Wheatgrass-wheat partial amphiploids as a novel source of stem rust and Fusarium head blight resistance

A THESIS
SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL
OF THE UNIVERSITY OF MINNESOTA
BY

Margaret Kathryn Turner

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
MASTER OF SCIENCE

James A. Anderson

February 2012

Acknowledgements

I would like to acknowledge Lee DeHaan and members of the Land Institute who inspired me and provided expertise to work on perennial crops. I would like to thank my advisor, Jim Anderson for his dedication and patience in helping me develop and write my thesis. I would also like to thank my committee members for their invaluable suggestions especially regarding the presentation of the data and development of the project. I would like to acknowledge my fellow graduate students for their support and Brian Seda particularly for his help and expertise with Fusarium head blight screening. I thank my family and friends for their constant support and encouragement and for being the kind of people who can do whatever is necessary. I thank my cat for his lap warming assistance for many hours. I thank my fiancé Paul who has taken responsibility to ensure that I take breaks to have fun and meet daily nutritional requirements throughout graduate school.

Abstract

Perennial wheatgrass (*Thinopyrum*) species are recognized sources of genetic variation for annual wheat improvement, as perennial forage crops with potential to be bred for grain production, and for utility in preventing soil and nutrient loss. Amphiploid lines made by crossing *Thinopyrum* species and *Triticum aestivum* (common wheat) can increase resilience of wheat to pathogens and abiotic stress and can improve the grain yield of the perennial crop. However, lack of pairing between chromosomes of *Thinopyrum* and *Triticum* species reduces genome stability, seed set, and perenniality. Fifty-three amphiploid wheat-wheatgrass lines from the perennials *Th. intermedium, Th. ponticum,* and *Th. junceum,* crossed with the annuals *T. aestivum, T. carthlicum, and T. turgidum,* were developed at the Land Institute in Salina, KS. Multiple plants of each line were evaluated for winter hardiness and perenniality, and screened for wheat stem rust (*Puccinia graminis*) and Fusarium head blight (FHB) (*Fusarium graminearum*) reaction. Two lines showed perenniality in Minnesota and may be valuable as cold-tolerant perennial wheat germplasm. Twenty-four of 48 amphiploid lines were resistant to all stem rust races screened, including TTKSK (syn. Ug99), TRTTF, and common US races. Of the 30 amphiploid lines point inoculated with *F. graminearum,* 21 were resistant based on the percentage of infected spikelets and the percent of visually scabby kernels. Three and four sources of potentially novel stem rust and FHB resistance, respectively were identified and may be useful for wheat improvement. Based on chromosome counts, seven lines representing two families showed genetic stability.

Table of Contents

List of Tables

List of Figures

Chapter 1. Literature Review

History of perennial wheat breeding

Annual crops account for the vast majority of the calories consumed in the world. However intensive annual production on erodible land depletes soil and nutrient resources (Glover, 2005). Perennials have many advantages over annual crops. Compared to annuals, perennial species retain higher soil fertility (Culman et al., 2010), prevent loss of nitrogen and phosphorus through surface runoff (Turner and Rabailas, 2003), protect against soil erosion, and they are more resilient to pathogens and abiotic stresses (Glover, 2005). Additional benefits that have yet to be quantified in comparison to an annual system include reducing fuel and labor inputs.

Of potential perennial crops, wheat is one of the most promising based on grain yield potential. There have been two strategies employed to develop a perennial crop resembling common bread wheat. The first involves amphiploidizing *Triticum* and *Thinopyrum* species. Perennial wheat development has employed multiple perennial wheatgrass (*Thinopyrum*) species including *Th. intermedium* (Host) Barkworth & D. R. Dewey (Intermediate wheatgrass) (2n=6x=42; JJJ^sJ^sSS (Chen et al., 1998)), *Th. elongatum* (Host) D. R. Dewey (2n=2x=14; EE), *Th. ponticum* (Podp.) Z.W. Liu & R.C. Wang (syn. *Agropyron elongatum* (Host) Beauv) Á. Löve (2n=10x=70; $JJJJJJJ^sJ^sJ^sJ^s$(Chen et al., 1998)), *Th. junceum* (L.) Löve (2n=4x=28; $JJSS=E^eE^eE^bE^b$ (Nieto-Lopez et al., 2003)), and *Th. junceiforme* (A. Löve & D. Löve) A. Löve (2n=6x=42; $JJJSS= E^eE^eE^eE^eE^bE^b$). The other approach involves improving *Th.*

intermedium per se for increased grain yield among other traits (Cox et al., 2002). Despite substantial progress to increase seed size and grain yield using both approaches, the resulting perennial crop does not achieve wheat grain yields (Murphy et al., 2009; Cox et al., 2010). As perennial breeders work to improve yields and other desirable traits, preliminary germplasm can be evaluated for novel disease resistance to improve wheat.

Early perennial wheat development

The first perennial wheat breeding program began in the former U.S.S.R. in the early 20th century with the goal to develop a new crop with dual grain and forage use (Tsitsin, 1965). Tsitsin and Lubimova (1959) crossed cultivated crops and wild perennial grass species, finding the most agronomic potential in *T. aestivum, T. durum, Th. intermedium,* and *Th. elongatum.* The researchers developed two new crops designated "Triticum agropyrotriticum perenne (Cicin)" (referenced as perennial wheat) and "Triticum-agropyrotriticum submittans," a forage crop that continued to tiller after seed production (Tsitsin and Lubimova, 1959). The perennial wheat lines regrew shoots, and were grown as a grain crop in Ukraine, Kazakhstan, and the southern regions of Russia and as a hay crop in the agricultural regions further north. These lines were highly variable, but some had favorable agronomic qualities. The best lines had large seed sizes with thousand kernel weights (TKW) up to 40g and good milling and baking properties. A particular perennial wheat variety, M2, produced grain for 2-3 years, had a TKW of 33g, and was cytologically stable with 56 chromosomes. Comparing the amphiploid M2 with its perennial and annual parents, only the annual parent succumbed to lodging and

disease pressure. The M2 amphiploid was intermediate in terms of many traits including number of stems, spacing of spikelets on the spike, duration of perenniality, form of pollination, and protein content (Tsitsin and Lubimova, 1959). The M2 amphiploid showed higher number of seed and spikelets per spike than either parent (Tsitsin and Lubimova, 1959). These researchers also noted top downward maturation in the perennial and the M2 amphiploid, as opposed to bottom up maturation of the annual parent. This observation could be useful in early evaluation of perenniality. Ultimately, the perennial germplasm developed in this program was considered unsuccessful due to decrease in grain yield after the establishment year resulting in its primary utility as forage (Cox et al., 2002).

In the United States, hundreds of perennial wheat lines involving *Triticum* and *Thinopyrum* species were developed from 1932 to 1935 by Dr. W. J. Sando, a United States Department of Agriculture breeder in Beltsville, MD (Vinall and Hein, 1937, p. 1059 in (Scheinost et al., 2001)). From this program, one small-seeded perennial wheat variety 'Montana-2' with good survival was released (Schultz-Schaeffer and Haller, 1987). Sando's germplasm would also be evaluated in later breeding programs in Kansas and at Washington State University (Schmidt et al., 1953).

Dr. Coit Suneson with the United States Department of Agriculture, in Davis, CA worked with perennial wheat from the 1940s through the 1960s. Five wheat varieties and two wheatgrass varieties (*Th. elongatum* and *Th. intermedium* ssp. *trichophorum* (Link) A. & Gr.) were crossed and improved using pedigree selection, bulk populations, and a backcrossing bulk selection scheme (Suneson and Pope, 1946; Van Deynze, accessed

2011). Suneson and Pope (1946) selected for plant vigor, large seed size, longevity, stem rust (caused by *Puccinia graminis* Pers.:Pers. f. sp. *tritici* Eriks. & E. Henn.) resistance, and leaf rust (caused by *Puccinia recondita* f. sp. *tritici*) resistance, ease of threshing, and non-fragile rachis. Undesirable traits including poor straw strength and off-type seeds were culled. Lines from this program yielded 60% as much as the best wheat cultivars, with 2-3 years of regrowth. Suneson noted that even after 6 cycles of selfing, all lines were still segregating for various agronomic traits. He also identified many lines highly resistant or immune to stem rust and leaf rust (Suneson and Pope, 1946). The project was eventually discontinued because yields were not competitive with wheat (Haag, 2011) and perennial persistence was inconsistent (Cox et al., 2002). While the program did not release a commercial variety, germplasm has been utilized as a wildlife food (Haag, 2011; Van Deynze, accessed 2011).

Intermediate Wheatgrass

In 1983, the Rodale Institute Research Center in Kutztown, PA began a program to identify the most promising species for development of a perennial grain crop. Large non-shattering seed and strong straw enabling mechanical harvest and threshing, as well as good grain flavor characteristics made *Th. intermedium* the most likely candidate (Wagoner, 1990). Using recurrent phenotypic selection, first year yields of 560 kg ha^{-1} were achieved but declined in subsequent years (Wagoner, 1994). Some of these improved *Th. intermedium* lines have been used by The Land Institute in Salina, KS in perennial wheat development.

Recent perennial wheat development

In 1989 the Washington State University (WSU) wheat program began introducing resistance to *Cephalosporium* stripe to winter wheat from *Th. elongatum*. When crosses produced progeny with regrowth after the sexual cycle, researchers recognized the opportunity to develop a separate perennial wheat breeding program to address erosion issues in the dry, windy region. Soil losses of 18.1 Mg acre^{-1} year^{-1} are not uncommon in eastern Washington where wheat crops are grown on 5˚ - 30˚ slopes (Haag, 2011).

Winter wheats and *Thinopyrum* sp. crosses were made from 1991 to 1997 at WSU. Performance of resulting lines was evaluated in the greenhouse for regrowth and tested at three field locations (Scheinost et al., 2001). Six weeks after harvest, the majority showed weak regrowth, however 152 of 524 showed vigorous regrowth, and a few showed no regrowth (Scheinost et al., 2001). The highest first year grain yields of 3082 kg ha^{-1} were approximately 65% as much as 'Madsen' (Allan et al, 1989), a popular common wheat in the region (Scheinost et al., 2001). The majority of lines were tall (97-145 cm), late senescing compared to wheat, resistant to shattering, free-threshing, and analogous to wheat in spike morphology, as opposed to the characteristic long rachis of wheatgrass species (Scheinost et al., 2001). Scheinost also noted that advanced lines were still segregating for head morphology, indicating additional potential for selection. Lines with vigorous regrowth survived subsequent winters for three years, although winters were more mild than normal (Scheinost et al., 2001). In a presentation at the Kellogg

Biological Station Perennial Grains Meeting in 2011, summarizing the progress of the WSU perennial wheat program, Dr. Kevin Murphy reported that many of the head rows survived 4-6 years, although some regrowth was potentially attributable to seed drop.

Perenniality is a very complex trait, with a variety of characteristics from prolonged growth (stay green) to continued tillering and seed production. However, research from WSU showed that post-sexual-cycle regrowth is simply inherited. Chinese spring addition and substitution lines with chromosomes from *Th. elongatum* were evaluated for post-sexual-cycle regrowth, which was defined as new growth from the crown exceeding 3 cm in length and tiller production after senescence (Lammer et al., 2004). Substitution lines with chromosome 4E, but not a substitution line with the long arm of 4E, showed post-sexual cycle regrowth and produced seed. However based on research in other cereal crops, it is unlikely that perenniality is controlled by a single locus. In sorghum, many unassociated quantitative trait loci are involved in rhizomatousness, tillering, and overwintering (Paterson et al., 1995). In rice, nine rhizome traits were controlled by 16 quantitative trait loci on 8 different chromosomes (Hu et al., 2003). In Lammer's study, full amphiploid lines demonstrated strong perenniality over multiple years, but regrowth in the 4E addition lines was variable and only observed in the first year (Lammer et al., 2004). While the 4E locus is sufficient for regrowth, other loci are necessary to contribute to more vigorous perenniality. Additional fine mapping could identify the region on 4ES contributing to post-sexual-cycle regrowth.

At WSU, researchers also have analyzed the grain quality of perennial wheat. Compared to bread wheat, perennial wheat has increased whole wheat flour protein and mineral nutrient concentration, but smaller seed size, loaf volume, lower test weight, lower flour yield, lower mixing time, and poorer threshability (Murphy et al., 2009). Variation in seed size and threshability indicated potential for improvement.

In the late 1970's, Dr. Wes Jackson founded The Land Institute in Salina, KS to address sustainable agricultural practices. Perennial crop development evolved as a primary focus. Strong breeding programs began in the mid 1990s when full-time plant breeders were hired to advance perennial sorghum, wheat, sunflower, and Illinois bundleflower germplasm. Breeders at The Land Institute pursued two approaches to develop a perennial crop resembling wheat. Similar to programs of Tstitsin, Suneson, and WSU, they developed perennial wheat through wide hybridization between annual *Triticum* species and perennial species. Because perennial wheat development had been limited by genome instability, sterility, and low yields, they also pursued *Th. intermedium* improvement based on the research of the Rodale Institute. While there has been significant progress in *Th. intermedium* improvement described by Cox et al. (2010), this review will focus on the wide hybridization approach.

Perennial wheat lines previously developed by other breeding programs showed no survival by September in KS, motivating breeders to develop lines more adapted to the hot weather (Cox et al., 2006). The perennial wheat breeding program began in 2002 and has involved hundreds of amphiploid lines (Cox et al., 2010). Many of the interspecific amphiploid lines had large seed size, high fertility, and regrew post-sexual-

cycle in the greenhouse, but very few survived field conditions (Cox et al., 2010). Some were crossed back to *Thinopyrum* species to improve perenniality, but many lines were male sterile (Cox et al., 2010). The few fertile lines were used to pollinate the male sterile lines, generating approximately 1,100 progeny (Cox et al., 2010). These progeny were generally low in fertility, demonstrated regrowth capacity in greenhouse trials, and had larger seed size than *Thinopyrum* parents (Cox et al., 2010).

Cytogenetic techniques have been employed to study the number of chromosomes involved in perenniality and the frequency of translocations. While successful crosses can be made with both hexaploid and tetraploid wheat, it is very difficult to produce full amphiploids with high ploidy levels (Cox et al., 2010). Stable wheat-wheatgrass partial amphiploids are expected to have 56 or fewer chromosomes (Dvorak, 1976). Chromosome numbers exceed 42 due to unreduced gametes and vary between lines due to chromosome loss over generations (Banks et al., 1993). Additionally, spontaneous chromosome doubling is common when generating hybrids between *T. carthlicum* and other grass species (Anamthawat-Jonsson et al., 1997). Stable lines produced by crosses between tetraploid *Thinopyrum* and *Triticum* spp. (2n=28) generally have 28 *Thinopyrum* and 28 *Triticum* chromosomes, and stable progeny of crosses between hexaploids *Thinopyrum* and *Triticum* sp. (2n=42) generally have 14 *Thinopyrum* and 42 *Triticum* chromosomes (Cox et al., 2002). In more complex crosses, the chromosome derivation tends to correspond to the most recently crossed parent, regardless of whether it was *Triticum* or *Thinopyrum* (unpublished data from Cindy Cox). Additionally, lines tend to become more stable with each generation of selfing; by the F_{5-6} generation lines are often

stable (Wang, S., personal communication 2011). Very few translocations between *Thinopyrum* and wheat chromosomes have been observed at The Land Institute or at Washington State, indicating that breeders cannot rely on recombination to reduce the size of deleterious segments from wheatgrass (Cai et al., 2001; Cox et al., 2010).

A growing number of research groups are studying agronomic practices, ecological effects, economics, and feasibility of perennial wheat. Dr. Sigmund Snapp at Michigan State's Kellogg Biological Research Station in Hickory Corners, MI is developing best management practices for perennial wheat and comparing the effects of perennial and annual wheats in organic and conventional systems on soil properties. Preliminary results indicate less nitrate leaching and overall water loss at deep soil horizons with perennial wheat, compared to annual wheat (Culman, S., personal communication 2011).

Researchers at Australia's Commonwealth Scientific and Industrial Research Organization in Wagga Wagga, New South Wales developed a feasibility study for perennial wheat in dry regions of the country. Models of perennial wheat in dryland sustainable agriculture systems show economic viability, assuming the current mixed cropping system and biomass forage yields of 800 $kgha^{-1}$ and grain yields 40% of conventional wheat (Bell et al., 2008; Braidotti, 2011). In field trials, perennial wheat lines developed by breeding programs in Russia, Washington State University, and The Land Institute have shown some success. Sixty percent of entries regrew after seed production and some lines have survived three growing seasons (Hayes, R., personal communication 2011).

