PRE-BREEDING TO IMPROVE YIELD AND DISEASE RESISTANCE OF HARD RED WINTER WHEAT

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MASTER OF SCIENCE

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ABSTRACT

Challenges to growing winter wheat in North Dakota include winter temperature and disease pressure. Fusarium head blight (FHB) is a devastating disease that necessitates breeding for resistance. In the NDSU breeding program FHB resistance genes are often associated with a decrease in performance. This study used single seed descent to advance lines while maintaining a near random population. Early generation (F_4) selection focused heavily on yield and the presence of FHB resistance quantitative trait loci to develop winter wheat lines with FHB resistance and high yield.

Stripe rust is a fungal disease that is becoming increasingly problematic in North Dakota. To assess the available stripe rust resistance in the NDSU winter wheat germplasm, two sets of diverse breeding lines were used for stripe rust resistance phenotyping and genotyping by sequencing. The phenotype and genotype data were then used to locate resistance genes through genome wide association study.

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Wheat Domestication

The earliest forms of cultivated wheat were the diploid species *Triticum monococcum* (einkorn) and the tetraploid species *Triticum dicoccon* (emmer wheat). It wasn't until around 10,000 years ago that the hexaploid bread wheat species *Triticum aestivum* first appeared (Feldman, 2001). *Triticum aestivum*, along with other small grains like barley, were among the first plants domesticated around 10,000 years ago in the Fertile Crescent. The center of origin for wheat can be pinpointed to the mountainous regions around the Tigris and Euphrates rivers (Braidwood, 1969).

Early humans began unconsciously breeding wheat by mass selection (Feldman, 2001). They did so by simply choosing the plants that had characteristics important to them such as the amount of seeds on a plant and taste, as well as plants that looked healthy. They would then save some of the harvested seeds to plant near their home the next year. Besides increased yield some of the other traits that were first bred for were domestication syndrome traits. Domestication syndrome traits are the traits that need to be modified in order for a plant species to be properly domesticated. Two common domestication traits are loss of spike shattering and a change from hulled to free-threshed spikes. In wheat the first of these traits that was bred for was non-brittle rachis (Charmet, 2011). Having brittle rachis results in spike shattering and loss of seed. It was important to get rid of spike shattering to decrease the amount of seed that is lost when spikes become mature (Nalam, 2006). The second trait that was bred for was naked grain (free-threshing) (Charmet, 2011). Making wheat free-threshing made it easy to remove the glumes from the grain (Jantasuriyarat, 2004). It was later determined that wheat plants that have non-brittle rachis and are free-threshing have a dominant allele at the *Q* locus (Jantasuriyarat, 2004).

Being qualitative traits made it possible for early farmers to breed for these two traits without having knowledge as to how the traits are genetically controlled. Later, more complex traits such as flowering time and grain size progressively improved favorably for farmers (Charmet, 2011). Since then, wheat has become the most widely cultivated crop with over 120 countries growing wheat (Langridge, 2017).

Economic Importance

Wheat has become one of the most widely grown crops in the world with over 214 million ha planted in 2018 with an average yield of 3425 kg/ha (FAOSTAT, 2018). Wheat has one of the largest cultivation regions of any crop being grown from Scandinavia to South America (CIMMYT-WHEAT, 2017). Wheat export quantity exceeds all other crops and reached 190 million tonnes in 2018 (FAOSTAT, 2018). China (131 million tonnes) and India 100 (million tonnes) are the two largest producers of wheat, accounting for 30% of the total wheat production in the world (FAOSTAT, 2018). The average per capita consumption of wheat around the world is 66 kg/year (Awika, 2011). Wheat is an even more important crop in developing countries where 80% of the wheat grown is used for food, compared to 50% in already developed countries. Wheat is an important part of a balanced diet as it is high in both carbohydrates and protein. Wheat is also a very important part of the North Dakota economy. North Dakota consistently ranks first in the United States in durum and spring wheat production (North Dakota Wheat Commission). In North Dakota 3.1 million hectares of wheat was grown in 2018. The average yield of wheat in North Dakota was 3201 kg/ha in 2018.

Types of Wheat

There are many different types of wheat with the main ones being hard red winter wheat (HRWW), soft red winter wheat (SRWW), hard red spring wheat (HRSW), hard white wheat

(HWW), soft white wheat (SWW), and durum (US Wheat Associates, 2020). Different types of wheat are used for different types of baking with HRWW being used mainly for bread, HRSW for rolls, SRWW for cookies, SWW for cakes, HWW for Asian style noodles, and durum for premium pasta. Different types of wheat also have different production regions within the United States. Hard red winter wheat is mainly grown in the Great Plains region with Kansas and Oklahoma being the two main producers. Soft red winter wheat production is spread throughout the eastern United States. Hard red spring wheat is mainly grown in the upper Midwest with most production occurring in North Dakota, Minnesota, Montana, and South Dakota. Hard white wheat is the least grown class of wheat with production occurring in the western United States as well as sporadically in the Great Plains. Soft white wheat is grown almost entirely in Washington, Oregon, and Idaho. Durum wheat is grown almost entirely in North Dakota. Ninety five percent of the wheat currently grown is of the hexaploid bread wheat types HRWW, SRWW, HRSW, HWW, and SWW (Shewry, 2009). Most of the remaining 5% grown is durum wheat. The many different uses in baking along with its ability to grow in drier environments than other crops like *Zea mays*, and *Glycine max* have made wheat a staple food crop throughout the world.

Wheat Breeding

Wheat breeding follows the same general five step plan as many other self-pollinating crops. Step 1 is to identify the problem and the objectives, step 2 is to select germplasm, step 3 choose selection type and breeding method, step 4 evaluate lines, and step 5 release cultivar (Baenziger, 2018). Common problems and objectives targeted in wheat breeding are increasing yield, improving disease resistance, increasing abiotic stress tolerance, and decreasing height (Grover, 2003). In wheat it is important to consider how improving one trait will affect other

important traits. Often times when one trait is improved another trait will decrease in value. An example of this in wheat is that when yield is increased protein content is decreased (Fettell, 2012). Another example that will be looked at in chapter 2 of this paper is that when exotic genes (such as FHB resistance genes) are incorporated into winter wheat, yield may be decreased (Bakhsh, 2013).

After the problem and objectives have been identified, the next step is to select germplasm that could achieve the objectives (Baenziger, 2018). In wheat the most common germplasm that is used is lines that have been developed by the breeding program, or lines that have been developed by other programs that are acclimated to similar conditions. If there are no lines in that group that could help achieve the objective, breeders will choose lines that are adapted to other regions. In rare circumstances if there are no developed lines that can help achieve the objective, breeding will get germplasm from wild relatives of wheat.

The next step is to choose the breeding method. In wheat common breeding methods used are pedigree selection, bulk selection, single seed descent, doubled haploids, and backcrossing (Mac Key, 1986). Pedigree selection involves planting F_2 seeds in rows, selecting the best plants in each row for replanting, and repeating these two actions in subsequent generations until near homozygosity is reached, which usually takes 5-6 generations (Osei, 2014). Pedigree selection focuses heavily on artificial selection (selection by humans) of the best genotypes and is therefore labor and resource intensive (Hill, 2001). The evaluation, selection and note taking done while performing pedigree selection provide a lot of useful information to the breeding program. Bulk selection involves planting F_2 seed in a bulk plot, harvesting plants in bulk, and replanting a sample of the bulked seed until near homozygosity is reached (5-6 generations) (Osei, 2014). Whereas pedigree selection involves selecting the best

genotypes, bulk selection relies more heavily on natural selection to get rid of inferior genotypes. Artificial selection does not occur until inbred lines have been developed in bulk selection. This makes bulk selection less labor and resource intensive than pedigree selection. Single seed descent is a method used to reach homozygosity rapidly (Snape, 1975). Single seed descent is most often performed in a greenhouse, because of this multiple generations can be grown every year. Single seed descent is initiated from a large F_2 and aims to keep as much of the variation in the population as possible (Mac Key, 1986). Maintaining variation prevents genotypes that are segregating from being prematurely discarded. Plants need to be handled individually when performing single seed descent, and since there is no selection, the population size is a constant making single seed descent labor intensive. Single seed descent is a more rapid procedure for spring wheat than winter wheat. This is because winter wheat requires a vernalization period, which reduces the number of generations that can be grown every year. Doubled haploid breeding is another method to reach homozygosity rapidly and can be initiated in the F_1. While haploids can be generated through androgenesis (Pandey, 1973), the F_1 is most commonly crossed with an unrelated species (usually maize); the chromosomes from the unrelated species are spontaneously eliminated shortly after fertilization leaving a haploid wheat zygote. Chromosome number can then be doubled by treating with colchicine (Niu, 2014). Doubled haploids produced in this manner are completely homozygous individuals.

While Pedigree breeding is the preferred breeding strategy of most wheat breeding programs, it has the disadvantage of being less suited to the improvement of quantitative traits such as yield (Nunes, 2008). This is because initial (early generation) selection is strongly focused on easily evaluated qualitative traits, resulting in rapid erosion of variability for complex quantitative traits that can most effectively be selected for in replicated trials conducted in the

advanced generations (Mac Key, 1986). A modified version of Pedigree breeding that is more accommodating of quantitative traits involves the use of single seed descent inbreeding steps rather than the conventional single plant selection steps. In this manner a large number of inbred lines are developed through single seed descent from the F_2 to generate a population of inbred lines that are more representative of the total variability for yield within the cross. Such lines can then be evaluated more thoroughly in yield trials. With winter wheat there is an added level of complexity when breeding for complex traits such as cold hardiness and yield.

Cold hardiness is a complex trait that is quantitatively inherited (Worzella, 1942). The complexity of cold hardiness is due to changes in every morphological, physiological, and chemical character when winter wheat goes through cold acclimation (Limin, 1991). Cold hardiness is mostly controlled by additive effect genes, with the cold hardiness of the F_1 population almost always equal to the midpoint of the two parents (Sutka, 1981). As a trait cold hardiness has not been improved upon much in the last century. This is likely due to all the genetic variability for cold hardiness in winter wheat having already been exploited (Limin, 1991). Further compounding the difficulty of breeding for cold hardiness is inconsistent winter temperatures (Fowler, 1979). If winter temperatures do not drop below the minimum killing temperature for a cultivar the cultivar could be considered cold tolerant when in fact its winter survival was due to mild winter temperatures. The same is true for extremely cold winters, which could result in complete winterkill. Variation in the cold hardiness of winter wheat genotypes makes selecting high yielding varieties of winter wheat more difficult as well.

Yield of a wheat plant is determined by the number of spikes per plant X the number of spikelets per spike X number of kernels per spikelet X weight per seed (Slafer, 2007). Being controlled by multiple associated traits and many genes with minor effects makes yield a very

complex trait (Erkul, 2010). Each yield component of wheat has higher heritability than yield itself, because of this it can be beneficial to select for a single yield component instead of yield when trying to improve yield (Erkul, 2010; Slafer, 2007). The problem with selecting for a single yield component is that yield components are often negatively correlated with each other (Slafer, 2003). In winter wheat selection for high yielding lines can be complicated by winterkill (Kergjord Olsen, 2017). A line that could be very high yielding could end up being discarded for low yield, when in fact a harsh winter could have resulted in an abnormal amount of winterkill. This can be especially problematic in North Dakota when trying to select for high yielding, cold hardy lines that also have Fusarium head blight (FHB) resistance. FHB resistance genes are often transferred from spring wheat germplasm which generally lacks cold tolerance and reduces yield if winter temperatures are low (Bakhsh, 2013).

Winter Wheat

The advantages of planting winter wheat include a higher yield potential than spring wheat, early spring growth which can help reduce competition with weeds, reducing the need for selective herbicides, and spreading the need of labor and equipment (Wiersma, 2006). Winter wheat can be classified into three different categories based on vernalization length requirements. It can be weak winter, semi-winter, and strong winter (Li, 2013). Weak winter types require two weeks to vernalize, semi-winter requires two to four weeks to vernalize, and strong winter types requires more than four weeks to vernalize. In order to vernalize, temperatures usually need to be under eight degrees Celsius for an extended period. The harsh winters in North Dakota can be problematic for growing winter wheat. Winter hardiness is controlled by many genes, and because of its complex inheritance there hasn't been any genetic improvement in the last 100 years (Fowler, 1999). Winter wheat can survive at soil temperatures

down to -26 degrees Celsius (Wiersma, 2005). In order to improve the likelihood of surviving the winter it is important to choose a cultivar with good genetics for winter survival, plant into previous crop residue, apply phosphorus, and plant at the recommended date (Ransom, 2016). Planting into taller residue from the previous crop can improve winter survival by helping retain snow cover on top of established winter wheat seedlings. Snow cover of three inches has been shown to greatly increase the likelihood of winter wheat survival (Larson, 1987). Planting winter wheat too early can lead to excessive fall growth and can diminish soil moisture (Ransom, 2016). Planting too late may result in the crown not being hardy enough to survive the winter. This makes it problematic to plant winter wheat in a wheat-soybean rotation as soybeans are often not harvested until after the optimum planting date for winter wheat. Soybean stubble also will not retain as much snow due to its short stature. In a study conducted by J.K. Larsen and L.J. Brun they showed that when planting into seven-inch-tall barley stubble winter wheat survival was near 100% (Larson, 1987).

Winter wheat represents 60-80% of the total wheat production in the United States (USDA-ERS). In the 2018/2019 growing season 61% of the 51.29 million metric tons of wheat produced in the United States was winter wheat; this means a total of 31.3 million metric tons of winter wheat were produced (FAOSTAT; USDA-ERS). The major growing region for winter wheat in the United States is the Great Plains. Kansas is the state that produces the most winter wheat (Plains Grains Inc., 2017). In the period, 2010-2017 Kansas planted an average of 3.3 million hectares of winter wheat every year and produced a total of 9.25 million metric tons. In North Dakota winter wheat is far less popular due to the harsh winters. In North Dakota, from 2010-2017, 132,000 hectares of winter wheat were grown per year. North Dakota produces an average of 427,000 metric tons of winter wheat per year. Winter wheat has however, shown

potential to be more widely grown in North Dakota. The average yield of winter wheat in North Dakota from 2010-2017 was 3090 kg/ha, higher than that of Kansas which had an average yield of 2890 kg/ha.

Winter Wheat Quality

In wheat quality, the most important traits are protein percent, milling properties, mixing properties, and baking properties. Protein percent is measured as the percentage of weight of a kernel at a specific moisture percent (Carson, 2009). Milling wheat removes the bran and germ from the wheat kernel leaving behind the endosperm (Kanojia, 2018). The endosperm is then crushed to make refined flour. For milling, high flour extraction percent is important. Flour extraction percent is determined by weighing the amount of flour obtained through milling and dividing by the weight of wheat that was used to obtain the flour. The amount of glutenin in wheat flour is an important characteristic that affects mixing properties of flour (Dhaka, 2015). Different ways that glutenin content and mixing properties are measured include solvent retention capacity, Mixograph, and GlutoPeak characteristics.

Mixographs are used to measure the mixing characteristics of flour (Hazelton, 1994). Mixographs involve adding wheat flower (35, 10, or 2g) into a bowl with water, pins are then used to produce a pull, stretch, and tear action on the dough (AACC, 1999; Hazelton, 1994). In a mixograph the increasing slope indicates water absorption, the peak indicates optimum dough development and dough strength, and the downward slope indicates the breakdown of gluten from excess mixing (Neacsu, 2009). The main traits that can be measured from a mixograph are mix time to optimum consistency, water absorption, dough extensibility, dough stability, mixing tolerance, and dough elasticity (Labushagne, 2016). Longer peak times also signify a higher glutenin content in the flour. The values provided from a mixograph such as peak value (%),

peak value time, tail slope, and tail width can be combined into a singular mixograph score ranging from 1 (worst) - 8 (best) (Olsen, 2020 Personal Communication).

Solvent retention capacity (SRC) involves adding different solvents to wheat flour to determine components of the flour (Slade, 1994; AACC, 2009). The SRC test measures the enhanced swelling behavior of polymer networks when put in a solvent. The solvents used for SRC tests are water, 5% sodium carbonate: 95% water, 50% sucrose: 50% water, and 5% lactic acid: 95% water (AACC, 2009). Different solvents cause different flour components to swell, because of this each solvent can be used to measure different components of flour (Guttieri, 2001). Sodium carbonate is used to measure damaged starch, sucrose is used to measure pentosans, and lactic acid is used to measure glutenin. Water causes all three components to swell. When performing an SRC test, 5 grams of flour is put in a test tube with 25 grams of solvent, the tube is then shaken until the flour is dissolved, the tube is then centrifuged, supernatant is then discarded, the test tube is drained, and the swollen flour that remains is weighed (AACC, 2009). The percent weight increase is then recorded as the SRC value. For bread wheat SRC values desired are 65-70 in water, 105-115 in sucrose, 80-90 in sodium carbonate, and greater than 140 in lactic acid (U.S. Wheat Associates). A high SRC in sucrose means that the bread made from the flour will have a higher water retention capacity (Kweon, 2011). If the SRC in sodium carbonate is high, the bread will stale faster (U.S. Wheat Associates). The high SRC in lactic acid means that the flour has more glutenin (Guttieri, 2001).

The GlutoPeak uses shear force to measure the aggregation behavior of gluten (Wang, 2018). In the GlutoPeak method flour and a solvent are mixed together, the gluten is then separated by a rotating paddle until aggregation. The gluten will then resist the force of the

paddle resulting in a torque curve. This curve can be used to measure how complex the structure of the gluten is as well as the breakdown behavior of the gluten structure.

Diseases Affecting Wheat

One of the main problems with growing wheat is that there are many diseases that do serious harm to the crop. These diseases include FHB, stem rust, leaf rust, stripe rust, tan spot, and stagonospora nodorum blotch (Wiese, 1987). These diseases along with other abiotic and biotic stress factors have led to farmers getting nowhere close to the absolute yield level for wheat (Curtis, 2002). Wheat is estimated to have an absolute yield of 20 tons/ha based on genetic potential, but the highest yield farmers have been able to obtain has been 14 tons/ha. This has led breeders to focus their research on disease resistance. In addition to the use of native resistance, many of the resistance genes in wheat have been transferred from wild relatives (Bakhsh, 2013). Doing this can sometimes co-introduce deleterious genes through linkage drag leading to reduced yield and lower quality. However, in many instances gene transfer may not influence other desirable traits, or may even increase yield and quality (Gill, 2008; Liu, 2009). If there is a yield or quality penalty associated with a resistance gene in a cultivar, then in years where the disease is absent yield or quality may be decreased.

Rust Diseases Affecting Wheat

Wheat rust diseases have been a major problem for wheat growers since the beginning of wheat cultivation - the Greeks and Romans prayed to specific gods for help against rust diseases (Peterson, 2001). Rust infection in the fall can leave plants weakened and reduce winter survival as well as increase the likelihood of other diseases such as powdery mildew (Wiese, 1987). Rust diseases are most harmful when the infection occurs during flowering. Rust resistance in wheat can be classified as either all-stage or adult-plant resistance (Sthapit, 2014). All-stage resistance

is usually race specific and more effective against a single or few races. The adult-plant resistance can be either race specific or non-race specific. Rust diseases have three ways of dispersion: single event across a very long distance, stepwise range progression, and extinction and recolonization (Sharma, 2012). Human assistance is usually needed for a single event, across a very long distance. Stepwise expansion is the gradual expansion of a disease to a new area. Extinction and recolonization occur in areas that aren't suited for year-round survival. In these areas the pathogen relies on wind to blow it to a more suitable area and then back to the original area when conditions are suitable there again. One of the main problems with breeding for rust resistance is that rust virulence genes can mutate fast, rendering race specific resistance genes useless (McIntosh, 1988). The three main types of rust diseases affecting wheat are stem rust, leaf rust, and stripe rust (yellow rust).

Stem rust is caused by the fungal pathogen *Puccinia graminis Pers.* f. sp. *tritici* (Sharma, 2012). *Puccinia graminis* is a heteroecious pathogen, meaning that it requires two hosts (wheat and barberry) to complete its life cycle (Wiese, 1987). *Puccinia graminis* is macrocyclic meaning that during its life cycle it will produce five different types of spores. These spores are basidiospores, pycniospores, aeciospores, urediniospores, and teliospores (Sharma, 2012). Stem rust can be identified by the red urediniospores that are seen on stems and by black teliospores that are seen on stems later in the season. Stem rust disrupts nutrient flow to the head which causes kernels to become shriveled. If stem rust is not controlled by either planting a resistant variety or by applying fungicide, it can lead to a yield reduction between 50 and 70%. In the early 1920s, stem rust destroyed 20% of the wheat grown in the United States. There are 50 established (named) genes that provide resistance to stem rust and another 22 genes that do not yet have established names (USDA-ARS Cereal Disease Lab, 2017).

Leaf rust is caused by the fungal pathogen *Puccinia triticina Erikss*. (Wiese, 1987). *Puccinia triticina* can survive winters by overwintering as mycelium on volunteer wheat. *Puccinia triticina* is spread by wind blowing the urediniospores from infected plants to uninfected plants. It develops most rapidly between 15 and 22 degrees Celsius when moisture is not limited. Leaf rust is so widespread that it is said to practically co-exist with wheat. Leaf rust can be identified by brown circular uredinia that occur on the top surface of leaves. Leaf rust is not as severe a disease as stem rust, but can lead to yield reductions of around 20%. There have been 77 resistance genes for leaf rust discovered and named (USDA-ARS Cereal Disease Lab, 2017).

Stripe rust that is present in North and South America is believed to have originated in Europe (Ali, 2014). Stripe rust was first described in Europe in 1827 (Line, 2002). Stripe rust was first known as *Uredo glumarum*, later changed to *Puccinia glumarum*, and in 1956 it was changed to its current scientific name *Puccinia striiformis* (Humphrey, 1916; Cummins, 1956). It was first recognized in the U.S. in 1915 in Arizona, but leaf samples revealed that stripe rust had been present since 1892 (Carleton, 1915; Line, 2002). Due to the increased demand for wheat during World War I, stripe rust research was rapidly increased (Line, 2002). Early research focused mainly on economic impact, origin, distribution, resistance, morphology, and infection process. Research on stripe rust stalled in 1930 when infection rates slowed (Coakley, 1979). Lack of research on stripe rust resulted in this disease becoming the most import disease in the Pacific Northwest in 1960. In 1960 and 1961, stripe rust resulted in tens of millions of dollars in damage in the Pacific Northwest alone. Stripe rust was found on experimental nurseries in Fargo, ND in 1958 (Gough, 1958). That same year it was also found on durum wheat fields near Valley City and Langdon, ND.

Wheat stripe rust (yellow rust) is caused by the fungal pathogen *P. striiformis* f. sp. *tritici* (Case, 2014). *P. striiformis* has five different spore stages (Hovmoller, 2011). These spore stages can be broadly categorized as sexual and asexual spore stages. The asexual spore stages are the infectious stages of *P. striiformis*. The sexual spore stages of *P. striiformis* are not infectious to wheat, instead they survive most often on *Berberis* spp. commonly known as barberry. Barberry is required for the sexual spore stages, because *P. striiformis* needs a living host to survive (Jin, 2010). The asexual spores can infect wheat at any stage of its growth cycle. The spores are usually dispersed through wind (Hovmoller, 2011).

Stripe rust is one of the most important leaf diseases of wheat with infections occurring everywhere that wheat is grown (CAHNRS & WSU Extension, 2020). Infections occur most often when temperatures are 10-16 degrees Celsius (UGA Extension, 2017). This makes stripe rust especially important in winter wheat where most of the growth cycle is completed when temperatures are cool in both the fall and spring. Signs of a stripe rust infection are yellow pustules occurring on the leaf surface that are often lined up resembling stripes (Chen, 2005). Stripe rust damages wheat plants by extracting nutrients from leaf tissue, increasing transpiration, increasing respiration, and decreasing photosynthesis. This leads to a decrease in floret number as well as grain weight. Stripe rust can destroy entire fields if infection is bad, genotypes without resistance are planted, and no fungicide is applied (Chen, 2010). Yield loss can range from 10% in lines with high resistance to 70% in lines with little resistance. It has been estimated that 5.47 million tonnes of wheat are lost to stripe rust infection each year, amounting to an economic loss of $979 million (Beddow, 2015).

