DIFFERENTIAL RESPONSE TO FOLIAR PATHOGENS IN WHEAT AS A CONSEQUENCE OF CYTOPLASMIC SUBSTITUTION

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Ryan Louis Burciaga

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Ryan Louis Burciaga

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

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SUPERVISORY COMMITTEE:

Shahryar Kianian
Chair

Steven Meinhardt

Maricelis Acevedo

Deland Myers

Approved:

January 31st, 2013
Date

Richard Horsely
Department Chair

ABSTRACT

Wheat alloplasmic lines are plants where the cytoplasmic genome of one wheat species was substituted by those of a wild relative, while maintaining the original nucleus. Our project studied differential responses of various alien cytoplasm in a specific nuclear background to various pathogens to identify NC interaction effects on biotic stress tolerance. This study analyzed fifty selected alloplasmic lines that were tested for disease response to *Pyrenophora tritici-repentis* isolates Br15 and Pti2. Results indicate that *Ae. bicornis* cytoplasm with nuclei donor of Chris and Selkirk provides increased resistance to tan spot isolate Br15. *Puccinia triticina* was used in determining differential responses between alloplasmic and euplasmic lines. A bulk set of four leaf rust isolates indicated *Aegilops heldreichii* cytoplasm with Chris nucleus provides resistance to the susceptible euplasmic line Chris and showed increased resistance to both tan spot isolates. These data indicates that cytoplasmic variability can improve resistance to plant diseases.

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DEDICATION

The thesis is dedicated to my parents, Robert and Un Yong Burciaga. Your love and support has inspired me to obtain a higher education away from home. Without you two, I would have still been in Hawaii, unknown to the plan God had for me outside the islands.

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CHAPTER 1. GENERAL INTRODUCTION

Wheat accounts for one fifth of the total calories consumed by the world's population (FAO stats 2011). To increase wheat production as an answer to an ever-growing seven billion world population, wheat breeding efforts have focused on increasing resistance to biotic and abiotic stresses. Cytoplasm substitution with elite cultivars may aid in this effort without affecting nuclear inherited agronomic traits such as grain yield or protein content. The interactions between genomes present in the cytoplasm and nucleus are critical to all eukaryotic organisms. These interactions allow gene regulation, production of ATP, and control a number of morpho-physiological functions (Tsunewaki 1980).

The nuclear-cytoplasmic (NC) interaction of common wheat (*Triticum aestivum* L.) with relative grass species of *Triticum* L. and *Aegilops* L. has received deliberation from wheat cytogeneticists and breeders (Kofoid and Maan 1981). The most sought after effect has been the introduction of cytoplasmic male sterility, but includes other effects such as delayed heading (Fukasawa 1959), variegation in leaf color and reduced vigor (Fukasawa 1959; Tsunewaki 1980). These observations demonstrated cytoplasmic diversity among various species. These species cannot be distinguished by their cytoplasmic component because alloplasmic plants appear to have normal fertility and plant growth (Maan 1975). The alien species that produce fertile alloplasmic lines may be considered as a new source of cytoplasmic-genetic variability to broaden the germplasm base of common wheat (Kofoid and Maan 1981).

Wheat alloplasmic lines are plants where the cytoplasmic genomes of one wheat species (*Triticum)* were substituted by those of a wild relative (*Triticum* or *Aegilops*), while the original nucleus is maintained. Utilizing these lines, the interaction between the nucleus and different

cytoplasm can be evaluated at the morpho-physiological and molecular level (Tsunewaki 1980). Alloplasmic wheat can be examined for agronomic suitability, disease reaction, grain quality, and other characteristics of economic importance (Washington and Maan 1974). The purpose of this project is to identify NC interactions that influence disease response in wheat by phenotyping differential responses in alloplasmic lines as compared to their respective euplasmic (true cytoplasm) lines. As most alloplasmic lines used in this work are of at least backcross 10, the nuclear genome should be more than 99% similar to the parental euplasmic line (Tsunewaki et al., 1996). This will allow us to analyze responses that are specific to the cytoplasm. An effective cytoplasmic genome may produce a foundation for development of cultivars conferring resistance to disease pathogens, although other agronomical traits must be considered.

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CHAPTER 2. LITERATURE REVIEW

Wheat Production

In the United States, wheat production ranks third among field crops in both planted acreage and value of production, behind corn and soybean (USDA baseline 2011). The Food and Agriculture Organization (FAO) estimated that the 2011 world wheat production stands at 676 million tones, a modest increase of 3.4 percent up from 2010 (FAO 2011). Wheat is grown on more than 200 million hectares of land worldwide (FAO 2003). In the upper Northern Plains of the US, spring wheat and durum wheat rank in the top percentile of small grain production in the US. As the population density increases, wheat production coupled with dietary habits in developing countries, global demand for high quality food, and the increased use of grains for livestock feed will proliferate the demand for grain production (Oerke 2004).

Since the beginning of agriculture, crops had to compete with other organisms for resources such as water, salt, iron (abiotic stresses) and pests such as fungi, small animals and bacteria (biotic stresses). Drought tolerance, salt tolerance, pest resistance are among quantative traits that are associated with improvements of wheat gains. Crops that adapt to environmental stresses provide quality traits that increase stability over time.

Modern breeding programs use conventional artificial selection. Plants are selected with the features believed to be desirable for future cultivation. After generations of selection, the combinations of best characteristics are selected for further breeding. The modification of both quality and quantitative traits are vital for food security across the globe. Important qualities such as protein content and overall yield are significant for breeding purposes, but abiotic and biotic stresses must also be recognized as co-significant factors in genetic wheat gains.

Alien Cytoplasm

In humans, aberrant nuclear-mitochondrial (NM) interactions are believed to cause cancer, infertility, blindness, Parkinson's, Alzheimer and mitochondrial neuropathies (Stokstad 2007). It would be very difficult to understand the complete mechanism behind these interactions in human (as opposed to *in vitro* culture studies), where sample population size is small and generating alloplasmic lines would be highly unethical and impossible. In other model animal species, the number of alloplasmic lines is limited or non-existent due to a lack of polymorphism in the cytoplasm and/or the viability of such lines. Wheat represents a model species with regard to studies of nuclear-cytoplasmic (NC) interactions due to the availability of hundreds of alloplasmic lines, by far the largest collection of its kind in any species. Plants contain three types of DNA; nuclear (nucleus), mitochondrial and chloroplast (cytoplasm). Understanding the molecular mechanism behind disease response as influenced by NC interactions in wheat may help understand the pathways that similarly affect other organisms. Identifying cytoplasmic sources of resistance may provide plant breeders with an unprecedented opportunity to introgress different mechanism of disease resistance into cultivated crops.

Wheat alloplasmic lines are preferred to understand NC interactions because of the high polymorphism within the cytoplasm and viability of these lines. The first attempt to obtain these lines was performed by Michaelis in 1954. Michaelis' objective was to perform an extensive investigation of the cytoplasmic factor of the genus *Epilobium* but was limited to *E. hirsutum* and *E. luteum* because of incompatibilities that exist between other species. The genus *Oenothera*, in which the plasmon is biparentally transmitted, was used to study NC interactions between species that belong to subgenus *Euoenothera* (Stubbe 1964). The phenotypes of their F1 hybrids were used as comparisons to their parents to exemplify NC interactions.

Throughout the years, the pertinent species for acquiring alloplasmic lines were *Triticum* (wheat genus) and the related *Aegilops* (goat grass genus). Most wheat alloplasmic lines can be created by using a wild relative cytoplasmic donor as female parent and a wheat cultivar as the recurrent pollen parent in a series of backcrosses (Tsunewaki et al., 1996). Along with the substitution of the cytoplasm, their plasmons are transmitted maternally to the offspring (Fukasawa 1959). These conditions allow the transfer of nucleus of the same wheat into the plasmon of most *Triticum* and *Aegilops* species. While maintaining the same nuclear donor and substituting the cytoplasm, in theory, the differences between the derived alloplasmic and parental lines should be the replaced plasmon of wild relative maternal species.

An immense assortment of alloplasmic wheat lines carrying different plasmons from other species, such as *Aegilops, Triticum, Secale,* and *Agropyron* has been developed (Maan 1975; Panayotov 1983; Suemoto 1983; Tsunewaki 1996). Sasakuma and Maan introduced the nuclear genomes of *T. durum* into the cytoplasms of six *Triticum* species, 14 *Aegilops*, one Rye (*Secale cereal* L) and one *Haynaladia* through backcrossing. Due to efforts of persistent and determined researchers, wheat has the largest collection of alloplasmic lines, larger than any other animal or plant system, making it an ideal model species to study NC interaction. Characterizing this collection for differential response to prevalent foliar pathogens could open possibilities to manipulate disease resistance pathways and develop improved varieties.

Vigor, biomass and productivity of alloplasmic lines in the *Triticum-Aegilops* collection tends to decrease with increasing genetic distance between the cytoplasm and nuclear donor species (Wilson and Driscoll 1983). Alloplasmic lines sharing cytoplasms from species such as *T. aestivum* and *T.dicoccoides* tend to have similar development to those of the euplasmic (true cytoplasm) lines. Hexaploid wheat lines with *Ae. Comosa* cytoplasm and other lines with similar

genetic distance are weak and have delayed maturity (Levin 2003). There are relatively a few studies that analyzed different alloplasmic lines for their reaction to plant disease. For example, wheat hybrids based on *Aegilops juvenalis* cytoplasm have greater resistance to powdery mildew (*Erysiphegraminis* E.J Marchal) and better seed germination than the hybrids based on *A. kotschyi* cytoplasm (Zhang 2001).

Aegilops sharonensis is proposed to have a rich source of genes providing resistance to important wheat diseases (Olivera et al. 2009). The wild relative source of nuclear resistance to new virulence types in pathogen population (Gill et al. 1985) is largely due to the ability to co-evolve with these pathogens before the domestication of wheat (Wahl et al. 1978). *Aegilops sharonensis* has a higher rate of seedling and adult plant resistant to leaf rust and lower level of resistance to spot blotch and tan spot as compared to leaf rust (Olivera et al.2007). *Aegilops sharonensis* possess a high level of diversity for disease resistance with widely variable environments and wider geographic range (Olivera et al.2009). Introducing *Ae. sharonensis* populations as a source of disease resistance provides adequate genetic variability for long-term evolution. Although the source of disease resistance genes originates from the nucleus, *Ae. sharonensis* could also be a good candidate for cytoplasmic genomes. There have been very few examples of cytoplasm substitution as a source of disease resistance; our objective in the following study was to identify particular wild relative species cytoplasms that may increase resistance to different foliar fungal pathogens to be introgressed into wheat cultivars.

Pyrenophora tritici-repentis **Died. (Tan Spot)**

Pyrenophora tritici-repentis (Ptr), the causal agent of tan spot, is an economically important fungal disease affecting wheat in the upper Great Plains of North America and

throughout the world (Lamari and Bernier 1989). Tan spot causes serious yield losses due to reducing the photosynthetic capacity of the leaves causing reduced grain fill, lower test weight, kernel shriveling, and reduced numbers of kernels per head (Shabeer and Bockus 1988). Tan spot may also cause significant loss in grain quality by red smudge and dark smudge. This disorder is called red smudge because the infected seeds have a reddish discoloration (Valder 1954). Symptoms of red smudge are most noticeable on wheat market types such as durum that lack a red color (Fernandez et al. 1994). *P. tritici-repentis* is also one of the pathogens associated with black point of wheat (Fernandez et al. 1994; Jordahl and Francl 1992).

Initially, tan spot was classified into pathotypes based on their ability to induce chlorosis and necrosis on appropriate susceptible differentials (Lamari and Bernier 1989). The individual reaction of the differential would specify which pathotype is being used. After years of research on tan spot, *P. tritici-repentis* classification was changed to races based on the production of three host-selective toxins (HSTs) [Ptr ToxA, Ptr ToxB and Ptr ToxC] that condition sensitivity to susceptible lines.

As wheat production has increased the methods of shortening rotations with continual wheat cultivation, and the increasing use of zero-tillage practices has lead to increased disease pressure from pathogens such as *P. tritici-repentis*. Tan spot can reside over winter in residues left by reduced or zero tillage practices and provide inoculums for the following years. Tan spot may be controlled with the application of fungicides, but concerns about the undesirable environmental effects and the subsequent rise in production costs make this choice less desirable. The production of disease resistant varieties is the most economical means of controlling this disease. The intent of the following studies is to investigate biologically altered cultivars,

alloplasmic lines, for resistance to tan spot that will be cost efficient, environmentally friendly and economically sustainable.

Disease Cycle

Pyrenophora tritici-repentis propagates both asexually by conidia and sexually by ascospores. Ascospores were thought to be the primary inoculum because of their ability to travel short distances from host to host (Hosford 1971). The initial infections from ascospores are released after overwintering on leaf stubble and affect the lower leaves of the plants. Subsequently, conidiophores and conidia are formed and then wind dispersed to create secondary infections that cycle several times during the growing season. *Pyrenophora tritici-repentis* is a diurnal sporulator and conidia are disseminated by wind (Ciuffetti and Tuori 1999).

