paul, MN race HKHJC in 2015, KEN\_15o for Njoro, Kenya *Pgt* TTKSK composite (includes races TTKSK, TTKST, TTKTK and TTKTT) in 2015 off-season, StP\_MC15 for St. Paul, MN race MCCFC in 2015, StP\_MC16 for St. Paul, MN race MCCFC in 2016, StP\_QC15 for St. Paul, MN race QCCJB in 2015, StP\_QC16 for St. Paul, MN race QCCJB in 2016, and SA\_16 for Greytown, South Africa race PTKST in 2016. Five statistics (bars) are represented in each box plot from bottom to top: the smallest observation, lower quartile, median, upper quartile, and largest observation, respectively. Data points positioned outside this range are extreme values and are represented as circles. .... 234

**Figure 4.8.** Boxplots of stem rust severity for recombinant inbred lines (RILs) and parents of the PI 382313/Hiproly (PH) population within each trial. The trial locations and years, along with the predominant *Puccinia graminis* f. sp. *tritici* race present, are abbreviated as follows: StP\_HK15 for St. Paul, MN race HKHJC in 2015, KEN\_15o for Njoro, Kenya *Pgt* TTKSK composite (includes races TTKSK, TTKST, TTKTK and TTKTT) in 2015 off-season, StP\_MC15 for St. Paul, MN race MCCFC in 2015, StP\_MC16 for St. Paul, MN race MCCFC in 2016, StP\_QC15 for St. Paul, MN race QCCJB in 2015, and StP\_QC16 for St. Paul, MN race QCCJB in 2016. Five statistics (bars) are represented in each box plot from bottom to top: the smallest observation, lower quartile, median, upper quartile, and largest observation, respectively. Data points positioned outside this range are extreme values and are represented as circles..... 236

**Figure 4.9.** Boxplots of stem rust severity for barley mapping parents (Hietpas-5, PI 382313, and Hiproly) and barley (Q21861, Q/SM20, Chevron, 80-TT-20, Quest, 80-tt-30, Steptoe, and PI 532013) and wheat (LMPG-6 and Red Bobs) stem rust controls averaged over all locations. Five statistics (bars) are represented in each box plot from bottom to top: the smallest observation, lower quartile, median, upper quartile, and largest observation,

respectively. Data points positioned outside this range are extreme values and are represented as circles..... 237

**Figure 4.10.** Severity of leaf rust infection by *Puccinia hordei* on the flag leaves of the resistant accession Hietpas-5 (left) and the susceptible accession Hiproly (right) in Greytown, South Africa in 2016. .... 238

**Figure 4.11.** Boxplots of leaf rust severity scores for recombinant inbred lines (RILs) and parents of the Hietpas-5/Hiproly (HH) population evaluated to *Puccinia hordei* at Greytown, South Africa in 2016. Up to five statistics (bars) are represented in each box plot from bottom to top: the smallest observation, lower quartile, median, upper quartile, and largest observation, respectively. .... 239

**Figure 4.12.** Seedling infection types of Hietpas-5, Hiproly, and selected recombinant inbred lines of the Hietpas-5/Hiproly population to South African *Puccinia hordei* isolate SAPH1601 in the greenhouse. .... 240

**Figure 4.13.** Linkage maps of the Hietpas-5/Hiproly recombinant inbred line population showing significant quantitative trait loci for stem rust severity, spike row type, heading date, leaf rust severity, leaf rust seedling infection type (IT) score, physiological leaf spotting, plant height, and seed cover. Traits, locations and years were abbreviated according to the following scheme “trait”.”location”.”year” where sr is stem rust, sp is spike row type, hd is heading date, lr is leaf rust severity as adults, lr.gh is seedling leaf rust IT score (converted to a coefficient of infection value as described by Zhou et al. (2014)) in the greenhouse, pls is physiological leaf spotting, ph is plant height, sc is covered or naked caryopsis, hk is the St. Paul race HKHJC nursery, mc is the St. Paul race MCCFC nursery, qc is the St. Paul race QCCJB nursery, ken is the Njoro, Kenya *Pgt* TTKSK composite (includes races TTKSK, TTKST, TTKTK and TTKTT) nursery, sa is the Greytown, South Africa race PTKST nursery, gh indicates greenhouse observations, and 15 and 16 represent the years of 2015 and 2016, respectively. .... 244

**Figure 4.14.** Linkage maps of the PI 382313/Hiproly recombinant inbred line population showing significant quantitative trait loci for stem rust severity, spike row type, heading date, physiological leaf spotting, plant height, and seed cover. Traits, locations and years

were abbreviated according to the following scheme “trait”.”location”.”year” where sr is stem rust, sp is spike row type, hd is heading date, pls is physiological leaf spotting, ph is plant height, sc is covered or naked caryopsis, hk is the St. Paul race HKHJC nursery, mc is the St. Paul race MCCFC nursery, qc is the St. Paul race QCCJB nursery, and ken is the Njoro, Kenya *Pgt* TTKSK composite (includes races TTKSK, TTKST, TTKTK and TTKTT) nursery, gh indicates greenhouse observations, and 15 and 16 represent the years of 2015 and 2016, respectively. .... 248

**Figure 4.15.** Average stem rust severity for recombinant inbred lines of the Hietpas-5/Hiproly population grouped according to the number of quantitative trait loci for resistance they carry. Bars with different letters above indicate statistically significant differences according to Tukey’s HSD test ( $p$ -value <0.05). The number of RILs in each group is shown in the box. Groups “+*Rpg2*” and “-*Rpg2*” comprise RILs that either possess or lack the QTLs *Rpg-qt1-HH-Hie-2H.2*, *Rpg-qt1-HH-Hie-2H.2 2H.3* (the putative underlying QTL for *Rpg2*), respectively. Five statistics (bars) are represented in each box plot from bottom to top: the smallest observation, lower quartile, median, upper quartile, and largest observation, respectively. Data points positioned outside this range are extreme values and are represented as circles. .... 249

**Figure 4.16.** Average stem rust severity for recombinant inbred lines of the PI 382313/Hiproly population grouped according to the number of quantitative trait loci for resistance they carry. Bars with different letters above indicate statistically significant differences according to Tukey’s HSD test ( $p$ -value <0.05). The number of RILs in each group is shown in the box. Groups “+*Rpg3*” and “-*Rpg3*” comprise RILs that either possess or lack the QTL *Rpg-qt1-PH-PI38-5H* (the putative underlying QTL for *Rpg3*), respectively. Five statistics (bars) are represented in each box plot from bottom to top: the smallest observation, lower quartile, median, upper quartile, and largest observation, respectively. Data points positioned outside this range are extreme values and are represented as circles. .... 250

**Appendix..... 280**

**Appendix A3.** Comparison of centiMorgan (cM) position to Mega Base Pair (MBP)

position in the Hietpas-5/Hiproly population map. The cM position plotted vs MBP position in the reference genome. Red lines indicate estimated centromere MBP range position based on the Morex reference genome (Matthew Haas, personal communication)..... 301

**Appendix A4.** Comparison of centiMorgan (cM) position to Mega Base Pair (MBP) position in the PI 382313/Hiproly population map. The cM position plotted vs MBP position in the reference genome. Red lines indicate estimated centromere MBP range position based on the Morex reference genome (Matthew Haas, personal communication).  
..... 303

# List of Online Resources

Online resources are stored on Dr. Brian Steffenson’s active directory at the University of Minnesota and are also available from Austin Case by request.

## **Chapter 2 Genome-Wide Association Study of Stem Rust Resistance in a World Collection of Cultivated Barley..... 25**

**Online Resource 2.1.** Accessions of the Barley iCore Collection (BCC) and their reaction to stem rust. .... 80

## **Chapter 3 Sources and Genetics of Stem Rust Resistance in a World Collection of Cultivated Barley ..... 81**

**Online Resource 3.1.** RGenePos an R package for postulation of resistance genes in crop plants. .... 186

**Online Resource 3.2.** Additional adult plant stem rust resistance nursery observations of the Barley iCore Collection Selects (BCCS)..... 186

## **Chapter 4 Mapping Adult Plant Stem Rust Resistance in Barley Accessions Hietpas-5 and GAW-79..... 187**

**Online Resource 4.1.** Hietpas-5/Hiproly and PI 382313/Hiproly recombinant inbred line population subsets used for genotyping and phenotyping and those used for genotyping only. In the Hietpas-5/Hiproly and the PI 382313/Hiproly populations, 280 and 278 RILs were genotyped, respectively, but only 200 RILs of each population were used in phenotyping. .... 265

**Online Resource 4.2.** Hietpas-5/Hiproly recombinant inbred line population raw genotyping by sequencing calls. Raw SNP data were generated as described in the Materials and Methods section where marker\_new, Chrom\_new and Pos\_new are based on the updated genome assembly. Ref\_seq is 30 bp before and 30 bp after the SNP in this reference genome version, whereas Marker\_old, Chrom\_old, and Pos\_old were based on an older



version of the reference genome where chromosomes were split into two parts..... 265

**Online Resource 4.3.** PI 382313/Hiproly recombinant inbred line population raw genotyping by sequencing calls. Raw SNP data were generated as described in the Materials and Methods section where marker\_new, Chrom\_new and Pos\_new are based on the updated genome assembly. Ref\_seq is 30 bp before and 30 bp after the SNP in this reference genome version, whereas Marker\_old, Chrom\_old, and Pos\_old were based on an older version of the reference genome where chromosomes were split into two parts..... 265

**Online Resource 4.4.** Hietpas-5/Hiproly recombinant inbred line population imputed genotyping by sequencing calls to fill in missing genotype data. Raw genotype by sequencing calls imputed using the LinkImpute method as described in the Materials and Methods section (Money et al. 2015)..... 265

**Online Resource 4.5.** PI 382313/Hiproly recombinant inbred line population imputed genotyping by sequencing calls to fill in missing genotype data. Raw genotype by sequencing calls imputed using the LinkImpute method as described in the Materials and Methods section (Money et al. 2015)..... 265

**Online Resource 4.6.** Hietpas-5/Hiproly recombinant inbred line population final genotyping by sequencing calls after imputation and filtering. The final set of genotype by sequencing marker calls were first imputed and then filtered for quality control as described in the Materials and Methods section. The total number of markers was 8,586, including the two morphological markers. Alleles calls were converted to A, B, H and N calls, where “A” alleles were Hietpas-5-like, “B” alleles Hiproly-like, “H” was heterozygous, and “N” was missing. .... 266

**Online Resource 4.7.** PI 382313/Hiproly recombinant inbred line population final genotyping by sequencing calls after imputation and filtering. The final set of genotype by sequencing marker calls were first imputed and then filtered for quality control as described in the Materials and Methods section. The total number of markers remaining was 6,985, including two morphological markers. Alleles calls were converted to A, B, H and N calls, where “A” alleles were PI 382313-like, “B” alleles Hiproly-like, “H” was heterozygous, and

|  |            |
|--|------------|
| “N” was missing.....   | 266        |
| <b>Online Resource 4.8.</b> Linkage maps of the Hietpas-5/Hiproly and PI 382313/Hiproly recombinant inbred line populations. Marker cM distance was calculated as described in the Materials and Methods section. Maps were constructed with 1,585 markers in the Hietpas-5/Hiproly population and 1,364 in the PI 382313/Hiproly population. .... | 266        |
| <b>Appendix.....</b>   | <b>280</b> |
| <b>Appendix A5.</b> Descriptions and names of datasets uploaded to the Triticeae Tool Box (T3) barley database ( <a href="https://triticeaetoolbox.org/barley/">https://triticeaetoolbox.org/barley/</a> ).....  | 304        |

# **Chapter 1**

## **General Introduction and Literature Review**

## **Barley Cultivation and Uses**

Barley (*Hordeum vulgare* ssp. *vulgare* L.) is an economically important crop in both the developed and developing world and ranks fourth in terms of global cereal production and acreage (FAO-STAT 2014). The top five global producers (by metric ton) are: Russia, France, Germany, Australia and Ukraine. Countries in North America rank globally as follows: Canada 6<sup>th</sup> (~7 million metric tons), United States 10<sup>th</sup> (~3 million metric tons) and Mexico 33<sup>rd</sup> (~800,000 metric tons). In the United States, barley is primarily produced in the northern and western states, with the majority of production occurring in Montana, North Dakota and Idaho (FAO-STAT 2014). However, with the recent boom in the local production of craft beer, some acreage of barley is produced in most states. Barley is extremely adaptable to a wide range of environments with cultivation occurring from the sub-arctic to the sub-tropics (Ullrich 2010). Indeed, the widespread cultivation of barley is due to its ability to adapt to extreme environments, including cold, frost, drought, and salinity (Newton et al. 2011). Diseases are a major limiting factor for both barley yield and quality. Diseases affecting barley vary by region. However, major diseases include: Fusarium head blight, powdery mildew, spot blotch, stem rust, leaf rust and Barley yellow dwarf or Cereal yellow dwarf (Mathre 1982). Globally, animal feed is the largest use of barley, accounting for about 60% of global consumption (Ullrich 2010). However, in the United States, the largest use of barley is in malting and brewing (FAO-STAT 2014). Barley is primarily grown as a spring annual, but is also cultivated in some regions as a winter annual (Ullrich 2010).

## **Barley Biology**

Barley is an annual cereal grain cultivated for its seeds. Its stems are erect and hollow, originating at the crown (Briggs 1978). Leaves are borne on the nodes that are separated by internodes, which make up the stem (Gomez-Macpherson 2001). At maturity, a barley plant will consist of a main stem and several side “tiller” stems. Productive stems terminate in spikes carrying flowers, which will develop into seeds. The spike has a

central rachis with spikelets at nodes on alternating sides. At each node on the rachis, there are three spikelets. Each spikelet is made up of two glumes that contain the flower consisting of the lemma, palea, pistil, and stamen. The lemma may or may not have an awn. In hulled accessions, the lemma and palea remain attached to the caryopsis at maturity, a trait controlled by the *Nud* gene (Franckowiak et al. 1997a). Hulled varieties are preferred in malting and brewing. Barley has either two-rowed spikes or six-rowed spikes, a trait controlled by the *Vrs1* gene (Franckowiak and Haus 1997a; Franckowiak et al. 1997b). All three spikelets are fertile at each node in six-rowed barley, whereas in two-rowed barley, only the center spikelet is fertile. In the *deficiens* type of two-rowed barley, the sterile lateral spikelets are absent (Franckowiak and Haus 1997b).

## **Barley Genetics**

Barley is a diploid inbreeding species with seven chromosomes ( $2n=2x=14$ ), designated as 1H to 7H according to their homeology to other species in the Triticeae (Graner et al. 2010; Linde-Laursen 1997). Barley has a large genome estimated at a haploid size of about 5.1 gigabases (International Barley Genome Sequencing et al. 2012). A large portion (>80%) of the genome consists of repetitive DNA sequences. The genome sequence of barley cultivar Morex was recently completed, providing a better insight into the genome of the crop (Mascher et al. 2017). The previous genome sequence was based on a whole-genome shotgun approach and is highly fragmented with the majority of the sequence representing the genic space (International Barley Genome Sequencing et al. 2012). The updated assembly is based on sequences of bacterial artificial chromosomes, allowing better assembly of the genic and non-genic space (Mascher et al. 2017). In addition to genes detected by sequencing, there is myriad of genes identified based on mutants with distinct phenotypes in the barley plant. Many of these genes have been described in the Barley Genetics Newsletter (<https://wheat.pw.usda.gov/ggpages/bgn/>). This includes genes controlling a large number of traits including flowering, plant morphology, fertility, resistance to diseases/pests, and abiotic stress tolerance (Cataloged in GrainGene, <https://wheat.pw.usda.gov/GG3>). This review will focus on the genes

related to biotic stress resistance. Most of these genes were described based on inheritance, mapping, allelism, or mutation. Some of the most important disease resistance genes described are those controlling the reaction to stem rust (i.e. Reaction to *Puccinia graminis* (*Rpg*)), leaf rust (Reaction to *Puccinia hordei* (*Rph*)), powdery mildew (Reaction to *Blumeria graminis* f. sp. *hordei* (*Rbg*), but the old nomenclature of *Mla* and *Mlo* prevails), spot blotch (Reaction to *Cochliobolus sativus* (*Rcs*)), and net blotch (Reaction to *Pyrenophora teres* f. *teres* and f. *maculata* (*Rpt*)) (Paulitz and Steffenson 2010). These genes are a valuable resource for breeding barley for resistance to biotic stresses.

### **Barley Genetic Resources**

The domestication of barley dates back to around 10,000 years ago, occurring in the Fertile Crescent region (Dai et al. 2012; Morrell et al. 2003). The direct progenitor of cultivated barley is wild barley (*Hordeum vulgare* ssp. *spontaneum* C. Koch Thell.) (von Bothmer and Komatsuda 2010). In terms of breeding, genetic diversity is paramount to the ability to make progress towards a breeding target and is a source of new beneficial alleles (Tanksley and Nelson 1996). In barley, the primary germplasm pool is comprised of cultivated barley (including landraces, breeding lines, and cultivars) and *H. vulgare* ssp. *spontaneum*; the secondary germplasm pool of a single species *Hordeum bulbosum* L. (bulbous barley grass), and the tertiary germplasm pool of about 30 different *Hordeum* species (Nevo 1992; Nevo et al. 1979; von Bothmer and Komatsuda 2010). No sterility barriers prevent the hybridization of cultivated barley within the primary germplasm pool. However, significant sterility barriers do limit hybridizations between cultivated barley and members of the secondary and tertiary pool, which must be overcome using advanced methodology (Nevo 1992; Nevo et al. 1979; von Bothmer and Komatsuda 2010)

In addition to the genetic diversity available in breeding germplasm, accessions held in *ex situ* collections are a valuable source of genetic diversity for barley breeding (Bockelman

and Valkoun 2010). Of these collections, landraces represent the largest component in barley and globally comprise about 44% of the 290,820 barley accessions held in genebanks around the world. The remainder of these collections include wild relatives (15%), breeding materials (17%), and genetic stocks (9%). Broadly speaking, these resources represent the best sources for finding novel genes or alleles. As an example, barley landraces from Switzerland carry a high frequency of stem rust resistance. The genes underlying this resistance include *Rpg1* and the *rpg4/Rpg5* gene complex (Steffenson et al. 2016). This result is suggestive of Switzerland being the place of origin for *Rpg1*, since the gene has not been found in other landrace germplasm or in wild barley. The *rpg4/Rpg5* complex was confirmed in wild barley, but is also present in relatively high frequency within Swiss landraces (Hulse et al. 2016; Mamo et al. 2015; Steffenson et al. 2017).

### **Genetics of Disease Resistance**

The classical view of how a plant host and pathogen interact at the genetic level was first described by Flor (1942) in the flax rust pathosystem (*Linus usitatissimum-Melampsora lini*). Flor (1942) demonstrated that host resistance to a pathogen is governed by genes that interact with pathogen virulence genes. Flor (1942) demonstrated this through inheritance studies on both the host and the pathogen side, showing that resistance and avirulence were both inherited as dominant single genes. We now know that in this system the resistance genes were producing proteins of the nucleotide binding site-leucine-rich repeat (NBS-LRR) class, a common motif of resistance proteins produced by resistance genes (Jones and Dangl 2006). Flor (1942) was successful in his research to dissect the host-parasite genetics of the flax rust pathosystem due, in part, to the fact that these genes were simply inherited and resistance proteins were interacting directly with the pathogen avirulence proteins (Ellis et al. 2007). These avirulence proteins are virulence factors or effectors, which aid in the infection of plants that do not have the corresponding resistance proteins. This is broadly categorized as a direct interaction model of plant immunity (Jones and Dangl 2006). Additional models for plant immunity

are the guard and decoy models (Dangl and Jones 2001; Van Der Biezen and Jones 1998; van der Hoorn and Kamoun 2008). Under the guard model, the plant resistance protein monitors the targets of the pathogen effectors and elicits a resistance response if modification of those targets is detected. Under the decoy model, modification of decoy effector targets by pathogen effectors elicits a resistance response. All three models are part of the Effector-Triggered Immunity (ETI) response. Typically, ETI triggers a Hypersensitive Reaction (HR), resulting in death of the infected tissue and the slowing of pathogen growth or the complete halting of infection. This type of response is usually effective against biotrophic pathogens that require a living host. However, such a response may not be as effective against necrotrophic pathogens that feed from dead tissue, where resistance to toxins or other pathogen effectors that kill or degrade plant tissue may be more important (Glazebrook 2005).

The most basic level of disease resistance in plants starts before ETI, with non-pathogen induced defense. At this initial stage of infection, plants may also perceive the presence of the pathogen based on conserved molecules called Pathogen-Associated Molecular Patterns (PAMPs), two examples being chitin from fungi and flagellin from bacteria (Jones and Dangl 2006). PAMPs are detected by Pattern Recognition Receptors (PRRs) in plants. These PRRs can then elicit PAMP-Triggered Immunity (PTI), which is a basal level of resistance sufficient to halt infection in non-host plants by non-adapted pathogens. In contrast to PTI, ETI is a more specific response between adapted pathogens and host plants (Dangl and Jones 2001; Jones and Dangl 2006).

### **Stem Rust Biology**

The stem rust pathogen, *Puccinia graminis*, is a biotrophic fungal parasite, causing disease in several small grain species including wheat (*Triticum* spp. L.), barley, rye (*Secale cereale* L.), oat (*Avena sativa* L.), and triticale ( $\times$ *Triticosecale*) (Bushnell and Roelfs 1985). *Puccinia graminis* is a heteroecious macrocyclic rust, producing urediniospores, teliospores, and basidiospores on the grass host and pycniospores and



aeciospores on the alternate hosts of *Berberis* spp. and *Mahonia* spp. (Jin et al. 2014; Leonard and Szabo 2005). Only the urediniospores and aeciospores are able to infect grasses, the former being most important for disease epidemics and the latter for initiation of infection early in the growing season in regions where the alternate host is present. Aeciospores represent an avenue for new virulence combinations to develop. The aeciospores arise from pycniospores, which in turn arise from basidiospores produced after karyogamy of the dikaryotic teliospores. During karyogamy meiosis can occur, which can lead to new to new virulence combinations. Therefore, aeciospores represent an avenue for new virulence development.

Although stem rust occurs wherever wheat is grown, disease development is favored in regions with frequent dew formation and warm weather (18-30°C)(Leonard and Szabo 2005). Urediniospores are the primary spores that cause disease and will only germinate in the presence of water or dew on the plant surface, requiring in addition, light for penetration into the stomata. Stem rust infection reduces plant vigor, causes lodging, and significantly reduces the yield of infected plants (Roelfs and Bushnell 1985). Total crop failure is possible under high disease pressure (Bushnell and Roelfs 1985; Leonard and Szabo 2005). Fungal haustoria ramify throughout the plant tissue, and then uredinia emerge and rupture leaf or stem tissue. Loss of epidermal tissue reduces the plant's ability to regulate transpiration, causing uncontrolled water loss and reduced ability to move water and nutrients in the plant vascular system, thus greatly reducing plant vigor (Leonard and Szabo 2005). Uredinia are somewhat oval in shape and occur primarily on the stems and leaf sheaths of plants, but also on the leaves, glumes, and awns.

### **Races of Stem Rust**

Several different *forma specialis* of *Puccinia graminis* exist based on the primary hosts they can infect. Both the wheat form, *Puccinia graminis* Pers.:Pers. f. sp. *tritici* Eriks. & E. Henn. (*Pgt*) and the rye form, *Puccinia graminis* Pers.:Pers. f. sp. *secalis* Eriks. & E. Henn. (*Pgs*), can infect barley (Roelfs 1982). Historically, the former has caused more

significant losses in both wheat and barley (Steffenson 1992). Isolates of *Pgt* are phenotyped for virulence and avirulence on a set of common wheat differential genotypes carrying different resistance genes (Leonard and Szabo 2005; Roelfs and Bushnell 1985). This forms the basis for assigning different isolates of *Puccinia graminis* into races based on their reaction pattern on this common set of wheat lines. In North America, the *Pgt* stem rust differential set consists of 20 wheat lines, which are divided into five groups of four lines each (Jin et al. 2008; Roelfs 1988; Roelfs et al. 1991). The virulence pattern on each group of four is represented by a unique alphabetical consonant, creating a five-letter code for race names, e.g. TTKSK, MCCFC, QCCJB, and HKHJC. At this time, the rye *Pgs* differential set is not available (Tan et al. 1976). Avirulence and virulence is assigned for *Pgt* isolates based on the reaction of the 20 differential wheat lines. Reactions are scored based on a 0-4 Infection Type (IT) scale, where ITs of 0 to 2 are considered indicative of pathogen avirulence (i.e. an incompatible response) and ITs of 3 to 4 of pathogen virulence (i.e. a compatible response) (Stakman et al. 1962). There is no comparable differential host set of barley for differentiating isolates of *Pgt* or *Pgs*. Instead, races of *Pgt* are typed for their virulence pattern on the aforementioned wheat differential hosts. However, the virulence pattern of these stem rust pathogens on the barley resistance genes of *Rpg1* and/or *rpg4/Rpg5* is considered important for resistance breeding in the crop. Races TTKSK and QCCJB are virulent for *Rpg1* and avirulent for *rpg4/Rpg5*. HKHJC is the only known race which is virulent for *rpg4/Rpg5*, but is avirulent for *Rpg1*. Finally, race MCCFC is avirulent for both *Rpg1* and *rpg4/Rpg5* (Steffenson 1992; Steffenson and Jin 2006; Sun and Steffenson 2005).

### **History of Stem Rust on Barley**

Stem rust is one of the most devastating diseases of small grains (Roelfs and Bushnell 1985). References to stem rust infection date back several millenia. The ancient Romans held a festival called the Robigalia to appease the rust god Robigus from sending the “plague” onto their crops (Chester 1946; Leonard and Szabo 2005). Historically, this disease has been one of the greatest biotic threats to wheat production in the Great Plains

region of North America (Roelfs and Bushnell 1985). Stem rust can make the production of susceptible cultivars impossible in regions with favorable environmental conditions. On barley, stem rust is less frequent and less severe than on wheat (Steffenson 1992). However, the disease does impact barley production in many regions of the world. Yield losses have been reported on barley in the United States, Canada, Australia, Kenya, Kazakhstan, Switzerland and other parts of Europe. (Dill-Macky et al. 1990; Mwando et al. 2012; Oehler 1950; Roelfs 1978; Schilperoord 2013; Steffenson 1992; Steffenson et al. 2016; Turuspekov et al. 2016).

Sporadic stem rust epidemics have occurred in North America as significant losses were reported during the 1920's and the 1930's (Roelfs 1978; Steffenson 1992). In Australia and in North America in the 1990's, stem rust epidemics occurred, resulting in losses up to 50% in isolated fields (Dill-Macky et al. 1990; Roelfs et al. 1991; Roelfs et al. 1990). However, stem rust losses as high as these are typically infrequent and occur only when there is a large inoculum load coming from a susceptible wheat crop. Yield losses alone are not the primary risk to barley from stem rust as even moderate infection levels can reduce kernel plumpness, kernel weight, and germination, all factors which may cause the crop to fail malting grade specifications and receive a discounted price at the grain elevator (Dill-Macky et al. 1990).

In most previous cases of widespread stem rust infection on barley, a susceptible wheat crop generating ample inoculum was present (Dill-Macky et al. 1990; Roelfs 1978; Steffenson 1992). During the epidemics of the 1920's and 1930's in North America, major losses were reported in both the United States and Canada, where up to 50% yield loss was reported on wheat in North Dakota and Minnesota during the *Pgt* race 56 (MCCFC) epidemics. During this epidemic, losses to barley were as high as 15% (Roelfs 1978). This spurred research into stem rust resistance of barley (Steffenson 1992). In the 1950's when wheat again suffered another epidemic due to race 15B (TPMKC), barley did not suffer any measurable yield loss because many cultivars in the region carried the *Rpg1* resistance gene. However, in the late 1980s, a race (QCC, now designated QCCJB)

with virulence for *Rpg1* emerged (Roelfs et al. 1991; Steffenson 1992). From 1989 to 1993, QCCJB was the most common race in the United States stem rust population (Jin 2005; Roelfs et al. 1990; Roelfs et al. 1993). During this time, stem rust losses on barley were widespread in Minnesota and North Dakota with up to 60% yield loss reported in isolated fields. Only a few winter wheat varieties in the central Great Plains were susceptible to race QCCJB. Once these cultivars were removed from production, race QCCJB became a rare component of the United States stem rust population (Jin 2005). Since the early 1990s, race QCCJB has only been detected once (in 2011 from North Dakota), and barley losses from stem rust have been minimal (USDA-ARS Cereal Disease Lab, Cereal Rust Bulletin 2003-current).

### **Breeding for Stem Rust Resistance in Barley**

Breeding for resistance to stem rust in barley has been an emphasis only in regions where stem rust infection has occurred with some frequency in the past (Friedt et al. 2010). Breeding for rust resistance in cereals focused on two major types: adult plant resistance (APR) and seedling (often called all-stage or major gene resistance) resistance (Ellis et al. 2014; Roelfs and Bushnell 1985). Seedling resistance is typically controlled by one or a few genes with major effects that can be readily discerned at the seedling stage. This type of resistance often remains effective through to the adult plant stage. Typically seedling resistance is associated with a HR characterized by chlorotic and/or necrotic flecks and greatly reduced uredinia, if they develop at all (Leonard and Szabo 2005). Additionally, seedling resistance is often “race-specific” conferring high levels of resistance to a defined set of races. This differs from APR, which is typically only effective at the adult plant stage and recognized by a reduced disease severity or slower rate of disease development (Ellis et al. 2014; Roelfs and Bushnell 1985). APR is typically effective against a broad spectrum of races and as such is thought to be more durable (Johnson 1984). However, there are reported cases where APR has lost effectiveness (Krattinger et al. 2013; Yildirim et al. 2012). As APR is expressed as a quantitative trait, breeding for it is more complex and is typically done in the field. APR and seedling resistance can both

be durable and also confer a high level of resistance. The complexity of breeding for these two types of resistance is markedly different. An example of this contrasting complexity can be found in comparing stem rust resistance breeding in barley in the Upper Midwest versus what is done for wheat at the Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT). In the Upper Midwest, breeders have incorporated the single gene *Rpg1* into all six-rowed (and some two-rowed) barleys over the last 70 years. The *Rpg1* gene has been highly effective and durable and is easy to select and retain in breeding germplasm (Steffenson 1992). The CIMMYT wheat breeding program has achieved resistance approaching “near-immune” levels by combining 4-5 minor effect genes for APR. However, selection for such types is complex and requires very large population sizes (Singh et al. 2014). In barley, most stem rust resistance breeding has focused on seedling resistance conferred by *Rpg1* and to some extent *rpg4/Rpg5*, both of which are highly effective seedling genes.

A successful resistance gene deployment strategy is important for long-term disease control. Several different deployment strategies have been tried in cereal crops. The most basic is the deployment of single seedling resistance genes. This deployment strategy has the benefits of being simple and quick to achieve because such resistance is easy to select for due to its large phenotypic effect and straightforward introgression due to its simple Mendelian inheritance (Johnson 1984; Kolmer et al. 2009). However, genes deployed using this method are vulnerable to being overcome by single step virulence changes in the pathogen, especially when a monoculture of a cultivar is sown over a wide area. Single gene resistance has been overcome in as little as three years after deployment in some rust pathosystems under high disease pressure (Chen 2005). Although *Rpg1* in barley is widely effective against many *Pgt* races and has protected barley from widespread losses for almost 70 years, the underlying basis of its durability has been aided by a variety of factors unique to North America, including the elimination of the sexual host for *Pgt* in most production areas, a largely resistant wheat crop, and barley’s mostly northern production range and early maturity time (Steffenson 1992). A better strategy is to deploy multiple seedling resistance genes in stacks or pyramids in the same

cultivar. This method has been widely adopted in most cereal rust breeding programs and has been highly effective at protecting wheat from stem rust losses in regions of North America and Australia that are prone to stem rust infection (Line and Chen 1995; Park 2007; Roelfs 1988). The deployment of this strategy is dependent on having a relatively large number of seedling genes to breed with and also markers to aid in the stacking since phenotypic selection of each individual gene is not possible because multiple genes mask the effect of each other (Ellis et al. 2014). APR deployment is relatively simple because selection can be made in the field, and lines with the highest level of resistance are then advanced in the program and ultimately released as a cultivar (Kolmer et al. 2009). However, achieving high levels of APR in a cultivar release candidate may require very large breeding populations (Singh et al. 2014). APR can be simply inherited in some cases. Such is the case with the stem rust resistance gene *Sr2* in wheat and leaf rust resistance gene *Rph20* in barley, both of which do not confer seedling resistance (Hickey et al. 2011; Mago et al. 2011). In these cases, linked molecular markers can greatly facilitate the efficient transfer of such APR (Hickey et al. 2011; Mago et al. 2011). The best strategy for long-term disease control is the deployment of both seedling and APR together. However, selection for these types of resistance may be difficult because seedling resistance genes may mask the effects of APR. For this reason, it is important to have molecular markers for the selection of APR in combination with seedling resistance genes.

### **Resistance Gene *Rpg1***

The stem rust resistance gene *Rpg1* (originally designated T) was the first gene described in barley for reaction to *Pgt* (Powers and Hines 1933; Shands 1939) and was derived from the Swiss landraces of Chevron (PI 38061) and Peatland (CIho 2613) (Steffenson 1992). *Rpg1* was mapped at about 7 cM on the short arm of chromosome 7H (Dahleen et al. 2003; Kleinhofs et al. 1993; Steffenson 1992; Steffenson et al. 1985). This gene has been used extensively in breeding in several United States (University of Minnesota and North Dakota State University) and Canadian (Agriculture and Agri-Food Canada-

Manitoba and The Crop Development Centre at the University of Saskatchewan) breeding programs. Although used in breeding now for almost 70 years, *Rpg1* continues to be effective against the majority of *Pgt* races in the United States (CDL 2016). This durability is aided by several factors, including the largely resistant United States wheat crop, which reduces the overall inoculum load on barley and selection pressure for *Rpg1* virulence; the early maturity of barley and its mostly northern production range, which can effectively limit stem rust build-up on the crop; and the removal of the alternate host barberry, which has greatly reduced the evolutionary potential of the pathogen (Steffenson 1992). *Rpg1* was the first stem rust resistance gene cloned from a small grain cereal crop and encodes a receptor kinase-like protein with tandem kinase domains (Brueggeman et al. 2002). As such, there are perfect markers to aid in the selection of *Rpg1* in breeding programs (Derevnina et al. 2014; Eckstein et al. 2003).

### **Resistance Gene Complex *rpg4/Rpg5***

In the late 1980s, a *Pgt* race (QCCJB) with virulence for *Rpg1* was detected in the Great Plains region and caused minor scattered epidemics on barley in the United States and Canada (Martens et al. 1989; Roelfs et al. 1991; Roelfs et al. 1990; Steffenson 1992). In reaction to this epidemic, new research efforts were initiated to identify sources of resistance, and the accession Q21861 (PI 584766) was found to carry high levels of resistance at the seedling and adult plant stage to race QCCJB (Dill-Macky et al. 1990; Dill-Macky and Rees 1992; Jin et al. 1994a; Jin et al. 1994b). Q21861 is a breeding line jointly from the International Center for Agricultural Research in the Dry Areas (ICARDA) and CIMMYT programs, and was selected for its agronomic phenotype in Mexico, and later observed as being highly resistant to stem rust at the adult plant and seedling stage in Queensland, Australia (Dill-Macky and Rees 1992). The pedigree of Q21861 is unknown. In a genetic study conducted on Q21861, a recessive gene (*rpg4*) was found to confer resistance to race QCCJB (Jin et al. 1994b). Later it was shown that *Rpg5*, a gene very closely linked to *rpg4*, conferred resistance to *Pgs* (Sun et al. 1996). Both *rpg4* and *Rpg5* are tightly linked on the long arm of chromosome 5H (145-155 cM),

and therefore are collectively known as the *rpg4/Rpg5* gene complex (Brueggeman et al. 2008; Brueggeman et al. 2009; Mamo et al. 2015; Mirlohi et al. 2008; Steffenson et al. 2009). Resistance to *Pgs* is mediated by *Rpg5*, which confers resistance to this *forma specialis* of *Puccinia graminis* independently of *rpg4* and the associated *Pgt* resistance (Brueggeman et al. 2008; Brueggeman et al. 2009). However, resistance to *Pgt* at this locus requires both *rpg4* and *Rpg5* (Arora et al. 2013; Wang et al. 2013). Positional cloning of *rpg4* has not yet been achieved--due to the complexity of the locus that may include at least four genes. However, genetic dissection of the *Rpg5* locus has identified at least three genes required for resistance: *Rpg5*, which encodes an NBS-LRR with a serine threonine protein kinase domain gene; *HvRga1*, encoding a protein with an NBS-LRR motif; and *HvAdf3*, a actin depolymerization factor-like gene. Q21861 has been used as a parent to introgress the *rpg4/Rpg5* gene complex in some United States and Canadian breeding programs. Although efforts have been expended in barley to enhance resistance against stem rust races with *Rpg1* virulence, only a small fraction of North American barley breeding germplasm is resistant to TTKSK or QCCJB at the seedling or adult plant stage (Steffenson et al. 2013; Zhou et al. 2014). Since *Rpg5* has been cloned, there are perfect markers for this gene that can aid in breeding (Brueggeman et al. 2008; Brueggeman et al. 2009; Wang et al. 2013). However, sequencing for the LRR domain of this gene is required to detect two rare non-functional, non-synonymous mutations in the kinase domain of *Rpg5* (Arora et al. 2013).

### **Other Resistance Genes**

Aside from *Rpg1* and *rpg4/Rpg5*, very little is known about other *Rpg* genes in barley. These other resistance genes include *Rpg2*, *Rpg3*, *rpg6*, *rpgBH* and *RpgU*. *Rpg2* is derived from Hietpas-5 (CIho 7124)(Patterson et al. 1957) and *Rpg3* from an Ethiopian landrace GAW-79 (PI 382313) (Jedel 1991; Jedel et al. 1989). These two genes confer a relatively high level of APR to *Pgt*, but exhibit little seedling resistance (Fox and Harder 1995; Franckowiak 1991; Franckowiak and Steffenson 1997; Jin et al. 1994a; Miller and Lambert 1955; Shands 1964; Sun and Steffenson 2005). The most recently identified *Rpg*



gene was derived from an introgression of *Hordeum bulbosum* into cultivated barley. This gene was transferred into line 212Y1, confers seedling resistance to race QCCJB, is recessive acting, and named *rpg6* (Fetch et al. 2009). However, this gene is ineffective against TTKSK at the seedling stage. Several provisionally designated genes for stem rust resistance have been identified, including *RpgU* from Peatland for APR to QCCJB and *rpgBH* from Black Hulless (CIho 666) for APR to *Pgs* (Fox and Harder 1995; Steffenson et al. 1984).

### **Stem Rust QTLs**

In addition to the genes mentioned above, a number of quantitative trait loci (QTL) for stem rust resistance have been identified by either genome-wide association study (GWAS) or bi-parental mapping. QTL for stem rust resistance to *Pgt* and/or *Pgs* have been identified on every chromosome of barley (Appendix A1)(Mamo 2013; Mamo et al. 2015; Moscou et al. 2011; Steffenson et al. 2007; Turuspekov et al. 2016; Zhou et al. 2014). Most of these studies have focused on resistance to race TTKSK at the seedling or adult stage with the exception of Turuspekov et al. (2016) and Steffenson et al. (2007) who focused on local races of *Pgt* in Kazakhstan and seedling resistance to *Pgt* races MCCFC and QCCJB and *Pgs* isolate 92-MN-90, respectively. The dataset generated by Steffenson et al. (2007) has been expanded to include *Pgt* races TTKSK and HKHJC in a GWAS based on high-density markers generated by genotype by sequencing (GBS) (Sallam et al. 2017). In the majority of these studies, a major effect QTL for TTKSK seedling resistance or APR was due to the *rpg4/Rpg5* gene complex on chromosome 5H (Mamo 2013; Mamo et al. 2015; Steffenson et al. 2009).

Aside from *rpg4/Rpg5*, other QTL for TTKSK resistance have been identified in barley; however, their effects are not as great as that conferred by the *rpg4/Rpg5* complex (Mamo 2013; Zhou et al. 2014). Zhou et al. (2014) found only a few lines resistant to race TTKSK at either the seedling or adult plant stage that was not due to *rpg4/Rpg5* from the evaluation of a large collection of United States barley breeding germplasm.

Additionally, when Mamo (2013) evaluated diverse barley landraces from Ethiopia and Eritrea, he found only one minor effect QTL for seedling resistance and none for APR to race TTKSK. The extreme vulnerability of *Hordeum* to race TTKSK was also demonstrated by Steffenson et al. (2017) who found that 96% of the 2,914 cultivated and wild barley accessions tested at the seedling stage were susceptible. Of the few resistant accessions identified from this study, most carried the *rpg4/Rpg5* gene complex. Turuspekov et al. (2016) mapped QTL for APR to Kazakhstan *Pgt* races on every chromosome; however, they were significant only at the pre-heading stage. Steffenson et al. (2007) mapped QTLs for seedling resistance to *Pgt* races MCCFC and QCCJB and *Pgs* isolate 92-MN-90 in the Wild Barley Diversity Collection, an assemblage of 318 accessions of *H. vulgare* ssp. *spontaneum* from across its native range. They found QTLs for race MCCFC seedling resistance on chromosomes 1H, 5H, and 6H. The one QTL on chromosome 5H mapped close to the *rpg4/Rpg5* gene complex. A QTL for combined resistance to *Pgt* race MCCFC and *Pgs* isolate 92-MN-90 was found on 3H at 15 cM, whereas two different QTLs for QCCJB resistance were found at about 50 and 150 cM on this chromosome. This, in addition to the results from the study by Sallam and Steffenson (2017), suggests that wild barley is an important resource for stem rust resistance QTLs. It is not known whether the QTL found in wild barley may also be present in cultivated barley, as was the case for *rpg4/Rpg5* (Steffenson et al. 2017; Steffenson et al. 2016). *Rpg1* has yet to be found in wild barley, suggesting the two taxa may not always share the same resistance genes. Stem rust resistance may have evolved in wild barley in response to its proximity to *Pgt*-infected barberry as accessions from Central Asia comprise the bulk of resistant accessions identified, this region being the native range of *Berberis* (Berlin et al. 2015; Hulse et al. 2016; Steffenson et al. 2016).

With respect to APR, Mamo (2013) is the only investigator to map APR QTL to a race other than TTKSK. In GWAS analysis of race MCCFC resistance in Ethiopian and Eritrean barley landraces, Mamo (2013) identified a QTL on chromosome 5H for APR, which was not located near *rpg4/Rpg5*. Remarkably, this QTL is near one found by Zhou et al. (2014) for TTKSK APR, suggesting this region could hold effective APR genes

against multiple races of *Pgt*.

## **Introduction to Genetic Mapping**

The identification of new resistance genes in crop plants is accomplished through an application of basic statistics, knowledge of genetic ratios, and also a bit of luck. Genes at independent loci in the genome will segregate in ratios described by Mendel (1866) for independent assortment. Morgan (1910) expanded on Mendel's theory and noted that recombination frequencies between two traits are related to their physical distance to each other and so began the idea of mapping the location of loci controlling traits (Sturtevant 1913). Indeed, the seminal importance of Morgan's early work is why the unit of recombination frequency is called a centiMorgan (cM). In general, this is the principal behind genetic mapping. We now know these traits are controlled by genes, the majority of which are located on chromosomes in the nucleus (in eukaryotes) (Klug et al. 2007; Watson and Crick 1953). When two genes are located on different chromosomes, or very distantly separated on the same chromosome, recombination between them will occur more frequently than recombination between genes located close together (Sleper and Poehlman 2006). Recombination between two linked genes occurs during prophase I of meiosis when homologous chromosomes pair and chiasmata form, exchanging DNA between non-sister chromatids (Klug et al. 2007). When recombination occurs, half of the resulting gametes will be recombinant. This is the basis for the expectation that linked genes must have a recombination frequency of no more than 0.5 (Sleper and Poehlman 2006). Linkage mapping can then be done using genetic markers to track recombination and therefore estimate statistical distances between points in the genome (Collard et al. 2005; Druka et al. 2010). Statistical distance will be related to, but not the same as, physical distance and will be inflated in regions of the genome with high recombination frequency (Collard et al. 2005; Klug et al. 2007).

## **Linkage Estimation**

Loci are linked when they are inherited physically on the same chromosome (Klug et al.

2007). Statistical linkage occurs when the fraction of recombinant loci is  $<0.5$  (Sleper and Poehlman 2006). A chi-square test can then be performed to test if loci deviate from this expectation. If the null hypothesis is rejected, then the two loci are said to be linked (Griffiths 2005). The recombination fraction between two loci is related to the physical distance between them, with recombinant types occurring more frequently as the distance between loci increase. However, as crossing over is not evenly distributed in the genome, the recombination frequency between loci is only a relative measure of physical distance (Klug et al. 2007). Recombination fraction is then converted to percent recombination expressed as cM, where 10 cM is equal to a recombination fraction of 0.1 (Collard et al. 2005). A variety of calculation methods are available to help increase the precision of cM distances by taking into account the biological effects of crossing over. The Haldane method takes into account unobserved double crossover events and is calculated with the formula below (Haldane 1919):

$$d = -\left(\frac{1}{2}\right) \ln(1 - 2r).$$

In this expression,  $d$  = map distance in cM and  $r$  = recombination fraction between two loci. The Kosambi function expands on the Haldane function and takes into account crossover interference, a phenomenon whereby one crossover event interferes with the coincident occurrence of another crossover event in a homologous pair of chromosomes (Collard et al. 2005; Kosambi 1943). The Kosambi function is given by the following expression:

$$d = -\left(\frac{1}{4}\right) \ln\left(\frac{1 + 2r}{1 - 2r}\right).$$

However, these methods may be unnecessary with the advent high-density molecular marker maps (Collard et al. 2005). The strength of linkage between markers is computed as a logarithm of odds score (LOD) (Risch 1992) as given below:

$$LOD = \text{Log}_{10} \frac{\textit{probability observation due to linkage}}{\textit{probability observation due to random assortment}} .$$

When many markers are linked together, they are referred to as comprising a linkage group and represent an individual chromosome (Collard et al. 2005). A minimum LOD cutoff value of 3 is usually used as a threshold value for creating linkage groups (Collard et al. 2005; Druka et al. 2010). To assign order to the group, methods such as maximum likelihood mapping and regression mapping are used (van Ooijen 2006). However, when the number of markers on a map becomes large (>5,000), these methods become computationally intensive. To deal with such large marker sets, methods such as the Minimum Spanning Tree (MST) method can be used (Wu et al. 2008). This method computes the order of markers based on the minimum spanning tree of a graph of the vectors of markers. This method was found to outperform other methods when datasets were large, but worked equally well with smaller datasets and was computationally much more efficient.

## **Genetic Markers**

There are three major classes of markers used in plants: morphological, biochemical, and DNA (Collard et al. 2005). Morphological markers are those that have a phenotypic effect on the plant (e.g. flower color), are visually assessed, and may be affected by the environment. Biochemical markers, such as isozymes, are more robust and plentiful, but also may be affected by the environment (Winter and Kahl 1995). The most commonly used marker system is based on DNA. There is a wide variety of DNA markers which can be utilized, including random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), simple sequence repeats (SSR), and single nucleotide polymorphism (SNP) (Druka et al. 2010; Somers and Humphreys 2009). SNP markers in barley have become very common with the release of genotyping arrays featuring SNPs developed by Close et al. (2009). These SNP arrays are popular because of the low-cost and very high density of markers that are generated. However, such arrays require upfront development and can have bias, for example when the SNPs are designed based on a single reference genotype. With continued advancements of high-throughput sequencing methods and publications

of high quality reference genomes, it has become possible to GBS individuals (Davey et al. 2011; International Barley Genome Sequencing et al. 2012; Mascher et al. 2017). In brief, GBS involves sequencing to find polymorphisms between individuals, using a reference genome to anchor sequences (Davey et al. 2011). GBS usually includes a complexity reduction step using restriction enzymes to create fragments of reduced length, and then these fragments are aligned to the reference genome (Poland et al. 2012). SNPs discovered by GBS may have the advantage of not being biased by a reference sample, and many can be discovered without the need to create a SNP array. However, a major drawback to GBS is that it requires a high quality reference genome sequence and expertise in sequence analysis and alignment. Inheritance of markers can then be used to create linkage maps and assign marker trait associations. Inheritance can be either directed as in the case of a bi-parental mapping population or be inferred based on shared ancestry, as is the case in GWAS panels.

### **Bi-parental Mapping Populations**

Mapping populations can be created by crossing two parents to create a segregating population, i.e. bi-parental mapping (Collard et al. 2005; Graner et al. 2010). Mapping can be done in either early (e.g.  $F_2$  and  $F_{2:3}$  families) or in later generations (e.g.  $F_5$  or later) after many cycles of inbreeding (recombinant inbred line (RIL) development) if the species can tolerate this process (Schneider 2005). The advantages of using RILs over early generation families are that the populations are “immortal” and replicated testing of genetically fixed lines can be conducted. One drawback to RILs is that they take a long time to develop. The production of double haploid (DH) lines is another method for generating genetically fixed lines. Using this method, chromosomes from  $F_2$  plants are artificially doubled, thereby generating immortal homozygous lines rapidly (Somers and Humphreys 2009). However, chromosome doubling may be technically difficult, and linkage disequilibrium (LD) may extend for longer distances in DH lines over RILs as fewer meiosis cycles have occurred. Extensions of these two basic types of populations are created if additional crosses are made to one parent, creating a backcrossed

population (Tanksley and Nelson 1996). The advantages of backcross populations are that if one parent is unadapted, the resulting population will be better adapted to the target environment, thereby allowing better phenotyping. Such populations allow for the simultaneous identification of QTL and progress toward cultivar development (Tanksley and Nelson 1996). However, additional crossing to one parent will increase the proportion of that parent's alleles, which may then require larger populations to detect significant QTL (Collard et al. 2005). The advantages of bi-parental mapping are that the pedigree is known, and all parental alleles are at a known frequency in the population. Thus, there is no constraint regarding the low frequency of beneficial alleles hampering detection of QTL. However, in bi-parental mapping populations, all lines are only a few generations advanced from the original cross and therefore only a few cycles of recombination have occurred, resulting in a larger extent of LD between causal genes and significantly associated markers (Rafalski 2010). There are several drawbacks to bi-parental mapping. The most significant being that effects of QTLs may be over estimated in the population they are detected in, and may not be as effective when transferred to other backgrounds in a breeding context (Bernardo 2008). Other drawbacks include the significant up front work required to identify the best mapping parents, the time needed to create a segregating population, and the ability to detect only two loci at a locus.

QTL can be detected using a variety of methods, the simplest of which is single marker analysis, which uses regression to assign significance between the trait of interest and marker haplotypes (Collard et al. 2005; Pierce 2005). Interval mapping is a more precise method of QTL mapping because it considers the linkage map as well as the intervals between markers on the map to define pseudo-marker regions and test significance of the intervals between markers (Lander and Botstein 1989). Composite interval mapping is an extension of interval mapping and includes additional markers other than those in the region being tested to control for background QTL effects. (Collard et al. 2005; Zeng 1993, 1994). QTL are then assigned as significant if their LOD values exceed a predetermined cutoff value (Collard et al. 2005). LOD cutoff values are usually assigned as  $LOD > 3$  or can be assigned using a permutation test, where marker and trait

relationships are randomly assigned, and QTL analysis is conducted to empirically estimate a type I error. In this latter analysis, between 500 to 1,000 replications are typically used when estimating this error (Churchill and Doerge 1994).

### **Genome-Wide Association Study**

GWAS is an alternative to bi-parental mapping that uses preexisting germplasm panels to detect associations between markers and traits of interest (Myles et al. 2009; Waugh et al. 2009; Yu and Buckler 2006). GWAS utilizes historical recombination within populations, which reduces LD, resulting in greater resolution and ability to detect significant QTL--if the density of markers is sufficient (Rafalski 2010). GWAS is based on LD, which is the non-independence of alleles in haplotypes of a population (Flint-Garcia et al. 2003; Gaut 2002). LD between alleles can result from linkage or by population drift. LD can be described using  $D'$  or  $r^2$  with the following equations:

$$D' = \frac{D}{D_{max}} \text{ if } D \geq 0$$

$$D' = \frac{D}{D_{min}} \text{ if } D < 0$$

$$r^2 = \frac{D^2}{p_1 p_2 q_1 q_2}$$

$$D = A_1 B_1 - p_1 q_1 .$$

$$A_x B_x = \text{frequency of } A_x B_x \text{ haplotype}$$

$$p_x = \text{frequency of } A_x B_y + A_x B_x \text{ haplotype}$$

$$q_x = \text{frequency of } A_x B_x + A_y B_x \text{ haplotype}$$

$$D_{max} = \max(p_1 q_1, q_2 p_1)$$



$$D_{min} = \max(p_1 q_1, q_2 p_1) .$$

Values of  $D'$  and  $r^2$  range between 0 and 1, which represent no LD to complete LD, respectively. LD calculated by  $r^2$  has the advantage of being more robust to low allele frequency as compared to  $D'$  and as such is the most commonly used estimate of LD in plants (Flint-Garcia et al. 2003; Gupta et al. 2005). Usually,  $r^2$  values of 0.1 or 0.2 are considered the minimum thresholds for declaring significant associations between pairs of loci (Zhu et al. 2008). LD can be caused by physical linkage, but also by population structure due to population drift and non-random mating between individuals in the population (Pritchard et al. 2000a; Pritchard et al. 2000b). If population structure is not corrected, it may lead to spurious associations due to heterogeneity of genetic backgrounds (Ziv and Burchard 2003). Statistical analysis methods have been developed to correct for population structure. The two main methods are structured association and Principal Components Analysis (PCA) (Graner et al. 2010). Corrections for structured associations using subpopulation estimations can be generated by a program called STRUCTURE that estimates the number of sub-populations and the probability of assignment to each population (Pritchard et al. 2000a; Pritchard et al. 2000b). However, STRUCTURE is computationally intensive. Population structure analysis by PCA has been shown to yield results similar to STRUCTURE and requires far less computational resources (Patterson et al. 2006; Price et al. 2006; Zhu et al. 2002). PCA is a spatial analysis technique, which attempts to reduce the dimensionality of data while retaining variation (Druka et al. 2010).

To determine significant marker-trait associations in GWAS, it is necessary to account for both population structure and also relatedness between individuals (known as kinship), otherwise false associations are found (Price et al. 2010; Yu et al. 2005). A common method for doing this is using mixed linear models (MLM) to assign  $p$ -values to marker-trait associations while taking into account both population structure (Q matrix)

and kinship (K matrix).

## **Chapter 2**

# **Genome-Wide Association Study of Stem Rust Resistance in a World Collection of Cultivated Barley**

## Introduction

Widely grown in North America and worldwide, barley (*Hordeum vulgare* ssp. *vulgare* L.) is an important cereal crop because of its wide array of end use products and ability to thrive in diverse climates (Newton et al. 2011). In 2015, approximately 1 million hectares of barley were harvested in the United States, the majority of which was malting barley used for beer production (USDA-NASS 2015). Stem rust, a relatively common disease of barley, is capable of destroying large plantings of the crop in a short period of time. Historically, this disease has been one of the major limiting factors of cereal crop production in the Great Plains region of North America. Although wheat (*Triticum* spp.) is typically more impacted by stem rust infection, barley can also suffer severe damage, particularly when infection occurs early in the growing season. Yield losses due to stem rust in barley have been reported in North America, Australia, eastern Africa, southern Africa, Europe, South America, Central Asia, southern Asia, and South America (Dill-Macky et al. 1990; Mwando et al. 2012; Oehler 1950; Roelfs 1978; Schilperoord 2013; Steffenson 1992; Steffenson et al. 2017; Steffenson et al. 2016; Turuspekov et al. 2016). During the stem rust epidemics in North America in the 1920's and 1930's, barley suffered significantly with yield losses of 15-20% (Roelfs 1978; Steffenson 1992). In Australia, losses up to 50% were reported under experimental conditions (Dill-Macky et al. 1990). In addition to yield losses, the primary risk to barley production from stem rust infection is a reduction in malting quality. Stem rust infection reduces kernel plumpness, kernel weight, and germination among other malting quality factors (Dill-Macky et al. 1990). Economic losses from failure to make malting grade are greater than yield losses alone.

Stem rust of barley is caused by *Puccinia graminis* Pers.:Pers. f. sp. *tritici* Eriks. & E. Henn. (*Pgt*) (wheat stem rust pathogen) or *Puccinia graminis* Pers.:Pers. f. sp. *secalis* Eriks. & E. Henn. (*Pgs*) (rye stem rust pathogen) (Roelfs 1982). The Upper Midwest region of the United States is a major center for both barley production and also the malting and brewing industries. Unfortunately, the climate in this region is conducive for

stem rust development. However, effective control of this disease has been achieved for many decades due chiefly to the deployment of effective stem rust resistance in many barley cultivars (Steffenson 1992). Several key factors have contributed to the longevity of this resistance and in reducing the pathogen population and its evolutionary potential, including the planting of a largely resistant wheat crop; removal of barberry plants (*Berberis* spp., an alternate host for *Pgt* and *Pgs*) in close proximity to barley fields; and the rapid maturation of barley in the northern latitudes where the first stem rust infections usually occur late in the season (Steffenson 1992). The stem rust resistance provided by the *Rpg1* (Reaction to *Puccinia graminis* (*Rpg*)) gene has been durable and highly effective at protecting barley production from major losses since its first deployment in the 1940's (Steffenson 1992). However, a minor epidemic on barley in the United States and Canada occurred in the late 1980's when *Pgt* race QCC (Roelfs 1988; Roelfs et al. 1991) (now designated QCCJB on the expanded differential set by Jin et al. (2008)) overcame *Rpg1*, causing some losses on cultivars carrying this resistance. During this time period, QCCJB was the most common race of *Pgt* in the United States (Roelfs et al. 1991; Steffenson 1992). Race QCCJB became a rare component of the *Pgt* population following the removal of susceptible wheat cultivars from the central Great Plains in the early 1990's. This episode demonstrated the danger of races which acquire virulence on both wheat and barley (McVey et al. 2002). Although a concerted effort to identify and incorporate additional sources of resistance to stem rust has been ongoing in several breeding programs since the QCCJB epidemic, *Rpg1* continues to be the primary source of stem rust resistance in North American barley cultivars.

A current threat to barley and wheat production is the Ug99 group of *Pgt* races from Africa of which TTKSK (isolate synonym Ug99) was the first described. The Ug99 race group is virulent on more than 80% of the world's wheat and more than 95% of the world's barley cultivars (Singh et al. 2008; Steffenson et al. 2017). Ug99 group races are particularly dangerous because of their virulence for a large number of stem rust resistance genes in wheat as well as *Rpg1* in barley. Wide virulence on cultivars of both species is uncommon and exceptionally dangerous due to the possibility for cross species

infection and inoculum buildup.

Since the detection of race TTKSK in Uganda in 1998, research in stem rust resistance breeding and genetics has been greatly expanded in wheat. This has led to a large number of newly described stem rust resistance genes in the crop. Currently, there are at least 73 genes described for stem rust resistance in wheat, 39 of which are effective against races in the Ug99 group (Singh et al. 2015). In barley, research on stem rust resistance has also expanded, initially after the race QCCJB epidemics in the early 1990s and more recently with the emergence of the Ug99 race group. One of the first genes described for QCCJB resistance was *rpg4*, identified from the accession Q21861 (Dill-Macky et al. 1990; Dill-Macky and Rees 1992; Jin et al. 1994a; Jin et al. 1994b). Later, the *rpg4* locus was found to be complex, consisting of at least three genes working in concert including *Rpg5*, a gene conferring resistance to *Pgs* (Brueggeman et al. 2008; Brueggeman et al. 2009; Mirlohi et al. 2008; Steffenson et al. 2009). In barley, the *rpg4/Rpg5* gene complex confers effective all-stage resistance to many cultures of *Pgt* and *Pgs*, including those in the Ug99 race group (Steffenson et al. 2009; Steffenson et al. 2017). Other stem rust resistance genes in barley including *Rpg2*, *Rpg3*, *rpg6*, *rpgBH*, and *RpgU* are all ineffective against TTKSK at the seedling stage (Fetch et al. 2009; Fox and Harder 1995; Jedel 1991; Jedel et al. 1989; Patterson et al. 1957; Shands 1939; Steffenson et al. 2009; Steffenson et al. 2017; Steffenson et al. 1984). However, *Rpg2* and *Rpg3* do confer adult plant resistance as described in Chapter 4.

In addition to the identification of effective genes for resistance, a successful deployment strategy is also important for long-term disease control. Several different gene deployment schemes have been tested in cereal crops to the rust diseases. The first one utilized was the deployment of single Mendelian genes with large phenotypic effects in individual cultivars (Johnson 1984; Kolmer et al. 2009). Genes deployed under this strategy were often short lived, with the breakdown of resistance being reported in as little as three years in some cereal rust pathosystems (Chen 2005). A notable exception with barley is *Rpg1*, a gene that has protected the crop from significant stem rust losses

since the 1940's (Steffenson 1992). A better strategy is to deploy resistance genes in “stacks” or “pyramids” of three or more in a single cultivar (Pink 2002). This method has been highly effective for controlling stem rust of wheat for many years in regions of North America and Australia that are prone to stem rust infection (Line and Chen 1995; Park 2007; Roelfs 1988). An alternative method to the deployment of large effect resistance genes is the use of adult plant resistance (APR). APR is expressed mostly at the adult plant stage and is incomplete, thereby allowing for some infection by the pathogen but at a reduced rate or severity. The Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT) breeding program has been successful in developing wheat cultivars with APR to the rust pathogens by pyramiding four to five genes, achieving resistance levels approaching immunity (Singh et al. 2014). Deploying a combination of APR and major effect genes may be the best strategy for achieving durable resistance. However, this will likely require the introgression of five to seven genes in a single cultivar to achieve long-term durability (Bowden 2015). To transfer such combinations into breeding lines, the resistance genes must first be identified and ideally tagged with molecular markers to facilitate selection.

Broadly speaking, there are two conventional ways to identify loci associated with resistance in plants and tag them with molecular markers: bi-parental mapping and association mapping. Bi-parental mapping utilizes the co-segregation between marker alleles and phenotypes of interest to identify quantitative trait loci (QTL) that are associated with the trait (reviewed by Collard et al. (2005)). However, as the progeny from these types of studies are typically just a few generations advanced from the original cross, only a few cycles of recombination have occurred, resulting in a larger extent of linkage disequilibrium (LD) with identified QTL (reviewed by Rafalski (2010)). Additionally, bi-parental mapping can only investigate the effect of two alleles at a locus, and extensive upfront experiments must be performed to select the best candidates to create the populations. An alternative to bi-parental mapping is genome-wide association mapping or genome-wide association study (GWAS) (reviewed by Myles et al. 2009; Waugh et al. 2009; Yu and Buckler 2006). GWAS has the advantage of utilizing pre-

existing germplasm to form the panel for analysis. It also has the advantages of utilizing historical recombinations, resulting in a faster rate of LD decay than bi-parental mapping populations and therefore greater mapping resolution (Rafalski 2010). Recently, a GWAS approach was successfully employed to map QTL for stem rust resistance in barley (Mamo 2013; Turuspekov et al. 2016; Zhou 2011; Zhou et al. 2014).

Extensive efforts have been advanced to identify new sources of stem rust resistance in barley, but with limited success. Zhou et al. (2014) evaluated the seedling and APR of North American barley breeding germplasm to *Pgt* race TTKSK and found only a few accessions with resistance at either the seedling or adult plant stage. Using GWAS, Zhou et al. (2014) identified a QTL (*Rpg-qt1-7H-12\_30528*) on chromosome 7H at 49 cM for seedling resistance and a QTL (*Rpg-qt1-5H-11\_11355*) on chromosome 5H at 86 cM for APR (Appendix A1). Although *Rpg-qt1-5H-11\_11355* explained very little of the variation, it reduced disease severity by 21-55%. Some accessions carried moderate to high levels of resistance at the adult stage; however, none had levels of seedling and APR (i.e. all-stage resistance) approaching that of Q21861. Wamalwa et al. (2016) investigated the genetics of resistance in selected barley accessions investigated by Zhou et al. (2014) and found that seedling resistance to race TTKSK was inherited as a single dominant gene or as two genes in epistasis.

Mamo et al. (2015) utilized bi-parental mapping to study the genetics of stem rust resistance in wild barley (*H. vulgare* ssp. *spontaneum* C. Koch Thell) and barley landraces from the Swiss Alps and found that these accessions all carried the *rpg4/Rpg5* gene complex at 152-168 cM on chromosome 5H (Appendix A1). Additionally, several QTL with small effects were identified on chromosomes 3H and 5H at 66 cM and 99 cM, respectively. Many landraces from the Swiss Alps carry the *rpg4/Rpg5* gene complex and are resistant to race TTKSK (Steffenson et al. 2016).

In another study, Mamo (2013) utilized GWAS on barley landraces from Ethiopia and Eritrea and found QTL for reaction to *Pgt*, race TTKSK and MCCFC at the seedling and



adult plant stages, respectively. No QTL were identified for TTKSK adult plant or MCCFC seedling resistance. However, QTL for TTKSK seedling resistance were identified on chromosome 2H at 41 and 172 cM, 3H at 152 cM, 4H at 54 cM, and 5H at 6 cM (Appendix A1). For APR to race MCCFC, Mamo (2013) also found one significantly associated marker (12\_10674) on chromosome 5H at 68 cM. This marker is very closely linked to the *Rpg-qt1-5H-11\_11355* QTL found by Zhou et al. (2014) for APR to race TTKSK according to the consensus map by Muñoz-Amatriaín et al. (2014). This result suggests that *Rpg-qt1-5H-11\_11355* may confer effective APR to multiple races of *Pgt*.

Moscou et al. (2011) found a QTL for TTKSK seedling reaction on chromosome 5H at 146 cM in the Q21861 x SM89010 doubled-haploid population. This QTL was confirmed to be *rpg4/Rpg5*. Moscou et al. (2011) also found a QTL for TTKSK APR on chromosome 2H bin 16 at 44 cM that enhanced the resistance of the *rpg4/Rpg5* locus (Appendix A1). Interestingly, this QTL (2H.16) was contributed by the susceptible parent and was only detected in the resistant subset of the population. This suggests that modification of *rpg4/Rpg5* may play a role in effective field-level expression of this resistance. This QTL also co-segregated with an eQTL for transcript accumulation during seedling infection by race TTKSK. This 2H trans-eQTL was thought to enhance resistance through transcriptional suppression of many genes.

Turuspekov et al. (2016) conducted GWAS with 96 spring barley cultivars from Kazakhstan and found QTL for resistance to local races of *Pgt* on every chromosome except 7H (Appendix A1). However, only two markers on chromosome 6H at 63.5 cM were found to be significant after multiple testing corrections and were only significant during the pre-heading severity assessment.

Barley is extremely vulnerable to races in the Ug99 race group as only the *rpg4/Rpg5* gene complex has been shown to confer high levels of resistance at both the seedling and adult plant stages. Despite extensive efforts to identify new sources of stem rust resistance in barley, recent works reported a low frequency of resistant accessions (Mamo

2013; Mamo et al. 2015; Steffenson et al. 2017; Steffenson et al. 2016; Turuspekov et al. 2016; Wamalwa et al. 2016; Zhou et al. 2014). In this investigation, a GWAS of stem rust resistance to *Pgt* TTKSK composite in Njoro, Kenya (races TTKST, TTKTK, and TTKTT also present) and QCCJB in St. Paul, MN was conducted using a curated collection of barley landraces and cultivars from around the world. Novel QTL for APR were identified on chromosomes 1H, 2H, 3H and 5H. Additionally, this study confirmed the importance of a previously identified QTL on chromosome 5H for APR to *Pgt* TTKSK composite and now also race QCCJB.

## Materials and Methods

### *Plant Materials*

The Barley Core Collection (BCC) held by the United States Department of Agriculture-Agricultural Research Service National Small Grains Collection (USDA-ARS NSGC) was utilized in this study. After curation to remove duplicates and accessions with low quality genotype data, an “informative core” or “iCore” collection of 1,860 accessions was assembled as described in detail by Muñoz-Amatriaín et al. (2014). The BCC consists of representatives from 94 countries, including 815 landraces, 781 cultivars, 243 accessions of unknown improvement status, and 21 genetic stocks (Online Resource 2.1). With respect to growth habit, 1,538 accessions are listed as spring or facultative types and 322 as winter types. Seed for all experiments was derived from a single plant selection by the USDA-ARS NSGC, which maintains the collection.

A number of susceptible and resistant barley accessions displaying different reactions to *Puccinia graminis* were used as stem rust controls in one or more of the experiments. PI 532013 is an extremely susceptible landrace from Egypt; Hiproly (PI 60693) is a highly susceptible landrace from Ethiopia; and Steptoe (CIho 15229) is a cultivar that is moderately susceptible to most races of *Pgt* (Muir and Nilan 1973; Steffenson et al. 2017; Sun and Steffenson 2005). Chevron (PI 38061) is a landrace from Switzerland and resistant to most races of *Pgt* due to the presence of *Rpg1* (Shands 1939; Steffenson

1992). 80-TT-29 (CIho 16129) and 80-tt-30 (CIho 16130) are near-isogenic lines with and without *Rpg1*, respectively (Steffenson et al. 1985). Hietpas-5 (CIho 7124) and GAW 79-3 (PI 382313) are the sources of resistance genes *Rpg2* and *Rpg3*, respectively (Franckowiak 1991; Franckowiak and Steffenson 1997; Jedel 1991; Jedel et al. 1989; Patterson et al. 1957; Shands 1964). Q21861 (PI 584766) is a highly resistant barley accession that originated from a single plant selection within a International Center for Agricultural Research in the Dry Areas (ICARDA)/CIMMYT nursery in Mexico. Its resistance to stem rust was then confirmed in Australia by Dill-Macky and Rees (1992). Genetic studies revealed that Q21861 carries both *Rpg1* as well as the *rpg4/Rpg5* resistance gene complex (Arora et al. 2013; Brueggeman et al. 2008; Brueggeman et al. 2009; Jin et al. 1994b; Steffenson et al. 2009; Steffenson et al. 1995; Sun et al. 1996; Wang et al. 2013). Q/SM20 is a doubled haploid accession produced by anther culture from a cross between Q21861 and SM89010, a susceptible breeding line from Canada (Steffenson et al. 1995). It carries only the *rpg4/Rpg5* gene complex derived from Q21861 (Steffenson et al. 2009). In addition, the susceptible wheat accessions of LMPG-6, and Red Bobs (CItr 6255) were also included as controls to assess the level of disease pressure in field nurseries at St. Paul, MN and Njoro, Kenya, respectively.

### *Genotyping*

Genotyping of the BCC was described in detail by Muñoz-Amatriaín et al. (2014). Briefly, the BCC was genotyped using the Illumina Infinium iSelect single nucleotide polymorphism (SNP) assay, and SNPs were called using a clustering algorithm in GenomeStudio software (Illumina, San Diego, CA). After quality control, a total of 6,224 SNP markers were called on the germplasm panel, 5,664 of which were placed on the consensus map of Muñoz-Amatriaín et al. (2014). Missing genotype information was imputed using Beagle v4.1 software (Browning and Browning 2016).

### *Adult Plant Rust Evaluations in Kenya*

The BCC was evaluated against stem rust at the adult stage in Njoro, Kenya, where the

predominant *Pgt* races at the time were TTKSK and TTKST (16 of 25 samples). However, races TTKTK and TTKTT were also present in the nursery (9 of 25 samples)(Newcomb et al. 2016). Since race TTKSK predominated, the population of the pathogen in the nursery will be referred to as *Pgt* TTKSK composite. Research plots in Kenya were maintained by the Kenya Agricultural Livestock Research Organization (KALRO) Njoro station, under the direction of CIMMYT as part of the African stem rust resistance coordination effort. About three grams of seed of each accession were sown in half-meter paired rows. Inoculum of *Pgt* was maintained on susceptible wheat spreader rows planted in and around the nursery, facilitating natural infection. In addition, up to five direct inoculations of the test entries were performed to ensure adequate disease pressure (Mwando et al. 2012; Njau et al. 2013). The nursery was designed as a single randomized complete block with one replication and replicated controls. The BCC germplasm was screened in the 2014 main-season (July to October) (KEN14m) and 2014 off-season (January to June) (KEN14o) nurseries. In both nurseries, the spring and facultative accessions were planted as described above. In the 2013 and 2014 main-season nurseries, the winter type accessions were planted as hill plots in a separate section of the field after five weeks of vernalization (at 4-10°C) in peat pots prior to planting, abbreviated as KEN13w and KEN14w, respectively. In all nurseries, the controls of Chevron, Q21861, and Hipoly were repeated every 50 and 200 rows in KEN14m and KEN14o, respectively. The wheat accession Red Bobs was planted every 50 rows in both seasons. Additionally, in the KEN14o nursery, Steptoe and Q/SM20 were repeated every 50 rows with susceptible barley (PI 532013) and wheat (LMPG-6) accessions repeated every 150 rows.

Disease severity (0-100%) on the stems and leaf sheaths of each accession was estimated visually using the modified Cobb scale (Peterson et al. 1948). The Infection Response (IR) (size and type of uredinia) was also scored on plants in addition to rust severity using the resistant (R), moderately resistant (MR), moderately susceptible (MS), and susceptible (S) scale as described by Peterson et al. (1948). IRs were converted to a numerical value using the following scale: R = 1, MR = 2, MS = 3, and S = 4. When

multiple IRs were observed on accessions, a weighted average was calculated as follows: for two IRs (IR 1 x 0.75) +(IR 2 x 0.25) and for three IRs (IR 1x 0.60)+(IR 2 x 0.30)+(IR 3 x 0.10). Disease evaluations were conducted when plants were between the heading to hard dough stages of development (Zadoks 55-87) (Zadoks et al. 1974). Only accessions reaching the hard dough stage or beyond (Zadoks 85+) during the final severity assessment were used in the final analysis. Accessions not reaching this developmental stage were treated as missing values. General adult plant reaction classifications were made based on the severity of rust infection. Accessions with 1% or less severity were classified into the highly resistant (Class 1), and those with severities greater than 1% and less than 5% were classified into the resistant (Class 2). These cutoff values were chosen based on the severity of accessions Q21861, defining the Class 1 reactions and Q/SM20, defining the Class 2 reactions. The bootstrap mean of these two accessions was individually calculated across all nurseries with 1,000 replications, where the threshold was defined as equal to or less than the third quartile of the bootstrap mean statistic. When these values were calculated, they were very close to 1% for Q21861 and 5% for Q/SM20. Since these percentage scores are common in rust severity estimations, they were used as the cutoff values for classifying accessions into the Class 1 and Class 2 categories. These strict selection criteria were implemented to identify accessions with resistance levels approaching those of the controls Q21816 or Q/SM20. Although some accessions known to have APR (like Hietpas-5 with *Rpg2*) may exhibit higher disease severities than the Class 2 cutoff, these values were chosen to provide a strict cut-off value to identify accessions which have valuable resistance in a breeding context.

#### *Adult Plant Rust Evaluations in the United States*

The BCC was also evaluated for APR to *Pgt* race QCCJB (isolate QCC-2 (Jin et al. 1994b)) during the summers of 2013 and 2014 in St. Paul, MN , with the resulting datasets abbreviated as StP13 and StP14. As races QCCJB and TTKSK are both virulent for *Rpg1*, the former served as a domestic “surrogate” race for the latter to identify resistance not conferred by *Rpg1*. The nurseries were planted at the Minnesota

Agricultural Experiment Station on the St. Paul campus of the University of Minnesota. About two grams of seed were sown in half-meter rows and maintained according to standard agricultural practices in the region. These nurseries included accessions of spring, facultative, and winter types. Accessions that did not head or were too late in maturity to allow for comparable rust infection scoring were discarded from the final analysis. The nursery was designed as a randomized complete block with one replication and replicated controls. The accessions Hiproly, Chevron, Q21861, and Q/SM20 were replicated every 50 rows, with susceptible barley accession PI 532013 and susceptible wheat accession LMPG-6 replicated every 150 rows. In StP13, Steptoe was included as a control every 50 rows, while in StP14 it was planted in a random position of every planting tray of 40 entries. Test entries were planted as two paired plots between continuous rows of a susceptible barley spreader row (a mixture of equal amounts of the cultivars Conlon, Stander, and Quest, all of which carry *Rpg1*), such that each plot was bordered on one side by a spreader row and on the other by the paired plot. The spreader row was needle-injected with *Pgt* inoculum at the tillering to early jointing stage (Zadoks 22-35). Inoculum was prepared by suspending 1 g of fresh urediniospores in 1 L of distilled water and six drops of 20% Tween 20 (Polyoxyethylene (20) sorbitan monolaurate). Then, 1.0 ml of inoculum was injected into two stems of the spreader row every meter. The foliage of the test entries and spreader rows was also directly inoculated three to four times with the rust pathogen. These inoculations were done by spraying a urediniospore suspension (1 g of urediniospores in 1 L of Soltrol 170 oil (Phillips Petroleum, Bartlesville, OK) directly onto the plants using a hand-held battery-operated sprayer (Mini-ULVA, Micron Group, Herefordshire, United Kingdom) at a rate of 1 L per 800 plots. The plants were inoculated on successive weeks when 75% or more of the entries were at the boot stage (Zadoks 40) or beyond. Disease ratings were then conducted as described above. Classification of accessions into Class 1 and Class 2 categories was based on stem rust terminal severity as described above.

### *Seedling Stem Rust Evaluations to Race TTKSK in the BSL-3*

Seedling tests against *Pgt* race TTKSK (isolate: 04KEN156/04) were conducted in the greenhouse or growth chamber at the Minnesota Agricultural Experiment Station / Minnesota Department of Agriculture Plant Growth Biosafety Level-3 (BSL-3) Containment Facility on the St. Paul campus of the University of Minnesota. For each entry, five seeds were sown in a round peat pot (7 x 9 cm) filled with a 1:1 mix of sterilized top soil and Sunshine MVP potting mix (vermiculite, Canadian sphagnum peat moss, nutrient charge, gypsum, and dolomitic limestone) (Sun Gro Horticulture, Quincy, MI). Sixteen peat pots were held in one plastic tray (28 x 32 cm) (Fertil, Slatington, PA). Plants were then placed in a cold room (4°C) for two to five days to break possible dormancy and then moved to the BSL-3 greenhouse and watered with a pH-buffering 15-0-15 fertilizer (Peters Dark Weather, Scott's Company, Marysville, OH) at 1/16 dilution (ca. 40 g/liter). Plants were watered from below to avoid wetting the foliage. When the first leaf was fully expanded (7-9 days after planting), the plants were inoculated with 12-15 mg of urediniospores suspended in 0.8 ml Soltrol 170 oil per tray. Inoculum was atomized onto the plants using a specialized inoculator pressured at 25-30 kPa, for a rate of about 0.01 ml of inoculum per plant. After inoculation, the carrier was allowed to off-gas for 90 minutes under lights with constant air circulation. The plants were then transferred to a misting chamber. Initially, plants were continuously misted by ultrasonic humidifiers for 30 minutes. Thereafter, the frequency of misting was reduced to two minutes of run time every 15 minutes. Plants were kept in the misting chambers for 16-18 hours in the dark at 22-25°C. Thereafter, lights (400 W sodium vapor lamps providing 150-250  $\mu\text{mol photons s}^{-1}\text{m}^{-2}$ ) were turned on to complete the final stages of infection by *Pgt*. After four hours, the misters were turned off and the doors opened to allow the plants to dry off slowly. When the plants were dry, they were moved to a greenhouse and fertilized with a water-soluble fertilizer (20-10-20, J.R. Peters, Inc., Allentown, PA) at 1/16 dilution (ca. 40 g/liter).

All experiments were designed as a randomized incomplete block of 18 trays with the

controls of Chevron, Steptoe, PI 532013, GAW 79-3, 80-tt-30, and 80-TT-29 planted once in every block and accessions Q/SM20 and Hiproly planted in every tray. A total of two complete replications of 18 trays were included in the experiment, and the resulting datasets were abbreviated as BSL3-1 and BSL3-2. Twelve to 14 days after inoculation, plants were assessed for their infection types (ITs) on the first leaves of plants using a 0 to 4 scale. This scale was originally developed by Stakman et al. (1962) for wheat and subsequently modified for barley (Miller and Lambert 1955; Stakman et al. 1962). Briefly, IT 0 = no visible sign of infection; IT 0; = hypersensitive flecks, but no uredinia; IT 1 = minute uredinia; IT 2 = small, restricted uredinia; IT 3- = medium-sized uredinia with some restriction; 3 = large uredinia showing no restriction; and IT 3+ = extremely large uredinia showing no restriction. Unlike wheat, barley does not often exhibit the classic “green island” reaction associated with IT 2 uredinia nor the classic diamond-shaped uredinia of IT 4. Since barley commonly exhibits a mesothetic reaction (a mixture of different ITs on the same leaf) to stem rust, the two to three most common ITs observed on leaves were scored in order of their prevalence. For analysis, these ITs were converted to a numerical Coefficient of Infection (CI) scale as described by Zhou et al. (2014) where IT “0” = 0, “0;” = 0.5, “1” = 2.0, “2” = 3, “3-” = 3.5, “3” = 4.0, and “3+” = 4.5. CI is a weighted average of the converted ITs by order of their frequency. The same bootstrap mean statistic was employed to call threshold values for seedling ITs as was employed for the severity assessments in the adult plant screening. The bootstrap mean of the reaction of lines Q21861 and Q/SM20 was calculated to define the Class 1 and Class 2 categories of accessions, respectively. After 1,000 replications, Class 1 ranged from IT 0; to 1 ( $CI \leq 2$ ), and Class 2 ranged from  $> IT 1$  to  $IT = 2$  ( $CI \text{ value } > 2 \text{ to } \leq 3$ ). The maximum CI values within the BSL3-1 and BSL3-2 datasets were used in the analysis to create a single datum for each accession in order to group it into either the Class 1 or Class 2 groups. If an accession was evaluated more than twice due to conflicting results across experiments, then the mode of the CI value was used.



### *Association Mapping*

Population structure and LD analysis of the BCC was previously reported by Muñoz-Amatriaín et al. (2014). Association mapping was conducted using a mixed linear model (MLM) as implemented in the software program TASSEL v.5.0 (Bradbury et al. 2007; Yu et al. 2005). For analysis of adult plant and seedling resistance, data for terminal rust severity and CI, respectively, were used to detect marker-trait associations. Association mapping was performed using the Q + K method explained in detail by Zhou et al. (2014). Parameter Q is population structure as estimated by the percentage of assignment to each subpopulation based on STRUCTURE analysis by Muñoz-Amatriaín et al. (2014) or a vector of the principal component values as determined by principal component analysis (Pritchard et al. 2000a). Muñoz-Amatriaín et al. (2014) assigned STRUCTURE values using the entire set of 1,860 BCC accessions; however, in each nursery only a subset of accessions could be evaluated. The main-season and off-season nursery in Kenya consisted of 1,520 spring-type accessions (Kenya\_Spring=KEN14m and KEN14o), and 313 winter-type accessions that were transplanted as hill-plots (after vernalization) into the winter section of the Kenya main-season nursery in 2013 and 2014 (Kenya\_Winter=KEN13w and KEN14w). In St. Paul, the nursery consisted of 1,484 spring-type accessions (Stp\_Spring=StP13 and StP14) (Online Resource 2.1). Because sub-dividing the population can affect the assignment of accessions to different subpopulations, a principal component analysis was conducted separately on each population subset. This analysis was also conducted in TASSEL v.5.0. Muñoz-Amatriaín et al. (2014) estimated that the entire BCC consists of five subpopulations. Overall, the principal component analysis showed clustering in a similar pattern to the STRUCTURE analysis as reported by Muñoz-Amatriaín et al. (2014) (Figure 2.1). In the Kenya\_Spring and StP\_Spring panels, the first four principal components explained most of the variation (28%) (PC1 = 12%, PC2 = 7%, PC3 = 5%, and PC4 = 4%), suggesting that the STRUCTURE results and principal components may be very similar. Therefore, the first four principal components were used in the analysis. In the Kenya\_Winter panel, the first two principal components explained most of the variation (32%) (PC1 = 20%, and PC2 =

12%) and were used in the analysis.  $K$  is the Kinship genetic relationship estimated by the proportion of shared alleles as executed in TASSEL v.5.0.

The formula used was  $y=xb+qv+u+e$ , where  $x$  is a vector of SNP genotypes,  $q$  is a matrix of vectors accounting for population structure,  $b$  is the coefficient of marker effects,  $v$  is the coefficient of population structure,  $u$  is the vector of random effects of kinship and genetic variance, and  $e$  is a vector of residuals. The variance of  $u$  is given by  $\text{Var}(u)=2KV_g$  where  $V_g$  is the genetic variance, and the variance of  $e$  is given by  $\text{Var}(e)=V_r$  where  $V_r$  is residual variance. A  $p$ -value was generated for each marker individually fit into the MLM. After analysis, a False Discovery Rate (FDR) correction was applied to the  $p$ -values to correct for multiple testing ( $q$ -value)(Benjamini and Hochberg 1995). A  $q$ -value  $<0.05$  was used to assign significant marker-trait associations.

Association mapping analysis was conducted separately for each dataset including: StP13, StP14, KEN14m, KEN14o, KEN13w, KEN14w, BSL3-1 and BSL3-2. Significant departure from the expected line in the Q-Q plots for the CI of BSL3-1 and BSL3-2 analyses indicated uncontrolled population structure or error. To correct for this, data from BSL3-1 and BSL3-2 were averaged, and the  $\log_{10}$  of this average was used in the final analysis, with the resulting dataset abbreviated as TTKSK\_Seedling (Balding 2006). Marker map position was based on the consensus map of Muñoz-Amatriaín et al. (2014). In cases where the marker was unmapped and significant, the map position was based on the POPSEQ map of Mascher et al. (2013).

To correct for field spatial variation and differences between seasons, the raw severity data were corrected using two methods. First, a moving average correction was calculated using the mvngGrAd v.0.1.5 package in R (Technow 2015). This correction used field row and column assignment to correct for spatial variability. Correction was assumed to be beneficial if the estimate of relative efficiency improved the corrected compared to the raw values. Relative efficiency was defined by the percent reduction in the mean squared error of the fitted values compared to the raw values. Second, mixed linear regression

was conducted to correct for differences between seasons in each environment (Kenya\_Spring, Kenya\_Winter, StP\_Spring) based on the repeated controls using the lme4 v 1.1-12 package in R (Bates et al. 2015). Environment was treated as a fixed effect. A correction factor was generated based on the solution of the fixed effects and applied to the moving average corrected values of the BCC accessions to correct for differences between seasons and create a single datum for each environment. Values were then averaged across environments. This created the datasets of Kenya\_Spring\_A, Kenya\_Winter\_A, and StP\_Spring\_A, which were corrected for field spatial variability and also differences between seasons (Sallam et al. 2015).

To estimate the allelic effect of identified QTL, mean severity was calculated based on what SNP allele each accession carries. The additive allelic effect was defined as half the difference between the two SNP classes. Then, the reduction in disease severity was calculated as the difference between the two classes divided by the susceptible class, known as the effect of allele substitution (Falconer 1960). This was done for both adult plant severity values as well as seedling CI values. If multiple markers were declared significant in the same genome region, they were assumed to be detecting the same QTL if the LD between them was high ( $R^2 > 0.7$ ). QTL were named according to the following convention: Reaction to *Puccinia graminis* (*Rpg*) - quantitative trait loci (*qtl*) - chromosome - most significant marker.

## **Results**

### *Adult Plant Rust Evaluations*

Correlation between IRs converted to a numerical scale and terminal rust severity across all locations for the BCC accessions were high ( $r^2 = 0.65$ ), and hence for simplicity only the rust severity values were used in further analyses. Infection levels in the four rust nurseries were moderate based on Hiproly. The primary controls of Hiproly, PI 532013, Chevron, Q21861, LMPG-6, and Red Bobs performed as expected based on previous research (Figure 2.2). Chevron, Hiproly, and Q21861 were included in every nursery and

exhibited mean severities (and ranges) of 14% (range of 3-30%), 30% (range of 5-70%), and 0.6% (range of 0-1%) in the Kenya\_Spring nurseries and 17% (range 5-35%), 35% (range 10-50%), and 0.7% (range 0.5-5%) in the St. Paul\_Spring nurseries. Due to poor establishment and growth of plants in the Kenya\_Winter nurseries, rust development was generally lower than in the spring nurseries: 11% (range 15-20%) on Chevron, 10% (range 10-15%) on Hiproly, and 3% (range 0.5-15%) on Q21861. With respect to the entire BCC panel, the overall mean rust severity in the four nurseries was similar ranging from a low of 13% in StP14 to a high of 24% for StP13 (Figure 2.3). Overall, the percentage of BCC accessions classified as highly resistant or resistant (i.e. Class 1 or 2) ranged from 7% to 25% (Table 2.1). The lowest percentage of resistance (7%) was found in StP13, which had the highest rust severity across all environments. The other spring nurseries had similar percentages of resistance ranging 22 to 25%. The percentage of resistance in the winter-type accessions of the BCC in Kenya was similar to the spring-type accessions in Kenya at 17% to 20%.

#### *Seedling Stem Rust Evaluations to Race TTKSK in the BSL-3*

In the BSL3-1 and BSL3-2 seedling evaluations to race TTKSK, the resistant controls of Q21861 and Q/SM20 gave highly resistant to resistant ITs, ranging from 0; to 10; (mode 0;, CI = 0.5) and 0; to 21 (mode 0;, CI = 0.5), respectively (Figure 2.4). ITs on the susceptible controls of Steptoe and Hiproly ranged from 23- to 3 (mode = 3-, CI = 3.5) and on the highly susceptible control of PI 532013 from 3 to 3+ (mode = 3, CI = 4.0). Accessions with *Rpg1* were included as controls to verify the virulence of TTKSK. The ITs on both Chevron and 80-TT-29 ranged from 23-1 to 3 (mode = 23-, CI = 3.125) (Figure 2.4). Whereas, the susceptible control of 80-tt-30 (near isogenic with 80-TT-29, but lacking *Rpg1*) ranged from 23- to 3 (mode = 3-, CI = 3.5). The majority of BCC accessions exhibited moderately susceptible to susceptible ITs (213- to 33+, CI > 3) (Figure 2.5). Only 5% (98) of the BCC accessions were classified into Class 1 with a resistance level similar to Q21861 (ITs 0; to 1, CI ≤ 2). Another 12% (227) of BCC accessions were classified into Class 2 with a resistance level similar to Q/SM 20 (ITs >1

to 2,  $CI > 2$  to  $\leq 3$ ) (Table 2.1).

### *Association Mapping*

Association mapping analyses were conducted separately for the BCC in each rust nursery or seedling experiment; thus, there were six separate analyses for APR with the raw rust severity data (StP13, StP14, KEN14m, KEN14o, KEN13w, and KEN14w) (Figure 2.6) and a single analysis for the seedling TTKSK evaluation (TTKSK\_Seedling) (Figure 2.7). To create a single datum for each adult plant environment, the corrected datasets of Kenya\_Spring\_A, StP\_Spring\_A, and Kenya\_Winter\_A were used (Figure 2.8).

In Kenya, the most significant markers identified were 11\_11355, 12\_10493, 12\_31427, SCRI\_RS\_205853 and SCRI\_RS\_177017, residing in the 71 -77 cM interval of chromosome 5H (Tables 2.2 and 2.3). Of these five markers, the most significant one was 11\_11355, which was detected in both spring nurseries (Table 2.3) in Kenya as well as in the Kenya\_Spring\_A corrected dataset (Table 2.2). Additionally, SCRI\_RS\_177017 was detected in both nurseries in Kenya and in the corrected Kenya\_Spring\_A dataset. Although SCRI\_RS\_177017 was unmapped in the consensus map by Muñoz-Amatriaín et al. (2014), the POPSEQ map of Mascher et al. (2013) positioned this marker on chromosome 5H very closely linked to 11\_11355, SCRI\_RS\_205853, and 12\_31427. The LD among all five of these markers was high ( $R^2 > 0.7$ ), suggesting that they are detecting the same QTL. This QTL was designated *Rpg-qt1-5H-11\_11355*, following the convention of Zhou et al. (2014).

In addition to *Rpg-qt1-5H-11\_11355*, other marker-trait associations were found for APR in Kenya. A marker on chromosome 1H (11\_11277) at 96 cM and another on chromosome 2H (12\_11278) at 69 cM were both found to be significantly associated with APR in Kenya (Table 2.3). These QTL were designated as *Rpg-qt1-1H-11\_11277* and *Rpg-qt1-2H-12\_11278*, respectively (Table 2.4). Both were identified in just one nursery using raw severity values and were not significant in the corrected

Kenya\_Spring\_A dataset.

In St. Paul to race QCCJB, no significant marker-trait associations were detected based on the raw severity values in 2013 (Table 2.3). In 2014, markers 12\_20613, SCRI\_RS\_199887, 11\_20388, and SCRI\_RS\_177017 were significantly associated with raw rust severity values. Using the corrected 2014 StP\_Spring\_A dataset, SCRI\_RS\_177017 was found significantly associated with rust severity as was 11\_11355, which was not detected based on the individual raw severity values (Table 2.2). Both 12\_31427 and SCRI\_RS\_177017 are very closely linked to and in high LD with 11\_11355 ( $R^2 > 0.7$ ); thus, it is possible that both SCRI\_RS\_177017 and 12\_31427 are detecting the same QTL as 11\_11355 and may be a part of the *Rpg-qt1-5H-11\_11355* QTL.

Additionally, markers detected on chromosome 1H (12\_20613), 3H (SCRI\_RS\_199887), and 5H (11\_20388) were also found significantly associated with resistance in StP14 (Table 2.3). The corresponding QTL were designated as *Rpg-qt1-1H-12\_20613*, *Rpg-qt1-3H-SCRI\_RS\_199887*, and *Rpg-qt1-5H-11\_20388*, respectively (Table 2.4). However, these markers were not detected in other nurseries and were not found in any of the corrected datasets. There were no significant markers detected after FDR correction in either the KEN13w or KEN14w nurseries (Tables 2.2 and 2.3).

For the GWAS of seedling resistance to race TTKSK, the raw CI values for each replicate and the average of the two replicates did not yield any significant marker-trait associations. However, the plot of the Q-Q values showed departure from the expected line, suggesting data transformation was needed (data not shown). To normalize the data, a  $\log_{10}$  transformation was used, creating the dataset TTKSK\_Seedling. Using the transformed data, markers SCRI\_RS\_167103 and 11\_10236 (residing at ~172 cM on chromosome 5H) were found significantly associated with seedling resistance (Figure 2.7 and Table 2.2). Both markers are in very high LD ( $R^2 = 1$ ) with each other and are likely detecting the same QTL (Table 2.4). This QTL was designated as *Rpg-qt1-5H-11\_10236*

for the most significant marker detected (Table 2.4).

In total, only about 1% (19) of the accessions were classified into Class 1 or Class 2 for APR in all field nurseries (Table 2.5, Online Resource 2.1). These accessions originated from: Australia (1), Chile (1), China (2), Colombia (1), Spain (2), Switzerland (3), Ukraine (2), United Kingdom (1), United States (4), and Yemen (2). Of these accessions, only nine also exhibited high levels of TTKSK seedling resistance: BCC2297, BCC1750, BCC1917, BCC1568, BCC1570, BCC1602, BCC1195, BCC2399, and BCC0131, originating from Australia (1), Colombia (1) Spain (1), Switzerland (3), Ukraine (2) and United States (1), respectively.

#### *Effect of Identified QTL*

In Kenya, the markers with the most significant effect were the unmapped marker 12\_10493 and the chromosome 5H marker SCRI\_RS\_205853, reducing rust severity by 39-51% and 33-47%, respectively (Table 2.6). SCRI\_RS\_205853 is closely linked to 11\_11355 and may be part of the *Rpg-qt1-5H-11\_11355* locus on chromosome 5H ( $R^2 > 0.7$ ). Marker 11\_11355 had a much larger  $-\log_{10}(p)$ , suggesting there was greater variance in the severity of accessions carrying the positive allele of this marker than with other markers (Table 2.2). Both 11\_11355 and SCRI\_RS\_177017 had a similar allele effect (32-40%) (Table 2.6).

In St. Paul, the marker with the most significant effect was 12\_20613 on chromosome 1H, which reduced disease severity by 48%, but only in the StP14 nursery (Table 2.6). Using the corrected StP\_Spring\_A dataset, markers 11\_11355 and SCRI\_RS\_177017 were also found to be significant. However, they only had a modest effect (9-18%) on disease severity reduction.

In response to race TTKSK at the seedling stage, two markers on chromosome 5H (11\_10236 and SCRI\_RS\_167103) representing the *Rpg-qt1-5H-11\_10236* locus reduced CI values by 10.8%, which is equivalent reduction from IT 3 to IT 3-.

## Discussion

Highly virulent races of the stem rust pathogen like TTKSK and others in the Ug99 race group are a serious threat to wheat and barley production worldwide. In this study, we evaluated the BCC for reaction to the *Rpg1* virulent races of the *Pgt* TTKSK composite from Africa and QCCJB from North America and identified a number of resistant accessions. We hypothesized that a diverse barley panel like the BCC would yield novel resistance loci that would be revealed by GWAS. The BCC is very diverse compared to most other barley collections recently evaluated, where germplasm from only specific geographic regions was used. In these previous studies, Zhou et al. (2014) evaluated 3,072 advanced breeding lines from 10 programs in the United States, Mamo (2013) evaluated 298 landraces from Ethiopia and Eritrea, Turuspekov et al. (2016) evaluated 92 spring cultivars from Kazakhstan, Steffenson et al. (2016) evaluated 73 landraces from Switzerland; and Sallam et al (2017) evaluated 318 accessions of wild barley (*H. vulgare* ssp. *spontaneum*) comprising the Wild Barley Diversity Collection (WBDC) (Steffenson et al. 2007). The most extensive barley collection screened to date was by Steffenson et al. (2017) who evaluated the reaction of 2,837 *Hordeum* accessions to race TTKSK at the seedling stage. This collection was comprised of a world collection of 1,924 accessions of cultivated barley (cultivars, breeding lines and landraces), 935 accessions of *H. vulgare* ssp. *spontaneum* from across its range and various introgression lines derived from wild *Hordeum* species.

