**CHAPTER 1**

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, 2005, Singh, 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 gene *Sr31* (Roelfs & 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, 2006). The discovery of two additional races similar to TTKS but also virulent on the resistance genes *Sr24* (TTKST) and *Sr36* (TTTSK) prompted the revision of the international nomenclature system, adding a fifth gene set in the differential series: *Sr24, Sr31, Sr38*, and *SrMcN* (Jin et al., 2008, Jin et al., 2009). 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 (Pretorius et al., 2012, Park, 2014, Singh et al., 2011).

The principal method employed to combat stem rust has been, and still is, genetic resistance{Johnson, 1981 #574}{Singh, 2011 #102}. There have been several historic examples of wheat cultivars maintaining resistance to stem rust over long periods of time but most cultivars complete their agronomic lifespan before resistance is overcome {Roelfs, 1992 #572}(Kilpatrick 1974 cited in {Kiyosawa, 1982 #586}. Currently, race specific seedling resistance and race-nonspecific resistance are often deployed in tandem with the expectation of creating more durable resistance {Singh, 2011 #102}{Singh, 2008 #101}. *P. graminis* f. sp. *tritici* is a macrocyclic heteroecious rust, meaning that it progresses through all 5 spores stages of ???? 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 the alternate hosts, particularly *Berberis vulgaris*, virtually eliminated sexual recombination within those regions’ *P. graminis* f. sp. *tritici* populations {Roelfs, 1982 #582}{Hermansen, 1968 #581}. 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 but the species of the pathogens have not been determined (Guthrie 1966 cited in {Green, 1970 #587}{Park, 2011 #585}. 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, 1992 #572}. Fungicides that inhibit sterol synthesis, both sterol biosynthesis inhibitors and demethylation inhibitors, are most effective but rarely deployed due to cost of application {Schumann, 2000 #590}. 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, 2010 #591}. 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 growing seasons per year for wheat making the this particular control impractical {Roelfs, 1992 #572}{Nagarajan, 2012 #592}. 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 {McIntosh, 1976 #577}{Borlaug, 1954 #578}. 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 #593}. 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, 1993 #594} 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, 2011 #102}. CIMMYT breeders believe that by combining 4 -5 minor resistance genes (how do they define a minor gene?) this will lead to durable resistance independent of effective major genes, safeguarding the major genes for use elsewhere.

Currently, there are 33 wheat stem rust resistance genes (*Sr*) 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 *Sr1RSAmigo* (Faris et al., 2008, Ghazvini et al., 2012, Hiebert et al., 2010, Jin et al., 2007, Jin & Singh, 2006, Kolmer et al., 2011, Liu et al., 2011a, Liu et al., 2011b, Niu et al., 2014b, Olson et al., 2013b, Olson et al., 2013a, Park, 2014, Qi et al., 2011, Rouse et al., 2014, Rouse et al., 2011b, Singh et al., 2013). 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 (Friebe et al., 1996, Jones et al., 1995, 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 & Mann, 1924, Gaines & 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 & Avery, 1937).

Laboratory production of haploid plants did not occur until the development of anther culture for *Datura innoxia* in 1964 (Guha & 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 & 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 pollen. In 1986, Laurie and Bennett developed a wide hybridization protocol based on this work that was highly efficient and did not seem hampered by the same parent genotype limitations as the *H. bulbosum* technique (Laurie & 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 (Inagaki & Tahir, 1990, Martins-Lopes et al., 2001, Suenaga & Nakajima, 1989).

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 & 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 & 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 & 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 (Bennett et al., 1976, Gupta, 1969, Houben et al., 2011, Kasha & Kao, 1970).

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: i) immediate culturing of spikelets for a 3 week period (Laurie & Bennett, 1988), ii) application of 0.5 mg/L dichlorophenoxyacetic acid (2,4-D) to pollinated spikelets for 2-3 weeks, iii) injecting 100 mg/L 2,4-D to the internode and/or the spikelets once or twice (Matzk & Mahn, 1994), iv) 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), v) 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 & vanLammeren, 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 (Suenaga & Nakajima, 1989, Cherkaoui et al., 2000, Murashige & Skoog, 1962). 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 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 (Thiebaut et al., 1979, Jensen, 1974, 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 (Inagaki, 1985, Niu et al., 2014a, Thiebaut et al., 1979). 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 (Friesen et al., 2006, Moieni et al., 1997, Steffenson et al., 1995, Druka et al., 2000, Fofana et al., 2008, Prins et al., 2011, Suenaga et al., 2003, Yang et al., 2005). The research presented in the second 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 i) locating the source of stem rust resistance and ii) 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 often 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 in use in breeding programs due to the 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 also 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 reassociation, highly repetitive regions reassociate faster and form bands of darkly stained heterochromatin (Gill & Kimber, 1974a). 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 (Seabright, 1971, Wang & Fedoroff, 1972, Merrick et al., 1973, Ray & Hamerton, 1973, Gill & Kimber, 1974a). 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 & Kimber, 1974b, Friebe et al., 1989, Friebe et al., 1991). Full wheat karyotypes were described in 1974 and 1983 but the karyotype and the accompanying nomenclature were not standardized until 1991 (Gill & Kimber, 1974b, Lukaszewski & Gustafson, 1983, Gill et al., 1991). In 1997 an alternative karyotype was developed using dual stain fluorescence in situ hybridization or FISH (Pedersen & 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 & 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 (Langer-Safer 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 imaging technology (Jiang & 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 squarrosa*, wheat’s D-genome donor(Rayburn & 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. We were able to combine FISH and GISH to simultaneously identify wheat chromosomes present in selected accessions as well as the number of *Thinopyrum ponticum* chromosomes present.

**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 inter-generic hybrids was created by W. J. Sando (employed by the United States Department of Agriculture (USDA)) during the first half of the 20th century. Species used for hybrid breeding with *T. aestivum* included *Aegilops* spp.*, Secale cereale*, *Thinopyrum intermedium*, *Th. ponticum*, *Triticum 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 (Reitz et al., 1945, Smith, 1942). 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* (Friebe et al., 1996, Smith et al., 1968). 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 Sando collection for seedling stem rust resistance. We therefore screened the 546 available accessions of the W.J. Sando collection, as of 2012, 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.

**Methods and Materials**

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) in 2012. The accessions were screened with 8 races of *P. graminis* f. sp. *tritici*. Foreign *P. graminis* f. sp. *tritici* races TTKSK, TTKST, TTTSK, and TRTTF were selected for their broad virulence and prevalence in African stem rust epidemics (Table 1). North American *P. graminis* f. sp. *tritici* races TTTTF, TPMKC, RKQQC, and QTHJC were also used for screening (Table 1). All *P. graminis* f. sp. *tritici* 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 minutes and placed in a rehydration chamber maintained at 80% relative humidity by a KOH solution for 2 to 4 hours. 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 assays were performed in duplicate.

