DEVELOPMENT OF A DIVERSE BASE POPULATION FOR HARD RED WINTER WHEAT RECURRENT MASS SELECTION

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

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ABSTRACT

Limited germplasm is available for a newly established NDSU hard red winter wheat breeding program. Therefore, a diverse pre-breeding base population was produced to broaden genetic variability through recurrent mass selection. A complex cross that involved approximately 150 diverse genotypes contained within five parental populations was used to incorporate the genetic male sterility gene, *Ms3*, plus a wide range of native and exotic disease resistance and adaptation genes. Phenotypic evaluations confirmed the presence of genes for resistance to leaf and stem rust, tan spot and Stagonospora nodorum blotch in the final base population. Markers specifically identified resistance genes *Lr19/Sr25*, *Lr21*, *Lr24/Sr24*, *Lr34*, *Lr37*, *Sr2*, and *Sr50*, yet could not detect *Fhb1*. An analysis of single nucleotide polymorphisms across the parental and hybrid genotypes indicated high diversity within the final base population. This population will be used to develop cold-hardy breeding parents with effective resistance against major diseases in North Dakota.

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1. INTRODUCTION

In the fall of 2013, approximately 352,077 hectares of winter wheat were planted in North Dakota; however only 226,624 hectares were harvested in the following growing season (Ransom et al., 2014; USDA, 2015). The loss of winter wheat acreage has been attributed to colder than normal weather experienced over the winter. Although winterkill was significant in the 2013-14 growing season, the winter wheat yield of 3295 kg/ha exceeded the previous year's yield of 2892 kg/ha (USDA-NASS, 2015). In four of the past five growing seasons, winter wheat has out-yielded spring wheat in North Dakota (USDA, 2015). This indicates that although winter survival is crucial to the production of winter wheat in North Dakota, it has the potential to be a profitable crop when compared to other small grain cereals.

Like with other crops grown in North Dakota, the successful cultivation of winter wheat is threatened by several pests and diseases. Among these are diseases such as Fusarium head blight, rust, and leaf spot diseases, including tan spot and Stagonospora nodorum blotch. Several species of Fusarium cause symptoms of Fusarium head blight (FHB) in wheat; however, the predominant causal agent of FHB in most areas of the world is *Fusarium graminearum* Scwabe. The fungal disease, Fusarium head blight has the ability to destroy wheat crops within weeks of harvest (McMullen et al., 1997). Similarly, the rust diseases can be extremely detrimental to wheat yields if the pathogen establishes itself early on and is allowed to build up over the season (Chen, 2005; Roelfs et al., 1992). Wheat leaf rust is caused by the fungus *Puccinia triticina* Erikss. (*Pg*), wheat stem rust is caused by *Puccinia graminis* Pers.:Pers. f. sp. *tritici* Erikss. E. Henn. (*Pgt*), and stripe rust is caused by *Puccinia striiformis* Westend. f. sp. *tritici* Erikss. The tan spot (caused by *Pyrenophora tritici-repentis* (Died.) Drechs) and S. nodorum blotch (caused by *Stagonospora nodorum* (Berk.)) leaf diseases are also potentially devastating threats to wheat

production and can cause significant yield losses in favorable environments (Friesen et al., 2003; Xu et al., 2004). In addition to the primary diseases affecting winter wheat production in North Dakota, the emergence of the wheat stem sawfly (*Cephus cinctus* Norton (Hymenoptera: Cephidae)) has prompted more effort to combat the pest by breeding for the solid-stem trait (Sherman et al., 2015).

Utilization of plant breeding techniques and methods to develop genetic resistance, or tolerance, to these parasites can be an effective manner to increase yield. Successful incorporation of desirable traits into adapted germplasm can provide more stability in overwintering winter wheat stands, resistance to plant diseases, and a better defense against pests migrating to the region, while maintaining a high level of grain quality and yield.

2. LITERATURE REVIEW

2.1. Winter wheat growth and development

Winter wheat is planted in the fall and maintains vegetative growth during winter. This protects the plant's growing point from cold exposure during winter. Winter wheat must undergo cold acclimation and vernalization to promote reproductive growth in the spring (Wiersma et al., 2006). Cold acclimation is the winter wheat's winter hardening response to cold temperatures in the fall. This allows the plants to survive the winter and continue growth in the spring. Vernalization requirement is the accumulated exposure to cold that is needed to initiate reproductive growth in the spring. Vernalization and overwintering allows the winter wheat plants to reach maturity earlier in the season than spring wheat. The wheat plants need to be well established in the fall to help combat severe winter conditions. Strong cold and freezing conditions can destroy the meristem and thus kill the plant.

The determination of winter versus spring growth habit has been known to be regulated primarily by the *Vrn1* genes. This homoeologous series of genes consists of the *Vrn-A1*, *Vrn-B1*, and the *Vrn-D1* homoeoloci (Galiba et al., 2009). The existence of a dominant allele at one or more of these homoeoloci will result in a spring type plant. However, there can be differences in spring growth habit of a plant dependent on which dominant homoeoalleles are present. *Vrn-A1* is the most significant inducer of spring type growth, while the presence of *Vrn-B1* or *Vrn-D1* will result in moderate spring growth and will still maintain a low vernalization requirement. A homozygous recessive wheat plant (*vrn-A1*, *vrn-B1*, and *vrn-D1*) will exhibit the winter growth habit and hence need to fulfil its vernalization requirement to initiate reproductive growth. The *Vrn* homoeoloci have been found to be linked to quantitative trait loci (QTL) for frost tolerance in the same chromosome region (Galiba et al., 2009).

Winter wheat plants undergo many physical and biochemical changes during cold acclimation. An increase of abscisic acid, soluble sugars, and the synthesis of antifreeze proteins are just a few of the physiological changes that the plant may experience during cold acclimation (Pearce, 1999). Among the many genes thought to be expressed during cold acclimation, tolerance to cold exposure has been related to the *Fr2* locus located near the *Vrn1* gene (Galiba et al., 2009). Within the *Fr2* locus, a collection of 11 *CBF* (C-repeat binding factors) genes were mapped within a 0.8 cM region in *T. monococcum* L. (Miller et al., 2006). The expression of these *CBF* genes was found to be associated with the *Fr2* locus, in achieving cold tolerance in cereals (Vagujfalvi et al., 2005). In addition, eight orthologous *CBF* genes located at the *Fr2* locus were examined in the *T. aestivum* genome. The analysis showed that three *CBF* genes (*Cbf1A, Cbf1C,* and *Cbf7*) exhibited higher transcript levels in relation to higher cold resistance. This discovery led to the understanding that the role of *CBF* genes linked to the *Fr2* locus might be an integral factor in a genotype's expression of frost tolerance.

Another genetic factor affecting cold acclimation and tolerance can be photoperiod sensitivity (*Ppd*). Although photoperiod sensitivity does not appear to affect the cold tolerance of winter habit genotypes (recessive *vrn1* gene), there was a significant difference among spring type genotypes grown under short versus long day length (Limin and Fowler, 2006). Short day length allows the plant to maintain its vegetative state and continue to express tolerance to low temperatures. In contrast, a plant experiencing a longer day length triggers a transition to reproductive growth, progressively eliminating the plant's ability to tolerate lower temperatures in the environment. In addition, a genotype that had been bred for adaptation to a cold environment overwhelmingly outperformed one that had not been bred for a cold environment

when both were exposed to colder conditions. This suggests that there are many other factors that can contribute to cold tolerance in wheat.

Several factors determine the actual winter hardiness of winter wheat in the field. As previously mentioned, the existence of the *Vrn*, *Fr*, and *Ppd* genes and their possible linkages and interactions all play a role in the expression of cold tolerance in wheat. Furthermore, environmental interactions and disease pressure (snow mold) can also have negative effects on winter wheat cold hardiness (Limin and Fowler, 2006; Galiba et al., 2009). The large numbers of genes combined with many different interactions with the environment can make the process to achieve cold hardiness difficult. Additionally, there appears to be a negative correlation of yield and maturity with increased cold tolerance (Gororo et al., 2001).

2.2. Fusarium head blight

Fusarium head blight, or scab, is a fungal disease that can affect small grains, including winter wheat (McMullen et al., 1997). The most common fungal pathogen to cause FHB is *Fusarium graminearum*; however other species of the *Fusarium* genus can be culprits of the disease. Severe decreases in yield and kernel quality can result from FHB infection. In 1993, a severe scab epidemic resulted in yield losses of approximately 95 million bushels of wheat in North Dakota (McMullen et al., 1997). Not only can FHB cost growers money in lost production, it can also lead to health hazards to humans and livestock that consume the damaged kernels. FHB infected kernels may contain mycotoxins, which can lead to health risks to consumers (Snijders, 1990).

Winter wheat varieties currently grown in North Dakota are very susceptible to FHB (Jin et al., 2006). There have been lines of spring wheat developed that show resistance to scab; these are mostly derivatives of the Chinese cultivar, Sumai 3 (Rudd et al., 2001). Other sources of

resistance have been identified among different elite germplasm; however, the specific resistance genes have not always been accurately identified. In addition, FHB resistance has been shown to be quantitative and of different types, thus making it difficult to introduce and optimally utilize resistance QTL within a winter wheat population. The different types of FHB resistance are thought to be associated with the different growth stages of the disease. Two initial physiological resistance types (type I and type II) have been postulated to understand and evaluate FHB resistance among wheat plants (Schroeder and Christensen, 1963). Type I and type II resistance are the most widely used sources of resistance in wheat. Type I resistance reduces the initial number of infections to the wheat spike. Type II resistance limits the spread of FHB across the spike from the initial infection point. Type I resistance can be difficult to assess, since its expression can be masked by the presence, or absence, of type II resistance. A wheat plant must contain type II resistance in order to obtain an accurate assessment of type I resistance (Rudd et al., 2001). Recently, other types of FHB resistance (III, IV, and V) were recognized which consist of the plant's ability to fight kernel infection; the level of mycotoxin accumulation; and tolerance as reflected in yield totals (Mesterhazy, 1995). These types of FHB resistance are difficult to evaluate in the early generations of a breeding program due to their generality and are best evaluated in uniform, non-segregating cultivars. Measurement of type III, IV, and V resistance should therefore be postponed to the later generations of a breeding program.

As previously mentioned, the Chinese spring wheat variety Sumai 3 has been identified as an effective source for FHB resistance in wheat. Three significant QTL, *Fhb1*, *Fhb2*, and *Fhb-5A*, have been sourced from Sumai 3 and are available in improved wheat germplasm (Buerstmayr et al., 2003; Waldron et al., 1999; Buerstmayr et al., 2009). The *Fhb1* (formerly designated *Qfhs.ndsu-3BS*) QTL has been extensively reviewed and properly mapped (Bakhsh et

al., 2013; Cuthbert, 2006). *Fhb1* is commonly found as a beacon of type II resistance in spring wheat varieties released in North America, however, it is absent in hard red winter wheat in the same region. Recently, the *Fhb1* resistance gene was evaluated in winter wheat, and proved to be a useful tool in battling FHB in winter wheat populations (Bakhsh et al., 2013). *Fhb2* and *Fhb-5A* (*Qfhs.ifa-5A*) were found to be consistent genes exhibiting resistance to FHB similar to *Fhb1* (Buerstmayr et al., 2009). Although located on different chromosomes, the QTL for FHB resistance in Sumai 3 provide an effective source for type II resistance.

The discovery and development of additional sources for FHB resistance for use in winter wheat breeding is essential. Many other QTL are being researched and identified to help aid in the defense against an FHB epidemic. The employment of multiple genes across different resistant types are essential to developing long-term FHB resistance in wheat. Ultimately, the incorporation of FHB resistance genes into adapted winter wheat germplasm will enhance winter wheat development within regions of high FHB pressure.

2.3. Rust diseases

All rust diseases can become an economic nightmare for all wheat types grown in North Dakota. The use of resistance genes to control rust is highly common in spring wheat. Some winter wheat varieties have shown resistance, however, there is a need for more and broader based resistance (Messmer et al., 2000). Two types of genetic resistance are generally considered in combating these diseases genetically: race specific resistance and race non-specific resistance.

Race-specific resistance, often known as vertical resistance, is due to single qualitative genes that defend against certain races of the pathogen. This source of resistance is generally more likely to be overcome by the evolution of pathogen virulence (Parlevliet, 2002). Race-specific resistance occurs when the product of a major R-gene recognizes the presence of a

pathogen and generates a hypersensitive defense response. When a pathogen evolves into a new virulent race, the major R-gene product will not be able to recognize the pathogen and susceptibility will result. The new race will effectively attack the plant tissue and inflict major damage. Thus, although race-specific genes provide strong resistance, it is generally short-lived and ineffective against the evolutionary changes in the pathogen. The durability of major gene resistance can be improved considerably by simultaneously pyramiding several effective genes in the same genotype.

Race non-specific resistance, or horizontal resistance, involves many quantitative minor genes (Parlevliet, 2002). These quantitative genes have a small but additive effect on the disease and provide only partial resistance. Horizontal resistance is equally effective against all races of the pathogen and is believed to be more durable and less affected by pathogen mutation. Quantitative resistance is more difficult to breed for, due to the difficulty to identify and manipulate the minor genes.

Leaf rust (caused by *Puccinia triticina*) is the most prevalent rust disease across the United States, including North Dakota (Kolmer, 2003). Leaf rust spores are not able to over-winter in North Dakota, but are blown north by wind from the southern wheat producing areas. The disease begins to appear in winter wheat plants in the southern parts of the state in late May, and progresses throughout the region. Although there are few known effective leaf rust resistance genes in local winter wheat varieties, approximately 77 identified resistance genes are available to wheat breeders (USDA-ARS, 2015). Many new major resistance genes were discovered/transferred from wild relatives in recent years (Bolton et al., 2008; Serfling et al., 2011). These genes occur primarily in spring wheat backgrounds and need to be incorporated into winter wheat germplasm. Three non-hypersensitive, durable resistance genes (*Lr34*, *Lr46*,

and *Lr68*) have been described and can be used in winter wheat breeding (Dyck, 1987; Singh et al., 1998; McIntosh et al., 1995; Herrera-Foessel et al., 2012). *Lr34* is a durable resistance gene that was described by Dyck et al. (1966).

Although stem rust (*Puccinia graminis*) is most commonly found on the stem, rust pustules may also be located on the leaves, glumes and awns of wheat plants (Roelfs et al., 1992). Severe infections may cause lodging and reduced yields. Over 50 identified stem rust resistance genes have been discovered and employed in small grains, providing an opportunity to combat this pathogen through wheat host resistance (USDA-ARS, 2008). The resistance gene, *Sr2*, provides partial, non-hypersensitive adult plant resistance to stem rust (but is ineffective until late in plant life). When *Sr2* is combined with other partial resistance genes, a higher level of resistance is achieved (Roelfs, 1988). The *Sr2* gene does not show very high resistance under high disease pressure.

Stripe rust, caused by the fungus *Puccinia striiformis*, is generally not very prevalent in North Dakota, yet it has the potential to become problematic in cool, moist environments (Singh et al., 2000). Most winter wheat varieties grown in North Dakota are susceptible to stripe rust, and under favorable conditions the disease can have a severe impact on production. Some stripe rust resistance genes have been discovered to be linked to leaf or stem rust resistance genes, providing an opportunity to enhance resistance to stripe rust with the pyramiding of other rust resistance genes. An example of this relationship can be found with the confirmed linkage of the *Lr37/Sr38/Yr17* rust resistance genes found on chromosome 2AS in wheat (Bariana and McIntosh, 1993).

New rust races will continue to evolve and elude the resistance mechanisms of current wheat lines. Since the rust resistance genes found in wheat either provide only partial resistance

to the disease or are easily overcome by pathogen evolution, the integration of multiple genes is necessary. Complex and diverse genetic resistance will need to be pursued and maintained in new varieties to provide substantial and stable resistance against the rust pathogen mutations.