While maintaining perenniality in high yielding germplasm remains a significant challenge, there are recent developments that may accelerate progress. Breeders are changing their approaches to develop locally adapted lines and new lines demonstrate perenniality in multiple locations. In addition to selection of the highest performing cultivated wheat lines, preliminary screening of wheatgrass accessions for local adaptation is becoming a new focus of perennial wheat breeding programs. Additionally, new technologies in genetics and plant breeding provide resources to understand the mechanisms of perenniality. Molecular markers, genetic mapping, and sequencing methodologies will promote more rapid crop development. Recent efforts at Washington State University by Dr. Stephen Jones and at The Land Institute by Drs. Lee Dehaan and Shuwen Wang have focused on the development of new lines and utilization of technology not available previously. Dr. Wang is currently working to identify genetically stable perennial wheat lines and is developing a marker system to efficiently identify each chromosome from *Th. intermedium*.

Significance of stem rust

Stem rust is caused by the pathogen *Puccinia graminis* Pers.:Pers. f. sp. *tritici* Eriks. & E. Henn.,which infects the leaves and stems of 365 species including wheat and barley (Leonard and Szabo, 2005). This heteroecious fungus reproduces asexually on grasses, completing the sexual portion of its life cycle on its alternate host barberry (*Berberis* or *Mahonia* spp.) (Leonard and Szabo, 2005). Severe infection of the stem limits the flow of nutrients to developing seed, resulting in small, shriveled seed. Infection also may weaken the stem and increase lodging, further reducing yield.

Historically, the highest yield losses in the U.S. exceeded 50% in MN and ND in 1935 (Leonard, 2001).

In the southern United States, rust overwinters on susceptible winter wheat, producing inoculum for the following summer. As wheat matures, northerly wind currents carry *Puccinia* spores from south to north, along the *Puccinia* pathway. Sexual reproduction on the alternate host, barberry, increases the genetic variation within the pathogen population (Craigie, 1927). Severe epidemics occurred in the 1930s due to the new race MCCF (race 56) and in the 1950s due to race TPMK (race 15) (Stakman and Rodenhiser, 1958). Since the late 1950s, major genes for seedling resistance have been introgressed into wheat (Stokstad, 2007). By growing resistant winter wheat varieties and eradicating the alternate host, the population size in North America has remained small since the 1950s (Kolmer et al., 2009). And with few exceptions stem rust has been controlled globally in the last 30 years with genetic resistance (Singh et al., 2011).

However in 1999, a new race of stem rust (Ug99) appeared in Uganda, exhibiting virulence to most resistance genes, including *Sr31* (Singh et al., 2006); this race was later designated as race TTKSK (Jin et al., 2008). The pathogen has continued to evolve new virulence to additional genes and quickly spread through much of Africa and into the Middle East (Singh et al., 2011). Since 1999, new variants of TTKS have been identified with additional virulence to *Sr24* (Jin et al., 2008) and *Sr36* (Singh et al., 2011). An estimated 80-90% of wheat cultivars around the world are susceptible to Ug99 or its variants (Stokstad, 2007). Continued spread of TTKS and other races require focused efforts to find novel resistance effective against the new races of stem rust.

Migration of rust races has been well documented and modeled. Spread of TTKS throughout western Africa was detected in Kenya in 2001, Ethiopia in 2003, Sudan and Yemen in 2006, Iran in 2007, and Tanzania in 2009 (Singh et al., 2011). Spread is projected using models based on atmospheric conditions and historic spread of other diseases. The most likely projected path for spread follows the spread of *Yr9 Puccinia striiformis* f. sp. *tritici* Westend. virulence which arose in Eastern Africa and migrated north to the Arabian peninsula in the 1990s (Singh et al., 2006).

Current strategies to mitigate the Ug99 threat include finding and introgressing resistance genes. Single, race-specific seedling genes can provide complete resistance and can be easily introgressed through backcrossing, but can also be defeated as races evolve new virulence. The preferred strategy involves combining multiple additive genes that are non-race specific and may confer adult plant resistance (Singh et al., 2006). These non-race specific genes could be pyramided together with race-specific genes or used in gene complexes to produce high levels of resistance that would be more durable. Pyramiding genes in a single cultivar requires the pathogen to evolve virulence to all genes simultaneously to infect the host. Evolving virulence becomes increasingly less probable in complexes with 2 to 3 or more effective genes (Schafer and Roelfs, 1985). Ultimately, the goal for controlling the pathogen is to keep population size small which will limit genetic diversity.

Stem rust resistance genes are named with permanent number designations in the order that they are identified, and given temporary designations until novel resistance can be confirmed. Of more 62 named and temporarily designated genes, a large number (30)

are from *T. aestivum* and the remaining genes are from related species (Singh et al., 2011). *Thinopyrum* species have contributed important stem rust resistance genes to wheat improvement. *Thinopyrum intermedium* is the source of stem rust resistance gene *Sr44* (Friebe, 1996) and *Th. ponticum* is the source of *Sr24* and *Sr25* (McIntosh et al., 1977), *Sr26* (Knott, 1961), and *Sr43* (Kibirige-Sebunya and Knott, 1983; Friebe, 1996) (Table 1).

Gene *Sr24* was transferred spontaneously along with *Lr24* from *Agropyron elongatum* (Host) Beauv. [*Thinopyrum ponticum* (Podp.) Barkw. & D.R. Dewey]) to wheat on a translocation between the long arm of 3Ae#1 and the long arm of chromosome 3D in wheat (Friebe, 1996). Additional translocations were induced using homeologous recombination and radiation treatment resulting in translocations in 3B, 3D, and the 1BS satellite (Friebe, 1996). Gene *Sr25* was transferred from the long arm of group 7 *Th. ponticum* from the cultivar 'Agatha' along with *Lr19* to the long arm of 7D in wheat with radiation (Friebe, 1996). Subsequent radiation treatment and homeologous recombination were used to eliminate negative flour color characteristics of the initial translocation (Friebe, 1996). Gene *Sr26* was transferred from the long arm of group 6 in *Th. ponticum* to the long arm of 6A in wheat using radiation. Gene *Sr43* was transferred from group 7 in *Th. elongatum* to the 7D in wheat with homoeologous recombination (Friebe, 1996). Gene *Sr44* was transferred to wheat by McIntosh with homoeologous recombination from the short arm of 7Ai#1 from the translocation line TAF2 (Friebe, 1996).

Resistance genes from wheatgrass have utility in protecting wheat crops against new races of stem rust, although some have now been defeated or have deleterious effects. Gene *Sr26* is effective against all known races of stem rust, including variants of TTKS and is currently being employed in breeding programs. Although used in cultivars, this translocation causes reduction in yield (Friebe, 1996). Recently, race TTKST was identified with virulence to *Sr24*, but had been previously used widely and is present in approximately 10% of stem rust resistant wheat grown globally (Singh et al., 2011). The *Sr24* gene still has utility in North America and Australia (Friebe, 1996). Virulence to *Sr25* was detected in India in 2009 (Jain et al., 2009). Gene *Sr43* is linked to yellow flour color and distorted inheritance making it undesirable for breeding efforts (Friebe, 1996). Neither gene *Sr43* nor *Sr44* are useful for wheat improvement as this time due to linkage with deleterious traits (Singh et al., 2011).

Significance of Fusarium head blight

In 1993, Fusarium head blight (FHB) decimated hard red wheat production in the Northern Great Plains resulting in a yield loss of 2.604 million tons (Nganje et al., 2004). The United States Department of Agriculture ranks FHB as the "worst plant disease of wheat and barley" since the stem rust epidemics in the 1950s (Wood et al., 1999). Currently there are no highly resistant FHB varieties of wheat and fungicide application is only partially effective.

Fusarium head blight is a fungal disease caused by the pathogen *Fusarium graminearum* Schwab (teleomorph: *Gibberella zeae*). The fungal perithcium which arise

from the hyphae overwinter on infected plant debris, mature and produce ascospores. These sticky airborne ascospores land on the flowering spikes of cereal plants, grow through xylem and pith, and induce necrosis (Trail, 2009). Additionally, asexual spores called conidia are produced throughout the season during moist periods. After initial onset of infection, the fungus produces mycotoxins including deoxynivalenol (DON), enabling the fungus to spread into the rachis, preventing the flow of nutrients into the top portion of the spike, and causing seed abortion. Seeds with accumulated DON are small, shriveled, and greatly reduced in economic value. If levels of DON are high, the grain cannot be consumed by humans or sold as livestock feed. In the upper Midwestern U.S., DON limits for human consumption are frequently exceeded (Trail, 2009).

Mesterhazy (1995) characterized five types of physiological resistance to FHB. Type I provides resistance from initial infection of a spike; Type II limits the spread of infection from the initial inoculated point to other spikelets on the spike; Type III reduces kernel abortion and infection; Type IV limits yield loss; and Type V promotes the decomposition or non-accumulation of mycotoxins. Spread of infection is most commonly used to evaluate disease presence in wheat for identifying potentially novel sources of resistance because it is easiest to measure accurately. After a single spikelet is inoculated, the spread of infection is assessed as bleached or grayed spikelets or a count of threshed visibly scabby kernels (VSK).

Although there are multiple species of *Fusarium* capable of inducing FHB, no host specificity has been observed. The main species in the U.S., *F. grainearum,* is most commonly used in screening for resistance. Frequently, multiple isolates of *F.*

grainearum are used in screening, but any “reasonably aggressive” strain should be appropriate for screening purposes (van Eeuwijk et al., 1995).

Resistance for wheat improvement has primarily been identified in *T. aestivum.* Of 52 mapping studies, 46 identified QTL in hexaploids wheat (Buerstmayr et al., 2009). The FHB resistance gene *Fhb1* on chromosome 3BS in *T. aestivum* explaining 25-30% of the phenotypic variation has been important to wheat improvement through the use of marker-assisted selection (Anderson, 2007).

Recently wheatgrass has been recognized as a potential source of resistance to FHB (Table 1). A segment of *Th. ponticum* 7el translocated to 7B in wheat showed a greater effect than the *Fhb1* gene on chromosome 3BS in an experiment measuring Type II resistance (Shen and Ohm, 2006). In 2007, the location of the 7el resistance was further mapped using substitution lines and associated with molecular markers to facilitate the development of smaller introgressions for wheat improvement (Shen and Ohm, 2007). Additionally, Oliver et al. (2005) evaluated 293 wheat-alien species derivatives for FHB resistance and identified 74 resistant derivatives with an average 2.5 spikelets or fewer infected 21 days after infection (dai), including amphiploid lines from *Th. intermedium, Th. ponticum,* and *Th. junceum*. Han et al. (2003) assessed six wheat x *Thinopyrum intermedium* ‘Zhong 5’ amphiploid lines for Type II resistance for FHB and identified resistance in a 2D substitution line.

.

Chapter 2. Resistance in wheat-wheatgrass partial amphiploids

Introduction

Perennial wheatgrass (*Thinopyrum* species) is a recognized source of genetic variation to improve annual wheat germplasm and as a potential perennial grain crop. Crossing *Thinopyrum* species and *Triticum* species (wheat) can improve both species. In wheat, two of the most destructive diseases currently threatening production are stem rust and Fusarium head blight (FHB), caused by the fungal pathogens *Puccinia graminis* Pers.:Pers. f. sp. *tritici* Eriks. & E. Henn and *Fusarium graminearum* Schwab [teleomorph *Gibberella zeae* (Schw.) Petch], respectively. After epidemics in the early 20th century, stem rust had declined globally to insignificant levels by the 1990s with the introgression of *Sr24, Sr26, Sr31,* and *Sr38* (Singh et al., 2006). However in 1998, an aggressive new race (Ug99) of stem rust appeared in Uganda, evolving virulence to most resistance genes and spreading throughout much of the wheat growing areas of Africa and into the Middle East (Singh et al., 2006).

Fusarium head blight (FHB) has been a very destructive disease. In 1993, FHB decimated hard red wheat production in the Northern Great Plains resulting in a yield loss of 2.604 million tons (Nganje et al., 2004). Currently, there are no wheat cultivars highly resistant to FHB and fungicides are only partially effective. Thus, finding novel sources of resistance to rapidly evolving races of stem rust and more effective resistance to FHB is increasingly important.

Resistance to many diseases of wheat has been identified in diverse *Thinopyrum* species, and several wheatgrass genes have been used to improve wheat. *Thinopyrum intermedium* is the source of stem rust resistance gene *Sr44* (Friebe et al., 1996).

Thinopyrum ponticum is the source of *Sr24* and *Sr25* (McIntosh et al., 1977), *Sr26* (Knott, 1961), *Sr43* (Friebe et al., 1996; Kibirige Sebunya and Knott, 1983), and leaf rust (*Puccinia recondita* f. sp. *tritici*) resistance genes *Lr24* (Li et al., 2003). *Thinopyrum elongatum* is the source of *Lr19* (Zhang et al., 2005). Resistance has also been identified in *Th. junceum* to powdery mildew caused by *Blumeria graminis* f. sp. *tritici* (Sepsi et al., 2008) and in *Th. intermedium to* eyespot caused by [*Tapesia yallundae* (Wallwork & Spooner) and *T. acuformis* (Boerma, Pieters & Hamers) Crous (anamorph *Pseudocercosporella herpotrichoides* (Fron.) Deighton)] (Cox et al., 2005), and barley yellow dwarf virus (Brettel et al., 1988; Banks et al., 1993). Recent studies showed high levels of resistance to FHB (Oliver et al., 2005) and stem rust (Xu et al., 2009) in several wheatgrass species. Further evaluation of *Thinopyrum* species as donors of disease resistance may provide additional novel genes for wheat improvement.

In addition to wheat improvement, there is a growing interest in developing perennial wheat as a grain crop. Compared to annuals, perennial species retain higher soil fertility (Culman et al., 2010), prevent loss of nitrogen and phosphorus through surface runoff (Turner and Rabalais, 2003), protect against soil erosion, and are more resilient to pathogens and abiotic stresses (Glover, 2005). Additional unevaluated potential benefits include lower labor, fuel, and pesticide inputs. Yet significant challenges to maintain fertility, yearly seed production, and high yields have limited progress (Suneson and Pope, 1946; Tsitsin and Lubimova, 1959; Scheinost et al., 2001; Cox et al., 2010) since the first program began in Russia in the 1920s (Scheinost et al., 2001). Many of these

issues can be attributed to genetic instability. Lack of pairing between perennial and annual species chromosomes limits genome stability and seed set (Cox et al. 2002).

While these challenges remain significant, newly developed lines from The Land Institute in Salina, KS and Washington State University produce up to 60% of annual wheat yield and show perenniality in multiple locations (Scheinost et al., 2001; S. Culman and R. Hayes, personal communications 2011). Molecular markers, genetic mapping, and sequencing methodologies are being used to understand the mechanisms of perenniality and to improve perennial wheat germplasm more rapidly. Cytological characterization has shown that genetically stable lines demonstrating perenniality have between 42 or 56 chromosomes (Tsitsin and Lubimova, 1959; Scheinost et al., 2001; R. Hayes, personal communication 2011). Already, one measure of perenniality, post-sexual-cycle regrowth, has been mapped to the short arm of chromosome 4E in *Th. elongatum* (Lammer et al., 2004). The 4E chromosome was sufficient for promoting post-sexual-cycle regrowth in Chinese Spring wheat (Lammer et al., 2004).

Based on disease threats to wheat production and recent work with perennial wheat, this study investigated the potential utility of new wheat-wheatgrass partial amphiploids developed at The Land Institute for disease resistance and as a perennial crop in Minnesota. The objective was to address the following questions:

1. Do any wheat-wheatgrass partial amphiploids show perenniality and winter hardiness in Minnesota?
2. Do wheatgrass-wheat lines possess resistance to Fusarium head blight or stem rust?

3. Is stem rust resistance identified in these materials novel, and therefore not conferred by previously identified stem rust resistance genes from *Thinopyrum* or *Triticum*?

Materials and Methods

Plant materials

Fifty-three F_2 to F_7 families from 17 amphiploid wheat-wheatgrass lines were developed at The Land Institute in Salina, KS by Dr. Lee DeHaan in 2001 (Table 2). Lines were created by crossing perennial wheatgrass species from the *Thinopyrum* genus [*Th. Intermedium* (Host) Barkworth & D. R. Dewey (Intermediate wheatgrass) (2n=6x=42; JJJ^sJ^sSS (Chen et al., 2001)), *Th. ponticum* (Podp.) Z.W. Liu & R.C. Wang (syn. *Agropyron elongatum* (Host) Beauv) Á. Löve (2n=10x=70; $JJJJJJJ^sJ^sJ^sJ^s$ (Chen et al., 1998)), *Th. junceum* (L.) Löve (2n=4x=28; $JJSS=E^eE^eE^bE^b$ (Nieto-Lopez et al., 2003)), and *Th. pycnanthum* (Godr.) Barkworth (2n = 6x = 42; $SSP^sP^sE^sE^s$ (Refoufi et al., 2001))] by annual wheat [*T. aestivum* L. (2n=6x=42; AABBDD), *T. carthlicum* Nevski (2n=4x=28; AABB), *T. durum* L. (2n=4x=28; AABB), and Triticale (2n=8x=42; AABBDDRR)]. The Land Institute lines provided by Dr. Lee DeHaan were initially developed for perenniality and high yield, with complex pedigrees involving one to two perennial wheatgrass species and one to four annual wheat lines using embryo rescue. The lines have been self pollinated producing seed ranging from F_2 to F_7 generations. Lines were advanced in the greenhouse and heads were not bagged, however the possibility of cross pollination is unlikely in the greenhouse due to limited pollen flow

and wide variation in pedigrees and chromosome constitution of the lines. Any seed production would therefore be very unlikely without embryo rescue.