In contrast studies by Krupinsky 1992 indicated conidia may play a primary role in the initiation of a tan spot epidemic in spring wheat in the Northern Great Plains. Conidiophores are erect and simple with a swollen base and give rise to conidia that are subhyaline, cylindrical typically four to seven septate, and multinucleate (Ciuffetti and Tuori 1999). Conidial production is thought to be followed by repeated cycles on diseased leaf caused by wind dispersal and said to travel longer distances than those of ascospores. Moisture, temperature, light, plant stage, plant genotype, and aggressiveness of the isolate all factor in the contribution to the amount of inoculum produced and the severity of the disease, either together or separately (Ciuffetti and Tuori 1999). As conditions vary across locations, varying combination of these factors in the wheat growing population can cause a tan spot epidemic. Spring wheat grown in the Great Plains has the optimum temperature, light, and moisture for tan spot to thrive during the summer months. Wheat disease surveys in North Dakota indicate that tan spot is one of the more

prevalent foliar diseases for wheat. Tan spot's ability to produce several cycles of non-sexual reproduction in a growing season results in multiple infection cycles, and survival on non-tillage stubble over winter makes *P. tritici-repentis* difficult to control.

Toxin Production

Pyrenophora tritici-repentis has been classified into a race structure and based on the expression of three toxins. All eight possible races have been described (Lamari et al. 2003). Race distinctions are attributed to the production of three host-selective toxins (HSTs) [Ptr ToxA, Ptr ToxB, and Ptr ToxC] (Ciuffetti 2010). Virulence data presented in Lamari et al. 2003 suggest some individual races possess more than these identified HSTs or virulence factors on hexaploid cultivars.

Table 1. Characterization of races one through eight of *P. tritici-repentis*[+]

P. tritici-repentis races	Toxin production	Symptomology	
		Necrosis	Chlorosis
Race 1	ToxA, ToxC	+	+
Race 2	ToxA	+	-
Race 3	ToxC	-	+
Race 4	ToxB*	-	-
Race 5	ToxB	-	+
Race 6	ToxB, ToxC	-	+
Race 7	ToxA, ToxB**	.	.
Race 8	ToxA, ToxB, ToxC	+	+

(+) Present, (-) absent, (.) undefined,
*Race 4-defined as non-virulent/avirulent
**Race 7-unidentified (possible combination)
[+] Information based on reactions of six wheat differentials to various isolates; Lamari et al. 2003

Race two, five, and three possess one host specific toxin (Ptr ToxA, Ptr ToxB, and Ptr ToxC), respectively. Race one appears to have both toxins produced from race two (Ptr ToxA) and race five (Ptr ToxB). Race six combines the toxin production from race five (Ptr ToxB) and race three (Ptr ToxC), while race eight combines all three toxin produced by race two, three, and five (Ptr ToxA, Ptr ToxC, and Ptr ToxB), respectively. The toxin combination of Ptr ToxA and Ptr ToxB, (race seven of *P. tritici-repentis*) has yet to be identified and/or characterized. Several scientific reports have indicated additional toxins, but none have been published. The additional toxins have characteristics that induce both chlorosis and necrosis, and both proteinaceous and non-protein like Ptr ToxC. An additional Ptr toxin, ToxD, is proposed to produce a necrotizing agent that has yet to be cataloged (Martinez et al. 2004; Ciuffetti 2010).

Pyrenophora tritici-repentis ToxA was first isolated by Ballance et al in 1989. Ballance identified a toxin that had been purified from a culture filtrate of a necrosis-only isolate of *P. tritici-repentis*. The toxin produced necrosis if it was infiltrated into the leaves of sensitive cultivars but had no effect to resistant lines. It was subsequently isolated by Tuori et al in 1995. Turoi confirmed the Ptr ToxA 13.2-kDA protein is a toxic agent that causes necrosis-only in sensitive cultivars. *Pyrenophora tritici-repentis* ToxA was characterized by Ciuffetti et al. 1998 as a necrotizing, proteinaceous host-selective toxin (HST) produced by *P. tritici-repentis*, and the causal agent of tan spot of wheat (Ciuffetti and Tuori 1999). The single gene encodes a selective toxin causal to the development of tan spot of wheat (Ciuffetti et al. 1997). The gene encodes a signal sequence for targeting to the secretory system (Ciuffetti et al. 1997), a pro-sequence (N-domain) necessary for proper folding that is cleaved prior to secretion (Tuori et al. 2000), and a mature peptide C-domain that corresponds to the native toxin. The native Ptr ToxA is a single domain protein consisting of a β-sandwich fold sharing a similar topography to the fibronectin

type III domain (Sarma et al. 2005). This is significant in that a conserved Arg-Gly-Asp (RGD)-sequence located on a solvent-exposed loop in both Ptr ToxA and fibronectin is important for their functional interactions respectively (Manning et al. 2004; Meinhardt et al. 2002; Peirschbacher and Ruoslahti 1984). One interaction mediated by the RGD-containing, solvent-exposed loop, is internalization into Ptr ToxA-sensitive mesophyll cells (Manning and Ciuffetti 2005; Manning et al. 2008).

The *Tsn1* locus in wheat is a single dominant gene, designated on the long arm of chromosome 5B (Faris et al 1996), which is required for disease manifestation of Ptr ToxA. Similar system of *SnTox1* locus (*Stagonospora nodorum*) is proposed to mimic the Ptr ToxA system by requiring a host gene product for sensitivity (Lui 2004). As the Tsn1 gene is important to display disease in Ptr ToxA sensitive cultivars, Friesen et al. 2003 demonstrated that insensitivity to Ptr ToxA in the host does not result in complete resistance to tan spot. The results described that Ptr ToxA is a virulence factor that is important for disease onset at days three and five post inoculation but non-significant after eight days post-inoculation.

Preliminary experiments done at NDSU in the summer of 2009, investigated the effects of Ptr ToxA on wheat cultivar Chris and 45 alloplasmic lines in an attempt to identify differences to Ptr ToxA sensitivity as a result of cytoplasm substitution. The cultivar Chris was insensitive to Ptr ToxA, including all sets of alloplasmic lines. Due to the nuclear donor being insensitive to Ptr ToxA, the alloplasmic lines followed the same orientation. The data of the experiment supports the likelihood that the sensitive component, *Tsn1*, was absent from the nucleus. This model demonstrates the inverse gene for gene hypothesis. In this scenario, because the *Tsn1* sensitivity gene is not present, no necrosis should form on leaves. If you were to observe necrosis, then the NC interaction would involve an additional susceptibility factor. Any role increasing or

decreasing sensitivity to the pathogens should be considered a critical NC interaction as the alloplasmic line would not behave the same as the euplasmic line.

In 1995, Orolazo et al identified an additional HST inducing extensive chlorosis on wheat cultivars from race five of *P. tritici-repentis*. Chlorosis toxins observed in races one and three were known to induce chlorosis in different wheat genotypes, but did not produce a detectable level of chlorosis toxin in several bioassays. When the additional race of *P. tritici-repentis* was culture filtrated and inoculated on a set of differentials, wheat (6B662 and Katepwa) and triticale (Banjo) developed a distinct level of chlorosis. The additional race was identified as producing a chlorosis-inducing toxin, which lead them to believe this chlorosis toxin differed from the previous races mentioned. The additional toxin was evaluated in F_2 progenies of 'race five-susceptible' and 'race five-resistant' wheat genotypes. Susceptibility of the seedlings cosegregated 1:3 (resistant/susceptible), suggesting a single dominant locus controlling the reaction to the fungus and toxin. Race five was designated to *P. tritici-repentis*, describing the toxins' pathogenicity factor for inducing chlorosis. Race five's chlorosis toxin was then isolated in 1999 by Strelkov et al, and introduced as Ptr ToxB (previously known as Ptr chlorosis-toxin). It was purified and characterized from culture filtrates of race five isolate Alg3-24 *P. tritici-repentis*. This generated the realization that other additional races would exist with Ptr ToxA and an unclassified toxin (race one and eight) (Strelkov et al. 2002; Lamari et al. 2003).

Pyrenophora tritici-repentis ToxB homologs were also found in races three and four (Strelkov and Lamari 2003; Martinez et al. 2004), which do not induce ToxB-related symptoms on ToxB-sensitive cultivars (Lamari et al. 1995). Two features differentiating the homologs of races three and four from races that produce ToxB symptoms are: (i) sequence variation and (ii) the presence of a gene in a single copy. Copy number variation is found in isolates that produce

Ptr ToxB symptoms, ranging from at least two to 10 copies (Lamari et al. 2003; Martinez et al. 2004; Strelkov et al. 2006). The number of isolates containing Ptr ToxB displays virulence proportional to the number of copies of genes producing the toxin (Strelkov et al. 2006). Unlike Ptr ToxA, where a single copy of the gene is sufficient to induce sensitivity, Ptr ToxB containing pathogenic races seems to require more than one copy to activate significant symptoms (Lamari et al. 2003; Martinez et al. 2004).

Several Ptr ToxB loci have been cloned from two race five isolates collected in two geographic regions, DW7 from North Dakota (Ali 1999) and Alg3-24 from Eastern Algeria (Strelkov and Lamari 2003). *Pyrenophora tritici-repentis* ToxB loci from DW7 showed high levels of conservation over >900 nucleotides, including identical Ptr ToxB ORFs compared to Alg3-24 (Martinez et al. 2004). Flanking these conserved regions are retrotransposon-like sequences along with inversions, inverted repeats and conserved insertions. These observations suggest that unequal crossing over with similar sequences in the genome has led to amplification of Ptr ToxB-containing loci (Ciuffetti 2010). *Pyrenophora tritici-repentis* ToxB loci present in a low-virulence race 5 isolate, 92-171R5 (Strelkov et al. 2002), appear to have significant differences in regions upstream of the Ptr ToxB ORF when compared to sequenced DW7 and Alg3-24 loci (Strelkov et al. 2006). Copy number variation of Ptr ToxB is associated with virulence, and more virulent isolates have a greater estimated copy number than less virulent counterparts (Strelkov et al. 2002; Martinez et al. 2004).

As previously stated, race one and three possessed low amounts of chlorosis-inducing toxin that differed from *P. tritici-repentis* race five's ToxB (Orolazo et al. 1995). In 1995, Lamari et al studies' identified *P. tritici-repentis* isolate 78-62 producing a toxin compound which was mimic chlorosis-inducing symptoms associated with wheat cultivar 6B365. The

previously identified Ptr ToxB does not induce chlorosis in wheat cultivar 6B365, suggesting that isolate 78-62 secretes an uncharacterized chlorosis toxin. Faris et al. 1997 discovered that quantitative trait locus (QTLs) on Chromosome 1AS, where *Tsc1* is, confers sensitivity to this unknown toxin. Several reports (Orolazo et al. 1995; Lamari et al. 1995; Faris et al. 1997; Strelkov et al. 1997; Ciuffetti et al. 1997) identified the uncharacterized chlorosis toxin, but it was Effertz et al. 2002 who characterized the low molecular weight, polar, nonionic, non-proteinaceous toxin. These physical properties make the toxin difficult to isolate. Though this molecule could not be fully characterized, it was designated a toxin because of the several essential criteria (Scheffer 1983) required to define the new toxin; Ptr ToxC is produced by the pathogen (*P. tritici-repentis*), causes damage to host (chlorosis), and is involved in disease development (Effertz et al. 2002). Several unpublished data reports other HSTs that are detected but uncharacterized, leaving one to ask, how many HSTs of *P. tritici-repentis* are not classified?

Resistance Mechanism

Resistance mechanisms associated with tan spot were designated as race specific following a gene-for-gene system (Lamari et al. 2003). This system involves the recognition of pathogen producing HSTs by specific genes in the host. The genes *Tsn1*, *Tsc1* and *Tsc2* which confer insensitivity to Ptr ToxA, Ptr ToxC, and Ptr ToxB, respectively, underlie major QTLs on chromosomes 5BL, 1AS, and 2BS for resistance to race two, three, and five, respectively (Effertz et al. 2002; Friesen and Faris 2004). Faris et al. 2005 suggests that recombinant inbred line (RI) lines of Br34 recognize multiple pathogenic races of *P. tritici-repentis*. Faris et al discussed the possibility of the race-nonspecific resistance mechanisms concealed by Br34 decreases toxin activity and render plants resistant before the onset of necrosis by the toxin. Although Faris et al. 2005 does not disprove the Lamari et al. 2003 conclusion of resistance

mechanism, it questions whether tan spot follows race-nonspecific resistance or race specific resistance mechanisms.