Statistical analysis of accessions and their race-specific resistance/susceptibility patterns was performed using R version 3.0.2 in RStudio® (RStudio, 2013, R Core Team, 2014). A multiple correspondence analysis (MCA) was performed using the R package ‘ca’ v. 0.53 (Nenadic & Greenacre, 2007). MCA 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 (Abdi & Valentin, 2007). 152 accessions with resistant ITs to one or more of the 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 as 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 *Puccinia graminis* f. sp. *tritici* and the observations (rows) were individual accessions and their reaction to each race. The analysis was run with default settings for both “lambda” and “nd” variables.

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 during 20-24 hours. 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 hrs. Root caps were removed with a razor 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 point and final compression performed manually. 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 (64). 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 (6) and *Th. ponticum* gDNA as a probe to identify alien chromosomes. We 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 crosslinked. The probe mixture (20 μl per slide) contained 50% formamide (Fisher, Cat. BP228-100), 2.75X 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 in 2SSC buffer twice: 5 min at room temperature, 10 min at 42° C and then in 1xSSC 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 *Puccinia 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 highest 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, TTTSK, and QTHJC, 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 and RKQQC. In comparison, reactions to TRTTF were correlated with reactions to races TTTTF and TPMKC. Reactions to races TTTTF and TPMKC were also highly correlated. The positive associations observed and the rejection of independence between the reactions to these races suggest the presence of resistance genes effective to multiple races.

While Pearson’s χ2 test allows pair-wise comparisons between races, MCA provides a means to visualize the relationships between the 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 (3). 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 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. Susceptibility to races possessing virulence to *Sr31* (TTKSK, TTKST, TTTSK) and those avirulent to *Sr31* (TRTTF, TTTTF, TPMKC, QTHJC, RKQQC) were also separated by the second dimension.

**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 infection type patterns (Table 4). 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). Races TTKSK, TTKST, TTTSK and RKQQC are all avirulent to stem rust resistance gene, *SrTmp*. This genewas 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 & McVey, 1979). However, race QTHJC, to which Group 1 accessions are susceptible, is also avirulent to *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 (Rouse et al., 2012, McIntosh, 1978, Y. Jin unpublished). *Sr57*/*Lr34* confers adult plant resistance only(Kolmer et al., 2011, Krattinger et al., 2009). However, *Sr42*, from the Japanese cultivar “Norin 40”, shares the same resistance/susceptibility pattern as Group 1 (Y. Jin unpublished, Ghazvini et al., 2012). The 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 resistance pattern of Group 1 accessions from the patterns displayed by *Sr42* and *Sr9h*.

Nine accessions resistant to the Ug99 race group were also resistant to the five remaining races*.* 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 4). Eight of the nine accessions in Group 2 have *Th. ponticum* listed in their pedigree, while the ninth contains *Th. intermedium*. *Th. ponticum* is the donor of stem rust resistance genes *Sr25*, *Sr26*, and *Sr43*; each effective against many races of *P. graminis* f. sp. *tritici* (Dundas et al., 2007, Liu et al., 2010, Niu et al., 2014b). *Sr25* provides marginal resistance to race TRTTF, expressing an IT of “2+3-“, however, this is still within the range of low ITs expected for this gene (McIntosh, 1978, 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 and colleagues (Prins et al., 2001) (data not shown). 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* conditions an IT that ranges from “;” to “2-“ and may also be a candidate for the gene conditioning 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* (Liu et al., 2010, Mago et al., 2005)(data not shown). Accession PI 604981 was screened with the same multiplex markers and 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* listed 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., 2011b). 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 shared the same race-specific resistance pattern and ITs as *SrTmp*. PI 605079 was more resistant to RKQQC than the expected low IT for *SrTmp* but this may be due to experimental variance. It is possible that resistance in these accessions is derived in part 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 to 7 display 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 they possess. Accessions PI 604924, 605103, 605132, 611887, and 611899 had chromosome counts of 2n = 54-56. The remaining accessions, PI 604981, PI 605057, PI 605286, and PI 611932, had chromosome counts 2n=42 and were selected for analysis using GISH and FISH (Figure 2). The alien parent in the four selected accessions is *Th. ponticum* [2n = 10x = 70; genome JJJJJJJsJsJsJs] (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).

*Th. ponticum* chromosomes replaced wheat D-genome chromosomes, including chromosome pairs 2D and 6D. Wheat chromosome pair 2D was absent in PI 604981, PI 605057, and PI 605286. Stem rust resistance genes *Sr25* and *Sr43* are derived from *Th. ponticum* group 7 chromosomes (Friebe et al., 1996, Knott, 1988). As none of the four accessions were missing wheat group 7 chromosomes it is unlikely that either *Sr25* or *Sr43* are the source of TTKSK resistance in these accessions.

*Sr24*, while effective against TTKSK (Ug99), is ineffective against TTKST to which these accessions 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 (Jiang et al., 1994, Mago et al., 2005, The et al., 1991). PI 605286 did not amplify the *Sr24*-associated fragment when screened with PCR marker *Xbarc71* identified by Mago and colleagues (Mago et al., 2005) (data not shown). 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 (except *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 PI605057 population.

*Sr26* was transferred from the long arm of a *Th. ponticum* group 6 chromosome to the wheat chromosome 6A (Knott, 1961, Knott, 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 from 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 604981, PI 605057, and PI 605286 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 by using a *ph1b* mutant developed at Kansas State University Wheat Genetics Resource Center (Friebe et al., 2011).

**Discussion**

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 but, to our knowledge, no published data exists characterizing the entire collection. We were able to characterize the resistance in the entire collection of accessions 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 will proceed more rapidly with the aid of this comprehensive screening. 25 of these accessions could not be distinguished from known *Sr* genes but more work may differentiate some of these as new genes or alleles. We postulate that the four accessions that could not be readily associated with any single *Sr* gene possess new genes with strong resistance to the Ug99 race group. Introgression of these resistance genes into adapted wheat germplasm will provide additional tools for breeding resistant wheat cultivars.

Nine accessions were selected and screened using cytogenetic techniques after preliminary results indicated that they possessed resistance to TTKSK. We combined the classic method of GISH with state-of-the-art fluorescence banding methods to quickly determine 1) how many alien chromosomes were present in each line and 2) which wheat chromosomes had been replaced by alien chromosomes. These methods allowed our pre-breeding work to focus on those accessions with the greatest likelihood of successful introgression. We identified three lines with likely new stem rust resistance and only a single pair of substituted chromosomes. The identification of these lines will be helpful in future studies introgressing new resistance into high-performing cultivars. Cytogenetic characterization also complemented phenotypic and molecular data and aided in the postulation of gene presence or absence. By combining classic phenotypic and cytogenetic techniques with modern microscopy and molecular markers we have shown that wheat-intra/intergeneric hybrids are a valuable and viable source of wheat stem rust resistance genes and are effective bridges between wheat’s tertiary gene pool and agronomic cultivars.