2.4. Tan spot and S. nodorum blotch

Tan spot (caused by *Pyrenophora tritici-repentis*) is usually more prevalent in the eastern part of North Dakota, but can be found in western North Dakota under high moisture and humidity conditions. Tan spot has been known to cause up to 50% yield losses in favorable environments throughout wheat growing regions (Wegulo, 2011). Tan spot populations have been found to contain eight races (named race 1 through 8) (Lamari et al., 2003). Races 2, 3, and 5 are considered to be 'base' races, in that each contains a single virulence gene. The remaining five races (races 1, 4, 6, 7, and 8) appear to contain a mixture of the virulence genes present in the base races. Race 1 is regarded as the most prevalent *Ptr* race in North America (Ali and Franc, 2003) and contains a combination of the virulences shown by race 2 and race 3 (Lamari et al., 2003). In wheat, susceptibility to *Ptr* has been explained in terms of a toxin/host interaction model (Lamari and Bernier, 1989). This explains that in order for sensitivity to *Ptr* to exist within the wheat host, the pathogen (depending on race) must contain a virulence gene, and the wheat genotype must contain its assumed receptor gene. Several host-selective toxins (HST) are produced during the development of *Ptr*, which will result in sensitivity to the disease (Chu et al., 2008). Specifically, two *Ptr* produced host-selective toxins (Ptr ToxA and Ptr ToxB) have been isolated and characterized across wheat cultivars. Ptr ToxA has been properly evaluated and its presence during infection was originally regarded to be an appropriate indicator of races 1 and 2 of the *Ptr* fungus (Friesen et al., 2003; Ciuffetti et al., 1998). Eventually, Ptr ToxA was also found to be secreted by the additional races 7 and 8, thus making it a significant factor in

determining *Ptr* susceptibility (Lamari et al., 2003). Sensitivity to Ptr ToxA has been found to be controlled by a dominant gene, *Tsn1,* located on the long arm of chromosome 5B (Faris et al., 1996). Although insensitivity to the Ptr ToxA HST usually signifies a resistant host reaction, it is not always the case (Friesen et al., 2003). Friesen et al. (2002) found that three Ptr ToxA insensitive mutants, were susceptible to two race 1 field isolates (Friesen et al., 2002). They concluded that although sensitivity to Ptr ToxA can be a general indicator of susceptibility, it does not absolutely equate to susceptibility within the wheat host. This data suggested that susceptibility to *Ptr* should be considered a quantitative trait that can involve several additive genes (Faris and Friesen, 2005; Noriel et al., 2011).

Ptr ToxB has also been identified as a prominent HST that can be used in screening for resistance to tan spot in wheat. Ptr ToxB was found to be produced by race 5 isolates of the *Ptr* pathogen (Martinez et al., 2001). *Ptr* toxin sensitivity screening with the use of multiple HSTs can be a useful tool to pyramid tan spot resistance genes in wheat genotypes. Similarly to Ptr ToxA, seedling insensitivity to Ptr ToxB does not definitively equate to a resistant reaction to *Ptr* on the adult wheat plant.

Stagonospora nodorum blotch (sexual stage *Phaeosphaeria nodorum* E. Müll. Hedjar.) (SNB) can be a potentially destructive foliar disease in wheat (Chu et al., 2008). Similarly to tan spot, this leaf spot disease also has the capability of causing up to 50% yield losses in wheat (Riede, et al., 1996). The interaction of *S. nodorum* and its wheat host appears to conform to a gene-for-gene model, similar to the one proposed for the wheat - tan spot interaction (Chu et al., 2008). Liu et al. (2004b) validated previous research that has suggested that resistance to SNB is quantitatively inherited.

The toxin Sn Tox1 has been isolated and Sn Tox1 sensitivity has been associated with susceptibility to the *S. nodorum* isolate Sn2000 (Liu et al., 2004b). A dominant gene, *Snn1*, has been discovered on the short arm of the 1B chromosome and shown to confer sensitivity to Sn Tox1 (Liu et al., 2004a). Additionally, Friesen et al. (2007) showed that certain lines known to be sensitive to Sn ToxA, were still resistant to a Sn ToxA producing *S. nodorum* isolate. This suggested the possibility of SNB resistance QTL that are not associated with toxin sensitivity (Friesen et al., 2007). Ultimately, the utilization of toxin screening for both tan spot and S. nodorum blotch can be an effective tool to pyramid resistance genes into winter wheat germplasm. Multiple sources for resistance need to be explored and utilized to provide a broad base of resistance (Oliver et al., 2008). As with the other wheat diseases, combinations of resistance genes will provide the most economical defense strategies.

2.5. Wheat stem sawfly

The wheat stem sawfly, *Cephus cinctus*, has been a major pest to wheat populations in Northern Great Plains for many years (Beres et al., 2011). The wheat stem sawfly impacts both spring and winter wheat, as well as durum wheat, barley, and winter rye. Generally found in the western part of North Dakota, wheat stem sawfly has had negative impacts on wheat production (Cook et al., 2004). The sawfly can cause up to 20 percent grain yield loss by mining the stem of the wheat plant. The female sawfly will lay eggs inside the stem of young wheat plants (Sherman et al., 2015). After the larva hatches, it begins to migrate toward the base of the plant. Along with yield reduction, the protein content of the grain will decrease as a result of vascular damage to the stem. Furthermore, severe lodging can occur due to the larva cutting a notch inside the stem.

Wheat stem sawfly resistance through solid-stem characteristics provides the best control method (Beres et al., 2011). The solid stem phenotype hinders the development of wheat stem

sawfly larvae, which ultimately reduces the damage to wheat plants through feeding and decreases lodging due to basal stem cutting (Sherman et al., 2015). Solid stemmed plants have been shown to be effective against wheat stem sawfly, but previously had been poor yielding. Conflicting studies have been published showing a negative correlation between solid-stem traits and yield (McNeal et al., 1965) and no correlation with yield (Cook et al., 2004). Additionally, research has been presented that showed that solid stemmed lines out-performed hollow-stem lines under water stressed conditions (Saint Pierre et al., 2010). Ultimately, the development of solid stem winter wheat will be necessary in areas that are infested with the wheat stem sawfly.

2.6. Recurrent mass selection

Recurrent mass selection (RMS) is the process of developing a base population, evaluating and selecting the superior genotypes that exhibit desirable traits, and inter-crossing them for future breeding cycles (Fehr, 1991). The inter-crossing of selected plants allows high rates of recombination and forges new gene combinations, while continuing to maintain genetic diversity within the population (Liu et al., 2007). Repeated cycles of inbreeding, selection, and inter-crossing can rapidly increase the frequencies of genes under selection. The recurrent selection strategy has been employed in wheat to develop populations containing desirable targeted traits, while maintaining genetic diversity and not exhausting non-targeted favorable attributes. Wiersma et al. (2001) found that kernel weight in wheat, a quantitative trait, can be increased through recurrent selection (Wiersma et al., 2001). Also, Jiang et al. (1994) used recurrent selection in an effort to obtain resistance to FHB in wheat populations (Jiang et al., 1994). Jiang et al. (1994) found that while recurrent selection effectively reduced FHB disease infection of both spikelets and seeds in successive cycles, most agronomic characteristics either remained the same or slightly progressed during the same period. Recurrent mass selection has

been established as an effective population improvement strategy to increase the frequencies of favorable quantitative trait alleles within a population. However, the maintenance of genetic diversity is critical to minimizing genetic vulnerability and not limiting the genetic improvement of the developed population (Fehr, 1991).

Development of the base population is important to ensure a successful recurrent selection scheme. Fehr (1991) observes two integral factors in proper population development: parents should demonstrate superior performance for the traits hoping to be improved through recurrent selection; and these parents should originate from different genetic backgrounds to maximize genetic diversity within the population (Fehr, 1991).

Once the base population has been developed, continued hybridization and population improvement can be achieved through two differing approaches: an evolving base population strategy; or by creating a fixed population. The Recurrent Introgressive Population Enrichment (RIPE) system in barley developed by Falk (2001) is a recurrent selection system involving an evolving base population (Falk, 2001). The population is improved over selection cycles with consistent selection pressure, and the introduction of new desirable traits from exotic sources to the population. To avoid the introduction of undesirable traits within the exotic sources, these sources are subjected to pre-breeding cycles involving recurrent backcrossing with elite parents. Along with the introduction of desirable traits to the evolving population, the population can be easily maintained as a smaller population due to less genetic diversity. The other approach to population improvement is by developing a fixed base population. By using a large number of genotypes as parents in the development of the population, the base population can be highly-diverse, and relatively complete (Marais and Botes, 2009). The introduction of new traits to the base population should be limited and based on backcrosses to the recurrent population.

Population improvement will be done by gradually increasing the genetic frequencies of desirable traits over recurrent cycles. This approach leads to the pyramiding of favorable genes within identified genotypes, but requires a more complex collection of parents used in the initial population development, as well as, the maintenance of large hybrid population sizes.

A primary objective of RMS is to increase the frequency of desirable genes in a population to support opportunities to obtain superior genotypes (Marais and Botes, 2009). Recurrent selection can thus aid in gene pyramiding as discussed previously. By continuously raising the frequencies of targeted genes in the base population, many desirable genes will more frequently be located in any particular genotype. However, recurrent selection strategies can often be inconvenient to employ in self-pollinated crops, such as wheat. The use of male sterility can aid in the development of a recurrent selection strategy in self-pollinating crops like winter wheat. The discovery and development of the dominant *Ms3* male-sterility gene (Maan and Williams, 1984) has provided opportunities to facilitate open pollination among wheat plants in a greenhouse environment (Marais et al., 2000). This creates optimal recombination of alleles within the wheat population in a cycle of the recurrent selection scheme. The resulting F_1 will then segregate between male fertile and sterile, allowing the male-fertile (*ms3ms3*) plants to be included into a pedigree breeding program, and the male-sterile (*Ms3ms3*) plants to continue on to the next cycle within the recurrent selection scheme. To allow for the selection of quantitatively inherited traits and increase genetic gain, male fertile plants can be selected in the field up to the F_5 or F_6 before being used as male parents in the RMS scheme. The proper use of this strategy can be an effective tool in the development of a strong pre-breeding program that can continue to develop desirable allelic combinations in wheat genotypes for multiple years.

The use of molecular markers has provided an alternative to basic phenotyping when attempting to measure genetic diversity within a recurrent selection population. Accurate identification of genotypes with targeted traits within a population through phenotyping can be successful; however, these traits are often influenced by the environment (Liu et al. 2007). Molecular markers can be used to accurately identify and select targeted genes within a population and can provide a tool to evaluate genetic diversity within the population. Liu et al. (2007) used simple sequence repeat (SSR) markers to measure genetic variation within a recurrent selection genepool. They found that the genepool had been improved and still maintained an adequate level of genetic variation suitable for recurrent selection. This result was comparable to what Yuan et al. (2004) found in a similar study using random amplification of polymorphic DNA (RAPD) markers in *Brassica napus* (Yuan et al., 2004, Liu et al., 2007). Thus, the use of molecular markers provide an opportunity to identify notable genes within the population, observe the approximate frequency of a specific gene, and recognize the genetic diversity maintained through a recurrent selection scheme.

2.7. Study objectives

This study had the following primary objectives:

a) To initiate the development of a genetically diverse hard red winter wheat base population suitable for recurrent mass selection (RMS) to supplement a newly established traditional pedigree breeding program.
b) To simultaneously establish a dominant gene for male sterility (*Ms3*) in the population so as to facilitate crosses among selected individuals following each selection cycle.
c) To incorporate a broad range of disease resistance, quality, and adaptation genes in the population.

d) To optimize a hybridization procedure that will allow near-random inter-mating of the selected male and female plants.

e) To assess the genetic variability within the population and it's potential for improvement through recurrent selection.

3. MATERIALS AND METHODS

3.1. Establishing a base population

A broad outline of the crossing strategy that was used to establish the base population is provided in Fig. 3.1. A complete list of the wheat varieties and breeding lines involved in the different phases of the strategy is provided in Table 3.1.

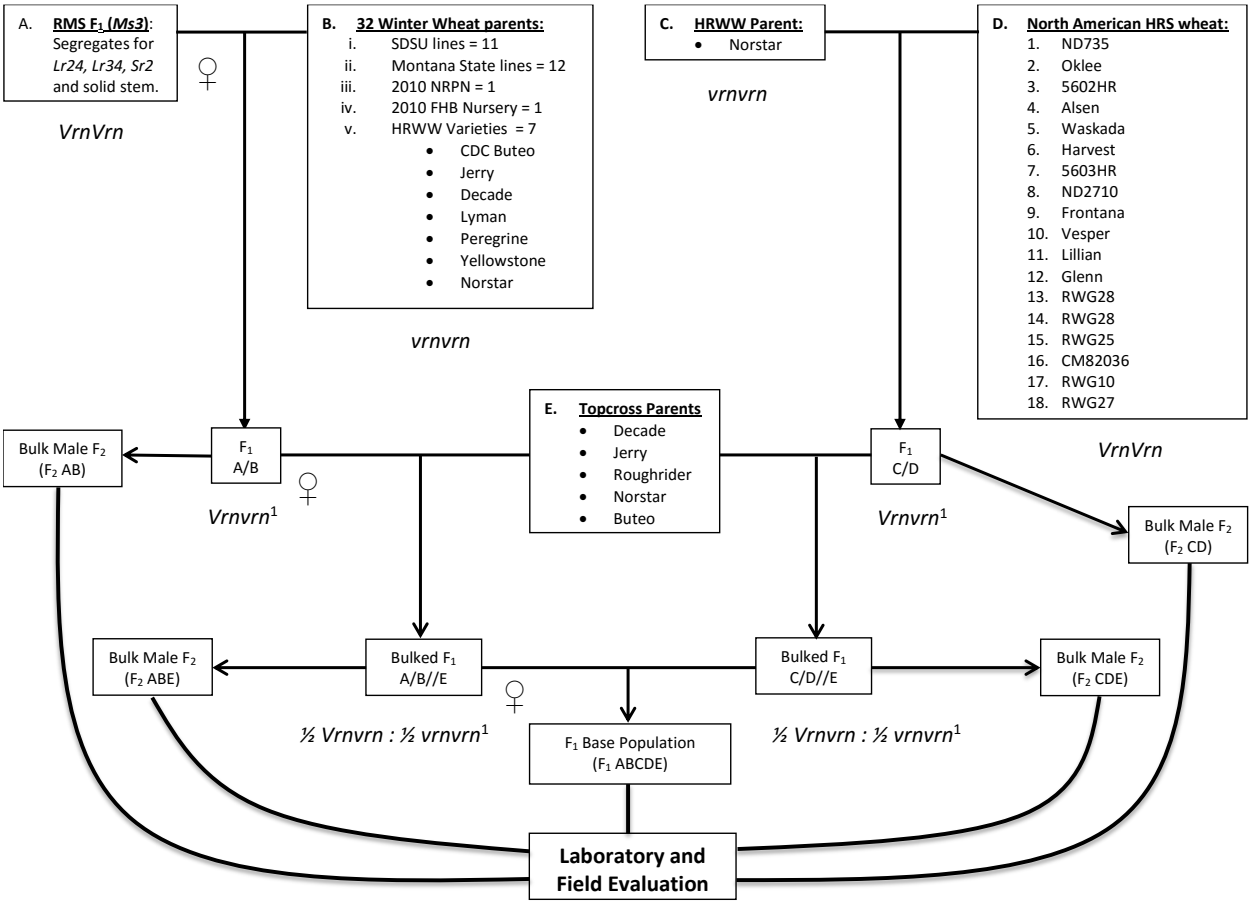

Figure 3.1. Crossing scheme to establish a hard red winter wheat base population for recurrent mass selection, while simultaneously introducing diverse genes for cold-hardiness, adaptation, quality and disease resistance.

[1] Expected segregation for winter habit based on a single dominant *Vrn* gene. Expected winter habit progeny will be lower with the occurrence of multiple dominant *Vrn* genes within the spring genotypes.

Table 3.1. Parental lines/varieties employed in making the complex cross.