Puccina triticina Eriks. (Leaf Rust)

Puccinia triticina, the causal agent of leaf rust, is among the most commonly occurring pathogen of all cereal rust. The wheat leaf rust is a heteroecious fungus in need of two hosts to complete its life cycle. The primary host is an asexual, uredinial host which is usually wheat and an alternative host that is a pycnial/aecial host (usually weeds). Leaf rust has adapted to a wide range of different climates and is found in wheat growing areas throughout the world. Wheat cultivars that are susceptible to leaf rust regularly suffer yield losses of 5-15% (Samborski 1985) or greater, depending on the stage of the crop when the onset of rust infections occurs. Epidemics of rusts, sometimes caused by new races, frequently affect wheat grain production and quality throughout the world. Sources of genetic resistance are valuable to increase the sustainability of cereal production, from both economic and environmental standpoints (Reynolds and Borlaug 2006). Several techniques, such as identifying resistant genes and introgressing them into modern cultivars, has reduced the impact of this disease worldwide.

Statler 1971 identified cultivars Chris and Selkirk as susceptible to leaf rust in several field plots in North Dakota's agriculture research stations. These lines were tested with races of leaf rust based on resistance or susceptibility of lines with host genes *Lr1*, *Lr2*, *Lr2*D and *Lr3*. In contrast to these findings, Washington and Maan 1974 identified leaf rust resistance using euplasmic and alloplasmic lines of Chris, Selkirk, and durum line 56-1 at the seedling stage to what was designated at that time as leaf rust races one, two, and 13.

Euplasmic Chris had a susceptible reaction to all three leaf rust isolates at the seedling stage; however it was resistant at the adult stage. Alloplasmic Chris with cytoplasms *T. araraticum*, *T. timopheevii*, and *T. monococcum* had susceptible reactions to all three races. Alloplasmic Chris with cytoplasms *T. macha* 140191, *T. dicoccoides* #2, *Ae. speltodies*, *Ae. ovata*, and *Ae. squarrosa* had a susceptible reaction to race 13. Alloplasmic Chris with cytoplasms *T. dicoccoides* #4 and *T. zhukovskyi* had a susceptible reaction to race two. However, though the two different accessions of *T. dicoccoides* are proposed to have the same cytoplasm, they differ in reaction to the two different races of leaf rust. *Triticum macha* cytoplasm with Chris nucleus had a susceptible reaction to race one and 13, but a resistant reaction to race two, indicating cytoplasm difference between *T. macha* and *T. aestivum* Chris.

Analysis of Selkirk indicated that the euplasmic and alloplasmic lines were resistant to all three races of leaf rust in Washington and Maan's experiment. This would validate the thought that the resistance gene(s) that are encoded in the nucleus adequately confers complete resistance in the host plant. Subsequently, the additional effects of the cytoplasm would be suppressed in Selkirk. Euplasmic and alloplasmic durum line 56-1 had ambiguous reactions to all three leaf rust isolates used (Washington and Maan 1974). At normal conditions of day time temperatures of 24 to 30°C and night time temperatures at 13 to 16°C post inoculations, these lines would be susceptible. At cooler day time temperature of 20 to 23°C and the same night temperatures, these lines would be resistant. In conclusion, the variability of infection types was credited to environmental effects. Considering this variability it was difficult to identify reliable evidence of resistance in alloplasmic durum lines. The evidence shown in these experiments lends confidence towards finding other sources of cytoplasm that would respond differently to modern leaf rust isolates.

Disease Characteristics and Life Cycle

Leaf rust is characterized by the uredinial stage. Uredinia are up to 1.5 mm in diameter, round, with brownish-orange uredinia that are dispersed on both the upper and lower leaf surfaces of the primary host (Bolton et al. 2008). Uredinia produce urediniospores that average 20 µm in diameter and are orange-brown, with up to eight germ pores scattered in thick, echinulate walls. Wheat varieties that are entirely susceptible have large uredinia without causing chlorosis or necrosis in the host (Long and Kolmer 2000). Resistant wheat varieties are characterized by responses that range from small hypersensitive flecks with no uredinia present to small or moderately sized uredinia that are surrounded by chlorotic and/or necrotic areas.

The life cycle of *P. triticina* was described by Bolton et al. 2008. *Puccinia triticina* produces urediniospores on the wheat hosts that are dikaryotic and will re-infect the telial host under appropriate environmental conditions. As the host plant matures, the uredinial infections develop into dikaryotic, brown-black, where two-celled teliospores that are produced in the uredinia. With optimal conditions, either one or both cells in the teliospore produce a hypal swelling called a promycelium (Anikster 1986). The diploid nuclei undergo meiosis into the promycelium which divides into four cells, each with one haploid nucleus. The sterigma forms on the distal wall of each cell and each haploid nucleus migrates through the sterigma into the basidiospore at the tip of the sterigma. The nucleus within each basidiospore undergoes mitosis, forming single-celled basidiospore, each with two identical haploid nuclei. Within hours after being formed, mature basidiospores are ejected from sterigma and carried from the telial host by wind dispersal for short distances to nearby alternative hosts. The basidiospores directly infect the epidermal cells of the host resulting in the development of the oval shaped pycnia that develop as yellow-orange pustules on both leaf surfaces. *Puccinia triticina* can cycle indefinitely

as uredinial infections on their hosts such as wheat. In the southern Great Plains of the US, leaf rust infects this region over-summer on volunteer wheat and can serve as reservoirs of inoculum for the autumn-planted winter wheat. In winter wheat, leaf rust can over-winter as mycelial or uredinial infections in areas with suitable temperature conditions (Roelfs 1989). Over time, leaf rust can accumulate and through wind dispersal return to the upper Great Plains when the conditions are favorable.

Resistance Mechanism

The gene-for-gene relationship between wheat and *Puccinia triticina* pathosystem has been studied throughout the years. For every resistance gene in the host, there is a corresponding locus in the pathogen that conditions avirulence (Samborski and Dyck 1968). Those wheat varieties that rely on race-specific resistance often lose effectiveness within a few years by forcing selection for virulent leaf rust races (Bolton et al. 2008). Race-specific resistance is usually displayed with a hypersensitive reaction of rapid cell death that occurs between the fungal uredinia and the host cells in the mesophyll layers. Different resistance genes condition a different characteristic response of resistant phenotypes or infection types (Bolton et al. 2008). However, the cause of differences between chlorosis and necrosis of a race-specific hypersensitive response is ambiguous. Different characteristics may be due to different infection time or colonization process of *P. triticina*.

There have been more than 60 leaf rust resistance (*Lr*) genes identified and were designated *Lr1* to *Lr60* by McIntosh et al. 2007. These genes have been characterized in hexaploid wheat on nearly all 42 chromosomes. Of these 60 *Lr* genes, four sets of allelic variations have also been described. For example, there are three allelic variations for *Lr2* [a, b,

and c] located on chromosome arm 2DS (McIntosh and Baker 1968). Dominant association of avirulence/virulence genes in the pathogen and leaf rust resistance genes in the host is determined by their individual genotypes. Kolmer 1996 describes that resistant genes in the host ranged from completely dominant to recessive, dependent on homozygous or heterozygous condition for avirulence in the pathogen. Likewise, expression of avirulence in the pathogen depended on whether the host was homozygous or heterozygous for resistance.

Introducing resistant genes from wild relatives of lower-ploidy into hexaploid bread wheat can be complicated by interactions between resistance genes and suppressor genes in the different genomes (Kolmer 1996). In 1992, Bai and Knott described crossing ten leaf rust resistant accessions of *T. dicoccoides* (tetraploid) and bread wheat (hexaploid). The F_1 plants from these crosses with bread wheat were susceptible. The F_2 progenies had fewer resistant plants with a lower number of D chromosomes as compared to the susceptible plants. Chromosomes 2B and 4B carried genes for leaf rust resistance, and 1D and 3D carried the suppressors of resistance. This suggests that the F_2 had far less suppressor genes for leaf rust indicating that *Lr* alleles were still segregating in a heterozygous condition.

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CHAPTER 3. NUCLEAR-CYTOPLASMIC INTERACTION AND ITS INFLUENCE ON DISEASE RESPONSE TO TAN SPOT (*PYRENOPHORA TRTITICI-REPENTIS*)

Abstract

Research was conducted to evaluate the potential of increased disease resistance to *Pyrenophora tritici-repentis* derived from substituted cytoplasmic genomes. Experimental treatments included parental wheat lines Chris, Selkirk, and 56-1 along with alloplasmic lines. Comparisons between euplasmic source and alloplasmic lines were used as a measure of differential response influenced by the cytoplasm. A mix model using days, treatments and seasons as fixed effects were used to verify the differential response of various lines to *P. tritici-repentis* isolates of Br15 and Pti2. A completely randomized design (CRD) allowed us to arrange our forty-six treatments and two replicates with two samples in each experimental block. Once the data was analyzed and specific alloplasmic lines representing a significant difference from their euplasmic line were identified, two more experiments were performed to ensure repeatability of results. The scoring system used followed Bernier and Lamari 1989 nomenclature for scoring tan spot disease with the additional score of zero. Several alloplasmic lines showed similar responses to that of their respective euplasmic donor when screened with Ptr isolate Br15. Fourteen different alloplasmic lines showed a significant increase in resistance to Ptr isolate Br15 when compared to their moderately susceptible euplasmic lines. Alloplasmic lines with an *Aegilops bicornis* cytoplasm (SSM0039, SSM0187, and SSM0237) in the Selkirk and Chris nuclear backgrounds manifested significant increases in resistance to Ptr isolate Br15 as compared to their euplasmic donor. There were no alloplasmic lines derived from Selkirk that demonstrated a differential response relative to their euplasmic donor to Ptr isolate Pti2. Alloplasmic lines with *Ae. bicornis* (SSM0187 and SSM0206), *Ae. variabilis* (SSM0237) and

Ae. Heldreichii (SSM0240) cytoplasms in the Chris nuclear backgrounds manifested a significant increase in disease resistance to Ptr isolate Pti2 as compared to their euplasmic donor. These results indicated that the appropriate cytoplasmic substitution may be able to increase disease resistance to tan spot isolates.

Introduction

Pyrenophora tritici-repentis (Ptr), the causal agent of tan spot, is an economically important fungal disease affecting wheat in the upper Great Plains of North America and throughout the world (Lamari and Bernier 1989). Tan spot causes serious yield losses due to reduction in the photosynthetic area of leaves causing reduced grain fill, lower test weight, kernel shriveling, and reduction in number of kernels per head (Shabeer and Bockus 1988). Though this disease may be controlled with the application of fungicides, this may result in undesirable environmental consequences and higher cost of production. Establishing biologically altered resistant cultivars with increased resistance will be cost efficient, environmentally friendly and economically sustainable for the control of tan spot. No research has been performed with alien cytoplasm substitution and their reaction to tan spot. Extensive research over the last 20 years has made tan spot a well characterized disease. Other research on crop diseases (i.e. *Stagonospora nodorum*) document similar resistance traits to those of *P. tritici-repentis*. With a well understood resistance mechanism, *P. tritici-repentis* is a model organism for traditional resistance methods for other uncharacterized organisms that rely on similar mechanics. Substitution of the cytoplasm of a wheat cultivar with a wild relative has been shown to influence several traits (Tsunewaki et al. 1996). The intent of this experiment was to measure the influence of this substitution on disease resistance. Initial screening of parental wheat lines

displayed susceptibility, conferring optimism of identifying possible resistance responses with alloplasmic wheat lines.

Materials and Methods

Pyrenophora tritici-repentis (Tan Spot) Screening

Of 335 alloplasmic lines available, approximately 50 lines were selected based on their characteristics of effective seed germination, good fertility and grains per spike. Paternal wheat lines Chris, Selkirk, and durum line 56-1 were used as our controls for the experiment. Comparisons between euplasmic sources and alloplasmic sources were used as a measure of differential responses influenced by the cytoplasm. A completely randomized design (CRD) was used for these experiments. A mix model using days, treatments and seasons as fixed effects were used to verify differential responses of various lines to selected races of tan spot. Isolate Br15 (race eight) contains all three HSTs of tan spot and was used to evaluate the effect of HSTs. Isolate Pti2 (race one), which contains HSTs ToxA and ToxC, is considered one of the most virulent isolates in NDSU collection and was used to look for pathogenic determinants in addition to the toxins.

Experimental Design

The initial experiment utilized a CRD, arranged in forty-six treatments and two replicates with two samples in each experimental block. Analysis of this data was used to select specific alloplasmic lines representing a significant difference from their euplasmic line. Two more experiments were performed on the selected lines to ensure repeatability of results. The scoring system used followed that of Lamari and Bernier 1989 for scoring tan spot disease with the additional score of zero. The qualitative rating system was developed to scale plant reactions to

tan spot as followed: 0=no sign of disease on leaves; 1=small dark-brown to black spots without any surrounding chlorosis or tan necrosis (resistant); 2=small dark-brown to black spots with very little chlorosis or tan necrosis (moderately resistant); 3=small dark-brown to black spots completely surrounded by distinct chlorotic or tan necrotic ring without merging into one another (moderately susceptible); 4=small dark-brown or black spots completely surrounded with chlorotic or tan necrotic zones, some of the lesion merged with one another (susceptible); and 5=dark-brown or black centers expanded, most lesions are merged with each other with severe chlorosis and necrosis throughout the leaves (susceptible).