From the seedling evaluations of the BCC to race TTKSK, 5% (98) of the accessions exhibited a resistance level comparable to Q21861 (i.e. IT 0; to 1) and 12% (227) exhibited a resistance level comparable to Q/SM20 (i.e. IT  $>1 \leq 2$ ) (Table 2.1). This is a higher frequency of resistance than has been reported in any previous study of *Hordeum* germplasm. Zhou et al. (2014) found  $< 1\%$  (12) of United States breeding lines had similar resistance levels at the seedling stage, and Mamo (2013) found no accessions with such a level of resistance. Steffenson et al. (2017) found only 1.7% (32) of cultivated and just 1.4% (13) of wild barley accessions resistant to TTKSK at the seedling stage in their



extensive screening effort of *Hordeum*. The higher frequency of resistance found in the BCC could be due to its greater diversity, highlighting the utility of this germplasm for stem rust resistance breeding. The collection evaluated by Steffenson et al. (2017) was also quite diverse. However, the greater frequency of resistance observed in the BCC could be due to the greater number of landraces comprising the germplasm panel. In the collection evaluated by Steffenson et al. (2017), about 84% of the cultivated barley evaluated had an improvement status listed as either a breeding line or cultivar and about 15% were listed as landraces. However, in the present study about 42% of the evaluated accessions were listed as breeding lines or cultivars and about 43% as landraces. A higher frequency of landraces in a collection could lead to greater overall diversity and may explain the higher frequency of resistance found in this collection. Selected accessions from the BCC with the highest level of resistance are currently being tested against a wider range of *Pgt* races to assess their resistance spectra. Those with the widest resistance spectra will be crossed with advanced breeding lines to investigate the genetic basis of resistance and also to transfer the resistance alleles into the barley improvement program.

APR to the *Pgt* TTKSK composite was, in general, more frequent than seedling resistance as 12% (218) and 9% (169) of the accessions carried resistance levels similar to Q21861 in the KEN14m and KEN14o nurseries, respectively (Table 2.1). This is a higher frequency than reported by Zhou et al. (2014) who found about 60 (4%) accessions with APR levels similar to Q21861. In the field screening of Ethiopian and Eritrean landraces, Mamo (2013) found no accessions with a resistance level similar to Q21861. Interestingly, only 2% (28) and 5% (84) of the BCC accessions were rated as having a high level of resistance to race QCCJB at the adult stage in the StP13 and StP14 nurseries, respectively. Overall, the correlation between the disease severities observed for the BCC in Kenya (*Pgt* TTKSK composite) and St. Paul (race QCCJB) was very low (0.27-0.3), suggesting that there may be different genes controlling resistance to these races. The different results obtained at the two field sites may also be due to genotype by environment interactions with respect to APR in this collection. Additionally, the lower

number of resistant accessions identified in St. Paul could be due to a heavier inoculum load from repeated direct inoculations of accessions in addition to differences in the prevailing environments during the growing season.

Taken together, our results indicate that the BCC contains valuable resistance against virulent foreign and domestic races like TTKSK and QCCJB, respectively. In addition to the accessions listed in Table 2.5, others with high levels of resistance are currently being evaluated against a diverse set of *Pgs* and *Pgt* races to elucidate the diversity of resistance genes they possess. This aspect is discussed in more detail in Chapter 3.

Previously, the BCC was used to map resistance to spot blotch (*Cochliobolus sativus*) and spot-form net blotch (*Pyrenophora teres* f. *maculata*), as well as the agronomic traits of naked caryopsis, spike row number and heading date (Leng et al. 2016; Muñoz-Amatriaín et al. 2014; Tamang et al. 2015). In this study, we identified loci associated with seedling resistance to race TTKSK, APR to *Pgt* TTKSK composite, and also APR to race QCCJB. Marker-trait associations were found on chromosomes 1H, 2H, 3H, and 5H. However, the most significant associations were detected on chromosome 5H. The most significant QTL identified for APR in this study was the *Rpg-qt1-5H-11\_11355* locus on chromosome 5H between 71 and 77 cM (Table 2.4). This very same marker of 11\_11355 was found significantly associated with TTKSK resistance in United States barley breeding germplasm by Zhou et al. (2014), who first designated this QTL. In this study, 11\_11355 and SCRI\_RS\_177017 were also the most significant markers identified for APR to *Pgt* TTKSK composite and were detected in both seasons in Kenya as well as in the corrected Kenya\_Spring\_A dataset. We conclude that the marker-trait associations found with 11\_11355 and SCRI\_RS\_177017 in this study likely identified the QTL *Rpg-qt1-5H-11\_11355* described by Zhou et al. (2014). Further validating the importance of this loci. Additionally, marker 12\_31427 was also identified as significantly associated with APR to race TTKSK by Zhou et al. (2014) and in this study. It is likely that 12\_31427 is also detecting the same *Rpg-qt1-5H-11\_11355* QTL, as all three of these markers were in high LD ( $R^2 > 0.7$ ). All markers in the vicinity of QTL *Rpg-qt1-5H-*

*11\_11355* had a large effect on adult plant severity (mean 40% reduction, range 32-51%)(Table 2.6). These values are very similar to those reported for APR to race TTKSK (3.7-55% reduction depending on year and breeding program) by Zhou et al. (2014). Moscou et al. (2011) also identified this same region as being important from a transcriptome analysis of the Q21861 x SM89010 mapping population for TTKSK seedling resistance, further supporting the contention that this region of chromosome 5H is important for TTKSK seedling and APR. Since *Rpg-qt1-5H-11\_11355* is located at 71-77 cM on 5H, it represents a genetically distinct locus from the *rpg4/Rpg5* complex at 158-165 cM (Brueggeman et al. 2008; Mamo et al. 2015; Moscou et al. 2011; Steffenson et al. 2009).

In this study, we also identified markers *11\_11355* and *SCRI\_RS\_177017* as being significantly associated with APR to race QCCJB. This suggests that *Rpg-qt1-5H-11\_11355* may be effective against multiple races of the stem rust pathogen. However, the allelic effect on disease severity reduction was lower for APR to race QCCJB compared to *Pgt* TTKSK composite (mean 14%, range 9-18%). Mamo (2013) found marker *12\_10674* significantly associated for APR to race MCCFC. This marker also maps to the *Rpg-qt1-5H-11\_11355* locus and is high LD with *11\_11355* ( $R^2 > 0.7$ ). This suggests that *Rpg-qt1-5H-11\_11355* or a closely linked QTL may be effective against this race as well. Similar clustering of stem rust resistance genes has been reported in wheat and also for powdery mildew resistance genes in barley (Cantalapiedra et al. 2016; Yu et al. 2014).

Zhou et al. (2014) suggested that the causal gene for resistance at *Rpg-qt1-5H-11\_11355* may be a RING-H2 gene or possibly a glycogen operon protein *glgX*, based on BLAST searches and exploitation of the synteny of barley with *Brachypodium*. However, recent advancements in barley genomic resources based on the cultivar Morex allowed for a more detailed analysis of this locus. Marker *11\_11355* lies on Morex contig 1559755, and the closest gene found was *MLOC\_10776.1*, also a glycogen debranching enzyme, as suggested by Zhou et al. (2014) (Matthew Haas, personal communication). Closely

linked nearby is MLOC\_6032.2, a gene postulated to produce a leucine-rich repeat protein, a common motif of plant disease resistance genes. However, the mechanisms or underlying genes at this QTL still need to be explored (Mascher et al. 2017).

The only markers significantly associated with the seedling response to race TTKSK in the BCC were SCRI\_RS\_167103 and 11\_10236 at 172 cM on chromosome 5H. These markers are in perfect LD with each other, and the identified QTL was named *Rpg-qt1-5H-11\_10236* for the most significant marker detected. The position of this QTL is relatively close to the map position given for *rpg4/Rpg5* by Mamo et al. (2015) at 158-165 cM. The closest gene model found to this marker was MLOC\_4406.1, which is predicted to produce a leucine-rich repeat protein. The resistance gene *Rpg5* (Gene bank number EU878778, Morex Genome Reference number HORVU5Hr1G113030.4) was BLAST searched against the Morex genome and found to be at 640.7 Mega Base Pairs (MBP) (Wang et al. 2013; Mascher et al. 2017), whereas markers 11\_10236 and SCRI\_RS\_167103 and gene MLOC\_4406.1 were all found to reside at 656.1 MPB. The close proximity to the *rpg4/Rpg5* gene complex is intriguing, suggesting that this gene may play a role in this interaction, possibly through epistasis. Alternatively, the association between MLOC\_4406.1 and *Rpg5* may be due to linkage, and the resistance detected at this QTL is actually an effect of this gene complex.

No previous studies have detected any significant marker-trait associations for stem rust resistance on chromosome 1H. In this study, we detected two markers, 11\_11277 and 12\_20613, for APR to *Pgt* TTKSK composite and QCCJB, respectively. Each marker was only detected in a single season. Given the genetic distance between 11\_11277 (96 cM) and 12\_20613 (104 cM) and the low LD in the region, it is likely that these markers are detecting different QTL. Thus, the respective QTL were designated as *Rpg-qt1-11\_11277* and *Rpg-qt1-12\_20613*.

Only one significant marker was detected on chromosome 2H at 69 cM. This QTL was named *Rpg-qt1-12\_11278* for the most significant marker associated with it and was only

detected in a single season for APR against *Pgt* TTKSK composite. Several previous studies have detected QTL for stem rust resistance on chromosome 2H. Mamo (2013) found significant associations for TTKSK seedling resistance with markers SCRI\_RS\_115905 and SCRI\_RS\_109266 at 41 and 172 cM, respectively. Additionally, Moscou et al. (2011) detected several eQTL between 44-75 cM on chromosome 2H for transcript accumulation during TTKSK seedling infection in the Q21861 x SM898019 population. Although *Rpg-qt1-12\_11278* at 69 cM is near several other identified QTL, it may be different because it conferred resistance at the adult plant stage and not the seedling stage. It is unclear how this QTL may be related to the QTL identified by Moscou et al. (2011) as that QTL was identified for transcript accumulation in response to infection and not resistance per se.

Stem rust is a serious disease affecting both wheat and barley. Although wheat is typically more receptive to stem rust infection, barley can be severely damaged by this disease under high inoculum pressure and favorable environmental conditions (Dill-Macky et al. 1990; Steffenson 1992; Steffenson et al. 2017). In wheat, this disease has been controlled effectively by a concerted effort to pyramid multiple genes for resistance. This strategy has been successfully used to control stem rust in North American wheat cultivars for many years. In barley, *Rpg1* has been the major genetic factor conferring stem rust resistance in North American cultivars for many years, although its longevity was aided by a variety of factors such as the low effective population size of the pathogen and also the late arrival of inoculum on an already maturing barley in its primary planted range of the northern latitudes (Steffenson 1992). In response to the QCCJB epidemics of the early 1990's when cultivars carrying *Rpg1* became vulnerable to stem rust losses, an effort was made to identify new sources of resistance to this virulent race. Jin et al. (1994a) evaluated 18,000 accessions of cultivated barley from around the world, including many accessions held by the USDA-ARS NSGC, for reaction to race QCCJB at the adult plant stage. A subset of resistant accessions from this field screening was also tested at the seedling stage. From these evaluations, only 13 accessions exhibited high levels of APR, and only one accession (Q21861) was shown to also possess a high level

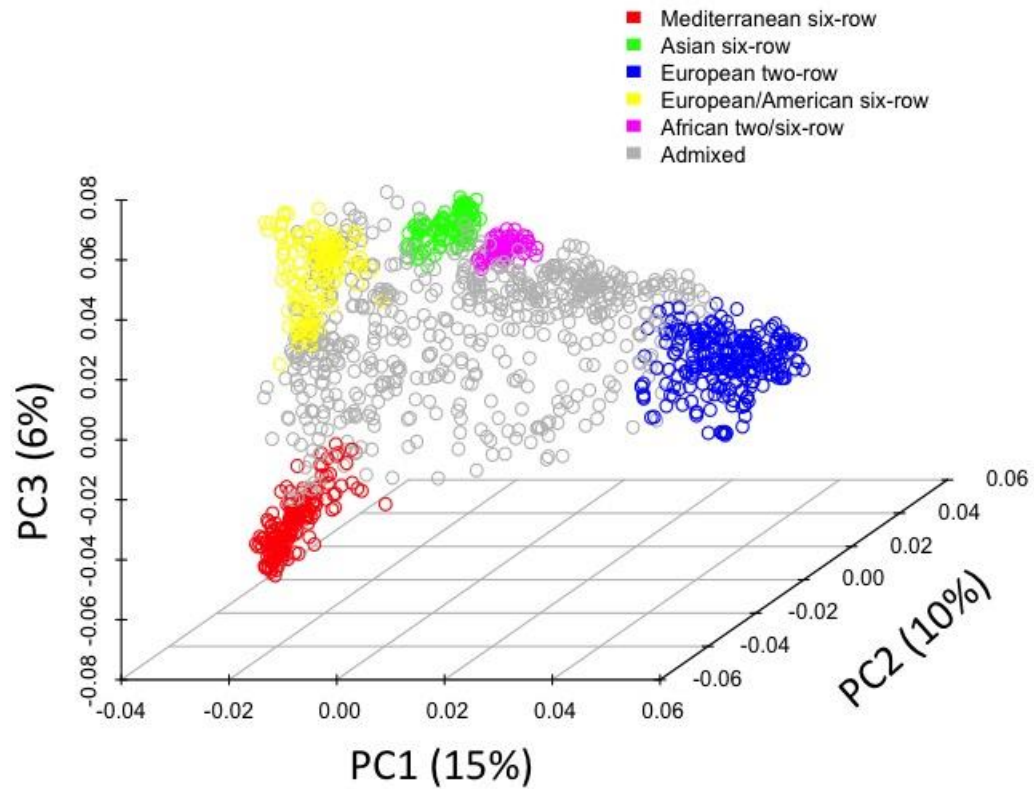
of seedling resistance. Our study included many of the same accessions from the USDA-ARS NSGC as investigated by Jin et al. (1994a); however, their original data were unavailable for comparison.

The *rpg4/Rpg5* gene complex was first identified from the accession Q21861, a selection made from a CIMMYT breeding line grown in Mexico. Subsequent evaluations in Australia (Dill-Macky and Rees 1992) and later the United States revealed that Q21861 was highly resistant to races of *Pgt*, including QCCJB (Jin et al. 1994a; Jin et al. 1994b). Q21861 and other accessions with resistance to QCCJB were used as parents in several breeding programs in both Canada and United States (Jin et al. 1994a; Steffenson and Smith 2006). However, nearly all North American cultivars and breeding lines remain vulnerable to *Rpg1*-virulent *Pgt* races such as QCCJB and TTKSK (Steffenson et al. 2017; Zhou et al. 2014). The best strategy for future gene deployment is to combine both *Rpg1* and *rpg4/Rpg5* together in cultivars to prolong the collective effectiveness of these resistance genes. However, there are known *Pgt* races that possess virulence for these genes at both the seedling and adult plant stages. Races like TTKSK (and others in the Ug99 lineage) and QCCJB possess virulence for *Rpg1*, while race HKHJC possesses virulence for *rpg4/Rpg5* (Sun and Steffenson 2005). Both QCCJB and HKHJC are from North America, demonstrating that virulence for the respective resistance genes are present in this *Pgt* population. Additionally, the stem rust pathogen continues to evolve new virulence combinations on barley as at least two additional isolates were described as having *Rpg1* virulence in a region of eastern Washington state where the alternate host (*Berberis* and *Mahonia*) of *Pgt* is present and often infected (Nirmala et al. 2015; Wang et al. 2015). To date, there is no known race that possesses combined virulence to both *Rpg1* and *rpg4/Rpg5*. However, if such a race does emerge, there will be very limited resistance sources for future breeding efforts.

A potential avenue for future stem rust resistance breeding is to utilize APR to achieve levels of disease resistance that would keep stem rust severity below economically damaging thresholds. Barley is known to possess a basal level of resistance to stem rust

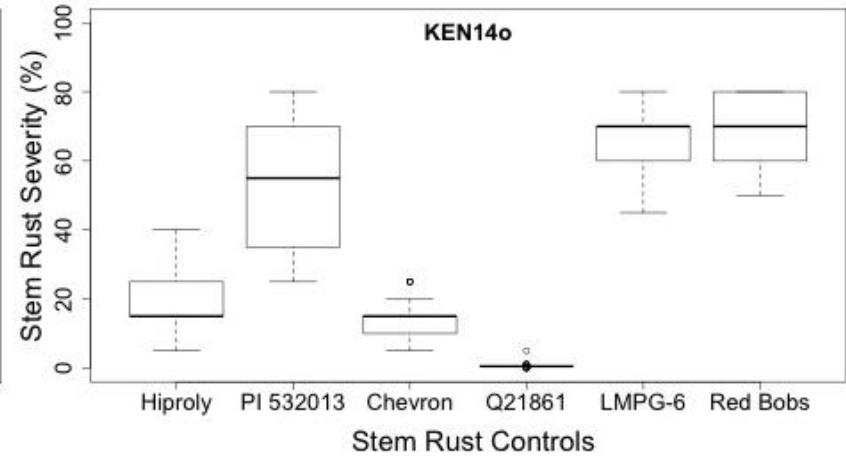
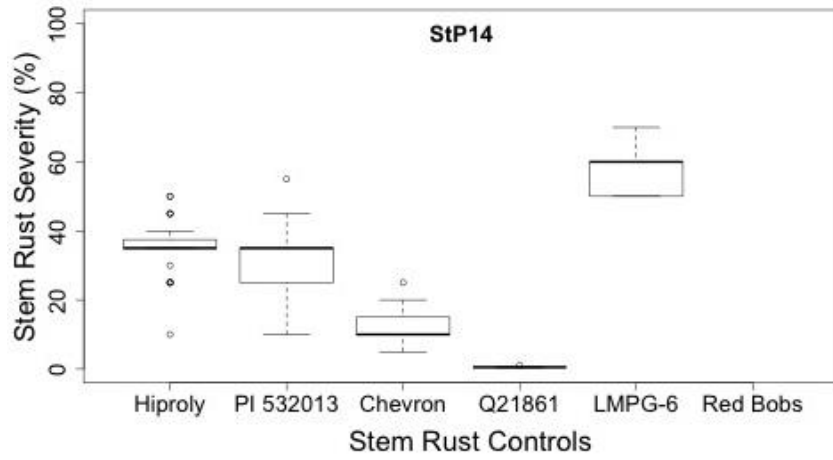
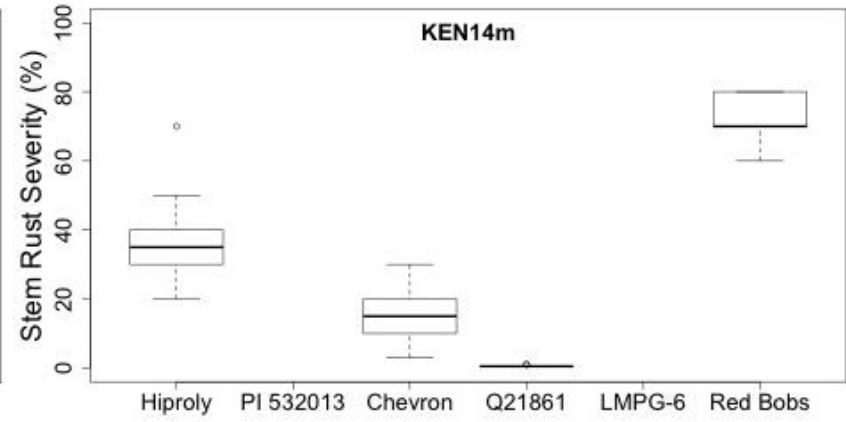
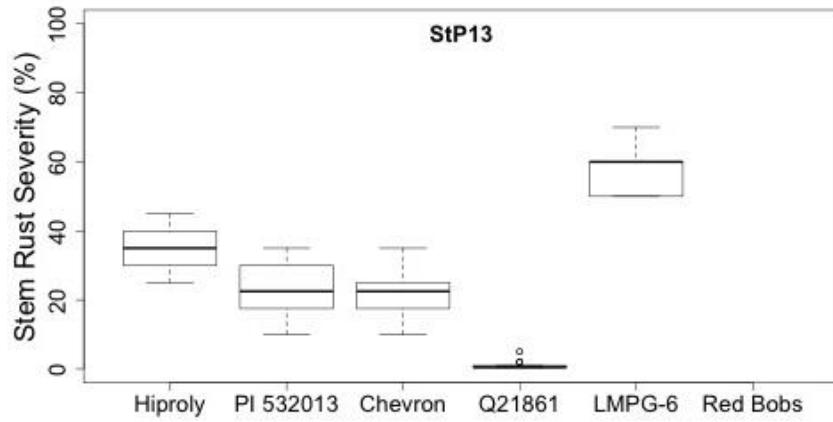
and does not succumb to heavy stem rust infection until heading or just prior to heading (Zadoks 55+) (Zhou et al. 2014). There are also various reports of APR in barley (Steffenson et al. 2017; Zhou et al. 2015). This basal resistance combined with the APR present in many materials evaluated in this and other studies suggest that future efforts focused on these types of stem rust resistance could be a fruitful strategy for managing this disease. Studies have been advanced to map the resistance genes *Rpg2* and *Rpg3*. These genes are not particularly effective at the seedling stage, but do confer a level of APR in the field to races such as TTKSK and QCCJB (Chapter 4). These two genes in addition to the new ones identified in this study may prove to be a useful resource for future breeding efforts if new races with wider virulence emerge. The markers identified in this research could be utilized as resources for stem rust resistance breeding in a marker-assisted selection scheme. The most promising markers for marker-assisted selection include 11\_11355 and SCRI\_RS\_177017, both of which are in high LD ( $R^2 > 0.7$ ) (resistant alleles at both loci  $n=674$ ) with each other. This haplotype is common in the European two-row (subpopulation 3,  $n=184$ ) and Mediterranean six-row (subpopulation 1,  $n=129$ ) subpopulations (Muñoz-Amatriaín et al. 2014), but very rare or absent in the European/American six-row (subpopulation 4,  $n=94$ ), Asian six-row (subpopulation 2,  $n=4$ ) and African two/six-row (subpopulation 5,  $n=0$ ) subpopulations. The 11\_11355 and SCRI\_RS\_177017 stem resistance haplotype was most common among admixed accessions ( $n=263$ ), suggesting that this locus is under selection in breeding programs as over 50% of accessions in the admixed group are listed as either breeding lines or cultivars. Breeders interested in utilizing this allele may wish to screen their germplasm for this marker haplotype prior to introgressing exotic germplasm. Of the 19 accessions listed in Table 2.5, only 5 (BCC2233 (China), 1750 (Colombia), 1568 (Switzerland), 1850 (Yemen) and 1851 (Yemen)) do not carry this resistance haplotype, making accessions listed in Table 2.5 good candidates for crossing in breeding programs.

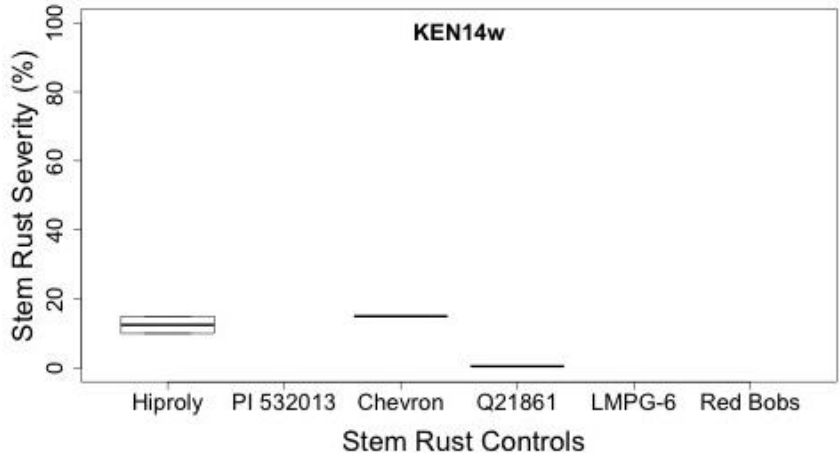
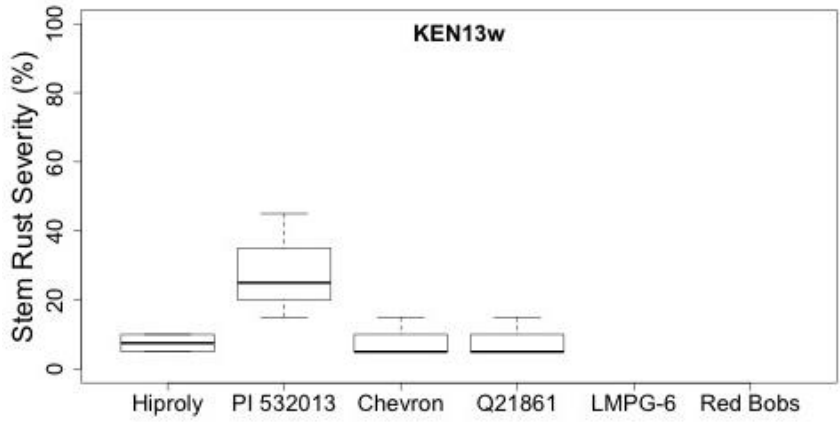
## Figures



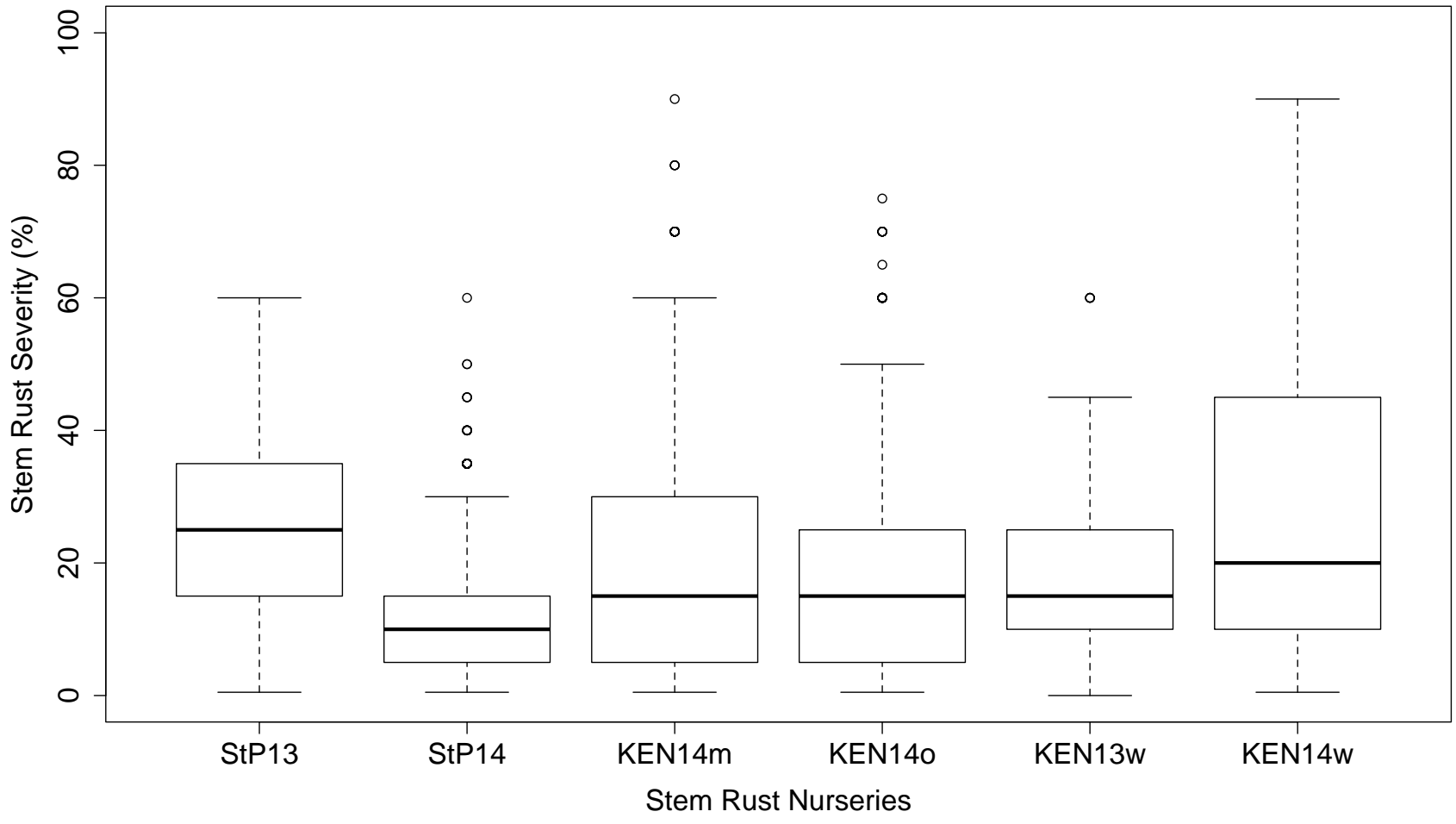
**Figure 2.1.** Principal component analysis of all accessions of the Barley iCore Collection (BCC) ( $n = 1,860$ ). Accessions are colored according to results from STRUCTURE analysis by Muñoz-Amatriaín et al. (2014).



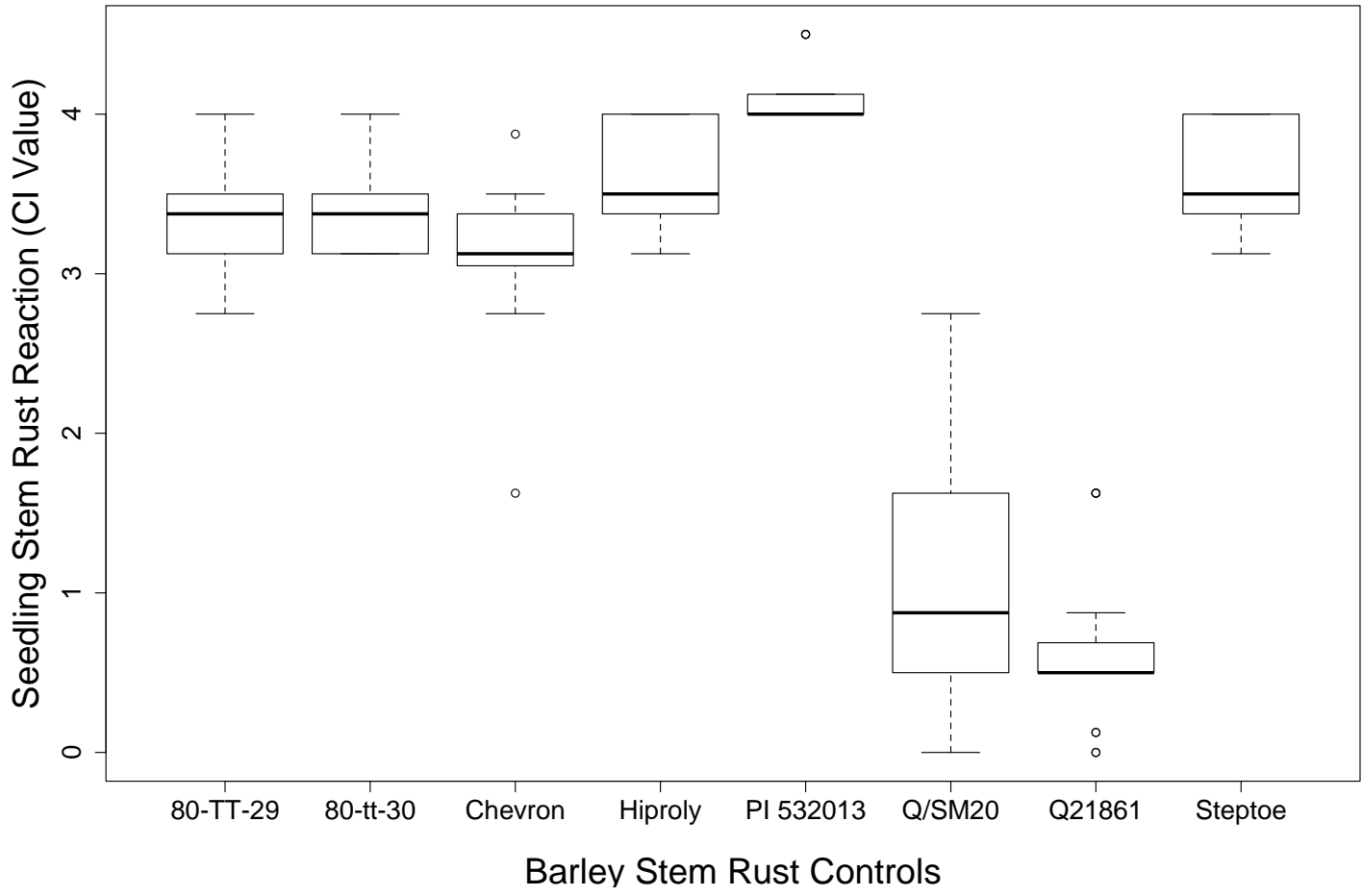




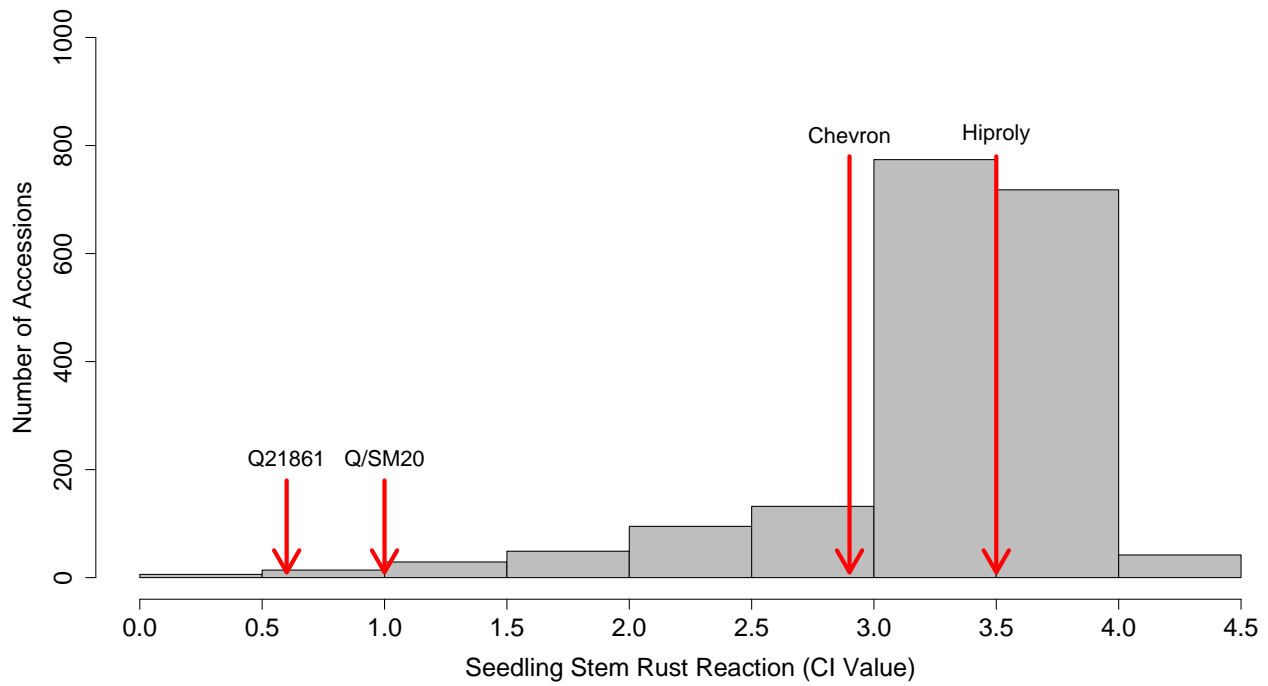
**Figure 2.2.** Performance of barley (Hiproly, PI 532013, Chevron, and Q21861) and wheat (LMPG-6 and Red Bobs) controls in multiple stem rust screening nurseries in Njoro, Kenya and St. Paul, MN, where nurseries are abbreviated as: 2013 St. Paul (StP13), 2014 St. Paul (StP14), 2014 Kenya main-season (KEN14m), 2014 Kenya off-season (KEN14o), 2013 Kenya winter (KEN13w) and 2014 Kenya winter (KEN14w). Five statistics (bars) are represented in each box plot from bottom to top: the smallest observation, lower quartile, median, upper quartile, and largest observation, respectively. Data points positioned outside this range are extreme values and are represented as circles.



**Figure 2.3.** Performance of all Barley iCore Collection (BCC) accessions in multiple stem rust screening nurseries in Njoro, Kenya and St. Paul, MN, where nurseries are abbreviated as: 2013 St. Paul (StP13), 2014 St. Paul (StP14), 2014 Kenya main-season (KEN14m), 2014 Kenya off-season (KEN14o), 2013 Kenya winter (KEN13w) and 2014 Kenya winter (KEN14w). Five statistics (bars) are represented in each box plot from bottom to top: the smallest observation, lower quartile, median, upper quartile, and largest observation, respectively. Data points positioned outside this range are extreme values and are represented as circles.

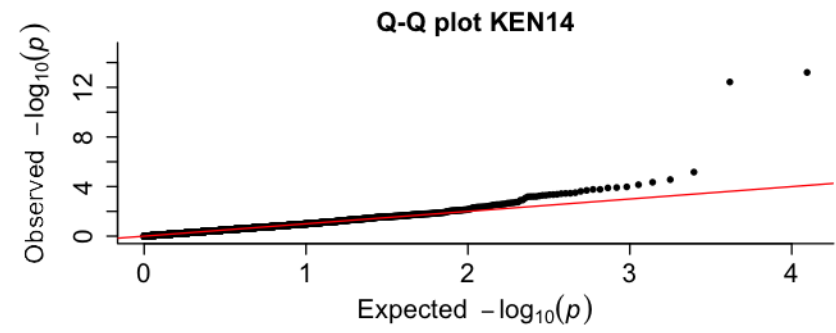
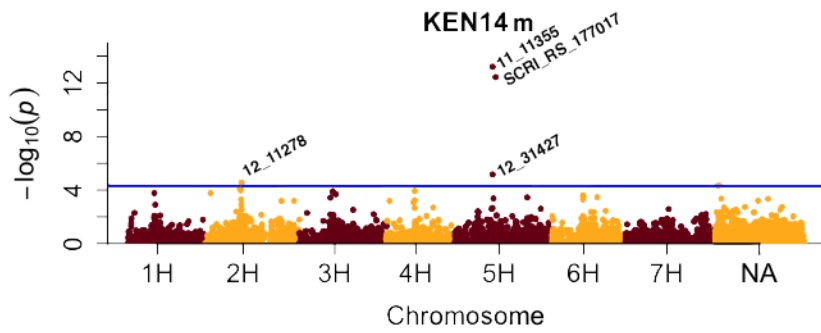
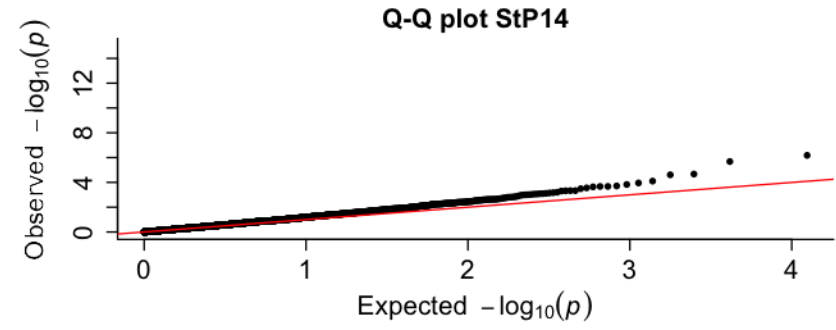
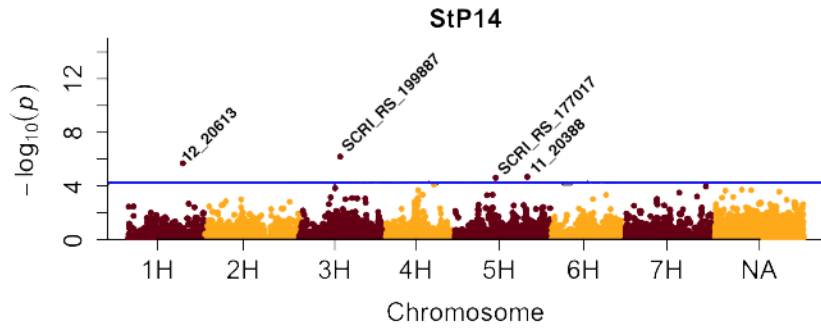
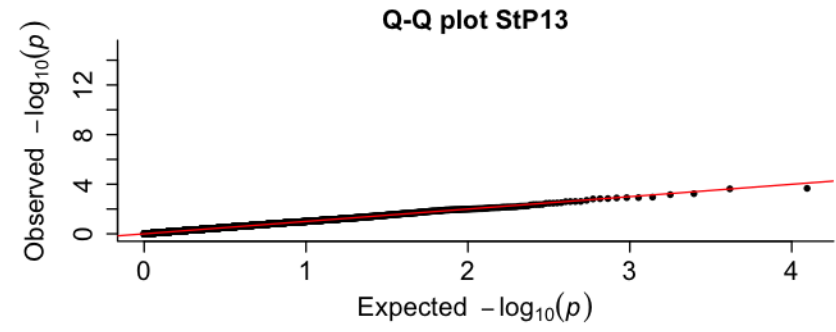
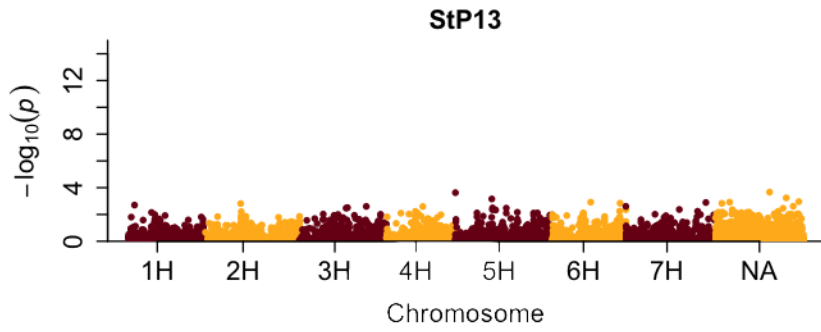


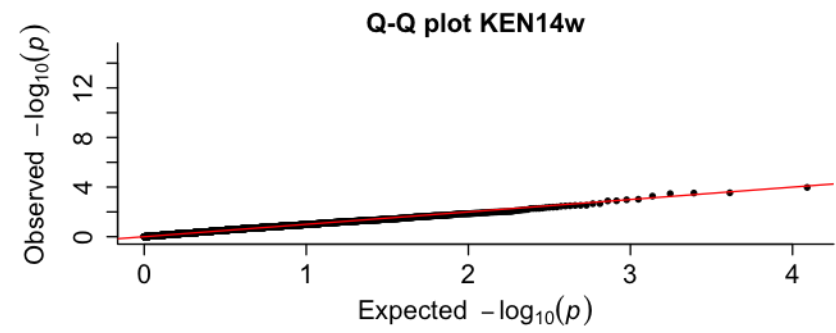
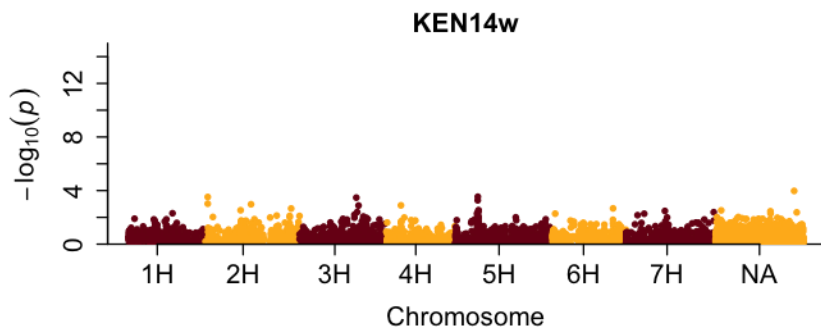
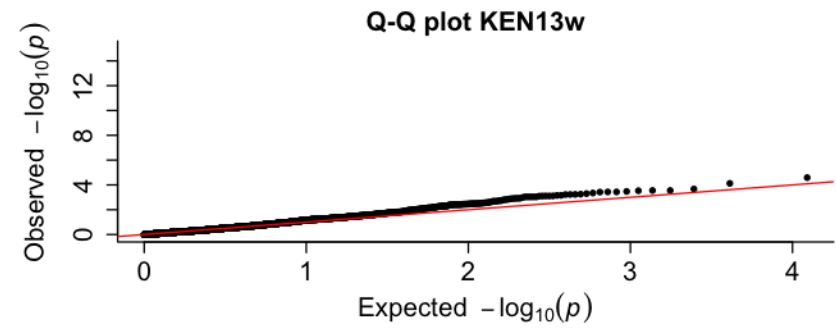
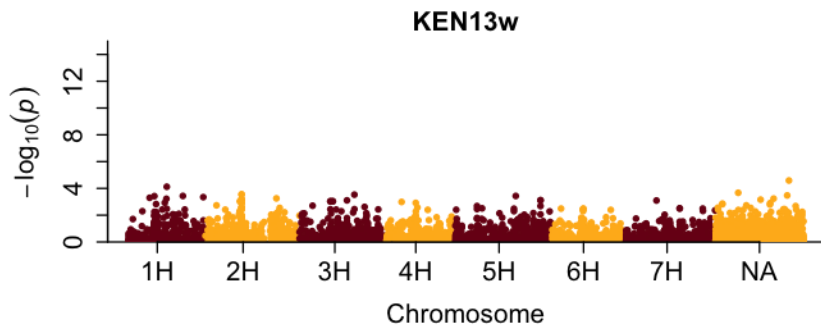
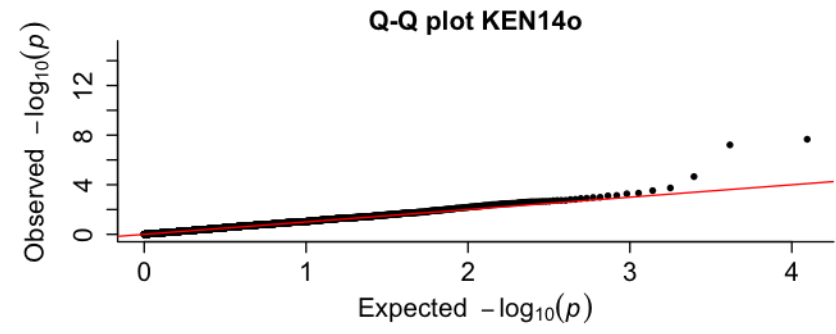
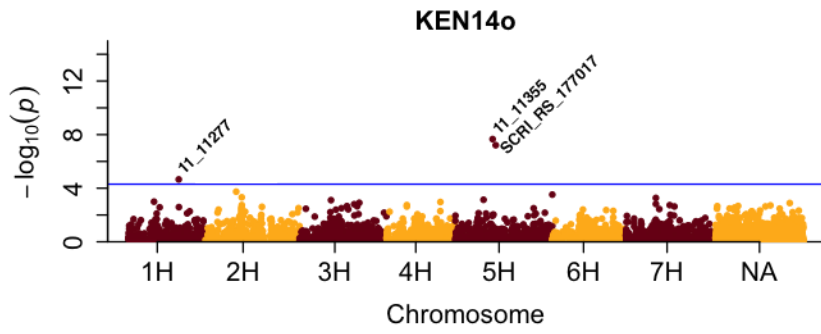
**Figure 2.4.** Response of all barley controls to seedling infection by *Puccinia graminis* f. sp. *tritici* race TTKSK. Raw infection type values were converted to Coefficient of Infection (CI) values as described by Zhou et al. (2014). Five statistics (bars) are represented in each box plot from bottom to top: the smallest observation, lower quartile, median, upper quartile, and largest observation, respectively. Data points positioned outside this range are extreme values and are represented as circles.



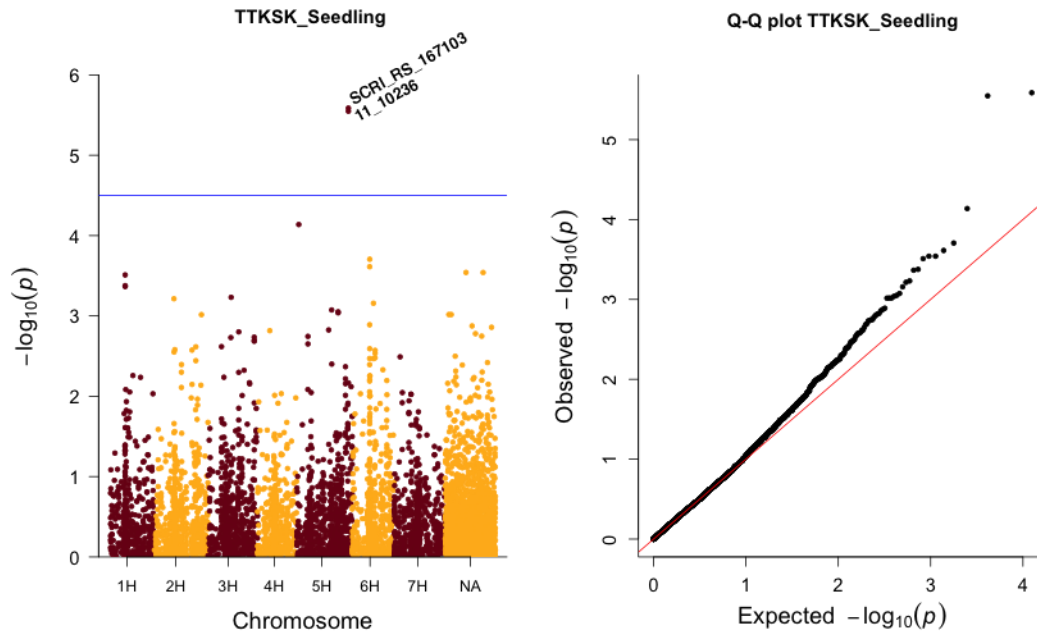


**Figure 2.5.** Distribution of seedling stem rust reactions to race TTKSK in the Barley iCore Collection (BCC). Raw infection type values were converted to Coefficient of Infection (CI) values as described by Zhou et al. (2014). The reactions of susceptible controls Chevron and Hipoly and resistant controls Q21861 and Q/SM20 are also shown for comparison.

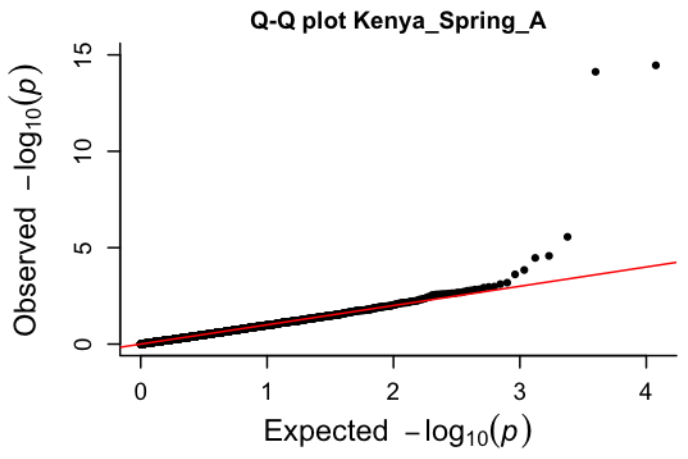
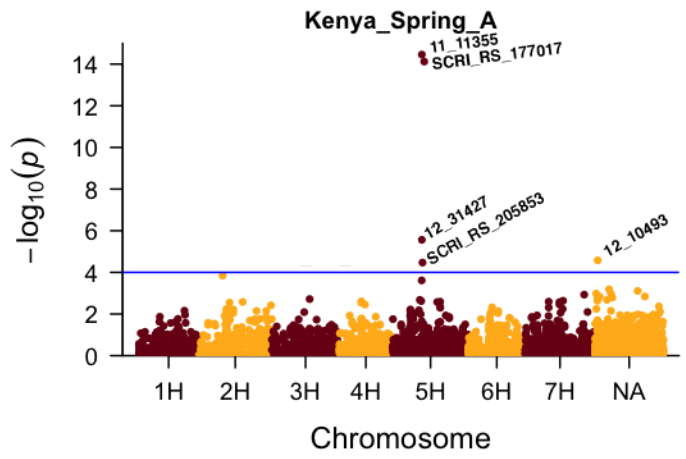
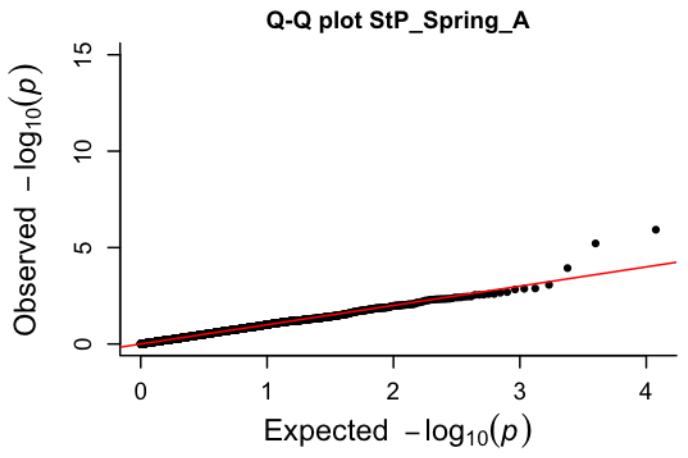
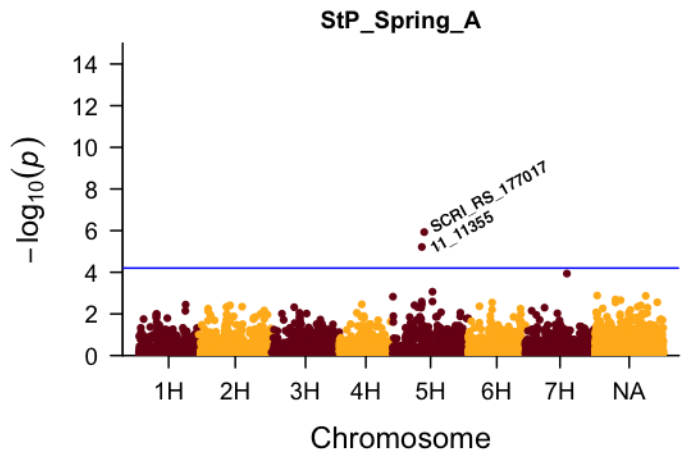


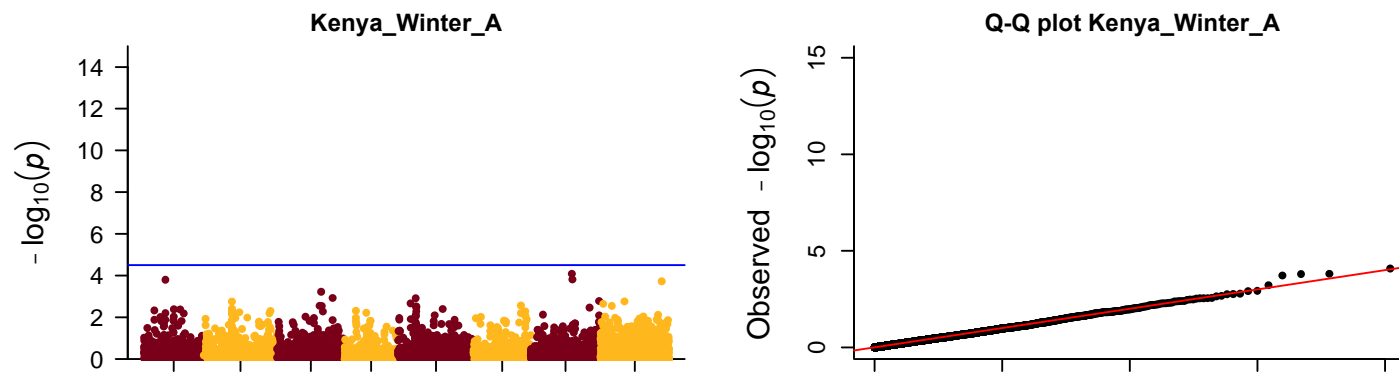


**Figure 2.6.** Manhattan plots showing significant marker-trait associations for stem rust severity (0-100%) in multiple stem rust screening nurseries in Njoro, Kenya and St. Paul, MN. Nurseries are abbreviated as: 2013 St. Paul (StP13), 2014 St. Paul (StP14), 2014 Kenya main-season (KEN14m), 2014 Kenya off-season (KEN14o), 2013 Kenya winter (KEN13w), and 2014 Kenya winter (KEN14w). Blue lines indicate threshold levels for declaring significant marker-trait associations using a false discovery rate correction of,  $q\text{-value} < 0.05$ . Q-Q plots are depicted to left of each Manhattan plot.



**Figure 2.7.** Manhattan plot showing significant marker-trait associations for stem rust reaction type at the seedling stage to *Puccinia graminis* f. sp. *tritici* race TTKSK. Raw infection type values were converted to Coefficient of Infection (CI) values as described by Zhou et al. (2014). The two replicates of BSL3-1 and BSL3-2 were averaged and then  $\log_{10}$  transformed to normalize the data (TTKSK\_Seedling). Blue lines indicate threshold levels for declaring significant marker-trait associations using a false discovery rate correction of,  $q\text{-value} < 0.05$ . A Q-Q plot is depicted to the left of the Manhattan plot.





**Figure 2.8.** Manhattan plots showing significant marker-trait associations for stem rust severity (0-100%) in multiple datasets corrected for field spatial and trial variability. Data sets were: Kenya Spring nurseries (Kenya\_Spring\_A), Kenya Winter nurseries (Kenya\_Winter\_A) and St. Paul Spring nurseries (StP\_Spring\_A). Blue lines indicate threshold levels for declaring significant marker-trait associations using a false discovery rate correction of,  $q\text{-value} < 0.05$ . Q-Q plots are depicted to left of each Manhattan plot.

## Tables

**Table 2.1.** Frequency of seedling and adult plant stem rust resistance in the Barley iCore Collection (BCC), with accessions categorized into the highly resistant (Class 1) or the resistant (Class 2) based on the reaction of Q21861 and Q/SM20, respectively.

|   | <b>StP13<sup>a</sup></b> | <b>StP14</b> | <b>KEN14m</b> | <b>KEN14o</b> | <b>KEN13w</b> | <b>KEN14w</b> | <b>TTKSK<sup>b</sup></b> |
|---|--------------------------|--------------|---------------|---------------|---------------|---------------|--------------------------|
| Class 1 (DS≤1) (IT 0; to 1) <sup>c</sup>  | 28 (2%)                  | 84 (5%)      | 218 (12%)     | 160 (9%)      | 11 (3%)       | 15 (5%)       | 98 (5%)                  |
| Class 2 (DS 5-1) (IT 1 to 2) <sup>d</sup> | 104 (6%)                 | 321 (17%)    | 210 (11%)     | 311 (17%)     | 55 (17%)      | 40 (13%)      | 227 (12%)                |
| Total                                     | 132 (7%)                 | 405 (22%)    | 428 (23%)     | 471 (25%)     | 66 (20%)      | 55 (17%)      | 325 (17%)                |

<sup>a</sup> Adult plant rust severity datasets were from the following field nurseries in St. Paul, MN and Njoro, Kenya: 2013 St. Paul (StP13), 2014 St. Paul (StP14), 2014 Kenya main-season (KEN14m), 2014 Kenya off-season (KEN14o), 2013 Kenya winter (KEN13w), and 2014 Kenya winter (KEN14w).

<sup>b</sup> Seedling reactions to *Pgt* race TTKSK in the greenhouse, the mode or maximum value from the BSL3-1 and BSL3-2 datasets in response to seedling infection by race TTKSK.

<sup>c</sup> Resistant category of phenotypic response, equivalent to Q/SM20.

<sup>d</sup> Highly resistant category of phenotypic response, equivalent to Q21861.



**Table 2.2.** Markers significantly associated with seedling and adult plant stem rust reactions in the Barley iCore Collection (BCC) using datasets corrected for seasonal and spatial variability.

| Marker         | Chr <sup>a</sup> | cM <sup>a</sup> | Chr <sup>b</sup> | cM <sup>b</sup> | StP_Spring_A <sup>c</sup> |                | Kenya_Spring_A <sup>d</sup> |                | Kenya_Winter_A <sup>e</sup> |                | TTKSK_Seedling <sup>f</sup> |                |
|----------------|------------------|-----------------|------------------|-----------------|---------------------------|----------------|-----------------------------|----------------|-----------------------------|----------------|-----------------------------|----------------|
|                |                  |                 |                  |                 | <i>p</i> -value           | R <sup>2</sup> | <i>p</i> -value             | R <sup>2</sup> | <i>p</i> -value             | R <sup>2</sup> | <i>p</i> -value             | R <sup>2</sup> |
| 11_11355       | 5H               | 68.58           | 5H               | 71.67           | 6 x 10 <sup>-6</sup>      | 0.01           | 4 x 10 <sup>-15</sup>       | 0.04           | -                           | -              | -                           | -              |
| 12_31427       | 5H               | 68.83           | 5H               | 71.67           | -                         | -              | 3 x 10 <sup>-6</sup>        | 0.02           | -                           | -              | -                           | -              |
| SCRI_RS_205853 | -                | -               | 5H               | 73.33           | -                         | -              | 3 x 10 <sup>-5</sup>        | 0.02           | -                           | -              | -                           | -              |
| SCRI_RS_177017 | -                | -               | 5H               | 77.52           | 1 x 10 <sup>-6</sup>      | 0.02           | 8 x 10 <sup>-15</sup>       | 0.04           | -                           | -              | -                           | -              |
| 12_10493       | -                | -               | -                | -               | -                         | -              | 3 x 10 <sup>-5</sup>        | 0.04           | -                           | -              | -                           | -              |
| SCRI_RS_167103 | 5H               | 172.13          | 5H               | 161.67          | -                         | -              | -                           | -              | -                           | -              | 3 x 10 <sup>-6</sup>        | 0.01           |
| 11_10236       | 5H               | 172.23          | 5H               | 161.67          | -                         | -              | -                           | -              | -                           | -              | 3 x 10 <sup>-6</sup>        | 0.01           |

<sup>a</sup> Chromosome (Chr) assignment and map position according to the consensus map of Muñoz-Amatriaín et al. (2014).

<sup>b</sup> Chromosome (Chr) assignment and map position according to the POPSEQ map of Mascher et al. (2013).

<sup>c</sup> St. Paul, MN (race QCCJB) rust severity values corrected for field spatial variability and seasonal differences and averaged between two replicates.

<sup>d</sup> Njoro, Kenya (*Pgt* TTKSK composite) spring rust severity values corrected for field spatial variability and seasonal differences and averaged between two replicates.

<sup>e</sup> Njoro, Kenya (*Pgt* TTKSK composite) winter rust severity values corrected for field spatial variability and seasonal differences and averaged between two replicates.

<sup>f</sup> TTKSK seedling coefficient of infection values as described by Zhou et al. (2014) were log<sub>10</sub> transformed and averaged between two replicates.

**Table 2.3.** Markers significantly associated with adult plant stem rust reactions in the Barley iCore Collection (BCC) using raw adult plant severity values.

| Marker         | Chr <sup>a</sup> | cM <sup>a</sup> | Chr <sup>b</sup> | cM <sup>b</sup> | StP14 <sup>c</sup>   |                | KEN14m                |                | KEN14o               |                |
|----------------|------------------|-----------------|------------------|-----------------|----------------------|----------------|-----------------------|----------------|----------------------|----------------|
|                |                  |                 |                  |                 | <i>p</i> -value      | R <sup>2</sup> | <i>p</i> -value       | R <sup>2</sup> | <i>p</i> -value      | R <sup>2</sup> |
| 11_11277       | 1H               | 96.25           | 1H               | 91.15           | -                    | -              | -                     | -              | 2 x 10 <sup>-5</sup> | 0.01           |
| 12_20613       | 1H               | 104.10          | 1H               | 95.61           | 2 x 10 <sup>-6</sup> | 0.02           | -                     | -              | -                    | -              |
| 12_11278       | 2H               | 69.55           | 2H               | 59.49           | -                    | -              | 3 x 10 <sup>-5</sup>  | 0.01           | -                    | -              |
| SCRI_RS_199887 | -                | -               | 3H               | 77.69           | 7 x 10 <sup>-7</sup> | 0.02           | -                     | -              | -                    | -              |
| 11_11355       | 5H               | 68.58           | 5H               | 71.67           | -                    | -              | 6 x 10 <sup>-14</sup> | 0.04           | 2 x 10 <sup>-8</sup> | 0.02           |
| 12_31427       | 5H               | 68.83           | 5H               | 71.67           | -                    | -              | 7 x 10 <sup>-6</sup>  | 0.02           | -                    | -              |
| 11_20388       | 5H               | 137.38          | 5H               | 137.22          | 2 x 10 <sup>-5</sup> | 0.01           | -                     | -              | -                    | -              |
| 12_10493       | -                | -               | -                | -               | -                    | -              | 3 x 10 <sup>-5</sup>  | 0.03           | -                    | -              |
| SCRI_RS_177017 | -                | -               | 5H               | 77.52           | 2 x 10 <sup>-5</sup> | 0.01           | 4 x 10 <sup>-13</sup> | 0.04           | 6 x 10 <sup>-8</sup> | 0.02           |

<sup>a</sup> Chromosome (Chr) assignment and map position according to the consensus map of Muñoz-Amatriaín et al. (2014)

<sup>b</sup> Chromosome (Chr) assignment and map position according to the POPSEQ map Mascher et al. (2013)

<sup>c</sup> Significant markers found using raw stem rust severity values of datasets from the following field nurseries in St. Paul, MN and Njoro, Kenya: 2013 St. Paul (StP13), 2014 St. Paul (StP14), 2014 Kenya main-season (KEN14m), 2014 Kenya off-season (KEN14o),

2013 Kenya winter (KEN13w), and 2014 Kenya winter (KEN14w). No significant markers were found in StP13, KEN13w or KEN14w.

**Table 2.4.** Quantitative trait loci (QTL), genomic positions, and associated markers detected in the Barley iCore Collection (BCC) for seedling and adult plant stem rust resistance.

| QTL Name                      | Traits <sup>a</sup>                                       | Chr <sup>b</sup> | cM <sup>b</sup> | Markers <sup>c</sup>                                     |
|-------------------------------|---|------------------|-----------------|--|
| Rpg-qt1-1H-11_11277           | KEN14o  | 1H               | 91.15           | 11_11277   |
| Rpg-qt1-1H-12_20613           | StP14   | 1H               | 95.61           | 12_20613   |
| Rpg-qt1-2H-12_11278           | KEN14m  | 2H               | 59.49           | 12_11278   |
| Rpg-qt1-3H-<br>SCRI_RS_199887 | StP14   | 3H               | 77.69           | SCRI_RS_199887   |
| Rpg-qt1-5H-11_11355           | KEN14m, KEN14o, StP14,<br>Kenya_Spring_A,<br>StP_Spring_A | 5H               | 71-75           | 11_11355, 12_31427,<br>SCRI_RS_205853,<br>SCRI_RS_177017 |
| Rpg-qt1-5H-11_20388           | StP14   | 5H               | 137.22          | 11_20388   |
| Rpg-qt1-5H-11_10236           | TTKSK_Seedling  | 5H               | 172.23          | SCRI_RS_167103, 11_10236                                 |

<sup>a</sup> Adult plant rust severity datasets were from the following field nurseries: 2013 St. Paul (StP13), 2014 St. Paul (StP14), St. Paul spring disease severity data values corrected for field spatial variability and season differences and averaged between two replicates (StP\_Spring\_A), 2014 Kenya main-season (KEN14m), 2014 Kenya off-season (KEN14o), 2013 Kenya winter (KEN13w), 2014 Kenya winter (KEN14w), and Kenya spring disease severity data values corrected for field spatial variability and seasonal differences and averaged between two replicates (Kenya\_Spring\_A), TTKSK seedling coefficient of infection values as described by Zhou et al. (2014) where log<sub>10</sub> transformed and averaged between two replicates (TTKSK\_Seedling).

<sup>b</sup> Chromosome (Chr) assignment and map position according to the POPSEQ map Mascher et al. (2013).

<sup>c</sup> Markers were grouped together to form a QTL if the linkage disequilibrium between the was high ( $r^2 > 0.7$ ).

**Table 2.5.** Accessions from the Barley iCore Collection (BCC) that were classified as either highly resistant (Class 1) or resistant (Class 2) to stem rust infection at the adult stage in all locations based on the performance of Q21861 or Q/SM20, respectively.

| BCC Name <sup>a</sup> | Accession ID <sup>b</sup> | Country        | State or Region   | QCCJB <sup>d</sup> |       | Pgt TTKSK composite |        | TTKSK            |                 |        |     |
|-----------------------|---------------------------|----------------|-------------------|--------------------|-------|---------------------|--------|------------------|-----------------|--------|-----|
|                       |                           |                |                   | StP13 <sup>c</sup> | StP14 | KEN14m              | KEN14o | BSL3-1           |                 | BSL3-2 |     |
| BCC2297               | PI 584766                 | Australia      | Western Australia | 1                  | 0.5   | 0.5                 | 0.5    | 0.5 <sup>e</sup> | 0; <sup>f</sup> | 0.5    | 0;  |
| BCC1891               | PI 436153                 | Chile          | Los Lagos         | 5                  | 3     | 5                   | 5      | 2.75             | 21              | 3.125  | 2-3 |
| BCC0138               | CIho 6496                 | China          | Xizang            | 5                  | 1     | 5                   | 1      | 3.125            | 2-3             | 3.375  | 3-2 |
| BCC2233               | PI 566497                 | China          | Yunnan            | 5                  | 5     | 1                   | 0.5    | 3                | -2              | 3.125  | 2-3 |
| BCC1750               | PI 402445                 | Colombia       | Cundinamarca      | 0.5                | 3     | 0.5                 | 1      | 0.875            | 0;1             | 0.875  | 0;1 |
| BCC1917               | PI 447191                 | Spain          | -                 | 5                  | 1     | 5                   | 5      | 2                | -1              | 0.875  | 0;1 |
| BCC1928               | PI 449279                 | Spain          | Zaragoza          | 0.5                | 0.5   | 0.5                 | 1      | 4                | -3              | 3.5    | 3-  |
| BCC1568               | PI 370852                 | Switzerland    | -                 | 5                  | 5     | 0.5                 | 3      | 0.875            | 0;1             | 0.5    | 0;  |
| BCC1570               | PI 370867                 | Switzerland    | Valais            | 5                  | 1     | 0.5                 | 5      | 1.625            | 10;             | 0.875  | 0;1 |
| BCC1602               | PI 371390                 | Switzerland    | Graubunden        | 1                  | 1     | 1                   | 0.5    | 0.5              | 0;              | 0.5    | 0;  |
| BCC1195               | PI 308142                 | Ukraine        | Vinnytsya         | 5                  | 5     | 0.5                 | 5      | 2.25             | 12              | 2.75   | 21  |
| BCC2399               | PI 611513                 | Ukraine        | Vinnytsya         | 5                  | 1     | 0.5                 | 0.5    | 0                | 0               | 0.5    | 0;  |
| BCC2345               | PI 592196                 | United Kingdom | -                 | 0.5                | 1     | 0.5                 | 3      | 3.5              | 3-              | 3.375  | 3-2 |
| BCC0131               | CIho 6371                 | United States  | South Dakota      | 0.5                | 0.5   | 1                   | 1      | 1.625            | 10;             | 0.875  | 0;1 |

|         |           |               |              |   |   |     |     |       |     |       |     |
|---------|-----------|---------------|--------------|---|---|-----|-----|-------|-----|-------|-----|
| BCC2549 | PI 643314 | United States | North Dakota | 5 | 1 | 0.5 | 1   | 3.5   | 3-  | 3.375 | 3-2 |
| BCC2552 | PI 643336 | United States | North Dakota | 5 | 3 | 0.5 | 0.5 | 3.375 | 3-2 | 3.5   | 3-  |
| BCC2559 | PI 643370 | United States | North Dakota | 1 | 1 | 0.5 | 0.5 | 3     | -2  | 3.375 | 3-2 |
| BCC1850 | PI 429311 | Yemen         | -            | 5 | 3 | 5   | 1   | 3.375 | 3-2 | 3.125 | 2-3 |
| BCC1851 | PI 429312 | Yemen         | -            | 1 | 1 | 0.5 | 1   | 2.75  | 21  | 3.5   | 3-  |

<sup>a</sup> Sequential number given to all lines in the Barley iCore Collection (BCC).

<sup>b</sup> Accession ID, and passport information as provided by the United States Department of Agriculture-Agricultural Research Service, Germplasm Resource Information Network.

<sup>c</sup> Adult plant raw stem rust severity datasets were from the following field nurseries in St. Paul, MN and Njoro, Kenya: 2013 St. Paul (StP13), 2014 St. Paul (StP14), 2014 Kenya main-season (KEN14m), 2014 Kenya off-season (KEN14o), 2013 Kenya winter (KEN13w), and 2014 Kenya winter (Ken14w).

<sup>d</sup> *Puccinia graminis* f. sp. *tritici* (*Pgt*) races QCCJB, TTKSK, and *Pgt* TTKSK composite (*Pgt* races TTKSK, TTKST, TTKTK, and TTKTT).

<sup>e</sup> Coefficient of infection values as described by Zhou et al. (2014).

<sup>f</sup> Raw infection type from (0-4) scale (Miller and Lambert 1955; Stakman et al. 1962).

**Table 2.6.** Disease reduction effects of significant markers by nursery for both adult plant stem rust severity (%) to *Pgt* races QCCJB and TTKSK and seedling TTKSK coefficient of infection values, identified by genome-wide association study in the Barley iCore Collection (BCC).

| <b>St. Paul</b>         |                |                 |                 |                                |                       |       |                   |                 |       |                   |  |
|-------------------------|----------------|-----------------|-----------------|--------------------------------|-----------------------|-------|-------------------|-----------------|-------|-------------------|--|
|                         |                | <b>11_11355</b> |                 |                                | <b>SCRI_RS_177017</b> |       |                   | <b>12_20613</b> |       |                   |  |
|                         |                | Allele          | DS <sup>c</sup> | Allele effect (%) <sup>c</sup> | Allele                | DS    | Allele effect (%) | Allele          | DS    | Allele effect (%) |  |
| <b>St13<sup>a</sup></b> | S <sup>b</sup> | A               | 25.42           | 1.22                           | T                     | 25.67 | 1.43              | G               | 25.0  | 0.27              |  |
|                         | R              | G               | 22.97           | (9.6%)                         | C                     | 22.81 | (11.1%)           | A               | 24.47 | (2.1%)            |  |
| <b>St14</b>             | S              | A               | 14.0            | 1.24                           | T                     | 14.11 | 1.3               | G               | 25.0  | 6.01              |  |
|                         | R              | G               | 11.53           | (17.6%)                        | C                     | 11.51 | (18.4%)           | A               | 12.99 | (48%)             |  |

| <b>Njoro</b>  |   |                 |       |                   |                       |       |                   |                       |       |                   |                 |       |                   |
|---------------|---|-----------------|-------|-------------------|-----------------------|-------|-------------------|-----------------------|-------|-------------------|-----------------|-------|-------------------|
|               |   | <b>11_11355</b> |       |                   | <b>SCRI_RS_177017</b> |       |                   | <b>SCRI_RS_205853</b> |       |                   | <b>12_10493</b> |       |                   |
|               |   | Allele          | DS    | Allele effect (%) | Allele                | DS    | Allele effect (%) | Allele                | DS    | Allele effect (%) | Allele          | DS    | Allele effect (%) |
| <b>KEN14m</b> | S | A               | 22.21 | 4.69              | T                     | 22.35 | 4.55              | G                     | 20.75 | 4.89              | C               | 36.30 | 9.37              |



|               |   |   |       |         |   |       |         |   |       |          |   |       |          |
|---------------|---|---|-------|---------|---|-------|---------|---|-------|----------|---|-------|----------|
|               | R | G | 12.84 | (42.4%) | C | 13.25 | (40.2%) | T | 10.97 | (47.12%) | G | 17.57 | (51.6%)  |
| <b>KEN14o</b> | S | A | 19.43 | 3.39    | T | 19.43 | 3.2     | G | 18.16 | 3.02     | C | 26.92 | 5.35     |
|               | R | G | 12.66 | (34.9%) | C | 13.02 | (32.8%) | T | 12.12 | (33.25%) | G | 16.22 | (39.72%) |

### BSL-3 St. Paul

| <b>11_10236</b>                   |               |           |                          |         | <b>SCRI_RS_167103</b> |           |                          |
|-----------------------------------|---------------|-----------|--------------------------|---------|-----------------------|-----------|--------------------------|
|                                   | <b>Allele</b> | <b>DS</b> | <b>Allele effect (%)</b> |         | <b>Allele</b>         | <b>DS</b> | <b>Allele effect (%)</b> |
| <b>TTKSK_Seedling<sup>d</sup></b> | S             | G         | 3.41                     | 0.18    | T                     | 3.41      | 0.18                     |
|                                   | R             | A         | 3.05                     | (10.8%) | C                     | 3.05      | (10.8%)                  |

<sup>a</sup> Adult plant raw stem rust severity datasets were from the following field nurseries: 2013 St. Paul (StP13), 2014 St. Paul (StP14), 2014 Kenya main-season (KEN14m), and 2014 Kenya off-season (KEN14o).

<sup>b</sup> Effect of alleles of the susceptible (S) and the resistant (R) classes.

<sup>c</sup> The additive allelic effect was defined as half the difference between the two SNP classes, on terminal stem rust disease severity (DS) (%). Then, the reduction in disease severity was calculated as the difference between the two classes divided by the susceptible class, known as the effect of allele substitution (Falconer 1960).

<sup>d</sup> TTKSK seedling coefficient of infection values as described by (Zhou et al. 2014) were  $\log_{10}$  transformed and averaged between two reps (TTKSK\_Seedling).

## Online Resource Captions

**Online Resource 2.1.** Accessions of the Barley iCore Collection (BCC) and their reaction to stem rust.

<sup>a</sup> Sequential number given to all lines in the Barley iCore Collection (BCC).

<sup>b</sup> Accession ID, line name, breeding history, and passport information as provided by the United States Department of Agriculture-Agricultural Research Service, Germplasm Resource Information Network.

<sup>c</sup> Stem rust severity (%) in datasets in St. Paul, MN and Njoro, Kenya: 2013 St. Paul (StP13), 2014 St. Paul (StP14), 2014 Kenya main-season (KEN14m), 2014 Kenya off-season (Ken14o), 2013 Kenya winter (Ken13w), and 2014 Kenya winter (Ken14w).

<sup>d</sup> Seedling coefficient of infection values as described by Zhou et al. (2014) to race TTKSK in two replicates (BSL3-1 and BSL3-2) with raw infection type values in parentheses.

<sup>e</sup> StP stem rust severity values corrected for field spatial variability and seasonal differences. 2013 St. Paul spring disease severity values corrected by moving average (StP13\_mvlg), 2013 St. Paul spring disease severity values corrected by multiple regression (StP13\_mlm), 2014 St. Paul disease severity values corrected by moving average (StP14\_mvlg), 2014 St. Paul disease severity values corrected by multiple regression (StP14\_mlm), St. Paul spring disease severity data values corrected for field spatial variability and seasonal differences and averaged between two reps (StP\_Spring\_A).

<sup>f</sup> Kenya spring stem rust severity values corrected for field spatial variability and seasonal differences, 2014 Kenya spring main-season disease severity values corrected by moving average (KEN14m\_mvlg), 2014 Kenya spring main-season disease severity values corrected by multiple regression (KEN14m\_mlm), 2014 Kenya spring off-season disease severity values corrected by moving average (KEN14o\_mvlg), 2014 Kenya spring off-season disease severity values corrected by multiple regression (KEN14o\_mlm), and disease severity averaged between the two Kenya nurseries after season effect correction and spatial variability correction (Kenya\_Spring\_A).

<sup>g</sup> Kenya winter stem rust severity values corrected for field spatial variability and seasonal differences, 2013 Kenya winter disease severity values corrected by moving average (KEN13w\_mvlg), 2013 Kenya winter disease severity values corrected by multiple regression (KEN13w\_mlm), 2014 Kenya winter disease severity values corrected by moving average (KEN14w\_mvlg), 2014 Kenya winter disease severity values corrected by multiple regression (KEN14w\_mlm), Kenya winter disease severity data values corrected for field spatial variability and seasonal differences and averaged between two reps (Kenya\_Winter\_A).

## **Chapter 3**

# **Sources and Genetics of Stem Rust Resistance in a World Collection of Cultivated Barley**

## Introduction

Stem rust is one of the most devastating diseases of cereal crops, capable of destroying large acreages in a short period of time. On barley (*Hordeum vulgare* L.), stem rust is caused by either the wheat stem rust pathogen *Puccinia graminis* Pers.:Pers. f. sp. *tritici* Eriks. & E. Henn. (*Pgt*) or the rye stem rust pathogen *Puccinia graminis* Pers.:Pers. f. sp. *secalis* Eriks. & E. Henn. (*Pgs*) (Roelfs 1982). The northern Great Plains region of the United States is historically prone to periodic stem rust outbreaks. Yield losses to stem rust were commonplace in wheat (*Triticum* spp.) during the first few decades of the 20<sup>th</sup> century (Roelfs 1978). In the mid-1930's, stem rust epidemics caused by *Pgt* race 56 (MCCFC) (race translations by personal communication with Yue Jin) resulted in yield losses of up to 50% and 15% in wheat and barley, respectively. The same region suffered another major epidemic in the 1950's when the virulent *Pgt* race 15B (TPMKC) emerged, overcoming many of the resistance genes in bred into wheat cultivars grown in the region (Roelfs 1978). However, barley cultivars did not suffer any yield losses during this epidemic due to the protection provided by the durable rust resistance (Reaction to *Puccinia graminis* (*Rpg*)) gene *Rpg1* (Steffenson 1992). In the late 1980's, the *Rpg1*-virulent *Pgt* race QCC (QCCJB) emerged in the northern Great Plains, causing a minor epidemic on barley (Roelfs et al. 1991; Steffenson 1992). During this time, QCCJB was a common component of the United States *Pgt* population and was widespread on barley, causing losses of up to 60% in some fields (Roelfs et al. 1990). Only a few wheat cultivars in the Great Plain region were susceptible to QCCJB and following their removal this race became a rare component of the *Pgt* population.

In the United States and Canada, *Rpg1* has been the primary gene used in breeding barley for stem rust resistance (Steffenson 1992). With the exception of the QCCJB epidemics of the late 1980's, *Rpg1* has protected barley from significant losses since it was first incorporated into cultivars in the 1940's and remains effective against the most common races in the United States *Pgt* population (CDL 2016). *Rpg1* was first identified from the landrace accessions Chevron (CIho 11526) and Peatland (CIho 2613) and later the

cultivar Kindred (CIho 6969)--a farmer selection from a field of susceptible Wisconsin 37 barley. Chevron and Peatland are derivatives of the same landrace sample from Canton Lucerne in Switzerland. This country is an important source of stem rust resistance in barley as *Rpg1* was thought to have originated there, and many Swiss landraces carry the *rpg4/Rpg5* resistance gene complex (Steffenson et al. 2016). The sequence of *Rpg1* in Kindred is identical to that of Chevron and Peatland. It is therefore likely that the single plant selection that became Kindred was actually due to an admixture from Chevron or Peatland because these landraces were being used in the Wisconsin breeding program of the time (Brueggeman et al. 2002; Steffenson 1992). Together, these accessions formed the base germplasm used for stem rust resistance breeding in North American barley. To some extent in Canada, crosses were made with the resistant cultivar Gartons (CIho 645), which is a landrace derivative of unknown origin. No studies have been advanced to determine the genetics of stem rust resistance in Gartons (Steffenson 1992).

In response to the QCCJB epidemics, various barley collections were evaluated for resistance (Jin et al. 1994a; Jin et al. 1994b). The most resistant accession identified from this research was Q21861 (PI 584766), a line originally selected from a joint International Center for Agricultural Research in the Dry Areas (ICARDA)/Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT) barley breeding nursery based in Mexico. The pedigree of Q21861 is not known, but its stem rust resistance was first noted by Dill-Macky and Rees (1992) in a nursery in Queensland, Australia. Jin et al. (1994b) investigated the genetics of this line and found that resistance to race QCCJB was due to a single recessive gene. Additionally, Q21861 also was found resistant to *Pgs*, conferred by a dominant gene (*Rpg5*) that co-segregates with *rpg4* on chromosome 5H (Sun et al. 1996). Later, it was shown that the resistance at this locus is due to tightly linked but separate *rpg4* and *Rpg5* resistance genes, known collectively as the *rpg4/Rpg5* gene complex (Brueggeman et al. 2008; Brueggeman et al. 2009; Mirlohi et al. 2008; Steffenson et al. 2009). The *Pgs* resistance mediated by *Rpg5* acts independent from *rpg4*; however, for *Pgt* resistance, both *rpg4* and *Rpg5*--plus at least one other gene at

this locus is required. Q21861 has been used as a parent in some North American barley breeding programs. In addition to Q21861, other accessions were identified as having resistance to race QCCJB at the seedling and adult plant stages. Several of these accessions (e.g. PC11 and PC84) also have been used in North American breeding programs (Jin et al. 1994a). The genetics of resistance to race QCCJB in PC11 and PC84 is unknown, but both accessions do not carry the *rpg4/Rpg5* gene complex as determined by *Rpg5* marker analysis (unpublished data).

Together, *Rpg1* and the *rpg4/Rpg5* gene complex comprise the primary targets for stem rust resistance breeding in barley. Relatively little is known about other sources of stem rust resistance in the crop. Hietpas-5 (CIho 7124), a farmer selection from the landrace Oderbrucker (CIho 1272), carries adult plant stem rust resistance conferred by the resistance gene *Rpg2* (Patterson et al. 1957). *Rpg2* confers moderate to high levels of Adult Plant Resistance (APR) to *Pgt* in the field, but is not effective at the seedling stage (Fox and Harder 1995; Franckowiak 1991; Franckowiak and Steffenson 1997; Jin et al. 1994a; Miller and Lambert 1955; Shands 1964; Sun and Steffenson 2005). *Rpg2* has not been widely used in any North American breeding program. *Rpg3*, from the Ethiopian landrace accession GAW-79 (PI 382313), confers moderate resistance at the adult plant stage to several *Pgt* races and mostly susceptible reactions at the seedling stage (Jedel 1991; Jedel et al. 1989; Sun and Steffenson 2005). This gene has been used to some extent in a few Canadian breeding programs. Both *Rpg2* and *Rpg3* were shown to confer APR to race QCCJB (Fox and Harder 1995). More recently, a gene (*rpg6*) for seedling resistance to race QCCJB was identified in a barley line (212Y1) with an introgression from *Hordeum bulbosum* L. (Fetch et al. 2009). This gene resides on chromosome 6H and was originally transferred from *H. bulbosum* accession Cb2920/4/Colch into the barley cultivar Golden Promise (PI 467829). Although line 212Y1 exhibits seedling resistance to race QCCJB, its late maturity and poor growth habit have hampered adult plant phenotyping and utilization in North American breeding programs. Other provisionally designated genes for stem rust resistance in barley include *RpgU* from Peatland for APR to race QCCJB and *rpgBH* from Black Hulless (CIho 666) for APR to

*Pgs* (Fox and Harder 1995; Steffenson et al. 1984). The resistance spectrum and utilization of these genes is unknown.

North American breeding programs have focused largely on resistance conferred by *Rpg1* and to some extent *rpg4/Rpg5* (Steffenson 1992; Steffenson et al. 2017). More is known about the sources and genetics of stem rust resistance in North American barley cultivars than those from other regions of the world. This is due, in part, to the historical importance of this disease in the region. In Australia, stem rust also occurs on barley; however, it was only considered a minor problem prior to 1982. This situation changed when several severe epidemics occurred in Queensland and New South Wales in 1983-1984 (Dill-Macky et al. 1990). However, as in North America, stem rust on barley in Australia tends to occur only when inoculum levels are high due to a susceptible wheat crop (Park 2007). From the analysis of stem rust resistance in Australian barley cultivars, Derevnina et al. (2014) found that *Rpg1* is common. However, the prevalent *Pgt* races in Australia carry virulence for this gene. Some Australian barley cultivars that are resistant to stem rust may carry the *rpg4/Rpg5* resistance gene complex in addition to potentially novel resistance (Derevnina et al. (2014). The first introduction of *Rpg1* into Australian germplasm is not known. With respect to the *rpg4/Rpg5* complex, cultivar Prior may be the donor because it was a parent in the background of many resistant cultivars that carry these genes (Derevnina et al. 2014; Dill-Macky et al. 1990).

In addition to cultivars and breeding lines, landraces and wild species can be extremely valuable sources of resistance. In particular, landrace accessions originating from the mountainous regions of Switzerland were shown to be valuable sources of resistance to stem rust in barley (Mamo et al. 2015; Steffenson et al. 2016). A landrace from this region was the original source of *Rpg1* in North American barley cultivars, and the *rpg4/Rpg5* gene complex was found in over 40% (n=73) of Swiss landraces tested races QCCJB and TTKSK (Steffenson 1992; Steffenson et al. 2016). Wild barley (*Hordeum vulgare* ssp. *spontaneum*) is also a valuable source of stem rust resistance in barley. The presence of *rpg4/Rpg5* was confirmed in a number of wild barley accessions resistant to

race TTKSK, indicating this gene complex likely originated in this barley progenitor (Steffenson et al. 2017). *Rpg1* has yet to be found in wild barley, leading to speculation that the origin of this gene is from cultivated barley, likely landraces from Switzerland (Hulse et al. 2016).

It is important to identify new genes for stem rust resistance in barley so the resistance spectrum of the crop can be broadened. In barley, only eight stem rust resistance genes have been described as compared to wheat where more than 73 genes have been reported (Singh et al. 2015). The urgency of identifying new resistance genes is highlighted by the threat posed from the rapidly evolving *Pgt* races in the “Ug99 race group” originating in Africa. This race group is virulent on more than 80% of the world’s wheat and more than 95% of the world’s barley cultivars (Singh et al. 2008; Steffenson et al. 2017). This includes wheat cultivars carrying the widely effective gene *Sr31* and barley cultivars carrying *Rpg1*. This wide virulence on cultivars of both crops is uncommon and exceptionally dangerous due to the possibility for cross species infection and inoculum buildup. Under this scenario, barley could become highly vulnerable to significant yield loss as demonstrated in Australia and North America (Dill-Macky et al. 1990; Steffenson 1992). Clearly, the threat these races pose is alarming. Fortunately, these races have failed to become established outside of Africa and West Asia. If they do spread to the stem rust prone areas of cereal production in South Asia, Australia, and North America, the losses could be substantial on both wheat and barley.

The evolution of new *Puccinia graminis* virulence types in domestic populations should not be underestimated. In North America, there are several *Pgt* races (e.g. QCCJB among others) and a number of *Pgs* isolates that are virulent on *Rpg1* but relatively avirulent or completely avirulent on wheat (CDL 2016; Nirmala et al. 2015; Steffenson 1992). In addition to *Rpg1* virulence, there is at least one described race (HKHJ now designated HKHJC) with virulence for the *rpg4/Rpg5* gene complex (Sun and Steffenson 2005). Races with virulence to one or the other of these major resistance genes are present in the United States stem rust population and could pose a threat to barley should they become



established and gain additional virulence for important wheat stem rust resistance genes.

Although a considerable effort has been expended on screening large collections of barley for resistance to stem rust, relatively few new sources of resistance have been described and their resistance loci characterized (Mamo 2013; Mamo et al. 2015; Steffenson et al. 2017; Steffenson et al. 2016; Turuspekov et al. 2016; Wamalwa et al. 2016; Zhou et al. 2014). Most of the previous studies have confirmed that the *rpg4/Rpg5* gene complex is important for stem rust resistance, particularly those focused on race TTKSK. In the study described herein, the United States Department of Agriculture-Agricultural Research Service, National Small Grains Collection (USDA-ARS NSGC) Barley iCore Collection (BCC) was analyzed for stem rust resistance. The BCC is a very diverse collection of landraces and cultivars from around the world and represents the diversity available within cultivated barley at the USDA-ARS NSGC (Muñoz-Amatriaín et al. 2014). In Chapter 2, this collection was subjected to a genome-wide association study (GWAS) for seedling reaction to race TTKSK and APR to races TTKSK and QCCJB. From this work, the most resistant accessions were assembled and analyzed further to elucidate and postulate what genes might be conferring resistance in this collection.

## **Materials and Methods**

### *Plant Materials*

The BCC is a subset of the large barley germplasm holdings of USDA-ARS NSGC in Aberdeen, ID. This collection was described in detail by Muñoz-Amatriaín et al. (2014). The entire BCC consists of 1,860 accessions from around the world including landraces, cultivars, and genetic stocks. In Chapter 2, GWAS was conducted for stem rust APR to races QCCJB and *Pgt* TTKSK composite and seedling resistance to TTKSK. From this experiment, 290 of the most resistant accessions were selected and designated as the BCC Selects (BCCS) (Appendix A2). Inclusion in the BCCS was based on whether an accession was ranked within the top 5% of the BCC panel for APR to race QCCJB or *Pgt*

TTKSK composite or seedling resistance to race TTKSK in at least two replications. Principal component analysis of the BCC with the BCCS highlighted showed that the selected resistant accessions were reasonably distributed across all of the sub-populations described by Muñoz-Amatriaín et al. (2014) (Figure 3.1).

Susceptible and resistant barley accessions were included as controls for germplasm evaluations at the seedling and adult plant stages. Hiproly (PI 60693), Steptoe (CIho 15229), and PI 532013 are susceptible to most races of *Pgt*. Chevron (PI 38061) is resistant to most races of the *Pgt* due to the presence of *Rpg1* (Steffenson 1992). Lines 80-TT-29 (CIho 16129) and 80-tt-30 (CIho 16130) are near-isogenic, differing for the presence and absence of *Rpg1*, respectively (Steffenson et al. 1985). The sources of *Rpg2* and *Rpg3* are Hietpas-5 (CIho 7124) and GAW 79-3 (PI 382313), respectively (Franckowiak 1991; Franckowiak and Steffenson 1997; Jedel 1991; Jedel et al. 1989; Patterson et al. 1957; Shands 1964). The most widely resistant control is Q21861 (PI 584766), which carries both *Rpg1* and *rpg4/Rpg5* (Arora et al. 2013; Brueggeman et al. 2008; Brueggeman et al. 2009; Jin et al. 1994b; Steffenson et al. 2009; Steffenson et al. 1995; Sun et al. 1996; Wang et al. 2013). The resistant control Q/SM20 carries only *rpg4/Rpg5* and was developed from a cross between Q21861 and SM89010, a susceptible breeding line from Canada (Steffenson et al. 1995). Additional lines with other *Rpg* genes include Diamond (PI 491573) carrying *Rpg1* and *RpgU*, 212Y1 carrying *rpg6*, and Black Hulless (PI 24849) carrying *rpgBH* (Fetch et al. 2009; Fox and Harder 1995; Steffenson et al. 1984). In addition to the susceptible barleys of Hiproly, Steptoe, and PI 532013, several susceptible wheat accessions were also included in the experiments. LMPG-6 and Red Bobs (CItr 6255) were included as controls in the adult plant experiments in the field, whereas McNair 701 (CItr 15288) and Line E (PI 357308) were included in seedling tests. Golden Promise (PI 467829) and Hv492 (PI 371249) were used as molecular marker controls for *Rpg1* and *Rpg5* genotyping due to their rare susceptible *rpg1* and *rpg5* alleles, respectively.

### *Stem Rust Isolates and Races*

The BCCS collection was evaluated to *Pgt* races TTKSK (isolate: 04KEN146/04), TTKST (06KEN19-v-3), TKTTF (13ETH18-1), TRTTF (06YEM34-1), QCCJB (QCC-2), MCCFC (A-5), HKHJC (CRL-1), and the *Pgs* isolate 92-MN-90 (Table 3.1). The *Pgt* race nomenclature is based on the North American stem rust differential system (Jin et al. 2008; Roelfs 1988; Roelfs et al. 1991). Races TTKSK and TTKST are from Kenya, are members of the Ug99 race group, and are virulent for *Rpg1* (Jin et al. 2007; Jin et al. 2008; Steffenson and Jin 2006). Races TKTTF and TRTTF are from Ethiopia and Yemen, respectively, but are not considered part of the Ug99 race group as revealed by their molecular marker profile and avirulence for *Sr31* (Olivera et al. 2015; Singh et al. 2011; Singh et al. 2015). *Pgt* races QCCJB, MCCFC, and HKHJC are from North America and are relatively avirulent on wheat. Additionally, they are not commonly detected in race surveys in the United States (CDL 2016). QCCJB is virulent for *Rpg1*, whereas MCCFC and HKHJC are avirulent for the gene. HKHJC is unique in that it is the only race of the panel which is virulent for *rpg4/Rpg5*. QCCJB and MCCFC are avirulent for *rpg4/Rpg5*. 92-MN-90 is a *Pgs* isolate from Minnesota, and is avirulent for *Rpg1* and *rpg4/Rpg5* at the seedling stage (Sun and Steffenson 2005). This isolate may have virulence for *Rpg1* at the adult plant stage (Steffenson et al. 1984).

### *Adult Plant Phenotyping*

The BCCS was evaluated for resistance to *Pgt* races QCCJB, MCCFC and HKHJC at the adult plant stage in St. Paul, MN in 2015 (datasets: StP15\_QC, StP15\_MC, and StP15\_HK). The three nurseries were planted in separate fields at the Minnesota Agricultural Experiment Station on the St. Paul campus of the University of Minnesota. Fields inoculated with *Pgt* races MCCFC and QCCJB were at least 0.25 km apart from each other, whereas the field inoculated with *Pgt* race HKHJC was 1 km away from the other two fields. About two grams of seed were sown in half-meter rows, and the resulting plots were maintained according to standard agricultural practices in the region. Two paired rows of test entries were planted between continuous rows of a susceptible

barley spreader row. This spreader row was comprised of cultivar Steptoe in the MCCFC and HKHJC nurseries and of equal proportions of cultivars Quest, Stander and Conlon in the QCCJB nursery. Each test entry plot was bordered on one side by a spreader row. Hiproly, Chevron, Q21861, and Q/SM20 were repeated every 50 and PI 532013 and LMPG-6 every 150 entries in the nurseries. Steptoe was planted in a random position of every planting tray of 40 entries. At the tillering stage, two stems of plants in the spreader row were injected with 1.0 ml of rust inoculum. This inoculum was prepared by mixing 1 g of fresh urediniospores into 1 L of distilled water to which six drops of 20% Tween 20 (Polyoxyethylene (20) sorbitan monolaurate) was added. The test entries were also directly spray-inoculated with a suspension of urediniospores in a lightweight mineral oil (1 g of urediniospores in 1 L of Soltrol 170 oil (Phillips Petroleum, Bartlesville, OK)) using a held-held, battery-operated sprayer (Mini-ULVA, Micron Group, Herefordshire, United Kingdom) at a rate 1 L inoculum per 800 plots. Two to three spray-inoculations were performed starting when 50-75% of test entries were at the booting stage (Zadoks 40+) or beyond (Zadoks et al. 1974). To prevent cross-contamination, only one race was inoculated per day. If this was not possible, all equipment was surface disinfested and clothing changed between inoculations with different races. Races HKHJC, QCCJB, and MCCFC were confirmed after the season by inoculating samples collected from the field onto key wheat differentials having differing reactions to these races. Disease severity (DS) was estimated using the modified Cobb scale for stem rust-infected stem and leaf sheath tissue (0-100%)(Peterson et al. 1948). In addition, the Infection Response (IR) was also scored based on the size and type (presence of chlorosis/necrosis) of uredinia observed. However, as highly significant correlations were observed between IR and DS (see Chapter 2), only the latter values were used in the final analysis. As stem rust development is strongly influenced by plant maturity, evaluations were conducted when plants were between the heading to hard dough stages of development (Zadoks 55-87) (Zadoks et al. 1974). In Chapter 2, the BCCS were evaluated for APR to race QCCJB in St. Paul in 2013 and 2014 (StP13\_QC and StP14\_QC) and also to *Pgt* TTKSK composite in Njoro, Kenya during the 2014 main and off-seasons (KEN14m and KEN14o). During

the screening trials in Njoro, the predominant *Pgt* races were TTKSK and TTKST (16 of 25 samples) with some TTKTK and TTKTT (9 of 25 samples) also present (Newcomb et al. 2016). These races will collectively be referred to as *Pgt* TTKSK composite to reflect the presence of races other than TTKSK. Disease ratings for these experiments were conducted as described above. The controls of Chevron, Q21861 and Hiproly were repeated every 50 entries in KEN14o and every 200 entries in KEN14m. The wheat accession Red Bobs was planted every 50 entries in both seasons in Kenya. The KEN14o nursery also included Steptoe and Q/SM20 repeated every 50 and PI 532013 and LMPG-6 repeated every 150 entries.