Parental lines used to develop the partial amphiploids were obtained through the National Plant Germplasm System from the Small Grains Collection in Aberdeen, ID and Western Region Plant Introduction Station in Pullman, WA. Additional lines were provided by Dr. Carl Griffey at Virginia Tech in Blacksburg, VA, Dr. Paul Murphy at North Carolina State University in Raleigh, NC, Ehmke Seed Co. in Healy, KS, and the Wheat Genomic Resource Center in Manhattan, KS. Big Flats Plant Materials Center (BFC) lines developed at the Rodale Institute and WD48 (winter durum from the Nebraska breeding program) were not accessible.

Field evaluation

On October 5, 2009, 52 amphiploid families were hill planted with five seeds per hill on 0.5m centers in two adjacent randomized replications on the St. Paul campus of the University of Minnesota in St. Paul, MN. Winter survival was assessed on April 7, 2010. Height was recorded on August 5, 2010, when plants were harvested and characterized by head counts, seed counts, and seed weight. Regrowth during spring 2011 was assessed April 12, 2011. Surviving plants were transplanted to a different field on campus April 17, 2011, measured for height and harvested again on September 11, 2011. Measurements of disease incidence were recorded monthly throughout the growing season in 2010 and 2011.

Stem rust screening

Stem rust screenings were similar to previously described screenings of wheatgrass species by Xu et al. (2009) and were based on methods described by Jin et al. (2007). Briefly, five seeds per genotype for each race were planted in the USDA-ARS Cereal Disease Laboratory greenhouses in St. Paul, MN. Seedlings were inoculated 10 d after planting, incubated for 16 h in a dark dew chamber at 18˚C, and scored 14 d after inoculation using a modified rating score of 0 - 4 (Stakman, 1962; Roelfs, 1988). Ratings of 0, ;, 1, 2, or any combination with these ratings, were considered resistant (Supplementary Figure 1). Ratings of 3 or 4 were considered susceptible. An 'X' rating describes a resistant response with a mixture of infection types.

Amphiploid lines were represented by five to ten seeds for each race screened. Parental lines were represented in total by 15 to 20 seeds for each race. Susceptible checks LMPG-6 and McNair were planted as controls in each screening. Sixteen of the 52 lines were preliminarily screened with U.S. races MCCFC, TPMKC, TTTTF, QTHJC, and RKQQC in October 2009 and with African race TTKSK in January 2010. Stem rust races TTTTF, TPMKC, TTKSK (Ug99), and TRTTF (Yemen) were selected due to high virulence and TPMKC and MCCFC races were selected due to historic prevalence in the U.S. (Kolmer et al., 2009). Forty-two amphiploid lines with adequate seed supply and 31 parental lines were screened with U.S. races MCCFC, TPMKC, TTTTF, QTHJC, and RKQQC in October 2010. The following year, 50 amphiploid lines and 33 parental lines were screened with TTTTF in January 2011 and TTKSK in February 2011. In May of 2011, 10 seeds from all parental lines were planted and re-screened with the five U.S.

races to confirm earlier readings. Eight of these lines were planted two days early to compensate for slow germination.

Fusarium head blight screening and seed production

All lines were planted in the greenhouse in November 2009 to assess seed production. Lines producing more than five seeds per plant on average were identified for further evaluation for Fusarium head blight resistance. Eight additional lines that had produced more than 100s seed per plant in past greenhouse trials in Kansas were also included.

Thirty amphiploid lines, 29 parental lines, resistant checks 'Alsen' (Frohberg et al., 2006) and 'BacUp' (Busch et al., 1998), and susceptible checks 'Roblin' (Campbell and Czarnecki, 1987), 'Wheaton' (Busch et al., 1984), and MN00269 were screened with a single isolate, Fg4, of *F. graminearum*. Plants were initially vernalized for seven weeks at 4.4˚C and moved to the greenhouse. The greenhouse was maintained at 20˚C with 16 h of light daily. In greenhouse trials during fall 2010 and spring 2011, five pots with four plants per pot were planted in complete blocks over two planting dates, one week apart. On average, 21 total heads per amphiploid line were inoculated over the two greenhouse seasons. Individual spikes were inoculated using the point inoculation technique described by Liu et al. (2006). Inoculations were made by injecting 10 µl of inoculum at a concentration of 100,000 spores/ml into the central spikelet of one spike per plant at anthesis. The number of infected spikelets was recorded 21 days after inoculation (dai) to measure spread of infection. Visually scabby kernel (VSK) counts were used as another

measure of disease severity. Since some lines were segregating for sterility, an additional non-inoculated spike from each inoculated plant was used to verify seed production. Tests of significance were conducted by ANOVA. The LSD test (α=0.05) was used for mean separation.

Marker screening for known stem rust resistance genes

Partial amphiploids and parents used in crosses were screened with molecular markers to determine whether stem rust resistance in the amphiploid lines was different from previously identified resistance genes from *Thinopyrum* species. Each line was represented by three bulk samples from four individuals. Bulk samples were harvested from the first replication of the fall 2010 FHB screening, the second replication of the 2010 fall FHB screening, and the January 2011 stem rust screenings. Young leaf tissue was harvested for DNA extraction with methods described by Riede and Anderson (1996) for samples harvested from the first replication of the fall 2010 FHB screening greenhouse and the January 2011 stem rust screenings. Samples harvested from the second replication of the fall 2010 FHB screening greenhouse were extracted using a Biosprint 96 DNA Plant Kit 571 (QIAGEN Inc., Valencia, CA) following the manufacturer instructions. Polymerase chain reaction (PCR) amplified DNA fragments linked with known wheatgrass resistance genes *Sr24, Sr25,* or *Sr26*. The *Sr24* and *Sr26* genes were detected with markers *Sr24#12* and *Sr26#43*, using PCR conditions described by Mago et al. (2005). The *Sr25* gene was detected using the diagnostic marker BF145935 with PCR conditions described by Liu et al. (2010).

Chromosome counts

Twenty-four partial amphiploids demonstrating perenniality, potentially novel stem rust resistance, or with *Thinopyrum* accessions showing previously uncharacterized FHB resistance were characterized genetically with chromosome counts. Seeds were germinated and root tips harvested after three to five days. Root tips were treated with N_2O to arrest meristematic cells in metaphase, based on modified methods described by Kato (1999). Root tips were placed inside a closed chamber with 155 psi NO_2 in moist Petri dishes for 2 h. Treated root tips were then fixed with 3:1 ethanol: acetic acid at 4˚C for a minimum of 24 h, stained with acetocarmine for a minimum of 72 h, and stored in 70% ethanol at 4˚C. Root tip squashes were viewed with a microscope.

Results and Discussion

Agronomic characterization

A total of 58 of the 106 perennial wheat replicated hill plots survived through their first winter from October 2009 to April 2010. Sixteen of the 52 amphiploid lines did not survive in 2010 in either replication (Supplemental Table 1). Nine of the 16 amphiploid lines not surviving the first winter contained spring *T. aestivum, T. carthlicum,* or Triticale parents, indicating that part of the observed winter kill could be attributed to lack of cold tolerance. Variability among amphiploid lines was apparent with height ranging from 31 to 135 cm and number of heads from 0 to 86 per hill plot. Thousand kernel weights (TKW) ranged from 3 to 40 g, with a mean of 21 g. The higher

TKW of the amphiploid lines were comparable to TKW of contemporary winter wheat cultivars grown in an adjacent field, ranging from 25-37g (data not shown).

Of the 52 amphiploid lines planted in 2009, two partial amphiploids B1016(8) and B1146(5) (Figure 1), demonstrated perenniality in St. Paul, MN as of November 2011 (Supplementary Table 1). Line B1016(8) was agronomically poor, with large variation in height among hill plots (78 and 110 cm). The line was largely infertile, averaging 34 heads with only two seeds per plot in 2010. Line B1146(5) was comparatively high yielding with an average of 65 heads and 403 seeds per plot in 2010. Neither line produced any seed in 2011 although B1016(8) produced 5 heads and B1146(5) produced 4 heads. The production of seed only in the first year, and none in the field in the second year or the greenhouse seed production trials indicate that these lines may be self incompatible. Parents of these lines *Th. intermedium* and *Th. ponticum* are self incompatible (Wang et al., 2003). Wind pollination in the 2010 field, with plants in 58 surviving hill plots, would have been more likely than in the 2011 field season, with only two hill plots surviving. Additionally lack of seed production could be attributed to linkage between sterility and perenniality, unbalanced gametes in meiosis, or environmental conditions.

Stem rust resistance in *Thinopyrum* sp.

All 13 parental *Thinopyrum* accessions were highly resistant to all races of stem rust screened, with infection types of 0 or ; (Figure 2, 3). Resistance to stem rust was segregating (ranging from 0; to 3) in the majority (11 of 13) of wheatgrass lines

(Supplemental Table 2). The difference in infection types within accessions indicates multiple resistance genes could be involved. Genes *Sr43* and *Sr44* may contribute resistance in *Thinopyrum* lines, but markers to screen for these genes have not been developed. However, infection types ;2- for *Sr43* (Jin et al., 2007; Xu et al., 2009) and ;2 for *Sr44* (Xu et al., 2009) alone cannot explain lower infection types of the wheatgrass parents (Supplemental Table 2). Xu (2009) identified *Th. junceum* lines AJAP7 and AJAP8 with infection types ranging from 2 to 2++ for races TTTTF and TTKSK. The *Th. junceum* accession PI414667 tested in this study had a lower infection type (0; to 3) and was negative for all markers (Supplementary Table 2) suggesting these lines represent a different source of resistance.

Stem rust resistance in *Triticum* sp.

Five of 20 parental annual wheats and Triticales were resistant to all stem rust races screened (Figure 2). These included wheat cultivars 'TAM 110' (Lazar et al., 1997), KS95WGRC33, and 'McCormick' (Griffey et al. 2005) and annual Triticale lines NE95T441 and 'NET422' (Baenziger and Vogel, 2003). The Chinese winter wheat accession PI531193 was resistant to all races screened except TTKSK (Supplemental Table 2). Pavon spring wheats PI519847 and PI520054, soft red winter wheat 'NC-Neuse' (Murphy et al., 2004), *T. carthlicum* PI573182, Presto and PI386154 Triticales, and PI634318 durum exhibited resistance to some races (Supplemental Table 2).

Only three of 23 annual wheat and Triticale parents tested positive for *Sr24* or *Sr25* markers (Supplemental Table 2). McCormick was positive for the *Sr24* marker and

Triticale lines NE95T441 and NE422T that were previously characterized as having moderate resistance (Baenziger and Vogel, 2003) were positive for the *Sr25* marker. Known stem rust resistance genes in McCormick include *Sr24* and *Sr1RS^{Amigo}* (Griffey et al., 2005). The gene *Sr25* has an expected infection type of 2 or 2+ when inoculated with TTKS (Jin et al., 2007). The low infection types in NE95T441 (;1 on TTKSK) and NE422T (; on TTKSK) cannot be accounted for by the presence of *Sr25* alone (Supplemental Table 2). Therefore the marker for gene *Sr25* in these two lines is likely a false positive as the lines in the pedigree of NE422T have not been associated with *Sr25*. It is possible that the presence of an additional unknown gene producing an infection type lower than that produced by *Sr25*.

Gene *Sr36* has been identified previously in NC-Neuse (Murphy et al., 2004). The resistance in TAM 110 is likely contributed by an undesignated gene on 1RS (Jin and Singh, 2006). The marker data for *Sr24* (Supplementary Table 2) agree with reported susceptibility to an isolate avirulent on *Lr24* (Jin and Singh, 2006) suggesting TAM 110 does not have the *Sr24* gene. No information on stem rust resistance for Triticale PI386154 was found in the literature, indicating the resistance may be uncharacterized and potentially novel.

Marker association with stem rust genes in *Thinopyrum* and amphiploid lines

While the markers for genes *Sr24*, *Sr25*, and *Sr26* are closely associated with resistance in wheat, their mapped genetic distance from the resistance gene and diagnostic value in *Thinopyrum* species are not known. Because there is little homology

(Chen et al., 2001) and limited recombination (Cox et al., 2010) between genomes from *Triticum* and *Thinopyrum*, markers could be diagnostic in wheat without tight linkage to the gene of interest. Gene *Sr24* provides resistance to all races used in this screening, but amphiploid family B373 showing susceptibility to races TTKSK, QTHJC, MCCFC, RKQQC, and TPMKC and line B1152(1) showing susceptibility to race MCCFC (Figure 4) showed positive marker results for *Sr24* (Supplemental Table 3). This result indicated that the *Sr24* marker may not be tightly linked to this gene or the presence of false positives. Lines with the *Sr25* markers had lower infection types than would be expected with only the presence of the genes *Sr25.*Therefore it was not possible to determine from this data whether the *Sr25* marker is diagnostic in *Thinopyrum* species.

Stem rust resistance in partial amphiploids

Many of the amphiploid families had high levels of resistance to stem rust. Thirteen of the 17 different pedigree families were resistant to TTKSK with highly resistant ratings (0,;) segregating in eight of these resistant families (Supplemental Table 3). Nine of the 17 families were resistant to all races screened (Figure 2). Of the 48 lines, 37 were resistant to TTKSK, and 14 were highly resistant with ratings of ; or 0 (Figure 5). All but two lines showed resistance to at least one race (Supplemental Table 3). *Thinopyrum intermedium* and *Th. junceum* were most prevalent *Thinopyrum* species in the amphiploids and showed the widest range in infection types (Figure 5).

While almost all amphiploid lines showed resistance to stem rust, some of the resistance may be contributed by annual wheats or genes *Sr24, Sr25*, and *Sr26* from

wheatgrass. Three amphiploid lines may be sources of novel resistance (Table 3). In the B1107 family involving *T. durum, T. aestivum* ('Jagger' (Sears et al., 1997a) or '2137' (Sears et al., 1997b)), and *Th. junceum*, novel resistance likely came from *Th. junceum.* The *T. durum* accession PI634318, Jagger, and 2137 are not as resistant to TTKSK, TPMKC, or TTTTF and molecular makers do not detect known stem rust resistance genes. Additionally, resistance to local races of stem rust in B1089 (ranging 0 to ;1- for races QTHJC, TPMKC, and TTTTF) resembled resistance of *Th. intermedium* PI314190. These ratings were lower than those of the annual wheat parents Thunderbolt and PI573182, which were susceptible to these races. The resistance in these B1089 lines was therefore likely derived from the *Th. intermedium* PI314190 or the *Th. intermedium* BFC line, which was not available for this study. Assuming marker results for gene *Sr24* accurately predict the presence of the gene, there were likely more genes contributing to resistance other than *Sr24,* which has an expected infection type of 2 or 2- on TTKSK (Jin et al., 2007). Finally, B1016(8) demonstrated a third source of potentially novel resistance either from *Th. ponticum* PI 508561 or PI578681 or Triticale NE422T or NE95T441, as Presto did not have resistance to TTKSK. Positive marker results for all three genes *Sr24, Sr25,* and *Sr26* from *Th. ponticum* accessions indicate probable contribution of some resistance to B1016(8). However, the *Sr24, Sr25,* and *Sr26* genes alone would not account for low infection types (0 and ;) in the B1016(8) family, indicating the presence of additional genes.

Fusarium head blight resistance in *Thinopyrum* sp.

Differences were observed among lines for number of infected spikelets, percentage of infected spikelets, and percentage VSK ($P < 0.001$). Of the 10 *Thinopyrum* accessions screened, nine were resistant based on the number and percentage of spikelets infected (Figure 2). Because outcrossing *Thinopyrum* accessions produced no seed in the greenhouse, the percentage of visually scabby kernels could not be assessed. All *Th. intermedium* and *Th. junceum* lines were resistant to FHB with percentage of infected spikelets ranging from 10 to 31% (Figure 6, Supplemental Table 2).

Fusarium head blight resistance has been mapped in *Th. ponticum* to chromosome 7E (Shen and Ohm, 2007) and identified in *Th. intermedium* and *Th. junceum* (Oliver et al., 2005). Amphiploid lines selected for FHB screening did not contain *Th. ponticum* parents, thus *Th. ponticum* lines were not screened. *Thinopyrum* accessions in this study differed from the ones screened by Oliver et al. (2005). Because FHB resistance is highly quantitative and controlled by many genes, resistance in *Thinopyrum* sp. is likely due to multiple genes.