Statistical analysis was conducted using SAS version 9.3 (SAS Institute 1999). A general linear model (GLM) was used to determine statistical significance at P value of ($P < 0.05$) and missing data were reported as no value in the Analysis of Variance (ANOVA) table. The error was calculated by using least square means instead of traditional means. The comparison of P values uses t-tests between each pair of treatments so significant separation could be observed. Type III sums of squares are preferred to Type I sum of squares in testing effects in unbalanced data sets. They test the function of the underlying parameters that is independent of the number of observations per treatment combination (SAS support).

Conidia Production

Conidia were produced by placing a mycelia plug in the center of V8/PDA plate. Plates were incubated for six days at 20°C under continuous darkness to allow for hyphae extension. The plates were then flooded with sterile distilled H_20 and mycelium were flatten with a flamed sterilized glass tube. Excess water was decanted and the plates were incubated under fluorescent light for 24 hours at 20°C. The following day, plates were transferred to the dark at 16°C for 24

hours to induce conidiophores and conidia production. Conidia were harvested by flooding the plates with sterile distilled H_20 and gently scraping the spores from the plate using a sterilized inoculating loop. The conidia were then filtered through two layers of cheesecloth into a beaker and mixed with a stir bar. Twenty microliters of conidial solution was placed on a slide and counted. Four counts were averaged to determine the number of spores per milliliter (20 microliters x 50 = 1000 microliters = 1 milliliter). The conidia solution was diluted to 650 spores/ml and one drop of Tween-20 per 125 ml of solution was used to separate the conidia prior to spraying. This concentration allowed us to measure disease progression for a total of five days post inoculation.

Plant Growth and Inoculation

Seeds of parental and alloplasmic lines (Table 2) were placed in cones that contained sunshine mix combined with soil (50% v/v) and grown to the two leaf stage. The seedlings were watered and fertilized with osmocote as needed. Plants were sprayed with the conidial suspension using compressed air until there was runoff. They were then placed in the mist chamber for 24 hours with misting for 20 seconds every four minutes. They were transferred to growth chamber under 14 hour light at 22°C and ten hour dark at 19°C.

Table 2. Germplasm screened for tan spot

Accession #	Cytoplasm Name	Nuclear Makeup	Generation	Constitution
SSM0003	*Ae. crassa* 6N	Selkirk	Self2-BC 7	Alloplasmic
SSM0004	*Ae. cyl. cw*	Selkirk	Self3- BC13	Alloplasmic
SSM0015	*T. dico* G1395	Selkirk	Self2-BC17	Alloplasmic
SSM0016	*T. dico* G1453	Selkirk	Self2-BC13	Alloplasmic
SSM0017	*T. dico* G1458	Selkirk	Self2-BC17	Alloplasmic
SSM0018	*T. dico* G1460	Selkirk	Self2-BC17	Alloplasmic
SSM0019	*T. dico* G671	Selkirk	Self2-BC13	Alloplasmic

(CONTINUED)

Table 2. Germplasm screened for tan spot (Continued)

Accession #	Cytoplasm Name	Nuclear Makeup	Generation	Constitution
SSM0020	*T. dico* G803	Selkirk	Self2-BC13	Alloplasmic
SSM0021	*T. dico* G1392	Selkirk	Self3-BC13	Alloplasmic
SSM0022	*T. dico* Okla 11140	Selkirk	Self3-BC18	Alloplasmic
SSM0023	*T. dico* Okla 11186	Selkirk	Self3-BC17	Alloplasmic
SSM0024	*T. dico* RL5207	Selkirk	Self2-BC16	Alloplasmic
SSM0027	*T. macha* 140191	Selkirk	Self3-BC19	Alloplasmic
SSM0028	*T. macha* 190923	Selkirk	Self3-BC11	Alloplasmic
SSM0029	*T.paleocolchicum* PI 330553	Selkirk	Self3-BC8	Alloplasmic
SSM0036	*T. turgidum*	Selkirk	Self3-BC11	Alloplasmic
SSM0039	*Ae. bicornis*	Selkirk	Self3-BC20	Alloplasmic
SSM0054	*Ae. crassa*	Selkirk	Self2-BC12	Alloplasmic
SSM0069	*Ae. longissimum khaplis*	56-1	Self3-BC2	Alloplasmic
SSM0076	*Ae. sharonensis*	56-1	Self2-op@BC10	Alloplasmic
SSM0085	*Ae. variabilis*	56-1	Self2-BC15	Alloplasmic
SSM0107	*T. dico.* G1453	56-1	Self2-BC2	Alloplasmic
SSM0187	*Ae. bicornis*	Chris	Self2-BC27	Alloplasmic
SSM0191	*Ae. crassa*	Chris	Self2-selk8-chr2	Alloplasmic
SSM0192	*Ae. crassa*	Chris	Self2-BC9	Alloplasmic
SSM0193	*Ae. crassa*	Chris	Self2-BC3	Alloplasmic
SSM0194	*Ae. cylindrical*	Chris	Self2-BC10	Alloplasmic
SSM0195	*Ae. cylindrical*	Chris	Self2-BC14	Alloplasmic
SSM0196	*Ae. juvenalis*	Chris	Self2-BC15	Alloplasmic
SSM0197	*Ae. kotschyi*	Chris	Self2-selk13-chr3	Alloplasmic
SSM0199	*Ae searsii*	Chris	Self2-Ae.sq.-selk5	Alloplasmic
SSM0202	*Ae. squarrosa*	Chris	Self2-BC18	Alloplasmic
SSM0205	*Ae. uni.* G633	Chris	Self2-BC10	Alloplasmic
SSM0206	*Ae. variabilis*	Chris	Self2-BC20	Alloplasmic
SSM0207	*Ae. vavilovi*	Chris	Self2-selk7-chr	Alloplasmic
SSM0208	*Ae. ventricosa*	Chris	Self2-BC9	Alloplasmic
SSM0210	*Haynaldia triticum* D.	Chris	Self2-BC10	Alloplasmic
SSM0229	*T. paleocolchium*	Chris	Self2-BC9	Alloplasmic
SSM0232	*T. t. nig.*	Chris	Self2-BC16	Alloplasmic
SSM0237	*Ae. bicornis*	Chris	Self2-BC25	Alloplasmic
SSM0240	*Ae. heldraichi*	Chris	Self2-BC10	Alloplasmic
SSM0253	*T. macha*	Chris	Self2-BC19	Alloplasmic
SSM0254	*T. macha* 140191	Chris	Self2-BC21	Alloplasmic
SSM0258	*T. aestivum* Chris	Chris	Chris parent	Euplasmic
SSM0317	*T. aestivum* Selkirk	Selkirk	Selkirk parent	Euplasmic
SSM0318	*T. turgidum* 56-1	56-1	56-1 parent	Euplasmic

Results and Discussion

Phenotypic Analysis of Isolate Br15 of Tan Spot

Table 3 summarizes the significant comparison amongst all genotypes (treatment) relative to their respective euplasmic donor. A mixed model was used to analyze three fixed effects including season, genotype, and day (Table A1). Season is defined as one replicated trial of each experimental block with isolate Br15. Each genotype is defined as the treatment. Genotype has a significant effect amongst other treatments. Day is defined as the average scores recorded on day three and day five post inoculation. Genotype × day and season × genotype × day are the non-significant interactions. The non-significant interactions are determined with a p-value that was higher than the 95% confidence level.

Significant differences are identified in the last column with p-values (Table A2). Genotype, season, and days were placed in the mix model to identify the average scores of disease response. Significant differences of increased resistance among alloplasmic versus euplasmic lines for disease response to isolate Br15 are listed in Table 3. The mixed model procedures included a general linear model (GLM) to compare average score estimation between euplasmic and alloplasmic scores. The average euplasmic scores were (Table A3), Chris= 2.61(+/-1.22), Selkirk= 2.41 (+/- 0.92), and durum line 56-1= 3.43 (+/- 1.52).

Fourteen different cytoplasm accessions showed a significant increase in resistance compared to the moderately susceptible euplasmic lines. Table 3 shows *Ae.bicornis* (SSM0039, SSM0187, and SSM0237) cytoplasm in the Selkirk and Chris nuclear background manifested a significant increase in the resistant response as compared to their euplasmic donor, unfortunately this cytoplasm background with durum line 56-1 nucleus was not tested.

Table 3. Significant responses of alloplasmic lines compared to euplasmic lines using isolate Br15 of tan spot

Genotype	Cytoplasm	Genotype	Cytoplasm	Nucleus	Pr > t	Reaction
SSM0004	*Ae. cylindric cw.*	SSM0317	PARENT	Selkirk	0.0460*	Susceptible
SSM0022	*T. dico Okla* 11140	SSM0317	PARENT	Selkirk	0.0055**	Resistant
SSM0039	*Ae. bicornis*	SSM0317	PARENT	Selkirk	<0.0001**	Resistant
SSM0045	*Ae. mutica*	SSM0317	PARENT	Selkirk	0.0003**	Resistant
SSM0076	*Ae. sharonensis*	SSM0318	PARENT	56-1	0.0004**	Resistant
SSM0085	*Ae. variabilis*	SSM0318	PARENT	56-1	0.0048**	Resistant
SSM0107	*T. dico* G1453	SSM0318	PARENT	56-1	0.0203*	Resistant
SSM0187	*Ae. bicornis*	SSM0258	PARENT	Chris	0.0037**	Resistant
SSM0193	*Ae. crassa*	SSM0258	PARENT	Chris	0.0126*	Susceptible
SSM0202	*Ae. squarrosa*	SSM0258	PARENT	Chris	0.0222*	Resistant
SSM0206	*Ae. variabilis*	SSM0258	PARENT	Chris	0.0006**	Resistant
SSM0237	*Ae. bicornis*	SSM0258	PARENT	Chris	0.0015**	Resistant
SSM0240	*Ae. heldraichi*	SSM0258	PARENT	Chris	0.0006**	Resistant
SSM0254	*T. macha* 140191	SSM0258	PARENT	Chris	0.0175*	Resistant

*Significance level at $p < 0.05$
**Significance level at $p < 0.01$

These results suggest a role for this cytoplasm in regard to resistance regardless of nuclear background. *Aegilops bicornis* alloplasmic line (SSM0187) develops more slowly to the two-leaf stage than other alloplasmic lines which may inhibit pathogen onset. The effectiveness of the pathogen may be inhibited by immature leaves by way of less surface area. Since these alloplasmic lines have a minimum of at least 20 generations of backcrossing, the nuclear component is isogenic and the influence of cytoplasmic genome(s) is evident.

The gene-for-gene hypothesis states that for every resistant gene in the host, the pathogen has a corresponding gene conditioning avirulence (Flor 1942). In this case, the resistance comes from the cytoplasm (i.e. extra-nuclear or maternally inherited) and does not follow this hypothesis. The gene for gene motif exemplifies a single gene that can provide complete resistance. Tan spot demonstrates multiple genes involved in complete resistance since there are

several different toxins interacting with the host-pathogen interaction. The host nuclear donors do not possess the required resistance genes against all three toxins of Ptr isolate Br15. We utilized several alloplasmic lines containing identical nuclei and differential cytoplasms to accurately display the different interactions provided by the plant to retard the pathogens progress. Although, the response did not result in immunity, or lack of disease, there was a decrease in the severity of the disease. We can conclude that the NC interactions affect the host ability to respond to Ptr isolate Br15.

Isolate Br15 toxin, ToxA, interacts with host *tsn*1 susceptibility gene. However, the absence of *Tsn1* gene in the host does not always confer complete resistance to Ptr ToxA (Friesen et al. 2003) as it should with the proposed inverse-gene-for-gene method. Depending on the race of tan spot, different symptoms often look the same as if *Tsn1* was present. This would indicate that there are more toxins and interactions in addition to *Tsn1*-ToxA. Ptr ToxA has been previously described as a necrotizing agent. The alloplasmic lines screened with isolate Br15 showed both symptoms of necrosis and chlorosis indicating a possible secondary necrotizing agent involved with virulence. In a similar assessment, ToxB seems to follow the inverse gene-for-gene hypothesis whereby infection is determined by pathogen recognition of host receptors or active host searching by pathogen toxins and interactions in addition to *Tsn1*-ToxA. As with ToxA, the presence of the susceptibility gene in the host leads to disease symptoms.