BCCS accessions were classified as highly resistant (Class 1) and resistant (Class 2) based on their comparative performance to the controls in individual nurseries. The maximum DS value for Q21861, was used as the upper cutoff value to classify accessions within Class 1, whereas values greater than the maximum DS for Q21861 but less than the minimum recorded DS values for Hiproly or Steptoe were used as the range for classifying accessions within Class 2 (Table 3.3).

### *Seedling Phenotyping*

Seedling phenotyping to races of North American origin was performed in a growth chamber on the St. Paul campus of the University of Minnesota, whereas phenotyping with foreign races took place inside a growth chamber or greenhouse in the Minnesota Agricultural Experiment Station / Minnesota Department of Agriculture Plant Growth Biosafety Level-3 (BSL-3) Containment Facility on the St. Paul campus of the University of Minnesota. All experiments were designed as a randomized complete block with twice-replicated controls of Q21861, Steptoe, PI 532013, 80-tt-30, and 80-TT-29. Hiproly, Q/SM20, and Chevron were replicated more frequently: once in each tray or cone rack. Barley entries were tested against each race with a minimum of two replications. Outside the BSL-3, experiments were planted in 96 count racks containing cones (5 cm diameter x 18 cm depth, Stuewe and Sons, Inc., Tangent, OR), and inside the BSL-3 experiments were planted in 16 count trays containing peat pots. In both types

of containers, the planting medium was a 1:1 mixture of sterilized topsoil and Sunshine MVP potting mix (vermiculite, Canadian sphagnum peat moss, nutrient charge, gypsum, and dolomitic limestone) (Sun Gro Horticulture, Quincy, MI). Plants were fertilized at planting with a pH buffering 15-0-15 formulation (Peters Dark Weather, Scott's Company, Marysville, OH) at 1/16 dilution (ca. 40 g/liter) and thereafter by a water-soluble formulation (20-10-20) at 1/16 dilution (ca. 40 g/liter) (J.R. Peters, Inc., Allentown, PA) on a weekly basis.

When the first leaves of plants were fully expanded (about 1 week), plants were inoculated with 12-15 mg of urediniospores suspended in 0.8 ml Soltrol 170 oil (Phillips Petroleum, Bartlesville, OK). The inoculum was contained in a gelatin capsule (size 00) fit inside a custom inoculator and sprayed as a fine mist onto plants pressured by a pump set at 25-30 kPa. One capsule was used per tray of 16 entries in the BSL-3, whereas outside the BSL-3 one capsule was used for every 48 entries, for a rate of about 0.05 and 0.015 ml of inoculum per plant, respectively. The oil carrier on plants was allowed to off gas for 90 minutes under lights with constant air circulation to reduce its phytotoxic effects. Plants were then incubated in mist chambers overnight at near 100% humidity for 16-18 hours in the dark at 22-25°C. The moist conditions for the infection period were produced by periodic misting from household ultrasonic humidifiers. In the morning, lights (400 W sodium vapor lamps providing 150-250  $\mu\text{mol photons s}^{-1}\text{m}^{-2}$ ) were switched on for four hours, after which the misters were turned off and the doors opened to allow plants to dry slowly. Once dry, plants were transferred back to either the growth chamber or greenhouse. Plants were then incubated with a 16-hour photoperiod in the greenhouse (400 W sodium vapor lamps emitting 300-350  $\mu\text{mol photons s}^{-1}\text{m}^{-2}$ ) or growth chamber (40 W fluorescent and incandescent lamps emitting 300-350  $\mu\text{mol photons s}^{-1}\text{m}^{-2}$ ). Incubation temperatures were set to optimize the infection responses of host genotypes to the specific rust cultures: either 18/22°C (night/day) for QCCJB and 92-MN-90 or 22/25°C for TTKSK, TTKST, TKTTF, TRTTF, MCCFC, and HKHJC (Sun and Steffenson 2005).

After 14 to 17 days of incubation, ITs were scored on the first leaves of plants. The second leaves of plants were clipped off three to five days before scoring to increase light penetration for maximum uredinia development. ITs were scored according to the 0-4 scale originally developed for wheat and modified for barley (Miller and Lambert 1955; Stakman et al. 1962). Briefly, IT 0 = no visible sign of infection; IT 0+ = hypersensitive flecks, but no uredinia; IT 1 = minute uredinia; IT 2 = small, restricted uredinia; IT 3- = medium-sized uredinia with some restriction; 3 = large uredinia showing no restriction; and IT 3+ = extremely large uredinia showing no restriction. Unlike wheat, barley does not exhibit the classic “green island” reaction associated with IT 2 nor the classic diamond-shaped uredinia of IT 4. Due to the typical mesothetic reaction of barley to stem rust (Miller and Lambert 1955), the three most common ITs observed were recorded in order of their prevalence. Raw IT values, data were transformed to a linear score (Table 3.4). Scores were converted to the nearest IT on the linear score. If the IT was not accounted for in Table 3.4, the third most common IT was dropped. For example, if an accession was given an IT of “213-“ this was converted to a “21” prior to being translated to the linear score. For comparison of reactions across replicates, the median linear score value (rounded-up to the nearest whole number) was calculated for each accession to each race. The linear score is a sequential progression of values representing the 15 most commonly observed ITs in the dataset from most resistant to most susceptible (Figure 3.2). Lines were considered resistant if the median IT  $\leq 2$  (linear score  $\leq 7$ ). Pearson Product-Moment correlation was calculated using the median linear score values between races.

### *Gene Postulation*

To postulate the resistance genes in the barley accessions, we followed the methods established in the Statistical Analysis System (SAS) computer program written by Wamishe et al. (2004). Since this program was written in an older version of SAS that is not widely supported, a new package called RGenePos was written in R (Team 2016). RGenePos executes all of the analysis steps defined by Wamishe et al. (2004) and is

available on GitHub (<https://github.com/austinjcase/RGenePos>) with the program source code given in Online Resource 3.1.

To postulate resistance genes, the reactions of the BCCS accessions were compared to the reactions of genetically characterized barley accessions carrying known resistance genes. The reaction of Chevron was used to define the reaction of *Rpg1*, Hietpas-5 the reaction of *Rpg2*, GAW-79 the reaction of *Rpg3*, and Q/SM20 the reaction of *rpg4/Rpg5*. Although these accessions were shown to carry these respective genes, it is not known whether they might also carry additional genes. Accession 212Y1, which carries *rpg6*, was removed from the analysis due to data quality concerns caused by its brachytic growth habit. Diamond, the control for *Rpg1+RpgU*, was removed because none of the races used could differentiate it from Chevron; thus, the presence of *RpgU* could not be specifically excluded in analysis. For the same reason, Black Hulless (with *rpgBH*) was also excluded as none of the available races were avirulent on this accession. Therefore, accessions Chevron, Hietpas-5, GAW-79, and Q/SM20 were treated as a differential panel to define the reactions of these *Rpg* genes in this R program, although they have some limitations for gene postulations. First, some of the accessions may carry more than one gene, complicating the postulation of individual resistance genes present in the BCCS. Second, the barley differentials are not near-isogenic as called for by Wamische et al. (2004). Near-isogenic lines are extremely useful for gene postulation studies because background genetic effects are eliminated, thereby giving clearer expression of the respective *Rpg* genes.

In the gene postulation analyses, BCCS accessions and differentials must be classified as either low (L) (i.e. resistant) or high (H) (i.e. susceptible). In the stem rust pathosystem, an IT of 2 is usually considered the upper cut-off value for classifying an accession as resistant, the relationship between IT and the linear score is given in Table 3.4 (Miller and Lambert 1955; Stakman et al. 1962). Therefore, the expected avirulence profile of the barley control *Rpg* genotypes was: *Rpg1* (TRTTF, MCCFC, HKHJC, and 92-MN-90); *Rpg2* (HKHJC and 92-MN-90); *Rpg3* (92-MN-90); *rpg4/Rpg5* (TTKSK, TTKST,



TKTTF, TRTTF, QCCJB, MCCFC, and 92-MN-90) (Table 3.1). In the wheat stem rust differentials, the common high and low ITs may differ for the respective resistance genes (Roelfs 1988; Roelfs et al. 1991; Jin et al. 2008). In barley, the common high and low ITs are less well-defined. Therefore, ITs of 2 or lower were considered as the cut-off value for resistance. In the RGenePos package, H or L reaction calls can be defined manually by the user or with one of several functions included in the package where the user can define IT scores as high or low based on the linear score shown in Table 3.4.

To postulate genes in the BCCS, the reactions of individual accessions were compared to those of the differential hosts across a panel of *Pgt* races that differed for virulence and avirulence on the described *Rpg* genes. When a BCCS accession and a differential host both exhibited resistance to the same suite of races, the former and the latter were postulated to carry at least one gene in common. This outcome was coded as “LL” (low low). When an accession exhibited susceptibility and a differential exhibited resistance to any of the suite of races, this was taken as strong evidence that the former did not carry the same gene as the latter. This outcome was coded as “HL” (high low). When both the accession and differential were susceptible to the suite of races, the relationship between their resistance genes is unknown as a particular race may be virulent for many different resistant genes. This outcome was coded as “HH” (high high). When both the accession and differential were resistant to the suite of races, the relationship between their resistance genes was also unknown as a race may be avirulent for many different resistance genes. This outcome was coded as “LL” (low low).

In the RGenePos package under outcome LL, was assigned a score of “1”, indicating that definitively rule out the presence of the known resistance gene in the differential being present in the accession, and the result was assigned a score of “-1”. Outcomes LH and HH were assigned “NA” for not applicable, since no useful data could be obtained from these results, i.e. the presence or absence of the known resistance gene in the differential could not be definitively established for the accession. The GenePos function in the package analyzes these data and for each accession will list the races and differentials

that never yield a -1 reaction on a per gene basis. The ITs exhibited by the accession will be displayed alongside the ITs of the respective differential, allowing the user to easily compare them side by side. If an accession gives a low reaction to races for which all known genes in the differential set give a high reaction, then a “+” symbol was assigned to the postulated gene(s), indicating the possible presence of additional genes in the accession.

### *Genotyping for Rpg1*

Genotyping for the presence of the *Rpg1* resistance gene was accomplished by Polymerase Chain Reaction (PCR) amplification of markers based on the cloned sequence of the gene. This marker is based on non-functional *rpg1* alleles having a 3 bp GTT insertion at amino acid position 320, resulting in a serine to arginine conversion and insertion of a phenylalanine residue (Brueggeman et al. 2002). This insertion is shared in susceptibility types 1, 2 and 3 (such as Steptoe) and is absent in resistant accessions (such as Chevron or Morex) with *Rpg1*.

Marker RPG1-S will amplify a product with group 1, 2, and 3 susceptible *rpg1* genotypes, but not with group 4 *rpg1* (such as Golden Promise) or *Rpg1* genotypes. RPG1-R or RPG1-N will amplify a product from the *Rpg1* genotypes, but not from group 1, 2, 3, or 4 susceptible *rpg1* genotypes (Table 3.5) (Eckstein et al. 2003). RPG1-N was used in this study to detect *Rpg1* genotypes. This marker is allele-specific and is based on the 3 bp insertion difference between the genotypes as described by Eckstein et al. (2003) and modified by Derevnina et al. (2014). RPG1-S was used to detect *rpg1* susceptible genotypes from groups 1, 2, and 3. One issue with the RPG1-S marker is that some wild barley (*Hordeum vulgare* ssp. *spontaneum* C. Koch Thell) accessions like OSU6 have a functional *Rpg1* gene, but also the 3bp insertion (Eckstein et al. 2003). Thus, it is possible for this marker to generate a false negative for *Rpg1*.

### *Genotyping for Rpg5*

Genotyping for the presence of a functional copy of *Rpg5* was used to determine if an accession has *Pgt* resistance conferred by the *rpg4/Rpg5* gene complex, as these two genes are very tightly linked (Steffenson et al. 2009). *Rpg5*-mediated *rpg4* resistance requires a functional *Rpg5* gene. The *rpg4/Rpg5* locus requires at least three genes to function: *HvRga1* (a NBS-LRR gene), the NBS-LRR-STPK gene that is *Rpg5*, and the actin depolymerizing factor-like gene *HvAdf3* (Brueggeman et al. 2008; Brueggeman et al. 2009). In susceptible cultivars (such as Steptoe and Morex), the serine/threonine protein kinase (STPK) domain of *Rpg5* is replaced by a protein phosphatase 2C gene (PP2C), called *HvPP2C*, rendering the accessions susceptible (Brueggeman et al. 2009; Wang et al. 2013). The marker LRK is designed to amplify the STPK domain of *Rpg5*, whereas the PP2C marker amplifies the inserted PP2C domain in susceptible *rpg5* cultivars (Table 3.6). These are updated markers from those reported by Wang et al. (2013) and modified by Arora et al. (2013) and R. Brueggeman (personal communication)(Derevnina et al. 2014; Steffenson et al. 2016).

There are at least two other non-functional *rpg5* genotypes that are not detected by the above-mentioned markers. Genotypes Hv492 (group 4S) and Golden Promise (group 2S) will give a false positive for the LRK marker as these genotypes have an intact but non-functional STPK domain (Arora et al. 2013). To detect group 2 and 4 *rpg5* genotypes, sequencing across the variant sequence was conducted. For group 2 *rpg5* alleles, an amplicon by PCR with the R5 marker was created and then sequenced using the R5-R1 primer (Table 3.7). Group 2 *rpg5* genotypes have the sequence (“GCAGGATCCCCCCCCATCACGG”) containing a single “C” insertion that is missing in *Rpg5* genotypes (“GCAGGATCCCCCCATCACGG”). To genotype for group 4 *rpg5* genotypes, PCR amplification using the R10 marker was conducted, and the amplicon was sequenced with the R1-seq-R1 primer (Table 3.7). Group 4 *rpg5* genotypes have amino acid change E1287A, resulting in the sequence “TCCTTCCCCGCGCGGG”, in contrast to *Rpg5* genotypes with the sequence “TCCTTCCCC(A/G)CGAGGG”. Thus, genotypes with a functional *Rpg5* gene would; 1) amplify the LRK marker, 2) not

amplify the PP2C marker, and 3) not have the group 2 or 4 *rpg5* sequence.

## Results

### *Adult Plant Resistance*

The BCCS were evaluated for APR to *Pgt* races HKHJC, QCCJB, MCCFC, and *Pgt* TTKSK composite (Table 3.8) and also to other races at various locations (Online Resource 3.1). Rust samples collected at the end of the season from the individual nurseries confirmed the presence of the inoculated races of HKHJC, QCCJB, and MCCFC. Only a few off-type pustules were observed on the abbreviated wheat stem rust differential panel, indicating a slight contamination of race QCCJB in the MCCFC nursery in 2015 (data not shown). Overall, the controls performed as expected with Chevron being highly resistant to races HKHJC and MCCFC, while exhibiting higher severities to races QCCJB and *Pgt* TTKSK composite (Figure 3.3). Q/SM20, carrying *rpg4/Rpg5*, was highly resistant to all races except HKHJC, one of the few described races with virulence for this gene complex. Q21861, carrying both *Rpg1* and *rpg4/Rpg5*, was highly resistant to all of the tested races as expected (Figure 3.3).

The correlation for adult plant rust severity of BCCS was highest between races MCCFC and HKHJC ( $r^2 = 0.47$ ) and lowest between HKHJC and TTKSK ( $r^2 = 0.01$ ) (Table 3.2). A high level of diversity was observed for both the terminal disease severity and also IR (Figure 3.4). To classify the level of resistance in the BCCS, the stem rust severity of accessions was compared to that of the controls. The Class 1 and Class 2 categories were defined based on the performance of the controls: i.e. the maximum severity of Q21861 defined the upper limit of the Class 1 category and any accession having a severity higher than this maximum, but lower than the minimum severity of Hiproly or Steptoe defined the Class 2 category. The number of accessions classified as Class 1 and Class 2 ranged from 15-162 and 26-146, respectively (Table 3.3). The largest number of accessions rated as Class 1 was found in KEN14m, whereas the smallest number was found in StP15\_QC. Multiple nurseries in Kenya (to Ug99 group races) and St. Paul (race QCCJB) allowed

for comparisons across seasons. In this regard, seventy-six accessions were rated as Class 1 across the two nurseries in Kenya, and five were rated as Class 1 across the three nurseries in St. Paul (Table 3.8). Twelve accessions were rated as either Class 1 or Class 2 across all nurseries (Table 3.9) and represent the highest and most stable resistance identified in the BCCS. Of these 12, three originated from Colombia and also the United States and one each from Australia, Ethiopia, Mexico, Saudi Arabia, Spain, and Ukraine.

### *Seedling Resistance*

The seedling ITs of barley controls carrying different *Rpg* genes and of the North American wheat stem rust differentials carrying different *Sr* genes were recorded in response to *Pgt* races TTKSK, TTKST, TKTTF, TRTTF, QCCJB, HKHJC, and MCCFC as well as *Pgs* isolate 92-MN-90 (Table 3.1). The reaction of the wheat differentials to most of these races has been well characterized and reported previously (Jin et al. 2007; Jin et al. 1994b; Jin et al. 2008; Olivera et al. 2015; Singh et al. 2011; Singh et al. 2015; Steffenson and Jin 2006; Sun and Steffenson 2005; Sun et al. 1996). In contrast, the reaction of barley controls carrying various *Rpg* genes, other than *Rpg1* and *rpg4/Rpg5*, has not been fully characterized (Table 3.1). For these experiments, the virulence | avirulence pattern of the *Pgt* races and *Pgs* culture was characterized on the barley control genotypes of Chevron (*Rpg1*), Hietpas-5 (*Rpg2*), GAW-79 (*Rpg3*), Q/SM20 (*rpg4/Rpg5*), 212Y1 (*rpg6*), Black Hulless (*rpgBH*), and Diamond (*Rpg1 + RpgU*) (Table 3.1). The virulence | avirulence pattern for races TTKSK and TTKST was *Rpg1*, *Rpg2*, *Rpg3*, *rpgBH*, *Rpg1+RpgU* | *rpg4/Rpg5*, *rpg6*. Race TKTTF had the same pattern as TTKSK and TTKST, but with a gain of virulence for *rpg6*. TRTTF had a similar pattern as TTKSK and TTKST, but with a loss of virulence for *Rpg1*. It is noteworthy that the reaction (IT = 21) elicited by race TRTTF on *Rpg1* genotypes was higher than is usually observed (0; to 10;) for a race with avirulence on this gene. Race QCCJB had a virulence | avirulence pattern of *Rpg1*, *Rpg2*, *Rpg3*, *rpgBH*, *Rpg1 + RpgU* | *rpg4/Rpg5*, *rpg6*. Race MCCFC had the same virulence pattern as QCCJB, but with a loss of virulence for *Rpg1* and a gain of virulence for *rpg6*. The pattern of race HKHJC was *Rpg3*, *rpg4/Rpg5*,

*rpgBH* | *Rpg1*, *Rpg2*, *rpg6*, *Rpg1+RpgU*. The *Pgs* isolate 92-MN-90 was the most avirulent culture used in the study and had a virulence | avirulence pattern of *rpgBH* | *Rpg1*, *Rpg2*, *Rpg3*, *rpg4/Rpg5*, *Rpg1+RpgU*. Diamond exhibits a reaction pattern similar to Chevron and 80-TT-29, suggesting that none of the *Puccinia graminis* cultures used carry virulence for *Rpg1+RpgU* at the seedling stage. Line 212Y1 with *rpg6* is very difficult to phenotype due to its brachytic growth habit. In our preliminary evaluations, it was clearly susceptible to races MCCFC and TKTTF, but gave clear resistant reactions to *Pgt* races QCCJB and HKHJC and *Pgs* isolate 92-MN-90. Line 212Y1 exhibited intermediate (IT=23-) or variable reactions to *Pgt* races TRTTF, TTKSK, and TTSKT. Due to the abnormal growth habit of 212Y1 and the difficulty in obtaining a reliable infection phenotype, it was removed from further analysis. Black Hulless was susceptible to all *Puccinia graminis* cultures used and was therefore removed from further analysis. Q21861, carrying both *Rpg1* and *rpg4/Rpg5*, was resistant to all races tested. The resulting resistance profiles of the barley genotypes carrying different *Rpg* genes were as follows: *Rpg1* effective against TRTTF, MCCFC, HKHJC, and 92-MN-90; *Rpg2* effective against HKHJC and 92-MN-90; *Rpg3* effective against 92-MN-90 only; and *rpg4/Rpg5* effective against TTKSK, TTKST, TKTTF, TRTTF, QCCJB, MCCFC, and 92-MN-90.

The seedling reactions of the BCCS were recorded to *Pgt* races TTKSK, TTKST, TKTTF, TRTTF, QCCJB, HKHJC, and MCCFC as well as *Pgs* isolate 92-MN-90 (Table 3.10). A high level of diversity for seedling ITs was observed among the accessions (Figure 3.5). The number (and percent) of accessions found resistant to each of the respective cultures was as follows: 36 (12%) for TTKSK, 22 (8%) for QCCJB, 17 (5%) for TTKST, 47 (16%) for TKTTF, 20 (7%) for MCCFC, 47 (16%) for TRTTF, 37 (13%) for HKHJC, and 91 (31%) for 92-MN-90. The fewest number of resistant accessions found was to race TTKST, and the largest number found was to isolate 92-MN-90. The correlation for ITs of the BCCS between races was relatively low in most cases. For this analysis, the raw seedling ITs were converted to a linear score as shown in Table 3.4. The highest correlation observed between two races was with MCCFC and HKHJC ( $R^2 =$

0.75) and the lowest correlation observed was between 92-MN-90 and HKHJC ( $R^2 = 0.03$ ). In general, the correlation between *Pgs* isolate 92-MN-90 and the *Pgt* races was very low ( $R^2 = 0.03$  to  $0.47$ ) (Table 3.11). Additionally, there was an overall low correlation between the seedling reaction to a race and the adult plant severity averaged across all locations for that race, where the  $r^2$  was 0.10 for QCCJB, 0.10 for TTKSK, 0.28 for MCCFC, and 0.26 for HKHJC.

The RGenePos package in R was used to postulate which genes might be present in the BCCS (Table 3.12). In total, there were 51 different resistance spectra identified to the eight rust cultures. No seedling resistance was observed in 140 of the 290 total accessions tested. Of the remaining lines, 39 were predicted to have a combination of known and unknown genes, 52 were predicted to have only known genes, and 59 were predicted to have only unknown genes. Of the 52 accessions with unknown genes, there were only 20 different resistance spectra identified of which the most common were: HKHJC and MCCFC = 14; TRTTF, HKHJC, and MCCFC = 9; and TRTTF = 8.

#### *Genotyping for Rpg1 and Rpg5*

Genotyping for *Rpg1* and *Rpg5* was conducted with all 290 accessions of the BCCS. In total, 118 accessions amplified the STPK domain of *Rpg5* with the LRK marker. Of these, 35 contained the non-functional *rpg5* allele characterized by a “C” insertion identified after sequencing with the R5 marker. The remaining 83 accessions were sequenced with the R10 marker to determine if any carried the E1287A mutation, resulting in a non-functional *rpg5* allele. Sixty-eight accessions had the E1287A mutation, resulting in just 15 possessing a functional *Rpg5* gene (Table 3.13). The *rpg4/Rpg5* control genotypes of Q21861 and Q/SM20 both amplified the LRK marker and did not have the C insertion or the E23187A allele. The Golden Promise control possesses the LRK marker, but has a non-functional allele due to the C insertion. The 212Y1 control for *rpg6* also had the C insertion as expected since this line is derived from Golden Promise. Control accession Hv492 possesses the LRK marker without the C insertion, but it contains the E1287A mutation, rendering the resistance gene non-

functional. PI 532013 also carries the E1287A mutation. The other controls of Chevron, 80-TT-29, Hietpas-5, GAW-79, Black Hulless, Diamond, 80-TT-29, 80-tt-30, and Hiproly all amplified the PP2C marker.

Only 40 accessions amplified a product from the RPG1-N marker for *Rpg1*, whereas 69 amplified a product from the RPG1-S marker. Additionally, 181 accessions did not amplify any product from either marker, and two accessions amplified a product from both markers. Thus, 40 accessions were found to carry a functional *Rpg1*. Just one accession (BCC2297) carried both *Rpg1* and *rpg4/Rpg5*. This accession was Q21861, which was part of the BCC and selected for the final 290 accessions of the BCCS due to its high level of stem rust resistance. The *Rpg1* controls of Chevron, 80-TT-29, Diamond, and Q21861 all amplified the marker for a functional *Rpg1* gene as expected. The 11 other controls of GAW-79, Q/SM20, Steptoe, Hietpas-5, 212Y1, Black Hulless, 80-tt-30, Hiproly, Golden Promise, PI 532013, and Hv492 all lack *Rpg1*. The first three amplified the RPG1-S marker, indicative of a non-functional *Rpg1*, whereas the last 8 failed to amplify either marker (RPG1-N or RPG1-S), also indicative of a non-functional *Rpg1*.

## Discussion

Stem rust is a challenging disease to combat in cereal crops. In Chapter 2, 1,860 accessions of the BCC were evaluated for stem rust resistance using a GWAS approach. From this work, 290 of the most resistant accessions (i.e. the BCCS) were selected for further evaluations against a diverse suite of many *Puccinia graminis* cultures at both the seedling and adult plant stages. To complement this analysis, molecular assays for the most common stem rust resistance genes in barley (i.e. *Rpg1* and *Rpg5*) were also conducted.

The BCCS was evaluated for APR to *Pgt* races HKHJC, QCCJB, and MCCFC in St. Paul and to race TTKSK in Kenya for a total of seven individual site-year locations. From these evaluations, 12 accessions were rated as Class 1 or Class 2 in all nurseries (Table 3.9). This select group included accession Q21861 (BCC2297) from Australia, which was



part of the original BCC and ultimately served as a “blind control” in this study. Q21861 carries *Rpg1* and was considered as the original source of *rpg4/Rpg5* until this gene complex was discovered in wild barley (Mamo et al. 2015). The molecular assays confirmed the presence of these genes both in this accession of the BCCS and also our control source of Q21861. With respect to the other 11 accessions in this group, only one other carried *rpg4/Rpg5*, a landrace (BCC2382) from Saudi Arabia. One accession (BCC2533) in this group of 12 was found to carry *Rpg1*. This was not surprising since BCC2533 is a breeding line from the North Dakota barley program where *Rpg1* is used extensively. Although both BCC2533 and BCC2382 tested positive for the *Rpg1* and *Rpg5* markers, respectively, their resistance spectra to various races do not match the pattern of the controls carrying these genes. Specifically, BCC2533 was susceptible to *Pgs* culture 92-MN-90, and BCC2382 was susceptible to *Pgt* races TRTTF and TKTTF, whereas the control cultivars with the respective genes were resistant to these *Puccinia graminis* cultures (Table 3.1). Using the RGenePos package, these accessions were predicted to have an unknown gene (+) and *Rpg3* in addition to an unknown gene “+”, respectively. Of the other accessions in Table 3.9 which did not test positive for either marker, one was predicted to have “+” (BCC1928), two were predicted to have *Rpg3* (BCC0908 and BCC1377), and one was predicted to have *Rpg3* + (BCC0131). The remaining accessions in Table 3.9 lacked seedling resistance to all of the *Puccinia graminis* cultures tested, indicating that they carry only APR.

It is interesting to note that the relationship between APR and presence of an underlying resistance gene was not always readily apparent. Of the 15 accessions that tested positive for the presence of *Rpg5*, only seven (BCC1568, BCC1600, BCC1602, BCC1603, BCC1859, BCC2297, and BCC2382) were rated as Class 1 or Class 2 as adult plants to races (*Pgt* TTKSK composite, QCCJB, and MCCFC) for which *rpg4/Rpg5* should be effective (Table 3.9 and 3.10). Of these seven, all were rated as Class 1 or Class 2 to all races that should be avirulent on *rpg4/Rpg5*, the exceptions being BCC1600, BCC1602, and BCC1859 which were moderately susceptible to race QCCJB in 2015 in St. Paul (StP15\_QC), at 15% MS, 20% MS, and 15% MR-MS, respectively. Of these seven, all

but one (BCC0524) was also resistant to race HKHJC at the adult plant stage but was seedling susceptible to this race. This suggests that these lines have additional genes for APR that enhance the expression of *rpg4/Rpg5*. The remaining eight accessions were either variable across seasons or were consistently susceptible to one or more *rpg4/Rpg5* avirulent races at the adult stage. Of the 15 accessions testing positive for the *Rpg5* marker, only four (BCC1859, BCC1600, BCC1602, and BCC2297) exhibited the expected seedling resistance spectrum to *Pgt* races TTKSK, TTKST, TKTTF, TRTTF, QCCJB, MCCFC, and *Pgs* isolate 92-MN-90 as the *rpg4/Rpg5* control of Q/SM20. The other 11 accessions exhibited resistance spectra not observed in the controls at the seedling stage, suggesting that they possibly carry different alleles of *Rpg5* that may be non-functional or that confer resistance to a different spectrum of races than the allele present in Q21816 and Q/SM20. However, if only the reaction to *Pgs* isolate 92-MN-90 is considered, all of the accessions that tested positive for the *Rpg5* marker were resistant to this isolate. This result indicates that the *Rpg5* marker used in this study effectively detected resistance to *Pgs*--the f. sp. of *P. graminis* for which this gene was originally described. Thus, the *Rpg5* marker may not have effectively detected the *rpg4*-mediated resistance to *Pgt* races that is controlled by this gene complex. This could possibly be due to additional non-functional *Rpg5* alleles or loss of the other genes at this complex that are required for resistance to *Pgt* as observed in control accessions Q21861 and Q/SM20 (Arora et al. 2013; Brueggeman et al. 2008; Brueggeman et al. 2009; Steffenson et al. 2009; Wang et al. 2013).

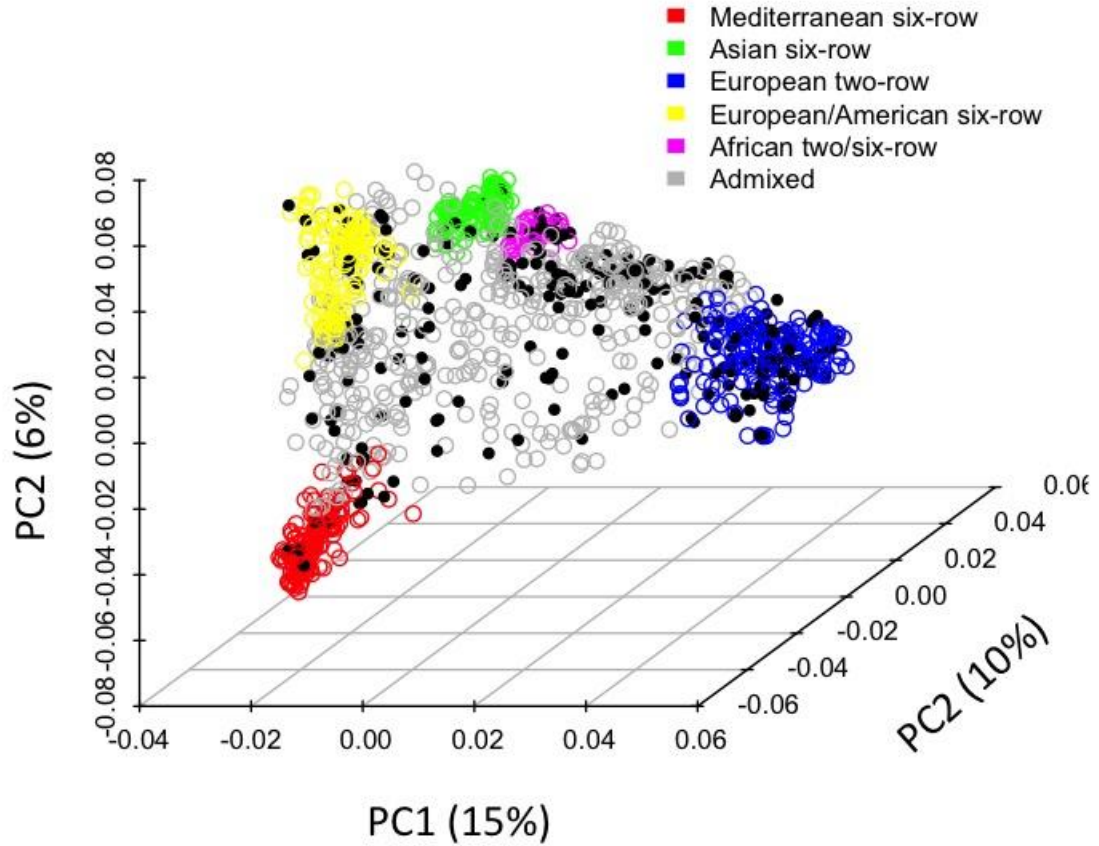
The level of resistance observed in accessions testing positive for *Rpg1* was markedly greater than that observed for accessions carrying *Rpg5*. Of the 40 accessions testing positive for *Rpg1*, all but six were consistently rated as Class 1 or Class 2 to both *Pgt* races HKHJC and MCCFC at the adult plant stage. In addition, 22 of these *Rpg1*-carrying accessions also gave consistent Class 1 or Class 2 reactions in Kenya, suggesting that they carry APR to *Pgt* TTKSK composite in addition to *Rpg1* resistance. However, in this and other studies done on the APR of barley to race TTKSK, accessions carrying *Rpg1* tend to have lower rust severities than those without the gene (unpublished data). Thus,

although races such as TTKSK are regarded as having virulence for *Rpg1*, the gene still may contribute a residual level of resistance.

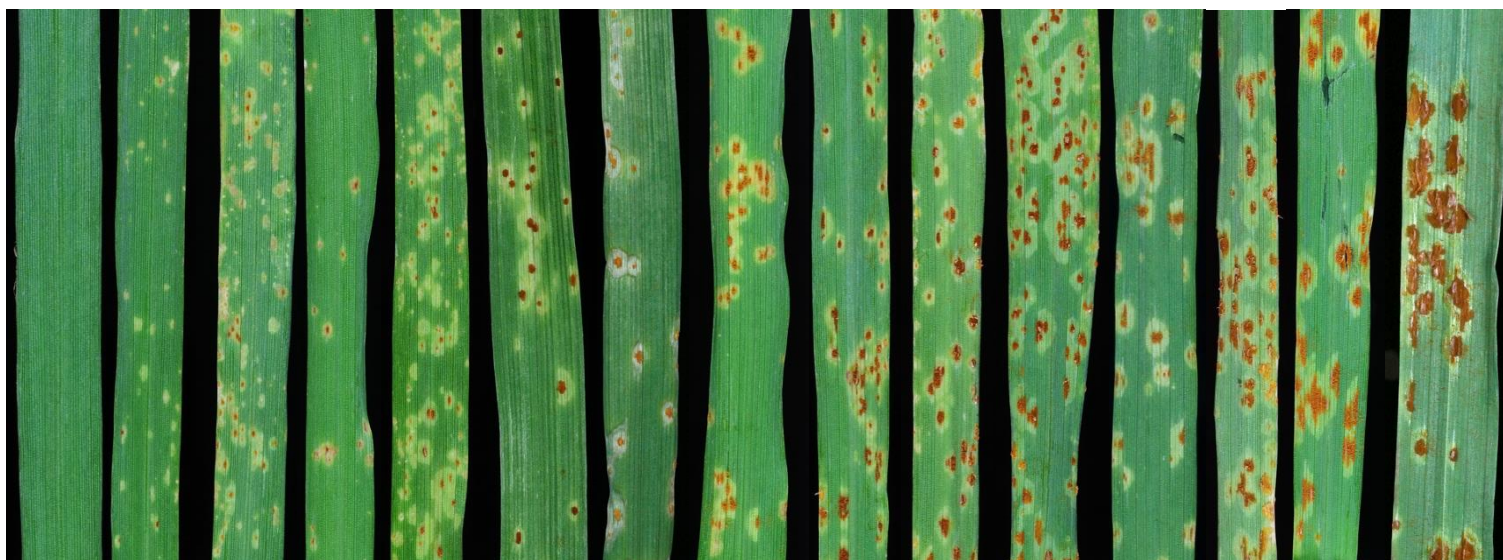
Based on the seedling race specificity pattern, just four accessions were predicted to carry *Rpg1* (BCC1209, BCC1822, BCC2544, and BCC2297) despite the fact that 40 accessions tested positive for this gene. The expected resistance spectrum for *Rpg1* was (TRTTF, HKHJC, MCCFC, and 92-MN-90) based on the reaction of the controls Chevron and 80-TT-29. Twenty-six of the 40 accessions mentioned above had a resistance spectrum similar to this, but were susceptible to one or two races. If one considers just the reaction to race MCCFC, only nine accessions that tested positive for the *Rpg1* marker did not have resistance to MCCFC. As this race is commonly used to detect *Rpg1* resistance, it is possible that there are additional genes in the controls of Chevron and 80-TT-29 that are providing resistance in addition to *Rpg1*. As only race MCCFC has been used in the mapping and analysis of *Rpg1* and also for selection of progeny with the gene in breeding programs, it is possible that Chevron carries other unidentified genes in addition to *Rpg1*. This may be one possible explanation for why Chevron confers resistance to additional races than would be expected for accessions carrying only *Rpg1* by marker analysis (Jin et al. 1993; Kilian et al. 1994; Steffenson 1992). This contention is further supported by the reaction data for 80-TT-29 and 80-tt-30, which exhibited very similar ITs in response to 92-MN-90. This suggests that additional genes were inherited from Chevron during the development of these near-isogenic lines, which were either linked to or unaffected by selection for race MCCFC resistance (Horvath et al. 1995; Steffenson et al. 1985; Sun and Steffenson 2005).

In summary, the extensive rust phenotype data collected in this study clearly demonstrate that the BCC is a valuable and diverse source of stem rust resistance in barley. Crosses are being made to validate and map resistance genes in selected accessions and should prove useful in future breeding efforts.

## Figures

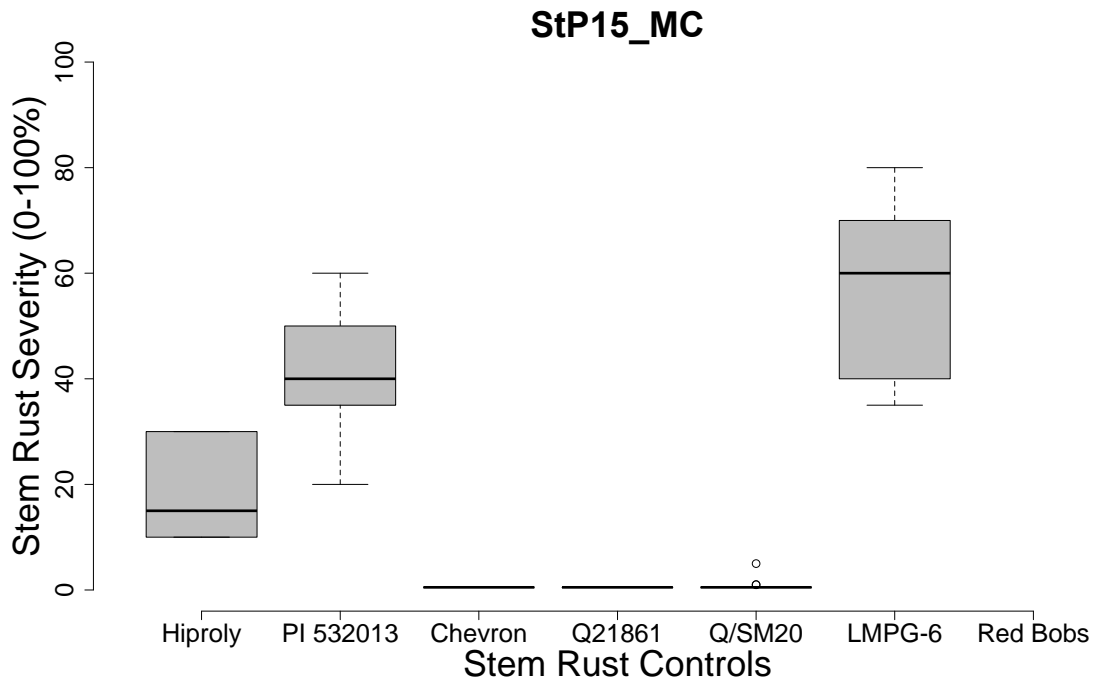
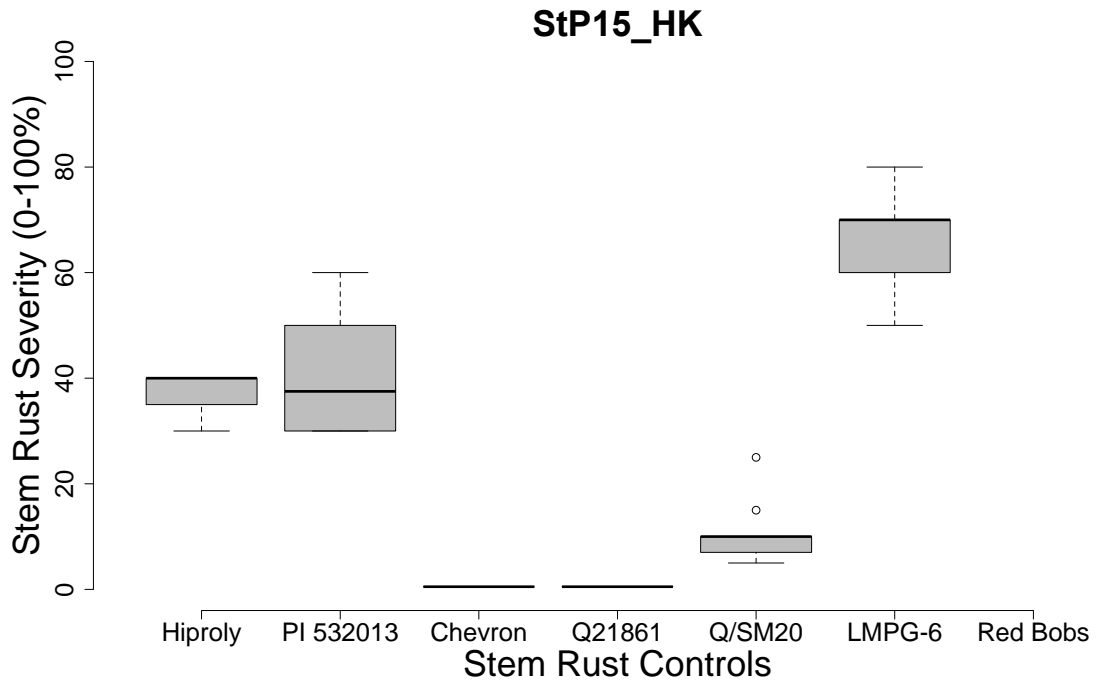


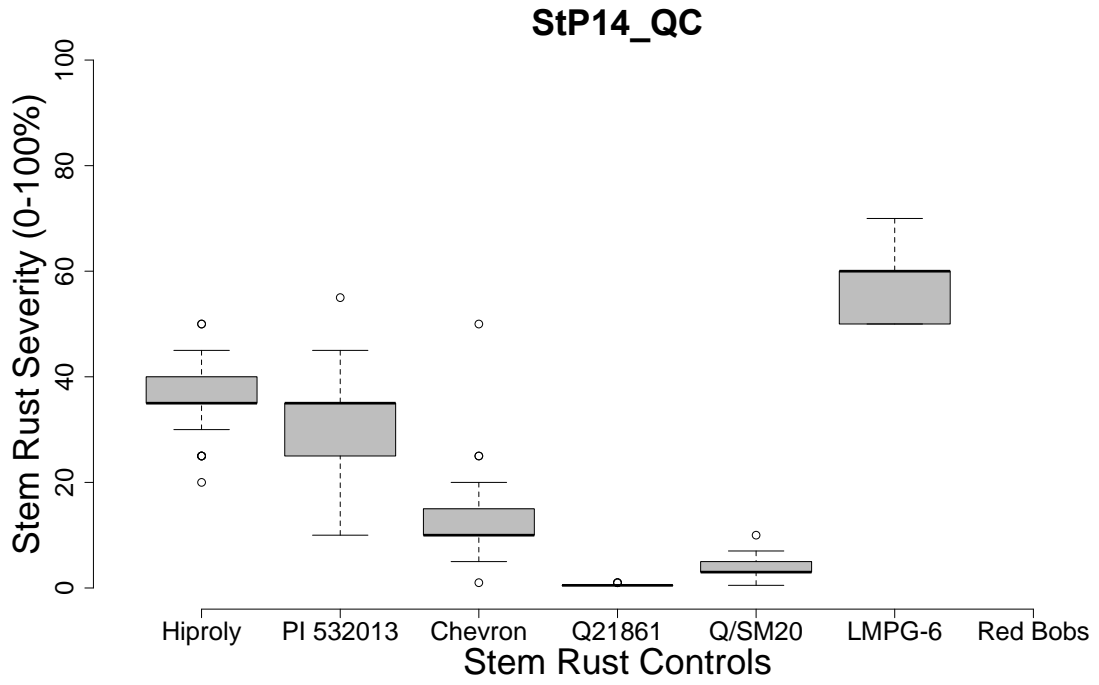
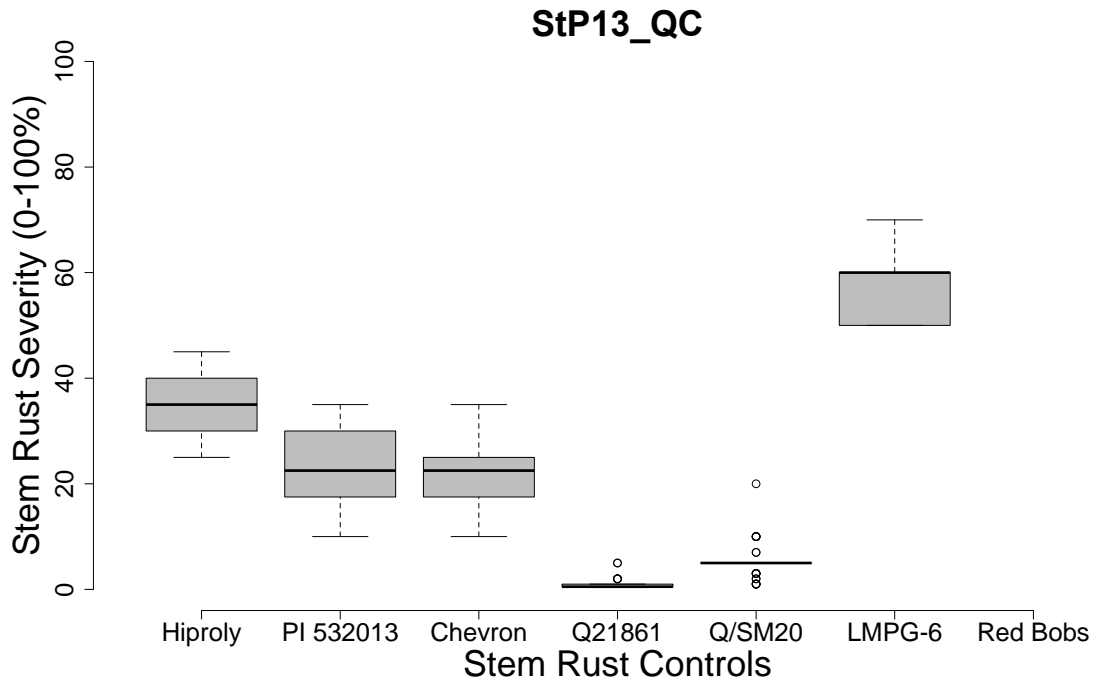
**Figure 3.1.** Principal Component Analysis of the 1,860 accessions in the Barley iCore Collection (BCC) showing the distribution of the 290 accessions of the Barley iCore Collection Selects (BCCS) among the population clusters. Accessions in the BCC are colored according to the STRUCTURE analysis results by Muñoz-Amatriaín et al. (2014) with BCCS accessions shown in black.

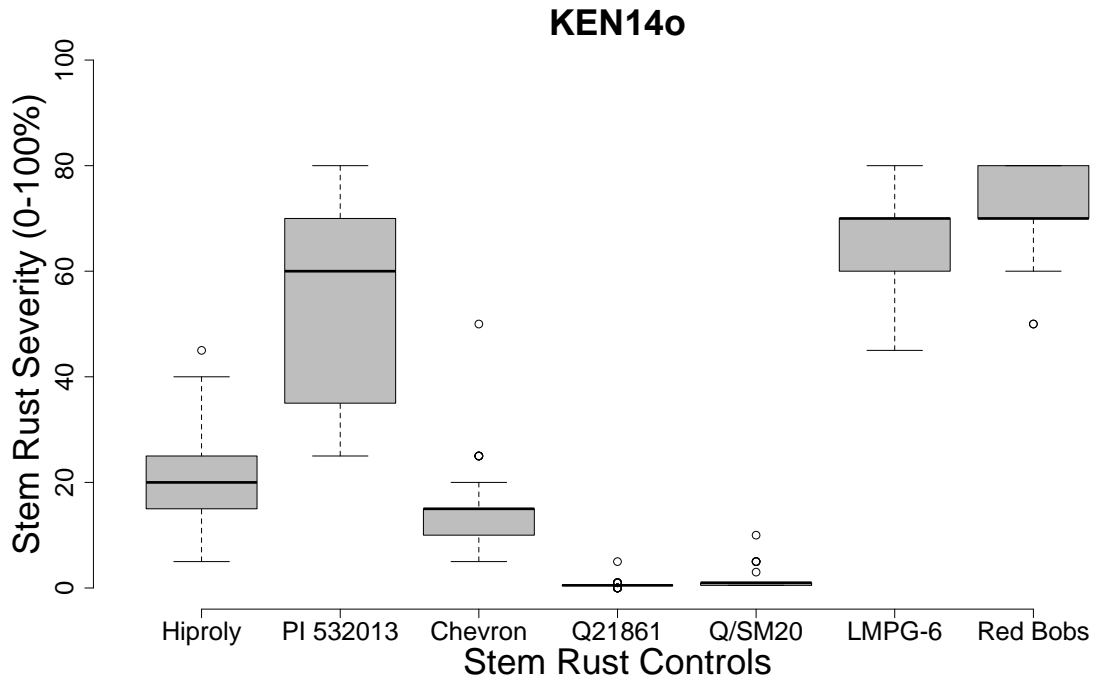
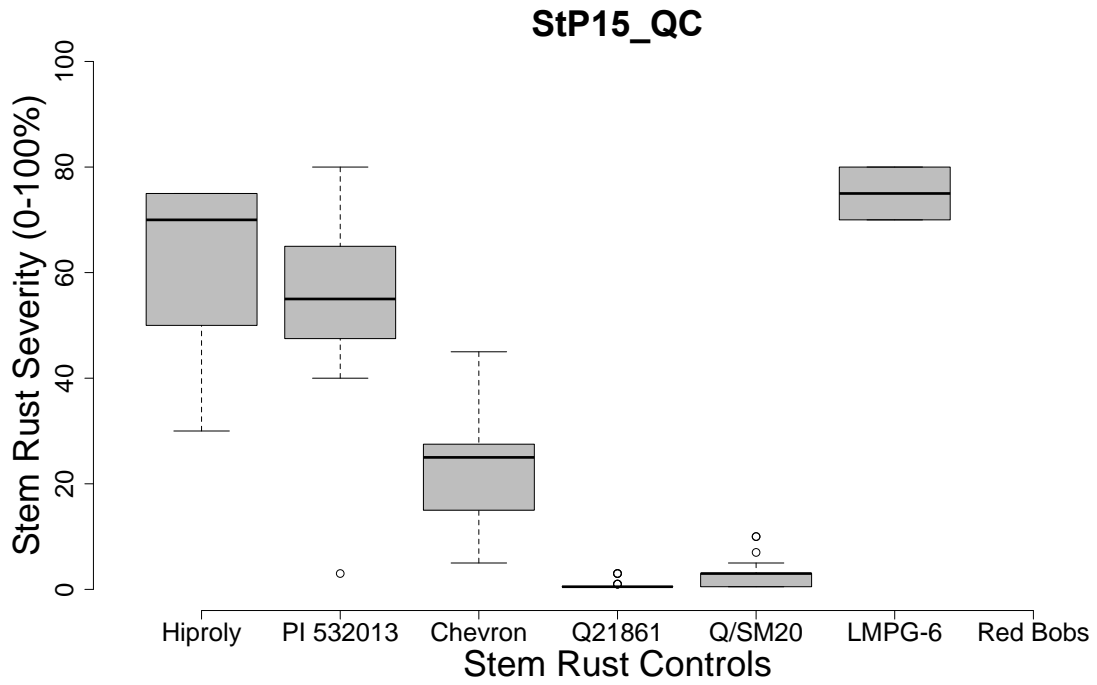


|         |    |     |     |   |      |     |    |      |     |    |     |     |    |     |    |
|---------|----|-----|-----|---|------|-----|----|------|-----|----|-----|-----|----|-----|----|
| Raw ITs | 0; | 0;1 | 10; | 1 | 120; | 21c | 2n | 23-c | 3-2 | 3- | 3-3 | 33- | 3  | 33+ | 3+ |
| LS      | 1  | 2   | 3   | 4 | 5    | 6   | 7  | 8    | 9   | 10 | 11  | 12  | 13 | 14  | 15 |

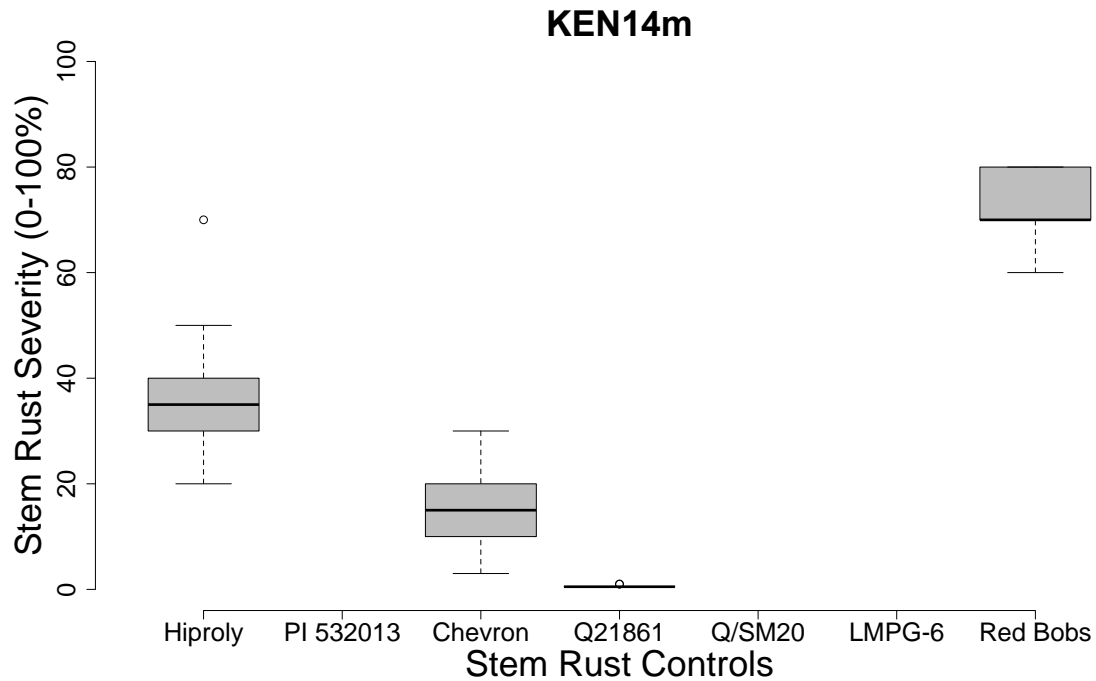
**Figure 3.2.** The range of stem rust infection types (ITs) observed on barley as assessed using the 0-4 scale of Stakman et al. (1962) in comparison to a linear score (LS) developed in this study. Barley accessions exhibiting a LS of  $< 8$  (IT  $< 23-$ ) were considered resistant, and those with a LS  $> 8$  were considered susceptible.



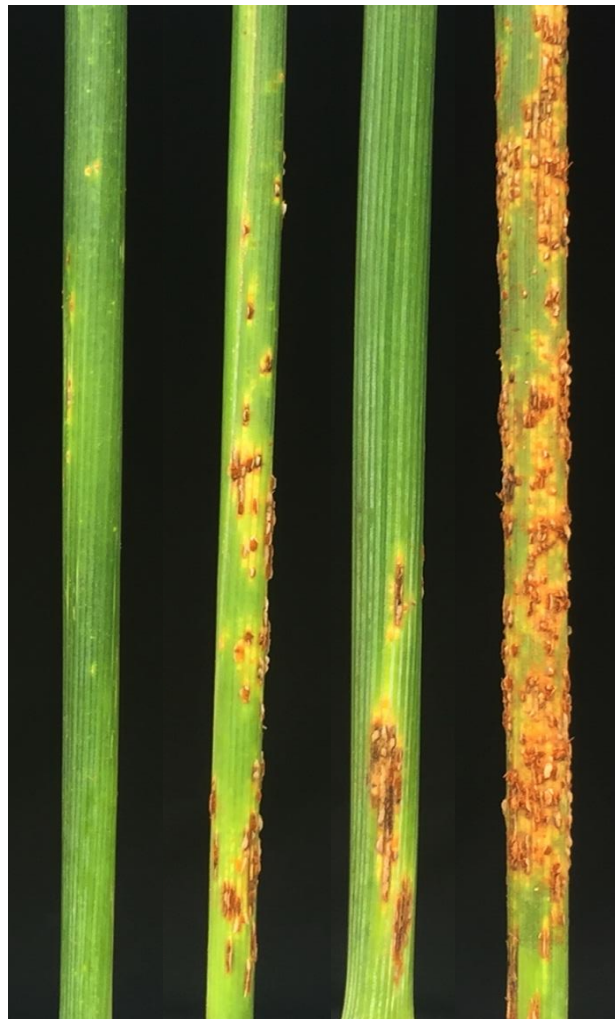








**Figure 3.3.** Performance of barley (Hiproly, PI 532013, Chevron, Q21861, and Q/SM20) and wheat (LMPG-6 and Red Bobs) stem rust controls in multiple stem rust screening nurseries in Njoro, Kenya and St. Paul, MN where nurseries are abbreviated as: 2015 St. Paul *Puccinia graminis* f. sp. *tritici* (*Pgt*) race HKHJC (StP15\_HK), 2015 St. Paul *Pgt* race MCCFC (StP15\_MC), 2013 St. Paul *Pgt* race QCCJB (StP13\_QC), 2014 St. Paul *Pgt* race QCCJB (StP14\_QC), 2015 St. Paul *Pgt* race QCCJB (StP15\_QC), 2014 Kenya off-season (KEN14o), 2014 Kenya main-season (KEN14m). *Pgt* races in Kenya were *Pgt* TTKSK composite (TTKSK, TTKST, TTKTK, and TTKTT). Five statistics (bars) are represented in each box plot from bottom to top: the smallest observation, lower quartile, median, upper quartile, and largest observation, respectively. Data points positioned outside this range are extreme values and are represented as circles.



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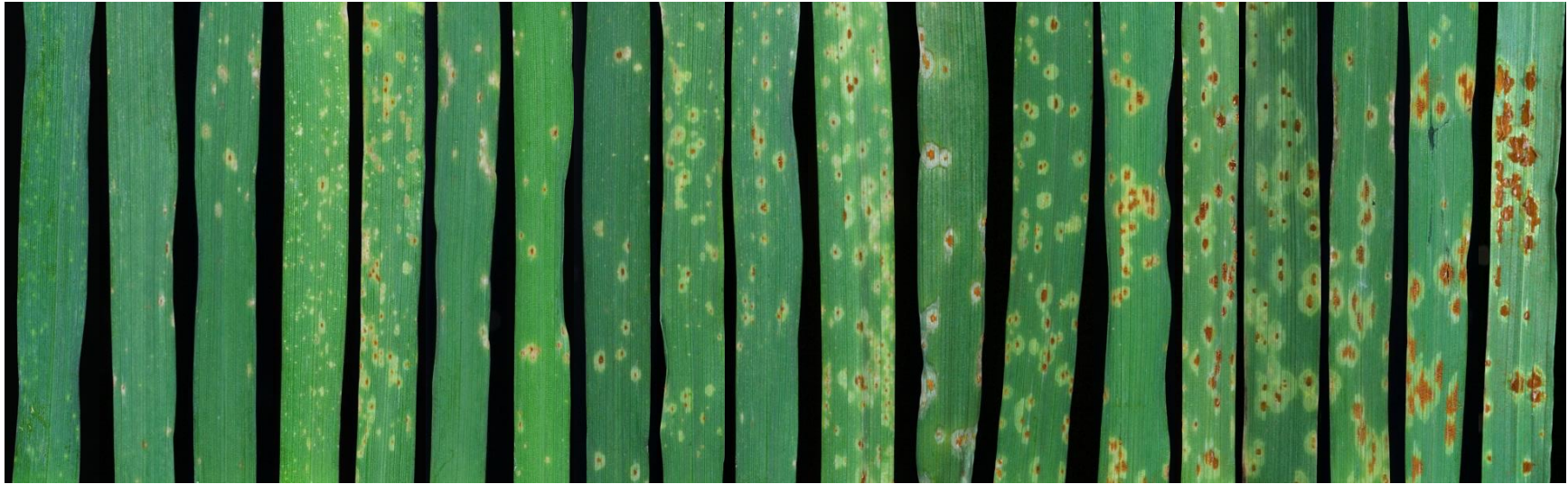
|   |    |    |   |
|---|----|----|---|
| R | MR | MS | S |
|---|----|----|---|

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Adult Plant Infection Response (IR)

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**Figure 3.4.** Examples of the diversity for adult plant infection response (IR) in barley accessions to *Puccinia graminis* f. sp. *tritici* in the field in Greytown, South Africa. IR ratings are R = Resistant, MR = Moderately Resistant, MS = Moderately Susceptible, and S = Susceptible.



**Figure 3.5.** Diversity of stem rust infection types observed on the first leaves (adaxial side) of accessions from the Barley iCore Collection Selects (BCCS). From left to right: BCC2297 (IT 0; to race TKTTF), BCC434 (0;n to TRTTF), BCC212 (0;1 to TTKST), BCC168 (0;1,2 to TRTTF), BCC1103 (10; to TTKST), BCC1578 (1n to TTKST), BCC1624 (1 to TTKST), BCC2558 (120; to TTKST), BCC1720 (12 to TKTTF), BCC1548 (21 to TRTTF), BCC654 (21n to TRTTF), BCC1917 (2n to TTKST), BCC1875 (23-1 to TRTTF), BCC394 (23-c to TTKST), BCC308 (3-2 to TRTTF), BCC1872 (3 to TKTTF), BCC2856 (3-3 to TTKST), BCC2134 (33+ to TTKST), and BCC2856 (3+ to TTKST on abaxial side).

## Tables

**Table 3.1.** Reaction mode of differential wheat, barley, and rye lines to specific races or isolates of the stem rust pathogen, *Puccinia graminis*.

|         |                 |                      | <i>Pgt<sup>a</sup></i>                             |     |             |     |                               |      |           |      |                |      | <i>Pgs</i> |      |       |      |          |   |
|---------|-----------------|----------------------|--|-----|-------------|-----|-------------------------------|------|-----------|------|----------------|------|------------|------|-------|------|----------|---|
|         |                 |                      | Race Group: African “Ug99” Race Group <sup>b</sup> |     |             |     | Foreign “non-Ug99” Race Group |      |           |      | North American |      |            |      |       |      |          |   |
|         |                 |                      | Isolate: 04KEN146/04 <sup>c</sup>                  |     | 06KEN19-v-3 |     | 13ETH18-1                     |      | 06YEM34-1 |      | QCC-2          |      | A-5        |      | CRL-1 |      | 92-MN-90 |   |
| Species | Resistance Gene | Line Name            | TTKSK <sup>d</sup>                                 |     | TTKST       |     | TKTTF                         |      | TRTTF     |      | QCCJB          |      | MCCFC      |      | HKHJC |      | -        |   |
| Wheat   | <i>Sr5</i>      | ISr5-Ra              | 4 <sup>e</sup>                                     | vir | 4           | vir | 4                             | vir  | 4         | vir  | 3              | vir  | 3          | vir  | 0;    | avir | -        | - |
| Wheat   | <i>Sr21</i>     | CnS_T_mono_<br>deriv | 3+4  | vir | 4           | vir | 4                             | vir  | 4         | vir  | 3              | vir  | 1          | avir | 3     | vir  | -        | - |
| Wheat   | <i>Sr9e</i>     | Vernstine            | 4  | vir | 3+          | vir | 3+                            | vir  | 4         | vir  | 0;             | avir | 0;         | avir | 1     | avir | -        | - |
| Wheat   | <i>Sr7b</i>     | ISr7b-Ra             | 4  | vir | 4           | vir | 4                             | vir  | 4         | vir  | 1              | avir | 3+         | vir  | 3     | vir  | -        | - |
| Wheat   | <i>Sr11</i>     | ISr11-Ra             | 4  | vir | 4           | vir | 1                             | avir | 4         | vir  | 0;             | avir | 1          | avir | 1     | avir | -        | - |
| Wheat   | <i>Sr6</i>      | ISr6-Ra              | 4  | vir | 4           | vir | 3+                            | vir  | 4         | vir  | 0;             | avir | 0;         | avir | 3     | vir  | -        | - |
| Wheat   | <i>Sr8a</i>     | ISr8a-Ra             | 4  | vir | 4           | vir | 4                             | vir  | 2         | avir | 1              | avir | 1          | avir | 3+    | vir  | -        | - |
| Wheat   | <i>Sr9g</i>     | CnSr9g               | 4  | vir | 4           | vir | 4                             | vir  | 4         | vir  | 3              | vir  | 3          | vir  | 3     | vir  | -        | - |

|        |              |                 |     |      |     |      |     |      |    |      |     |      |     |      |      |      |    |      |
|--------|--------------|-----------------|-----|------|-----|------|-----|------|----|------|-----|------|-----|------|------|------|----|------|
| Wheat  | <i>Sr36</i>  | W2691SrTt-1     | 0;  | avir | 0;  | avir | 4   | vir  | 4  | vir  | 0;  | avir | 0;  | avir | 0;   | avir | -  | -    |
| Wheat  | <i>Sr9b</i>  | W2691Sr9b       | 4   | vir  | 4   | vir  | 4   | vir  | 4  | vir  | 1   | avir | 0;  | avir | 3    | vir  | -  | -    |
| Wheat  | <i>Sr30</i>  | BtSr30Wst       | 3+4 | vir  | 4   | vir  | 4   | vir  | 4  | vir  | 1   | avir | 1   | avir | 1    | avir | -  | -    |
| Wheat  | <i>Sr17</i>  | Combination VII | 32+ | vir  | 3+  | vir  | 4   | vir  | 4  | vir  | 3   | vir  | 3+  | vir  | 3    | vir  | -  | -    |
| Wheat  | <i>Sr9a</i>  | ISr9a-Ra        | 3+4 | vir  | 4   | vir  | 4   | vir  | 4  | vir  | 1   | avir | 1   | avir | 1    | avir | -  | -    |
| Wheat  | <i>Sr9d</i>  | ISr9d-Ra        | 4   | vir  | 4   | vir  | 4   | vir  | 4  | vir  | 3   | vir  | 2   | avir | 3+   | vir  | -  | -    |
| Wheat  | <i>Sr10</i>  | W2691Sr10       | 4   | vir  | 4   | vir  | 4   | vir  | 4  | vir  | 3   | vir  | 3+  | vir  | 3+   | vir  | -  | -    |
| Wheat  | <i>SrTmp</i> | CnsSrTmp        | 2   | avir | 1   | avir | 3+  | vir  | 3+ | vir  | 2   | avir | 3+  | vir  | 2+3- | avir | -  | -    |
| Wheat  | <i>Sr24</i>  | LcSr24Ag        | 2   | avir | 3+  | vir  | 2   | avir | 2  | avir | 2   | avir | 1   | avir | 1    | avir | -  | -    |
| Wheat  | <i>Sr31</i>  | Sr31/6*LMPG     | 4   | vir  | 3+  | vir  | 10; | avir | 1  | avir | 2   | avir | 1   | avir | 1    | avir | -  | -    |
| Wheat  | <i>Sr38</i>  | VPM1            | 4   | vir  | 4   | vir  | 4   | vir  | 4  | vir  | 0;  | avir | 0;  | avir | 0;1  | avir | -  | -    |
| Wheat  | <i>SrMcN</i> | McNair 701      | 3+  | vir  | 3+  | vir  | 3+  | vir  | 4  | vir  | 1   | avir | 3+  | vir  | 3+   | vir  | 0; | avir |
| Wheat  | -            | Line E          | 3+  | vir  | 3+  | vir  | 3+  | vir  | 4  | vir  | 3+  | vir  | 3+  | vir  | 3+   | vir  | 3+ | vir  |
| Rye    | -            | Prolific        | -   | -    | -   | -    | -   | -    | -  | -    | -   | -    | -   | -    | -    | -    | 3+ | vir  |
| Barley | <i>Rpg1</i>  | 80-TT-29        | 3-2 | vir  | 3   | vir  | 3-  | vir  | 21 | avir | 33- | vir  | 10; | avir | 0;   | avir | 21 | avir |
| Barley | <i>Rpg1</i>  | Chevron         | 23- | vir  | 33- | vir  | 33- | vir  | 21 | avir | 3-2 | vir  | 0;  | avir | 0;   | avir | 12 | avir |

|        |                                   |               |     |      |     |      |     |      |     |      |     |      |     |      |     |      |     |      |
|--------|-----------------------------------|---------------|-----|------|-----|------|-----|------|-----|------|-----|------|-----|------|-----|------|-----|------|
| Barley | <i>Rpg1</i> +<br><i>RpgU</i>      | Diamond       | 3-  | vir  | 3   | vir  | 3   | vir  | 2   | avir | 3-  | vir  | 10; | avir | 0;  | avir | 1   | avir |
| Barley | <i>Rpg2</i>                       | Hietpas-5     | 3   | vir  | 33- | vir  | 3   | vir  | 3   | vir  | 23- | vir  | 3-3 | vir  | 2   | avir | 12  | avir |
| Barley | <i>Rpg3</i>                       | GAW-79        | 3-  | vir  | 3-2 | vir  | 33- | vir  | 23- | vir  | 3-  | vir  | 3-  | vir  | 23- | vir  | 21  | avir |
| Barley | <i>rpg4/Rpg5</i>                  | Q/SM20        | 0;1 | avir | 10; | avir | 0;1 | avir | 12  | avir | 0;  | avir | 0;1 | avir | 3-  | vir  | 0;  | avir |
| Barley | <i>rpgBH</i>                      | Black Hulless | 3   | vir  | 3   | vir  | 3   | vir  | 3   | vir  | 3-2 | vir  | 3   | vir  | 3   | vir  | 3-  | vir  |
| Barley | <i>Rpg1</i> +<br><i>rpg4/Rpg5</i> | Q21861        | 0;  | avir | 0;1 | avir | 0;1 | avir | 0;1 | avir | 0;  | avir | 0;  | avir | 0;  | avir | 0;  | avir |
| Barley | -                                 | Step toe      | 3-  | vir  | 3   | vir  | 3   | vir  | 33+ | vir  | 3   | vir  | 3   | vir  | 3-  | vir  | 3-2 | vir  |
| Barley | -                                 | PI 532013     | 3   | vir  | 33+ | vir  | 3   | vir  | 3   | vir  | 3   | vir  | 3   | vir  | 3   | vir  | 3   | vir  |
| Barley | -                                 | 80-tt-30      | 3-  | vir  | 3   | vir  | 33- | vir  | 3   | vir  | 3-  | vir  | 3   | vir  | 3-  | vir  | 23- | vir  |
| Barley | -                                 | Hiproly       | 3-  | vir  | 3   | vir  | 3   | vir  | 3   | vir  | 3   | vir  | 3   | vir  | 3   | vir  | 3   | vir  |

<sup>a</sup> Wheat stem rust pathogen *Puccinia graminis* f. sp. *tritici* (*Pgt*) or rye stem rust pathogen *Puccinia graminis* f. sp. *secalis* (*Pgs*)

<sup>b</sup> Origin of the *Puccinia graminis* race or isolate

<sup>c</sup> Isolate designation

<sup>d</sup> Race epithet is as given by the North American stem rust nomenclature system of wheat (Jin et al. 2008; Roelfs 1988; Roelfs et al. 1991)

<sup>e</sup> Stem rust seedling infection types were assessed based on the 0-4 Stakman scale (Miller and Lambert 1955; Stakman et al. 1962), “-“ indicates missing or no data”.

<sup>f</sup> Virulence (vir) or Avirulence (avir) of race.

**Table 3.2.** Pearson correlation for adult plant disease severities (averaged within race) of Barley iCore Collection Selects (BCCS) to *Puccinia graminis* f. sp. *tritici* races *Pgt* TTKSK Composite in Njoro, Kenya and QCCJB, MCCFC, and HKHJC in St. Paul, MN.

|       | <i>Pgt</i> TTKSK Composite <sup>a</sup> | QCCJB          | MCCFC |
|-------|---|----------------|-------|
| QCCJB | 0.17                                    | _ <sup>b</sup> | -     |
| MCCFC | 0.04                                    | 0.45           | -     |
| HKHJC | 0.02                                    | 0.32           | 0.47  |

<sup>a</sup> In addition to TTKSK, races TTKSK, TTKST, TTKTK, and TTKTT were also present in the nursery (Newcomb et al. 2016).

<sup>b</sup> “-“ indicates data omitted.

**Table 3.3.** Threshold values used for assigning accessions of the Barley iCore Collection Selects (BCCS) into adult plant stem rust reaction classes where highly resistant (Class 1) and resistant (Class 2).

| Nursery <sup>a</sup> | Accession classifications                         |   | Number of accessions |         |         |
|----------------------|---|---|----------------------|---------|---------|
|                      | Class 1 disease severity (%) maximum <sup>b</sup> | Class 2 disease severity (%) range <sup>c</sup> | Class 1 <sup>e</sup> | Class 2 | Missing |
| StP15_QC             | 3   | 3 to 7  | 15                   | 26      | 14      |
| StP14_QC             | 1   | 1 to 10   | 64                   | 146     | 18      |
| StP13_QC             | 3   | 3 to 20   | 36                   | 134     | 10      |
| KEN14m               | 1   | 1 to 7  | 162                  | 60      | 12      |
| KEN14o               | 1   | 1 to 10   | 119                  | 92      | 17      |
| StP15_MC             | 0.5   | 0.5 to 7  | 55                   | 117     | 10      |
| StP15_HK             | 3   | 3 to 10   | 140                  | 46      | 10      |

<sup>a</sup> Nurseries where accessions were evaluated. Abbreviations are as follows for locations in St. Paul, MN and Njoro, Kenya: StP13\_QC = dataset resulting from adult plant evaluations to race QCCJB in St. Paul in 2013; StP14\_QC = dataset resulting from adult plant evaluations to race HKHJC in St. Paul in 2014; StP15\_QC = dataset resulting from adult plant evaluations to race QCCJB in St. Paul in 2015; StP15\_MC = dataset resulting from adult plant evaluations to race MCCFC in St. Paul in 2015; StP15\_HK = dataset resulting from adult plant evaluations to race HKHJC in St. Paul in 2015; and KEN14m = dataset resulting from adult plant evaluations to race TTKSK composite in Njoro in 2014 main-season; KEN14o = dataset resulting from adult plant evaluations to *Pgt* TTKSK composite in Njoro in 2014 off-season.

<sup>b</sup> Disease severity was estimated visually on the stem and leaf sheath tissue of accessions on a 0-100% scale. Maximum value for assigning accessions as highly resistant (Class 1) was based on performance the of Q21861 or Chevron according to which had the lowest disease severity in the nursery.



<sup>c</sup> Disease severity estimated visually on the stem and leaf sheath tissue of accessions on a 0-100% scale. Range for calling accessions as resistant (Class 2) was based on the range between the severity cutoff values for calling Class 1 and the minimum recorded severity values for Hiproly or Steptoe.

<sup>d</sup> Disease severity estimated visually on the stem and leaf sheath tissue of accessions on a 0-100% scale. Minimum value for calling accessions susceptible was based on performance of Hiproly or Steptoe according to which had the lowest disease severity in the nursery.

<sup>e</sup> The number of accessions which were classified as Class 1, Class 2 or Missing (no data was obtained from the respective nurseries).

**Table 3.4.** Linear score for conversion of the raw seedling infection type scores.

| <b>Linear Score<sup>a</sup></b> | <b>Raw IT</b> |
|---------------------------------|---------------|
| 1                               | 0;            |
| 2                               | 0;1           |
| 3                               | 10;           |
| 4                               | 1             |
| 5                               | 12            |
| 6                               | 21            |
| 7                               | 2             |
| 8                               | 23-           |
| 9                               | 3-2           |
| 10                              | 3-            |
| 11                              | 3-3           |
| 12                              | 33-           |
| 13                              | 3             |
| 14                              | 33+           |
| 15                              | 3+            |

<sup>a</sup> The linear score is a sequential progression of values representing the 15 most commonly observed ITs in the dataset from most resistant (incompatible LS = 1) to most susceptible (compatible Ls = 15).

<sup>b</sup> Stem rust seedling infection types were assessed based on the Stakman scale as modified from barley by Miller and Lambert (1955) (Stakman et al. 1962).

**Table 3.5.** Molecular markers used to detect functional *RpgI* genotypes.

| Marker | Primer pair <sup>a</sup> | Marker Sequence      | Annealing (°C) | Control genotype                      | Amplicon (bp)  | Result <sup>e</sup> |
|--------|--------------------------|----------------------|----------------|---------------------------------------|----------------|---------------------|
| RPG1R  | RPG1R-F <sup>b</sup>     | CGGCTAATCACATCAAGTAA | 51-66          | Chevron ( <i>RpgI</i> )               | 610            | <i>RpgI</i> +       |
|        | RPG1R-R                  | TTCTCCATTGTCCAACACTC |                | Steptoe ( <i>rpgI</i> group 1-3)      | - <sup>c</sup> | <i>RpgI</i> -       |
|        |                          |                      |                | Golden Promise ( <i>rpgI</i> group 4) | -              | <i>RpgI</i> -       |
| RPG1-N | RPG1-N-F                 | CGGCTAATCACATCAAGTAA | 61             | Chevron ( <i>RpgI</i> )               | 669            | <i>RpgI</i> +       |
|        | RPG1-N-R                 | AGCCCATCATCAATAGACAA |                | Steptoe ( <i>rpgI</i> group 1-3)      | -              | <i>RpgI</i> -       |
|        |                          |                      |                | Golden Promise ( <i>rpgI</i> group 4) | -              | <i>RpgI</i> -       |
| RPG1-S | RPG1-S-F                 | GGCTAATCACATCAAGGTT  | 57             | Chevron ( <i>RpgI</i> )               | -              | <i>RpgI</i> +       |
|        | RPG1-S-R                 | CCACGACCAATTATGTTCTG |                | Steptoe ( <i>rpgI</i> group 1-3)      | 487            | <i>RpgI</i> -       |
|        |                          |                      |                | Golden Promise ( <i>rpgI</i> group 4) | -              | <i>RpgI</i> +       |

<sup>a</sup> Markers based on previously reported *RpgI* markers (Derevnina et al. 2014; Eckstein et al. 2003).

<sup>b</sup> Corrected sequence. The original sequence was missing a “A” at position 17 (Eckstein et al. 2003).

<sup>c</sup> No amplification expected.

<sup>e</sup> Result indicates interpretation of marker reaction, i.e. “*RpgI* +” indicates the accession has a functional *RpgI*, whereas, “*RpgI* -” indicates the accession does not have a functional *RpgI*.

**Table 3.6.** Molecular markers used to detect common functional *Rpg5* and non-functional *rpg5* genotypes.

| Marker <sup>a</sup> | Primer pair | Sequence                  | Annealing (°C) | Control genotype                      | Amplicon (bp) | Result <sup>b</sup> |
|---------------------|-------------|---------------------------|----------------|---------------------------------------|---------------|---------------------|
| LRK                 | Rpg5-LRK-F1 | CTGCTGGCACAGAGTCTGCCTTGAG | 62             | Q21861 ( <i>Rpg5</i> group 1 or 3)    | 1,045         | <i>Rpg5</i> +       |
|                     | Rpg5-LRK-R1 | ACTCTCGGGTCTGAAGTTCCGTGTG |                | Hv492 ( <i>rpg4</i> group 4)          | 1,045         | <i>Rpg5</i> +       |
| PP2C                | Rpg5-LRK-F1 | CTGCTGGCACAGAGTCTGCCTTGAG | 62             | Golden Promise ( <i>rpg4</i> group 1) | 1,045         | <i>Rpg5</i> +       |
|                     | PP2C-R2     | CCCGAGGTTTGCGATGAAGAGAGTC |                | Steptoe ( <i>rpg4</i> group 2)        | -             | <i>Rpg5</i> -       |
| PP2C                | Rpg5-LRK-F1 | CTGCTGGCACAGAGTCTGCCTTGAG | 62             | Q21861 ( <i>Rpg5</i> group 1 or 3)    | -             | <i>rpg5</i> -       |
|                     | PP2C-R2     | CCCGAGGTTTGCGATGAAGAGAGTC |                | Hv492 ( <i>rpg4</i> group 4)          | -             | <i>rpg5</i> -       |
|                     |             |                           |                | Golden Promise ( <i>rpg4</i> group 1) | -             | <i>rpg5</i> -       |
|                     |             |                           |                | Steptoe ( <i>rpg4</i> group 2)        | 839           | <i>rpg5</i> +       |

<sup>a</sup> Marker sequence based on previously reported markers for *Rpg5* genotyping and modified by Robert Brueggeman (personal communication) (Derevnina et al. 2014; Steffenson et al. 2016; Wang et al. 2013).

<sup>b</sup> Result indicates interpretation of marker reaction, i.e. “*Rpg5* +” indicates marker that this accession has a functional *Rpg5*, the, “*Rpg5* -” indicates this accession does not have a functional *Rpg5*, “*rpg5* +” indicates accession has a non-functional *Rpg5*, and “*rpg5* -” indicates this accession does not have a non-functional *Rpg5*.

**Table 3.7.** Molecular markers used to detect rare non-functional *rpg5* genotypes.

| Marker <sup>a</sup> | Non-functional allele tested | Primer pair | Sequence                          | Sequencing primer                            | Control genotype  | Hallmark sequence                      | Result <sup>b</sup>                |
|---------------------|------------------------------|-------------|-----------------------------------|--|---|--|------------------------------------|
| R5                  | Group 1 <i>rpg5</i>          | R5-F1       | CCGCCTACCACA<br>CCTCCGATTCCAC     | R5-R1:<br>TCAGGTTTG<br>ATGGCTGTC<br>TCTGGAG  | Q21861<br>( <i>Rpg5</i> group 1 or 3)<br>Hv492<br>( <i>rpg4</i> group 4)          | GCAGGATCCCCCATCACGG                    | <i>rpg5</i> -<br><br><i>rpg5</i> - |
|                     |                              | R5-R1       | TCAGGTTTGATGG<br>CTGTCTCTGGAG     |  | Golden Promise<br>( <i>rpg4</i> group 1)  | GCAGGATCCCCCATCACG<br>G                | <i>rpg5</i> +                      |
|                     |                              |             |                                   |  | Q21861<br>( <i>Rpg5</i> group 1 or 3)   | GCAGGATCCCCCATCACGG                    | <i>rpg5</i> -                      |
|                     |                              |             |                                   |  |   |  |                                    |
| R10                 | Group 4 <i>rpg5</i>          | Rpg5-R10    | AACAATATTCAC<br>CTGCGGCACCAA<br>C | Rpg5-seq-R1:<br>AGTGGCTTG<br>AGAGCTTCA<br>AC | Q21861<br>( <i>Rpg5</i> group 1 or 3)<br>Hv492<br>( <i>rpg4</i> group 4)          | TCCTTCCCCGCGAGGG /<br>TCCTTCCCCACGAGGG | <i>rpg5</i> -<br><br><i>rpg5</i> + |
|                     |                              | Rpg5-F10    | TGCATCTATCTGC<br>TCATGCAAGGAG     |  | Golden Promise<br>( <i>rpg4</i> group 1)<br>Q21861<br>( <i>Rpg5</i> group 1 or 3) | TCCTTCCCCGCGAGGG                       | <i>rpg5</i> -<br><br><i>rpg5</i> - |
|                     |                              |             |                                   |  |   |  |                                    |

<sup>a</sup> Marker sequence based on previously reported markers for *Rpg5* genotyping and modified by Robert Brueggeman (personal communications) (Arora et al. 2013 ; Derevnina et al. 2014; Steffenson et al. 2016; Wang et al. 2013).

<sup>b</sup> Result indicates interpretation of marker reaction, i.e. “*Rpg5* +” indicates marker suggests accession has a functional *Rpg5*, whereas, “*Rpg5* -“ indicates accession does not have a functional *Rpg5*, and “*rpg5* +” indicates marker suggests that accession has a non-functional *Rpg5*, whereas, “*rpg5* -“ indicates this accession does not have a non-functional *Rpg5*.