***Fusarium* head blight resistance in *Triticum* sp.**

Six of 19 *Triticum* lines screened were resistant based on all three measures of disease severity (Figure 2). Within each *Triticum* species, there was a range in percentage of infected spikelets from less than 20% infected in some accessions to greater than 50% infected in other accessions, with the exception of *T. durum* which was only represented by one accession (Figure 6). Lines considered resistant based on number of spikelets

infected, percentage of infected spikelets, and percentage of VSK included ‘Karl 92’ (Sears et al., 1997c), NC-Neuse, Pavon spring wheat PI519847, *T. carthlicum* lines PI532505 and PI573182, and Triticale Presto (Supplemental Table 2). Though not statistically different from resistant checks, the percentage of infected spikelets was high in Karl 92 (.40) and NC-Neuse (.44) (Supplemental Table 2). Results were consistent with other reports of moderate FHB resistance in Karl 92 (Sears et al., 1997) and NC-Neuse (Murphy et al., 2004), and FHB resistance in Presto (Arseniuk et al., 1999), PI532505, PI532506, and PI573182 (Oliver et al., 2008). Resistance to FHB in Pavon F76 spring wheat PI519847 was not found in the literature.

Fusarium head blight resistance in partial amphiploids

Of the 30 amphiploid lines inoculated with *F. graminearum,* 21 were resistant, three intermediate, and six susceptible (Supplemental Table 3). These 30 lines represented 11 families, 7 of which were resistant to all measures (Figure 2). Lines within a family were similar in resistance or susceptibility (Supplemental Table 3). All three measures of FHB severity (number of spikelets infected, percentage of infected spikelets, and percentage of VSK) were generally consistent across amphiploid lines. Resistance based on percentage of spikelets infected was reported because it was the most discriminatory among amphiploid lines. The level and range of resistance conferred by different *Thinopyrum* species was similar among amphiploid lines (Figure 7).

Three *Thinopyrum* accessions showed previously uncharacterized resistance (Table 4, Supplemental Table 3). Resistance in the families B875 (17-48% infected),

B913 (16-43%), and B1107 (14-28%) was probably at least partially derived from PI414667 (31%). Other parents of these crosses: *T. carthlicum* Do1 (100% infected), *T. durum* PI634318 (54% infected), 2137 (88%), and Jagger (64%) were susceptible (Table 4). Additionally, *Th. intermedium* PI401201 likely contributed the majority of the resistance in amphiploid families B373 and B1146. PI410201 had higher resistance (30% infected spikelets) than the susceptible wheat lines in the pedigree, 2137 (88%), Jagger (64%), and TAM110 (60%). Remaining amphiploid lines were analyzed in the same way, finding resistance potentially contributed by *Th. intermedium* PI401129 in B938. While *T. aestivum* PI520054 showed low % infection (23%), it could not explain the low %VSK observed in the amphiploid line. McCormick winter wheat is also present in the background of B938, but was not screened in this study. McCormick is moderately resistant to FHB (Griffey et al., 2005), but alone probably could not account for the highly resistant lines in the B938 family.

Wheat is most susceptible to FHB at anthesis (Osborne and Stein, 2007). Thus, infertility could prevent spread of infection in an otherwise susceptible line. Sterility was assessed by counting the number of seeds in non-inoculated spikes of inoculated plants. No amphiploid lines were completely sterile, but eight of 30 had low seed production (on average less than 5 seed per spike) (data not shown). Low fertility, however, did not prevent spread of infection. Susceptible lines with low fertility included *T. carthlicum* Do1 (one seed per spike on average) and winter wheat PI531193 (four seeds per spike on average).

Genomic stability

Genetically characterized partial amphiploid lines ranged in chromosome number from 40 to 60 with the majority of families differing in chromosome number between lines and individuals (Figure 7, Supplementary Table 1). Early generation families were expected to have higher variation in chromosome number, but this was not observed in the F_2 family B1146 (Figure 7) although sample size may have been limiting with only two individuals counted (Supplemental Table 1). Chromosome counts from F_3 and F_4 generation families varied between individuals, but the later generation F_7 B373 family were more stable (Figure 7, Supplementary Table 1). Lines from the B373 family had 56 chromosomes (Figure 8) (7 individuals) and 54 (1 individual) (Supplemental Table 1). This family has been independently characterized as stable, in addition to other families with 2n=42, 56, 44, 52, or 54 chromosomes at The Land Institute (Wang, S., personal communication 2011). The B913 family had 42 chromosomes (Figure 8) based on six individuals counted from two lines (Supplemental Table 1). Fifteen of 24 lines were considered unstable based on either varying chromosome numbers among individuals or chromosome counts differing by more than two from the previously characterized stable counts of 42 or 56 (Supplemental Table 1). Unstable lines could be self-pollinated and selected to achieve stability for use as chromosome addition lines. Genome instability has been attributed to non-homology with a low pairing frequency of 4.6% between *Triticum* and *Thinopyrum* genomes (Chen et al., 2001).

Conclusions

Two lines showed perenniality in Minnesota. Seven lines representing two families showed potential genetic stability. Forty-six of the 48 amphiploid lines screened with stem rust were resistant to at least one race. Twenty-one of the 30 amphiploid lines screened with *F. graminearum* were resistant. The two amphiploid lines exhibiting stem rust resistance and perenniality may be targeted for future perennial wheat breeding efforts. This study identified three sources of potentially novel stem rust resistance and three previously uncharacterized accessions with FHB resistance. To utilize this resistance in wheat improvement, isolation of small introgression segments containing resistance genes would be necessary. A strategy similar to the one described by Niu et al. (2011) could be employed by backcrossing alien lines to wheat lines containing the *ph1* mutant to induce non-homologous recombination.

Table 1. Thinopyrum sources of resistance to stem rust and Fusarium head blight

Donor species	Disease resistance	Gene	Locus identified	Donor line	Size of alien translocation	Reference
Th. intermedium	Stem rust	*Sr44*	7Ai#1	TAF2		Friebe et al. 1996
	Fusarium head blight		2Ai	Zhong 5		Han et al. 2003
Th. junceum	Stem rust		Not designated			Xu et al. 2009
	Fusarium head blight		Not designated			Oliver et al. 2005
Th. ponticum	Stem rust	*Sr24*	7Ae#1	Agent	1.26 µm	Friebe et al. 1996
		Sr25	7Ae#1L	Agatha	2.55 µm	Friebe et al. 1996
		Sr26	3Ae#1L	K2046	2.48 µm	Friebe et al. 1996
		Sr43	7Ae#2			Friebe et al. 1996
	Fusarium head blight		7Ae			Shen 2006

Table 2. Pedigrees, generation, and number of lines in partial amphiploid families

Family	Generation	Pedigree[†]	No. lines
B307	F4	Tam110/**PI314190**//WGRC33	4
B373	F7	Tam110/**PI401201**//Jagger or 2137	5
B875	F4	Do1/**PI414667**	5
B913	F4	PI634318/**PI414667**	3
B938	F3	PI520172/**PI261099**//PI520054/3/McCormick/4/**PI401129**	10
B1016	F2	NE422T/**PI578681**//Presto/3/NE95T441/4/**PI508561**	1
B1037	F3	PI386154/**BFC2**//PI386154/3/**PI380639**	2
B1085	F3	PI573182/**PI314190**//McCormick/3/**PI314189**	3
B1089	F3	Thunderbolt/PI573182//**PI314190**/3/**BFC1**	2
B1094	F3	WD48/**PI414667**	1
B1100	F3	Neuse/**BFC1**	1
B1107	F3	PI634318/**PI414667**//Jagger or 2137	4
B1126	F2	Tam110/**PI401201**//Jagger or 2137/3/PI520054/4/ **PI401168**/5/(Tam110/**PI401201**//Jagger or 2137)	1
B1129	F2	PI532505/**BFC1**//Jagger/3/PI531193/4/**IWG120**/5/(WD46/**BFC2**//**PI314189**)	1
B1139	F2	PI532506/**BFC2-19**//Karl92/3/PavonF76/4/WGRC33/5/ **PI401176**/6/(Tam110/**PI401201**//Jagger or 2137)	1
B1146	F2	Tam110/**PI401201**//Jagger or 2137/3/Jagger	2
B1152	F2	PI573182/**BFC2-4**//**BFC2-N**/3/**PI440048**/4/(Tam110/**PI401201**//Jagger or 2137)	2

[†] *Thinopyrum* species are indicated by bold text in the pedigree

Table 3. Novel resistance to stem rust in wheat-wheatgrass partial amphiploids

		Stem rust infection types for races							Stem rust markers[‡]		
Line[†]	**Description**	**TRTTF**	**TTKSK**	**QTHJC**	**MCCFC**	**RKQQC**	**TPMKC**	**TTTTF**	***Sr24***	***Sr25***	***Sr26***
B1107(2)-14	**amphiploid**	**;1/;2-**	**;1**	**1/;**	**;1-**	**;1**	**0**	**1**	**-**	**-**	**-**
2137	*T. aestivum*	4	3	3/4;	4	3	2+	3	-	-	-
Jagger	*T. aestivum*	3-	3+	;3+	2/3+	;3+	4	;3	-	-	-
PI634318	*T. durum*	;2,2+	3+/;3+	;1-	;1-/;1	1-	3/4	3+	-	-	-
PI414667	*Th. junceum*		0;/ ;2	;1-/1/3	0/1/3	0/;/3	0/;1/2/3	;1-/3	-	-	-
B1089(11)-4	**amphiploid**	**;3**	**;**	**;**	**0**	**0**	**0**	**;1-**	**+**	**-**	**-**
Thunderbolt	*T. aestivum*	;1,3	0/ 0,3	3+	3	3	3	3	-	-	-
PI573182	*T. carthlicum*	3	---	3+	1+	;2-	2/3	;3	-	-	-
PI314190	*Th. intermedium*	;/ 2/ 3	0;/ ;2-	;/;1	;/1	;/;1	;/;1/3	;/2	+	-	-
B1016(8)	**amphiploid**	**0**	**;**	**---**	**---**	**---**	**---**	**---**	**+**	**+**	**+**
PI573182	*T. carthlicum*	3	---	3+	1+	;2-	2/3	;3	-	-	-
NE95T441	Triticale	;1-	;1	;	;	;	;	0/;1	-	+	-
NET422	Triticale	;	;	;	;	;	;	0/1-	-	+	-
Presto	Triticale	;1,3	3	;1-	1/2-/;3	;	3+	;1	-	-	-
PI508561	*Th. ponticum*	0	0;	0/;	0/;	0/;	0/;/1	;	+	-	+
PI578681	*Th. ponticum*	0	0	0/;	0/;	0/;/1	0/1/3	0/;	+	+	+

[†] Amphiploid lines are designated in bold, followed by *Triticum* and *Thinopyrum* parents used to generate the amphiploid.

[‡] PCR based marker results for stem rust resistance genes *Sr24, Sr 25, and Sr26*. "+" indicates the same band as the positive check

Table 4. Previously uncharacterized resistance to Fusarium head blight in wheat-wheatgrass partial amphiploid accessions

Line[†]	Description	FHB ratings[‡]			
		No. infected	% infected	% VSK	FHB Rating
B875-12-1-13	**414667IP/1oD**	**1.3**	**17**	**17**	**R**
B913(3)-12-7	**PI634318/PI414667**	**1.8**	**16**	**4**	**R**
B1107(2)-14	**PI634318/PI414667// Jagger or 2137**	**1.3**	**14**	**1**	**R**
PI414667	*Th. junceum*	1.4	31		R
Do1	*T. carthlicum*	9.5	100	100	S
PI634318 (Afuwan)	*T. durum*	5.5	54	44	I
2137	*T. aestivum*	8.8	88	91	S
Jagger	*T. aestivum*	5.3	64	56	S
B373-4-30-3-6-1-1	Tam110/**PI401201**//Jagger or 2137	**2.1**	**27**	**14**	**R**
B1146(5)	Tam110/**PI401201**//Jagger or 2137/3/Jagger	**5.1**	**50**	**1**	**R**
PI401201	*Th. intermedium*	2.1	30		R
2137	*T. aestivum*	8.8	88	91	S
Jagger	*T. aestivum*	5.3	64	56	S
TAM 110	*T. aestivum*	3.9	60	60	S
B938(15)-12	PI520172/**PI261099**//PI520054/3/ McCormick/4/**PI401129**	**1.6**	**18**	**10**	**R**
PI261099	*Th. intermedium* x *Th. pycnanthum*	3	60		S
PI401129	*Th. intermedium*	1.5	19		R
PI520054	*T. aestivum*	3	23	74	S
PI520172	*T. aestivum*	5.5	58	45	S

[†] Amphiploid lines are designated in bold, followed by *Triticum* and *Thinopyrum* parents used to generate the amphiploid.

[‡] Mean disease severity ratings of single heads point inoculated with *Fusarium graminerum*. Number infected describes the number of spikelets bleached, 21 dai. Percent infected describes the percentage of spikelets bleached, 21 dai. Percent VSK describes the percentage of visually infected kernals from the inoculated head. FHB rating characterizes resistant lines (R) not statistically different from resistant checks for any of the three disease severity measures (Number infected, percent infected, and percent VSK); susceptible lines (S) not statistically different from susceptible checks; and intermediate lines (I) with statistically higher disease severity than resistant checks, but lower severity than susceptible checks, or inconsistent between measures of severity.

Supplemental Table 1. 2010 and 2011 field data and chromosome counts for 52 wheat-wheatgrass partial amphiploids

Hybrid line	2010 survival ¶	No. heads†		Seed no. †		Seed wt.(g) †		TKW (g) †‡		Height (cm) †		2011 survival ¶	Chromosome count	No. counted§
		avg	st dev	avg	st dev	avg	st dev	avg	st dev	avg	st dev			
B307-13-23-3	2	31	26	13	4	0.4	0.1	33	1	66	16	0		
B307-55-10-1	0													
B307-67-7-1	2	30	21	245	221	5.5	5.5	21	4	88	19	0		
B307-67-7-5	2	10	11	56	76	1.3	1.9	17	10	67	50	0		
B373-4-30-3-6-1-1	2	23	9	158	6	4.5	0.1	29	0	77	8	0	56	2
B373-4-30-3-6-3-4	2	43	16	923	107	29.7	0.4	32	4	82	4	0	56	1
B373-4-30-3-6-2-11	2	31	5	337	86	10.4	2.9	30	1	83	4	0	54, 56	2
B373-4-30-3-6-3-2	2	40	16	635	559	19.9	22	26	11	78	4	0	56	1
B373-4-30-3-6-3-5	2	32	14	630	107	19.2	3.9	30	1	77	4	0	56	2
B875-12-12-12	0												50, 44	2
B875-12-1-27	0												46	1
B875-12-1-13	1	8		56		0.6		11		50		0	50	1
B875-12-1-9	0												44	1
B875-12-1-29	Not planted												40, 46, 48	3
B913(3)-14-5	1	18		190		2.8		15		71		0	42	3
B913(3)-12-7	1	30		740		14.8		20		85		0	42	3
B913(3)-6-8	0													
B938(13)-18	1	34		1272		22.7		18		61		0		
B938(15)-12	1	8		200		4.5		22		57		0	44, 48	2
B938(16)-8	0												46	1
B938(17)-14	1	16		405		9.2		23		69		0	48, 50, 52	3
B938(23)-8	0												44, 46.5, 48	3
B938(24)-7	2	6	1	12	1	0.2	0	16	1	52	5	0		

Continued on next page

Supplemental Table 1. Continued

Hybrid line	2010 survival [¶]	No. heads [†]		Seed no. [†]		Seed wt.(g) [†]		TKW (g) [†‡]		Height (cm) [†]		2011 survival [¶]	Chromosome count	No. counted [§]
		avg	st dev	avg	st dev	avg	st dev	avg	st dev	avg	st dev			
B938(24)-8	1	4		3		0.1		17		31		0	44, 46	2
B938(24)-9	1	13		17		0.4				52		0	46	1
B947(5)-9	0													
B947(5)-10	0													
B1016(8)	2	34	32	2	3	0		16		94	23	1	~60	1
B1037(1)-9	2	86	10	1808	70	40.5	1.7	22	0	125	0	0		
B1037(1)-19	2	77	4	1974	713	51.6	18.6	26	0	135	4	0		
B1085(3)-17	2	30	7	845	223	14.3	4.7	17	1	100	3	0		
B1085(3)-18	2	21	18	596	495	12	9.7	20	1	100	15	0		
B1085(3)-20	2	36	1	1043	2	20.4	3.2	20	3	108	2	0		
B1089(5)-3	1	0										0	44	1
B1089(11)-4	1	0										0	60	2
B1100(1)-19	1	14		0						65		0		
B1094(1)-14	2	28	5	1003	78	39.8	4.4	40	1	77	4	0		
B1094(1)-22	2	8	2	287	283	10	9.2	37	4	71	4	0		
B1094(1)-25	0													
B1107(2)-3	0												42, 43	2
B1107(2)-14	0												43, 44	3
B1107(4)-4	0													
B1107(4)-29	1											0	41	1
B1121(2)	1	4		30		0		3		44		0		
B1126(3)	2	6	4	6	4	0.4	0.1	15	0	69	4	0		