Two different accessions of *T. dicoccoides* showed a significant decrease in disease response when compared to their respective euplasmic donors; *Triticum dicoccoides* Okla 11140 cytoplasm with Selkirk nucleus and *T. dicoccoides* G1453 cytoplasm with durum line 56-1. These two accessions give resistance with two different nuclei suggesting the two accessions are sufficiently different to respond to different nuclei, yet a similar phenotype is obtained. This

would propose the NC interaction of this cytoplasm retards disease development. The interaction of *T. dicoccoides* Okla 11140 (SSM0022) Selkirk alloplasmic line may imply resistance is introduced through the cytoplasm without interference of nuclear susceptible alleles. This response could indicate a specific NC interaction increasing resistance to Br15 of tan spot. Introgressing lower-ploidy species with higher-ploidy species such as bread wheat (*Triticum aestivum*) can cause incompatibilities (Kolmer 1996). The *T. dicoccoides* G1453 (SSM0107) 56-1 alloplasmic line conferred resistance. Durum line 56-1 (tetraploid) appears to have a highly susceptible reaction to the Br15 isolate of tan spot. The decreased sensitivity in the alloplasmic line demonstrates the introduction of resistance through the cytoplasm. However, the average score given to the *T. dicoccoides* G1453 (SSM0107) 56-1 alloplasmic line was identified as moderately susceptible (3) in one experiment and somewhat resistant (2) in the other; the overall score indicated a significant decrease in the value given that of the euplasmic donor (3.5).

Five of the fourteen-alloplasmic lines indicated increase resistances as compared to their respective nuclear donors. These alloplasmic lines represent possible unique NC interactions. *Aegilops mutica* (SSM0045) Selkirk alloplasmic line, *Ae. sharonensis* (SSM0076) 56-1 alloplasmic line, *Ae. Squarossa* (SSM0202) Chris alloplasmic line, *Ae. heldreichii* (SSM0240) Chris alloplasmic line, and *T. macha* 140191 (SSM0254) Chris alloplasmic line demonstrated increased resistance. The hypothesis stated here is that specific NC interactions are the source of increased resistance would suggest that the resistance mechanisms of these alloplasmic lines may not follow the conventional models.

Triticum macha 140191 (SSM0254) Chris alloplasmic line is the only *T. macha* accession that showed increased resistance to tan spot isolate Br15. *Triticum macha* 140191 (SSM0027) Selkirk alloplasmic line had a P value of 0.2379, a non-significant response relative

to the euplasmic donor. *Triticum macha* 190923 (SSM0028) Selkirk alloplasmic line had a P value of 0.6805 indicating response similar to that of the euplasmic donor. *Triticum macha* (SSM0253) Chris alloplasmic line had a P value of 0.5458 indicating a reaction similar to the euplasmic donor. Three different nuclei and accession combinations of different *T. macha* showed a similar response as their respective euplasmic donors. The cytoplasm of susceptible accessions may not contain the resistance mechanism previously described in other accessions. The three accessions of *T. macha* do not appear to influence disease resistance to isolate Br15. The increased resistance of SSM0254 suggests that the different *T. macha* accessions have negative or no influences on the resistance mechanism against tan spot isolate Br15. The NC interactions should be characterized as a means to increase disease resistance. It is apparent that the different cytoplasmic accessions may interact with the nuclei uniquely resulting in increased susceptibility and increased resistance.

In similar assessments, two cytoplasms showed a significant increase in disease susceptibility when compared to their corresponding euplasmic donors; *Ae. cylindrica* and *Ae. crassa*. The resulting susceptible lines may indicate incompatibility of the mitochondrial component involved with a hypersensitive response influencing disease resistance. This accession of *Ae. crassa* cytoplasm's interaction with the Chris nucleus makes this specific combination more susceptible. It is important to point out that this NC interaction can result in all three scenarios of increased resistance, increased susceptibility and/or no effect. The euplasmic line displays moderate levels of susceptibility that are not affected by the cytoplasm. The influence of increased susceptibility may result with the incompatibility of NC interactions against pathogen attacks. This describes the importance of the cytoplasm accessions involved in influencing resistance as a critical component. The specific combinations of cytoplasm and

nucleus provide a visual indication that there is an underlying mechanism involved in disease response.

The Selkirk nucleus and cytoplasm of *Ae. cylindrica* (SSM0004) has a sufficient amount of backcrosses (13) that constitutes an isogenic nuclei and cytoplasmic genome. However, since the Chris nucleus and cytoplasm of *Ae. crassa* (SSM0193) went through only three generations of backcrossing before selfing, it is difficult to know if the nuclear genome has been sufficiently replaced to represent only the nuclear donor (~87.5%). If neither the nucleus nor the cytoplasm is exhibiting true form, it may not result in a demonstration of increased susceptibility or resistance.

Alternative cytoplasms are the only variation within the experiment. We can conclude the motif of disease resistance could be more complex than previously stated. The mitochondria effects and multiple nuclear genes could be involved in differential responses. Although the parental lines display susceptibility, the alloplasmic lines show a wide range of both increased and decreased susceptibility. To determine which genetic material is responsible for delaying progress of the disease, in depth genetic studies are required. To identifying disease responses as localized or systemic would require additional observations. Those observations would include plants infected at the four-leaf stage and at the lower leaves of the host. Several days after the initial inoculation, a second inoculation would be performed on the same host plant of the upper leaf with the pathogen or an additional pathogen. In theory, if the plant is more resistant, then the possibility of systemic acquired resistance (SAR) could be observed. Identifying pathogenesis-related proteins (PR proteins) within the host would support this finding of resistance. The toxin model of resistance is very complex and there are several hypotheses about disease resistance. A hypersensitive response is a candidate response that results in localized necrotic lesions at the site of infection. Observation of the rapid uptake of oxygen during the initial infection would

indicate whether the alternative hypothesis supports the HR mechanism. The rapid uptake of oxygen is due to the mitochondrial respiratory chain signaling along a cellular pathway in response to the plant being attacked by a pathogen.

We have observed delayed disease progression, and partial resistance, which has not been observed previously in other alloplasmic studies. This resistance mechanism is introduced as an alternative hypothesis that does not fit the conventional disease resistance model. We have observed characteristics that decreased sensitivity to the tan spot isolates tested, different than both Faris et al. 2005 and Lamari et al. 2003.

Phenotypic Analysis of Isolate Pti2 of Tan Spot

Table 4 summarizes the significant comparison amongst all genotypes (treatment) relative to their respective euplasmic donor. A mixed model was used to analyze three fixed effects including season, genotype, and day (Table A4). Season is defined as one replicated trial of each experimental block with isolate Pti2. Genotype is defined as the treatment. Each genotype is defined as the treatment. Genotype has a significant influence in comparison to the other treatments. Day is defined as the average scores recorded on day three and day five post inoculation. Genotype × day and season × genotype × day are the non-significant interactions. The non-significant interactions are determined with a p-value that was higher than the 95% confidence level.

Significant differences are identified in the last column with p-values (Table A5). Genotype, season, and days were placed in the mix model to identify the average scores of disease response. Significant differences were observed among alloplasmic versus euplasmic lines for disease response to isolate Pti2. The scoring system used followed that of the Bernier

and Lamari 1989 for scoring tan spot disease with the additional score of zero. The mixed model procedures included a general linear model (GLM) to compare average score estimation between euplasmic and alloplasmic scores. The average euplasmic scores were (Table A6), Chris=2.41(+/-1.11), Selkirk=1.95 (+/- 0.74), and durum line 56-1=3.57 (+/- 1.47).

Table 4. Significant responses of alloplasmic lines compared to euplasmic lines using isolate Pti2 of tan spot

Genotype	Cytoplasm	Genotype	Cytoplasm	Nucleus	Pr > t	Reaction
SSM0069	*Ae. longissimum*	SSM0318	PARENT	56-1	<0.0001**	Resistant
SSM0076	*Ae. sharonensis*	SSM0318	PARENT	56-1	<0.0001**	Susceptible
SSM0085	*Ae. variabilis*	SSM0318	PARENT	56-1	<0.0001**	Susceptible
SSM0107	*T. dico.* G1453	SSM0318	PARENT	56-1	0.0022**	Resistant
SSM0187	*Ae. bicornis*	SSM0318	PARENT	Chris	0.0046**	Resistant
SSM0206	*Ae. variabilis*	SSM0258	PARENT	Chris	0.0233*	Resistant
SSM0240	*Ae. heldraichi*	SSM0258	PARENT	Chris	0.0458*	Resistant

*Significance level of 5% (α=0.05)
**Significance level of 1% (α=0.01)

Several alloplasmic lines showed responses similar to that of their respective euplasmic donor when screened with Ptr isolate Pti2. There were no alloplasmic lines with the Selkirk nucleus that demonstrated a differential response relative to their euplasmic donor. One difference between the isolate Pti2 and Br15 is that Pti2 does not produce ToxB. However, Pti2 is one of the most virulent strains in our collection of isolates. Several alloplasmic lines of Chris seem to have increased resistance to both Ptr isolates Pti2 and Br15. Nuclear donor Chris in the cytoplasmic background of *Ae. bicornis* (SSM0187), *Ae. variabilis* (SSM0206), and *Ae. heldreichii* (SSM0240) displayed resistance to Pti2. These three cytoplasm accessions seem to have a less sensitive response than that of the euplasmic donor. The new cytoplasmic component, containing mitochondria and chloroplast DNA, may condition a different source of disease resistance. *Aegilops variabilis* (SSM0085) durum line 56-1 alloplasmic line confers

susceptibility to Pti2 while *Aegilops variabilis* (SSM0206) Chris alloplasmic line confers resistance. Although they share the same cytoplasm, the NC interaction may function differently in tetraploid wheat (56-1) than hexaploid wheat (Chris).

The cytoplasm backgrounds of *Ae. longissimum khaplis* and *T. dicoccoides* G1453 with the nuclear donor of 56-1 (tetraploid) showed a significant increase in resistance as compared to the euplasmic donor. *Triticum dicoccoides* G1453 with cv. 56-1 nucleus showed significantly increased resistance to both isolates of tan spot, Br15 and Pti2. The other cytoplasm accessions of 56-1, *Ae. sharonensis* and *Ae. variabilis*, showed significant increases of susceptibility when compared to the euplasmic donor. *Aegilops sharonensis* (SSM0076) with cv. 56-1 has contrasting responses to the isolate screening. SSM0076 was observed as showing increased resistance to Br15, but increased susceptibility to Pti2.

The aggressiveness of Pti2 could correspond to similar responses of alloplasmic lines to the euplasmic donor Selkirk. The toxins appear to follow the inverse gene for gene model and therefore the loss of either the susceptibility gene or the effectors would result in no disease. Since this conversion agrees to the inverse gene-for-gene hypothesis, this method of resistance is inherited by the nucleus. All of the alloplasmic lines of Selkirk were resistant possibly due to the absence of a susceptibility locus in the nucleus. Although it is apparent that the cytoplasm does induce resistance in alloplasmic lines of Chris, the resistance mode of action does not seem to affect the alloplasmic lines of Selkirk.

In the case of the nuclear donor Chris in the cytoplasmic background of *Ae. bicornis* (SSM0187), *Ae. variabilis* (SSM0206), and *Ae. heldreichii* (SSM0240), they demonstrated an increased resistance response to two different isolates of Ptr isolates Br15 and Pti2. The

euplasmic donors were moderately susceptible, indicating the absence of the traditional model for resistance mechanism. Therefore, there is an alternative resistance response to the tan spot isolates. The role of inducing higher ROS accumulation may fit this hypothesis. The production of ROS belongs to the mitochondrial respiratory chain which is located in the cytoplasm. The introduction of a new source of cytoplasm, containing mitochondria and chloroplast genomes, may influence a different mechanism than cultivated wheat, which increases the rate of ROS accumulation at the site of infection. The new NC interaction may compose an alternate hypothesis for disease resistance.

An explanation of differential responses between two different polyploidy wheat could be the introduction of additional chromosomes. An alternative resistance explanation could indicate the NC interaction between hexaploid wheat (Chris) and tetraploid (56-1) is due to the additional D chromosomes of *Triticum aestivum* (hexaploid). The additional chromosome would make the alloplasmic pairing of the new cytoplasm more stable during meiosis. The combination that confers resistance would be *Ae. variabilis* (SSM0206) Chris alloplasmic line. This would indicate that this particular NC interaction would influence disease resistance through the cytoplasmic component. Future testing of this cytoplasm with modern/elite cultivars needs to be conducted.

Durum line 56-1 shows variable degrees of resistance and susceptibility as it does not agree with previous NC interactions. This contrasting interaction could have resulted from an absence of virulence genes that are typically present in the pathogen. The absence of ToxB in Pti2 could also suggest that susceptibility is due to the lack of recognition of a necessary elicitor produced by the pathogen. The two different accessions conferring increased resistance may suggest that the cytoplasmic component, though different, may have a similar resistance

mechanism involving ROS accumulation. As for the two accessions of alloplasmic lines demonstrating increased sensitivity, the cytoplasm background may not contain the resistance mechanism of those resistant lines.