**Table 3.8.** Adult plant disease severity and infection responses of Barley iCore Collection Selects (BCCS) to *Puccinia graminis* f. sp. *tritici* races HKHJC, QCCJB, and MCCFC in St. Paul, MN and Pgt TTKSK composite in Njoro, Kenya.

| Name <sup>b</sup> | StP15_HK <sup>a</sup> |                 |                 | StP15_MC |    |       | StP13_QC |    |       | StP14_QC |    |       | StP15_QC |    |       | KEN14o |    |       | KEN14m |    |       |
|-------------------|-----------------------|-----------------|-----------------|----------|----|-------|----------|----|-------|----------|----|-------|----------|----|-------|--------|----|-------|--------|----|-------|
|                   | CL <sup>c</sup>       | DS <sup>d</sup> | IR <sup>e</sup> | CL       | DS | IR    | CL       | DS | IR    | CL       | DS | IR    | CL       | DS | IR    | CL     | DS | IR    | CL     | DS | IT    |
| BCC0007           | 1                     | 1               | MR              | -        | 10 | MS    | -        | 30 | S     | 2        | 7  | S     | -        | 25 | S-MS  | 2      | 5  | S     | 1      | T  | R     |
| BCC0015           | -                     | 30              | MS              | -        | 10 | MS    | -        | -  | -     | 2        | 7  | MS    | -        | 35 | S     | 2      | 5  | MS-S  | 1      | T  | MS    |
| BCC0057           | 1                     | T               | MS-MR           | -        | 35 | MS    | 2        | 5  | MS    | 2        | 3  | MR    | -        | 30 | MS    | 2      | 5  | S     | -      | 30 | MS-S  |
| BCC0058           | 2                     | 7               | MS              | -        | 25 | S     | -        | 30 | S     | 2        | 7  | MS-S  | -        | 45 | S     | -      | 15 | S     | 1      | 1  | MS-MR |
| BCC0077           | -                     | -               | -               | -        | -  | -     | -        | -  | -     | -        | -  | -     | -        | -  | -     | -      | -  | -     | -      | -  | -     |
| BCC0131           | 1                     | 1               | MR              | 2        | 3  | MR-MS | 1        | T  | R-MS  | 1        | T  | R-MR  | 2        | 5  | MR    | 1      | 1  | R     | 1      | 1  | R-MR  |
| BCC0138           | 1                     | 1               | MR              | 2        | 1  | MR    | 2        | 5  | MR-MS | 1        | 1  | S     | -        | 45 | S     | 1      | 1  | R-MR  | 2      | 5  | MR-MS |
| BCC0156           | 2                     | 10              | S               | 2        | 3  | MR-MS | -        | 25 | S-MS  | -        | 15 | S     | -        | 20 | S     | -      | -  | -     | 1      | T  | R-MR  |
| BCC0157           | -                     | 30              | MS              | -        | 30 | S     | 2        | 15 | MS-S  | -        | 25 | MS-S  | -        | 70 | S     | 2      | 3  | S     | 1      | T  | MR-R  |
| BCC0168           | 1                     | T               | MR-MS           | -        | 10 | MS-S  | 1        | 1  | MR-MS | 1        | 1  | MR-MS | 1        | 1  | R-MR  | 2      | 5  | MS-MR | 2      | 7  | MS    |
| BCC0169           | 1                     | 3               | MS              | 1        | T  | MS    | 2        | 5  | MS-MR | 2        | 5  | MS    | 1        | 3  | MR-MS | 1      | 1  | MR-MS | 1      | T  | R     |
| BCC0174           | 1                     | 1               | MS              | 2        | 3  | MS    | 2        | 10 | S     | 2        | 7  | MS-S  | -        | 20 | S     | 2      | 3  | MS-S  | -      | 50 | S-MS  |
| BCC0175           | -                     | 15              | MS              | -        | 35 | S     | -        | 30 | S     | -        | 15 | MS    | -        | 60 | S     | 1      | T  | MS    | 1      | 1  | MR-R  |

|         |   |    |       |   |    |    |   |    |         |   |    |       |   |    |       |   |    |    |   |    |      |
|---------|---|----|-------|---|----|----|---|----|---------|---|----|-------|---|----|-------|---|----|----|---|----|------|
| BCC0180 | 1 | 1  | MR    | 2 | 1  | MR | 2 | 5  | MR-MS-S | 2 | 3  | MR-MS | - | 15 | S-MS  | 2 | 3  | MS | 1 | T  | MR-R |
| BCC0212 | 2 | 5  | MR    | - | 20 | MS | - | 30 | S-MS    | 2 | 10 | S-MS  | - | 60 | S     | 1 | T  | MS | 2 | 3  | MR   |
| BCC0231 | - | 15 | MS    | 2 | 7  | MS | 2 | 10 | MS-MR   | 2 | 5  | S-MS  | - | 25 | MS-MR | 1 | 1  | R  | 1 | T  | MR-R |
| BCC0240 | 1 | 1  | MS    | 2 | 1  | MR | - | 30 | S-MS    | 2 | 3  | MR-MS | - | 10 | MR-S  | 1 | 1  | MS | 2 | 7  | MS   |
| BCC0241 | 1 | 1  | -MS   | 2 | 3  | MS | - | 35 | MS-MS   | 2 | 5  | MS    | - | 15 | MS    | 2 | 5  | S  | 1 | T  | R    |
| BCC0242 | 2 | 10 | S     | - | 10 | MR | 2 | 10 | MS-MR   | - | 15 | S     | - | 50 | S     | 1 | T  | MS | 1 | T  | MR-R |
| BCC0244 | 1 | T  | R     | 1 | T  | R  | - | 35 | S-MS    | 2 | 7  | MS    | - | 30 | S     | 1 | 1  | S  | 1 | 1  | MR   |
| BCC0255 | - | 30 | S     | - | 30 | S  | - | 30 | S       | - | 40 | S     | - | 40 | S     | 1 | 1  | S  | - | 15 | MS   |
| BCC0260 | 2 | 10 | S-MS  | - | 15 | MS | 2 | 5  | MR-R-MS | 2 | 10 | S     | - | 25 | S-MS  | 1 | T  | R  | 1 | T  | R    |
| BCC0261 | 2 | 7  | MS    | 2 | 3  | MS | 2 | 15 | MS-MS   | 1 | 1  | MR-MS | - | -  | -     | 2 | 5  | S  | 1 | 1  | MR-R |
| BCC0266 | - | 15 | S     | 2 | 1  | MS | - | 35 | S-MS    | - | 25 | S     | 2 | 5  | S     | 1 | T  | S  | 1 | T  | MS?  |
| BCC0270 | 2 | 10 | MS    | 1 | T  | MS | 1 | 2  | MR-MS-R | 2 | 3  | MS-MR | - | 20 | S     | 2 | 5  | S  | - | 15 | MS-S |
| BCC0308 | - | -  | -     | - | -  | -  | - | -  | -       | - | -  | -     | - | 25 | S-MS  | - | -  | -  | - | 30 | MS-S |
| BCC0318 | 2 | 10 | MR-MS | - | 10 | MS | 2 | 5  | MS-MR   | 1 | 1  | MR-MS | - | 30 | MS    | - | 30 | S  | - | 30 | MS   |
| BCC0324 | 1 | 3  | MR    | 2 | 5  | MS | 1 | 1  | MR-MS   | 1 | 1  | MR-MS | - | 10 | S     | - | 15 | MS | 2 | 5  | MR   |
| BCC0333 | 2 | 5  | MS    | 2 | 3  | MS | - | 30 | S       | 1 | 1  | MR-MS | - | 15 | MS    | 2 | 5  | MR | - | 15 | MS   |
| BCC0334 | 1 | T  | MR    | 2 | 3  | MS | 2 | 15 | MS      | 2 | 5  | MS    | 2 | 5  | MS    | 1 | T  | MS | 1 | T  | MS   |



|         |   |    |       |   |    |       |   |    |         |   |    |       |   |    |       |   |    |       |   |    |      |
|---------|---|----|-------|---|----|-------|---|----|---------|---|----|-------|---|----|-------|---|----|-------|---|----|------|
| BCC0376 | - | 15 | S     | 2 | 3  | MS    | 2 | 10 | MR-MS-S | - | 15 | MS    | 2 | 5  | MS-MR | 1 | 1  | MS-MR | 1 | T  | MS   |
| BCC0377 | 1 | T  | R     | 1 | T  | MS    | - | 30 | MS-S    | 2 | 3  | S     | - | 10 | MS    | 1 | 1  | MS-MR | - | 35 | MS   |
| BCC0392 | 2 | 5  | MS    | - | 10 | MS    | - | 35 | MS-S    | 2 | 3  | MS-MR | - | 10 | MS    | 1 | 1  | MR-R  | 1 | 1  | MR-R |
| BCC0394 | - | 15 | S     | - | 10 | MS    | - | 30 | MS-S    | - | 25 | S     | - | 60 | S     | 2 | 5  | S     | 2 | 3  | MS   |
| BCC0395 | 2 | 10 | MS    | 2 | 5  | MS    | - | 30 | MS-S    | 2 | 7  | MS    | - | 10 | MS    | 1 | 1  | MS    | 2 | 3  | MS   |
| BCC0422 | 1 | 3  | MR-MS | 1 | T  | MS    | 2 | 20 | MS-S    | 2 | 3  | MS    | - | 15 | MS    | 1 | 1  | MR-MS | 2 | 5  | S    |
| BCC0434 | - | -  | -     | - | -  | -     | - | -  | -       | - | -  | -     | - | -  | -     | - | -  | -     | - | -  | -    |
| BCC0435 | 1 | 3  | S-MS  | 1 | T  | MS-MR | 1 | 1  | MS-MR-S | 1 | 1  | MS    | - | 15 | MS-S  | 1 | 1  | MS    | 2 | 5  | M    |
| BCC0444 | - | -  | -     | - | -  | -     | - | -  | -       | - | -  | -     | - | -  | -     | - | -  | -     | - | -  | -    |
| BCC0476 | 2 | 5  | MS    | - | 15 | S     | 2 | 5  | MS-MR-R | 2 | 7  | MS    | - | 15 | S-MS  | 1 | 1  | MR-R  | - | 15 | MS-S |
| BCC0490 | 2 | 7  | MS    | 2 | 5  | MR    | 1 | 2  | MR-MS-R | 2 | 5  | S     | - | 15 | MS-S  | 1 | 1  | MR    | - | 15 | MS-S |
| BCC0524 | 2 | 10 | MS    | - | 15 | MS    | - | 35 | MS-MR   | 2 | 3  | MR    | 2 | 5  | MR-R  | - | 50 | MS    | - | 30 | S    |
| BCC0579 | 2 | 10 | MS    | - | 10 | MS    | 2 | 5  | MS-MR-R | 2 | 3  | MR-MS | 2 | 5  | S-MS  | 2 | 10 | S     | - | 30 | MS-S |
| BCC0592 | 2 | 5  | MR-MS | 1 | T  | MR    | 2 | 10 | MS-MR   | 2 | 3  | MS    | - | 15 | MS    | 2 | 3  | MR    | 2 | 3  | MS   |
| BCC0597 | 2 | 10 | MR-MS | 2 | 1  | MR    | 1 | 1  | MS-MR   | 2 | 2  | MR    | 1 | 3  | MR-MS | 1 | T  | MR-R  | - | 10 | M    |
| BCC0603 | - | 20 | MS-S  | - | 20 | MS    | - | 25 | MS-S    | 2 | 10 | MS    | - | 15 | MS    | 1 | T  | MR    | 1 | T  | MR   |

|         |   |    |      |   |    |       |   |    |         |   |    |       |   |    |       |   |    |      |   |    |       |
|---------|---|----|------|---|----|-------|---|----|---------|---|----|-------|---|----|-------|---|----|------|---|----|-------|
| BCC0654 | - | 40 | S    | - | 20 | MS    | 2 | 20 | S-MS    | 2 | 7  | MS    | - | 45 | S-MS  | 1 | 1  | MS   | 2 | 5  | R     |
| BCC0669 | 2 | 5  | S-MS | 2 | 5  | MS    | - | 25 | S       | 2 | 5  | S-MS  | - | 50 | S     | 1 | T  | -MS  | 1 | T  | MR-R  |
| BCC0673 | - | 20 | MS   | 2 | 1  | MR    | - | 25 | MS-S    | 2 | 7  | S     | - | 45 | S     | - | 15 | S    | 1 | T  | MR    |
| BCC0694 | - | -  | -    | - | 20 | MS    | - | 25 | S       | - | -  | -     | - | 20 | MS    | 2 | 10 | MR   | 1 | T  | -S    |
| BCC0709 | 1 | 1  | MR   | - | 10 | MS-MR | - | 35 | S-MS    | - | 15 | MS-MR | 2 | 5  | MS    | 1 | 1  | -MS  | 1 | T  | -R    |
| BCC0713 | 1 | 1  | MR   | 2 | 5  | MS    | 2 | 15 | MS-S    | 2 | 5  | MS    | - | -  | -     | 2 | 3  | S    | - | 40 | S     |
| BCC0718 | - | 20 | MS   | - | 15 | MS    | 1 | 1  | MS-MR-R | 2 | 5  | MS-S  | 1 | 3  | MS-MR | 2 | 3  | S-MS | 2 | 5  | S-MS  |
| BCC0728 | - | 15 | MS   | - | 30 | MS    | - | 35 | S-MS    | 2 | 5  | MS-S  | - | 70 | S     | 2 | 3  | MS-S | 1 | T  | S     |
| BCC0736 | 2 | 10 | MS   | - | 30 | S     | - | 25 | MS-S    | 2 | 3  | MS    | - | 35 | S     | 2 | 5  | MS-S | 1 | T  | MS-S  |
| BCC0738 | 2 | 7  | MS   | 2 | 3  | MR    | 2 | 5  | MS-S-MR | 2 | 10 | S     | 1 | T  | MS    | 1 | T  | MR   | 2 | 5  | S     |
| BCC0740 | - | 15 | S-MS | 2 | 5  | MS    | 2 | 5  | MS-S    | 1 | 1  | MS    | - | -  | -     | - | 15 | S    | - | 25 | S-MS  |
| BCC0741 | 1 | T  | R    | 2 | 1  | MR    | 2 | 15 | MS-MR   | 1 | 1  | MS    | - | 25 | S     | 1 | T  | R-MR | 1 | T  | R     |
| BCC0743 | 1 | 1  | MS   | 2 | 3  | MS    | 1 | 1  | MR-MS   | 1 | 1  | MR    | - | 10 | S-MS? | 1 | T  | R    | 1 | T  | MR-MS |
| BCC0746 | 2 | 10 | MS   | - | 20 | MS-S  | 2 | 10 | MS      | 2 | 3  | S     | - | 25 | S     | 2 | 5  | MS-S | 1 | 1  | S     |
| BCC0747 | - | 20 | MS-S | - | 15 | MS    | 2 | 15 | S-MS    | 2 | 7  | S-MS  | - | 35 | S-MS  | 1 | T  | S-MS | 1 | T  | MR-R  |
| BCC0750 | 1 | 1  | MS   | 2 | 1  | MS    | 2 | 15 | S       | 2 | 3  | S-MS  | - | 35 | S-MS  | - | -  | -    | 1 | T  | R-MR  |
| BCC0762 | - | 30 | S-MS | - | 10 | MS-MR | - | 25 | S-MS    | 2 | 3  | S-MS  | - | 10 | MS-MR | 1 | 1  | MR   | - | 10 | MS-S  |

|         |   |    |         |   |    |    |   |    |         |   |    |       |   |    |       |   |    |         |   |   |      |
|---------|---|----|---------|---|----|----|---|----|---------|---|----|-------|---|----|-------|---|----|---------|---|---|------|
| BCC0771 | 2 | 5  | S-MS    | 2 | 5  | MR | 2 | 10 | MS      | 2 | 3  | MR-MS | - | 25 | S     | 2 | 5  | MS-MR   | 1 | T | R-MR |
| BCC0773 | 1 | 1  | MS      | 2 | 5  | MS | - | 25 | S-MS-MR | - | 25 | S-MS  | - | 10 | S-MS  | 1 | T  | R       | 1 | T | R    |
| BCC0774 | 2 | 7  | MS      | - | 10 | MS | - | 35 | S-MS    | 2 | 3  | S-MS  | - | 25 | S-MS  | 1 | 1  | MR-R    | 2 | 7 | S    |
| BCC0777 | 1 | 1  | MR-MS-S | - | 15 | MS | - | 35 | S-MS    | - | 25 | S     | - | 35 | S-MS  | 1 | T  | S       | 1 | T | R-MR |
| BCC0821 | 2 | 10 | S       | - | 30 | MS | - | 35 | S-MS    | - | 20 | MS    | - | 15 | S-MS  | - | -  | -       | - | - | -    |
| BCC0822 | 2 | 5  | MS      | 2 | 7  | MS | 2 | 20 | S-MS    | 2 | 10 | MS    | - | 15 | S-MS  | 2 | 3  | S       | 1 | T | R    |
| BCC0829 | 2 | 10 | S       | - | 15 | MS | - | 35 | S-MS    | 1 | 1  | MS    | 2 | 5  | MS    | 1 | 1  | MR-MS   | 1 | T | MR-R |
| BCC0842 | - | 15 | MR-MS   | - | 30 | S  | 2 | 20 | S-MS    | 2 | 10 | S-MS  | - | 50 | S     | - | 15 | S-MS    | 1 | T | R-MR |
| BCC0856 | 2 | 10 | MS      | 1 | T  | MR | 2 | 5  | S-MS-MR | 1 | 1  | MS    | 2 | 5  | S-MS  | 1 | 1  | R-MR    | 1 | T | MS   |
| BCC0862 | 2 | 7  | MS      | - | 20 | MS | 2 | 10 | MS      | 1 | 1  | MR-MS | - | 50 | S     | 1 | T  | R       | 1 | T | MS?  |
| BCC0875 | 1 | 3  | S-MS    | - | 30 | MS | 2 | 15 | S-MS    | - | 15 | S-MS  | - | 35 | S     | 1 | 1  | MS-MR   | 1 | T | MR?  |
| BCC0884 | 1 | 3  | MS      | - | 15 | MS | 2 | 5  | MR-MS   | 2 | 3  | S-MS  | - | 45 | S-MS  | 2 | 10 | MR      | 2 | 5 | M    |
| BCC0889 | 1 | 3  | MR      | 2 | 3  | MS | 2 | 15 | MS      | 2 | 3  | MS-MR | - | 10 | MS    | 1 | T  | R-MR-MS | 2 | 5 | MR-R |
| BCC0899 | 1 | T  | R       | 2 | 3  | MS | 2 | 5  | MR-MS-S | 1 | T  | MR-R  | - | 20 | S     | 1 | T  | MR      | 1 | T | R-MR |
| BCC0908 | 1 | T  | R       | 1 | T  | R  | 2 | 5  | MS-S    | 1 | 1  | MR-MS | 1 | 3  | MS-MR | 2 | 5  | S-MS    | 2 | 5 | R-MR |
| BCC0950 | - | -  | -       | - | -  | -  | - | -  | -       | - | -  | -     | - | -  | -     | - | -  | -       | - | - | -    |
| BCC0985 | - | -  | -       | - | -  | -  | - | -  | -       | - | -  | -     | - | -  | -     | - | -  | -       | - | - | -    |

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|---------|---|----|-------|---|----|------|---|----|---------|---|----|-------|---|----|------|---|----|-------|---|----|-------|
| BCC0991 | - | 20 | S-MS  | - | 15 | MS   | - | 35 | MS-S    | 2 | 10 | S-MS  | - | 17 | S    | 2 | 10 | S     | 1 | 1  | R-MR  |
| BCC0994 | 2 | 10 | S-MS  | 2 | 7  | MS   | - | 35 | S-MS    | - | 15 | MS    | - | 20 | MS   | 1 | T  | MR    | 1 | 1  | MR-R  |
| BCC1003 | 2 | 10 | S     | 2 | 5  | MS   | 2 | 5  | MS-MR   | 1 | 1  | MR-MS | - | 10 | MS   | 1 | T  | MS-MR | 2 | 3  | MR    |
| BCC1060 | 2 | 10 | MS    | 2 | 7  | MS   | 2 | 20 | MS-S    | 2 | 3  | MS    | - | 45 | MS   | - | 15 | MS-MR | 1 | 1  | MS    |
| BCC1061 | 2 | 5  | MS-MR | - | 20 | MS   | 2 | 10 | S-MS    | 2 | 5  | MS    | - | 40 | MS   | 2 | 3  | MS-MR | 1 | 1  | MS-MR |
| BCC1062 | 1 | 3  | S-MS  | 2 | 3  | MS   | 2 | 5  | MS-MR   | - | -  | -     | - | 15 | MS   | 1 | T  | MR    | 1 | 1  | MR-R  |
| BCC1069 | 2 | 10 | MS-S  | - | 25 | S    | 2 | 20 | MS-S    | 2 | 3  | MS    | - | 35 | MS   | 2 | 5  | S     | 1 | T  | MR-R  |
| BCC1081 | - | 20 | S     | - | 40 | S-MS | - | 35 | MS-S    | - | 25 | S     | - | 35 | MS   | - | 15 | MS    | 2 | 3  | MS-MR |
| BCC1100 | - | 30 | S     | - | 35 | S    | 2 | 20 | S-MS    | 2 | 7  | MS    | - | 30 | MS   | 2 | 3  | MS-MR | - | 15 | MS-S  |
| BCC1101 | 1 | 1  | MR    | - | 25 | S-MS | - | 35 | S-MS    | 2 | 10 | MS    | - | 50 | S    | 1 | T  | MR    | - | 15 | S     |
| BCC1103 | 2 | 5  | MS    | 2 | 1  | MS   | 2 | 10 | MS      | 2 | 3  | S     | - | -  | -    | - | 50 | S     | - | 40 | S     |
| BCC1131 | - | -  | -     | - | -  | -    | - | -  | -       | - | -  | -     | - | -  | -    | - | -  | -     | - | -  | -     |
| BCC1135 | 2 | 10 | MS    | - | -  | -    | 1 | T  | MS      | - | 15 | MS    | - | -  | -    | 2 | 3  | MS    | 1 | T  | MR-R  |
| BCC1142 | 1 | T  | R     | 2 | 5  | MS   | 2 | 5  | MS-MR   | 2 | 3  | MR    | - | 10 | S-MS | 1 | T  | MR    | 1 | T  | MR    |
| BCC1156 | 1 | 1  | MR    | - | 20 | MS-S | 1 | 1  | R-MR-MS | - | -  | -     | 2 | 5  | S-MS | 2 | 5  | S     | 2 | 5  | MR-MS |
| BCC1157 | 2 | 10 | S     | - | 20 | MS   | - | 30 | MS-S    | - | 15 | MS-S  | - | 10 | S    | 2 | 10 | S     | 1 | T  | MR-R  |
| BCC1158 | 2 | 10 | S-MS  | 2 | 1  | MS   | 2 | 15 | MS-S    | 1 | 1  | MR    | - | 10 | MS   | 2 | 3  | MS    | 1 | 1  | S     |

|         |   |    |        |   |    |       |   |    |         |   |    |       |   |    |      |   |    |         |   |    |      |
|---------|---|----|--------|---|----|-------|---|----|---------|---|----|-------|---|----|------|---|----|---------|---|----|------|
| BCC1160 | 1 | T  | R      | 2 | 3  | MS    | 1 | 2  | MR-MS-R | 1 | 1  | MR    | - | 35 | S-MS | 1 | 1  | MR      | 1 | T  | R-MR |
| BCC1182 | 1 | T  | R      | 1 | T  | MR    | 2 | 10 | MS      | 2 | 3  | MS    | - | 25 | S-MS | 2 | 3  | MR-R    | - | 10 | MS   |
| BCC1195 | 2 | 10 | S-SM-S | - | 10 | S-MS  | 2 | 5  | MS-MR-R | 2 | 5  | MS-MR | - | 45 | S    | 2 | 5  | S-MS    | 1 | T  | R-MR |
| BCC1206 | 1 | T  | R      | - | 15 | MS    | 2 | 5  | MS-MR-S | 2 | 5  | S     | - | 45 | S    | 2 | 3  | MS      | - | 20 | S    |
| BCC1207 | 1 | T  | R      | 1 | T  | MR    | 1 | 2  | MS-MR-R | - | -  | -     | - | 20 | S    | 1 | T  | R-MR    | 2 | 5  | R-MR |
| BCC1209 | 1 | 1  | MR     | - | 10 | MS    | 2 | 5  | MR-MS-R | 2 | 10 | S     | - | 60 | S-MS | - | 30 | S       | - | 20 | S    |
| BCC1215 | 2 | 5  | S-MS   | 1 | T  | MR-R  | 1 | 1  | MR-R-MS | 2 | 3  | MS    | - | 40 | MS   | 1 | 1  | MR-MS-R | 1 | T  | R    |
| BCC1219 | 2 | 10 | MS     | 2 | 3  | MS    | - | 25 | S-MS    | 2 | 3  | MS    | - | 40 | S    | 2 | 5  | MS      | 2 | 7  | MS   |
| BCC1222 | 2 | 10 | S      | 2 | 3  | MR-MS | - | 25 | S-MS    | 2 | 3  | MR-MS | - | 45 | S    | 2 | 3  | MR-MS   | 1 | 1  | S-MS |
| BCC1229 | 1 | 3  | MS     | - | 20 | MS    | 2 | 15 | S-MS    | 2 | 5  | MS    | - | 15 | MS   | 2 | 3  | MS-MR   | 2 | 3  | MR   |
| BCC1310 | 1 | T  | R      | - | 15 | S     | 2 | 20 | MS      | 2 | 3  | MS-S  | - | 10 | MS-S | 2 | 10 | S-MS    | 2 | 5  | M    |
| BCC1324 | 2 | 5  | MS     | - | 20 | S     | - | 25 | S       | 2 | 5  | MS    | - | 35 | S    | 2 | 10 | MS      | 1 | T  | -S   |
| BCC1337 | 2 | 10 | S-MS   | - | 15 | S-MS  | 2 | 15 | S-MS    | 2 | 3  | MS-MR | - | 35 | S-MS | - | 15 | MS-MR   | 1 | T  | MR   |
| BCC1375 | 1 | T  | R      | 2 | 5  | MS    | 2 | 15 | MS-S    | - | -  | -     | - | 35 | S-MS | 1 | 1  | MR-R    | 1 | T  | R    |

|         |   |    |       |   |    |       |   |    |         |   |    |       |   |    |       |   |    |       |   |    |       |
|---------|---|----|-------|---|----|-------|---|----|---------|---|----|-------|---|----|-------|---|----|-------|---|----|-------|
| BCC1377 | 2 | 5  | S-MS  | 2 | 1  | MS    | 1 | 2  | MR-MS-R | 1 | 1  | MR-MS | 1 | 3  | MR    | 1 | T  | MR-MS | 1 | 1  | MR-MS |
| BCC1390 | - | 20 | S-MS  | - | 20 | MS    | 2 | 5  | MS-S    | 2 | 5  | S-MS  | - | 70 | S     | 1 | 1  | R-MR  | - | 15 | S     |
| BCC1391 | 1 | 1  | MS-MR | - | -  | -     | 2 | 20 | S-MS    | 2 | 10 | S     | - | 35 | S     | 1 | 1  | MR    | 1 | 1  | MR-MS |
| BCC1392 | 1 | 3  | S-MS  | 2 | 3  | MR-MS | - | 40 | S-MS    | 2 | 3  | MS-MR | - | 45 | S     | 1 | 1  | MR    | 1 | 1  | MR-MS |
| BCC1393 | - | 25 | MS-S  | - | 15 | MS    | - | 35 | S-MS    | 2 | 7  | S-MS  | - | 50 | S     | 1 | 1  | R-MR  | 1 | T  | MR-MS |
| BCC1394 | 2 | 10 | S-MS  | - | 10 | S-MS  | - | 25 | MS-S    | 2 | 5  | S     | - | 10 | S     | 1 | T  | MR    | 1 | 1  | MS    |
| BCC1399 | 2 | 5  | MS    | 2 | 3  | MR    | 2 | 10 | S-MS    | 2 | 7  | MS    | - | 15 | MS-MR | 2 | 5  | MS    | 1 | T  | MS-MR |
| BCC1404 | 1 | T  | MR    | - | 15 | MS    | 1 | T  | R-MR-S  | 2 | 3  | S-MS  | - | 20 | MS-S  | 2 | 10 | S     | - | 10 | M     |
| BCC1408 | 2 | 7  | MS    | - | 15 | MS    | - | 35 | MS-S    | 2 | 10 | S     | - | 20 | MS    | 1 | 1  | MR    | 1 | T  | S     |
| BCC1427 | 2 | 10 | MS    | - | 10 | MS    | 2 | 5  | MR-MS   | 2 | 3  | S-MS  | - | 25 | MS    | 1 | 1  | R-MR  | 2 | 5  | M     |
| BCC1450 | - | 20 | MS    | - | 20 | MS    | - | 30 | S-MS    | 2 | 10 | MS-S  | - | 30 | MS-S  | 2 | 3  | MS-MR | 1 | T  | MR    |
| BCC1465 | 1 | 3  | S-MS  | 2 | 7  | MS    | - | 45 | S       | 2 | 7  | MS    | - | 20 | MS    | 2 | 3  | MS    | 1 | 1  | R-MR  |
| BCC1469 | 1 | 1  | MR    | - | 10 | MS    | - | 30 | MS      | - | 15 | S     | - | -  | -     | 1 | 1  | MR    | 1 | T  | R     |
| BCC1472 | 1 | 3  | MS    | 2 | 3  | S-MS  | - | 30 | MS-S    | 2 | 7  | MS-S  | - | 35 | S     | 2 | 3  | MS    | 2 | 5  | MR-MS |
| BCC1474 | 2 | 5  | MR    | - | 10 | MS    | - | 30 | S-MS    | 2 | 10 | MS-S  | - | 40 | S-MS  | 2 | 3  | MS-MR | - | 10 | MS    |
| BCC1485 | 1 | T  | MR    | - | 10 | MS    | - | 45 | S       | 1 | 1  | MS-S  | 1 | 1  | MR    | 2 | 3  | MS-   | 2 | 7  | MS    |
| BCC1495 | 1 | 3  | MS    | 2 | 1  | MS    | 1 | T  | R-MR-S  | 1 | 1  | MR-R  | 1 | T  | MS    | 1 | 1  | R-MR  | - | 15 | M     |

|         |   |    |       |   |    |      |   |    |         |   |    |       |   |    |       |   |    |       |   |   |       |
|---------|---|----|-------|---|----|------|---|----|---------|---|----|-------|---|----|-------|---|----|-------|---|---|-------|
| BCC1510 | 1 | T  | MR-MS | - | 20 | MS   | 1 | 2  | MR-R-MS | 1 | T  | MS    | 1 | 1  | MS-MR | 1 | T  | MR-MS | 1 | T | MR    |
| BCC1515 | 1 | 3  | MS    | - | 10 | S    | - | 35 | S       | - | 30 | S     | - | 35 | S-MS  | 1 | 1  | MR    | 1 | T | MR    |
| BCC1522 | 1 | 3  | MS-MR | 2 | 7  | MS   | - | 25 | MS-S    | 1 | 1  | MR    | 2 | 7  | S-MS  | - | -  | -     | 1 | T | MR    |
| BCC1525 | 2 | 10 | MS    | 2 | 5  | MS   | 1 | 3  | MR-MS-R | 2 | 3  | MR-MS | - | 10 | MR-MS | 2 | 3  | MR-MS | 1 | T | MR-R  |
| BCC1529 | - | 30 | S     | - | 20 | S-MS | - | 40 | S-MS    | - | 25 | S-MS  | - | 45 | S     | 1 | 1  | MR-R  | 1 | T | MR-R  |
| BCC1535 | 1 | 3  | MR    | 2 | 7  | MS   | 2 | 5  | MS-MR-S | 2 | 3  | MR-MS | - | 15 | S     | 2 | 10 | S-MS  | 2 | 3 | MS    |
| BCC1548 | 2 | 10 | MS    | 2 | 5  | MS   | 2 | 15 | MS      | 2 | 7  | S     | - | 35 | S-MS  | 2 | 10 | S-MS  | 1 | T | MR    |
| BCC1556 | 1 | T  | MR    | 2 | 3  | R-MR | 2 | 5  | MR-R-MS | 1 | 1  | MR-R  | 1 | 3  | MR    | 1 | 1  | R     | 2 | 5 | R-MR  |
| BCC1562 | 2 | 5  | MS    | 1 | T  | MR   | 2 | 5  | MS-MR-S | 2 | 5  | MS    | - | 25 | S-MS  | 2 | 10 | S     | 2 | 5 | S-MS  |
| BCC1568 | 2 | 10 | MS    | 2 | 5  | MS   | 2 | 5  | MS-MR-R | 2 | 5  | MR    | 2 | 5  | MR-R  | 2 | 3  | MR-R  | 1 | T | R     |
| BCC1570 | 1 | 3  | MR    | 2 | 5  | MS   | 2 | 5  | MS-MR-S | 1 | 1  | MR-R  | - | 10 | MS-MR | 2 | 5  | MS    | 1 | T | R     |
| BCC1571 | - | 20 | MS    | - | 10 | S    | - | 25 | MS-S    | 1 | 1  | MS    | - | 10 | MS    | 2 | 10 | S-MS  | 2 | 3 | MR-MS |
| BCC1576 | 2 | 10 | S-MS  | 2 | 5  | MS   | 2 | 10 | MS-S    | 2 | 3  | MR-MS | - | 20 | MS-MR | 2 | 5  | MS-MR | 1 | T | R     |
| BCC1577 | 1 | 1  | MS    | - | 10 | MS   | - | 25 | MS-S    | 2 | 5  | MR    | - | 45 | S-MS  | 1 | 1  | MR    | 1 | T | R     |
| BCC1578 | 1 | 1  | MS-S  | - | 15 | MS   | - | 25 | MS-S    | 2 | 3  | MS    | - | 15 | S-MS  | 2 | 5  | MS-MR | 1 | T | MR-MS |

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|---------|---|----|-------|---|----|-------|---|----|-------|---|----|-------|---|----|-------|---|----|-------|---|----|-------|
| BCC1582 | - | 25 | MS    | - | 10 | S     | - | 25 | MS-S  | 2 | 7  | MS    | - | 30 | MS-S  | 2 | 10 | MS-S  | 2 | 3  | MS-MR |
| BCC1584 | 2 | 7  | MS    | 2 | 3  | MS    | - | 25 | MS-S  | 2 | 7  | MS    | - | 35 | S     | 1 | 1  | MS-MR | 1 | 1  | MS    |
| BCC1585 | 2 | 5  | S-MS  | 2 | 3  | MS    | - | 35 | MS-S  | - | 15 | MS-S  | 2 | 7  | MS-S  | 2 | 5  | MS-S  | 1 | T  | MR    |
| BCC1592 | 2 | 5  | MS-S  | - | 10 | MS    | - | 25 | MS-S  | 2 | 5  | MR    | - | 10 | MR    | - | 15 | MS-S  | 1 | T  | MR-MS |
| BCC1595 | - | 20 | S     | - | 20 | MS-MR | - | 30 | MS-S  | 2 | 3  | MS    | - | 25 | MS-S  | 2 | 5  | MS-S  | - | 10 | M     |
| BCC1600 | 1 | 1  | MS    | 2 | 3  | MS    | 1 | 1  | MR-MS | - | -  | -     | - | 15 | MS    | 1 | 1  | R-MR  | 2 | 5  | R-MR  |
| BCC1602 | 1 | T  | R     | 1 | T  | MR    | 1 | 1  | MR-MS | 1 | 1  | MR-R  | - | 30 | MS    | 1 | T  | R-MR  | 1 | 1  | MS-MR |
| BCC1603 | 1 | T  | MR    | 1 | T  | R     | 2 | 5  | MS-MR | - | -  | -     | 2 | 5  | MR-MS | 1 | T  | R     | 1 | T  | R-MR  |
| BCC1614 | 1 | T  | MR-MS | 2 | 7  | MS    | - | 25 | MS-S  | 2 | 10 | MS-S  | - | 35 | S     | - | 35 | S     | 1 | T  | MR-MS |
| BCC1620 | 1 | T  | R     | 2 | 1  | MR    | 2 | 5  | MS-R  | 1 | T  | MR    | - | 10 | MR-MS | - | -  | -     | - | -  | -     |
| BCC1623 | 2 | 5  | S     | - | 15 | MS    | 2 | 15 | MS-S  | 2 | 3  | MS    | - | 35 | MS-S  | 2 | 3  | MR-R  | 2 | 5  | MS-MR |
| BCC1624 | - | -  | -     | - | -  | -     | - | -  | -     | - | -  | -     | - | -  | -     | - | -  | -     | - | -  | -     |
| BCC1625 | 2 | 10 | MS    | - | 35 | MS    | 2 | 5  | MS-S  | 1 | 1  | MS-MR | - | 10 | MS    | - | 25 | MS-S  | - | 10 | M     |
| BCC1627 | - | 20 | MS-S  | - | 10 | S     | - | 25 | S     | 2 | 10 | MS    | - | 25 | MS-S  | - | 15 | MS-S  | 1 | T  | R-MR  |
| BCC1636 | 2 | 10 | MS    | 2 | 7  | MS-MR | 2 | 20 | MS-S  | 2 | 3  | MS    | - | 15 | MS-S  | 2 | 10 | MS-MR | 2 | 3  | MR    |
| BCC1639 | - | 15 | S     | 2 | 3  | MS    | 2 | 15 | MS-S  | 2 | 3  | MS    | - | 60 | S     | - | 20 | MS-S  | 1 | T  | R     |
| BCC1640 | 2 | 5  | MS    | - | 10 | MS    | 1 | 3  | MS-S  | - | -  | -     | - | 10 | MS-S  | - | 35 | MS    | 2 | 3  | MS    |



|         |   |    |                |   |    |           |   |    |                             |   |    |                       |   |    |                      |   |    |                      |   |    |                |
|---------|---|----|----------------|---|----|-----------|---|----|-----------------------------|---|----|-----------------------|---|----|----------------------|---|----|----------------------|---|----|----------------|
| BCC1655 | 2 | 5  | MR<br>-MS      | - | 10 | S         | 1 | 1  | R-<br>MR-<br>MS             | 2 | 3  | MR-<br>MS             | - | 25 | MS-<br>S             | 2 | 10 | MR<br>-MS            | - | 40 | S              |
| BCC1661 | 1 | 3  | MS             | 2 | 1  | MR        | - | 45 | S                           | 2 | 3  | MS                    | 1 | T  | MS                   | - | 15 | S-<br>MS             | 1 | T  | MR-<br>MS      |
| BCC1665 | 2 | 10 | S              | - | 10 | MS        | - | 35 | S                           | 2 | 5  | MS                    | - | -  | -                    | 1 | T  | S                    | 2 | 5  | M              |
| BCC1691 | 1 | T  | MR<br>-MS      | 2 | 5  | MS        | - | 25 | S-<br>MS                    | - | 20 | S                     | - | 10 | MS                   | 1 | 1  | MR<br>-R             | 1 | T  | R-<br>MR       |
| BCC1694 | - | 30 | S              | - | 30 | MS        | 2 | 10 | MS                          | 2 | 10 | S-<br>MS              | - | 40 | S-<br>MS             | - | 25 | MS-<br>S             | 1 | T  | MR-<br>MS      |
| BCC1695 | - | 25 | MS-<br>S       | 1 | T  | MR        | 2 | 5  | MR-<br>MS                   | 2 | 5  | S-<br>MS              | 2 | 5  | MR<br>-MS            | - | 15 | MR<br>-MS            | 2 | 5  | MS             |
| BCC1702 | 1 | T  | MS             | 2 | 1  | MR        | 2 | 15 | S-<br>MS                    | 2 | 10 | MS-<br>S              | - | 35 | S                    | 1 | T  | S-<br>MS             | 1 | T  | MS-<br>S       |
| BCC1703 | 2 | 10 | S              | - | 15 | MS        | - | 30 | S-<br>MS                    | - | 15 | S-<br>MS              | - | 10 | S-<br>MS             | 1 | 1  | R-<br>MR             | 1 | T  | R              |
| BCC1712 | 1 | T  | MR<br>S-<br>MS | 2 | 1  | MS        | 1 | T  | R-<br>MR-<br>MS<br>S-<br>MS | 1 | 1  | MR-<br>MS<br>S-<br>MS | - | 30 | S-<br>MS             | 1 | 1  | R-<br>MR<br>S-<br>MS | 2 | 5  | MR-<br>MS      |
| BCC1719 | - | 30 | MS             | 2 | 7  | MS        | 2 | 15 | MS                          | 2 | 3  | MS                    | - | 70 | S                    | 2 | 5  | MS                   | 1 | T  | S              |
| BCC1720 | 1 | T  | R              | 1 | T  | R         | 1 | T  | R-<br>MR-<br>MS<br>S-<br>MS | 2 | 3  | MR-<br>MS             | - | 15 | MS                   | - | 15 | S-<br>MS<br>MS-<br>S | 1 | T  | MR             |
| BCC1721 | 2 | 10 | S              | 2 | 5  | MS-<br>MR | 2 | 5  | S-<br>MS                    | 2 | 3  | MS                    | 2 | 5  | MS-<br>MR            | - | 20 | MS-<br>S             | 1 | T  | MR-<br>MS      |
| BCC1724 | 1 | 1  | MR             | 1 | T  | MR        | 2 | 10 | S<br>MS-<br>MS              | 2 | 3  | S-<br>MS<br>MS-<br>MR | 2 | 5  | S-<br>MS<br>S-<br>MS | 1 | T  | MS-<br>MR            | 1 | T  | S<br>MR-<br>R  |
| BCC1732 | 1 | T  | MR             | 1 | T  | MR        | 2 | 15 | S                           | 1 | 1  | MR                    | - | 15 | MS                   | 2 | 10 | MS<br>S-<br>MS       | 1 | T  | R-<br>MR-<br>R |
| BCC1737 | 1 | T  | R              | - | 15 | MS        | - | 30 | S<br>MR-<br>MS-<br>S        | 2 | 3  | MR                    | - | 35 | S                    | - | 15 | S-<br>MS             | 1 | 1  | MR-<br>R       |
| BCC1738 | 2 | 5  | MS             | - | 15 | MS        | 2 | 5  | MR-<br>MS-<br>S             | 2 | 10 | S-<br>MS              | - | 70 | S                    | 2 | 5  | MR<br>-MS            | 2 | 3  | MR             |

|         |   |    |           |   |    |           |   |    |                 |   |   |           |   |    |          |   |    |           |   |    |           |
|---------|---|----|-----------|---|----|-----------|---|----|-----------------|---|---|-----------|---|----|----------|---|----|-----------|---|----|-----------|
| BCC1741 | 1 | 3  | MS        | - | 15 | S         | 2 | 5  | MS-<br>MR-<br>S | 2 | 3 | MS-<br>S  | - | 10 | MS-<br>S | 2 | 10 | MS-<br>S  | 1 | T  | R-<br>MR  |
| BCC1743 | 1 | 1  | MR        | 1 | T  | R         | 2 | 5  | MR-<br>R-<br>MS | 2 | 5 | MR-<br>MS | - | 10 | S-<br>MS | 1 | 1  | R-<br>MR  | - | 25 | MS-<br>S  |
| BCC1744 | 2 | 10 | MS        | - | 20 | MS        | 2 | 15 | S-<br>MS        | 2 | 7 | MS-<br>S  | - | 35 | MS-<br>S | - | 15 | MS-<br>S  | 2 | 3  | MS        |
| BCC1745 | 1 | T  | MR        | 1 | T  | MR        | 1 | 1  | R-<br>MR-<br>MS | 1 | 1 | MR-<br>MS | 1 | 1  | MR       | 2 | 5  | MR-<br>R  | 1 | T  | R         |
| BCC1750 | 1 | 1  | MR        | 2 | 3  | MR-<br>R  | 1 | T  | R-<br>MR-<br>MS | 2 | 3 | MR        | - | 30 | S-<br>MS | 1 | 1  | MR-<br>MS | 1 | T  | R         |
| BCC1751 | - | 15 | S         | 2 | 3  | MS-<br>MR | 2 | 15 | MS-<br>S        | 2 | 7 | MS        | - | 70 | S        | 2 | 5  | S         | 2 | 3  | MS        |
| BCC1755 | 2 | 5  | MR-<br>MS | - | 15 | MS        | 2 | 15 | S-<br>MS        | 2 | 5 | MS        | - | 20 | MS-<br>S | 2 | 3  | MS-<br>MR | 1 | T  | MR        |
| BCC1783 | 2 | 7  | MS-<br>S  | - | 10 | MS        | 2 | 10 | MS-<br>MR       | 1 | 1 | MS        | - | 30 | S        | - | 25 | S         | 1 | T  | MR-<br>MS |
| BCC1789 | 1 | T  | MR        | 2 | 1  | MR-<br>MS | 1 | 3  | MR-<br>MS-<br>R | 2 | 5 | MS-<br>S  | - | 25 | S-<br>MS | 1 | 1  | MR-<br>R  | - | 10 | MR        |
| BCC1796 | 1 | T  | MS        | 2 | 5  | MS        | 2 | 15 | MS              | 2 | 3 | MR-<br>MS | - | 30 | S        | 1 | 1  | MR-<br>R  | - | 20 | MS-<br>S  |
| BCC1797 | 1 | T  | R         | 1 | T  | MS-<br>MR | - | 30 | MS-<br>S        | 2 | 3 | MR-<br>MS | - | 45 | S        | 1 | 1  | M-<br>MS  | - | 20 | MS-<br>S  |
| BCC1822 | 1 | T  | R         | 2 | 3  | MR        | 2 | 5  | MS-<br>S        | 1 | 1 | MR        | - | 10 | MS       | 1 | T  | R-<br>MR  | 2 | 5  | MS        |
| BCC1823 | 1 | T  | MR        | 2 | 3  | MS        | 2 | 15 | S-<br>MS        | 2 | 3 | MS        | - | 45 | S        | 1 | T  | MR        | 2 | 7  | MS        |
| BCC1830 | - | 15 | MS-<br>S  | 2 | 7  | MS-<br>MR | 2 | 5  | MR-<br>MS       | 2 | 3 | MS-<br>MR | - | 30 | MS-<br>S | - | 15 | MS-<br>S  | - | 10 | MS-<br>S  |
| BCC1831 | - | 20 | MS        | - | 10 | MS        | 2 | 10 | MS-<br>S        | 1 | 1 | MR        | - | 45 | S        | 2 | 5  | MR-<br>MS | 1 | 1  | R-<br>MR  |

|         |   |    |           |   |    |          |   |    |                 |   |    |           |   |    |           |   |    |           |   |    |           |
|---------|---|----|-----------|---|----|----------|---|----|-----------------|---|----|-----------|---|----|-----------|---|----|-----------|---|----|-----------|
| BCC1832 | 2 | 10 | S-<br>MS  | 2 | 1  | MR       | 2 | 5  | MR-<br>MS-<br>S | 2 | 3  | S-<br>MS  | - | 25 | MS        | 2 | 2  | MR<br>-MS | 2 | 7  | MS        |
| BCC1839 | 1 | 1  | MR<br>-MS | 2 | 3  | MR       | - | 45 | MS-<br>S        | 2 | 7  | S-<br>MS  | - | 25 | S-<br>MS  | - | -  | -         | 1 | T  | MR        |
| BCC1850 | 1 | 1  | MR        | 2 | 5  | MS       | 2 | 5  | MR-<br>R-<br>MS | 2 | 3  | S-<br>MS  | - | 15 | S-<br>MS  | 1 | 1  | MS-<br>MR | 2 | 5  | S         |
| BCC1851 | 1 | T  | MR        | 2 | 3  | MS       | 1 | 1  | MR-<br>MS-<br>R | 1 | 1  | MR        | - | 10 | MS-<br>S  | 1 | 1  | MR<br>-MS | 1 | T  | R         |
| BCC1852 | 1 | T  | MR        | 1 | T  | MS       | 2 | 5  | MS-<br>MR       | 2 | 10 | S         | - | 10 | MS        | 2 | 10 | S-<br>MS  | 1 | T  | R         |
| BCC1857 | 2 | 10 | S-<br>MS  | - | 20 | MS       | 2 | 5  | MR-<br>MS       | 1 | T  | MR        | - | 15 | MS        | - | 15 | MS-<br>S  | - | 15 | S<br>MS-  |
| BCC1859 | 1 | T  | R         | 2 | 7  | MS       | 2 | 15 | MS-<br>S        | 2 | 3  | MS        | - | 15 | MR<br>-MS | 1 | T  | S         | - | 20 | S<br>MS   |
| BCC1872 | 1 | T  | R         | 2 | 1  | MS       | 2 | 5  | MR-<br>S-R      | 2 | 7  | S         | - | 40 | S         | 2 | 5  | MS-<br>MR | 1 | T  | MS        |
| BCC1875 | 1 | T  | R         | 1 | T  | R        | 2 | 5  | MS-<br>MR-<br>S | - | 15 | MS-<br>S  | - | 10 | MR<br>-MS | - | 15 | MR<br>-MS | 1 | T  | R         |
| BCC1876 | 2 | 7  | MS        | 1 | T  | R        | 2 | 5  | MR-<br>R-<br>MS | 2 | 3  | MS-<br>MR | - | 20 | S         | 2 | 5  | MR<br>-MS | 1 | T  | R         |
| BCC1879 | 1 | 1  | MS        | 1 | T  | MS       | 2 | 5  | MR-<br>MS-<br>R | 2 | 3  | MS-<br>MR | - | 20 | S-<br>MS  | 2 | 5  | S-<br>MS  | 2 | 5  | M         |
| BCC1886 | 2 | 10 | S-<br>MS  | - | 30 | MS       | 2 | 20 | S-<br>MS        | 2 | 3  | MS        | - | 45 | S-<br>MS  | 2 | 3  | MR<br>-MS | 2 | 5  | MR-<br>MS |
| BCC1889 | 1 | T  | R         | 2 | 3  | MS       | 2 | 5  | MR-<br>MS       | 2 | 5  | S-<br>MS  | - | 35 | S         | 1 | 1  | MS-<br>MR | 1 | T  | MR        |
| BCC1891 | 2 | 5  | MS-<br>S  | 2 | 7  | S-<br>MS | 2 | 5  | MS-<br>MR-<br>S | 2 | 3  | MS-<br>MR | - | 20 | S-<br>MS  | 2 | 5  | MR        | 2 | 5  | MR-<br>MS |
| BCC1917 | 1 | T  | MS        | 1 | T  | MR       | 2 | 5  | MS-<br>S        | 1 | 1  | MR-<br>MS | - | 25 | MS-<br>S  | 2 | 5  | M         | 2 | 5  | S<br>MS   |

|         |   |    |       |   |    |       |   |    |         |   |    |       |   |    |       |   |    |       |   |   |       |
|---------|---|----|-------|---|----|-------|---|----|---------|---|----|-------|---|----|-------|---|----|-------|---|---|-------|
| BCC1918 | - | 30 | MS    | 2 | 7  | MS-MR | - | 25 | MS-MR-S | 2 | 3  | MS-MR | 2 | 5  | MS    | - | 15 | MS-MR | 1 | T | R-MR  |
| BCC1928 | 1 | T  | MR    | 2 | 7  | MS    | 1 | T  | MR-R-MS | 1 | T  | R     | 2 | 5  | MR-MS | 1 | 1  | MR-R  | 1 | T | R-MR  |
| BCC1938 | 1 | T  | R     | 1 | T  | MR    | 2 | 5  | MS-MR-S | - | 15 | S-MS  | - | 15 | S-MS  | - | 25 | S-MS  | 1 | T | MS-MR |
| BCC1971 | 2 | 10 | S     | - | 25 | MS    | - | 30 | MS-S    | 2 | 3  | MS    | - | 10 | MS    | - | 35 | S-MS  | 1 | T | MR-R  |
| BCC2013 | - | 15 | S     | 2 | 3  | MS    | 2 | 20 | MS-S    | 2 | 3  | MR    | - | 45 | S     | 2 | 10 | MR-MS | 1 | T | MR-R  |
| BCC2016 | 1 | 1  | MR-MS | 1 | T  | R     | 2 | 5  | MR-R-MS | - | -  | -     | - | 25 | S     | 2 | 5  | MS-MR | 2 | 3 | MR-MS |
| BCC2017 | - | 15 | MS    | - | 20 | S     | - | 30 | S-MS    | 2 | 3  | MS    | - | 50 | S     | 2 | 10 | MS-MR | 1 | 1 | MR-MS |
| BCC2019 | 2 | 10 | MS    | - | 10 | S-MS  | 2 | 10 | MS      | 2 | 3  | S-MS  | - | 25 | S     | 1 | 1  | MR-MS | 1 | T | R-MR  |
| BCC2022 | 1 | T  | R     | 1 | T  | R     | - | 25 | MS-S    | 2 | 3  | MS    | - | 35 | MS    | 1 | 1  | MR-MS | 1 | 1 | MR-R  |
| BCC2023 | 2 | 10 | MS    | - | 10 | MS    | - | 30 | S       | 2 | 10 | MS    | - | 35 | MS    | 1 | T  | R-MR  | 1 | 1 | MS    |
| BCC2024 | 2 | 5  | MR    | - | 15 | S     | - | 30 | MS-S    | 2 | 10 | MS    | - | 30 | S     | 2 | 3  | MR-R  | 2 | 3 | MS    |
| BCC2026 | 2 | 10 | S     | - | 35 | MS    | 2 | 15 | MS-S    | - | 15 | MS    | - | 45 | S     | 2 | 5  | MS    | 1 | T | S     |
| BCC2027 | 2 | 5  | MR-MS | - | 20 | MS    | 2 | 10 | S-MS    | 2 | 10 | S     | - | 35 | S     | 2 | 5  | MR-MS | 2 | 3 | MS    |
| BCC2028 | 1 | 3  | MS    | 2 | 7  | MS-S  | 2 | 15 | MS-S    | 2 | 5  | S     | - | 25 | MS    | 2 | 5  | S-MS  | 1 | T | S-MS  |
| BCC2065 | 1 | T  | MR    | 1 | T  | MR    | - | 25 | S-MS    | 2 | 3  | MS    | 2 | 5  | S-MS  | 1 | T  | MR    | 1 | 1 | MS    |
| BCC2066 | - | 20 | MS    | - | 10 | MS    | - | 45 | S-MS    | 2 | 7  | MS    | - | 15 | MS    | 1 | T  | R-MR  | 2 | 3 | MS    |

|         |   |    |           |   |    |           |   |    |                 |   |    |           |   |    |          |   |    |           |   |    |            |
|---------|---|----|-----------|---|----|-----------|---|----|-----------------|---|----|-----------|---|----|----------|---|----|-----------|---|----|------------|
| BCC2068 | 1 | 1  | MS        | 1 | T  | MR        | 2 | 10 | MS              | 2 | 3  | MS        | - | 15 | MS       | 1 | 1  | MS-<br>MR | 2 | 5  | MS-<br>S   |
| BCC2069 | 2 | 10 | S-<br>MS  | 2 | 3  | MR-<br>MS | 2 | 5  | MR-<br>MS       | - | 15 | S         | - | 45 | S-<br>MS | - | -  | -         | - | -  | -          |
| BCC2074 | 2 | 10 | S-<br>MS  | 2 | 3  | MS        | - | 25 | S-<br>MS        | 2 | 10 | S-<br>MS  | - | 20 | S        | 1 | T  | MS-<br>MR | 1 | T  | -MR-<br>MS |
| BCC2083 | 1 | T  | MS        | - | 10 | MS        | 2 | 5  | MR-<br>MS-<br>R | 2 | 5  | MS        | 2 | 5  | MS       | 2 | 5  | MS-<br>S  | 1 | 1  | MR         |
| BCC2084 | 1 | 1  | MS        | 2 | 5  | MS        | 2 | 15 | MS-<br>S        | 1 | 1  | MR        | - | 20 | S-<br>MS | - | 15 | MS-<br>S  | 2 | 3  | MS-<br>S   |
| BCC2091 | - | 35 | MS        | 2 | 7  | S-<br>MS  | 2 | 5  | MR-<br>MS-<br>S | 2 | 3  | MS        | - | 15 | S-<br>MS | 2 | 5  | S-<br>MS  | - | 10 | MS         |
| BCC2092 | 1 | 3  | MS        | 2 | 1  | MR-<br>MS | 2 | 15 | MS              | 2 | 7  | MS-<br>S  | - | 15 | S-<br>MS | 2 | 3  | MS        | 2 | 3  | MS         |
| BCC2093 | 1 | 1  | MR        | 2 | 1  | MS        | 2 | 5  | MR-<br>MS-<br>S | - | -  | -         | - | 10 | MS       | 1 | T  | R-<br>MR  | 1 | T  | R          |
| BCC2118 | 2 | 7  | MS        | - | 10 | MS        | 2 | 10 | S-<br>MS        | 1 | 1  | MS        | - | 45 | S        | 2 | 5  | S         | 1 | 1  | MR         |
| BCC2142 | 1 | T  | R         | 2 | 7  | MS        | - | 30 | S-<br>MS        | 2 | 3  | MR        | - | 20 | S        | - | 35 | S-<br>MS  | 1 | 1  | MS         |
| BCC2144 | - | 15 | MS        | - | 10 | MS        | - | 45 | S               | 1 | 1  | S         | - | 20 | S-<br>MS | - | -  | -         | - | -  | -          |
| BCC2152 | 2 | 7  | MS        | 2 | 3  | MR        | 2 | 5  | MR-<br>MS       | 1 | 1  | MS        | - | 50 | S        | - | -  | -         | - | -  | -          |
| BCC2162 | 1 | 3  | MR-<br>MS | 2 | 5  | MS        | 2 | 5  | MS-<br>S-<br>MR | 1 | 1  | MR-<br>MS | - | 15 | S-<br>MS | 2 | 5  | MR-<br>MS | 1 | 1  | MR-<br>R   |
| BCC2168 | 1 | T  | R         | 1 | T  | MR        | - | 35 | S               | 1 | T  | MR        | 2 | 5  | S        | 2 | 3  | S-<br>MS  | - | 35 | S-<br>MS   |
| BCC2233 | 2 | 5  | MS-<br>MR | 2 | 5  | MR-<br>MS | 2 | 5  | S               | 2 | 5  | MS        | - | 10 | S        | 1 | T  | MR-<br>MS | 1 | 1  | MS-<br>S   |
| BCC2236 | 1 | T  | MR        | 1 | T  | MS        | 2 | 15 | MS              | 1 | T  | MS        | - | 25 | S-<br>MS | 1 | T  | MR        | 2 | 3  | MS-<br>MR  |

|         |   |    |      |   |    |    |   |    |         |   |    |       |   |    |       |   |    |       |   |    |      |
|---------|---|----|------|---|----|----|---|----|---------|---|----|-------|---|----|-------|---|----|-------|---|----|------|
| BCC2241 | 2 | 5  | S    | 2 | 7  | MS | - | 30 | MS      | 2 | 5  | MR-MS | - | 20 | S-MS  | 2 | 3  | MS-MR | 1 | T  | MR   |
| BCC2242 | 2 | 10 | MS   | - | 10 | S  | - | 25 | MS      | - | 15 | S     | - | 45 | S-MS  | 2 | 10 | S-MS  | 1 | T  | R-MR |
| BCC2254 | 1 | T  | R    | 2 | 3  | MS | - | 25 | S-MS    | 1 | T  | R-MR  | 1 | 3  | MR    | 1 | T  | R     | 1 | T  | S    |
| BCC2259 | - | -  | -    | 2 | 1  | MS | - | -  | -       | 1 | T  | MS    | 2 | 5  | MS    | - | 15 | S     | 2 | 3  | MS   |
| BCC2265 | 2 | 5  | S-MS | - | 10 | MS | 2 | 5  | S-MS    | 1 | T  | R-MR  | - | 15 | MS    | 2 | 5  | MR    | - | 20 | S    |
| BCC2291 | 2 | 10 | MS   | - | 10 | MS | 2 | 20 | MS      | 1 | 1  | MS    | - | 15 | MS-S  | 2 | 5  | MR-MS | - | 10 | S    |
| BCC2297 | 1 | T  | R    | 1 | T  | R  | 1 | 1  | R-MR-MS | 1 | T  | MR-R  | 2 | 5  | MR-MS | 1 | T  | R     | 1 | T  | R    |
| BCC2336 | 1 | T  | MR   | - | 10 | MS | 2 | 20 | S       | 1 | T  | MR    | - | 15 | MS-   | 1 | 1  | MR-R  | 1 | T  | R    |
| BCC2342 | 2 | 5  | S-MS | 2 | 5  | MS | 2 | 10 | MS-MR-S | 2 | 3  | MS-MR | - | 30 | S-MS  | 2 | 5  | MS    | 1 | T  | R-MR |
| BCC2345 | 1 | T  | R    | 1 | T  | MR | 1 | T  | R-MR-MS | 1 | 1  | MS-MR | - | 50 | S     | 2 | 3  | MS-MR | 1 | T  | R-MR |
| BCC2352 | 2 | 10 | MS   | 2 | 1  | MR | 2 | 20 | MS      | 2 | 3  | S-MS  | 1 | 3  | MS-MR | 2 | 3  | MS-MR | 1 | 1  | MS   |
| BCC2362 | 2 | 10 | MS   | 2 | 3  | MR | 2 | 20 | MS-S    | 1 | 1  | MS-MR | - | 10 | S-MS  | 1 | T  | R-MR  | 1 | T  | R    |
| BCC2371 | 2 | 7  | MS   | 2 | 5  | MR | - | 35 | S-MS    | 2 | 7  | MS    | - | 25 | S-MS  | 2 | 3  | MR-MS | - | 15 | MS   |
| BCC2373 | 2 | 10 | MS   | 1 | T  | R  | - | 30 | MS      | 2 | 3  | S-MS  | - | 40 | S-MS  | 1 | T  | MR-R  | 2 | 5  | MS   |
| BCC2378 | 1 | 1  | MR   | 2 | 1  | MR | - | 35 | MS      | 2 | 3  | MS    | - | 35 | S     | 1 | T  | MR    | 1 | 1  | MS   |
| BCC2380 | 2 | 5  | MS   | 2 | 3  | MS | - | 30 | S       | - | 25 | S     | - | 35 | S     | 1 | T  | MR    | 1 | T  | R    |
| BCC2381 | 1 | T  | MR   | 2 | 3  | MS | 2 | 15 | S-MS    | 2 | 3  | S-MS  | - | 35 | MS-S  | - | 15 | MS    | 1 | 1  | S    |

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|---------|---|----|-------|---|----|-------|---|----|---------|---|----|-------|---|----|-------|---|----|-------|---|----|-------|
| BCC2382 | 2 | 5  | MS    | 1 | T  | MS    | 2 | 15 | MS-S    | 1 | T  | MR-R  | 2 | 5  | S     | 2 | 5  | MS-MR | 2 | 5  | MS    |
| BCC2384 | 1 | T  | R     | 1 | T  | MR    | 2 | 5  | MR-MS-R | 2 | 7  | S-MS  | - | 10 | MS-S  | 1 | T  | R     | 1 | T  | R-MR  |
| BCC2397 | 1 | 3  | MR    | 2 | 1  | MS-MR | - | 35 | S-MS    | 2 | 10 | MS-S  | - | 20 | MS-S  | 1 | 1  | MS-MR | - | 10 | MR-MS |
| BCC2399 | 2 | 7  | S-MS  | - | 20 | MS    | 2 | 5  | MS-S-MR | 1 | 1  | MS    | - | 15 | MS    | 1 | T  | R-MR  | 1 | T  | R     |
| BCC2400 | 1 | 3  | MS    | 2 | 3  | MS    | 2 | 5  | MS-MR-S | 2 | 7  | MS-S  | - | 10 | MS    | - | 35 | S-MS  | 1 | 1  | MR    |
| BCC2401 | - | 30 | MS    | - | 15 | MS    | - | 40 | S-MS    | - | 15 | MS    | - | 10 | S-MS  | 1 | 1  | MR-MS | 1 | T  | MR-R  |
| BCC2408 | - | 15 | MS-S  | - | 30 | S     | 2 | 20 | S-MS    | 2 | 10 | MS    | - | 15 | MR-MS | 2 | 5  | MR    | 1 | T  | MR    |
| BCC2413 | 1 | T  | R     | 1 | T  | R     | 2 | 5  | MS-MR   | 2 | 7  | S     | - | 20 | S     | 2 | 5  | S     | 2 | 5  | MS    |
| BCC2414 | 1 | T  | R     | - | 15 | S     | - | 25 | S-MS    | 2 | 10 | MR    | - | 10 | S     | 2 | 5  | S-MS  | 2 | 3  | MR    |
| BCC2415 | 1 | T  | R     | 1 | T  | R     | 2 | 10 | MS-MR   | 1 | T  | MS    | - | 25 | S     | - | 35 | MS    | 1 | 1  | MR-MS |
| BCC2423 | 1 | 3  | MR-MS | - | 10 | MS    | 2 | 15 | MS      | 2 | 5  | S     | - | 15 | S     | 2 | 3  | R-MR  | 1 | 1  | MR    |
| BCC2424 | 1 | T  | MR    | - | 10 | MS    | 1 | 1  | MR-R-MS | 2 | 3  | MS-MR | - | 20 | MS-S  | 2 | 5  | MR-MS | 2 | 5  | M     |
| BCC2430 | 1 | T  | R     | 1 | T  | R     | - | 35 | S-MS    | 2 | 7  | S     | - | 45 | S     | 2 | 3  | MR-MS | - | 15 | S-MS  |
| BCC2431 | 1 | 1  | MR    | 1 | T  | R     | 1 | 3  | MS-S-R  | 1 | 1  | MR-MS | - | 10 | MS    | - | 15 | S-MS  | - | 15 | MS    |
| BCC2462 | 1 | T  | R     | 2 | 1  | MR    | 1 | 3  | MS-MR-R | 2 | 10 | S-MS  | - | 35 | S     | 1 | 1  | MS-MR | 2 | 7  | S-MS  |
| BCC2505 | 1 | T  | R     | 1 | T  | MR    | - | 35 | S-MS    | 2 | 3  | MS    | - | 75 | S     | 2 | 5  | S     | 1 | 1  | MR-MS |

|         |   |   |    |   |    |       |   |    |         |   |    |       |   |    |       |   |    |       |   |    |       |
|---------|---|---|----|---|----|-------|---|----|---------|---|----|-------|---|----|-------|---|----|-------|---|----|-------|
| BCC2527 | 1 | T | R  | - | 15 | MS    | - | 40 | S-MS    | 2 | 5  | S     | - | 15 | S-MS  | 1 | T  | R-MR  | 1 | 1  | R     |
| BCC2530 | 1 | T | MR | 2 | 5  | MS    | 2 | 15 | S-MS    | 2 | 3  | S     | - | 20 | S-MS  | 1 | T  | MR    | 2 | 5  | S-MS  |
| BCC2533 | 1 | T | R  | 1 | T  | R     | 2 | 10 | MS      | 1 | 1  | MR-R  | 2 | 7  | S-MS  | 1 | T  | MR    | 1 | T  | R     |
| BCC2536 | 1 | 1 | MR | 2 | 5  | MS    | 2 | 15 | MS      | 1 | 1  | MS-MR | - | 30 | S-MS  | 2 | 10 | S-MS  | - | 30 | S     |
| BCC2537 | 1 | 1 | MR | 2 | 1  | MR    | 2 | 10 | MS-S    | - | 15 | S-MS  | - | 10 | MR-MS | 1 | 1  | R-MR  | - | 30 | MS-S  |
| BCC2544 | 1 | T | R  | 1 | T  | MR    | 2 | 10 | MS-S    | 2 | 10 | S     | - | 45 | S     | 2 | 3  | MR-MS | - | 25 | MS    |
| BCC2545 | 1 | T | MR | 2 | 1  | MS-MR | 2 | 15 | MS-MR-S | 1 | 1  | MS    | - | 35 | S-MS  | 2 | 2  | MR    | 2 | 5  | MR-MS |
| BCC2548 | 1 | T | MR | 1 | T  | R     | 2 | 15 | S-MS    | 2 | 5  | MS-MR | - | 10 | MS-S  | 2 | 3  | MR    | 1 | T  | R-MR  |
| BCC2549 | 1 | T | R  | 1 | T  | R     | 2 | 5  | MS-MR-R | 1 | 1  | MR-MS | - | 35 | S-MS  | 1 | 1  | MR-R  | 1 | T  | MR    |
| BCC2552 | 1 | T | R  | 1 | T  | R     | 2 | 5  | MS      | 2 | 3  | MS-MR | - | 70 | S-MS  | 1 | T  | MR-MS | 1 | T  | R     |
| BCC2553 | 1 | T | R  | 2 | 1  | MR    | 2 | 5  | MS-MR-S | - | 15 | S     | - | 40 | S-MS  | 2 | 3  | MR    | 1 | T  | MR    |
| BCC2555 | 1 | T | R  | 1 | T  | MR    | 2 | 5  | MS-MR-S | 2 | 3  | MS    | - | 35 | S-MS  | 1 | 1  | MR    | - | 35 | MS    |
| BCC2556 | 1 | T | R  | 1 | T  | R     | 2 | 5  | MS-MR   | - | 15 | S     | - | 15 | S     | 2 | 10 | S     | 1 | T  | MS    |
| BCC2557 | 1 | T | R  | 1 | T  | R     | 2 | 10 | S       | 2 | 5  | S     | - | 65 | S     | 2 | 5  | S-MS  | 1 | 1  | MS    |
| BCC2558 | 1 | T | R  | 2 | 1  | R     | 2 | 10 | MS-MR   | 2 | 7  | S     | - | 40 | S-MS  | 1 | 1  | MR    | 1 | T  | R     |
| BCC2559 | 1 | T | MR | 2 | 5  | MS    | 1 | 1  | MS-MR-S | 1 | 1  | MR-MS | - | 15 | S-MS  | 1 | T  | R     | 1 | T  | R-MR  |



|         |   |   |   |   |   |    |   |    |                 |   |    |           |   |    |          |   |   |            |   |   |           |
|---------|---|---|---|---|---|----|---|----|-----------------|---|----|-----------|---|----|----------|---|---|------------|---|---|-----------|
| BCC2560 | 1 | T | R | 1 | T | R  | 2 | 7  | MS-<br>S        | 2 | 5  | MS        | - | 15 | S-<br>MS | 1 | T | R-<br>MR   | 1 | T | MS-<br>MR |
| BCC2561 | 1 | T | R | 1 | T | R  | - | 35 | S-<br>MS        | - | 15 | MS        | - | 60 | S        | 2 | 5 | MR-<br>-MS | 2 | 3 | MS        |
| BCC2562 | 1 | T | R | 1 | T | MR | 2 | 5  | MS-<br>MR-<br>S | 2 | 3  | MR-<br>MS | - | 35 | S-<br>MS | 1 | 1 | MR<br>-R   | 1 | 1 | S         |

<sup>a</sup> Nurseries where accessions were evaluated. Abbreviations are as follows in St. Paul, MN and Njoro, Kenya: StP13\_QC = dataset resulting from adult plant evaluations to race QCCJB in St. Paul in 2013; StP14\_QC = dataset resulting from adult plant evaluations to race HKHJC in St. Paul in 2014; StP15\_QC = dataset resulting from adult plant evaluations to race QCCJB in St. Paul in 2015; StP15\_MC = dataset resulting from adult plant evaluations to race MCCFC in St. Paul in 2015; StP15\_HK = dataset resulting from adult plant evaluations to race HKHJC in St. Paul in 2015; KEN14m = dataset resulting from adult plant evaluations to *Pgt* TTKSK composite in Njoro in 2014 main-season; KEN14o = dataset resulting from adult plant evaluations to *Pgt* TTKSK composite in Njoro in 2014 off-season.

<sup>b</sup> Sequential number given to all accessions from the Barley iCore Collection (BCC), see Appendix A2 or Online Resource 2.1 for more information.

<sup>c</sup> Stem rust reaction classes according to table 3.3 where “1” is highly resistant (Class 1), and “2” resistant (Class 2).

<sup>d</sup> Disease severity (DS) estimated visually on the stem and leaf sheath tissue of accessions on 0-100% scale, where T designates Trace: DS values less than 1%.

<sup>e</sup> Infection responses (IRs) are based on size and type of uredinia observed where R = Resistant, MR = Moderately Resistant, MS = Moderately Susceptible, and S = Susceptible.

**Table 3.9.** Barley iCore Collection Selects (BCCS) exhibiting the highest levels of adult plant stem rust resistance over multiple years and races.

| Name <sup>b</sup> | GRIN Name <sup>c</sup> | County <sup>c</sup> | HKHJC <sup>a</sup><br>StP15_HK |                 | MCCFC<br>StP15_MC |       | StP13_QC |         | QCCFC<br>StP14_QC |       | StP15_QC |       | <i>Pgt</i> TTKSK composite |       |        |       |
|-------------------|------------------------|---------------------|--------------------------------|-----------------|-------------------|-------|----------|---------|-------------------|-------|----------|-------|----------------------------|-------|--------|-------|
|                   |                        |                     | DS <sup>d</sup>                | IR <sup>e</sup> | DS                | IR    | DS       | IR      | DS                | IR    | DS       | IR    | DS                         | IR    | KEN14o |       |
| BCC1377           | PI 328976              | Ethiopia            | 5                              | S-MS            | 1                 | MS    | 2        | MR-MS-R | 1                 | MR-MS | 3        | MR    | T                          | MR-MS | 1      | MR-MS |
| BCC1745           | PI 402354              | Colombia            | T                              | MR              | T                 | MR    | 1        | R-MR-MS | 1                 | MR-MS | 1        | MR    | 5                          | MR-R  | T      | R     |
| BCC2297           | PI 584766              | Australia           | T                              | R               | T                 | R     | 1        | R-MR-MS | T                 | MR-R  | 5        | MR-MS | T                          | R     | T      | R     |
| BCC2533           | PI 643232              | United States       | T                              | R               | T                 | R     | 10       | MS      | 1                 | MR-R  | 7        | S-MS  | T                          | R-MR  | T      | R     |
| BCC1928           | PI 449279              | Spain               | T                              | MR              | 7                 | MS    | T        | MR-R-MS | T                 | R     | 5        | MR-MS | 1                          | MR-R  | T      | R-MR  |
| BCC0169           | CIho 7492              | Mexico              | 3                              | MS              | T                 | MS    | 5        | MS-MR   | 5                 | MS    | 3        | MR-MS | 1                          | MR-MS | T      | R     |
| BCC0131           | CIho 6371              | United States       | 1                              | MR              | 3                 | MR-MS | T        | R-MS    | T                 | R-MR  | 5        | MR    | 1                          | R     | 1      | R-MR  |
| BCC1724           | PI 401973              | Colombia            | 1                              | MR              | T                 | MR    | 10       | MS      | 3                 | S-MS  | 5        | S-MS  | T                          | MS-MR | T      | S     |
| BCC0334           | CIho 15203             | United States       | T                              | MR              | 3                 | MS    | 15       | MS      | 5                 | MS    | 5        | MS    | T                          | MS    | T      | MS    |
| BCC1556           | PI 369732              | Ukraine             | T                              | MR              | 3                 | R-MR  | 5        | MR-R-MS | 1                 | MR-R  | 3        | MR    | 1                          | R     | 5      | R-MR  |
| BCC0908           | PI 237571              | Colombia            | T                              | R               | T                 | R     | 5        | MS-S    | 1                 | MR-MS | 3        | MS-MR | 5                          | S-MS  | 5      | R-MR  |

|         |           |              |   |    |   |    |    |      |   |      |   |   |   |       |   |    |
|---------|-----------|--------------|---|----|---|----|----|------|---|------|---|---|---|-------|---|----|
| BCC2382 | PI 606305 | Saudi Arabia | 5 | MS | T | MS | 15 | MS-S | T | MR-R | 5 | S | 5 | MS-MR | 5 | MS |
|---------|-----------|--------------|---|----|---|----|----|------|---|------|---|---|---|-------|---|----|

<sup>a</sup> Nurseries where accession were evaluated. Abbreviations as in St. Paul, MN and Njoro, Kenya: StP13\_QC = dataset resulting from adult plant evaluations to race QCCJB in St. Paul in 2013; StP14\_QC = dataset resulting from adult plant evaluations to race HKHJC in St. Paul in 2014; StP15\_QC = dataset resulting from adult plant evaluations to race QCCJB in St. Paul in 2015; StP15\_MC = dataset resulting from adult plant evaluations to race MCCFC in St. Paul in 2015; StP15\_HK = dataset resulting from adult plant evaluations to race HKHJC in St. Paul in 2015; KEN14m = dataset resulting from adult plant evaluations to *Pgt* TTKSK composite in Njoro in 2014 main-season; KEN14o = dataset resulting from adult plant evaluations to *Pgt* TTKSK composite in Njoro in 2014 off-season

<sup>b</sup> Sequential number given to all accessions from the Barley iCore Collection (BCC), see Appendix A2 or Online Resource 2.1 for more information.

<sup>c</sup> Accession name and passport and improvement status as reported by the United States Department of Agriculture Germplasm Resources Information Network

<sup>e</sup> Disease severity (DS) estimated visually on stem tissue as 0-100%, where T designates Trace: DS values less than 1%.

<sup>f</sup> Infection response (IR) based on size and type of uredinia pustules, scored as R (resistant), MR (moderately resistant) , MS (moderately susceptible), and S (susceptible).

**Table 3.10.** Seedling reactions of Barley iCore Collection Selects (BCCS) to *Puccinia graminis* f. sp. *tritici* races TTKSK, TTKST, TKTTF, TRTTF, QCCJB, HKHJC, and MCCFC and also *Puccinia graminis* f. sp. *secalis* isolate 92-MN-90.

| Name <sup>a</sup> | GRIN Name <sup>b</sup> | Resistance spectrum <sup>c</sup>  | TTKSK <sup>d</sup> | TTKST             | TKTTF                | TRTTF               | QCCJB             | HKHJC            | MCCFC          | 92-MN-90    |
|-------------------|------------------------|---|--------------------|-------------------|----------------------|---------------------|-------------------|------------------|----------------|-------------|
| BCC2297           | PI 584766              | TTKSK<br>TTKST<br>TKTTF<br>TRTTF<br>QCCJB<br>HKHJC<br>MCCFC<br>92-MN-90 | 2<br>(0;2,1,3-)    | 3<br>(0;1/2,1,3-) | 3<br>(0;1/1,0;2)     | 3<br>(0;1/1,0;2)    | 2<br>(0;1-/1,0;)  | 3<br>(0;1,2)     | 2<br>(0;1,0;)  | 1<br>(0;0;) |
| BCC1600           | PI 371377              | TTKSK<br>TTKST<br>TKTTF<br>TRTTF<br>QCCJB<br>MCCFC<br>92-MN-90          | 2<br>(0;1,c)       | 3<br>(0;1,2-/1,c) | 4<br>(0;1/2,1,3-,0;) | 2<br>(0;1/0;1,2-)   | 4<br>(1,0;2/2,1)  | 8<br>(2/3)       | 5<br>(0;2,3-)  | 1<br>(0;0;) |
| BCC1602           | PI 371390              | TTKSK<br>TTKST<br>TKTTF<br>TRTTF<br>QCCJB<br>MCCFC<br>92-MN-90          | 2<br>(0;1,0;)      | 3<br>(0;1--/1,0;) | 4<br>(0;1/1,2,0;)    | 3<br>(0;1/1,0;2)    | 5<br>(0;1/2,3-)   | 8<br>(2,3-/3-,2) | 3<br>(0;1/2,1) | 1<br>(0;0;) |
| BCC1603           | PI 371392              | TTKSK<br>TTKST<br>TKTTF<br>TRTTF<br>QCCJB<br>MCCFC<br>92-MN-90          | 1<br>(0;0;1)       | 6<br>(0;1/3-)     | 2<br>(0;0;1,2)       | 3<br>(0;1/1,0;2,3-) | 3<br>(1,0;2,1,3-) | 9<br>(2,3-/3)    | 2<br>(0;1,0;)  | 1<br>(0;0;) |

|         |           |   |                    |                     |                       |                          |                   |                  |                   |                      |
|---------|-----------|---|--------------------|---------------------|-----------------------|--------------------------|-------------------|------------------|-------------------|----------------------|
| BCC0524 | PI 76285  | TTKSK<br>TTKST<br>TKTTF<br>QCCJB<br>MCCFC<br>92-MN-90 | 6<br>(1,0;/3-,2,c) | 5<br>(0;:,1,c/2,1)  | 6<br>(1,2/2,3-)       | 9<br>(2,3-,c/3-,2)       | 3<br>(0;:,1/1,2)  | 8<br>(2,3-/3-,2) | 7<br>(0;:,1/2)    | 1<br>(0;/0;)         |
| BCC1568 | PI 370852 | TTKSK<br>TTKST<br>TKTTF<br>TRTTF<br>QCCJB<br>92-MN-90 | 3<br>(0;/2,1,3-)   | 4<br>(0;:,1-/2,1,c) | 4<br>(0;:,1/2,1,c)    | 4<br>(0;:,1/2,1,c)       | 6<br>(2,1/2,1,3-) | 13<br>(3,3-/3)   | 9<br>(0;:,1/3,3-) | 1<br>(0;/0;)         |
| BCC1822 | PI 422230 | TKTTF<br>TRTTF<br>HKHJC<br>MCCFC<br>92-MN-90          | 8<br>(2,1/3,3-)    | 8<br>(2,1,3-/3,3-)  | 6<br>(1,0;:,2/2,3-,1) | 6<br>(1,2-/2,1,0;)       | 9<br>(2,3-/3-)    | 1<br>(0;/0;)     | 3<br>(0;:,1/1,0;) | 6<br>(0;:,1/3-)      |
| BCC2431 | PI 640006 | TTKSK<br>TKTTF<br>TRTTF<br>HKHJC<br>MCCFC             | 7<br>(0;/3-,2)     | 9<br>(2,1,3-/3,3-)  | 5<br>(0;:,1,2/2,3-)   | 3<br>(0;:,1,2-/1,0;,-2-) | 9<br>(2,1/3,3-)   | 3<br>(0;:,1/1)   | 2<br>(0;/0;:,1)   | 8<br>(2,3-/2,3-)     |
| BCC1103 | PI 289811 | TTKSK<br>TKTTF<br>TRTTF<br>QCCJB<br>92-MN-90          | 5<br>(0;:,1/3)     | 10<br>(2,1,3-/3)    | 4<br>(1,0;:,2/1,2,0;) | 6<br>(1,2/3)             | 6<br>(1,2/2,3-)   | 13<br>(3-/3)     | 8<br>(0;:,1/2,3-) | 2<br>(0;:,1/0;:,1)   |
| BCC2544 | PI 643288 | TTKSK<br>TRTTF<br>HKHJC<br>MCCFC<br>92-MN-90          | 6<br>(0;/3,3+)     | 12<br>(2,1,0;/3)    | 12<br>(3,3-,2/3,3-)   | 6<br>(2,1,3-/2,3-,1)     | 12<br>(3-/3)      | 6<br>(2,1/2)     | 5<br>(1,0;/2)     | 5<br>(1,2,3-/1,2,3-) |
| BCC2560 | PI 643376 | TTKSK<br>TTKST<br>HKHJC<br>MCCFC<br>92-MN-90          | 5<br>(0;:,1/3-)    | 3<br>(0;:,1-/2,3-)  | 9<br>(2,3-,1/3,3-)    | 8<br>(2,1/3,3-,2)        | 13<br>(3/3)       | 7<br>(1,2/3-,2)  | 7<br>(1,0;/3)     | 4<br>(1,0;:,2/1,2)   |

|         |              |                                     |                     |                      |                        |                        |                     |                  |                   |                      |
|---------|--------------|-------------------------------------|---------------------|----------------------|------------------------|------------------------|---------------------|------------------|-------------------|----------------------|
| BCC1209 | PI<br>320219 | TRTTF<br>HKHJC<br>MCCFC<br>92-MN-90 | 8<br>(2,1/3+)       | 12<br>(2,3-,1/3+)    | 12<br>(3,3-,2/3,3-)    | 5<br>(1/3)             | 8<br>(2,1,3-/3-)    | 3<br>(0;/1,0;)   | 5<br>(1,0;/2,1)   | 6<br>(2,1,3-/2,1,3-) |
| BCC0174 | Clho<br>7556 | TRTTF<br>QCCJB<br>HKHJC<br>92-MN-90 | 12<br>(2,3-,c/3,3+) | 13<br>(3/3)          | 12<br>(3,3-,2/3,3-)    | 5<br>(1,2,0;3-/1,2,3-) | 6<br>(1,2/3,3-)     | 7<br>(2,1,3-/3-) | 9<br>(2,3-/3-,2)  | 5<br>(1,2/1,2)       |
| BCC1851 | PI<br>429312 | TTKSK<br>TKTTF<br>TRTTF<br>92-MN-90 | 5<br>(0;/3-)        | 8<br>(2,1,3-/3-)     | 5<br>(1,2,0;/1,2,3-,c) | 4<br>(0;1,2/2,1)       | 9<br>(1,0;/3-)      | 8<br>(2/2,3-)    | 8<br>(1,2/2,3-)   | 6<br>(2,1/2,1)       |
| BCC1743 | PI<br>402335 | TTKSK<br>TRTTF<br>HKHJC<br>MCCFC    | 7<br>(0;1/3,3-)     | 10<br>(3-,2/3-)      | 12<br>(3-/3)           | 6<br>(0;1,2-/2)        | 8<br>(1,2,3-/3-,2)  | 5<br>(1,0;/1,2)  | 3<br>(0;1/1,0;)   | 9<br>(2,3-/3-,2)     |
| BCC2065 | PI<br>483048 | TTKSK<br>TTKST<br>HKHJC<br>92-MN-90 | 6<br>(1/2,3-)       | 7<br>(2-,1,c/2,3-)   | 8<br>(2,3-,c/2,3-,c)   | 10<br>(2,3-,c/3,3-)    | 9<br>(2,1,c/3-,2,c) | 7<br>(2,1/2,c)   | 11<br>(3-,2/3)    | 3<br>(1,0;2/1,0;2)   |
| BCC2382 | PI<br>606305 | TTKSK<br>TTKST<br>QCCJB<br>92-MN-90 | 6<br>(0;1/2,1,3-)   | 7<br>(2,1,0;/2,3-,1) | 9<br>(2,1,3-/3,3-,c)   | 11<br>(2,3-,1/3)       | 7<br>(2/2,c)        | 8<br>(2/3,3-)    | 10<br>(3-,2/3)    | 1<br>(0;/0;)         |
| BCC2536 | PI<br>643247 | TTKSK<br>TTKST<br>TRTTF<br>92-MN-90 | 5<br>(0;1/3-,3)     | 6<br>(0;1,2/2,1,3-)  | 8<br>(2,1,3-/3,3-)     | 7<br>(2,1,0;/2,3-)     | 8<br>(2,3-/3-)      | 8<br>(2/3-,2)    | 10<br>(2,3-/3,3-) | 5<br>(1,2/1,2)       |
| BCC1917 | PI<br>447191 | TTKST<br>TKTTF<br>TRTTF<br>92-MN-90 | 9<br>(0;1/3)        | 6<br>(1,2,n/2,cn)    | 6<br>(1,2,n/2,1,n)     | 5<br>(1,n/1,2,n)       | 13<br>(3/3)         | 13<br>(3-/3)     | 10<br>(3-,2/3)    | 6<br>(1,2/2,1)       |
| BCC2233 | PI<br>566497 | TTKST<br>TRTTF<br>HKHJC<br>92-MN-90 | 8<br>(0;1/3-,3)     | 7<br>(2,1/2,3-)      | 9<br>(2,3-,1/3-,2)     | 5<br>(1,0;2/2,1,3-)    | 13<br>(3,3-/3)      | 6<br>(1,2/2,3-)  | 12<br>(3-/3)      | 6<br>(2,1/2,1)       |

|         |               |                                     |                     |                     |                     |                         |                  |                  |                 |                      |
|---------|---------------|-------------------------------------|---------------------|---------------------|---------------------|-------------------------|------------------|------------------|-----------------|----------------------|
| BCC0991 | PI<br>265462  | TTKST<br>TRTTF<br>QCCJB<br>92-MN-90 | 8<br>(1,2,3-/2,3,c) | 4<br>(1,0;2/1,2)    | 8<br>(2,1/3,3-)     | 6<br>(2,1/2,3-)         | 7<br>(2,1/3-)    | 9<br>(2/3-,2)    | 11<br>(3-,2/3)  | 6<br>(2,1/2,1)       |
| BCC2384 | PI<br>608667  | HKHJC<br>MCCFC<br>92-MN-90          | 9<br>(2,3-/3)       | 13<br>(3,3-/3)      | 13<br>(3,3-,2/3)    | 8<br>(2,3-,1/2,3-)      | 9<br>(2,3-/3-,2) | 4<br>(0;/1,2)    | 3<br>(0;/1,0;)  | 6<br>(2,1/2,1)       |
| BCC2413 | PI<br>620640  | HKHJC<br>MCCFC<br>92-MN-90          | 9<br>(0;1/3)        | 11<br>(3-,2/3)      | 13<br>(3-,2,3/3)    | 9<br>(2-,1/3,3-)        | 13<br>(3/3)      | 4<br>(1,0;/2,3-) | 3<br>(0;1/2)    | 5<br>(1,2,3-/1,2,3-) |
| BCC2254 | PI<br>573662  | QCCJB<br>HKHJC<br>92-MN-90          | 10<br>(1/3)         | 10<br>(3-/3-)       | 9<br>(2,3-/3-,2,1)  | 10<br>(2,3-/3,3-)       | 6<br>(1,0;/2)    | 7<br>(1,2/3-,2)  | 13<br>(3/3)     | 6<br>(2,1,3-/2,1,3-) |
| BCC0750 | PI<br>182661  | QCCJB<br>HKHJC<br>92-MN-90          | 10<br>(2,1/3)       | 12<br>(3,3-/3,3-)   | 11<br>(2,3-/3)      | 13<br>(3,3-,2/3)        | 6<br>(2,1/3-,2)  | 4<br>(1/3-)      | 9<br>(2,3-/3-)  | 6<br>(2,1/2,1)       |
| BCC2026 | PI<br>467836  | TKTTF<br>TRTTF<br>92-MN-90          | 11<br>(1,0;/3)      | 10<br>(1,2,3-/3,3-) | 5<br>(0;1,2/2,3-)   | 7<br>(2,1,3-/2,3-)      | 11<br>(2,3-/3)   | 11<br>(2,3-/3)   | 11<br>(3-,2/3)  | 5<br>(1,2/1,2)       |
| BCC1720 | PI<br>401952  | TRTTF<br>HKHJC<br>MCCFC             | 9<br>(1,0;/3)       | 11<br>(2,3-,1/3)    | 12<br>(3,3-,2/3,3-) | 6<br>(0;1,2/2,1,3-)     | 10<br>(3-,2/3-)  | 1<br>(0;/0;)     | 2<br>(0;1/1,0;) | 9<br>(2,3-/3-,2)     |
| BCC1797 | PI<br>415014  | TRTTF<br>HKHJC<br>MCCFC             | 10<br>(2/3)         | 12<br>(3-/3)        | 10<br>(2,3-/3,3-)   | 5<br>(1,2-,n/1,2)       | 8<br>(2/3-,2,1)  | 2<br>(0;1/1,0;)  | 2<br>(0;/0;1)   | 12<br>(3,3-/3,3-)    |
| BCC0244 | Clho<br>11864 | TRTTF<br>HKHJC<br>MCCFC             | 8<br>(0;1/3)        | 9<br>(2,3-,1/3-)    | 11<br>(2,3-/3)      | 6<br>(2,1,3-/2,1,3-,0;) | 9<br>(2,3-/3-)   | 2<br>(0;/1,0;)   | 3<br>(0;/1,0;)  | 12<br>(3,3-/3,3-)    |
| BCC2527 | PI<br>643212  | TRTTF<br>HKHJC<br>MCCFC             | 12<br>(2,3-/3,3+)   | 13<br>(3,3-/3)      | 11<br>(3-/3,3-,2)   | 7<br>(2,1/2,3-,1)       | 12<br>(3-/3)     | 3<br>(0;/1)      | 4<br>(1,0;/1,2) | 9<br>(2,3-/3-,2)     |
| BCC2537 | PI<br>643257  | TRTTF<br>HKHJC<br>MCCFC             | 9<br>(1,0;2/3)      | 12<br>(2,1,3-/3)    | 13<br>(3,3-/3)      | 3<br>(1,0;2/1,0;2)      | 11<br>(3-,2/3)   | 3<br>(0;1/1,2)   | 6<br>(1,2/2,1)  | 8<br>(2,3-,1/2,3-,1) |
| BCC2548 | PI<br>643310  | TRTTF<br>HKHJC<br>MCCFC             | 9<br>(0;/3,3-)      | 8<br>(0;1-/3-,2)    | 12<br>(0;1,2/3)     | 4<br>(0;1/1,2)          | 12<br>(3-/3)     | 5<br>(1,0;/2)    | 5<br>(1,0;/2)   | 8<br>(2,3-,1/2,3-,1) |

|         |               |                            |                      |                           |                       |                         |                    |                   |                   |                      |
|---------|---------------|----------------------------|----------------------|---------------------------|-----------------------|-------------------------|--------------------|-------------------|-------------------|----------------------|
| BCC2549 | PI<br>643314  | TRTTF<br>HKHJC<br>MCCFC    | 9<br>(0,;1/3)        | 10<br>(1,2/3,3-,2)        | 13<br>(3,3-,2/3)      | 4<br>(1,0,;2/1,2,3-)    | 12<br>(3-/3)       | 4<br>(1/1)        | 4<br>(1,0;/2,1)   | 9<br>(2,3-/3-,2)     |
| BCC2558 | PI<br>643369  | TRTTF<br>HKHJC<br>MCCFC    | 9<br>(1,2,c/3)       | 10<br>(2,3-/3,3-,2)       | 11<br>(2,3-/3)        | 6<br>(2,1,3-/2,1,3-)    | 11<br>(3-/3-,3)    | 5<br>(1,0;/2)     | 3<br>(0,;1/1,2)   | 8<br>(2,3-/2,3-)     |
| BCC2561 | PI<br>643377  | TRTTF<br>HKHJC<br>MCCFC    | 12<br>(1,0;/3,3+)    | 11<br>(3-,2/3)            | 14<br>(3/3,3+)        | 4<br>(0,;1,2-/2,1,3-)   | 13<br>(3,3-/3)     | 5<br>(1,0,;2/2)   | 6<br>(1,0;/2,3-)  | 9<br>(2,3-/3-,2,1)   |
| BCC0333 | CIho<br>14978 | TRTTF<br>QCCJB<br>92-MN-90 | 8<br>(1,0;/3,3-)     | 10<br>(2/3,3-)            | 9<br>(2,1,3-/3,3-)    | 7<br>(2,1,3-/2,3-)      | 6<br>(2,1/3-)      | 9<br>(3-,2/3-,2)  | 11<br>(3-,2/3,3-) | 6<br>(1,2/2,1)       |
| BCC1665 | PI<br>386838  | TTKSK<br>QCCJB<br>92-MN-90 | 6<br>(0,;1/2,3-)     | 8<br>(2,3-/2,3-)          | 12<br>(3,3-,2/3,3-,2) | 9<br>(2,1,3-/3,3-)      | 7<br>(2,1/3-)      | 10<br>(2,3-/3)    | 9<br>(2,3-/3,3-)  | 6<br>(1,2/2,1)       |
| BCC1592 | PI<br>371248  | TTKSK<br>TKTTF<br>92-MN-90 | 5<br>(0;/3)          | 8<br>(1,0;/3)             | 4<br>(0,;1/2,1,3-)    | 8<br>(2,3-,1/2,3-,1)    | 8<br>(1,0,;2/3-,2) | 13<br>(3,3-/3)    | 8<br>(0,;1/3)     | 1<br>(0;/0,;)        |
| BCC0131 | CIho<br>6371  | TTKSK<br>TRTTF<br>92-MN-90 | 5<br>(0,;1-/3)       | 10<br>(2,3-/3,3-)         | 12<br>(3-/3)          | 6<br>(2,1,0,;3-/2,1,3-) | 11<br>(3-,2/3)     | 9<br>(2,3-/3-)    | 13<br>(3/3)       | 5<br>(1,2/1,2)       |
| BCC1195 | PI<br>308142  | TTKSK<br>TTKST<br>92-MN-90 | 7<br>(1,2/3-)        | 7<br>(2,1/2,3-)           | 11<br>(3-,2/3,3-)     | 9<br>(2/3,3-)           | 9<br>(2,3-/3-)     | 9<br>(2,3-/3-)    | 13<br>(3/3)       | 6<br>(2,1,3-/2,1,3-) |
| BCC2397 | PI<br>611511  | TTKSK<br>TTKST<br>TRTTF    | 5<br>(0,;1,2,n/3,3-) | 5<br>(0,;1,2,3-<br>/2,3-) | 12<br>(2,3-,c/3,3-,c) | 7<br>(1,2,3-/2,3-,1)    | 8<br>(2,1/3-)      | 10<br>(3-,2/3,3-) | 12<br>(3-,2/3)    | 8<br>(2,3-,1/2,3-)   |
| BCC2092 | PI<br>506295  | HKHJC<br>92-MN-90          | 10<br>(0,;1/3)       | 13<br>(3/3)               | 13<br>(3/3)           | 11<br>(3-,2,1/3,3-,2)   | 11<br>(2,3-/3)     | 7<br>(2/2,3-)     | 12<br>(3-/3)      | 5<br>(1,2/1,2,3-)    |
| BCC0308 | CIho<br>14291 | HKHJC<br>92-MN-90          | 10<br>(2,1/3)        | 11<br>(3-,2/3)            | 13<br>(3,3-/3)        | 8<br>(1,2,c/3)          | 9<br>(2,3-/3-)     | 7<br>(2/2,3-)     | 11<br>(2,3-/3)    | 5<br>(1,2/1,2,3-)    |
| BCC1737 | PI<br>402164  | HKHJC<br>MCCFC             | 12<br>(2,3-/3,3+)    | 12<br>(3,3-/3,3-)         | 13<br>(3/3)           | 9<br>(2,3-/3-,2,2-)     | 13<br>(3/3)        | 5<br>(1/2,1)      | 5<br>(0,;1/2,1)   | 9<br>(2,3-/3-,2)     |
| BCC1872 | PI<br>434760  | HKHJC<br>MCCFC             | 10<br>(2,3-/3,3+)    | 14<br>(3/3,3+)            | 13<br>(3,3-/3)        | 11<br>(3-,2/3)          | 10<br>(3-/3-)      | 5<br>(1,2/2,1)    | 5<br>(1,2/1,2)    | 11<br>(3-,2/3,3-)    |
| BCC1875 | PI<br>434814  | HKHJC<br>MCCFC             | 13<br>(3-/3,3+)      | 13<br>(3/3)               | 13<br>(3/3)           | 13<br>(3,3-/3)          | 13<br>(3,3-/3)     | 5<br>(1/2,1)      | 3<br>(1,0;/2,1)   | 13<br>(3/3)          |



|         |               |                   |                     |                    |                       |                         |                   |                    |                  |                      |
|---------|---------------|-------------------|---------------------|--------------------|-----------------------|-------------------------|-------------------|--------------------|------------------|----------------------|
| BCC2415 | PI<br>632274  | HKHJC<br>MCCFC    | 12<br>(0;/3)        | 10<br>(1,2,3-/3)   | 10<br>(2,3-,1/3,3-)   | 8<br>(2,3-,1/2,3-)      | 9<br>(2/3-)       | 4<br>(1,0;/1,2)    | 6<br>(1,0;/2,3-) | 11<br>(3-,2/3,3-)    |
| BCC2430 | PI<br>639999  | HKHJC<br>MCCFC    | 12<br>(2,1/3,3+)    | 13<br>(3,3-/3)     | 13<br>(3,3-,c/3)      | 9<br>(2,1,c/3,3-)       | 9<br>(3-,2/3-,2)  | 3<br>(0,;1/1,2)    | 3<br>(0;/1,2)    | 13<br>(3,3-/3)       |
| BCC2462 | PI<br>640166  | HKHJC<br>MCCFC    | 9<br>(1,0;/3-,2)    | 9<br>(3-,2/3-,2)   | 12<br>(3,3-,2/3,3-)   | 9<br>(2,1,3-/3,3-)      | 9<br>(2/3-)       | 3<br>(1,0;/1,2)    | 6<br>(1,0;/2,3-) | 9<br>(2,3-,1/3-)     |
| BCC2505 | PI<br>640366  | HKHJC<br>MCCFC    | 10<br>(3-,2/3)      | 13<br>(3,3-/3)     | 13<br>(3/3)           | 10<br>(3-,2,c/3-)       | 10<br>(3-/3-)     | 3<br>(1,0;/1)      | 3<br>(1,0;/2,1)  | 10<br>(3-/3-)        |
| BCC2533 | PI<br>643232  | HKHJC<br>MCCFC    | 9<br>(2,1/3)        | 10<br>(2,1,3-/3)   | 13<br>(3,3-/3)        | 9<br>(2,3-/3-,2)        | 12<br>(3-/3)      | 5<br>(1,2/2,1)     | 5<br>(1,0;/2)    | 8<br>(2,3-/2,3-)     |
| BCC2552 | PI<br>643336  | HKHJC<br>MCCFC    | 10<br>(0,;2,1/3,3+) | 11<br>(3-/3,3-)    | 12<br>(1,2,3-/3)      | 9<br>(2,3-,1/3-,2)      | 10<br>(3-,2/3-)   | 3<br>(0,;1/1,2)    | 6<br>(1,0;/2,3-) | 8<br>(2,3-,1/2,3-,1) |
| BCC2553 | PI<br>643339  | HKHJC<br>MCCFC    | 12<br>(3-,2/3,3+)   | 14<br>(3/3,3+)     | 13<br>(3,3-/3,3+)     | 11<br>(3-,2,1/3,3-,2)   | 12<br>(3-/3)      | 5<br>(1,0,;2/2,1)  | 6<br>(0,;1/2)    | 9<br>(3-,2/3-,2)     |
| BCC2556 | PI<br>643364  | HKHJC<br>MCCFC    | 10<br>(3-,2/3)      | 13<br>(3,3-/3)     | 13<br>(3,3-/3,3+)     | 8<br>(1,0,;2/3,3-,2)    | 9<br>(2,3-/3-,2)  | 3<br>(0,;1/1,0,;)  | 5<br>(0;/2,1)    | 9<br>(2,3-,1/3-,2)   |
| BCC2557 | PI<br>643368  | HKHJC<br>MCCFC    | 10<br>(0,;1/3,3-)   | 12<br>(3,3-/3,3-)  | 13<br>(3/3)           | 10<br>(2,3-,1/3,3-,2)   | 12<br>(3-/3)      | 2<br>(0;/2)        | 7<br>(1,0;/2,3-) | 14<br>(3/3,3+)       |
| BCC2562 | PI<br>643383  | HKHJC<br>MCCFC    | 10<br>(1,0,;2,3-/3) | 9<br>(3-,2,1/3-,2) | 12<br>(3,3-,2/3,3-)   | 8<br>(2,3-,1,0;/2,3-)   | 12<br>(3-/3)      | 5<br>(0,;1/1,2)    | 5<br>(1,0;/2,3-) | 8<br>(2,3-,1/2,3-,1) |
| BCC0377 | CIho<br>15624 | HKHJC<br>MCCFC    | 8<br>(2,1,c/3)      | 11<br>(2,3-/3)     | 10<br>(2,3-/3,3-)     | 9<br>(2,3-,1/3-,2)      | 10<br>(3-,2/3-)   | 4<br>(1,0;/1)      | 3<br>(1,0;/1,2)  | 8<br>(2,3-,1/2,3-)   |
| BCC0740 | PI<br>181113  | MCCFC<br>92-MN-90 | 8<br>(1,2,cn/3,3-)  | 12<br>(3,3-/3,3-)  | 10<br>(2,3-,3/3,3-,2) | 9<br>(2,3-/3-,2)        | 9<br>(2,1/3-,2)   | 9<br>(2/3,3-)      | 6<br>(1,0;/2,3-) | 3<br>(1,0;/1,0,;)    |
| BCC1721 | PI<br>401954  | QCCJB<br>HKHJC    | 12<br>(0;/3,3+)     | 11<br>(3-/3,3-)    | 13<br>(3,3-/3)        | 13<br>(3,3-/3)          | 7<br>(2,1,0;/3)   | 6<br>(1,0,;2/3-,3) | 12<br>(3-/3)     | 8<br>(2,3-,1/2,3-,1) |
| BCC2024 | PI<br>467831  | TKTTF<br>TRTTF    | 8<br>(2,1/3)        | 12<br>(1,2,3-/3)   | 7<br>(2,1/2,3-)       | 6<br>(1,0,;2-,n/2,3-,1) | 10<br>(2,3-/3,3-) | 9<br>(2,3-/3-)     | 12<br>(3-/3)     | 8<br>(2,3-/2,3-)     |
| BCC1472 | PI<br>349359  | TRTTF<br>92-MN-90 | 8<br>(0;/3,3-)      | 9<br>(2,3-/3-)     | 11<br>(3-/3,3-)       | 7<br>(2,1,3-/2,3-)      | 12<br>(3-/3)      | 9<br>(2,3-/3-,2)   | 9<br>(2,3-/3-)   | 6<br>(2,1/2,1)       |
| BCC1627 | PI<br>374420  | TRTTF<br>92-MN-90 | 10<br>(0;/3)        | 10<br>(2,1/3)      | 13<br>(3,3-/3)        | 7<br>(2,1,3-/2,3-)      | 13<br>(3,3-/3)    | 9<br>(2/3-,2)      | 12<br>(3-/3)     | 6<br>(2,1/2,1)       |
| BCC1823 | PI<br>422232  | TRTTF<br>92-MN-90 | 8<br>(1,n/3)        | 11<br>(2,3-/3)     | 11<br>(2,3-,c/3)      | 7<br>(2,1,c/2,3-)       | 9<br>(2,1,3-/3-)  | 10<br>(2/3)        | 10<br>(3-/3-)    | 5<br>(1,2/1,2,3-)    |
| BCC2068 | PI<br>485536  | TRTTF<br>92-MN-90 | 9<br>(0,;1/3,3-)    | 9<br>(2,1,3-/3,3-) | 10<br>(2,3-,1/3,3-,2) | 7<br>(2,1/2,3-,1)       | 11<br>(2,3-/3)    | 13<br>(3/3)        | 12<br>(3-/3)     | 2<br>(0;/1,0,;)      |

|         |               |                   |                     |                        |                        |                       |                     |                   |                   |                      |
|---------|---------------|-------------------|---------------------|------------------------|------------------------|-----------------------|---------------------|-------------------|-------------------|----------------------|
| BCC0241 | CIho<br>11849 | TRTTF<br>92-MN-90 | 8<br>(2,1/3)        | 10<br>(3-,2/3-)        | 10<br>(2,3-,1/3,3-)    | 6<br>(2,1/2,1,3-)     | 9<br>(2,3-/3-,2)    | 9<br>(2,3-/3-)    | 8<br>(2,3-/2,3-)  | 6<br>(1,2/2,1)       |
| BCC0709 | PI<br>174478  | TRTTF<br>92-MN-90 | 13<br>(3-/3)        | 13<br>(2,1/3)          | 9<br>(2,1,3-,n/3,3-,2) | 6<br>(1,0;-,3-,n/3)   | 9<br>(2,3-/3-)      | 8<br>(2/3-)       | 13<br>(3,3-/3)    | 6<br>(1,2/2,1)       |
| BCC2545 | PI<br>643292  | TRTTF<br>HKHJC    | 8<br>(1,0;-,2/3)    | 11<br>(2,3-,1/3)       | 12<br>(2,1,3-/3)       | 4<br>(0;-,1,2-/1,2)   | 13<br>(3/3)         | 6<br>(1,2/2,1)    | 9<br>(2,3-/3-)    | 9<br>(3-,2/3-,2)     |
| BCC1570 | PI<br>370867  | TTKSK<br>92-MN-90 | 5<br>(0;/3,3-)      | 11<br>(3-,2/3,3-)      | 12<br>(2,1,3-/3)       | 9<br>(1,2/3)          | 9<br>(3-,2/3-,2)    | 9<br>(2,3-/3-,2)  | 12<br>(3-/3)      | 5<br>(1,2/1,2)       |
| BCC1585 | PI<br>371149  | TTKSK<br>92-MN-90 | 3<br>(0;-,1/3,3-)   | 9<br>(2,1/3,3-)        | 13<br>(3,3-/3)         | 11<br>(3-,2/3,3-)     | 8<br>(2,1,3-/2,3-)  | 10<br>(3-,2/3,3-) | 10<br>(2,3-/3,3-) | 5<br>(1,2/1,2)       |
| BCC1703 | PI<br>392501  | TTKSK<br>92-MN-90 | 6<br>(0;/3)         | 10<br>(2,1/3)          | 9<br>(2,1,3-/3,3-,c)   | 12<br>(3,3-,2/3,3-,2) | 10<br>(3-/3-)       | 9<br>(2,3-/3-,2)  | 9<br>(2,3-/3-)    | 6<br>(2,1,3-/2,1,3-) |
| BCC1938 | PI<br>466726  | TTKSK<br>92-MN-90 | 6<br>(1,0;/3)       | 8<br>(2,1,3-/3-)       | 12<br>(3,3-/3,3-,c)    | 9<br>(1,2/3,3-)       | 13<br>(3/3)         | 9<br>(2,3-/3-)    | 10<br>(3-/3-)     | 6<br>(1,2/2,1)       |
| BCC2345 | PI<br>592196  | TTKSK<br>92-MN-90 | 6<br>(0;-,1/3)      | 8<br>(2,3-/3)          | 8<br>(2,3-/2,3-,n)     | 13<br>(3,3-/3)        | 10<br>(3-/3-)       | 10<br>(3-/3-)     | 11<br>(2,3-/3)    | 5<br>(1/1,2)         |
| BCC2408 | PI<br>611576  | TTKSK<br>92-MN-90 | 6<br>(1,2,0;/3,3-)  | 11<br>(3-,2/3)         | 8<br>(2,3-,1/3-,2,c)   | 14<br>(3/3+)          | 13<br>(3/3)         | 12<br>(3-/3)      | 12<br>(3-/3)      | 4<br>(1,0;-,2/1,2)   |
| BCC1750 | PI<br>402445  | TTKSK<br>HKHJC    | 6<br>(0;-,1/3)      | 13<br>(3,3-/3)         | 11<br>(3-,2,1/3)       | 11<br>(2,3-/3)        | 11<br>(2,3-/3)      | 6<br>(1/2,3-)     | 12<br>(3-/3)      | 9<br>(2,3-/3-,2,1)   |
| BCC1156 | PI<br>295442  | TTKSK<br>QCCJB    | 6<br>(1,0;/2,3-)    | 9<br>(2,3-/3-,2)       | 8<br>(2,3-,1/2,3-)     | 11<br>(3-,2/3,3-)     | 6<br>(1,0;-,2/2,3-) | 8<br>(2,3-/3-)    | 9<br>(2,1/3-,3)   | 8<br>(2,3-,1/2,3-)   |
| BCC1755 | PI<br>404204  | TTKSK<br>TKTTF    | 7<br>(0;/3,3-)      | 12<br>(1,2/3)          | 7<br>(2,1,3-/2,3-,3)   | 11<br>(2,3-/3)        | 10<br>(3-,2/3-)     | 9<br>(2,3-/3-)    | 12<br>(3-/3)      | 10<br>(3-,2/3-)      |
| BCC0434 | PI 39397      | TTKSK<br>TRTTF    | 7<br>(0;-,1,n/3-,2) | 8<br>(2,3-,1/2,3-)     | 10<br>(2,3-/3,3-)      | 6<br>(2,1,3-/2,1,3-)  | 10<br>(3-,2/3-)     | 11<br>(3-,2/3)    | 12<br>(3-/3)      | 9<br>(2,3-/3-,2)     |
| BCC1639 | PI<br>382275  | TTKSK<br>TTKST    | 7<br>(1,0;/3-)      | 6<br>(1,0;-,3-/2,3-,1) | 11<br>(2,3-/3)         | 11<br>(3-,2/3,3-,2)   | 8<br>(2,1/3)        | 10<br>(2,3-/3-)   | 12<br>(3-/3)      | 8<br>(2,3-/2,3-)     |
| BCC0212 | CIho<br>10661 | TTKSK<br>TTKST    | 6<br>(1,0;/3,3-)    | 7<br>(2,1,3-/2,3-,1)   | 8<br>(2,3-,1/2,3-)     | 10<br>(2,3-/3,3-,2)   | 10<br>(3-,2/3-)     | 8<br>(2,3-/2,3-)  | 11<br>(3-,2/3,3-) | 9<br>(2,3-/3-,2)     |
| BCC2069 | PI<br>485548  | TTKST<br>92-MN-90 | 8<br>(0;/3,3-)      | 7<br>(1,2,0;/2,3-,1)   | 10<br>(2,1,3-/3)       | 8<br>(2/3)            | 11<br>(2,3-/3)      | 9<br>(2/3)        | 10<br>(3-/3-)     | 6<br>(2,1/2,1,3-)    |