**CHAPTER 3:**

**Ug99 resistance in accession PI 410954**

The threat of the Ug99 race group has led to a large coordinated effort to find and deploy new sources of resistance to this disease (Newcomb et al., 2013, Rouse et al., 2011b, Xu et al., 2009, Turner et al., 2013, Aghaee-Sarbarzeh et al., 2013, Fedak et al., 2012, Endresen et al., 2012, Rouse et al., 2011a, Rouse & Jin, 2011, Njau et al., 2010). Rouse et al. (2011a) screened over 700 spring wheat lines with stem rust race TTKSK and found 88 accessions that conferred some degree of resistance at the seedling stage. One accession from this study with broad-spectrum resistance to various races of *P. graminis* f. sp. *tritici* is 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 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 our research was to locate and introgress the unknown resistance gene in PI 410954 using doubled haploid technology.

**Methods and Materials**

**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. A cross between PI 410954 (male) and ‘Faller’ (female) was made in the spring of 2012. F1 progeny from this cross, given laboratory identifier 12XR031, were used as male parents for a top cross to ‘RB07’ in summer of 2012.

Generation F2 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). F2 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.

Generation F2 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. F3 seed was harvested and at least 15 plants of each F2:3 family (*n* = 100)were screened using two *P. graminis* f. sp. *tritici* races, TTKSK and TRTTF, 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 on Edwards et al. (1991). PI 410954 was screened with PCR markers *Xbarc71*, multiplex Sr26#43/BE518379, and Gb associated with resistance genes *Sr24*, *Sr26*, and *Sr25*, respectively, based on pedigree information indicating the presence of *Agropyron elongatum* synm. *Thinopyrum ponticum*, the donor of the three listed *Sr* genes (Prins et al., 2001, Mago et al., 2005, Liu et al., 2010). PI 410954, Faller, and RB07 were also screened with markers listed in Table 5. Published PCR procedures were followed for all markers.

Generation TC1F1 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, selected TC1F1 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 *Pgt* race TTKST (virulent to lines with *Sr24*) as described above. All DH lines were screened using *Sr24* associated PCR markers *Xbarc71* and *Sr24#12* (Mago et al. 2005). Lines lacking *Sr24* (n = 109) and the parents (RB07, Faller, and PI 410954) were genotyped at the USDA Small Grains Genotyping Laboratory in Fargo, North Dakota using a custom Illumina GoldenGate 90K SNP chip (Wang et al., 2014).

**Data Analysis**

All statistics were performed in R v. 3.1.1 within the RStudio GUI (R Core Team, 2014, RStudio, 2013). SNP data generated using Illumina chip 90K was inspected manually in GenomeStudio. Manual inspection using in-program filters identified multiple SNPs on chromosome 6D that appeared to be associated with non-*Sr24* race TTKST-resistant DH lines. All SNPs mapped to chromosome 6D and the corresponding genotype calls were exported into a .csv file appropriate for analysis using the R package, /qtl. Parameters for the single QTL analysis were as follows: genotypes=c(“AA”, “BB”); alleles=c(“A”, “B”); crosstype = “dh”. After manual conversion of SNPs that had been designated within GenomeStudio indicating that AB should be converted to either AA or BB, any genotypes listed as NC (no call) or AB (inappropriate call) were treated as missing data. The function jittermap() was performed to avoid multiple markers at the same map location and the parameter, amount, was left at the default value of 1e-06. Standard interval mapping was used as the method of QTL analysis because it allows the inspection of positions between markers (Broman & Karl, 2009). The scanone() function was used with model= “binary” and method = “em” (standard interval mapping). Significant LOD thresholds were set using operm() with 1000 permutations and alpha=0.05. The resulting LOD scores were plotted using the plot function of R. Two individual lines lacked phenotypic data and were ignored in the QTL analysis.

To determine the level of dissimilarity between the DH lines and Faller/RB07, the R package, cluster was used (Maechler et al., 2014). All genotype calls for the 37496 SNPs mapped to wheat chromosomes were used in the analysis. Three genotype classes, AA, AB, and BB, were transformed to the numeric values 1, 0, and -1 respectively. No calls were treated as missing data and coded as NA within the data frame. The data frame was transposed to fit the R package data requirements and the command daisy() was used with default settings. Dissimilarity was measured in Euclidean distance.

**Results**

**Stem Rust screening**

Stem rust screening results are displayed in Table 6. Generation F2 populations, 12XR019 and 12XR020, segregated at a 15:1 ratio (χ2 = 1.38 *P* = 0.2395; χ2 = 0.041 *P* = 0.8406, respectively), indicating resistance controlled by 2 genes. However, the 11XR188 F2 population did not segregate at the same ratio (χ2 = 22.90 *P* = 1.71 × 106) but instead segregated at a 3:1 ratio (χ2 = 0.0035 *P* = 0.953). The F2:3 population, 11XR188-3 was screened with races TTKSK and TRTTF. 11XR188-3 F2:3 families segregated in a 15:1 manner when screened with TTKSK (χ2 = 0 *P* = 1) 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 (χ2 = 4.26 P = 0.03894). While 11XR188-3 F2:3 families showed slightly different segregation ratios between the two screenings, the ratios of resistant:susceptible individuals were not independent (χ2 = 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 of the 24 TC1F1 plants selected for production of DH progeny can be seen in Table 7.

**Molecular Marker Screening**

PI 410954 failed to yield the *Sr25*-positive product for molecular marker *Gb* (*Sr25*) and the multiplex marker Sr26#43/BE518379 (*Sr26*). 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. Neither 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 8.

**SNP Genotyping and Mapping**

The genotype data of 109 DH lines and parents (*n*=112) for all markers on chromosome 6D was exported and transposed to conform to file parameters required for R/qtl. Initially, 497 SNPs were exported for analysis, after quality control for poor quality SNPs (no calls > 10 or majority of calls with norm R < 0.20) and uninformative SNPs (SNPs at which no distinct binomial distribution was observed), 482 SNPs were used for futher marker analyses. The use of the function jittermap created an additional 331 markers across the entire chromosome (total = 813). These markers were not used for downstream analysis beyond the construction of the LOD plot. The plot of the calculated LOD scores can be seen in Figure 3. **This section will depend on the results of new screenings involving the following DH individuals: 158-13-4, 158-13-8, 158-13-6,162-3-3, 168-5-4, 168-5-6, 169-11-9, 169-13-5, 169-13-14**

When phenotypes for the above lines are not corrected all LOD scores above 2.12 are significant (*P* < 0.05). Out of 482 total SNPs, there are 22 SNPs between 18.20 cM and 27.21 cm that meet this significance threshold. However, 14 SNPs are monomorphic and the 8 remaining SNPs are between 18.20 cM and 24.77 cM on the 90K consensus map with adjusted distances (Wang et al., 2014).