Summary

The effects of alien cytoplasm substitution on the response of wheat to *Pyrenophora tritici-repentis* were examined, using alloplasmic lines of two hexaploid cultivars, Chris and Selkirk, and one tetraploid line, 56-1. The conventional model of disease resistance does not seem to fit the model of cytoplasm substitution. Cytoplasm substitution caused unidirectional effects on *P. tritici-repentis* -response, alloplasmic lines of three cultivars expressed increased resistance in comparison to the corresponding euplasmic lines. The reduced resistance observed in the alloplasmic lines was associated with the parental lines displaying moderate susceptibility to both tan spot isolates. The susceptible parental lines indicated that the resistant alleles are not present in the cultivar. Specific cytoplasms employed differential responses of resistance in the parental cultivars. This would suggest an alternative model of resistance that is induced by cytoplasm substitution. The cytoplasmic genome of both mitochondria and chloroplast has been replaced resulting in an altered NC interaction. One possible hypothesis is that the mitochondrial respiratory chain is more prone to ROS accumulation in response to the pathogen attack due to altered NC interaction. The increase in susceptible lines may indicate cytoplasmic incompatibility that disrupts this important function. Future testing of the cytoplasmic component is needed to support this hypothesis, but it is apparent that the introduction of an alien cytoplasm confers resistance. The potential for propagating *P. tritici-repentis* -tolerant cultivars by incorporating alien cytoplasm has been observed, although further experiments are needed with regard to agronomic traits and suitability of use in modern cultivars.

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CHAPTER 2. CYTOPLASMIC SUBSTITUTION EFFECT ON LEAF RUST (*PUCCINIA TRITICINA*)

Abstract

Experiments were conducted to evaluate the potential of increased disease resistance to *Puccinia triticina* as a result of cytoplasmic substitution. This experiment used alloplasmic lines of two spring wheat cultivars (*T.aestivum*), Chris and Selkirk. Four rust isolates [THBL, MCDL, MFPS, and TDBG] were used as a bulk inoculation on a set of euplasmic and alloplasmic stock. Twelve treatments (genotype) with two replicates (blocks) with three plants per treatment were used in the initial screening. A supplementary experiment with an additional number of Chris alloplasmic lines were expanded from eight to 26 different cytoplasm accessions. Randomized complete block design (RCBD) with three replicates (blocks) and one genotype (treatment) per replicate that included four plants was used to identify the symptomology of each genotype. Scoring followed the nomenclature system for designating virulence combinations of cultures of leaf rust in North America accepted by the North American Wheat Leaf Rust Research Workers Committee in 1986. Euplasmic Chris displayed susceptibility and euplasmic Selkirk was resistant to the bulk inoculum. Chris alloplasmic lines *Ae. crassa* (SSM0193), *Ae. cylindrica* (SSM0195), *Ae. juvenalis* (SSM0196), *Ae. kotschyii* (SSM0197), *T. araraticum* Th (SSM0215), *Ae. bicornis* (SSM0237), *Ae. heldreichii* (SSM0240), and *Ae. mutica* (SSM0242) were identified as resistant out of the 26 alloplasmic lines screened. *Aegilops heldreichii* (SSM0240) Chris alloplasmic line had the most resistant reaction (flecking) and should pursue additional experiments as a cytoplasm candidate for leaf rust resistance.

Introduction

Puccinia triticina Eriks., the causal agent of leaf rust, is among the most commonly occurring pathogen of all cereals. Sources of genetic resistance are valuable to increase the sustainability of cereal production, from both economic and environmental standpoints (Reynolds and Borlaug 2006). Throughout the years there have been several techniques involving identifying resistant genes and introgressing them into modern cultivars. The gene-for-gene (Samborski and Dyck 1968) relationship between wheat (*Triticum aestivum*) and *Puccinia triticina* pathosystem has been studied thoroughly throughout the years. For every resistance gene in the host, there is a corresponding locus in the pathogen that condition avirulence ((Flor 1947; Flor 1956). Those wheat varieties that rely on race-specific resistance often lose effectiveness within a few years by forcing the selection for virulent leaf rust races (Bolton et al. 2008). Race-specific resistance is usually displayed with a hypersensitive response (HR) of rapid cell death that occurs between the fungal uredinia and the host cells in the mesophyll layers. Washington and Maan (1974) conducted experiments involving alloplasmic wheat lines and their reaction to leaf rust isolates. They concluded that certain alien cytoplasms may alter the expression of host nuclear genes for resistance to certain races of leaf rust. In this study, we followed these early experiments to determine if an alternative source of the cytoplasm will confer resistance to multiple rust isolates in the susceptible euplasmic line Chris.

Material and Methods

Puccinia triticina (Leaf Rust) Screening

This experiment used alloplasmic lines of two spring wheat cultivars (*T.aestivum*), Chris and Selkirk. The cytoplasm donors were those of genus *Aegilops* and *Triticum* (Table 5). Four

rust isolates [THBL, MCDL, MFPS, and TDBG] were used as a bulk for inoculation on the euplasmic and alloplasmic stock. Twelve treatments (genotype) with two replicates (blocks) with three plants per treatment were used in the initial screening. A supplementary experiment with an additional number of Chris alloplasmic lines were expanded from eight to 29 different cytoplasm accessions (Table 5) with a susceptible control (Chris euplasmic) and resistant control (Selkirk euplasmic).

Experimental Design

Randomized complete block design (RCBD) with three replicates (blocks), and one genotype (treatment) per replicate that included four plants were used to evaluate the symptomology of each genotype. Additional replicates were used to validate the results. Scoring followed the nomenclature system for designating virulence combinations of cultures of leaf rust in North America accepted by the North American Wheat Leaf Rust Research Workers Committee in 1986 (Long et al. 2000). Scoring was based on three criteria; the various sizes of uredinia, presence or absence of chlorosis/necrosis, and distribution of uredina.

Plant Growth and Inoculation

Seeds of parental and alloplasmic lines were placed in trays that contained sunshine mix combined with soil (50% v/v) and grown to the two leaf stage. The seedlings were watered and fertilized with osmocote as needed. Fourteen day old plants were inoculated with a bulk set of four leaf rust isolates THBL, MCDL, MFPS, and TDBG; each letter indicating a set of virulence combination differential. Inoculum contained urediospores from all four isolates suspended in a clear gel capsule using isoparaffin oil (sotrol170) to allow for a consistent distribution among wheat varieties. Inoculation was done with compressed air at 2 psi. Before misting began, the oil

based substance was allowed to dry to prevent the urediospores from running off the cuticle of the leaves when they were being misted. Blocks were then placed in the misting chamber for 24 hours with misting occurring for 20 seconds every four minutes. Plants were then placed in the greenhouse after inoculation and scored for disease reaction 10-14 days post-inoculation.

Table 5. Germplasm screened for leaf rust

Accession #	Nucleus	Cytoplasm	Generation	Constitution
SSM0187	Chris	*Ae. bicornis*	Self2-BC27	Alloplasmic
SSM0192	Chris	*Ae. crassa*	Self2-BC9	Alloplasmic
SSM0193	Chris	*Ae. crassa*	Self2-BC3	Alloplasmic
SSM0194	Chris	*Ae. cylindrica*	Self2-BC10	Alloplasmic
SSM0195	Chris	*Ae. cylindrica*	Self1-BC14	Alloplasmic
SSM0196	Chris	*Ae. juvenalis*	Self2-BC15	Alloplasmic
SSM0197	Chris	*Ae. kotschyii*	Self2-Sk13-Chr3	Alloplasmic
SSM0198	Chris	*Ae. longissimum*	Self2-BC6	Alloplasmic
SSM0199	Chris	*Ae. searsii*	Self2-Ae.sq-Sk5-Chr	Alloplasmic
SSM0202	Chris	*Ae. squarrosa*	Self2-BC18	Alloplasmic
SSM0205	Chris	*Ae. uni* G633	Self2-BC10	Alloplasmic
SSM0206	Chris	*Ae. variabilis*	Self2-BC20	Alloplasmic
SSM0207	Chris	*Ae. vavilovi*	Self2-Sk7-Chr	Alloplasmic
SSM0208	Chris	*Ae. ventricosa*	Self2-BC9	Alloplasmic
SSM0210	Chris	*Haynaldia triticum* D.	Self2-BC10	Alloplasmic
SSM0211	Chris	*T. araraticum* 5E	BC22	Alloplasmic
SSM0215	Chris	*T. araraticum* Th	BC15	Alloplasmic
SSM0217	Chris	*T. dico.* G1396	BC14	Alloplasmic
SSM0229	Chris	*T. paleocolchium*	Self2-BC9	Alloplasmic
SSM0237	Chris	*Ae. bicornis*	Self2-BC25	Alloplasmic
SSM0240	Chris	*Ae. heldreichii*	Self2-BC10	Alloplasmic
SSM0241	Chris	*Ae. longissimum*	Self2-BC5	Alloplasmic
SSM0242	Chris	*Ae. mutica*	Self2-BC9	Alloplasmic
SSM0253	Chris	*T. macha*	Self2-BC19	Alloplasmic
SSM0254	Chris	*T. macha* 140191	Self2-BC21	Alloplasmic
SSM0255	Chris	*T. macha* 140191	Self2-BC21	Alloplasmic
SSM0258	Chris	*Cv. Chris*	Chris Parent	Euplasmic
SSM0317	Selkirk	*Cv. Selkirk*	Selkirk Parent	Euplasmic

Results and Discussion

Puccina triticina (Leaf Rust)

Two experiments were performed to determine the response of experimental lines to leaf rust. The first experiment was done to confirm the resistant and susceptible reaction of paternal controls, Selkirk and Chris. In addition, this experiment included 12 different sets of alloplasmic lines showing resistance to tan spot (Table 6). A bulk of four common leaf rust isolates identified in the upper Great Plains of the US was used as the inoculum. Four alloplasmic lines of cv. Selkirk with *Ae. cylindrica* (SSM0004), *T. dicoccoides* G671 (SSM0021), *T. dicoccoides* Okla 11140 (SSM0022), and *Ae. bicornis* (SSM0039) were resistant, similar to their respective euplasmic donor. This reaction was expected due to the resistance genes found in the nucleus. Similarities between alloplasmic and euplamsic lines demonstrate the substituted cytoplasms did not alter disease resistance, and the nucleus has a dominant resistance effect on these lines. Cultivar Selkirk presents a resistance response of small-to medium-size uredinia along with chlorosis, an apparent hypersensitive reaction (HR). However, *T. dicoccoides* G671 presented a small variation in resistance response, displaying small uredinia and small necrotic halos. This response could involve the NC interaction inducing a necrotizing response rather than a chlorotic response influenced by the cytoplasm.

Six of the eight Chris alloplasmic lines with *Ae. bicornis* (SSM0187), *Ae. cylindrica* (SSM0194), *Ae. variabilis* (SSM0206), *Ae. bicornis* (SSM0237), *Ae. mutica* (SSM0242), and *T. macha* 140191 (SSM0254), showed similar susceptible disease responses as their euplasmic parent, Chris. Two alloplasmic lines with cytoplasms from *Ae. crassa* (SSM0191) and *Ae. heldreichii* (SSM0240) , showed a differential disease resistance response. With an average

disease response score 2++, *Ae. crassa* (SSM0191) showed responses similar to cv. Chris. *Aegilops heldreichii* cytoplasm however, exhibited a highly resistant response. The leaves displayed flecking with no uredinia present, along with very small uredinia and adjacent necrosis. This observation indicates that the NC interaction is conferring resistance to a bulk inoculum of wheat leaf rust. The highly resistant line was observed at the seedling stage, but whether it confers adult resistance will need to be determined. A thorough investigation is needed to characterize these interactions. An additional experiment was done with additional Chris alloplasmic lines (Table 7) to confirm the initial results.

Table 6. Scoring of alloplasmic lines for initial leaf rust screening

Treatment #	Nucleus	Cytoplasm	Rep 1	Rep 2	Results
SSM0004	Selkrik	*Ae. cylindrica*	2+	2+	Resistant
SSM0019	Selkirk	*T.dico* G671	1n	2+	Resistant
SSM0022	Selkirk	*T.dico* Okla 11140	2++	2++	Resistant
SSM0039	Selkirk	*Ae. bicornis*	2++	2++	Resistant
SSM0187	Chris	*Ae. bicornis*	3	3	Susceptible
SSM0191	Chris	*Ae. crassa*	2++	2++	Resistant
SSM0194	Chris	*Ae. cylindrica*	3	3-	Susceptible
SSM0206	Chris	*Ae. variabilis*	3	3	Susceptible
SSM0237	Chris	*Ae. bicornis*	3	3	Susceptible
SSM0240	Chris	*Ae. heldreichii*	;,1	1,;	Resistant
SSM0242	Chris	*Ae. mutica*	3	3	Susceptible
SSM0254	Chris	*T. macha* 140191	3	3	Susceptible
SSM0258*	Chris	Cv. Chris	3	3	Susceptible
SSM0317*	Selkirk	Cv. Selkirk	2++	2+	Resistant

Scoring based on the North American nomenclature for *Puccinia triticina* (Long and Kolmer 2000)
*Paternal nuclear donor lines

The second experiment was conducted with 26 alloplasmic lines derived from cv. Chris (Table 7). These alloplasmic lines were chosen to validate the initial results, and to identify other possible combinations that confer a differential response. Disease screenings were carried out as previously described. Disease resistance symptomology varied from chlorosis and necrosis

denoting different interactions. Alloplasmic lines derived from Selkirk were not pursued due to resistance within the parental line. A range of variation between infection types is recorded by indicating the range, with the most prevalent infection type listed first.