No.	Parent Group	Name	Pedigree	Known genes/ traits
1	SDSU	SDSU 1	SD02024/Intrada	
2	SDSU	SDSU 2	AP02T4342/SD97W609	
3	SDSU	SDSU 3	OK00618W/SD98W175-1	
4	SDSU	SDSU 5	N02Y5075/Wendy	
5	SDSU	SDSU 8	Expedition/Sturdy2K	
6	SDSU	SDSU 12	W03-20/SD02W129	
7	SDSU	SDSU 14	Arapahoe/SD97W609	
8	SDSU	SDSU 15	Expedition/Wendy	
9	SDSU	SDSU 17	OK00611W/SD97W609	
10	SDSU	SDSU 19	Falcon/SD01W064	
11	SDSU	SDSU 20	Ransom/Sturdy2K	
12	MSU	MSU 21	Yellowstone//MT03177/W99-194	
13	MSU	MSU 22	Yellowstone//MT9982/SD02W011	
14	MSU	MSU 23	Yellowstone//Jerry/MT0419	
15	MSU	MSU 24	Yellowstone//MT03177/W98-159-7	
16	MSU	MSU 25	MT06126//MT0403/NE99533-5	*Sr24*
17	MSU	MSU 27	MT0403/MT0650	
18	MSU	MSU 30	MT0097/NE01481	*Lr16, Lr24, Lr34, Lr50*
19	MSU	MSU 34	MT06110/MT0664	
20	MSU	MSU 35	MT03176/MT0685	
21	MSU	MSU 38	MT06138/SD01W062-4	
22	MSU	MSU 39	MT02136//MT03177/NE00658	
23	MSU	MSU 40	MT0565//HV9W00-B361R	Unknown rust resistance
24	2010 NRPN	SDSU 45	SD97250/SD99W006//Avalanche	*Lr16, Lr17, Lr24, Sr24*
25	2010 FHB[1]	KSU 51	KS08FHB-31	
26	HRWW	Lyman	KS93U134/Arapahoe	
27	HRWW	Yellowstone	F_2 composite of Promontory/Judith and Judith-dwarf/Promontory	
28	HRWW	Peregrine	McClintock/S86-808	
29	HRWW	Jerry	Roughrider//(ND7571, Winoka/NB66425)/3/ Arapahoe	
30	HRWW	Buteo	Norstar*2/Vona(S-86-808)//Abilene	
31	HRWW	Decade	N-95-L-159/CDC-Clair	
32	HRWW	Norstar	Winalta/Alabaskaya	
33	HRWW	Roughrider	SeuSeun/CI12500//RedChief/Pawnee/3/Cheyenne/4//Hume/5/Yogo/…	
34	HRWW	Accipiter	CDC-Raptor/CDC-Falcon	
35	HRWW	Falcon	Norstar*2/Vona(S-86-808)//Abilene	
36	HRWW	Moats	McClintock/CDC-Falcon	
37	HRWW	Sunrise	McClintock/CDC-Ptarmigan	
38	HRWW	Flourish	RL-4746/Blizzard//CDC-Kestrel/3/CDC-Falcon	
39	HRWW	AC Emerson	CDC-Osprey/McClintock	
40	HRWW	AC Broadview	KS-92-WGRC-15/CDC-Kestrel//CDC-Falcon	
41	HRWW	Ideal	Wesley/NE-93613	
42	HRWW	Expedition	Tomahawk/Bennett	
43	HRWW	Wesley	KS-831936-3/NE-86501	
44	HRWW	Overland	NE-94482/ND-8974	
45	HRWW	Robidoux	NE-96644/(SIB)Wahoo	
46	HRWW	McGill	NE-92458/Ike	
47	HRWW	SY Wolf	W-99-331/97-X-0906-8	
48	HRWW	Art	Jagger/W-94-244-132	
49	HRWW	Boomer	Jerry/CDC-Falcon	
50	HRWW	WB Matlock	CDC-Falcon/Jerry	
51	HRWW	WB-Grainfield	G982231/G982159//KS920709W	
52	N.A. HRSW	ND 735	ND 2907/3/Grandin*3//Ramsey/ND 622/4/ND 2809	*Lr34, Fhb1?*
53	N.A. HRSW	Oklee	Pioneer-2375/SBF-0670	*Lr34*
54	N.A. HRSW	5602HR	AC Barrie/Norpro	*Lr34, Lr16, Sr23*
55	N.A. HRSW	Alsen	ND-674/ND-2710//ND-688	*Lr34, Fhb1*
56	N.A. HRSW	Waskada	BW-278/2*BW-252	*Lr16, Sr23, Fhb2?*
57	N.A. HRSW	Harvest	AC-Domain*2/ND-640	*Lr16, Sr23*
58	N.A. HRSW	5603HR	McKenzie//FHB5227/Lars	*Lr16, Lr21, Sr23, Fhb2?*
59	N.A. HRSW	ND 2710	Sumai-3/Wheaton//Grandin	*Lr34, Fhb1, Fhb2*
60	N.A. HRSW	Frontana	Fronteira/Mentana	*Lr34*
61	N.A. HRSW	Vesper	Augusta/Hard-White-Alpha//3*AC-Barrie/3/BW-150*2//TP/TM/3/2...	*Lr21*
62	N.A. HRSW	Lillian	BW-621*3/90-B-07-AU-2-B	*Lr34*
63	N.A. HRSW	Glenn	ND-2831/Steele-ND	*Lr21*
64	N.A. HRSW	RWG21	Russ 2*/PI277012	
65	N.A. HRSW	RWG28	(BG290/3* Alsen)/(BG282/3* Alsen)	*Lr34, tsn1, snn2, Fhb1*
66	N.A. HRSW	RWG25	Russ 2*/PI277012	
67	N.A. HRSW	CM82036	Sumai3/Thornbird	*Lr34, Fhb1, Fhb-5A*
68	N.A. HRSW	RWG10	BG282/3* Alsen	*Lr34, Fhb1*
69	N.A. HRSW	RWG27	4152-60/3* Alsen	

[1] 2010 Fusarium Head Blight nursery

A spring wheat F_1 population segregating 1:1 for the male sterility gene, *Ms*3, was used as the female parent in the first leg of a complex cross (Fig. 3.1.) to derive a base population. The *Ms*3 segregating population derives from a former spring wheat breeding program at the University of Stellenbosch, South Africa which was based on a recurrent mass selection system (Marais and Botes, 2009). Apart from many un-identified resistance genes, the population segregates for the rust resistance genes *Lr24*/*Sr24*, *Lr*34/*Yr18* and *Sr*2, as well as solid stem. The spring wheat *Ms3ms3* females (approximately 60 individuals) were hand-pollinated with a male population consisting of 32 winter wheat parents (numbered 1 to 32 in Table 3.1.). These winter wheat parents derive from segregating populations donated by South Dakota State University and Montana State University, as well as selected entries from the 2010 Hard Winter Wheat Northern Regional Performance Nursery, the 2010 Fusarium Head Blight nursery, and released commercial varieties (Table 3.1). Approximately 125 crosses were made between parents groups A and B in Fig. 3.1. Each F_1 plant that was produced segregated for male sterility (1:1 ratio).

The latter F_1 (designated AB in Fig. 3.1.) was used in a top-cross with a group of five winter-hardy wheat varieties, i.e. Jerry (NDSU), Buteo (University of Saskatchewan), Decade (NDSU and Montana State University), Norstar (Ag Canada), and Roughrider (NDSU) and are located in Table 3.1. Six sets were planted of each winter wheat variety; a set consisting of five 12-inch pots and five seeds planted per pot. The six sets were planted ten days apart to ensure that adequate pollen was available for an extended period of time. Twenty-four hours after planting, the seeded pots were moved into a cold chamber (4° C) for 56 days of vernalization. After the cold treatment, the plants were returned to the greenhouse.

The first set of un-vernalized spring X winter F_1 AB (1:1; female: male) were planted in cones in unison with the removal of the third set of winter parents from the cold chamber. Since

the spring habit is dominant, the plants did not need to be vernalized. The use of cones allowed individual plants to be easily separated into males and females. The male fertile F_1 population was placed aside and allowed to self-pollinate. The resulting F_2 seed (designated F_2 AB in Fig. 3.1.) was bulked for evaluation. The female plants were pollinated by the five winter wheat parents (Decade, Jerry, Roughrider, Norstar, and Buteo). For this purpose receptive florets on the female plants were cut open to facilitate pollination. Flowering tillers collected from the winter wheat males were arranged randomly (in specially prepared plastic containers) around and raised above the female spikes to effect pollination. The cut ends of the tillers were kept in water while in the containers (Fig. 3.2.), so that they could stay alive for several days, ensuring the continuation of pollination for an extended period of time. Male spikes were cut and placed in the holder at random to effect random pollination of the fertile female spikes. Following pollination, female plants were kept in isolation for the seeds (F_1 ABE; Fig. 3.1.) to ripen.

Figure 3.2. Pollination of male sterile plants by male fertile tillers. The male tillers were cut and placed in water containers above the female plants to ensure effective pollination for several days.

The second leg of the complex cross (Fig. 3.1) involved the crossing of the very winter-hardy wheat variety Norstar with 18 North American HRSW lines (52 to 69 in Table 2.1). The North American HRSW lines contain many different resistance genes for various diseases, as well as genes for superior quality, pre-harvest sprouting resistance, adaptation, and yield as is outlined in Table 3.1.

In order to increase the frequency of genes for cold hardiness, the 18 spring X winter F_1 progenies (F_1 CD in Fig. 3.1.) were crossed with the same five winter wheat parents used in the top-cross of the first leg of this strategy (Decade, Jerry, Norstar, Roughrider, & Buteo). For this second top-cross, hand pollination was used. Two sets of three seeds of each F_1 CD progeny were planted into six-inch pots. The sets of pots were planted ten days apart. The first set was planted at the same time the 3rd set of winter parents were removed from the cold chamber, in order to provide adequate pollen for hand pollination.

The hand-pollinated spikes were harvested separately, but together made up the F_1 CDE population. The remaining tillers/spikes that were not used for crossing, and allowed to self-pollinate were harvested as a bulk population (F_2 CD). The bulk F_2 CD population was used for lab testing and evaluation.

The top-cross F_1 from the two legs of the complex cross were then intercrossed (Fig. 3.1.). Both of the F_1 populations (ABE & CDE) were planted into cones. A similar pollination procedure to that used for first leg top-cross was utilized in this cross. The ABE F_1 segregated 1:1 for male sterility. For this reason, the first leg (ABE) F_1 population was larger than the second leg (CDE) F_1 population. The F_1 ABE population was divided into three plantings of 200 seeds each. Each set was planted ten days apart.

The F_1 CDE population was planted across five plantings to provide adequate pollen across the flowering spectrum of the male-sterile female population. When making the crosses, the F_1 ABE males were removed and only the F_1 ABE female plants pollinated. Each set of male parents (F_1 CDE = 18 X 5 = 90 individual cross combinations) consisted of 90 cones (each cone contained one F_1 seed from a different cross). The first and final plantings of the CDE F_1 population consisted of one set of 90 plants. The 2^{nd}, 3^{rd}, and 4^{th} plantings all consisted of two sets of 90 plants. The 2^{nd}, 3^{rd} and 4^{th} plantings of F_1 CDE were planted in unison with the F_1 ABE plantings. The 1^{st} and 5^{th} F_1 CDE sets were planted ten days before and after the male-sterile population, respectively.

In order to also harvest selfed seed of the male parent F_1 CDE, the male tillers were not cut prior to pollination. Instead, the entire plants, inside the cones, were arranged around and above the male sterile plants (Fig. 3.3.). Once again, the florets on the male sterile plants were cut open to allow pollen to enter and fertilize the spikes.

The F_1 (designated F_1 ABCDE in Fig. 3.1.) resulting from this final cross constituted a genetically diverse base population in which the *Ms*3 male sterility gene segregated. This base population was randomly interbred for one further cycle to disperse the specific target genes contributed by each of the eighteen subsets of F_1 CDE males and to increase the volume of seed. Approximately 15,000 kernels (240 g) of hybrid ABCDE F_1 seed was obtained from the crossing scheme. In addition, the male ABCDE plants were allowed to self-pollinate and were bulk to establish an ABCDE F_2 population for evaluation. This F_2 hybrid population consisted of approximately 50,000 F_2 kernels (2,360 g).

Figure 3.3. Modified open pollination system, consisting of male fertile plants in cones raised above the female (male-sterile) plants.

3.2. Evaluation of the genetic diversity contained within the base population

Tests were done to assess the genetic variability contained within the new base population. In order to do the assessments, one or more of the following were utilized: the parents involved in the composite cross; the various intermediate F_1 populations (or F_2 derived from them); as well as the final base population. The specific material (or combination) that was employed in each analysis differed depending on the stage in the development of the population when the assessment was done, the number of plants that had to be analyzed, as well as the costs involved.

3.2.1. Leaf and stem rust seedling tests

These tests were done using the F_2 populations AB and CD from the complex cross (Fig. 3.1.). Five *Puccinia triticina* (*Pt*) (Table 3.2.) and four *P. graminis tritici* (*Pgt*) pathotypes (Table 3.3.) prevalent in North Dakota were used for screening. Pure cultures of the *Pt* pathotypes were provided by Dr. Maricelis Acevedo (NDSU Dept. of Plant Pathology) whereas the *Pgt* cultures were provided by Dr. Tim Friesen (USDA-ARS, Cereal Crops Research Unit, Fargo, ND). The

virulence/avirulence formulae for the *Puccinia triticina* and *P. graminis tritici* isolates that were used can be found in Tables 3.2. and 3.3., respectively.

Table 3.2. Avirulence and virulence interactions of five *Puccinia triticina* (*Pt*) pathotypes prevalent in North Dakota with known common wheat resistance genes.

Race	Avirulent	Virulent
MFPS	*Lr2a, Lr2c, Lr9, Lr16, Lr11, Lr18*	*Lr1, Lr3a, Lr24, Lr26, Lr3ka, Lr17, Lr30, LrB, Lr10, Lr14a*
TBDJQ	*Lr3ka, Lr9 Lr11, Lr16, Lr18, Lr24, Lr26, Lr30, LrB, Lr41, Lr42*	*Lr1, Lr2a, Lr2c, Lr3, Lr10, Lr17, Lr14a, Lr21, Lr28*
THBL	*Lr3ka, Lr9, Lr10, Lr11, Lr14a, Lr17, Lr18, Lr24, Lr30*	*Lr1, Lr2a, Lr2c, Lr3, Lr16, Lr26, LrB*
TDBG	*Lr3ka, Lr9, Lr11, Lr14a, Lr16, Lr17, Lr18, Lr26, Lr30, LrB*	*Lr1, Lr2a, Lr2c, Lr3, Lr10, Lr24*
MCDL	*Lr2a, Lr2c, Lr3ka, Lr9, Lr10, Lr11, Lr14a, Lr16, Lr18, Lr24, Lr30*	*Lr1, Lr3, Lr17, Lr26, LrB*

Table 3.3. Avirulence/virulence formulae of four *Puccinia graminis tritici* (*Pgt*) pathotypes prevalent in North Dakota with respect to known common wheat resistance genes.

