New resistance in old places: resistance to the Ug99 race group of *Puccinia graminis* f. sp. *tritici* in wheat intra/inter-generic hybrids and historic germplasm

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Dedication

This thesis is dedicated to plant pathologists everywhere who continue the good fight of protecting the world’s food from devastating disease.

Abstract

Wheat stem rust caused by the rust fungus, *Puccinia graminis* f. sp. *tritici*, threatens global wheat (*Triticum aestivum*) production. New races originating in Eastern Africa have overcome many existing stem rust resistance genes. The W. J. Sando collection of wheat intra/inter-generic hybrids is a valuable source of stem rust resistance. The entire collection was characterized for seedling stem rust resistance to 8 races of the stem rust pathogen and cytogenetic analysis was performed on select lines. Several accessions are postulated to contain new sources of resistance. Full screening results are displayed in Supplementary Table S1 and the pedigrees of 29 resistant lines are displayed in Supplementary Table S2. South African accession PI 410954 displayed strong resistance to stem rust race TTKSK at the seedling stage and under field conditions. The source of new resistance was located and material suitable for integration into modern spring wheat breeding programs was produced.

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CHAPTER 1:

Literature Review

Despite more than a century of research, wheat stem rust, caused by *Puccinia graminis* Pers. f. sp. *tritici* Erikss. & E. Henning, continues to threaten global wheat (*Triticum aestivum* L.) production. While this disease has been effectively controlled via genetic resistance in Europe and North America since 1951 and 1974, respectively, localized severe epidemics of stem rust in East Africa, caused by new races of *P. graminis* f. sp. *tritici*, serve to remind wheat scientists that indeed "rust never sleeps" (Leonard and Szabo, 2005; Singh et al., 2006).

The stem rust races responsible for these epidemics are predominately members of the Ug99 race group, so-called due to a *P. graminis* f. sp. *tritici* isolate discovered in the highlands of Uganda in 1998 and named in 1999 (Pretorius et al., 2000). This isolate was typed as race TTKS, in accordance with the international stem rust nomenclature system, and was particularly alarming due to its virulence on the widely deployed stem rust resistance (*Sr*) gene *Sr31* (Roelfs and Martens, 1988; Wanyera et al., 2006). Subsequent wheat rust nursery screenings at the Kenya Agricultural Research Institute revealed that the majority of wheat cultivars in countries threatened by potential race TTKS migration were susceptible (Singh et al., 2006). The discovery of two additional races similar to TTKS but with additional virulence towards the resistance genes *Sr24* (TTKST) and *Sr36* (TTTSK) prompted a revision of the international nomenclature system, adding a fifth gene set in the differential series: *Sr24, Sr31, Sr38*, and *SrMcN* (Jin et al., 2008; 2009). Currently, the Ug99 race group is comprised of 8 races: PTKSK, PTKST, TTKSF, TTKSK (Ug99), TTKSP, TTKST, TTTSK, TTKSF+ (Park et al., 2011;

Pretorius et al., 2012). One or more of these races have been found in South Africa, Zimbabwe, Mozambique, Tanzania, Rwanda, Kenya, Uganda, Ethiopia, Sudan, Eritrea, Yemen, and Iran (Singh et al., 2011; Pretorius et al., 2012; Park, 2014).

The principal method employed to combat stem rust has been, and still is, genetic resistance (Johnson, 1981; Singh et al., 2011). There have been several historic examples of wheat cultivars maintaining resistance to stem rust over long periods of time but most cultivars are retired or replaced before their resistance fails (Kiyosawa, 1982; Roelfs et al., 1992). Currently, race specific seedling resistance and race-nonspecific resistance are often deployed in tandem with the expectation of creating more durable resistance (Singh et al., 2008; 2011). *P. graminis* f. sp. *tritici* is a macrocyclic heteroecious rust, meaning that it progresses through 5 distinct spore stages and reproduces sexually on an alternate host. The alternate hosts of *P. graminis* f. sp. *tritici* include multiple *Berberis* spp. and *Mahonia* spp. In the USA and northern Europe the eradication of alternate hosts, particularly *B. vulgaris*, virtually eliminated the fungus' sexual recombination within these regions (Hermansen, 1968; Roelfs, 1982). However, this control measure requires a large financial investment on the part of participating governments and is not feasible in many parts of the world. Previous studies indicated that *Berberis* spp. did not play a role in stem rust epidemics in East Africa but recent surveys have found aecial infection on *Berberis* spp. in Kenya with the species of the pathogen not yet determined (Guthrie 1966 cited in Green et al., 1970; Park et al., 2011). Chemical control of stem rust can be achieved via appropriate application of fungicides and this is often used when yields and wheat prices are expected to be high (Roelfs et al., 1992). Fungicides that inhibit sterol synthesis, both sterol biosynthesis inhibitors and demethylation inhibitors, are most

effective but rarely deployed due to the cost of application (Schumann and Leonard, 2000). Farmers affected by the Ug99 race group are often smallholder farmers without the necessary capital or equipment for effective fungicide application. Commercial farmers in East Africa and South Africa have resorted to multiple chemical applications throughout the season to protect their crops (Wanyera et al., 2009). Finally, cultural methods can be employed to counteract stem rust. It was recognized early in the history of stem rust research that the elimination of "green bridges" between fields and/or seasons significantly reduced stem rust infections, however, due to East Africa's equatorial location there are two wheat seasons per year making this particular control impractical (Roelfs et al., 1992; Nagarajan et al., 2012). Early maturing varieties may allow crops to escape severe losses caused by stem rust infection and have been a recognized cultural practice since the 1930's (Borlaug, 1954; McIntosh, 1976). Again, the dual season in East Africa renders this cultural method impractical.

As opposed to "major" resistance genes, "minor" resistance genes are generally recessive, have a small effect on overall infection, and only act in adult plants (Knott, 1982). Two well known minor genes are *Sr2* and *Lr34*, both have been used in CIMMYT stock and have maintained resistance over several decades (Van Ginkel and Rajaram, 1993). The lack of regulation regarding the use and deployment of cultivars carrying single resistance genes has prompted CIMMYT to focus its breeding efforts on adult plant resistance (Singh et al., 2011). CIMMYT breeders believe that by combining 4 -5 minor resistance genes (i.e. lacking a race-specific effect) this will lead to durable resistance independent of effective major genes, safeguarding the major genes for use elsewhere.

Currently, there are 33 *Sr* genes that provide protection against various members of the Ug99 race group: *Sr2*, *Sr9h, Sr13*, *Sr21*, *Sr22*, *Sr24*, *Sr25*, *Sr26*, *Sr27*, *Sr28*, *Sr32*, *Sr33*, *Sr35*, *Sr36*, *Sr37*, *Sr39*, *Sr40*, *Sr42*, *Sr43*, *Sr44, Sr45*, *Sr46*, *Sr47*, *Sr50, Sr51*, *Sr52*, *Sr53*, *Sr57*(*Lr34*), *SrTA10171*, *SrTA10187*, *SrTA1662*, *SrTmp*, and *Sr1RS*^{Amigo} (Olson et al., 2013a; Jin and Singh, 2006; Jin et al., 2007; Faris et al., 2008; Hiebert et al., 2010; Kolmer et al., 2011; Rouse et al., 2011a; Liu et al., 2011a; Qi et al., 2011; Liu et al., 2011b; Ghazvini et al., 2012; Olson et al., 2013b; Singh et al., 2013; Park, 2014; Niu et al., 2014b; Rouse et al., 2014). Of these 33 genes, only five are derived from *T. aestivum*. Species that are the sources of the remaining 27 genes include *Triticum turgidum* Flaksb., *T. monoccocum* subsp. *monoccocum* L. Flaksb. , *T. timopheevii* subsp. *timopheevii* (Zhuk.) Zhuk., *T. timopheevii* subsp. *armeniacum* (Jakubz.) MacKey, *Aegilops comosa* Sm. in Sibth. & Sm. var. *comosa, Ae. ventricosa* Tausch*, Ae. speltoides* Tausch var. *speltoides*, *Ae. tauschii* Cross, *Thinopyrum ponticum* (Podp.) Barkworth and D. R. Dewey (syn. *Agropyron elongatum* (Host) Beauvois), and *Th. intermedium* (Host) Barkworth and D. R. Dewey (syn. *A. intermedium* (Host) Beauvois). Singh et al. (2011) have listed the barriers to the large-scale deployment of these genes, which include linkage with undesirable agronomic traits, known virulence in other races of *P. graminis* f. sp. *tritici*, and/or ineffective levels of resistance conferred under high inoculum loads. Despite the continued erosion of resistance and significant barriers to gene deployment, host genetic resistance remains the most effective form of disease control available and several groups have reviewed the importance of alien gene transfer for disease resistance in wheat (Jones, 1995; Friebe et al., 1996; Xu et al., 2009). Several technological advancements have improved researchers' ability to both identify and rapidly introgress

resistance from both alien species and *T. aestivum*. Doubled haploid technology and modern cytogenetic techniques are key tools used in the research presented in this thesis and to that end they will be reviewed below.

A Review of Doubled Haploid Technology

The term 'haploid' has come to mean many things in the realm of plant and agricultural sciences. In the widest sense, haploid is used to denote any sporophyte possessing the gametic chromosome number. Bergner's group, working with *Datura* s*tromonium* L., was the first to report the natural occurrence of sphorophytic haploidy (Blakeslee et al., 1922). Soon after, reports of the haploid phenomenon were confirmed in both *Nicotiana tabacum* and *T. aestivum* (Clausen and Mann, 1924; Gaines and Aase, 1926). However, the technology to develop haploid plants *in vitro* was to remain elusive until the 1970's (Forster et al., 2007). The lack of technology notwithstanding, naturally occurring haploids in maize (*Zea mays* L.) had been observed in the 1930's and breeders quickly recognized their potential in the rapid development of homozygous lines (Chase, 1969). Blakeslee and Avery's discovery of colchicine's effect on chromosome doubling provided maize breeders with the necessary tool to make use of naturally occurring haploid plants (Blakeslee and Avery, 1937).

Laboratory production of haploid plants did not occur until the development of anther culture for *Datura innoxia* in 1964 (Guha and Maheshwari, 1964). Bread wheat proved to be amenable to anther culture and several cultivars have been released using this method (Niu et al., 2014a). Unfortunately, anther culture in wheat faces a number of challenges including high rates of albinism, detrimental gametoclonal variation, and mixed ploidy plants (Tuvesson et al., 1989; Kisana et al., 1993). Soon after the

development of anther culture, haploid induction via interspecific fertilization was discovered in the *Hordeum vulgare*/*Hordeum bulbosum* system (Kasha and Kao, 1970). Fertilization of *H. vulgare* emasculated florets with pollen from *H. bulbosum* induced haploidy in the resulting embryos. Pollination of emasculated *T. aestivum* florets with pollen from *H. bulbosum* was also able to induce haploid development in *T. aestivum* embryos (Barclay, 1975). Again, barriers to widespread adoption of this technique developed due to the interaction between *H. bulbosum* and wheat's dominant crossability inhibitor genes *Kr1* and *Kr2*, limiting effective haploid production to only those wheat genotypes amenable to fertilization with *H. bulbosum* (Niu et al., 2014a). Zenkteler and Nitzsche (1984) tested the viability of wide hybridization for plant breeding in cereals and observed that embryos were formed when wheat (*T. aestivum*) was fertilized with maize (*Z. mays*) pollen. In 1986, Laurie and Bennett developed a highly efficient wide hybridization protocol based on this work that did not seem hampered by the same parent genotype limitations as the *H. bulbosum* technique (Laurie and Bennett, 1986). Further studies indicated that the percentage of haploid embryos recovered from pollinated florets is, in fact, influenced by both the maize and wheat genotypes involved in the cross (Suenaga and Nakajima, 1989; Inagaki and Tahir, 1990; Martins-Lopes et al., 2001).

Haploid production using wide hybridization is completely dependent on the elimination of the male chromosomes during embryo and endosperm development following fertilization. Seed set (a sign of successful fertilization) in wide hybridization involving crosses between *H. bulbosum* and both *H. vulgare* and *T. aestivum* have been reported to range from 13% to 63% (Kasha and Kao, 1970; Barclay, 1975). In both systems, seeds began to show signs of abortion soon after fertilization and embryo

excision and culture methods were necessary to obtain haploid plantlets. Laurie and Bennet reported embryo development in roughly 27% of crosses between wheat and maize using various methods but were only able to recover haploid plantlets at a rate of 17% using spikelet culture (Laurie and Bennett, 1988). They also observed that maize chromosomes were eliminated quickly after fertilization as all embryos with six or more cells only contained micronuclei (Laurie and Bennett, 1986). The mechanisms for chromosome elimination observed by these pioneering researchers remain unclear. In a recent review (see Niu et al., 2014a), a thorough list of possible mechanisms is presented: timing of mitotic processes, genomic balance, failure of parental chromosomes to congregate during metaphase, failure of migration at anaphase, peripheral locations of maize chromosomes on metaphase plates, and/or genome-specific fragmentation based on self recognition (Gupta, 1969; Kasha and Kao, 1970; Bennett et al., 1976; Houben et al., 2010).

Doubled haploid production in wheat has improved significantly in the last decade but still remains a labor-intensive process. The two main barriers are the low ratio of successful embryo formation and the absence of endosperm leading to embryo abortion (Niu et al., 2014a). To overcome these barriers there are several stages that a successful doubled haploid program must develop. After fertilization of the selected wheat plants researchers must choose among several post-pollination treatments, develop efficient embryo rescue protocols, and apply the appropriate colchicine treatment to the haploid plants.

There are 5 post-pollination techniques listed in the literature: 1) immediate culturing of spikelets for a 3 week period (Laurie and Bennett, 1988), 2) application of

0.5 mg/L dichlorophenoxyacetic acid (2,4-D) to pollinated spikelets for 2-3 weeks, 3) injecting 100 mg/L 2,4-D to the internode and/or the spikelets once or twice (Matzk and Mahn, 1994), 4) applying a combination of an auxin (picloram, 2,4-D, or 2,4,5-T) with 6-benzylaminopurine (6-BA) or a combination of 2,4-D and gibberellic acid in the florets at 24-30 h after pollination (Singh et al., 2001), 5) or applying dicamba (3,6-dichloro-o-anisic acid) or zearalenone (Biesaga-Kościelniak et al., 2003). The fourth treatment generates the highest percentage of viable embryos (Niu et al., 2014a). Post-pollination treatment with 2,4-D increases pollen tube lengths and the number of sperms cells within pollen tubes, likely contributing to higher rates of successful intergeneric fertilization (Wedzony and Van Lammeren, 1996).

As mentioned above, maize chromosomes are eliminated from developing embryos but the accompanying endosperm also undergoes chromosome elimination and aborts early in development. For this reason it is necessary to use embryo rescue methods to produce viable haploid plantlets (Forster et al., 2007). Seeds containing embryos can be identified efficiently by placing the seeds on a transparent surface, illuminating them from above, and viewing the seeds from below or via a mirror (Bains et al., 1998). Two different methods are used for embryo rescue depending on the size of the embryo. Large embryos can be cultured directly on MS, ½ MS, or B5 media and maintained in a dark growth chamber for 1-2 weeks until germination (Murashige and Skoog, 1962; Suenaga and Nakajima, 1989; Cherkaoui et al., 2000). Smaller embryos can be cultured using the nurse endosperm technique on MS media in which the embryos are placed on 20 day old endosperm cultured in MS media (Niu et al., 2014a).