Table 7. Scoring of alloplasmic lines for second leaf rust screening

Treatment #	Nucleus	Cytoplasm	Rep 1	Rep 2	Rep 3	Results
SSM0187	Chris	*Ae. bicornis*	3n,3	3,3+	2	Susceptible
SSM0192	Chris	*Ae. crassa*	3	3	3	Susceptible
SSM0193	Chris	*Ae. crassa*	2n,2	2n	2+	Resistant
SSM0194	Chris	*Ae. cylindrica*	3,3n	3+	3	Susceptible
SSM0195	Chris	*Ae. cylindrica*	2-,2	2,2n	2++	Resistant
SSM0196	Chris	*Ae. juvenalis*	2,2n	2+	3	Resistant
SSM0197	Chris	*Ae. kotschyii*	2+	2++	3	Resistant
SSM0198	Chris	*Ae. longissimum*	3,3n	3	3	Susceptible
SSM0199	Chris	*Ae. searsii*	3	3	2-,3	Susceptible
SSM0202	Chris	*Ae. squarrosa*	3-,3	3+	3n,3	Susceptible
SSM0205	Chris	*Ae. uni* G633	3+	3	2++	Susceptible
SSM0206	Chris	*Ae. variabilis*	3	3+	3	Susceptible
SSM0207	Chris	*Ae. vavilovi*	3n,2+	2++,3	3,2n	Susceptible
SSM0208	Chris	*Ae. ventricosa*	3,2++	3,2++	3,2n	Susceptible
SSM0210	Chris	*Haynaldia triticum* D.	3	3	3	Susceptible
SSM0211	Chris	*T. araraticum* 5E	3+	3	3	Susceptible
SSM0215	Chris	*T. araraticum* Th	2+,3	2+	2,2n	Resistant
SSM0217	Chris	*T. dico.* G1396	3,3n	2++,3	3-,3n	Susceptible
SSM0229	Chris	*T. paleocolchium*	3	3-	3	Susceptible
SSM0237	Chris	*Ae. bicornis*	3,3+	2	2++	Resistant
SSM0240	Chris	*Ae. heldreichii*	2-,;	2,2-	2-,;	Resistant
SSM0241	Chris	*Ae. longissimum*	3	3n,3-	3	Susceptible
SSM0242	Chris	*Ae. mutica*	2+	2+,3-	2++,3	Resistant
SSM0253	Chris	*T. macha*	3	3	3	Susceptible
SSM0254	Chris	*T. macha* 140191	3-	2++,3-	3-	Susceptible
SSM0255	Chris	*T. macha* 140191	3n,3-	3-,3	3,3-	Susceptible
SSM0258*	Chris	Cv. Chris	3,3n	3,3+	3,2++	Susceptible
SSM0317*	Selkirk	Cv. Selkirk	2++	2+	2+,2++	Resistant

Scoring based on the North American nomenclature for *Puccinia triticina* (Long and Kolmer 2000)
*Parental nuclear donor lines

Euplasmic Chris displayed susceptibility and euplasmic Selkirk was resistant to the bulk inoculum of leaf rust isolates. This experiment agrees with the study performed by Washington and Maan (1974). In their study, they concluded that certain alien cytoplasms may alter the expression of host nuclear genes for resistance to certain races of leaf rust. Also, the host pathogen interaction was influenced by host cytoplasm and nuclear genes and rust fungus. The experiments in this study indicate that cytoplasm substitution alters the expression of resistance genes with four races of leaf rust. The cytoplasm directly influences the fungal interaction that introduces resistance in the alloplasmic line versus the euplasmic line. The resistance can also be credited to the effect on the mitochondria that predisposes the defense response of the alloplasmic line but does not directly alter the gene expression.

Eight alloplasmic lines of cv. Chris indicated resistance to the bulk inoculation of four leaf rust isolates. Throughout the scoring analyses some scores indicated susceptibility (3's). Overall, most of the lines appeared resistant, and a HR reaction was indicated by the presence of necrosis. This does not suggest a conclusive interaction indicating complete resistance to all four leaf rust isolates. Single isolate treatments would indicate the level of resistance to each leaf rust isolate, identifying which resistance gene(s) are present in the nuclear genome. The appearance of both necrosis and chlorosis would support an underlying resistance mechanism that is influenced by the addition of a substituted cytoplasm. Alloplasmic lines with cytoplasms from *Aegilops crassa* (SSM0193), *Ae. cylindrica* (SSM0195), *Ae. juvenalis* (SSM0196), and *T. araraticum* Th. (SSM0215), showed a resistant response with small-to medium-uredinia surrounded by necrosis, which is not the most common response of chlorosis. Additionally, in the *Ae. mutica* (SSM0242) cytoplasm background, uredinia were larger than some of the individual responses as indicated with a (+) symbol. Alloplasmic line with cytoplasm *Aegilops*

kotschyi (SSM0197) conferred a resistance response with uredinia sizes larger than normal infection type surrounded by a HR response. This alloplasmic line was identified as resistant. Although the parental line was susceptible, certain cytoplasmic genomes influence the response to leaf rust isolates in the host plant. The eight cytoplasm backgrounds may have an altered expression of host nuclear genes. These altered expressions may provide resistance by influencing the cytoplasmic component to produce higher amounts of ROS.

Although one replicate of *Ae. bicornis* (SSM0237) Chris alloplasmic line displayed susceptibility, the other two replicates showed a resistant response. A susceptible response is possible because certain alien cytoplasms may alter the expression of host nuclear genes for resistance to certain leaf rust races (Washington and Maan 1974). The expression of the susceptible response could be the plants' inability to express those resistance genes in the host at the time. With two out of three replicates expressing resistance, *Aegilops bicornis* (SSM0237) Chris alloplasmic line was classified as resistant to all four isolates of leaf rust. This particular NC interaction is a candidate for horizontal resistance because it displays significant differential response to the tan spot isolates tested and to the four leaf rust races. *Aegilops bicornis* (SSM0237) Chris alloplasmic line seems to delay development to the two leaf stage similar to that of *Ae.bicornis* (SSM0187) Chris alloplasmic line. Nevertheless, these species cannot be identified by their individual cytoplasmic component because alloplasmic plants appear to have normal fertility and plant growth (Maan 1975). *Aegilops heldreichii* (SSM0240) Chris alloplasmic line was the only line that expressed fleck-type disease response, with no uredinia and traces of small HR across the leaves. The two minus (2-) rating indicates that the uredinia were somewhat smaller than normal for the infection type. The fleck-type disease response is indicative of a highly resistant response, which suggests a cytoplasmic involvement in disease

resistance that could lead to its use in elite cultivars. The resistance in seedling stage does not automatically suggest transfer to the adult stage, additional studies should be considered.

The other 18 alloplasmic lines showed variable levels of pathogenic susceptibility, none showing a disease score higher than 3+. *Aegilops squarrosa* (SSM0202), *Haynaldia triticum* D. (SSM0210), *T. paleocolchium* (SSM0229), and *T. macha* (SSM0253), are four Chris alloplasmic lines, representing alloplasmic lines whose score was similar to their parental line. The *Ae. cylindrica* (SSM0194) Chris alloplasmic line was classified as susceptible. The *Ae. cylindrica* (SSM0194) Chris alloplasmic line shares a cytoplasm obtained from the same species as *Ae. cylindrica* (SSM0195) Chris alloplasmic line. These lines are derived from different accessions of *Ae. cylindrica,* one being susceptible (SSM0194) and other being resistant (SSM0195). Similar observations can be said about Chris alloplasmic lines *Ae. crassa* (SSM0192) being susceptible and *Ae. crassa* (SSM0193) as resistant. The same could be said about susceptible *T. araraticum* 5E (SSM0211) and resistant *T. araraticum* Th (SSM0215). It should be noted that *T. araraticum* 5E (SSM0211), *T. araraticum* Th (SSM0215) and *T. dicoccoides* G1396 (SSM0217) are all male sterile. This incompatibility may not directly influence the onset or resistance of the host to the disease, but is an important physiological consideration. *Aegilops bicornis* (SSM0187) Chris alloplasmic line is a slow maturing line that displays susceptibility, both undesirable agronomic traits. Maturing slower than other alloplasmic lines, *Ae. bicornis* SSM0187 Chris alloplasmic line is susceptible to leaf rust but is resistant to the tan spot isolates tested. This may represent the development of the host as an important factor in the onset of these foliar diseases.

Aegilops longissimum (SSM0198) had normal infection type, though the first replicate indicated low amounts of necrosis surrounded by a few uredinia. This observation is not a

common occurrence with a susceptible reaction, although they do arise. The *Ae. longissimum* (SSM0241) accession showed susceptible reaction excluding necrosis, which is a normal infection type similar to that of the euplasmic line. *Aegilops searsii* (SSM0199) Chris alloplasmic line showed normal infection type as the susceptible line. The third replicate however, had more resistant symptomology displaying smaller uredinia than normal infection type. *Aegilops uniaristata* G633 (SSM0205) Chris alloplasmic line had larger uredinia than typical infection type with no chlorosis in the first replicate; but, showed nearly the same sized uredinia with chlorosis in the third replicate. The susceptible reaction was more frequent than the resistant reaction. *Aegilops variabilis* (SSM0206) Chris alloplasmic line was susceptible to all four leaf rust isolates. Though it is unclear if *Ae. variabilis* could be resistant to one or two of the component isolates, future experiments could determine which specific isolates elicit disease response. This cytoplasm confers resistance to tan spot, and apparently does not do the same for leaf rust.

In comparison to the previous experiment done by Washington and Maan (1974), alloplasmic Chris with *T. macha* 140191 had a mesothetic reaction to one leaf rust isolate (race 13). In this data set, *T. macha* 140191 in Chris background had a susceptible reaction to the bulked rust isolates. Individual testing with single isolates would determine whether this line has resistance to one or more of the isolates/races of leaf rust tested. As a conclusion to this data set, all three accessions of *T. macha* are susceptible to leaf rust. Two alloplasmic lines, *Ae. vavilovi* (SSM0207) and *Ae. ventricosa* (SSM0208) Chris alloplasmic lines, exhibited both susceptibility and resistance. Though there is no clear means of determining a specific level of susceptibility, a score of (3) indicates medium-sized uredinia without chlorosis or necrosis in every replicate. A score of (2) was given to the same leaf indicating it had an underlying mechanism of resistance

with signs of chlorosis and necrosis. This experiment needs to be replicated, possibly with individual isolates and bulks, to substantiate the reaction response.

Leaf rust resistance follows the classical gene-for-gene hypothesis. On the other hand, necrotrophic fungi appear to follow the inverse-gene-for-gene hypothesis based on the toxins they produced. At this time the cytoplasmic influences on disease resistance appear to fit either of these models. There are several pathways along which this alternative system may function. An alternative explanation for the increased disease resistance would be that the alien cytoplasm results in either a higher or more rapid accumulation of ROS that is induced with a hypersensitive reaction (HR). It appears that the euplasmic (parental) line Chris does not express resistance genes. With the introduction of the new cytoplasm, it may allow the expression of some normally silenced resistance gene(s) in the nucleus. The new cytoplasm can also produce higher than normal levels of ROS so that when the host is invaded, other events can stimulate the HR or release of pre-accumulated ROS. This is a proposed alternative system that responds more quickly to pathogen attacks without the need of the classical signaling. Although this hypothesis has not been tested, it is possible that the mitochondria are responsible for increased disease response.

These experiments display an increased disease resistance response influenced by the cytoplasm. This response suggests that cytoplasmic variability would increase the role of resistance mechanism which would improve the wheat cultivar Chris. The absence of resistance alleles in the paternal donor would indicate the cytoplasmic contribution of an increased resistance mechanism. The euplasmic line Chris does not display resistance, yet these eight alloplasmic lines display various levels of resistance. The specific combination of Chris nucleus and alien cytoplasms confers some level of disease resistance to leaf rust. The data presented

agrees with the studies previously visited by Washington and Maan 1974 and concludes that the cytoplasm has a critical role in host-pathogen interaction. These experiments identify important characteristics of alloplasmic lines and their significant role towards increasing disease resistance.