Stripe rust can be controlled by applying fungicide. However, spraying fungicide is costly, because of this, lines with resistance to stripe rust are preferred (Singh, 2016). When

stripe rust genes are named they are usually given a name that starts with "*Yr*" for yellow rust followed by a number. There are two main types of resistance genes to stripe rust (Case, 2014). These are all-stage resistance genes and adult-stage resistance genes. All-stage resistance genes provide resistance to stripe rust throughout a plant's life cycle. The resistance offered by all-stage resistance genes is often race specific. Resistance to race specific resistance genes can be easily overcome. Oftentimes only a single gene mutation is needed in the fungus to render the gene useless (Flor, 1971). There are a few all-stage resistance genes such as *Yr5*, *Yr15*, and *Yr45* that are effective against many races (Chen, 2002; Chen, 2007; Li, 2011). Adult-stage resistance genes are usually only effective at the later stages of plant development (Chen, 2005). Adult-stage resistance genes offer low levels of resistance to multiple races of stripe rust. Adult-stage resistance genes that are not specific to a single race of stripe rust are the most durable. Stacking multiple genes that provide resistance to multiple races of a pathogen is one of the most effective ways to provide resistance to a certain pathogen (Singh, 2000). Most of the stripe rust genes that have been identified to date are race specific genes. Due to smaller resistance effects, quantitative inheritance and masking by the race-specific genes, the non-race specific genes have been less well studied and mapped (Liu, 2015).

In the study outlined in chapter two the rust resistance genes *Lr37*/*Sr38*/*Yr17*, *Lr24*/*Sr24*, *Lr46*/*Yr29*, *Lr56*, *Lr16*, and *Lr77* were implemented in a winter wheat population. The *Lr37*/*Sr38*/*Yr17* translocation is located on the short arm of chromosome 2A in wheat (Blaszczyk, 2004). The translocation is originally from *Triticum ventricosum* chromosome 2N. *Lr37* is a race-specific gene for leaf rust resistance (Cristina, 2015). *Sr38* is a widely used seedling resistance gene for stem rust (Flath, 2017). Some studies have shown that resistances from *Yr17* is only present in the adult-stage, whereas others state that resistance is present in the

one-leaf stage (Milus, 2015). *Lr24* and *Sr24* co-segregate as they are located on a *Thinopyrum ponticum*-derived translocation that occurs on the long arm of chromosome 3D in bread wheat (Mago, 2005). *Sr24* offers resistance to many different races of stem rust. *Lr24* provides resistance to many leaf rust races (Zhang, 2011). *Lr46* and *Yr29* are located on chromosome 1B in wheat and often co-segregate (William, 2003). *Lr46* does not provide active resistance to leaf rust, it instead slows down the infection process of leaf rust. *Yr29* is an adult stage resistance gene that provides a moderate level of resistance to stripe rust. *Lr56* previously designated as *LrS12* is a leaf rust resistance gene derived from *Aegilops sharonensis* (Marais, 2006). *Lr56* is a widely-effective race-specific gene located on the short arm of chromosome 6A (Marais, 2010). *Lr16* is a widely utilized leaf rust resistance gene located on the short arm of chromosome 2B. *Lr16* is an all-stage resistance gene that leads to a necrotic ring being formed around *Puccinia triticina* uredinia spores when lines carrying *Lr16* are infected with leaf rust (Kassa, 2017). *Lr77* is a leaf rust resistance gene located on the long arm of chromosome 3B (Kolmer, 2018). *Lr77* is an adult plant resistance gene.

Tan Spot and Stagonospora Nodorum Blotch

Tan spot is caused by the fungal pathogen *Pyrenophora tritici-repentis* (Wegulo, 2011). Tan spot can occur in both tetraploid and hexaploid wheat. *Pyrenophora tritici-repentis* overwinters in stubble residue (Faris, 2013). Signs of a *Pyrenophora tritici-repentis* infection include tan colored lesions surrounded by chlorotic circles. Persistent wet weather during the growing season will increase tan spot infection. In the worst-case scenario, tan spot can lead to a yield reduction of 49% (Rees, 1982). Yield losses are mainly due to reduced kernel weight and kernels per head (Shabeer, 1988).

Stagonospora nodorum blotch is caused by the fungal pathogen *Stagonospora nodorum* (Mehra, 2019). Stagonospora nodorum blotch is also commonly referred to as septoria nodorum blotch and glume blotch. The primary inoculum of Stagonospora nodorum blotch are infected seeds and ascospores (Solomon, 2006). Like *Pyrenophora tritici-repentis, Stagonospora nodorum* also overwinters as pseudothecia and pycnidia in stubble (Mehra, 2019). Initial signs of a *Stagonospora nodorum* infection include yellowing at the infection site and necrosis at the leaf tip (Solomon, 2006). Chlorotic tissue then expands to form oval shaped lesions. Necrotic tissue can then be seen within the chlorotic lesion; in advanced infections the entire leaf can become chlorotic. Yield losses are highest when the flag, F-1, or F-2 leaves are infected. Yield losses are due to a decrease in thousand-kernel-weight.

Fusarium Head Blight

Fusarium head blight (FHB) is a fungal disease caused primarily by the pathogen *Fusarium graminearum* (Wiese, 1987). It is known by many different names such as scab, pink mold, whiteheads, and tombstone scab. FHB prefers wet conditions and temperatures between 20 and 30 degrees Celsius (Bakhsh, 2013). It can affect many small grains including wheat and barley (Wiese, 1987). *Fusarium graminearum* can overwinter as perithecium on crop residues (Manstretta, 2016). During anthesis *Fusarium graminearum* can infect crops when its ascospores are blown from an infected plant to a plant that is not infected. FHB is more severe in spring wheat than in winter wheat. Winter wheat can avoid infection in some cases, because it flowers earlier than spring wheat in a drier period (Wiersma, 2005).

Symptoms of FHB include premature bleaching of the heads and shriveled kernels (Wiese, 1987). This can lead to a massive yield reduction of between 20 and 80% in susceptible varieties. The reduction in yield can be attributed to the reduced size of kernels, and the

decreased number of kernels produced when infection occurs early in kernel development (Aakre, 2005). Nganje et al. 2004 found that the cumulative economic cost due to yield loss from FHB from 1993-2001 in Illinois, Indiana, Kentucky, Michigan, Missouri, Minnesota, Ohio, South Dakota, and North Dakota was $2.491 billion. They also found that annual economic losses over that time period varied from 6.4% in 2000 to 18.7% in 1998. Economic losses from 1993-2001 due to FHB were greatest in North Dakota with a loss of $1.1 billion, nearly double that of Minnesota, the second worst hit state. The reduction of yield, however, is not the only major problem associated with fusarium.

Fusarium graminearum produces a mycotoxin known as deoxynivalenol (DON) that is poisonous to both humans and animals at very low quantities. The FDA recommends DON levels in finished food products not exceeding 1 ppm, 10 ppm for cattle feed, and 5 ppm for swine and other animals (U.S. Food and Drug Administration, 2010).

These problems have led to wheat breeders trying to incorporate resistance genes into almost every new variety that they release. Two well-characterized and commonly used resistance genes for FHB are *Fhb1* and *Qfhs.ifa-5A* (Steiner, 2017). *Fhb1* is located on the short arm of chromosome 3B, and *Qfhs.ifa-5A* is located on chromosome 5A (Waldron, 1999; Buerstmayr, 2002). It was recently determined by Steiner et al. (2019) that *Qfhs.ifa-5A* is actually two separate QTL. One of these QTL is a major effect QTL referred to as *Qfhs.ifa-5Ac* and was mapped across the centromere; the other QTL, *Qfhs.ifa-5AS,* is a minor effect QTL and was mapped to the distal half of the short arm on chromosome 5A. Other QTL that have been successfully implemented in breeding programs include *Fhb2, Fhb4, Fhb5,* and *Qfhs.nau-2DL* (Anderson, 2001; Xue, 2010; Xue, 2011; Jiang, 2007). These QTL were all identified in Chinese germplasm, with *Fhb1, Fhb2,* and *Qfhs.ifa-5A* all coming from the Chinese variety Sumai-3.

There are five different mechanisms of FHB resistance (Mesterhazy, 1999). Type I resistance is resistance to the initial infection (Schroeder, 1963), type II resistance is resistance to disease spread within spikes, type III is resistance to DON accumulation (Miller, 1986), type IV is resistance to kernel damage (Mesterhazy, 1995), and type V is tolerance (Mesterhazy, 1999). Different resistance mechanisms have different broad sense heritabilities with type II being the most heritable (.73), followed by type I (.70), type IV (.68), and type III with .54 (Ma, 2019). Major resistance genes that confer type I resistance are *Fhb4*, *Fhb5*, *Qfhs.ifa-5A*, and *Qfhb.nau-2b* (Lin, 2006; Buerstmayr, 2003; Lin, 2004). Major resistance genes that confer type II resistance are *Fhb1*, *Fhb2*, *Qfhs.ndsu-3AS*, and *Qfhb.nau-2B* (Liu, 2006; Cuthbert, 2007; Lin, 2006). Major resistance genes for type III resistance are *QFhs.nau-2DL* and *QFhs.nau-5AS* (Jiang, 2007).

Along with the use of partially resistant cultivars, additional cultivation strategies that producers can use to decrease DON levels include implementing an effective crop rotation, applying fungicide, and tillage (Aakre, 2005). A post-harvest strategy to reduce DON content is to use screens and air pressure to clean the wheat (DON infected kernels have a lighter test weight than uninfected kernels). However, cleaning wheat is expensive (around 40 cents per bushel), and this expenditure has to be less than the discount DON infected wheat has at the time of sale.

Wheat Genome

Common wheat (*Triticum aestivum* L.) is an allohexaploid meaning that it is a hexaploid derived from the genomes of unrelated species (Haider, 2013). Wheat has 42 chromosomes and is functionally a diploid giving it a genetic formula of 2n=6x=42. It has two complete sets of each of the A, B, and the D genomes. It was formed from a hybrid between emmer wheat

(*Triticum turgidum* L.), which has a genetic formula of 2n=4x=AABB=28, and *Aegilops tauschii*, which has a genetic formula of 2n=2x=DD=14. *Triticum turgidum* in turn originated through the hybridization of two diploid species, i.e. *T. urartu* Thumanian ex Gandilvan (2n=2x=AA=14) and an unknown species closely related to the S-genome species of the *Sitopsis* section (possibly *Ae. speltoides* Tausch) of the genus *Aegilops* L. that contributed the B genome. The average wheat chromosome contains 810 Mb DNA, making it 25 times larger than the average rice chromosome (Gupta, 1999). Hexaploid wheat has 48 regions that are gene rich regions (GRR) (Erayman, 2004). Eighteen of the gene rich regions are major gene rich regions, the other 30 gene rich regions are minor gene rich regions. The major gene rich regions cover 11% of the genome, but contain 60% of the genes. Every chromosome group contains gene rich regions with group 1, 5, and 7 chromosomes (8 gene rich regions in each) containing the most gene rich regions. Gene rich regions occur on both the long and short arm of the chromosomes with 27 gene rich regions occurring on the long arm of chromosomes, and 21 gene rich regions occurring on the short arm of chromosomes. The five largest gene rich regions (1S0.8, 2L1.0, 4S0.7, 6S1.0 and 6L0.9) span 3% of the wheat genome and contain 26% of wheat genes.

Molecular Markers

Molecular markers can be broadly broken down into two types; hybridization-based and PCR-based (Nadeem, 2018). Hybridization-based markers where the first markers used (He, 2014). Restriction fragment length polymorphism (RFLP) is the only hybridization-based marker. PCR is a technique used to amplify a DNA fragment by denaturation of template DNA into single strands, annealing of primers to each original strand, and extension of new DNA strands (Delidow, 1993). There are many types of molecular markers that are PCR-based with randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphism

(AFLP), sequence-characterized amplified regions (SCAR), cleaved amplified polymorphic sequence (CAPS), simple sequence repeat (SSR), kompetitive allele specific PCR (KASP), and single nucleotide polymorphism (SNP) being among the most common (Nadeem, 2018; Zhao, 2017). SNP, SSR, KASP, CAPS, and SCAR markers are based on actual sequence information; RAPD and AFLP markers are based on more randomly obtained sequences (Pardok, 2014). Of these, single nucleotide polymorphisms are the most common type used.

A single nucleotide polymorphism is a single base change in a strand of DNA. Single nucleotide polymorphisms are the most common type of genetic variation (Johnson, 2009). Single nucleotide polymorphisms are most commonly the result of two types of genetic mutations (Robert, 2018). These mutations are transversions or transitions. Single nucleotide polymorphisms can be used to develop markers. These markers can then be used to map useful genes that are linked to the marker. Single nucleotide polymorphism markers have many advantages over other types of markers. The advantage of SNP markers are that they are abundant, highly heritable, cost effective, are convenient to work with (Koopaee, 2014).

Genotyping by Sequencing

Genotyping by sequencing (GBS) is a form of next-generation sequencing (NGS) using the Illumina NGS platform for large genome species like wheat (Elshire, 2011). GBS reveals large numbers of SNPs that can be used in genetic studies (Wickland, 2017). GBS has been successfully implemented in genome-wide association studies (Babu, 2020). The general steps of GBS are 1: obtain plant tissue sample; 2: grind tissue and isolate DNA; 3: digest DNA with the use of enzymes; 4: attach adaptors and barcoding region (short, unique DNA sequence) to DNA segment (amplified fragments from different samples at this stage represent the GBS library); 5: analyze library using NGS program for GBS (the program used is usually either

MiSeq or HiSeq2500); 6: analyze sequenced data; 7: implement results for GWAS, QTL mapping, constructing genome maps, genomic selection, and SNP identification among others (He, 2014). Advantages of using GBS to genotype include low cost, fewer PCR steps, it is easy to scale up, and requires fewer samples than other genotyping methods (Davey, 2011).

Genetic Mapping

The goal of genetic mapping studies is to identify QTL that contribute to the phenotypic expression of a trait (Flint-Garcia, 2003). QTL can be identified after recombination events rearrange chromosome regions. The different rearranged regions will then result in different levels of phenotypic expression of a trait. There are two main types of genetic mapping studies. These are association mapping and linkage mapping (Alvarez, 2014). Association mapping and linkage mapping both rely on recombination events to identify QTL responsible for the variation in phenotypic expression of a trait (Zhu, 2008). They differ however, in the control the experimenter has over the amount of recombination.

Linkage mapping is often conducted by performing a bi-parental cross to develop a mapping population (Li, 2010). This means that the recombination in linkage mapping is highly controlled, but that there is only one recombination event. In linkage mapping only a few molecular markers need to be used to identify QTL because there will only be a few recombination regions.

The first step in linkage mapping is to select parental strains that express differing phenotypes for the trait of interest (Xu, 2017). The next step is to choose which type of molecular marker is going to be used (SNP, SSR, KASP, etc.). The third step is to make a bi-parental cross to develop a mapping population. There are different types of mapping populations. The most common are F_2-derived, backcross, doubled haploid, recombinant inbred

lines, and near isogenic lines. Each has positive and negative attributes when used as a mapping population. The positives for F_2-derived lines are that they can be developed quickly and can estimate additive and dominance effects; the negatives are that F_2-derived lines have low power to detect QTL and limited recombination. Backcross lines work well when trying to map the trait that is being incorporated, but backcross lines take a long time to develop and don't estimate dominance effects. Doubled haploid lines can be developed rapidly and the results of a mapping experiment are easy to replicate; the disadvantages for doubled haploids is the cost to develop a population and they do not allow the estimation of dominance effects. Recombinant inbred lines allow for abundant recombination, but take a long time to be developed. After a mapping population has been developed the next step is to genotype and phenotype the population. The final step is to find QTL. The power to detect QTL is affected by the amount that a QTL contributes to phenotypic variation, the allele frequency, and the mapping population. Linkage mapping has been successfully used to map many QTL (Morrell, 2012). The need for higher resolution maps however, has led to an increase in the use of linkage disequilibrium mapping.

Linkage disequilibrium is the nonrandom association of alleles at different loci (Qanbari, 2020). Linkage disequilibrium means that there is a correlation between multiple alleles. Linkage disequilibrium is used to map QTL in association mapping (linkage disequilibrium mapping) studies. There are many factors that affect linkage disequilibrium in a population. The common factors are recombination, the mating system, subdivisions within the population, admixture, population size, mutation, and selection (Rafalski, 2004). Self-pollinating plant species have an extended linkage disequilibrium. Plant populations that have been selected such as commercial cultivars have a more extended linkage disequilibrium decay than populations that have not been selected such as landraces. High recombination rate, high mutation rate, and large

populations all have decreased linkage disequilibrium. The rate of decrease in linkage disequilibrium in a population determines the number of markers that are needed to scan the genome (Xu, 2017). If linkage disequilibrium rapidly decreases more markers are needed, and the resulting map will have higher resolution. If linkage disequilibrium decreases slowly, less markers are needed, but the resulting map will be lower resolution.

In the association mapping experiment discussed in chapter 3, junior and senior winter wheat lines were used as the mapping population. Since a self-pollinating species was used and the population had previously been selected, the linkage disequilibrium is expected to decay slowly. This means that fewer markers will be needed to cover the genome in the population, but the map will be low resolution.

Genome wide association mapping (linkage disequilibrium mapping) takes advantage of all the recombination events that have occurred while the population has been developing (Flint-Garcia, 2003). Consequently, maps developed by genome wide association mapping are of a much higher resolution than maps that have been developed from linkage mapping (Yu, 2006). In order to obtain high resolution association maps the following prerequisites must be considered: population structure, genotyping, and accurate phenotyping (Balding, 2006). Another advantage of genome wide association mapping is that multiple traits can be tested at the same time as long as phenotypic data are available. Genome wide association mapping does; however, have some disadvantages including false-positive and false-negative results (Zegeye, 2014).

False-positives are defined as the association of a SNP with the expression of a phenotype when no association exists (Li, 2012). False-positives can result from the population structure, due to many neutral markers being significantly correlated with trait differences among

subpopulations (Yu, 2006). Mixed linear models with population structure set as a fixed effect can be used to correct for population structure. The population structure can be obtained by using principal component analysis (Price, 2006). Principal component analysis summarizes the original genotype data as a small number of underlying components. Relatedness is another problem that needs to be corrected for in order to obtain accurate results. Mixed linear models can also correct for relatedness by using a marker-genotype kinship matrix (Yu, 2006).

False-negatives are defined as finding no association between a SNP and the phenotypic expression of a trait when an association exists (Li, 2012). False-negatives can happen because GWAS has a low power to detect rare alleles (Kaler, 2020). GWAS can only detect rare alleles if they contribute a significant amount of variation in phenotypic expression. Using mixed linear models to correct for false-positives can lead to an increase in false-negatives due to overfitting of the model (Liu, 2016). Although a single, minor allele does not contribute much to phenotypic variation; quantitative traits such as yield are controlled by many minor alleles (Korte, 2013). Most association mapping studies would not consider any of these alleles of minor effect to be significant. Linkage mapping is a much better option when trying to locate rare alleles than association mapping (Xu, 2017).

Another limitation of association mapping is that testing the same population multiple times can lead to false results. In order to correct for false results from multiple testing, use of the Bonferroni's correction, permutation test, or false discovery rate can be done (Muller, 2011; Benjamini, 1995). Bonferroni's correction is a highly conservative conventional way to correct for multiple testing (Muller, 2011). The permutation test provides reliable results, but can take a long time. False discovery rate is simpler than the other two methods, but can be less precise (Benjamini, 1995).

The first step when performing a genome wide association study is the selection of germplasm (Gomez, 2011). The germplasm that is selected should be as diverse as possible in order to maximize the number of different recombination events that have occurred in each line. The selected germplasm should then be phenotyped for the trait of interest. Some traits that association mapping studies are commonly conducted on are disease resistance, abiotic stress tolerance, and height. The next step is to genotype the population. This is done using a gene chip or library (Rimbert, 2018). In wheat, multiple gene chips of different marker densities have been created. The next step is to determine the linkage disequilibrium. There are three different models that can be used to determine linkage disequilibrium; they are the P model, K model, and PK model. The P model accounts for just the population structure, the K model accounts for the relatedness (kinship) within the population, and the PK model accounts for both the population structure and the relatedness. The best model is then determined by calculating the mean square difference between the observed and the expected P value. The model with the lowest mean square difference is the best model for that trait within that population. The chosen model can be used to determine the markers that have a significant effect on the expression of a trait based on the P-value of each marker. A Manhattan plot can then be constructed to show what chromosome each significant marker is on as well as how likely that marker is associated with the trait of interest.

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EARLY GENERATION SELECTION OF HARD RED WINTER WHEAT LINES TO IMPROVE YIELD AND FHB RESISTANCE

Abstract

Incorporating *Fhb1* and *Qfhs.ifa-5A* into NDSU winter wheat lines has historically resulted in lines that had lower grain yield than lines without the resistance. This necessitated pre-breeding to develop higher yielding, Fusarium head blight (FHB) resistant breeding stock. Towards this goal, a double cross was made; modified single seed descent (SSD) inbreeding was initiated; early generation selection (F_4) focused primarily on yield and marker screening results for *Fhb1* and *Qfhs.ifa-5A* was done; and the selected lines were planted in a replicated yield trial. The highest yielding lines from the replicated yield trial were selected and marker screened for FHB and rust resistance QTL. A final round of selection was done on single plants with the best gene pyramids from the highest yielding families. These plants will be used as cross parents to further introgress *Fhb1* and *Qhfs.ifa-5A* into NDSU winter wheat germplasm.

Introduction

The main problem with growing winter wheat in the United States upper Midwest is the cold, unpredictable winters (Karki, 2019). Despite the harsh winters, winter wheat has been successfully grown in North Dakota. From 2010-2017 the average yield of winter wheat in North Dakota was 3090 kg/ha. This was higher than the average yields from Kansas and Oklahoma, the two largest winter wheat producing states (Plains Grains Inc., 2017). Variety trials conducted by NDSU extension services have also shown the potential of winter wheat. The three most commonly planted varieties in North Dakota, SY Wolf, Jerry, and Willow Creek, account for 46% of the winter wheat acres planted (Ransom, 2019a). Jerry had a three-year average yield (2017-2019) of 3630 kg/ha in western (Dickinson, Hettinger, Minot, Williston)

North Dakota, and 4100 kg/ha in eastern (Carrington, Prosper) North Dakota. SY Wolf had a three-year average yield of 3690 kg/ha in western North Dakota, and 4500 kg/ha in eastern North Dakota.

Apart from the severe winters, winter wheat production in North Dakota is compounded by the prevalence of many diseases, with FHB being one of the most damaging. Besides FHB, other diseases of importance in North Dakota include leaf rust, stem rust, and tan spot (Ransom, 2019a). FHB in North America is primarily caused by the fungal pathogen *Fusarium graminearum* (Dweba, 2017). In susceptible cultivars, yield losses up to 80% occur if no fungicide is applied (McMullen, 2012). Yield loss is not the only problem associated with FHB. *Fusarium graminearum* produces the mycotoxin deoxynivalenol in infected wheat kernels (Sobrova, 2010). Deoxynivalenol is a highly toxic chemical and grain with a low deoxynivalenol concentration can be rejected at elevators.

Worldwide, across crops, *Fusarium graminearum* is considered the fourth most important fungal pathogen in terms of economic importance (Dean, 2012). As far as fungal pathogens affecting wheat, *Fusarium graminearum* is the second most important pathogen after *Puccinia* spp. if all rust diseases (leaf, stem, stripe rust) are grouped together. FHB re-emerged as a serious threat to US wheat production in the 1990s (Del Ponte, 2017). One reason for the re-emergence of FHB is the increase in maize production. The casual organism that causes FHB, *Fusarium graminearum* can infect maize causing Gibberella ear rot (Kuhnem, 2015). This along with the increased adoption of conservation tillage and climatic variability are the most likely reasons for the re-emergence of FHB.

The U.S. Wheat and Barley Scab Initiative was established in 1997 to combat the disease through discovery and implementation of effective control measures that would minimize the

threat to producers, processors, and consumers of wheat and barley (Anderson, 2020). FHB is considered the number one disease of spring wheat in North Dakota and it is highly unlikely that a hard-red spring wheat (HRSW) cultivar without moderate resistance to FHB will be grown in North Dakota (Ransom, 2019b). Winter wheat flowers earlier than spring wheat and during a drier period (Wiersma, 2005) and as a result the crop often escapes infection in FHB epidemic seasons. Hence, compared to spring wheat, the general level of FHB resistance in ND winter wheat cultivars is low.