Continued on next page

Supplemental Table 1. Continued

Hybrid line	2010 survival [¶]	No. heads [†]		Seed no. [†]		Seed wt.(g) [†]		TKW (g) [†‡]		Height (cm) [†]		2011 survival [¶]	Chromosome count	No. counted [§]
		avg	st dev	avg	st dev	avg	st dev	avg	st dev	avg	st dev			
B1129(1)	2	28	6	5	4	0.1	0.1	20	0	67	1	0		
B1139(3)	1			31		0.5		15		62		0		
B1139(8)	2	17	0	19	17	0.3	0.2	12	4	86	13	0		
B1146(1)	2	53	9	341	210	6.9	3.9	21	1	97	6	1		
B1146(5)	2	65	16	403	539	8	11	16	6	80	24	0	~42	2
B1152(1)	2	23		156		1.6		10						
B1152(2)	0													
B1179(33)	0													

[†] No. heads, no. seed, estimated thousand kernel weight (TKW), and height averaged over two hill plots

[‡] Estimated thousand kernel weight (g) was calculated by dividing weight of kernels from single plot by number of seed from plot and multiplying by 1000

[§] No. counted is the number of individuals from each line counted; individuals were characterized by a minimum of 3 cells with consistent chromosome counts with the exception of line B1016(8) which was only characterized by one cell

[¶] Survival is the number of hillplots (maximum of 2) with surviving plants on April 7, 2010 and April 12, 2011

Supplemental Table 2. Stem rust infection types, stem rust DNA marker profile, and FHB ratings of parental perennial wheatgrass species used to generate wheat-wheatgrass partial amphiploids, and checks

		Stem rust infection types for races [†]							Stem rust markers [‡]			FHB ratings [§]			
Line	**Description**	**TRTTF**	**TTKSK**	**QTHJC**	**MCCFC**	**RKQQC**	**TPMKC**	**TTTTF**	***Sr24***	***Sr25***	***Sr26***	**No. infected**	**% infected**	**% VSK**	**FHB Rating**
PI261099	*Th. intermedium* x *Th. pycnanthum*	0	0;	0/;	0/;	0/;	0/;	0/;	+	+	+	3.0	60		S
PI314189	*Th. intermedium*	0	;1	0/;/1+	0/;	;	0/;/1,3	0/;/1	+	-	-	1.5	27		R
PI314190	*Th. intermedium*	;/ 2/ 3	0;/ ;2-	;/;1	;/1	;/;1	;/;1/3	;/2	+	-	-	---	---		---
PI380639	*Th. intermedium*	0		0/;1/1/2	;/;1/1/3	0/;/1	;/1	0/;/;1/1	+	-	-	1.0	18		R
PI401129	*Th. intermedium*	;/ 2+	;1/ 0	0/;/;1/2-/3	0/;/1-/1	;/;1/2/3	;/;1/2-	;/2	+	-	-	1.5	19		R
PI401201	*Th. intermedium*	0/3	0;	0/;1-/2	;/;1-	0/;/1	;/1/2-	;/1/1+/3	+	-	-	2.1	30		R
PI383564	*Th. intermedium*	0	0;	;/1/3	;/;1/3	;/;1-	1/3	;/;1/1+	+	-	-	1.2	11		R
PI401168	*Th. intermedium*	;	0;	;/1	0/;1-	;/;1/3	;1	;/1+	+	-	-	1.0	14		R
PI401176	*Th. intermedium*	;1-	;1-	;/;1-/1+	0/;/;1-	0/;/3	;/1+	;/;1/2	+	-	-	1.0	10		R
PI440048	*Th. intermedium*	;1-/ 2+	0;	0/;/;1/1+/3	0/;/;1/1-/3	0/;/1	;/;1/1/;2	;/1	+	-	-	1.1	21		R
PI414667	*Th. junceum*	escape	0;/ ;2	;1-/1/3	0/1/3	0/;/3	0/;/;1/2/3	;1-/3	-	-	-	1.4	31		R
PI508561	*Th. ponticum*	0	0;	0/;	0/;	0/;	0/;/1	;	+	-	+	---	---		---
PI578681	*Th. ponticum*	0	0	0/;	0/;	0/;/1	0/1/3	0/;	+	+	+	---	---		---
2137	*T. aestivum,* winter	4	3	3/4;	4	3	2+	3	-	-	-	8.8	88	91	S
Jagger	*T. aestivum,* winter	3-	3+	;3+	2/3+	;3+	4	;3	-	-	-	5.3	64	56	S
Karl 92	*T. aestivum,* winter	2/2+	3	1,2,3	2+,3/3	3-	3	3	-	-	-	3.6	40	32	R
McCormick	*T. aestivum,* winter	;1/ ;2-	;1	2	1	;1	2	1	+	-	-	-	---	---	---
NC-Neuse	*T. aestivum,* winter	3	;1-	;3	;	3	;1/3	3	-	-	-	4.6	44	22	R
TAM 110	*T. aestivum,* winter	;1,3/;1,2	2-	2	1,2-	1-/3	2	2	-	-	-	3.9	60	60	S

Continued on next page

Supplemental Table 2. Continued

		Stem rust infection types for races †							Stem rust markers‡			FHB ratings §			
Line	**Description**	**TRTTF**	**TTKSK**	**QTHJC**	**MCCFC**	**RKQQC**	**TPMKC**	**TTTTF**	***Sr24***	***Sr25***	***Sr26***	**No. infected**	**% infected**	**% VSK**	**FHB Rating**
Thunderbolt	*T. aestivum,* winter	;1,3	0/ 0,3	3+	3	3	3	3	-	-	-	10.5	77	64	S
KS95WGRC33	*T. aestivum,* winter	1/3+	2-	;1	;1	;	2	1+	-	-	-	4.1	52	52	I
PI519847	*T. aestivum,* spring	2-	3	2,2+	;	;2	;2+	2+	-	-	-	1.6	14	24	R
PI520054	*T. aestivum,* spring	1,2-	3-	;2+	;/1-	;/2+	2+	;3	-	-	-	3.0	23	74	S
PI520172	*T. aestivum,* spring	;1,2-/ ;2	3	3	;	;	3/4	3	-	-	-	5.5	58	45	S
PI531193	*T. aestivum,*winter	;1	3	;2	;1	0	2-	1	-	-	-	3.4	32	69	S
Do1	*T. carthlicum,* spring	---	---	---	---	---	---	---	-	-	-	9.5	100	100	S
Do1 8	*T. carthlicum,* spring	---	;	---	---	---	---	---	-	-	-	-	---	---	---
Do1 JD	*T. carthlicum,* spring	---	---	---	---	---	---	---	-	-	-	10.7	100	94	S
PI532505	*T. carthlicum,* spring	3	2	;1,2,3	;2-	;1	0	3	-	-	-	5.6	34	5	R
PI532506	*T. carthlicum,* spring	---	---	2+	3	2+	3	3	-	-	-	2.0	11	49	I
PI573182	*T. carthlicum,* spring	3	---	3+	1+	;2-	2/3	;3	-	-	-	3.8	32	24	R
PI634318	*T. durum,* winter	;2,2+	3+/ ;3+	;1-	;1-/;1	1-	3/4	3+	-	-	-	5.5	54	44	I
NE95T441	Triticale, winter	;1-	;1	;	;	;	;	0/;1	-	+	-	-	---	---	---
NET422	Triticale, winter	;	;	;	;	;	;	0/1-	-	+	-	-	---	---	---
Presto	Triticale, winter	;1,3	3	;1-	1/2-/;3	;	3+	;1	-	-	-	0.9	10	0	R
PI386154	Triticale, winter	2	;2-/ 2/ 2+	2	2+/3	2-	3-	2	-	-	-	10.7	54	48	S

Continued on next page

Supplemental Table 2. Continued

		Stem rust infection types for races †							Stem rust markers‡			FHB ratings §			
Line	**Description**	**TRTTF**	**TTKSK**	**QTHJC**	**MCCFC**	**RKQQC**	**TPMKC**	**TTTTF**	***Sr24***	***Sr25***	***Sr26***	**No. infected**	**% infected**	**% VSK**	**FHB Rating**
LMPG-6	*T. aestivum*	4	4	4	3	3+	3+	3+							
McNair	*T. aestivum*	4	4	3+	3	3	3+	3+							
Alsen	*T. aestivum*											3.0	22	29	R
Bacup	*T. aestivum*											3.1	26	26	R
MN00269	*T. aestivum*											12.7	83	83	S
Roblin	*T. aestivum*											11.5	95	83	S
Wheaton	*T. aestivum*											13.2	89	100	S
LSD(.05)¶												3.7	31	37	

† Stem rust seedling screening infection types ;, 1, or 2 indicate resistance and 3 or 4 indicate susceptibility. "N" represents a necrotic resistant response. "," separates multiple infection types for an individual seedling and "/" separates multiple infection types for individuals of the same line.

‡ PCR based marker results for stem rust resistance genes *Sr24, Sr 25, and Sr26*. "+" indicates the same band as the positive check.

§ Mean disease severity ratings of single heads point inoculated with *Fusarium graminerum*. Number infected describes the number of spikelets bleached, 21 dai. Percent infected describes the percentage of spikelets bleached, 21 dai. Percent VSK describes the percentage of visually infected kernals from the inoculated head. FHB rating characterizes resistant lines (R) not statistically different from resistant checks for any of the three disease severity measures (Number infected, percent infected, and percent VSK); susceptible lines (S) not statistically different from susceptible checks; and intermediate lines (I) with statistically higher disease severity than resistant checks, but lower severity than susceptible checks, or inconsistent between measures of severity.

¶LSD test includes all hybrid, parental lines, and checks

"-" indicates negative result; "---" indicates line not tested

Supplemental Table 3. Stem rust infection types, stem rust DNA marker profile, and FHB ratings of wheat-wheatgrass partial amphiploids, and checks

	Stem rust infection types for races [†]							Stem rust markers [‡]			FHB ratings [§]			
Line	TRTTF	TTKSK	QTHJC	MCCFC	RKQQC	TPMKC	TTTTF	*Sr24*	*Sr25*	*Sr26*	No.	%	% VSK	FHB
B307-13-23-3	;1-/;2,3/;2	;/;1/1/;1+/3,2-	---	---	---	---	---	-	-	-	---	---	---	---
B307-55-10-1	2	;2-/2-	;/1-	;1-	1+	1+	1-	-	-	-	---	---	---	---
B307-67-7-1	2-/3	2-	---	---	---	---	---	+	-	-	---	---	---	---
B307-67-7-5	2/3	;/2-/0	2	1	2	;1	2	+	-	-	4.4	52	48	I
B373-4-30-3-6-1-1	;2+	3	3+	3,4	3	3+	;2+,3	+	-	-	2.1	27	14	R
B373-4-30-3-6-2-11	;2+,4	3+	3	3	3	3+	;2+,3	+	-	-	1.9	11	18	R
B373-4-30-3-6-3-2	;1,3	;2/4/2+/3	3	3	3	3	;2,3	+	-	-	---	---	---	---
B373-4-30-3-6-3-4	;2+/4	3+	3/3+	3+	3+	4	;2,3	+	-	-	1.1	21	28	R
B373-4-30-3-6-3-5	;2,4	3,2+/3+	3,4	3+	3+	4	;1,3	+	-	-	1.0	11	7	R
B875-12-1-13	-	3	3	3,4	3	3/4	3	-	-	-	1.3	17	17	R
B875-12-12-12	-	3/2-/2,3/2	2+	2+	3	3/4	3	-	-	-	1.8	24	16	R
B875-12-1-27	;1,3	3	3	;1+/3	2+/3	3-	3	-	-	-	---	---	---	---
B875-12-1-29	2,3	;/;2	1;	;1	;1/2+	0	1,2+				---	---	---	---
B875-12-1-9	2+/3/4	2+	3	2/4	2/3	4/3	;/2/3	-	-	-	3.7	48	26	R
B913(3)-12-7	;1,3-/3	;1/2/;	;1-	;1+	;/2+	2+/3	2+/3	-	-	-	1.8	16	4	R
B913(3)-14-5	;2-	2/3	;1-	;	;1	;1+	3	-	-	-	3.9	38	35	R
B913(3)-6-8	;1/3	;1/3-	;2-	;	;1-/3	;/3-	;1-	-	-	-	4.6	43	18	R
B938(13)-18	0/;1-	0	0/;	0/;	;	0	0	+	-	-	7.4	71	43	I
B938(15)-12	2-	1+,2-	2	1	1/3-	;1	1-N	+	+	-	1.6	18	10	R
B938(16)-8	;1/2/;1+/3	;1/2-/0	2+	1	1-/3-	;	1-	+	+	-	1.7	20	6	R
B938(17)-14	0/0;	0	0	0/;	0	;	0	+	+	-	1.4	16	1	R
B938(23)-8	0/0;	;1-/0	0/;	0	;	;	;1-	+/-	-	-	2.2	24	1	R
B938(24)-10	0	;/0	;	0	0	0	0	+	-	-	1.6	15	10	R
B938(24)-7	0/0;	;1/;	0/;	0/1	1+	0/1-	;/3	+/-	-	-	2.3	20	6	R
B938(24)-8	0;/0	0	0	0/;	;	0/;	0	-	+	-	1.6	21	15	R
B938(24)-9	0/2	;1-/2-	;/3	;/1	1/3	1/1-	;/1/1+	-	-	-	5.0	54	45	I
B1016(8)	0	;	---	---	---	---	---	+	+	+	---	---	---	---
B1037(1)-19	2N/2	;2-/2	1+	2+/3/4	1/2+	2/3	1+	-	-	-	---	---	---	---
B1037(1)-9	2-/2	;1+	1	3	2+	2+	2-	-	-	-	10.5	50	32	S
B1085(3)-17	;1,2-/;1,2-	3	3	;1-/2	1/2/3	2-/3	3	-	-	-	7.7	77	81	S
B1085(3)-18	1/2-N	3	3	1+/2	3	2+/3	3	-	-	-	9.9	83	82	S

Continued on next page

Supplemental Table 3. Continued

	Stem rust infection types for races †							Stem rust markers ‡			FHB ratings §			
Line	**TRTTF**	**TTKSK**	**QTHJC**	**MCCFC**	**RKQQC**	**TPMKC**	**TTTTF**	***Sr24***	***Sr25***	***Sr26***	**No.**	**%**	**% VSK**	**FHB**
B1085(3)-20	2N	3+/3	3	2-/2	3	3-	3	-	-	-	6.0	69	68	S
B1089(11)-4	;3	;	;	0	0	0	;1-	+	-	-	1.7	11	0	R
B1089(5)-3	;1-	;/;1	1-	;1-	1	;	0/1	+	-	-	---	---	---	---
B1094(1)-14	4	3-	3+	4	;2-	3+	3,2+	-	-	-	8.7	87	52	S
B1094(1)-22	3+/4	3	3/;2-	3	;2-	3	3	-	-	-	10.1	95	79	S
B1094(1)-25	4	3	3/;2,1	3	;1,2	3+	;3-	-	-	-	---	---	---	---
B1100(1)-19	3+	;2-	3	3/;	;1+,2+	3	-	-	-	-	---	---	---	---
B1107(2)-14	;1/;2-	;1	1/;	;1-	;1	0	1	-	-	-	1.3	14	1	R
B1107(2)-3	;1/2/3N	;2-/1	---	---	---	---	---	-	-		---	---	---	---
B1107(4)-29	2	;/;1	0/;1-	;/1-	;1-	;1-,2	1+	-	-	-	2.3	28	2,0	R
B1126(3)	3-	;1	---	---	---	---	---	+	-					
B1129(1)	;1-	;2-/2	---	---	---	---	---	-	-	-	---	---	---	---
B1139(3)	;1+,1-/N	;1-/0	1+/;	;/1-	;	0/1	1	+	-	-	---	---	---	---
B1146(1)	;1/2+N/2	;2-	3-	3/;1+	;	2/3	;/3	+	-	-	---	---	---	---
B1146(5)	3/2+	;1	2+/3	3	;/2	0	2-	+	-	-	5.1	50	1	R
B1152(1)	;3/2-	;1	1/3	3/2-	1/2	2-/3+	;1+	+	-	-	---	---	---	---
B1152(2)	2/;1	;1	2	3	3/;	2/3	1	+	-	-	---	---	---	---
LMPG-6	4	4	3+	3+	3+	4	4							
McNair	4	4	4	4	3+	4	3+							
Alsen											3.0	22	29	R
Bacup											3.1	26	26	R
MN00269											12.7	83	83	S
Roblin											11.5	95	83	S
Wheaton											13.2	89	100	S
LSD(.05)¶											3.7	31	37	

† Stem rust seedling screening infection types ;, 1, or 2 indicate resistance and 3 or 4 indicate susceptibility. "N" represents a necrotic resistant response.