When phenotypes for the above lines are corrected all LOD scores above 2.0 are significant (*P* < 0.05). Out of 482 total SNPs, the same 22 SNPs between 18.20 cM and 27.21 cM are above the significance threshold. However, 13 SNPs are monomorphic and the remaining 9 SNPs are between 18.20 cM and 24.77 cM. Only one SNP is located outside the range of 23-24 cM in both analyses, IACX9471 yet this SNP has the highest LOD score in both analyses, 8.94 and 10.54, respectively. In the analyses without the corrected phenotypes there are three resistant lines that consistently possess the non-PI 410954 alleles (see Table 9). In the analysis with the corrected phenotypes this issue is avoided (see Table 10). When susceptible and resistant haplotypes are compared there are 5 markers that are consistent with the phenotypes in that the resistant lines have all PI 410954 alleles and the susceptible lines all lack PI 410954 alleles. In the susceptible category there are 7 lines that posses PI 410954 alleles at all but two SNPs. **Redo these along with others listed above: 153-13-3, 153-13-4, 158-13-7, 158-13-8, 158-13-12, 158-13-14, 158-13-16.**

Results of the pairwise dissimilarity analysis using non-corrected phenotypes revealed that only one DH line was significantly dissimilar (*P* < 0.05) from either RB07 or Faller. 16 of 22 and 20 of 22 lines were more distant from PI 410954 than either Faller or RB07, respectively. For all pairwise Euclidean distances see Table 11.

**Discussion**

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, usually phenotypic, to minimize undesired traits. As can be imagined, 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 as elite parents. Faller, released by the North Dakota Agricultural Experiment Station 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 are both excellent cultivars. 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 (Anderson et al., 2009, Mergoum et al., 2008).

We used a genome-wide pairwise dissimilarity analysis to determine the degree of dissimilarity between the elite parental lines and the derived DH lines. The pairwise dissimilarity analysis revealed that the parents were more dissimilar to each other than was any DH line compared to the elite parents. Because the DH lines are derived from the elite cultivars this result is to be expected. The majority of DH lines were more distant from PI 410954 than from either elite parent. Lines with the lowest amount of dissimilarity represent lines possessing a higher amount of elite parental genomic material and will be preferred lines for breeders. Preliminary field trials have provided some evidence based on number of tillers and plant height to support this hypothesis.

In total, 9(8) SNPs on chromosome 6D were polymorphic and had significant (*P*<0.05) LOD scores. SNPs IACX9471, BS00021983\_51, BS00022523\_51, Ra\_c42576\_780, and RAC875\_c3996\_851 have been mapped to the short arm of chromosome 6D (Wang et al., 2014). The remaining 4(3) SNPs have not been conclusively mapped to a specific arm of 6D, however, their interspersed placement among markers on 6DS and their map location suggest that they are located on 6DS. Thus far, no unambiguous SNPs have been identified. The resistant phenotype in the non-*Sr24* resistant DH lines was difficult to distinguish due to the scoring ambiguity between ITs 23 and 3. Also, several DH lines showed inconsistent reactions between the two screenings. We are in the process of screening ambiguous lines with race TTKST. While it is clear that non-*Sr24* resistance is controlled by a single qualitative gene, using a single-QTL model for analysis allowed us to ascertain the approximate location of the gene and identify informative SNPs. The TC1DH population is not ideal for gene mapping due to the potential of 3 distinct alleles at each locus instead of only 2. SNP genotyping treats each loci as bi-allelic, therefore it is difficult to assign specific alleles to a single parent when parents may share SNP genotypes but possess different alleles. Despite the ambiguity introduced by phenotyping and population structure the significant LOD scores strongly indicate that the non-*Sr24* resistance gene is located distally on chromosome 6DS. We are currently in the process of converting informative SNPs to KASP markers that will be used to screen 11XR188-3 F4:5 families to verify the location of the resistance gene in a biparental population.

There are 2 named stem rust resistance genes effective against TTKSK known to be located on 6DS: *Sr42* and *SrTA10187* (McIntosh et al., 1995, Ghazvini et al., 2012, Olson et al., 2013a). *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). *SrTA10187* was discovered in *Aegilops tauschii*, a species not listed in any parental pedigree. Therefore *A. tauschii* is likely not the source of the resistance observed in the DH lines. The low infection type for *SrTmp*, 2- to 23, is very similar to the resistance we observed in the DH lines when screened with pgt race TTKST (McIntosh et al., 1995). PI 410954 has the cultivar Triumph listed in its pedigree. *SrTmp* is found in the Triumph background quite often (Roelfs & 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 pgt 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 epidemic of stem rust emerging from the southern Great Plains (Roelfs & McVey, 1979).

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 four 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 |
| RKQQ | 99KS76A | 5,6,7b,8a,9a,9b,9d,9g,21,36/9e,10,11,17,30,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 associations of the 152 resistant accessions’ reactions among *Puccinia graminis* f. sp. *tritici* races in pair-wise comparisons measured by χ2 values and probability. All associations shown are positive.

|  |  |  |  |
| --- | --- | --- | --- |
| **Association between** | |  |  |
| **Race** | **Race** | **χ2** | ***P* value (<0.05)** |
| TTKSK | TTKST | 37.4683 | 9.29E-10 |
| TTKSK | TTTSK | 35.0852 | 3.16E-09 |
| TTKST | TTTSK | 20.2154 | 6.92E-06 |
| TTKSK | QTHJC | 5.7579 | 1.64E-02 |
| TTTSK | RKQQC | 7.4455 | 6.36E-03 |
| TRTTF | TTTTF | 13.0779 | 2.99E-04 |
| TRTTF | TPMKC | 24.5119 | 7.39E-07 |
| TTTTF | TPMKC | 22.8337 | 1.77E-06 |
| TPMKC | QTHJC | 8.2589 | 4.06E-03 |
| TPMKC | RKQQC | 4.8075 | 2.83E-02 |