The four most commonly grown winter wheat varieties in North Dakota in 2018 were Jerry, Decade, Peregrine, and SY Wolf. Using a 1-9 scale to rate FHB with 9 being very susceptible Jerry has a score of 8, Decade has a score of 9, Peregrine has a score of 6, and SY Wolf has a score of 6 (Ransom, 2018). With the implementation of a NDSU winter wheat breeding program in 2011 (Marais 2020, Personal communication) FHB resistance was a primary breeding objective. To acquire breeding stock with notable FHB resistance, effective resistance QTL are being transferred from HRSW and integrated into adapted, winter-hardy and high yielding winter wheat genetic backgrounds. It is to be expected that HRSW genes closely linked to the targeted FHB resistance QTL will be co-transferred to winter wheat. Some of the linkage-dragged genes from HRSW could detrimentally affect hardiness and yield of winter wheat.

One way to increase the likelihood of combining multiple favorable traits that are sometimes negatively correlated is by relying on random recombination and segregation, as first proposed by Kaufmann in 1971 for oat breeding. This involves advancing elite crosses using single seed descent and not performing any selection. This allows homozygosity to increase while maintaining a near-random population to ensure maximum variation in F_3 - F_4 lines where

early generation selection can then take place (Mac Key, 1986). When performing early generation selection there usually will not be enough seed to plant a replicated yield trial; however, yield trials without replication that utilize multiple checks or moving means are possible. In 1973, Townley-Smith and Hurd found that when doing early generation yield selection in wheat, moving means methods had less experimental error than the multiple checks method. Having the maximum amount of variation in a population when selection takes place increases the likelihood that a genotype with the most favorable traits is present in the population. This is especially true when considering traits such as winter survival and yield that may correlate negatively with FHB resistance in winter wheat (Kaufmann, 1971). Kaufmann found that under normal pedigree selection, high yielding lines that are segregating might be discarded before they are recognized as such.

The main problem with using single seed descent (SSD) inbreeding is that no genetic advancement occurs during the inbreeding process (Snape, 1975). Using SSD to reduce segregation while maintaining maximum variation would be the ideal method to use for crosses designed to improve traits that may be negatively correlated, but logistics prevent it from being more widely used (Mac Key, 1986).

When using SSD to maintain maximum variation it is still very unlikely that the perfect genotype will be present. A selection index weighing traits based on their importance to the objectives of the breeding program can be used to select lines that best meet the needs of the breeding program (Vieira, 2016). In this study, a double cross that segregated for target traits of primary importance was explored. To maximize chances of selecting lines that are winter-hardy, high yielding and FHB resistant, a large population of near random inbred lines were first developed and evaluated in a yield trial without replication. Following this, index selection was

done considering adjusted yield, height, test weight, winter survival, lodging, and marker information. Yield was weighted the heaviest, followed by FHB resistance (based on marker screening), then winter survival.

Selected lines were then grown in a replicated field trial using a randomized complete block design. Lines were scored for the same traits as in the trial without replication with the addition of quality traits. Marker screening for rust and FHB resistance was conducted on the highest yielding lines.

Materials and Methods

Starting Material

An appropriate segregating population (cross 16M10) was derived from the double cross that is outlined in Fig. 1. Resistance genes believed to occur in each of the parental lines are given in Table 1. The average genetic composition of the cross 16M10 F_1 in terms of the parents that were used was calculated to be approximately 62.5% Jerry, 12.5% CM82036, and 25% SD09227. Due to the excellent winter survival of Jerry, it was important to have a greater relative contribution of Jerry to the double-cross population. The spring wheat line CM82036 was crossed with Jerry and the progeny was crossed with Jerry-*Lr56* to produce the parental line 14K456-K-1 for this study. CM82036 was the initial donor of *Fhb1* and *Qfhs.ifa-5A*. 14K456-K-1 is homozygous for *Fhb1* and *Qfhs.ifa-5A* (based on marker presence as well as FHB resistance response). As a result, it was expected that the F_1 of cross 16M10 would segregate 1:2:1 with respect to both QTL. SD09227 contributed stem rust resistance through the *Sr24* translocation and stripe rust resistance from the *Yr17* translocation. Major gene leaf rust resistance is contributed by the *Lr56* gene from Jerry-*Lr56*. Line 14K456-K-1 is homozygous for *Lr56* and this gene was also expected to segregate 1:2:1 in the 16M10 F_1.

Table 1: Resistance genes believed to occur in the parental lines of double cross 16M10

Parents	Resistance genes contributed
14K456-K-1	*Fhb1, Qfhs.ifa-5A, Lr56*
Jerry	*Sr24/Lr24*[1]*, Lr77*
Jerry-Lr56	*Lr46/Yr29, Lr56*
SD09227	*Lr16, Sr24/Lr24, Lr37/Sr38/Yr17*

[1] Marker tests have shown Jerry to be heterogeneous for the translocation

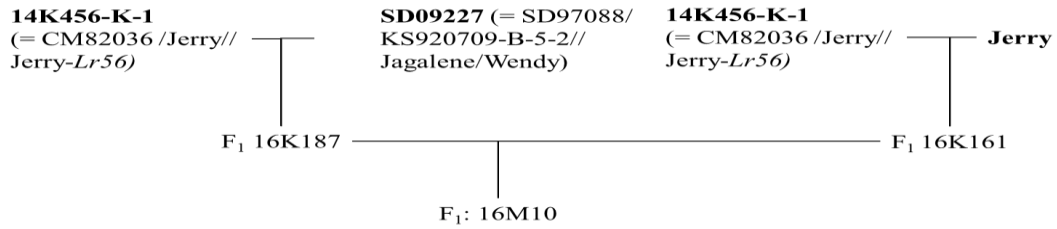

Figure 1: Outline of the double cross F_1: 16M10.

In March 2017, SSD inbreeding was initiated in a greenhouse with approximately 200 16M10 F_1 seeds. In September 2017 the $F_{1:2}$ seeds of 151 of the initial families were re-planted. In March 2018, six F_3 seeds of each of 259 F_3 families that stemmed from 151 of the initial F_1 plants were planted. Families that were excessively tall were discarded, as well as those that were diseased and had very low seed set (a greenhouse FHB trial was conducted in part of the greenhouse and the disease appeared to have spread to the seed increase pots). Individual plants were harvested from the remaining 141 families in August 2018 and their F_4 seeds provided the starting material for this study.

Broad Outline of the Study

A field trial without replication was conducted at Casselton in 2018/19 in which the 141 F_4 families were compared for yield. All plots were evaluated for winter survival, height, flowering date, and yield. Marker tests were performed to determine the possible presence of effective disease resistance alleles. Following the assessment of the combined data, the best selections were advanced to a replicated yield trial with appropriate controls that was again

planted at Casselton in 2019/20. Following the second season's assessment for agronomic performance, disease resistance and marker presence, and associations with yield were studied and the most productive and promising pyramided genotypes were identified. When testing for disease resistance based solely on molecular markers, recombination may break the marker-trait associations resulting in false positive tests. When possible, in this empirical experiment, natural infection in the field was used for verification of the marker data.

Yield Trial Without Replication

In August 2018 the four best plants (based on phenotype) from each of 141 pots of F_3 plants were harvested from the greenhouse. Marker analyses (*Fhb1, Qfhs.ifa-5A, Sr24,* 1BL.1RS, *Lr56* and *Yr17*) were done by the USDA-ARS Small Grains Genotyping Center (located at the Red River Valley Agricultural Research Center in Fargo, ND) on two random seeds from each of the 141 F_4 families that were planted in the field. The parental lines were also included in the marker analyses. Samples were cut and placed into sampling plates provided by the Genotyping Center. The Genotyping Center protocols for performing routine marker analyses can be accessed through the link: https://wheat.pw.usda.gov/GenotypingLabs/fargo. Assuming random segregation, the expected ratio of homozygous plus heterozygous resistant plants to susceptible plants in the F_4 was expected to conform to a 0.531: 0.469 ratio with respect to each of the *Fhb1, Qfhs.ifa-5A* and *Lr56* loci (since the F_1: 16M10 was heterozygous for each locus). The expected incidences of *Sr24, Yr17* and the 1BL.1RS translocation in the F_4 could not be predicted from the available data. The marker data for *Fhb1* and *Qfhs.ifa-5A* were used to assign an FHB resistance score (MS) for each of the lines based on the number of resistance QTL markers that were detected (none = 0; *Qfhs.ifa-5A* marker = 1; *Fhb1* marker = 2; both *Fhb1* and *Qfhs.ifa-5A* markers = 3).

Two and a half grams of seed from each plant was then used to plant a row in a 4-row by two-meter plot. The trial consisted of 141 plots of F_3-derived F_4-seed rows with no checks. In the spring, winter-survival (WS) scores were taken based on a 0-10 scale with 0 equal to 0% of the plants emerged in the spring, and 10 equal to 100% of the plot emerged. Days (Julian) to flowering (DF) reflected the number of days that lapsed until 50% of the heads in a plot flowered. Plant height (PH; cm) was the average height of plants in a plot taken from the base of the plant to the tip of the tallest spike (awns not included). Lodging scores (LS) were taken as no lodging (recorded as 0), partially lodged (.5), and completely lodged (1). All of the plots were harvested by combine. Plot weights (Y; g) and test weights (TW; kg/hl) were recorded. Test weights were adjusted to the industry standard moisture content for wheat at 13.5%, based on the moisture content at the time of recording. Next, the winter survival, plant height, flowering date, plot yield and test weight data were adjusted using the moving means method that compares a plot to its surrounding plots. For example, if a plot had a winter survival score of 10 and the closest eight plots had an average score of 9, the adjusted value of the plot would be 1. The moving means method helped reduce the experimental error caused by changes in the environment without having to use multiple checks throughout the field which makes a trial much larger. The data gathered throughout the summer as well as the marker data were then used to select single rows from the 60 best genotypes. An index score (Figure 2) was calculated for every line to facilitate selection. The index was designed to emphasize high yielding plots the most (adjusted yield needed to be divided by 15 because it was measured in grams/plot and some adjusted values were high), but also favor plots that had good marker scores and did not lodge. Winter survival was not weighed heavily in the selection index due to winter survival scores being high for every line. The index discriminated against lines that were later, taller and

lodged more severely than the average. Days to flowering and test weight were also not weighed heavily as variation among the lines was minimal. The 40 best lines based on their index score were selected and planted in a replicated trial in Casselton, North Dakota in 2019-2020 using an RCBD design with four replications. Cultivars Jerry, SY Wolf, Emerson, Keldin, Ideal, SY Monument, Oahe, and Northern were used as checks.

$$WS * 2 - \frac{DF}{2} - \frac{PH}{2} + \frac{TW}{2} + \frac{Y}{15} + MS * 5 - LS * 10$$

Figure 2: Selection index equation. WS= adjusted winter survival; DF=adjusted days to flower; PH= adjusted plant height; TW=adjusted test weight; Y=adjusted yield; MS=marker score; LS=lodging score

Seedling Screening

The 40 selected lines were also tested for seedling resistance to six races of leaf rust, four races of stem rust, one tan spot race, and one *Stagonospora nodorum* race. This was done by planting three seeds from each line into a pot. Ten lines were grown in each pot. Seedlings were grown in a growth chamber, and inoculated using a suspension of spores in distilled water and a small amount of wetting agent (Triton). The parental lines Jerry, SD09227, and 14K456-K-1 were used as controls. The six leaf rust races used were TBDJQ, MFPS, TDBG, THBL, MCDL, and an unidentified race collected in the field in the summer of 2017. The four stem rust races used were isolates 72-41-SP2 (TMLK), 64E(1)-SP-1 (QTHJ), TNMK-SP-1 (TPMKC), and A48 (QFCQ). The tan spot isolate used was Pti2 and the *S. nodorum* isolate used was Sn4.

Rust seedling reactions were recorded using a 0-4 scale with 0=immune, ;= nearly immune, 1= very resistant, 2= moderate resistant, 3= moderately susceptible, and 4= very susceptible (Stakman, 1962). X was used to indicate if a genotype was heterogeneous. Within the scale + and – were used to indicate if a line was slightly less or more resistant/susceptible. Lines were evaluated on a single plant basis if obvious variation was present between plants of

the same line. If variable infection types were present on a single plant, multiple infection types were recorded (i.e. if a single plant expressed both infection types 1 and 2, the infection type was recorded as 12). Chlorosis (C) and necrosis (N) were also recorded when present.

The scoring scale used for tan spot was a 1-5 scale (Lamari, 1989). A score of 1 (resistant) meant that there were resistant specks present on the leaves; 2 (moderately resistant) meant that there were lesions with little necrosis and chlorosis; 3 (moderately susceptible) meant that there were lesions with distinct necrosis and chlorosis; 4 (susceptible) meant that there were coalescing type 3 lesions, while 5 (susceptible) meant that there was extensive necrosis/chlorosis, and an absence of borders between lesions. The scoring scale used for *S. nodorum* was similar to that of tan spot but it also included a 0 (Liu, 2004). A 0 (highly resistant) meant that there was no visible lesions; 1 (resistant) meant there was some flecking and a few dark spots; 2 (moderately resistant) meant there was a few dark spots and a little surrounding necrosis/chlorosis; 3 (moderately susceptible) meant lesions were 2-3 mm with necrosis/chlorosis; 4 (susceptible) meant there were large lesions with necrosis/chlorosis; and 5 (highly susceptible) meant that there was major leaf damage with little green leaf tissue remaining.

Replicated Yield Trial

In September, 2019 a replicated yield trial was planted at Casselton, ND. The replicated trial consisted of the forty selected lines as well as nine checks. Four replications were planted for each entry. Plots were five meters long with seven rows, and seven-inch row spacing.

In the summer, data were collected on winter survival, flowering date, plant height, lodging, yield, and test weight using the same procedures and scales employed for the trial without replication. A 200-gram bulk sample was then taken for each entry by pooling 50 grams

of seed from each replicate of that entry. The 200-gram samples were used to test for wheat quality traits. Quality analysis was performed by the Cereal and Food Biochemistry Research Laboratory at North Dakota State University. Milling quality (flour extraction %), protein % (12% moisture basis), mixing characteristics, and baking quality were evaluated. Mixograph measurements included: mixograph score, envelope right slope (%/min), mid line peak time (min), mid line tail slope (%/min), and mid line timex width (%). Lactic acid SRC and water SRC were measured on the flour samples. The Glutopeak measurements were: peak maximum time (sec), maximum torque (GPU), torque 15 seconds before maximum (GPU), torque 15 seconds after maximum (GPU), startup energy (cm^2), plateau energy (cm^2), and aggregation energy (cm^2). Baking characteristics measured were: mix time (min), baking absorption 14% mb (%), dough optimization (1-10), loaf volume (cc), crumb color (1-10), and crust color (1-10).

Data analysis was performed using SAS 9.4 software (SAS Institute, Cary, NC). Analyses of variance (PROC GLM) were done for winter survival, flowering date, height, yield, and test weight. Mean separation was determined using Tukey's test with an alpha level of .05. The highest yielding lines were selected to be marker screened for *Fhb1*, *Qfhs.ifa-5A*, and multiple rust resistance genes.

Marker Screening

Selected high yielding 16M10 lines with the parental lines Jerry, 14K456-K-1, and CM82037 used as checks were planted to be screened for the presence of expected segregating markers. Twenty F_6 seeds were planted of each line. Five cm of leaf samples were taken from each plant when seedlings were ~25 cm tall. Tissue samples were placed into a silica coated cell of a 96-cell tray. The trays were then sent to the Small Grains Genotyping Center of the USDA-ARS in Fargo, ND. Marker tests were performed to test for the presence of *Fhb1*, *Qfhs.ifa-5A*,

and several rust resistance genes. For *Fhb1* the Fhb1-FM227 and the TaHRC markers were used. For *Qfhs.ifa-5A* the markers barc180_Gene-3371_56 and barc186_80018 were used. The Fhb1-FM227 (UMN10) marker is a codominant marker that is highly diagnostic for *Fhb1* (Liu, 2008). The TaHRC marker is based on a deletion mutation in the TaHRC protein coding region of the *Fhb1* gene (Su, 2018). Barc180 and barc186 are flanking markers for *Qfhs.ifa-5A* that are located 1.7 centimorgans apart (Buerstmayr, 2018). The rust resistance genes tested for were *Lr56* (Dupw217), and *Sr24*/*Lr24* (Sr24#12) whereas *Lr34*, 1B1R, *Lr46*/*Yr29*, and *Lr37*/*Sr38*/*Yr17* were tested for employing internal (Genotyping Center) markers.

Results

Trial Without Replication

Data were recorded for MS, WS, DF, PH, LS, TW, and Y. Barring MS and LS, the plot values of all traits were adjusted, based on their moving means. The adjusted plot values and MS were then used to calculate a selection index. The forty best lines, based on the selection index were selected. The data pertaining to the index are given in Table 2.

The ranges and averages recorded for the agronomic measurements of the yield trial without replication are given in Table 2 together with the corresponding values of the 40 selected lines and the remaining lines that were not selected. The mean of the selected entries was 1330 g/plot compared to 1120 g/plot for the original population and 1040 g/plot for lines that were not selected. The use of moving means to provide a control value for each plot allowed for adjustment of the data for soil trends. Adjustment gave extra weight to a plot value if the surrounding plots performed comparatively poorly and vice versa, thus correcting for local environmental differences. The overall index was designed to give most weight to yield performance. The 40 selected lines had a mean selection index score of 21.04 (range = 14.38 to

37.36). The average index value for the remaining (rejected) lines was .05 (range = -49.89 to 14.07). Table 2 also gives the size ranges of the different component traits of the index following adjustment for environmental effects and weighting according to the importance of each trait.

Table 2: Summary of the variation in un-adjusted and adjusted trait vales that were used for the calculation of the selection index.

	Range of variation and mean values among lines with respect to:							
	Winter Survival (WS)[1]	**Flowering Date (DF)[2]**	**Plant height (PH)**	**Test weight (TW)**	**Yield (Y)**	**Marker Score (MS)**	**Lodging Scores (LS)**	**Index**
All lines	6 to 10	18 to 26	71 to 96	56.6 to 70.9	512 to 1,715	0 to 3	0 to 1	-49.9 to 37.4
Average (all lines)	9.3	22	84	64.5	1,122	2.3	.57	6
Selected group	8 to 10	18 to 25	74 to 96	60.8 to 70.9	954 to 1,715	0 to 3	0 to 1	14.4 to 37.4
Average (selected)	9.4	21	86	65.4	1,328	2.1	.56	21
Lines not selected	6 to 10	18 to 26	71 to 95	56.6 to 68.8	512 to 1,451	0 to 3	0 to 1	-49.9 to 14.1
Average (not selected)	9.2	22	83.6	64.1	1,040	2.3	.57	.05
Calculated index[3]	-6.5 to 2.9	-2.1 to 2.3	-7.1 to 5.9	-3.3 to 2.5	-44 to 39.1	0 to 15	0 to 10	-49.9 to 37.4

[1] Winter survival was evaluated on a 1(worst)-10(best) scale based on percent emergence in the spring

[2] The entire population flowered within the range of June 18 to 26.

[3] Trait values were adjusted for surrounding plots using moving means and multiplied with weight constant as in formula (Fig. 1)

Winter survival, DF, PH and TW showed limited variation within the original population of lines and therefore had only small effects on the index. The index discriminated against taller and later lines and those that lodged whereas better WS was an advantage. For WS the average of the selected group was 9.4 compared to the average of the original set, which was 9.3. The selected group did; however, exclude the most sensitive lines. The lines flowered within a range of only eight days (June 18 to 26), which was similar for both the selected (mean = June 21st) and the original population (June 22nd). The adjusted DF means for the lines in the selected population was negative for 57.5% of the selected lines, meaning that lines that flowered earlier were slightly favored by the selection index. The selected lines were on average slightly (2 cm) taller than the original group. Although the selected lines had a slightly higher mean height, 14 of the 40 selected lines had a negative adjusted height; meaning that they were shorter than the surrounding plots. The selected population being slightly taller than the lines that were not selected was also due to the index favoring other traits more than height. Lodging was a general problem due to frequent rain and high winds around harvest time which affected most of the breeding material. In this trial, 73% of plots experienced some degree of lodging. The lines that were not selected were completely lodged in 40.4% of plots, and 32.6% of plots were partially lodged. Among the selected lines, 40% of the plots were completely lodged, and 32.5% of plots were partially lodged, thus selection is not expected to improve the population for this trait. With regard to TW the selected population had an average of 65.5 kg/hl compared to 64.5 kg/hl for the original population. The marker score was calculated based on marker data for *Fhb1* and *Qfhs.ifa-5A*. With regard to the 141 F_4 families that were marker tested, it was expected that approximately 53% will have the diagnostic marker while 47% will lack it. With regard to *Qfhs.ifa-5A*, 55% of the lines were positive for the marker's presence and 24% lacked the marker

(in 21% of lines that allele couldn't be determined); however, for *Fhb1* the observed presence of its marker was 74% and absence was 11% (in 15% of lines the allele couldn't be determined). As this was unexpected, a rerun of the marker was done by the Genotyping Center which produced the same result. A Chi-square test to determine goodness of fit was not conducted due to the number of lines where the presence or absence of *Fhb1*/*Qfhs.ifa-5A* could not be determined. The strong deviation in expected versus observed segregation of the *Fhb1* marker is likely due to unintended selection against FHB susceptibility in the F_3 increase pots. Among the lines that were not selected, 80% had the *Fhb1* positive marker allele and 55% had the *Qfhs.ifa-5A* positive marker allele. In the selected group, 68% had the *Fhb1* marker, whereas 58% had the *Qfhs.ifa-5A* positive marker allele. Thus, it is possible that the selection index, which favored high yielding lines more strongly, resulted in the inclusion of a lower frequency of lines with *Fhb1*. A comparison of the raw and adjusted yield data with the *Fhb1* marker data for the selected population and the population before selection is provided in Table 3. The data suggest that lines without the *Fhb1* marker on average yielded better than lines with the *Fhb1* marker. While it was not possible to attach a measure of experimental error to the mean comparisons, the results imply a high likelihood that within the total population of lines, the introgression of *Fhb1* was accompanied with a deleterious effect on yield.

Table 3: Raw and adjusted yield of selected lines and entire population with or without *Fhb1*.

Yield data	Group	*Fhb1* Marker	Range	Mean
Raw	All lines (141)	+ (105)[1]	512 to 1601	1097
		- (16)	997 to 1715	1303
	Selected (40)	+ (28)	954 to 1601	1301
		- (8)	1153 to 1715	1401
Adjusted	All lines (141)	+	-567 to 383	-20
		-	-128 to 586	195
	Selected (40)	+	-34 to 383	193
		-	128 to 586	337

[1] Number indicates the number of lines that were positive/negative for *Fhb1* marker

Seedling Resistance Screening

The parents plus 40 lines selected for inclusion in a follow-up replicated trial were evaluated for seedling resistance to leaf rust and stem rust. The seedling resistance data pertaining to all of the entries are provided in Tables A4 (leaf rust) and A5 (stem rust) of the Appendix. Among the parents, SD09227 showed good resistance to five of the six *P. triticina* races with the lone exception being MCDL which could not be scored (seedlings did not emerge) (Table 6). Jerry was also resistant to all six races. The 14K456-K-1 seed had low viability and results were obtained with only two of the races (THBL and MFPS). 14K456-K-1 was resistant to both of these races. The seedling leaf rust resistance data pertaining to the 40 lines that were evaluated in the replicated yield trial (Table 4) revealed very strong resistance (Infection Type (IT) = 0 to 1- in 42% - 76% of the inbred lines), strong resistance (IT = 1 to 12 in 17% - 39% of the lines) and intermediate (IT = 12+ to 2++3, X in 0% - 18% of lines). Susceptible ratings (IT = 3 to 34) were obtained in 13% of the lines to race TBDJQ, 6% of the lines to race MFPS, 4% of the lines to race TDBG, 4% of the lines to the unidentified race, 11% of the lines to race THBL, and 3% of the lines to race MCDL. Thus, the 16M10 population segregates for resistance to all six leaf rust races tested.

Table 4: Number of seedlings within each infection type to six *Puccinia triticina* races.