‡ PCR based marker results for stem rust resistance genes *Sr24, Sr 25, and Sr26*. "+" indicates the same band as the positive check.

§ Mean disease severity ratings of single heads point inoculated with *Fusarium graminerum*. Number infected describes the number of spikelets bleached,

¶LSD test includes all hybrid, parental lines, and checks

"-" indicates negative result; "---" indicates line not tested

Thinopyrum species lines are bolded in the pedigree

Figure 1. *T. aestivum* parents (TAM110, 2137, and Jagger) and *Th. intermedium* parent (PI401201) of partial amphiploid line B1146(5).

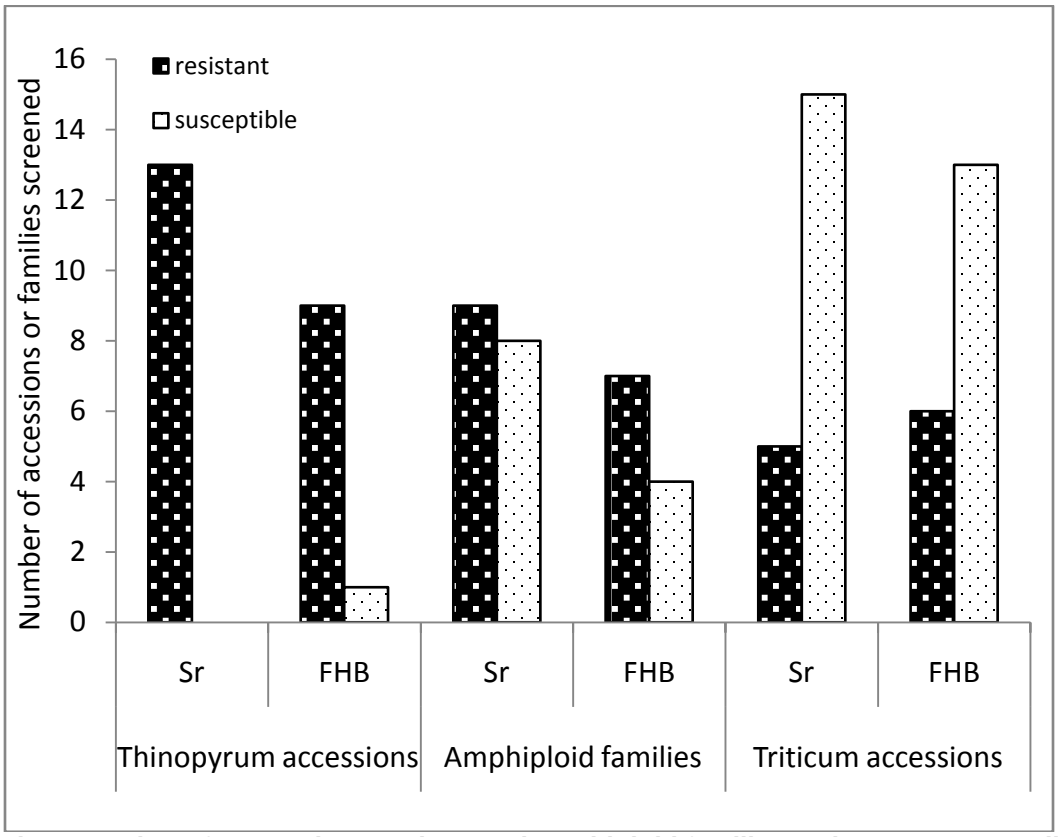


Fig 2. Number of parental accessions and amphiploid families resistant or susceptible to stem rust and Fusarium head blight. Accessions or families categorized as resistant to stem rust demonstrated resistance to all 7 races used in screening. Accessions or families categorized as resistant to Fusarium head blight demonstrated resistance to all measures of severity including number of infected spikelets, percentage of infected spikelets, and percentage VSK, with the exception of *Thinopyrum* accessions which do not include percentage of VSK kernels assessment. Susceptible lines included any line susceptible to at least one race of stem rust or one measure of FHB severity.

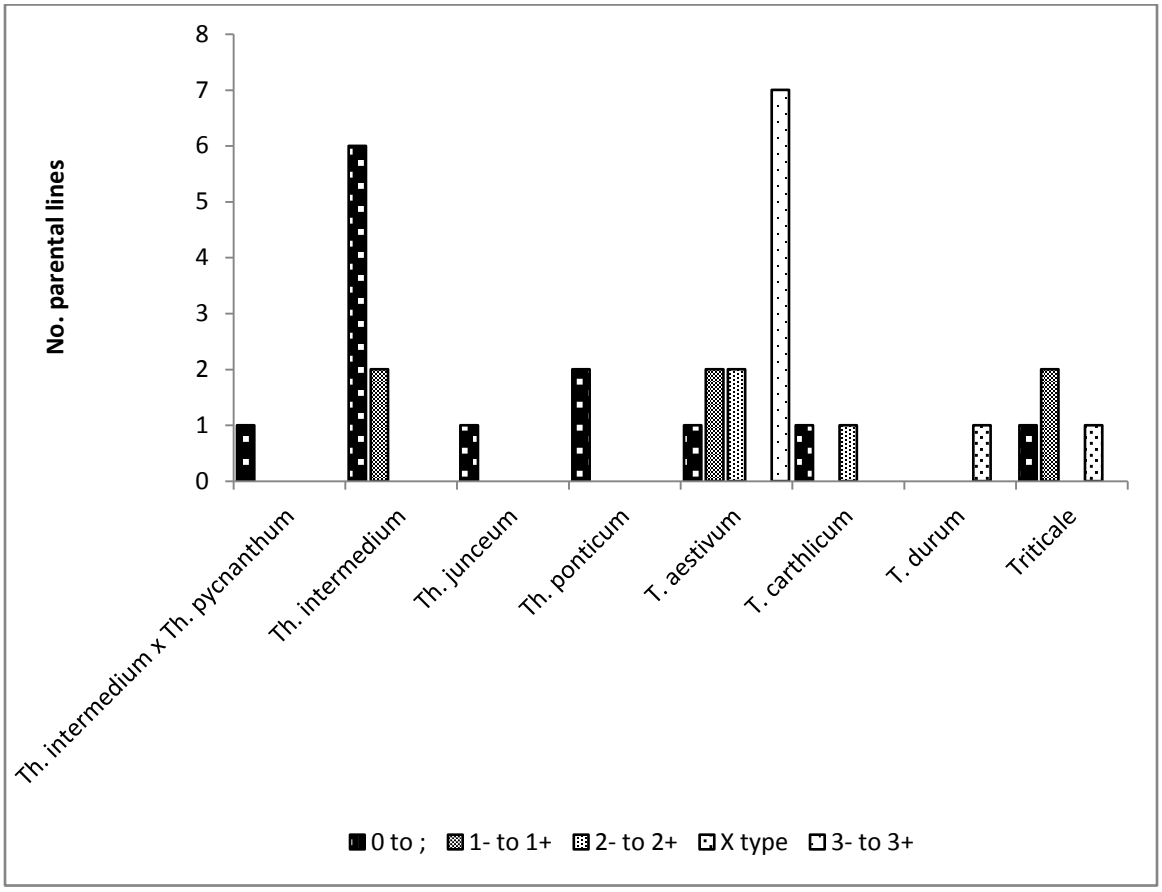


Figure 3. Number of parental lines used to create partial amphiploid lines with infection types of 0 to ;, 1- to 1+, 2- to 2+, x type, or 3- to 3+ when inoculated with stem rust race TTKSK. These ratings reflect the lowest recorded rating of multiple individuals.

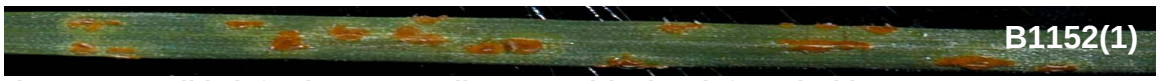


Fig 4. Susceptible infection type on line B1152(1) when infected with stem rust race MCCFC.

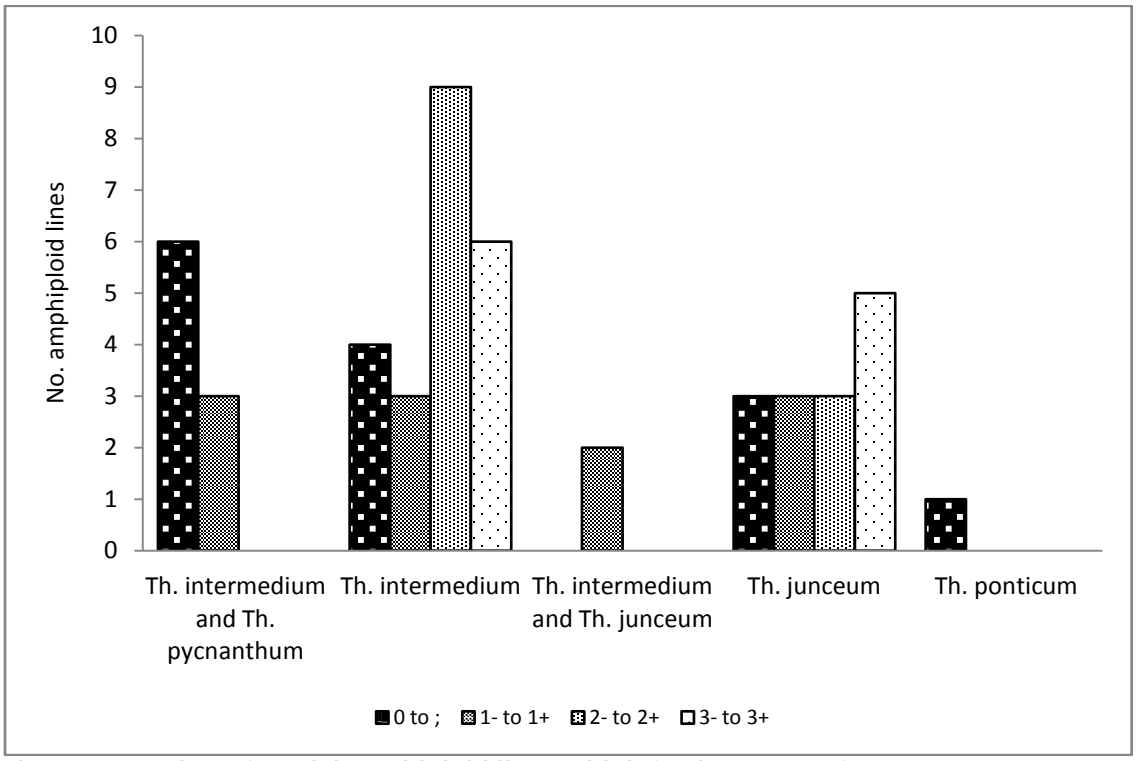


Figure 5. Number of partial amphiploid lines with infection types of 0 to ;, 1- to 1+, 2- to 2+, x type, or 3- to 3+ when inoculated with stem rust race TTKSK, grouped by *Thinopyrum* species present in the pedigree. These ratings reflect the lowest recorded rating of multiple individuals.

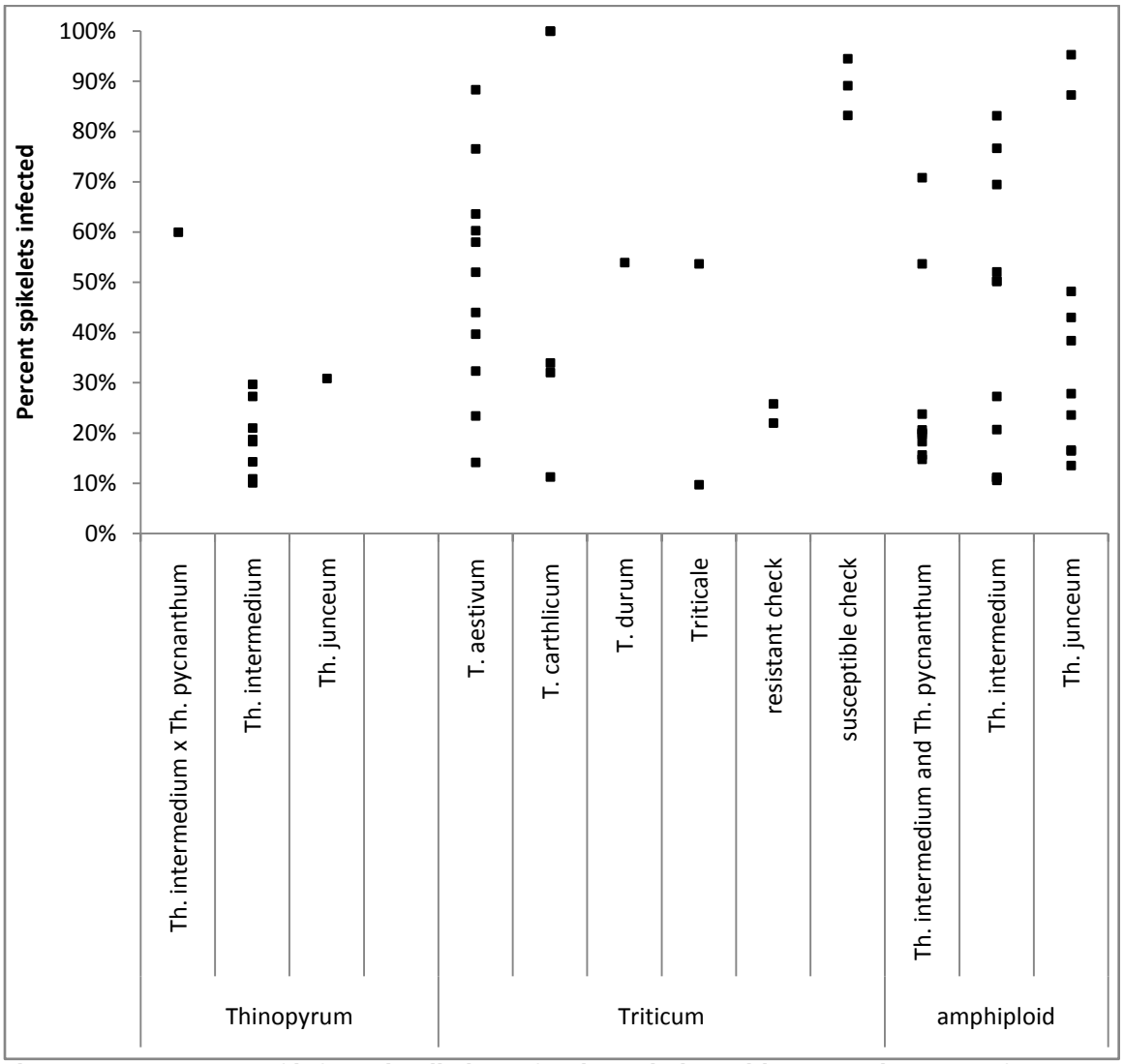


Figure 6. Percentage of infected spikelets after inoculation with *F. graminearum* of *Thinopyrum* and *Triticum* parental accessions used to generate partial amphiploid lines, and partial amphiploid lines, grouped by *Thinopyrum* species present in the pedigree LSD(.05) = 31%.