While various chromosome doubling agents have been explored for use in the wheat-maize system, colchicine has been the most common agent used for this procedure (Niu et al., 2014a). Colchicine's effect on chromosomes, as stated above, was discovered by Blakeslee and Avery (1937) but the mechanism whereby the chromosome doubling occurs was described in 1974 (Jensen, 1974). Jensen (1974) described colchicine as disrupting the formation of spindle fibers during mitosis therefore disturbing normal chromosome polar migration leading to the doubling of identical chromosomes within the same cell. Colchicine's effect is dose and plant stage dependent and many researchers have worked to find the optimal combination (Jensen, 1974; Thiebaut et al., 1979; Inagaki, 1985; Sood et al., 2003). Most recommendations are that colchicine be applied between the 3- and 4- tiller stage and at doses ranging from 0.00045% to 0.1% colchicine (Thiebaut et al., 1979; Inagaki, 1985; Niu et al., 2014a). Colchicine solutions contain dimethyl sulfoxide, gibberellic acid, and tween in addition to colchicine. The root system of the plants to be treated are soaked in the solution for 5-8 hours, rinsed overnight with deionized water, and finally planted into soil and maintained at 14-16° C with a light regimen of 16/8 h day/night until new tillers emerge. Using this method, plant survival and chromosome doubling rates can be higher than 95% (Inagaki, 1985; Niu et al., 2014a).

As outlined above significant investments must be made to develop an efficient DH method, however, when compared to the time required to reach a homozygous state using traditional single-seed descent, the advantages of DH lines become obvious. Doubled haploid techniques have been incorporated into both barley and wheat breeding programs to varying degrees (Devaux, 2003; Inagaki, 2003). The cost and level of

expertise needed have prevented some programs from adopting these techniques but the potential applications of doubled haploid techniques remains undisputed. Doubled haploid techniques have also been used in pre-breeding efforts to introduce disease resistant loci or develop markers for marker-assisted selection in both wheat and barley (Steffenson et al., 1995; Moieni et al., 1997; Druka et al., 2000; Suenaga et al., 2003; Yang et al., 2005; Friesen et al., 2006; Fofana et al., 2008; Prins et al., 2011). The research presented in the third chapter of this thesis is well aligned with the goal of exploiting doubled haploid technology but may be unique in its development of doubled haploid lines intended to be used directly in a wheat breeding program. Most studies in the literature use germplasm ideal for mapping resistance loci, however, we chose to use a unique backcross scheme to achieve the combined goals of 1) locating the source of stem rust resistance and 2) introgressing this resistance into adapted hard red spring wheat germplasm for the Northern Plains.

A Review of Cytogenetic Techniques

As mentioned above, alien species that are closely related to *T. aestivum* serve as a large reservoir of beneficial traits that can be incorporated into wheat breading programs (Friebe et al., 1996; Kilian et al., 2011). Transferring genes from the wild and distant relatives of wheat into agronomically acceptable material is a significant challenge. The portion of alien chromatin introduced into a *T. aestivum* background must be reduced to the smallest portion possible, limiting the effect of deleterious linkage drag. Many genes have been transferred to *T. aestivum* from various species but few of these are used in breeding programs due to associated linkage drag (Friebe et al., 1996). Ideally, a gene transfer strategy should allow both the introgression of alien chromatin

possessing the gene of interest and compensate for the replaced wheat chromatin (Danilova et al., 2014). Accessions in the W. J. Sando collection that have been examined cytogenetically contain a variety of alien chromosome complements, ranging from wheat-alien whole-arm translocations to full genomes derived from the parental alien species (Cox et al., 2002). To successfully introgress an alien gene from this heterogeneous material it is necessary to determine the number and identity of wheat chromosomes that have been replaced. Several methods exist to accomplish this.

Giemsa C-banding takes advantage of the repetitive, adenine/thymine enriched nature of constitutive heterochromatin as a target for Giemsa staining. By denaturing the DNA of the chromosomes of interest and allowing for their re-association, highly repetitive regions re-associate faster and form bands of darkly stained heterochromatin (Gill and Kimber, 1974). The darkly stained regions result in a banding pattern that allows researchers to identify individual chromosomes and even chromosome arms. Giemsa C-banding was first developed in mammalian systems and subsequently applied to plant systems (Wang and Fedoroff, 1972; Müller and Rosenkranz, 1972; Ray and Hamerton, 1973; Merrick et al., 1973; Gill and Kimber, 1974). Giemsa C-banding is able to identify species-specific chromosomes and has been used to identify segmented and whole alien chromosomes in hybrid wheat-alien lines (Gill and Kimber, 1974; Friebe et al., 1989; 1991). Full wheat karyotypes were described in 1974 and 1983 but the karyotype and the accompanying nomenclature were not standardized until 1991 (Gill and Kimber, 1974; Lukaszewski and Gustafson, 1983; Gill et al., 1991). In 1997 an alternative karyotype was described using dual stain fluorescence in situ hybridization or FISH (Pedersen and Langridge, 1997). All of these developments were huge steps

forward in wheat cytogenetic research but exhibited limitations such as the inability to simultaneously identify chromosomes and map DNA sequences of interest.

In situ DNA hybridization (ISH) was first developed in the late 1960's (Gall and Pardue, 1969; John et al., 1969). The first ISH techniques depended on radiation for probe labeling and signal detection but were soon replaced by enzymatic methods that allowed for higher resolution, decreased time requirements, and long-term stability of the labels (Langersafer et al., 1982). Fluorescence-based ISH methods, now known as FISH, have several advantages over enzymatic-based ISH in that multiple probes can be imaged at the same time and analyzed using digital imaging technology (Jiang and Gill, 1994). ISH was first used in plants by Rayburn and Gill (1985) to map specific DNA sequences to their positions on wheat chromosomes. A FISH karyotype of wheat chromosomes was developed by Pedersen and Langridge (1997). This karyotype was based on fluorescent probes designed to hybridize with GAA-satellite sequences and pAs1 sequences. GAA-satellite DNA probes created major bands in all B genome chromosomes with minor sites in A and D genome chromosomes, excluding chromosomes 1A, 4D, 5D, and 6D (Dennis et al., 1980). The paucity of major GAA-banding sites in the D genome led to the development of the pAs1 probe detected in *Aegilops tauschii* (synm. *Ae. squarrosa*), wheat's D-genome progenitor (Rayburn and Gill, 1986).

Genomic in situ hybridization (GISH) was developed soon after the advent of FISH (Durnam et al., 1985; Pinkel et al., 1986; Le et al., 1989). GISH allows the detection of species specific chromosomes by using total genomic DNA of one of the parents of the hybrid offspring to determine which chromosomes or portions of chromosomes belong to that specific parent. By combining GISH, FISH, and phenotypic

screening, the objective of the research presented in the following chapter was to identify promising stem rust resistant accessions in the W. J. Sando collection.

CHAPTER 2:

Stem Rust Resistance in the W.J. Sando Collection and Cytogenetic Characterization of Select Resistant Lines

An effective strategy to discover and rapidly develop new sources of resistance should be the focused stem rust screening of existing collections of wheat-intra/intergeneric hybrids. The W. J. Sando collection of intra- and intergeneric hybrids was created by W. J. Sando [employed by the United States Department of Agriculture, Beltsville, Maryland (USDA)] during the first half of the 20th century. Species used for hybrid breeding with *T. aestivum* included *Aegilops* spp.*, Secale cereale*, *Th. intermedium*, *Th. ponticum*, *T. timopheevii*, and *T. turgidum* subsp. *durum*. Early research on wheat-intergeneric hybrids, including that of W. J. Sando, was spurred by the pursuit of perennial grain and forage crops (Smith, 1942; Reitz et al., 1945). While perennial material of agronomic value eluded early researchers, disease resistant material did result from this work, including material developed by W. J. Sando (Reitz et al., 1945). Of note, crosses made with selections from a Sando line resulted in the wheat cultivar 'Agent', the modern source of *Sr24* (Smith et al., 1968; Friebe et al., 1996). In more recent work, accessions from the Sando collection have shown resistance to eyespot, Cephalosporium stripe, scab, Stagnospora blotch, tan spot, wheat streak mosaic, barley yellow dwarf, and stem rust (Banks et al., 1993; Cox et al., 2002; Oliver et al., 2006; Xu et al., 2009).

To our knowledge there is no published work characterizing the W. J. Sando collection for seedling stem rust resistance. We therefore screened the 546 available accessions of the W.J. Sando collection from the USDA National Small Grains Collection in Aberdeen, ID. Our goal was to identify accessions resistant to the Ug99

race group and characterize these accessions cytogenetically in order to select promising material for further manipulation and introgression.

Materials and Methods

Plant material and stem rust screening. A total of 546 accessions of the W. J. Sando Collection were obtained from the United States Department of Agriculture National Small Grains Collection (Aberdeen, ID). The accessions were screened with 8 races of *P. graminis* f. sp. *tritici*. Stem rust races TTKSK, TTKST, TTTSK, and TRTTF were selected for their broad virulence and prevalence in African stem rust epidemics (Table 1). North American stem rust races TTTTF, TPMKC, RKQQC, and QTHJC were also used for screening (Table 1). All stem rust races used are maintained at the USDA Agricultural Research Service (ARS) Cereal Disease Laboratory in St. Paul, MN.

Urediniospores were collected from infected wheat seedlings (for race TTKST) or removed from storage in gelatin capsules at -80°C (for all other races). Urediniospores removed from storage were heat shocked at 45°C for 15 min and placed in a rehydration chamber maintained at 80% relative humidity by a KOH solution for 2 to 4 h. Fresh urediniospores were collected into gelatin capsules and immediately inoculated onto seedlings following their suspension in a light mineral oil, Soltrol 170 (Chevron Phillips Chemical Company LP). Fresh and stored urediniospores were inoculated onto seedlings following previously described methods (Jin et al., 2007). Plants were scored for Stakman seedling infection types (ITs) at 14 days post inoculation (Stakman et al., 1962). Accessions with ITs of ‘0’, ‘;’, ‘1’, and ‘2’ or a combination thereof were considered resistant. Accessions with ITs of ‘3’ or ‘4’ were considered susceptible. Each assay

contained five to eight plants of each accession. If plants of a single accession segregated for resistance, the accession was considered heterogeneous. All screenings were performed in duplicate.

Data analysis. Statistical analysis of accessions and their race-specific resistance/susceptibility patterns was performed using R v. 3.0.2 in RStudio® (R is available online at http://www.r-project.org, RStudio is available from RStudio, Inc.). A multiple correspondence analysis (MCA) was performed using the R package 'ca' v. 0.53 (Nenadic and Greenacre, 2007). MCA, based on the work of Benzérci and colleagues, can explain underlying patterns in complex data sets and is an appropriate alternative to principal coordinate analysis when the data to be analyzed is categorical, as in "Resistant" or "Susceptible", instead of quantitative (Blasius and Greenacre, 2006). It can be used to visualize the spatial relationships among the "responses", namely susceptible (S) and resistant (R), of accessions to individual races of *P. graminis* f. sp. *tritici*, i.e., how resistance to TTKSK correlates with resistance to all other races. A total of 152 accessions displaying resistant ITs to one or more races were analyzed. For simplicity, only those accessions resistant in all available replications were coded as resistant, "R", all other accessions were coded as susceptible, "S", even if the mixed reactions included a resistant and susceptible IT. ITs of '1+3' and '2+3' were considered susceptible reactions, and both were coded as "S" in the spreadsheet used for MCA analysis. The MCA was performed using a dataframe in which the qualitative variables (columns) were the eight races of *P. graminis* f. sp. *tritici* and the observations (rows) were individual accessions and their reaction to each race. The analysis was run with the variable lambda as "lambda=adjusted" and the nd as "nd=5".

Molecular marker screening of selected accessions. Accessions PI 604981, PI 605057, PI 605286, and PI 611932 were screened with the molecular markers *Xbarc71* (*Sr24*), *Gb* (*Sr25*), and *BE518379/Sr26#43* (*Sr26*). Leaf tissue for each accession was collected at the seedling stage (~7 days after germination) and total DNA was extracted using a modified CTAB method. Published protocols were followed for all PCR reactions involving these markers (Mago et al., 2005; Liu et al., 2010).

Cytogenetic examination of selected accessions. Nine resistant accessions were examined using the root squash method to count the number of chromosomes present. Briefly, rootlets were cut when 1.5 to 2 cm long and placed in 2 mL glass vials cooled to 1°C in an ice-water bath for 20-24 h. Roots were fixed in 2 mL Carnoy's solution (1:3, glacial acetic acid : absolute ethanol) and stored at 4°C until examined. For chromosome examination, roots were stained in a 1% acetocarmine solution for 1 to 3 h. Root caps were removed with a razor blade and meristematic tissue squeezed out with a lancet needle. Meristematic tissue was placed on a glass slide in a single drop of 1% acetocarmine, carefully compressed, and covered with a glass slide. Prepared slides were heated to just below boiling and final compression performed manually. A minimum of 3 rootlets were examined for each accession. Observations were made using a Zeiss Photomicroscope III (Carl Zeiss AG, Germany).

Accessions resistant to race TTKSK and initially found to possess 42 chromosomes were assessed for the presence of *Th. ponticum* DNA using genomic in situ hybridization (GISH) with genomic DNA (gDNA) from *Th. ponticum* as a probe (Zhang et al., 2001). To detect the homoeologous group of *Th. ponticum* chromosomes, these accessions were submitted to combined fluorescence in situ hybridization (FISH) and

GISH procedures, using GAA and pAs1 oligonucleotide probes to identify individual wheat chromosomes and *Th. ponticum* gDNA probe to identify alien chromosomes (Danilova et al., 2012). It was assumed that the missing wheat chromosomes were substituted by *Th. ponticum* homoeologs. The FISH+GISH procedure followed modified protocols from Zhang et al. (2001). After removing cover slips from frozen squashed preparations, slides were immersed in 100% ethanol for 5 min, dried and UV cross-linked. The probe mixture (20 µl per slide) contained 50% formamide (Fisher, Cat. BP228-100), 2.75X saline-sodium citrate (SSC) buffer, 13.75% dextran sulfate, 2.4 µg of wheat blocking gDNA, 40 ng of *Th. ponticum* gDNA probe, 1 ng of Cy5-$(GAA)_9$ and 60 ng of TEX615-pAs1- oligonucleotide probes (Integrated DNA Technologies, Inc., Coralville, IA, USA). The mixture of probes and the slide preparations were denatured separately in 100°C water baths. The remainder of the FISH+GISH procedures followed Kato et al. (2006). Slides were incubated at 37°C overnight and washed twice in 2X SSC buffer: 5 min at room temperature, 10 min at 42°C and then in 1X SSC buffer for 5 min at room temperature. Chromosome preparations were mounted and counterstained with 4',6-diamidino-2-phenylindole solution (DAPI) or propidium iodide (PI) in Vectashield (Vector Laboratories, cat # H-1200, H-1300). Images were captured with a Zeiss Axioplan 2 microscope using a cooled charge-coupled device camera CoolSNAP HQ2 (Photometrics, Tuscon, AZ) and AxioVision 4.8 software (Carl Zeiss AG, Germany). Images were processed using the Adobe Photoshop software (Adobe Systems Incorporated, San Jose, CA, USA).

Results

Seedling resistance to stem rust. The W. J. Sando collection was found to harbor 152 accessions with resistance to one or more races of *P. graminis* f. sp. *tritici*. The number of accessions resistant to the individual races in the Ug99 race group, TTKSK, TTKST, and TTTSK, ranged from 52 to 64 (Table 2). Races TRTTF and TPMKC were virulent on the greatest number of accessions, with only 25 of 546 accessions resistant to each. Race RKQQC was the least virulent with 79 accessions displaying resistance in both replications. Full results of the screening are available in Supplementary Table S1.