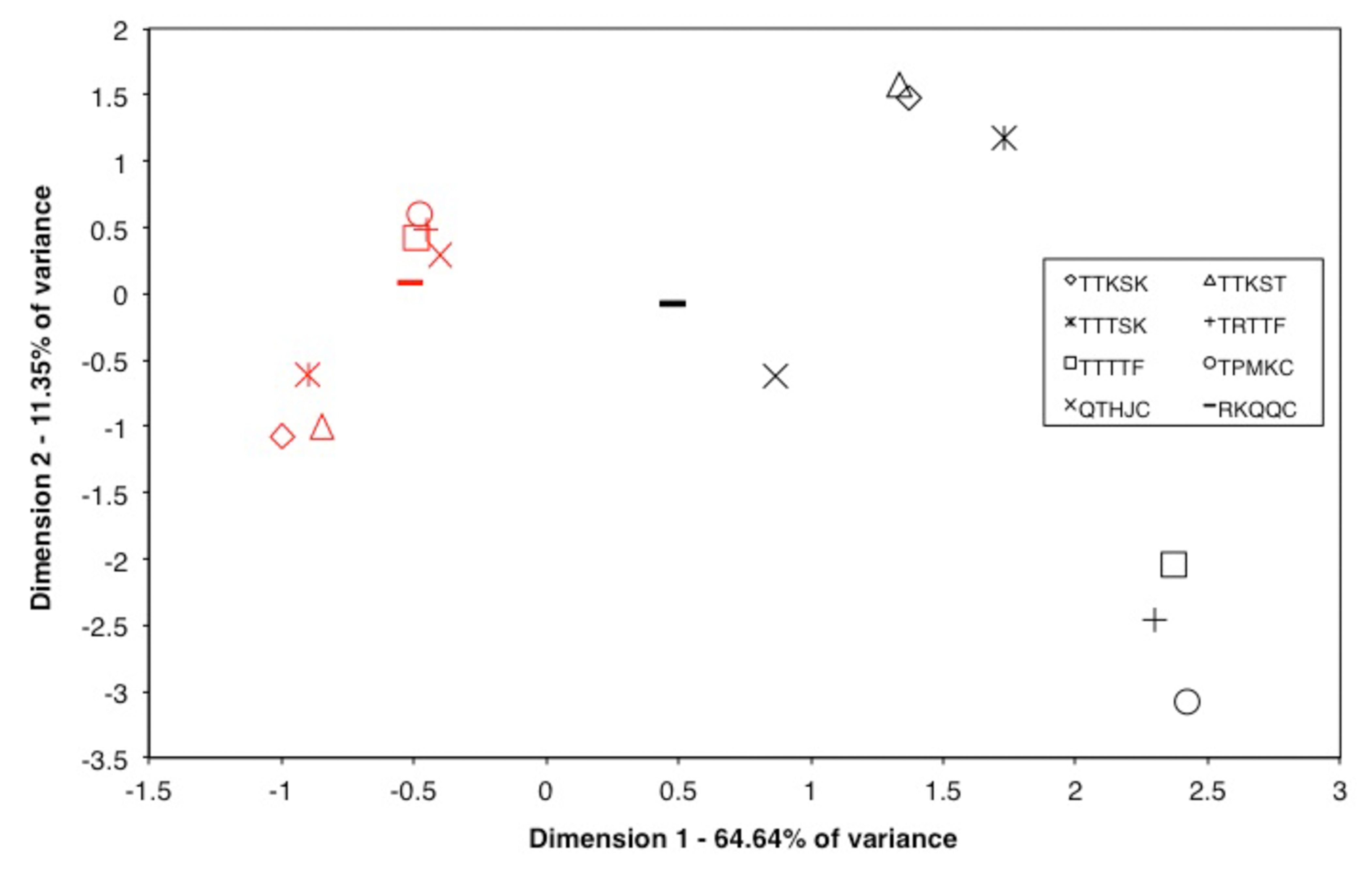
|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | | | | | | | | | |
|  |  | **Race** | | | | | | | |
|  |  | **TTKSK** | | **TTKST** | | **TTTSK** | | **TRTTF** | |
| **Accession** | **Grp.** | **Rep 1** | **Rep 2** | **Rep 1** | **Rep 2** | **Rep 1** | **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 4.**  Infection types clustered by group of the 29 accessions resistant to *P. 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”)

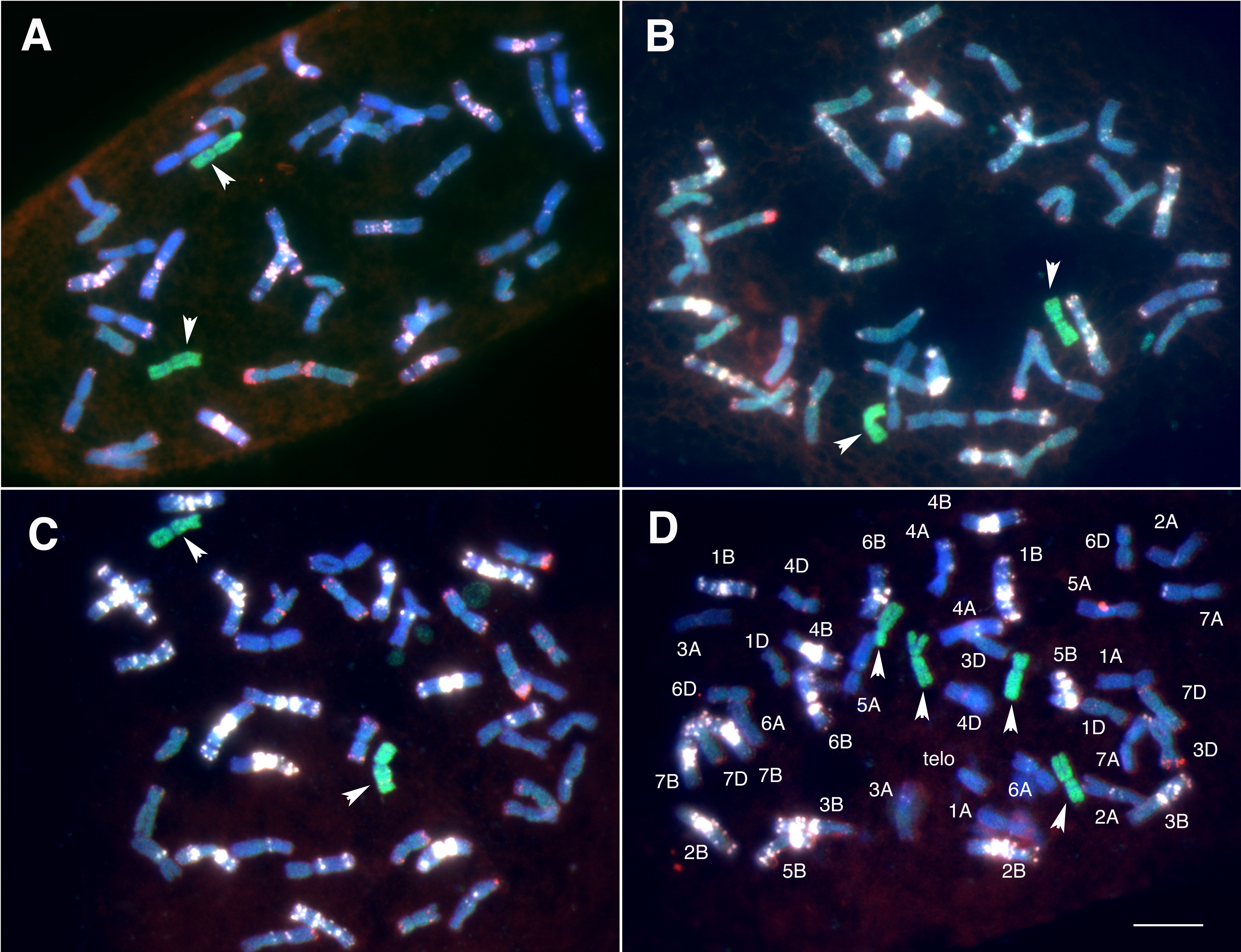
**Table 4.** *(continued)*

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | | **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 | |

**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.** FISH-GISH images of A. PI 604981, B. PI 611932, C. PI 605286, D. PI 605057 identifying *Th. ponticum* chromosomes by GISH (green stain) and individual *T. aestivum* chromosomes by chromosome specific GAA and pAs1 pattern. Chromosomes are counterstained with DAPI (blue), GAA repeats are white and pAs1 repeats are red. Bar corresponds to 10 μm.