Race	Avirulent	Virulent
QFCQ	*Sr9e, Sr7b, Sr11, Sr6, Sr36, Sr9b, Sr30, Sr10, SrTmp*	*Sr5, Sr21, Sr8a, Sr9g, Sr17(+13), Sr9a, Sr9d*
QTHJ	*Sr9e, Sr7b, Sr36, Sr30, Sr9a, SrTmp*	*Sr5, Sr21, Sr11, Sr6, Sr8a, Sr9g, Sr9b, Sr17(+13), Sr9d, Sr10*
TPMKC	*Sr6, Sr9b, Sr30, Sr9a, Sr24, Sr31, Sr38*	*Sr5, Sr21, Sr9e, Sr7b, Sr11, Sr8a, Sr9g, Sr36, Sr17(+13), Sr9d, Sr10, SrTmp, SrMcN*
TMLK	*Sr6, Sr8a, Sr9b, Sr30, Sr17(+13), Sr9a*	*Sr5, Sr21, Sr9e, Sr7b, Sr11, Sr9g, Sr36, Sr9d, Sr10, SrTmp*

A total of 150 seeds of each of populations F_2 AB and F_2 CD were planted in two sets for each pathotype. In each set, twenty-five seeds were planted in each of three 6" pots. Rust inoculation was done when the plants were 2-week old seedlings, or at approximately the 2.5 leaf stage. To prepare the inoculum, 5-6 oz. of distilled water (room temperature) was mixed with ½ droplet of surfactant (Tween 20). Pathotype spores (or harvested leaves containing sporulating colonies) were mixed with the water to suspend the spores. The inoculum was then sprayed onto

the seedlings. Next, moistened 1 gallon plastic bags were placed over the plants and secured with a rubber band. This provided an environment with high humidity, which aided disease development. The covered seedlings were then placed in indirect light source for 24 hours (room temperature). After 24 hours the bags were removed, and the plants were placed in a controlled environment growth chamber (24^{o}C constant temperature; day/night cycle = 16/8 hours). After 7-14 days, the plants were scored on disease symptoms using the scale developed by Stakman et al., 1962 (McIntosh et. al., 1995). Ratings ranged from 0 to 4, with 0 indicating complete immunity and 4 as severe susceptibility. The scale also contains non-numerical scores including the very resistant ; IT, and intermediate resistant X and Z ITs. The leaves with significant sporulation were harvested and set aside to provide inoculum for the 2nd set of testing.

3.2.2. Sensitivity to tan spot and Stagonospora nodorum blotch

Seedling screening was done to test for tan spot and Stagonospora nodorum blotch insensitivity within the AB and CD F_2 populations (broad seedling inoculation and toxin infiltration). The evaluations were done under guidance of Dr. Tim Friesen (USDA-ARS, Cereal Crops Research Unit, Fargo, ND). Two different procedures were used to discover resistance within the populations; the first being a general application of inoculum across seedlings of each population, and the other involving toxin infiltrations directly in the leaves of individual plants.

The process of inoculating the populations with the causal fungi for tan spot and S. nodorum blotch was the same for both diseases. The tan spot test was done employing approximately 60 seedlings per population, planted individually in small cones. The SNB inoculation was done with approximately 150 plants per population, planted among six 6” pots.

Inoculation was done when the plants were 2 weeks old or when the second leaf had fully emerged. The inoculum for both tan spot (race 1 isolate Pti2) and SNB (isolate Sn2000) was

prepared by the USDA-ARS (Friesen et al., 2002, Liu et al., 2004b, and Oliver et al., 2008). Inoculation was performed by spraying the inoculum mixture equally across the seedlings. The plants were then placed in a humidity chamber for 24 hours following which they were moved to a growth chamber for disease development. The plants were scored approximately seven days later. A qualitative numerical rating scale based on lesion-type (Liu et al. 2004b) was used in scoring the plants. The scale ranged from 0 to 5, with 0 (highly resistant) showing the absence of visible lesions, and 5 (highly susceptible) exhibiting large lesions with very little green tissue remaining.

One-hundred seeds from each of population F_2 AB and F_2 CD were planted into small cones for toxin infiltration tests. Four toxins were used in the infiltrations; Sn-4, Ptr ToxA, Sn Tox1, and Sn Tox3. The toxin was prepared and provided by the USDA-ARS Cereal Research Unit. The infiltrations were performed on the secondary (2+ leaf) leaves, primarily on the 2nd leaf (Chu et al.). Each toxin was infiltrated into the leaf tissue of each plant using a 1mL syringe without needle. After 3 days, the infiltrations were scored based on lesion size and description (Friesen and Faris, 2012).

3.2.3. Solid stem characteristics

The male portion of the F_1 ABCDE population within the crossing scheme was evaluated for solid stem characteristics (produced the bulk male F_2 ABCDE population). This population was planted into cones in a greenhouse, and was used to provide male plants in the crossing scheme. This provided an opportunity for the evaluation of solid stem characteristics. Upon harvest, the main stems of 392 male plants were cut to determine solid-stem features. The stem was cut in the middle of each of the bottom five internodes. The cross sections were rated on a scale of 1 through 5, with 1 being hollow and 5 being completely solid (McNeal et al., 1965;

Sherman et al., 2015). The ratings were then added to give a solid stem score for the plant ranging from 5 to 25.

3.2.4. Molecular marker analyses of specific, useful genes

Leaf tissue of 96 hybrid individuals (F_2 Population ABCDE) were harvested and sent to the USDA Genotyping Center on the campus of North Dakota State University in Fargo, ND for DNA extraction. The DNA extraction protocol can be found at <http://wheat.pw.usda.gov/GenotypingLabs/fargo.html>. After extraction, the DNA was analyzed with a number of molecular markers (STS, SSR, and CAPS markers). The USDA Genotyping Center provided marker data for the resistance genes *Fhb1* and *Lr34*. The csLV34 primer was used to identify the presence or absence of the *Lr34* gene among the 96 F_2 ABCDE genotypes. The UMN10 molecular marker was used to identify *Fhb1* across 96 ABCDE F_2 genotypes. In addition, polymerase chain reactions (PCR) were performed across all 96 hybrids using markers for the genes *Lr21* (Talbert et al., 1994), *Lr19/Sr25* (Yu et al., 2010), *Lr37/Sr38/Yr17* (Helguera et al., 2003), *Sr2* (Mago et al., 2011), *Lr24*/*Sr24* (Mago et al., 2005), and *Sr50* (Mago et al., 2002). Each PCR involved a 20µL total volume consisting of 4µL 5X GoTaq Flexi PCR Buffer (Promega), 1.6µL 25mM $MgCl_2$ (Promega), 2µL 10mM dNTP, 1µL 10Pmol primer (both forward and reverse, mixed), 0.2µL Taq DNA polymerase (Promega), 3µL template DNA, and 8.2µL d.d.H_2O. The mixtures were then amplified using a BIORAD T100TM Thermal Cycler (Life Sciences, Hercules, CA). The amplification settings were dependent upon specific individual marker protocols, and can be found in Table 3.4. The PCR products were then separated by gel electrophoresis in a 3% (w/v) agarose gel, treated with 12µL ethidium bromide.

Table 3.4. PCR markers (STS, SSR, and CAPS) and their appropriate amplification settings.

Gene	Marker(s)	Initial Denaturation	Amplification Cycle	Final extension	Reference
Lr21	D14	95°C, 5 min.	30X 95ºC, 1 min. 50ºC, 1 min. 72ºC, 2 min.	72ºC, 5 min.	Talbert et al., 1994
Lr19/Sr25	Gb	94ºC, 4 min.	30X 94ºC, 45 sec. 60ºC, 45 sec. 72ºC, 1 min.	72ºC, 7 min.	Yu et al., 2010
Lr37/Sr38/Yr17	F: VENTRIUP R: LN2	95ºC, 5 min.	30X 95ºC, 45 sec. 65ºC, 30 sec. 72ºC, 1 min.	72ºC, 7 min.	Helguera et al., 2003
	F: URIC R: LN2	95ºC, 5 min.	38X 95ºC, 45 sec. 64ºC, 30 sec. 72ºC, 1 min.	72ºC, 7 min.	
Sr2	csSr2	95ºC, 5 min.	30X 95ºC, 35 sec. 60ºC, 40 sec. 72ºC, 50 sec.	72ºC, 5 min.	Mago et al., 2011
Lr24/Sr24	*Xbarc71*	95ºC, 5 min.	37X 95ºC, 30 sec. 56ºC, 30 sec. 72ºC, 30 sec.	72ºC, 10 min.	Mago et al., 2005
	Sr24 #12	95ºC, 5 min.	30X[1] 95ºC, 30 sec. 59ºC, 30 sec. 72ºC, 30 sec.	72ºC, 10 min.	
	Sr24 #50	95ºC, 3 min.	35X 95ºC, 30 sec. 63ºC, 30 sec. 72ºC, 40 min.	72ºC, 10 min.	
Sr50	IB-267	95ºC, 3 min.	30X 95ºC, 30 sec. 55ºC, 1 min. 72ºC, 1 min.	25ºC, 1 min.	Mago et al., 2002

[1] Includes a touchdown step before the amplification cycle; starting with 95ºC for 30 sec., then 65ºC for 30 sec., followed by 72ºC for 30 sec. This step was run 7 times, and 1ºC down each cycle.

3.2.5. General genomic diversity: SNP analyses

A total of 113 winter and spring wheat lines were used in a further attempt to assess the genetic diversity within the newly-developed hybrid population. Selected lines for this experiment included the various parents that were used in the complex cross and are listed in Table 3.1. and Fig. 3.1. (12 SDSU experimental lines; 12 MSU experimental lines; one entry from the 2010 Northern Fusarium Head Blight Nursery; 26 released winter wheat varieties; 18 North American hard red spring wheat lines as well as 24 random plants from the South African spring wheat recurrent selection population) plus 25 random F_1 plants from the final ABCDE hybrid population in Fig. 3.1.

Leaf tissue was cut from seedlings of each entry at approximately the two leaf stage. The tissue was then sent to the USDA Genotyping Center located on the NDSU campus in Fargo, North Dakota for DNA extraction and genotyping. DNA was extracted at the USDA-ARS Biosciences Research Lab on the NDSU campus, in Fargo, ND. Their protocol can be found at <http://wheat.pw.usda.gov/GenotypingLabs/fargo.html>. The extracted DNA was then genotyped by employing Illumina's Infinium Assay, using the wheat 9K SNP array (Cavanagh et al., 2013). Using Illumina's Genome Studio software, the genotypes were called and reviewed to reject poor SNP data (Mohamed et al., 2014). Each SNP was reviewed and individual genotypes were properly aligned into three distinct classifications; AA, AB, and BB. If some of the genotypes could not be appropriately placed, they were labeled as a no-call and the affected markers were omitted from the data. Due to the substantial number of SNPs developed, all SNPs showing non-polymorphism and missing calls were excluded from the final analysis. In order to obtain an appropriate number of SNPs to be used for cluster analysis, only 228 SNP markers were selected based on the minor allele frequencies among the data.

A dendrogram (tree diagram) was created using the cluster procedure in the SAS statistical software program (SAS institute Inc., Cary, NC). The SAS code was created by Su Hua and Curt Doetkott, NDSU Statistics Dept., with strong input from Dr. James Hammond, NDSU Plant Sciences. This hierarchical clustering structure was constructed to identify clusters of entries across multiple anonymous loci.

4. RESULTS

4.1. Leaf rust and stem rust seedling tests

The data obtained in the leaf rust and stem rust screening tests are summarized in Tables 4.1. and 4.2., respectively. Both populations (F_2 AB and F_2 CD) showed a wide range of infection types (ITs) across multiple pathotypes. With respect to the leaf rust resistance data, infection types 0 to 2++, and also including ITs Z and X, were considered to reflect strong to intermediate resistance to *Puccinia triticina* pathotypes. The infection types 3 and 4 were regarded as susceptible phenotypes. Susceptibility percentages were calculated for both populations across multiple pathotypes.

In the *P. triticina* screening, the F_2 AB population displayed susceptibility percentages of 77, 71, 31, 58, and 32, across the pathotypes MFPS, TBDJQ, THBL, TDBG, and MCDL, respectively. The F_2 CD population displayed susceptibility percentages of 45, 80, 81, 66, and 59, among the pathotypes MFPS, TBDJQ, THBL, TDBG, and MCDL, respectively. The Z infection type was observed on three plants within the F_2 AB population tested with the MFPS pathotype, and also on two plants within the F_2 CD population tested with the TBDJQ pathotype.

The percentages of plants showing very resistant reactions (ITs 0 to 1-) across the five pathotypes ranged from 2.7 to 13.8%, and 0 to 4.8%, for population AB and population CD, respectively. Percentages for strong resistance reactions (ITs 1 to 1++) ranged slightly more than for very strong reactions. Across the five *Puccinia triticina* pathotypes, strong resistance percentages varied from 2.7 to 15.7%, and 0 to 6.9%, for populations AB and CD, respectively. Intermediate resistance reactions (ITs 2 to 2++; X, Z) were much more frequent for all leaf rust pathotypes screened in this project. Percentages of intermediate reactions ranged from 17.6 to 55.2%, and 17.4 to 44.5%, for the populations AB and CD, respectively.

Table 4.1. A summary of leaf rust infection types (ITs) observed following the inoculation of random samples of F_2 seedlings from hybrid populations AB and CD. The number of seedlings within each of six ranges of infection types is provided for each hybrid population inoculated with one of five *Puccinia triticina* pathotypes.

Range of ITs	***Puccinia triticina* pathotype:**									
	MFPS		**TBDJQ**		**THBL**		**TDBG**		**MCDL**	
	F_2 AB	**F_2 CD**	**F_2 AB**	**F_2 CD**	**F_2 AB**	**F_2 CD**	**F_2 AB**	**F_2 CD**	**F_2 AB**	**F_2 CD**
0 to 1-[1,2]	4	7	7	1	7	0	19	7	7	0
1 to 1++[1,2]	4	8	5	3	13	0	14	10	23	2
2 to 2++[1,2]	23	20	31	23	60	23	20	28	69	59
Z[2]	3	0	0	2	0	0	0	0	0	0
X[2]	0	45	0	0	20	5	5	5	0	0
3 to 4[3]	114	66	107	115	45	119	79	95	47	87
Total	148	146	150	144	145	147	137	145	146	148
Susceptible (%)	77	45	71	80	31	81	58	66	32	59

[1] With or without associated flecking

[2] Resistant phenotypes

[3] Susceptible phenotypes

Table 4.2. A summary of stem rust infection types (ITs) observed following the inoculation of random samples of F_2 seedlings from hybrid populations AB and CD. The number of seedlings within each of four ranges of infection types is provided for each hybrid population inoculated with one of four *Puccinia graminis* pathotypes.

Range of ITs	***Puccinia graminis* pathotype:**							
	QFCQ		**QTHJ**		**TPMKC**		**TMLK**	
	F_2 AB	**F_2 CD**	**F_2 AB**	**F_2 CD**	**F_2 AB**	**F_2 CD**	**F_2 AB**	**F_2 CD**
0 to 1-[1,2]	56	4	22	20	49	8	43	22
1 to 1++[1,2]	0	18	23	12	8	18	19	19
2 to 2++[1,2]	83	80	24	9	13	23	5	10
3 to 4[3]	4	35	4	29	1	24	7	22
Total	143	137	73	70	71	73	74	73
Susceptible (%)	3	26	1	41	0	33	1	30

[1] With or without associated flecking

[2] Resistant phenotypes

[3] Susceptible phenotypes

In the *P. graminis* resistance evaluations, susceptibility percentages for each population were calculated with respect to each stem rust pathotype. Population F_2 AB showed very little susceptibility to all pathotypes, with susceptibility percentages of 3, 1, 0, and 1, for pathotypes QFCQ, QTHJ, TPMKC, and TMLK, respectively. Population F_2 AB showed generally very strong resistance with respectively 56 (39% of the total observed genotypes), 22 (31%), 49 (69%), and 43 (58%) of the genotypes exhibiting an IT of less than (1-). The F_2 CD population showed more susceptibility than the F_2 AB population across the stem rust pathotypes. Susceptibility percentages of 26, 41, 33, and 30 were observed following evaluation with pathotypes QFCQ, QTHJ, TPMKC, and TMLK, respectively. The resistant genotypes observed showed a wide range in ITs across the stem rust pathotypes.

4.2. Tan spot and Stagonospora nodorum blotch

The data observed from the tan spot and Stagonospora nodorum blotch seedling tests are summarized in Table 4.3. The genotypes were determined to be resistant (IT = 0 to 2 for both SNB and tan spot) or susceptible (IT = 2.5 to 5 for SNB or IT = 2.5 to 4 for tan spot). Similar to the rust screenings, a susceptibility percentage was calculated for each population based on the number of susceptible phenotypes observed. SNB inoculation and evaluation showed that a vast majority of the genotypes were susceptible to the pathogen. In populations F_2 AB and F_2 CD, the susceptibility percentages were 88 and 97, respectively. However, resistance was observed in 17 F_2 AB genotypes, including two plants that exhibited very strong resistance to SNB (IT = 1 or less). Additionally, four genotypes were recorded as showing moderate resistance to *S. nodorum* inoculation within the F_2 CD population.