The reactions of accessions to race TTKSK were correlated with their reactions to races TTKST (r = 0.496), TTTSK (r = 0.480), and QTHJC (r = 0.210), more so than can be expected under the assumption of independence (Table 3). Reactions to race TTTSK were also significantly correlated with reactions to races TTKST (r = 0.365) and RKQQC (r = 0.221). In comparison, reactions to TRTTF were correlated with reactions to races TTTTF (r = 0.317) and TPMKC (r = 0.425). Reactions to races TTTTF and TPMKC (r = 0.411) were also highly correlated.

The result of the MCA is shown in Figure 1. The first dimension (x-axis) explained 64.6% of the variance and separated resistant reactions from susceptible reactions, designating resistant reactions with positive values and susceptible reactions with negative values. This dimension also separated resistance to less virulent races, QTHJC and RKQQC, from resistance to the six more virulent races. The second dimension (y-axis) explained 11.4% of the variance and discriminated resistance into 3 clusters: 1) the Ug99 race group, 2) races RKQQC and QTHJC, and 3) races TTTTF, TPMKC, and TRTTF. The second dimension also distinguished susceptibility to races

possessing virulence on *Sr31* (TTKSK, TTKST, TTTSK) from susceptibility to races avirulent on *Sr31* (TRTTF, TTTTF, TPMKC, QTHJC, RKQQC).

Molecular markers. The results for the molecular marker screening of accessions PI 604981, PI 605057, PI 605286, and PI 611932 are displayed in Table 4. None of the four accessions amplified the *Sr24* amplicon , PI 604981 amplified the *Sr25* amplicon, and PI 611932 amplified the *Sr26* amplicon.

Resistance to the Ug99 race group. Of the 152 resistant accessions, 29 were resistant to races TTKSK (Ug99), TTKST, and TTTSK combined. The pedigrees for these accessions are available in Supplementary Table S2. The 29 Ug99 resistant accessions clustered into 7 different race-specific IT patterns (Table 5). The most common pattern, exhibited by 14 of 29 accessions, combined resistance to the Ug99 race group with resistance to the North American race RKQQC. Accessions sharing this pattern are referred to as Group 1. Resistant ITs in Group 1 ranged from '2-' to '2+' (PI 605023 exhibited IT '2+3' in one replication with TTKSK). Nine accessions resistant to the Ug99 race group were also resistant to all other races used for screening. These accessions are referred to as Group 2. Resistant ITs in Group 2 ranged from '0;' to '2-' (PI's 604981, 604986, 611887, 611915 displayed higher ITs in some replications to some races, see Table 5). Group 3 accessions PI 605079 and PI 605321 displayed expected race-specific resistance patterns and ITs for lines possessing the resistance gene, *SrTmp*. Groups 4 to 7 displayed unique resistance/susceptibility patterns that are not readily associated with any known, single *Sr* gene.

Cytogenetics of selected resistant accessions. Nine accessions, selected for their resistance to race TTKSK, were analyzed using the root squash method to determine the

number of chromosomes present. Accessions PI 604924, 605132, 611887, and 611899 had chromosome counts of 2n = 54. PI 605103 had individual root tips that displayed chromosome counts of 2n = 54, 55, and 56. The remaining accessions, PI 604981, PI 605057, PI 605286, and PI 611932, had chromosome counts 2n=42 in initial examinations and were selected for analysis using GISH and FISH (Figure 2). The ITs of these accessions are displayed in Table 6. The alien parent in the four selected accessions is the decaploid tall wheatgrass, *Th. ponticum* [2n = 10x = 70; genome $JJJJJJ^sJ^sJ^sJ^s$] (Chen et al., 1998). GISH-FISH analysis revealed that three accessions (PI 604981, PI 605286, and PI 611932) were disomic substitutions (20" + 1" E) (Figure 2a, c, d). PI 604981 was a mixture of plants with 2n = 42 or 41; some had a pair of *Th. ponticum* chromosomes substituting the wheat 2D pair, some contained no alien chromosomes. All analyzed plants had rearranged wheat chromosomes: telosomes and dicentric or translocation chromosomes involving 7A and an unknown D-chromosome. Only one plant each of PI 605286 and PI 611932 were analyzed. Each had a pair of *Th. ponticum* chromosomes substituting for wheat chromosome pairs 2D and 6D, respectively (Figure 2c, d). PI 605057 was a mixture of plants with chromosome numbers 2n=44, 43, and 41. Four of six plants analyzed were disomic substitution/additions (20" + 2" E) with 2n=44 and chromosome pair 2D absent (Figure 2.b). Wheat chromosome pair 2D was absent in PI 604981, PI 605057, and PI 605286.

Discussion

Race specific associations and reactions. The analysis of 152 resistant accessions and their reactions to 8 races of *P. graminis* f. sp. *tritici* revealed multiple

positive correlations. However, while Pearson's Product Moment Correlation (Pearson's *r*) allows pair-wise comparisons between races, MCA provides a means to visualize the relationships between all reactions, namely susceptible (S) and resistant (R), to all races of *P. graminis* f. sp. *tritici*. MCA is based on the work of Benzérci and colleagues and designed for the analysis of categorical data (Blasius and Greenacre, 2006). It can be used to visualize the spatial relationships among the "responses" of accessions to individual races of *P. graminis* f. sp. *tritici*, i.e., how resistance to TTKSK correlates with resistance to all other races.

The results of the analysis using Pearson's *r* and those of the MCA tease apart different relationships among the races of stem rust used in this study. The key factors affecting race clustering in MCA were virulence (more virulent and less virulent) and whether a race was virulent/avirulent on *Sr31*. While resistance to races RKQQC and QTHJC were distinctly clustered in the MCA, reactions to both races were correlated with one or more races in the Ug99 race group using Pearson's *r*. Also, North American races (TTTTF, TPMKC, QTHJC, and RKQQC) separated into distinct clusters in the MCA but showed varying degrees of correlation when compared using Pearson's *r*. The genetic relationship between these races has not yet been determined but recent advances in the genetics of *P. graminis* f. sp. *tritici*, such as a published genome and SNP assay may reveal underlying relationships that could explain the phenotypic relationships observed here (Szabo et al., 2014). Overall, the various degrees of clustering and correlation indicate that *Sr* gene/s effective against multiple races may be present in the W. J. Sando collection.

Resistance to the Ug99 race group. Accessions that displayed resistance to each of three races in the Ug99 race group (TTKSK, TTKST, and TTTSK) segregated into 7 groups based on race-specific IT patterns. Some accessions displayed different ITs between replications of a single *P. graminis* f. sp. *tritici* race. Multiple accessions within this collection that were examined cytogenetically displayed inter-seedling chromosomal variation (see discussion of cytogenetics below). The different ITs displayed by a single accession to a single race could be explained by the presence of different chromosome complements within the same accession. It is also possible that slight environmental variability between replicates may have influenced individual accessions' reactions to a single race of the stem rust pathogen. Group 1 accessions are characterized by their additional resistance to race RKQQC and susceptibility to the remaining races. Races TTKSK, TTKST, TTTSK and RKQQC are all avirulent to stem rust resistance gene, *SrTmp*. This gene was introduced into US winter wheat germplasm with the arrival of Turkey hard red winter wheat in 1874 and is a widely distributed *Sr* gene in hard red winter wheat germplasm (Roelfs and McVey, 1979). However, race QTHJC, to which Group 1 accessions are susceptible, is also avirulent on *SrTmp*. All Group 1 accessions exhibited ITs of '3+' or greater when screened with QTHJC except accessions PI 605016 and PI 611927, which exhibited ITs of '1+' and '2-', respectively, in one of two replicates. Phenotypic data indicates that *SrTmp* is not the *Sr* gene conferring resistance in Group 1. Accessions in Group 1 have several alien species listed in their pedigrees including *Th. ponticum*, *S. cereale*, *T. turgidum* subsp. *dicoccum*, and *Ae. ventricosa* (Table S2). The lack of a single, consistent alien parent in Group 1 pedigrees suggests that the *Sr* gene/s conditioning resistance in this group may be from *T. aestivum*.

In addition to *SrTmp*, there are four *T. aestivum*-derived *Sr* genes effective against the Ug99 race group: *Sr28*, *Sr42*, *Sr57/Lr34*, and *Sr9h* (formerly *SrWeb*) (Rouse et al., 2014). *Sr28*, from the US cultivar 'Kota', is not effective against RKQQC and conditions an IT response of ';3', not '2', to TTKSK, TTKST, and TTTSK (McIntosh et al., 1995; Rouse et al., 2012). *Sr57/Lr34* confers adult plant resistance only (Krattinger et al., 2009; Kolmer et al., 2011). However, *Sr42*, from the Japanese cultivar 'Norin 40', shares the same resistance/susceptibility pattern as Group 1 (Ghazvini et al., 2012). The expected low IT for *Sr42* ranges from ';1' to '2' (Ghazvini et al., 2012). Lopez-Vera et al. (2014) suggested that *Sr42* and *SrTmp* may be the same gene or alleles of the same gene. However, *SrTmp* is effective against race QTHJC to which Norin 40, donor of *Sr42*, is susceptible (Ghazvini et al., 2012). *Sr9h* also shares the same pattern as Group 1 (Hiebert et al., 2010; Rouse et al., 2014). Therefore, current data cannot differentiate the race-specific IT pattern of Group 1 accessions from the patterns displayed by *Sr42* and *Sr9h*.

Eight of nine accessions in Group 2 have *Th. ponticum* listed in their pedigree, while one contains *Th. intermedium*. *Th. ponticum* is the donor of stem rust resistance genes *Sr24*, *Sr25*, *Sr26*, and *Sr43*; each effective against many races of *P. graminis* f. sp. *tritici* (McIntosh et al., 1995; Dundas et al., 2007; Liu et al., 2010; Niu et al., 2014b). *Sr24* is not effective against race TTKST and each Group 2 accession displayed resistant ITs against this race. *Sr25* provides marginal resistance to race TRTTF, expressing an IT of '2+3-' in a 'Little Club' background, however, this is still within the range of low ITs expected for this gene (McIntosh et al., 1995; Olivera et al., 2012). PI 604981 displayed mixed reactions to race TRTTF and also amplified the *Sr25*-associated amplicon when

screened with the PCR marker, *Gb*, developed by Prins et al. (2001) (see Table 4). PI 611899 also displayed a higher IT to race TRTTF in one replication but has not been screened with *Sr25*-linked molecular markers. All other accessions in Group 2 displayed much lower ITs than that conferred by *Sr25* when challenged with race TRTTF.

Sr43 is a temperature sensitive gene, becoming ineffective at 26°C, and also displays a higher IT ('12;/;12') to race QTHJC than that exhibited by Group 2 accessions except PI's 611899 and 604986 (Niu et al., 2014b). Because the screenings did not occur at temperatures above 26°C we cannot rule out the possibility that Group 2 accessions may possess *Sr43*.

Sr26 has an expected low IT that ranges from ';' to '2-' and may also be a candidate for the gene providing the resistance observed in accessions derived from *Th. ponticum* (McIntosh et al., 1995). Accession PI 611932 amplified the *Sr26*-associated fragment when screened with the multiplex PCR markers *BE518379/Sr26#43* (Mago et al., 2005; Liu et al., 2010) (see Table 4). PI 604981 was the only other Group 2 accession screened with the same multiplex marker and it failed to amplify the *Sr26*-associated amplicon. Further screening at temperatures above 26°C as well as more extensive genotyping will need to be conducted to determine the resistance gene/s present in Group 2. However, molecular and phenotypic analyses, as well as cytogenetic results to be discussed later, indicate that Group 2 accession PI 611932 likely possesses *Sr26*.

PI 605132 is the only accession in Group 2 with *Th. intermedium* in its pedigree. *Th. intermedium* is the donor of *Sr44*, a resistance gene effective against races TTKSK, TTKST, and TTTSK but susceptible to TRTTF (Liu et al., 2013). Liu et al. (2013) postulated that *Th. intermedium* chromosome segment 7J#1L harbors an unknown stem

rust resistance gene that does confer resistance to race TRTTF. PI 605132 was found to have a chromosome composition of 2n=54 and may possess multiple unknown stem rust resistance genes located on *Th. intermedium* chromatin.

Group 3 accessions, PI 605079 and PI 605321, displayed IT patterns that were indistinguishable from *SrTmp*. PI 605079 was more resistant (IT = '1;') to RKQQC than the expected low IT ('2' to '23') for *SrTmp* but this may be due to experimental variance. It is possible that resistance in these accessions is derived from *SrTmp*, however we cannot exclude the possibility that additional *Sr* genes may be present in these accessions. Allelism tests could determine if *SrTmp* is the source of resistance in these accessions.

Groups 4 through 7 each contained a single accession whose IT pattern could not be matched to any known single stem rust resistance gene. Each accession has a unique alien parent within its pedigree and more work would need to be done to determine the nature of each line's resistance (Table S2). However, we postulate that accessions PI 604884, PI 605094, PI 605098, and PI 605246 possess new resistance genes effective against the Ug99 race group (Table 5).

Molecular marker analysis of selected accessions. Only two of four accessions analyzed using GISH-FISH amplified alleles associated with known stem rust resistance genes derived from *Th. ponticum*. Recent work with *Thinopyrum* spp. or *Thinopyrum*-wheat partial amphiploids have found that false positives are common when using molecular markers designed from hexaploid bread wheat to screen *Thinopyrum* material (Turner et al., 2013; Zheng et al., 2014). Zheng and colleagues (2014) found that some markers were species or genus specific when used to screen five *Thinopyrum* spp. PI 604981 amplified the *Sr25* associated aplicon when screened with marker *Gb*. Marker *Gb*

has been verified in multiple studies as amplifying a 130-bp product only in wheat cultivars known to possess *Sr25* (Liu et al., 2010; Yu et al., 2010). However, no cultivars tested in these studies possessed whole chromosomes from a *Thinopyrum* species as does PI 604981. Additionally, all species of *Thinopyrum* tested by Zheng and colleagues, except *Th. junceum*, possessed some accessions that were positive for the *Sr25* amplicon (Zheng et al., 2014). Because *Sr25* has been shown to be derived from *Th. ponticum*, this may indicate that *Gb* detects a common *Thinopyrum* locus regardless of the presence of *Sr25* (McIntosh et al., 1977; Kim et al., 1993). Phenotypic evidence indicates that the resistance observed in PI 604981 (';' to ';3') differs from that expected of *Sr25* ('1' to '23') (McIntosh et al., 1995). The variability in both molecular and phenotypic evidence indicates that PI 604981 either does not carry *Sr25* or possesses a unique allele of this resistance gene. Accession PI 611932, which amplified the *Sr26*-associated amplicon, is discussed extensively both in the previous subsection and below.

Cytogenetics of selected accessions. Cytogenetic analysis of selected W. J. Sando accessions confirmed previous reports of the mixed ploidy and chromosome complements present in this collection (Cox et al., 2002; Oliver et al., 2006). Each accession that was analyzed using FISH possessed *Th. ponticum* as the alien species in the listed pedigree. Stem rust resistance genes *Sr24*, *Sr25*, *Sr26*, and *Sr43* are all derived from *Th. ponticum* (Knott, 1988; McIntosh et al., 1995; Friebe et al., 1996). Both, *Sr25* and *Sr43* are derived from *Th. ponticum* group 7 chromosomes (Knott, 1988; Friebe et al., 1996). Only PI 604981 amplified the expected product when screened with *Sr25* marker *Gb* (see Table 4). *Sr25* is located on a *Th. ponticum* group 7 chromosome, and while only chromosome pair 2D is missing from PI 604981, each plant of this accession

that was examined also possessed telosomes and dicentric or translocation chromosomes involving 7A and an unknown D-chromosome (Friebe et al., 1994). As we were able to identify the unknown chromosome involved as belonging to the D genome, the only *Th. ponticum* genetic material in this accession is the chromosome pair replacing 2D. As marker *Gb* has only been tested in adapted *T. aestivum* lines, it is possible that *Gb* amplifies non-*Sr25* loci located in other regions of the *Th. ponticum* genome. Also, see the above subsection for recent work with *Thinopyrum* germplasm and molecular markers. Cytogenetic evidence supports our statement that PI 604981 does not possess *Sr25*.