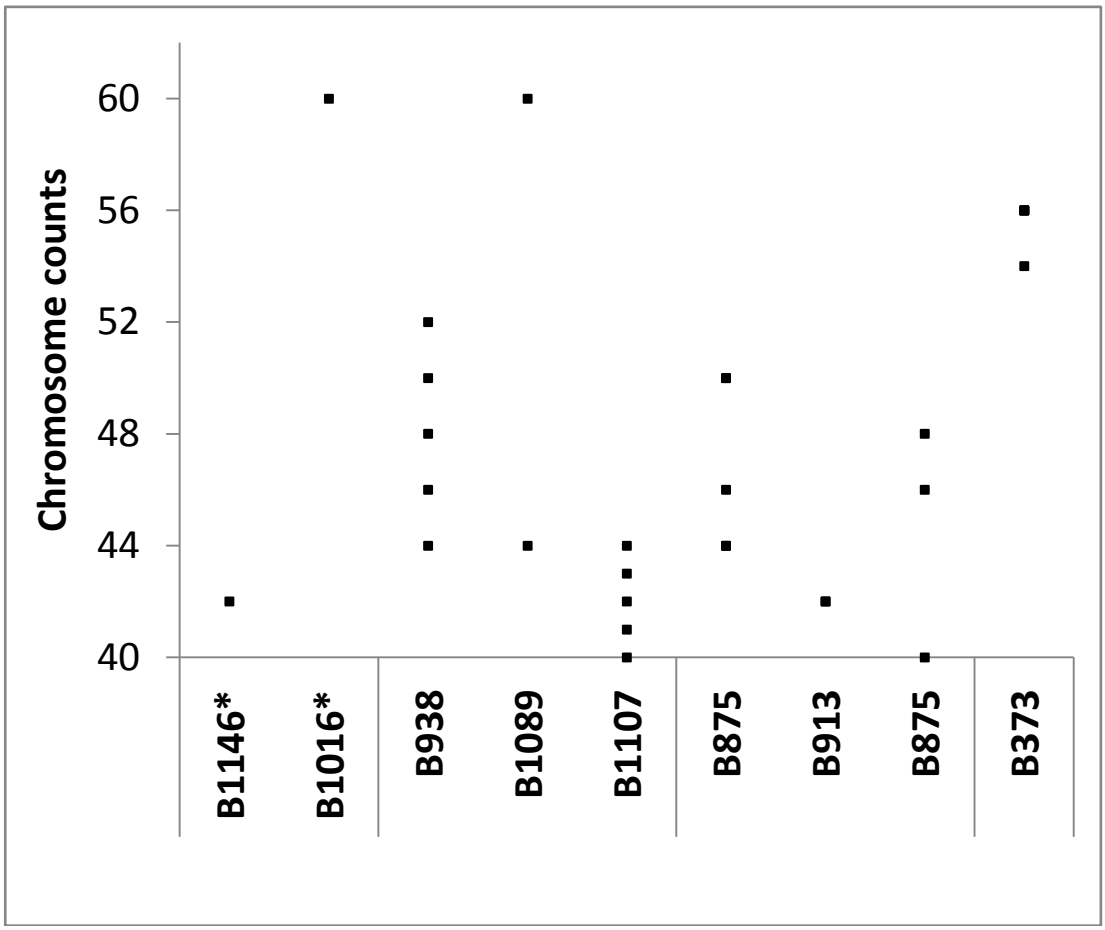


Figure 7. Chromosome counts of F_2 to F_7 amphiploid lines showing perenniality or potentially novel disease resistance. * indicates line demonstrated perenniality.

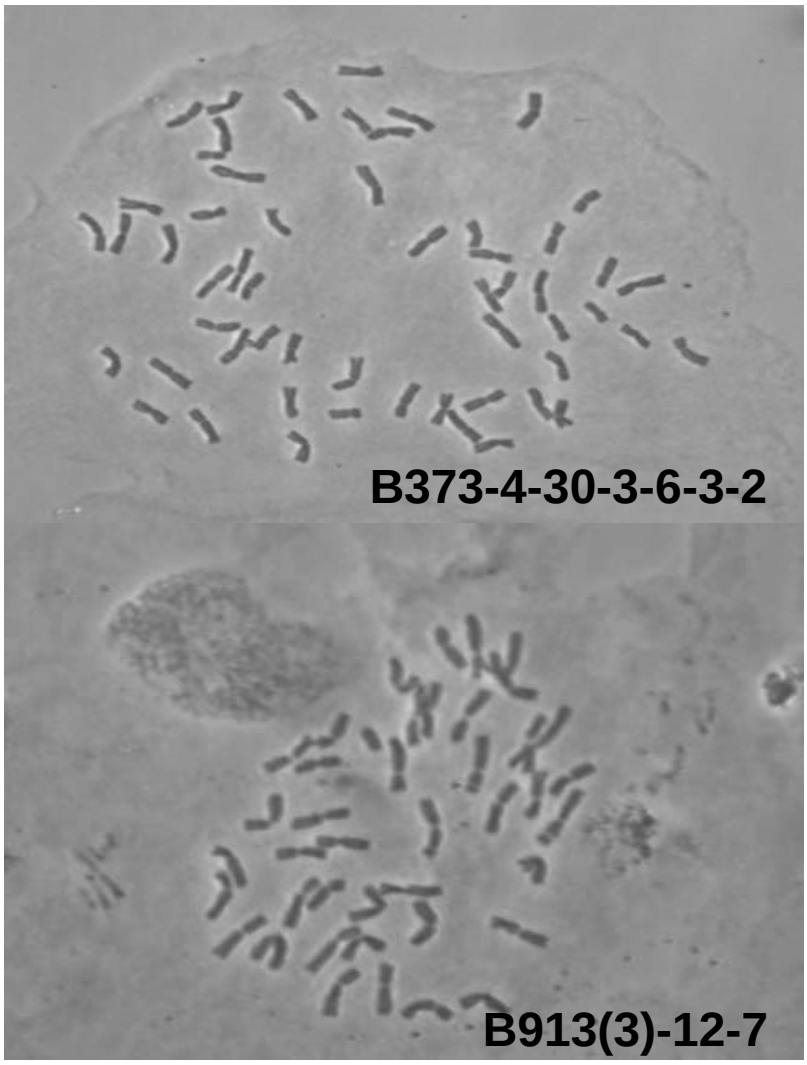
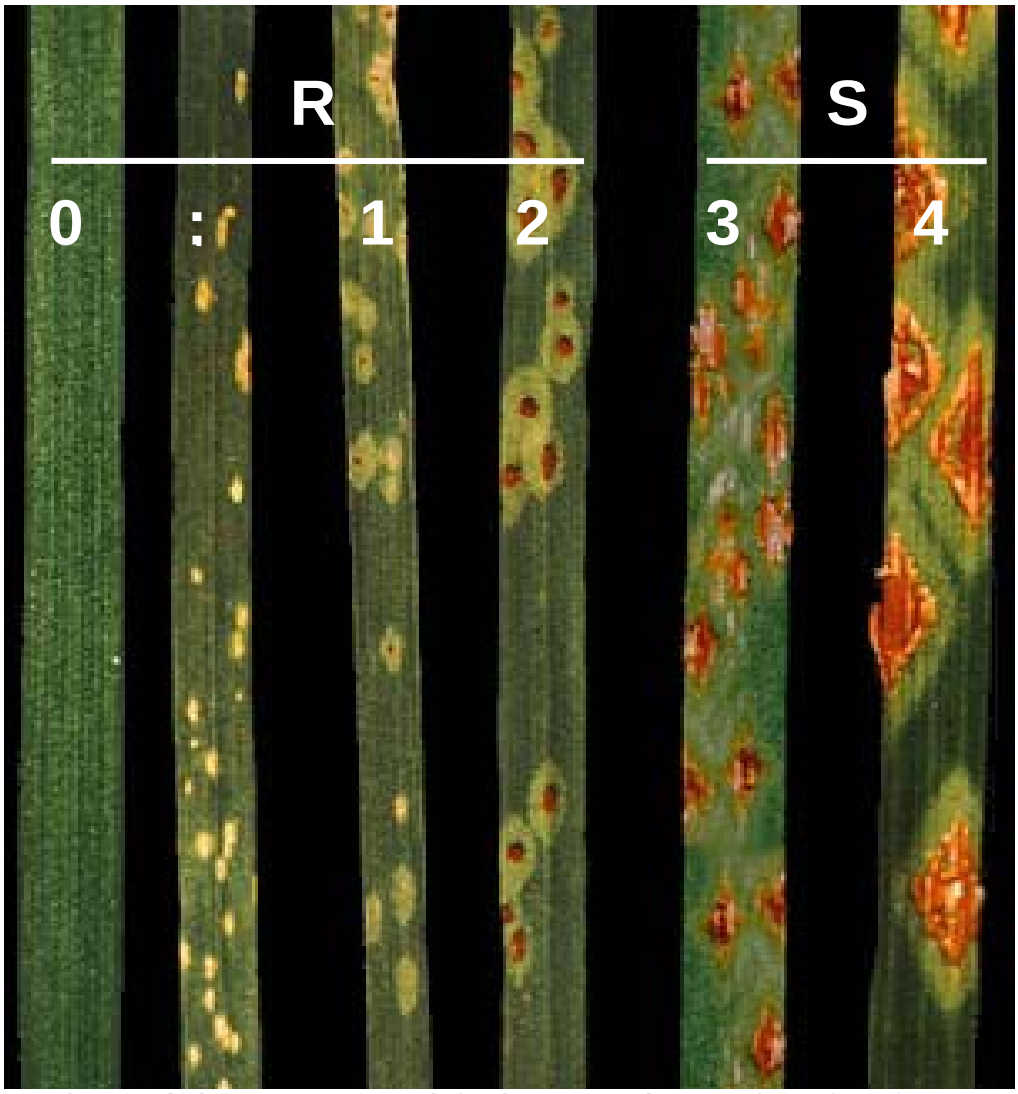
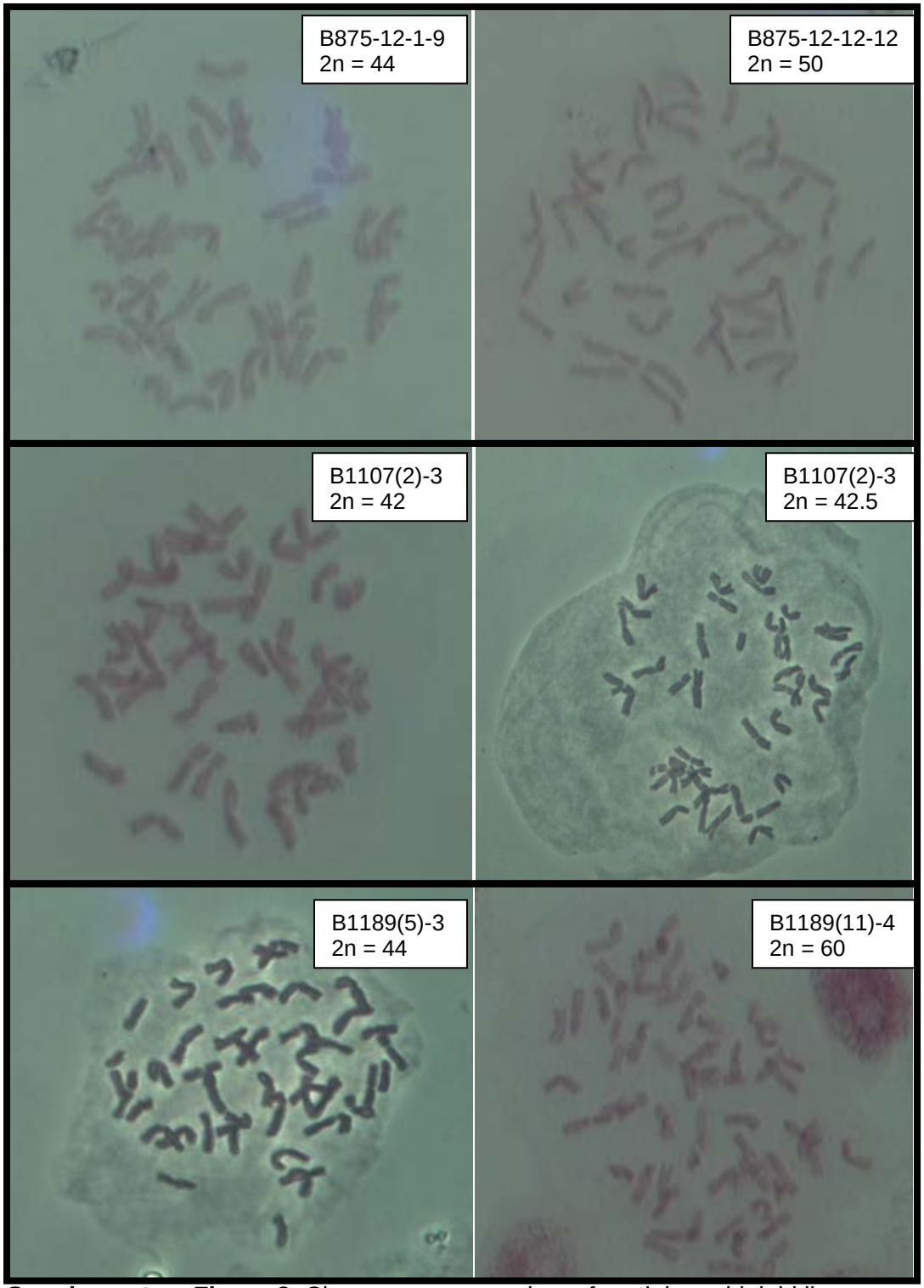


Figure 8. Chromosome squashes of potentially stable partial amphiploid lines. Line B373-4-30-3-6-3-2 has 2n=56 chromosomes and line B913(3)-12-7 has 2n=42.



Supplemental Figure 1. Stem rust infection type ratings. Ratings of 2 or lower are resistant and ratings of 3 or 4 are susceptible.
http://www.ars.usda.gov/SP2UserFiles/ad_hoc/36400500Cerealrusts/inf_set.jpg



Supplementary Figure 2. Chromosome squashes of partial amphiploid lines demonstrating perenniality and cold headiness or potentially novel or previously uncharacterized disease resistance.

Bibliography

Allan, R.E., Peterson, C.J., Jr., Rubenthaler, G.L., Line, R.F., and Roberts, D.E. 1989. Registration of Madsen wheat. Crop Sci. 29:1575-1576.

Anamthawat-Jonsson, K., S.K. Bodvarsdottir, B.T. Bragason, J. Gudmundsson, and R.M.D. Koebner. 1997. Wide hybridization between wheat (*Triticum* L.) and Lymegrass (*Leymus* Hochst.). *Euphytica* 93:293–300.

Anderson, J.A. Marker-assisted selection for Fusarium head blight resistance in wheat. 2007. Int. J. Food Microbiol. 119:51-53.

Arseniuk, E., E. Foremska, T. Goral, and J. Chelkowski. 1999. Fusarium head blight reactions and accumulation of deoxynivalenol (DON) and some of its derivatives in kernels of wheat, Triticale and rye. J. Phytopathol. 147:577-590.

Baenziger, P.S., and K.P. Vogel. 2003. Registration of 'NE422T'winter Triticale. Crop Sci. 43:434-435.

Banks, P.M., S.J. Xu, R.R.C. Wang, and P.J. Larkin. 1993. Varying chromosome composition of 56-chromosome wheat *Thinopyrum intermedium* partial amphiploids. Genome 38:395-405.

Bell, L.W., F. Byrne, M.A. Ewing, and L.J. Wade. 2008. A preliminary whole-farm economic analysis of perennial wheat in an Australian dryland farming system. Agric. Syst. 96:166-174.

Braidotti, G. 2011. Perennial wheat: The next frontier. *In:* Ground Cover. [Online]. [3 p.] Available at http://www.grdc.com.au/director/events/groundcover?item_id=6ECA059EAC658F0B4DB5C93B9BD14611&article_id=7DE44D560D65AA0772666997E3BD6426. (Accessed 10/21/2011). Australian Government, Grains Reserach and Development Corporation.

Brettel, R.I.S., E.M. Banks, Y. Cauderon, X. Chen, Z.M. Cheng, J. Larkin, and P.M. Waterhouse. 1988. A single wheatgrass chromosome reduces the concentration of barley yellow dwarf virus in wheat. Ann. Appl. Biol. 113:599-603.

Buerstmayr, H., T. Ban, and J.A. Anderson. 2009. QTL mapping and marker-assisted selection for Fusarium head blight resistance in wheat: A review. Plant Breed. 128:1–26.

Busch, R.H., D.V. McVey, T. Rauch, J. Baumer, and F. Elsayed. 1984. Registration of 'Wheaton' wheat. Crop Sci. 24:622.

Busch, R.H., D.V McVey, G.L. Linkert, J.V. Wiersma, D.D. Warnes, R.D. Wilcoxson, R. Dill-Macky, G.A. Hareland, I. Edwards, and H.J. Schmidt. 1998. Registration of 'BacUp' wheat. Crop Sci. 38:550-551.

Campbell, A.B. and E. Czarnecki. 1987. Roblin hard red spring wheat. Can. J. of Plant Sci. 67:803-804.

Cai, X., S.S. Jones, and T.D. Murray. 2001. Molecular cytogenetic characterization of *Thinopyrum* genomes conferring perennial growth habit in wheat-*Thinopyrum* amphiploids. Plant Breed. 120:21-26.

Chen, Q., R.L. Conner, A. Laroche, and J.B. Thomas. 1998. Genome analysis of *Thinopyrum intermedium* and *Thinopyrum ponticum* using genomic in situ amphiploidization. Genome 41:580-586.

Chen, Q., R.L. Conner, A. Laroche, and F. Ahmad. 2001. Molecular cytogenetic evidence for a high level of chromosome pairing among different genomes in *Triticum aestivum-Thinopyrum intermedium* amphiploids. Theor. Appl. Genet. 102:847-852.