	Puccinia triticina Race					
Infection Type Range	TBDJQ	MFPS	TDBG	Field	THBL	MCDL
0 to 1-[1]	13	25	13	18	16	21
1 to 12[1,2]	12	6	11	5	8	11
12+ to 2++3[1]	2	0	4	4	0	1
X	0	0	0	1	0	0
3 to 34[2]	4	2	1	1	3	1
4	0	0	0	0	0	0
Total	31	33	29	29	27	36
Susceptible (%)	12.9	6	3.4	3.4	11.1	2.8

Lines that segregated for infection type were not counted

[1]Resistant phenotype

[2]Two numbers (12 or 34) indicate that both infection types were present on the same plant

Of the three parents, SD09227 showed the strongest resistance to the four *P. graminis* races (Table 5). 14K456-K-1 was also resistant to all four races. Jerry was susceptible to race QTHJ and marginally resistant to race TMLK. Eleven of the 40 inbred lines showed a high level of resistance to all four races. A breakdown of how the population scored against each race according to the Stakman scale can be seen in Table 5.

For tan spot and *S. nodorum* none of the parental lines or any of the experimental lines tested showed any level of resistance.

Table 5: Number of seedlings within each infection type to four *Puccinia graminis* races.

	Puccinia graminis Race			
Infection Type Range	TMLK	QTHJ	TPMKC	QFCQ
0 to 1-[1]	4	1	17	10
1 to 12[1,2]	13	15	7	6
12+ to 2++3[1]	14	6	12	10
3 to 34[2]	2	4	0	11
4	0	2	0	0
Total	33	28	36	37
Susceptible (%)	6	21.4	0	29.7

Lines that segregated for infection type were not counted

[1] Resistant phenotype

[2] Two numbers (12 or 34) indicate that both infection types were present on the same plant

Disease resistance scores to the six *Puccinia triticina* races and the four *Puccinia graminis* races for the 16M10 lines selected from the replicated yield trial and the parental lines can be seen in Table 6.

Table 6: Seedling leaf and stem rust screening scores for selected lines and checks

	Leaf Rust Race						Stem Rust Race			
Line	TBDJQ	MFPS	TDBG	Field	THBL	MCDL	TMLK	QTHJ	TPMKC	QFCQ
143-2	1P12 1P3	2P; 1P1	1+	;	1	;	2+	4	;	2++3
136-2	1P; 2P2	12	12	1P1CN 2P2CN	2P; 1P2CN	12CN	2P1 1P3	2++3CN	12+	12++CN
17	1CN	;1	2	12	2P1 1P2	1	2++3CN	1P1 2P2++3	1P1 1P2+	3
67	12CN	;	;	12CN	2P; 1P2+CN	;	23	12CN	1P1 1P2+	3
143-1	2P12 2P3	2P12 1P3	2+	2++	12	1=	2++3	2++3	2+CN	12++
73	3	3	3	3	3	3	12	1P1 2P2++3	2++3	3
22	;	;1	2++	2++	12	3P12 1P3	;1	2P1CN 1P3	;	;
105-1	;	1P; 1P2	1P; 1P12	;	;12	;	1CN	1CN	;	;
123-1	12	;CN	12	12CN	1P; 1P3	12CN	1P1 1P2++	1P1CN 2P34	1P0 1P2++	1/3
Jerry	2	;	;	;1=	;	2CN	2++3CN	3	12	;1
SD09227	1CN	;1=	;1=	1CN	1P0 1P2CN	-	1-CN	1-CN	;1=	1=CN
14K456-K-1	-	;	-	-	1CN	-	1=CN	2	;	0

Scoring Scale: Resistant=0<;<1<2. Susceptible=3<4

Two numbers (i.e. 12) indicate that both infection types occurred on the same plant

Key: - = No data; +/- = slightly higher/lower; CN = Chlorosis/Necrosis; P = # of plants

Replicated Yield Trial

A summary of the analysis of variance results for all traits is given in Table 7. There were significant (Tukey's p=.05) differences among entries for all five traits (WS, H, FD, TW, and Y). The mean values for traits WS, H, FD, TW, and Y and outcomes of Tukey's test for significance of differences among the 40 lines are given in Figures A1 through A5 of the Appendix.

Table 7: Analysis of Variance results for five winter wheat traits

	Entry			Rep		
Trait	F Value	p-Value	Interpretation	F Value	p-Value	Interpretation
Winter Survival	1.73	0.008	Significant	2.09	0.105	Not Significant
Flowering Date	4.79	<.0001	Significant	6.26	0.0005	Significant
Height	6.31	<.0001	Significant	8.27	<.0001	Significant
Test Weight	12.23	<.0001	Significant	3.95	0.0096	Significant
Yield	3.43	<.0001	Significant	2.25	0.085	Not Significant

The agronomic data of all the trial entries are given in Table A1 of the Appendix. The average plot yield of the trial was 2690 g/plot. The entries ranged in yield from 2253 to 3350 g/plot with the yield of the eight control varieties ranging from 2560 g/plot (Northern) to 3350 g/plot (Ideal). The minimum yield difference to be considered significant using Tukey's multiple comparison test (p=.05) was 654 g/plot. Nine 16M10 lines had yields statistically similar to the best yielding entry, Ideal (Figure A5). For selection purposes, yield was considered the single most important trait, and hence the nine best yielding 16M10 selections were retained. The selections ranged in yield from 2750 g/plot to 2970 g/plot.

The agronomic and marker data pertaining to the eight control varieties and nine selected entries are summarized in Table 8. The non-yield data were used for further characterization of the selected entries rather than for direct selection. The marker data were used to identify superior plants within each of the highest yielding families.

Table 8: Summary of agronomic traits and marker screening results for the selected 16M10 lines and controls

Entry	Winter Survival 0-10	Flowering Date June-	Plant Height cm	Test weight Kg/hl	Grain Yield grams	Frequency of F_6 plants per family with the critical marker allele[2]						
						Lr46/ Yr29	*Lr37/Sr38/ Yr17*	*Lr24/ Sr24*	*Fhb1*	*Qfhs.ifa-5A*	*1B1R*	*Lr56*
16M10-143-2	9	14	83	74.25	2972	0.4	0.3	0	0.9	1	0	0.25
16M10-136-2	9.3	14	84	72.55	2939	0	0	0	0	1	0	0.45
16M10-17	9.4	14	84	73.24	2893	0	0.15	0.05	0.05	0.5	0.25	0
16M10-67	9	16	84	71.24	2850	0	0.75	0	0.6	0.45	0	0.85
16M10-143-1	9.3	15	83	74.44	2826	1	0.35	0	0.35	1	0	0
16M10-73	9.3	15	85	72.9	2807	0.56	0.65	0	0.85	1	0	0
16M10-22	8.9	14	84	72.38	2788	0	0	0.6	0.90	1	0.5	0.4
16M10-105-1	9.6	14	85	72.63	2777	0	0.95	1	1	1	0.15	0.65
16M10-123-1	8.9	17	83	72.14	2748	0	0.33	0	0.83	0.15	0.05	0.2
Jerry	9.1	16	87	72.76	2996	1	0	0	0	0	0	0
SY Wolf	9.4	15	71	73.5	2989							
Ideal	9.8	16	77	74.52	3350							
Monument	9.8	16	69	71.52	2931							
Oahe	9.6	15	80	74.04	2897							
Keldin	9.3	17	77	72.54	2822							
Emerson	9	15	86	75.04	2860							
Northern	8.9	18	80	70.25	2564							
CM82036[1]						0	0	0	1	1	1	0
14K456-K-1[1]						0	0	0	1	1	1	0
Trial Average	9.1	15	83	72.16	2695							

[1]CM82036 (spring wheat) and 14K456-K-1 (lack of seed) are parental lines that were excluded from the field trial

[2]Twenty samples were screened for each line, samples where an allele could not be determined were not included

With respect to WS, the control scores ranged from 8.9 to 9.8 whereas the selections had values ranging from 8.9 to 9.6. Based on Tukey's multiple comparison test (p=.05), almost every 16M10 line tested was statistically similar to the hardiest checks Ideal and Monument (score 9.8) (Figure A1). Lines 16M10-105-1 and 16M10-126-2 had the highest WS score (9.6) in the selected group. For FD the average date when 50% of the plants in a plot were flowering was June 15th. The controls flowered between June 15th and 18th whereas the nine selections had a slightly broader range (June 14th to 17th). The earliest flowering check was SY Wolf with an average FD of June 15th. Using Tukey's test (p=.05), the minimum difference to be considered significant was 2.7 days. The 16M10 selections were statistically similar to the majority of the check varieties (Figure A2). The range for height of all entries was 69 to 92 cm. The controls varied in height from 69 cm (Monument) to 87 cm, whereas the nine selections were all within a taller category (83 to 85 cm). The TWs of all trial entries varied from 68.9 to 75.0 kg/hl. The test weights of the nine selections (71.2 to 74.4) were within the range of test weights produced by the controls (70.3 to 75 (Emerson)). The minimum difference to be considered significantly different using Tukey's (p=.05) test was 3.1 kg/hl. Using this value for mean separation meant that eight of the nine selected lines were not significantly lower in TW than Emerson (Figure A4). Thus, based on their agronomic features in comparison with the controls, the selected group merited continued testing in yield trials.

The complete quality data with respect to the forty lines that were evaluated in the replicated trial are given in Tables A2 and A3 of the Appendix. The data were obtained from bulked (replications) samples and it was not possible to test for significance of differences among lines. The general trial statistics (data range, mean, standard deviation and coefficient of variation) for those quality traits that were deemed most informative are summarized in Table 9.

Table 9: Summary of statistics for the quality tests conducted on 16M10 lines and controls

			Mixograph			SRC		GlutoPeak			Baking		
	Flour Extraction	Protein	Mixo Score	Peak Time	Peak Value	Lactic Acid	Water	Max Torque	Peak Max Time	Aggregation Energy	Loaf Volume	Crumb Color	Crust Color
	%	12% Mb	1-8	Minutes	%	%	%	GPU[a]	Seconds	cm^2	cc	0-10[b]	1-10[b]
Mean	50.12	13.28	2.10	6.73	36.99	129.73	62.12	43.39	194.55	1323.86	176.24	5.49	9.51
Minimum	44.77	12.09	1.00	4.03	27.48	97.00	58.00	13.00	80.00	1112.50	127.00	0.00	7.00
Maximum	54.89	14.69	5.00	8.00	47.66	150.00	69.00	49.00	417.00	4761.95	221.00	8.00	10.00
StDev	2.34	0.59	1.01	1.24	4.01	11.24	2.12	4.85	77.14	505.11	20.05	2.36	0.89
CV	0.05	0.04	0.48	0.18	0.11	0.09	0.03	0.11	0.40	0.38	0.11	0.43	0.09

[a]GPU= GlutoPeak Units

[b]Scale for crumb color and crust color is 0/1(worst) – 10(best)

With regard to the nine best-yielding selections and eight control cultivars, the relevant data were extracted and summarized in Table 10. For flour protein content, the controls ranged from 12.3% (Ideal) to 13.7% (Emerson) and the nine selections fell within a similar range (12.2% to 13.5%). The flour extraction percentage of the controls ranged from 46.7% (Keldin) to 50.1% whereas the selected group had higher-tending values (49% to 53%). The range in mixograph peak time values for the control and selected groups were 4 to 8 and 5.5 to 7, respectively. For mixograph peak value the ranges were 28 to 48 and 32 to 41, respectively. While for both peak time and peak value, the selections were within the ranges of the controls, the mixograph scores (visual assessment) suggested that three lines, 16M10-17, -22 and -105-1, had weaker (score = 1) mixing properties than the controls. Mixing properties were also measured with a Glutopeak instrument. The maximum torque values of the controls ranged from 13 (Monument) to 47 (Northern) whereas the selections ranged from 41 to 46. Maximum peak time ranged from 82 (Oahe) to 417 (Monument) for the controls and from 175 to 300 for the selections. Aggregation energy of the controls ranged from 1134 to 1374 for seven of the controls (Monument with 4762 being the outlier) and from 1177 to 1321 for the selected group. Thus, for the Glutopeak measurements, the selections had mixing properties in the same range as seven of the released cultivars; however, Monument appeared to have clearly weaker mixing properties (low max torque, long max time, and high energy) than both the selections and remaining controls. The ranges of solvent retention capacities of the selected group fell within the ranges set by the eight controls. The loaf volumes of the controls varied from 127 to 221 ml whereas the selected group ranged from 147 to 195 ml. The averages of both groups were 175 ml. Overall, the preliminary data are not indicative of serious quality defects within the nine selected families.

Table 10: Summary of important quality traits for the selected 16M10 lines and checks

			Mixograph			SRC[1]		Glutopeak			Baking		
	Flour Extraction	Protein	Mixo Score	Mixo Time	Peak Value	Lactic Acid	Water	Max Torque	Peak Max Time	Aggregation Energy	Loaf Volume	Crumb Color	Crust Color
Entry	%	12% Mb	1-8	Min	%	%	%	GPU	Sec	cm^2	ml	0-10	1-10
16M10-143-2	53.11	12.6	2	5.72	35.3	124	63	42	194	1230	166	6	10
16M10-136-2	52.7	13.5	3	6.75	41	127	61	42	250	1197	190	6	9
16M10-17	50.81	12.2	1	6.39	31.6	124	63	41	175	1177	187	6	9
16M10-67	52.86	13.2	4	8	41.3	136	62	42	300	1221	172	6	10
16M10-143-1	50.65	12.6	2	6.31	39.8	127	60	43	201	1232	175	8	10
16M10-73	52.27	13.3	2	5.48	40.6	124	61	44	190	1223	195	6	10
16M10-22	48.99	13.3	1	6.55	37.8	121	63	43	175	1222	187	6	10
16M10-105-1	50.84	12.7	1	7.51	32.4	134	62	46	182	1321	147	0	8
16M10-123-1	51.3	12.7	3	7.25	36.4	130	60	42	241	1210	155	1	8
Jerry	50.06	13	2	5.13	38.7	130	59	44	185	1226	206	7	10
SY Wolf	46.85	13.5	2	8	36.4	110	61	43	146	1168	192	7	10
Ideal	49.77	12.3	2	7.97	31.2	131	60	39	374	1135	127	1	7
Monument	48.9	12.5	4	7.88	29.5	142	66	13	417	4762	131	1	7
Oahe	50.1	13.2	4	4.03	41.5	118	63	45	82	1292	166	8	10
Keldin	46.71	12.6	3	8	36.7	140	63	44	243	1256	170	1	8
Emerson	49.97	13.7	2	8	27.5	148	60	41	381	1195	187	7	10
Northern	46.85	13.3	5	5.25	47.7	134	69	47	109	1374	221	8	10
Trial Average	50.12	13.28	2.1	6.73	36.99	129.73	62.12	43.39	194.55	1324	176	5.49	9.51

[1]SRC=solvent retention capacity

Marker Screening

Twenty plants per selected 16M10 family were tested with the marker panel and the individual plant data are provided in Appendix Table A4. The marker data are also summarized in Table 8. The results showed that some 16M10 lines derived from the 16M10 population are still segregating for many of the markers that were tested. For each line the proportion of the twenty samples that had the resistance allele for each of the markers tested are shown in Table 8. Only two of the nine selected lines completely lacked the *Fhb1* marker; in three lines 90-100% of the 20 plants sampled tested positive for the *Fhb1* marker; whereas the presence of the marker ranged from 0.35 to 0.85 among the four remaining lines. Thus, the data did not reflect a negative effect on yield in plants with the *Fhb1* marker. Table 11 provides a summary of the single, apparently homozygous plants within each selected family that have the *Fhb1* and *Qfhs-ifa-5A* markers together with markers for additional resistance genes. The latter plants will be selfed for seed increase, continued evaluation in yield trials and use in new crosses.

Table 11: Individual plants with the best gene pyramids in the selected 16M10 families

		Resistance Gene						
Entry	Plant #	*Lr46/Yr29*	*Lr37/Sr38/Yr17*	*Lr24/Sr24*	*Fhb1*	*Qfhs.ifa-5A*	1B1R	*Lr56*
16M10-143-2	12	Present	Present		Present	Present		Present
16M10-143-2	14		Present		Present	Present		Present
16M10-143-2	18		Present		Present	Present		Present
16M10-67	14		Present		Present	Present		Present
16M20-143-1	11	Present	Present		Present	Present		
16M10-73	2	Present	Present		Present	Present		
16M10-73	6	Present	Present		Present	Present		
16M10-73	11	Present	Present		Present	Present		
16M10-22	14			Present	Present	Present	Present	Present
16M10-105-1	3		Present	Present	Present	Present	Present	Present
16M10-105-1	7		Present	Present	Present	Present	Present	Present
16M10-105-1	11		Present	Present	Present	Present	Present	Present
16M10-123-1	6		Present		Present	Present		

Discussion

Hard red winter wheat has not been commonly accepted by growers in North Dakota. In North Dakota winter wheat was the fifteenth most widely grown crop in 2018 with a total of 34,398 hectares planted (USDA-NASS, 2019). Most of the winter wheat planted in North Dakota is in the Southwest (34.4% of total area planted) and Northwest (11.5% of total area planted) regions of the state. The Southwest and Northwest regions have historically been some of the least productive regions for all types of wheat. Winter wheat had an average yield of 2500 kg/ha in the Northwest region, and 2720 kg/ha in the Southwest region in 2018. Yields of winter wheat in these regions are comparable to 5-year average (2013-2017) yields of spring wheat in these regions (2840 kg/ha in Northwest, 2520 kg/ha in Southwest region). However, the market price for spring wheat is consistently higher than that of winter wheat. One reason for this is the higher protein percentage in spring wheat (Ransom, 2019a, Ransom, 2019b). Variety trials conducted by NDSU extension found that the average protein percent of winter wheat varieties in North Dakota is 13.6% (12% mb), spring wheat had an average protein percent of 15% (12% mb). Another problem with winter wheat is the lack of FHB resistance genes in the genome. Most FHB resistance genes originate in spring wheat, because of this winter wheat needs to be crossed with spring wheat to introduce FHB resistance genes (Buerstmayr, 2009). Crossing winter wheat with spring wheat can lead to a decrease in winter hardiness and lower yield, because of this most winter wheat varieties grown in North Dakota have low/no resistance to FHB (von der Ohe, 2010). The lower protein percentage than spring wheat, comparable yields to spring wheat, risk of winter kill, lack of FHB resistance, and height are some of the main reasons why winter wheat has not become a more widely grown crop in North Dakota. The main goal of this study was to select a high yielding winter wheat line(s) with FHB resistance QTL *Fhb1* and

Qfhs.ifa-5A, acceptable agronomic and quality traits and possibly also resistance to the wheat rusts. Such material will be very valuable for continued cross-breeding and improvement of the winter wheat breeding population.

The most widely grown winter wheat varieties in North Dakota are SY Wolf (24% of acreage in 2018-19 growing season) and Jerry (11% of acreage) (Ransom, 2019a); because of this SY Wolf and Jerry were the main varieties that the 16M10 lines were compared to. In the replicated field trial, the highest yielding entries were control varieties Ideal (3350 g/plot), Jerry (3000 g/plot) and SY Wolf (2990 g/plot). The yields of the nine highest yielding 16M10 lines were not statistically different from that of Jerry, SY Wolf and Ideal with 16M10-143-2 (2970 g/plot) being the best. There were no significant differences between the nine highest yielding 16M10 lines, Jerry, and SY Wolf for winter survival. Previous studies have found that winter wheat can survive temperatures as low as -26°C with snow cover increasing the likelihood that winter wheat will survive lower temperatures (Wiersma, 2005; Larson, 1987). Temperatures in December, January, February, and March of 2020 were above the 100-year average from 1901-2000 (NOAA, 2020). This was a likely contributing factor to the winter survival scores being high. Height has been shown to be correlated with lodging tolerance in wheat (Navabi, 2006). The highest yielding 16M10 lines ranged in height from 83 cm for 16M10-123-1 to 84.75 for 16M10-73. All were all significantly taller than SY Wolf (70.75 cm) but slightly shorter than Jerry (87 cm). The likely reason for the 16M10 lines being tall is due to 62.5% of the genetic makeup of the 16M10 lines coming from Jerry. It is important for hard red winter wheat to be U.S. Grade No.2 or better, because if a wheat sample is lower than U.S. Grade No.2 it is ineligible for protein premiums (USDA-FSA, 2019). For hard red winter wheat to be considered a U.S. Grade No.2 the test weight must be at least 72.5 kg/hl. Both Jerry (72.8 kg/hl) and SY

Wolf (73.5 kg/hl) are considered U.S. Grade No.2 based on test weight. Of the 16M10 lines 16M10-143-2, 16M10-136-2, 16M10-17, 16M10-143-1, 16M10-73, and 16M10-105-1 were all above a test weight of 72.5 kg/hl. Summarized, the field agronomic data recorded for the 16M10 lines during the 2019-2020 growing season suggest that the nine selections include potentially good lines that, while having comparable yield to the leading North Dakota varieties, have the added (marker predicted) advantage of incorporating excellent FHB and rust resistance gene pyramids. Based on the preliminary trial data, the most serious agronomic concern is with regard to the height and lodging resistance of the final selections.

No major diseases occurred in the field in 2020, and thus, the resistance of the lines to natural infection could not be evaluated. Resistance differences were, however, seen with regard to the seedling resistance evaluations. As is reflected by the diverse infection types that were observed as well the variation in virulent/ avirulent interaction patterns produced in the inbred lines by the six races, several resistance genes are segregating in the cross. Every parent in the double cross contributed leaf rust resistance genes. The population is therefore expected to segregate for the all stage leaf rust resistance gene *Lr56* (Marais, 2010) that derives from 14K456-K-1 and appears to give universal resistance; adult plant resistance gene *Lr46* derived from Jerry-*Lr56*; and seedling resistance genes *Lr16*, *Lr24* and *Lr37* from SD09227 (Table 1). *Lr16* is a seedling resistance gene that is highly effective against North American leaf rust races when pyramided with other genes (Kassa, 2017). SD09227 also contributed the *Aegilops ventricosa*-derived *Lr37/Sr38/Yr17* translocation (Blaszczyk, 2004) and the *Lr24/Sr24* translocation from *Thinopyrum ponticum* (Mago, 2005). Both *Lr37* and *Lr24* have been overcome by mutation in the pathogen and are no longer effective against all races of *P. triticina* (Kolmer, 2017; Hanzalova, 2016). *Lr46* is a slow rusting gene that expresses only in the adult

stage and it can reduce the symptoms caused by the pathogen (William, 2003). *Lr77* is another adult-stage resistance gene, so it would not have had any effect on the results from the seedling screenings (Kolmer, 2018). Based on the leaf rust seedling screenings 16M10-136-2, 16M10-17, 16M10-67, and 16M10-105-1 were resistant to all races of leaf rust tested. In all of these families besides 16M10-17, *Lr56* was present in some plants. The resistance in 16M10-17 is likely due to *Lr37* and 1B1R being present in some plants in the family. 16M10-105-1 had the best leaf rust gene pyramid with *Lr37*, *Lr24*, 1B1R, and *Lr56* occurring at varying frequencies in the family. All F_6 plants in the 16M10-105-1 family also had *Fhb1* and *Qfhs.ifa-5A*.

The resistance to stem rust in the 16M10 population (Table 5) is inherited mostly from the parental line SD09227. SD09227 contains the *Sr24/Lr24* translocation as well as the *Lr37/Sr38/Yr17* translocation. Jerry also appears to be a heterogeneous cultivar reported to have *Sr24/Lr24* (USDA-ARS, 2019); however, *Sr24* does not appear to occur in all plants of the cultivar (Marais 2020, Personal communication). *Sr24* is a seedling resistance gene that is effective against multiple races (Flath, 2017). Recent variants of the Ug99 race have proven to be virulent to *Sr24* (Xu, 2017). *Sr24* segregates with the leaf rust resistance gene *Lr24*. However, the diversity in ITs observed among the inbred lines and the diverse responses to the panel of four races, suggest that additional, unknown major stem rust resistance genes are likely segregating in the population. The marker data (Table 8) suggest that the 1BL.1RS translocation occurs in a low frequency of lines. The 14K456-K-1 parent is not completely pure-breeding; is known to segregate for the presence of the translocation; and is probably the source. The marker data also corroborate the likely presence of the *Sr24/Lr24*, *Lr37/Sr38/Yr17*, and *Lr56* translocations as well as resistance genes *Lr46/Yr29*. Based on marker screenings of the F_6 families only 16M10-22 and 16M10-105-1 showed the presence of *Sr24*. 16M10-105-1 was

highly resistant to all four stem rust races tested, 16M10-22 was highly resistant to all races but QTHJ. 16M10-22 also had a 50% frequency of plants with 1B1R. 16M10-105-1 had a 95% frequency of *Sr38*. The results from the marker screening and seedling screening suggest that the presence of *Sr24* along with one other SR gene provides a high level of resistance to the stem rust races tested. Lines with just *Sr38* ranged with moderate resistance to susceptible to the stem rust races tested. Both 16M10-22 and 16M10-105-1 had *Fhb1* and *Qfhs.ifa-5A* in a high frequency of F_6 plants (90% for 16M10-22 and 100% for 16M10-105-1).