Susceptibility of the genotypes to tan spot also varied between the populations with the F_2 AB and F_2 CD showing susceptibility percentages of 85 and 63, respectively. In comparison to

the SNB evaluation, more genotypes were resistant to tan spot based on a percentage of the total plants from the appropriate population.

Table 4.3. A summary of *Stagonospora nodorum* (Sn2000) and *Pyrenophora tritici-repentis* (race 2, isolate 86-124) infection types (ITs) observed following the inoculation of random samples of F_2 seedlings from hybrid populations AB and CD.

Stagonospora nodorum			***Pyrenophora tritici-repentis***		
Range of	**Population**		**Range of**	**Population**	
ITs	**F_2 AB**	**F_2 CD**	**ITs**	**F_2 AB**	**F_2 CD**
0 to 1[1]	2	0	**0 to 1[1]**	1	4
1.5 to 2[1]	15	4	**1.5 to 2[1]**	8	17
2.5 to 3[2]	39	28	**2.5 to 3[2]**	25	31
3.5 to 4[2]	59	71	**3.5 to 4[2]**	26	5
4.5 to 5[2]	31	42			
Total	146	145		60	57
Susceptible (%)	88	97		85	63

[1] Resistant phenotypes

[2] Susceptible phenotypes

The data resulting from toxin infiltration screening among seedlings from the hybrid populations F_2 AB and F_2 CD are shown in Table 4.4. Toxin infiltration reactions (TIRs) with rating of 0 or 1 were regarded as insensitive to the toxin, whereas ratings of 2 or 3 were deemed sensitive Ptr ToxA is produced by both pathogens. The Ptr ToxA infiltrations revealed the highest percentages of sensitivity for populations F_2 AB and F_2 CD with 63% and 88%, respectively. Population F_2 CD included only 11 genotypes with insensitive reactions, while the F_2 AB population included 34 genotypes with insensitivity to the Ptr ToxA toxin. With respect to toxins produced by *S. nodorum*, the Sn-Tox1 infiltrations showed the smallest percentages of genotypes with sensitivity at 18 and 15 for populations F_2 AB and F_2 CD, respectively. Sn Tox3 produced the largest difference in percentages for sensitivity between the F_2 AB and F_2 CD

populations at 56 and 22, respectively. For the toxin Sn- Tox4, both populations F_2 AB and F_2 CD showed relatively low sensitivity percentages of 27% and 30%, respectively. A majority of the genotypes from both populations (53 per population) scored TIR of 1. Overall, both populations appeared to be the most sensitive to the Ptr ToxA toxin infiltrations, and generally insensitive to the Sn Tox1 infiltrations.

Table 4.4. A summary of toxin infiltration reactions (TIRs) observed following the infiltration of random samples of F_2 seedlings from hybrid populations AB and CD. The number of seedlings within each of the four ranges of toxin infiltration types is provided.

	Toxin:							
Range of	**Ptr ToxA**		**Sn Tox1**		**Sn Tox3**		**Sn Tox4**	
TIRs	**F_2 AB**	**F_2 CD**	**F_2 AB**	**F_2 CD**	**F_2 AB**	**F_2 CD**	**F_2 AB**	**F_2 CD**
0[1]	9	6	12	30	10	30	14	15
1[1]	25	5	54	44	35	39	53	53
2[2]	22	18	14	8	40	15	20	25
3[2]	36	66	0	5	18	4	5	4
Total	92	95	80	87	103	88	92	97
Sensitive (%)	63	88	18	15	56	22	27	30

[1] Insensitive phenotype

[2] Sensitive phenotype

4.3. Solid stem

The data resulting from the evaluation of the solid stem trait among plants derived from the F_1 ABCDE population are indicated in Table 4.5. A vast majority of the genotypes exhibited a solid stem rating of 10 or less. Although no completely solid stem genotypes were observed, 5 plants were identified as having a rating of 10 or better.

Table 4.5. A summary of solid-stem scores of individual plants in the F_1 ABCDE hybrid population.

Score	No. of plants
5	183
6	101
7	60
8	30
9	9
10	4
11	1
12	1
13	1
14	1
15	1
Total	**392**

4.4. SNP comparison

A dendrogram constructed from the analysis of 228 single nucleotide polymorphisms from the experimental lines, varieties, and populations used in this project (Fig. 3.1.) are shown in Fig. 4.1. The primary objective of this evaluation was to study the genetic diversity in the hybrid base population with reference to the diversity within and among the parental populations that were used to derive it. It was expected that the different parental groups (HRSW South Africa; HRSW North America; HRWW Northern Prairies and HRWW Southern Prairies) will show broader inter-group as compared to intra-group variability.

In Fig. 4.1., five distinct clusters (Groups 1-5) are noted. Group 1 consists of mainly winter wheat experimental lines and southern winter wheat varieties. Within group 1, another

sub-group can also be identified which encompasses the Montana State University experimental lines. Group 2 largely consists of the North American spring wheat lines. Group 3 primarily consists of the Canadian released winter wheat varieties, including a small sub-group of nearly exclusive Canadian varieties (Sub-group 2). Group 4 includes a large number of entries from the ABCDE hybrid population. Finally, lines from the South African spring wheat population constitute group 5.

4.5. Molecular markers

Results obtained from the USDA Genotyping Center for identification of molecular markers for the FHB resistance gene *Fhb1*, showed that in the sample of 96 plants no genotypes contained the *Fhb1* locus. In addition, only 1 genotype (ABCDE 50) showed homozygosity of the *Lr34* resistance gene. However, 29 genotypes were heterozygous for *Lr34*. Therefore, the *Lr34* gene is included in the final hybrid population, and occurs in approximately 31% of the plants.

The PCR analysis of leaf and stem rust resistance genes (*Lr21*, *Lr19/Sr25*, *Lr37/Sr38/Yr17*, *Sr2*, *Lr24/Sr24*, and *Sr50*) across 96 F_2 ABCDE genotypes showed varied results. The *Lr21* gene was detected in five of the hybrid genotypes (Fig. 4.2.). *Lr21* is confirmed by an 885-bp band following digestion (Talbert et al., 1994). Four of the hybrid genotypes (ABCDE 45, 65, 80, and 87) showed heterozygosity for *Lr21*, and one genotype (ABCDE 81) showed a homozygous presence of *Lr21*.The observation of a 130-bp band amplified by the dominant marker *Gb* indicates the presence of the *Lr19/Sr25* translocation (Yu et al., 2010). Only three genotypes (ABCDE 24, 53, and 81) displayed a polymorphic band at 130-bp through PCR analysis (Fig. 4.3.).

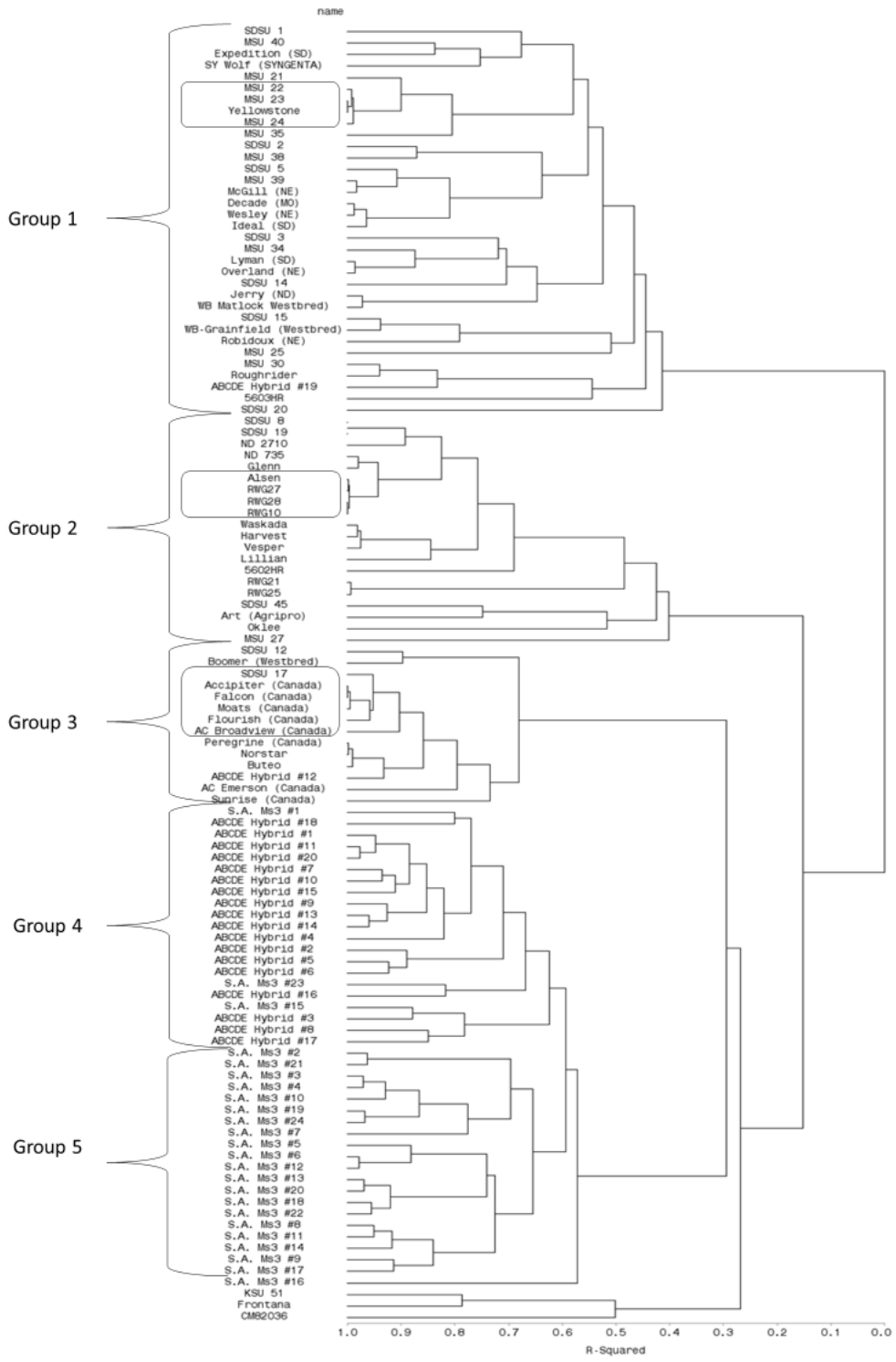

Figure 4.1. A tree diagram based on 228 single nucleotide polymorphisms (SNPs) identified among 113 spring and winter habit genotypes, and analyzed using the cluster procedure from the SAS statistical software. Five groups (1-5) are identified and labeled to indicate suggested clusters within the analysis.

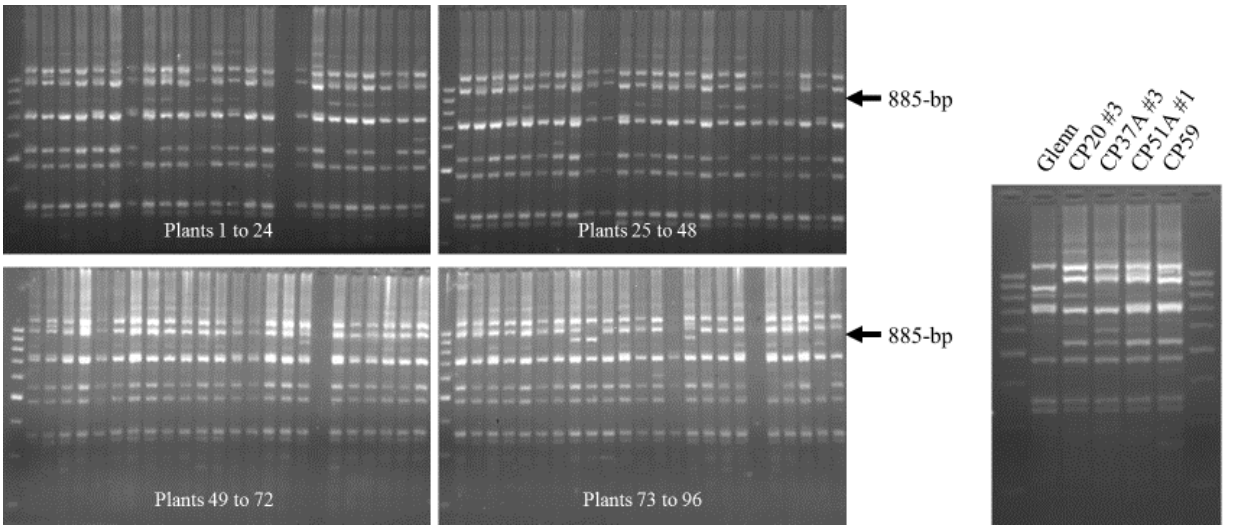

Figure 4.2. PCR amplification of the D14 marker on genotypes ABCDE 1-96 and a control panel containing the experimental line Glenn, which is homozygous for *Lr21*, with separation on 3% agarose gels and visualization with EtBr. The first lane in each picture indicates a 1000-bp ladder, followed by subsequent hybrid ABCDE genotypes. The approximate location of the 885-bp band that is expected in the presence of the *Lr21* gene is indicated with an arrow. Five genotypes (ABCDE 45, 65, 80, 81, and 87) produced the 885-bp band characteristic of *Lr21* in this experiment.

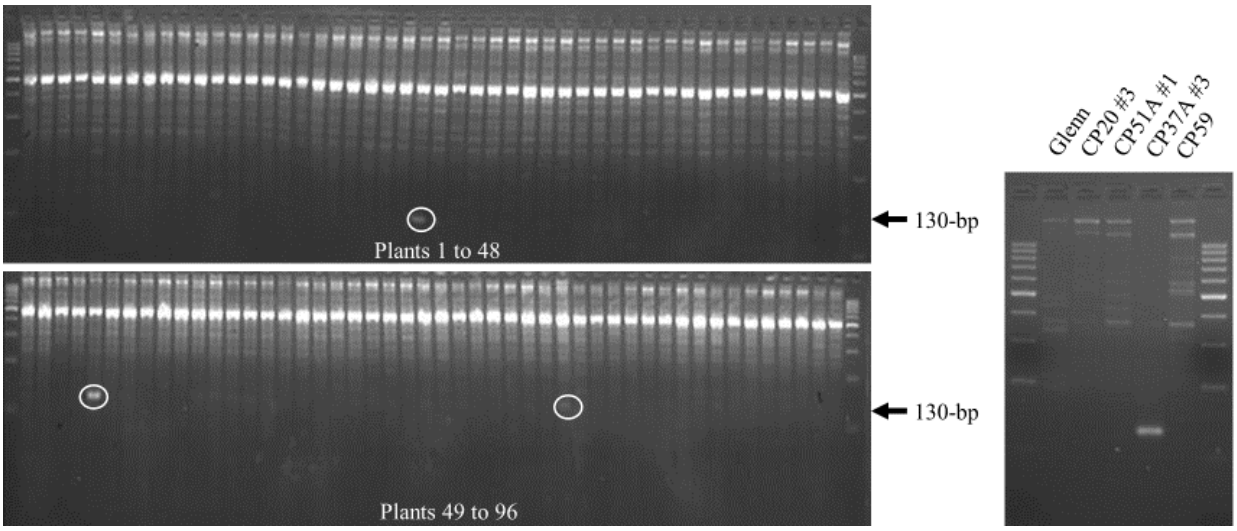

Figure 4.3. PCR amplification of *Gb* marker on genotypes ABCDE 1-96 and a control panel containing the experimental line CP37A #3, which is homozygous for *Lr19*, with separation on 3% agarose gels and visualization with EtBr. The first lane in each picture indicates a 1000-bp ladder, followed by the subsequent 96 ABCDE genotypes. The presence of a 130-bp band indicates the presence of the *Lr19/Sr25* genes. The three genotypes with the *Lr19/Sr25* translocation are circled in the figure.