Cox, T.S., M.H. Bender, C. Picone, D.L. Van Tassel, J.H. Holland, and E.C. Brummer. 2002. Breeding perennial graincrops. Crit. Rev. Plant Sci. 21:59-91.

Cox, C.M., K.A. Garrett, T.S. Cox, W.W. Bockus, and T. Peters. 2005. Reactions of perennial grain accessions to four major cereal pathogens of the Great Plains. Plant Dis. 89:1235-1240.

Cox, T.S., J.G. Glover, D.L. Van Tassel, C.M. Cox, L.R. DeHaan. 2006. Prospects for developing perennial grain crops. Bioscience 56:649-659.

Cox, T.S., D.L. Van Tassel, C.M. Cox, and L.R. DeHaan. 2010. Progress in breeding perennial grains. Crop and Pasture Science 61:513-521.

Craigie, J.H. 1927. Discovery of the function of the pycnia of the rust fungi. Nature 120:765-767.

Culman, S.W., S.T. DuPont, J.D. Glover, D.H. Buckley, G.W. Fick, H. Ferris, and T.E. Crews. 2010. Long-term impacts of high-input annual cropping and unfertilized perennial grass production on soil properties and belowground food webs in Kansas, USA. Agric. Ecosyst. Environ. 137:13-24.

Dvorak, J. 1976. The cytogenetic structure of a 56–chromosome derivative from a cross between *Triticum aestivum* and *Agropyron elongatum* (2n=70). Can. J. Genet. Cytol 18:271-279.

Friebe, B. J. Jiang , W. J. Raupp, R. A. Mclntosh, and B. S. Gill. 1996. Characterization of wheat-alien translocations conferring resistance to diseases and pests: Current status. Euphytica 91:59-87.

Frohberg, R. C., R. W. Stack, and M. Mergoum. 2006. Registration of 'Alsen' wheat. Crop Sci. 46:2311-2312.

Glover, J.D. 2005. The necessity and possibility of perennial grain production systems. Renewable Agriculture and Food Systems. 20:1-4.

Griffey, C.A., Rohrer W.L., Pridgen T.H., Brooks W.S., Chen J. , Wilson J.A., Nabati D., Brann D.E., Rucker E.G., Berl H.D., Vaughn M.E., Sisson W.L., Randall T.R., Corbin R.A., Kenner J.C., Dunaway D.W., Pitman R.M., Bockelman H.E., Gaines C., Long D.L., McVey D.V., Cambron S.E., and Whitcher L. 2005. Registration of 'McCormick'wheat. Crop Sci. 45:417-419.

Haag, E. 2011. Potential for perennial wheat. [Online]. [3 p]. Available at: http://www.agriculture.com/crops/wheat/production/potential-f-perennial-wheat_145-ar16924. (Accessed 10/21/2011). Meredith Corporation.

Han, F.P., A. Fedak, K. Armstrong, and T. Ouellet. 2003. Characterization of six wheat x *Thinopyrum intermedium* derivatives by GISH, RFLP, and multicolor GISH. Genome. 46:490-495.

Hu, F.Y., D.Y. Tao, E. Sacks, B.Y. Fu, P. Xu, J. Li, Y. Yang, K. McNally, G.S. Khush, A.H. Paterson, and Z. Li. 2003. Convergent evolution of perenniality in rice and sorghum. Proc Natl. Acad. Sci. USA 100:4050-4054.

Jain, S.K., M. Prashar, S. C. Bhardwaj, S. B. Singh, and Y. P. Sharma. 2009. Emergence of Virulence to *Sr25* of *Puccinia graminis* f. sp. *tritici* on Wheat in India. Plant Dis. 93:840.

Jin, Y., and R. Singh. 2006. Resistance to recent eastern African stem rust isolates with virulence to *Sr31* in U.S. wheat. Plant Dis. 90:476-480.

Jin, Y., R.P. Singh, Ward, R.W., Wanyera, R., M. Kinyua, P. Njau, T. Fetch, Z.A. Pretorius, and A. Yahyaoui. 2007. Characterization of seedling infection types and adult plant infection responses of monogenic *Sr* gene lines to race TTKS of *Puccinia graminis* f. sp. *tritici*. Plant Dis. 91:1096-1099.

Jin, Y., and L.J. Szabo. 2008. Detection of virulence to resistance gene Sr24 within race TTKS of *Puccinia graminis* f. sp. *tritici*. Plant Dis. 92:923-926.

Kato, A. 1998. Air drying method using nitrous oxide for chromosome counting in maize. Biotechnic & Histochemistry. 743:160-166.

Kibirige Sebunya, I., and D.R. Knott. 1983. Transfer of stem rust resistance to wheat from an *Agropyron* chromosome having a gametocidal effect. Can. J. Genet. Cytol. 25:215-221.

Knott, D.R. 1961. The inheritance of rust resistance. VI. The transfer of stem rust resistance from *Agropyron elongatum* to common wheat. Can. J. Plant Sci. 41:109-123.

Kolmer, J.A., X. Chen, and Y. Jin. 2009. Diseases which challenge global wheat production - the cereal rusts. *In*: Carver, Brett F., editor. wheat: Science and trade. Hoboken, NJ: Wiley press. p. 84-124.

Lammer, D., X. Cai, M. Arterburn, J. Chatelain, T. Murray, and S. Jones. 2004. A single chromosome addition from *Thinopyrum elongatum* confers a polycarpic, perennial habit to annual wheat. J. Exp. Bot. 55:1715-1720.

Lazar, M. D., W. D. Worrall, G. L. Peterson, K. B. Porter, L. W. Rooney, N. A. Tuleen, D. S. Marshall, M. E. McDaniel, and L. R. Nelson. 1997. Registration of TAM110. Crop Sci. 37: 1978-1979.

Leonard, K.J. 2001. Stem rust—future enemy? p. 119-146. *In* P.D. Peterson (ed.) Stem rust of wheat: From ancient enemy to modern foe. APS Press, St. Paul, MN.

Leonard, K.J., and L.J. Szabo. 2005. Stem rust of small grains and grasses caused by *Puccinia graminis*. Molecular Plant Pathology 6:99-111.

Li, H., Q. Chen, R.L. Conner, B. Guo, Y. Zhang, R.J. Graf, A. Laroche, X. Jia, G. Liu, and C. Chu. 2003. Molecular characterization of a wheat-*Thinopyrum ponticum* partial amphiploid and its derivatives for resistance to leaf rust. Genome 46:906-913.

Liu, S., X. Zhang, M.O. Pumphrey, R.W. Stack, B.S. Gill, and J.A. Anderson. 2006. Complex microcolinearity among wheat, rice, and barley revealed by fine mapping of the genomic region harboring a major QTL for resistance to Fusarium head blight in wheat. Functional Integrative Genomics 6:83-89.

Liu, S., L. Yu, R.P. Singh, Y. Jin, M.E. Sorrells, and J.A. Anderson. 2010. Diagnostic and co-dominant PCR markers for wheat stem rust resistance genes *Sr25* and *Sr26*. Theor. Appl. Genet. 691-697.

Mago, R., H.S. Bariana, I.S. Dundas, W. Spielmeyer, G.J. Lawrence, A.J. Pryor, and J.G. Ellis. 2005. Development of PCR markers for the selection of wheat stem rust resistance genes *Sr24* and *Sr26* in diverse wheat germplasm. Theor. Appl. Genet. 111:496-504.

McIntosh, R.A., P.L. Dyck, and G.J. Green. 1977. Inheritance of leaf rest and stem rust resistance in wheat cultivars Agent and Agatha. Aust. J. Agric. Res 28:37-45.

Mesterhazy, A. 1995. Types and components of resistance to Fusarium head blight of wheat. Plant Breed. 114:377-386.

Murphy, J.P., R.A. Navarro, S. Leath, D.T. Bowman, P.R. Weisz, L.G. Ambrose, M.H. Pate, and M.O. Fountain. 2004. Registration of 'NC-Neuse'wheat. Crop Sci. 44:1479-1480.

Nganje,W.E., Bangsund, D.A., Leistritz, F.L.,Wilson,W.W., Tiapo, N.M, 2004. Regional economic impacts of Fusarium head blight in wheat and barley. Review of Agricultural Economics 26: 332–347.

Nieto-Lopez, R.M., C. Soler, and P. Garcia. 2003. Genetic diversity in wild Spanish populations of *Thinopyrum junceum* and *Thinopyrum junceiforme* using endosperm proteins and PCR-based markers. Hereditas 139:18-27.

Niu Z.,D.L. Klindworth, T.L. Friesen, S. Chao, Y. Jin, X. Cai, and S.S. Xu. 2011. Targeted introgression of a wheat stem rust resistance gene by DNA marker-assisted chromosome engineering. Genetics 187:1011–1021.

Oliver, R.E., X. Cai, S. S. Xu, X. Chen, and R. W. Stack. 2005. Wheat-alien species derivatives: A novel source of resistance to Fusarium head blight in wheat. Crop Sci. 45:1353-1360.

Oliver, R.E., X. Cai, T.L. Friesen, S. Halley, R.W. Stack, and S.S. Xu. 2008. Evaluation of Fusarium head blight resistance in tetraploid wheat (*Triticum turgidum* L.). Crop Sci. 48:213-222.

Osborne, L.E. and J.M. Stein. 2007. Epidemiology of Fusarium head blight on small-grain cereals. Int. J. Food Microbiol. 119:103-108.

Paterson, A.H., K.F. Schertz, Y.R. Lin, S.C. Liu, and Y.L. Chang. 1995. The weediness of wild plants: Molecular analysis of genes influencing dispersal and persistence of johnsongrass, *Sorghum halepense* (L.) pers. Proc. Natl. Acad. Sci. USA 92:6127–6131.

Refoufi, A., J. Jahier, and M.A. Esnault. 2001. Genome analysis of *Elytrigia pycnantha* and *Thinopyrum junceiforme* and of their putative natural amphiploid using the GISH technique. Genome 44:708-715.

Riede, C.R., and J.A. Anderson. 1996. Linkage of RFLP markers to an aluminium tolerance gene in wheat. Crop Sci. 36:905-909.

Roelfs, A.P. 1988. Genetic control of phenotypes in wheat stem rust. Annu. Rev. Phytopathol. 26:351-367.

Schafer, J.F., and A.P. Roelfs. 1985. Estimated relation between numbers of uridiniospores of *P. graminis* f. sp. tritici and rates of occurence of virulence. Phytopathology 75:749-750.

Scheinost, P.L., D.L. Lammer, X. Cai, T.D. Murray, and S.S. Jones. 2001. Perennial wheat: The development of a sustainable cropping system for the U.S., pacific northwest. American Journal of Alternative Agriculture 16:147-151.

Schmidt, J.W., E.G. Heyne, C.O. Johnston, and E.D. Hansing. 1953. Progress in *Agrotriticum* breeding in Kansas. Transactions of the Kansas Academy of Science 56:29-45.

Schultz-Schaeffer, J., and S.E. Haller. 1987. Registration of Montana-2 perennial *Agrotriticum intermeiodurum* Khizhnyak. Crop Sci. 27:822-823.

Sears, R.G., J.M. Moffatt, T.J. Martin, T.S. Cox, R.K. Bequette, S.P. Curran, O.K. Chung, W.F. Heer, J.H. Long, and M.D. Witt. 1997a. Registration of 'Jagger' wheat. Crop Sci. 37:1010.

Sears, J.M., T.J. Martin, T.S. Cox, R.K. Bequette, S.P. Curran, O.K. Chung, W.F. Heer, J.H. Long, and M.D. Witt. 1997b. Registration of '2137' wheat. Crop Sci. 37:628.

Sears, R.G., T.J. Martin, T.S. Cox, O.K. Chung,S.P. Curran, W.F. Heer, and M.D. Witt. 1997c. Registration of Karl 92 wheat. Crop Sci. 37:628.

Sepsi, A., I. Molnar, D. Szalay, M. Molnar-Lang. 2008**.** Characterization of a leaf rust-resistant wheat–*Thinopyrum ponticum* partial amphiploid BE-1, using sequential multicolor GISH and FISH. Theor. Appl. Genet. 116:825-834.

Shen, X., and H. Ohm. 2006. Fusarium head blight resistance derived from Lophopyrum elongatum chromosome 7E and its augmentation with Fhb1 in wheat. Plant Breed. 125:424-429.

Shen, X., and H. Ohm. 2007. Molecular mapping of *Thinopyrum*-derived Fusarium head blight resistance in common wheat. Mol. Breeding 20:131-140.

Singh, R.P., D.P. Hodson, Y. Jin, J. Huerta-Espino, and M.G. Kinyua. 2006. Current status, likely migration and strategies to mitigate the threat to wheat production from race Ug99 (TTKS) of stem rust pathogen. CAB Rev. Perspect. Agric. Vet. Sci. Nutr. Nat. Res. 54:1-13.

Singh, R.P., J. Hodson, Y. Huerta-Espino, Y. Jin, S. Bhavani, P. Njau, S. Herrera-Foessel, P.K. Singh, S. Singh, and V. Govindan. 2011. The emergence of Ug99 races of the stem rust fungus is a threat to world wheat production. Annu. Rev. Phytopathol. 49:465-481.

Stakman, E.C., and H.A. Rodenhiser. 1958. Race 15B of wheat stem rust-what it is and what it means. Adv. Agron. 10:143-165

Stakman, E.C. 1962. Identification of physiologic races of puccinia graminis var. tritic. p.53. Edited by E. C. Stakman, D.M. Stewart, and W. Q. Loegering. U.S. Agricultural Research Service, Entomology Research Branch, E617, Washington, DC.

Stokstad, E. 2007. Plant pathology - Deadly wheat fungus threatens world's breadbaskets. Science 315:1786-1787.

Suneson, C.A., and W.K. Pope. 1946. Progress with *Triticum* x *Agropyron* crosses in California. J. Amer. Soc. Agron. 38:956-963. 38:956-963.

Trail, F. 2009. For blighted waves of grain: *Fusarium graminearum* in the postgenomics era. Plant Physiol. 149:103-110.

Tsitsin, N.V., and V.F. Lubimova. 1959. New species and forms of cereals derived from amphiploidization between wheat and couch grass. Am. Nat. 93:181-191.

Tsitsin, N.V. 1965. Remote amphiploidization as a method of creating new species and varieties of plants. Euphytica326--330.

Turner, R.E., and N. N. Rabalais. 2003. Linking landscape and water quality in the Mississippi river basin for 200 years. Bioscience 53:563-572.

Van Deynze, A.(ed). 100 years of breeding: UC Davis plant breeding program. [Online]. [52p]. Available at: http://sbc.ucdavis.edu/files2/57360.pdf (Accessed 11/23/2011). University of California.

Van Eeuwijk, F.A., A. Mesterhazy, C.I. Kling, P. Ruckenbauer, L. Saur, H. Burstmayr, M. Lemmens, L.C.P. Keizer, N. Maurin, and C.H.A. Snijders. 1995. Assessing non-specificity of resistance in wheat to head blight caused by inoculation with European strains of *Fusarium culmorum, F. graminearum* and *F. nivale* using a multiplicative model for interaction. Theor. Appl. Genet. 90:221-228.

Vinall, H.N., and M.A. Hein, 1937. Breeding miscellaneous grasses. Yearbook of Agriculture. U.S. Dept. of Agriculture. U.S. Govt. Printing Office, Washington, DC. p.1032-1102.

Wagoner, P. 1990. Perennial grain new use for Intermediate wheatgrass. J. Soil Water Conserv. 45:81-82.

Wagoner, P. 1994. Perennial grain. *In:* The Newsletter of the Indiana Center for New Crops and Plant Products 4(2). [Online]. [11 p.] Available at: http://www.hort.purdue.edu/newcrop/newcropsnews/94-4-2/fall94.html. (Accessed 10/21/2011). Department of Horticulture, Purdue University.

Wang, Z.Y., J. Bell, and A. Hopkins. 2003. Establishment of a plant regeneration system for wheatgrasses (*Thinopyrum, Agropyron* and *Pascopyrum*). Plant Cell Tissue and Organ Cult. 73:265.

Wood, M., D. Comis, D. Harden, L. McGraw, and K.B. Stelljes. 1999. Fighting fusarium. Agric. Res. 47:18-21.

Xu, S.S., Y. Jin, D.L. Klindworth, R. R.-C. Wang, and X. Cai. 2009. Evaluation and characterization of seedling resistances to stem rust Ug99 races in wheat-alien species derivatives. Crop Sci. 49:2167-2175.

Zhang W.J., A.J. Lukaszewski, J. Kolmer, M.A. Soria, S.Goyal, and J. Dubcovsky. 2005. Molecular characterization of durum and common wheat recombinant lines carrying leaf rust resistance (*Lr19*) and yellow pigment (Y) genes from *Lophopyrum ponticum*. Theor. Appl. Genet. 111:573-582.