**Table 5.** 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 et al., 2005) |
| SR Resistance | *Sr24* | Sr24#12 | F: CACCCGTGACATGCTCGTA  R: AACAGGAAATGAGCAACGATGT | (Mago et al., 2005) |
| SR Resistance | *Sr25* | Gb | F: CATCCTTGGGGACCTC  R: CCAGCTCGCATACATCCA | (Liu et al., 2010) |
| SR Resistance | *Sr26* | Sr26#43/  BE518379 | Sr26#43-F: AATCGTCCACATTGGCTTCT  Sr26#43-R: CGCAACAAAATCATGCACTA  BE518379-F: AGCCGCGAAATCTACTTTGA  BE518379-R: TTAAACGGACAGAGCACACG | (Liu et al., 2010) |
| LR Resistance | *Lr34* | csLV34 | csLV34F: GTTGGTTAAGACTGGTGATGG csLV34R: TGCTTGCTATTGCTGAATAGT | (Lagudah et al., 2006) |
| Plant Height WT | *Rht-B1a* | BF/WR1 | BF: GGTAGGGAGGCGAGAGGCGAG WR1:CATCCCCATGGCCATCTCGAGCTG | (Ellis et al., 2002) |
| Plant Height Dwarfing | *Rht-B1b* | BF/MR1 | F: CCAGATACACAACTGCTGGC R: TGATCTTGAGGTTCTCGTCG |  |
| Plant Height WT | *Rht-D1a* | DF2/WR2 | DF2: GGCAAGCAAAAGCTTCGCG WR2: GGCCATCTCGAGCTGCAC |  |
| Plant Height Dwarfing | *Rht-D1b* | DF/MR2 | DF: CGCGCAATTATTGGCCAGAGATAG MR2:CCCCATGGCCATCTCGAGCTGCTA |  |
| HMW-Gs | *Glu-A1* | UMN19 | UMN19F:CGAGACAATATGAGCAGCAAG UMN19R: CTGCCATGGAGAAGTTGGA | (Liu et al., 2008) |
| HMW-Gs | *Glu-D1* | UMN25 | UMN25F: GGGACAATACGAGCAGCAAA UMN25R: CTTGTTCCGGTTGTTGCCA | (Liu et al., 2008) |

**Table 6a.** Segregation of multiple PI 410954 F2 populations when screened with *P. graminis* f. sp. *tritici* race TTKSK. χ2 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** | **χ2** | ***P* value (<0.05)** |
| 11XR188 | 147 | 47 | 22.9 | 1.71 x 106 |
| 12XR019 | 327 | 29 | 0.625 | 0.4044 |
| 12XR020 | 96 | 8 | 0.041 | 0.8406 |

**Table 6b.** Segregation of 100 11XR188-3 F2:3 families when screened with two races of *P. graminis* f. sp.*tritici*. TTKSK:TRTTF is the comparison of the ratio resistant:susceptible in the two screenings.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Race** | **Resistant** | **Susceptible** | **χ2** | ***P* value (<0.05)** |
| TTKSK | 94 | 6 | 0 | 1 |
| TRTTF | 88 | 12 | 4.26 | 0.0389 |
| TTKSK:TRTTF | NA | NA | 1.53 | 0.2167 |

**Table 7.** Infection types (IT) of TC1F1 individuals selected from the cross, RB07//Faller/PI 410954, for doubled haploid (DH) production using *P. graminis* f. sp. *tritici* race TRTTF. “- -“ indicates pustules were much smaller than expected for the given IT.

|  |  |
| --- | --- |
| **Line** | **IT** |
| 12XR132-6 | 2--; |
| 12XR134-4 | 2--; |
| 12XR137-3 | 22-; |
| 12XR140-2 | 2-; |
| 12XR140-5 | 2-; |
| 12XR147-1 | 2--; |
| 12XR150-8 | 2-; |
| 12XR150-17 | 2-; |
| 12XR153-13 | ;12-- |
| 12XR154-10 | ;1 |
| 12XR158-13 | ;1 |
| 12XR158-14 | ;1 |
| 12XR162-3 | 2-; |
| 12XR163-4 | 2--; |
| 12XR163-22 | 2-; |
| 12XR163-30 | ;2- |
| 12XR164-4 | ;1 |
| 12XR166-18 | 2-; |
| 12XR168-5 | ;2- |
| 12XR168-8 | ;1 |
| 12XR169-1 | 2-; |
| 12XR169-11 | ;2-- |
| 12XR169-13 | ;1 |
| 12XR170-5 | 0 |
| Faller | 3+ |
| RB07 | 3+ |
| PI 410954 | 2-; |

**Table 8.** 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 | **+** | **+** | **+** |

Figure 3. Plot of LOD scores for SNPs on chromosome 6D derived using the multiple imputation method in the program R/qtl. The X-axis shows the mapped SNP locations and the Y-axis displays the corresponding LOD score at each SNP. The dotted red line indicates a significance of *P* < 0.05.



**Table 9.** Genotypes of non-*Sr24* resistant DH lines and parents at significant (*P* < 0.05), polymorphic SNPs (non-corrected phenos)

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | SNP | IACX9471 | BS00021983\_51 | BS00022523\_51 | Ra\_c42576\_780 | BobWhite\_rep\_c52808\_186 | Ex\_c882\_534 | IAAV1942 | BS00093063\_51 |
|  |  | chr |  | 6D | 6D | 6D | 6D | 6D | 6D | 6D |
|  | **Phenotype (R=1,S=0)** | cM | 18.20 | 23.84 | 23.84 | 23.84 | 24.77 | 24.77 | 24.77 | 24.77 |
| **Sample ID** | LOD | 8.94 | 5.22 | 5.22 | 5.22 | 4.70 | 4.70 | 4.70 | 4.70 |
| 12XR140-2-1 | 1 |  | BB | BB | BB | BB | BB | BB | BB | AA |
| 12XR140-2-2 | 1 |  | BB | BB | BB | BB | BB | BB | BB | AA |
| 12XR140-2-13 | 1 |  | BB | BB | BB | BB | BB | BB | BB | AA |
| 12XR147-1-1 | 1 |  | BB | BB | BB | BB | BB | BB | BB | AA |
| 12XR147-1-7 | 1 |  | BB | BB | BB | BB | BB | BB | BB | BB |
| 12XR147-1-10 | 1 |  | BB | BB | BB | BB | BB | BB | BB | NC |
| 12XR158-13-4 | 1 |  | AA | AA | AA | AA | AA | AA | AA | BB |
| 12XR158-13-8 | 1 |  | BB | BB | BB | BB | BB | BB | BB | AA |
| 12XR158-13-10 | NA |  | BB | BB | BB | BB | BB | BB | BB | BB |
| 12XR158-13-16 | 1 |  | BB | BB | BB | BB | BB | BB | BB | AA |
| 12XR158-14-5 | NA |  | BB | BB | BB | BB | BB | BB | BB | BB |
| 12XR158-14-9 | 1 |  | BB | BB | BB | BB | BB | BB | BB | AA |
| 12XR162-3-1 | 1 |  | BB | BB | BB | BB | BB | BB | BB | AA |
| 12XR164-4-3 | 1 |  | BB | BB | BB | BB | BB | BB | BB | BB |
| 12XR168-5-2 | 1 |  | BB | BB | BB | BB | BB | BB | BB | AA |
| 12XR168-5-7 | 1 |  | BB | BB | BB | BB | BB | BB | BB | AA |
| 12XR169-1-1 | 1 |  | BB | BB | BB | BB | BB | BB | BB | BB |
| 12XR169-1-4 | 1 |  | BB | BB | BB | BB | BB | BB | BB | AA |
| 12XR169-11-9 | 1 |  | AA | AA | AA | AA | AA | AA | AA | BB |
| 12XR169-13-2 | 1 |  | BB | BB | BB | BB | BB | BB | NC | AA |
| 12XR169-13-5 | 1 |  | AA | AA | AA | AA | BB | BB | AA | BB |
| 12XR169-13-8 | 1 |  | BB | BB | BB | BB | BB | BB | BB | BB |
| 12XR169-13-12 | 1 |  | BB | BB | BB | BB | BB | BB | BB | AA |
| RB07 | 0 |  | AA | AA | AA | AA | BB | BB | AA | AA |
| Faller | 0 |  | AA | AA | AA | AA | AA | AA | AA | BB |
| PI 410954 | 1 |  | BB | BB | BB | BB | BB | BB | BB | BB |