Sr43 is derived from a *Th. ponticum* chromosome $7el_2$ and was transferred to wheat chromosome 7D (Knott et al., 1977; Kibirige-Sebunya and Knott, 1983). Because all accessions examined are fertile and show no loss in vigor, we assume with Knott and colleagues (1977) that the *Th. ponticum* chromosomes found in these accessions are homeologous with the chromosomes they replaced. As no accession was missing wheat group 7 chromosomes, it is unlikely that the *Th. ponticum* chromosome carrying *Sr43* is the source of TTKSK resistance in these accessions.

Sr24, while effective against TTKSK (Ug99), is ineffective against TTKST to which these accessions, minus PI 605057, were resistant. However, PI 605286 exhibited a mixed reaction, '0;/3' to race TTKST. In contrast, PI 604981 and PI 611932 exhibited highly resistant ITs when inoculated with TTKST. *Sr24* was transferred from a group 3 *Th. ponticum* chromosome to chromosome 3D of wheat and has also been transferred to the short arm of wheat chromosome 1B (The et al., 1991; Jiang et al., 1994; Mago et al., 2005). PI 605286 did not amplify the *Sr24*-associated fragment when screened with PCR

marker *Xbarc71* identified by Mago et al. (2005) (Table 4). Yu et al. (2010) have successfully used this marker to genotype 228 wheat lines from CIMMYT, ICARDA, China, and other miscellaneous origins for the presence of *Sr24*. However, despite the absence of molecular data, screening results remain inconclusive in regards to whether PI 605286 possesses *Sr24* due to potential false negatives when using molecular markers designed from a specific *Th. ponticum* translocation.

PI 605057 was susceptible to races TTKST, TTTSK, TPMKC and exhibited a resistant reaction and a susceptible reaction to race RKQQC in separate replicates. All known *Sr* genes derived from *Th. ponticum* are resistant to these races (*Sr24* is not effective against TTKST). To our knowledge no known *Sr* gene shares this resistance/susceptibility pattern and this may indicate either a novel *Sr* gene/s or a new allele/s of known *Sr* genes or a heterogeneous structure of the PI 605057 population.

Sr26 was transferred from the long arm of a *Th. ponticum* group 6 chromosome to wheat chromosome 6A (Knott, 1961; 1968). In GISH analysis, PI 611932 was shown to possess a single pair of *Th. ponticum* chromosomes and lacked wheat chromosome group 6D, indicating that the *Th. ponticum* chromosomes may be group 6 chromosomes possessing *Sr26*. PI 611932 also possessed a possible T6AS·6AL/6DL translocation. Phenotypic, molecular, and cytogenetic data indicate that *Sr26* is the resistance gene in PI 611932 but no allelism tests have been conducted to confirm this. We postulate that PI 605057 and PI 605286, and possibly PI 604981, have uncharacterized stem rust resistance genes effective against the Ug99 race group. Chromosome engineering efforts are currently underway to reduce the size of alien chromatin in these accessions using a

homozygous *ph1b* line developed at Kansas State University Wheat Genetics Resource Center (Friebe et al., 2012).

Conclusions

The W. J. Sando collection is known to harbor valuable resistance genes to multiple diseases effecting wheat production. Though individual lines had been characterized for their reaction to stem rust, to our knowledge, no published data exists characterizing the entire collection. We were able to characterize the entire collection for stem rust resistance using 8 races of *P. graminis* f. sp. *tritici*. Furthermore, the 29 accessions identified with resistance to three races within the Ug99 race group are a valuable resource in the fight against stem rust. Future work with this germplasm will proceed more efficiently with the aid of this screening. Of the 29 accessions resistant to Ug99 races, 25 could not be distinguished from known *Sr* genes but future work may show that some accessions possess new genes or alleles. Cytogenetic techniques allowed us to identify promising resistant accessions, three of which are postulated to contain new resistance. Introgression of these resistance genes into adapted wheat germplasm will provide additional tools for breeding resistant wheat cultivars. Altogether seven accessions were identified as candidates possessing novel stem rust resistance.

CHAPTER 3:

Ug99 resistance in accession PI 410954

The threat posed by the Ug99 race group of *P. graminis* f. sp. *tritici* has led to a large coordinated effort to find and deploy new sources of resistance to this pathogen (Xu et al., 2009; Rouse and Jin, 2011; Rouse et al., 2011b; a; Endresen et al., 2012; Fedak et al., 2012; Turner et al., 2013; Newcomb et al., 2013; Njau et al., 2010; Aghaee-Sarbarzeh et al., 2013). Rouse et al. (2011b) screened over 700 spring wheat lines with stem rust pathogen race TTKSK and found 88 accessions that conferred some degree of resistance at the seedling stage. One accession from this study displaying broad-spectrum resistance to various races of *P. graminis* f. sp. *tritici* was PI 410954. PI 410954 was developed in South Africa and deposited in the USDA National Small Grains Collection in 1975 (USDA, 1978). The pedigree of PI 410954 consists of a cross between CI 13523 and the US cultivar 'Triumph', crossed again with a Uruguayan cultivar, 'Klein Impacto'. CI 13523 is the accession number of the US cultivar, 'Agent', the source of the stem rust resistance gene *Sr24* (Friebe et al., 1996). Initial screening indicated that PI 410954 possessed two genes conferring resistance to *P. graminis* f. sp. *tritici* race TTKSK. The goal of this research was to use doubled haploid technology to locate and introgress the unknown resistance gene in PI 410954.

Materials and Methods

Plant materials and stem rust screening. Seed for accession PI 410954 was obtained from the USDA National Small Grains Collection in Aberdeen, ID. All seed for

susceptible wheat lines ‘LMPG-6’, North Dakota spring wheat cultivar ‘Faller’, and Minnesota spring wheat cultivar ‘RB07’, are maintained at the USDA Cereal Disease Laboratory in St. Paul, MN (Mergoum et al., 2008; Anderson et al., 2009). Initial seed for Faller and RB07 were obtained from North Dakota State University (NDSU) and the University of Minnesota (UM), respectively. A cross between PI 410954 (male) and Faller (female) was made in the spring of 2012. F_1 progeny from this cross, given laboratory identifier 12XR031, were used as male parents for a top cross to RB07 in the summer of 2012.

Generation F_2 progeny of a Faller/PI 410954 population (11XR188) were evaluated at the seedling stage with stem rust race TTKSK in two replications (n=98 and n=96, respectively). In short, seedlings were inoculated between 7-9 days after germination using a suspension of *P. graminis* f. sp. *tritici* urediniospores in a light mineral oil, Soltrol 170 (Chevron Phillips Chemical Company LP). Inoculated seedlings were placed in dew chambers overnight and grown in a greenhouse maintained at 18±2°C until 14 days after inoculation at which point they were scored for seedling infection types (ITs) according to the scale developed by Stakman et al. (1962). F_2 progeny from two different LMPG-6/ PI 410954 populations (12XR019 and 12XR020) were screened with race TTKSK (n = 356 and n = 104) and assessed for seedling infection types. Seedling infection types of ‘2+’ or lower were categorized as resistant and those of ‘3’ or higher as susceptible.

Generation F_2 seed from a Faller/PI 410954 population (11XR188-3) were planted at the University of Minnesota St. Paul Campus Experimental Fields during the 2012 field season. F_3 seed was harvested and at least 15 plants of each $F_{2:3}$ family (*n* =

100) were screened using two *P. graminis* f. sp. *tritici* races, TTKSK (isolate: 04KEN156/04) and TRTTF (isolate: 06YEM 34-1), to confirm segregation ratios observed in previous screenings. After each individual in a family was scored, all families were placed into one of three categories: homozygous resistant, homozygous susceptible, or segregating. All seedling screenings were performed at the jointly managed University of Minnesota/Minnesota Department of Agriculture BioSafety-Level 3 facility (race TTKSK) or at the USDA Cereal Disease Laboratory (race TRTTF) during the winter months of December to February.

Molecular marker screening. Leaf tissues for all molecular analyses were collected from seedlings and homogenized in liquid nitrogen. DNA extractions were performed using a modified CTAB method or a DNA microprep for PCR designed by Edwards et al. (1991). Based on pedigree information indicating the presence of *A. elongatum* (synm. *Th. ponticum*), PI 410954 was screened with PCR markers *Xbarc71*, multiplex Sr26#43/BE518379, and *Gb* associated with resistance genes *Sr24*, *Sr26*, and *Sr25*, respectively. *Th. ponticum* is the donor of these three *Sr* genes (Prins et al., 2001; Mago et al., 2005; Liu et al., 2010). PI 410954, Faller, and RB07 were also screened with the markers listed in Table 7. Published PCR protocols were followed for all markers.

Generation TC_1F_1 progeny of an RB07//Faller/PI 410954 population (n = 425) were screened with race TRTTF to select resistant plants for doubled haploid (DH) production at Washington State University's Wheat Doubled Haploid facility. Twenty-four plants were selected based on their low IT and the results of marker analysis for agronomically desirable traits. After removing all infected plant tissue and treating plants with the fungicide Tilt® (propiconazole; Syngenta AG), selected TC_1F_1 plants were

mailed from the Cereal Disease Laboratory to Washington State University. DH progeny (n = 213) were received in spring of 2014 and screened with *P. graminis* f. sp. *tritici* race TTKST (isolate: 06KEN19-V 3) as described above. DH progeny were screened with race TTKST in two replications with 5-8 plants per line in each replicate. All DH lines were screened, following published protocols, using *Sr24* associated PCR markers *Xbarc71* and *Sr24#12* (Mago et al., 2005). Lines lacking *Sr24* (n = 108) and the parents (RB07, Faller, and PI 410954) were genotyped using a custom Illumina GoldenGate 90K SNP chip at the USDA Small Grains Genotyping Laboratory in Fargo, North Dakota (Wang et al., 2014).

Data analysis. All statistical analysis was performed in R v. 3.1.1 within the RStudio GUI (RStudio, 2013; R Core Team,). SNP data generated using the 90K SNP chip was inspected manually in GenomeStudio. All SNPs mapped by Wang et al. (2014) were exported as a tab-delimited text file (*n* = 38800). SNPs with missing data greater than 10% (no calls > 12) were removed from analysis leaving a total of 36,068 SNPs. SNP data was analyzed using R package ‘rrBLUP’ (Endelman, 2011). The SNP data set was passed through the function ‘Amat’ in order to develop an appropriate additive relationship matrix for genome wide association study (GWAS). ‘Amat’ automatically removes monomorphic markers and the SNP data set was trimmed from 36,068 to 27,196 SNPs. The number of SNPs per individual chromosome, (according to the mapped locations of SNP by Wang et al., 2014), is displayed in Table 8. Phenotypes for the 111 non-*Sr24* lines were coded as follows 0 = susceptible and 1 = resistant. All genotypes were converted from the form AA, AB, and BB to 1, 0, and -1, respectively. No calls were coded as NA. The command ‘GWAS’ conducts a genome wide association analysis

using phenotype and genotype data using a mixed model developed by Yu et al. (2006). The command line for this analysis was entered as follows:

```
GWAS(pheno, geno, K=A, n.core=16, P3D=TRUE, n.PC=2)
```

'Pheno' and 'geno' are data frames containing the phenotypic and genotypic data respectively. 'K' is the kinship matrix for covariance between lines and was assigned the matrix, A, developed via the function 'A.mat'. 'n.core' divides the SNPs into 16 groups to allow them to be analyzed in parallel on a single machine. 'P3D' (population parameters previously determined) when TRUE is equivalent to the expedited efficient mixed-model association (EMMAX) developed by Kang et al. (2010). EMMAX is able to correct for sample structure by taking into account the pairwise relationship between individuals in a population. The final term, 'n.PC', determines the number of principle components to include as fixed effects. The number used for this setting was determined by conducting an eigenvalue decomposition of matrix A developed via the function 'A.mat'. The first two principal components accounted for ~18% of the total spectrum and were included as covariates in the GWAS (see Figure 3).

For each line, the percentage of SNPs shared with Faller and/or RB07 was calculated in Excel to determine the level of similarity between the DH lines and the adapted parents. Only SNPs unique to PI 410954 were used for this analysis (n = 6451). Unique SNPs were defined as those SNPs in which PI 410954 did not share a genotype with either Faller or RB07. Missing SNP data (NA) was excluded from this analysis. A boxplot and a "beanplot" were created in R, the beanplot was created using R package 'beanplot' (Kampstra, 2008).

Results

Stem rust screening. Stem rust screening results for early generation populations are displayed in Table 9. Generation F_2 populations, 12XR019 and 12XR020, segregated for resistance to race TTKSK at a 15:1 ratio ($\chi^2 = 1.38$ $P = 0.2395$; $\chi^2 = 0.041$ $P = 0.8406$, respectively), indicating resistance controlled by 2 genes. However, the 11XR188 F_2 population did not segregate at the same ratio ($\chi^2 = 22.90$ $P = 1.71 \times 10^{-6}$) but instead segregated at a 3:1 ratio ($\chi^2 = 0.0035$ $P = 0.953$). The $F_{2:3}$ population, 11XR188-3 was screened with races TTKSK and TRTTF. 11XR188-3 $F_{2:3}$ families segregated in a 15:1 manner when screened with TTKSK ($\chi^2 = 0.0107$ $P = 0.9177$) but did not segregate in this ratio when screened with TRTTF when significance was set at $P < 0.05$ but did so when $P < 0.01$ ($\chi^2 = 4.26$ P = 0.03894). While 11XR188-3 $F_{2:3}$ families showed slightly different segregation ratios between the two race evaluations, the ratios of resistant:susceptible individuals were not independent ($\chi^2 = 1.53$ $P = 0.2167$). While the number of families within the categories homozygous resistant, segregating, and homozygous susceptible did not differ, a *t*-test revealed that the results from the two screenings were significantly different (df= 99, $t = 3.34$, $P = 0.001$). The ITs and number of progeny for the 24 TC_1F_1 plants selected for production of DH progeny can be seen in Table 10.

Molecular marker screening. PI 410954 failed to yield the *Sr*-associated product for molecular marker *Gb* (*Sr25*) and the multiplex marker Sr26#43/BE518379 (*Sr26*). In turn, the expected products for the *Sr24* SSR marker *Xbarc71* and the AFLP marker *Sr24#12* were amplified in PI 410954. The expected products for the presence of two dwarfing genes, *Rht-B1b* and *Rht-D1b*, were not amplified in PI 410954. Both RB07 and

Faller possess the dwarfing gene *Rht-B1b*. None of the three lines amplified the expected product when screened with the marker *csLV34* linked with the pleiotropic rust and mildew resistance gene, *Lr34/Sr57/Yr18/Pm38* (Lagudah et al., 2006). Results of the molecular screenings are displayed in Table 11.