To detect the presence of *Lr37* within the F_2 ABCDE hybrid population, three primer sets (VENTRIUP, LN2; URIC, LN2) were used to identify two loci (Helguera et al., 2003). The first marker combination (VENTRIUP-LN2) for *Lr37* is a dominant marker for the 2NS segment, and amplifies a 259-bp fragment within the translocation (Fig. 4.4.). Eleven genotypes exhibited a clear band at 259-bp (ABCDE 2, 5, 17, 28, 33, 37, 46, 78, 87, 90, and 92), as well as faint bands for four other genotypes (ABCDE 1, 25, 48, and 56). The second marker, a cleavage amplified polymorphic sequence (CAPS), was used to validate the detection of *Lr37* within the F_2 ABCDE population genotypes (Fig. 4.5.). The presence and absence of the *Lr37* translocation is revealed through the amplification of 285-bp and 275-bp fragments, respectively. Due to similarity in size of the 275-bp fragments generated from the corresponding common wheat and translocated chromosome segments, a restriction enzyme (*Dpn* II) is used to digest the 275-bp fragment (absence of *Lr37*) into two smaller fragments (109-bp and 166-bp). Since the 275-bp fragment derived from the alien segment lacks the restriction site, the intact 285-bp fragment (*Lr37* present) can be observed in the same eleven genotypes (ABCDE 2, 5, 17, 28, 33, 37, 46, 78, 87, 90, and 92) that tested positive for the first dominant marker PCR. In addition, the genotype ABCDE 56 produced a faint band for the first marker (VENTRIUP-LN2) and a 285-bp band for the CAPS marker, suggesting that *Lr37* is indeed present in this genotype. However, the remaining three faint bands (F_2 ABCDE 1, 25, and 48) for the VENTRIUP-LN2 marker did not show a 285-bp fragment for the CAPS marker, and should be deemed to lack *Lr37*. Also, the presence of all three bands (285-bp, 166-bp, and 109-bp) in the CAPS marker PCR, indicates that all of the plants that tested positive for *Lr37* are heterozygotes.

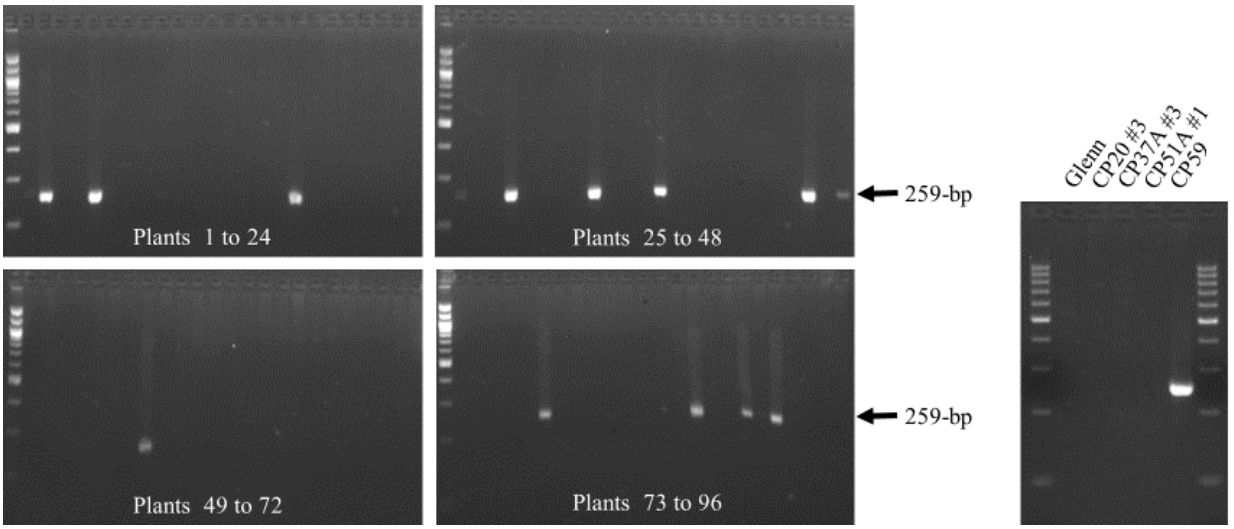

Figure 4.4. PCR amplification of the VENTRIUP-LN2 marker on genotypes ABCDE 1-96 and a control panel containing the experimental line CP59, which is homozygous for *Lr37*, with separation on 3% agarose gels and visualization with EtBr. The first lane in each picture indicates a 1000-bp ladder, followed by the subsequent 96 ABCDE genotypes. The amplification of a 259-bp band signifies the presence of the *Lr37* resistance gene and is identified with arrows in the figure.

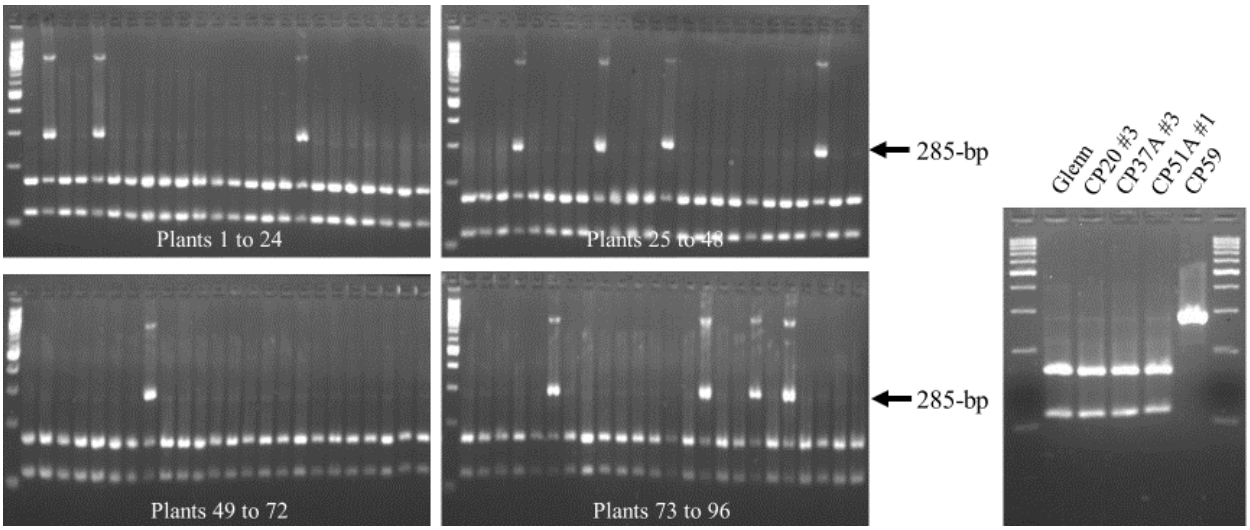

Figure 4.5. PCR amplification of the URIC-LN2 marker on genotypes ABCDE 1-96 and a control panel containing the experimental line CP59, which is homozygous for *Lr37*, with separation on 3% agarose gels and visualization with EtBr. The first lane in each picture indicates a 1000-bp ladder, followed by the subsequent 96 ABCDE genotypes. The amplification of a 285-bp band signifies the presence of the *Lr37* resistance gene and is identified with arrows in the figure. All 12 ABCDE genotypes identified by the URIC-LN2 marker were found with a positive *Lr37* presence using the VENTRIUP-LN2 marker.

The marker results for the stem rust resistance gene *Sr2* can be found in Fig. 4.6. Three different alleles can be detected using the *csSr2* molecular marker (Mago et al., 2011). The complete lack of amplification within the PCR represents a "null allele, and indicates the absence of the *Sr2* gene (identified as III in Fig. 4.6). Seven F_2 ABCDE genotypes displayed a null type allele. A "Marquis" type allele is characterized by the presence of bands at 225-bp and 112-bp, following amplification and digestion by the restriction enzyme (*Bsp*HI) (Identified as I in Fig. 4.6.). The "Marquis" allele is another indication of the absence of *Sr2*, and was fairly common throughout the F_2 ABCDE population. The third polymorphism is a "Hope" type allele, which indicates the presence of *Sr2*, and is characterized by the appearance of three bands at 172-bp, 112-bp, and 53-bp, respectively (Identified as IV in Fig. 4.6.). There were eleven F_2 ABCDE genotypes (ABCDE 7, 11, 20, 25, 31, 42, 51, 54, 61, 71, and 88) that showed the "Hope" allele polymorphic bands. Therefore, *Sr2* occurred among genotypes within the F_2 ABCDE population, albeit at a low frequency. However, there have been reports of potential "false positives" associated with the *csSr2* marker (Mago et al., 2011). Although this marker may not be perfect, it is still relatively more accurate than other *Sr2* markers available in wheat.

PCR analysis of three different molecular markers for *Lr24*/*Sr24* yielded similar results for the 96 F_2 ABCDE individuals. The molecular marker *Xbarc71* was the most difficult to distinguish the presence/absence of the gene (Mago et al., 2005) (Fig. 4.7.). Fortunately, the two other markers can be used to validate and confirm the presence of the *Lr24/Sr24* translocation. The dominant Sr24#12 marker amplifies a single polymorphic band at 500-bp (Fig 4.8.). Similarly to Sr24#12, the Sr24#50 marker amplifies a single band at 200-bp (Fig. 4.9.). Ultimately, 56 (out of 96) genotypes proved to carry *Lr24/Sr24*, indicating a fairly frequent incidence within the F_2 ABCDE population. The genotype ABCDE 64 did test positive for

Lr24/Sr24 with the markers *Xbarc71* and Sr24#12, however the 200-bp band was absent for the Sr24#50 marker, suggesting that the latter amplification failed and that it does in fact carry the translocation.

The results of the PCR analysis for the presence of *Sr50* among F_2 ABCDE genotypes can be found in Fig. 4.10. The IB-267 molecular marker amplifies a band at 210-bp when this rye translocation is present (Mago et al., 2002). Eight genotypes showed a polymorphic band at 210-bp (ABCDE 17, 26, 44, 55, 64, 73, 84, and 89). This confirms the presence of *Sr50* within the F_2 ABCDE population, although at relatively low frequency.

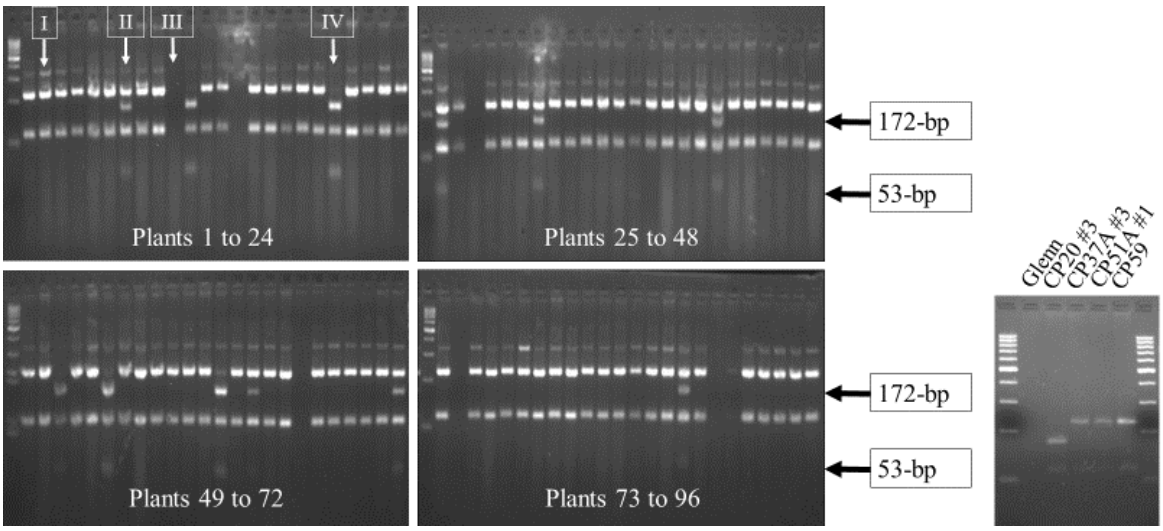

Figure 4.6. PCR amplification of the *csSr2* marker for *Sr2* on genotypes ABCDE 1-96 and a control panel containing the experimental line CP20 #3, which is homozygous for *Sr2*, with separation on 3% agarose gels and visualization with EtBr. The first lane in each picture indicates a 1000-bp ladder, followed by the subsequent 96 ABCDE genotypes. I = "Marquis" type allele (susceptible), II = Both "Marquis" and "Hope" type alleles present (heterozygous *Sr2*), III = null allele (susceptible), and IV = "Hope" type allele (homozygous *Sr2*).

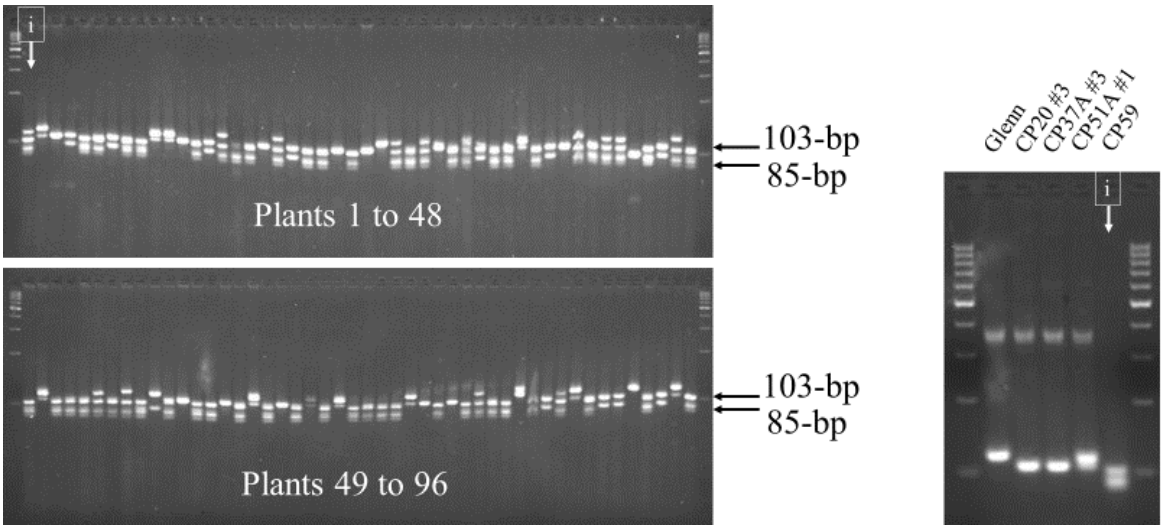

Figure 4.7. PCR amplification of the X*barc71* marker for *Lr24*/*Sr24* on genotypes ABCDE 1-96 and a control panel containing the experimental line CP59, which is homozygous for *Lr24*/*Sr24*,with separation on 3% agarose gels and visualization with EtBr. The first lane in each picture indicates a 1000-bp ladder, followed by the subsequent 96 ABCDE genotypes. The presence of 103-bp and 85-bp bands (pattern "i") is associated with *Lr24*/*Sr2*4.