|         |               |                   |                     |                    |                       |                       |                      |                   |                   |                      |
|---------|---------------|-------------------|---------------------|--------------------|-----------------------|-----------------------|----------------------|-------------------|-------------------|----------------------|
| BCC0773 | PI<br>184872  | TTKST<br>92-MN-90 | 10<br>(2,3-,1/3,3+) | 7<br>(2,1,3-/2,3-) | 12<br>(1,2,3-,n/3,3-) | 10<br>(3-/3-)         | 10<br>(3-,2/3-)      | 12<br>(3-/3)      | 9<br>(2,3-/3-)    | 5<br>(1,2/1,2)       |
| BCC1160 | PI<br>295960  | TTKST<br>QCCJB    | 12<br>(3-,2,c/3,3-) | 7<br>(1,0;,2/3-)   | 13<br>(3,3-/3)        | 9<br>(2,1,3-/3,3-,c)  | 6<br>(1,0;,2,n/3-,2) | 8<br>(2/3)        | 10<br>(2,3-/3,3-) | 8<br>(2,3-/2,3-)     |
| BCC0395 | CIho<br>16676 | TTKST<br>QCCJB    | 8<br>(1,2/3)        | 5<br>(1,2/3)       | 13<br>(3,3-/3)        | 9<br>(2,1,3-,0;/3,3-) | 7<br>(2,1,3-/2,3-)   | 9<br>(2,3-/3-,2)  | 11<br>(3-,2/3)    | 11<br>(3-,2/3,3-)    |
| BCC1069 | PI<br>283397  | 92-MN-90          | 9<br>(0;,1/3,3+)    | 13<br>(3,3-/3,3+)  | 13<br>(3,3-/3)        | 11<br>(3-,2,1/3,3-)   | 11<br>(2,3-/3)       | 10<br>(3-/3-)     | 13<br>(3/3)       | 5<br>(1,2/1,2)       |
| BCC1142 | PI<br>294739  | 92-MN-90          | 11<br>(2,3-/3)      | 14<br>(3/3,3+)     | 13<br>(3/3)           | 13<br>(3,3-,2/3)      | 11<br>(3-,2/3)       | 12<br>(3-/3)      | 13<br>(3/3)       | 3<br>(1,0;/1,0;,2)   |
| BCC1182 | PI<br>306439  | 92-MN-90          | 9<br>(2,3-/3)       | 13<br>(3/3)        | 10<br>(2,3-,1,c/3,3-) | 10<br>(2,3-/3,3-)     | 12<br>(3-/3)         | 9<br>(2/3-)       | 10<br>(3-,2/3-)   | 6<br>(1,2/2,1)       |
| BCC1206 | PI<br>320213  | 92-MN-90          | 11<br>(2,3-/3)      | 13<br>(3,3-,2/3)   | 8<br>(2,3-,1,2,3-)    | 13<br>(3/3)           | 13<br>(3/3)          | 10<br>(2,3-/3,3-) | 12<br>(3-/3)      | 5<br>(1,2/1,2)       |
| BCC1337 | PI<br>328327  | 92-MN-90          | 11<br>(2,3-/3)      | 13<br>(3/3)        | 13<br>(3,3-/3)        | 13<br>(3,3-/3)        | 12<br>(3-/3)         | 9<br>(2,1/3,3-)   | 12<br>(3-/3)      | 6<br>(2,1/2,1)       |
| BCC1377 | PI<br>328976  | 92-MN-90          | 11<br>(2,3-/3)      | 12<br>(3-/3)       | 13<br>(3,3-,2/3)      | 12<br>(3,3-,2/3,3-)   | 8<br>(2/3)           | 8<br>(2/3-)       | 10<br>(3-,2/3-)   | 6<br>(1,2/2,1)       |
| BCC1394 | PI<br>330400  | 92-MN-90          | 12<br>(2,3-/3,3+)   | 10<br>(2,1,3-/3)   | 12<br>(3,3-,2/3,3-)   | 13<br>(3,3-/3,3+)     | 13<br>(3/3)          | 9<br>(2,3-/3-,2)  | 11<br>(3-/2/3)    | 3<br>(1,0;,2/1,0;,2) |
| BCC1515 | PI<br>361036  | 92-MN-90          | 8<br>(1,2,3-/3)     | 10<br>(2,3-/3,3-)  | 10<br>(2,3-/3,3-)     | 13<br>(3,3-/3,3+,2)   | 13<br>(3,3-/3)       | 12<br>(3-/3)      | 12<br>(3-/3)      | 6<br>(2,1,3-/2,1,3-) |
| BCC1529 | PI<br>361672  | 92-MN-90          | 9<br>(2,3-/3)       | 11<br>(2,3-/3)     | 11<br>(2,3-/3)        | 13<br>(3/3)           | 13<br>(3/3)          | 13<br>(3/3)       | 12<br>(3-/3)      | 6<br>(1,2/2,1)       |
| BCC1548 | PI<br>365547  | 92-MN-90          | 8<br>(0;,1/3)       | 11<br>(3-,2/3)     | 12<br>(2,3-,1/3)      | 11<br>(3-,2/3,3-,2)   | 10<br>(2,3-/3)       | 13<br>(3-/3+)     | 13<br>(3/3)       | 5<br>(1,2/1,2)       |
| BCC1584 | PI<br>371111  | 92-MN-90          | 9<br>(2,3-/3)       | 13<br>(3/3)        | 12<br>(3,3-,2/3,3-)   | 13<br>(3/3)           | 12<br>(3-/3)         | 13<br>(3/3)       | 13<br>(3/3)       | 6<br>(2,1,3-/2,1,3-) |
| BCC1595 | PI<br>371320  | 92-MN-90          | 8<br>(1/3)          | 11<br>(2,3-/3)     | 9<br>(2,1/3,3-,2)     | 9<br>(2,1,3-/3,3-)    | 11<br>(3-/3,3-)      | 10<br>(2,3-/3,3-) | 9<br>(2,3-/3-)    | 5<br>(1,2/1,2)       |
| BCC1789 | PI<br>412946  | 92-MN-90          | 9<br>(2,1/3)        | 13<br>(3/3)        | 9<br>(2,1,3-/3,3-,2)  | 13<br>(3/3)           | 13<br>(3/3)          | 12<br>(3-/3)      | 12<br>(3-/3)      | 6<br>(1,2/2,1)       |
| BCC0180 | CIho<br>8054  | 92-MN-90          | 10<br>(2/3,3-)      | 9<br>(2,3-/3-,2)   | 13<br>(3,3-/3)        | 11<br>(3-/3,3-)       | 13<br>(2,1/3)        | 10<br>(1/3,3-,2)  | 11<br>(3-/3,3-)   | 6<br>(2,1,3-/2,1,3-) |
| BCC1832 | PI<br>428491  | 92-MN-90          | 8<br>(2,3-,1/3)     | 12<br>(3-,3/3)     | 11<br>(2,3-/3)        | 13<br>(3/3)           | 13<br>(3/3)          | 9<br>(2,3-/3-)    | 13<br>(3/3)       | 6<br>(2,1,2/1,3-)    |

|         |               |          |                    |                     |                     |                       |                   |                     |                   |                      |
|---------|---------------|----------|--------------------|---------------------|---------------------|-----------------------|-------------------|---------------------|-------------------|----------------------|
| BCC1859 | PI<br>429526  | 92-MN-90 | 13<br>(3-/3)       | 11<br>(3-,2/3)      | 13<br>(3/3)         | 13<br>(3,3-/3)        | 13<br>(3,3-/3)    | 13<br>(3-/3)        | 11<br>(3-/3,3-)   | 3<br>(0,1/1,0;)      |
| BCC2013 | PI<br>467749  | 92-MN-90 | 13<br>(3-,2/3,3+)  | 13<br>(3,3-/3)      | 13<br>(3/3)         | 13<br>(3/3)           | 13<br>(3/3)       | 11<br>(2,3-/3)      | 13<br>(3/3)       | 6<br>(2,1,3-/2,1,3-) |
| BCC2017 | PI<br>467789  | 92-MN-90 | 11<br>(2,3-/3,3-)  | 9<br>(2,1/3,3-)     | 13<br>(3/3)         | 10<br>(2,3-/3,3-)     | 13<br>(3,3-/3)    | 12<br>(3-/3)        | 10<br>(3-,2/3-)   | 5<br>(1,2/1,2)       |
| BCC2019 | PI<br>467797  | 92-MN-90 | 11<br>(2,3-/3,3+)  | 11<br>(3-,2/3)      | 11<br>(2,3-/3)      | 14<br>(3/3,3+)        | 13<br>(3,3-/3)    | 13<br>(3/3)         | 13<br>(3/3)       | 5<br>(1,2/1,2)       |
| BCC2091 | PI<br>506293  | 92-MN-90 | 12<br>(2,3-/3)     | 13<br>(3/3)         | 13<br>(3,3-/3)      | 13<br>(3/3)           | 13<br>(3/3)       | 12<br>(3-/3)        | 13<br>(3/3)       | 5<br>(1,2/1,2)       |
| BCC2093 | PI<br>506299  | 92-MN-90 | 9<br>(2,1,3-/3,3+) | 11<br>(2,3-,1/3)    | 13<br>(3,3-/3)      | 12<br>(3-,3/3)        | 12<br>(3-/3)      | 13<br>(3/3)         | 13<br>(3/3)       | 5<br>(1/1,2)         |
| BCC2144 | PI<br>548736  | 92-MN-90 | 9<br>(1/3)         | 10<br>(2,1/3)       | 13<br>(3/3)         | 12<br>(3,3-,2/3,3-,2) | 10<br>(3-/3-)     | 11<br>(3-/3,3-)     | 12<br>(3-/3)      | 6<br>(1,2/2,1)       |
| BCC2265 | PI<br>573703  | 92-MN-90 | 13<br>(3-,2/3)     | 12<br>(3-/3)        | 13<br>(3/3)         | 13<br>(3/3)           | 13<br>(3,3-/3)    | 11<br>(2,3-/3)      | 13<br>(3/3)       | 6<br>(2,1,3-/2,1,3-) |
| BCC2291 | PI<br>574094  | 92-MN-90 | 10<br>(2,1/3,3+)   | 13<br>(3,3-/3,3+)   | 10<br>(2,3-/3,3-)   | 12<br>(3,3-/3,3-)     | 8<br>(1,2/3)      | 9<br>(3-,2/3-,2)    | 10<br>(3-,2/3,3-) | 5<br>(1/1,2)         |
| BCC2336 | PI<br>591928  | 92-MN-90 | 11<br>(1,2,3-/3)   | 13<br>(3/3)         | 11<br>(2,3-/3)      | 13<br>(3,3-/3)        | 13<br>(3/3)       | 13<br>(3,3-/3)      | 13<br>(3/3)       | 6<br>(2,1/2,1,3-)    |
| BCC2342 | PI<br>592173  | 92-MN-90 | 9<br>(2,3-/3,3+)   | 11<br>(3-/3,3-)     | 13<br>(3/3)         | 12<br>(3,3-,2/3,3-)   | 13<br>(3,3-/3)    | 9<br>(2,3-/3-)      | 13<br>(3/3)       | 6<br>(2,1/2,1,3-)    |
| BCC2373 | PI<br>599640  | 92-MN-90 | 9<br>(1,2/3,3+)    | 11<br>(3-/3,3-,2,1) | 11<br>(2,3-,1/3)    | 13<br>(3,3-,c/3)      | 9<br>(2,3-/3-,2)  | 10<br>(2,3-,1/3-,3) | 12<br>(3-/3)      | 6<br>(1,2/2,1,3-)    |
| BCC2378 | PI<br>601065  | 92-MN-90 | 11<br>(3-,2/3)     | 13<br>(3/3)         | 13<br>(3,3-,2/3)    | 13<br>(3,3-/3)        | 12<br>(3,3-/3,3-) | 9<br>(2,3-/3)       | 11<br>(3-,2/3)    | 5<br>(1,2/1,2,3-)    |
| BCC2380 | PI<br>605472  | 92-MN-90 | 9<br>(1,2/3)       | 12<br>(1,2-/3)      | 10<br>(2,3-,1/3,3-) | 13<br>(3,3-/3)        | 14<br>(3/3+)      | 10<br>(3-,2/3)      | 11<br>(3-,2/3)    | 7<br>(2,1,3-/2,3-)   |
| BCC0240 | CIho<br>11845 | 92-MN-90 | 11<br>(3-/3)       | 13<br>(3/3)         | 13<br>(3,3-/3)      | 11<br>(3-,2/3)        | 10<br>(3-/3-)     | 10<br>(3-/3-)       | 12<br>(3-/3)      | 6<br>(2,1/2,1)       |
| BCC0266 | CIho<br>13743 | 92-MN-90 | 9<br>(2,3-/3,3-)   | 9<br>(3-,2/3-,2)    | 14<br>(3/3,3+)      | 11<br>(2,3-/3,3+)     | 12<br>(3-/3)      | 12<br>(3-/3)        | 11<br>(3-/3,3-)   | 6<br>(2,1,3-/2,1,3-) |
| BCC0324 | CIho<br>14395 | 92-MN-90 | 13<br>(3-/3,3+)    | 14<br>(3,3+/3,3+)   | 13<br>(3/3)         | 14<br>(3/3,3+)        | 11<br>(3-/3,3-)   | 12<br>(3-/3)        | 13<br>(3/3)       | 6<br>(2,1,3-/2,1,3-) |
| BCC0476 | PI 60663      | 92-MN-90 | 11<br>(3-,2/3)     | 13<br>(3,3-/3)      | 10<br>(2,3-/3,3-)   | 11<br>(3-,2/3)        | 9<br>(2,3-/3-)    | 9<br>(2/3,3-)       | 9<br>(2,3-/3-)    | 5<br>(1,2/1,2)       |

|         |              |          |                    |                    |                       |                     |                   |                |                  |                       |
|---------|--------------|----------|--------------------|--------------------|-----------------------|---------------------|-------------------|----------------|------------------|-----------------------|
| BCC0654 | PI<br>155303 | 92-MN-90 | 9<br>(0;/3,3-,2)   | 8<br>(1/3)         | 10<br>(2,3-/3,3-)     | 9<br>(2,3-/3-,2)    | 9<br>(2,1,3-/3)   | 9<br>(2/3-,2)  | 10<br>(3-/3-)    | 2<br>(0;/0,;1)        |
| BCC0694 | PI<br>168328 | 92-MN-90 | 10<br>(2,1/3)      | 11<br>(3-,2/3)     | 13<br>(3,3-/3)        | 13<br>(2,1,3-,c/3)  | 13<br>(3,3-/3)    | 13<br>(3/3)    | 13<br>(3/3)      | 5<br>(1,2/1,2,3-)     |
| BCC0738 | PI<br>181102 | 92-MN-90 | 11<br>(3-,2/3)     | 13<br>(3/3)        | 13<br>(3,3-,2/3)      | 11<br>(3-,2/3,3-)   | 12<br>(3-/3)      | 13<br>(3/3)    | 13<br>(3/3)      | 5<br>(1,2/1,2)        |
| BCC0747 | PI<br>182645 | 92-MN-90 | 8<br>(1,2/3)       | 13<br>(3/3)        | 10<br>(2,3-/3,3-)     | 13<br>(3,3-/3)      | 9<br>(2,3-/3-)    | 9<br>(2,3-/3-) | 11<br>(2,3-/3)   | 6<br>(2,1/2,1)        |
| BCC0771 | PI<br>184103 | 92-MN-90 | 11<br>(2,3,c/3)    | 13<br>(3,3-/3)     | 11<br>(2,3-,1,c/3)    | 10<br>(2,3-,c/3,3-) | 12<br>(3-/3)      | 12<br>(3-/3)   | 11<br>(3-,2/3)   | 4<br>(1,0,;2/1,2)     |
| BCC0774 | PI<br>184873 | 92-MN-90 | 10<br>(2,1/3)      | 11<br>(3-,2/3,3-)  | 12<br>(2,3-/3)        | 11<br>(3-,2/3)      | 13<br>(3,3-/3)    | 10<br>(3-/3-)  | 11<br>(3-,2/3)   | 3<br>(1,0,;2/1,0,;3-) |
| BCC0777 | PI<br>184880 | 92-MN-90 | 11<br>(0,;1/3)     | 8<br>(1,2/3)       | 12<br>(3,3-,2/3,3-)   | 9<br>(2,3-,1/3-,2)  | 11<br>(3-,2/3)    | 10<br>(3-/3-)  | 10<br>(3-/3-)    | 6<br>(2,1/2,1)        |
| BCC0822 | PI<br>193063 | 92-MN-90 | 8<br>(1,2,0;/3,3+) | 10<br>(2,1,3-/3)   | 13<br>(3,3-/3)        | 13<br>(3,3-/3)      | 10<br>(3-,2/3-)   | 11<br>(3-,2/3) | 10<br>(3-/3-)    | 7<br>(2,1,3-/2,3-,1)  |
| BCC0829 | PI<br>194556 | 92-MN-90 | 9<br>(2,1/3,3-)    | 8<br>(1,0,;2/3)    | 8<br>(2,3-,1/2,3-)    | 11<br>(3-,2,1/3,3-) | 10<br>(2/3)       | 10<br>(2/3,3-) | 12<br>(3-/3)     | 3<br>(1,0,;2/1,0,;2)  |
| BCC0842 | PI<br>201097 | 92-MN-90 | 10<br>(2,1/3)      | 9<br>(2,3-/3-,2,1) | 8<br>(2,3-/3,3-)      | 11<br>(2,3-/3)      | 12<br>(3-/3)      | 11<br>(2,3-/3) | 10<br>(3-/3-)    | 6<br>(2,1/2,1)        |
| BCC0856 | PI<br>221326 | 92-MN-90 | 11<br>(2,3-/3,3+)  | 11<br>(2,3-,1/3)   | 13<br>(3,3-,c/3)      | 14<br>(3/3+)        | 12<br>(3-/3)      | 12<br>(3-/3)   | 12<br>(3-/3)     | 6<br>(2,1/2,1,3-)     |
| BCC0908 | PI<br>237571 | 92-MN-90 | 11<br>(3-,2/3)     | 14<br>(3/3,3+)     | 13<br>(3/3)           | 13<br>(3,3-/3)      | 12<br>(3,3-/3,3-) | 12<br>(3-/3)   | 10<br>(3-/3-)    | 6<br>(1,2/2,1)        |
| BCC1131 | PI<br>290353 | HKHJC    | 8<br>(2/3)         | 10<br>(2,3-/3,3-)  | 10<br>(2,3-,c/3,3-)   | 13<br>(3,3-/3)      | 11<br>(2,3-/3)    | 6<br>(1/2)     | 9<br>(2,3-/3-,2) | 10<br>(3-/3-)         |
| BCC1310 | PI<br>327902 | HKHJC    | 9<br>(2,1/3)       | 13<br>(3/3)        | 9<br>(2,3-/3-,2,1)    | 13<br>(3,3-/3)      | 8<br>(2,3-/2,3-)  | 7<br>(2/3-,2)  | 11<br>(2,3-/3)   | 9<br>(3-,2/3-,2)      |
| BCC0138 | CIho<br>6496 | HKHJC    | 9<br>(2,1/3,3-)    | 11<br>(3-/3,3-)    | 11<br>(3-,2,1/3,3-,2) | 11<br>(3-,2/3,3-)   | 13<br>(3/3)       | 7<br>(1/3)     | 11<br>(2,3-/3)   | 8<br>(2,3-/2,3-)      |
| BCC1571 | PI<br>370869 | HKHJC    | 11<br>(3-,2,1/3)   | 13<br>(3/3)        | 12<br>(3-/3)          | 12<br>(3,3-/3,3-)   | 11<br>(3-,2/3)    | 7<br>(2/3,3-)  | 13<br>(3/3)      | 13<br>(3/3)           |
| BCC2555 | PI<br>643362 | HKHJC    | 13<br>(3-,2/3+)    | 15<br>(3,3+/3+)    | 13<br>(3,3-/3,3+)     | 9<br>(2,1,3-/3,3-)  | 13<br>(3/3)       | 6<br>(1/2,1)   | 8<br>(1,0;/3)    | 8<br>(2,3-/2,3-)      |
| BCC1100 | PI<br>286388 | QCCJB    | 10<br>(2,1,c/3)    | 13<br>(3/3)        | 9<br>(2,1,3-/3,3-)    | 12<br>(3,3-,2/3,3-) | 7<br>(2,1/2)      | 11<br>(3-,2/3) | 10<br>(3-/3-)    | 13<br>(3/3)           |

|         |               |       |                     |                     |                     |                          |                  |                   |                   |                      |
|---------|---------------|-------|---------------------|---------------------|---------------------|--------------------------|------------------|-------------------|-------------------|----------------------|
| BCC2152 | PI<br>559516  | QCCJB | 9<br>(2,1,3-/3)     | 12<br>(3-/3)        | 11<br>(2,3-,3/3)    | 12<br>(3,3-/3,3-)        | 7<br>(2,1/2,3-)  | 12<br>(3-/3)      | 10<br>(3-,2/3-)   | 10<br>(3-,2/3-)      |
| BCC1783 | PI<br>410865  | TKTTF | 10<br>(0;/3)        | 11<br>(2,3-/3)      | 7<br>(2,1,n/2,3-)   | 13<br>(3-/3)             | 8<br>(2,3-/2,3-) | 8<br>(2,3-/2,3-)  | 12<br>(3-,3/3,3-) | 8<br>(2,3-/2,3-)     |
| BCC0422 | PI 38320      | TKTTF | 11<br>(3-,2/3)      | 13<br>(3/3)         | 7<br>(2,1,3-/2,3-)  | 11<br>(3-,2/3,3-,2)      | 11<br>(3-,2,1/3) | 12<br>(2/3)       | 11<br>(2,3-/3)    | 9<br>(2,3-/3-,2)     |
| BCC1157 | PI<br>295581  | TRTTF | 8<br>(1,2,c/3,3-,2) | 11<br>(3-/3-,3,2)   | 13<br>(3,3-,2/3)    | 6<br>(1,2,3-,0;/3,3-)    | 10<br>(3-/3-)    | 9<br>(2,3-/3-)    | 11<br>(3-,2/3)    | 9<br>(3-,2/3-,2)     |
| BCC1661 | PI<br>386601  | TRTTF | 8<br>(2,3-/3,3-)    | 9<br>(2,3-/3-,2)    | 10<br>(2,3-/3,3-)   | 7<br>(2,1,3-/2,3-)       | 10<br>(3-,2/3-)  | 12<br>(3-/3)      | 10<br>(3-/3-)     | 9<br>(2,3-/3-)       |
| BCC1850 | PI<br>429311  | TRTTF | 8<br>(0;,1/3,3-,2)  | 8<br>(2,1,3-/3-,c)  | 10<br>(2,3-/3,3-)   | 7<br>(2,1,3-/2,3-)       | 13<br>(3/3)      | 8<br>(2/3-)       | 10<br>(3-,2/3-)   | 9<br>(3-,2/3-,2)     |
| BCC1928 | PI<br>449279  | TRTTF | 12<br>(0;,1/3)      | 10<br>(3-,2/3-)     | 13<br>(3,3-/3)      | 2<br>(0;,1,2-/0;,1,2,3-) | 11<br>(3-,2/3)   | 9<br>(2,3-/3-)    | 10<br>(3-/3-)     | 8<br>(2,3-/2,3-)     |
| BCC2084 | PI<br>498437  | TRTTF | 8<br>(2,3-,1/3)     | 10<br>(2,3-/3,3-,2) | 11<br>(3-,2/3,3-,2) | 5<br>(1,2-,0;/1,3-)      | 10<br>(3-,2/3-)  | 8<br>(2,1/2,3-)   | 9<br>(2,3-/3-)    | 9<br>(2,3-/3-,2)     |
| BCC2142 | PI<br>548724  | TRTTF | 8<br>(2,1/3)        | 11<br>(3-,2/3)      | 12<br>(3,3-,2/3,3-) | 2<br>(0;,1,2/0;,1,2,3-)  | 12<br>(3-/3)     | 11<br>(2,3-/3)    | 13<br>(3/3)       | 10<br>(3-,2/3-,3)    |
| BCC2168 | PI<br>564502  | TRTTF | 11<br>(2,3-/3)      | 12<br>(3-,3/3)      | 13<br>(3,3-,2/3)    | 7<br>(2,1,3-/2,3-)       | 13<br>(3/3)      | 15<br>(3,3+/3+)   | 13<br>(3/3)       | 9<br>(3-,2/3-,2)     |
| BCC2401 | PI<br>611527  | TRTTF | 9<br>(2,1/3)        | 11<br>(2,3-/3)      | 12<br>(3,3-/3,3-)   | 7<br>(2,1,3-/2,3-)       | 10<br>(3-/3-)    | 10<br>(3-,2/3-)   | 13<br>(3/3)       | 9<br>(3-,2/3-,2)     |
| BCC1219 | PI<br>321784  | TTKSK | 6<br>(0;/3)         | 12<br>(1,2/3,3-)    | 11<br>(3-,2,1/3)    | 9<br>(2,3-,1/3-,2)       | 10<br>(2,3-/3)   | 10<br>(3-/3-)     | 12<br>(3-,2/3)    | 9<br>(3-,2/3-,2)     |
| BCC1578 | PI<br>371056  | TTKSK | 6<br>(0;,1/3)       | 11<br>(3-,2,c/3)    | 8<br>(2,1,3-/3)     | 8<br>(2,3-,1/3)          | 10<br>(3-/3)     | 10<br>(3-,2/3,3-) | 10<br>(3-/3)      | 8<br>(2,3-/2,3-)     |
| BCC1624 | PI<br>372107  | TTKSK | 7<br>(0;,1/3)       | 10<br>(2,1/3)       | 12<br>(3-,2/3,3-)   | 9<br>(2,3-,1/3-,2)       | 9<br>(2,3-/3-)   | 9<br>(2/3-)       | 12<br>(3-/3)      | 8<br>(2,3-,1/2,3-,1) |
| BCC2023 | PI<br>467815  | TTKSK | 5<br>(0;/3)         | 13<br>(3/3)         | 13<br>(3,3-/3)      | 11<br>(3-,2/3)           | 9<br>(2,3-/3-)   | 10<br>(2,3-/3)    | 12<br>(3-,2/3)    | 9<br>(3-,2/3-,2)     |
| BCC0394 | CIho<br>16671 | TTKST | 9<br>(0;/3)         | 7<br>(2,1/2,3-)     | 9<br>(2,3-/3-)      | 8<br>(2,3-,1/2,3-)       | 13<br>(3/3)      | 9<br>(2,3-/3-)    | 11<br>(2,3-/3)    | 9<br>(2,3-/3-,2)     |
| BCC1003 | PI<br>268167  |       | 13<br>(2,3-/3)      | 13<br>(3/3)         | 13<br>(3,3-,2/3)    | 13<br>(3/3)              | 13<br>(3/3)      | 13<br>(3/3)       | 13<br>(3,3-/3)    | 10<br>(3-,2/3-)      |
| BCC1060 | PI<br>280441  |       | 12<br>(3-,2/3)      | 13<br>(3,3-/3)      | 12<br>(3,3-/3,3-,c) | 13<br>(3,3-/3)           | 13<br>(3/3)      | 13<br>(3/3)       | 13<br>(3/3)       | 9<br>(2,3-/3-,2)     |

|         |              |  |                   |                   |                       |                      |                   |                   |                   |                   |
|---------|--------------|--|-------------------|-------------------|-----------------------|----------------------|-------------------|-------------------|-------------------|-------------------|
| BCC1061 | PI<br>281524 |  | 12<br>(3-,2/3,3+) | 13<br>(3/3)       | 13<br>(3,3-/3,3+)     | 13<br>(3,3-/3)       | 13<br>(3/3)       | 12<br>(3-/3)      | 13<br>(3/3)       | 10<br>(3-,2/3-,3) |
| BCC1062 | PI<br>281525 |  | 10<br>(2,3-/3)    | 13<br>(3/3)       | 13<br>(3,3-/3)        | 12<br>(3-/3)         | 8<br>(2/3-,2)     | 8<br>(2,3-/2,3-)  | 10<br>(3-/3-)     | 9<br>(3-,2/3-,2)  |
| BCC1081 | PI<br>283429 |  | 10<br>(2,1/3)     | 10<br>(2,1/3)     | 11<br>(3-/3,3-)       | 11<br>(3-,2/3)       | 9<br>(2,3-/3-)    | 11<br>(2,3-/3)    | 12<br>(3-/3)      | 11<br>(3-,2/3)    |
| BCC1101 | PI<br>286389 |  | 9<br>(2,1,3-/3)   | 13<br>(3/3)       | 11<br>(2,3-/3)        | 12<br>(3,3-,2/3,3-)  | 10<br>(3-,2/3-)   | 11<br>(2,3-/3)    | 12<br>(3-/3,3+)   | 11<br>(2,3-/3)    |
| BCC1135 | PI<br>292016 |  | 8<br>(2,1,3-/3)   | 14<br>(3/3+)      | 12<br>(3,3-/3,3-)     | 13<br>(2,3-/3)       | 14<br>(3/3,3+)    | 10<br>(3-/3-)     | 12<br>(3-/3,3+)   | 9<br>(3-,2/3-,2)  |
| BCC1158 | PI<br>295956 |  | 11<br>(3-,2/3)    | 12<br>(3-/3)      | 13<br>(3,3-,c/3)      | 14<br>(3/3+)         | 13<br>(3/3)       | 11<br>(3-,2/3,3-) | 13<br>(3/3)       | 9<br>(2,3-/3-,2)  |
| BCC1207 | PI<br>320216 |  | 11<br>(3-,2/3)    | 11<br>(3-,2/3)    | 13<br>(3/3)           | 13<br>(3,3-,2/3)     | 12<br>(2/3,3+)    | 8<br>(2/3-,2)     | 9<br>(2,3-/3-)    | 9<br>(3-,2/3-,2)  |
| BCC1215 | PI<br>321770 |  | 11<br>(2,3-/3)    | 11<br>(2,3-,1/3)  | 12<br>(3-/3)          | 14<br>(3/3,3+)       | 13<br>(3/3)       | 11<br>(2,3-/3)    | 13<br>(3/3)       | 10<br>(3-,2/3-)   |
| BCC1222 | PI<br>321797 |  | 9<br>(2,1/3)      | 12<br>(3-/3)      | 12<br>(3,3-/3,3-)     | 9<br>(2,1,3-/3,3-)   | 13<br>(3/3)       | 12<br>(3-/3)      | 11<br>(3-,2/3)    | 8<br>(2,3-/2,3-)  |
| BCC1229 | PI<br>321845 |  | 9<br>(1,2,3-/3)   | 11<br>(3-,2,1/3)  | 10<br>(2,1,3-/3)      | 10<br>(2,3-,1/3,3-)  | 10<br>(2,3-/3,3-) | 10<br>(3-,2/3-)   | 12<br>(3-/3)      | 9<br>(2,3-/3-,2)  |
| BCC1324 | PI<br>328154 |  | 13<br>(2,3-/3,3+) | 10<br>(2,1,3-/3)  | 13<br>(3,3-/3)        | 14<br>(3/3,3+)       | 14<br>(3/3,3+)    | 13<br>(3,3-/3)    | 13<br>(3/3)       | 9<br>(3-,2/3-,2)  |
| BCC1375 | PI<br>328950 |  | 9<br>(1,0;/3)     | 11<br>(2,3-,1/3)  | 13<br>(3,3-/3)        | 11<br>(3-,2,1/3)     | 11<br>(3-,2/3)    | 12<br>(3-/3)      | 12<br>(3-,2/3,3+) | 8<br>(2,3-/2,3-)  |
| BCC1390 | PI<br>330326 |  | 11<br>(2,3-/3,3+) | 12<br>(3-/3,3+)   | 12<br>(3,3-,2/3,3-)   | 13<br>(3,3-,2/3,3+)  | 12<br>(3-/3)      | 12<br>(3-/3)      | 13<br>(3/3)       | 11<br>(3-/3,3-)   |
| BCC1391 | PI<br>330397 |  | 9<br>(0;/3,3+)    | 13<br>(3,3-/3,3+) | 12<br>(3,3-/3,3-)     | 13<br>(3/3)          | 13<br>(3/3)       | 14<br>(3/3+)      | 13<br>(3/3)       | 8<br>(2,3-/2,3-)  |
| BCC1392 | PI<br>330398 |  | 8<br>(0;,1/3,3-)  | 11<br>(3-/3,3-)   | 10<br>(2,1,3-/3)      | 12<br>(3,3-/3,3-)    | 12<br>(3-/3)      | 13<br>(3,3-/3)    | 12<br>(3,3-/3,3-) | 8<br>(2,3-/2,3-)  |
| BCC1393 | PI<br>330399 |  | 11<br>(1,0-,2/3)  | 11<br>(3-,2/3)    | 10<br>(2,3-/3,3-)     | 11<br>(3-,2/3,3-)    | 12<br>(3-/3)      | 12<br>(3-/3)      | 12<br>(3-/3)      | 8<br>(2,3-/2,3-)  |
| BCC1399 | PI<br>331217 |  | 8<br>(0;/3)       | 11<br>(3-,2/3)    | 10<br>(2,3-,1/3,3-,2) | 8<br>(2,1,3-/3-,2,1) | 12<br>(3-/3)      | 10<br>(3-,2/3-)   | 13<br>(3/3)       | 8<br>(2,3-/2,3-)  |
| BCC1404 | PI<br>331895 |  | 13<br>(3/3,3+)    | 13<br>(3/3)       | 13<br>(3,3-/3)        | 13<br>(3/3)          | 12<br>(3-/3)      | 13<br>(3/3)       | 14<br>(3/3,3+)    | 14<br>(3,3+/3,3+) |

|         |              |  |                    |                      |                     |                     |                    |                  |                 |                      |
|---------|--------------|--|--------------------|----------------------|---------------------|---------------------|--------------------|------------------|-----------------|----------------------|
| BCC1408 | PI<br>337143 |  | 9<br>(1/3)         | 12<br>(3,3-/3,3-)    | 13<br>(3/3)         | 9<br>(2,3-,c/3-,2)  | 13<br>(3/3)        | 9<br>(2,3-,1/3-) | 10<br>(3-/3-)   | 10<br>(3-,2/3-)      |
| BCC1427 | PI<br>342219 |  | 13<br>(3-,2/3+)    | 13<br>(3/3)          | 12<br>(3-,2/3)      | 13<br>(3/3)         | 10<br>(3-,2/3)     | 13<br>(3-/3,3+)  | 13<br>(3-/3)    | 12<br>(3-,3/3,3-)    |
| BCC1450 | PI<br>344920 |  | 12<br>(3-/3)       | 14<br>(3/3,3+)       | 13<br>(3,3-/3)      | 13<br>(3,3-/3)      | 10<br>(3-/3-)      | 11<br>(3-/3-,3)  | 13<br>(3/3)     | 10<br>(3-,2/3-)      |
| BCC1465 | PI<br>345618 |  | 11<br>(2,3-/3)     | 11<br>(3-,2/3)       | 11<br>(2,3-/3)      | 12<br>(3-/3)        | 9<br>(2,3-,1/3-,2) | 13<br>(3/3)      | 12<br>(3-/3)    | 8<br>(2,3-/2,3-)     |
| BCC1469 | PI<br>346390 |  | 10<br>(2,3-/3)     | 13<br>(3,3-/3)       | 12<br>(3,3-/3,3-)   | 13<br>(3/3)         | 12<br>(3-/3)       | 13<br>(3/3)      | 12<br>(3-/3)    | 9<br>(2,3-/3-,2)     |
| BCC1474 | PI<br>349896 |  | 12<br>(2,1/3,3+)   | 13<br>(3/3)          | 13<br>(3/3)         | 11<br>(3-,2,c/3,3-) | 9<br>(2,3-/3-)     | 11<br>(2,3-/3)   | 12<br>(3-/3)    | 9<br>(3-,2/3-,2)     |
| BCC1485 | PI<br>356580 |  | 10<br>(1,2/3)      | 13<br>(3/3)          | 13<br>(3,3-/3)      | 12<br>(2,3-/3)      | 12<br>(3-/3)       | 13<br>(3/3)      | 13<br>(3/3)     | 10<br>(3-/3-)        |
| BCC1495 | PI<br>356715 |  | 8<br>(1,c/3)       | 8<br>(1,0;,c/3-,2,c) | 10<br>(2,3-,3/3,3-) | 8<br>(2,1,c/3-,2,c) | 11<br>(3-/3,3-)    | 13<br>(3-,2/3)   | 10<br>(3-/3,3+) | 9<br>(3-,2/3-,2)     |
| BCC0015 | CIho<br>1604 |  | 10<br>(2,1/3)      | 13<br>(3/3)          | 10<br>(2,3-/3,3-,2) | 13<br>(3,3-/3)      | 13<br>(3/3)        | 10<br>(2/3)      | 11<br>(3-,2/3)  | 9<br>(3-,2,1/3-,2)   |
| BCC1510 | PI<br>356775 |  | 12<br>(2,1,3-/3)   | 14<br>(3/3,3+)       | 12<br>(3,3-,2/3,3-) | 13<br>(3/3)         | 12<br>(3-/3)       | 10<br>(3-,2/3-)  | 11<br>(3-/3-,3) | 9<br>(3-,2,1/3-,2)   |
| BCC1522 | PI<br>361641 |  | 11<br>(3-,2/3)     | 10<br>(2,3-/3,3-,2)  | 12<br>(3,3-/3,3-)   | 13<br>(3,3-/3)      | 12<br>(3-/3)       | 11<br>(3-/3,3-)  | 12<br>(3-/3)    | 10<br>(3-,2/3-)      |
| BCC1525 | PI<br>361665 |  | 13<br>(2,3-/3)     | 13<br>(3,3-/3)       | 12<br>(3,3-/3,3-)   | 13<br>(3/3)         | 11<br>(3-/3,3-)    | 10<br>(3-/3-)    | 12<br>(3-/3)    | 8<br>(2,3-,1/2,3-,1) |
| BCC1535 | PI<br>361695 |  | 13<br>(3-,2/3)     | 13<br>(3,3-/3)       | 13<br>(3,3-,2/3)    | 11<br>(3-,2/3,3-,2) | 10<br>(2,3-/3,3-)  | 13<br>(3/3)      | 11<br>(2,3-/3)  | 9<br>(3-,2/3-,2)     |
| BCC1556 | PI<br>369732 |  | 9<br>(2,1,3-/3,3-) | 13<br>(3,3-/3)       | 8<br>(2,3-,1/2,3-)  | 11<br>(2,3-,1/3)    | 10<br>(3-/3-)      | 9<br>(2/3)       | 10<br>(3-/3-)   | 9<br>(2,3-/3-,2)     |
| BCC0156 | CIho<br>7127 |  | 12<br>(1,0;,2/3)   | 11<br>(2,3-/3)       | 13<br>(3/3)         | 13<br>(3,3-/3)      | 12<br>(3,3-/3,3-)  | 9<br>(2,3-/3-)   | 13<br>(3,3-/3)  | 9<br>(2,3-/3-,2)     |
| BCC1562 | PI<br>369747 |  | 10<br>(1,2/3)      | 13<br>(3,3-,2/3)     | 14<br>(3,3+/3,3+)   | 14<br>(3,3+/3,3+)   | 13<br>(3,3-/3)     | 14<br>(3/3,3+)   | 12<br>(3-/3)    | 9<br>(3-,2/3-,2)     |
| BCC0157 | CIho<br>7153 |  | 12<br>(2,3-/3+)    | 15<br>(3+/3+)        | 14<br>(3/3,3+)      | 14<br>(3/3,3+)      | 13<br>(3,3-/3)     | 10<br>(3-/3-)    | 13<br>(3/3)     | 11<br>(3-,2/3,3-)    |
| BCC1576 | PI<br>370994 |  | 13<br>(2,3-/3)     | 13<br>(3/3)          | 13<br>(3,3-/3)      | 14<br>(3,3-/3+)     | 11<br>(3-/3,3-)    | 12<br>(3-/3)     | 13<br>(3/3)     | 12<br>(3-/3)         |



|         |              |  |                     |                    |                     |                        |                 |                    |                   |                      |
|---------|--------------|--|---------------------|--------------------|---------------------|------------------------|-----------------|--------------------|-------------------|----------------------|
| BCC1577 | PI<br>371017 |  | 13<br>(3-/3,3+)     | 12<br>(3-/3)       | 14<br>(3/3,3+)      | 14<br>(3/3,3+)         | 13<br>(3/3)     | 13<br>(3/3)        | 14<br>(3/3,3+)    | 13<br>(3/3)          |
| BCC1582 | PI<br>371102 |  | 12<br>(2,1/3)       | 12<br>(3-/3)       | 13<br>(3,3-,2/3)    | 8<br>(2,3-,1/2,3-,1,n) | 11<br>(3-/3,-)  | 11<br>(2,3-/3,3+)  | 13<br>(3,3-/3)    | 10<br>(3-,2/3-)      |
| BCC1614 | PI<br>372084 |  | 11<br>(0,;1/3)      | 13<br>(3,3-/3)     | 12<br>(3-/3,3+)     | 12<br>(3,3-,2/3)       | 13<br>(3-/3+)   | 9<br>(2,3-/3-,2)   | 12<br>(3-/3)      | 9<br>(3-,2/3-,2)     |
| BCC1620 | PI<br>372099 |  | 13<br>(3-/3,3+)     | 13<br>(3/3)        | 14<br>(3/3,3+)      | 12<br>(3-/3)           | 13<br>(3/3)     | 12<br>(3-/3)       | 13<br>(3/3)       | 11<br>(3-/3,3-)      |
| BCC1623 | PI<br>372102 |  | 9<br>(2,3-/3,3-,2)  | 10<br>(2,3-/3,3-)  | 13<br>(3,3-/3)      | 13<br>(3,3-/3)         | 13<br>(3,3-/3)  | 12<br>(3-/3)       | 12<br>(3-/3)      | 11<br>(3-,2/3,3-)    |
| BCC1625 | PI<br>374413 |  | 12<br>(3-,2/3,3+)   | 13<br>(3/3)        | 13<br>(3,3-/3)      | 13<br>(3/3)            | 13<br>(3/3)     | 13<br>(3/3)        | 13<br>(3/3)       | 11<br>(2,3-/3)       |
| BCC1636 | PI<br>378218 |  | 10<br>(1/3)         | 13<br>(3,3-/3)     | 13<br>(3,3-,c/3,3+) | 14<br>(3,3-/3++)       | 12<br>(3-/3)    | 12<br>(3-,2/3+)    | 12<br>(3-/3)      | 9<br>(3-,2/3-,2)     |
| BCC1640 | PI<br>382296 |  | 8<br>(2,1,c/3-,2)   | 9<br>(2,3-/3-,2)   | 13<br>(3,3-/3)      | 13<br>(3,3-/3)         | 13<br>(3/3)     | 9<br>(2,3-,1/3-,2) | 11<br>(3-,2/3)    | 9<br>(3-,2/3-,2)     |
| BCC1655 | PI<br>386406 |  | 11<br>(2,3-/3,3-,2) | 9<br>(2,3-,1/3-,2) | 12<br>(3,3-,2/3,3-) | 10<br>(2,1/3)          | 12<br>(3-/3)    | 9<br>(3-,2/3-,2)   | 13<br>(3/3)       | 8<br>(2,3-,2/3-)     |
| BCC0168 | CIho<br>7491 |  | 9<br>(2,3-/3,3-)    | 13<br>(3,3-/3,3+)  | 13<br>(3,3-,2/3)    | 14<br>(3/3,3+)         | 12<br>(3-/3)    | 12<br>(3-/3)       | 11<br>(3-/3,3-)   | 9<br>(3-,2/3-,2)     |
| BCC0169 | CIho<br>7492 |  | 10<br>(2,3-/3,3+)   | 12<br>(3-,2/3,3+)  | 13<br>(3/3)         | 13<br>(3/3)            | 11<br>(3-/3,3-) | 9<br>(2,3-/3-)     | 13<br>(3/3)       | 9<br>(3-,2/3-,2)     |
| BCC1691 | PI<br>392464 |  | 9<br>(1,0,;2,3-/3)  | 12<br>(1,2,3-/3)   | 8<br>(2,3-/2,3-,c)  | 11<br>(2,3-/3)         | 12<br>(3-/3)    | 11<br>(3-/3,3-)    | 11<br>(3-,2/3,3-) | 8<br>(2,3-/2,3-)     |
| BCC1694 | PI<br>392471 |  | 10<br>(0;/3)        | 13<br>(3,3-,2/3)   | 13<br>(3,3-/3)      | 11<br>(2,3-/3)         | 10<br>(3-/3-)   | 9<br>(2,3-/3-,2)   | 9<br>(2,3-/3-,2)  | 9<br>(2,3-/3-)       |
| BCC1695 | PI<br>392478 |  | 8<br>(0;/3)         | 9<br>(2,3-/3-,2)   | 13<br>(3,3-/3)      | 13<br>(3,3-/3)         | 10<br>(2/3-,3)  | 10<br>(3-/3-)      | 11<br>(2,3-/3)    | 10<br>(3-,2/3-)      |
| BCC1702 | PI<br>392499 |  | 8<br>(0,;1/3-,3)    | 8<br>(1,2,0;/3,3-) | 13<br>(3,3-/3)      | 11<br>(2,3-/3,3+)      | 11<br>(3-,2/3)  | 11<br>(2,3-/3)     | 10<br>(3-,2/3-)   | 11<br>(2,3-/3)       |
| BCC1712 | PI<br>399482 |  | 12<br>(3-,2/3)      | 13<br>(3,3-,2/3)   | 13<br>(3,3-/3)      | 12<br>(3-,2/3,3-)      | 13<br>(3/3)     | 12<br>(3-/3)       | 13<br>(3/3)       | 8<br>(2,3-,1/2,3-,1) |
| BCC1719 | PI<br>401939 |  | 13<br>(0,;1/3,3+)   | 13<br>(3/3)        | 10<br>(2,3-/3,3-)   | 13<br>(3/3)            | 8<br>(2/3,3-)   | 10<br>(3-,2/3-)    | 11<br>(3-/3,3-)   | 9<br>(3-,2/3-,2)     |
| BCC1724 | PI<br>401973 |  | 11<br>(2,1/3,3+)    | 13<br>(3,3-,2/3)   | 13<br>(3,3-/3,3+)   | 13<br>(3,3-,2/3)       | 12<br>(3-,3/3)  | 13<br>(3/3)        | 13<br>(3/3)       | 9<br>(3-,2/3-,2)     |

|         |              |  |                   |                   |                     |                     |                  |                   |                   |                    |
|---------|--------------|--|-------------------|-------------------|---------------------|---------------------|------------------|-------------------|-------------------|--------------------|
| BCC1732 | PI<br>402098 |  | 10<br>(0;/3)      | 11<br>(2,3-/3)    | 8<br>(2,1,3-/3)     | 11<br>(3-,2/3,3-)   | 10<br>(3-,2/3-)  | 10<br>(3-/3-)     | 10<br>(3-/3-)     | 9<br>(2,3-/3-,2)   |
| BCC1738 | PI<br>402168 |  | 13<br>(2,3-/3+)   | 13<br>(3/3)       | 11<br>(3-/3,3-)     | 13<br>(3,3-/3)      | 10<br>(3-,2/3-)  | 9<br>(2,3-/3-)    | 10<br>(3-/3-)     | 11<br>(3-,2/3,3-)  |
| BCC1741 | PI<br>402264 |  | 9<br>(1,0;2/3)    | 12<br>(3-/3)      | 11<br>(2,3-/3)      | 14<br>(3,3-/3+)     | 13<br>(3/3)      | 13<br>(3/3)       | 13<br>(3/3)       | 9<br>(3-,2,1/3-,2) |
| BCC1744 | PI<br>402352 |  | 12<br>(2,1/3,3+)  | 14<br>(3/3,3+)    | 13<br>(3,3-/3)      | 14<br>(3/3,3+)      | 13<br>(3/3)      | 10<br>(3-,2/3-)   | 12<br>(3-/3)      | 9<br>(3-,2/3-,2)   |
| BCC1745 | PI<br>402354 |  | 8<br>(2,1/3,3-)   | 9<br>(2,3-/3-,2)  | 11<br>(2,3-/3)      | 8<br>(1,0;2/3,3-)   | 11<br>(3-/3,3-)  | 9<br>(2,3-/3-)    | 10<br>(3-/3-)     | 9<br>(2,3-/3-,2)   |
| BCC0175 | CIho<br>7782 |  | 10<br>(2,3-/3,3+) | 11<br>(3-,2/3)    | 12<br>(3-/3)        | 10<br>(2,3-/3,3-)   | 13<br>(3/3)      | 14<br>(3/3,3+)    | 13<br>(3,3/3)     | 9<br>(3-,2/3-,2)   |
| BCC1751 | PI<br>402468 |  | 10<br>(2,1/3)     | 13<br>(3,3-/3)    | 13<br>(3,3-/3)      | 13<br>(3,3-/3)      | 9<br>(2,3-/3-,2) | 11<br>(2,3-/3)    | 12<br>(3-,2/3,3+) | 9<br>(2,3-/3-,2)   |
| BCC1796 | PI<br>415012 |  | 11<br>(3-,2/3)    | 13<br>(3/3)       | 10<br>(2,3-/3,3-)   | 13<br>(3/3)         | 13<br>(3/3)      | 12<br>(3-/3)      | 13<br>(3/3)       | 9<br>(3-,2/3-,2)   |
| BCC1830 | PI<br>428411 |  | 12<br>(3-,2/3)    | 13<br>(3/3)       | 13<br>(3,3-,2/3)    | 10<br>(2,3-/3,3-)   | 13<br>(3,3-/3)   | 10<br>(2,3-/3,3-) | 13<br>(3/3)       | 9<br>(2,3-/3-,2)   |
| BCC1831 | PI<br>428413 |  | 10<br>(2,3-/3,3+) | 13<br>(3-,3/3,3+) | 13<br>(3,3-/3)      | 13<br>(3/3)         | 13<br>(3/3)      | 13<br>(3,3-/3)    | 13<br>(3/3)       | 10<br>(3-,2/3-)    |
| BCC1839 | PI<br>428628 |  | 10<br>(1,n/3)     | 11<br>(3-,2,1/3)  | 10<br>(3-,2,c/3,3-) | 13<br>(3,3-/3)      | 12<br>(3-/3)     | 9<br>(2,3-/3-,2)  | 13<br>(3/3)       | 12<br>(3,3-/3,3-)  |
| BCC1852 | PI<br>429313 |  | 9<br>(2,3-/3)     | 13<br>(3,3-/3)    | 12<br>(3,3-/3,3-)   | 9<br>(1,2/3,3-)     | 12<br>(3-/3)     | 9<br>(2,3-/3-,2)  | 12<br>(3-/3)      | 9<br>(2,3-/3-)     |
| BCC1857 | PI<br>429519 |  | 13<br>(3-,2/3+)   | 14<br>(3/3,3+)    | 13<br>(3/3)         | 15<br>(3+/3+)       | 11<br>(2,3-/3)   | 9<br>(2,3-/3-)    | 12<br>(3-/3)      | 9<br>(3-,2/3-,2)   |
| BCC1876 | PI<br>434815 |  | 12<br>(3-/3+)     | 14<br>(3/3+)      | 14<br>(3/3,3+)      | 15<br>(3+/3+)       | 13<br>(3/3)      | 12<br>(3-/3)      | 13<br>(3/3)       | 12<br>(3-,3/3,3-)  |
| BCC1879 | PI<br>436135 |  | 11<br>(1,0;2/3)   | 10<br>(2,1,3-/3)  | 12<br>(3-/3)        | 10<br>(2,3-,1/3,3-) | 13<br>(3/3)      | 8<br>(2,3-/2,3-)  | 13<br>(3/3)       | 9<br>(3-,2/3-,2)   |
| BCC1886 | PI<br>436146 |  | 9<br>(2,1/3,3+)   | 13<br>(3,3-/3,3+) | 12<br>(3,3-/3,3-,c) | 11<br>(2,3-/3)      | 13<br>(3,3-/3)   | 14<br>(3/3+)      | 13<br>(3/3)       | 10<br>(3-/3-)      |
| BCC1889 | PI<br>436150 |  | 9<br>(2,1/3)      | 11<br>(3-,2/3)    | 13<br>(3,3-/3,3+)   | 11<br>(2,3-,1/3)    | 9<br>(2,3-/3-)   | 13<br>(3/3)       | 13<br>(3/3)       | 9<br>(3-,2/3-,2)   |
| BCC1891 | PI<br>436153 |  | 12<br>(0;1/3)     | 9<br>(1,2,3-/3)   | 13<br>(3,3-/3)      | 11<br>(3-/3,3-)     | 10<br>(3-/3-)    | 12<br>(3-/3)      | 12<br>(3-/3)      | 10<br>(3-,2/3-)    |

|         |               |  |                   |                  |                       |                     |                  |                   |                |                      |
|---------|---------------|--|-------------------|------------------|-----------------------|---------------------|------------------|-------------------|----------------|----------------------|
| BCC1918 | PI<br>447207  |  | 9<br>(2,3-/3)     | 11<br>(2,3-/3)   | 11<br>(3-,2/3,3-)     | 10<br>(2,3-/3,3-,c) | 13<br>(3,3-/3)   | 10<br>(3-,2/3-)   | 12<br>(3-/3)   | 10<br>(3-/3-)        |
| BCC1971 | PI<br>467454  |  | 9<br>(2,3-/3)     | 9<br>(3-,2/3-,2) | 12<br>(3,3-,2/3,3-,c) | 11<br>(2,3-/3)      | 9<br>(2,3-/3-)   | 12<br>(3-/3)      | 13<br>(3/3)    | 9<br>(2,3-/3-)       |
| BCC2016 | PI<br>467786  |  | 11<br>(3-/3)      | 13<br>(3,3-/3)   | 13<br>(3,3-/3)        | 11<br>(2,3-,1/3)    | 12<br>(3-/3)     | 13<br>(3-,2/3)    | 12<br>(3-,3/3) | 9<br>(2,3-/3-,2)     |
| BCC2022 | PI<br>467814  |  | 11<br>(1,2/3,3+)  | 13<br>(3/3)      | 13<br>(3,3-/3)        | 13<br>(3/3,c)       | 13<br>(3/3)      | 12<br>(3-/3)      | 13<br>(3/3)    | 10<br>(3-/3-)        |
| BCC2027 | PI<br>467839  |  | 9<br>(0,;1/3)     | 11<br>(3-,2/3)   | 13<br>(3,3-/3)        | 11<br>(2,3-,1/3)    | 14<br>(3/3,3+)   | 11<br>(3-,2/3)    | 12<br>(3-/3)   | 9<br>(2,3-/3-,2)     |
| BCC2028 | PI<br>467840  |  | 9<br>(0,;1/3)     | 13<br>(3/3)      | 8<br>(2,3-/3,3-)      | 12<br>(1,2,0/3,3-)  | 10<br>(3-,2/3-)  | 12<br>(3-/3)      | 12<br>(3-/3)   | 9<br>(3-,2/3-,2)     |
| BCC2066 | PI<br>485524  |  | 12<br>(2,1/3)     | 12<br>(3-/3)     | 13<br>(3/3)           | 13<br>(3/3)         | 13<br>(3/3)      | 10<br>(3-/3-)     | 12<br>(3-/3)   | 10<br>(3-/3-)        |
| BCC2074 | PI<br>494099  |  | 13<br>(2,3-/3)    | 13<br>(3/3)      | 14<br>(3/3,3+)        | 14<br>(3/3,3+)      | 12<br>(3-/3)     | 12<br>(3-/3)      | 13<br>(3/3)    | 8<br>(2,3-/2,3-)     |
| BCC2083 | PI<br>498436  |  | 10<br>(2/3)       | 13<br>(3/3)      | 11<br>(2,3-/3,3+)     | 11<br>(2,3-,1/3)    | 13<br>(3/3)      | 10<br>(2/3)       | 12<br>(3-/3)   | 8<br>(2,3-,1/2,3-,1) |
| BCC2118 | PI<br>531896  |  | 13<br>(2,3-/3)    | 13<br>(3/3)      | 13<br>(3/3)           | 14<br>(3/3+)        | 11<br>(3-,2/3)   | 9<br>(2,3-/3-)    | 12<br>(3-/3)   | 10<br>(2,3-/3,3-,2)  |
| BCC2162 | PI<br>564477  |  | 10<br>(2,3-/3)    | 9<br>(2,3-/3-)   | 13<br>(3,3-/3)        | 11<br>(2,3-/3)      | 9<br>(2,3-/3-)   | 10<br>(3-/3-)     | 12<br>(3-/3)   | 8<br>(2,3-/2,3-)     |
| BCC2236 | PI<br>572588  |  | 10<br>(3-,2/3,3-) | 11<br>(2,3-/3)   | 12<br>(3,3-/3,3-)     | 13<br>(3,3-/3,3+)   | 10<br>(3-/3-)    | 11<br>(3-,2/3)    | 12<br>(3-/3)   | 8<br>(2,3-,1/2,3-,1) |
| BCC2241 | PI<br>573594  |  | 10<br>(2,3-/3)    | 12<br>(3-/3)     | 12<br>(3,3-,2/3,3-)   | 12<br>(3,3-/3,3-)   | 13<br>(3/3)      | 10<br>(3-,2/3-)   | 12<br>(3-/3)   | 8<br>(2,3-/2,3-)     |
| BCC2242 | PI<br>573598  |  | 8<br>(0/3)        | 8<br>(0,;1/3)    | 9<br>(2,1,3-/3,3-)    | 10<br>(2,3-/3,3-,2) | 14<br>(3/3+)     | 13<br>(3/3)       | 13<br>(3,3-/3) | 11<br>(3-,3/3-,3)    |
| BCC2259 | PI<br>573682  |  | 11<br>(2,3-/3)    | 13<br>(3/3)      | 13<br>(3,3-,2/3,3+)   | 13<br>(3/3)         | 12<br>(3-/3,3+)  | 10<br>(3-/3-)     | 13<br>(3/3)    | 12<br>(3,3-/3,3-)    |
| BCC0231 | Clho<br>11789 |  | 8<br>(1,0;/3-)    | 9<br>(2,3-/3-,2) | 8<br>(2,1,3-/3,3-)    | 9<br>(2,3-,1/3-,c)  | 11<br>(2,3-,c/3) | 10<br>(3-,2/3-,c) | 12<br>(3-/3)   | 9<br>(2,3-/3-,2)     |
| BCC2352 | PI<br>592240  |  | 12<br>(3-,2/3)    | 11<br>(2,3-,1/3) | 13<br>(3,3-/3)        | 14<br>(3/3,3+)      | 11<br>(3-/3,3-)  | 13<br>(3,3-/3)    | 13<br>(3/3)    | 9<br>(2,3-/3-,2)     |
| BCC2362 | PI<br>592282  |  | 9<br>(0,;1,2,n/3) | 13<br>(3/3)      | 10<br>(2,3-,1/3,3-,2) | 13<br>(3,3-/3)      | 11<br>(3-,2/3)   | 10<br>(3-,2/3-)   | 12<br>(3-/3)   | 9<br>(2,3-/3-,2)     |

|         |               |  |                     |                   |                     |                       |                |                   |                   |                      |
|---------|---------------|--|---------------------|-------------------|---------------------|-----------------------|----------------|-------------------|-------------------|----------------------|
| BCC2371 | PI<br>599633  |  | 10<br>(1/3)         | 12<br>(3-/3)      | 12<br>(3,3-/3,3-)   | 13<br>(3,3-/3)        | 10<br>(3-/3-)  | 13<br>(3/3)       | 12<br>(3-/3)      | 10<br>(3-/3-)        |
| BCC2381 | PI<br>605699  |  | 12<br>(2,3-,1/3,3+) | 11<br>(2,3-/3)    | 12<br>(3,3-,2/3,3-) | 13<br>(3/3)           | 12<br>(3-/3)   | 9<br>(2,3-/3-)    | 12<br>(3-/3)      | 8<br>(2,3-/2,3-)     |
| BCC2399 | PI<br>611513  |  | 10<br>(0;/3,3-)     | 9<br>(2,3-/3-)    | 9<br>(2,1,3-/3,3-)  | 11<br>(2,3-/3)        | 13<br>(3/3)    | 10<br>(2/3-)      | 13<br>(3/3)       | 12<br>(3-/3)         |
| BCC2400 | PI<br>611526  |  | 11<br>(3-/3)        | 11<br>(2,3-/3)    | 12<br>(3,3-/3,3-)   | 11<br>(3-,2/3)        | 11<br>(3-,2/3) | 12<br>(3-/3)      | 12<br>(3-/3)      | 8<br>(2,3-/2,3-)     |
| BCC2414 | PI<br>629116  |  | 9<br>(1,0;/3,3-,2)  | 12<br>(3-/3)      | 11<br>(2,3-,1/3)    | 11<br>(2,3-,1,c/3,3+) | 12<br>(3-/3)   | 10<br>(3-,2/3-)   | 10<br>(2,3-/3-,3) | 8<br>(2,3-/2,3-)     |
| BCC0242 | CIho<br>11852 |  | 11<br>(2,3-/3)      | 13<br>(3,3-/3)    | 13<br>(3,3-/3)      | 13<br>(3,3-/3)        | 13<br>(3/3)    | 13<br>(3,3-/3)    | 12<br>(3-/3)      | 11<br>(2,3-/3)       |
| BCC2423 | PI<br>639299  |  | 13<br>(3-/3,3+)     | 12<br>(3-,2/3,3+) | 11<br>(3-,c/3,3-)   | 11<br>(3-,2/3,3-,2)   | 13<br>(3/3)    | 10<br>(3-/3-)     | 13<br>(3/3)       | 14<br>(3,3+/3,3+)    |
| BCC2424 | PI<br>639300  |  | 11<br>(3-,2/3,3+)   | 14<br>(3/3,3+)    | 13<br>(3,3-/3)      | 11<br>(2,3-,c/3)      | 13<br>(3/3)    | 11<br>(3-/3-,3)   | 13<br>(3,3-/3)    | 12<br>(3-/3)         |
| BCC2530 | PI<br>643227  |  | 13<br>(0;/3+)       | 13<br>(3/3)       | 13<br>(3,3-/3)      | 13<br>(3,3-/3,3+)     | 9<br>(2/3)     | 13<br>(3/3)       | 12<br>(3-/3)      | 10<br>(3-,2/3-)      |
| BCC0255 | CIho<br>13453 |  | 9<br>(2,1/3)        | 10<br>(2,1,3-/3)  | 10<br>(2,3-/3,3-)   | 13<br>(3,3-/3)        | 13<br>(3,3-/3) | 10<br>(2,3-/3,3-) | 10<br>(3-/3-)     | 13<br>(3/3)          |
| BCC2559 | PI<br>643370  |  | 12<br>(1,0;/3,3+)   | 10<br>(2,3-/3,3-) | 12<br>(3,3-,2/3,3-) | 13<br>(3/3)           | 11<br>(2,3-/3) | 10<br>(2,3-/3,3-) | 11<br>(3-,2/3)    | 9<br>(2,3-/3-,2)     |
| BCC0260 | CIho<br>13651 |  | 12<br>(3-/3,3+)     | 13<br>(3/3)       | 13<br>(3/3)         | 11<br>(3-,2/3)        | 12<br>(3-/3)   | 11<br>(2,3-/3)    | 12<br>(3-/3)      | 13<br>(3,3-/3)       |
| BCC0261 | CIho<br>13653 |  | 12<br>(2,3-/3)      | 13<br>(3/3)       | 13<br>(3/3)         | 13<br>(3/3)           | 12<br>(3-/3)   | 12<br>(3-/3)      | 13<br>(3/3)       | 10<br>(2,3-/3,3-)    |
| BCC0270 | CIho<br>13824 |  | 11<br>(3-/3)        | 13<br>(3,3-/3)    | 13<br>(3,3-/3)      | 13<br>(3/3)           | 12<br>(3-/3)   | 12<br>(3-/3)      | 13<br>(3/3)       | 8<br>(2,3-,1/2,3-,1) |
| BCC0318 | CIho<br>14334 |  | 13<br>(2,3-/3,3+)   | 13<br>(3/3)       | 13<br>(3,3-/3)      | 11<br>(3-,2/3)        | 12<br>(3-/3)   | 10<br>(2,3-/3)    | 12<br>(3-/3)      | 8<br>(2,3-/2,3-)     |
| BCC0334 | CIho<br>15203 |  | 9<br>(2,3-/3)       | 8<br>(1,0;;2-/3)  | 13<br>(3,3-,2/3)    | 11<br>(2,3-,1/3)      | 12<br>(3-/3)   | 10<br>(3-/3-)     | 11<br>(3-,2/3)    | 8<br>(2,3-/2,3-)     |
| BCC0376 | CIho<br>15616 |  | 13<br>(2,3-/3)      | 11<br>(2,3-/3)    | 12<br>(3,3-/3,3-)   | 13<br>(3,3-/3,3+)     | 13<br>(3/3)    | 13<br>(3,3-/3)    | 13<br>(3/3)       | 8<br>(2,3-/2,3-)     |
| BCC0392 | CIho<br>16612 |  | 11<br>(2,3-/3,3+)   | 13<br>(3/3)       | 13<br>(3,3-/3,c)    | 11<br>(2,3-/3)        | 13<br>(3/3)    | 9<br>(2,3-/3)     | 13<br>(3,3-/3)    | 9<br>(2,3-/3-,2)     |

|         |              |  |                     |                    |                       |                     |                 |                  |                   |                   |
|---------|--------------|--|---------------------|--------------------|-----------------------|---------------------|-----------------|------------------|-------------------|-------------------|
| BCC0435 | PI 39590     |  | 8<br>(2,1/3)        | 10<br>(3-,2/3-)    | 12<br>(3,3-,2/3,3-,2) | 11<br>(2,3-/3)      | 10<br>(3-/3-)   | 9<br>(2,1/3-)    | 12<br>(3-/3)      | 9<br>(2,3-/3-,2)  |
| BCC0444 | PI 47541     |  | 13<br>(2,3-/3)      | 13<br>(3,3-/3)     | 11<br>(2,3-,c/3)      | 13<br>(3/3)         | 10<br>(3-/3-)   | 9<br>(3-,2/3-,2) | 11<br>(3-,2/3,3-) | 9<br>(2,3-/3-,2)  |
| BCC0490 | PI 61589     |  | 12<br>(2,3-/3)      | 13<br>(3/3)        | 13<br>(3,3-/3)        | 14<br>(3/3,3+)      | 13<br>(2,1/3)   | 12<br>(3-/3)     | 13<br>(3/3)       | 10<br>(3-/3-)     |
| BCC0057 | CIho<br>3240 |  | 13<br>(3-/3)        | 14<br>(3/3,3+)     | 14<br>(3/3,3+)        | 13<br>(3,3-/3)      | 13<br>(3/3)     | 9<br>(2,3-/3-)   | 10<br>(3-/3-)     | 8<br>(2,3-/2,3-)  |
| BCC0579 | PI 94806     |  | 13<br>(3,3-/3)      | 13<br>(3/3)        | 13<br>(3/3)           | 13<br>(3/3)         | 11<br>(3-/3,3-) | 12<br>(3-/3)     | 13<br>(3/3)       | 14<br>(3,3+/3,3+) |
| BCC0058 | CIho<br>3387 |  | 12<br>(1,2/3,3+)    | 13<br>(3/3)        | 12<br>(3,3-,2/3,3-)   | 13<br>(3,3-/3)      | 11<br>(3-,2/3)  | 10<br>(3-/3-)    | 13<br>(3/3)       | 8<br>(2,3-/2,3-)  |
| BCC0592 | PI 95167     |  | 11<br>(3-,2/3)      | 13<br>(3/3)        | 12<br>(3,3-/3,3-)     | 14<br>(3/3,3+)      | 13<br>(3/3)     | 9<br>(2,3-/3)    | 13<br>(3/3)       | 10<br>(3-,2/3-)   |
| BCC0597 | PI 95198     |  | 9<br>(2,1,3-/3)     | 13<br>(3,3-/3)     | 13<br>(3/3)           | 13<br>(3,3-/3)      | 11<br>(2,3-/3)  | 10<br>(3-/3-)    | 11<br>(3-/3,3-)   | 9<br>(2,3-/3-,2)  |
| BCC0603 | PI 95270     |  | 9<br>(2,3-/3,3+)    | 11<br>(2,3-/3,3+)  | 13<br>(3,3-,2/3,3+)   | 13<br>(3/3)         | 14<br>(3/3,3+)  | 12<br>(3-/3)     | 14<br>(3/3,3+)    | 9<br>(2,3-/3-,2)  |
| BCC0669 | PI<br>157890 |  | 12<br>(2,3-,1/3,3+) | 9<br>(2,3-,1/3-,2) | 12<br>(3,3-,2/3,3-)   | 13<br>(3,3-,2/3)    | 10<br>(3-,2/3-) | 10<br>(3-,2/3-)  | 13<br>(3/3)       | 8<br>(2,1/3-,2)   |
| BCC0673 | PI<br>159126 |  | 8<br>(2,3-/3,3-)    | 12<br>(2/3,3-)     | 11<br>(2,3-/3)        | 8<br>(1,2/3,3-)     | 10<br>(3-/3-)   | 9<br>(2/3,3-)    | 9<br>(2,3-/3-,2)  | 9<br>(2,3-/3-,2)  |
| BCC0007 | CIho<br>1388 |  | 12<br>(1,0:/3)      | 12<br>(3-/3)       | 12<br>(3,3-,2/3,3-)   | 12<br>(3,3-,2/3,3-) | 13<br>(3,3-/3)  | 10<br>(3-,2/3-)  | 10<br>(3-,2/3-)   | 8<br>(2,3-/2,3-)  |
| BCC0713 | PI<br>175506 |  | 11<br>(0:/3,3+)     | 13<br>(3,3-/3)     | 12<br>(3,3-,2/3,3-)   | 12<br>(3,3-,2/3,3-) | 13<br>(3/3)     | 13<br>(3/3)      | 11<br>(2,3-/3)    | 13<br>(3/3)       |
| BCC0718 | PI<br>176042 |  | 12<br>(3-,2/3)      | 11<br>(3-,2,1/3)   | 11<br>(3-,2/3)        | 14<br>(3/3,3+)      | 12<br>(3-/3)    | 13<br>(3/3)      | 13<br>(3/3)       | 11<br>(3-/3,3-)   |
| BCC0728 | PI<br>178285 |  | 13<br>(3-/3+)       | 14<br>(3/3+)       | 13<br>(3,3-/3,3+)     | 14<br>(3/3,3+)      | 13<br>(3/3)     | 13<br>(3/3)      | 12<br>(3-/3)      | 9<br>(2,3-/3-,2)  |
| BCC0736 | PI<br>180670 |  | 8<br>(0:,1/3,3-)    | 9<br>(2,1,3-/3,3-) | 13<br>(3,3-,2/3)      | 9<br>(2-,1/3,3-,2)  | 12<br>(3-/3)    | 10<br>(3-/3-)    | 12<br>(3-/3)      | 9<br>(3-,2/3-,2)  |
| BCC0741 | PI<br>181148 |  | 11<br>(1,2,n/3,3-)  | 11<br>(3-/3,3-)    | 13<br>(3/3)           | 12<br>(3,3-/3,3-)   | 11<br>(2,3-/3)  | 11<br>(3-,2/3)   | 12<br>(3-/3)      | 11<br>(3-,2/3,3-) |
| BCC0743 | PI<br>182373 |  | 12<br>(2,3-/3)      | 10<br>(3-,2/3-)    | 13<br>(3/3)           | 13<br>(3,3-/3,3+)   | 13<br>(3/3)     | 9<br>(2,3-/3-)   | 13<br>(3,3-/3)    | 9<br>(2,3-/3-,2)  |

|         |              |  |                    |                  |                     |                      |                 |                  |                   |                    |
|---------|--------------|--|--------------------|------------------|---------------------|----------------------|-----------------|------------------|-------------------|--------------------|
| BCC0746 | PI<br>182625 |  | 13<br>(3/3,3+)     | 13<br>(3/3)      | 12<br>(3,3-/3)      | 13<br>(3/3)          | 13<br>(3/3)     | 10<br>(2/3)      | 14<br>(3,3+/3,3+) | 14<br>(3/3,3+)     |
| BCC0762 | PI<br>183590 |  | 11<br>(2,3-/3,3+)  | 13<br>(3/3)      | 12<br>(3,3-/3,3-,c) | 12<br>(3,3-/3,3-)    | 13<br>(3,3-/3)  | 12<br>(3-/3)     | 14<br>(3/3,3+)    | 10<br>(3-,2/3-)    |
| BCC0077 | CIho<br>3886 |  | 12<br>(2,3-/3)     | 11<br>(2,3-,c/3) | 10<br>(2,3-/3,3-)   | 10<br>(2,3-,3/3,3-)  | 12<br>(3-/3)    | 9<br>(2/3-)      | 10<br>(3-,2/3-)   | 11<br>(3-,2/3)     |
| BCC0821 | PI<br>193062 |  | 13<br>(1/3,3+)     | 13<br>(3/3)      | 13<br>(3,3-/3,3+)   | 13<br>(3,3-/3)       | 12<br>(3-/3)    | 14<br>(3/3+)     | 13<br>(3/3)       | 8<br>(2,3-/2,3-)   |
| BCC0862 | PI<br>223134 |  | 12<br>(2,1,3-,0/3) | 13<br>(3,3-/3)   | 12<br>(1,2,3-,n/3)  | 13<br>(3/3)          | 13<br>(3,3-/3)  | 11<br>(2,3-/3)   | 12<br>(3-/3)      | 8<br>(2,3-,1/2,3-) |
| BCC0875 | PI<br>223879 |  | 13<br>(3,3-/3)     | 12<br>(3-/3)     | 12<br>(3,3-/3,3-)   | 13<br>(3/3)          | 13<br>(3/3)     | 12<br>(3-/3,3+)  | 14<br>(3/3,3+)    | 13<br>(3,3-/3,3+)  |
| BCC0884 | PI<br>226612 |  | 12<br>(2,3-/3)     | 12<br>(3-/3)     | 12<br>(3-/3)        | 11<br>(3-,2/3)       | 10<br>(3-,2/3-) | 13<br>(3/3)      | 12<br>(3-/3)      | 9<br>(2,3-/3-,2)   |
| BCC0889 | PI<br>231151 |  | 11<br>(3-,2/3)     | 12<br>(3-/3)     | 13<br>(3,3-/3)      | 14<br>(3/3,3+)       | 13<br>(3/3)     | 13<br>(3,3-/3)   | 14<br>(3/3,3+)    | 8<br>(2,3-/2,3-)   |
| BCC0899 | PI<br>235172 |  | 12<br>(3-/3)       | 11<br>(3-,2/3)   | 13<br>(3/3)         | 11<br>(3-,2/3)       | 13<br>(3,3-/3)  | 10<br>(3-/3-)    | 13<br>(3,3-/3)    | 9<br>(2,3-/3-,2)   |
| BCC0950 | PI<br>259878 |  | 8<br>(2,1,3-/3,3-) | 11<br>(3-/3,3-)  | 8<br>(2,3-/2,3-)    | 13<br>(3,3-/3)       | 12<br>(3-/3)    | 8<br>(2,3-/2,3-) | 10<br>(3-/3-)     | 8<br>(2,3-/2,3-)   |
| BCC0985 | PI<br>264916 |  | 13<br>(3-/3,3+)    | 11<br>(2,3-/3)   | 10<br>(2,3-/3,3-)   | 9<br>(2,1,0;3-/3,3-) | 13<br>(3-/3)    | 9<br>(2,3-/3,3-) | 10<br>(3-/3-)     | 13<br>(3/3)        |
| BCC0994 | PI<br>266132 |  | 12<br>(2,1,3-/3)   | 13<br>(3,3-/2/3) | 10<br>(2,3-,1/3,3-) | 11<br>(3-,2,1/3)     | 11<br>(2,3-/3)  | 10<br>(3-/3-)    | 12<br>(3-/3)      | 10<br>(3-,2/3-)    |

<sup>a</sup> Sequential number given to all accessions from the Barley iCore Collection (BCC).

<sup>b</sup> Number assigned by the United States Department of Agriculture-Agricultural Research Service, Germplasm Resources Information Network (GRIN).

<sup>c</sup> Races for which the accession gave a median infection type (IT)  $\leq 2$  (linear score  $\leq 7$ ).

<sup>d</sup> Seedling infection type (IT) based on size of uredinia using the 0-4 scale. Shown here is the median linear score conversion of the IT given in Table 3.4., and the minimum and maximum ITs observed shown as “median linear score (minimum raw IT/ maximum raw IT)” (Miller and Lambert 1955; Stakman et al. 1962).

**Table 3.11.** Pearson correlation coefficient among seedling infection types of Barley iCore Collection Selects (BCCS) to *Puccinia graminis* f. sp. *tritici* races TTKSK, TTKST, TKTTF, TRTTF, QCCJB, HKHJC, and MCCFC and also *Puccinia graminis* f. sp. *secalis* isolate 92-MN-90.

|                 | <b>TTKSK</b>      | <b>TTKST</b>   | <b>TRTTF</b> | <b>TKTTF</b> | <b>QCCJB</b> | <b>HKHJC</b> | <b>MCCFC</b> |
|-----------------|-------------------|----------------|--------------|--------------|--------------|--------------|--------------|
| <b>TTKST</b>    | 0.63 <sup>a</sup> | - <sup>b</sup> | -            | -            | -            | -            | -            |
| <b>TRTTF</b>    | 0.58              | 0.51           | -            | -            | -            | -            | -            |
| <b>TKTTF</b>    | 0.52              | 0.53           | 0.42         | -            | -            | -            | -            |
| <b>QCCJB</b>    | 0.47              | 0.42           | 0.47         | 0.40         | -            | -            | -            |
| <b>HKHJC</b>    | 0.21              | 0.17           | 0.13         | 0.48         | 0.31         | -            | -            |
| <b>MCCFC</b>    | 0.35              | 0.29           | 0.30         | 0.59         | 0.40         | 0.76         | -            |
| <b>92-MN-90</b> | 0.47              | 0.41           | 0.41         | 0.29         | 0.34         | 0.03         | 0.19         |

<sup>a</sup> Linear score conversion of infection types used in analysis, see Table 3.4.

<sup>b</sup>“-“ indicates data omitted.

**Table 3.12.** Resistance genes in the Barley iCore Collection Selects (BCCS) postulated by comparison with the reaction of barley stem rust controls carrying known resistance genes as executed in the RGenePos package in R.

| <b>Predicted Resistance Genes<sup>a</sup></b> | <b>Count</b> | <b>Expected Resistance Spectrum</b>                   |
|---|--------------|---|
| <i>Rpg3</i>                                   | 45           | 92-MN-90  |
| <i>Rpg2 Rpg3</i>                              | 2            | HKHJC 92-MN-90  |
| <i>Rpg1 Rpg2 Rpg3</i>                         | 1            | TRTTF HKHJC MCCFC 92-MN-90                            |
| <i>Rpg3 rpg4/Rpg5</i>                         | 3            | TTKSK TTKST TKTTF TRTTF QCCJB<br>MCCFC 92-MN-90       |
| <i>Rpg1 Rpg2 Rpg3 rpg4/Rpg5</i>               | 1            | TTKSK TTKST TKTTF TRTTF QCCJB<br>HKHJC MCCFC 92-MN-90 |
| + <sup>b</sup>                                | 59           | Varies <sup>c</sup>                                   |
| <i>Rpg3</i> +                                 | 29           | 92-MN-90 +  |
| <i>Rpg2 Rpg3</i> +                            | 8            | HKHJC 92-MN-90 +                                      |
| <i>Rpg1 Rpg2 Rpg3</i> +                       | 2            | TKTTF TRTTF HKHJC MCCFC 92-MN-90 +                    |
| None  | 140          | -   |

<sup>a</sup> Genes predicted using the RGenePos package in R

<sup>b</sup> “+” used to denote when an accession has a unknown or additional resistance gene(s) alone or in combination with known genes

<sup>c</sup> 20 different resistance spectra were found, the most common were HKHJC and MCCFC =14; TRTTF, HKHJC, and MCCFC = 9; and TRTTF = 8.



**Table 3.13.** Genotype outcomes of the *Rpg1* and *rpg4/Rpg5* markers for the Barley iCore Collection Selects (BCCS) and barley stem rust controls carrying different resistance genes.

| <b>Barley <i>Rpg</i> controls</b> |                  |                          |                          |                          |               |   |                                 |                                  |        |                                  |     |    |     |
|-----------------------------------|------------------|--------------------------|--------------------------|--------------------------|---------------|---|---------------------------------|----------------------------------|--------|----------------------------------|-----|----|-----|
| Control                           | Known Gene       | <i>Rpg1</i> <sup>d</sup> | <i>Rpg5</i> <sup>c</sup> | Improvement <sup>e</sup> | Country       | Resistance spectrum <sup>f</sup>                                | Predicted genes <sup>g</sup>    | <i>Rpg1</i> markers <sup>i</sup> |        | <i>Rpg5</i> markers <sup>h</sup> |     |    |     |
|                                   |                  |                          |                          |                          |               |   |                                 | RPG1-N                           | RPG1-S | PP2C                             | LRK | R5 | R10 |
| Chevron                           | <i>Rpg1</i>      | +                        | -                        | Landrace                 | Switzerland   | MCCFC<br>HKHJC<br>TRTTF 92-<br>MN-90                            | <i>Rpg1 Rpg2</i><br><i>Rpg3</i> | +                                | -      | +                                | -   |    |     |
| 80-TT-29                          | <i>Rpg1</i>      | +                        | -                        | Genetic Stock            | United States | MCCFC<br>HKHJC<br>TRTTF 92-<br>MN-90                            | <i>Rpg1 Rpg2</i><br><i>Rpg3</i> | +                                | -      | +                                | -   |    |     |
| Hietpas-5                         | <i>Rpg2</i>      | -                        | -                        | Cultivar                 | United States | HKHJC 92-<br>MN-90  | <i>Rpg2 Rpg3</i>                | -                                | -      | +                                | -   |    |     |
| GAW-79                            | <i>Rpg3</i>      | -                        | -                        | Landrace                 | Ethiopia      | 92-MN-90  | <i>Rpg3</i>                     | -                                | +      | +                                | -   |    |     |
| Q/SM20                            | <i>rpg4/Rpg5</i> | -                        | +                        | Genetic Stock            | United States | TTKSK<br>TTKST<br>TKTTF<br>TRTTF<br>QCCJB<br>MCCFC 92-<br>MN-90 | <i>Rpg3</i><br><i>rpg4/Rpg5</i> | -                                | +      | -                                | +   | -  | -   |
| 212Y1                             | <i>rpg6</i>      | -                        | -                        | Genetic Stock            | Canada        | QCCJB<br>HKHJC 92-<br>MN-90                                     | -                               | -                                | -      | -                                | +   | +  |     |
| Black Hulless                     | <i>rpgBH</i>     | -                        | -                        | Landrace                 | China         |   | -                               | -                                | -      | +                                | -   |    |     |

|                |                                   |   |   |               |                |  |   |   |   |   |   |   |   |
|----------------|-----------------------------------|---|---|---------------|----------------|--|---|---|---|---|---|---|---|
| Diamond        | <i>Rpg1</i> +<br><i>RpgU</i>      | + | - | Cultivar      | Canada         | MCCFC<br>HKHJC<br>TRTTF 92-<br>MN-90                                     | -   | + | - | + | - |   |   |
| Q21861         | <i>Rpg1</i> +<br><i>rpg4/Rpg5</i> | + | + | Breeding Line | Mexico         | TTKSK<br>TTKST<br>TKTTF<br>TRTTF<br>QCCJB<br>HKHJC<br>MCCFC 92-<br>MN-90 | <i>Rpg1 Rpg2</i><br><i>Rpg3</i><br><i>rpg4/Rpg5</i> | + | - | - | + | - | - |
| 80-tt-30       | none                              | - | - | Genetic Stock | United States  |  | none  | - | - | + | - |   |   |
| Step toe       | none                              | - | - | Cultivar      | United States  |  | none  | - | + | + | - |   |   |
| Hiproly        | none                              | - | - | Landrace      | Ethiopia       |  | none  | - | - | + | - |   |   |
| Golden Promise | none                              | - | - | Cultivar      | United Kingdom |  | none  | - | - | - | + | + |   |
| PI 532013      | none                              | - | - | Landrace      | Egypt          |  | none  | - | - | - | + | - | + |
| Hv492          | none                              | - | - | Landrace      | Switzerland    |  | none  | - | - | - | + | - | + |