SNP genotyping and mapping. All genetic distances used are those reported by Wang et al. (2014) using scaled distances based on the SynOP DH genetic map as described in Cavanagh et al. (2013). SNP data was visually inspected within Illumina's GenomeStudio using in-program filtering. Filtering was defined to select SNPs at which PI 410954 and the resistant DH progeny shared a genotype and Faller, RB07, and susceptible DH progeny shared a different genotype. All filtered SNPs were located on chromosome 6D. However, to avoid user bias, all SNPs with reported map positions were exported and stringently filtered for missing data. Figure 4 shows the Manhattan plot of all 27,196 SNPs created using the GWAS function in the R package 'rrBLUP'. A total of 15 SNPs with q values < 0.05 were discovered, these SNPs and their associated $-log_{10}p$ values are listed in Table 12. The haplotype of each line for the 15 SNPs as well as each SNPs chromosome assignment and map location is listed in Table 13. Two of these SNPs, BS00009514_51 and BS00022094_51 map to both chromosome 6A and 6D in the consensus map developed by Wang et al. (2014). Kukri_rep_c105406_308 maps to chromosome 6A exclusively. All remaining SNPs are located within a 6 cM region of chromosome 6D between 18.2 cm and 24.8 cm, using scaled map distances in Wang et al. (2014).

Similarity between DH lines and elite parents. The percentage of shared unique SNPs between DH lines and the elite parents ranged from 43.45% (12XR170-5-3) to

92.20% (12XR163-22-6), with a mean of 75.5%, median of 76.77%, and standard deviation of 8.51%. The mean percentage for resistant lines was slightly higher, 76.53%, but insignificant (p = 0.29) compared to that of the population. In resistant lines, 60% (15/25), had shared SNP percentages above the population average compared with 55% of the whole population. Figure 5 shows a boxplot (5a) and beanplot (5b) of the distribution of the shared SNP percentages.

Discussion

Similarity of DH lines to elite parents. Backcross conversion is a standard breeding process used to improve advanced lines that are deficient in one or more traits (Forster et al., 2007). The method has traditionally consisted of an initial cross between the elite line and a donor line possessing the desired characteristic followed by subsequent backcrosses to the elite line coupled with selection to minimize undesired traits. This process takes multiple generations to achieve the desired goals of introgression and donor genome reduction. Doubled haploid production can improve this process by allowing the breeder to forego as few or as many natural generations as needed while still achieving the goals above, shortening the introgression process considerably. The research presented here adopted this process for the purpose of introgressing unknown stem rust resistance into elite spring wheat cultivars. While unfamiliar with Forster's article at the time, we followed the outline provided in Figure 3 of Forster et al. (2007) with slight modifications. In lieu of a single elite parent, two high quality hard red spring wheat cultivars from the northern Great Plains were selected. Faller, released by the North Dakota Agricultural Experiment Station (NDAES) in 2008,

combines high yield, good end-use quality, and resistance to Fusarium head blight (FHB) (Mergoum et al., 2008). Faller was derived from multiple NDAES experimental lines and released cultivars from both the NDAES and the University of Minnesota Agricultural Experiment Station/USDA-ARS. RB07 was released by the University of Minnesota Agricultural Experiment Station in 2007 due to its high and consistent yield, earliness, and disease resistance (Anderson et al., 2009). RB07 and Faller were the two most commonly grown wheat cultivars in Minnesota during 2010 and 2011. Faller yields are slightly higher than RB07's, 4693 kg ha^{-1} and 4467 kg ha^{-1} respectively, and RB07 has slightly higher protein than Faller, 15% and 14.6% respectively (Mergoum et al., 2008; Anderson et al., 2009).

One of the primary objectives of this research was to make this new source of resistance available to breeders. For this reason, we adopted a rapid germplasm advancement strategy incorporating top crosses and DH production. The percentage of unique SNPs (those in which PI 410954 did not share a genotype with either Faller or RB07) shared by each DH line with Faller and/or RB07 is a direct measure of genotypic similarity and provides insight into the amount of donor germplasm within the DH lines. The average percentage of SNPs shared with Faller/RB07 was 75.5%. This average matches the genotypic ratios predicted based on the crossing scheme employed. However, six lines carrying the unknown resistance had shared SNP percentages ≥ 85%: 12XR162-3-3, 12XR168-5-2, 12XR168-5-4, 12XR168-5-6, 12XR168-5-7, and 12XR169-13-8. Lines 12XR168-5-2, 12XR168-5-7, and 12XR169-13-8 displayed consistent levels of resistance to *Sr24*-virulent race TTKST. The remaining lines were scored as susceptible (IT ≥ '3') in one of two screenings; however, resistance phenotypes

for this population did range as high as '2+3'. Figure 6 displays the ITs displayed by various lines when screened with race TTKSK. Due to the high degree of SNPs shared with elite cultivars, these lines may be promising material for breeders but field trials will need to be conducted to confirm their agronomic potential.

GWAS. The R package 'rrBLUP' was developed as a genomic selection tool by Jeffrey Endelman (2011). The GWAS function within this package is based on the mixed model developed by Yu et al. (2006). This mixed model reduces Type I and Type II errors that occur due to unaccounted population structure within the population of interest. Below is the equation used for the mixed model in the 'rrBLUP' package:

$$y = X\beta + Zg + \varepsilon \quad [1]$$

The term $X\beta$ is a vector of fixed effects that can model environmental variables and population structure (Endelman, 2011; Yu et al., 2006). The term g models the genetic background of each line as a random effect (Endelman, 2011). Both X and Z are indices matrices that relate y to β and g, respectively. Finally, ε accounts for residual variance. The advantage of the mixed model is its ability to account for various levels of relatedness within individuals contained in the sample (Yu et al., 2006; Kang et al., 2008; 2010). Mixed models have been used in both maize and wheat to perform association analysis for various traits of interest, including disease resistance (Parisseaux and Bernardo, 2004; Breseghello and Sorrells, 2006).

The results of the GWAS function in the package rrBLUP are reported via a Manhattan plot, a QQ plot, and a table listing all SNPs and their associated $-log_{10}p$

value. The key feature of this analysis is the Manhattan plot (see Figure 4) showing the location of significant SNPs and the relative level of their significance. The dashed line in the plot represents a *q* value of 0.05, which corresponds to a false discovery rate (FDR) of 5% (Storey and Tibshirani, 2003). In contrast, *p* values correspond to a false positive rate, therefore a *p* value of 0.05 would mean that one would expect 5% of truly null SNPs to be called significant. This would correspond to more than 1300 SNPs being "significant" in the current study. On the other hand, a *q* value of 0.05 means that one can expect, on average, that 5% of SNPs found to be significantly associated with the phenotype of interest would actually be null. In the current data set this corresponds to a single SNP out of 15 significant SNPs.

Table 12 lists 15 SNPs with significant associations with the resistance phenotype observed in 25 DH lines. Two SNPs, BS00009514_51 and BS00022094_51, mapped to both chromosomes 6A and 6D in Wang et al. (2014) consensus map. However, these SNPs are listed as occurring only on chromosome 6A in SNP data analyzed in Illumina's GenomeStudio. Both SNPs are mapped to the same position, 23.84 cM, on chromosome 6D (see supplementary table S13 in Wang et al., 2014), placing them within the interval of the 12 significant SNPs mapped to chromosome 6D. We do not know why only the 6A SNP variants are included in the GoldenGate 90K assay. The third SNP mapped to 6A, Kukri_rep_c105406_308, is only mapped to this location. It is possible that this SNP is a false positive given an FDR of 5% or that this SNP corresponds to polymorphism in 6D in our germplasm. Overall, evidence strongly suggests that the resistance gene in PI 410954 is located on the short arm of chromosome 6D.

The haplotypes of all 108 DH lines and elite parents are displayed in Table 13. A single susceptible line, 12XR169-13-4, (IT = 3 LIF, low infection frequency) possessed the full resistance haplotype. Unfortunately, due to seed shortages this line was only screened once. It is possible that due to the difficult nature of scoring in this population that this line would prove to be resistant in subsequent screenings. Three additional susceptible lines, 12XR153-13-4, 158-13-10, and158-13-14, had the resistant alleles at 12 or 13 of 15 resistance-associated SNPs.

All resistant DH lines derived from the TC_1F_1 plant 12XR158-3 lacked the resistant haplotype at SNPs Excalibur_rep_c66622_1066 and Excalibur_s114066_247. However, these lines possessed the resistant haplotype at SNPs mapped to the same location. A single resistant DH line, 12XR169-13-2 lacked SNP Excalibur_rep_c66622_1066 as well. These ambiguities could be associated with genotyping errors or recombination. We are currently in the process of converting informative SNPs to KASP markers that will be used to screen 11XR188-3 $F_{4:5}$ families to verify the location of the resistance gene in a biparental population.

Gene postulation. There are three named stem rust resistance genes effective against TTKSK known to be located on 6DS: *Sr42* , *SrCad*, and *SrTA10187* (Olson et al., 2013a; McIntosh et al., 1995; Hiebert et al., 2011; Ghazvini et al., 2012). *SrTmp*, while unconfirmed, has also been postulated to be located on 6DS (Lopez-Vera et al., 2014). The resistance response of the DH lines was much higher than the low infection type for *Sr42*, which has a low infection type that ranges from 1- to 2 (Ghazvini et al., 2012). Currently there is no data distinguishing *SrCad* and *Sr42* and further evidence is needed to determine the allelic relationship between these two genes (Lopez-Vera et al., 2014;

Yu et al., 2014). *SrTA10187* was discovered in *Aegilops tauschii*, a species not listed in any parental pedigree. The resistant phenotype of '2+' observed in this study is much higher than that observed for *SrTA10187* (';1+') (Olson et al., 2013a). However, this difference in infection type could be due to reduced effectiveness of the same resistance gene at greater ploidy levels. Because *Ae. tauschii* is the postulated progenitor of the D genome in hexaploid wheat, it is possible that another *Ae. tauschii* gene may be responsible for the resistance in this work (Kihara, 1944; McFadden and Sears, 1946). In the 90K assay there 4,427 SNPs that are functional markers developed from the resequencing of two *Ae. tauschii* accessions (You et al., 2011; Wang et al., 2014). Each SNP derived in this way has a label beginning with "D_". Within the interval containing the significant SNPs there are two such markers: D_contig14230_361 (19.05 cM) is polymorphic but both PI 410954 and RB07 share the same allele at this locus and D_contig17056_55 (23.84 cM) is monomorphic. This is consistent with Wang et al. (2014) finding that only 18% of SNPs derived from *Ae. tauschii* were polymorphic within a diverse wheat panel of 550 lines. While a gene from an initial hybridization event between tetraploid wheat and *Ae. tauschii* may be the gene of interest here, it is likely that this gene has undergone evolutionary changes that may render it undetectable when using SNPs from extant *Ae. tauschii* accessions.

The low infection type for *SrTmp*, '2-' to '23', is very similar to the resistance observed in the DH lines when screened with *P. graminis* f. sp. *tritici* race TTKST (McIntosh et al., 1995). PI 410954 has the cultivar Triumph listed in its pedigree and *SrTmp* is found in the Triumph background quite often (Roelfs and McVey, 1979). If the gene conferring resistance in the DH lines does prove to be *SrTmp*, this will be the first

report of this gene's introgression into United States hard red spring wheat. It is questionable whether this is a good thing due to two facts: 1) historic *P. graminis* f. sp. *tritici* races 15 and 56, both responsible for severe crop losses, are virulent on lines with *SrTmp* (McIntosh et al., 1995); 2) *SrTmp* is widely deployed in the southern Great Plains hard red winter wheat regions and the possible dependence on this gene in hard red spring wheat may make this crop more vulnerable to future epidemics of stem rust emerging from the southern Great Plains (Roelfs and McVey, 1979).

Conclusions

Regardless of the utility of this resistance gene in hard red spring wheat germplasm, we believe this research serves as strong proof of concept for the ability of DH technology to significantly shorten pre-breeding cycles. The resistance in PI 410954 was first identified in 2011 and within a span of five wheat generations we were able introgress this resistance into elite germplasm and elucidate the location of the unknown resistance gene. The combination of DH technology with current genotyping capabilities is a powerful tool for plant pathologists and breeders interested in the rapid discovery and deployment of effective resistance.

Table 1. Virulence/avirulence patterns of *Puccinia graminis* f. sp. *tritici* races used to screen the W. J. Sando collection of wheat-intra/intergeneric hybrids and derivatives. All *Sr* genes listed are part of the International Wheat Stem Rust differential series.

Race	Isolate	Virulence/Avirulence
TTKSK	04KEN156/04	5,6,7b,8a,9a,9b,9d,9e,9g,10,11,17,21,30,31,38,McN/24,36,Tmp
TTKST	06 KEN 19-V 3	5,6,7b,8a,9a,9b,9d,9e,9g,10,11,17,21,24,30,31,38,McN/36,Tmp
TTTSK	07 KEN 24-4	5,6,7b,8a,9a,9b,9d,9e,9g,10,11,17,21,30,31,36,38,McN/24,Tmp
TRTTF	06 YEM 34-1	5,6,7b,9a,9b,9d,9e,9g,10,11,17,21,30,36,38,McN,Tmp/8a,24,31
TPMKC	74 MN 1409	5,7b,8a,9d,9e,9g,10,11,17,21,36,McN,Tmp/6,9a,9b,24,30,31,38
TTTTF	01MN84A-1-2	5,6,7b,8a,9a,9b,9d,9e,9g,10,11,17,21,30,36,38,McN,Tmp/24,31
QTHJC	75 ND 717-C	5,6,8a,9b,9d,9g,10,11,17,21,McN/9a,9e,7b,24,30,31,36,38,Tmp
RKQQC	99KS76A	5,6,7b,8a,9a,9b,9d,9g,21,36,McN/9e,10,11,17,24,30,31,38,Tmp

Table 2. Percentages of W. J. Sando accessions resistant, heterogeneous, and susceptible to 8 races of *P. graminis* f. sp. *tritici*. Totals are not equal due to lack of germination in some replications.

Race	**Total**	**Resistant**	**Heterozygous**	**Susceptible**
TTKSK	546	64 (11.72%)	67 (12.27%)	415 (76.01%)
TTKST	542	59 (10.89%)	70 (12.92%)	413 (76.20%)
TTTSK	544	52 (9.56%)	89 (16.36%)	403 (74.08%)
TRTTF	544	25 (4.60%)	51 (9.38%)	468 (86.03%)
TTTTF	545	26 (4.77%)	102 (18.72%)	417 (76.51%)
TPMKC	545	25 (4.59%)	37 (6.79%)	483 (88.62%)
QTHJC	536	50 (9.33%)	53 (9.89%)	433 (80.78%)
RKQQC	542	79 (14.58%)	115 (21.22%)	348 (64.21%)

Table 3. Significant correlations between resistant accessions' of the W. J. Sando collection and their reactions to different races of *Puccinia graminis* f. sp. *tritici*. Analyzed using Pearson's Product Moment Correlation in pair-wise comparisons. All correlations shown are positive.