The 16M10 lines had similar milling properties as SY Wolf and Jerry, based primarily on flour extraction percent. For the 16M10 lines flour extraction ranged from 44.8-54.9% whereas Jerry had a flour extraction of 50.1%, and SY Wolf had a flour extraction of 46.8%. The nine highest yielding 16M10 lines had higher flour extraction percentages than both Jerry and SY Wolf. Protein percent is a very important trait for hard red winter wheat as premiums are given to higher protein wheat. For hard red winter wheat, the standard grain protein percent is usually 11% with premiums occurring above this level (USDA-FSA, 2019). Protein content of the nine highest yielding 16M10 lines compared relatively well with Jerry (13%) and SY Wolf (13.5%). 16M10-136-2 along with 16M10-39, 16M10-22, 16M10-73, and 16M10-67 all had higher protein contents than Jerry. The Mixograph test indicated that the 16M10 lines had mixing qualities similar to that of Jerry and SY Wolf. 16M10 lines had Mixograph scores between 1-4, Jerry and SY Wolf had a score of 2. The solvent retention capacity test showed that the 16M10 lines had more glutenin than that of SY Wolf, and three lines were higher than Jerry. The glutopeak test showed that 16M10 lines had gluten strengths similar to that of Jerry and SY Wolf, based on maximum torque. To determine baking quality; bread loaf volume, crumb color, and crust color were scored. The baking test results indicated that the 16M10 lines produced

bread that is visually similar to Jerry and SY Wolf, but the average bread volume of the 16M10 lines was 16.4% less than that of SY Wolf. Generally, the quality characteristics of the nine selections were within the boundaries set by the control varieties.

The preliminary marker screening done on just two F_4 samples from each 16M10 line detected the *Fhb1* and *Qfhs.ifa-5A* markers in the 16M10 population. The second marker screening done on nine high yielding 16M10 lines using twenty samples (F_6) of each line confirmed the results from the first marker screening. The second marker screen also allowed for the identification of individual plants that appeared to be homozygous for desirable marker pyramids.

Fusarium head blight (FHB) is one of the most prevalent wheat diseases in North Dakota, because of this wheat varieties that have tolerance to FHB are preferred (Friskop, 2018). Neither Jerry nor SY Wolf contain *Fhb1* or *Qfhs.ifa-5A*. The lack of *Fhb1* and *Qfhs.ifa-5A* resistant alleles results in SY Wolf scored as susceptible to FHB, and Jerry scored as very susceptible to FHB (Ransom, 2019). The marker data of the 16M10 lines showed that six of the highest yielding 16M10 lines had the *Fhb1* marker in >50% of plants, and seven of the 16M10 lines had the *Qfhs.ifa-5A* marker in >50% of plants, while 16M10-105-1, 16M10-73, 16M10-143-2, and 16M10-22 had both markers. Winter wheat often flowers early enough to partially avoid FHB infection. This coupled with the presence of *Fhb1* and *Qfhs.ifa-5A* could strongly reduce fungicide application for the control of FHB.

One objective of this study to determine if *Fhb1* and *Qfhs.ifa-5A* could be stacked in hard red winter wheat lines in the NDSU breeding program while maintaining a high yield. The results obtained in the yield trial without replication suggested that a general decrease in yield was a correlated response to the introgression of *Fhb1*. Results from the replicated yield trial

suggested that by targeting selection for yield beginning in the early generations it is possible to incorporate FHB resistance while maintaining high yield. Four of the ten highest yielding 16M10 lines contained both the *Fhb1* and *Qfhs.ifa-5A* markers, which is evidence that it is possible to maintain high yields while incorporating FHB resistance genes. There was also no significant difference between the highest yielding check (Ideal) and any of the high yielding 16M10 lines with the FHB resistance stack. Continued intercrosses with/of the best yielding, *Fhb1*-carrying breeding lines followed by strict yield selection of the progeny might further improve the yield of FHB resistant material.

Marker screening was done to detect rust resistance genes/translocations *Lr34*/*Yr18*, *Lr46*/*Yr29*, *Lr37*/*Sr38*/*Yr17*, *Lr24*/*Sr24*, 1B1R, and *Lr56* among 20 random F_6 plants from each of the nine highest yielding selections. Only plants that had both the *Fhb1* and *Qfhs.ifa-5A* markers plus additional resistance markers are listed in Table 11. Only four plants (three from 16M10-105-1) with the 1BL.1RS translocation were selected due to the negative connotation with processing quality (Zhao, 2012). Plant 16M10-143-2-12 is the most promising plant (derived from the highest yielding line) with three additional positive marker results. Plants in the 16M10-105-1 family had the most additional resistance genes with four rust resistance genes occurring in plant numbers 3, 7, 11. 16M10-17 and 16M10-136-2 were the only families that did not have any plants with *Fhb1*, because of this no plants in these families were selected. The thirteen selected F_6 plants are expected to be highly homozygous (97% for loci that were heterozygous in the F_1) and will continue to be evaluated in advanced yield trials.

Conclusion

The results of the field trial, the quality tests, and the marker screens suggested that the nine highest yielding 16M10 selections were very comparable to the commercial control

varieties and barring plant height (which was comparable to the tall variety, Jerry) exhibited no major production or processing defects. The pure lines being developed from the F_6 single plant selections will have a high level of within-line uniformity and will continue to be tested for possible commercialization. However, the preliminary data suggest that it is unlikely that the selections will significantly out-yield the leading commercial varieties. The selections will, however, have the advantage of possessing pyramids of *Fhb1* and *Qfhs.ifa-5A* and rust resistance markers which could provide a benefit when disease epidemics occur. Since the expression of FHB resistance is strongly influenced by genetic background, it will be necessary to confirm that the marker pyramids actually translate into resistance in the field. The selections will nonetheless be valuable resources for continued crosses to introgress *Fhb1* and *Qfhs.ifa-5A* in the breeding population and develop pyramided germplasm with broader disease resistance. Selection 16M10-143-2-12 derives from the highest yielding 16M10 line and is the single, most promising selection with an excellent marker pyramid (*Fhb1, Qfhs.ifa-5A, Lr46/Yr29, Lr37/Sr38/Yr17,* and *Lr56*). Finally, the study suggested that while the incorporation of *Fhb1* in this double cross was accompanied by detrimental yield effects, it appeared possible to select recombined genotypes with both *Fhb1* and high yield.

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GENOME WIDE ASSOCIATION MAPPING OF STRIPE RUST RESISTANCE QTL

Abstract

Historically in the United States, stripe rust infections have been problematic in the Pacific Northwest. In recent years however, stripe rust infections have become more common in North Dakota, creating an increasing need to breed for stripe rust resistance. The first step was to survey and assess the genetic variability for resistance within the NDSU hard red winter wheat (HRWW) breeding population. Of similar importance was to determine how many different resistance loci might be present in the more resistant lines. One way to map and study genes is through genome wide association study (GWAS). GWAS locates single nucleotide polymorphisms (SNPs) that are closely associated with quantitative trait loci that contribute to the phenotypic variation of a trait. In this study, genome wide association mapping was performed on two different populations of breeding material. There were no SNPs found that were associated with stripe rust resistance in either population.

Introduction

Association mapping is a quantitative trait locus (QTL) mapping method that exploits historical recombination events (Zhu, 2008). Instead of using crossover and recombination information such as from a conventional bi-parental mapping experiment, association mapping uses linkage disequilibrium to find associations between markers and phenotypes. Maps created by association mapping have higher resolution than maps created by bi-parental mapping (Yu, 2006). Association mapping is often used to locate disease resistance genes as well as genes affecting other agronomic traits. Association mapping has been used to map traits such as spot blotch resistance, tan spot resistance, drought stress tolerance, as well as stripe rust resistance in bread wheat (Ahirwar, 2018; Gurung, 2011; Qaseem, 2018; Naruoka, 2015).

Agriculture is very important to North Dakota having contributed $8.2 billion to the economy in 2016 (U of A-Research & Extension, 2020). Three crops, soybean, corn, and wheat contributed over 50% of the 8.2 billion dollars. Wheat is the second largest agricultural commodity contributing $1.6 billion to the North Dakota economy. Wheat exports were just under $1 billion in 2016, second to that of soybeans.

Stripe rust used to mainly be a problem in the Pacific Northwest of the United States, but spreading significantly increased beginning in 1960 (Coakley, 1979; Beddow, 2015). Eighty-eight percent of wheat production in the world is now vulnerable to stripe rust infections (Beddow, 2015). The most likely explanations for the expansion are changing climatic conditions and newer races of stripe rust that are adapted to warmer temperatures and are more virulent (Lyon, 2017). The climatic factors that affect the ability of stripe rust to infect crops are moisture, temperature, and wind. In North Dakota, the average annual temperature has been rising at a rate of .26°F per decade (Frankson, 2017). Five-year averages for precipitation in North Dakota have been above the average yearly precipitation of 38.5 cm since 1990. It is likely that increasing temperatures and more precipitation will increase the likelihood of stripe rust infection and cause stripe rust infections to occur earlier in the growing season (Lyon, 2017).

Stripe rust reduces yield by slowing grain filling and decreasing seed weight (He, 2019). Yield loss can be up to 70% if a susceptible cultivar is planted, conditions are favorable for infection, and no fungicide is applied (Chen, 2005). Although fungicide treatments are an effective option to control stripe rust, it is costly. A much cheaper way to control stripe rust is by planting resistant cultivars. Eighty-two stripe rust resistance genes have been mapped to date (Gevreslasie, 2020). Many of these resistance genes are all-stage resistance genes. All-stage resistance genes can be easily mapped and implemented in lines, because they often provide high

levels of resistance to specific races of a pathogen (Yuan, 2018). Resistance to specific races provided by all-stage resistance genes is often short lived as pathogens can mutate to make the resistance ineffective (Hou, 2013). An alternate approach to using all-stage resistance genes is stacking multiple adult-stage resistance genes (Yuan, 2018). Adult-stage resistance genes provide low levels of resistance to many races of a pathogen, so by pyramiding adult-stage resistance genes, or by combining adult stage and all stage resistance genes, a high level of stable resistance to many races can be achieved.

An integrated genetic map of wheat was produced that incorporates 78 permanently named stripe rust resistance genes, 67 temporarily designated stripe rust resistance genes, and 327 resistance QTL (Wang, 2017). Previous studies have shown that GWAS is a strong tool to locate novel adult-plant stripe rust resistance genes (Ye, 2019; Li 2019). Well-studied stripe rust resistance genes are *Yr29*, *Yr5*, *Yr15*, *Yr17* (Cobo, 2019; Zhang, 2020; Klymiuk, 2018; Milus, 2015). *Yr29* is a durable, moderately effective, adult stage, non-race specific gene (Cobo, 2019). *Yr5* and *Yr15* are all-stage resistance genes that provides resistance to a broad range of stripe rust races (Murphy, 2009). *Yr17* is an all-stage race specific gene (Milus, 2015). *Yr17* has been widely used since it was introduced into wheat in 1967 from *Aegilops ventricosa* (Maia, 1967). Virulence to *Yr17* appeared in 1994 in the United Kingdom and Denmark due to large acreages of wheat with *Yr17* as the only source of stripe rust resistance utilized by resistance cultivars (Bayles, 2000). However, incorporating *Yr17* along with other race specific and/or race non-specific genes remains an effective management strategy for stripe rust (Paillard, 2012; Aviles, 2015).

In the United States, the most common stripe rust races are PSTv-37, PSTv-47, PSTv-41, PSTv-4, and PSTv-52 (Chen, 2019). PSTv-47, PSTv-41, PSTv-4, and PSTv-52 occurred only in

wheat grown in the western United States (Washington, Oregon, Idaho, California) in 2019. PSTv-37 has the largest infection range and this race was present in twelve states in 2019. No significant stripe rust infection was reported in North Dakota in 2019. However, previous studies have shown that both PSTv-37 and PSTv-52 have occurred in North Dakota (Wan, 2016). Both of these races are virulent to a number of stripe rust resistance genes including *Yr17* (Chen, 2019).

To evaluate and map the available and currently effective resistance to stripe rust within the NDSU breeding germplasm, two populations of inbred lines from the junior, senior, and elite trials of the 2018 and 2019 seasons were used for genome wide association mapping. Such resistance genes could be of either the all stage or adult stage types. This will serve as a first step in discovering useful and different sources of resistance that can be employed in crosses and breeding.

Materials and Methods

Mapping Populations

Two different mapping populations were used for this study; one based on inbred lines developed in 2018 and the other consisting of inbred lines developed in 2019. The 2018 mapping population consisted of 162 senior and elite winter wheat lines from the NDSU winter wheat breeding program. The 2019 mapping population consisted of 270 junior and senior lines from the NDSU winter wheat breeding program.

Data Recorded

In 2018 and 2019 resistance phenotype data for the 2018 and 2019 inbred lines were obtained by Dr. Kimberly A. Garland-Campbell; USDA-ARS Research Geneticist (Plants); Wheat Health, Genetics, and Quality Research; Pullman, WA 99164. Lines were evaluated for

stripe rust race PSTv-37 infection type and disease severity. Scores for infection type were based on the Qayoum and Line scale (Line, 1992). Signs and symptoms of each infection type base on the Qayoum and Line scale are shown in Table 12. PSTv-37 is by far the most common race in the United States. The virulence/avirulence formula for race PSTv-37 is provided in Table 13.

Table 12: Stripe rust infection type scale of Qayoum and Line (1992)

Infection Type	Signs and symptoms
0	No visible signs of symptoms
1	Necrotic and/or chlorotic flecks; no sporulation
2	Necrotic and/or chlorotic blotches or stripes; no sporulation
3	Necrotic and/or chlorotic blotches or stripes; trace sporulation
4	Necrotic and/or chlorotic blotches or stripes; light sporulation
5	Necrotic and/or chlorotic blotches or stripes; intermediate sporulation
6	Necrotic and/or chlorotic blotches or stripes; moderate sporulation
7	Necrotic and/or chlorotic blotches or stripes; abundant sporulation
8	Chlorosis behind sporulating area; abundant sporulation
9	No necrosis or chlorosis; abundant sporulation

Infection types were only recorded as 2, 5, and 8 in this study

Stripe rust severity was recorded as the percentage of leaf area that was affected. Disease severity and infection type were also multiplied to get a disease index. Data normalization was conducted using sqrt and log transformation for infection type and index, and for severity arcsine sqrt was used. To test for normality the Shapiro-Wilk test was used (Shapiro, 1965).

Table 13: Virulence/avirulence data for *Puccinia striiformis* race PSTv-37.

	Stripe Rust Resistance Genes	
Stripe Rust Race	Virulent	Avirulent
PSTv-37	*Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr17*, *Yr27*, *Yr43*, *Yr44*, *YrTr1*, *YrExp2*	*Yr1*, *Yr5*, *Yr10*, *Yr15*, *Yr24*, *Yr32*, *YrSP*, *Yr76*

Environments

Field evaluations were conducted at two localities in Washington (Central Ferry and Pullman) in 2018 and 2019. Two replications were planted at each location. At Pullman in 2019, disease ratings were taken on two different dates (July 1st, and July 12th). At Central Ferry in 2019, ratings were taken on June 12th. Due to data being collected in two years from two locations, four environments were available for genome wide association mapping. However, none of the inbred lines or checks used in 2018 and 2019 were the same, because of this the lines used in 2018 and 2019 needed to be analyzed separately.

Calculating BLUEs

Phenotype data collected from Washington State were used to calculate best linear unbiased estimators (BLUEs) for infection type, severity, and disease index. RStudio packages lme4 version 1.1-21 and LMERConvenianceFunctions were used to calculate BLUEs (Bates, 2019), using a linear mixed model with genotype as a fixed effect and location and replication as random effects.

Genotyping

Plants that were phenotyped were also planted in the Agricultural Experiment Station Research Greenhouse Complex at the North Dakota State University campus in 2018 and 2019. Plants were grown to the 3-leaf stage when a 3.8-5-cm leaf segment was cut from each sample and placed into a 96-deep-well block filled with silica gel. The last well in each block was left empty to help make sure the blocks were orientated correctly for genotyping. After each block was filled it was sealed tightly with the cap-mat and inverted multiple times to ensure that leaf tissue was in contact with the silica gel. Genotyping by sequencing was performed by Dr.

Xuehui Li's laboratory at North Dakota State University. Wheat genotype libraries GBS30 and GBS50 were used for genotype calling. The raw genotype file consisted of 447,310 sites.

Association Mapping

Filtering

The 2018 and 2019 genotype files both had a large proportion of missing alleles. In an attempt to lower the proportion of missing alleles, genotype filtering was done on sites with high proportions of missing alleles as well as genotypes with high proportions of missing alleles. For both the sets of lines developed in 2018 and 2019, sites were filtered so that sites with 50% missing data or more were not included in the analysis. For the 2018 genotypes there was one outlier genotype that was near 100% missing data that was filtered out. For the 2019 genotypes there were four genotypes that had over 90% missing data that were filtered out. A final filtering for minor allele frequency (MAF) of .05 was performed before imputation.

Imputation

The remaining missing genotypes were imputed using LD KnnI imputation in TASSEL 5.0. LD Knni is based on nearest neighbor imputation. LD KnnI factors in linkage disequilibrium between SNPs when choosing the nearest neighbor (Money, 2015). LD KnnI Imputation was set to include the ten nearest neighbors in the imputation.

Population Structure and Kinship

Principal component analysis was done (using TASSEL 5.0) to control population structure and remove false positives due to population structure. Principal component analysis reduces the dimensionality of a data set while still maintaining maximum variation (Lever, 2017). It does this by identifying directions along which variation is maximal. The number of principal components that will be used in an analysis is determined by the total amount of

variance the researcher wants the principal components to include. In this experiment the number of principal components used accounted for 25% of the total variation. The identity by state relative K-matrix was estimated by measuring relatedness between pairs of entries.

Regression Models

In order to find marker-trait associations, four different models were tested using TASSEL 5.0 and RStudio. The models tested were the simple model, P (principal component) model, K (kinship) model, and the PK (principal component + kinship) model. The simple and the P models are general linear models with only fixed effects. The simple model had an equation of phenotype is explained by the fixed effect of the SNPs plus residual effects. The P model had an equation of phenotype is explained by the fixed effects of the SNPs plus principal component matrix multiplied by the fixed effects of population structure plus residual effects. The K model and the PK model were mixed linear models with both fixed and random effects. The K model had an equation of phenotype is explained by fixed effects of the SNPs plus kinship matrix multiplied by random effects of individuals relatedness plus residual effects. The PK model had an equation of phenotype is explained by the fixed effect of SNPs plus principal component matrix multiplied by the fixed effect of population structure plus kinship matrix multiplied by the random effects of individuals relatedness plus residual effects. P values were calculated for each SNP for the four models using TASSEL 5.0. Mean square differences were then calculated to find deviations for observed P-values from the uniform distribution using RStudio. The uniform distribution was found by calculating the expected p-values using the ppoints function in the stats (version v3.6.1) package in RStudio (R Core Team). The model that had the lowest mean square difference is the best model to use for genome wide association study for stripe rust resistance in this study.

Determining Significance

Manhattan plots were constructed in RStudio using -log10 p-values as the y-axis and chromosome number (chromosome numbers were labeled as 1-21 instead of 1A-7D) as the x-axis. Bonferroni's correction with a genome wide error rate of $\alpha=.1$ was used to reduce the number of false-positive results. To determine if a single marker is significantly associated with a trait when using Bonferroni's correction, the genome-wide error rate is divided by the number of markers tested.

Results

Phenotype Results

Infection type and severity of stripe rust were evaluated at Central Ferry and Pullman in Washington. For each genotype a mean infection type, severity, and disease index was calculated for each location. Genotype means were then used to derive the range, mean, and standard deviation for infection type, severity, and disease index at each location (summarized in Table 14). Disease severity, infection type, and disease index scores were higher at Pullman than at Central Ferry in 2019. For severity recorded in 2018, severity in 2019, and disease index in 2019 there was a significant interaction between location and genotype. Heritability was high for all three traits evaluated for both years. For infection type 2018 the heritability was 84%, and in 2019 it was 80%. For severity in 2018 the heritability was 92%, and in 2019 it was 84%. For disease index in 2018 the heritability was 92%, and in 2019 it was 83%. Severity, infection type, and disease index data were not normally distributed. In an attempt to normalize infection type and disease index both log and sqrt (square root) transformation were performed. In attempt to normalize disease severity arcsine sqrt transformation was used. The attempts to normalize the phenotype data however, did not result in any of the data being close to normally distributed.

Table 14: Summary of stripe rust resistance traits in NDSU hard red winter wheat breeding material

	2018 CF	2018 P	2019 CF	2019 P
Infection type: Range	2-8 (6.5)	2-8 (6.3)	2-8 (5)	2-8 (6.2)
Infection type: SD (CV)	1.95 (0.3)	1.8 (0.29)	2.25 (0.45)	1.69 (0.27)
Severity: Range (mean)	10-90 (57.4)	15-100 (57.3)	5-90 (29.1)	5-90 (46.7)
Severity: SD (CV)	21.5 (0.37)	19.67 (0.34)	23.8 (0.82)	24.93 (0.53)
Disease index: Range	20-720	30-800	10-720	10-720
Disease index: SD (CV)	216.7 (0.54)	208.3 (0.54)	200.2 (1.07)	217.5 (0.66)

CF=Central Ferry, Washington
P=Pullman, Washington

To minimize environmental effects, Best Linear Unbiased Estimates (BLUEs) were calculated for disease severity, infection type, and disease index for the 2018 and 2019 breeding populations. The calculated BLUEs were then used for GWAS. A summary of the distribution of BLUEs can be seen in Table 15.

Table 15: Distribution of stripe rust infection type and severity in the 2018 and 2019 winter wheat populations based on BLUEs

		2018		2019	
Trait	Classification	Number	Percentage	Number	Percentage
Infection Type[a]	1-3	14	8.7%	21	7.9%
	4-6	76	47.2%	140	52.4%
	7-9	71	44.1%	106	39.7%
Severity	0-20	1	0.6%	69	25.8%
	20-40	36	22.4%	86	32.2%
	40-60	48	29.8%	69	25.8%
	60-80	63	39.1%	38	14.2%
	80-100	13	8.1%	5	1.9%
Disease Index	0-250	48	29.6%	153	57.3%
	250-500	55	34%	89	33.3%
	500-750	59	36.4%	25	9.4%

[a] BLUEs that fell between the ranges of 3-4 and 6-7 were rounded to the nearest whole number and included in that classification

Filtering/Imputation

The 2018 raw genotype file consisted of 138 taxa and 447,310 sites for a total of 6.17×10^7 sites x taxa (alleles) with 58.2% of those alleles missing before filtering and imputation. By filtering out taxa and sites that had high proportions of missing data, filtering out

MAF .05, and by performing LD KnnI Imputation, the proportion of missing alleles was reduced to 3%. After filtering and imputation, the 2018 genotype file consisted of 137 taxa and 139,994 sites. The 2019 genotype file consisted of 271 taxa and 447,310 sites for a total of $1.2x10^8$ alleles, 67.3% of which alleles were missing before filtering and imputation. After filtering and imputation, 5.3% of the alleles were missing. The resulting genotype file consisted of 267 taxa and 89,967 sites.

Principal Component Analysis

To reduce false-positives, principal component analysis was performed to account for population structure. Principal components that explained up to 25% of the total variation were used. The 2018 genotype data had seven principal components that explained approximately 25% of the genetic variation. The 2019 genotype data had nine principal components that explained approximately 25% of the genetic variation.

Regression Models

The mean squared difference of the four different models were calculated in RStudio. The results for the simple, P, K, and PK models for the six association studies (2018 infection type, 2018 severity, 2018 disease index, 2019 infection type, 2019 severity, and 2019 disease index) are reported in Table 16. The bold, mean squared difference indicates the model that was used for that association.