BCCS accessions

| Name <sup>a</sup> | GRIN Name <sup>b</sup> | Rpg1 <sup>d</sup> | Rpg5 <sup>c</sup> | Improvement <sup>e</sup> | County    | Resistance spectrum <sup>f</sup>                            | Predicted genes <sup>g</sup>                        | RPG1-N | RPG1-S | PP2C | LRK | R5 | R10 |
|-------------------|------------------------|-------------------|-------------------|--------------------------|-----------|---|---|--------|--------|------|-----|----|-----|
| BCC2297           | PI 584766              | +                 | +                 | Breeding                 | Australia | TTKSK TTKST<br>TKTTF TRTTF<br>QCCJB HKHJC<br>MCCFC 92-MN-90 | <i>Rpg1 Rpg2</i><br><i>Rpg3</i><br><i>rpg4/Rpg5</i> | +      | -      | -    | +   | -  | -   |
| BCC0654           | PI 155303              | -                 | +                 | Landrace                 | Yemen     | 92-MN-90  | <i>Rpg3</i>   | -      | -      | -    | +   | -  | -   |

|         |              |   |   |          |              |   |                                 |   |   |   |   |   |   |
|---------|--------------|---|---|----------|--------------|---|---------------------------------|---|---|---|---|---|---|
| BCC1548 | PI<br>365547 | - | + | Cultivar | Netherlands  | 92-MN-90  | <i>Rpg3</i>                     | - | + | - | + | - | - |
| BCC1859 | PI<br>429526 | - | + | Landrace | Nepal        | 92-MN-90  | <i>Rpg3</i>                     | - | + | - | + | - | - |
| BCC2291 | PI<br>574094 | - | + | Landrace | Nepal        | 92-MN-90  | <i>Rpg3</i>                     | - | + | - | + | - | - |
| BCC1600 | PI<br>371377 | - | + | Landrace | Switzerland  | TTKSK TTKST<br>TKTTF TRTTF<br>QCCJB MCCFC<br>92-MN-90 | <i>Rpg3</i><br><i>rpg4/Rpg5</i> | - | - | - | + | - | - |
| BCC1602 | PI<br>371390 | - | + | Landrace | Switzerland  | TTKSK TTKST<br>TKTTF TRTTF<br>QCCJB MCCFC<br>92-MN-90 | <i>Rpg3</i><br><i>rpg4/Rpg5</i> | - | + | - | + | - | - |
| BCC1603 | PI<br>371392 | - | + | Landrace | Switzerland  | TTKSK TTKST<br>TKTTF TRTTF<br>QCCJB MCCFC<br>92-MN-90 | <i>Rpg3</i><br><i>rpg4/Rpg5</i> | - | - | - | + | - | - |
| BCC0524 | PI<br>76285  | - | + | Landrace | Iraq         | TTKSK TTKST<br>TKTTF QCCJB<br>MCCFC 92-MN-<br>90      | <i>Rpg3,+</i>                   | - | + | - | + | - | - |
| BCC1568 | PI<br>370852 | - | + | Landrace | Switzerland  | TTKSK TTKST<br>TKTTF TRTTF<br>QCCJB 92-MN-90          | <i>Rpg3,+</i>                   | - | + | - | + | - | - |
| BCC1103 | PI<br>289811 | - | + | Landrace | Iran         | TTKSK TTKST<br>TRTTF QCCJB<br>92-MN-90                | <i>Rpg3,+</i>                   | - | - | - | + | - | - |
| BCC2382 | PI<br>606305 | - | + | Landrace | Saudi Arabia | TTKSK TTKST<br>QCCJB 92-MN-90                         | <i>Rpg3,+</i>                   | - | + | - | + | - | - |
| BCC1665 | PI<br>386838 | - | + | Landrace | Ethiopia     | TTKSK QCCJB<br>92-MN-90                               | <i>Rpg3,+</i>                   | - | - | - | + | - | - |
| BCC1592 | PI<br>371248 | - | + | Landrace | Switzerland  | TTKSK TTKST<br>92-MN-90                               | <i>Rpg3,+</i>                   | - | - | - | + | - | - |
| BCC0740 | PI<br>181113 | - | + | Landrace | India        | MCCFC 92-MN-<br>90                                    | <i>Rpg3,+</i>                   | - | - | - | + | - | - |

|         |               |   |   |           |                  |                            |   |   |   |   |   |   |   |
|---------|---------------|---|---|-----------|------------------|----------------------------|---|---|---|---|---|---|---|
| BCC1743 | PI<br>402335  | + | - | Uncertain | Colombia         | TTKSK TRTTF<br>HKHJC MCCFC | + | + | - | + | - |   |   |
| BCC0244 | Clho<br>11864 | + | - | Breeding  | United<br>States | TRTTF HKHJC<br>MCCFC       | + | + | - | + | - |   |   |
| BCC1720 | PI<br>401952  | + | - | Uncertain | Colombia         | TRTTF HKHJC<br>MCCFC       | + | + | - | + | - |   |   |
| BCC1797 | PI<br>415014  | + | - | Cultivar  | Mexico           | TRTTF HKHJC<br>MCCFC       | + | + | - | + | - |   |   |
| BCC2527 | PI<br>643212  | + | - | Breeding  | United<br>States | TRTTF HKHJC<br>MCCFC       | + | + | - | + | - |   |   |
| BCC2537 | PI<br>643257  | + | - | Breeding  | United<br>States | TRTTF HKHJC<br>MCCFC       | + | + | - | + | - |   |   |
| BCC2548 | PI<br>643310  | + | - | Breeding  | United<br>States | TRTTF HKHJC<br>MCCFC       | + | + | - | + | - |   |   |
| BCC2549 | PI<br>643314  | + | - | Breeding  | United<br>States | TRTTF HKHJC<br>MCCFC       | + | + | - | + | - |   |   |
| BCC2558 | PI<br>643369  | + | - | Breeding  | United<br>States | TRTTF HKHJC<br>MCCFC       | + | + | - | + | - |   |   |
| BCC2561 | PI<br>643377  | + | - | Breeding  | United<br>States | TRTTF HKHJC<br>MCCFC       | + | + | - | + | - |   |   |
| BCC0377 | Clho<br>15624 | + | - | Cultivar  | Canada           | HKHJC MCCFC                | + | + | - | + | - |   |   |
| BCC1737 | PI<br>402164  | + | - | Uncertain | Colombia         | HKHJC MCCFC                | + | + | - | + | - |   |   |
| BCC1872 | PI<br>434760  | + | - | Breeding  | Canada           | HKHJC MCCFC                | + | + | - | + | - |   |   |
| BCC1875 | PI<br>434814  | + | - | Breeding  | Canada           | HKHJC MCCFC                | + | + | - | + | - |   |   |
| BCC2415 | PI<br>632274  | + | - | Cultivar  | United<br>States | HKHJC MCCFC                | + | + | - | + | - |   |   |
| BCC2430 | PI<br>639999  | + | - | Breeding  | United<br>States | HKHJC MCCFC                | + | + | - | + | - |   |   |
| BCC2462 | PI<br>640166  | + | - | Breeding  | United<br>States | HKHJC MCCFC                | + | + | - | - | + | - | + |
| BCC2505 | PI<br>640366  | + | - | Breeding  | United<br>States | HKHJC MCCFC                | + | + | - | + | - |   |   |

|         |              |   |   |           |                 |  |                             |   |   |   |   |   |   |
|---------|--------------|---|---|-----------|-----------------|--|-----------------------------|---|---|---|---|---|---|
| BCC2533 | PI<br>643232 | + | - | Breeding  | United States   | HKHJC MCCFC                            | +                           | + | - | + | - |   |   |
| BCC2552 | PI<br>643336 | + | - | Breeding  | United States   | HKHJC MCCFC                            | +                           | + | - | + | - |   |   |
| BCC2553 | PI<br>643339 | + | - | Breeding  | United States   | HKHJC MCCFC                            | +                           | + | - | + | - |   |   |
| BCC2556 | PI<br>643364 | + | - | Breeding  | United States   | HKHJC MCCFC                            | +                           | + | - | + | - |   |   |
| BCC2557 | PI<br>643368 | + | - | Breeding  | United States   | HKHJC MCCFC                            | +                           | + | - | + | - |   |   |
| BCC2562 | PI<br>643383 | + | - | Breeding  | United States   | HKHJC MCCFC                            | +                           | + | - | + | - |   |   |
| BCC2555 | PI<br>643362 | + | - | Breeding  | United States   | HKHJC                                  | +                           | + | - | + | - |   |   |
| BCC2142 | PI<br>548724 | + | - | Uncertain | Afghanist<br>an | TRTTF                                  | +                           | + | - | + | - |   |   |
| BCC0673 | PI<br>159126 | + | - | Landrace  | Mexico          |  | none                        | + | - | + | - |   |   |
| BCC1003 | PI<br>268167 | + | - | Cultivar  | Ukraine         |  | none                        | + | - | - | + | - | + |
| BCC1495 | PI<br>356715 | + | - | Landrace  | Morocco         |  | none                        | + | - | + | - |   |   |
| BCC1209 | PI<br>320219 | + | - | Uncertain | Australia       | TRTTF HKHJC<br>MCCFC 92-MN-<br>90      | <i>Rpg1 Rpg2<br/>Rpg3</i>   | + | - | + | - |   |   |
| BCC1822 | PI<br>422230 | + | - | Landrace  | Yemen           | TKTTF TRTTF<br>HKHJC MCCFC<br>92-MN-90 | <i>Rpg1 Rpg2<br/>Rpg3 +</i> | + | - | + | - |   |   |
| BCC2544 | PI<br>643288 | + | - | Breeding  | United States   | TTKSK TRTTF<br>HKHJC MCCFC<br>92-MN-90 | <i>Rpg1 Rpg2<br/>Rpg3 +</i> | + | - | + | - |   |   |
| BCC2560 | PI<br>643376 | + | - | Breeding  | United States   | TTKSK TTKST<br>HKHJC MCCFC<br>92-MN-90 | <i>Rpg2 Rpg3<br/>+</i>      | + | - | + | - |   |   |
| BCC2384 | PI<br>608667 | + | - | Cultivar  | United States   | HKHJC MCCFC<br>92-MN-90                | <i>Rpg2 Rpg3<br/>+</i>      | + | - | - | + | - | + |

|         |               |   |   |           |                  |                                     |                       |   |   |   |   |   |   |
|---------|---------------|---|---|-----------|------------------|-------------------------------------|-----------------------|---|---|---|---|---|---|
| BCC2413 | PI<br>620640  | + | - | Cultivar  | Canada           | HKHJC MCCFC<br>92-MN-90             | <i>Rpg2 Rpg3</i><br>+ | + | - | + | - |   |   |
| BCC2380 | PI<br>605472  | + | - | Cultivar  | United<br>States | 92-MN-90                            | <i>Rpg3</i>           | + | + | + | - |   |   |
| BCC1851 | PI<br>429312  | + | - | Landrace  | Yemen            | TTKSK TKTTF<br>TRTTF 92-MN-90       | <i>Rpg3,+</i>         | + | + | + | - |   |   |
| BCC1917 | PI<br>447191  | + | - | Uncertain | Spain            | TTKST TKTTF<br>TRTTF 92-MN-90       | <i>Rpg3,+</i>         | + | - | + | - |   |   |
| BCC1195 | PI<br>308142  | + | - | Cultivar  | Ukraine          | TTKSK TTKST<br>92-MN-90             | <i>Rpg3,+</i>         | + | - | - | + | + |   |
| BCC2431 | PI<br>640006  | - | - | Breeding  | United<br>States | TTKSK TKTTF<br>TRTTF HKHJC<br>MCCFC | +                     | - | + | + | - |   |   |
| BCC2397 | PI<br>611511  | - | - | Cultivar  | Ukraine          | TTKSK TTKST<br>TRTTF                | +                     | - | - | + | - |   |   |
| BCC1721 | PI<br>401954  | - | - | Uncertain | Colombia         | QCCJB HKHJC                         | +                     | - | - | + | - |   |   |
| BCC2024 | PI<br>467831  | - | - | Cultivar  | Poland           | TKTTF TRTTF                         | +                     | - | + | + | - |   |   |
| BCC2545 | PI<br>643292  | - | - | Breeding  | United<br>States | TRTTF HKHJC                         | +                     | - | - | + | - |   |   |
| BCC1750 | PI<br>402445  | - | - | Uncertain | Colombia         | TTKSK HKHJC                         | +                     | - | - | + | - |   |   |
| BCC1156 | PI<br>295442  | - | - | Landrace  | Ethiopia         | TTKSK QCCJB                         | +                     | - | + | + | - |   |   |
| BCC1755 | PI<br>404204  | - | - | Cultivar  | Ukraine          | TTKSK TKTTF                         | +                     | - | + | + | - |   |   |
| BCC0434 | PI<br>39397   | - | - | Uncertain | Australia        | TTKSK TRTTF                         | +                     | - | - | + | - |   |   |
| BCC0212 | CIho<br>10661 | - | - | Breeding  | United<br>States | TTKSK TTKST                         | +                     | - | - | - | + | - | + |
| BCC1639 | PI<br>382275  | - | - | Landrace  | Ethiopia         | TTKSK TTKST                         | +                     | - | + | + | - |   |   |
| BCC0395 | CIho<br>16676 | - | - | Genetic   | United<br>States | TTKST QCCJB                         | +                     | - | + | - | + | - | + |
| BCC1160 | PI<br>295960  | - | - | Cultivar  | Israel           | TTKST QCCJB                         | +                     | - | + | + | - |   |   |

|         |              |   |   |           |                    |       |   |   |   |   |   |   |   |
|---------|--------------|---|---|-----------|--------------------|-------|---|---|---|---|---|---|---|
| BCC0138 | CIho<br>6496 | - | - | Uncertain | China              | HKHJC | + | - | + | + | - |   |   |
| BCC1131 | PI<br>290353 | - | - | Cultivar  | Kyrgyzstan         | HKHJC | + | - | - | + | - |   |   |
| BCC1310 | PI<br>327902 | - | - | Cultivar  | Bulgaria           | HKHJC | + | - | + | + | - |   |   |
| BCC1571 | PI<br>370869 | - | - | Landrace  | Switzerland        | HKHJC | + | - | - | + | + | - | + |
| BCC1100 | PI<br>286388 | - | - | Landrace  | Eritrea            | QCCJB | + | - | + | - | - |   |   |
| BCC2152 | PI<br>559516 | - | - | Landrace  | Nepal              | QCCJB | + | - | - | + | - |   |   |
| BCC0422 | PI<br>38320  | - | - | Landrace  | Russian Federation | TKTTF | + | - | - | - | + | - | + |
| BCC1783 | PI<br>410865 | - | - | Cultivar  | Netherlands        | TKTTF | + | - | + | + | - |   |   |
| BCC1157 | PI<br>295581 | - | - | Landrace  | Ethiopia           | TRTTF | + | - | + | + | - |   |   |
| BCC1661 | PI<br>386601 | - | - | Landrace  | Ethiopia           | TRTTF | + | - | + | + | - |   |   |
| BCC1850 | PI<br>429311 | - | - | Landrace  | Yemen              | TRTTF | + | - | - | + | - |   |   |
| BCC1928 | PI<br>449279 | - | - | Breeding  | Spain              | TRTTF | + | - | - | + | - |   |   |
| BCC2084 | PI<br>498437 | - | - | Cultivar  | New Zealand        | TRTTF | + | - | - | + | + | - | + |
| BCC2168 | PI<br>564502 | - | - | Cultivar  | Bolivia            | TRTTF | + | - | - | + | - |   |   |
| BCC2401 | PI<br>611527 | - | - | Cultivar  | Ukraine            | TRTTF | + | - | + | + | - |   |   |
| BCC1219 | PI<br>321784 | - | - | Cultivar  | Belgium            | TTKSK | + | - | - | + | + | + |   |
| BCC1578 | PI<br>371056 | - | - | Landrace  | Switzerland        | TTKSK | + | - | - | - | + | - | + |
| BCC1624 | PI<br>372107 | - | - | Cultivar  | Moldova            | TTKSK | + | - | - | - | + | - | + |

|         |               |   |   |           |                   |                               |                       |   |   |   |   |   |   |
|---------|---------------|---|---|-----------|-------------------|-------------------------------|-----------------------|---|---|---|---|---|---|
| BCC2023 | PI<br>467815  | - | - | Cultivar  | Finland           | TTKSK                         | +                     | - | - | + | - |   |   |
| BCC0394 | CIho<br>16671 | - | - | Genetic   | United<br>States  | TTKST                         | +                     | - | - | - | + | - | + |
| BCC0308 | CIho<br>14291 | - | - | Landrace  | China             | HKHJC 92-MN-90                | <i>Rpg2 Rpg3</i>      | - | + | + | - |   |   |
| BCC2092 | PI<br>506295  | - | - | Breeding  | United<br>Kingdom | HKHJC 92-MN-90                | <i>Rpg2 Rpg3</i>      | - | - | + | - |   |   |
| BCC0174 | CIho<br>7556  | - | - | Breeding  | Argentina         | TRTTF QCCJB<br>HKHJC 92-MN-90 | <i>Rpg2 Rpg3</i><br>+ | - | - | + | - |   |   |
| BCC2065 | PI<br>483048  | - | - | Cultivar  | Australia         | TTKSK TTKST<br>HKHJC 92-MN-90 | <i>Rpg2 Rpg3</i><br>+ | - | - | - | + | - | + |
| BCC2233 | PI<br>566497  | - | - | Uncertain | China             | TTKST TRTTF<br>HKHJC 92-MN-90 | <i>Rpg2 Rpg3</i><br>+ | - | - | + | - |   |   |
| BCC0750 | PI<br>182661  | - | - | Landrace  | Lebanon           | QCCJB HKHJC<br>92-MN-90       | <i>Rpg2 Rpg3</i><br>+ | - | + | + | - |   |   |
| BCC2254 | PI<br>573662  | - | - | Landrace  | Georgia           | QCCJB HKHJC<br>92-MN-90       | <i>Rpg2 Rpg3</i><br>+ | - | + | + | + | - | + |
| BCC0180 | CIho<br>8054  | - | - | Breeding  | United<br>States  | 92-MN-90                      | <i>Rpg3</i>           | - | - | + | - |   |   |
| BCC0240 | CIho<br>11845 | - | - | Uncertain | Israel            | 92-MN-90                      | <i>Rpg3</i>           | - | - | + | + | - | + |
| BCC0266 | CIho<br>13743 | - | - | Landrace  | Eritrea           | 92-MN-90                      | <i>Rpg3</i>           | - | + | + | - |   |   |
| BCC0324 | CIho<br>14395 | - | - | Landrace  | Armenia           | 92-MN-90                      | <i>Rpg3</i>           | - | - | - | + | - | + |
| BCC0476 | PI<br>60663   | - | - | Landrace  | Libya             | 92-MN-90                      | <i>Rpg3</i>           | - | - | + | - |   |   |
| BCC0694 | PI<br>168328  | - | - | Landrace  | Turkey            | 92-MN-90                      | <i>Rpg3</i>           | - | - | + | - |   |   |
| BCC0738 | PI<br>181102  | - | - | Landrace  | India             | 92-MN-90                      | <i>Rpg3</i>           | - | - | + | - |   |   |
| BCC0747 | PI<br>182645  | - | - | Cultivar  | Japan             | 92-MN-90                      | <i>Rpg3</i>           | - | - | - | + | - | + |
| BCC0771 | PI<br>184103  | - | - | Landrace  | Monteneg<br>ro    | 92-MN-90                      | <i>Rpg3</i>           | - | - | + | - |   |   |



|         |              |   |   |           |                   |          |             |   |   |   |   |   |   |
|---------|--------------|---|---|-----------|-------------------|----------|-------------|---|---|---|---|---|---|
| BCC0774 | PI<br>184873 | - | - | Cultivar  | Sweden            | 92-MN-90 | <i>Rpg3</i> | - | - | + | - |   |   |
| BCC0777 | PI<br>184880 | - | - | Breeding  | Sweden            | 92-MN-90 | <i>Rpg3</i> | - | - | + | - |   |   |
| BCC0822 | PI<br>193063 | - | - | Cultivar  | Belgium           | 92-MN-90 | <i>Rpg3</i> | - | - | + | - |   |   |
| BCC0829 | PI<br>194556 | - | - | Breeding  | Germany           | 92-MN-90 | <i>Rpg3</i> | - | + | + | - |   |   |
| BCC0842 | PI<br>201097 | - | - | Cultivar  | Austria           | 92-MN-90 | <i>Rpg3</i> | - | + | + | - |   |   |
| BCC0856 | PI<br>221326 | - | - | Uncertain | Serbia            | 92-MN-90 | <i>Rpg3</i> | - | - | + | - |   |   |
| BCC0908 | PI<br>237571 | - | - | Breeding  | Colombia          | 92-MN-90 | <i>Rpg3</i> | - | + | + | - |   |   |
| BCC1069 | PI<br>283397 | - | - | Landrace  | Armenia           | 92-MN-90 | <i>Rpg3</i> | - | + | + | + | - | + |
| BCC1142 | PI<br>294739 | - | - | Uncertain | Western<br>Asia   | 92-MN-90 | <i>Rpg3</i> | - | - | - | + | - | + |
| BCC1182 | PI<br>306439 | - | - | Uncertain | Romania           | 92-MN-90 | <i>Rpg3</i> | - | - | + | - |   |   |
| BCC1206 | PI<br>320213 | - | - | Uncertain | Australia         | 92-MN-90 | <i>Rpg3</i> | - | - | + | - |   |   |
| BCC1337 | PI<br>328327 | - | - | Landrace  | Turkey            | 92-MN-90 | <i>Rpg3</i> | - | + | + | - |   |   |
| BCC1377 | PI<br>328976 | - | - | Landrace  | Ethiopia          | 92-MN-90 | <i>Rpg3</i> | - | - | + | - |   |   |
| BCC1394 | PI<br>330400 | - | - | Cultivar  | Czech<br>Republic | 92-MN-90 | <i>Rpg3</i> | - | + | + | - |   |   |
| BCC1515 | PI<br>361036 | - | - | Breeding  | United<br>Kingdom | 92-MN-90 | <i>Rpg3</i> | - | - | + | - |   |   |
| BCC1529 | PI<br>361672 | - | - | Cultivar  | Denmark           | 92-MN-90 | <i>Rpg3</i> | - | + | + | - |   |   |
| BCC1584 | PI<br>371111 | - | - | Landrace  | Switzerlan<br>d   | 92-MN-90 | <i>Rpg3</i> | - | - | - | + | + |   |
| BCC1595 | PI<br>371320 | - | - | Landrace  | Switzerlan<br>d   | 92-MN-90 | <i>Rpg3</i> | - | - | - | + | - | + |

|         |               |   |   |           |                   |                               |                 |   |   |   |   |   |   |
|---------|---------------|---|---|-----------|-------------------|-------------------------------|-----------------|---|---|---|---|---|---|
| BCC1789 | PI<br>412946  | - | - | Uncertain | South<br>Africa   | 92-MN-90                      | <i>Rpg3</i>     | - | - | - | + | - | + |
| BCC1832 | PI<br>428491  | - | - | Cultivar  | Netherlan<br>ds   | 92-MN-90                      | <i>Rpg3</i>     | - | - | - | + | - | + |
| BCC2013 | PI<br>467749  | - | - | Cultivar  | Netherlan<br>ds   | 92-MN-90                      | <i>Rpg3</i>     | - | - | + | + | - | + |
| BCC2017 | PI<br>467789  | - | - | Cultivar  | Netherlan<br>ds   | 92-MN-90                      | <i>Rpg3</i>     | - | - | - | + | - | + |
| BCC2019 | PI<br>467797  | - | - | Cultivar  | Greece            | 92-MN-90                      | <i>Rpg3</i>     | - | - | + | - |   |   |
| BCC2091 | PI<br>506293  | - | - | Cultivar  | United<br>Kingdom | 92-MN-90                      | <i>Rpg3</i>     | - | + | - | + | + |   |
| BCC2093 | PI<br>506299  | - | - | Breeding  | United<br>Kingdom | 92-MN-90                      | <i>Rpg3</i>     | - | - | - | + | + |   |
| BCC2144 | PI<br>548736  | - | - | Uncertain | Ethiopia          | 92-MN-90                      | <i>Rpg3</i>     | - | - | + | - |   |   |
| BCC2265 | PI<br>573703  | - | - | Landrace  | Georgia           | 92-MN-90                      | <i>Rpg3</i>     | - | - | - | + | - | + |
| BCC2336 | PI<br>591928  | - | - | Cultivar  | Australia         | 92-MN-90                      | <i>Rpg3</i>     | - | - | + | - |   |   |
| BCC2342 | PI<br>592173  | - | - | Cultivar  | United<br>Kingdom | 92-MN-90                      | <i>Rpg3</i>     | - | - | + | + | + |   |
| BCC2373 | PI<br>599640  | - | - | Cultivar  | Czech<br>Republic | 92-MN-90                      | <i>Rpg3</i>     | - | + | + | - |   |   |
| BCC2378 | PI<br>601065  | - | - | Cultivar  | United<br>States  | 92-MN-90                      | <i>Rpg3</i>     | - | - | + | - |   |   |
| BCC2536 | PI<br>643247  | - | - | Breeding  | United<br>States  | TTKSK TTKST<br>TRTTF 92-MN-90 | <i>Rpg3</i> , + | - | - | + | - |   |   |
| BCC0991 | PI<br>265462  | - | - | Cultivar  | Finland           | TTKST TRTTF<br>QCCJB 92-MN-90 | <i>Rpg3</i> , + | - | - | + | - |   |   |
| BCC2026 | PI<br>467836  | - | - | Cultivar  | Poland            | TKTTF TRTTF<br>92-MN-90       | <i>Rpg3</i> , + | - | + | - | + | + |   |
| BCC0333 | Clho<br>14978 | - | - | Landrace  | Ethiopia          | TRTTF QCCJB<br>92-MN-90       | <i>Rpg3</i> , + | - | - | + | - |   |   |
| BCC0131 | Clho<br>6371  | - | - | Breeding  | United<br>States  | TTKSK TRTTF<br>92-MN-90       | <i>Rpg3</i> , + | - | - | - | + | - | + |

|         |               |   |   |           |                                  |                |                 |   |   |   |   |   |   |
|---------|---------------|---|---|-----------|----------------------------------|----------------|-----------------|---|---|---|---|---|---|
| BCC0241 | CIho<br>11849 | - | - | Landrace  | Ethiopia                         | TRTTF 92-MN-90 | <i>Rpg3</i> , + | - | - | + | - |   |   |
| BCC0709 | PI<br>174478  | - | - | Cultivar  | France                           | TRTTF 92-MN-90 | <i>Rpg3</i> , + | - | - | + | - |   |   |
| BCC1472 | PI<br>349359  | - | - | Breeding  | United<br>Kingdom                | TRTTF 92-MN-90 | <i>Rpg3</i> , + | - | - | - | + | + |   |
| BCC1627 | PI<br>374420  | - | - | Landrace  | Bosnia<br>and<br>Herzegovi<br>na | TRTTF 92-MN-90 | <i>Rpg3</i> , + | - | - | + | - |   |   |
| BCC1823 | PI<br>422232  | - | - | Landrace  | Yemen                            | TRTTF 92-MN-90 | <i>Rpg3</i> , + | - | + | + | - |   |   |
| BCC2068 | PI<br>485536  | - | - | Breeding  | United<br>Kingdom                | TRTTF 92-MN-90 | <i>Rpg3</i> , + | - | - | - | + | + |   |
| BCC1570 | PI<br>370867  | - | - | Landrace  | Switzerlan<br>d                  | TTKSK 92-MN-90 | <i>Rpg3</i> , + | - | - | - | + | - | + |
| BCC1585 | PI<br>371149  | - | - | Landrace  | Switzerlan<br>d                  | TTKSK 92-MN-90 | <i>Rpg3</i> , + | - | - | + | + | - | + |
| BCC1703 | PI<br>392501  | - | - | Breeding  | South<br>Africa                  | TTKSK 92-MN-90 | <i>Rpg3</i> , + | - | - | + | - |   |   |
| BCC1938 | PI<br>466726  | - | - | Cultivar  | Sweden                           | TTKSK 92-MN-90 | <i>Rpg3</i> , + | - | - | + | - |   |   |
| BCC2345 | PI<br>592196  | - | - | Cultivar  | United<br>Kingdom                | TTKSK 92-MN-90 | <i>Rpg3</i> , + | - | - | - | + | - | + |
| BCC2408 | PI<br>611576  | - | - | Cultivar  | Ukraine                          | TTKSK 92-MN-90 | <i>Rpg3</i> , + | - | - | + | - |   |   |
| BCC0773 | PI<br>184872  | - | - | Genetic   | Sweden                           | TTKST 92-MN-90 | <i>Rpg3</i> , + | - | - | + | - |   |   |
| BCC2069 | PI<br>485548  | - | - | Breeding  | United<br>Kingdom                | TTKST 92-MN-90 | <i>Rpg3</i> , + | - | - | + | - |   |   |
| BCC0007 | CIho<br>1388  | - | - | Cultivar  | Denmark                          |                | none            | - | - | - | + | + |   |
| BCC0015 | CIho<br>1604  | - | - | Landrace  | Ethiopia                         |                | none            | - | - | + | - |   |   |
| BCC0057 | CIho<br>3240  | - | - | Uncertain | Egypt                            |                | none            | - | - | + | - |   |   |

|         |               |   |   |           |                           |      |   |   |   |   |   |   |
|---------|---------------|---|---|-----------|---------------------------|------|---|---|---|---|---|---|
| BCC0058 | CIho<br>3387  | - | - | Uncertain | Algeria                   | none | - | - | + | - |   |   |
| BCC0077 | CIho<br>3886  | - | - | Landrace  | India                     | none | - | - | - | - |   |   |
| BCC0156 | CIho<br>7127  | - | - | Cultivar  | United<br>Kingdom         | none | - | - | - | + | + |   |
| BCC0157 | CIho<br>7153  | - | - | Breeding  | United<br>States          | none | - | - | - | + | + |   |
| BCC0168 | CIho<br>7491  | - | - | Landrace  | Mexico                    | none | - | - | + | - |   |   |
| BCC0169 | CIho<br>7492  | - | - | Landrace  | Mexico                    | none | - | - | + | - |   |   |
| BCC0175 | CIho<br>7782  | - | - | Cultivar  | Slovakia                  | none | - | - | - | + | + |   |
| BCC0231 | CIho<br>11789 | - | - | Uncertain | Saudi<br>Arabia           | none | - | - | - | + | - | + |
| BCC0242 | CIho<br>11852 | - | - | Genetic   | United<br>States          | none | - | - | + | - |   |   |
| BCC0255 | CIho<br>13453 | - | - | Uncertain | Romania                   | none | - | - | + | - |   |   |
| BCC0260 | CIho<br>13651 | - | - | Breeding  | Israel                    | none | - | - | + | - |   |   |
| BCC0261 | CIho<br>13653 | - | - | Cultivar  | Former<br>Soviet<br>Union | none | - | - | - | + | - | + |
| BCC0270 | CIho<br>13824 | - | - | Cultivar  | United<br>States          | none | - | - | + | - | + |   |
| BCC0318 | CIho<br>14334 | - | - | Landrace  | Azerbaija<br>n            | none | - | - | + | - |   |   |
| BCC0334 | CIho<br>15203 | - | - | Breeding  | United<br>States          | none | - | + | + | - |   |   |
| BCC0376 | CIho<br>15616 | - | - | Breeding  | Canada                    | none | - | - | - | + | - | + |
| BCC0392 | CIho<br>16612 | - | - | Genetic   | United<br>States          | none | - | - | - | + | + |   |
| BCC0435 | PI<br>39590   | - | - | Landrace  | Algeria                   | none | - | - | + | - |   |   |

|         |              |   |   |           |                              |  |      |   |   |   |   |   |   |
|---------|--------------|---|---|-----------|------------------------------|--|------|---|---|---|---|---|---|
| BCC0444 | PI<br>47541  | - | - | Landrace  | Iran                         |  | none | - | - | - | + | + |   |
| BCC0490 | PI<br>61589  | - | - | Landrace  | Mongolia                     |  | none | - | - | + | - |   |   |
| BCC0579 | PI<br>94806  | - | - | Landrace  | The<br>country of<br>Georgia |  | none | - | - | - | + | - | + |
| BCC0592 | PI<br>95167  | - | - | Landrace  | Unknown                      |  | none | - | - | - | + | - | + |
| BCC0597 | PI<br>95198  | - | - | Landrace  | Ukraine                      |  | none | - | - | - | + | - | + |
| BCC0603 | PI<br>95270  | - | - | Landrace  | Unknown                      |  | none | - | - | + | - |   |   |
| BCC0669 | PI<br>157890 | - | - | Uncertain | Italy                        |  | none | - | - | + | - |   |   |
| BCC0713 | PI<br>175506 | - | - | Cultivar  | Finland                      |  | none | - | - | - | + | - | + |
| BCC0718 | PI<br>176042 | - | - | Landrace  | India                        |  | none | - | - | + | - |   |   |
| BCC0728 | PI<br>178285 | - | - | Landrace  | Turkey                       |  | none | - | - | + | - |   |   |
| BCC0736 | PI<br>180670 | - | - | Cultivar  | Germany                      |  | none | - | + | + | - |   |   |
| BCC0741 | PI<br>181148 | - | - | Cultivar  | Netherlan<br>ds              |  | none | - | + | - | + | - | + |
| BCC0743 | PI<br>182373 | - | - | Landrace  | Turkey                       |  | none | - | - | - | + | - | + |
| BCC0746 | PI<br>182625 | - | - | Cultivar  | Japan                        |  | none | - | - | + | - |   |   |
| BCC0762 | PI<br>183590 | - | - | Cultivar  | Austria                      |  | none | - | - | - | + | - | + |
| BCC0821 | PI<br>193062 | - | - | Cultivar  | Belgium                      |  | none | - | - | - | + | - | + |
| BCC0862 | PI<br>223134 | - | - | Uncertain | Jordan                       |  | none | - | - | + | - |   |   |
| BCC0875 | PI<br>223879 | - | - | Landrace  | Afghanist<br>an              |  | none | - | + | + | - |   |   |

|         |              |   |   |           |                       |  |      |   |   |   |   |   |   |
|---------|--------------|---|---|-----------|-----------------------|--|------|---|---|---|---|---|---|
| BCC0884 | PI<br>226612 | - | - | Uncertain | Cyprus                |  | none | - | - | + | - |   |   |
| BCC0889 | PI<br>231151 | - | - | Cultivar  | Colombia              |  | none | - | - | + | - |   |   |
| BCC0899 | PI<br>235172 | - | - | Breeding  | Turkey                |  | none | - | - | - | + | - | + |
| BCC0950 | PI<br>259878 | - | - | Breeding  | Croatia               |  | none | - | + | + | - |   |   |
| BCC0985 | PI<br>264916 | - | - | Landrace  | Croatia               |  | none | - | - | - | + | - | + |
| BCC0994 | PI<br>266132 | - | - | Cultivar  | Sweden                |  | none | - | - | - | - |   |   |
| BCC1060 | PI<br>280441 | - | - | Cultivar  | Russian<br>Federation |  | none | - | - | - | + | - | + |
| BCC1061 | PI<br>281524 | - | - | Cultivar  | Argentina             |  | none | - | - | - | + | - | + |
| BCC1062 | PI<br>281525 | - | - | Cultivar  | Argentina             |  | none | - | - | + | - |   |   |
| BCC1081 | PI<br>283429 | - | - | Cultivar  | Denmark               |  | none | - | - | + | - |   |   |
| BCC1101 | PI<br>286389 | - | - | Landrace  | Eritrea               |  | none | - | - | + | - |   |   |
| BCC1135 | PI<br>292016 | - | - | Cultivar  | Israel                |  | none | - | - | + | - |   |   |
| BCC1158 | PI<br>295956 | - | - | Breeding  | Israel                |  | none | - | - | + | - |   |   |
| BCC1207 | PI<br>320216 | - | - | Uncertain | Australia             |  | none | - | - | + | - |   |   |
| BCC1215 | PI<br>321770 | - | - | Cultivar  | Slovenia              |  | none | - | + | - | + | - | + |
| BCC1222 | PI<br>321797 | - | - | Cultivar  | Netherlan<br>ds       |  | none | - | + | - | + | - | + |
| BCC1229 | PI<br>321845 | - | - | Uncertain | Slovenia              |  | none | - | + | - | + | - | + |
| BCC1324 | PI<br>328154 | - | - | Landrace  | Bulgaria              |  | none | - | + | + | + | - | + |

|         |              |   |   |          |                        |      |   |   |   |   |   |   |
|---------|--------------|---|---|----------|------------------------|------|---|---|---|---|---|---|
| BCC1375 | PI<br>328950 | - | - | Cultivar | Netherlands            | none | - | + | - | + | - | + |
| BCC1390 | PI<br>330326 | - | - | Breeding | Colombia               | none | - | - | + | - |   |   |
| BCC1391 | PI<br>330397 | - | - | Cultivar | Czechoslovakia         | none | - | - | + | - |   |   |
| BCC1392 | PI<br>330398 | - | - | Cultivar | Slovakia               | none | - | - | - | + | + |   |
| BCC1393 | PI<br>330399 | - | - | Cultivar | Czech Republic         | none | - | - | - | + | - | + |
| BCC1399 | PI<br>331217 | - | - | Landrace | Eritrea                | none | - | + | + | - |   |   |
| BCC1404 | PI<br>331895 | - | - | Landrace | Afghanistan            | none | - | - | - | + | - | + |
| BCC1408 | PI<br>337143 | - | - | Cultivar | Argentina              | none | - | - | - | + | - | + |
| BCC1427 | PI<br>342219 | - | - | Landrace | Turkey                 | none | - | - | - | + | - | + |
| BCC1450 | PI<br>344920 | - | - | Landrace | Bosnia and Herzegovina | none | - | - | - | + | - | + |
| BCC1465 | PI<br>345618 | - | - | Cultivar | Russian Federation     | none | - | + | - | + | + |   |
| BCC1469 | PI<br>346390 | - | - | Breeding | Argentina              | none | - | - | - | + | - | + |
| BCC1474 | PI<br>349896 | - | - | Landrace | Serbia                 | none | - | + | + | - |   |   |
| BCC1485 | PI<br>356580 | - | - | Landrace | Ethiopia               | none | - | - | + | - |   |   |
| BCC1510 | PI<br>356775 | - | - | Landrace | Morocco                | none | - | - | - | + | - | + |
| BCC1522 | PI<br>361641 | - | - | Cultivar | France                 | none | - | - | - | + | + |   |
| BCC1525 | PI<br>361665 | - | - | Cultivar | Denmark                | none | - | + | - | + | - | + |
| BCC1535 | PI<br>361695 | - | - | Cultivar | Finland                | none | - | - | + | - |   |   |

|         |              |   |   |          |              |  |      |   |   |   |   |   |   |
|---------|--------------|---|---|----------|--------------|--|------|---|---|---|---|---|---|
| BCC1556 | PI<br>369732 | - | - | Cultivar | Ukraine      |  | none | - | - | - | + | - | + |
| BCC1562 | PI<br>369747 | - | - | Cultivar | Ukraine      |  | none | - | + | + | + | - | + |
| BCC1576 | PI<br>370994 | - | - | Landrace | Switzerland  |  | none | - | - | - | + | - | + |
| BCC1577 | PI<br>371017 | - | - | Landrace | Switzerland  |  | none | - | - | - | + | - | + |
| BCC1582 | PI<br>371102 | - | - | Landrace | Switzerland  |  | none | - | - | - | + | + |   |
| BCC1614 | PI<br>372084 | - | - | Cultivar | Estonia      |  | none | - | - | + | - |   |   |
| BCC1620 | PI<br>372099 | - | - | Cultivar | Turkmenistan |  | none | - | - | + | + | - | + |
| BCC1623 | PI<br>372102 | - | - | Cultivar | Belarus      |  | none | - | + | - | + | + |   |
| BCC1625 | PI<br>374413 | - | - | Landrace | Macedonia    |  | none | - | - | - | + | + |   |
| BCC1636 | PI<br>378218 | - | - | Landrace | Serbia       |  | none | - | - | + | - |   |   |
| BCC1640 | PI<br>382296 | - | - | Landrace | Ethiopia     |  | none | - | - | + | - |   |   |
| BCC1655 | PI<br>386406 | - | - | Landrace | Eritrea      |  | none | - | + | + | - |   |   |
| BCC1691 | PI<br>392464 | - | - | Breeding | South Africa |  | none | - | + | + | - |   |   |
| BCC1694 | PI<br>392471 | - | - | Breeding | South Africa |  | none | - | - | - | + | + |   |
| BCC1695 | PI<br>392478 | - | - | Breeding | South Africa |  | none | - | + | - | + | + |   |
| BCC1702 | PI<br>392499 | - | - | Breeding | South Africa |  | none | - | - | + | - |   |   |
| BCC1712 | PI<br>399482 | - | - | Cultivar | Netherlands  |  | none | - | - | - | + | + |   |



|         |              |   |   |           |                |  |      |   |   |   |   |   |  |
|---------|--------------|---|---|-----------|----------------|--|------|---|---|---|---|---|--|
| BCC1719 | PI<br>401939 | - | - | Uncertain | Colombia       |  | none | - | - | + | - |   |  |
| BCC1724 | PI<br>401973 | - | - | Uncertain | Colombia       |  | none | - | - | - | - |   |  |
| BCC1732 | PI<br>402098 | - | - | Uncertain | Colombia       |  | none | - | - | + | - |   |  |
| BCC1738 | PI<br>402168 | - | - | Uncertain | Colombia       |  | none | - | - | + | - |   |  |
| BCC1741 | PI<br>402264 | - | - | Uncertain | Colombia       |  | none | - | - | + | - |   |  |
| BCC1744 | PI<br>402352 | - | - | Uncertain | Colombia       |  | none | - | - | + | - |   |  |
| BCC1745 | PI<br>402354 | - | - | Uncertain | Colombia       |  | none | - | - | + | - |   |  |
| BCC1751 | PI<br>402468 | - | - | Uncertain | Colombia       |  | none | - | - | + | - |   |  |
| BCC1796 | PI<br>415012 | - | - | Cultivar  | Mexico         |  | none | - | - | + | - |   |  |
| BCC1830 | PI<br>428411 | - | - | Cultivar  | Mexico         |  | none | - | - | + | - |   |  |
| BCC1831 | PI<br>428413 | - | - | Cultivar  | France         |  | none | - | - | - | + | + |  |
| BCC1839 | PI<br>428628 | - | - | Cultivar  | Czechoslovakia |  | none | - | - | + | - |   |  |
| BCC1852 | PI<br>429313 | - | - | Landrace  | Yemen          |  | none | - | - | + | - |   |  |
| BCC1857 | PI<br>429519 | - | - | Landrace  | Nepal          |  | none | - | - | + | - |   |  |
| BCC1876 | PI<br>434815 | - | - | Breeding  | Canada         |  | none | - | - | + | + | + |  |
| BCC1879 | PI<br>436135 | - | - | Landrace  | Chile          |  | none | - | - | - | + | + |  |
| BCC1886 | PI<br>436146 | - | - | Landrace  | Chile          |  | none | - | + | + | - |   |  |
| BCC1889 | PI<br>436150 | - | - | Landrace  | Chile          |  | none | - | + | + | - |   |  |
| BCC1891 | PI<br>436153 | - | - | Landrace  | Chile          |  | none | - | + | + | - |   |  |

|         |              |   |   |           |                |      |   |   |   |   |   |   |
|---------|--------------|---|---|-----------|----------------|------|---|---|---|---|---|---|
| BCC1918 | PI<br>447207 | - | - | Uncertain | Spain          | none | - | + | + | - |   |   |
| BCC1971 | PI<br>467454 | - | - | Uncertain | Hungary        | none | - | + | + | + | - | + |
| BCC2016 | PI<br>467786 | - | - | Cultivar  | Netherlands    | none | - | - | + | + | + |   |
| BCC2022 | PI<br>467814 | - | - | Cultivar  | Croatia        | none | - | + | - | + | + |   |
| BCC2027 | PI<br>467839 | - | - | Cultivar  | Poland         | none | - | - | + | - |   |   |
| BCC2028 | PI<br>467840 | - | - | Cultivar  | Poland         | none | - | + | + | + | - | + |
| BCC2066 | PI<br>485524 | - | - | Cultivar  | United Kingdom | none | - | - | + | + | + |   |
| BCC2074 | PI<br>494099 | - | - | Cultivar  | United States  | none | - | - | + | - |   |   |
| BCC2083 | PI<br>498436 | - | - | Cultivar  | New Zealand    | none | - | - | - | + | + |   |
| BCC2118 | PI<br>531896 | - | - | Uncertain | Egypt          | none | - | - | + | - |   |   |
| BCC2162 | PI<br>564477 | - | - | Cultivar  | Bulgaria       | none | - | + | + | - |   |   |
| BCC2236 | PI<br>572588 | - | - | Cultivar  | China          | none | - | + | + | - |   |   |
| BCC2241 | PI<br>573594 | - | - | Cultivar  | Lithuania      | none | - | - | - | + | + |   |
| BCC2242 | PI<br>573598 | - | - | Cultivar  | Ukraine        | none | - | + | + | + | - | + |
| BCC2259 | PI<br>573682 | - | - | Landrace  | Georgia        | none | - | - | - | + | - | + |
| BCC2352 | PI<br>592240 | - | - | Cultivar  | United Kingdom | none | - | + | - | + | - | + |
| BCC2362 | PI<br>592282 | - | - | Cultivar  | United Kingdom | none | - | - | - | + | - | + |
| BCC2371 | PI<br>599633 | - | - | Cultivar  | Czech Republic | none | - | - | - | + | + |   |
| BCC2381 | PI<br>605699 | - | - | Cultivar  | Canada         | none | - | - | + | - |   |   |

|         |              |   |   |          |                  |  |      |   |   |   |   |   |   |
|---------|--------------|---|---|----------|------------------|--|------|---|---|---|---|---|---|
| BCC2399 | PI<br>611513 | - | - | Cultivar | Ukraine          |  | none | - | + | + | - |   |   |
| BCC2400 | PI<br>611526 | - | - | Cultivar | Ukraine          |  | none | - | - | - | + | - | + |
| BCC2414 | PI<br>629116 | - | - | Cultivar | United<br>States |  | none | - | - | + | - |   |   |
| BCC2423 | PI<br>639299 | - | - | Landrace | Kazakhstan       |  | none | - | + | + | + | + |   |
| BCC2424 | PI<br>639300 | - | - | Landrace | Kazakhstan       |  | none | - | + | - | + | + |   |
| BCC2530 | PI<br>643227 | - | - | Breeding | United<br>States |  | none | - | - | + | - |   |   |
| BCC2559 | PI<br>643370 | - | - | Breeding | United<br>States |  | none | - | - | + | - |   |   |

<sup>a</sup> Sequential number given to all accessions from the Barley iCore Collection (BCC)

<sup>b</sup> Number assigned by the United States Department of Agriculture-Agricultural Research Service, Germplasm Resources Information Network (GRIN)

<sup>c</sup> If accession has functional *Rpg5*, “+” if functional and “-“ if non-functional

<sup>d</sup> If accession has functional *Rpg1*, “+” if functional and “-“ if non-functional

<sup>e</sup> Improvement status and passport information as provided by the United States Department of Agriculture-Agricultural Research Service, Germplasm Resources Information Network

<sup>f</sup> Races which the accession gave a median infection type (IT)  $\leq 2$  (linear score  $\leq 7$ )

<sup>g</sup> Resistance genes predicted using the RGenePos Package in R

<sup>h</sup> Markers used to determine if accession has functional *Rpg5* gene, markers PP2C, LRK, R5, R10 are given in tables 5 and 6, “+” indicates a presence of marker and “-“ indicates absence

<sup>i</sup> Markers used to determine if accession has functional *Rpg1* gene, markers RPG1-N, RPG1-s are given in Table 3.5., “+” indicates a presence of marker and “-“ indicates absence

## **Online Resource Captions**

**Online Resource 3.1.** RGenePos an R package for postulation of resistance genes in crop plants.

**Online Resource 3.2.** Additional adult plant stem rust resistance nursery observations of the Barley iCore Collection Selects (BCCS).

## **Chapter 4**

# **Mapping Adult Plant Stem Rust Resistance in Barley Accessions Hietpas-5 and GAW-79**

## Introduction

Stem rust is an extremely devastating disease of cereal crops, capable of destroying large acreages in a short period of time. Barley (*Hordeum vulgare* ssp. *vulgare* L.) is vulnerable to stem rust infection from both the wheat stem rust pathogen, *Puccinia graminis* Pers.:Pers. f. sp. *tritici* Eriks. & E. Henn, (*Pgt*) and the rye stem rust pathogen, *Puccinia graminis* Pers.:Pers. f. sp. *secalis* Eriks. & E. Henn, (*Pgs*) (Roelfs 1982). Stem rust is an episodic, but major threat to barley and wheat (*Triticum* spp.) in regions of North America where prevailing environmental conditions are conducive for infection and spread, including Minnesota, North Dakota, South Dakota, and Washington in the United States and Alberta, Saskatchewan, and Manitoba in Canada (CDL 2016; Roelfs 1978; Steffenson 1992). In addition to North America, stem rust was reported to cause yield losses of barley in other parts of the world, including Australia, Africa (Eritrea, Ethiopia, Kenya and Uganda), the Middle East (Yemen, Iran, Iraq), Russia, far eastern Europe (Ukraine and Georgia), central Europe (Switzerland), Central Asia (Kazakhstan and Tajikistan), South Asia (Bhutan, Nepal and Pakistan), and South America (Uruguay) (Dill-Macky et al. 1990; Mwando et al. 2012; Oehler 1950; Schilperoord 2013; Steffenson 1992; Steffenson et al. 2017; Steffenson et al. 2016; Turuspekov et al. 2016). Sporadic, but nonetheless significant stem rust epidemics have been reported on barley in several countries, including significant episodes in the 1920's, 1930's, and 1990's in United States and also in the 1990's in Australia (Dill-Macky et al. 1990; Roelfs et al. 1991; Roelfs 1978; Steffenson 1992). During these epidemics, barley suffered both yield and also malting quality losses (Dill-Macky et al. 1990). Yield losses ranging from 15 to 60% have been reported for barley infected with stem rust (Dill-Macky et al. 1990; Roelfs 1978; Steffenson 1992). Malting quality is adversely affected through a reduction in kernel plumpness, kernel weight, and germination, resulting in additional economic losses to producers because a premium is paid for malting grade barley (Dill-Macky et al. 1990; Roelfs et al. 1991; Roelfs 1978; Steffenson 1992). The discovery of races in the

“Ug99 race group” from Africa have exposed a significant vulnerability in both wheat and barley. Races in this lineage, such as *Pgt* TTKSK, possess virulence for many deployed stem rust (*Sr*) resistance genes in wheat and also for *Rpg1* (Reaction to *Puccinia graminis* (*Rpg*)), the most widely used stem rust resistance gene in North American barley cultivars (Pretorius et al. 2000; Steffenson and Jin 2006; Steffenson et al. 2007). Races in this group are virulent on more than 80% of the world’s wheat and more than 95% of the world’s barley cultivars (Singh et al. 2011; Singh et al. 2008; Singh et al. 2015; Steffenson et al. 2017). The wide virulence of a single *Pgt* race on such a large percentage of cultivated germplasm of both crop species is extremely rare and exceptionally dangerous.

The most widely used *Rpg* genes in barley breeding are *Rpg1* and the *rpg4/Rpg5* gene complex (Steffenson 1992; Steffenson et al. 2017). These genes confer all-stage resistance, where the phenotypic effect is apparent from the seedling through to the adult plant stage. *Rpg1* was the first described gene for resistance to stem rust in barley. It was initially identified in the Swiss landrace accessions of Chevron (PI 38061) and Peatland (CIho 2613), but a farmer selection that became the cultivar Kindred (CIho 6969) is also a source of the gene (Brueggeman et al. 2002; Shands 1939; Steffenson 1992). *Rpg1* confers resistance to most of the common *Pgt* races in North America and is the primary resistance target for many breeding programs in the United States and Canada. The *rpg4/Rpg5* gene complex confers resistance to many *Pgt* races, including those in the Ug99 race group from Africa (Steffenson et al. 2009; Steffenson et al. 2013; Steffenson et al. 2017). It also confers resistance to cultures of *Pgs* (Sun and Steffenson 2005; Sun et al. 1996). This locus is a complex of at least three genes combining resistance to both *Pgs* and *Pgt* by *rpg4*- and *Rpg5*-mediated actions, respectively (Brueggeman et al. 2008; Brueggeman et al. 2009; Mirlohi et al. 2008; Steffenson et al. 2009). As these two genes are very tightly linked, they are commonly referred to as the *rpg4/Rpg5* gene complex. This gene complex was originally described from Q21861 (PI 584766), a line of unknown parentage selected from an International Center for Agricultural Research in the

Dry Areas (ICARDA)/Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT) breeding nursery in Mexico. The stem rust resistance of Q21861 was first described in evaluations conducted in Queensland, Australia. Subsequent genetic studies on Q21861 revealed that it carries both *Rpg1* and *rpg4/Rpg5* (Dill-Macky et al. 1990; Dill-Macky and Rees 1992; Jin et al. 1994a; Jin et al. 1994b). Relatively little is known about other described *Rpg* genes in barley, including *Rpg2*, *Rpg3*, *rpg6*, *rpgBH*, and *RpgU* (Fetch et al. 2009; Fox and Harder 1995; Jedel 1991; Jedel et al. 1989; Patterson et al. 1957; Shands 1939; Steffenson et al. 2009; Steffenson et al. 2013; Steffenson et al. 2017; Steffenson et al. 1984). Of these genes, *Rpg2*, *Rpg3*, and *RpgU* have the most promise for breeding because they confer moderate to high levels of Adult Plant Resistance (APR) to *Pgt* race QCCJB, which is virulent for *Rpg1* (Fox and Harder 1995; Roelfs et al. 1991; Steffenson 1992). The resistance gene *rpg6* was transferred from bulbous barley grass (*H. bulbosum* L.) into barley line 212Y1, which has a Golden Promise (PI 467829) genetic background (Fetch et al. 2009). This gene was described based on its seedling reaction to *Pgt* race QCCJB; however, it is moderately susceptible to race TTKSK at the seedling stage. The growth phenotype of accessions with a Golden Promise background has made evaluations for APR in North America challenging. The resistance gene *rpgBH* was described from the accession Black Hulless (CIho 666) and confers APR to *Pgs* (Fox and Harder 1995; Steffenson et al. 1984). In our evaluations, Black Hulless exhibits little to no APR or seedling resistance to *Pgt* races or *Pgs* isolate 92-MN-90 (Table 3.1 in Chapter 3) (Steffenson et al. 2017).

*Rpg2* was described from Hietpas-5 (CIho 7124), a stem rust resistant selection made by a Wisconsin farmer from the barley landrace Oderbrucker (CIho 1272) (Patterson et al. 1957). The resistance spectrum of *Rpg2* is not well-characterized, but it has been shown to possess high levels of APR to races QCCJB and MCCFC, despite being mostly susceptible at the seedling stage (Fox and Harder 1995; Franckowiak 1991; Franckowiak and Steffenson 1997; Jin et al. 1994a; Miller and Lambert 1955; Shands 1964; Sun and Steffenson 2005). *Rpg2* segregates independently from *Rpg1* based on the results of a



Hietpas-5/Chevron F<sub>2:3</sub> population tested for APR to a bulk of *Pgt* races 17, 19 and 56 (17=HFLGC, 19=G----, 56=MCCFC, Yue Jin, personal communication) (Miller and Lambert 1955; Patterson 1955; Shands 1964). In a previous study, we showed that Hietpas-5 has a moderate to high level of APR to several *Pgt* races, including QCCJB and MCCFC and has seedling resistance to *Pgt* race HKHJC and *Pgs* isolate 92-MN-90 (Table 3.1 in Chapter 3). The past use of *Rpg2* in breeding programs is not known; however, it may be present in some Midwest breeding germplasm where Oderbrucker was a founder.

*Rpg3* was derived from the Ethiopian landrace accession GAW-79 (PI 382313), referred to hereafter as PI 382313. Like Heitpas-5, PI 382313 exhibits a moderate to high level of APR to races QCCJB and MCCFC among others, and a mostly susceptible seedling phenotype (Table 3.1 in Chapter 3) (Fox and Harder 1995; Jedel 1991; Jedel et al. 1989; Sun and Steffenson 2005). In Chapter 3, we showed that PI 382313 also has a moderate level of seedling resistance to *Pgs* isolate 92-MN-90 and in addition APR to *Pgt* races QCCJB and MCCFC (Steffenson et al. 2017). This gene has been introgressed into several Canadian breeding programs to enhance the level of resistance to race QCCJB.

The challenge of breeding for stem rust resistance in barley is that there is a paucity of resistance genes in this species compared to wheat where more than 70 *Sr* genes have been described (Singh et al. 2015). The majority of barley stem rust research has focused on the resistance genes *Rpg1* and *rpg4/Rpg5*. Aside from these few well-described genes, there is a dearth of resistant accessions from which to conduct breeding or genetic analyses. Swiss landraces have been an important source of stem rust resistance for barley with the discovery of *Rpg1* in Chevron and Peatland. A relatively high frequency of Swiss landraces were also found to carry the *rpg4/Rpg5* gene complex (Mamo et al. 2015; Steffenson et al. 2016). However, no additional new *Rpg* genes have been described from this unique germplasm. From the evaluation of North American barley breeding germplasm, Zhou et al. (2014) found only 12 and 64 accessions (out of 3,840)

resistant to race TTKSK at the seedling and adult stage, respectively. Mamo (2013) screened 298 barley landraces from Ethiopia and Eritrea and identified only 12 accessions with APR to North American races of *Pgt* and none resistant to TTKSK. The most diverse new sources of resistance identified to date are from a world collection of *Hordeum* accessions (Steffenson et al. 2017). Steffenson et al. (2017) focused on seedling resistance to race TTKSK in a world collection of cultivated and wild barley (*H. vulgare* ssp. *spontaneum* C. Koch Thell) and found 17 highly resistant accessions (out of 2,914) that did not carry either *Rpg1* or *rpg4/Rpg5*. In Chapter 3, we evaluated a different world collection of barley held by the United States Department of Agriculture-Agricultural Research Service (USDA-ARS), National Small Grains Collection and identified as many as 98 accessions (out of 1,860) with resistance to a diverse suite of seven *Pgt* races (TTKSK, TTKST, TKTTF, TRTTF, QCCJB, MCCFC, and HKHJC) and also an isolate of *Pgs* (92-MN-90). These accessions were postulated to carry either unknown resistance genes or unknown resistance genes in combination with previously described *Rpg* genes. None of the previous studies identified any accessions with a resistance level as high or a resistance spectrum as wide as Q21861.

Due to the lack of stem rust resistance genes in barley, it is imperative to fully characterize all identified resistance sources and to pyramid their resistance genes together to prolong their effective lifespan. The urgency of this objective is highlighted by the broad virulence spectrum of races in the Ug99 lineage, which may eventually spread to North America. In the study described herein, quantitative trait loci (QTL) mapping was used to dissect the APR of Hietpas-5, the source of *Rpg2*, and also PI 382313, the source of *Rpg3*. These accessions were crossed to the highly susceptible barley accession Hiproly (PI 60693), and two recombinant inbred line (RIL) populations were created. We hypothesized that these accessions carry heritable resistance to stem rust, whose genetic architecture can be characterized using a bi-parental mapping approach.

## Materials and Methods

### *Stem Rust Controls*

Hiproly, Steptoe (CIho 15229), 80-tt-30 (CIho 16130), and PI 532013 were used as the susceptible controls in the seedling evaluations conducted in the greenhouse and in the adult plant evaluations conducted in the field. Chevron (PI 38061) is a source of *Rpg1* and was used as a resistant control for this gene. Line 80-TT-29 (CIho 16129) also carries *Rpg1* and is near-isogenic with 80-tt-30, providing an unequivocal genetic comparison for the effect of this gene (Steffenson 1992; Steffenson et al. 1985). The Minnesota cultivar Quest (PI 663183) also carries *Rpg1* (Smith et al. 2013). The controls for *Rpg2* and *Rpg3* were Hietpas-5 and PI 382313, respectively (Franckowiak 1991; Franckowiak and Steffenson 1997; Jedel 1991; Jedel et al. 1989; Patterson et al. 1957; Shands 1964). Neither Hietpas-5 nor PI 382313 carry *Rpg1* or *rpg4/Rpg5* based on molecular analyses with markers generated from the sequences of these cloned genes (Table 3.13 in Chapter 3,) (Steffenson et al. 2017). The resistant control Q21861 (PI 584766) carries *Rpg1* and the *rpg4/Rpg5* gene complex and is resistant to all cultures of *Puccinia graminis* tested to date (Arora et al. 2013; Brueggeman et al. 2008; Brueggeman et al. 2009; Jin et al. 1994b; Steffenson et al. 2009; Steffenson et al. 1995; Sun et al. 1996; Wang et al. 2013). The line Q/SM20 is a resistant control carrying only the *rpg4/Rpg5* gene complex and was selected from a cross between Q21861 and SM89010, a susceptible breeding line from Canada (Steffenson et al. 2009; Steffenson et al. 1995). Wheat is more receptive to stem rust infection than barley. To assess the development of rust infection in the field experiments, the susceptible bread wheat (*Triticum aestivum* L.) accessions LMPG-6 and Red Bobs (CItr 6255) were also included.

### *Mapping Population Development*

To achieve greater uniformity in the parental accessions, single plant selections were made based on the rust phenotype and used in all hybridizations and genetic analyses.

Selection PI 382313-2B was derived from PI 382313, Hietpas-5-1C from Hietpas-5, and Hiproly-2C from Hiproly. Crosses were made between Hietpas-5-1C and Hiproly-2C creating the Hietpas-5/Hiproly (HH) population and between PI 382313-2B and Hiproly-2C creating the PI 382313/Hiproly (PH) population. In all experiments, the seed source for Hietpas-5, PI 382313, and Hiproly were derived from these single plant selections. Single seed descent was then used to advance the populations. Approximately 300 F<sub>2</sub> seeds were obtained from selfed F<sub>1</sub> plants. Each F<sub>2</sub> seed was then planted in a small volume cone (5 cm diameter x 18 cm depth, Stuewe and Sons, Inc., Tangent, OR) filled with a 50:50 mixture of steam-sterilized field soil and Sunshine MVP potting mix (vermiculite, Canadian sphagnum peat moss, nutrient charge, gypsum, and dolomitic limestone) (Sun Gro Horticulture, Quincy, MI). Plants were watered at planting with a pH buffering 15-0-15 fertilizer (Peters Dark Weather, Scott's Company, Marysville, OH) at 1/16 dilution (ca. 40 g/liter), and then Osmocote (Scott's Company) slow release fertilizer (14-14-14) was applied. Plants were also fertilized with another water-soluble formulation (20-10-20 at 1/16 dilution (ca. 40 g/liter) (J.R. Peters, Inc., Allentown, PA) on a weekly basis. A single spike was obtained from each F<sub>2</sub> plant, and a single seed from each spike was used to produce the next generation. This same protocol was used to advance the population to the F<sub>5</sub> generation. From each RIL, a single F<sub>5</sub> seed was grown in a large square plastic pot (13.3 x 13.3 x 10.2 cm) filled with the soil mixture described above and fertilized with the same regime. Tissue from the second leaves of F<sub>5:6</sub> plants was harvested for DNA extraction, and the seed was harvested for the F<sub>5:6</sub> generation seed stock. In the next generation, five F<sub>5:6</sub> seeds from each RIL were planted in a large pot and grown in the greenhouse as described above. All spikes from this generation were bulk harvested to obtain maximum seed production for subsequent experiments, resulting in the F<sub>5:7</sub> generation seed stock that was used in the majority of the experiments. All greenhouse seed increases were done in the Plant Growth Facility on the St. Paul campus of the University of Minnesota in a greenhouse at 18-25°C with a 16-hr photoperiod (supplemental sodium vapor lamps emitting ~300 μmol photons s<sup>-1</sup> m<sup>-2</sup>). Spikes were

harvested at maturity, dried in a forced air dryer for two days at 35°C and subsequently threshed and cleaned for the next generation advance and seed storage.

PI 382313 has a two-rowed *deficiens* (lacking sterile lateral florets, controlled by the *Vrs1.t* allele of the *Vrs1* gene) spike type with covered caryopsis (glumes remain attached to caryopsis at maturity, controlled by the *Nud* allele of the *nud* gene) (Franckowiak and Haus 1997b; Franckowiak et al. 1997a). Hietpas-5 has a six-rowed (lateral florets fertile, controlled by the *vrs1.a* allele of *Vrs1* gene) spike type also with a covered caryopsis (*Nud*) (Franckowiak et al. 1997b). Hiproly has a two-rowed (lateral florets present but sterile, controlled by the *Vrs1.b* allele of *Vrs1* gene) spike type with naked caryopsis (glumes thresh free of caryopsis at maturity, controlled by the *nud* allele of the *nud* gene) (Franckowiak and Haus 1997a). Therefore, the PH population segregates for covered and naked caryopsis and also two-rowed *deficiens* type lateral floret morphology, whereas the HH population segregates for covered and naked caryopsis as well as two-rowed and six-rowed spike morphology. In total, there were 280 RILs in the HH population and 278 RILs in the PH population. All RILs were used in genotyping and linkage map analysis.

Additionally, the populations were also scored for several agro-morphological traits in the 2015 winter greenhouse season (GH). These traits included plant height at maturity (in cm), physiological leaf spotting at heading (percentage of flag leaf area affected on a 0-100% basis), spike morphology scored as “2” for two-rowed types with normal lateral floret development, “3” for a two-rowed *deficiens* lateral floret morphology, and “6” for six-rowed spike morphology. Covered and naked caryopsis seed types were designated with a “c” for covered and “n” for naked.

### *Rust Phenotyping*

The two populations were phenotyped for APR to the North American *Pgt* races MCCFC, QCCJB, and HKHJC in the summer of 2015 (StP\_MC15, StP\_QC15, and StP\_HK15) and to races MCCFC and QCCJB in the summer of 2016 (StP\_MC16 and

StP\_QC16) in St. Paul, MN. The three nurseries were planted in separate fields at the Minnesota Agricultural Experiment Station on the St. Paul campus of the University of Minnesota. Fields inoculated with *Pgt* races MCCFC and QCCJB were separated from each other by at least 0.25 km, and the one inoculated with *Pgt* race HKHJC by 1 km. Seed for all experiments was derived from the F<sub>5:7</sub> source produced in the greenhouse. Due to space restrictions in the field nurseries, only two hundred (selected at random) RILs from each population were screened for APR (Online Resource 4.1). The populations were also phenotyped for APR to Ug99 group races of *Pgt* in Njoro, Kenya during the 2015 off-season (KEN\_15o). The predominant races of *Pgt* in this nursery were TTKSK and TTKST (16 of 25 samples); however, races TTKTK and TTKTT were also present in the nursery (9 of 25 samples)(Newcomb et al. 2016). The *Pgt* race composition in Njoro will collectively be referred to as *Pgt* TTKSK composite, since some infections could be due to the other races. Finally, the HH population was also phenotyped for APR to *Pgt* race PTKST in Greytown, South Africa in 2016 (SA\_16). The expected reaction of these races on differential bread wheat and barley genotypes are listed in Table 3.1. in Chapter 3. Races MCCFC and HKHJC are avirulent on *Rpg1*, and races QCCJB, TTKSK, TTKST, and PTKST are avirulent on *rpg4/Rpg5*, but virulent on *Rpg1*. Race HKHJC is virulent on *rpg4/Rpg5*, and race MCCFC is avirulent on both *Rpg1* and *rpg4/Rpg5* (Sun and Steffenson 2005). The *Pgt* stem rust race nomenclature is based on the North American stem rust differential system (Jin et al. 2008; Roelfs 1988; Roelfs et al. 1991).

In the 2016 South Africa nursery, natural infections of the barley leaf rust pathogen (*Puccinia hordei* Otth) developed uniformly on all of the RILs and parents of the HH population. Since Hietpas-5 and Hiproly exhibited highly resistant and highly susceptible reactions to leaf rust, respectively, the entire RIL population was assessed for reaction to *P. hordei* to determine the genetic basis of resistance. For these assessments, the severity of infection was recorded on flag leaves at the mid-dough stage of development using a 1 to 9 scale, where 1 was highly resistant (low severity) and 9 was highly susceptible (high

severity) (dataset SA\_16LR). An isolate (SAPh1601) of *P. hordei* from this nursery was collected for virulence characterization on the barley leaf rust differential set in the greenhouse (Brooks et al. 2000). The isolate was increased on the susceptible cultivar Moore (CIho 7251) and inoculated onto the RILs of the HH population to assess their seedling infection types (ITs). Three seeds of each RIL plus the respective parents were planted in 96 count racks containing cones, where the planting medium and fertilizer regime were as described above for RIL development. When the first leaves were fully expanded (about 1 week), plants were inoculated with 7 mg of urediniospores suspended in 0.8 ml Soltrol 170 oil (Phillips Petroleum, Bartlesville, OK). The inoculum was contained in a gelatin capsule (size 00) fit inside a custom inoculator and sprayed onto plants using a pump set at 25-30 kPa. One capsule was used per 48 entries, for a rate of about 0.01 ml inoculum per plant. The oil carrier was allowed to off gas for 90 minutes under lights with constant air circulation. Then, plants were incubated in chambers overnight at near 100% humidity for 24 hours in the dark at 22-25°C. The saturated moisture conditions were produced by periodic misting from household ultrasonic humidifiers. After 24 hours, the doors were opened and misters turned off to allow plants to dry. Once dry, plants were transferred to a greenhouse at 22/26°C (night/day) with a 16-hour photoperiod (minimum 300-350  $\mu\text{mol photons s}^{-1}\text{m}^{-2}$ , from 400 W sodium vapor lamps). At 12 days post-inoculation, the ITs on each entry were scored using a 0 to 4 scale (Park and Karakousis 2002). Both the HH RIL population and the leaf rust differential set were evaluated in two replications over time (dataset LR\_GH).

In St. Paul, all experiments were designed as randomized complete blocks with controls and two replications per race. Hiproly, Chevron, Q/SM20, Q21861, and the resistant parents (Hietpas-5 or PI 382313) were planted every 50 rows within the nursery. Susceptible barley and wheat accessions of PI 532013 and LMPG-6, respectively, were replicated every 150 rows to assess disease development in the nursery. The experiments were planted in isolated nurseries at the Minnesota Agricultural Experiment Station on the St. Paul campus of the University of Minnesota. Two grams of seed were sown in

half-meter rows and maintained according to local agronomic practices. Plots were planted between continuous rows of a susceptible barley spreader row, such that every plot had a spreader row on one side. The spreader row in the QCCJB nursery consisted of equal parts of cultivars Quest, Stander (PI 564743), and Conlon (PI 597789), which all carry *Rpg1*. In the MCCFC and HKHJC nurseries, the spreader rows consisted of a 50:50 mix of Steptoe and 80-tt-30, neither of which carries *Rpg1* or any other described *Rpg* gene. To ensure sufficient disease development for assessing APR in the field trials, individual plants at 1 m intervals in the spreader rows were needle-injected at the second tiller stage (Zadoks 22-29) with 1.0 ml of inoculum (Zadoks et al. 1974). Inoculum for needle injection was prepared by mixing 1 g of fresh or frozen and heat-shocked urediniospores in 1 L of distilled water and six drops of 20% Tween 20 (Polyoxyethylene (20) sorbitan monolaurate). Test plots were also directly inoculated with urediniospores suspended in Soltrol 170 oil dispersed by a hand-held, battery-powered Mini-ULVA sprayer (Mini-ULVA, Micron Group, Herefordshire, United Kingdom). Two to three direct inoculations were performed starting when 50-75% of the plots were at the heading stage (Zadoks 51-59) and continuing until all plots had headed. Inoculations took place during the evening when dew was predicted to form. Inoculum was prepared by mixing 1 g of urediniospores in 1 L of Soltrol 170 oil. The inoculation rate was approximately 1 L of inoculum per 500 plots. To prevent cross-contamination, only one race was inoculated per day. If this was not possible, all equipment was surface disinfested and clothing changed between inoculations with different races. In addition, samples of rust were taken at the end of the season to confirm race purity by inoculation onto key wheat differentials giving differing reactions to the races used. Disease symptoms were monitored on the susceptible controls, and when they reached a rust incidence of ~10%, the first rust severity ratings were taken on the test plots. Disease severity was estimated visually on the stem and leaf sheath tissue using the modified Cobb scale (0-100%) (Peterson et al. 1948). Additionally, infection responses (IRs) (size and type of uredinia) were also scored on each line according to the scale of R = Resistant; MR = Moderately



Susceptible; MS = Moderately Susceptible; and S = Susceptible (Peterson et al. 1948). However, as both parents and progeny in both populations show predominantly susceptible IRs (i.e. IRs of MS or S) and the correlation between IRs and disease severity was found to be very high ( $R^2 = 0.65$ ) (see Chapter 2), only disease severity values were used in analysis. Barley shows strong maturity effects with respect to rust development. Thus, evaluations were conducted over several sequential dates when plants were between the heading to hard-dough (Zadoks 55-87) stages of development. Any lines that did not head were discarded from analysis. Heading date (recorded as days after planting) data were recorded in St. Paul in 2015 (both the HKHJ and MCCFC nurseries) and also in 2016 (race MCCFC nursery). Raw stem rust severity data were used to create a second rust severity dataset corrected for differences in heading date among lines at each location. This was done using linear regression with the `lm` function in R (Chambers 1992; Team 2016). In this analysis, heading date and line identity were used as predictor variables for stem rust severity, and coefficients of the model were used to generate a correction factor to correct for heading date within each location. This created corrected datasets for StP\_HK15, StP\_MC15 and StP\_MC16 in both populations where heading date was taken. The  $R^2$  of the model with and without heading date included was recorded for comparison. Correlations between stem rust severities across locations and with heading date and spike type were also calculated. Significant correlations were assigned using a Pearson's product-moment correlation ( $p$ -value  $< 0.05$ ) executed using the `cor.test` function in R (Best and Roberts 1975).

In Njoro and Greytown, experiments were conducted using an un-replicated complete block design with replicated controls. In Njoro, the controls (Red Bobs, Q21816, Steptoe, and Quest) and parents (Hipoly and either Hietpas-5 or PI 382313) were planted every 50 entries, and the susceptible accession PI 532013 was planted every 100 entries. In Njoro, Quest was used in place of Chevron for assessing the effect of *Rpg1*. In Greytown, the controls and parents (Q21861, Q/SM20, Chevron, Hipoly, Hietpas-5, and PI 382313) were planted every 100 entries. At both African evaluation sites, the inoculum infecting

barley was produced on susceptible wheat spreader rows planted in and around the nursery. Additionally, the test entries were also directly inoculated with a urediniospore/oil suspension using hand-held, battery-powered sprayer. The broad sense heritability ( $H^2$ ) of stem rust resistance was calculated using the equation:

$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_e^2}{n}}$$

where  $\sigma_g^2$  is genetic variance,  $\sigma_e^2$  is the pooled error variance, and  $n$  is the number of locations. Variance was calculated using a mixed linear model lmer function in the lme4 package in R (Bates et al. 2015). To calculate heritability on a global basis and include the effect of individual experiments, both line identity and location were treated as random effects, and stem rust severity was averaged over replications within each location. Heritability was also calculated on an individual location basis. A chi-square test for inheritance of APR to each race within each population was conducted using an expected segregation ratio of 1 : 1 for resistant : susceptible lines (single gene ratio for a RIL population), since Patterson (1955) reported that a single gene (*Rpg2*) conferred resistance in Heitpas-5. These general disease reaction categories were based on the average stem rust severity on lines across locations within a race. RILs were classified as resistant if their average severity was less than the maximum severity of the resistant parent across seasons. If the average severity of a RIL was higher than the maximum severity of the resistant parent, the RIL was classified as susceptible.

### *Genotyping*

All 280 HH and 278 PH RILs were used in genotyping and linkage map construction. The young second or third leaf tissue of  $F_{5:6}$  RILs was collected for DNA extraction and placed in 96 well tissue-harvesting plates. Tissue samples were dried over silica gel prior to DNA extraction. All accessions were subject to genotyping by sequencing (GBS).

DNA was extracted using the Mag-Bind<sup>®</sup> Plant DNA Plus kit from Omega Bio-tek (Norcross, GA), following the manufacturer's instructions. Genomic DNA was quantified using Quant-iT<sup>™</sup> PicoGreen<sup>®</sup> dsDNA Assay Kit and normalized to 20 ng/μl buffer. GBS libraries were created using the *PstI*-*MspI* restriction enzyme combination as described in Poland et al. (2012). The samples were pooled together at 96-plex to create libraries, and each library was sequenced on a single lane of Illumina Hi-Seq 2500. Single nucleotide polymorphism (SNP) calling was performed with the TASSEL v.5.0 GBS v.2.0 pipeline using 64 base kmer length and minimum kmer count of 5 (Bradbury et al. 2007). Reads were aligned to the new Morex barley reference genome assembly (Mascher et al. 2017) using the file "Barley\_Reference\_Genome\_2-3-16.fa" using the file "150831\_barley\_pseudomolecules .fasta.gz": DOI:10.5447/IPK/2016/34). Reads were aligned using the aln method of Burrows-Wheeler aligner, version 0.7.10 (Li and Durbin 2009). Alleles were called according to IUPAC naming convention of: G (Guanine), A (Adenine), T (Thymine), C (cytosine), R (G or A), Y (T or C), M (A or C), K (G or T), S (G or C), W (A or T), H (A or C or T), B (G or T or C), V (G or C or A), D (G or A or T), or N (missing). Marker naming convention was "S(Chromosome)\_(allele position in reference sequence)". Raw SNP data generated from the TASSEL pipeline was filtered to remove taxa with > 90% missing data. Genotypic data was then filtered to select for biallelic SNPs with minor allele frequency  $\geq$  5%, missing data  $\leq$  50%, and heterozygosity  $\leq$  10%. A custom script was used to extract 100 base pairs (bp) of reference sequence SNPs of interest and 60 bp around all SNPs. This tag sequence was then used to BLAST search the current barley reference assembly available on Ensembl Plants to identify genes around the SNP (McLaren et al. 2016).

Markers were then imputed using the LinkImpute method based on the k-nearest neighbor genotype imputation method to fill in missing genotype data (Money et al. 2015). In addition, several morphological markers were assessed on the population: the covered vs. naked caryopsis trait (Naked\_Seed) was scored on both the PH and HH populations, the two- vs. six-rowed spike type on the HH population (Spike\_Row\_26),

and the two-rowed vs. two-rowed deficient lateral floret morphology on the PH population (Spike\_Row\_23). All markers where either parent was called as heterozygous were dropped from the analysis. Moreover, all monomorphic markers were also dropped. Markers were then filtered once more for segregation distortion, based on a chi-square fit of a 1:1 ratio ( $p$ -value  $<0.05$ ). Markers were then filtered by adjacent linkage disequilibrium (LD) analysis based on sequence position. Markers found in perfect LD ( $R^2=1$ ) with adjacent markers were dropped so that only one marker represented each locus. The markers chosen to represent each locus had the least amount of missing data. For QTL analysis and map construction, the markers were converted to “A”, “B”, and “H” calls, where “A” was either PI 382313 or Hietpas-5 and Hipoly was always the “B” parent. “H” denotes a heterozygous marker, and “N” denotes missing values.

#### *Linkage Map Construction*

To facilitate linkage map construction using high-density markers, the Minimum Spanning Tree (MST) mapping function was used and executed in the ASMap package in R (Taylor and Butler 2016; Wu et al. 2008). To simplify the map, linkage groups representing each chromosome were constructed independently according to the chromosome assignment based on sequence position. This created individual linkage groups with a very high density of markers. Then, 200 evenly spaced markers per chromosome were chosen based on physical distance as determined by sequence position. Next, an additional evenly spaced 200 markers were selected based on centiMorgan (cM) (Morgan 1910) position of the high-density mapping of each chromosome. Even spacing of markers based on sequence and cM position was achieved using a custom script in R that executed linear programming to solve a transportation problem by selecting markers that optimized even spacing of sequence and cM position using the `lp.transport` function in the `lpSolve` package of R (Berkelaar and Buttrey 2015). The two marker sets were then combined, resulting in 200-300 markers per chromosome. If less than 200 markers were called on an individual chromosome, the entire marker set was used. A  $p$ -value of  $1 \times 10^{-7}$

<sup>10</sup> was used as minimum threshold for grouping markers. All heterozygous markers were set to missing. The Kosambi mapping function was used in map construction to estimate cM values (Kosambi 1943).

### *QTL Detection*

QTLs were detected using WinQTLCart v2.5 (Wang et al. 2012). Composite interval mapping (CIM) was performed with forward and backward stepwise regression, with a window size of 10 cM, five control markers, and a walk speed of 1 cM (Zeng 1993, 1994). Cutoff LOD (logarithm (base 10) of odds) values for declaring significant QTLs were derived using a permutation test with 1,000 permutations. QTLs were established as significant if they exceeded the minimum LOD value as set by permutation testing. QTL locations were based on overlapping significant QTL hits for individual traits. A “QTL boundary” was established as the cM position where the trace of the LOD value intersects the LOD cutoff value. QTL confidence intervals were defined as the location of the trace of the LOD curve where it drops one LOD from the peak LOD value (Case et al. 2014a; Case et al. 2014b). Diagnostic markers for QTLs were those closest to the peak of the LOD value. QTL were named using the convention: “trait”-“qtl”-“population”-“parental allele which reduced the trait value”-“chromosome”, where trait was abbreviated as stem rust (sr), spike row (sp), heading date (hd), leaf rust (lr), physiological leaf spotting (pls), plant height (ph), and covered or naked caryopsis (sc). Terminal disease severity values (0-100%) were used in the stem rust QTL analysis, as artificial inoculation of test entries was necessary to ensure adequate disease pressure. For the leaf rust assays on seedling plants, the raw IT values were converted to numerical coefficient of infection (CI) values for QTL analysis using the scale described by Zhou et al. (2014). Covered and naked caryopsis traits were transformed to numeric values, where a covered caryopsis was 1 and a naked caryopsis was 0. For the QTL analysis of spike row type, the values of 2 and 6 were used for two-rowed and six-rowed spike row morphology, respectively, in the HH population. In the PH population, the RILs segregated for normal and deficiens type of

lateral floret morphology within a two-rowed spike type. Normal floret types were given a value of 2 and deficiens types a value of 3, following convention of USDA-ARS, National Small Grains Collection. Hietpas-5 or PI 382313 was set as the A parent and Hiproly as the B parent in the QTL analysis. Then, Tukey's HSD test was performed on the mean stem rust severity values across all locations using the total number of QTLs each RIL contained and major effect QTLs (those proposed to be *Rpg2* or *Rpg3*). Tukey's HSD test was executed in R using the agricolae package ( $p$ -value<0.05) (De Mendiburu 2016).

## **Results**

### *Genotyping*

The raw SNP genotype data were first filtered using the criteria described above (Online Resource 4.2 and Online Resource 4.3). The initial number of markers called in the HH population was 20,787. The raw SNP data were then imputed to reduce the number of missing values in the dataset using the LinkImpute method (Money et al. 2015)(Online Resource 4.4 and Online Resource 4.5). LD before and after imputation was 0.794 and 0.789 in the HH population and 0.820 and 0.808 in the PH population, respectively. Based on average  $R^2$  LD estimation with a five marker-sliding window executed in TASSEL (Bradbury et al. 2007), this result indicated that imputation was appropriate, as it did not change allele frequencies. These markers were then converted to "A", "B", "H", and "N" calls as described above (Online Resource 4.6 and Online Resource 4.7).

Then, 7,049 markers were removed based on the filtering criteria. Additionally, there were 5,154 SNPs that mapped to the same loci. These markers were filtered out to include only one per locus, which brought the marker total in the HH population to 8,586, including the morphological traits. In the PH population, a total of 15,503 markers were initially called. These were then filtered, removing 6,436 markers based on the quality

criteria described above. In addition, 2,084 duplicate loci were removed, leaving a total of 6,985 markers that passed quality control, including the morphological traits.

Within the HH RILs, the mean frequency of the “A” parent (Hietpas-5) allele was 0.48 (range 0.16-0.84), and the mean frequency of the “B” parent (Hiproly) allele was 0.48 (range 0.14-0.81). The mean number of heterozygous loci and missing values was 0.02 (range 0-0.13) and 0.01 (range 0-0.2), respectively. By sequence alignment to the barley reference genome (cv. Morex), 880 markers were assigned to chromosome 1H, 2,117 to 2H, 2,141 to 3H, 1,077 to 4H, 412 to 5H, 539 to 6H, and 1,292 to 7H. One hundred and twenty-six markers could not be aligned back to the reference genome.

Within the PH RILs, the mean frequency of the “A” parent (PI 382313) allele was 0.48 (range 0.05-0.98), and the mean frequency of the “B” parent (Hiproly) allele was 0.47 (range 0.07-0.93). The mean number of heterozygous loci and missing values was 0.01 (range 0-0.14) and 0.02 (range 0-0.18), respectively. By sequence alignment, 186 markers were assigned to chromosome 1H, 1,943 to 2H, 1,591 to 3H, 712 to 4H, 431 to 5H, 284 to 6H, and 1,741 to 7H. Ninety-five markers could not be aligned back to the reference genome.

### *Map Construction*

Initially, a very high-density map was constructed using all available markers. However, this resulted on chromosomes often exceeding 500 cM in length. To reduce overall map distance, markers were filtered as described above to about 200-300 evenly spaced markers per chromosome based on their physical distance and initial cM distance. After this filtering step, maps were constructed based on 1,528 and 1,364 markers in the HH and PH populations, respectively (Online Resource 4.8). In the end, all seven chromosomes were well saturated with markers across their lengths (Figures 4.1 and 4.2). Correlation of cM position and Mega Base Pair (MBP) based on the reference sequence were high. In the HH population, correlations for the respective seven chromosomes (1H

to 7H) were 0.91, 0.91, 0.92, 0.87, 0.89, 0.87, and 0.92 and in the PH population 0.85, 0.91, 0.92, 0.83, 0.88, 0.88, and 0.93 (Appendix A3 and A4).

The average number of markers per chromosome was 218.2 in the HH population and 194.8 in the PH population with total map distances of 2,167.9 cM and 2,090.9 cM, respectively (Table 4.1). The average chromosome length was 309.7 cM and 298.7 cM in the HH and PH populations, respectively. On average, there was one marker every 1.4 and 1.5 cM in the HH and PH populations, respectively. Two-rowed vs. six-rowed spike type scored as marker *Spike\_Row\_26* mapped to 216 cM on chromosome 2H in the HH population (Online Resource 4.7). Two-rowed vs. two-rowed deficiens type of lateral floret morphology scored as marker *Spike\_row\_23* mapped nearby at 214 cM in the PH population (Online Resource 4.8) (Franckowiak et al. 1997a; Franckowiak et al. 1997b; Wenzl et al. 2006). The naked caryopsis trait scored as marker *Naked\_Seed* mapped at 232 and 263 cM on chromosome 7H in the HH and PH populations, respectively (Online Resource 4.7 and 4.8).

### *Rust Resistance Phenotyping*

In total, there were five stem rust APR evaluations of the PH and HH populations with North American races of *Pgt* (StP\_MC15, StP\_QC15, StP\_HK15, StP\_QC16, and StP\_HK16). The QCCJB, MCCFC, and HKHJC nurseries were confirmed to be pure for the respective races based on samples collected at the end of the season and phenotyped on an abridged set of diagnostic wheat differentials (data not shown). Both populations were also evaluated in Njoro (KEN\_15o), but only the HH population was additionally evaluated in Greytown (SA\_16). Thus, the number of APR phenotype datasets was seven and six for the HH and PH populations, respectively. Overall, the controls performed as expected with Chevron being highly resistant to races HKHJC and MCCFC, while exhibiting higher severities to races QCCJB, PTKST, and *Pgt* TTKSK composite (Figure 4.3). Additionally, Q/SM20 (with *rpg4/Rpg5* only) and Q21861 (with both *Rpg1* and



*rpg4/Rpg5*) performed as expected with the former being highly resistant to all races except HKHJC and the latter resistant to all races tested (Figure 4.3). Hietpas-5 and PI 382313 showed reduced disease severities compared to Hiproly to all races except *Pgt* TTKSK composite (KEN\_15o) (Figure 4.3). These two accessions also exhibited lower adult plant IRs on stem tissue in comparison to Hiproly (Figure 4.4). Overall, both populations were skewed toward lower stem rust severities (i.e. higher resistance) across all locations (Figures 4.5 and 4.6). The overall mean and standard deviation (SD) of RILs for the HH and PH populations were 23.0% and 17.5% and 18.6% and 16.5%, respectively. The mean severity for the resistant parents of Hietpas-5 and PI 382313 were 8.3% and 7.6%, respectively. In contrast, the susceptible parent of Hiproly had a markedly higher mean severity of 47.1% and 48.3% in the HH and PH populations, respectively. Within each location, the resistant parent always exhibited a much lower stem rust severity than the susceptible parent Hiproly, the exception being to *Pgt* TTKSK composite (KEN\_15o) where the differences were smaller (Figures 4.7 and 4.8). The mean difference in severity between the two parents was 36.2% (range 16.5-56.2%) in the HH population and 39.8% (range 7.1-55.9%) in the PH population. The smallest severity difference observed between the two parents was in KEN\_15o for both populations. Overall, the stem rust controls performed as expected across all locations (Figure 4.9).

Variation for stem rust severity was observed among locations with the lowest scores found in KEN\_15o and StP\_MCC15 based on the susceptible controls (Table 4.2). The mean rust severity of the susceptible barley controls across locations was: 15.4% (range 3.0-35%) for 80-tt-30, 50.3% (range 20.0-80.0%) for Hiproly, 60.3% (range 20.0-100%) for PI 532013, and 36.1% (range 5.0-80.0%) for Steptoe. The mean severity of the susceptible wheat controls of LMPG-6 was 63.3% (range 40.0-80.0%) in St. Paul and for Red Bobs 85.0% (range 60.0-100%) in Njoro. The mean severity of the barley controls with *Rpg1* was 8.7% for 80-TT-29 (range 0.5-45.0%) and 10.8% for Chevron (range 0.5-35.0%). These accessions showed a wider range of severity across locations because they

were challenged against races that were virulent and avirulent for *Rpg1*. The mean severity of Quest was moderately high at 35.0% (range 20.0-50.0%) in KEN\_15o; it was used in place of Chevron in Njoro to assess the performance of *Rpg1* and as such showed mostly susceptible reactions. The mean rust severities for the resistant parents of Hietpas-5 and PI 382313 were 8.90% (range 0.5-30.0%) and 12.8% (0.5-25.0%), respectively. These two parents typically had low to moderately low disease severities (range 0.5-25.0), but occasionally higher severities (range 10.0-45.0%) were observed in KEN\_15o and SA\_16. Of the checks with *rpg4/Rpg5*, Q/SM20 had a mean severity of 3.1% (range 0.0-30.0%) and Q21861 a mean of 1.9% (range 0.0-30.0%). These accessions exhibited low rust severities (range 0.5-10.0%) across most locations with some higher severities (range 5.0-30.0%) recorded on Q/SM20 in StP\_HK15 and on Q21861 in KEN\_15o.

$H^2$  was calculated on a global basis treating experiment and line identity as a random effect within each population. From this analysis, the global  $H^2$  for the HH and PH populations was 0.85 and 0.88, respectively (Table 4.3). Within each experiment,  $H^2$  ranged from 0.33 to 0.63 for the HH population and 0.65 to 0.75 for the PH population. The experiments in KEN\_15o and SA\_16 were unreplicated; thus,  $H^2$  could not be calculated.

A chi-square test for inheritance of APR in both populations was also conducted (based on a 1 : 1 ratio for resistance : susceptibility) using the maximum value of Hietpas-5 or PI 382313 as the cutoff for classifying a RIL as resistant when compared to the mean stem rust severity of a RIL within a race. In the HH population, resistance to races MCCFC, QCCJB, and HKHJC all fit a 1:1 ratio for single gene inheritance (Table 4.4). A clear Mendelian inheritance pattern was not observed, however, for APR to *Pgt* TTKSK composite or PTKST. In the PH population, resistance to races QCCJB and HKHJC fit a single gene ratio (Table 4.4). A Mendelian inheritance pattern was not observed in response to race MCCFC nor to *Pgt* TTKSK composite.

Correlation analyses revealed some associations between various agro-morphological traits segregating in the populations and stem rust severity (Table 4.5). In the HH population, there was only one location where the correlation between stem rust severity and heading date was significant (0.149) (StP\_MC16). However, there were significant correlations between spike row type and stem rust severity across most locations. Heading date corrected values for stem rust severity in both populations were created for StP\_HK15, StP\_MC15, and StP\_MC16. The correlation between the corrected and non-corrected datasets was very high (0.98), suggesting that heading data was not altering stem rust severity values. Additionally,  $R^2$  values of the mixed linear model with and without heading date included were nearly identical (range 0.79-0.98). In the PH population, two locations had significant correlations between stem rust severity and heading date (St\_MC15 and StP\_MC16). The highest coefficient found was for StP\_MC16 (-0.440).

In Greytown, a severe epidemic of leaf rust caused by *P. hordei* occurred, revealing striking reaction differences between the HH parents (SA\_16LR). Hietpas-5 was very resistant with a mean leaf rust score of 1.5 (range 1-3), and Hiproly was very susceptible with a mean score of 7.5 (range 7-8) (Figure 4.10). At the adult plant stage, the stem rust controls of Q21861, Q/SM20, Chevron, 212Y1, and Steptoe were resistant (range 1-3), whereas PI 532013 was highly susceptible (range 7-8). PI 382313, Black Hulless, and Quest were moderately susceptible (range 4-6). The overall mean of the HH RILs was 5 with a range of 1-9 and SD of 2.3. The mean difference for leaf rust score between Hietpas-5 and Hiproly was 6.1 (Figure 4.11). Virulence profiling of *P. hordei* isolate SAPH1601 collected from the nursery revealed the following virulence | avirulence pattern on the provisional leaf rust differential set (Brooks et al. 2000) at the seedling stage: Sudan (*Rph1*), Peruvian (*Rph2*), Estate (*Rph3*), Gold (*Rph4*), Magnif 102 (*Rph5*), Bolivia New (*Rph6*), Egypt 4 (*Rph8*), Hor 2596 (*Rph9*), Clipper BC8 (*Rph10*), Triumph (*Rph12*), Hor 15560 (*Rph16*), Prior (*Rph19*), Flagship (*Rph20*), Yerong (*Rph23*) | Cebada Capa (*Rph7*), Clipper BC67 (*Rph11*), PI 531849 (*Rph13*), PI 584760 (*Rph14*),

I95-282-2 (*Rph15*), 81882/BS1 (*Rph17*), 38P18/8/1/10 (*Rph18*), Ricardo (*Rph2+Rph21*). To isolate SAPH1601, Hietpas-5 exhibited an intermediate IT of 21c, whereas Hiproly exhibited a high IT of 33+ (Figure 4.12). Seedling evaluations of the HH population revealed clear and unequivocal segregation: 41 RILs exhibited ITs ranging from 12c to 23-c, whereas 158 exhibited ITs ranging 3 to 3+. This segregation fits a two gene complementary model, where two genes are required for resistance (*p*-value 0.14) (Table 4.4).

CIM was used to scan the genome for significant QTLs for traits assessed in the field (stem rust severity and days to heading) and greenhouse (physiological leaf spotting, naked caryopsis and spike row type). Only the greenhouse dataset for spike row type was used in QTL mapping since this trait could be easily and reliably scored under this controlled environment. QTL locations were based on overlapping QTL boundaries for individual traits. QTLs for stem rust resistance were detected on chromosomes 2H, 3H, 4H, and 7H in the HH population and on chromosomes 2H, 3H, 4H, 5H, and 7H in the PH population (Figures 4.13 and 4.14). In all locations where a QTL was detected based on the corrected stem rust severity data, a coincident QTL was also identified based on the raw severity data from that respective location. Therefore, the raw severity data was used in all subsequent analyses.

In the HH population, the most significant QTLs identified for stem rust resistance were in chromosome 2H (Figure 4.13, Table 4.6). In this chromosome, significant QTLs were detected with all rust severity datasets, except for those from Africa (KEN\_15o and SA\_16). However, the positions of these QTL were not static as some did not overlap with one another. As such, there were four QTL regions called on chromosome 2H: *Rpg-qt1-HH-Hie-2H.1*, *Rpg-qt1-HH-Hie-2H.2*, *Rpg-qt1-HH-Hie-2H.3*, and *Rpg-qt1-HH-Hie-2H.4* (Table 4.6). All of these resistance QTLs were contributed by Hietpas-5 with peak LOD values ranging from 5.0 to 11.9,  $R^2$  from 0.08 to 0.21, and additive effects from -9.2 to -4.16. The most significant QTL identified on chromosome 2H was *Rpg-qt1-HH-Hie-*

2H.3, which had the highest overall LOD,  $R^2$ , and additive effect value. This QTL was the only one detected in multiple years to multiple races, including StP\_QC15, StP\_QC15, StP\_MC15, and StP\_MC16. In contrast *Rpg-qt1-HH-Hie-2H.1*, *Rpg-qt1-HH-Hie-2H.2* and *Rpg-qt1-HH-Hie-2H.4* were all detected in just one season and to a single rust race with datasets, StP\_QC16, StP\_MC15, and StP\_HK15, respectively. Additionally, a QTL for heading date, *Hd-qt1-HH-Hip-2H*, mapped near these resistance QTLs. Minor effect QTLs also were detected on chromosomes 3H, 4H and 7H and designated as *Rpg-qt1-HH-Hip-3H*, *Rpg-qt1-HH-Hie-4H*, and *Rpg-qt1-HH-Hie-7H*, respectively (Figure 4.13, Table 4.6). Hiproly was the donor of the positive allele for resistance for the first QTL and Hietpas-5 for the last two QTLs. The only significant QTL detected in the KEN\_15o dataset was on chromosome 4H. The QTL on 7H was detected only from locations in 2016. QTLs for spike-row type (*Sp-qt1-Hip-7H*) and naked caryopsis (*Sc-qt1-HH-Hip-7H*) were detected at 210 and 237 cM on chromosomes 2H and 7H, respectively (Figure 4.13, Table 4.6). These QTLs mapped very near the mapping locations for these traits as a marker in this population at 216 cM on 2H and 232 cM on 7H, respectively. A Tukey's HSD test was performed on the HH population, with RILs grouped by the number of QTL they carry and if they were carrying *Rpg-qt1-HH-Hie-2H.2*, *Rpg-qt1-HH-Hie-2H.3* haplotype representing the most likely QTLs for *Rpg2* (Figure 4.15). Stem rust severity was averaged over all locations, and just two significantly different groups were detected. RILs carrying zero to two QTLs were significantly different from those carrying four to seven QTLs, although RILs carrying all 7 QTL had the lowest mean severity. If one considers only RILs with *Rpg-qt1-HH-Hie-2H.2*, *Rpg-qt1-HH-Hie-2H.3* as likely candidates for carrying the previously described APR gene of *Rpg2* by Patterson (1955), then the overall rust severity reduction in these RILs is 50% compared to those carrying no resistance QTLs. RILs lacking the *Rpg-HH-Hie-2H.2*, *Rpg-HH-Hie-2H.3* QTLs were not significantly different from those with no QTLs. Moreover, RILs carrying only the *Rpg-HH-Hie-2H.2*, *Rpg-HH-Hie-2H.3* QTLs were not significantly different from those carrying all seven QTLs.

Two QTL for leaf rust APR were detected in the HH population: one at 110 cM on chromosome 2H (*Rph-qt1-HH-Hie-2H.1*) and the other at 64 cM on 6H (*Rph-qt1-HH-Hie-6H*) (Figure 4.13, Table 4.6). Both of these QTL were contributed by Hietpas-5. LOD values ranged from 6.8 to 17.7,  $R^2$  from 0.09 to 0.25, and additive effect from -1.2 to -0.7 with *Rph-qt1-HH-Hie-6H* having the higher value in each case. In addition, QTL analyses also were conducted for the seedling leaf rust reaction to isolate SAPH1601 in the greenhouse where the raw seedling IT data were converted into a linear CI scale as described by Zhou et al. (2014) and also a binary classification of resistant and susceptible. Two major effect QTL on chromosomes 1H and 2H were detected at identical loci using both datasets. To simplify the analysis, only the binary dataset was considered further. The QTL on chromosome 1H (*Rph-qt1-HH-Hie-1H*) mapped at 7.5 cM and the one on 2H (*Rph-qt1-HH-Hie-2H.2*) mapped at 234.1 cM (Figure 4.13, Table 4.6). The positive alleles for increased leaf rust resistance were both contributed by Hietpas-5. For *Rph-qt1-HH-Hie-1H* and *Rph-qt1-HH-Hie-2H.2*, the LOD,  $R^2$ , and additive effect values were 23.1 and 20.2, 0.31 and 0.26, and -0.31 and -0.28, respectively (Table 4.6). Resistance QTLs identified at the seedling stage did not co-localize with resistance QTLs identified at the adult plant stage in the field (Figure 4.13).

In the PH population, a QTL was detected in the 148-171 cM interval on chromosome 5H at all locations except for KEN\_15o (Figure 4.14, Table 4.7). This QTL was named *Rpg-qt1-PH-PI38-5H* using the convention described above. No other traits were detected at this locus. LOD values for this QTL ranged from 4.5 to 8.7,  $R^2$  from 0.12 to 0.20, and additive effect from -6.7 to -4.1. An additional large effect QTL for stem rust severity was detected in the 41-50 cM interval on chromosome 4H and named *Rpg-qt1-PH-PI38-4H*. This locus was significant in the StP\_QC15, StP\_QC16, StP\_MC16, and KEN\_15o datasets. No other traits were detected at this locus. The LOD,  $R^2$ , and additive effect values for *Rpg-qt1-PH-PI38-4H* ranged from 4.3 to 5.6, from 0.07 to 0.10 and from -4.7 to -4.1, respectively. Several minor effect QTLs for stem rust resistance were also detected on chromosomes 2H, 3H, and 7H and named *Rpg-qt1-PH-PI38-2H*, *Rpg-qt1-PH-*

*Hip-3H*, and *Rpg-qt1-PH-PI38-7H*, respectively (Figure 4.14, Table 4.7). *Hip* contributed the positive allele for increased resistance with the chromosome 3H QTL, whereas PI 383313 contributed the positive allele for resistance with the chromosome 2H and 7H QTLs. The latter two QTL appeared to be associated with heading date because they mapped within the vicinity of *Hd-qt1-PH-Hip-2H.2* and *Hd-qt1-PH-Hip-7H.1*. Significant QTLs for spike row type (*Sp-qt1-PH-Hip-2H*) at 216 cM on chromosome 2H and seed cover (*Sc-qt1-PH-Hip-7H*) at 260-267 cM on chromosome 7H were detected very near the mapping locations for these traits as a marker in this population at 214 cM in 2H and 263 cM in 7H, respectively. As with the HH population, Tukey's HSD test was performed on the PH population RILs grouped by the number of QTL they possess, and and if they were carrying *Rpg-qt1-PH-PI38-5H* QTL representing the most likely QTL for *Rpg3* (Figure 4.16). RILs carrying five resistance QTLs exhibited the lowest overall rust severity. The rust severities of RILs carrying three or four QTLs were not significantly different from those carrying five QTLs. RILs with one or two resistance QTL had significantly lower rust severities than those carrying no resistance QTLs. If one considers only RILs with *Rpg-PH-PI38-5H* as likely candidates for carrying the previously described APR gene of *Rpg3* (Jedel et al. 1989), then the overall rust severity of these RILs is 52% lower than those with no resistance QTLs. RILs lacking the *Rpg-PH-PI38-5H* QTL were significantly different from those with no QTLs, but were not significantly different from those with one or two QTLs. However, RILs carrying only the *Rpg-PH-PI38-5H* QTL were not significantly different from those carrying all five QTLs.

All markers nearest to the peak LOD values for resistance QTLs were BLAST searched against the Morex reference assembly ([http://webblast.ipk-gatersleben.de/barley\\_ibsc/](http://webblast.ipk-gatersleben.de/barley_ibsc/)) (Mascher et al. 2017) to identify possible hits to genes with known function in disease resistance (Deng et al. 2007). However, no noteworthy hits of such genes were found.

## Discussion

In this study, we successfully mapped QTLs for stem rust APR originating from Hietpas-5 and PI 382313, the sources of *Rpg2* and *Rpg3*, respectively. Major effect QTLs from Hietpas-5 were mapped on chromosomes 2H and 7H, whereas those from PI 382313 were mapped on chromosomes 4H and 5H. The most important stem rust genes identified in barley include *Rpg1* on chromosome 7H at 7 cM and *rpg4/Rpg5* on chromosome 5H at about 152-168 cM (Dahleen et al. 2003; Kleinhofs et al. 1993; Mamo et al. 2015; Steffenson et al. 2009; Steffenson 1992; Steffenson et al. 2007; Steffenson et al. 1985). As Hietpas-5, PI 382313, and Hiproly all tested negative for the presence of *Rpg1* and *rpg4/Rpg5* (Table 3.13 in Chapter 3) and were mostly susceptible at the seedling stage (Table 3.1 in Chapter 3), it is highly unlikely that these genes were responsible for the described resistance. However, APR or seedling resistance QTLs have been reported near some of the QTLs identified in this study (Mamo 2013; Mamo et al. 2015; Moscou et al. 2011; Turuspekov et al. 2016; Zhou et al. 2014). As the majority of recent research has focused on the response of barleys to race TTKSK, comparison of QTLs not significant in KEN\_150 with those reported in the literature is difficult. In previous testing, we found that Hietpas-5 and PI 382313 have high to moderate levels of APR to TTKSK, but mostly susceptible reactions to this race at the seedling stage (Table 3.1 in Chapter 3)(Steffenson et al. 2017). Identified QTLs conferring resistance to *Pgt* races other than TTKSK can be compared to those found in a GWAS of wild barley (seedling reaction to *Pgt* races MCCFC and QCCJB and also *Pgs* isolate 92-MN-90) and in a GWAS of Ethiopian and Eritrean landraces (APR to *Pgt* race MCCFC)(Mamo 2013; Steffenson et al. 2007). Recently, Steffenson et al. (2017) expanded on the study from Steffenson et al. (2007) by including the above-mentioned races as well as *Pgt* races TTKSK and HKHJC in an analysis that included over 50,000 GBS markers (Sallam and Steffenson 2017).

Hietpas-5 was reported to carry APR to race QCCJB in addition to *Pgt* races 17, 19 and 56 (17=HFLGC, 56=MCCFC, personal communication Yue Jin) (Fox and Harder 1995;



Jin et al. 1994a; Miller and Lambert 1955; Patterson 1955; Patterson et al. 1957). Given the association with resistance to race QCCJB, it is likely that *Rpg2* is associated with the *Rpg-qt1-HH-Hie-2H.3* QTL on chromosome 2H at 179-192 cM. In a Hietpas-5/Chevron population, Patterson (1955) reported a 15 (homogeneous or heterogeneous resistant family row) : 1 (homogeneous susceptible family row) inheritance ratio for APR in F<sub>2:3</sub> families screened in the field to a bulk of stem rust races 17, 19, and 56 (Miller and Lambert 1955; Shands 1964). This suggests that two dominant, independently segregating genes conferred resistance to these races, one being *Rpg1* and the other *Rpg2*. Indeed, in the HH population, a chi-square test revealed that resistance to races MCCFC, QCCJB, and HKHJC was inherited as a single locus. As the major effect QTL in the HH population (*Rpg-qt1-HH-Hie-2H.3*) mapped to chromosome 2H, it is certain that *Rpg1* is not responsible for the resistance reported here. The resistance QTL, *Rpg-qt1-HH-Hie-2H.2*, effective against race MCCFC and mapping at 140 cM, is closely linked to *Rpg-qt1-HH-Hie-2H.3* and also conditions resistance against races MCCFC and QCCJB, suggesting these QTL may be detecting the same genes. The chromosome 2H QTL (*Rpg-qt1-HH-Hie-2H.1*) also was detected in response to race QCCJB, but is distant from other QTLs at 61 cM. This result suggests that it is detecting a different gene from *Rpg-qt1-HH-Hie-2H.1* or *Rpg-qt1-HH-Hie-2H.2*. It is not known what association the QTL *Rpg-qt1-HH-Hie-2H.4* at 219 cM may have to *Rpg2* as HKHJC was not one of the original races used to describe this resistance. Mixed linear models with and without heading date as a covariant had nearly identical R<sup>2</sup> values, showing that heading date was not an important descriptor of the data for this model and was a less important factor in stem rust severity than line identity. Therefore, QTLs for heading date near stem rust resistance QTLs may be coincidental. Several resistance QTLs on chromosome 2H were mapped by both Moscou et al. (2011), Mamo (2013), and in this dissertation (Table 2.2 in Chapter 2), to race TTKSK at the seedling or adult stages. However, as none of the QTLs identified here were significant in KEN\_15o, the possible relationships among these QTLs are not known

The most likely QTLs representing the *Rpg2* resistance originally described by Patterson (1955) are *Rpg-qt1-HH-Hie-2H.3* at 179-192 cM and *Rpg-qt1-HH-Hie-2H.2* at 140 cM because they confer APR to races QCCJB and MCCFC. It seems plausible to conclude that the location of *Rpg2* is between 140-192 cM on chromosome 2H. Using GWAS in a wild barley panel, Sellam and Steffenson (2017) mapped QTLs for seedling resistance to races MCCFC and QCCJB at about 110 cM on this chromosome (Sallam and Steffenson 2017; Steffenson et al. 2007). However, as Hietpas-5 is susceptible to races QCCJB and MCCFC at the seedling stage, it is unlikely that these QTL are the same (Table 3.1 in Chapter 3)(Sun and Steffenson 2005). As no previous studies have mapped any QTLs for APR to these races in this region, it is unlikely that the *Rpg2* resistance had been mapped previously. The *Vrs1* gene that controls the row type of spikes is located on chromosome 2H at around 100 cM (Franckowiak and Haus 1997a, b; Komatsuda et al. 1999; Komatsuda et al. 2007; Muñoz-Amatriaín et al. 2014; Wenzl et al. 2006). However, here we mapped the Mendelian locus controlling row type (Spike\_Row26, presumably *Vrs1*) to 216 cM on chromosome 2H. Additionally, QTL analysis identified a major effect locus for spike row type (*Sp-qt1-HH-Hip-2H*) that mapped at 209 cM on 2H, near the described Mendelian locus defined by the dataset Spike\_Row26. The large difference in cM position for the previously described *Vrs1* locus (Franckowiak and Haus 1997a, b; Komatsuda et al. 1999; Komatsuda et al. 2007; Muñoz-Amatriaín et al. 2014; Wenzl et al. 2006) and spike row type in this study could be due to the high density of the current map. Interestingly, a QTL for APR to race HKHJC (*Rpg-qt1-HH-Hie-2H.4*) at 219 cM on 2H co-localized with a QTL for row type in this population. The effect of row type on stem rust severity is unknown, but may be related to differences in the stem characteristics of two-rowed and six-rowed barley. Six-rowed types generally have thicker stems than two-rowed types, thereby facilitating greater uredinia expansion.

Of the other QTLs identified for stem rust resistance in the HH population, the most significant one was *Rpg-qt1-HH-Hie-7H* at 228 cM on chromosome 7H. This QTL was also detected in response to races MCCFC and QCCJB, both of which are associated with

*Rpg2* resistance. However, it was only detected in 2016, suggesting that it is not likely the primary QTL for *Rpg2*. *Rpg1* is a major gene on chromosome 7H that resides at about 7 cM and confers resistance to race MCCFC, but not QCCJB (Dahleen et al. 2003; Kleinhofs et al. 1993; Steffenson 1992; Steffenson et al. 1985). However, Hietpas-5 does not have this gene based on molecular marker analysis (Table 3.13 in Chapter 3) and multi-race seedling stem rust analysis (Table 3.1 in Chapter 3). No previous study has reported a stem rust resistance QTL in this region. *Rpg-qt1-HH-Hie-7H* lies near the *nud* gene (Franckowiak and Haus 1997a) controlling naked caryopsis at 232 cM, and the QTL (*Sc-qt1-HH-Hip-7H*) for this trait lies nearby at 236 cM. The association between the *nud* gene and stem rust severity is likely coincidental.

The resistance QTL on chromosome 4H (*Rpg-qt1-HH-Hie-4H*) at 49 cM was detected only in the KEN\_15o dataset. It is not known whether *Rpg2* or another gene present in Hietpas-5 is conferring resistance to race TTKSK. As TTKSK was not one of the original races used to describe *Rpg2*, it is unlikely that this QTL is a primary candidate for *Rpg2* and therefore represents an additional resistance gene present in Hietpas-5. Two previous studies have reported QTLs for stem rust resistance on chromosome 4H. Using GWAS, Mamo (2013) described a seedling resistance QTL in Ethiopian and Eritrean barley landraces for TTKSK seedling resistance, and Turuspekov et al. (2016) reported resistance QTL in spring barleys from Kazakhstan to local races of *Pgt*. However, the QTLs reported by Turuspekov et al. (2016) were only significant at the pre-heading stage to local races of *Pgt* and therefore are likely not related to QTL detected here. The QTL reported by Mamo (2013) for TTKSK seedling resistance is near *Rpg-qt1-HH-Hie-4H*; however, it was not significant at the adult plant stage and is therefore unlikely to be related to *Rpg-qt1-HH-Hie-4H* for APR to TTKSK.