Correlation between			
Race	**Race**	***r***	***P* value (<0.05)**
TTKSK	TTKST	0.496	7.76×10^{-11}
TTKSK	TTTSK	0.480	3.75×10^{-9}
TTKST	TTTSK	0.365	3.85×10^{-6}
TTKSK	QTHJC	0.210	0.009
TTTSK	RKQQC	0.221	0.006
TRTTF	TTTTF	0.317	6.96×10^{-5}
TRTTF	TPMKC	0.425	4.63×10^{-8}
TTTTF	TPMKC	0.411	1.48×10^{-7}
TPMKC	QTHJC	0.263	0.001
TPMKC	RKQQC	0.178	0.028

Table 4. Results of molecular marker screening of accessions selected for cytogenetic characterization using genomic in situ hybridization and fluorescence in situ hybridization (GISH-FISH). All accessions contained the decaploid tall wheatgrass, *Thinopyrum ponticum*, in their pedigree. The molecular markers used were those associated with stem rust resistance genes derived from *Th. ponticum*: *Sr24* (*Xbarc71*), *Sr25* (*Gb*), and *Sr26* (*BE518379/Sr26#43*)

	Marker		
Accession	***Xbarc71***	***Gb***	***BE518379/Sr26#43***
PI 604981	-	-	-
PI 605057	-	+	-
PI 605286	-	-	-
PI 611932	-	-	+

Table 5. Infection types clustered by group of the 29 accessions resistant to *Puccinia graminis* f. sp. *tritici* races TTKSK, TTKST, and TTTSK combined. (Different ITs in a replication are separated by "/", "+" = pustules larger than expected for specific IT, "-" = pustules smaller than expected for specific IT, C = more chlorosis than expected, N = more necrosis than expected, LIF = Low Infection Frequency)

		Race							
		TTKSK		**TTKST**		**TTTSK**		**TRTTF**	
Accession	**Grp.**	**Rep 1**	**Rep 2**	**Rep 1**	**Rep 2**	**Rep 1**	**Rep 2**	**Rep 1**	**Rep 2**
PI 604868	1	22+	2	2	2	2	2	-	3+
PI 604890	1	2	2	2-C	-	2-C	2	3+	3
PI 604905	1	2	22+	2	2	2-	2	3+	3
PI 605016	1	2C	2/22+	2-	2	-	2-	3+	3
PI 605022	1	2/2C	2	2-	-	2-	2-	3+4	3+
PI 605023	1	2	2+3	2	2	2-	2-	3+	3
PI 605039	1	22+	2	2	2	2-	2	3+	3+
PI 605106	1	2	2 CN	2	2	2-	2-	3+	3+
PI 605107	1	2	2 CN	2-	2	2	2	3+	3+
PI 605185	1	2-LIF	22+	2	2	2	2	3+	3+
PI 605188	1	0;/2	2	2	2	2	2	3+	3+
PI 605245	1	2	2	2-	2	2	2	3+	3+
PI 605317	1	2	2	2	2	2-	2	3+	3+
PI 611927	1	2	2	2	2	2	2	3	2
PI 604924	2	0	0;	0;	0;	0;	0;	0;	0;
PI 604981	2	0;	;1	0;	;1	2	0;	3-;/0;	0;1/;13
PI 604986	2	2	2	1NC	2	1	2-	22-	;1N
PI 605103	2	0;	0	0	0;	0;	0;	;1/;13	0;
PI 605132	2	0;	0;	0;	0;	0;	0;	0;	0
PI 611887	2	0;1	0;	0	0;	0;	0;	0;	0;
PI 611899	2	2-	2--	;1 CN	1;	2-	1C	22-3	1;
PI 611915	2	1	0;	0;	0;	1	1	31;	1;
PI 611932	2	1N	0;/1	0;	0;	;1	2-	1;	2-C
PI 605079	3	22+	-	2	-	2	2+	3+	3+
PI 605321	3	2	2	2	2	2	2-	-	3+
PI 604884	4	22-	22-	1C	2	1	-	22+	2+2
PI 605094	5	0;1N	22--CN	2-CN	2	2	2-C	3+	3
PI 605098	6	22+	2	22-LIF	2	2	2+	2+	2+
PI 605246	7	2/0;	2	2-	2	2-	2	3+	01;

Table 5. *(continued)*

		Race							
		TTTTF		TPMKC		QTHJC		RKQQC	
Accession	Grp.	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2
PI 604868	1	3+	3+	4	3+	3+	-	2	-
PI 604890	1	2	3-	3+	3	4	4	-	2
PI 604905	1	23	3+	3+	4	3+	4	2	2
PI 605016	1	3	3	-	3	1+	3+	2+	2
PI 605022	1	2+3	-	3+	4	3+ LIF	3+	2	2
PI 605023	1	2+	3	-	4	3+	3+	2	2
PI 605039	1	3	3+	3+	3+	3	-	2	2
PI 605106	1	2-	3+4	3+	3+	4	4	2	2+
PI 605107	1	2	3	3	3+	3+	3	2	2
PI 605185	1	2	3	4	4	3+	4	2	2
PI 605188	1	2	3	3	4	-	4	2	2+
PI 605245	1	3-	3	3	3+	3+	4	2	2+
PI 605317	1	4	3	3+	3	3+	3+	2	2
PI 611927	1	31	3	3	3	2-	3	2	2+
PI 604924	2	0	0;	0;	0;	-	0;	0;	0;
PI 604981	2	;	0;	;3	0;3	1;	1	0;	0;
PI 604986	2	2	2CN	1N/3	1C	2-C/3	1C	2-/;1	2-CN
PI 605103	2	0	0	0;	0;	0;	0;	0;	0;
PI 605132	2	0;	0;	;	0;	0;	0;	0;	0;
PI 611887	2	0/1;/3	0	0;/3-	0;	0;	0;/1;	0;	0;
PI 611899	2	2-	2-	1;N	2-	2-	2-C	2-	2-
PI 611915	2	0/1	;1+/0;	;1+	0;	0;	;1	0;	0;
PI 611932	2	;	2-	;1	;	0;	0;	-	2-
PI 605079	3	32	3+	3	4	2	2+	1;	1;
PI 605321	3	4	3	3	2	2	2	2	2
PI 604884	4	2	3	3	-	1;	-	-	2+
PI 605094	5	2-;	2	3+	3+	3	2+3	2/3+	2-C/3
PI 605098	6	2;C	2+	2	2	3+	3	2	2
PI 605246	7	3	4	3+	3+	3+	-	3+	2/0;/4

Table 6. The infection types (IT) of accessions PI 604981, PI 605057, PI 605286, and PI 611932 to eight races of *Puccinia graminis* f. sp. *tritici*. These accessions were examined using genomic in situ hybridization and fluorescence in situ hybridization (GISH-FISH)

Accession	TTKSK Rep 1	TTKSK Rep 2	TTKST Rep 1	TTKST Rep 2	TTTSK Rep 1	TTTSK Rep 2	TRTTF Rep 1	TRTTF Rep 2
PI 604981	0;	;1	0;	;1	2	0;	3-;/0;	0;1/;13
PI 605057	0;3	0;	-	31	3	3	2 LIF	2--/2-;/3
PI 605286	0;3	0;	-	0;/3	1	1	0;/0;1+	0;/1;
PI 611932	1N	0;/1	0;	0;	;1	2-	1;	2-C

Accession	TTTTF Rep 1	TTTTF Rep 2	TPMKC Rep 1	TPMKC Rep 2	QTHJC Rep 1	QTHJC Rep 2	RKQQ Rep 1	RKQQ Rep 2
PI 604981	;	0;	;3	0;3	1;	1	0;	0;
PI 605057	2	1+N	3	3	1;	1+	1+;	3 LIF
PI 605286	3	;1/1+/4	;11+	0;	0;	0;	;1	0;
PI 611932	;	2-	;1	;	0;	0;	-	2-

Figure 1. Biplot showing relationships between resistance and susceptibility of 152 resistant accessions to eight races of *Puccinia graminis* f. sp. *tritici*. Data points based on MCA analysis using R package "ca" (lambda = "adjusted", nd = 5). The X and Y-axes explain 64.64% and 11.35%, respectively, of the variation. Red symbols = susceptibility to the associated race, Black symbols = resistance to the associated race.

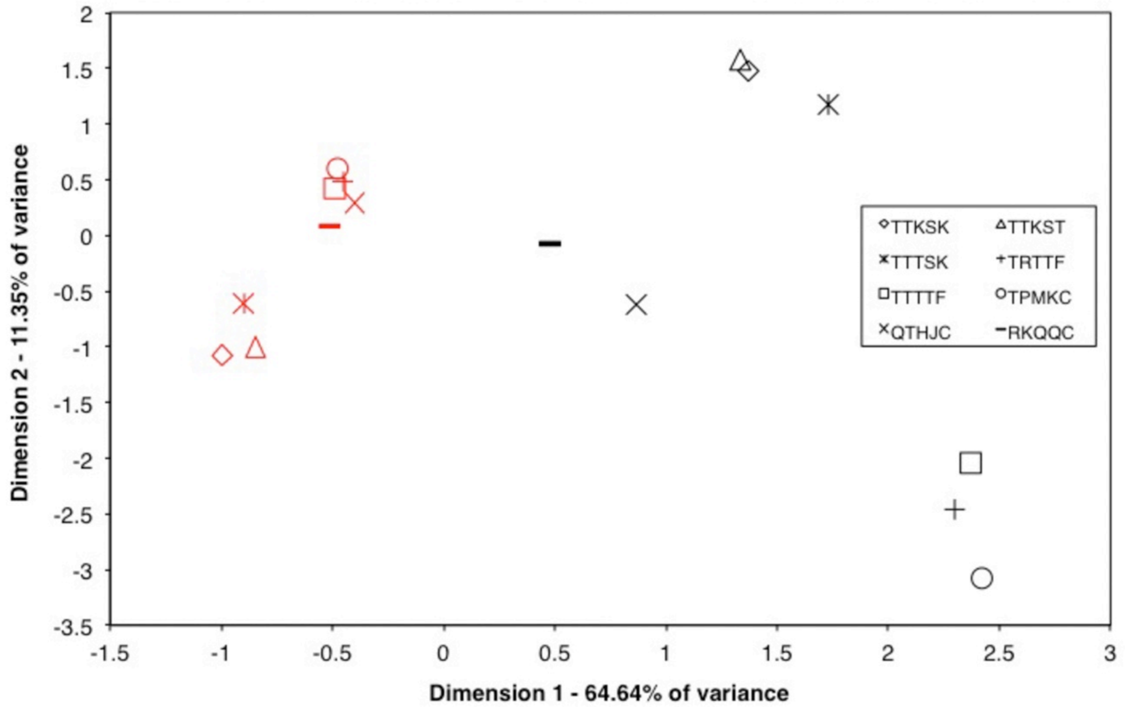

Figure 2. Genomic in situ hybridization (GISH) with *Th. ponticum* genomic DNA as a probe detected one pair of *Th. ponticum* chromosomes (marked by arrowheads) in accessions with 2n=42 chromosomes PI 604981 (A), PI 611932 (B) and PI 605286 (C), and two pairs in accession with 2n=44 PI 605057 (D). Oligonucleotide probes GAA and pAs1 produces chromosome-specific pattern and allowed to identify individual wheat chromosomes (6). In accessions PI 604981, PI 605286, PI 605057 wheat chromosome pair 2D is missing; in PI 611932 chromosome pair 6D is missing. We assumed that the missing wheat chromosomes were substituted by *Th. ponticum* homoeologs. In PI 605057 the additional *Th. ponticum* pair belongs to an unknown homoeologous group. Chromosomes were counterstained with DAPI and fluoresce blue, GAA repeats are white, pAs1 repeats are red, *and Th. ponticum chromosomes* are painted in green. Bar corresponds to 10 μm

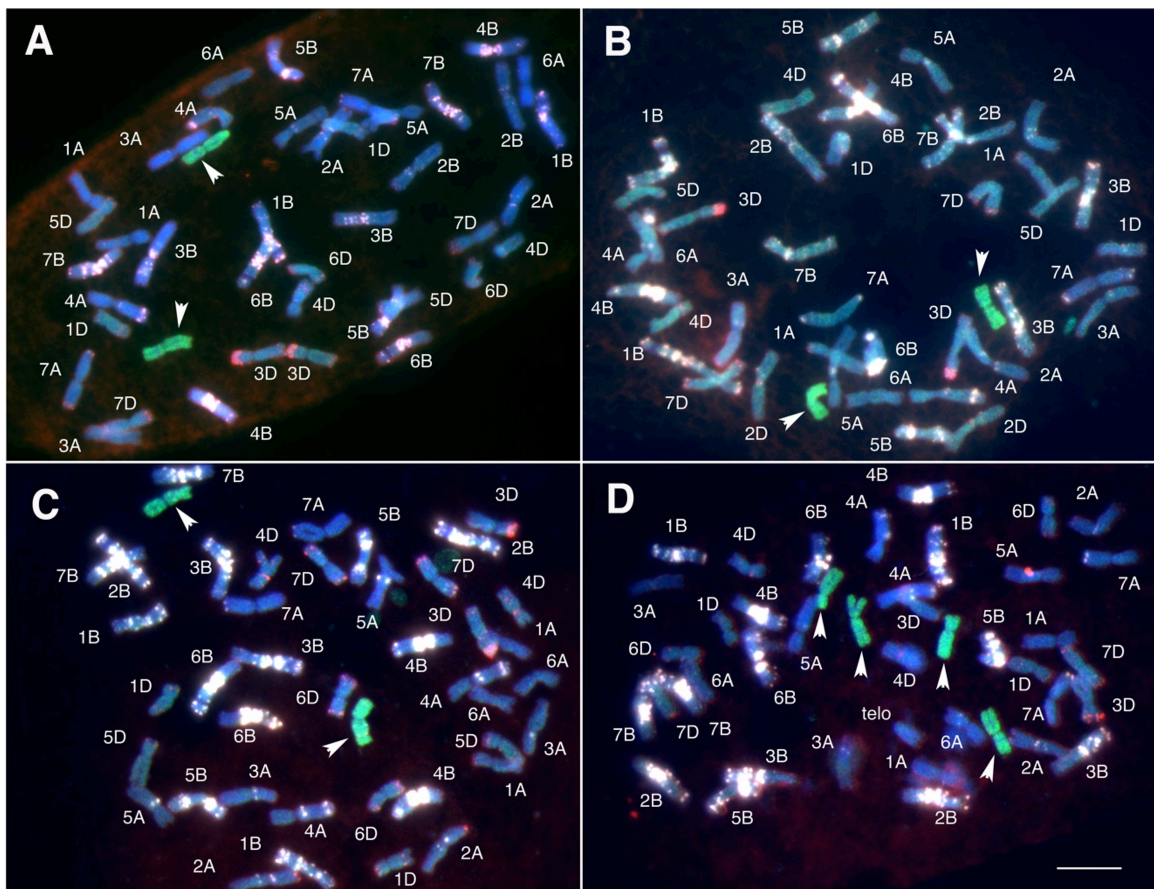

Table 7. Molecular markers used to screen PI 410954, Faller, and RB07. SR = stem rust; LR = leaf rust; WT = wild type; HMW-Gs = high molecular weight glutenin subunits

Trait	Gene	Marker	Sequence	Reference
SR Resistance	*Sr24*	Xbarc71	F: GCGCTTGTTCCTCACCTGCTCATA R: GCGTATATTCTCTCGTCTTCTTGT TGGTT	(Mago, Bariana, et al., 2005)
SR Resistance	*Sr24*	Sr24#12	F: CACCCGTGACATGCTCGTA R: AACAGGAAATGAGCAACGATGT	(Mago, Bariana, et al., 2005)
SR Resistance	*Sr25*	Gb	F: CATCCTTGGGGACCTC R: CCAGCTCGCATACATCCA	(Liu, Yu, et al., 2010)
SR Resistance	*Sr26*	Sr26#43/ BE518379	Sr26#43-F: AATCGTCCACATTGGCTTCT Sr26#43-R: CGCAACAAAATCATGCACTA BE518379-F: AGCCGCGAAATCTACTTTGA BE518379-R: TTAAACGGACAGAGCACACG	(Liu, Yu, et al., 2010)
LR Resistance	*Lr34*	csLV34	csLV34F: GTTGGTTAAGACTGGTGATGG csLV34R: TGCTTGCTATTGCTGAATAGT	(Lagudah, McFadden, et al., 2006)
Plant Height WT	*Rht-B1a*	BF/WR1	BF: GGTAGGGAGGCGAGAGGCGAG WR1:CATCCCCATGGCCATCTCGAGCTG	(Ellis, Spielmeyer, et al., 2002)
Plant Height Dwarfing	*Rht-B1b*	BF/MR1	F: CCAGATACACAACTGCTGGC R: TGATCTTGAGGTTCTCGTCG	(Ellis, Spielmeyer, et al., 2002)
Plant Height WT	*Rht-D1a*	DF2/WR2	DF2: GGCAAGCAAAAGCTTCGCG WR2: GGCCATCTCGAGCTGCAC	(Ellis, Spielmeyer, et al., 2002)
Plant Height Dwarfing	*Rht-D1b*	DF/MR2	DF: CGCGCAATTATTGGCCAGAGATAG MR2:CCCCATGGCCATCTCGAGCTGCTA	(Ellis, Spielmeyer, et al., 2002)
HMW-Gs	*Glu-A1*	UMN19	UMN19F:CGAGACAATATGAGCAGCAAG UMN19R: CTGCCATGGAGAAGTTGGA	(Liu, Chao, et al., 2008)
HMW-Gs	*Glu-D1*	UMN25	UMN25F: GGGACAATACGAGCAGCAAA UMN25R: CTTGTTCCGGTTGTTGCCA	(Liu, Chao, et al., 2008)

Table 8. The number of SNPs per chromosome (as mapped by Wang et al., 2014) used for the genome wide association study of doubled haploid progeny lacking *Sr24* from the cross RB07//Faller/PI 410954 in the R package 'rrBLUP'

SNPs per chromosome by genome and group								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**Total**
A	1556	1385	1325	1355	1734	1438	1783	10576
B	2244	2387	1788	952	2049	1779	1692	12891
D	650	907	504	155	542	364	607	3729
Total	4450	4679	3617	2462	4325	3581	4082	27196

Figure 3. The eigenvalue decomposition of the realized additive relationship matrix developed by the analysis of doubled haploid accessions lacking *Sr24* and their genotypes at 27,196 SNPs from the Illumina GoldenGate 90K SNP chip (Endelman, 2011, Wang, Wong, et al., 2014). The x-axis represents each of 108 principal components, those accounting for more than 5% of variance (1 and 2) were included as covariates in the genome wide association analysis.