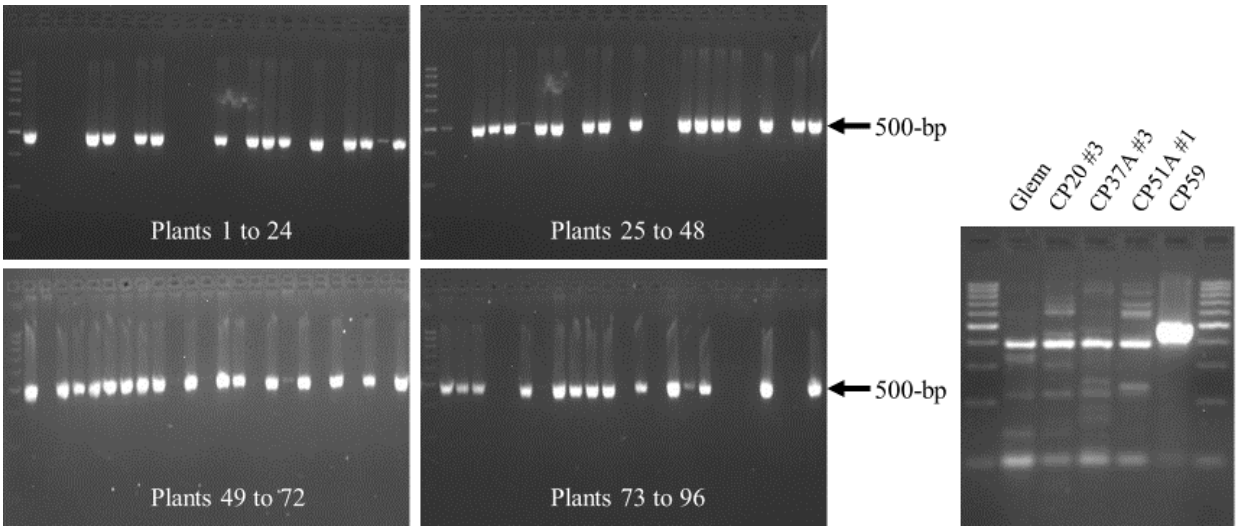

Figure 4.8. PCR amplification of the Sr24#12 marker for *Lr24*/*Sr24* on genotypes ABCDE 1-96 and a control panel containing the experimental line CP59, which is homozygous for *Lr24*/*Sr24*, with separation on 3% agarose gels and visualization with EtBr. The first lane in each picture indicates a 1000-bp ladder, followed by the subsequent 96 ABCDE genotypes. The presence of a 500-bp band signifies the presence of the *Lr24/Sr24* genes.

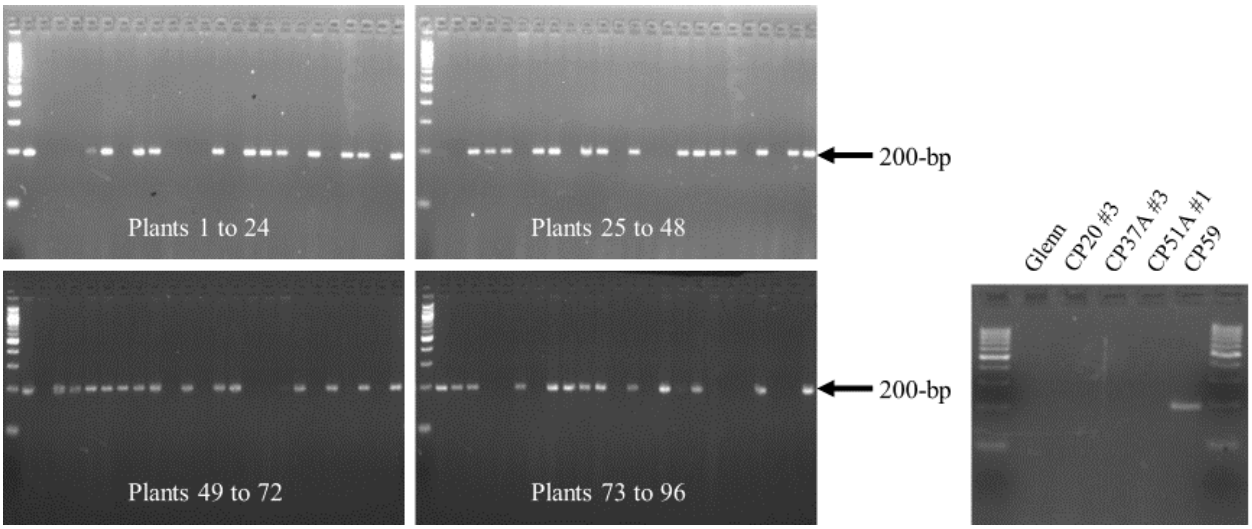

Figure 4.9. PCR amplification of the Sr24#50 marker for *Lr24/Sr24* on genotypes ABCDE 1-96 and a control panel containing the experimental line CP59, which is homozygous for *Lr24*/*Sr24*, with separation on 3% agarose gels and visualization with EtBr. The first lane in each picture indicates a 1000-bp ladder, followed by the subsequent 96 ABCDE genotypes. The presence of a 200-bp band signifies the presence of the *Lr24/Sr24* genes.

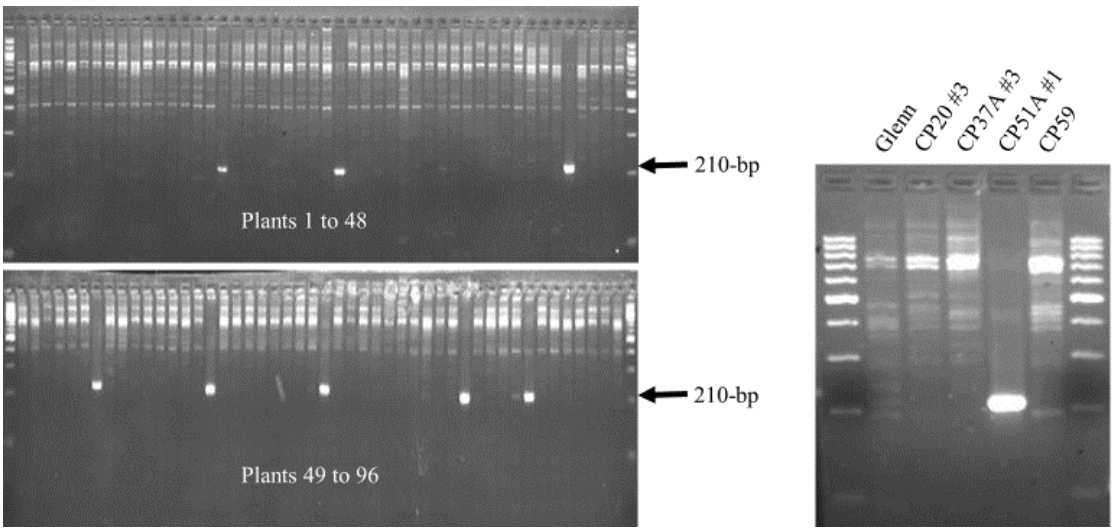

Figure 4.10. PCR amplification of the IB-267 marker for *Sr50* on genotypes ABCDE 1-96 and a control panel containing the experimental line CP51A #1, which is homozygous for *Sr50*, with separation on 3% agarose gels and visualization with EtBr. The first lane in each picture indicates a 1000-bp ladder, followed by the subsequent 96 ABCDE genotypes. The presence of a 210-bp band signifies the presence of the *Sr50* gene.

There were three F_2 ABCDE genotypes (ABCDE 17, 81, and 87) that showed the presence of three separate translocations. Additionally, there were 15 genotypes that showed the presence of two resistance sources by molecular marker analysis. Each of these had *Lr24/Sr24*, plus one of the other remaining sources (*Lr19/Sr25*, *Lr37/Sr38/Yr17*, *Sr2*, or *Sr50*). A summary of the F_2 ABCDE genotypes and their corresponding rust resistance gene combinations can be found in Table 4.6.

Table 4.6. F_2 ABCDE genotypes and the presence (+) or absence (-) of the indicated leaf and/or stem rust resistance genes.

	Resistance Gene					
Genotype	*Lr37/Sr38/Yr17*	*Lr19/Sr25*	*Sr2*	*Lr24/Sr24*	*Sr50*	*Lr21*
ABCDE 5	+	-	-	+	-	-
ABCDE 17*	+	-	-	+	+	-
ABCDE 24	-	+	-	+	-	-
ABCDE 28	+	-	-	+	-	-
ABCDE 31	-	-	+	+	-	-
ABCDE 42	-	-	+	+	-	-
ABCDE 51	-	-	+	+	-	-
ABCDE 53	-	+	-	+	-	-
ABCDE 54	-	-	+	+	-	-
ABCDE 55	-	-	-	+	+	-
ABCDE 56	+	-	-	+	-	-
ABCDE 61	-	-	+	+	-	-
ABCDE 72	-	-	+	+	-	-
ABCDE 73	-	-	-	+	+	-
ABCDE 78	+	-	-	+	-	-
ABCDE 81*	-	+	-	+	-	+
ABCDE 87*	+	-	-	+	-	+
ABCDE 89	-	-	-	+	+	-
Total (% of plants)	6 (6.3)	3 (3.1)	6 (6.3)	18 (18.8)	4 (4.2)	2(2.1)

* Three genotypes exhibiting three of the resistance genes.

5. DISCUSSION

The primary objective of this project was to develop a highly diverse hard red winter wheat pre-breeding population to be used as a source of future germplasm, complementing a main pedigree breeding program. In addition, subsequent objectives were described to generate the population by incorporating the male-sterility gene, *Ms3*, thus facilitating near-random inter-mating and improved hybridization between the selected male and female plants. The remaining study objectives were detailed to assess the variability contained within the final ABCDE hybrid population and to decide whether continued introgression of specific genes might be necessary.

5.1 Development of a base population suitable for RMS using *Ms3*

A general drawback to the application of recurrent mass selection in wheat is the necessity of maintaining large populations to maintain a high amount of diversity. Hand emasculation is laborious and impractical and not only limits population size but also restricts the ability to randomly intercross selected genotypes in order to derive the next generation. The introduction of dominant genetic male sterility (*Ms3* in this study) has provided an opportunity to successfully utilize the recurrent mass selection system in wheat. Since male-sterile plants can be identified easily, emasculation is not necessary. Additionally, there is no need to collect pollen from male-fertile plants. Instead male fertile plants can be placed slightly above the female plants and allowed to cross pollinate naturally (slight wind agitation). The only labor-intensive step of this system is to cut open each floret for male-sterile plants and the rotation/replacement of male-fertile plants around the females. The use of cones allowed for plants to be easily separated and distributed during pollination which promoted randomization and open pollination, while maintaining an organized system. Compared to the difficulty and time-consuming nature of employing individual crosses in wheat, this task is relatively minor and undemanding.

Therefore, labor and time spent to develop and improve the hybrid population is greatly reduced. Another benefit of this inter-crossing system was the randomization of pollen throughout the pollination process. As a result the final hybrid population contains numerous desirable genes inherited from the multiple male and female parental genotypes.

5.2. Evaluation of the genetic variability within the hybrid population

5.2.1. Cold-hardiness and adaptation

One feature of the complex crossing system was to combine desirable genetic characteristics from both spring and winter wheat backgrounds, while maintaining an acceptable level of winter hardiness. The first crosses in both legs of the complex scheme involved a spring wheat by noteworthy winter hardy winter wheat (32 winter wheat parents for the first leg, and Norstar in the second leg). Additionally, a top-cross using winter-hardy, locally adapted winter wheat (Buteo, Jerry, Norstar, Decade, and Roughrider) was used to provide further winter hardiness within the hybrid genetic background. Therefore, the final F_1 hybrid population is expected to represent approximately 75 percent winter habit and 25 percent spring habit backgrounds. The 75 percent winter habit can be attributed to 50 percent due to the winter-hardy top-cross parents (Population E in Fig. 1.), 12.5 percent from the (less cold-hardy) winter wheat parents used in the first leg of the scheme (designated population B in Fig. 1.), and finally 12.5 percent from the very winter hardy variety Norstar in the second leg of the crossing system (Population C in Fig. 1.). The 25 percent spring habit parentage can be credited to the South African RMS population (Population A in Fig. 1.) and the North American adapted spring wheat lines (Population D in Fig. 1.). By using very winter hardy parents in each leg of the crossing scheme, intermediate variability for winter hardiness can be maintained. As a result, the

introgression of desirable genes from spring habit genotypes into regionally adapted, cold hardy progeny had been buffered.

5.2.2. Rust resistance

With respect to the leaf rust seedling resistance results of Table 4.1. the following general observations can be made:

1. Strong (1-1^{++}) to very strong (0-1^{-}) ITs: For population AB, the frequencies of such reactions ranged from 5% (MFPS) to 24% (TDBG). For population CD, the corresponding range was 0% (THBL) to 12% (TDBG). Some of the very strong reactions could reflect the presence of *Lr19* which is resistant to all North American leaf rust races (McCallum and Seto-Goh, 2008; Ordonez and Kolmer, 2008; Mohamed et al., 2014) and typically produce a 0; infection type. This gene occurs in about 3% of the seedlings of the final hybrid, ABCDE. The absence of very strong infection types in population CD following infection with races TBDJQ, THBL and MCDL would suggest that *Lr19* did not occur in parental groups C, D and E. It was most likely contributed by parental groups A and B. *Lr24* is another gene that produces a very strong (0;) infection type and probably contributed strongly to the observed resistance. Molecular markers showed that *Lr24* occurred in 18% of the final hybrid seedlings, which is in accord with the relatively wide use in the past of the *Lr24/Sr24* translocation (Olson et al., 2010) in many wheat breeding programs. *Lr24* is ineffective against races MFPS and TDBG (Table 3.2.); however, a fairly high proportion of seedlings tested with the two races showed strong resistance, suggesting the presence of additional, and unknown major hypersensitive response gene(s) with strong effect. Resistance gene *Lr21* typically also produces a

strong infection type (0;, 12^{-}), and it was detected (marker) in five genotypes of the final ABCDE hybrid population and may therefore occur at very low frequency.

2. Intermediate ($2\text{-}2^{++}$) ITs: In both populations AB and CD these infection types were regularly seen (Table 4.1.). With respect to population AB, 15% (TDBG) to 47% (MCDL), and with respect to population CD, 14% (MFPS) to 41% (MCDL) of the seedlings exhibited intermediate infection types. The wide variation across pathotypes in the percentage of seedlings with intermediate ITs would suggest that more than a single gene is at stake (with only one gene involved, it can be expected that for a virulent race, all seedlings will be susceptible, rather than just a proportion). It is not known which major genes are involved although it could include *Lr16*.
3. Mesothetic infection types: The percentages of mesothetic ITs varied widely (0% to 31%) across races (Table 4.1). *Lr37* is an adult plant resistance gene (McIntosh et al., 1995) and generally gives a X^{+}-3N seedling reaction (Kolmer, 2014), thus it is possible that seedlings with *Lr37* were sometimes included with the susceptible classes (ITs ≥3). Marker analyses confirmed that the *Lr37/Sr38/Yr17* translocation occurs in approximately 6% of the final ABCDE population plants.

The seedling infection type data were therefore indicative of the presence of multiple leaf rust resistance genes, relatively few of which could be identified/ postulated. In addition to these it is likely that further previously defeated major genes that continue to provide resistance to some races only, also segregate in the population. Combinations of such defeated genes may be able to complement to provide broader resistance than is afforded by the individual genes, and thus, if pyramided could significantly aid attempts to breed for resistance. The adult plant rust resistance gene *Lr34* was identified within the hybrid population by the use of a molecular

marker. Although only one genotype was identified to be a resistant homozygote, twenty-nine showed the existence of both resistant and susceptible alleles. The relatively low frequency of *Lr34* alleles (= .16) was expected, since the resistance gene can be traced back to spring wheat genotypes used in the first crosses, which ultimately only consist of less than 25 percent of the hybrid genetic background. Due to the high level of heterozygosity in the F_1 ABCDE plants that result from the near-random intercrosses, it is to be expected that segregation of a gene which occur at such a low frequency will result in a preponderance of heterozygotes in the progeny. The detection of *Lr34* within the hybrid population provides another leaf rust resistance gene to supplement and enhance gene pyramiding in developing resistant genotypes.

In addition to the leaf rust resistance genes already within the hybrid genepool, several other resistance genes could be included to boost the gene pyramiding effort of this research. The adult plant resistance genes *Lr46* and *Lr68* have proven to be effective when coupled with other slow rusting resistance genes, such as *Lr34* (Singh et al., 1998; Herrera-Foessel et al., 2012). Furthermore, hypersensitive adult plant resistance genes, *Lr48* and *Lr49*, have been found to be useful in leaf rust resistance gene combinations (Saini et al., 2002).Unfortunately, perfect or near–perfect markers have not been found for the detection of the latter genes (due to the high level of heterogeneity within the base population, imperfect flanking markers are of limited use). Other hypersensitive response (HR) seedling resistance genes such as *Lr50*, *Lr51*, *Lr53*, *Lr56*, *Lr59*, and *Lr62*, also provide an opportunity to enrich the leaf rust resistance available within hybrid base population (Brown-Guedira et al., 2003; Helguera et al., 2005; Marais et al., 2005; Marais et al., 2006; Marais et al., 2008; Marais et al., 2009).