**Table 10.** Genotypes of non-*Sr24* resistant DH lines and parents at significant (*P* < 0.05), polymorphic SNPs (corrected phenos)

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | SNP | IACX9471 | BS00021983\_51 | Ra\_c42576\_780 | BS00022523\_51 | Ex\_c882\_534 | IAAV1942 | BobWhite\_rep\_c52808\_186 | RAC875\_c67463\_300 | BS00093063\_51 |
|  |  | chr | 6D | 6D | 6D | 6D | 6D | 6D | 6D | 6D | 6D |
|  | **Phenotype (R=1,S=0)** | cM | 18.20 | 23.84 | 23.84 | 23.84 | 24.77 | 24.77 | 24.77 | 23.32 | 24.77 |
| **Sample ID** | LOD | 11.27 | 7.73 | 7.73 | 7.73 | 7.31 | 7.31 | 7.31 | 7.72 | 7.31 |
| 12XR140-2-1 | 1 |  | BB | BB | BB | BB | BB | BB | BB | AA | AA |
| 12XR140-2-2 | 1 |  | BB | BB | BB | BB | BB | BB | BB | AA | AA |
| 12XR140-2-13 | 1 |  | BB | BB | BB | BB | BB | BB | BB | AA | AA |
| 12XR147-1-1 | 1 |  | BB | BB | BB | BB | BB | BB | BB | AA | AA |
| 12XR147-1-7 | 1 |  | BB | BB | BB | BB | BB | BB | BB | BB | BB |
| 12XR147-1-10 | 1 |  | BB | BB | BB | BB | BB | BB | BB | AA | NC |
| 12XR158-13-10 | NA |  | BB | BB | BB | BB | BB | BB | BB | BB | BB |
| 12XR158-14-5 | NA |  | BB | BB | BB | BB | BB | BB | BB | BB | BB |
| 12XR158-14-9 | 1 |  | BB | BB | BB | BB | BB | BB | BB | BB | AA |
| 12XR162-3-1 | 1 |  | BB | BB | BB | BB | BB | BB | BB | BB | AA |
| 12XR162-3-3 | 1 |  | BB | BB | BB | BB | BB | BB | BB | BB | AA |
| 12XR164-4-3 | 1 |  | BB | BB | BB | BB | BB | BB | BB | BB | BB |
| 12XR168-5-2 | 1 |  | BB | BB | BB | BB | BB | BB | BB | AA | AA |
| 12XR168-5-4 | 1 |  | BB | BB | BB | BB | BB | BB | BB | AA | AA |
| 12XR168-5-6 | 1 |  | BB | BB | BB | BB | BB | BB | BB | AA | AA |
| 12XR168-5-7 | 1 |  | BB | BB | BB | BB | BB | BB | BB | AA | AA |
| 12XR169-1-1 | 1 |  | BB | BB | BB | BB | BB | BB | BB | BB | BB |
| 12XR169-1-4 | 1 |  | BB | BB | BB | BB | BB | BB | BB | AA | AA |
| 12XR169-13-2 | 1 |  | BB | BB | BB | BB | BB | NC | BB | AA | AA |
| 12XR169-13-8 | 1 |  | BB | BB | BB | BB | BB | BB | BB | AA | BB |
| 12XR169-13-12 | 1 |  | BB | BB | BB | BB | BB | BB | BB | AA | AA |
| 12XR169-13-14 | 1 |  | BB | BB | BB | BB | BB | BB | BB | AA | AA |
| RB07 | 0 |  | AA | AA | AA | AA | BB | AA | BB | AA | AA |
| Faller | 0 |  | AA | AA | AA | AA | AA | AA | AA | BB | BB |
| PI 410954 | 1 |  | BB | BB | BB | BB | BB | BB | BB | BB | BB |

**Table 11.**  Pairwise dissimilarity distances (Euclidean) between non-*Sr24* resistant DH lines and PI 410954, Faller, RB07. Analysis peformed in R using the package cluster. Distances in bold italics are significant (*P* < 0.05).

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **12XR140-2-1** | **12XR140-2-2** | **12XR140-2-13** | **12XR147-1-1** | **12XR147-1-7** | **12XR147-1-10** | **12XR158-13-4** | **12XR158-13-8** |
| **12XR140-2-1** |  |  |  |  |  |  |  |  |
| **12XR140-2-2** | 88.57 |  |  |  |  |  |  |  |
| **12XR140-2-13** | 82.30 | 87.63 |  |  |  |  |  |  |
| **12XR147-1-1** | 104.99 | 93.38 | 111.51 |  |  |  |  |  |
| **12XR147-1-7** | 97.04 | 100.63 | 106.51 | 82.91 |  |  |  |  |
| **12XR147-1-10** | 98.93 | 95.85 | 99.88 | 78.41 | 80.46 |  |  |  |
| **12XR158-13-4** | 98.97 | 97.04 | 98.88 | 90.84 | 95.31 | 93.68 |  |  |
| **12XR158-13-8** | 105.58 | 101.69 | 106.37 | 103.90 | 111.44 | 108.71 | 107.55 |  |
| **12XR158-13-16** | 108.63 | 107.63 | 111.84 | 108.53 | 111.27 | 113.34 | 110.03 | 82.09 |
| **12XR158-14-5** | 102.21 | 94.63 | 104.24 | 88.78 | 95.14 | 95.84 | 91.29 | 115.42 |
| **12XR158-14-9** | 100.32 | 95.85 | 87.65 | 107.16 | 105.66 | 101.17 | 94.79 | 112.48 |
| **12XR162-3-1** | 100.26 | 95.81 | 87.67 | 107.13 | 105.58 | 101.13 | 94.71 | 112.47 |
| **12XR164-4-3** | 90.37 | 107.31 | 98.72 | 106.68 | 102.32 | 106.44 | 99.36 | 113.27 |
| **12XR168-5-2** | 85.13 | 91.52 | 96.50 | 93.71 | 83.60 | 90.70 | 85.04 | 104.79 |
| **12XR168-5-7** | 85.04 | 91.47 | 96.41 | 93.71 | 83.57 | 90.67 | 85.05 | 104.79 |
| **12XR169-1-1** | 94.46 | 99.70 | 82.01 | 110.58 | 93.66 | 102.10 | 95.61 | 112.82 |
| **12XR169-1-4** | 87.21 | 92.74 | 100.71 | 97.94 | 102.48 | 92.90 | 93.11 | 108.56 |
| **12XR169-11-9** | 85.26 | 89.32 | 85.11 | 101.12 | 91.38 | 94.71 | 96.18 | 108.98 |
| **12XR169-13-2** | 93.63 | 87.54 | 84.21 | 105.49 | 93.44 | 93.11 | 95.43 | 109.69 |
| **12XR169-13-5** | 91.14 | 94.34 | 102.16 | 101.73 | 95.49 | 90.49 | 97.64 | 113.13 |
| **12XR169-13-8** | 85.55 | 89.73 | 102.91 | 95.78 | 96.30 | 92.84 | 89.52 | 106.88 |
| **12XR169-13-12** | 90.02 | 91.74 | 92.33 | 97.89 | 95.58 | 88.59 | 90.25 | 110.24 |
| **PI 410954** | 112.43 | 109.42 | 106.63 | 111.35 | 108.04 | 113.52 | ***120.57*** | 113.24 |
| **RB07** | 89.25 | 89.28 | 92.05 | 91.57 | 91.98 | 84.98 | 86.80 | 116.38 |
| **Faller** | 105.83 | 103.02 | 106.83 | 106.98 | 107.06 | 106.99 | 99.27 | 103.20 |