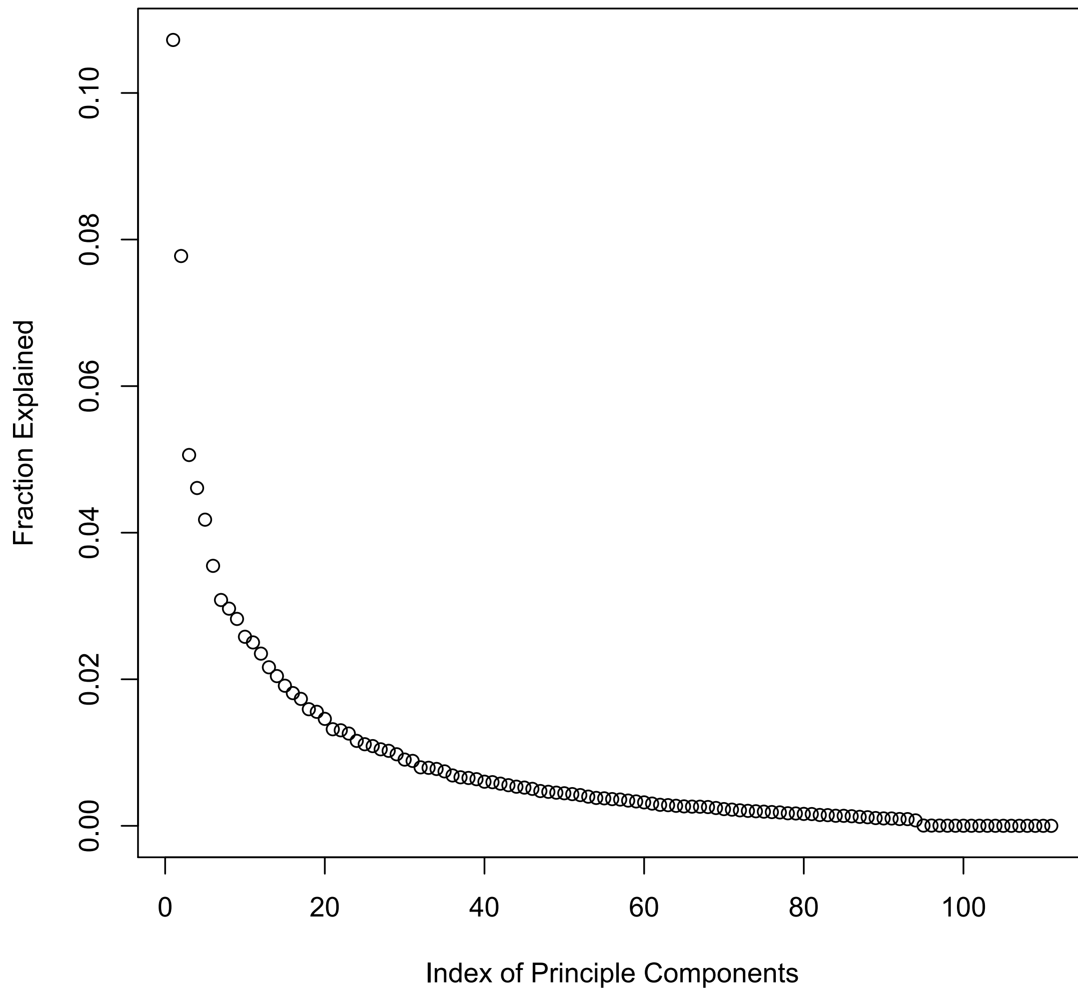

Table 9a. Segregation of multiple PI 410954 F_2 populations when screened with *P. graminis* f. sp. *tritici* race TTKSK. χ^2 values are based on comparison with an expected segregation ratio of 15:1 (resistant:susceptible). LMPG-6 is the female parent in 12XR019 and 12XR020 and Faller is the female parent in 11XR188-3

Population	Resistant	Susceptible	χ^2 (15:1)	χ^2 (3:1)	*P* value (<0.05)
11XR188	147	47	22.9	0.0035	1.71×10^{-6} (0.953)
12XR019	327	29	0.625	NA	0.4044
12XR020	96	8	0.041	NA	0.8406

Table 9b. Segregation of 100 11XR188-3 $F_{2:3}$ families when screened with two races of *P. graminis* f. sp. *tritici*. χ^2 values are based on comparison with an expected segregation ratio of 15:1 (resistant:susceptible)

Race	Resistant	Susceptible	χ^2 (15:1)	*P* value (<0.05)
TTKSK	94	6	0.0107	0.9177
TRTTF	88	12	4.26	0.0389

Table 10. Infection types (IT) of TC_1F_1 individuals selected from the cross, RB07//Faller/PI 410954, for doubled haploid (DH) production using *P. graminis* f. sp. *tritici* race TRTTF and the number of DH progeny derived from each plant. “- -” indicates pustules were much smaller than expected for the given IT

Line	IT	# of Progeny
12XR132-6	2--;	12 (5.6%)
12XR134-4	2--;	5 (2.4%)
12XR137-3	22-;	0
12XR140-2	2-;	13 (6.1%)
12XR140-5	2-;	13 (6.1%)
12XR147-1	2--;	15 (7.0%)
12XR150-8	2-;	7 (3.3%)
12XR150-17	2-;	5 (2.4%)
12XR153-13	;12--	6 (2.8%)
12XR154-10	;1	0
12XR158-13	;1	21 (9.9%)
12XR158-14	;1	12 (5.6%)
12XR162-3	2-;	10 (4.7%)
12XR163-4	2--;	11 (5.2%)
12XR163-22	2-;	13 (6.1%)
12XR163-30	;2-	7 (3.3%)
12XR164-4	;1	3 (1.4%)
12XR166-18	2-;	0
12XR168-5	;2-	8 (3.8%)
12XR168-8	;1	8 (3.8%)
12XR169-1	2-;	13 (6.1%)
12XR169-11	;2--	9 (4.2%)
12XR169-13	;1	17 (8.0%)
12XR170-5	0	5 (2.4%)
Faller	3+	NA
RB07	3+	NA
PI 410954	2-;	NA
		Total = 213

Table 11. Results of molecular marker screenings for PI 410954, Faller, and RB07. "+" = presence of expected product; "-"indicates absence of expected product

Gene	Marker	PI 410954	Faller	RB07
Sr24	Xbarc71	+	-	-
Sr24	Sr24#12	+	-	-
Sr25	Gb	-	-	-
Sr26	Sr26#43/BE518379	-	-	-
Lr34	csLV34	-	-	-
Rht-B1a	BF/WR1	+	-	-
Rht-B1b	BF/MR1	-	+	+
Rht-D1a	DF2/WR2	+	+	+
Rht-D1b	DF/MR2	-	-	-
Glu-A1	UMN19	+	+	+
Glu-D1	UMN25	+	+	+

Table 12. Single nucleotide polymorphisms (SNPs) from Illumina GoldenGate 90K assay with significant associations with the resistant phenotype in non-*Sr24* doubled haploid (DH) lines derived from RB07//Faller/PI 410954 TC_1F_1 plants. SNPs were identified using the GWAS function in Jeffrey Endelman's R package 'rrBLUP'. All SNPs listed have a q value ≤ 0.05, corresponding to a false discovery rate (FDR) of less than 5%.

SNP	Chromosome	cM	$-log_{10}p$ value
Kukri_rep_c105406_308	6A	13.45	32.01
BS00009514_51	6A/6D	16.96(23.84)	32.05
BS00022094_51	6A/6D	16.96(23.84)	18.28
IACX9471	6D	18.20	32.08
BS00074495_51	6D	19.00	28.50
wsnp_Ku_c2637_5009091	6D	19.00	27.93
BobWhite_c7090_2001	6D	20.75	10.38
Excalibur_rep_c66622_1066	6D	20.75	8.03
Excalibur_s114066_247	6D	20.75	12.89
Kukri_rep_c68823_696	6D	20.75	29.49
BobWhite_c11808_975	6D	21.83	27.61
BS00021983_51	6D	23.84	31.59
BS00022523_51	6D	23.84	32.07
Ra_c42576_780	6D	23.84	32.07
IAAV1942	6D	24.77	29.69

Figure 4. Manhattan plot of SNPs associated with non-*Sr24* resistance developed from genome wide association study in R package 'rrBLUP'. A total of 108 DH lines and 3 parents were used in the analysis. The dashed line represents a *q* value of 0.05, all points above this line have *q* values ≤ 0.05. The X-axis represents the haploid chromosome composition (n = 21) of *Triticum aestivum*.

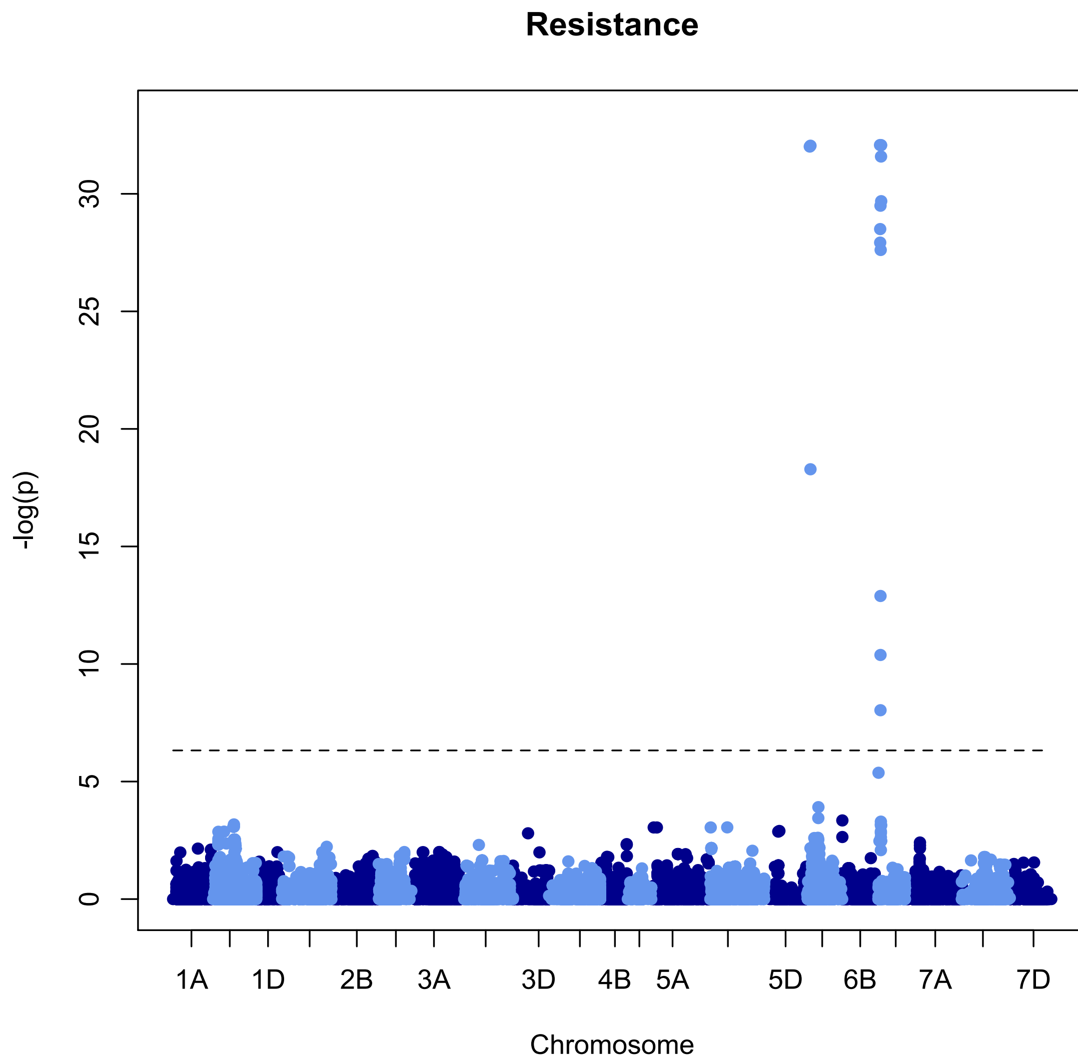

Table 13. Haplotypes of non-*Sr24* doubled haploid lines, RB07, Faller, and PI 410954 at 15 significant ($q \leq 0.05$) SNPs

		Kukri_rep_c105406_308[*]	BS00009514_51	BS00022094_51	IACX9471	BS00074495_51	wsnp_Ku_c2637_5009091	BobWhite_c7090_2001	Excalibur_rep_c66622_1066	Excalibur_s114066_247	Kukri_rep_c68823_696	BobWhite_c11808_975	BS00021983_51	BS00022523_51	Ra_c42576_780	IAAV1942
		6A[†]	6A	6A	6D	6D	6D	6D	6D	6D	6D	6D	6D	6D	6D	6D
Line		13.45[‡]	16.96	16.96	18.20	19.00	19.00	20.75	20.75	20.75	20.75	21.83	23.84	23.84	23.84	24.77
12XR132-6-1	S[§]	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB
12XR132-6-3	S	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB
12XR132-6-4	S	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB
12XR132-6-5	S	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB
12XR132-6-6	S	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB
12XR132-6-8	S	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB
12XR132-6-11	S	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB
12XR132-6-12	S	BB	BB	BB	BB	NA	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB
12XR134-4-1	S	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB
12XR134-4-2	S	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB
12XR134-4-3	S	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB
12XR134-4-4	S	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB
12XR134-4-5	S	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB
12XR140-2-1	R	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA
12XR140-2-2	R	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA
12XR140-2-8	S	BB	BB	BB	BB	NA	BB	AA	BB	BB	BB	BB	BB	BB	BB	BB
12XR140-2-9	S	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB

[*] SNP name on wheat 90K Illumina GoldenGate Assay
[†] Wheat chromosome arm to which corresponding SNP is mapped
[‡] Map position of SNP in centimorgans (Wang et al., 2014)
[§] The corresponding line's reaction to *P. graminis* f. sp. *tritici* race TTKST; S = susceptible, R = resistant

Table 13. (*continued*)

Line		Kukri_rep_c105406_308	BS00009514_51	BS00022094_51	IACX9471	BS00074495_51	wsnp_Ku_c2637_5009091	BobWhite_c7090_2001	Excalibur_rep_c66622_1066	Excalibur_s114066_247	Kukri_rep_c68823_696	BobWhite_c11808_975	BS00021983_51	BS00022523_51	Ra_c42576_780	IAAV1942
		6A	6A	6A	6D	6D	6D	6D	6D	6D	6D	6D	6D	6D	6D	6D
		13.45	16.96	16.96	18.20	19.00	19.00	20.75	20.75	20.75	20.75	21.83	23.84	23.84	23.84	24.77
12XR140-2-11	S	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB
12XR140-2-13	R	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA
12XR140-5-8	S	BB	BB	BB	BB	BB	BB	AA	AA	BB	BB	BB	BB	BB	BB	BB
12XR140-5-8	S	BB	BB	BB	BB	BB	BB	AA	AA	BB	BB	BB	BB	BB	BB	BB
12XR140-5-9	S	BB	BB	BB	BB	BB	BB	BB	NA	BB	BB	BB	BB	BB	BB	BB
12XR140-5-10	S	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB
12XR140-5-12	S	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB
12XR147-1-1	R	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA
12XR147-1-3	S	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	AA	BB	BB	BB	BB
12XR147-1-7	R	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA
12XR147-1-10	R	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA
12XR150-8-1	S	BB	BB	BB	BB	BB	BB	AA	BB	BB	BB	BB	BB	BB	BB	BB
12XR150-8-2	S	BB	BB	BB	BB	BB	BB	AA	BB	BB	BB	BB	BB	BB	BB	BB
12XR150-8-6	S	BB	BB	BB	BB	BB	BB	AA	BB	BB	BB	BB	BB	BB	BB	BB
12XR150-8-7	S	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB
12XR150-17-3	S	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB
12XR150-17-5	S	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB
12XR153-13-3	R	AA	AA	AA	AA	AA	AA	AA	BB	BB	AA	AA	AA	AA	AA	AA
12XR153-13-4	S	AA	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	AA	AA	AA	AA
12XR153-13-6	S	BB	BB	BB	BB	NA	BB	AA	BB	BB	BB	BB	BB	BB	BB	BB
12XR158-13-1	S	BB	BB	BB	BB	BB	BB	BB	NA	BB	BB	BB	BB	BB	BB	BB

Table 13. (*continued*)

Line		Kukri_rep_c105406_308	BS00009514_51	BS00022094_51	IACX9471	BS00074495_51	wsnp_Ku_c2637_5009091	BobWhite_c7090_2001	Excalibur_rep_c66622_1066	Excalibur_s114066_247	Kukri_rep_c68823_696	BobWhite_c11808_975	BS00021983_51	BS00022523_51	Ra_c42576_780	IAAV1942
		6A	6A	6A	6D	6D	6D	6D	6D	6D	6D	6D	6D	6D	6D	6D
		13.45	16.96	16.96	18.20	19.00	19.00	20.75	20.75	20.75	20.75	21.83	23.84	23.84	23.84	24.77
12XR158-13-4	S	BB	BB	BB	BB	BB	BB	AA	AA	BB	BB	BB	BB	BB	BB	BB
12XR158-13-7	R	AA	AA	AA	AA	AA	AA	AA	BB	BB	AA	AA	AA	AA	AA	AA
12XR158-13-8	R	AA	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	AA	AA	AA	AA
12XR158-13-9	S	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB
12XR158-13-10	S	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	AA	AA	AA	AA	AA
12XR158-13-12	R	AA	AA	AA	AA	AA	AA	AA	BB	BB	AA	AA	AA	AA	AA	AA
12XR158-13-13	S	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB
12XR158-13-14	S	AA	AA	AA	AA	AA	AA	AA	BB	BB	AA	AA	AA	AA	AA	AA
12XR158-13-16	R	AA	AA	AA	AA	AA	AA	AA	BB	BB	AA	AA	AA	AA	AA	AA
12XR158-13-17	S	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB
12XR158-13-19	S	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB
12XR158-14-1	S	BB	BB	BB	BB	BB	BB	AA	BB	BB	BB	BB	BB	BB	BB	BB
12XR158-14-2	S	BB	BB	BB	BB	BB	BB	AA	BB	BB	BB	BB	BB	BB	BB	BB
12XR158-14-4	S	BB	BB	BB	BB	BB	BB	AA	BB	BB	BB	BB	BB	BB	BB	BB
12XR158-14-5	R	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA
12XR158-14-6	S	BB	BB	BB	BB	NA	BB	AA	BB	BB	BB	BB	BB	BB	BB	BB
12XR158-14-9	R	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA
12XR158-14-11	S	BB	BB	BB	BB	BB	BB	AA	BB	BB	BB	BB	BB	BB	BB	BB
12XR158-14-12	S	BB	BB	BB	BB	BB	BB	AA	BB	BB	BB	BB	BB	BB	BB	BB
12XR162-3-1	R	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA
12XR162-3-2	S	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB

Table 13. (*continued*)

Line		Kukri_rep_c105406_308	BS00009514_51	BS00022094_51	IACX9471	BS00074495_51	wsnp_Ku_c2637_5009091	BobWhite_c7090_2001	Excalibur_rep_c66622_1066	Excalibur_s114066_247	Kukri_rep_c68823_696	BobWhite_c11808_975	BS00021983_51	BS00022523_51	Ra_c42576_780	IAAV1942
		6A	6A	6A	6D	6D	6D	6D	6D	6D	6D	6D	6D	6D	6D	6D
		13.45	16.96	16.96	18.20	19.00	19.00	20.75	20.75	20.75	20.75	21.83	23.84	23.84	23.84	24.77
12XR162-3-3	R	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA
12XR162-3-5	S	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB
12XR162-3-6	S	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB
12XR163-4-1	S	BB	BB	BB	BB	BB	BB	AA	BB	BB	BB	BB	BB	BB	BB	BB
12XR163-4-4	S	BB	BB	BB	BB	BB	BB	AA	AA	BB	BB	BB	BB	BB	BB	BB
12XR163-4-7	S	BB	BB	BB	BB	BB	BB	AA	BB	BB	BB	BB	BB	BB	BB	BB
12XR163-4-11	S	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB
12XR163-22-3	S	BB	BB	BB	BB	NA	BB	AA	AA	BB	BB	BB	BB	BB	BB	BB
12XR163-22-4	S	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB
12XR163-22-5	S	BB	BB	BB	BB	NA	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB
12XR163-22-6	S	BB	BB	BB	BB	NA	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB
12XR163-22-7	S	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB
12XR163-22-8	S	BB	BB	BB	BB	BB	BB	AA	AA	BB	BB	BB	BB	BB	BB	BB
12XR163-22-9	S	BB	BB	BB	BB	BB	BB	AA	AA	BB	BB	BB	BB	BB	BB	BB
12XR163-22-10	S	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB
12XR163-22-11	S	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB
12XR163-22-12	S	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB
12XR163-22-13	S	BB	BB	BB	BB	BB	BB	AA	AA	NA	BB	BB	BB	BB	BB	BB
12XR164-4-1	S	BB	BB	BB	BB	BB	BB	AA	BB	BB	BB	BB	BB	BB	BB	BB
12XR164-4-2	S	BB	BB	BB	BB	BB	BB	AA	BB	BB	BB	BB	BB	BB	BB	BB
12XR164-4-3	R	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA

Table 13. (*continued*)

Line		Kukri_rep_c105406_308	BS00009514_51	BS00022094_51	IACX9471	BS00074495_51	wsnp_Ku_c2637_5009091	BobWhite_c7090_2001	Excalibur_rep_c66622_1066	Excalibur_s114066_247	Kukri_rep_c68823_696	BobWhite_c11808_975	BS00021983_51	BS00022523_51	Ra_c42576_780	IAAV1942
		6A	6A	6A	6D	6D	6D	6D	6D	6D	6D	6D	6D	6D	6D	6D
		13.45	16.96	16.96	18.20	19.00	19.00	20.75	20.75	20.75	20.75	21.83	23.84	23.84	23.84	24.77
12XR168-5-2	R	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA
12XR168-5-4	R	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA
12XR168-5-6	R	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA
12XR168-5-7	R	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA
12XR168-8-2	S	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB
12XR168-8-3	S	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB
12XR168-8-6	S	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB
12XR169-1-1	R	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA
12XR169-1-4	R	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA
12XR169-1-5	S	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB
12XR169-1-10	S	BB	BB	BB	BB	BB	BB	NA	BB	BB	BB	BB	BB	BB	BB	BB
12XR169-1-11	S	BB	BB	BB	BB	BB	BB	AA	BB	BB	BB	BB	BB	BB	BB	BB
12XR169-1-13	S	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB
12XR169-11-1	S	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB
12XR169-11-4	S	BB	BB	BB	BB	BB	BB	NA	AA	BB	BB	BB	BB	BB	BB	BB
12XR169-11-7	S	BB	BB	BB	BB	BB	BB	AA	BB	BB	BB	BB	BB	BB	BB	BB
12XR169-11-9	S	BB	BB	BB	BB	BB	BB	NA	AA	BB	BB	BB	BB	BB	BB	BB
12XR169-13-2	R	AA	AA	AA	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	NA
12XR169-13-8	R	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA
12XR169-13-9	S	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB
12XR169-13-12	R	AA	AA	AA	AA	AA	NA	AA	AA	AA	AA	AA	AA	AA	AA	AA

Table 13. (*continued*)

Line		Kukri_rep_c105406_308	BS00009514_51	BS00022094_51	IACX9471	BS00074495_51	wsnp_Ku_c2637_5009091	BobWhite_c7090_2001	Excalibur_rep_c66622_1066	Excalibur_s114066_247	Kukri_rep_c68823_696	BobWhite_c11808_975	BS00021983_51	BS00022523_51	Ra_c42576_780	IAAV1942
		6A	6A	6A	6D	6D	6D	6D	6D	6D	6D	6D	6D	6D	6D	6D
		13.45	16.96	16.96	18.20	19.00	19.00	20.75	20.75	20.75	20.75	21.83	23.84	23.84	23.84	24.77
12XR169-13-14	S	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA
12XR169-13-15	S	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB
12XR169-13-16	S	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB
12XR170-5-1	S	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB
12XR170-5-2	S	BB	BB	BB	BB	BB	BB	AA	AA	BB	BB	BB	BB	BB	BB	BB
12XR170-5-3	S	BB	AA	NA	BB	AA	NA	BB	BB	NA	NA	BB	BB	NA	NA	BB
12XR170-5-4	S	BB	BB	NA	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB
12XR170-5-5	S	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	NA	BB	BB	BB
RB07	S	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB
FALLER	S	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB
PI 410954	R	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA

Figure 5. a. Boxplot showing the distribution of the DH lines based on the percentage of unique SNPs shared with elite parents, RB07 and Faller. **b**. Beanplot of the distribution of DH lines based on the percentage of unique SNP shared with elite parents. All SNPs were polymorphic between PI 410954 and the elite parents (n = 6451). Plots represent 108 DH lines.

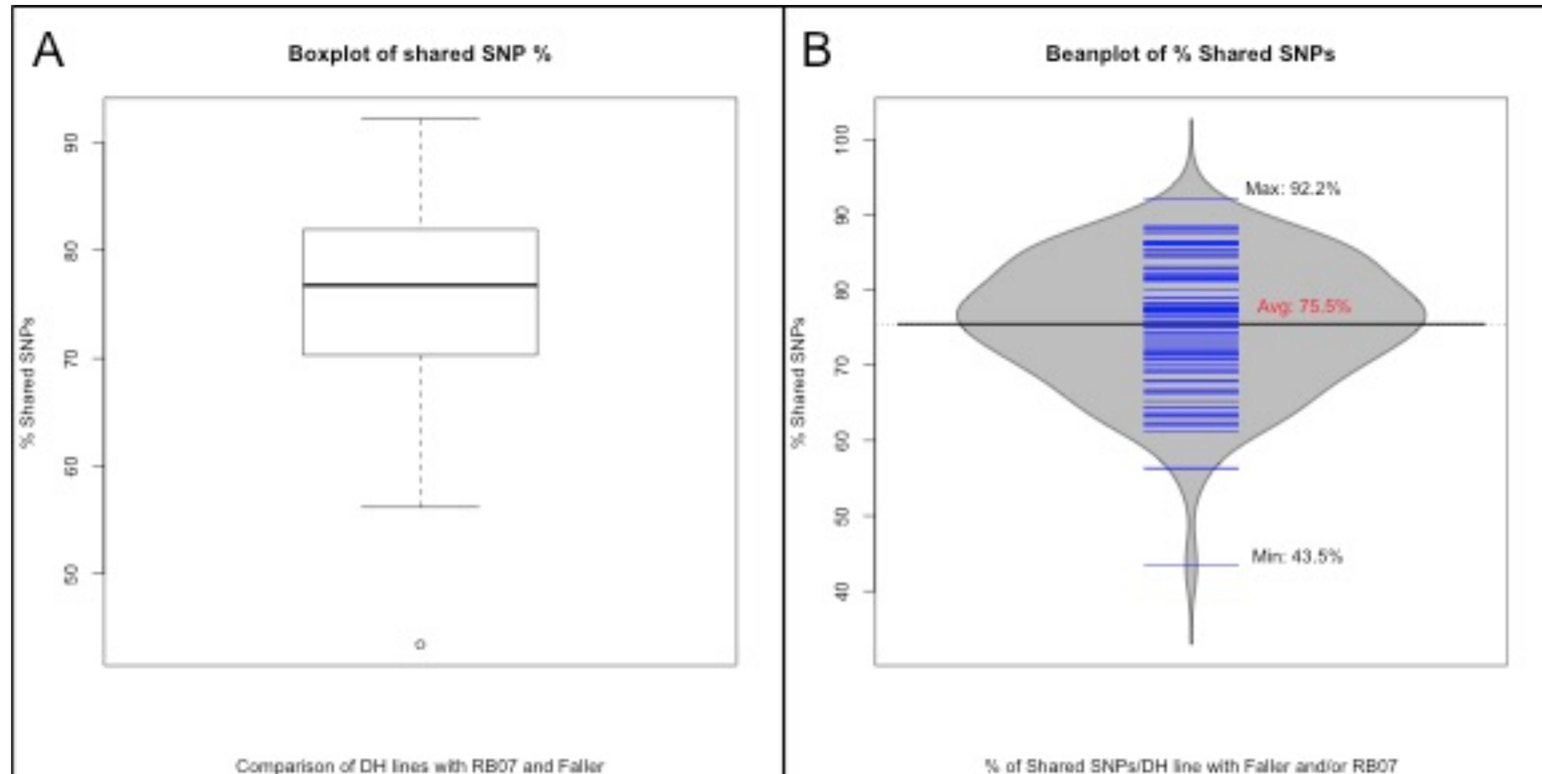

Figure 6. Infection types of lines when screened with *P. graminis* f. sp. *tritici* race TTKSK (04KEN156/04). Photo taken 14 days post inoculation. From left to right: PI 410954, Faller, RB07, 12XR158-14-5, and *Sr24* differential (LcSr24Ag).

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