Interestingly, Hiproly, the susceptible parent, contributed the resistance allele for the QTL (*Rpg-qt1-HH-Hip-3H*) at 214 cM on chromosome 3H in response to race QCCJB.

This QTL was only detected in the StP\_QC15 dataset. No previously reported QTL lie near *Rpg-qt1-HH-Hip-3H*.

PI 382313 is the source of *Rpg3* and has been shown to have high to moderate levels of field resistance to race QCCJB and a composite of Canadian races (C17=MCCFC, C25=QTHJC, C35=RKQQC, 49, 61, and 67, personal communication Yue Jin) (Fox and Harder 1995; Jedel 1991; Jedel et al. 1989). As *Rpg3* confers resistance to races MCCFC and QCCJB, we focused our investigation on these races. Two major effect QTLs for APR were detected in PI 382313. *Rpg-qt1-PH-PI38-5H* was mapped at 148-172 cM on chromosome 5H and was significant at all locations except for KEN\_15o. As this QTL conferred resistance against races QCCJB and MCCFC in both seasons, it likely represents the *Rpg3* gene. Chi-square analysis of resistance to races QCCJB and HKHJC revealed the presence of a single gene (Table 4.4). Since PI 382313 does not carry *rpg4/Rpg5* complex on chromosome 5H as determined by molecular analyses (Table 3.13 in Chapter 3) and multi-race seedling stem rust analysis (Table 3.1 in Chapter 3), it is highly unlikely that the identified QTLs is due to these genes. Chromosome 5H is particularly important for stem rust resistance in barley. In addition to the *rpg4/Rpg5* gene complex at about 145-152 cM, many QTLs have been reported for both seedling and APR (Mamo et al. 2015; Steffenson et al. 2009). Zhou et al. (2014), Mamo (2013), Moscou et al. (2011), Case 2017 (Chapter 2 in this dissertation), and Turuspekov et al. (2016) have all mapped QTL for seedling resistance or APR on chromosome 5H. Mamo (2013) and Moscou et al. (2011) reported QTL close to *Rpg-qt1-PH-PI38-5H* at 145-152 cM; however, all of these were shown to be an effect of the *rpg4/Rpg5* complex locus and as such are not likely the same as any QTLs mapped in this population. Therefore, it is likely that *Rpg3* resides somewhere near, but not at the *rpg4/Rpg5* gene complex on chromosome 5H. The resistance spectra of lines carrying these respective genes are clearly different. The resistance gene *Rpg5* (Gene bank number EU878778, Morex Genome Reference number HORVU5Hr1G113030.4) was BLAST searched against the Morex genome and found to be at 640.7 MBP (Wang et al. 2013; Mascher et al. 2017).

Whereas, the most significant markers for *Rpg-qt1-PH-PI38-5H* were positioned at 461.0 MBP. This result demonstrates that *Rpg5* and *Rpg-qt1-PH-PI38-5H* are distinct loci physically separated from each other. Allelism studies may not be needed to confirm the independence of *Rpg3* from *rpg4/Rpg5*, although possible epistatic interactions between the genes would be interesting. In Chapter 2, we detected a QTL (*Rpg-qt1-5H-11\_20388*) at 137 cM for race QCCJB APR, which may be related to *Rpg-qt1-PH-PI38-5H* as both were detected at the adult plant stage in response to race QCCJB. The possible association between these two QTLs warrants further investigation. However, the physical location (619.1 MBP) of marker 11\_20388 is distant from the physical location for the peak marker of *Rpg-qt1-PH-PI38-5* (467.6 MBP).

Another major QTL for stem rust resistance, *Rpg-qt1-PH-PI38-4H*, was found on chromosome 4H at 41-50 cM, associated with QCCJB and MCCFC resistance in addition to TTKSK resistance. However, this QTL was not stable across seasons. Since *Rpg-qt1-PH-PI38-4H* was associated with TTKSK resistance, it is not related to the original described *Rpg3* resistance and warrants further investigation as it may be novel. As mentioned above, Mamo (2013) reported a QTL for TTKSK seedling resistance near *Rpg-qt1-PH-PI38-4H*. However, since this QTL was not significant for TTKSK or MCCFC APR, it is unlikely related to *Rpg-qt1-PH-PI38-4H*.

QTLs for stem rust resistance donated by PI 382313 were also detected on chromosome 2H (*Rpg-qt1-PH-PI38-2H*) and 7H (*Rpg-qt1-PH-PI38-7H*), but only in 2016. Since these QTLs were not stable across seasons, they likely do not represent the major resistance loci in the population and are not primary candidates for *Rpg3*. Both of these QTLs appear to be associated with QTLs for heading date: *Hd-qt1-PH-Hip-2H.2* at 35-43 cM on chromosome 2H and *Hd-qt1-PH-Hip-7H.1* at 71 cM on chromosome 7H. The close proximity of QTL for heading date with those for stem rust resistance suggests a possible pleiotropic effect of the former trait on the latter. The closest QTLs to *Rpg-qt1-PH-PI38-2H* that have previously been mapped are those reported by Mamo (2013) and Moscou et

al. (2011) for TTKSK seedling resistance and APR, respectively. The closest QTLs to *Rpg-qt1-PH-PI38-7H* are those mapped by Turuspekov et al. (2016) to local races of *Pgt* in Kazakhstan. Both *Rpg-qt1-PH-PI38-2H* and *Rpg-qt1-PH-PI38-7H* were identified for APR to races MCCFC and QCCJB; thus it is unlikely that they are related to previously mapped QTLs.

The only QTL found for TTKSK APR in the PH population was *Rpg-qt1-PH-Hip-3H* at 100 cM on chromosome 3H in the KEN\_15o dataset. Interestingly, this QTL was contributed by the susceptible parent Hiproly. Several previously reported QTL were mapped near this QTL. Mamo et al. (2015) reported a QTL for TTKSK seedling resistance at 66 cM, and in Chapter 2 (Table 2.2) of this dissertation, we found a QTL for race QCCJB APR at 77 cM. As discussed above, a QTL (*Rpg-qt1-HH-Hip-3H*) for stem rust resistance, also donated by Hiproly, was detected in the HH population at 213 cM. Both of these QTLs explained only a small portion of the genetic variation for the trait and were significant in only one location. Although surprising, it is not uncommon for resistance alleles of QTL to be contributed by the susceptible parent. Moscou et al. (2011) reported a QTL for TTKSK APR contributed by the susceptible parent that enhanced the effect of *rpg4/Rpg5* resistance. This same phenomenon was reported in other rust pathosystems. Case et al. (2014a) reported a QTL in wheat for yellow rust (*Puccinia striiformis* f. sp. *tritici*) APR contributed by the susceptible parent that enhanced the effect of QTL donated by the resistant parent.

In the 2016 Greytown nursery, only the HH population was evaluated for stem rust resistance (SA\_16), and no significant QTL were detected. Leaf rust also occurred in this nursery, so severity data (SA\_16LR) were taken for this disease. Interestingly, Hietpas-5 was highly resistant and Hiproly was highly susceptible to this natural leaf rust population (Figure 4.10 and 4.11). Two QTLs for leaf rust APR were found in the HH population: one at 110 cM on chromosome 2H (*Rph-qt1-HH-Hie-2H.1*) and a second at 64 cM on chromosome 6H (*Rph-qt1-HH-Hie-6H*) (Figure 4.13, Table 4.6). The QTL on

chromosome 6H was the most significant with an  $R^2$  value of 0.25. An isolate of *P. hordei* (SAPh1601) was taken from Greytown nursery and used in seedling evaluations of the HH population (Figure 4.12). From this evaluation, two QTLs were found: one on chromosome 1H (*Rph-qt1-HH-Hie-1H*) at 7.5 cM and the other on 2H (*Rph-qt1-HH-Hie-2H.2*) at 234.1 cM (Figure 4.13, Table 4.6). Interestingly, the QTLs for seedling resistance did not co-localize with any of the identified QTLs for APR. Many leaf rust resistance (*Reaction to Puccinia hordei* (*Rph*)) genes have been described in barley. Race typing revealed that isolate SAPh1601 is avirulent for the genes of *Rph7*, *Rph11*, *Rph13*, *Rph14*, *Rph15*, *Rph17*, *Rph18*, and *Rph21*. *Rph-qt1-HH-Hie-6H* maps about 50 cM proximal to *Rph11* at ~120 cM on 6H, so it cannot be this gene (Feuerstein et al. 1990; Ziemis et al. 2014). However, allelism tests should be made to confirm this. QTL *Rph-qt1-HH-Hie-6H* may be close to ones identified in the L94/Vada RIL population for seedling and APR to *P. hordei* isolate 1.2.1 (Marcel et al. 2007; Qi et al. 1998). The possible relatedness between Vada, a leaf rust resistant cultivar from Western Europe, and Hietpas-5 is unknown. There are a number of *Rph* genes and QTLs described on chromosome 2H. The major genes are *Rph1* at about 20 cM and *Rph8*, *Rph14*, and *Rph15*--all at about 50 cM (Ziemis et al. 2014). As *Rph-qt1-HH-Hie-2H.1* was found at about 110 cM and *Rph-qt1-HH-Hie-2H.2* at 234 cM, it is highly unlikely that these QTLs are represented by one of these previously reported genes. A QTL for seedling resistance to isolate 1.2.1 was identified in the Oregon Wolfe Barley Population at about 110 cM and therefore may be close to *Rph-qt1-HH-Hie-2H* (Costa et al. 2001; Marcel et al. 2007; Spaner et al. 1998). The major effect QTL *Rph-qt1-HH-Hie-1H* at 7.5 cM on 1H maps very close to *Rph4* at about 5 cM; however, SAPh1601 is virulent for *Rph4*.

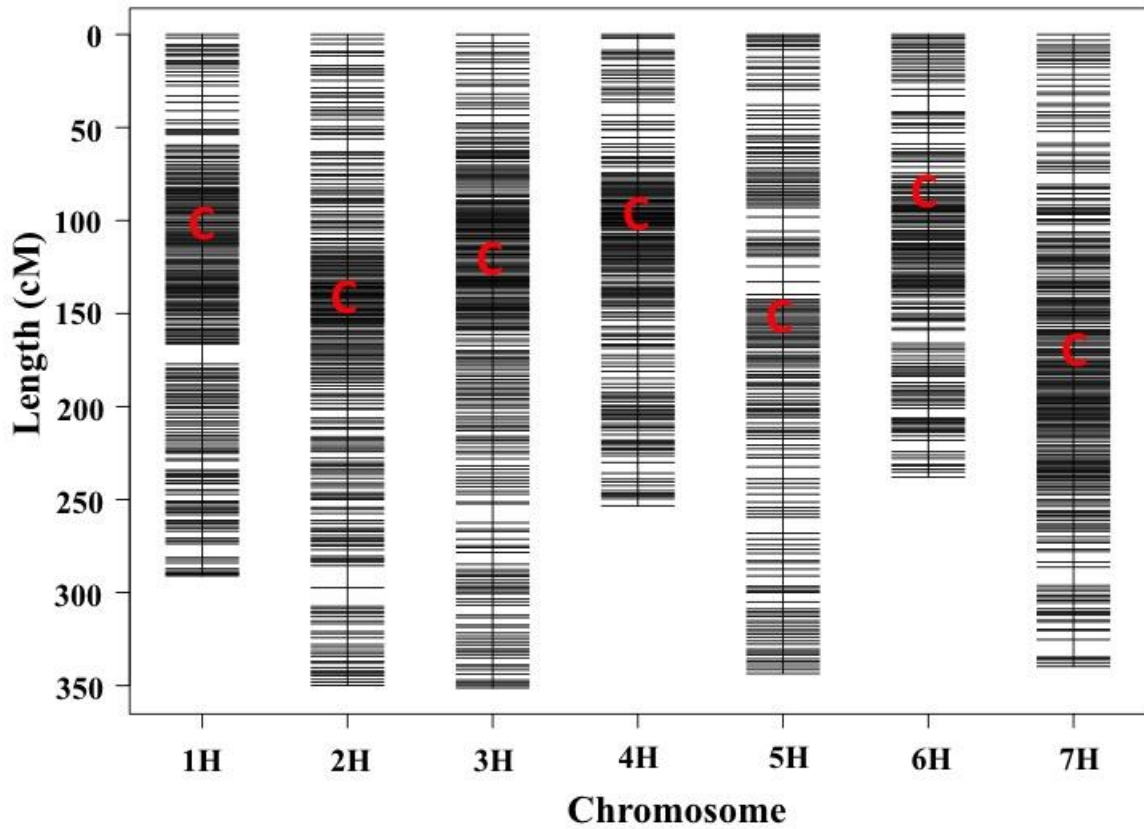
In total, there were seven QTLs for stem rust resistance mapped in the HH population and five in the PH population. RILs carrying multiple QTLs had significantly lower severities than those carrying two or less (Figures 4.15 and 4.16). As subdividing the population reduced the RIL numbers in each group drastically, it was not appropriate to compare all possible QTL combinations. In the HH population, RILs carrying all seven QTLs had a

mean severity of 9.1%, which is similar to that of Hietpas-5 at 8.3%. In the PH population, RILs carrying all five QTLs had a mean severity of 8.2%, a value close to the mean severity of 7.3% found for PI 382313. RILs were recovered with APR similar to or higher than that of the resistant parents. This was expected because in both populations Hiproly contributed a minor effect QTL for stem rust resistance. This result demonstrates that breeding for APR by accumulating several minor effect genes is possible in barley and can result in progeny with enhanced levels of stem rust resistance. This is a strategy that the CIMMYT wheat breeding program has followed, resulting in the development of resistant breeding lines carrying four to five minor effect genes and sustaining rust severities of 10% or less (Singh et al. 2014). The 10% level of stem rust severity is considered acceptable for preventing economic damage in the CIMMYT wheat program. It is likely that similar stem rust resistance levels will be acceptable for avoiding yield loss in barley because many accessions also carry pre-heading resistance and mature earlier than wheat (Steffenson 1992). Combining *Rpg1* and *rpg4/Rpg5* is the best strategy in the short term for reducing the vulnerability of barley to stem rust infection. In the long-term, breeding for stem rust resistance using APR QTLs like *Rpg2* and *Rpg3* will be an important strategy as these sources of resistance may be sufficient to keep rust severities below damaging levels. Pyramiding all-stage resistance genes such as *Rpg1* and *rpg4/Rpg5* together with the APR QTLs represented by *Rpg2* and *Rpg3* will be an important backstop should any race emerge that is capable of overcoming both *Rpg1* and *rpg4/Rpg5* resistance. Indeed, at least one stem rust race in the United States population carries virulence for *rpg4/Rpg5* (HKHJC), and multiple races with *Rpg1* virulence have been reported in the past (Nirmala et al. 2015; Sun and Steffenson 2005). Pyramiding all-stage resistance genes like *Rpg1* and *rpg4/Rpg5* together with APR QTLs is difficult due to the masking of the latter by the former. The use of markers in a marker-assisted selection scheme will allow pyramiding of both all-stage and APR genes like *Rpg2* and *Rpg3*. The markers identified in this study may be used in such a scheme. Additionally, as the GBS markers reported here have a known genome position, these regions can be

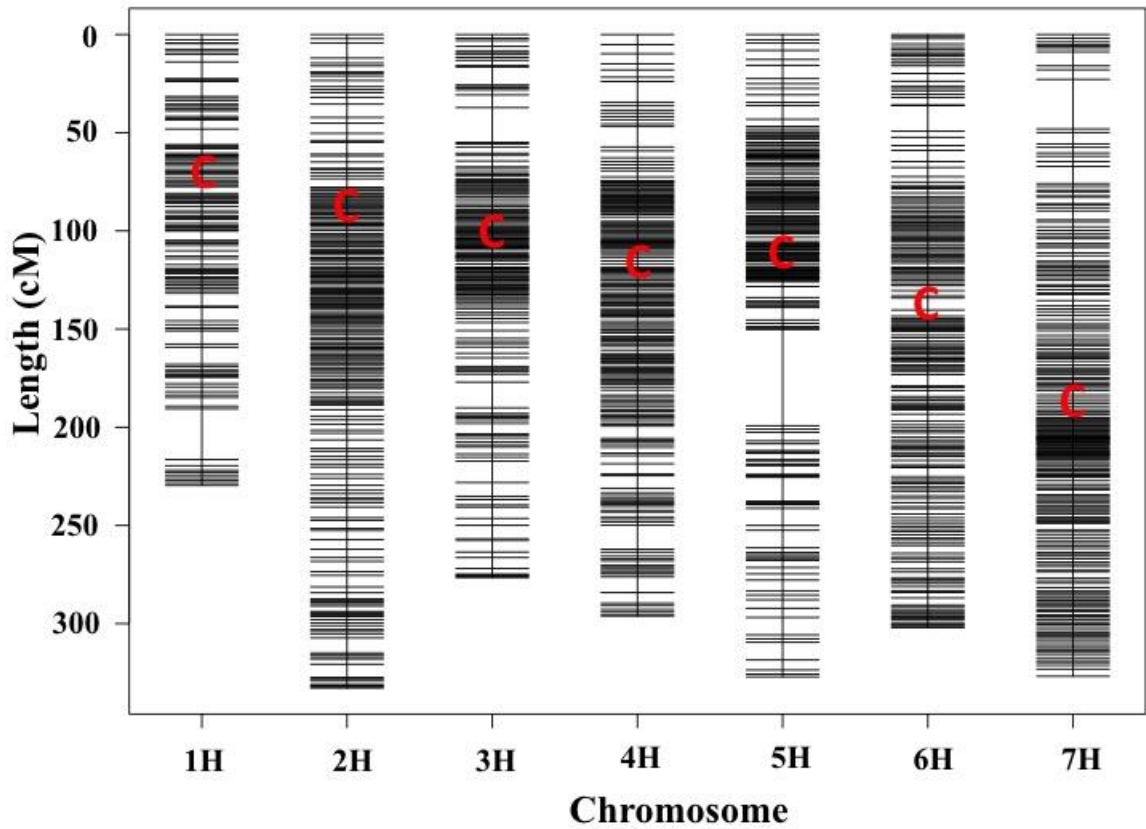


enriched with markers to facilitate fine mapping and also design breeder-friendly markers targeted to these regions.

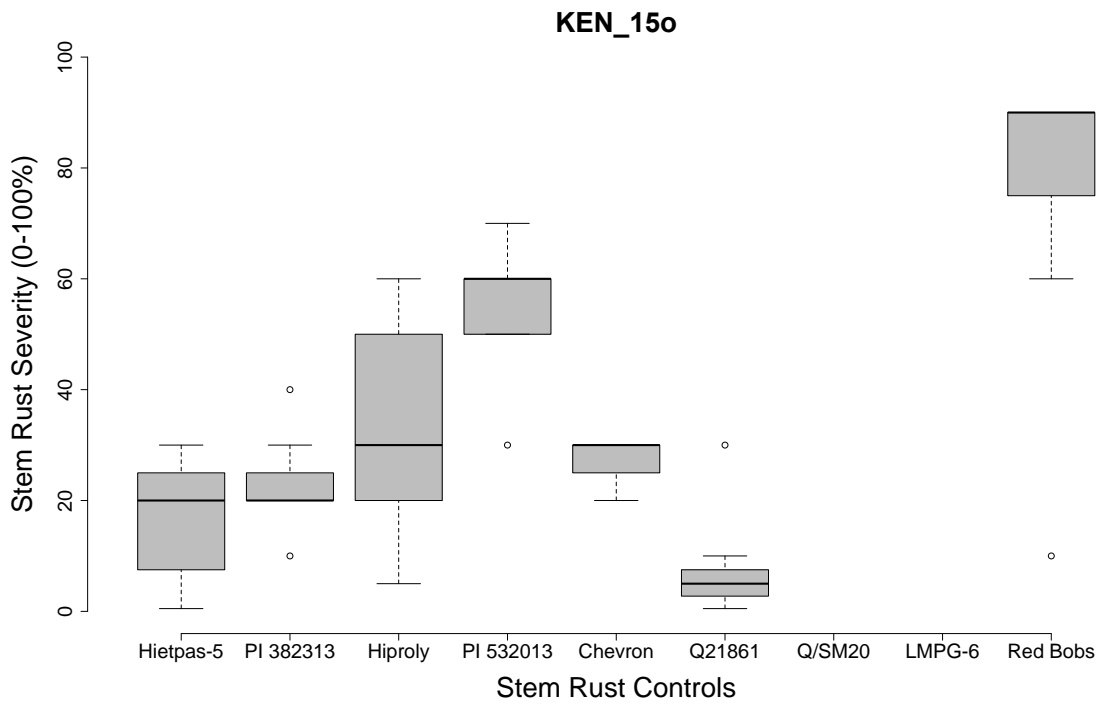
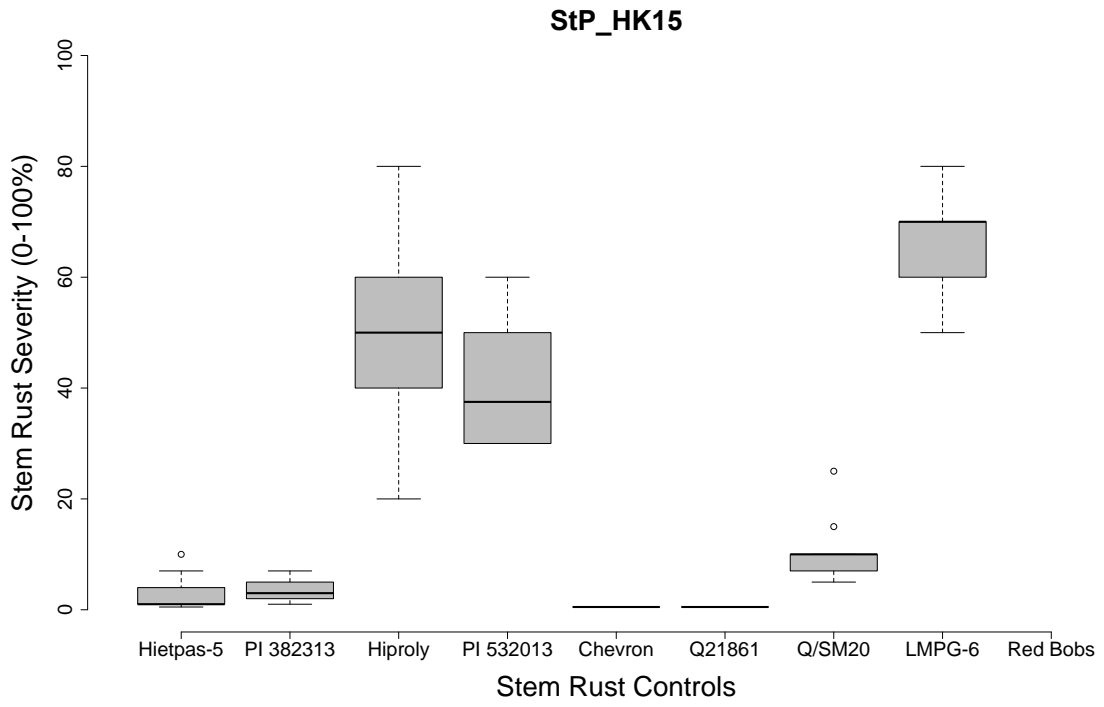
## Figures

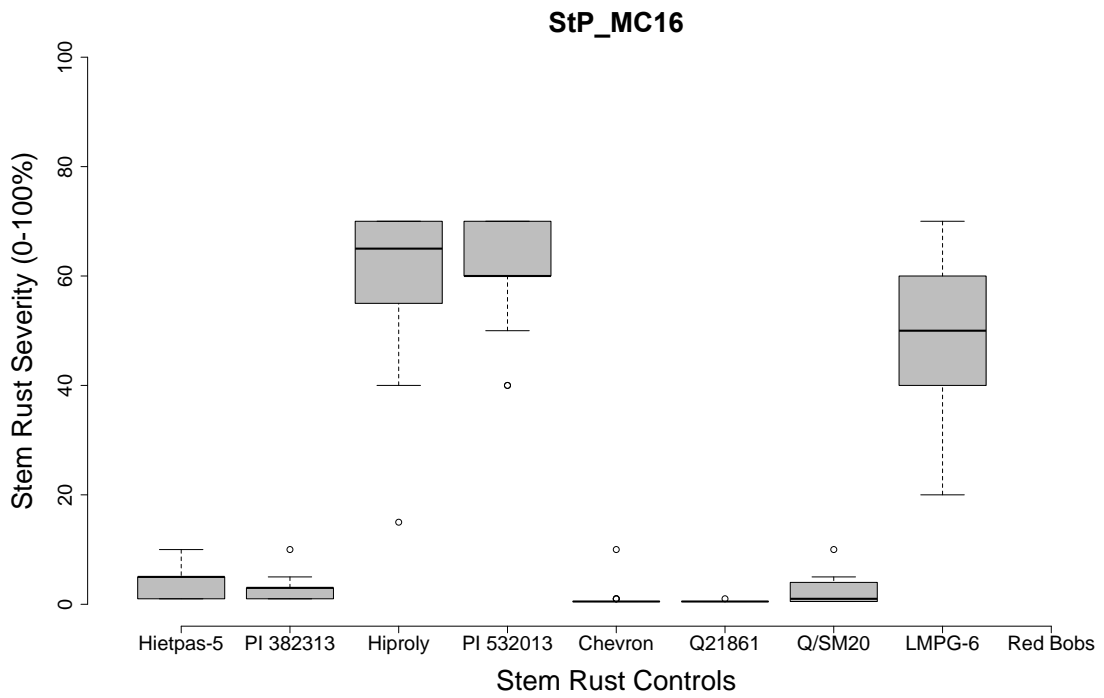
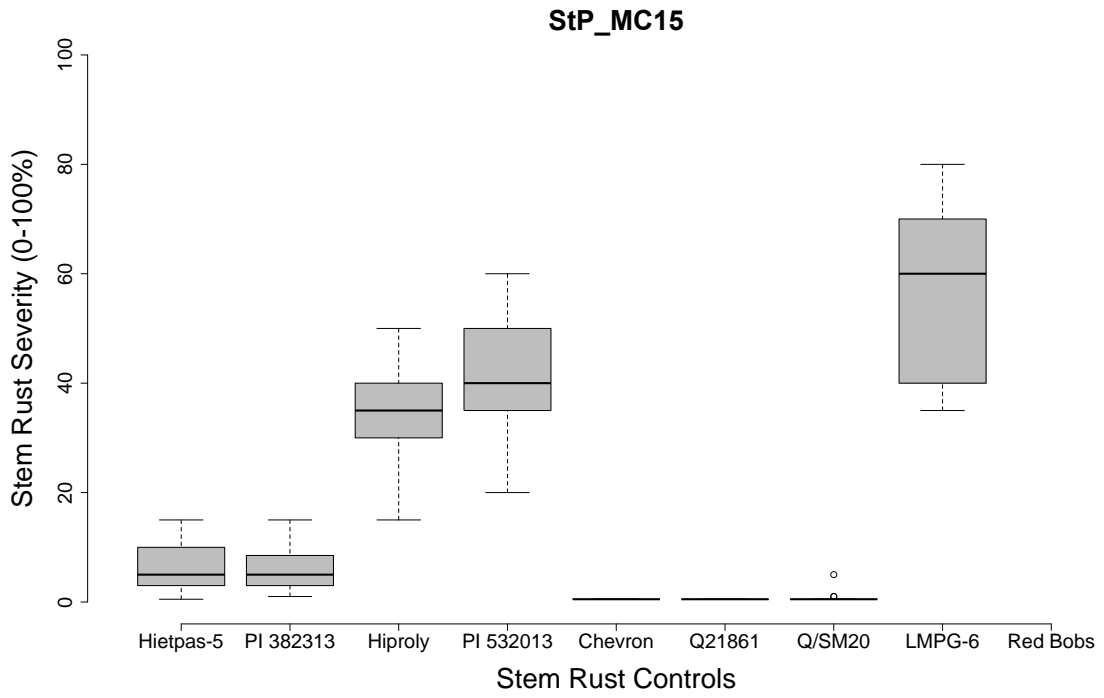


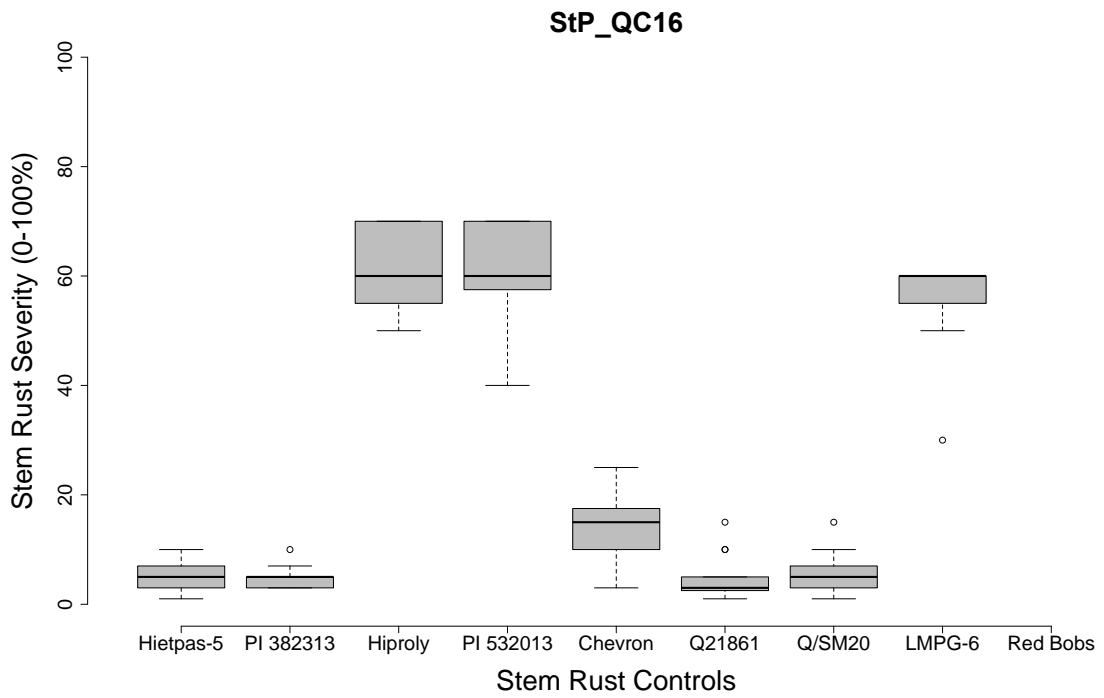
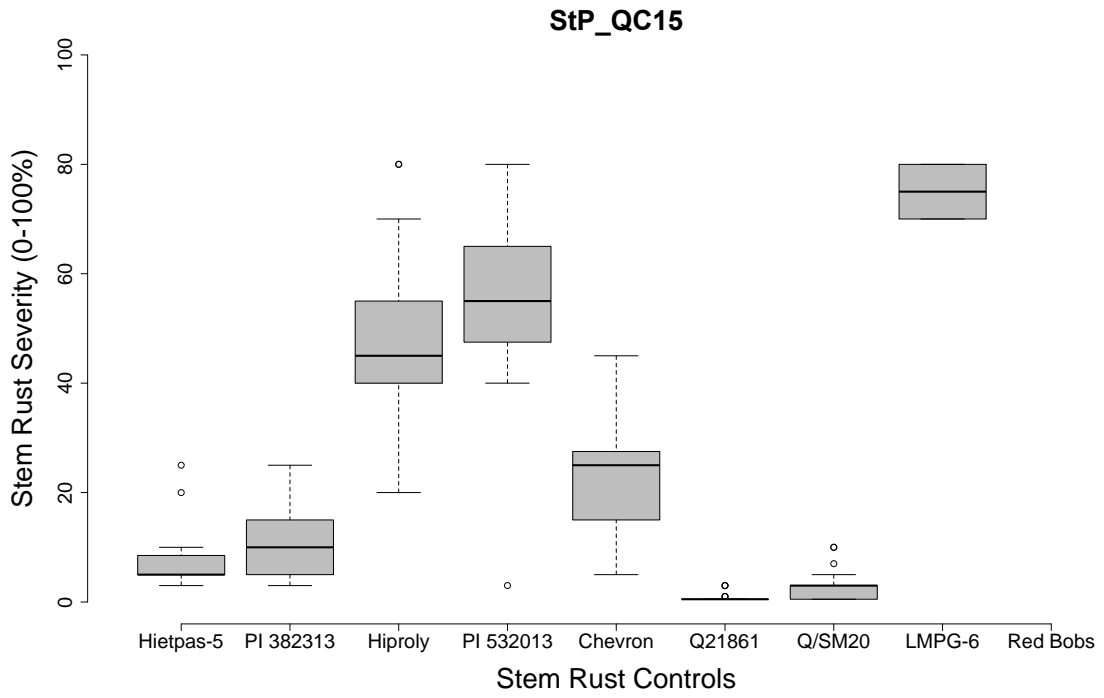
**Figure 4.1.** Linkage map of the Hietpas-5/Hiproly recombinant inbred line population showing calculated centiMorgan (cM) lengths for the seven chromosomes of barley. The red “C” indicates the approximate centromere position.

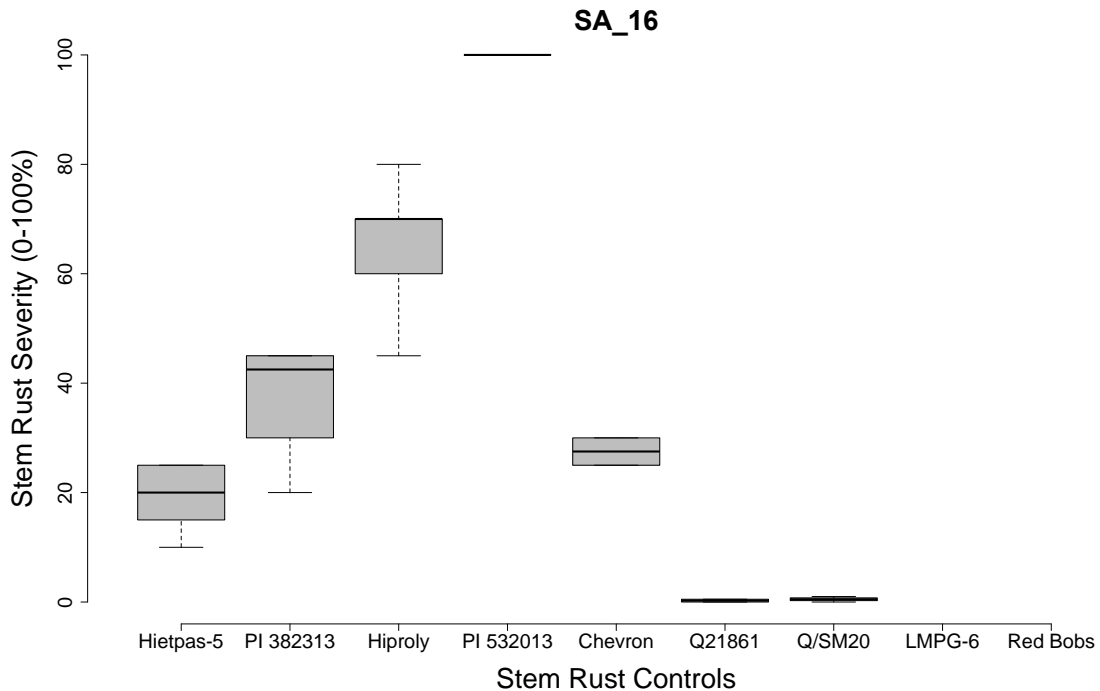


**Figure 4.2.** Linkage map of the PI 382313/Hiproly recombinant inbred line population showing calculated centiMorgan (cM) lengths for the seven chromosomes of barley. The red “C” indicates the approximate centromere position.

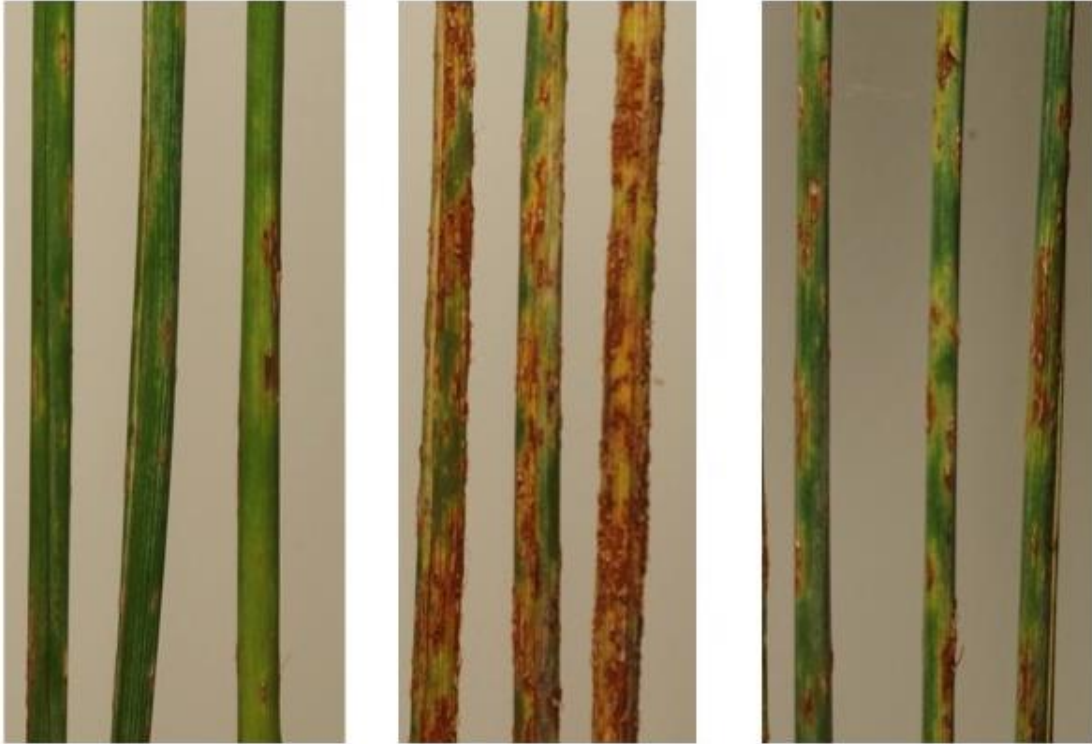






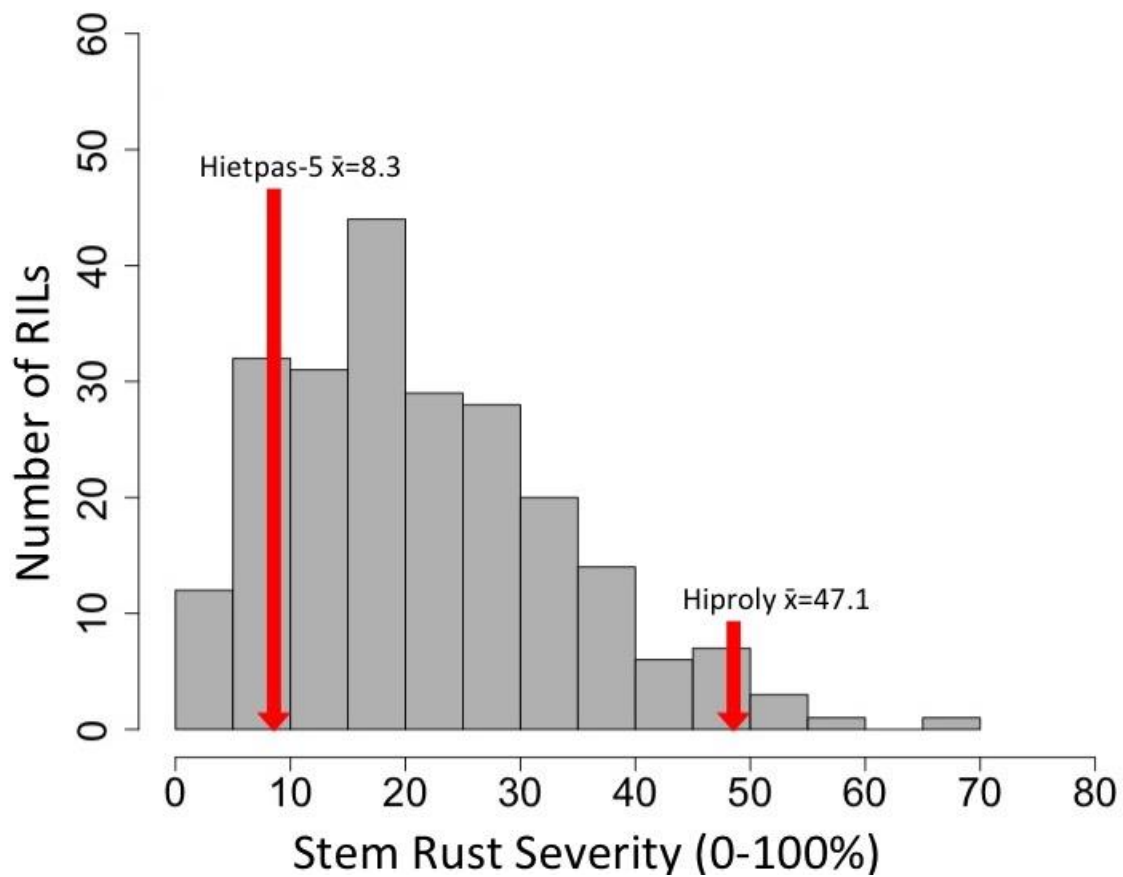


**Figure 4.3.** Performance of barley mapping parents (Hietpas-5, PI 382313, and Hiproly) and barley (PI 532013, Chevron, Q21861, and Q/SM20) and wheat (LMPG-6 and Red Bobs) stem rust controls in multiple stem rust screening nurseries in Njoro, Kenya, Greytown, South Africa, and St. Paul, MN. Nurseries are abbreviated as: 2015 St. Paul *Puccinia graminis* f. sp. *tritici* (*Pgt*) race HKHJC (StP\_HK15), 2015 Kenya off-season *Pgt* TTKSK composite (includes races TTKSK, TTKST, TTKTK and TTKTT) (KEN\_15o), 2015 St. Paul *Pgt* race MCCFC (StP\_MC15), 2016 St. Paul *Pgt* race MCCFC (StP\_MC16), 2015 St. Paul *Pgt* race QCCJB (StP\_QC15), 2016 St. Paul *Pgt* race QCCJB (StP\_QC16), and 2016 Greytown (SA\_16) *Pgt* race PTKST. Five statistics (bars) are represented in each box plot from bottom to top: the smallest observation, lower quartile, median, upper quartile, and largest observation, respectively. Data points positioned outside this range are extreme values and are represented as circles.

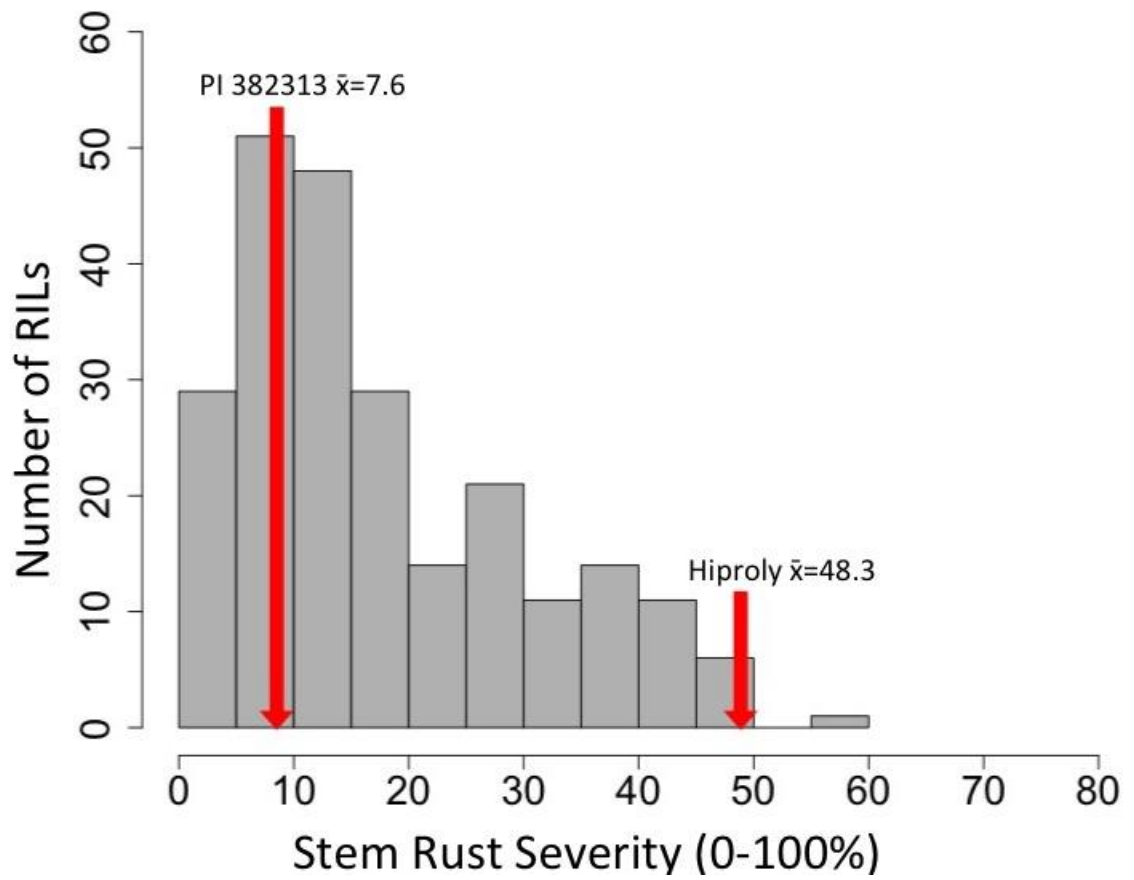


**Figure 4.4.** Adult plant infection responses of Hietpas-5 (left), Hiproly (center), and PI 382313 (right) to *Puccinia graminis* f. sp. *tritici* race QCCJB in St. Paul, MN in 2015.

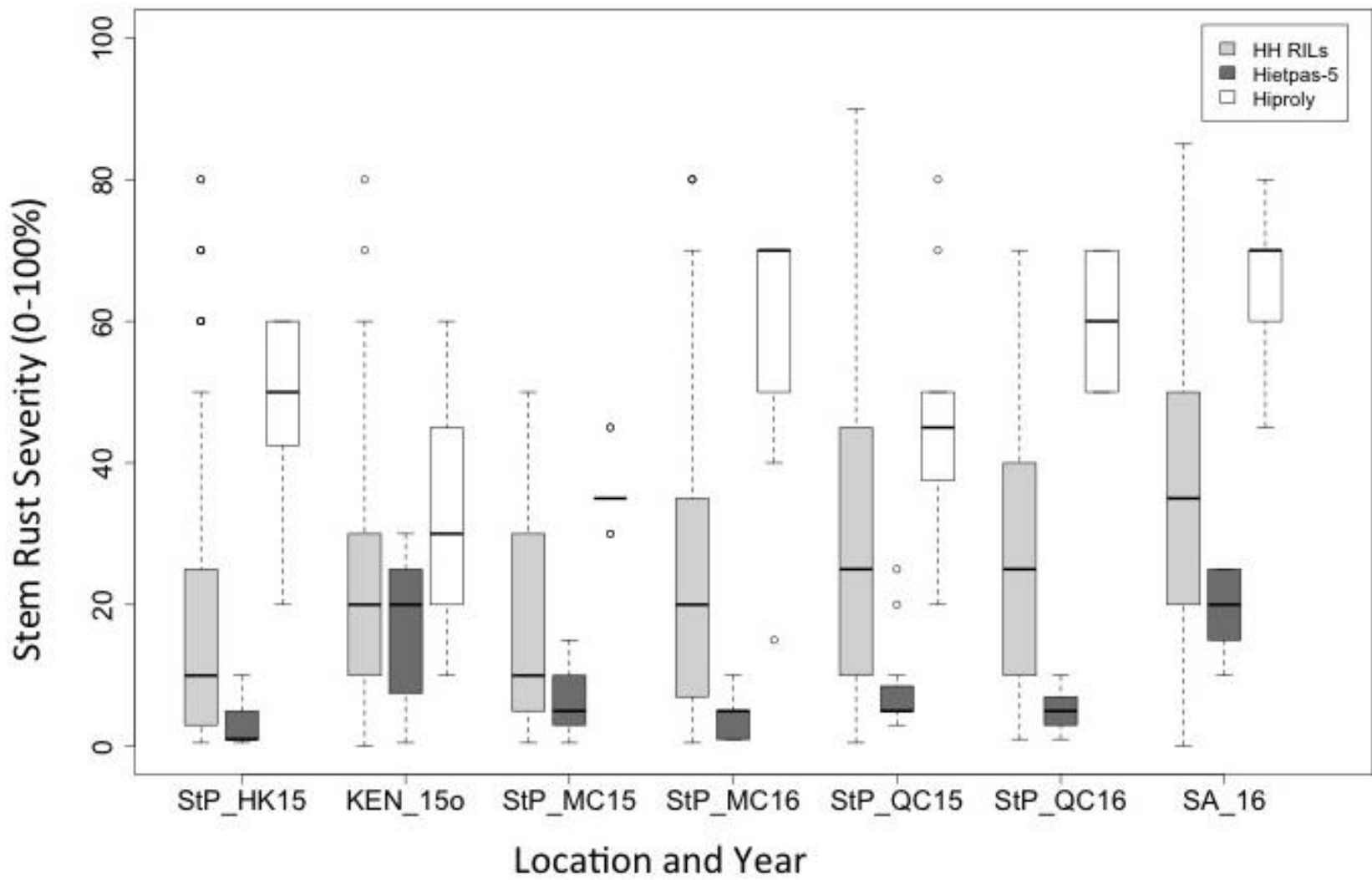




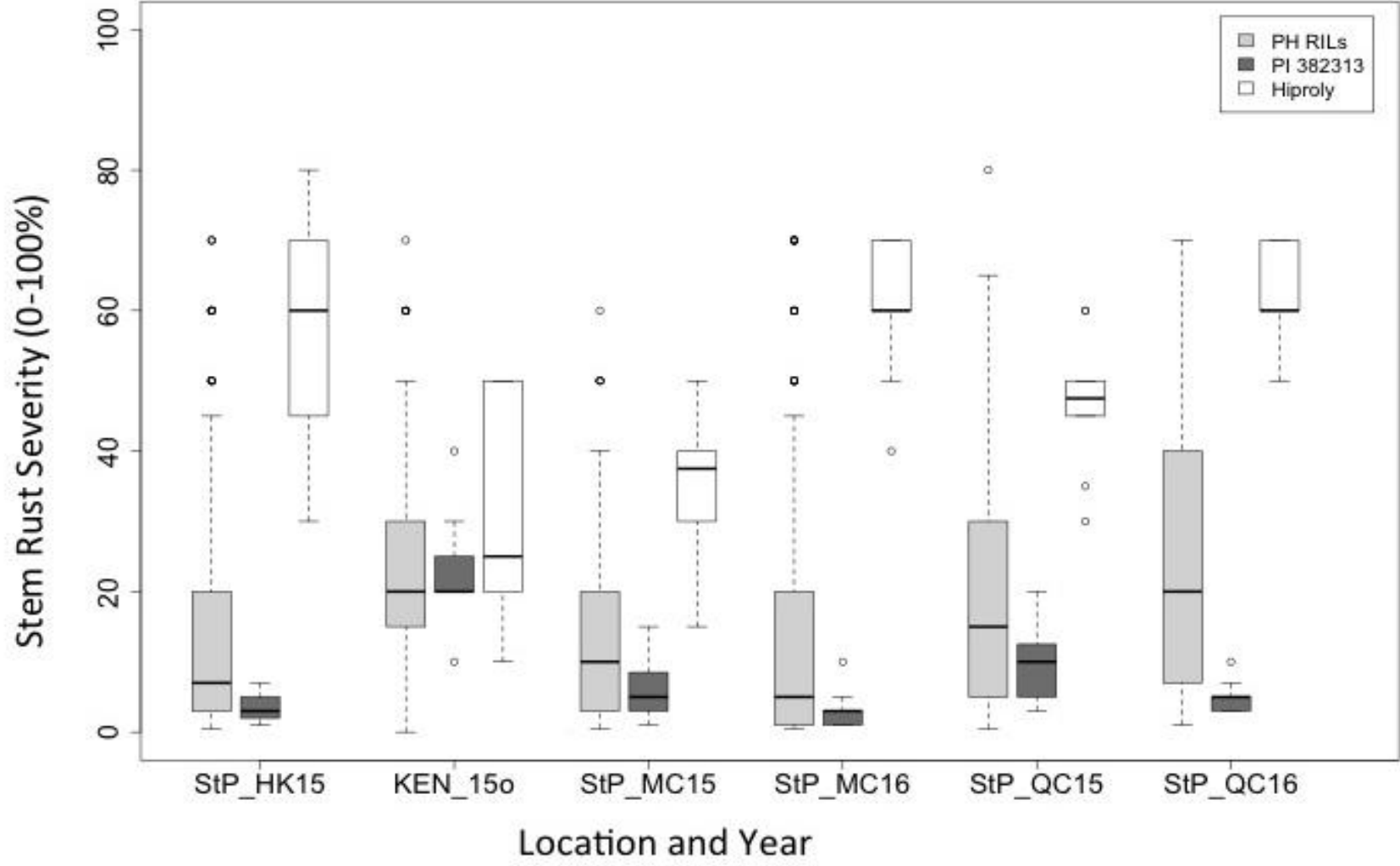
**Figure 4.5.** Histogram of stem rust severity averaged over all locations for 200 recombinant inbred lines of the Hietpas-5/Hiproly population.



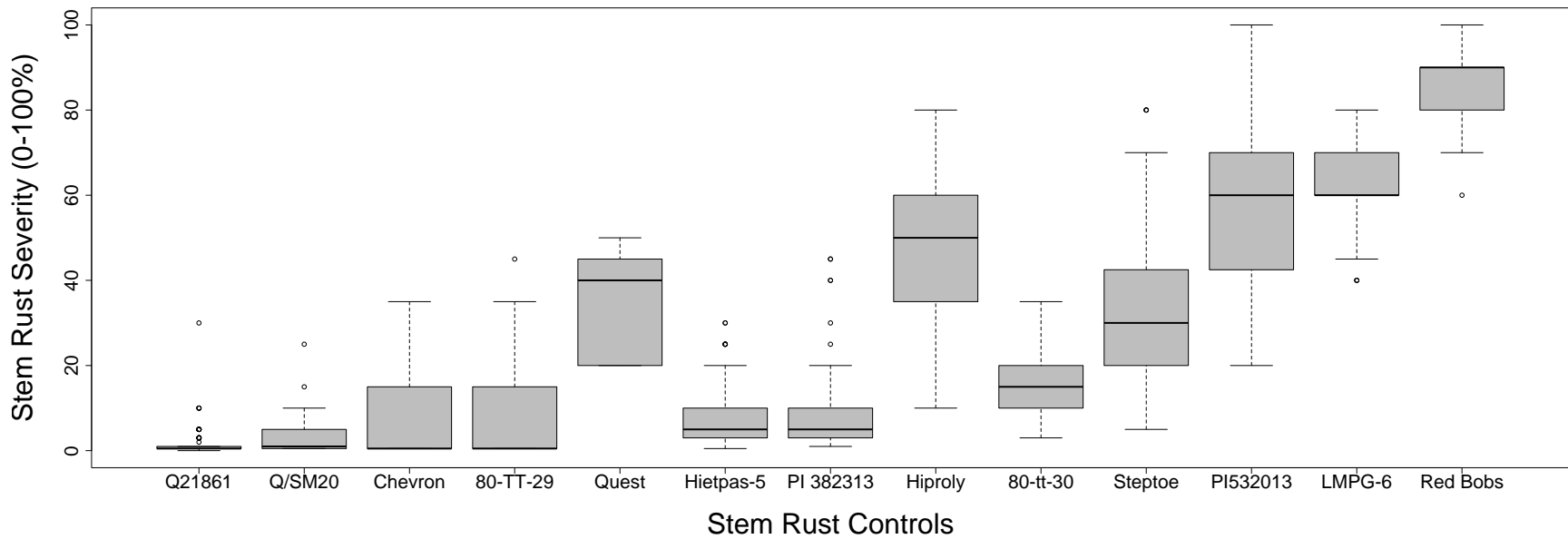
**Figure 4.6.** Histogram of stem rust severity averaged over all locations for 200 recombinant inbred lines of the PI 382313/Hiproly population.



**Figure 4.7.** Boxplots of stem rust severity for recombinant inbred lines (RILs) and parents of the Hietpas-5/Hiproly (HH) population within each trial. The trial locations and years, along with the predominant *Puccinia graminis* f. sp. *tritici* race present, are abbreviated as follows: StP\_HK15 for St. Paul, MN race HKHJC in 2015, KEN\_15o for Njoro, Kenya *Pgt* TTKSK composite (includes races TTKSK, TTKST, TTKTK and TTKTT) in 2015 off-season, StP\_MC15 for St. Paul, MN race MCCFC in 2015, StP\_MC16 for St. Paul, MN race MCCFC in 2016, StP\_QC15 for St. Paul, MN race QCCJB in 2015, StP\_QC16 for St. Paul, MN race QCCJB in 2016, and SA\_16 for Greytown, South Africa race PTKST in 2016. Five statistics (bars) are represented in each box plot from bottom to top: the smallest observation, lower quartile, median, upper quartile, and largest observation, respectively. Data points positioned outside this range are extreme values and are represented as circles.



**Figure 4.8.** Boxplots of stem rust severity for recombinant inbred lines (RILs) and parents of the PI 382313/Hiproly (PH) population within each trial. The trial locations and years, along with the predominant *Puccinia graminis* f. sp. *tritici* race present, are abbreviated as follows: StP\_HK15 for St. Paul, MN race HKHJC in 2015, KEN\_15o for Njoro, Kenya *Pgt* TTKSK composite (includes races TTKSK, TTKST, TTKTK and TTKTT) in 2015 off-season, StP\_MC15 for St. Paul, MN race MCCFC in 2015, StP\_MC16 for St. Paul, MN race MCCFC in 2016, StP\_QC15 for St. Paul, MN race QCCJB in 2015, and StP\_QC16 for St. Paul, MN race QCCJB in 2016. Five statistics (bars) are represented in each box plot from bottom to top: the smallest observation, lower quartile, median, upper quartile, and largest observation, respectively. Data points positioned outside this range are extreme values and are represented as circles.

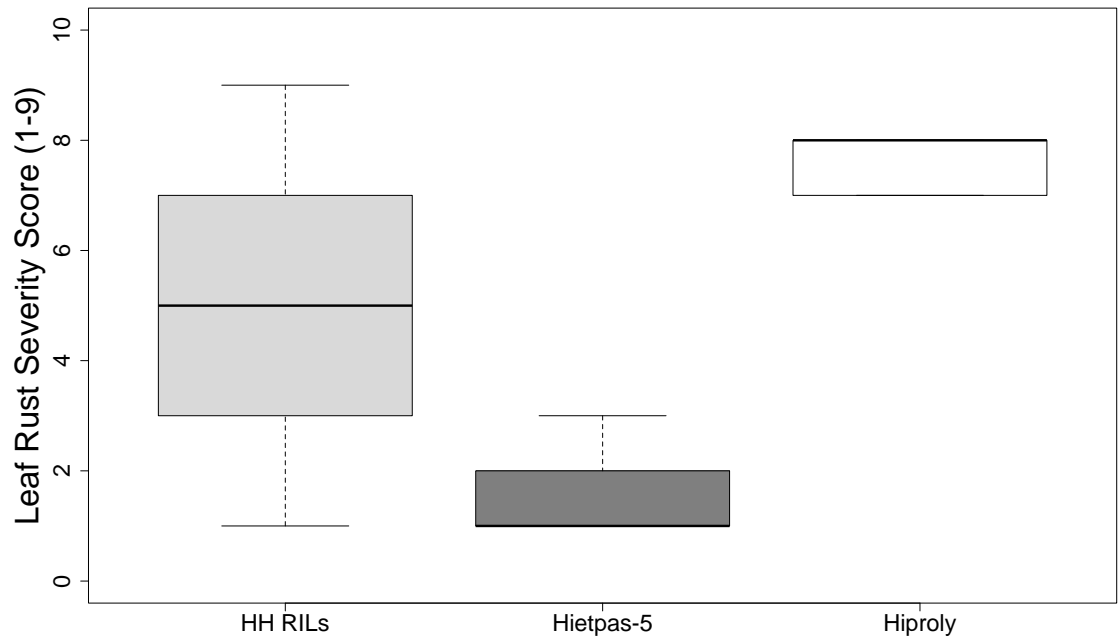


**Figure 4.9.** Boxplots of stem rust severity for barley mapping parents (Hietpas-5, PI 382313, and Hiproly) and barley (Q21861, Q/SM20, Chevron, 80-TT-20, Quest, 80-tt-30, Steptoe, and PI 532013) and wheat (LMPG-6 and Red Bobs) stem rust controls averaged over all locations. Five statistics (bars) are represented in each box plot from bottom to top: the smallest observation, lower quartile, median, upper quartile, and largest observation, respectively. Data points positioned outside this range are extreme values and are represented as circles.

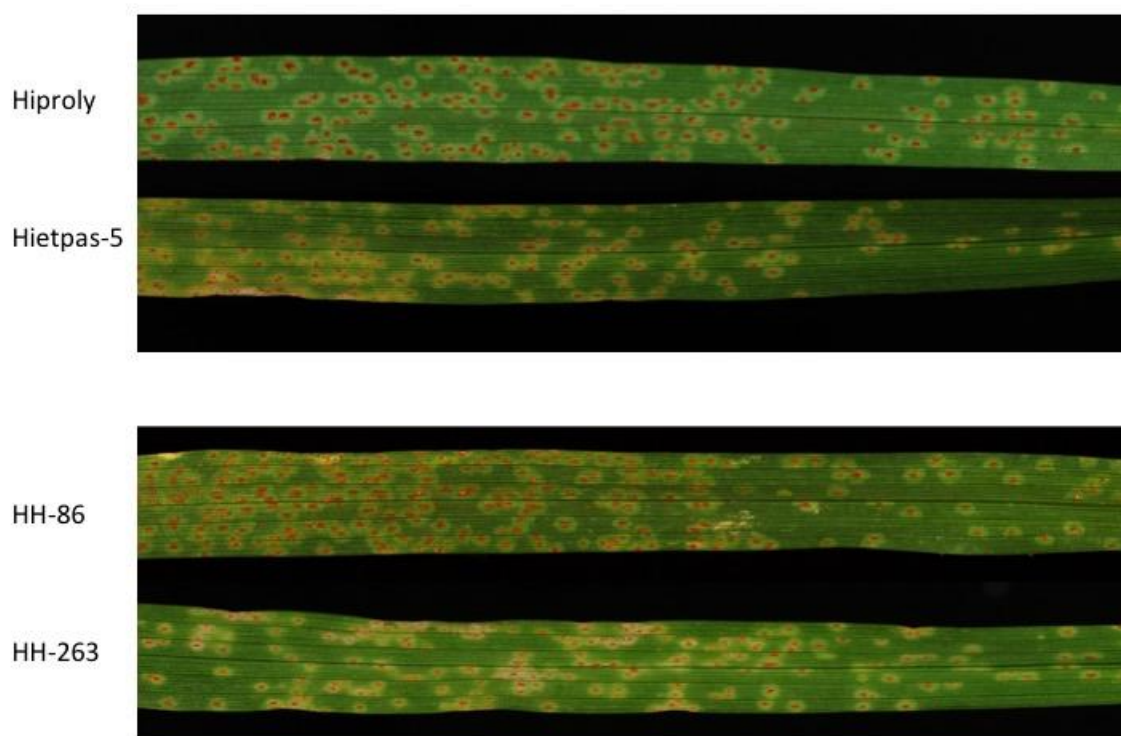


**Figure 4.10.** Severity of leaf rust infection by *Puccinia hordei* on the flag leaves of the resistant accession Hietpas-5 (left) and the susceptible accession Hiproly (right) in Greytown, South Africa in 2016.

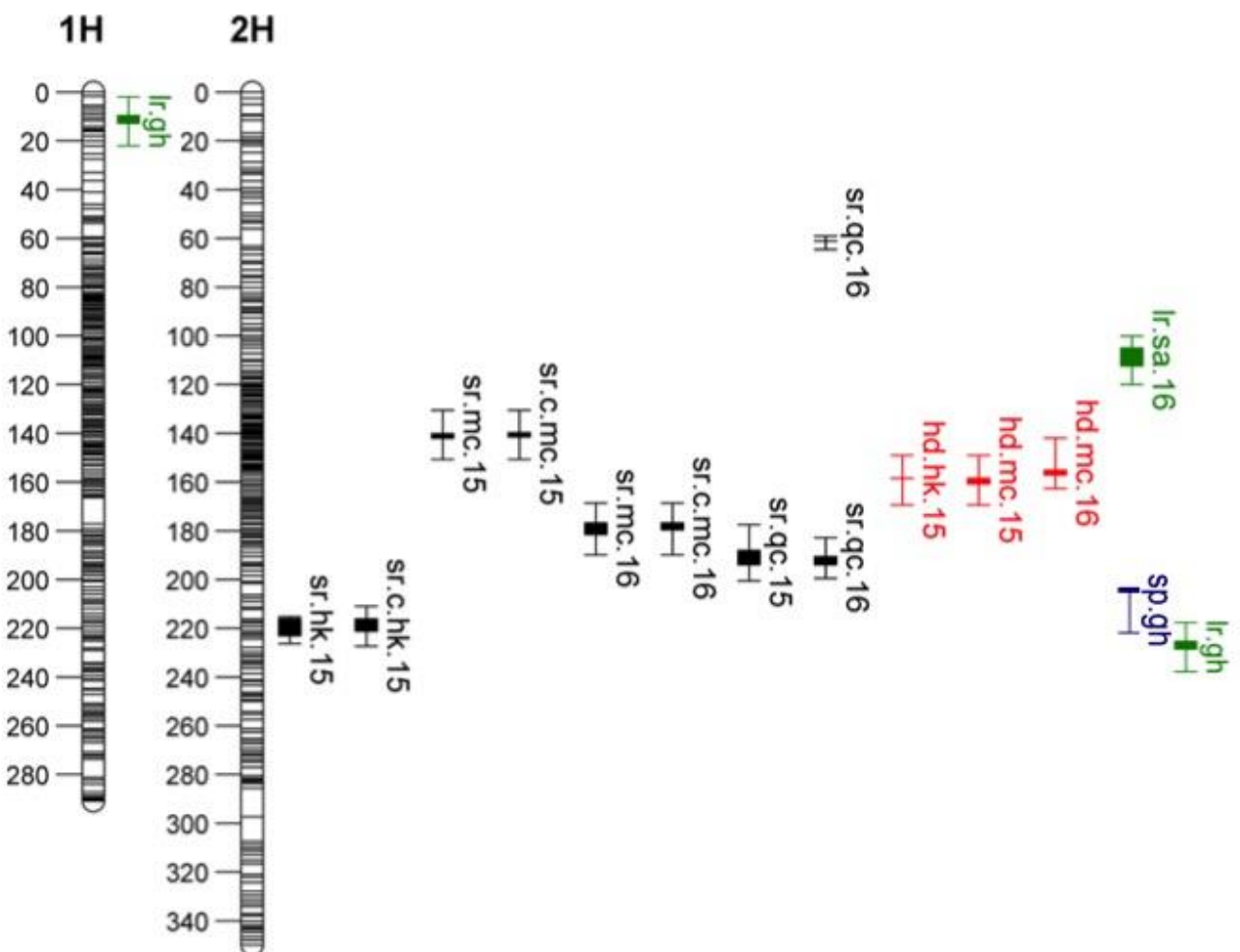


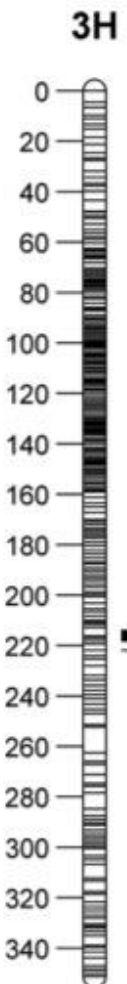


**Figure 4.11.** Boxplots of leaf rust severity scores for recombinant inbred lines (RILs) and parents of the Hietpas-5/Hiproly (HH) population evaluated to *Puccinia hordei* at Greytown, South Africa in 2016. Up to five statistics (bars) are represented in each box plot from bottom to top: the smallest observation, lower quartile, median, upper quartile, and largest observation, respectively.

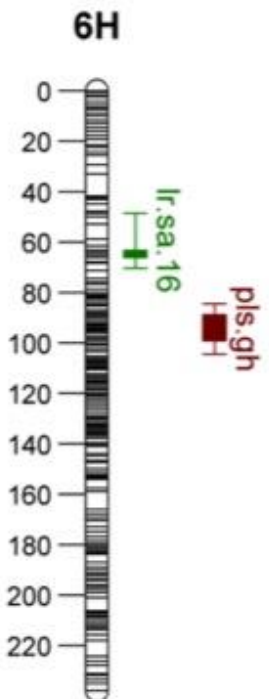
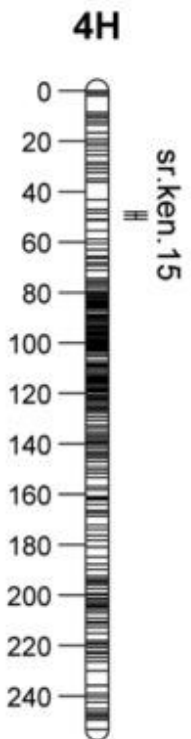


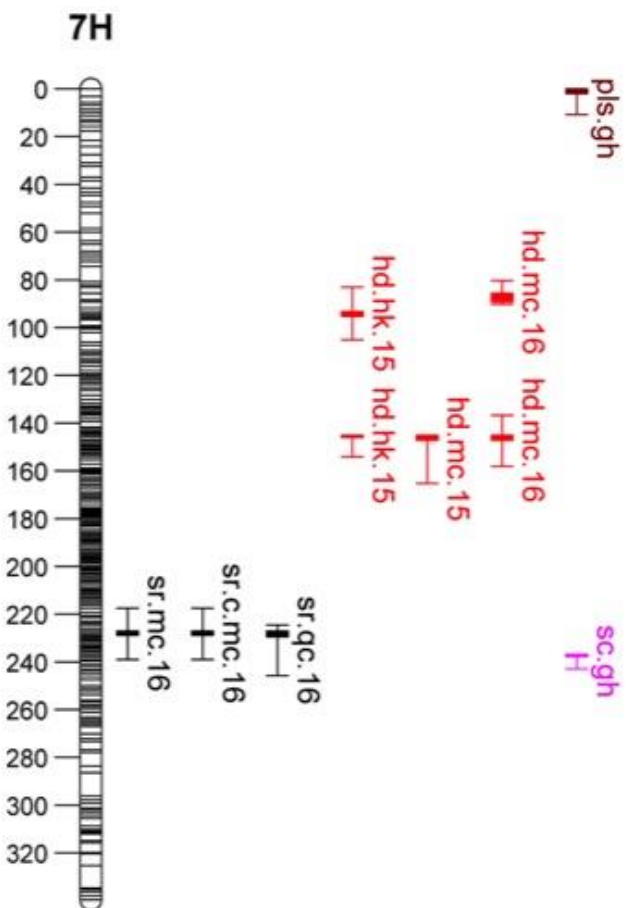
**Figure 4.12.** Seedling infection types of Hietpas-5, Hiproly, and selected recombinant inbred lines of the Hietpas-5/Hiproly population to South African *Puccinia hordei* isolate SAPh1601 in the greenhouse.



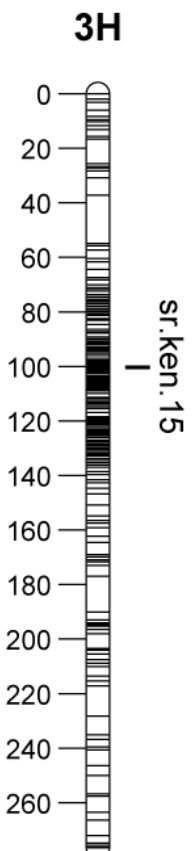
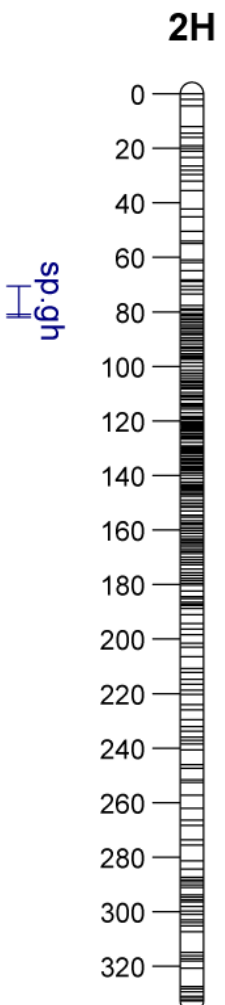
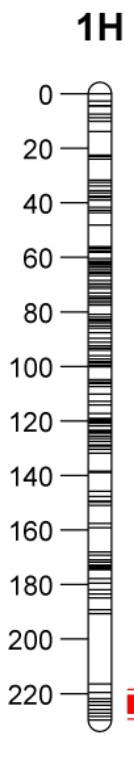


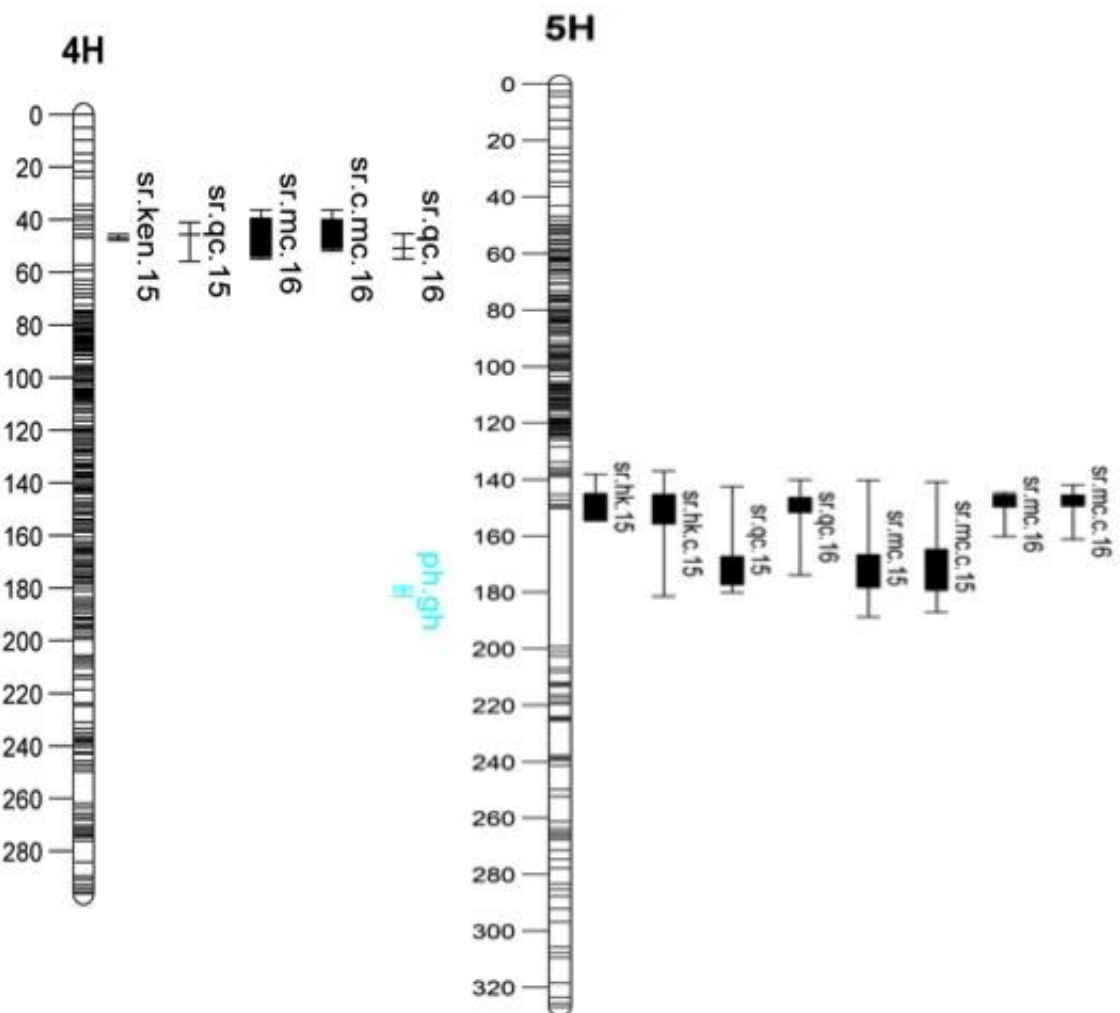
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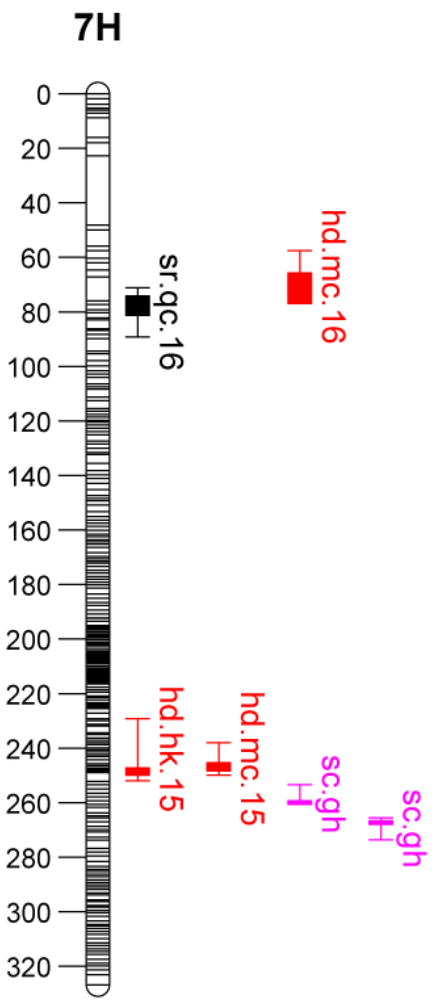


**Figure 4.13.** Linkage maps of the Hietpas-5/Hiproly recombinant inbred line population showing significant quantitative trait loci for stem rust severity, spike row type, heading date, leaf rust severity, leaf rust seedling infection type (IT) score, physiological leaf spotting, plant height, and seed cover. Traits, locations and years were abbreviated according to the following scheme “trait”.”location”.”year” where sr is stem rust, sp is spike row type, hd is heading date, lr is leaf rust severity as adults, lr.gh is seedling leaf rust IT score (converted to a coefficient of infection value as described by Zhou et al. (2014)) in the greenhouse, pls is physiological leaf spotting, ph is plant height, sc is covered or naked caryopsis, hk is the St. Paul race HKHJC nursery, mc is the St. Paul race MCCFC nursery, qc is the St. Paul race QCCJB nursery, ken is the Njoro, Kenya *Pgt* TTKSK composite (includes races TTKSK, TTKST, TTKTK and TTKTT) nursery, sa is the Greytown, South Africa race PTKST nursery, gh indicates greenhouse observations, and 15 and 16 represent the years of 2015 and 2016, respectively.

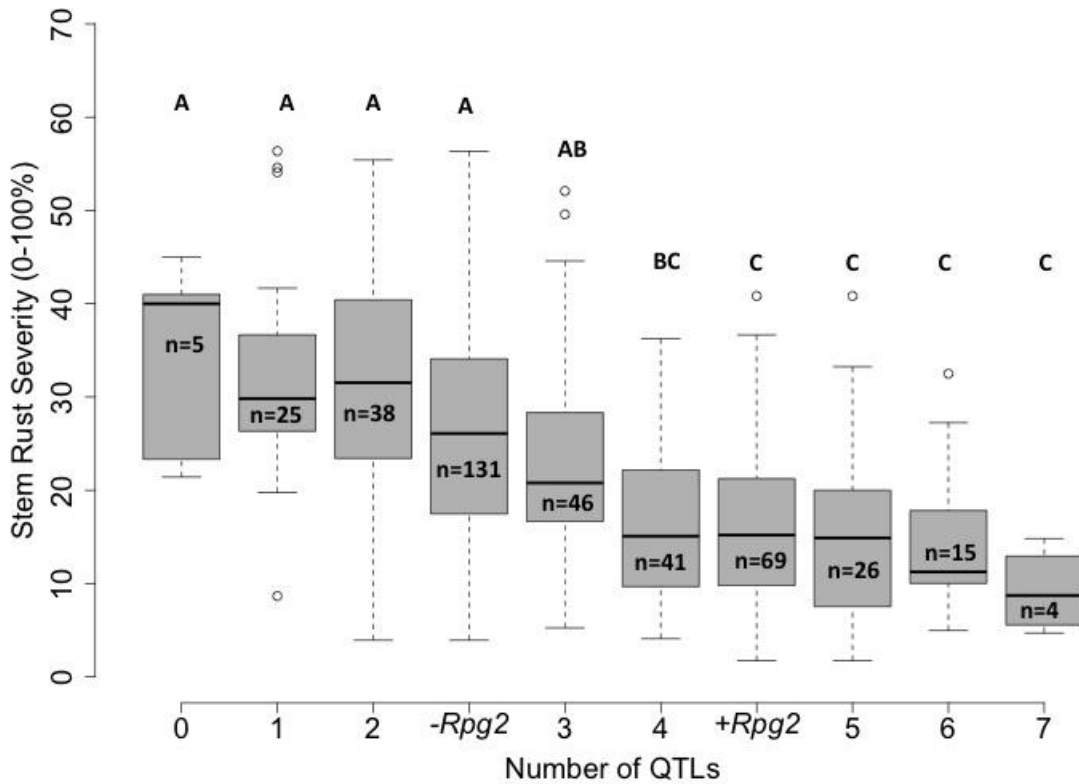




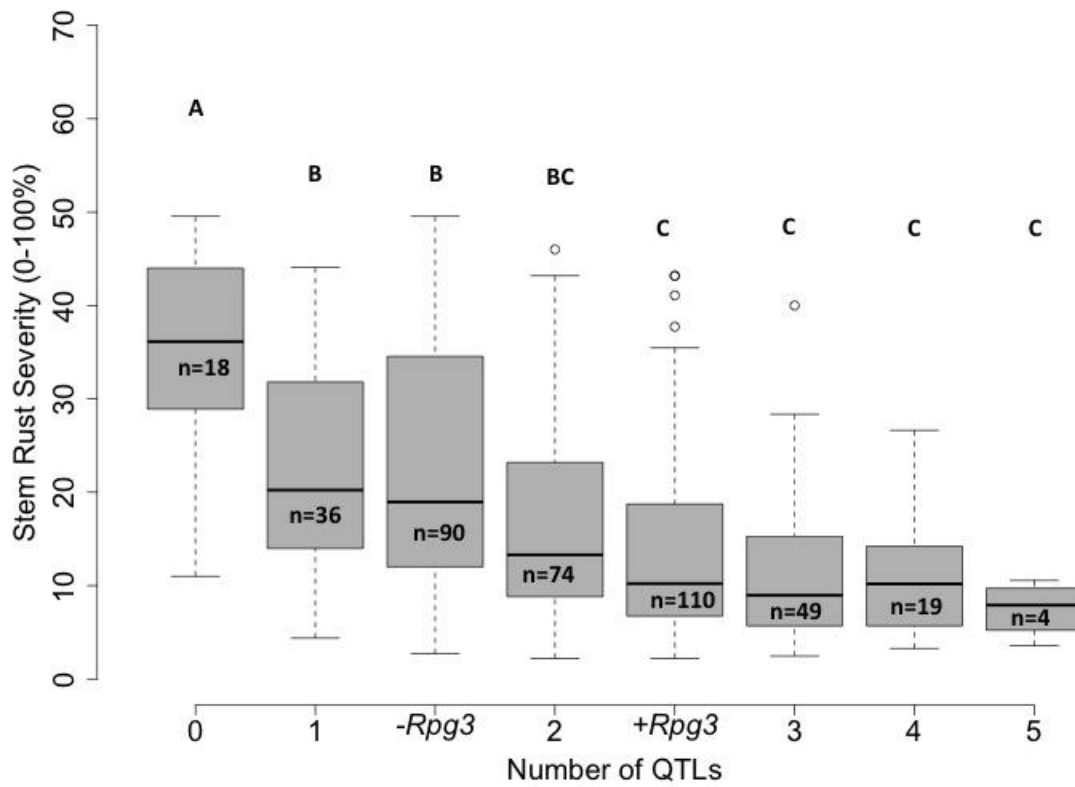




**Figure 4.14.** Linkage maps of the PI 382313/Hiproly recombinant inbred line population showing significant quantitative trait loci for stem rust severity, spike row type, heading date, physiological leaf spotting, plant height, and seed cover. Traits, locations and years were abbreviated according to the following scheme “trait”.”location”.”year” where sr is stem rust, sp is spike row type, hd is heading date, pls is physiological leaf spotting, ph is plant height, sc is covered or naked caryopsis, hk is the St. Paul race HKHJC nursery, mc is the St. Paul race MCCFC nursery, qc is the St. Paul race QCCJB nursery, and ken is the Njoro, Kenya *Pgt* TTKSK composite (includes races TTKSK, TTKST, TTKTK and TTKTT) nursery, gh indicates greenhouse observations, and 15 and 16 represent the years of 2015 and 2016, respectively.



**Figure 4.15.** Average stem rust severity for recombinant inbred lines of the Hietpas-5/Hiproly population grouped according to the number of quantitative trait loci for resistance they carry. Bars with different letters above indicate statistically significant differences according to Tukey’s HSD test ( $p$ -value  $<0.05$ ). The number of RILs in each group is shown in the box. Groups “+Rpg2” and “-Rpg2” comprise RILs that either possess or lack the QTLs *Rpg-qt1-HH-Hie-2H.2*, *Rpg-qt1-HH-Hie-2H.2 2H.3* (the putative underlying QTL for *Rpg2*), respectively. Five statistics (bars) are represented in each box plot from bottom to top: the smallest observation, lower quartile, median, upper quartile, and largest observation, respectively. Data points positioned outside this range are extreme values and are represented as circles.



**Figure 4.16.** Average stem rust severity for recombinant inbred lines of the PI 382313/Hiproly population grouped according to the number of quantitative trait loci for resistance they carry. Bars with different letters above indicate statistically significant differences according to Tukey’s HSD test ( $p$ -value  $<0.05$ ). The number of RILs in each group is shown in the box. Groups “+Rpg3” and “-Rpg3” comprise RILs that either possess or lack the QTL *Rpg-qt1-PH-PI38-5H* (the putative underlying QTL for *Rpg3*), respectively. Five statistics (bars) are represented in each box plot from bottom to top: the smallest observation, lower quartile, median, upper quartile, and largest observation, respectively. Data points positioned outside this range are extreme values and are represented as circles.

## Tables

**Table 4.1.** Summary statistics of linkage maps for the Hietpas-5/Hiproly and PI 382313/Hiproly recombinant inbred line populations.

| Chr <sup>a</sup> | Hietpas-5/Hiproly Population |                           |                      | PI 382313/Hiproly Population |                     |                      |
|------------------|------------------------------|---------------------------|----------------------|------------------------------|---------------------|----------------------|
|                  | Number of Markers            | Length <sup>b</sup>       | Density <sup>c</sup> | Number of Markers            | Length <sup>b</sup> | Density <sup>c</sup> |
| 1H               | 230                          | 290.9                     | 1.3                  | 122                          | 229.7               | 1.9                  |
| 2H               | 242                          | 349.9                     | 1.4                  | 225                          | 332.7               | 1.5                  |
| 3H               | 253                          | 351.2                     | 1.4                  | 176                          | 276.5               | 1.6                  |
| 4H               | 198                          | 253.3                     | 1.3                  | 210                          | 296.1               | 1.4                  |
| 5H               | 182                          | 344.9                     | 1.8                  | 194                          | 327.1               | 1.6                  |
| 6H               | 174                          | 237.9                     | 1.4                  | 203                          | 302.0               | 1.5                  |
| 7H               | 249                          | 339.7                     | 1.4                  | 234                          | 326.8               | 1.4                  |
| <b>Mean</b>      | <b>218.2</b>                 | <b>309.7</b>              | <b>1.4</b>           | <b>194.8</b>                 | <b>298.7</b>        | <b>1.5</b>           |
| <b>Total</b>     | <b>1528</b>                  | <b>2167.9<sup>d</sup></b> | <b>-</b>             | <b>1364</b>                  | <b>2090.9</b>       | <b>-</b>             |

<sup>a</sup> Chromosome (Chr).

<sup>b</sup> Total length of the linkage group in cM.

<sup>c</sup> Number of markers per cM.

<sup>d</sup> Total centiMorgan (cM) length across all linkage groups.

**Table 4.2.** The mean and range of stem rust severities for barley and wheat controls infected with various races of *Puccinia graminis* f. sp. *tritici* in St. Paul, MN and Njoro, Kenya, 2015-2016.

| Name      | Recognized Genes | Global <sup>a</sup><br>Mean <sup>b</sup><br>(Range) | SA_16<br>Mean<br>(Range) | StP_HK15<br>Mean<br>(Range) | KEN_15o<br>Mean<br>(Range) | StP_MC15<br>Mean<br>(Range) | StP_MC16<br>Mean<br>(Range) | StP_QC15<br>Mean<br>(Range) | StP_QC16<br>Mean<br>(Range) |
|-----------|------------------|---|--------------------------|-----------------------------|----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| Chevron   | <i>Rpg1</i>      | 10.8<br>(0.5-35)                                    | 27.5<br>(25-30)          | 0.5<br>(0.5 - 0.5)          | - <sup>c</sup>             | 0.5<br>(0.5 - 0.5)          | 0.5<br>(0.5 - 0.5)          | 20.4<br>(7 - 35)            | 15.3<br>(5 - 25)            |
| 80-TT-29  | <i>Rpg1</i>      | 8.7<br>(0.5-45)                                     | -                        | 0.6<br>(0.5 - 1)            | -                          | 0.5<br>(0.5 - 0.5)          | 0.5<br>(0.5 - 0.5)          | 27.9<br>(15 - 45)           | 14.2<br>(10 - 20)           |
| Quest     | <i>Rpg1</i>      | 35<br>(20-50)                                       | -                        | -                           | 35.0<br>(20 - 50)          | -                           | -                           | -                           | -                           |
| Hietpas-5 | <i>Rpg2</i>      | 8.9<br>(0.5-30)                                     | 19.1<br>(10-25)          | 3.0<br>(0.5 - 10)           | 16.5<br>(0.5 - 30)         | 6.4<br>(0.5 - 15)           | 4.6<br>(1 - 10)             | 8.3<br>(3 - 25)             | 4.9<br>(1 - 10)             |
| Hiproly   | -                | 50.3<br>(20-80)                                     | 65.0<br>(45-80)          | 52.0<br>(20 - 80)           | 32.0<br>(10 - 60)          | 35<br>(15 - 50)             | 59.8<br>(15 - 70)           | 46.2<br>(20 - 80)           | 61.0<br>(50 - 70)           |
| PI 382313 | <i>Rpg3</i>      | 12.8<br>(0.5-25)                                    | 38.8<br>(25-45)          | 3.7<br>(1 - 7)              | 22.9<br>(10 - 40)          | 6.3<br>(1 - 15)             | 3.2<br>(1 - 10)             | 9.4<br>(3 - 20)             | 5.1<br>(3 - 10)             |
| PI 532013 | -                | 60.3<br>(20-100)                                    | 100<br>(100-100)         | 45<br>(30 - 60)             | 54.0<br>(30 - 70)          | 44.2<br>(20 - 60)           | 61.7<br>(40 - 70)           | 55.8<br>(30 - 80)           | 61.7<br>(40 - 70)           |
| Q/SM20    | <i>rpg4/Rpg5</i> | 3.1<br>(0-30)                                       | 0.8<br>(0.5-1)           | 9.4<br>(5 - 25)             | -                          | 0.5<br>(0.5 - 1)            | 0.9<br>(0.5 - 3)            | 2.2<br>(0.5 - 5)            | 4.7<br>(1 - 10)             |

|          |                         |                 |                   |                    |                    |                    |                   |                   |                   |
|----------|-------------------------|-----------------|-------------------|--------------------|--------------------|--------------------|-------------------|-------------------|-------------------|
| Q21861   | <i>Rpg1 + rpg4/Rpg5</i> | 1.9<br>(0-30)   | 0.4<br>(0.1-0.75) | 0.5<br>(0.5 - 0.5) | 6.9<br>(0 - 30)    | 0.5<br>(0.5 - 0.5) | 0.5<br>(0.5 - 1)  | 0.9<br>(0.5 - 3)  | 3.9<br>(1 - 10)   |
| Step toe | -                       | 36.1<br>(5-80)  | 50.0<br>(45-55)   | 21.0<br>(10 - 35)  | 44.6<br>(5 - 80)   | 18.9<br>(7 - 40)   | 35.4<br>(15 - 60) | 42.9<br>(10 - 80) | 40.2<br>(20 - 60) |
| 80-tt-30 | -                       | 15.4<br>(3-35)  | -                 | 15.4<br>(3 - 25)   | -                  | 15.0<br>(10 - 30)  | 11.7<br>(10 - 15) | 27.1<br>(10 - 35) | 10.3<br>(5 - 20)  |
| LMPG-6   | -                       | 63.3<br>(40-80) | -                 | 71.7<br>(60 - 80)  | -                  | 63.3<br>(40 - 80)  | 49.2<br>(40 - 60) | 75.8<br>(70 - 80) | 56.7<br>(50 - 60) |
| Red Bobs | -                       | 85<br>(60-100)  | -                 | -                  | 85.0<br>(60 - 100) | -                  | -                 | -                 | -                 |

<sup>a</sup> Locations in St. Paul, MN, Njoro, Kenya, and Gerytown, South Africa, abbreviated: Global (all locations), StP\_HK15 (St. Paul race HKHJC in 2015), KEN\_15o (Njoro *Pgt* TTKSK composite (races TTKSK, TTKST, TTKTK, and TTKTT) in 2015 off-season), StP\_MC15 (St. Paul race MCCFC in 2015), StP\_MC16 (St. Paul race MCCFC in 2016), StP\_QC15 (St. Paul race QCCJB in 2015), StP\_QC16 (St. Paul race QCCJB in 2016), and SA\_16 (Greytown, South Africa race PTKST in 2016).

<sup>b</sup> Mean and range for terminal stem rust severity (0-100%).

<sup>c</sup> no data not applicable “-“.

**Table 4.3.** Broad sense heritability ( $H^2$ ) of stem rust resistance in the Hietpas-5/Hiproly and PI 382313/Hiproly recombinant inbred line populations.

|                             | <b>Hietpas-5/Hiproly<br/>Population</b> | <b>PI 382313/Hiproly<br/>Population</b> |
|-----------------------------|---|---|
| <b>Location<sup>a</sup></b> | <b><math>H^2</math></b>                 | <b><math>H^2</math></b>                 |
| Global                      | 0.85                                    | 0.88                                    |
| StP_HK15                    | 0.33                                    | 0.75                                    |
| StP_MC15                    | 0.63                                    | 0.74                                    |
| StP_MC16                    | 0.58                                    | 0.69                                    |
| StP_QC15                    | 0.61                                    | 0.65                                    |
| StP_QC16                    | 0.58                                    | 0.73                                    |
| KEN_15o                     | unreplicated                            | unreplicated                            |
| SA_16                       | unreplicated                            | — <sup>b</sup>                          |

<sup>a</sup> Locations in St. Paul, MN, Njoro, Kenya, and Greytown, South Africa abbreviated as: Global (all locations), StP\_HK15 (St. Paul race HKHJC in 2015), KEN\_15o (Njoro *Pgt* TTKSK composite (races TTKSK, TTKST, TTKTK, and TTKTT) in 2015 off-season), StP\_MC15 (St. Paul race MCCFC in 2015), StP\_MC16 (St. Paul race MCCFC in 2016), StP\_QC15 (St. Paul race QCCJB in 2015), StP\_QC16 (St. Paul race QCCJB in 2016), and SA\_16 (Greytown, South Africa race PTKST in 2016).

<sup>b</sup> no data is “—”.



**Table 4.4.** Chi-square test for inheritance of stem rust (*Puccinia graminis* f. sp. *tritici*) adult plant resistance in the Hietpas-5/Hiproly and PI 382313/Hiproly recombinant inbred line populations and leaf rust (*Puccinia hordei*) seedling and adult plant resistance in the Hietpas-5/Hiproly population.

| <b>Hietpas-5/Hiproly Population</b> |                              |                    |  |  |
|-------------------------------------|------------------------------|--------------------|--|--|
| <b>Race or Isolate<sup>a</sup></b>  | <b>Resistant<sup>b</sup></b> | <b>Susceptible</b> | <b>One Gene Model<br/><i>p</i>-value (<math>\chi^2</math> value)<sup>c</sup></b> | <b>Two Gene Complementary Model<br/><i>p</i>-value (<math>\chi^2</math> value)</b> |
| MCCFC                               | 91                           | 109                | 0.20 (1.6)   | <0.01 (44.8)   |
| QCCJB                               | 95                           | 105                | 0.48 (0.5)   | <0.01 (54.0)   |
| HKHJC                               | 107                          | 93                 | 0.32 (0.9)   | <0.01 (86.6)   |
| <i>Pgt</i> TTKSK composite          | 174                          | 26                 | <0.01 (109.5)  | <0.01 (410.0)  |
| PTKST                               | 72                           | 128                | <0.01 (15.6)   | <0.01 (12.9)   |
| South Africa Leaf Rust <sup>d</sup> | 70                           | 128                | <0.01 (16.8)   | <0.01 (11.2)   |
| SAPh1601 <sup>e</sup>               | 41                           | 158                | <0.01 (68.5)   | 0.14 (2.0)   |
| <b>PI 382313/Hiproly Population</b> |                              |                    |  |  |
| MCCFC                               | 131                          | 69                 | <0.01 (19.2)   | <0.01 (174.9)  |
| QCCJB                               | 111                          | 89                 | 0.12 (2.4)   | <0.01 (99.2)   |
| HKHJC                               | 90                           | 110                | 0.15 (2.0)   | <0.01 (42.6)   |
| <i>Pgt</i> TTKSK composite          | 178                          | 22                 | <0.01 (121.8)  | <0.01 (436.9)  |

<sup>a</sup> Data for each respective race or isolate is the mean stem rust severity of the locations in St. Paul, MN, Njoro, Kenya, and Greytown, South Africa: abbreviated as StP\_MC15 and StP\_MC16 datasets (MCCFC), StP\_HK15 dataset (HKHJC), StP\_QC15 and StP\_QC16

datasets (QCCJB), KEN\_15o dataset (*Pgt* TTKSK composite (Races TTKSK, TTKST, TTKTK, and TTKTT), SA\_16 dataset (PTKST), SA\_16 dataset (South Africa Leaf Rust), and LR\_GH dataset (SAPh1601).

<sup>b</sup> A progeny line was considered resistant if the mean severity or infection type was less than or equal to the maximum value of Hietpas-5 or PI 382313 in that dataset.