**Table 11.** *(continued)*

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **12XR158-13-16** | **12XR158-14-5** | **12XR158-14-9** | **12XR162-3-1** | **12XR164-4-3** | **12XR168-5-2** | **12XR168-5-7** | **12XR169-1-1** |
| **12XR140-2-1** |  |  |  |  |  |  |  |  |
| **12XR140-2-2** |  |  |  |  |  |  |  |  |
| **12XR140-2-13** |  |  |  |  |  |  |  |  |
| **12XR147-1-1** |  |  |  |  |  |  |  |  |
| **12XR147-1-7** |  |  |  |  |  |  |  |  |
| **12XR147-1-10** |  |  |  |  |  |  |  |  |
| **12XR158-13-4** |  |  |  |  |  |  |  |  |
| **12XR158-13-8** |  |  |  |  |  |  |  |  |
| **12XR158-13-16** |  |  |  |  |  |  |  |  |
| **12XR158-14-5** | ***119.40*** |  |  |  |  |  |  |  |
| **12XR158-14-9** | 111.73 | 76.93 |  |  |  |  |  |  |
| **12XR162-3-1** | 111.67 | 76.95 | 1.45 |  |  |  |  |  |
| **12XR164-4-3** | 114.44 | 93.83 | 95.40 | 95.36 |  |  |  |  |
| **12XR168-5-2** | 105.61 | 95.77 | 96.97 | 96.87 | 97.37 |  |  |  |
| **12XR168-5-7** | 105.55 | 95.69 | 96.89 | 96.81 | 97.33 | 1.03 |  |  |
| **12XR169-1-1** | 114.81 | 97.62 | 91.92 | 91.85 | 95.26 | 95.11 | 95.03 |  |
| **12XR169-1-4** | 112.17 | 87.11 | 94.86 | 94.85 | 91.15 | 89.72 | 89.72 | 85.76 |
| **12XR169-11-9** | 110.26 | 87.97 | 87.90 | 87.85 | 88.20 | 83.97 | 83.84 | 79.66 |
| **12XR169-13-2** | 108.32 | 100.85 | 88.66 | 88.58 | 101.20 | 93.37 | 93.24 | 84.07 |
| **12XR169-13-5** | 113.30 | 97.11 | 97.39 | 97.31 | 99.09 | 94.17 | 94.08 | 98.65 |
| **12XR169-13-8** | 111.93 | 93.15 | 98.62 | 98.54 | 89.76 | 91.11 | 91.08 | 92.92 |
| **12XR169-13-12** | 109.72 | 99.60 | 100.71 | 100.67 | 101.55 | 91.12 | 91.08 | 91.68 |
| **PI 410954** | 117.43 | 108.88 | 106.77 | 106.68 | 111.98 | 116.90 | 116.89 | 101.14 |
| **RB07** | ***118.96*** | 80.12 | 88.70 | 88.67 | 92.20 | 80.11 | 80.08 | 90.99 |
| **Faller** | 107.71 | 114.24 | 107.58 | 107.48 | 105.53 | 104.60 | 104.49 | 108.60 |

**Table 11.** *(continued)*

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **12XR169-1-4** | **12XR169-11-9** | **12XR169-13-2** | **12XR169-13-5** | **12XR169-13-8** | **12XR169-13-12** | **PI 410954** | **RB07** |
| **12XR140-2-1** |  |  |  |  |  |  |  |  |
| **12XR140-2-2** |  |  |  |  |  |  |  |  |
| **12XR140-2-13** |  |  |  |  |  |  |  |  |
| **12XR147-1-1** |  |  |  |  |  |  |  |  |
| **12XR147-1-7** |  |  |  |  |  |  |  |  |
| **12XR147-1-10** |  |  |  |  |  |  |  |  |
| **12XR158-13-4** |  |  |  |  |  |  |  |  |
| **12XR158-13-8** |  |  |  |  |  |  |  |  |
| **12XR158-13-16** |  |  |  |  |  |  |  |  |
| **12XR158-14-5** |  |  |  |  |  |  |  |  |
| **12XR158-14-9** |  |  |  |  |  |  |  |  |
| **12XR162-3-1** |  |  |  |  |  |  |  |  |
| **12XR164-4-3** |  |  |  |  |  |  |  |  |
| **12XR168-5-2** |  |  |  |  |  |  |  |  |
| **12XR168-5-7** |  |  |  |  |  |  |  |  |
| **12XR169-1-1** |  |  |  |  |  |  |  |  |
| **12XR169-1-4** |  |  |  |  |  |  |  |  |
| **12XR169-11-9** | 87.52 |  |  |  |  |  |  |  |
| **12XR169-13-2** | 98.40 | 84.21 |  |  |  |  |  |  |
| **12XR169-13-5** | 101.32 | 94.57 | 82.04 |  |  |  |  |  |
| **12XR169-13-8** | 81.96 | 90.12 | 90.38 | 84.74 |  |  |  |  |
| **12XR169-13-12** | 91.75 | 87.76 | 71.57 | 77.18 | 89.43 |  |  |  |
| **PI 410954** | 113.80 | 108.99 | 109.29 | 111.31 | 115.32 | 112.03 |  |  |
| **RB07** | 79.58 | 82.88 | 96.71 | 91.23 | 80.22 | 89.65 | ***124.56*** |  |
| **Faller** | 108.66 | 109.68 | 96.53 | 104.60 | 102.74 | 105.28 | ***129.00*** | ***121.49*** |

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