Seedling stem rust resistance tests done with populations AB and CD (Table 4.2.) showed higher frequencies of resistance as compared to the leaf rust seedling data (97-99% for population AB and 59-74% for population CD).

1. Strong (1-1^{++}) to very strong (0-1^{-}) ITs: Across the four races, percentages of 39% (QFCQ) to 84% (TMLK) of the population AB seedlings fell in this category, whereas for population CD the corresponding range was 16% (QFCQ) to 56% (TMLK). Resistance genes *Sr38* (IT = ;1) and *Sr50* (IT = 1) could have contributed to the observed resistance in both populations and were found to occur at frequencies of 6% and 4%, respectively, in the final ABCDE hybrid population. It is therefore very likely that additional HR genes with strong expression were present in the two hybrid populations.
2. Intermediate (2-2^{++}) ITs: Across races, the percentages of resistant seedlings in this category ranged from 7% (TMLK) to 58% (QFCQ) for population AB and 13% to 58% (QFCQ) for population CD. Resistance genes *Sr24* and *Sr25* produce IT = 2 disease phenotypes and likely contributed to the variation seen in this category. *Sr24* was present in 18% of the final hybrid ABCDE seedlings whereas *Sr25* was present in 3%. Race TPMKC is virulent on *Sr24*. Approximately 18% (population AB) and 32% (population CD) of seedlings infected with TPMKC produced intermediate infection types. Since the *Lr19/Sr25* translocation was most likely contributed by the population B cross parents, the results suggest that additional, unknown resistance genes probably contribute to the resistance in this category.

The adult plant resistance gene, *Sr2*, was detected in about 6% of the ABCDE population. This gene does not produce a recognizable seedling response reaction and therefore did not contribute to the interactions of Table 4.2. While *Sr2* gives insufficient resistance when used

singly, it is very useful when pyramided with HR genes due to its durability and ubiquitous resistance.

The UG99 race has proven to be devastating in Africa and the Middle East, and has the potential to impact global wheat production. In an effort to prepare for the capability of UG99's fast spread over the world, many wheat breeding programs are incorporating resistance genes known to be effective against UG99. Resistance genes *Sr2, Sr25,* and *Sr50* are effective against UG99 and its variants (Singh et al., 2015). However, both *Sr25* and *Sr50* have been shown to be susceptible to other stem rust races. An evolved race from UG99 has shown virulence on *Sr24*, and therefore it is no longer considered effective to fight UG99. Although the UG99 race has not reached North America, it is important that breeding efforts are made to develop host resistance to this devastating disease.

Other useful stem rust resistance genes have been discovered and may provide additional resources to defend against the UG99 race and its variants. The resistance gene *Sr22* was found to show effectiveness against all UG99 race variants (Jin et al., 2007). In addition, resistance genes *Sr26* and *Sr35* have proven to potentially be the most effective race-specific resistance genes when used in gene pyramiding backgrounds (Singh et al., 2015). Other broadly UG99 effective stem rust resistance genes, such as *Sr39* and *Sr47*, have also proved to be useful in wheat breeding programs (Singh et al., 2015; Klindworth et al., 2012). However, many of these alternative resistance genes have not been used in breeding programs due to possible negative agronomic effects (Olson et al., 2010). Recent research, aimed at removing detrimental linkage drag, is allowing these resistance genes originating from alien species to be more acceptable in wheat breeding programs (Ellis et al., 2014; Niu et al., 2011; Mago et al., 2013). This will provide more sources of stem rust resistance while maintaining good agronomic genotypes. In

regard to the developed hybrid population in this study, these new stem rust resistance genes could possibly be incorporated into the population. Introgression of new stem rust resistance genes would provide a higher incidence of general genetic resistance, while providing a more complex gene pyramiding network to combat stem rust.

5.2.3. Leaf spot resistance

The tan spot and *S. nodorum* seedling evaluations revealed much lower frequencies of resistant phenotypes than was the case with the rust screenings.

1. Following inoculation with *Stagonospora nodorum* isolate 86-124 only 12% of population AB and only 3% of population CD individuals were resistant. Testing with tan spot race 2 showed that somewhat more - approximately 15% of the population AB individuals and 37% of the population CD individuals, were resistant. Both the SNB and tan spot races used in the screenings were selected to reflect the predominant isolates of the diseases in North Dakota.
2. When tested with host selective toxins, the percentage of population AB individuals showing toxin insensitivity ranged from 37% (Ptr ToxA) to 82% (Sn Tox1). For population CD the corresponding range was from 12% (Ptr ToxA) to 85% (Sn Tox1). Ptr ToxA strongly enhances the ability of both pathogens to colonize their wheat host and is widely prevalent in pathogen populations across the Great Plains region in the United States (Friesen et al., 2003). Insensitivity to ToxA occurs at relatively low yet useful levels in the two populations. Insensitivity to the three SNB host selective toxins on the other hand is present at fairly high levels in both populations.

Few individuals showed strong resistance to these leaf spot pathogens, reflecting a lower frequency and diversity of resistance genes in the intermediate populations. However, it is

important to note that insensitivity is a recessive trait and that the frequency of insensitive F_2 individuals that were detected equals the occurrence of recessive homozygotes. Due to the heterogeneous nature of the AB and CD populations, many of the sensitive individuals may have been heterozygotes, and thus, the actual frequencies of the insensitivity alleles may be at intermediate levels.

Tan spot and *S. nodorum* resistance genes and mechanisms are not as well characterized as is the situation with the cereal rusts. Major QTL such as *tsn1*, *tsn2*, *snn1*, and *snn2*, and their associated molecular markers were discovered fairly recently (Faris and Friesen, 2005; Singh et al., 2008; Liu et al., 2004a; Friesen et al., 2007) and now allows for more rigorous selection and resistance breeding than previously possible. In addition, resistance and toxin insensitivity to the leaf spot diseases have complex inheritance and is affected by multiple QTL that makes it difficult to improve through conventional phenotyping and breeding (Singh et al., 2010). At the onset of the study, very little was known regarding parental lines/ varieties with utilizable levels of diverse resistance. Also, the current winter and spring wheat varieties in North Dakota have generally low levels of resistance (Liu et al., 2015). The current genetic variability for resistance/ toxin insensitivity that was detected within the base population should support significant initial selection progress. However, as additional sources of useful resistance are obtained in future, it will be necessary to continue to diversify and enrich the base population so as to sustain longer term genetic progress.

5.2.4. Solid stem characteristics

The results from solid-stem scoring in this study indicated low levels of solid stem traits within the hybrid population. This can be explained by the low incidence of the solid stem trait within the winter and spring wheats used to create the complex cross (Fig. 3.1.). The primary

sources of solid stem plants are the South African HRSW population (Parent group A) and some of the lines obtained from the Montana State University program included in the parent group B. Earlier wheat breeding attempts determined that hollow-stemmed cultivars were generally better performing than their solid-stemmed counterparts (Sherman et al., 2015). Due to the inferiority of the solid stem genotypes, the solid-stemmed trait was often disregarded and not employed in wheat backgrounds in the United States. With the rise of the wheat stem sawfly, a need for solid-stemmed genotypes has become fundamental in wheat breeding programs. Cook et al. (2004) discovered a single major QTL, *Qss.msub-3BL* that was shown to produce solid-stemmed genotypes while maintaining a superior agronomic performance comparable to hollow-stemmed cultivars. Although this identified QTL provides a new avenue for solid stem development, it may not be successful by itself. Other unidentified major and minor genes could help strengthen the solid stem background in wheat genotypes. Furthermore, the environment has proved to be a great limiting factor in solid-stem traits (Weiss and Morrill, 1992). Although the solid stem trait appears to exist in a very low frequency with the hybrid population, the identification of a few intermediately solid stemmed genotypes represents an opportunity for improvement. Careful selection of solid-stemmed wheat genotypes for future recurrent selection cycles can help improve the solid stem incidence within the population and provide valuable lines exhibiting desirable stem solidness.

5.2.5. Fusarium head blight

The major Fusarium head blight resistance gene *Fhb1* was not identified by molecular markers in 96 ABCDE population genotypes. Although it was surprising to not identify a hybrid genotype with *Fhb1*, it is possible that it simply occurs at a very low frequency in the hybrid cross. From Table 3.1. it can be seen that only six of 18 parents, all from the North American

spring wheat parental population (Parent group D. in Fig. 3.1.), contained the *Fhb1* resistance gene. Considering that the North American spring wheat germplasm comprised only 12.5% of the final hybrid population, only 4% of the genetic background of the final hybrid derives from parents with *Fhb1*. Although *Fhb1* was not found in this study's marker analysis, it should not be assumed that it is completely absent from the final population genepool. Other FHB resistance genes, *Fhb2* and *Fhb-5A,* as well as unknown native resistance from spring wheat parents that could also contribute to FHB genetic resistance within the final population, may similarly occur at very low frequencies. At the onset of the project, no winter wheat germplasm/varieties with significant FHB resistance were available for their inclusion as parents. However, in the past five years several resistance QTL have been introduced through backcrosses from spring wheat donors and are now available for introgression into the hybrid population. The recent discovery of other FHB resistance genes, such as *Fhb3* and *Fhb6,* provide another opportunity to enhance FHB genetic resistance within winter wheat germplasm (Qi et al., 2008; Cainong et al., 2015). Due to the low frequency of known FHB resistance within the final hybrid population of this study, future introgression of FHB resistance genes will be a vital key to improvement of this RMS population.

5.3. Genetic diversity within and among the parental and final hybrid populations

A breeding program normally targets a specific class of wheat, delineates a potential production region and is directed at the development of varieties with similar phenotype and adaptation traits. For example, a HRWW breeding program for North Dakota will foremost focus on elevated cold-hardiness, a specific rate of development to reach the reproductive stage, a particular plant height range, resistance/ tolerance to a subset of prevalent biotic and abiotic stresses, etc. These shared 'ideotype' features make it more difficult to evaluate 'non-ideotype'

related genetic variability. While pedigree information can reveal relationships among commercial varieties, breeding parents and advanced generation breeding lines, it is less informative regarding the effect on overall genetic variability of the recurrent use of breeding material over numerous cycles of conventional breeding. The purpose of this part of the study was to sample a large number of random, anonymous SNP loci and use this information as an indicator of overall genetic variability within the contributing parental populations. Only the SNPs showing the highest amount of polymorphism between lines were therefore employed for the cluster analysis.

The dendrogram (Fig. 4.1.) indicates that lines from similar backgrounds (spring or winter plus geographic region) tend to be more closely related than lines from different backgrounds. On this basis it is possible to distinguish five different pre-dominant clusters of genotypes. Group 1 consists primarily of HRWW varieties and breeding lines developed in the USA. Group 2 includes mostly USA and Canadian bred HRSW genotypes. Group 3 includes most of the Canadian bred HRWW varieties. Group 4 almost exclusively contains the final ABCDE hybrid genotypes whereas group 5 is composed of South African derived (recurrent selection) HRSW genotypes. A subgroup of three experimental North American spring wheat lines (RWG27, RWG28, and RWG10) and Alsen are contained within Group 2 (Fig. 4.1.) and provides a perspective of the clustering of highly related material. From the pedigree data (Table 3.1.) it is clear that the three experimental lines are BC_2-derived Alsen near-isogenic lines and thus share approximately 87.5% of their genetic background with Alsen. Within group 2, a subset consisting of MSU 22, MSU 23, MSU 24 and Yellowstone forms a narrow cluster that can be explained by the fact that Yellowstone was one of the parents of each line (thus, a commonality of 50% of each with Yellowstone). In group 3 the close cluster of Accipiter, Falcon

and Moats is once again explained by the fact that Falcon is one of the parents of each of Accipiter and Moats. Thus, it is clear that as compared to these closer relationships, there is substantial genetic variation contained within each of the five groups, and moreover, the genetic make-up of the five groups is obviously very different. From Fig. 4.1. it is evident that there are no extremely closely related genotypes within the ABCDE population cluster or South African spring wheat population (Groups 4 and 5 in Fig. 4.1.). This should be expected, since these genotypes were developed as recurrent selection populations and not through a typical pedigree breeding program. Both of these populations (hybrid ABCDE and South African HRSW) would be highly heterogeneous, unlike the inbred lines developed from the traditional pedigree breeding systems. Figure 4.1. also shows that the genotypes from the final ABCDE population (Group 4) tend to form a distinct cluster. By not being "closely" related to any specific parental background, but instead creating a unique independent cluster the results imply that the hybrid population has acquired diversity from all of the parental groups, making it uniquely more diverse than any other single group.

The SNP comparison across genotypes from different genetic backgrounds proved to be a powerful tool in reflecting genetic diversity other than that targeted in routine breeding programs. This confirms that substantial genetic diversity contained within each of four groups of local and exotic germplasm had been successfully combined in the formation of a highly diverse base population. Based on their commercial performance in North Dakota and Canada, it is known that winter wheat varieties with exceptional cold-hardiness and broad adaptation to the region contributed significantly to the hybrid gene pool.

On the contrary, the resulting contribution of spring type wheat parents (Parent groups A and D) was reduced in the hybrid population. It can be estimated that on average, only .2% and

.7% of final hybrid ABCDE genotype was contributed from individual genotypes within the parental groups A and D, respectively. Therefore, the genetic screening (molecular markers) of a large number of individuals from the hybrid population would be essential to determine the contribution from specific parental genotypes. However, this endeavor was not explored since the focus of this study was to create a diverse population to be utilized as a source for germplasm in a main pedigree program and not intended to identify the contribution of specific parental genotypes in the final hybrid population. Therefore, the parental genotypes were excluded from the molecular marker screening to reduce labor and cost.

A survey of disease resistance phenotypes and marker-aided testing for the presence of key rust resistance genes, revealed a strong presence of useful genetic variability. The latter survey also revealed the need to continue to introgress additional useful resistance genes into the base population as they become available. There is a particularly strong need to broaden the variability for FHB resistance within the new population. Such future introgressions will ideally be achieved through backcrossing to the hybrid population so as to conserve its overall genetic variability.

5.4. Future endeavors to improve the population through RMS

Recurrent selection is a population improvement strategy and a base population can be improved in numerous ways. Two very different approaches were outlined by Marais and Botes (2009) and by Falk (2002). The closed population approach (Marais and Botes, 2009) aims to preserve both the overall genetic diversity contained within a base population and the genetic improvement realized in earlier selection cycles. New variation is introduced through backcrosses to the base population and the aim is to consistently raise the frequency of desired genes in the population. When the frequencies of specific target genes are raised to 0.75 and

higher, randomly derived inbred lines will carry pyramids of such target genes while being highly diverse in other respects. In contrast, the concept behind an evolving base population strategy (RIPE), is to improve the recurrent population through continuous introgression (through limited backcrosses) of new genes (Falk, 2002). This strategy allows for rapid integration of new variability to effect faster change in the population, however, will give lower retention of prior genetic gain. Obviously, many alternatives exist, for example a sub population from a closed population can be handled in parallel as a faster changing evolving population, drawing on the advantages of both concepts. The objective with the evolving sub population may then be the shorter term pursuit and more rapid improvement of a specific trait such as FHB resistance.

The use of a recurrent mass selection system for doing pre-breeding in wheat can provide a powerful tool to enhance a breeding program. This study demonstrated that large numbers of plants can readily be intercrossed with a high degree of randomness and very modest labor input. In the immediate future a primary objective will therefore be to annually plant large numbers of male fertile F_2 in the field and subject them to winter-kill so as to get rid of spring habit genes and raise the frequency of genes for winter survival. In parallel additional genes for rust and FHB resistance will be introduced. Selection in field planted populations will produce future male parents whereas F_1 populations of the preceding season will be screened for seedling resistance and used to annually provide new female parents for crosses. The frequency of desirable alleles should therefore be increased in successive crossing cycles, and the population as a whole will be improved over time.

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