Table 16: Mean squared differences for simple, p, k, and pk models for the years 2018 and 2019 infection type, disease severity, and disease index

	Mean Squared Difference					
Model	2018 IT	2018 Sev	2018 Index	2019 IT	2019 Sev	2019 Index
Simple	0.0028	0.004	.004	0.021	0.022	.0197
P	0.0009	0.0015	.001	0.0058	0.0072	.006
K	0.0002	0.0002	6.8E-05	2.77E-05	**1.96E-05**	**5.08E-05**
PK	**1.45E-05**	**8.24E-06**	**3.8E-06**	**2.29E-05**	2.15E-05	7.88E-05

Bold indicated model used for each year and trait
It=Infection Type, Sev=Disease Severity

Significant Markers

SNPs were considered significant at the experiment wise Bonferroni level of α=.1. For the 2018 GWAS experiments 139,994 markers were tested. For a single marker to be considered significant it must have a p value of 7.1×10^{-7}. There were no markers that were considered significant for 2018 infection type, disease severity, or disease index at this level. For 2019 GWAS experiments 89,967 markers were tested, for a single marker to be considered significantly associated with a trait is must have a p value of 1.1×10^{-6}. There were no markers that were considered significant at this level for 2019 infection type, disease severity, or disease index. The Manhattan plots that were constructed are shown in Figures 3-8 with $-\log_{10}(p)$ plotted against chromosome location. Different colors signify the change from one chromosome to another.

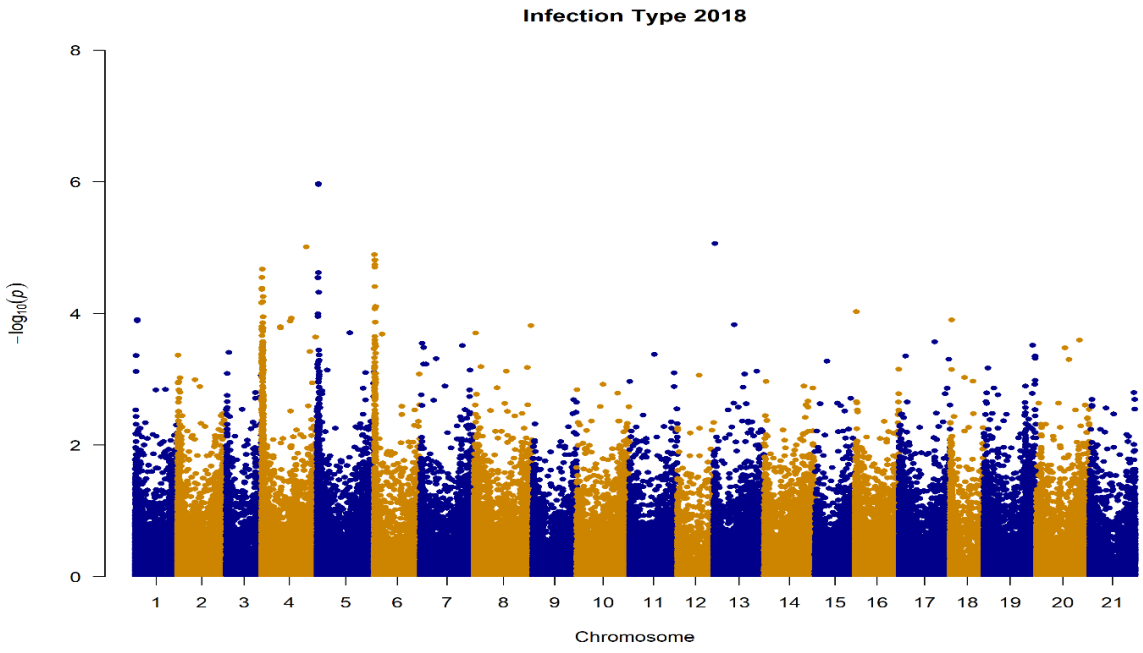

Figure 3: Manhattan Plot for Infection Type 2018 based on -log10(p)

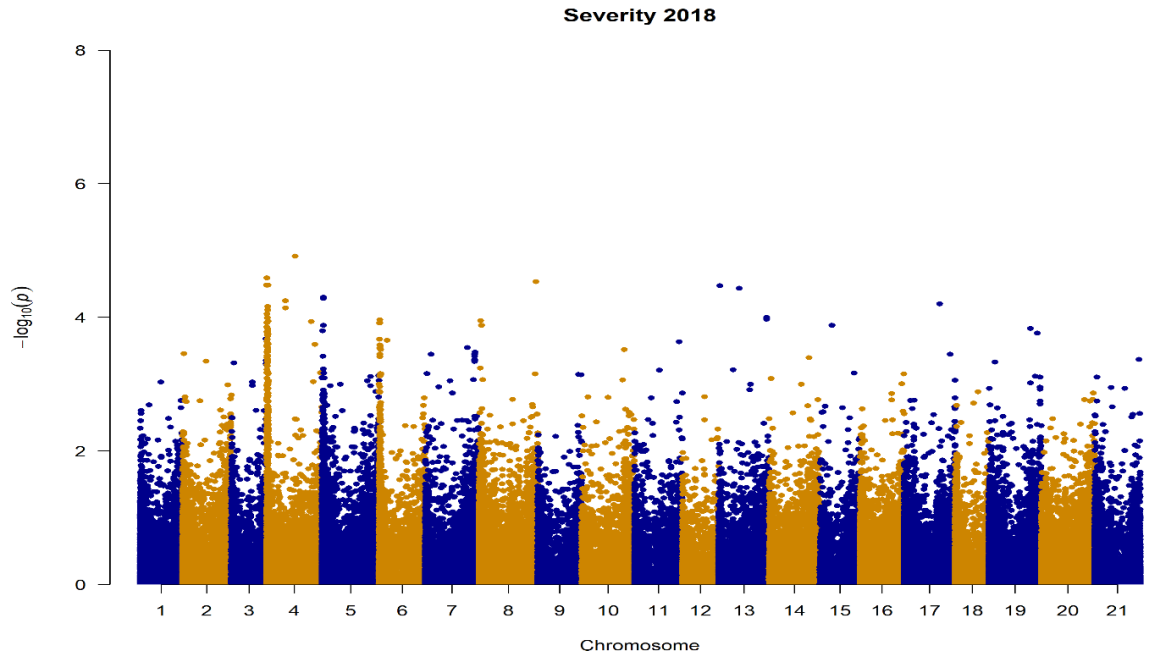

Figure 4: Manhattan plot for Severity 2018 based on -log10(p)

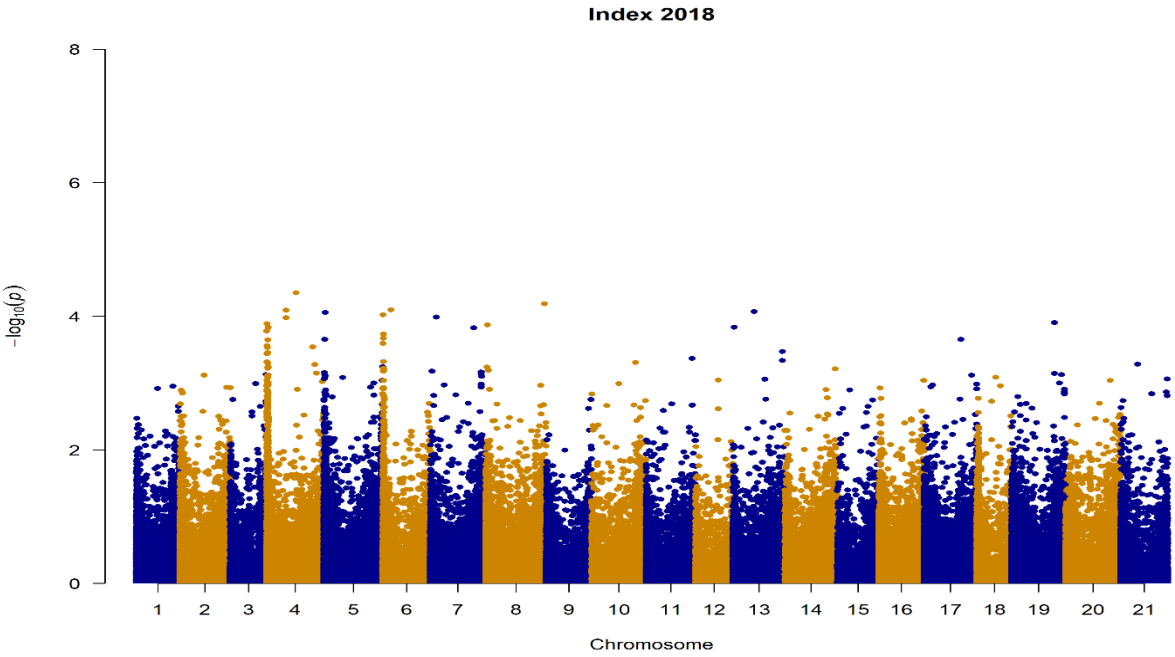

Figure 5: Manhattan plot for Index 2018 based on -log10(p)

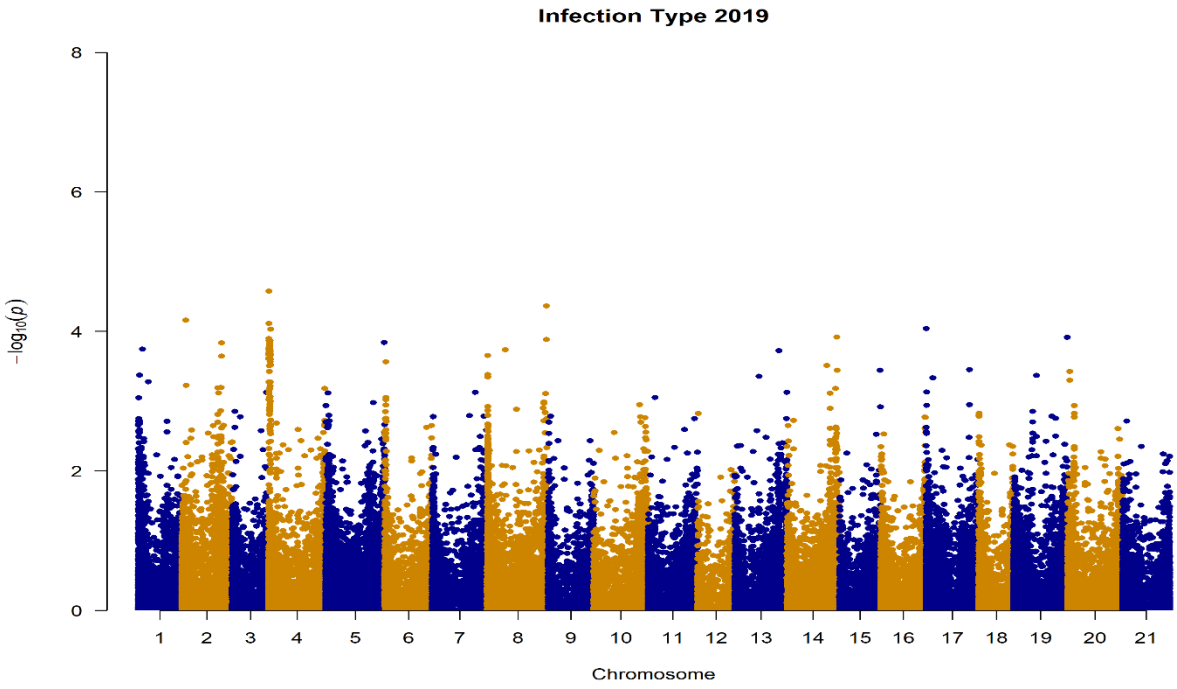

Figure 6: Manhattan plot for Infection Type 2019 based on -log10(p)

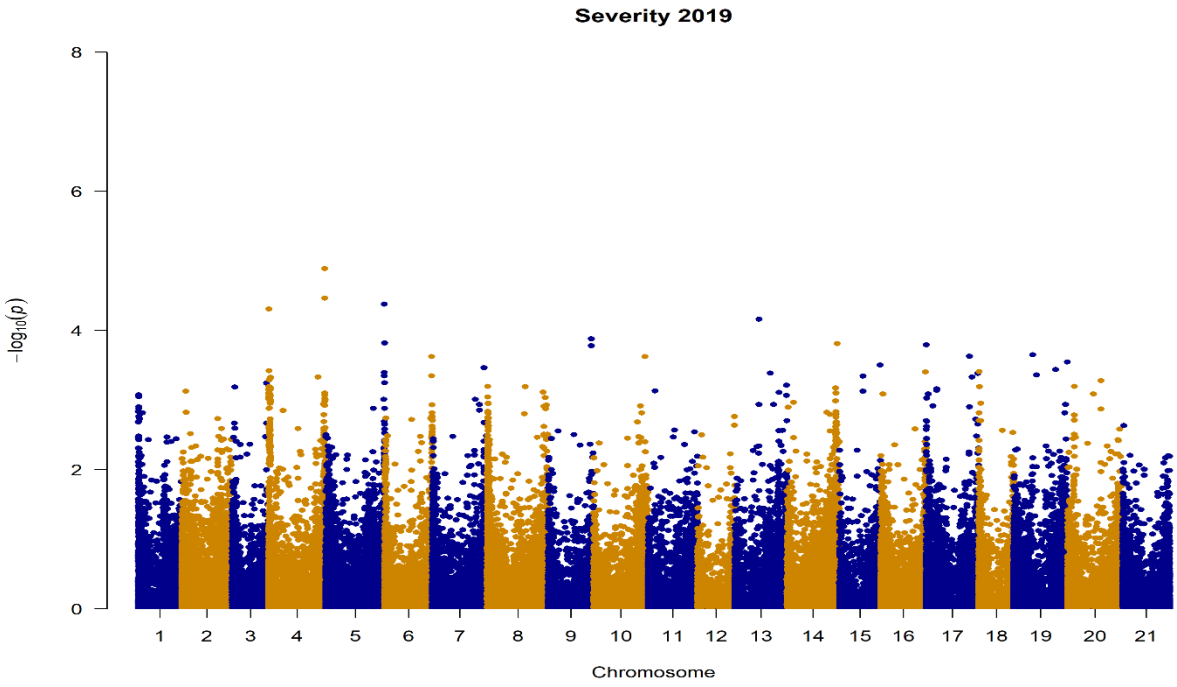

Figure 7: Manhattan plot for Severity 2019 based on -log10(p)

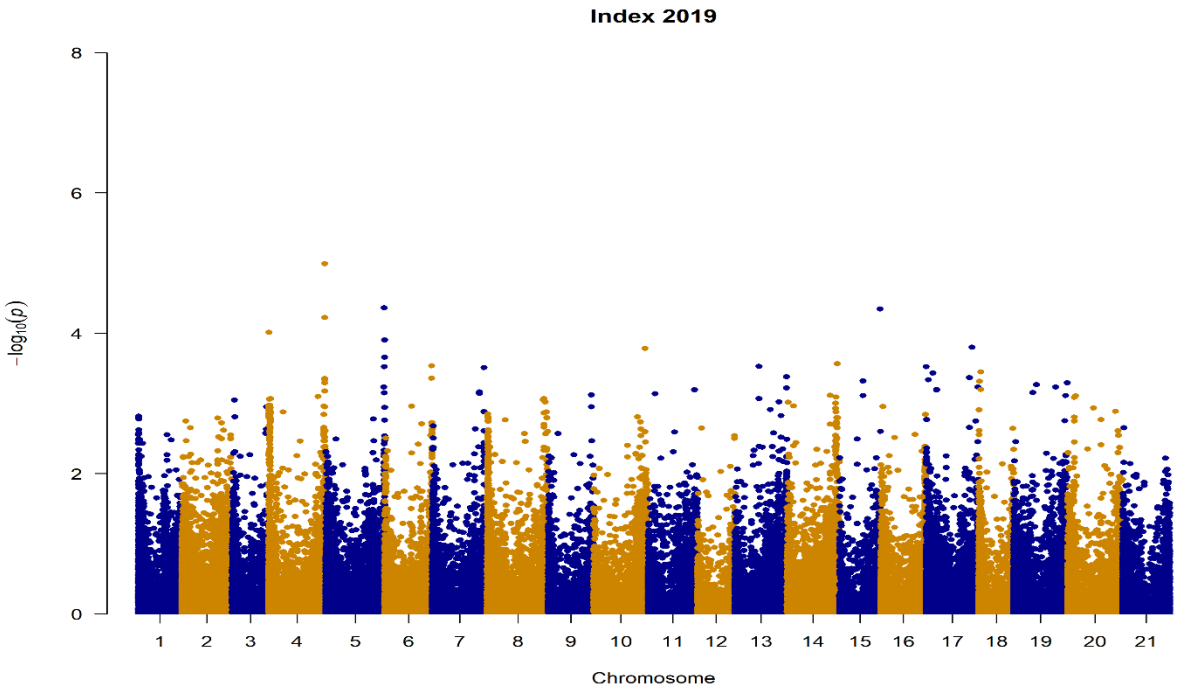

Figure 8: Manhattan plot of Index 2019 based on -log10(p)

Discussion

Stripe rust is still an uncommon disease in North Dakota with infections rarely occurring (Friskop, 2015). This resulted in little effort being put into the collection of stripe rust resistant material when the hard red winter wheat breeding program was started at NDSU (Marais personal communication, 2020). Therefore, it was not expected that many, if any, effective stripe rust resistance QTL would occur in the breeding material. The results from the phenotyping conducted at Washington State University showed that there is very little resistance to stripe rust race PSTv-37. One reason for this is that in the breeding material, the most commonly used race-specific resistance gene *Yr17* is not effective against stripe rust race PSTv-37 (Wan, 2016). Despite this, 8.7% of the lines tested in 2018 had the resistant infection type, and 23% of lines were partially resistant (severity less than 40%). In 2019, 7.9% of the lines had the resistant infection type, and 58% of lines were partially resistant, based on severity. In an

attempt to identify markers that correlate with resistance in the winter wheat breeding material a genome wide association study (GWAS) was conducted.

When conducting GWAS, false-positive results are common (Kaler, 2019). In an attempt to limit false-positive results, kinship analysis and principal component analysis were conducted to correct for relatedness of genotypes and population structure. In all six of the GWAS data sets (2018 and 2019 infection type, disease severity, and disease index) kinship needed to be accounted for, and population structure needed to be accounted for in all datasets except for 2019 severity and 2019 disease index. Even when kinship and population structure are accounted for, false-positive results can still occur due to the number of marker-trait associations that are tested in a GWAS (Johnson, 2010). In the 2018 and 2019 GWAS, totals of 139,884 and 89,967 markers were tested respectively. Since each one of these markers can result in a false-positive association, a statistical method that considers experiment-wise Type I (false-positive) error rate needed to be implemented. One common method used to account for experiment-wise Type I error is Bonferroni's correction (Johnson, 2010). When performing multiple tests simultaneously, the risk of a Type I error increases. Bonferroni's correction takes this into account by dividing the experiment-wise α level needed to be considered significant by the total number of tests being conducted to get the p-value that is needed to be considered significant for a single marker. When using Bonferroni's correction in this experiment a marker needed to have a p-value of 7.14×10^{-7} for 2018 data and 1.11×10^{-6} for 2019 data to be considered significant.

There were no markers that were significantly associated with stripe rust resistance at these p-values in any of the six GWAS conducted. It is however, important to note that Bonferroni's correction is a very conservative approach to minimize Type I errors that often results in an increase in Type II errors (Kaler, 2019). This coupled with each line only being

grown in two environments, the phenotype data not being normally distributed, and a high amount of missing genotype data could be reasons why no marker-trait associations were found. Ideally, phenotype data for GWAS should be gathered over multiple years and multiple locations as more observations for each entry helps minimize the environmental error. In this experiment phenotype data were only gathered for one year at two locations for each line. When analyzing QTL, it is assumed that the phenotypic data is normally distributed (Goh, 2009).

If the phenotype data is not normally distributed it can severely reduce the power of a GWAS to identify marker-trait associations. Attempts to normalize the stripe rust phenotype data using arcsine, log, and square root transformation methods did not improve normality. These factors could have contributed to no significant markers being found. Previous marker screening has shown that the stripe rust resistance genes *Yr29* and *Yr18* are present in NDSU hard red winter wheat germplasm. *Yr29* and *Yr18* are both race-nonspecific resistance genes that would provide resistance to stripe rust race PSTv-37 (Cobo, 2019; Wu, 2015). Possible reasons why *Yr29* and *Yr18* were not identified in this study could be that their individual contributions to stripe rust resistance is not significant enough to be identified by GWAS, or the frequency at which the two QTL occur in the germplasm is too low.

Conclusion

This experiment failed to identify stripe rust resistance genes that provide significant resistance to stripe rust race PSTv-37 in the NDSU winter wheat breeding germplasm. The ability of GWAS to detect minor stripe rust resistance QTL in the NDSU winter wheat germplasm can be improved through more comprehensive phenotyping employing multiple years and multiple races of stripe rust. However, lack of resources and facilities does not make this possible, especially because the disease still shows very sporadic incidence and regular,

annual field evaluations within North Dakota are currently not possible. An obvious, primary reason for the paucity of effective resistance QTL is that in previous years stripe rust has not been considered a significant threat to winter wheat production in North Dakota. More frequent incursions of the disease into North Dakota in recent years means that concerted attempts should be made to introgress resistance genes into the breeding population drawing on known resistance sources from elsewhere and utilizing established molecular markers. If stripe rust infections become more common in North Dakota it is likely that it will be race PSTv-37 or PSTv-52 as these races have already shown the ability to survive and infect wheat in North Dakota. Race specific stripe rust genes that are currently effective against PSTv-37 (Table 13) and could be targeted for introgression include the major resistance genes *Yr5* and *Yr15* among others.