<sup>c</sup> Resistance gene models tested were: single gene = 100 Resistant (R) : 100 Susceptible (S); two-gene complementary = 50 Resistant (R) : 150 Susceptible (S); and two-gene epistatic = 150 Resistant (R) : 50 Susceptible (S). None of the data for any of the pathogen races or isolates fit a two-gene epistatic model and are not shown in the table.

<sup>d</sup> A natural barley leaf rust (*Puccinia hordei*) epidemic developed in the field in Greytown, South Africa in 2016.

<sup>e</sup> An isolate of barley leaf rust (*Puccinia hordei*) from Greytown, South Africa, nursery in 2016.

**Table 4.5.** Correlations between stem rust severity and agro-morphological traits in the Hietpas-5/Hiproly and PI 382313/Hiproly recombinant inbred line populations.

| Locations <sup>a</sup> | Traits <sup>b</sup> | Hietpas-5/Hiproly Population |                |           | PI 382313/Hiproly Population |              |           |
|------------------------|---------------------|------------------------------|----------------|-----------|------------------------------|--------------|-----------|
|                        |                     | Stem Rust                    | Heading Date   | Spike Row | Stem Rust                    | Heading Date | Spike Row |
| StP_HK15               | Heading Date        | 0.004                        | - <sup>d</sup> | -         | -0.051                       | -            | -         |
|                        | Spike Row           | -<br>0.307*** <sup>c</sup>   | 0.004          | -         | -0.01                        | -0.051       | -         |
| StP_MC15               | Heading Date        | 0.071                        | -              | -         | -0.121 *                     | -            | -         |
|                        | Spike Row           | 0.197**                      | 0.071          | -         | 0.062                        | -0.121*      | -         |
| StP_MC16               | Heading Date        | -0.149*                      | -              | -         | -0.440**                     | -            | -         |
|                        | Spike Row           | -                            | -              | -         | na                           | na           | -         |
| StP_QC15               | Heading Date        | -0.154                       | -              | -         | -0.117                       | -            | -         |
|                        | Spike Row           | -0.340**                     | -0.154         | -         | -0.025                       | -0.117       | -         |
| StP_QC16               |                     | -                            | -              | -         | -                            | -            | -         |
| KEN_15o                |                     | -                            | -              | -         | -                            | -            | -         |

|       |              |       |   |   |   |   |   |
|-------|--------------|-------|---|---|---|---|---|
| SA_16 | Heading Date | -     | - | - | - | - | - |
|       | Spike Row    | 0.012 | - | - | - | - | - |

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<sup>a</sup> Locations in St. Paul, MN, Njoro, Kenya, and Gerytown, South Africa abbreviated as: Global (all locations), StP\_HK15 (St. Paul race HKHJC in 2015), KEN\_15o (Njoro *Pgt* TTKSK composite (races TTKSK, TTKST, TTKTK, and TTKTT) in 2015 off-season), StP\_MC15 (St. Paul race MCCFC in 2015), StP\_MC16 (St. Paul race MCCFC in 2016), StP\_QC15 (St. Paul race QCCJB in 2015), StP\_QC16 (St. Paul race QCCJB in 2016), and SA\_16 (Greytown, race PTKST in 2016).

<sup>b</sup> Stem rust (0-100% terminal stem rust severity), heading date (days after planting), and spike row type (two-rowed (2), six-rowed (6), and two-rowed deficient lateral floret morphology (3)).

<sup>c</sup> \*Significant correlation at  $p$ -value <0.05 and \*\*significant correlation at  $p$ -value <0.01,

<sup>d</sup> no data or data omitted “-“

**Table 4.6.** Quantitative trait loci detected for stem rust resistance and agro-morphological traits in the Hietpas-5/Hiproly recombinant inbred line population.

| QTL <sup>a</sup>    | Trait <sup>b</sup>          | Location <sup>c</sup> | Chr <sup>d</sup> | Peak <sup>e</sup> | Confidence Interval <sup>f</sup> | QTL Boundary <sup>g</sup> | LOD Cutoff <sup>h</sup> | LOD Peak <sup>i</sup> | R <sup>2j</sup> | Additive Effect <sup>k</sup> | Nearest Marker <sup>l</sup> |
|---------------------|-----------------------------|-----------------------|------------------|-------------------|----------------------------------|---------------------------|-------------------------|-----------------------|-----------------|------------------------------|-----------------------------|
| Hd-qtl-HH-Hie-7H.2  | Heading Date                | StP_HK15              | 7H               | 145.1             | 145.1-146                        | 145.1-145.1               | 4.51                    | 43.6                  | 0.52            | -2.86                        | S7H_119763886               |
|                     |                             | StP_MC15              | 7H               | 146.0             | 145.1-147.2                      | 145.1-165.3               | 4.52                    | 43.4                  | 0.5             | -3.9                         | S7H_122941988               |
|                     |                             | StP_MC16              | 7H               | 147.0             | 145.1-147.2                      | 136.7-158.1               | 4.27                    | 42                    | 0.48            | -3.12                        | S7H_122941988               |
| Hd-qtl-HH-Hip-2H    | Heading Date                | StP_HK15              | 2H               | 159.6             | 158.4-158.4                      | 149-169.4                 | 4.51                    | 21.2                  | 0.16            | 1.53                         | S2H_517149832               |
|                     |                             | StP_MC15              | 2H               | 159.6             | 158.4-160.7                      | 149-169.4                 | 4.52                    | 11.7                  | 0.09            | 1.66                         | S2H_517149832               |
|                     |                             | StP_MC16              | 2H               | 156.0             | 155.2-157                        | 142-162.7                 | 4.27                    | 19.7                  | 0.17            | 1.73                         | S2H_513221241               |
| Hd-qtl-HH-Hip-3H    | Heading Date                | StP_MC15              | 3H               | 60.5              | 56.6-61.5                        | 53.9-61.5                 | 4.52                    | 5.4                   | 0.04            | 1.08                         | S3H_45721880                |
| Hd-qtl-HH-Hip-7H.1  | Heading Date                | StP_HK15              | 7H               | 94.3              | 93.3-95.3                        | 83.1-105.1                | 4.51                    | 8.3                   | 0.06            | 0.98                         | S7H_45370288                |
|                     |                             | StP_MC16              | 7H               | 88.3              | 85.6-89.2                        | 80.3-90.2                 | 4.27                    | 6.4                   | 0.05            | 0.98                         | S7H_41103850                |
| Pls-qtl-HH-Hip-6H   | Physiological Leaf Spotting | GH                    | 6H               | 92.8              | 88.8-99.1                        | 84.5-104.5                | 4.40                    | 6.2                   | 0.1             | 5.91                         | S6H_426714892               |
| Pls-qtl-HH-Hip-7H   | Physiological Leaf Spotting | GH                    | 7H               | 0.0               | 0-2                              | 0-10.8                    | 4.40                    | 9.4                   | 0.15            | 8.55                         | S7H_2466757                 |
| Rpg-qtl-HH-Hie-2H.1 | Stem Rust                   | StP_QC16              | 2H               | 61.1              | 61.1-61.1                        | 59.1-64.6                 | 4.53                    | 5                     | 0.08            | -4.26                        | S2H_26426904                |
| Rpg-qtl-HH-Hie-2H.2 | Stem Rust                   | StP_MC15              | 2H               | 140.7             | 140.2-142                        | 130.5-150.7               | 4.56                    | 7.6                   | 0.12            | -4.2                         | S2H_333344265               |
|                     |                             | StP_MC15*             | 2H               | 140.7             | 139.8-141.4                      | 130.5-150.7               | 4.06                    | 7.3                   | 0.12            | -4.16                        | S2H_333344265               |
| Rpg-qtl-HH-Hie-2H.3 | Stem Rust                   | StP_QC15              | 2H               | 191.6             | 188.1-193.9                      | 177.5-200.5               | 4.34                    | 11.9                  | 0.21            | -9.17                        | S2H_626235770               |
|                     |                             | StP_MC16              | 2H               | 179.2             | 176.7-181.5                      | 168.6-189.9               | 3.81                    | 9.5                   | 0.15            | -6.46                        | S2H_607795630               |

|                     |                 |           |    |       |             |             |      |       |      |       |               |
|---------------------|-----------------|-----------|----|-------|-------------|-------------|------|-------|------|-------|---------------|
|                     |                 | StP_MC16* | 2H | 179.2 | 176.7-179.5 | 168.6-189.9 | 4.41 | 8.9   | 0.14 | -6.24 | S2H_607795630 |
|                     |                 | StP_QC16  | 2H | 192.9 | 190.6-193.9 | 182.9-199.5 | 4.53 | 7.2   | 0.11 | -4.82 | S2H_632608376 |
| Rpg-qt1-HH-Hie-2H.4 | Stem Rust       | StP_HK15  | 2H | 219.5 | 216.2-223   | 215.3-226.3 | 4.74 | 6.5   | 0.11 | -4.89 | S2H_650628734 |
|                     |                 | StP_HK15* | 2H | 219.5 | 216.2-221.1 | 211-227.3   | 4.01 | 6.8   | 0.12 | -5.05 | S2H_650628734 |
| Rpg-qt1-HH-Hie-4H   | Stem Rust       | KEN_15o   | 4H | 49.4  | 49.4-49.4   | 48-50.9     | 4.20 | 5.2   | 0.11 | -6.83 | S4H_18920429  |
| Rpg-qt1-HH-Hip-3H   | Stem Rust       | StP_QC15  | 3H | 214.0 | 214-218.2   | 214-221.9   | 4.34 | 4.8   | 0.09 | 6.32  | S3H_582311657 |
| Rpg-qt1-HH-Hie-7H   | Stem Rust       | StP_MC16  | 7H | 228.0 | 227.1-228.9 | 217.6-239   | 3.81 | 6.4   | 0.1  | -5.22 | S7H_534521877 |
|                     |                 | StP_MC16* | 7H | 228.0 | 227.1-228.9 | 217.6-239   | 4.41 | 6.9   | 0.11 | -5.46 | S7H_534521877 |
|                     |                 | StP_QC16  | 7H | 228.0 | 227.1-229.5 | 224.6-245.7 | 4.53 | 8.7   | 0.13 | -5.17 | S7H_534521877 |
| Rph-qt1-HH-Hie-2H.1 | Leaf Rust       | SA_16LR   | 2H | 110.6 | 104.9-112.3 | 100.1-119.9 | 4.60 | 6.8   | 0.09 | -0.7  | S2H_96851362  |
| Rph-qt1-HH-Hie-6H   | Leaf Rust       | SA_16LR   | 6H | 64.1  | 63.3-66.1   | 48.6-70.4   | 4.60 | 17.7  | 0.25 | -1.18 | S6H_38384837  |
| Rph-qt1-HH-Hie-1H   | Leaf Rust       | GH        | 1H | 7.5   | 7.1-10.2    | 0-18.5      | 4.2  | 23.1  | 0.31 | -0.31 | S1H_2362240   |
| Rph-qt1-HH-Hie-2H.2 | Leaf Rust       | GH        | 2H | 234.1 | 232.3-234.7 | 222.1-244.3 | 4.2  | 20.2  | 0.26 | -0.28 | S2H_752081396 |
| Sc-qt1-HH-Hip-7H    | Naked Caryopsis | GH        | 7H | 236.9 | 236.9-237.7 | 236.9-242.9 | 4.50 | 64.7  | 0.79 | 0.44  | S7H_566336223 |
| Sp-qt1-HH-Hip-2H    | Spike Row       | GH        | 2H | 210.0 | 209-211     | 209-229.8   | 4.31 | 142.4 | 0.97 | 1.98  | S2H_646238075 |

<sup>a</sup> QTL were named using the convention “trait”-“qt1”-“population”-“parental allele which reduced trait value”-“chromosome”.

<sup>b</sup> Traits were heading date (days to heading), naked caryopsis, spike row type (two-rowed or six-rowed), lateral floret type (normal or deficiens), plant height (cm at maturity), stem rust (terminal severity, 0-100%), and physiological leaf spotting (0-100% of flag leaf covered at heading).

<sup>c</sup> Locations in St. Paul, MN, Njoro, Kenya, and Gerytown, South Africa abbreviated as: StP\_HK15 (St. Paul race HKHJC in 2015), KEN\_15o (Njoro *Pgt* TTKSK composite (races TTKSK, TTKST, TTKTK, TTKTT) in 2015 off-season), StP\_MC15 (St. Paul race MCCFC in 2015), StP\_MC16 (St. Paul race MCCFC in 2016), StP\_QC15 (St. Paul race QCCJB in 2015), StP\_QC16 (St. Paul race QCCJB in 2016), and SA\_16 (Greytown, South Africa race PTKST in 2016), SA\_16LR (leaf rust in Greytown, South Africa 2016), GH (observations made in the greenhouse), \* indicates locations with values corrected for heading date.

<sup>d</sup> Chr is barley chromosomes: 1H, 2H, 3H, 4H, 5H, 6H and 7H.

<sup>e</sup> centiMorgan (cM) position of the peak LOD (Logarithm, base 10, of odds) value.

<sup>g</sup> Confidence interval defined as the cM position of a 1 LOD drop-off from the peak LOD value.

<sup>h</sup> QTL boundary defined as the cM position of the intersection of the trace of the LOD value with the LOD cutoff value.

<sup>i</sup> Significant LOD cutoff value as determined by permutation testing.

<sup>j</sup> Percent of variance explained by the QTL.

<sup>k</sup> Additive effect of the resistant parent (Hietpas-5) allele.

<sup>l</sup> Marker nearest to the peak LOD value.

**Table 4.7.** Quantitative trait loci detected for stem rust resistance and agro-morphological traits in the PI 382313/Hiproly recombinant inbred line population.

| QTL <sup>a</sup>   | Trait <sup>b</sup> | Location <sup>c</sup> | Chr <sup>d</sup> | Peak <sup>e</sup> | Confidence Interval <sup>f</sup> | QTL Boundary <sup>g</sup> | LOD Cutoff <sup>h</sup> | LOD Peak <sup>i</sup> | R <sup>2j</sup> | Additive Effect <sup>k</sup> | Nearest Marker <sup>l</sup> |
|--------------------|--------------------|-----------------------|------------------|-------------------|----------------------------------|---------------------------|-------------------------|-----------------------|-----------------|------------------------------|-----------------------------|
| Hd-qtl-PH-PI38-1H  | Heading Date       | StP_MC15              | 1H               | 225.4             | 220.6-228.1                      | 206.7-229.3               | 3.96                    | 6.6                   | 0.12            | -1.11                        | S1H_558521120               |
|                    |                    | StP_HK15              | 1H               | 225.4             | 220.6-227.1                      | 218.5-229.3               | 4.22                    | 5.9                   | 0.08            | -0.53                        | S1H_558521120               |
| Hd-qtl-PH-Hip-2H.1 | Heading Date       | StP_MC16              | 2H               | 23.1              | 23.1-23.1                        | 21-26.6                   | 4.42                    | 5.5                   | 0.06            | 0.9                          | S2H_19561762                |
| Hd-qtl-PH-Hip-2H.2 | Heading Date       | StP_MC16              | 2H               | 35.0              | 33-35.5                          | 32-53.4                   | 4.42                    | 21.1                  | 0.36            | 1.91                         | S2H_24051156                |
|                    |                    | StP_MC15              | 2H               | 42.2              | 39.5-44.2                        | 32-53.4                   | 3.96                    | 9.5                   | 0.16            | 1.38                         | S2H_29157898                |
|                    |                    | StP_MC15              | 2H               | 43.2              | 39.5-47.1                        | 32-53.4                   | 4.22                    | 9.3                   | 0.13            | 0.69                         | S2H_29157898                |
| Hd-qtl-PH-PI38-2H  | Heading Date       | StP_HK15              | 2H               | 237.1             | 236-238.6                        | 228-241.6                 | 4.22                    | 5.6                   | 0.07            | -0.51                        | S2H_681301684               |
| Hd-qtl-PH-Hip-7H.1 | Heading Date       | StP_MC16              | 7H               | 71.2              | 65.7-76.9                        | 57.5-76.9                 | 4.42                    | 7                     | 0.07            | 0.87                         | S7H_32873913                |
| Hd-qtl-PH-Hip-7H.2 | Heading Date       | StP_HK15              | 7H               | 248.4             | 247.2-249.9                      | 229.2-251.9               | 4.22                    | 13.5                  | 0.19            | 0.84                         | S7H_529894869               |
|                    |                    | StP_MC15              | 7H               | 247.2             | 245.3-248.4                      | 238-249.9                 | 3.96                    | 5.5                   | 0.08            | 1.03                         | S7H_531689694               |
| Ph-qtl-PH-Hip-4H   | Plant Height       | GH                    | 4H               | 181.1             | 181.1-181.1                      | 179.5-183.1               | 3.82                    | 4.1                   | 0.08            | 5.2                          | S4H_567761521               |
| Sc-qtl-PH-Hip-7H   | Naked Caryopsis    | GH                    | 7H               | 260.6             | 259.3-260.6                      | 253.4-260.6               | 3.58                    | 44.1                  | 0.69            | 0.43                         | S7H_546612869               |
|                    |                    | GH                    | 7H               | 267.0             | 266.6-268                        | 265.6-273.6               | 3.58                    | 48.2                  | 0.69            | 0.42                         | S7H_557408398               |
| Sp-qtl-PH-PI38-1H  | Spike Row          | GH                    | 1H               | 80.9              | 80.9-80.9                        | 70.5-82                   | 3.64                    | 4.1                   | 0.02            | -0.07                        | S1H_419469059               |
| Sp-qtl-PH-Hip-2H   | Spike Row          | GH                    | 2H               | 216.6             | 215.7-217.6                      | 206.5-228                 | 3.64                    | 63.8                  | 0.97            | 0.44                         | S2H_655393766               |
| Rpg-qtl-PH-PI38-2H | Stem Rust          | StP_MC16              | 2H               | 45.1              | 41.5-47.1                        | 33-60.9                   | 4.13                    | 12.3                  | 0.19            | -6.8                         | S2H_29881258                |
|                    |                    | StP_QC16              | 2H               | 43.2              | 40.5-47.1                        | 35-60.9                   | 3.63                    | 9.1                   | 0.13            | -6.45                        | S2H_29157898                |



|                    |           |           |    |       |             |             |      |     |      |       |               |
|--------------------|-----------|-----------|----|-------|-------------|-------------|------|-----|------|-------|---------------|
|                    |           | StP_MC16* | 2H | 45.1  | 42.2-47.1   | 35-58.9     | 4.03 | 9.8 | 0.15 | -5.93 | S2H_29881258  |
| Rpg-qt1-PH-Hip-3H  | Stem Rust | KEN_15o   | 3H | 100.7 | 100.3-100.7 | 99.9-100.7  | 4.01 | 4.6 | 0.09 | 8.4   | S3H_210225402 |
| Rpg-qt1-PH-PI38-4H | Stem Rust | StP_MC16  | 4H | 41.9  | 39.5-53.9   | 36.3-54.9   | 4.13 | 5.6 | 0.09 | -4.39 | S4H_12761761  |
|                    |           | StP_MC16* | 4H | 41.9  | 40-50.9     | 36.3-51.9   | 4.03 | 5.5 | 0.08 | -4.33 | S4H_12761761  |
|                    |           | StP_QC15  | 4H | 45.6  | 45.6-45.6   | 41-55.9     | 3.66 | 5.1 | 0.10 | -4.08 | S4H_15106305  |
|                    |           | StP_QC16  | 4H | 50.9  | 50.9-50.9   | 45.4-54.9   | 3.63 | 4.3 | 0.07 | -4.7  | S4H_15638350  |
|                    |           | KEN_15o   | 4H | 46.9  | 46.9-46.9   | 45.6-47.9   | 4.01 | 4.6 | 0.09 | -4.46 | S4H_15638350  |
| Rpg-qt1-PH-PI38-5H | Stem Rust | StP_QC15  | 5H | 171.1 | 167.2-177.4 | 142.5-180.2 | 3.66 | 5.0 | 0.15 | -5.63 | S5H_461048085 |
|                    |           | StP_MC16  | 5H | 148.4 | 145.5-149.7 | 144.7-160.2 | 4.13 | 4.5 | 0.14 | -5.96 | S5H_105563530 |
|                    |           | StP_MC16* | 5H | 148.8 | 145.5-149.4 | 142.0-161.1 | 4.03 | 4.6 | 0.14 | -5.78 | S5H_105563530 |
|                    |           | StP_HK15  | 5H | 147.2 | 145.0-154.6 | 138.2-154.6 | 4.32 | 8.2 | 0.20 | -6.54 | S5H_116258695 |
|                    |           | StP_HK15* | 5H | 147.5 | 145.3-155.7 | 137.0-181.5 | 4.1  | 8.2 | 0.20 | -6.5  | S5H_242404417 |
|                    |           | StP_MC15  | 5H | 171.8 | 166.6-178.4 | 140.3-188.9 | 3.98 | 8.7 | 0.12 | -4.16 | S5H_461048085 |
|                    |           | StP_MC15* | 5H | 172.0 | 164.8-179.3 | 140.9-187.1 | 4.1  | 8.3 | 0.12 | -4.08 | S5H_467610878 |
|                    |           | StP_QC16  | 5H | 149.9 | 146.4-151.6 | 140.1-173.8 | 3.63 | 5.2 | 0.14 | -6.71 | S5H_327036744 |
| Rpg-qt1-PH-PI38-7H | Stem Rust | StP_QC16  | 7H | 79.2  | 74.2-81.3   | 71.2-89.2   | 3.63 | 6.0 | 0.08 | -5.22 | S7H_47374714  |

<sup>a</sup> QTL were named using the convention “trait”-“qt1”-“population”-“parental allele which reduced trait value”-“chromosome”.

<sup>b</sup> Traits were heading date (days to heading), naked caryopsis, spike row type (two-rowed or six-rowed), lateral floret type (normal or deficiens), plant height (cm at maturity), stem rust (terminal severity, 0-100%), and physiological leaf spotting (0-100% of flag leaf covered at heading).

<sup>c</sup> Locations in St. Paul, MN, and Njoro, Kenya: StP\_HK15 (St. Paul race HKHJC in 2015), KEN\_15o (Njoro *Pgt* TTKSK composite (races TTKSK, TTKST, TTKTK, TTKTT) in 2015 off-season), StP\_MC15 (St. Paul race MCCFC in 2015), StP\_MC16 (St. Paul race MCCFC in 2016), StP\_QC15 (St. Paul race QCCJB in 2015), StP\_QC16 (St. Paul race QCCJB in 2016), GH (observations made in the greenhouse), \* indicates locations with values corrected for heading date.

<sup>d</sup> Chr is barley chromosomes: 1H, 2H, 3H, 4H, 5H, 6H, and 7H.

<sup>e</sup> centiMorgan (cM) position of the peak LOD (Logarithm, base 10, of odds) value.

<sup>f</sup> Confidence interval defined as the cM position of a 1 LOD drop-off from the peak LOD value.

<sup>h</sup> QTL boundary defined as the cM position of the intersection of the trace of the LOD value with the LOD cutoff value.

<sup>i</sup> Significant LOD cutoff value as determined by permutation testing.

<sup>j</sup> Percent of variance explained by the QTL.

<sup>k</sup> Additive effect of the resistant parent (PI 382313) allele.

<sup>l</sup> Marker nearest to the peak LOD value.

## Online Resource Captions

**Online Resource 4.1.** Hietpas-5/Hiproly and PI 382313/Hiproly recombinant inbred line population subsets used for genotyping and phenotyping and those used for genotyping only. In the Hietpas-5/Hiproly and the PI 382313/Hiproly populations, 280 and 278 RILs were genotyped, respectively, but only 200 RILs of each population were used in phenotyping.

**Online Resource 4.2.** Hietpas-5/Hiproly recombinant inbred line population raw genotyping by sequencing calls. Raw SNP data were generated as described in the Materials and Methods section where marker\_new, Chrom\_new and Pos\_new are based on the updated genome assembly. Ref\_seq is 30 bp before and 30 bp after the SNP in this reference genome version, whereas Marker\_old, Chrom\_old, and Pos\_old were based on an older version of the reference genome where chromosomes were split into two parts.

**Online Resource 4.3.** PI 382313/Hiproly recombinant inbred line population raw genotyping by sequencing calls. Raw SNP data were generated as described in the Materials and Methods section where marker\_new, Chrom\_new and Pos\_new are based on the updated genome assembly. Ref\_seq is 30 bp before and 30 bp after the SNP in this reference genome version, whereas Marker\_old, Chrom\_old, and Pos\_old were based on an older version of the reference genome where chromosomes were split into two parts.

**Online Resource 4.4.** Hietpas-5/Hiproly recombinant inbred line population imputed genotyping by sequencing calls to fill in missing genotype data. Raw genotype by sequencing calls imputed using the LinkImpute method as described in the Materials and Methods section (Money et al. 2015).

**Online Resource 4.5.** PI 382313/Hiproly recombinant inbred line population imputed genotyping by sequencing calls to fill in missing genotype data. Raw genotype by sequencing calls imputed using the LinkImpute method as described in the Materials and Methods section (Money et al. 2015).

**Online Resource 4.6.** Hietpas-5/Hiproly recombinant inbred line population final genotyping by sequencing calls after imputation and filtering. The final set of genotype by sequencing marker calls were first imputed and then filtered for quality control as described in the Materials and Methods section. The total number of markers was 8,586, including the two morphological markers. Alleles calls were converted to A, B, H and N calls, where “A” alleles were Hietpas-5-like, “B” alleles Hiproly-like, “H” was heterozygous, and “N” was missing.

**Online Resource 4.7.** PI 382313/Hiproly recombinant inbred line population final genotyping by sequencing calls after imputation and filtering. The final set of genotype by sequencing marker calls were first imputed and then filtered for quality control as described in the Materials and Methods section. The total number of markers remaining was 6,985, including two morphological markers. Alleles calls were converted to A, B, H and N calls, where “A” alleles were PI 382313-like, “B” alleles Hiproly-like, “H” was heterozygous, and “N” was missing.

**Online Resource 4.8.** Linkage maps of the Hietpas-5/Hiproly and PI 382313/Hiproly recombinant inbred line populations. Marker cM distance was calculated as described in the Materials and Methods section. Maps were constructed with 1,585 markers in the Hietpas-5/Hiproly population and 1,364 in the PI 382313/Hiproly population.

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## Appendix

**Appendix A1.** Previously identified quantitative trait loci (QTL) stem rust resistance.

| Marker or loci | Chromosome | cM Position | Trait            | Commnet | Reference <sup>a</sup> | Materials                        |
|----------------|------------|-------------|------------------|---------|------------------------|----------------------------------|
| 12_10674       | 5H         | 68.83       | MCCFC - Adult    | -       | Mamo 2013              | Ethiopian and Etrian Barley GWAS |
| SCRI_RS_115905 | 2H         | 41.67       | TTKSK - Seedling | -       | Mamo 2013              | Ethiopian and Etrian Barley GWAS |
| SCRI_RS_109266 | 2H         | 172.15      | TTKSK - Seedling | -       | Mamo 2013              | Ethiopian and Etrian Barley GWAS |
| SCRI_RS_180847 | 3H         | 152.69      | TTKSK - Seedling | -       | Mamo 2013              | Ethiopian and Etrian Barley GWAS |
| 12_30995       | 4H         | 54.95       | TTKSK - Seedling | -       | Mamo 2013              | Ethiopian and Etrian Barley GWAS |
| SCRI_RS_10929  | 5H         | 2.62        | TTKSK - Seedling | -       | Mamo 2013              | Ethiopian and Etrian Barley GWAS |
| 11_20206       | 5H         | 6.55        | TTKSK - Seedling | -       | Mamo 2013              | Ethiopian and Etrian Barley GWAS |
| 11_21472       | 3H         | 66.62       | TTKSK - Seedling | -       | Mamo et al. 2015       | Swiss Landrace F2's              |
| 11_20501       | 5H         | 51.51       | TTKSK - Seedling | -       | Mamo et al. 2015       | Swiss Landrace F2's              |
| 11_21061       | 5H         | 99.39       | TTKSK - Seedling | -       | Mamo et al. 2015       | Swiss Landrace F2's              |

|              |    |        |                  |                       |                    |                                     |
|--------------|----|--------|------------------|-----------------------|--------------------|-------------------------------------|
| 11_10901     | 5H | 152.93 | TTKSK - Seedling | -                     | Mamo et al. 2015   | Wild Barley F2's                    |
| 11_10528     | 5H | 155.45 | TTKSK - Seedling | -                     | Mamo et al. 2015   | Wild Barley F2's                    |
| 11_21024     | 5H | 155.66 | TTKSK - Seedling | -                     | Mamo et al. 2015   | Wild Barley F2's                    |
| 11_10336     | 5H | 157.61 | TTKSK - Seedling | -                     | Mamo et al. 2015   | Swiss Landrace and Wild Barley F2's |
| 11_20646     | 5H | 157.61 | TTKSK - Seedling | -                     | Mamo et al. 2015   | Swiss Landrace and Wild Barley F2's |
| 11_21018     | 5H | 157.61 | TTKSK - Seedling | -                     | Mamo et al. 2015   | Swiss Landrace F2's                 |
| 11_11464     | 5H | 158.28 | TTKSK - Seedling | -                     | Mamo et al. 2015   | Wild Barley F2's                    |
| 11_11216     | 5H | 162.98 | TTKSK - Seedling | -                     | Mamo et al. 2015   | Swiss Landrace and Wild Barley F2's |
| 11_20546     | 5H | 163.72 | TTKSK - Seedling | -                     | Mamo et al. 2015   | Swiss Landrace F2's                 |
| 11_20686     | 5H | 163.72 | TTKSK - Seedling | -                     | Mamo et al. 2015   | Wild Barley F2's                    |
| 11_20644     | 5H | 164.15 | TTKSK - Seedling | -                     | Mamo et al. 2015   | Swiss Landrace and Wild Barley F2's |
| 11_10869     | 5H | 165.28 | TTKSK - Seedling | -                     | Mamo et al. 2015   | Swiss Landrace F2's                 |
| 11_20536     | 5H | 168.44 | TTKSK - Seedling | -                     | Mamo et al. 2015   | Swiss Landrace F2's                 |
| Bin-16/17/18 | 2H | 44-55  | TTKSK - Seedling | eQTL, Mock            | Moscou et al. 2011 | QSM Population DH Mapping           |
| Bin-21/22    | 2H | 63-64  | TTKSK - Seedling | eQTL, Inoc            | Moscou et al. 2011 | QSM Population DH Mapping           |
| Bin-28/29    | 2H | 75-76  | TTKSK - Seedling | eQTL, Mock            | Moscou et al. 2011 | QSM Population DH Mapping           |
| Bin-40       | 6H | 6H.40  | TTKSK - Seedling | eQTL, Inoc            | Moscou et al. 2011 | QSM Population DH Mapping           |
| Bin-44       | 1H | 154.8  | TTKSK- Adult     | Infection Coefficient | Moscou et al. 2011 | QSM Population DH Mapping           |

|        |    |       |              |   |                    |                              |
|--------|----|-------|--------------|---|--------------------|------------------------------|
| Bin-47 | 1H | 165.6 | TTKSK- Adult | Severity,<br>Infection<br>Coefficient                                       | Moscou et al. 2011 | QSM Population<br>DH Mapping |
| Bin-16 | 2H | 44.2  | TTKSK- Adult | TTKSK -<br>Adult -<br>Lesion Size,<br>Infection<br>Coefficient,<br>Severity | Moscou et al. 2011 | QSM Population<br>DH Mapping |
| Bin-53 | 3H | 178.2 | TTKSK- Adult | Lesion Size   | Moscou et al. 2011 | QSM Population<br>DH Mapping |
| Bin-53 | 3H | 182.2 | TTKSK- Adult | Lesion Size   | Moscou et al. 2011 | QSM Population<br>DH Mapping |
| Bin-25 | 5H | 72.2  | TTKSK- Adult | Lesion Size   | Moscou et al. 2011 | QSM Population<br>DH Mapping |
| Bin-27 | 5H | 74.9  | TTKSK- Adult | TTKSK -<br>Adult -<br>Lesion Size,<br>Infection<br>Coefficient,<br>Severity | Moscou et al. 2011 | QSM Population<br>DH Mapping |
| Bin-45 | 5H | 141.3 | TTKSK- Adult | Severity  | Moscou et al. 2011 | QSM Population<br>DH Mapping |
| Bin-46 | 5H | 141.4 | TTKSK- Adult | TTKSK -<br>Adult -<br>Lesion Size,<br>Infection<br>Coefficient,<br>Severity | Moscou et al. 2011 | QSM Population<br>DH Mapping |
| Bin-48 | 5H | 145.4 | TTKSK- Adult | TTKSK -<br>Adult -<br>Lesion Size,<br>Infection<br>Coefficient,<br>Severity | Moscou et al. 2011 | QSM Population<br>DH Mapping |
| Bin-50 | 5H | 148.1 | TTKSK- Adult | Lesion Size   | Moscou et al. 2011 | QSM Population<br>DH Mapping |

|           |    |        |                                       |                                       |                           |   |
|-----------|----|--------|---------------------------------------|---------------------------------------|---------------------------|---|
| Bin-4     | 6H | 6.7    | TTKSK- Adult                          | Lesion Size                           | Moscou et al. 2011        | QSM Population<br>DH Mapping                |
| Bin-14    | 6H | 33.8   | TTKSK- Adult                          | Severity                              | Moscou et al. 2011        | QSM Population<br>DH Mapping                |
| Bin-32    | 7H | 76.8   | TTKSK- Adult                          | Severity,<br>Infection<br>Coefficient | Moscou et al. 2011        | QSM Population<br>DH Mapping                |
| Bin-48    | 5H | 145.43 | TTKSK- Seedling                       | -                                     | Moscou et al. 2011        | QSM Population<br>DH Mapping                |
| Bin-49    | 5H | 146.78 | TTKSK- Seedling                       | -                                     | Moscou et al. 2011        | QSM Population<br>DH Mapping                |
| Bin-49    | 5H | 146.78 | TTKSK- Seedling                       | -                                     | Moscou et al. 2011        | QSM Population<br>DH Mapping                |
| Bin-49    | 5H | 146.78 | TTKSK- Seedling                       | -                                     | Moscou et al. 2011        | QSM Population<br>DH Mapping                |
| lcal      | 1H | 55     | MCCFC-seedling                        | -                                     | Steffenson et al.<br>2007 | Wild Barley<br>Diversity Collection<br>GWAS |
| ABG474    | 6H | 75     | MCCFC-seedling                        | -                                     | Steffenson et al.<br>2007 | Wild Barley<br>Diversity Collection<br>GWAS |
| DAk133    | 5H | 5      | MCCFC-seedling                        | -                                     | Steffenson et al.<br>2007 | Wild Barley<br>Diversity Collection<br>GWAS |
| MWG920.1A | 5H | 15     | MCCFC-seedling                        | -                                     | Steffenson et al.<br>2007 | Wild Barley<br>Diversity Collection<br>GWAS |
| ABG496    | 5H | 170    | MCCFC-seedling                        | -                                     | Steffenson et al.<br>2007 | Wild Barley<br>Diversity Collection<br>GWAS |
| WMG503    | 2H | 110    | MCCFC, QCCJB -<br>seedling            | -                                     | Steffenson et al.<br>2007 | Wild Barley<br>Diversity Collection<br>GWAS |
| JS195F    | 3H | 15     | MCCFC, QCCJB,<br><i>Pgs</i> -seedling | -                                     | Steffenson et al.<br>2007 | Wild Barley<br>Diversity Collection<br>GWAS |

|                |    |       |                |   |  |                        |                                       |
|----------------|----|-------|----------------|---|--|------------------------|---------------------------------------|
| ABC807A        | 6H | 135   | Pgs-seedling   | -   |  | Steffenson et al. 2007 | Wild Barley Diversity Collection GWAS |
| BCD1532        | 3H | 50    | QCCJB-seedling | -   |  | Steffenson et al. 2007 | Wild Barley Diversity Collection GWAS |
| ABG004         | 3H | 145   | QCCJB-seedling | -   |  | Steffenson et al. 2007 | Wild Barley Diversity Collection GWAS |
| SCRI_RS_216088 | 1H | 122.2 | Pgt - Adult    | Heading, not multiple testing significant, GLM                |  | Turuspekov et al. 2016 | Kazakhstan Spring Barley GWAS         |
| SCRI_RS_216088 | 1H | 122.2 | Pgt - Adult    | Seed Maturity, Heading, not multiple testing significant, MLM |  | Turuspekov et al. 2016 | Kazakhstan Spring Barley GWAS         |
| 11_20714       | 2H | 132.6 | Pgt - Adult    | Heading, not multiple testing significant, GLM                |  | Turuspekov et al. 2016 | Kazakhstan Spring Barley GWAS         |
| 12_21386       | 3H | 131.3 | Pgt - Adult    | Seed Maturity, not multiple testing significant, GLM          |  | Turuspekov et al. 2016 | Kazakhstan Spring Barley GWAS         |

|                |    |       |             |  |                        |                               |
|----------------|----|-------|-------------|--|------------------------|-------------------------------|
| SCRI_RS_189710 | 3H | 135.6 | Pgt - Adult | Seed Maturity, not multiple testing significant, GLM | Turuspekov et al. 2016 | Kazakhstan Spring Barley GWAS |
| SCRI_RS_96016  | 4H | 59.5  | Pgt - Adult | Heading, not multiple testing significant, GLM       | Turuspekov et al. 2016 | Kazakhstan Spring Barley GWAS |
| 11_10785       | 4H | 90    | Pgt - Adult | Heading, not multiple testing significant, GLM       | Turuspekov et al. 2016 | Kazakhstan Spring Barley GWAS |
| 12_30158       | 4H | 97    | Pgt - Adult | Heading, not multiple testing significant, GLM       | Turuspekov et al. 2016 | Kazakhstan Spring Barley GWAS |
| 12_10844       | 5H | 93.9  | Pgt - Adult | Heading, not multiple testing significant, GLM       | Turuspekov et al. 2016 | Kazakhstan Spring Barley GWAS |
| SCRI_RS_189878 | 6H | 28.5  | Pgt - Adult | Heading, not multiple testing significant, GLM       | Turuspekov et al. 2016 | Kazakhstan Spring Barley GWAS |
| 11_20936       | 6H | 44.1  | Pgt - Adult | Heading, not multiple testing significant, GLM       | Turuspekov et al. 2016 | Kazakhstan Spring Barley GWAS |

|                |    |      |             |   |                           |                                  |
|----------------|----|------|-------------|---|---------------------------|----------------------------------|
| 11_21225       | 6H | 63.5 | Pgt - Adult | Heading,<br>multiple<br>testing<br>significant,<br>GLM                          | Turuspekov et al.<br>2016 | Kazakhstan Spring<br>Barley GWAS |
| 11_21225       | 6H | 63.5 | Pgt - Adult | Seed<br>Maturity,<br>Heading, not<br>multiple<br>testing<br>significant,<br>MLM | Turuspekov et al.<br>2016 | Kazakhstan Spring<br>Barley GWAS |
| 11_21293       | 6H | 63.5 | Pgt - Adult | Heading, not<br>multiple<br>testing<br>significant,<br>GLM                      | Turuspekov et al.<br>2016 | Kazakhstan Spring<br>Barley GWAS |
| 12_30637       | 6H | 63.5 | Pgt - Adult | Heading, not<br>multiple<br>testing<br>significant,<br>GLM                      | Turuspekov et al.<br>2016 | Kazakhstan Spring<br>Barley GWAS |
| SCRI_RS_152841 | 6H | 63.5 | Pgt - Adult | Heading,<br>multiple<br>testing<br>significant,<br>GLM                          | Turuspekov et al.<br>2016 | Kazakhstan Spring<br>Barley GWAS |
| SCRI_RS_152841 | 6H | 63.5 | Pgt - Adult | Seed<br>Maturity,<br>Heading, not<br>multiple<br>testing<br>significant,<br>MLM | Turuspekov et al.<br>2016 | Kazakhstan Spring<br>Barley GWAS |



|          |    |       |                  |  |                        |                               |
|----------|----|-------|------------------|--|------------------------|-------------------------------|
| 11_21069 | 6H | 63.9  | Pgt - Adult      | Heading, not multiple testing significant, GLM | Turuspekov et al. 2016 | Kazakhstan Spring Barley GWAS |
| 11_10781 | 6H | 71    | Pgt - Adult      | Heading, not multiple testing significant, GLM | Turuspekov et al. 2016 | Kazakhstan Spring Barley GWAS |
| 11_20287 | 6H | 72.79 | Pgt - Adult      | Heading, not multiple testing significant, GLM | Turuspekov et al. 2016 | Kazakhstan Spring Barley GWAS |
| 11_11355 | 5H | 86.6  | TTKSK - Adult    | -  | Zhou et al. 2014       | North American Breeding GWAS  |
| 12_31427 | 5H | 90.8  | TTKSK - Adult    | -  | Zhou et al. 2014       | North American Breeding GWAS  |
| 12_10930 | 5H | 94.4  | TTKSK - Adult    | -  | Zhou et al. 2014       | North American Breeding GWAS  |
| 12_11106 | 5H | 94.4  | TTKSK - Adult    | -  | Zhou et al. 2014       | North American Breeding GWAS  |
| 12_21497 | 5H | 94.4  | TTKSK - Adult    | -  | Zhou et al. 2014       | North American Breeding GWAS  |
| 11_21491 | 7H | 48.9  | TTKSK - Seedling | -  | Zhou et al. 2014       | North American Breeding GWAS  |
| 12_30528 | 7H | 49.7  | TTKSK - Seedling | -  | Zhou et al. 2014       | North American Breeding GWAS  |

<sup>a</sup> References for stem rust QTL (Mamo 2013; Mamo et al. 2015; Moscou et al. 2011; Steffenson et al. 2007; Turuspekov et al. 2016; Zhou et al. 2014).

**Appendix A2.** Two-hundred and ninety accessions of the Barley iCore Collection Selects (BCCS) chosen on the basis of their stem rust resistance from the 1,860 member Barley iCore Collection (BCC).

| Name <sup>a</sup> | Accession <sup>b</sup> | Spike Row <sup>d</sup> | Improvement Status | County         | State or Region   | Population Assignment <sup>e</sup>           |
|-------------------|------------------------|------------------------|--------------------|----------------|-------------------|--|
| BCC0007           | CIho 1388              | NA                     | cultivar           | Denmark        | NA                | Admixed                                      |
| BCC0015           | CIho 1604              | 6                      | landrace           | Ethiopia       | NA                | population 5<br>African two/six-row          |
| BCC0057           | CIho 3240              | 6                      | uncertain          | Egypt          | NA                | population 1<br>Mediterranean six-row        |
| BCC0058           | CIho 3387              | 6                      | uncertain          | Algeria        | NA                | population 1<br>Mediterranean six-row        |
| BCC0077           | CIho 3886              | 6                      | landrace           | India          | Jammu and Kashmir | population 2 Asian<br>six-row                |
| BCC0131           | CIho 6371              | NA                     | breeding           | United States  | South Dakota      | Admixed                                      |
| BCC0138           | CIho 6496              | 6                      | uncertain          | China          | Xizang            | Admixed                                      |
| BCC0156           | CIho 7127              | 2                      | cultivar           | United Kingdom | England           | Admixed                                      |
| BCC0157           | CIho 7153              | 6                      | breeding           | United States  | Idaho             | Admixed                                      |
| BCC0168           | CIho 7491              | 6                      | landrace           | Mexico         | Hidalgo           | population 1<br>Mediterranean six-row        |
| BCC0169           | CIho 7492              | 6                      | landrace           | Mexico         | Hidalgo           | population 1<br>Mediterranean six-row        |
| BCC0174           | CIho 7556              | 6                      | breeding           | Argentina      | NA                | population 2 Asian<br>six-row                |
| BCC0175           | CIho 7782              | NA                     | cultivar           | Slovakia       | East Slovakia     | population 3<br>European two-row             |
| BCC0180           | CIho 8054              | 6                      | breeding           | United States  | Utah              | Admixed                                      |
| BCC0212           | CIho 10661             | 6                      | breeding           | United States  | Texas             | Admixed                                      |
| BCC0231           | CIho 11789             | 2                      | uncertain          | Saudi Arabia   | NA                | Admixed                                      |
| BCC0240           | CIho 11845             | 2                      | uncertain          | Israel         | NA                | population 3<br>European two-row             |
| BCC0241           | CIho 11849             | 6                      | landrace           | Ethiopia       | NA                | population 5<br>African two/six-row          |
| BCC0242           | CIho 11852             | 2                      | genetic            | United States  | California        | Admixed                                      |
| BCC0244           | CIho 11864             | 6                      | breeding           | United States  | North Dakota      | population 4<br>European/American<br>six-row |
| BCC0255           | CIho 13453             | 3                      | uncertain          | Romania        | NA                | Admixed                                      |

|         |            |    |           |                        |                 |  |
|---------|------------|----|-----------|------------------------|-----------------|--|
| BCC0260 | CIho 13651 | 6  | breeding  | Israel                 | NA              | population 1<br>Mediterranean six-row        |
| BCC0261 | CIho 13653 | 3  | cultivar  | Former Soviet Union    | NA              | Admixed                                      |
| BCC0266 | CIho 13743 | 6  | landrace  | Eritrea                | Asmara          | population 5<br>African two/six-row          |
| BCC0270 | CIho 13824 | 6  | cultivar  | United States          | California      | population 1<br>Mediterranean six-row        |
| BCC0308 | CIho 14291 | 6  | landrace  | China                  | Sichuan         | population 2 Asian<br>six-row                |
| BCC0318 | CIho 14334 | 2  | landrace  | Azerbaijan             | NA              | Admixed                                      |
| BCC0324 | CIho 14395 | 2  | landrace  | Armenia                | NA              | Admixed                                      |
| BCC0333 | CIho 14978 | 3  | landrace  | Ethiopia               | Tigre           | population 5<br>African two/six-row          |
| BCC0334 | CIho 15203 | 2  | breeding  | United States          | Oregon          | Admixed                                      |
| BCC0376 | CIho 15616 | 2  | breeding  | Canada                 | Quebec          | population 3<br>European two-row             |
| BCC0377 | CIho 15624 | 6  | cultivar  | Canada                 | Ontario         | population 4<br>European/American<br>six-row |
| BCC0392 | CIho 16612 | 2  | genetic   | United States          | Montana         | Admixed                                      |
| BCC0394 | CIho 16671 | 2  | genetic   | United States          | Montana         | Admixed                                      |
| BCC0395 | CIho 16676 | 6  | genetic   | United States          | Montana         | Admixed                                      |
| BCC0422 | PI 38320   | NA | landrace  | Russian Federation     | Leningrad       | Admixed                                      |
| BCC0434 | PI 39397   | 6  | uncertain | Australia              | New South Wales | population 1<br>Mediterranean six-row        |
| BCC0435 | PI 39590   | 6  | landrace  | Algeria                | Mascara         | population 1<br>Mediterranean six-row        |
| BCC0444 | PI 47541   | NA | landrace  | Iran                   | NA              | population 2 Asian<br>six-row                |
| BCC0476 | PI 60663   | 6  | landrace  | Libya                  | Tarabulus       | population 1<br>Mediterranean six-row        |
| BCC0490 | PI 61589   | 6  | landrace  | Mongolia               | NA              | population 2 Asian<br>six-row                |
| BCC0524 | PI 76285   | 6  | landrace  | Iraq                   | Baghdad         | Admixed                                      |
| BCC0579 | PI 94806   | NA | landrace  | The country of Georgia | NA              | Admixed                                      |
| BCC0592 | PI 95167   | 2  | landrace  | Unknown                | NA              | population 3<br>European two-row             |
| BCC0597 | PI 95198   | NA | landrace  | Ukraine                | NA              | Admixed                                      |
| BCC0603 | PI 95270   | 6  | landrace  | Unknown                | NA              | population 5<br>African two/six-row          |

|         |           |    |           |             |                  |  |
|---------|-----------|----|-----------|-------------|------------------|--|
| BCC0654 | PI 155303 | NA | landrace  | Yemen       | NA               | Admixed                                      |
| BCC0669 | PI 157890 | 6  | uncertain | Italy       | Latium           | population 1<br>Mediterranean six-row        |
| BCC0673 | PI 159126 | 6  | landrace  | Mexico      | Guanajuato       | population 1<br>Mediterranean six-row        |
| BCC0694 | PI 168328 | 6  | landrace  | Turkey      | Balikesir        | Admixed                                      |
| BCC0709 | PI 174478 | NA | cultivar  | France      | Yvelines         | Admixed                                      |
| BCC0713 | PI 175506 | NA | cultivar  | Finland     | NA               | population 3<br>European two-row             |
| BCC0718 | PI 176042 | 6  | landrace  | India       | Uttar Pradesh    | population 2 Asian<br>six-row                |
| BCC0728 | PI 178285 | 6  | landrace  | Turkey      | Adana            | population 1<br>Mediterranean six-row        |
| BCC0736 | PI 180670 | NA | cultivar  | Germany     | Bavaria          | population 3<br>European two-row             |
| BCC0738 | PI 181102 | 6  | landrace  | India       | Himachal Pradesh | population 2 Asian<br>six-row                |
| BCC0740 | PI 181113 | 6  | landrace  | India       | Gujarat          | Admixed                                      |
| BCC0741 | PI 181148 | NA | cultivar  | Netherlands | South Holland    | population 3<br>European two-row             |
| BCC0743 | PI 182373 | NA | landrace  | Turkey      | Bayburt          | Admixed                                      |
| BCC0746 | PI 182625 | 6  | cultivar  | Japan       | Kyoto            | population 2 Asian<br>six-row                |
| BCC0747 | PI 182645 | 6  | cultivar  | Japan       | Fukuoka          | Admixed                                      |
| BCC0750 | PI 182661 | 6  | landrace  | Lebanon     | El Beqaa         | Admixed                                      |
| BCC0762 | PI 183590 | 2  | cultivar  | Austria     | NA               | Admixed                                      |
| BCC0771 | PI 184103 | NA | landrace  | Montenegro  | NA               | Admixed                                      |
| BCC0773 | PI 184872 | 2  | genetic   | Sweden      | Malmohus         | population 3<br>European two-row             |
| BCC0774 | PI 184873 | NA | cultivar  | Sweden      | Malmohus         | population 3<br>European two-row             |
| BCC0777 | PI 184880 | NA | breeding  | Sweden      | Malmohus         | population 3<br>European two-row             |
| BCC0821 | PI 193062 | 6  | cultivar  | Belgium     | Namur            | population 4<br>European/American<br>six-row |
| BCC0822 | PI 193063 | 2  | cultivar  | Belgium     | Namur            | population 3<br>European two-row             |
| BCC0829 | PI 194556 | NA | breeding  | Germany     | Saxony           | population 3<br>European two-row             |
| BCC0842 | PI 201097 | 2  | cultivar  | Austria     | NA               | population 3<br>European two-row             |
| BCC0856 | PI 221326 | 6  | uncertain | Serbia      | NA               | Admixed                                      |

|         |           |    |           |                       |                          |  |
|---------|-----------|----|-----------|-----------------------|--------------------------|--|
| BCC0862 | PI 223134 | 6  | uncertain | Jordan                | NA                       | population 1<br>Mediterranean six-row        |
| BCC0875 | PI 223879 | 6  | landrace  | Afghanistan           | Baghlan                  | population 2 Asian<br>six-row                |
| BCC0884 | PI 226612 | 6  | uncertain | Cyprus                | NA                       | population 1<br>Mediterranean six-row        |
| BCC0889 | PI 231151 | 6  | cultivar  | Colombia              | NA                       | Admixed                                      |
| BCC0899 | PI 235172 | 6  | breeding  | Turkey                | Istanbul                 | population 4<br>European/American<br>six-row |
| BCC0908 | PI 237571 | 6  | breeding  | Colombia              | Cundinamarca             | Admixed                                      |
| BCC0950 | PI 259878 | NA | breeding  | Croatia               | NA                       | population 4<br>European/American<br>six-row |
| BCC0985 | PI 264916 | 6  | landrace  | Croatia               | Dubrovacko-<br>neretvans | Admixed                                      |
| BCC0991 | PI 265462 | 2  | cultivar  | Finland               | NA                       | population 3<br>European two-row             |
| BCC0994 | PI 266132 | 2  | cultivar  | Sweden                | Malmohus                 | population 3<br>European two-row             |
| BCC1003 | PI 268167 | 2  | cultivar  | Ukraine               | Krym                     | Admixed                                      |
| BCC1060 | PI 280441 | 2  | cultivar  | Russian<br>Federation | Irkutsk                  | population 3<br>European two-row             |
| BCC1061 | PI 281524 | 6  | cultivar  | Argentina             | Buenos Aires             | population 1<br>Mediterranean six-row        |
| BCC1062 | PI 281525 | 6  | cultivar  | Argentina             | Buenos Aires             | Admixed                                      |
| BCC1069 | PI 283397 | 2  | landrace  | Armenia               | NA                       | population 3<br>European two-row             |
| BCC1081 | PI 283429 | 2  | cultivar  | Denmark               | NA                       | population 3<br>European two-row             |
| BCC1100 | PI 286388 | 3  | landrace  | Eritrea               | Asmara                   | population 5<br>African two/six-row          |
| BCC1101 | PI 286389 | 3  | landrace  | Eritrea               | NA                       | population 5<br>African two/six-row          |
| BCC1103 | PI 289811 | 6  | landrace  | Iran                  | Khuzestan                | Admixed                                      |
| BCC1131 | PI 290353 | 6  | cultivar  | Kyrgyzstan            | NA                       | Admixed                                      |
| BCC1135 | PI 292016 | 2  | cultivar  | Israel                | NA                       | population 1<br>Mediterranean six-row        |
| BCC1142 | PI 294739 | 2  | uncertain | Western Asia          | NA                       | Admixed                                      |
| BCC1156 | PI 295442 | 5  | landrace  | Ethiopia              | Shewa                    | population 5<br>African two/six-row          |
| BCC1157 | PI 295581 | 6  | landrace  | Ethiopia              | Shewa                    | population 5<br>African two/six-row          |

|         |           |   |           |                        |                   |   |
|---------|-----------|---|-----------|------------------------|-------------------|---|
| BCC1158 | PI 295956 | 6 | breeding  | Israel                 | NA                | population 1<br>Mediterranean six-row     |
| BCC1160 | PI 295960 | 6 | cultivar  | Israel                 | NA                | Admixed                                   |
| BCC1182 | PI 306439 | 2 | uncertain | Romania                | NA                | population 3<br>European two-row          |
| BCC1195 | PI 308142 | 3 | cultivar  | Ukraine                | Vinnytsya         | population 3<br>European two-row          |
| BCC1206 | PI 320213 | 6 | uncertain | Australia              | Western Australia | population 1<br>Mediterranean six-row     |
| BCC1207 | PI 320216 | 6 | uncertain | Australia              | Western Australia | population 1<br>Mediterranean six-row     |
| BCC1209 | PI 320219 | 6 | uncertain | Australia              | Western Australia | Admixed                                   |
| BCC1215 | PI 321770 | 2 | cultivar  | Slovenia               | NA                | population 3<br>European two-row          |
| BCC1219 | PI 321784 | 2 | cultivar  | Belgium                | Namur             | population 3<br>European two-row          |
| BCC1222 | PI 321797 | 2 | cultivar  | Netherlands            | Groningen         | population 3<br>European two-row          |
| BCC1229 | PI 321845 | 2 | uncertain | Slovenia               | NA                | population 3<br>European two-row          |
| BCC1310 | PI 327902 | 6 | cultivar  | Bulgaria               | NA                | Admixed                                   |
| BCC1324 | PI 328154 | 6 | landrace  | Bulgaria               | NA                | population 4<br>European/American six-row |
| BCC1337 | PI 328327 | 6 | landrace  | Turkey                 | Balikesir         | Admixed                                   |
| BCC1375 | PI 328950 | 2 | cultivar  | Netherlands            | South Holland     | population 3<br>European two-row          |
| BCC1377 | PI 328976 | 6 | landrace  | Ethiopia               | NA                | Admixed                                   |
| BCC1390 | PI 330326 | 6 | breeding  | Colombia               | Cundinamarca      | Admixed                                   |
| BCC1391 | PI 330397 | 2 | cultivar  | Czechoslovakia         | NA                | population 3<br>European two-row          |
| BCC1392 | PI 330398 | 2 | cultivar  | Slovakia               | West Slovakia     | population 3<br>European two-row          |
| BCC1393 | PI 330399 | 2 | cultivar  | Czech Republic         | East Bohemia      | population 3<br>European two-row          |
| BCC1394 | PI 330400 | 2 | cultivar  | Czech Republic         | NA                | population 3<br>European two-row          |
| BCC1399 | PI 331217 | 2 | landrace  | Eritrea                | Asmara            | population 5<br>African two/six-row       |
| BCC1404 | PI 331895 | 6 | landrace  | Afghanistan            | NA                | Admixed                                   |
| BCC1408 | PI 337143 | 2 | cultivar  | Argentina              | Buenos Aires      | population 3<br>European two-row          |
| BCC1427 | PI 342219 | 2 | landrace  | Turkey                 | Kutahya           | Admixed                                   |
| BCC1450 | PI 344920 | 2 | landrace  | Bosnia and Herzegovina | NA                | Admixed                                   |

|         |           |     |          |                    |              |  |
|---------|-----------|-----|----------|--------------------|--------------|--|
| BCC1465 | PI 345618 | 2   | cultivar | Russian Federation | Omsk         | population 3<br>European two-row             |
| BCC1469 | PI 346390 | 2   | breeding | Argentina          | Buenos Aires | population 3<br>European two-row             |
| BCC1472 | PI 349359 | 2   | breeding | United Kingdom     | England      | population 3<br>European two-row             |
| BCC1474 | PI 349896 | 2   | landrace | Serbia             | NA           | population 3<br>European two-row             |
| BCC1485 | PI 356580 | 5   | landrace | Ethiopia           | NA           | population 5<br>African two/six-row          |
| BCC1495 | PI 356715 | 3,6 | landrace | Morocco            | NA           | population 5<br>African two/six-row          |
| BCC1510 | PI 356775 | 6   | landrace | Morocco            | NA           | Admixed                                      |
| BCC1515 | PI 361036 | 2   | breeding | United Kingdom     | England      | population 3<br>European two-row             |
| BCC1522 | PI 361641 | 2   | cultivar | France             | Yvelines     | population 3<br>European two-row             |
| BCC1525 | PI 361665 | 2   | cultivar | Denmark            | NA           | Admixed                                      |
| BCC1529 | PI 361672 | 2   | cultivar | Denmark            | NA           | population 3<br>European two-row             |
| BCC1535 | PI 361695 | 6   | cultivar | Finland            | NA           | population 4<br>European/American<br>six-row |
| BCC1548 | PI 365547 | 2   | cultivar | Netherlands        | Groningen    | population 3<br>European two-row             |
| BCC1556 | PI 369732 | 2   | cultivar | Ukraine            | Kharkiv      | Admixed                                      |
| BCC1562 | PI 369747 | 6   | cultivar | Ukraine            | Odesa        | Admixed                                      |
| BCC1568 | PI 370852 | 2   | landrace | Switzerland        | Graubunden   | Admixed                                      |
| BCC1570 | PI 370867 | 2   | landrace | Switzerland        | Valais       | Admixed                                      |
| BCC1571 | PI 370869 | 2   | landrace | Switzerland        | Valais       | Admixed                                      |
| BCC1576 | PI 370994 | 2   | landrace | Switzerland        | Valais       | Admixed                                      |
| BCC1577 | PI 371017 | 2   | landrace | Switzerland        | Bern         | Admixed                                      |
| BCC1578 | PI 371056 | NA  | landrace | Switzerland        | Valais       | Admixed                                      |
| BCC1582 | PI 371102 | 2   | landrace | Switzerland        | Valais       | Admixed                                      |
| BCC1584 | PI 371111 | 2   | landrace | Switzerland        | Valais       | Admixed                                      |
| BCC1585 | PI 371149 | 2   | landrace | Switzerland        | Valais       | Admixed                                      |
| BCC1592 | PI 371248 | 2   | landrace | Switzerland        | Graubunden   | Admixed                                      |
| BCC1595 | PI 371320 | 2   | landrace | Switzerland        | Uri          | Admixed                                      |
| BCC1600 | PI 371377 | 2   | landrace | Switzerland        | Graubunden   | Admixed                                      |
| BCC1602 | PI 371390 | 2   | landrace | Switzerland        | Graubunden   | Admixed                                      |
| BCC1603 | PI 371392 | 2   | landrace | Switzerland        | Graubunden   | Admixed                                      |

|         |           |   |           |                           |               |  |
|---------|-----------|---|-----------|---------------------------|---------------|--|
| BCC1614 | PI 372084 | 2 | cultivar  | Estonia                   | NA            | population 3<br>European two-row             |
| BCC1620 | PI 372099 | 6 | cultivar  | Turkmenistan              | NA            | Admixed                                      |
| BCC1623 | PI 372102 | 2 | cultivar  | Belarus                   | Minsk         | population 3<br>European two-row             |
| BCC1624 | PI 372107 | 6 | cultivar  | Moldova                   | NA            | Admixed                                      |
| BCC1625 | PI 374413 | 2 | landrace  | Macedonia                 | NA            | Admixed                                      |
| BCC1627 | PI 374420 | 2 | landrace  | Bosnia and<br>Herzegovina | NA            | population 3<br>European two-row             |
| BCC1636 | PI 378218 | 2 | landrace  | Serbia                    | NA            | Admixed                                      |
| BCC1639 | PI 382275 | 3 | landrace  | Ethiopia                  | Tigre         | population 5<br>African two/six-row          |
| BCC1640 | PI 382296 | 2 | landrace  | Ethiopia                  | Tigre         | population 5<br>African two/six-row          |
| BCC1655 | PI 386406 | 3 | landrace  | Eritrea                   | NA            | population 5<br>African two/six-row          |
| BCC1661 | PI 386601 | 6 | landrace  | Ethiopia                  | Arusi         | population 5<br>African two/six-row          |
| BCC1665 | PI 386838 | 6 | landrace  | Ethiopia                  | Gonder        | population 5<br>African two/six-row          |
| BCC1691 | PI 392464 | 2 | breeding  | South Africa              | Cape Province | Admixed                                      |
| BCC1694 | PI 392471 | 2 | breeding  | South Africa              | Cape Province | Admixed                                      |
| BCC1695 | PI 392478 | 2 | breeding  | South Africa              | Cape Province | Admixed                                      |
| BCC1702 | PI 392499 | 2 | breeding  | South Africa              | Cape Province | Admixed                                      |
| BCC1703 | PI 392501 | 2 | breeding  | South Africa              | Cape Province | Admixed                                      |
| BCC1712 | PI 399482 | 2 | cultivar  | Netherlands               | South Holland | population 3<br>European two-row             |
| BCC1719 | PI 401939 | 6 | uncertain | Colombia                  | Cundinamarca  | Admixed                                      |
| BCC1720 | PI 401952 | 6 | uncertain | Colombia                  | Cundinamarca  | Admixed                                      |
| BCC1721 | PI 401954 | 6 | uncertain | Colombia                  | Cundinamarca  | Admixed                                      |
| BCC1724 | PI 401973 | 6 | uncertain | Colombia                  | Cundinamarca  | Admixed                                      |
| BCC1732 | PI 402098 | 6 | uncertain | Colombia                  | Cundinamarca  | Admixed                                      |
| BCC1737 | PI 402164 | 6 | uncertain | Colombia                  | Cundinamarca  | population 4<br>European/American<br>six-row |
| BCC1738 | PI 402168 | 6 | uncertain | Colombia                  | Cundinamarca  | Admixed                                      |
| BCC1741 | PI 402264 | 6 | uncertain | Colombia                  | Cundinamarca  | Admixed                                      |
| BCC1743 | PI 402335 | 6 | uncertain | Colombia                  | Cundinamarca  | Admixed                                      |
| BCC1744 | PI 402352 | 6 | uncertain | Colombia                  | Cundinamarca  | Admixed                                      |
| BCC1745 | PI 402354 | 6 | uncertain | Colombia                  | Cundinamarca  | Admixed                                      |



|         |           |   |           |                |                  |  |
|---------|-----------|---|-----------|----------------|------------------|--|
| BCC1750 | PI 402445 | 6 | uncertain | Colombia       | Cundinamarca     | Admixed                                      |
| BCC1751 | PI 402468 | 6 | uncertain | Colombia       | Cundinamarca     | Admixed                                      |
| BCC1755 | PI 404204 | 2 | cultivar  | Ukraine        | Chernihiv        | population 3<br>European two-row             |
| BCC1783 | PI 410865 | 2 | cultivar  | Netherlands    | Gelderland       | Admixed                                      |
| BCC1789 | PI 412946 | 2 | uncertain | South Africa   | Cape Province    | population 3<br>European two-row             |
| BCC1796 | PI 415012 | 6 | cultivar  | Mexico         | Federal District | Admixed                                      |
| BCC1797 | PI 415014 | 6 | cultivar  | Mexico         | Federal District | Admixed                                      |
| BCC1822 | PI 422230 | 3 | landrace  | Yemen          | NA               | Admixed                                      |
| BCC1823 | PI 422232 | 3 | landrace  | Yemen          | NA               | Admixed                                      |
| BCC1830 | PI 428411 | 6 | cultivar  | Mexico         | Federal District | Admixed                                      |
| BCC1831 | PI 428413 | 2 | cultivar  | France         | Yvelines         | population 3<br>European two-row             |
| BCC1832 | PI 428491 | 2 | cultivar  | Netherlands    | NA               | population 3<br>European two-row             |
| BCC1839 | PI 428628 | 2 | cultivar  | Czechoslovakia | NA               | population 3<br>European two-row             |
| BCC1850 | PI 429311 | 2 | landrace  | Yemen          | NA               | Admixed                                      |
| BCC1851 | PI 429312 | 2 | landrace  | Yemen          | NA               | Admixed                                      |
| BCC1852 | PI 429313 | 2 | landrace  | Yemen          | NA               | Admixed                                      |
| BCC1857 | PI 429519 | 6 | landrace  | Nepal          | Kosi             | population 2 Asian<br>six-row                |
| BCC1859 | PI 429526 | 6 | landrace  | Nepal          | Kosi             | population 2 Asian<br>six-row                |
| BCC1872 | PI 434760 | 6 | breeding  | Canada         | Quebec           | population 4<br>European/American<br>six-row |
| BCC1875 | PI 434814 | 6 | breeding  | Canada         | Quebec           | population 4<br>European/American<br>six-row |
| BCC1876 | PI 434815 | 6 | breeding  | Canada         | Quebec           | population 4<br>European/American<br>six-row |
| BCC1879 | PI 436135 | 2 | landrace  | Chile          | La Araucania     | population 3<br>European two-row             |
| BCC1886 | PI 436146 | 2 | landrace  | Chile          | La Araucania     | population 3<br>European two-row             |
| BCC1889 | PI 436150 | 2 | landrace  | Chile          | La Araucania     | population 3<br>European two-row             |
| BCC1891 | PI 436153 | 2 | landrace  | Chile          | Los Lagos        | population 3<br>European two-row             |
| BCC1917 | PI 447191 | 6 | uncertain | Spain          | NA               | population 1<br>Mediterranean six-<br>row    |

|         |           |     |           |                |               |   |
|---------|-----------|-----|-----------|----------------|---------------|---|
| BCC1918 | PI 447207 | 2   | uncertain | Spain          | Zaragoza      | population 3<br>European two-row          |
| BCC1928 | PI 449279 | 6   | breeding  | Spain          | Zaragoza      | Admixed                                   |
| BCC1938 | PI 466726 | 2   | cultivar  | Sweden         | Malmöhus      | population 3<br>European two-row          |
| BCC1971 | PI 467454 | 2   | uncertain | Hungary        | NA            | population 3<br>European two-row          |
| BCC2013 | PI 467749 | 2   | cultivar  | Netherlands    | NA            | population 3<br>European two-row          |
| BCC2016 | PI 467786 | 2   | cultivar  | Netherlands    | South Holland | population 3<br>European two-row          |
| BCC2017 | PI 467789 | 2   | cultivar  | Netherlands    | NA            | population 3<br>European two-row          |
| BCC2019 | PI 467797 | 2   | cultivar  | Greece         | Macedonia     | population 1<br>Mediterranean six-row     |
| BCC2022 | PI 467814 | 2   | cultivar  | Croatia        | NA            | population 3<br>European two-row          |
| BCC2023 | PI 467815 | 6   | cultivar  | Finland        | NA            | Admixed                                   |
| BCC2024 | PI 467831 | 2   | cultivar  | Poland         | NA            | population 3<br>European two-row          |
| BCC2026 | PI 467836 | 2   | cultivar  | Poland         | NA            | population 3<br>European two-row          |
| BCC2027 | PI 467839 | 2   | cultivar  | Poland         | NA            | population 3<br>European two-row          |
| BCC2028 | PI 467840 | 2   | cultivar  | Poland         | NA            | population 3<br>European two-row          |
| BCC2065 | PI 483048 | 2   | cultivar  | Australia      | Queensland    | Admixed                                   |
| BCC2066 | PI 485524 | 2   | cultivar  | United Kingdom | England       | population 3<br>European two-row          |
| BCC2068 | PI 485536 | 2   | breeding  | United Kingdom | England       | population 3<br>European two-row          |
| BCC2069 | PI 485548 | 2   | breeding  | United Kingdom | England       | population 3<br>European two-row          |
| BCC2074 | PI 494099 | 2   | cultivar  | United States  | Colorado      | population 3<br>European two-row          |
| BCC2083 | PI 498436 | 2   | cultivar  | New Zealand    | NA            | population 3<br>European two-row          |
| BCC2084 | PI 498437 | 2   | cultivar  | New Zealand    | NA            | population 3<br>European two-row          |
| BCC2091 | PI 506293 | 2   | cultivar  | United Kingdom | England       | population 3<br>European two-row          |
| BCC2092 | PI 506295 | 2   | breeding  | United Kingdom | England       | population 3<br>European two-row          |
| BCC2093 | PI 506299 | 2   | breeding  | United Kingdom | England       | population 3<br>European two-row          |
| BCC2118 | PI 531896 | 5,6 | uncertain | Egypt          | NA            | Admixed                                   |
| BCC2142 | PI 548724 | 6   | uncertain | Afghanistan    | NA            | population 4<br>European/American six-row |

|         |           |    |           |                   |                      |                                     |
|---------|-----------|----|-----------|-------------------|----------------------|-------------------------------------|
| BCC2144 | PI 548736 | NA | uncertain | Ethiopia          | NA                   | population 5<br>African two/six-row |
| BCC2152 | PI 559516 | NA | landrace  | Nepal             | NA                   | population 2 Asian<br>six-row       |
| BCC2162 | PI 564477 | 2  | cultivar  | Bulgaria          | Plovdiv              | population 3<br>European two-row    |
| BCC2168 | PI 564502 | 2  | cultivar  | Bolivia           | NA                   | population 5<br>African two/six-row |
| BCC2233 | PI 566497 | 6  | uncertain | China             | Yunnan               | Admixed                             |
| BCC2236 | PI 572588 | 6  | cultivar  | China             | Shaanxi              | Admixed                             |
| BCC2241 | PI 573594 | 2  | cultivar  | Lithuania         | NA                   | population 3<br>European two-row    |
| BCC2242 | PI 573598 | 2  | cultivar  | Ukraine           | Odesa                | population 3<br>European two-row    |
| BCC2254 | PI 573662 | 2  | landrace  | Georgia           | NA                   | Admixed                             |
| BCC2259 | PI 573682 | 2  | landrace  | Georgia           | NA                   | Admixed                             |
| BCC2265 | PI 573703 | 2  | landrace  | Georgia           | NA                   | Admixed                             |
| BCC2291 | PI 574094 | 6  | landrace  | Nepal             | Mechi                | population 2 Asian<br>six-row       |
| BCC2297 | PI 584766 | 2  | breeding  | Australia         | Western<br>Australia | Admixed                             |
| BCC2336 | PI 591928 | 2  | cultivar  | Australia         | New South<br>Wales   | population 3<br>European two-row    |
| BCC2342 | PI 592173 | 2  | cultivar  | United<br>Kingdom | Wales                | population 3<br>European two-row    |
| BCC2345 | PI 592196 | 2  | cultivar  | United<br>Kingdom | NA                   | population 3<br>European two-row    |
| BCC2352 | PI 592240 | 2  | cultivar  | United<br>Kingdom | England              | Admixed                             |
| BCC2362 | PI 592282 | 2  | cultivar  | United<br>Kingdom | England              | population 3<br>European two-row    |
| BCC2371 | PI 599633 | 2  | cultivar  | Czech<br>Republic | South Moravia        | population 3<br>European two-row    |
| BCC2373 | PI 599640 | 2  | cultivar  | Czech<br>Republic | NA                   | Admixed                             |
| BCC2378 | PI 601065 | NA | cultivar  | United States     | California           | Admixed                             |
| BCC2380 | PI 605472 | 2  | cultivar  | United States     | Idaho                | population 3<br>European two-row    |
| BCC2381 | PI 605699 | 6  | cultivar  | Canada            | Alberta              | Admixed                             |
| BCC2382 | PI 606305 | NA | landrace  | Saudi Arabia      | NA                   | Admixed                             |
| BCC2384 | PI 608667 | 6  | cultivar  | United States     | California           | Admixed                             |
| BCC2397 | PI 611511 | NA | cultivar  | Ukraine           | Odesa                | Admixed                             |
| BCC2399 | PI 611513 | NA | cultivar  | Ukraine           | Vinnytsya            | population 3<br>European two-row    |
| BCC2400 | PI 611526 | NA | cultivar  | Ukraine           | Dnipropetrovsk       | Admixed                             |

|         |           |    |          |               |                |  |
|---------|-----------|----|----------|---------------|----------------|--|
| BCC2401 | PI 611527 | NA | cultivar | Ukraine       | Dnipropetrovsk | population 3<br>European two-row             |
| BCC2408 | PI 611576 | NA | cultivar | Ukraine       | NA             | Admixed                                      |
| BCC2413 | PI 620640 | 6  | cultivar | Canada        | Manitoba       | Admixed                                      |
| BCC2414 | PI 629116 | 6  | cultivar | United States | Montana        | Admixed                                      |
| BCC2415 | PI 632274 | 6  | cultivar | United States | Idaho          | population 4<br>European/American<br>six-row |
| BCC2423 | PI 639299 | NA | landrace | Kazakhstan    | Alma-Ata       | Admixed                                      |
| BCC2424 | PI 639300 | 2  | landrace | Kazakhstan    | Alma-Ata       | Admixed                                      |
| BCC2430 | PI 639999 | 6  | breeding | United States | Texas          | population 4<br>European/American<br>six-row |
| BCC2431 | PI 640006 | 6  | breeding | United States | Texas          | Admixed                                      |
| BCC2462 | PI 640166 | 6  | breeding | United States | Texas          | population 4<br>European/American<br>six-row |
| BCC2505 | PI 640366 | 6  | breeding | United States | Texas          | Admixed                                      |
| BCC2527 | PI 643212 | 6  | breeding | United States | North Dakota   | population 4<br>European/American<br>six-row |
| BCC2530 | PI 643227 | 2  | breeding | United States | North Dakota   | Admixed                                      |
| BCC2533 | PI 643232 | 2  | breeding | United States | North Dakota   | Admixed                                      |
| BCC2536 | PI 643247 | 2  | breeding | United States | North Dakota   | Admixed                                      |
| BCC2537 | PI 643257 | 2  | breeding | United States | North Dakota   | Admixed                                      |
| BCC2544 | PI 643288 | 2  | breeding | United States | North Dakota   | Admixed                                      |
| BCC2545 | PI 643292 | 2  | breeding | United States | North Dakota   | Admixed                                      |
| BCC2548 | PI 643310 | 2  | breeding | United States | North Dakota   | Admixed                                      |
| BCC2549 | PI 643314 | 2  | breeding | United States | North Dakota   | Admixed                                      |
| BCC2552 | PI 643336 | 2  | breeding | United States | North Dakota   | Admixed                                      |
| BCC2553 | PI 643339 | 2  | breeding | United States | North Dakota   | Admixed                                      |
| BCC2555 | PI 643362 | 2  | breeding | United States | North Dakota   | Admixed                                      |
| BCC2556 | PI 643364 | 2  | breeding | United States | North Dakota   | Admixed                                      |
| BCC2557 | PI 643368 | 2  | breeding | United States | North Dakota   | Admixed                                      |
| BCC2558 | PI 643369 | 2  | breeding | United States | North Dakota   | Admixed                                      |
| BCC2559 | PI 643370 | 2  | breeding | United States | North Dakota   | Admixed                                      |
| BCC2560 | PI 643376 | 2  | breeding | United States | North Dakota   | Admixed                                      |

|         |           |   |          |               |              |         |
|---------|-----------|---|----------|---------------|--------------|---------|
| BCC2561 | PI 643377 | 2 | breeding | United States | North Dakota | Admixed |
| BCC2562 | PI 643383 | 2 | breeding | United States | North Dakota | Admixed |

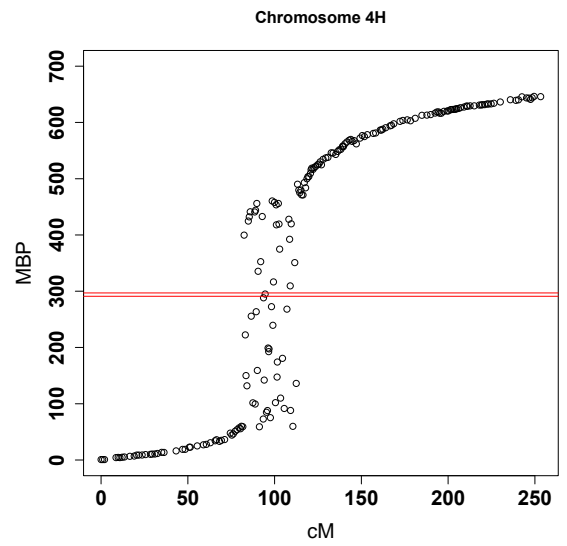
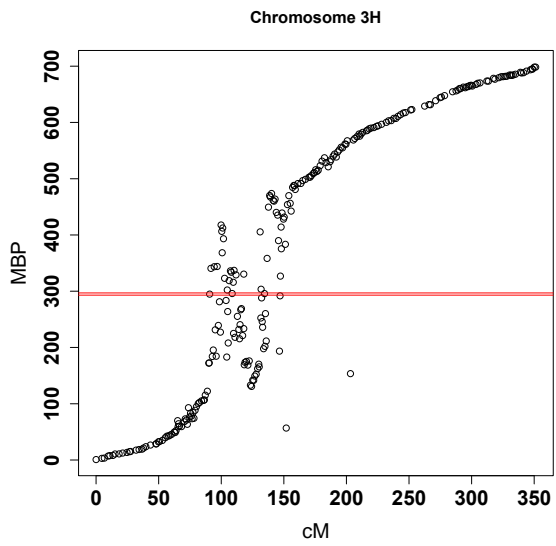
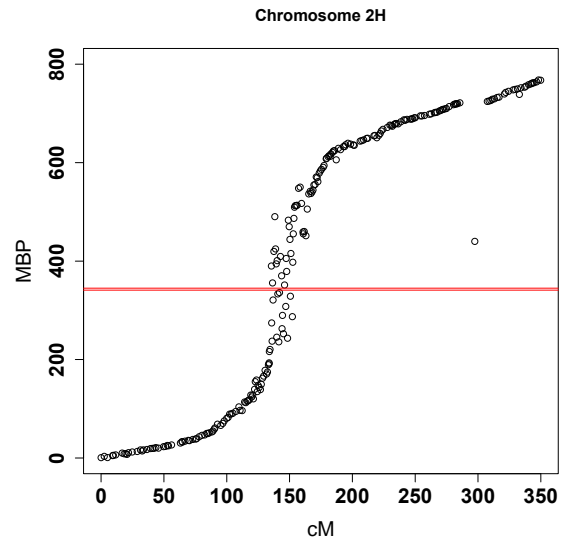
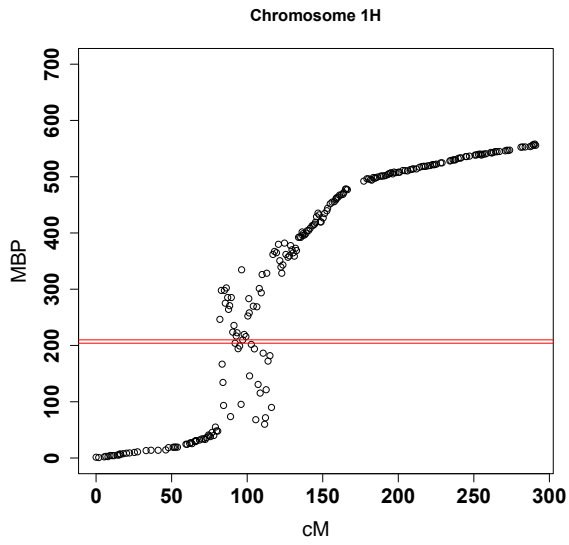
<sup>a</sup> Sequential number given to all lines in the Barley iCore Collection (BCC)

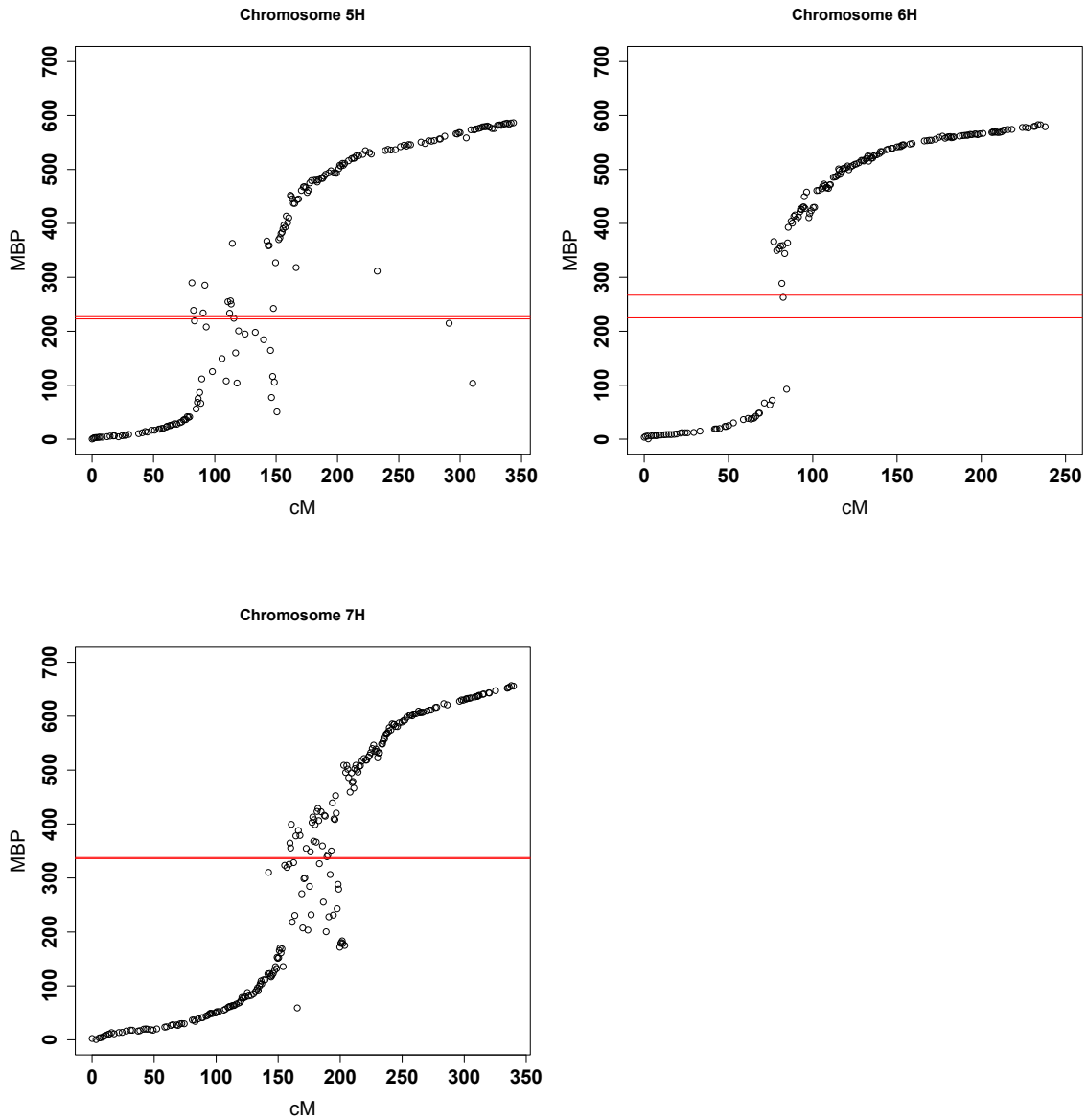
<sup>b</sup> Accession, Local Name, Aliases, Growth Habit as provided by the United States Department of Agriculture-Agricultural Research Service, Germplasm Resource Information Network.

<sup>c</sup> Spring growth habit if accession will grow reproductively in either St. Paul, MN or Njoro, Kenya

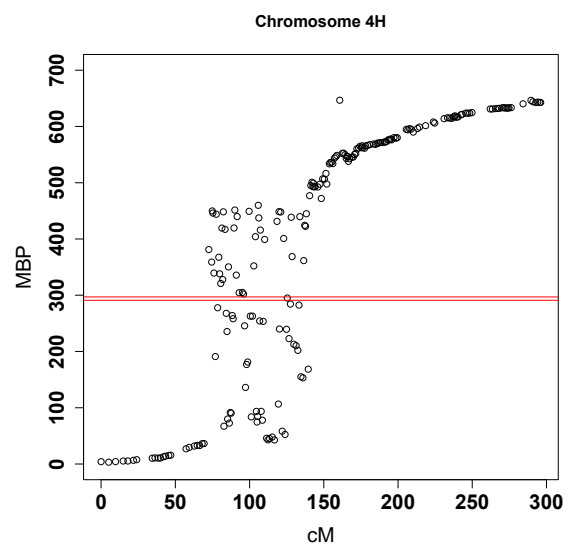
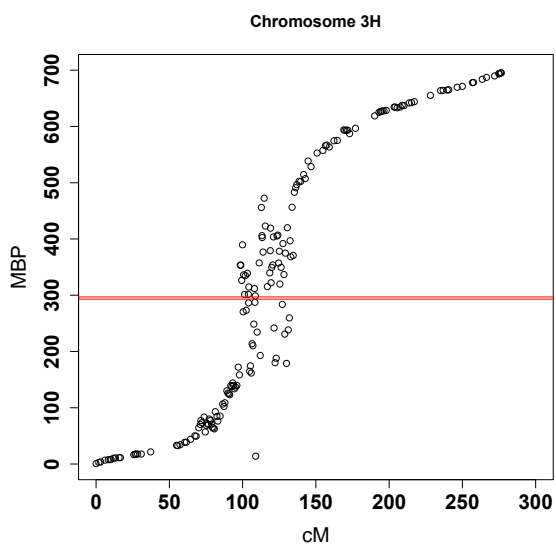
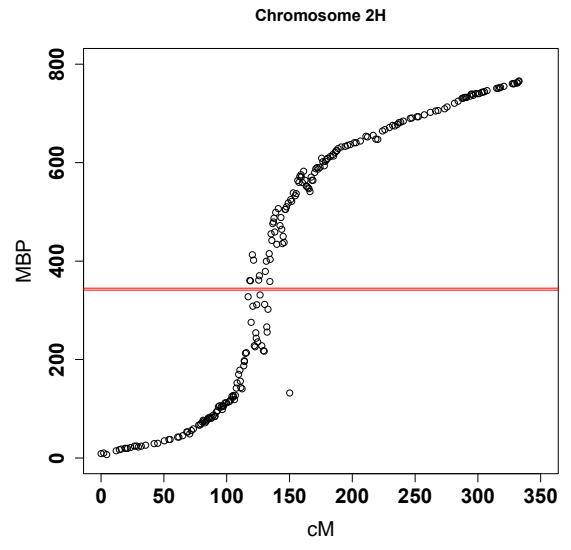
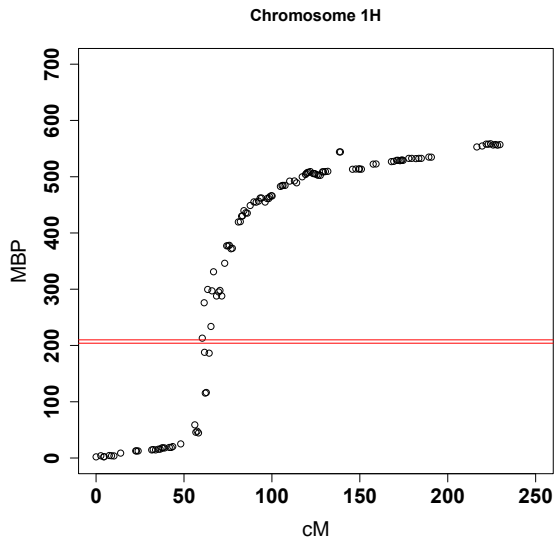
<sup>d</sup> Spike row number, where spike row “2”=two-rowed, “3”= two-rowed deficiens type, “5”= variable, “6”= six-rowed, as provided by the United States Department of Agriculture-Agricultural Research Service, Germplasm Resource Information Network.

<sup>e</sup> Sub-population as given by Muñoz-Amatriaín et al. (2014).

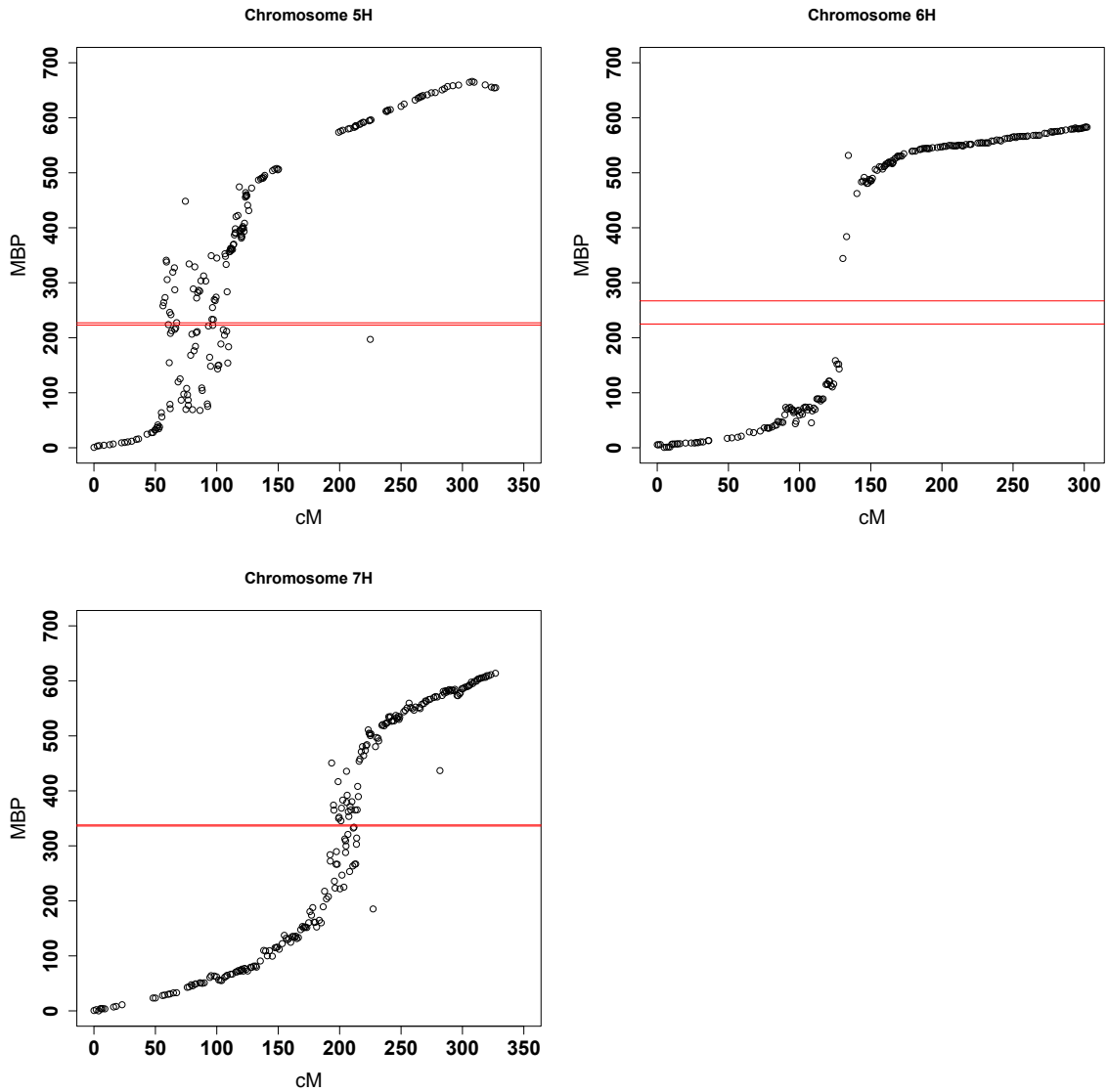




**Appendix A3.** Comparison of centiMorgan (cM) position to Mega Base Pair (MBP) position in the Hietpas-5/Hiproly population map. The cM position plotted vs MBP position in the reference genome. Red lines indicate estimated centromere MBP range position based on the Morex reference genome (Matthew Haas, personal communication).







**Appendix A4.** Comparison of centiMorgan (cM) position to Mega Base Pair (MBP) position in the PI 382313/Hiproly population map. The cM position plotted vs MBP position in the reference genome. Red lines indicate estimated centromere MBP range position based on the Morex reference genome (Matthew Haas, personal communication).

**Appendix A5.** Descriptions and names of datasets uploaded to the Triticeae Tool Box (T3) barley database (<https://triticeaetoolbox.org/barley/>).

| <b>T3 Data Set Name</b>  | <b>Description</b>  |
|--------------------------|---|
| SR_TTKSK_2015_ETH        | Adult plant stem rust severity data from Debre Zeit, Ethiopia 2015 main-season        |
| SR_TTKSK_2012_M_KEN      | Adult plant stem rust severity data from Njoro, Kenya 2012 main-season                |
| SR_TTKSK_2013_W_KEN      | Adult plant stem rust severity data from Njoro, Kenya 2013 main-season winter nursery |
| SR_TTKSK_2014_M_KEN      | Adult plant stem rust severity data from Njoro, Kenya 2014 main-season                |
| SR_TTKSK_2014_O_KEN      | Adult plant stem rust severity data from Njoro, Kenya 2014 off-season                 |
| SR_TTKSK_2014_W_KEN      | Adult plant stem rust severity data from Njoro, Kenya 2014 main-season winter nursery |
| SR_PTKST_2012_SA         | Adult plant stem rust severity data from Greytown, South Africa 2012 season           |
| SR_PTKST_2013_SA         | Adult plant stem rust severity data from Greytown, South Africa 2013 season           |
| SR_PTKST_2014_SA         | Adult plant stem rust severity data from Greytown, South Africa 2014 season           |
| SR_PTKST_2015_SA         | Adult plant stem rust severity data from Greytown, South Africa 2015 season           |
| SR_PTKST_2016_SA         | Adult plant stem rust severity data from Greytown, South Africa 2016 season           |
| SR_MCCFC_2013_StPaul     | Adult plant stem rust severity data from St. Paul, MN 2013 season, race MCCFC nursery |
| SR_QCCJB_2013_StPaul     | Adult plant stem rust severity data from St. Paul, MN 2013 season, race QCCJB nursery |
| SR_MCCFC_2014_StPaul     | Adult plant stem rust severity data from St. Paul, MN 2014 season, race MCCFC nursery |
| SR_QCCJB_2014_StPaul     | Adult plant stem rust severity data from St. Paul, MN 2014 season, race QCCJB nursery |
| SR_HKHJC_2015_StPaul     | Adult plant stem rust severity data from St. Paul, MN 2015 season, race HKHJC nursery |
| SR_MCCFC_2015_StPaul     | Adult plant stem rust severity data from St. Paul, MN 2015 season, race MCCFC nursery |
| SR_QCCJB_2015_StPaul     | Adult plant stem rust severity data from St. Paul, MN 2015 season, race QCCJB nursery |
| SR_MCCFC_2016_StPaul     | Adult plant stem rust severity data from St. Paul, MN 2016 season, race MCCFC nursery |
| SR_QCCJB_2016_StPaul     | Adult plant stem rust severity data from St. Paul, MN 2016 season, race QCCJB nursery |
| SR_BSR1_TTKSK_13/14_BSL3 | Seedling stem rust data from the BSL-3 2013/14 season, race TTKSK                     |
| SR_BSR2_TTKSK_13/14_BSL3 | Seedling stem rust data from the BSL-3 2013/14 season, race TTKSK                     |
| SR_BSR3_TTKSK_13/14_BSL3 | Seedling stem rust data from the BSL-3 2013/14 season, race TTKSK                     |

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| SR_TCAP_92MN90_16/17_GH    | Seedling stem rust data from the greenhouse 2016/17 season, isolate 92-MN-90 |
| SR_TCAP_HKHJC_15/16_GH     | Seedling stem rust data from the greenhouse 2015/16 season, race HKHJC       |
| SR_TCAP_HKHJC_16/17_GH     | Seedling stem rust data from the greenhouse 2016/17 season, race HKHJC       |
| SR_TCAP_MCCFC_15/16_GH     | Seedling stem rust data from the greenhouse 2015/16 season, race MCCFC       |
| SR_TCAP_MCCFC_16/17_GH     | Seedling stem rust data from the greenhouse 2016/17 season, race MCCFC       |
| SR_TCAP_QCCJB_15/16_GH     | Seedling stem rust data from the greenhouse 2015/16 season, race QCCJB       |
| SR_TCAP_QCCJB_16/17_GH     | Seedling stem rust data from the greenhouse 2016/17 season, race QCCJB       |
| SR_TCAP_TKTTF_14/15_BSL3   | Seedling stem rust data from the BSL-3 2014/15 season, race TKTTF            |
| SR_TCAP_TKTTF_15/16_BSL3   | Seedling stem rust data from the BSL-3 2015/16 season, race TKTTF            |
| SR_TCAP_TRTTF_14/15_BSL3   | Seedling stem rust data from the BSL-3 2014/15 season, race TRTTF            |
| SR_TCAP_TRTTF_15/16_BSL3   | Seedling stem rust data from the BSL-3 2015/16 season, race TRTTF            |
| SR_TCAP_TTKSK_13/14_BSL3   | Seedling stem rust data from the BSL-3 2013/14 season, race TTKSK            |
| SR_TCAP_TTKSK_14/15_BSL3   | Seedling stem rust data from the BSL-3 2014/15 season, race TTKSK            |
| SR_TCAP_TTKSK_15/16_BSL3   | Seedling stem rust data from the BSL-3 2015/16 season, race TTKSK            |
| SR_TCAP_TTKST_14/15_BSL3   | Seedling stem rust data from the BSL-3 2014/15 season, race TTKST            |
| SR_TCAP_TTKST_15/16_BSL3   | Seedling stem rust data from the BSL-3 2015/16 season, race TTKST            |
| LR_HHRIL_SAPh1601_16/17_GH | Adult plant leaf rust severity data from Greytown, South Africa 2016 season  |
| LR_SAPh1601_16_SA          | Seedling leaf rust data from the greenhouse 2016/17 season, isolate SAPh1601 |