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APPENDIX

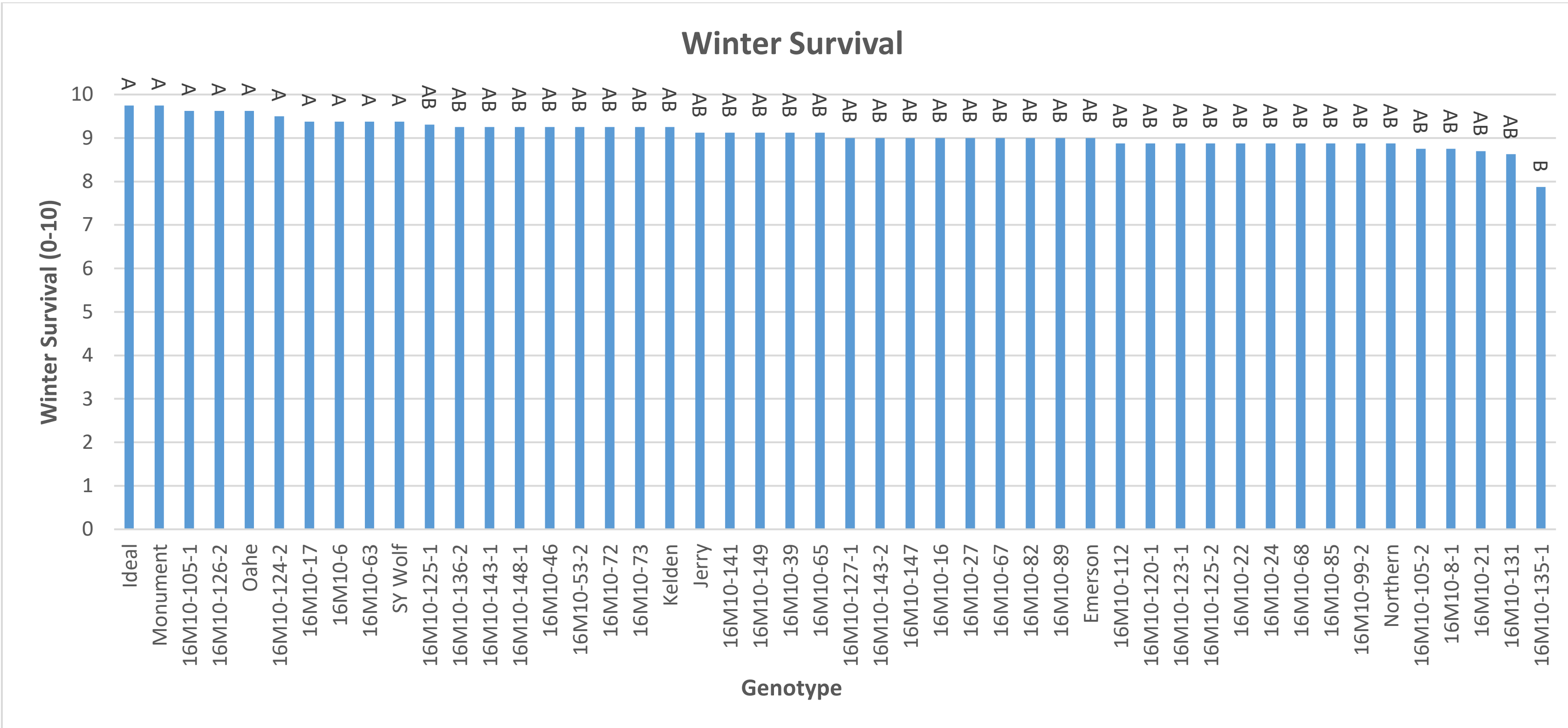

Figure A1: Mean separation of 16M10 lines and checks for winter survival

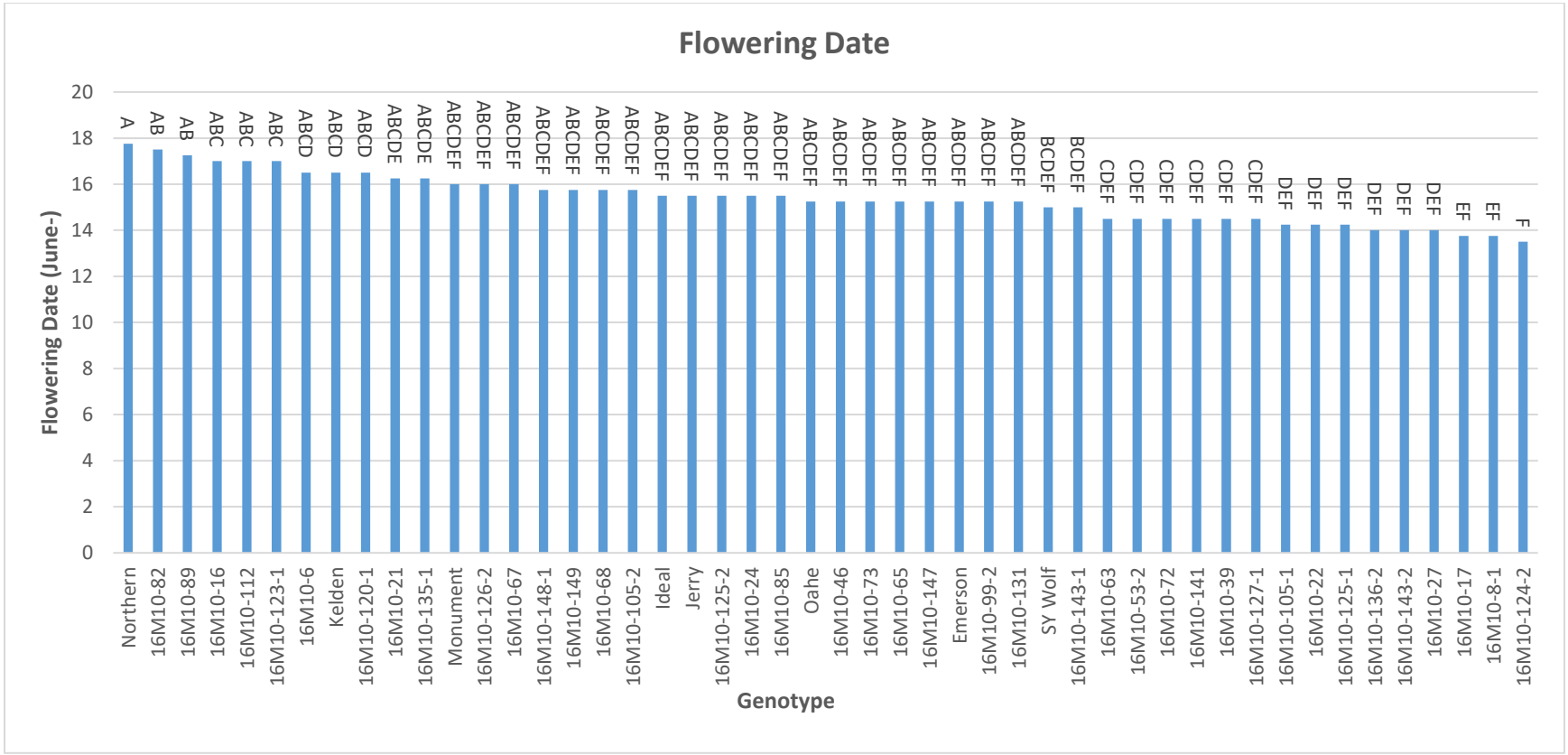

Figure A2: Mean separation of 16M10 lines and checks for flowering date

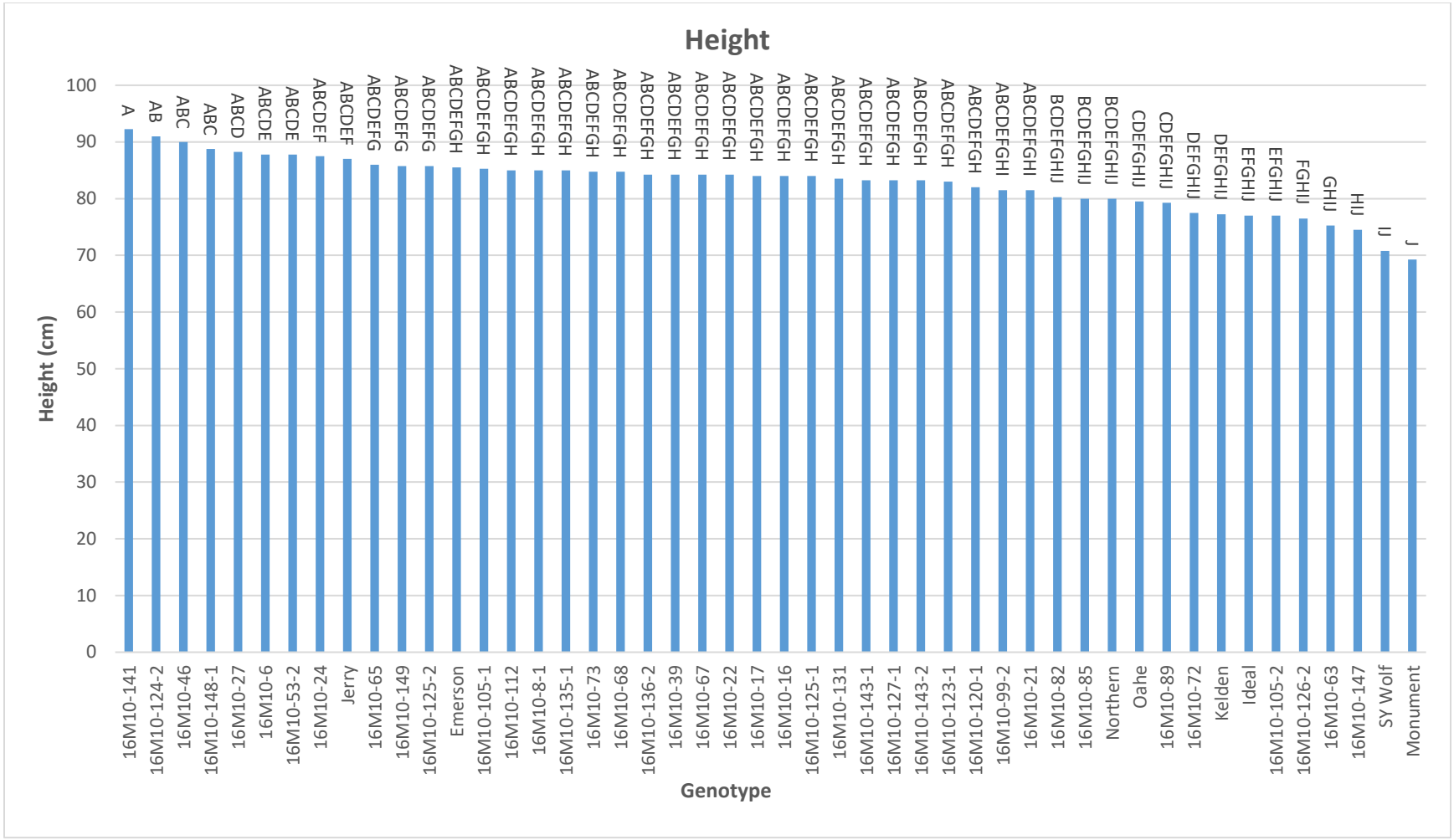

Figure A3: Mean separation of 16M10 lines and checks for height

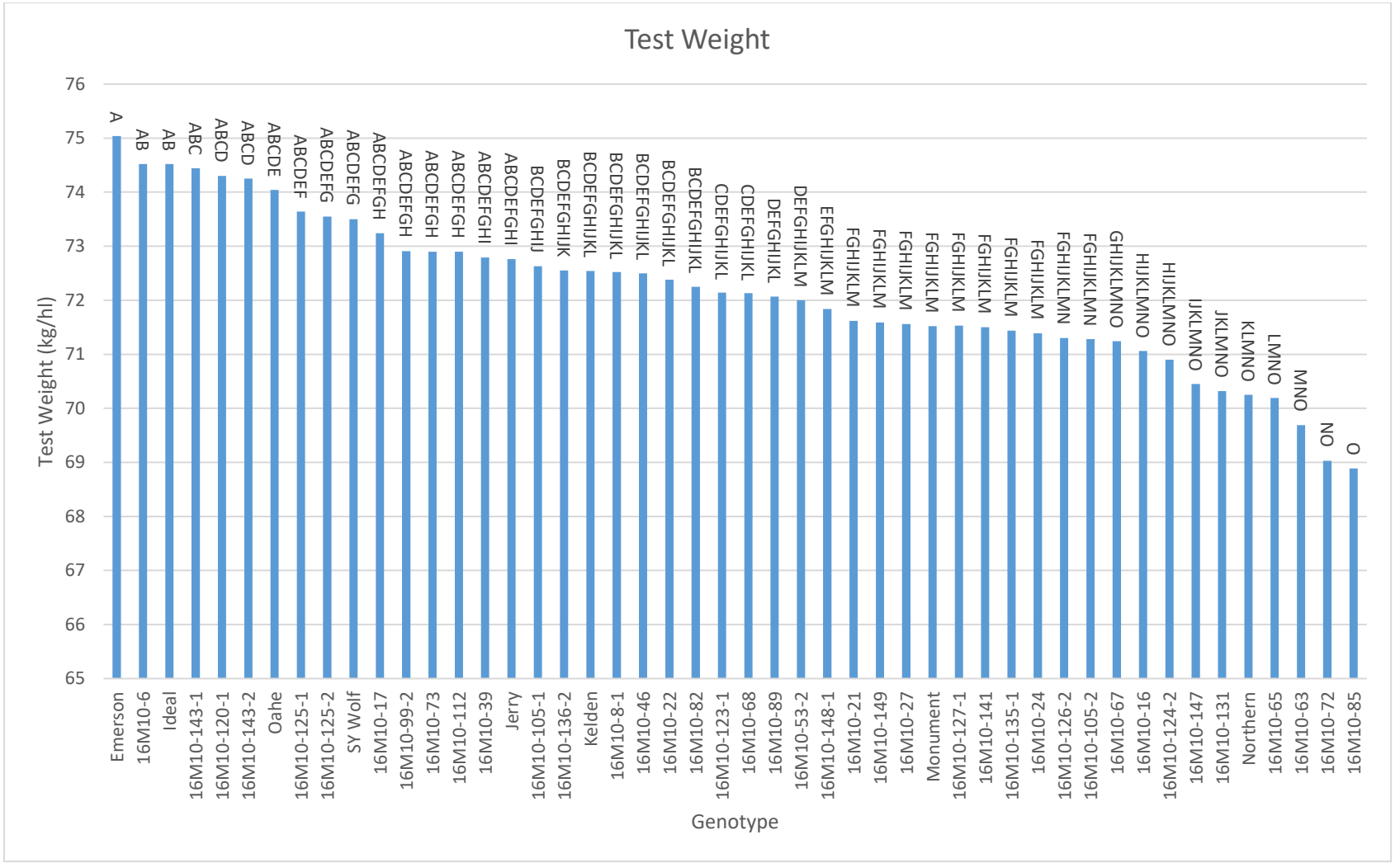

Figure A4: Mean separation of 16M10 lines and checks for test weight

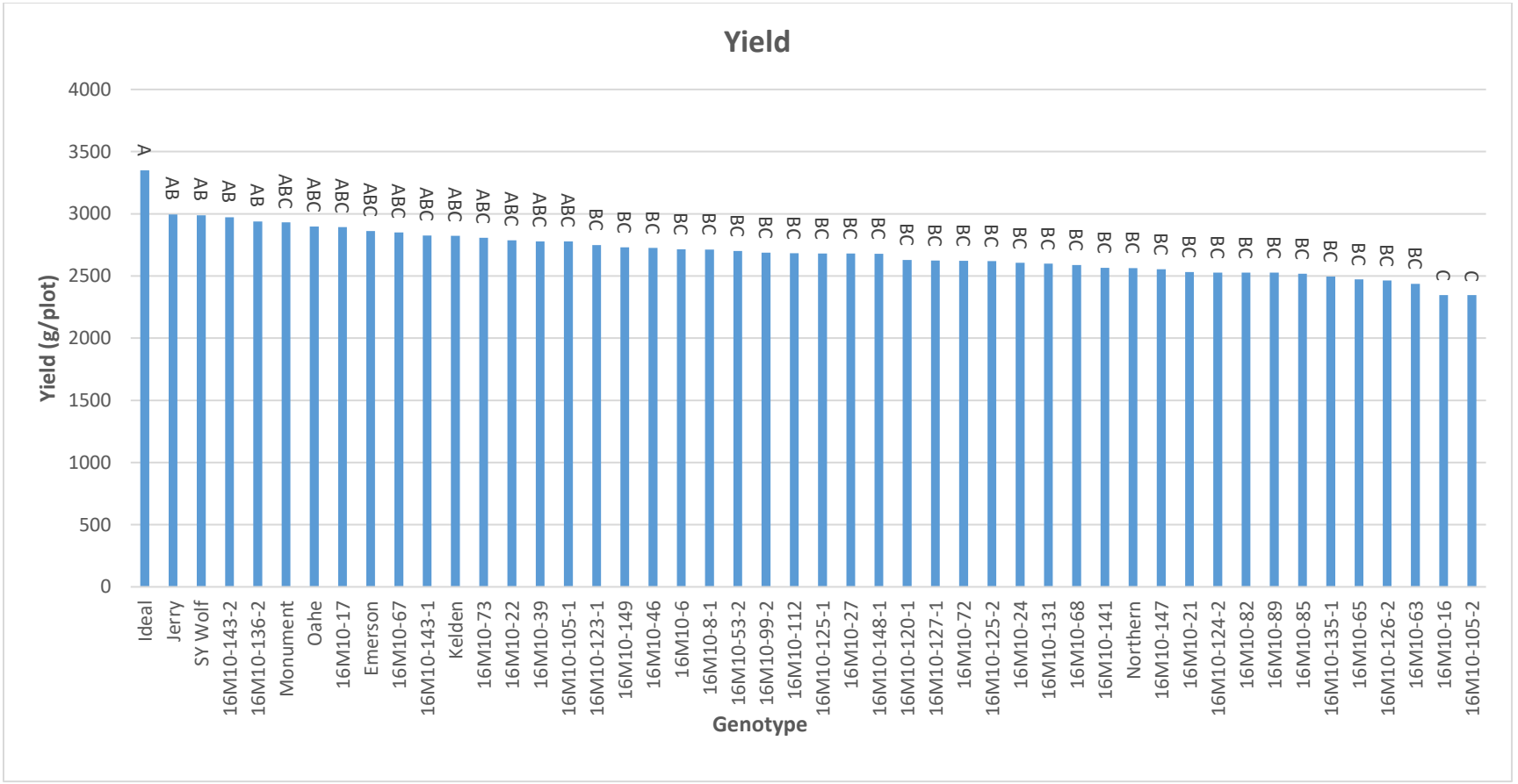

Figure A5: Mean separation of 16M10 lines and checks for yield

Table A1: Mean value for agronomic traits for 40 16M10 lines and eight checks

	Entry											
Trait	65	125-2	53-2	72	46	39	105-1	16	143-2	120-1	125-1	73
Winter Survival	9.125	8.875	9.25	9.25	9.25	9.125	9.625	9	9	8.875	9.31	9.25
Flowering Date	15.25	15.25	14.5	14.5	15.25	14.5	14.25	17	14	16.5	14.25	15.25
Height	86	86	87.75	77.5	90	84.25	85.25	84	83.25	82	84	84.75
Test Weight	70.19	73.55	72.00	69.03	72.50	72.79	72.63	71.06	74.25	74.30	73.64	72.90
Yield	2471.8	2471.8	2701.6	2621.6	2725	2777.7	2777	2346.5	2972.1	2628.6	2681.3	2806.9
	Entry											
Trait	8-1	112	123-1	136-2	149	124-2	22	147	82	126-2	127-1	85
Winter Survival	8.75	8.875	8.875	9.25	9.125	9.5	8.875	9	9	9.625	9	8.875
Flowering Date	13.75	17	17	14	15.75	13.5	14.25	15.25	17.5	16	14.5	15.5
Height	85	85	83	84.25	85.75	91	84.25	74.5	80.25	76.5	83.25	80
Test Weight	72.52	72.90	72.14	72.55	71.59	70.9	72.38	70.45	72.25	71.30	71.53	68.89
Yield	2711.9	2683.1	2747.9	2938.6	2731.6	2527.8	2788	2553.2	2527.3	2464.2	2624.5	2517.9
	Entry											
Trait	89	27	141	99-2	131	67	17	105-2	6	135-1	148-1	68
Winter Survival	9	9	9.125	8.875	8.625	9	9.375	8.75	9.375	7.875	9.25	8.875
Flowering Date	17.25	14	14.5	15.25	15.25	16	13.75	15.75	16.5	16.25	15.75	15.75
Height	79.25	88.25	92.25	81.5	83.5	84.25	84	77	87.75	85	88.75	84.75
Test Weight	72.07	71.56	71.50	72.91	70.32	71.24	73.24	71.28	74.52	71.44	71.84	72.13
Yield	2526.1	2681	2565.1	2686.7	2598.7	2849.8	2893.2	2345.9	2715.1	2495.6	2679.2	2587.9
	Entry											
Trait	24	143-1	63	21	Jerry	Ideal	Northern	Monument	Oahe	Keldin	SY Wolf	Emerson
Winter Survival	8.875	9.25	9.375	8.7	9.125	9.75	8.875	9.75	9.625	9.25	9.375	9
Flowering Date	15.5	15	14.5	16.25	15.5	15.5	17.75	16	15.25	16.5	15	15.25
Height	87.5	83.25	75.25	81.5	87	77	80	69.25	79.5	77.25	70.75	85.5
Test Weight	71.39	74.44	69.69	71.62	72.76	74.52	70.25	71.52	74.04	72.54	73.5	75.04
Yield	2605.1	2825.7	2436.7	2532	2995.5	3349.6	2564.3	2930.7	2897.3	2822.4	2989.1	2860.4

Table A2: Milling and baking characteristics for 16M10 lines and checks

Entry	Extraction (%)	Protein (12% Mb)	Loaf Volume (cc)	Crumb Color (1-10)	Crust Color (1-10)
16M10-65	51.98	13.5	189	5	10
16M10-125-2	54.57	12.8	183	6	10
16M10-53-2	50.32	13.6	192	7	10
16M10-72	49.38	14.7	160	1	10
16M10-46	51.98	12.9	143	3	8
16M10-39	52.90	13.1	148	7	10
16M10-105-1	50.84	12.7	147	0	8
16M10-16	50.55	14.1	196	7	10
16M10-143-2	53.11	12.6	166	6	10
16M10-120-1	54.12	12.9	198	7	10
16M10-125-1	51.95	13.5	190	7	10
16M10-73	52.27	13.3	195	6	10
16M10-8-1	49.45	13.4	167	7	10
16M10-112	50.68	13.6	180	7	10
16M10-123-1	51.30	12.7	155	1	8
16M10-136-2	52.70	13.5	190	6	9
16M10-149	48.41	13.6	166	5	10
16M10-124-2	50.81	13.2	148	5	8
16M10-22	48.99	13.3	187	6	10
16M10-147	51.43	14.1	167	5	9
16M10-82	52.44	14.0	167	5	8
16M10-126-2	50.58	13.3	189	7	10
16M10-127-1	51.40	13.9	199	7	10
16M10-85	47.34	13.8	194	6	10
16M10-89	48.28	13.7	210	6	10
16M10-27	51.62	13.4	171	7	10
16M10-141	51.95	12.1	163	6	9
16M10-99-2	48.99	13.0	188	7	10
16M10-131	47.37	12.5	171	7	10
16M10-67	52.86	13.2	172	6	10
16M10-17	50.81	12.2	187	6	9
16M10-105-2	47.40	14.2	183	7	10
16M10-6	54.89	13.8	183	7	10
16M10-135-1	49.68	13.7	150	6	8
16M10-148-1	50.39	12.9	171	7	10
16M10-68	46.01	13.3	177	6	10
16M10-24	47.91	12.8	160	0	10
16M10-143-1	50.65	12.6	175	8	10
16M10-63	47.66	13.3	188	8	10
16M10-21	46.10	13.1	170	6	10
Jerry	50.06	13.0	206	7	10
14K456-K-1	44.77	14.6	201	0	10
Ideal	49.77	12.3	127	1	7
Northern	46.85	13.3	221	8	10
Monument	48.90	12.5	131	1	7
Oahe	50.10	13.2	166	8	10
Keldin	46.71	12.6	170	1	8
SY Wolf	46.85	13.5	192	7	10
Emerson	49.97	13.7	187	7	10

Table A3: Mixing quality test results for 16M10 lines and checks

		Mixograph		SRC		GlutoPeak		
Entry	Mixo Score	Peak time (min)	Peak value (%)	Lactic Acid	Water	PMT (sec)	BEM (GPU)	Aggregation Energy (cm^2)
16M10-65	3	5.11	41.4	127	61	114	46	1325.8
16M10-125-2	2	8.00	39.1	130	58	211	43	1212.3
16M10-53-2	1	5.37	37.5	133	62	103	46	1341.5
16M10-72	1	8.00	31.6	150	62	207	46	1323.2
16M10-46	1	8.00	33.8	140	63	246	44	1302.8
16M10-39	3	7.43	40.5	139	63	205	46	1289.7
16M10-105-1	1	7.51	32.4	134	62	182	46	1321.2
16M10-16	2	7.41	32.1	148	59	175	46	1323.7
16M10-143-2	2	5.72	35.3	124	63	194	42	1230.2
16M10-120-1	2	5.81	39.0	133	59	241	44	1245.7
16M10-125-1	1	7.49	35.6	129	62	185	44	1241.7
16M10-73	2	5.48	40.6	124	61	190	44	1222.9
16M10-8-1	1	7.37	33.4	119	61	184	44	1235.6
16M10-112	2	6.27	37.8	126	63	260	43	1219.1
16M10-123-1	3	7.25	36.4	130	60	241	42	1209.7
16M10-136-2	3	6.75	41.0	127	61	250	42	1196.6
16M10-149	1	7.73	35.3	124	62	150	45	1255.3
16M10-124-2	2	8.00	32.7	139	62	280	44	1285.6
16M10-22	1	6.55	37.8	121	63	175	43	1221.8
16M10-147	3	7.46	39.4	142	62	296	45	1274.0
16M10-82	4	7.87	40.5	137	64	263	44	1294.7
16M10-126-2	2	6.02	39.8	131	63	151	45	1244.8
16M10-127-1	3	4.26	43.3	131	62	147	42	1249.8
16M10-85	1	6.74	37.4	111	65	81	47	1262.3
16M10-89	3	5.38	42.2	140	61	173	45	1276.1
16M10-27	2	7.78	34.0	138	64	225	44	1264.0
16M10-141	2	6.84	36.1	117	66	209	40	1135.1
16M10-99-2	2	8.00	37.7	131	63	201	44	1231.2
16M10-131	1	4.22	33.9	108	63	87	42	1112.5
16M10-67	4	8.00	41.3	136	62	300	42	1221.3
16M10-17	1	6.39	31.6	124	63	175	41	1177.0
16M10-105-2	2	7.62	36.8	146	67	161	48	1383.8
16M10-6	3	4.42	42.0	129	61	123	45	1268.7
16M10-135-1	2	7.65	37.5	150	61	226	45	1331.0
16M10-148-1	2	8.00	36.7	129	60	227	44	1251.7
16M10-68	2	6.52	40.2	120	63	106	46	1268.2
16M10-24	1	7.96	32.1	125	64	166	44	1263.9
16M10-143-1	2	6.31	39.8	127	60	201	43	1231.8
16M10-63	1	5.67	35.7	119	60	118	43	1207.3
16M10-21	1	5.52	32.5	97	61	87	42	1144.7
Jerry	2	5.13	38.7	130	59	185	44	1225.5
14K456-K-1	1	5.41	40.2	119	61	80	49	1365.6
Ideal	2	7.97	31.2	131	60	374	39	1134.5
Northern	5	5.25	47.7	134	69	109	47	1374.3
Monument	4	7.88	29.5	142	66	417	13	4762.0
Oahe	4	4.03	41.5	118	63	82	45	1292.1
Keldin	3	8.00	36.7	140	63	243	44	1256.3
SY Wolf	2	8.00	36.4	110	61	146	43	1167.5
Emerson	2	8.00	27.5	148	60	381	41	1194.6

Table A4: Seedling leaf rust resistance scores for 16M10 lines and checks

	Leaf Rust Race					
Line	TBDJQ	MFPS	TDBG	Field	THBL	MCDL
Jerry	2	;	;	;1=	;	2CN
SD09227	1CN	;1=	;1=	1CN	1P0 1P2CN	-
14K456-K-1	-	;	-	-	1CN	-
120-1	;1=	;	;	;	;	;
126-2	3	3	12	2P12 1P2++3	3-	1P1 2P3
131	;CN	;1=	-	;	2P; 1P1CN	1=CN
99-2	3	1P; 1P2 1P3	2P; 1P2++ 1P3	2++3	3	1P; 1P23
6	;1=	;CN	;	;	;	;
125-2	;	;	;	;	;	;
27	;	;	;	;	0;	;
82	;1=	;CN	;	;	;	;
68	12CN	1P;1= 2P2	12-	1P; 1P1 1P2CN	;	1P; 1P2
148-1	12	;	2P; 2P1	;1-	;	12CN
22	;	;1	2++	2++	12	3P12 1P3
112	1P12CN 1P3	;1=	12CN	1-CN	1	1=CN
17	1CN	;1	2	12	2P1 1P2	1
89	3	2P12 1P2	3P2++3	1P1 1P2	12CN	;1-
85	2	;	12	;	1CN	;
105-2	2++3	1-CN	2P; 1P12	2P1 1P12CN	;	;
143-2	1P12 1P3	2P; 1P1	1+	;	1	;
24	;	;	;	;	;	;
147	;1=	;	;CN	;	1P12 1P3	2
124-2	;	1P; 2P2+CN	3P; 1P2	1P; 1P3	1P1 2P12	12
21	-	;1-	;	;	-	2P; 1P2
143-1	2P12 2P3	2P12 1P3	2+	2++	12	1=
67	12CN	;	;	12CN	2P; 1P2+CN	;
46	12	;1-	2P; 1P12	-	;1=	1=
72	;1-	;1-	;1=	;1=	;1=	;
135-1	;1+	;	;	;1=	-	;
39	;	;	;	;1-	-	;
123-1	12	;CN	12	12CN	1P; 1P3	12CN
16	-	1+	1P; 1P12	1P12 1P2++	2P3 1P1CN	1P; 2P34
53-2	-	12CN	2	X(;3)	2P12 1P3	1CN
149	12CN	1CN	1=CN	1P1 2P12CN	1P1 1P2	;1-
125-1	;1-	;	;	;	0;	;
127-1	1P; 1P12CN	;1=	-	1P12CN 1P;	;1-	;
8-1	2P1CN 1P2	;1	1=	1CN	;1=	;1-

Table A4: Seedling leaf rust resistance scores for 16M10 lines and checks (continued)

	Leaf Rust Race					
Line	TBDJQ	MFPS	TDBG	Field	THBL	MCDL
136-2	1P; 2P2	12	12	1P1CN 2P2CN	2P; 1P2CN	12CN
73	3	3	3	3	3	3
63	12CN	1CN	1=CN	1+CN	1=CN	;
141	1P;1= 2P3	;1=	1P; 1P2++3	1P; 1P2++3	;	;
65	1CN	1CN	1=CN	2CN	;1-	1
105-1	;	1P; 1P2	1P; 1P12	;	;12	;

Scoring scale from most to least resistant 0>;>1>2>3>4

Two numbers such as “12” indicates that both infection types occurred on a single plant

X indicates a heterogeneous genotype

CN chlorosis/necrosis; P plants; = variation between +/- for the infection type

Table A5: Seedling stem rust resistance scores for 16M10 lines and checks

	Stem Rust Race			
Line	TMLK	QTHJ	TPMKC	QFCQ
Jerry	2++3CN	3	12	;1
SD09227	1-CN	1-CN	;1=	1=CN
14K456-K-1	1=CN	2	;	0
120-1	1/3	2++3CN	12CN	2++3
126-2	12	0	12	3
131	;1-	1=CN	;1=	;
99-2	1P; 2P3	1P1CN 2P3	12++	1P2 1P3
6	3	3	12+	3
125-2	2++3	2++CN	12+	12++CN
27	3	34	;1	34
82	2++3	3	2++3	2++
68	;1	1CN	;	1
148-1	1P1 1P3	12	;1=	3
22	;1	2P1CN 1P3	;	;
112	2++3	1P1-CN 1P2++CN	;1=	0
17	2++3CN	1P1 2P2++3	1P1 1P2+	3
89	;1	1P1CN 2P34CN	;1=	1
85	1=	1CN	;1-	1=
105-2	12	1CN	;1=	12
143-2	2+	4	;	2++3
24	2+	12CN	;	3
147	12++CN	2P12CN 1P2++CN	;	0
124-2	12	1CN	;1=	;1=
21	1	1CN	;	;
143-1	2++3	2++3	2+CN	12++
67	23	12CN	1P1 1P2+	3
46	12++CN	1CN	12++	0
72	1	1CN	1-	1
135-1	12	2P2 1P3	12CN	3
39	12++CN	2++3CN	;	2++3
123-1	1P1 1P2++	1P1CN 2P34	1P0 1P2++	1/3
16	1=/3	4	2+	3
53-2	23	1P1CN 2P3	1=	2
149	12	1=CN	1CN	1=
125-1	;/3	2++CN	2++3	2++3
127-1	22++CN	2++CN	2++	0
8-1	1	0/1	;1=	;1
136-2	2P1 1P3	2++3CN	12+	12++CN
73	12	1P1 2P2++3	2++3	3
63	1-	1CN	1	12
141	1/3	34	12++	3
65	2++3	1CN	1P;1 2P2++	2++3
105-1	1CN	1CN	;	;

Scoring scale from most to least resistant o>;>1>2>3>4
Two numbers such as “12” indicates that both infection types occurred on a single plant
CN chlorosis/necrosis, P plants, = variation between +/- for the infection type

Table A6: Marker screening results for individual plants in F_6 16M10 families.

Name	Sample #	Leaf Rust 1B Lr46/Yr29 C = Sus G = Res	Leaf Rust 2A Lr37/Sr38/Yr17 G = Sus T = Res	Rust 1B, 3D Lr24/Sr24 G = Sus T = Res	Fhb 3B Fhb1-FM227 T = Res C = Sus	Fhb 3B TaHRC T = Res G = Sus	Fhb 5A barc180 A = Res G = Sus	Fhb 5A barc186 A = Res C = Sus	1B1R G = Absent A = Present	Lr56 dupw217
16M10-105-1	1	C	T	T	T	T	A	A	G	251
16M10-105-1	2	C	T	T	T	T	A	A	G	
16M10-105-1	3	C	T	T	T	T	A	A	A	251
16M10-105-1	4	C	T	T	T	T	A	A	G	251
16M10-105-1	5	C	G	T	T	T	A	A	G	251
16M10-105-1	6	C	T	T	T	T	A	A	G	251
16M10-105-1	7	C	T	T	T	T	A	A	A	251
16M10-105-1	8	C	T	T	T	T	A	A	G	
16M10-105-1	9	C	T	T	T	T	A	A	G	
16M10-105-1	10	C	T	T	T	T	A	A	G	251
16M10-67	1	C	T	G	C	G	A	A	G	251
16M10-67	2	C	T	G	T	T	G	C	G	251
16M10-67	3	C	G	G	C	G	G	C	G	251
16M10-67	4	C	T	G	T	T	A	A	G	251
16M10-67	4-2	C	G	G	T	T	G	C	G	251
16M10-67	6	C	T	G	T	T	A	A	G	251
16M10-67	7	C	Het	G	T	T	G	C	G	
16M10-67	8	C	T	G	T	G	Het	Het	G	251
16M10-67	9	C	T	G	C	G	A	A	G	251
16M10-67	10	C	T	G	T	T	A	A	G	251
16M10-136-2	1	C	G	G	C	G	A	A	G	251
16M10-136-2	2	C	G	G	C	G	A	A	G	251
16M10-136-2	3	C	G	G	C	G	A	A	G	251
16M10-136-2	4	C	G	G	C	G	A	A	G	
16M10-136-2	5	C	G	G	C	G	A	A	G	251
16M10-136-2	6	C	G	G	C	G	A	A	G	
16M10-136-2	7	C	G	G	C	G	A	A	G	251
16M10-136-2	8	C	G	G	C	G	A	A	G	
16M10-136-2	9	C	G	G	C	G	A	A	G	
16M10-136-2	10	C	G	G	C	G	A	A	G	251
16M10-17	1	C	G	G	C	G	G	C	G	
16M10-17	2	C	G	G	C	G	A	A	A	
16M10-17	3	C	G	G	C	G	G	C	A	
16M10-17	4	C	G	G	C	G	G	C	G	
16M10-17	5	C	G	G	C	G	G	C	A	
16M10-17	5-2	C	G	G	C	G	A	A	G	
16M10-17	7	C	G	G	C	G	A	A	G	
16M10-17	8	C	G	G	C	G	A	A	G	

Table A6: Marker screening results for individual plants in F_6 16M10 families (continued).

Name	Sample #	Leaf Rust 1B Lr46/Yr29 C = Sus G = Res	Leaf Rust 2A Lr37/Sr38/Yr17 G = Sus T = Res	Rust 1B, 3D Lr24/Sr24 G = Sus T = Res	Fhb 3B Fhb1-FM227 T = Res C = Sus	Fhb 3B TaHRC T = Res G = Sus	Fhb 5A barc180 A = Res G = Sus	Fhb 5A barc186 A = Res C = Sus	1B1R G = Absent A = Present	Lr56 dupw217
16M10-17	9	C	G	G	C	G	G	C	G	
16M10-17	10	C	G	G	C	G	A	A	G	
16M10-73	1	Unk	T	G	T	T	A	A	G	
16M10-73	2	G	T	G	T	T	A	A	G	
16M10-73	3	G	T	G	T	T	A	A	G	
16M10-73	4	G	G	G	T	G	A	A	G	
16M10-73	5	G	T	G	T	T	A	A	G	
16M10-73	6	G	T	G	T	T	A	A	G	
16M10-73	7	C	G	G	T	T	A	A	G	
16M10-73	8	Het	G	G	Unk	T	A	A	G	
16M10-73	9	C	Het	G	T	G	A	A	G	
16M10-73	10	C	T	G	T	T	A	A	G	
16M10-143-2	1	C	G	G	T	T	A	A	G	
16M10-143-2	2	C	G	G	T	T	A	A	G	251
16M10-143-2	3	C	G	G	T	T	A	A	G	
16M10-143-2	4	G	T	G	T	T	A	A	G	
16M10-143-2	5	C	T	G	C	G	A	A	G	
16M10-143-2	6	G	G	G	T	T	A	A	G	
16M10-143-2	7	G	G	G	T	T	A	A	G	
16M10-143-2	8	C	Het	G	T	T	A	A	G	
16M10-143-2	9	C	G	G	T	T	A	A	G	
16M10-143-2	10	C	T	G	T	T	A	A	G	251
16M10-123-1	1	C	G	G	T	T	G	C	G	
16M10-123-1	2	C	G	G	T	T	G	C	G	
16M10-123-1	3	C	G	G	T	T	A	A	G	
16M10-123-1	4	C	T	G	T	T	Het	Het	G	
16M10-123-1	5	C	G	G	T	T	G	C	G	
16M10-123-1	6	C	T	G	T	T	A	A	G	
16M10-123-1	7	C	G	G	T	T	G	C	G	
16M10-123-1	8	C	T	G	T	G	G	C	G	251
16M10-123-1	9	C	G	G	C	G	G	C	A	251
16M10-123-1	10	C	Het	G	T	T	A	A	G	
16M10-143-1	1	G	T	G	C	G	A	A	G	
16M10-143-1	2	G	G	G	C	G	A	A	G	
16M10-143-1	3	G	G	G	T	T	Unk	A	G	
16M10-143-1	4	G	G	G	C	G	A	A	G	
16M10-143-1	5	G	G	G	T	T	A	A	G	
16M10-143-1	6	G	T	G	C	G	A	A	G	

Table A6: Marker screening results for individual plants in F_6 16M10 families (continued).

Name	Sample #	Leaf Rust 1B Lr46/Yr29 C = Sus G = Res	Leaf Rust 2A Lr37/Sr38/Yr17 G = Sus T = Res	Rust 1B, 3D Lr24/Sr24 G = Sus T = Res	Fhb 3B Fhb1-FM227 T = Res C = Sus	Fhb 3B TaHRC T = Res G = Sus	Fhb 5A barc180 A = Res G = Sus	Fhb 5A barc186 A = Res C = Sus	1B1R G = Absent A = Present	Lr56 dupw217
16M10-143-1	7	G	G	G	T	T	A	A	G	
16M10-143-1	8	G	G	G	T	T	A	A	G	
16M10-143-1	9	G	T	G	C	G	A	A	G	
16M10-143-1	10	G	T	G	C	G	A	A	G	
16M10-22	1	C	G	G	T	T	A	A	G	
16M10-22	2	C	G	T	T	T	A	A	A	251
16M10-22	3	C	G	T	T	Unk	A	A	G	
16M10-22	4	C	G	T	T	T	A	A	A	
16M10-22	5	C	G	T	T	T	A	A	G	251
16M10-22	6	C	G	T	T	T	A	A	A	251
16M10-22	7	C	G	G	T	T	A	A	G	
16M10-22	8	C	G	G	T	T	A	A	G	251
16M10-22	9	C	G	T	T	T	A	A	A	
16M10-22	10	C	G	G	T	T	A	A	A	
16M10-123-1	1	C	T	G	T	T	G	C	G	
16M10-123-1	2	C	T	G	T	T	Het	Het	G	
16M10-123-1	3	C	G	G	T	T	G	C	G	
16M10-123-1	4	C	T	G	T	T	G	C	G	
16M10-123-1	5	C	G	G	C	Unk	G	C	G	251
16M10-123-1	6	C	T	G	T	T	A	A	G	
16M10-123-1	7	C	T	G	T	T	G	C	G	
16M10-123-1	8	C	G	G	T	T	G	C	G	
16M10-123-1	9	C	G	G	T	T	G	C	A	251
16M10-123-1	10	C	T	G	C	G	G	C	G	251
Jerry	1	G	G	G	C	G	G	Het	G	
CM82036	1	C	G	G	T	T	A	A	A	
14K456-K-1	1	C	G	G	T	T	A	A	A	
Jerry	2	G	G	G	C	G	G	C	G	
CM82036	2	C	G	G	T	T	A	A	A	
14K456-K-1	2	C	G	G	T	T	A	A	A	
16M10-136-2	11	C	G	G	C	G	A	A	G	
16M10-136-2	12	C	G	G	C	G	A	A	G	
16M10-136-2	13	C	G	G	C	G	A	A	G	
16M10-136-2	14	C	G	G	C	G	A	A	G	
16M10-136-2	15	C	G	G	C	G	A	A	G	
16M10-136-2	16	C	G	G	C	G	A	A	G	251
16M10-136-2	17	C	G	G	C	G	A	A	G	
16M10-136-2	18	C	G	G	C	G	A	A	G	

Table A6: Marker screening results for individual plants in F_6 16M10 families (continued).

Name	Sample #	Leaf Rust 1B Lr46/Yr29 C = Sus G = Res	Leaf Rust 2A Lr37/Sr38/Yr17 G = Sus T = Res	Rust 1B, 3D Lr24/Sr24 G = Sus T = Res	Fhb 3B Fhb1-FM227 T = Res C = Sus	Fhb 3B TaHRC T = Res G = Sus	Fhb 5A barc180 A = Res G = Sus	Fhb 5A barc186 A = Res C = Sus	1B1R G = Absent A = Present	Lr56 dupw217
16M10-136-2	19	C	G	G	C	G	A	A	G	251
16M10-136-2	20	C	G	G	C	G	A	A	G	251
16M10-67	11	C	T	G	C	G	G	C	G	251
16M10-67	12	C	T	G	C	G	G	C	G	
16M10-67	13	C	T	G	C	G	A	A	G	251
16M10-67	14	C	T	G	T	T	A	A	G	251
16M10-67	15	C	T	G	T	T	G	C	G	251
16M10-67	16	C	G	G	C	G	G	C	G	251
16M10-67	17	C	G	G	T	T	A	A	G	251
16M10-67	18	C	T	G	T	T	G	C	G	251
16M10-67	19	C	T	G	T	T	A	A	G	
16M10-67	20	C	T	G	T	T	G	C	G	251
16M10-73	11	G	T	G	T	T	A	A	G	
16M10-73	12	Unk	G	G	T	T	A	A	G	
16M10-73	13	G	T	G	T	T	A	A	G	
16M10-73	14	C	T	G	T	G	A	A	G	
16M10-73	15	C	T	G	T	T	A	A	G	
16M10-73	16	C	G	G	T	T	A	A	G	
16M10-73	17	G	T	G	T	T	A	A	G	
16M10-73	18	G	T	G	T	T	A	A	G	
16M10-73	19	G	T	G	T	T	A	A	G	
16M10-73	20	C	Het	G	T	T	A	A	G	
16M10-17	11	C	T	G	C	G	G	C	G	
16M10-17	12	C	T	G	C	G	Het	Het	G	
16M10-17	13	C	G	G	C	G	A	A	G	
16M10-17	14	C	G	G	C	G	A	A	G	
16M10-17	15	C	G	G	C	G	Het	Het	G	
16M10-17	16	C	G	G	C	G	G	C	A	
16M10-17	17	C	G	G	C	G	A	A	G	
16M10-17	18	C	G	G	C	G	A	A	G	
16M10-17	19	C	Het	T	T	T	Het	Het	G	
16M10-17	20	C	T	G	C	G	A	A	A	
16M10-123-1	11	C	G	G	T	T	A	Het	G	
16M10-123-1	12	C	T	G	T	T	A	A	G	
16M10-123-1	13	C	G	G	T	G	G	C	G	251
16M10-123-1	14	C	T	G	T	T	G	C	G	
16M10-123-1	15	C	T	G	T	T	A	A	G	
16M10-123-1	16	C	G	G	T	T	Unk	C	G	

Table A6: Marker screening results for individual plants in F_6 16M10 families (continued).

Name	Sample #	Leaf Rust 1B Lr46/Yr29 C = Sus G = Res	Leaf Rust 2A Lr37/Sr38/Yr17 G = Sus T = Res	Rust 1B, 3D Lr24/Sr24 G = Sus T = Res	Fhb 3B Fhb1-FM227 T = Res C = Sus	Fhb 3B TaHRC T = Res G = Sus	Fhb 5A barc180 A = Res G = Sus	Fhb 5A barc186 A = Res C = Sus	1B1R G = Absent A = Present	Lr56 dupw217
16M10-123-1	17	C	G	G	T	T	G	C	G	
16M10-123-1	18	C	G	G	T	T	G	C	G	
16M10-123-1	19	C	T	G	T	T	G	C	G	
16M10-123-1	20	C	G	G	T	G	G	C	G	
16M10-143-2	11	C	G	G	T	T	A	A	G	
16M10-143-2	12	G	T	G	T	T	A	A	G	251
16M10-143-2	13	G	G	G	T	T	A	A	G	
16M10-143-2	14	C	T	G	T	T	A	A	G	251
16M10-143-2	15	G	G	G	T	T	A	A	G	
16M10-143-2	16	C	G	G	T	T	A	A	G	
16M10-143-2	17	G	G	G	T	Unk	A	A	G	
16M10-143-2	18	C	T	G	T	T	A	A	G	251
16M10-143-2	19	C	G	G	C	G	A	A	G	
16M10-143-2	20	G	G	G	T	T	A	A	G	
16M10-105-1	11	C	T	T	T	T	A	A	A	251
16M10-105-1	12	C	T	T	T	T	A	A	G	
16M10-105-1	13	C	T	T	T	T	A	A	G	
16M10-105-1	14	C	T	T	T	T	A	A	G	
16M10-105-1	15	C	T	T	T	T	A	A	G	251
16M10-105-1	16	C	T	T	T	T	A	A	G	251
16M10-105-1	17	C	T	T	T	T	A	A	G	251
16M10-105-1	18	C	T	T	T	T	A	A	G	251
16M10-105-1	19	C	T	T	T	T	A	A	G	
16M10-105-1	20	C	T	T	T	T	A	A	G	251
16M10-143-1	11	G	T	G	T	T	A	A	G	
16M10-143-1	12	G	G	G	C	G	A	A	G	
16M10-143-1	13	G	T	G	C	G	A	A	G	
16M10-143-1	14	G	Het	G	C	G	A	A	G	
16M10-143-1	15	G	G	G	C	G	A	A	G	
16M10-143-1	16	G	G	G	T	T	A	A	G	
16M10-143-1	17	G	G	G	T	T	A	A	G	
16M10-143-1	18	G	G	G	C	G	A	A	G	
16M10-143-1	19	G	T	G	C	G	A	A	G	
16M10-143-1	20	G	G	G	C	G	A	A	G	
16M10-22	11	C	G	G	T	G	A	A	G	251
16M10-22	12	C	G	G	T	T	A	A	G	
16M10-22	13	C	G	T	T	G	A	A	G	
16M10-22	14	C	G	T	T	T	A	A	A	251

Table A6: Marker screening results for individual plants in F_6 16M10 families (continued).

Name	Sample #	Leaf Rust 1B Lr46/Yr29 C = Sus G = Res	Leaf Rust 2A Lr37/Sr38/Yr17 G = Sus T = Res	Rust 1B, 3D Lr24/Sr24 G = Sus T = Res	Fhb 3B Fhb1-FM227 T = Res C = Sus	Fhb 3B TaHRC T = Res G = Sus	Fhb 5A barc180 A = Res G = Sus	Fhb 5A barc186 A = Res C = Sus	1B1R G = Absent A = Present	Lr56 dupw217
16M10-22	15	C	G	T	T	T	A	A	A	
16M10-22	16	C	G	T	T	T	A	A	A	
16M10-22	17	C	G	T	T	T	A	A	G	
16M10-22	18	C	G	G	T	T	A	A	A	251
16M10-22	19	C	G	G	T	T	A	A	A	
16M10-22	20	C	G	T	T	T	A	A	G	251
16M10-123-1	11	C	G	G	C	G	G	C	G	251
16M10-123-1	12	C	Het	G	T	T	G	C	G	
16M10-123-1	13	C	G	G	T	T	G	C	G	
16M10-123-1	14	C	Unk	G	T	T	G	C	G	
16M10-123-1	14-2	C	G	G	T	T	G	C	G	
16M10-123-1	16	C	G	G	T	T	G	C	G	
16M10-123-1	17	C	G	G	T	T	G	C	G	251
16M10-123-1	18	C	G	G	T	T	G	C	G	
16M10-123-1	19	C	G	G	T	T	G	C	G	
16M10-123-1	20	C	G	G	T	T	Het	Het	G	