Summary

The effects of cytoplasmic substitution with alloplasmic wheat in response to *Puccinia triticina* were examined using hexaploid cultivar Chris and 29 Chris alloplasmic lines. The objective of this experiment was to observe any variable responses of Chris alloplasmic lines to determine if cytoplasmic substitution has any effect on the disease reaction to leaf rust. The Chris parental line expressed a susceptible response. Eight of the 29 Chris alloplasmic lines screened resulted in resistance as compared to the parental Chris line to the bulk inoculums of leaf rust isolates. Although it does not appear that the resistant alleles are not absent in the Chris cultivar, there is an alternative mode of resistance influenced by cytoplasm substitution. The retarded development and size of uredinia expresses a differential response unequivocal to conventional disease resistance mechanisms. Substituting the cytoplasmic genome of both mitochondria and chloroplast favors the hypothesis that the altered NC interactions give rise to an alternative mode of resistance. Although future experiments with additional isolates and alloplasmic lines are needed, it is apparent that substituting cytoplasms may positively alter disease response.

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CHAPTER 5. GENERAL CONCLUSION

This study of differential responses to tan spot was conducted with three identical experiments containing the same number of replicates, treatments and isolates. Comparisons were done with a set of alloplasmic lines featuring the same nuclear donor (euplasmic line). The results confirmed that the cytoplasm can provide increased resistance in the host-pathogen interaction. Three scenarios of increased resistance during host-pathogen interaction have been described i) The ability of one cytoplasm inducing resistance to any nuclear donor; ii) two different cytoplasm accessions with identical nuclear donor conferring resistance, and; iii) one cytoplasmic accession presents increased resistances while the other accessions fail to do so.

Aegilops bicornis (SSM0039, SSM0187, and SSM0237) cytoplasm in the Selkirk and Chris nuclear background manifested significant disease resistance response as compared to euplasmic donor species. We can hypothesize that the cytoplasmic component is conferring resistance to two different nuclear donors. The compatibility with *Ae. bicornis* to the cultivars used in this study allows for uniform increase of disease resistance through the cytoplasm. This cytoplasm may be used to generate a general increased resistance independent of the nucleus. Additional experiments would need to test other nuclear donors to confirm such observation. Although the specific mechanism is unclear, the hypothesis of high ROS accumulation introduced through the cytoplasm can be considered. As the ROS is involved in important signaling pathways, the host response to the disease could develop a high number of ROS that reduces further development of disease. The cytoplasm assists in pathogen defense contributing to an alternative role of resistance.

Several alloplasmic lines displayed significant differential responses to one or the other isolates. *Aegilops bicornis* (SSM0187), *Ae. Variabilis* (SSM0206), and *Ae. heldreichii* (SSM0240) with cv. Chris nucleus were resistant to both Br15 and Pti2 isolates of tan spot. This may indicate that the combination of the nucleus and cytoplasm may be the optimum combination to combat all races of tan spot. *Aegilops bicornis* (SSM0187) cytoplasm with Chris nucleus is the slow maturing alloplasmic line with significant resistance to both tan spot isolates tested. For propagating new cultivars, slower maturing cultivars would be considered a deleterious side effect. *Aegilops heldreichii* does not have those phenotypes involved in delayed maturity. This particular NC interaction is a candidate for horizontal resistance because it displays a significant differential response to tan spot and leaf rust pathogens. The phenotype of *Ae. heldreichii* (SSM0240) Chris alloplasmic line appears to be no different than other alloplasmic or euplasmic lines in the collection of germplasm. *Aegilops heldreichii* should be taken into strong consideration as a new source of cytoplasm. *Triticum macha* 140191 (SSM0254) Chris alloplasmic line cytoplasmic accession presents increased resistances while the other accessions fails to do so. This specific nuclei and cytoplasmic accession seems to alter the NC interactions and increase the resistance to the tan spot isolates tested. Although the other accession do not have a significant effect to tan spot isolates tested, additional experiments should determine whether this accession improves disease resistance while the others ones do not.

Regarding cytoplasmic substitution and leaf rust resistance, certain alien cytoplasm combinations may alter the expression of host nuclear genes for resistance to certain races of wheat leaf rust (Washington and Maan 1974). We failed to reject the notion that no single cytoplasmic genome in any nuclear background conferred resistance to all the pathogens tested.

We observed several differential responses to tan spot and leaf rust isolates within our collection of euplasmic and alloplasmic lines. The data representing tan spot isolates identified three scenarios that display resistance with an alien cytoplasm. The leaf rust data confirms those finding conducted by Washington and Maan in 1974. The alternative hypothesis of high ROS accumulation would need to be tested with future experiments. The cytoplasm does contain both mitochondria and chloroplast DNA which is important for cellular signaling controlling ROS production. Therefore, it is important to disprove the observed hypothesis that the mitochondria respiratory chain producing ROS influences the disease response in alloplasmic lines.

APPENDIX

Table A1. Type III tests of fixed effects to tan spot isolate Br15

Effect	Num DF	Den DF	F Value	Pr > F
Season	2	33	7.65	0.0019**
Genotype	47	172	4.40	<.0001**
Season × genotype	94	204	4.98	<.0001**
Day	1	172	586.45	<.0001**
Season × day	2	204	14.87	<.0001**
Genotype × day	47	172	1.20	0.2042
Season × genotype × day	94	204	1.15	0.1993

*Significance level at $p < 0.05$
**Significance level at $p < 0.01$

Table A2. Differences of Least Squares Means (LSmeans) comparing the average score of the euplasmic lines compare to the alloplasmic lines inoculated with isolate Br15 of tan spot

Effect	Alloplasmic	Euplasmic	Estimate	Standard Error	DF	t Value	Pr > |t|
genotype	SSM0003	SSM0317	-0.1001	0.2235	172	-0.45	0.6546
genotype	SSM0004	SSM0317	-0.4457	0.2218	172	-2.01	0.0460
genotype	SSM0015	SSM0317	0.2835	0.2265	172	1.25	0.2124
genotype	SSM0016	SSM0317	0.1251	0.2282	172	0.55	0.5844
genotype	SSM0017	SSM0317	0.07517	0.2218	172	0.34	0.7351
genotype	SSM0018	SSM0317	0.1879	0.2235	172	0.84	0.4016
genotype	SSM0019	SSM0317	-0.1262	0.2265	172	-0.56	0.5781
genotype	SSM0020	SSM0317	-0.04983	0.2218	172	-0.22	0.8225
genotype	SSM0021	SSM0317	-0.04983	0.2218	172	-0.22	0.8225
genotype	SSM0022	SSM0317	-0.6282	0.2235	172	-2.81	0.0055
genotype	SSM0023	SSM0317	-0.09149	0.2218	172	-0.41	0.6805
genotype	SSM0024	SSM0317	-0.1332	0.2218	172	-0.60	0.5490

(CONTINUED)

Table A2. Differences of Least Squares Means (LSmeans) comparing the average score of the euplasmic lines compare to the alloplasmic lines inoculated with isolate Br15 of tan spot (Continued)

Effect	Alloplasmic	Euplasmic	Estimate	Standard Error	DF	t Value	Pr > |t|
genotype	SSM0027	SSM0317	0.2627	0.2218	172	1.18	0.2379
genotype	SSM0028	SSM0317	-0.09149	0.2218	172	-0.41	0.6805
genotype	SSM0029	SSM0317	-0.3832	0.2218	172	-1.73	0.0858
genotype	SSM0036	SSM0317	-0.07066	0.2218	172	-0.32	0.7504
genotype	SSM0039	SSM0317	-0.9366	0.2342	172	-4.00	<.0001
genotype	SSM0045	SSM0317	-0.8207	0.2235	172	-3.67	0.0003
genotype	SSM0049	SSM0317	-0.1957	0.2218	172	-0.88	0.3789
genotype	SSM0050	SSM0317	-0.1814	0.2261	172	-0.80	0.4236
genotype	SSM0054	SSM0317	-0.1332	0.2218	172	-0.60	0.5490
genotype	SSM0069	SSM0318	-0.3962	0.2407	172	-1.65	0.1015
genotype	SSM0076	SSM0318	-0.9568	0.2087	172	-4.59	<.0001
genotype	SSM0085	SSM0318	-0.8103	0.2125	172	-3.81	0.0002
genotype	SSM0107	SSM0318	-0.4970	0.2122	172	-2.34	0.0203
genotype	SSM0187	SSM0258	-0.8183	0.1976	172	-4.14	<.0001
genotype	SSM0191	SSM0258	0.05882	0.2131	172	0.28	0.7829
genotype	SSM0192	SSM0258	-0.3253	0.1932	172	-1.68	0.0941
genotype	SSM0193	SSM0258	0.4872	0.1932	172	2.52	0.0126
genotype	SSM0194	SSM0258	-0.2003	0.1932	172	-1.04	0.3013
genotype	SSM0195	SSM0258	-0.2137	0.1951	172	-1.10	0.2749
genotype	SSM0196	SSM0258	-0.3893	0.2049	172	-1.90	0.0591
genotype	SSM0197	SSM0258	-0.2150	0.1951	172	-1.10	0.2720
genotype	SSM0199	SSM0258	-0.3910	0.2137	172	-1.83	0.0690
genotype	SSM0202	SSM0258	-0.4558	0.1976	172	-2.31	0.0222
genotype	SSM0206	SSM0258	-0.6794	0.1932	172	-3.52	0.0006
genotype	SSM0207	SSM0258	0.3622	0.1932	172	1.88	0.0625

(CONTINUED)

Table A2. Differences of Least Squares Means (LSmeans) comparing the average score of the euplasmic lines compare to the alloplasmic lines inoculated with isolate Br15 of tan spot (Continued)

Effect	Alloplasmic	Euplasmic	Estimate	Standard Error	DF	t Value	Pr > |t|
genotype	SSM0210	SSM0258	-0.2419	0.1932	172	-1.25	0.2121
genotype	SSM0229	SSM0258	-0.2782	0.1995	172	-1.39	0.1649
genotype	SSM0232	SSM0258	0.09964	0.2208	172	0.45	0.6524
genotype	SSM0237	SSM0258	-0.6441	0.1995	172	-3.23	0.0015
genotype	SSM0240	SSM0258	-0.7118	0.2034	172	-3.50	0.0006
genotype	SSM0253	SSM0258	-0.1169	0.1932	172	-0.61	0.5458
genotype	SSM0254	SSM0258	-0.5108	0.2129	172	-2.40	0.0175
genotype	SSM0258	SSM0317	-0.2037	0.1614	172	-1.26	0.2086
genotype	SSM0258	SSM0318	-0.4483	0.1555	172	-2.88	0.0045
genotype	SSM0317	SSM0318	-0.2446	0.1635	172	-1.50	0.1365

Mixed model procedures using a general linear model (GLM) compare average score estimation between euplasmic and alloplasmic scores.

Table A3. Day five scores of euplasmic lines with standard deviation (Ptr isolate Br15)

Trial	Reps	Chris	Trial	Reps	Selkirk	Trial	Reps	56-1
1	20	2.25	1	20	2	1	10	4
2	15	3.31	2	15	3.07	2	8	1.75
3	16	2.56	3	16	2.31	3	10	4.2
Total Avg		2.61	Total Avg		2.41	Total Avg		3.43
St.dv		1.22	St.dv		0.92	St.dv		1.55

Table A4. Day five scores of euplasmic lines with standard deviation (Ptr isolate Pti2)

Trial	Reps	Chris	Trial	Reps	Selkirk	Trial	Reps	56-1
1	20	2.65	1	20	1.90	1	10	2.10
2	8	2.60	2	8	1.50	2	8	4.75
3	16	2.00	3	16	2.25	3	10	4.10
Total Avg		2.41	Total Avg		1.95	Total Avg		3.57
St.dv		1.11	St.dv		0.74	St.dv		1.47

Table A5. Type III tests of fixed effects to tan spot isolate Pti2

Effect	Num DF	Den DF	F Value	Pr > F
Season	2	33	17.56	<.0001**
Genotype	47	174	7.26	<.0001**
Season × genotype	94	205	4.69	<.0001**
* Day	1	174	321.03	<.0001**
Season × day	2	205	13.43	<.0001**
Genotype × day	47	174	1.27	0.1334
Season × genotype × day	94	205	1.07	0.3420

*Significance level at $p < 0.05$
**Significance level at $p < 0.01$

Table A6. Differences of Least Squares Means (LSmeans) comparing the average score of the euplasmic lines compare to the alloplasmic lines inoculated with isolate Pti2 of tan spot

Effect	Alloplasmic	Euplasmic	Estimate	Standard Error	DF	t Value	Pr > |t|
genotype	SSM0003	SSM0317	-0.3326	0.2066	174	-1.61	0.1092
genotype	SSM0004	SSM0317	-0.2417	0.2066	174	-1.17	0.2436
genotype	SSM0015	SSM0317	0.1900	0.2095	174	0.91	0.3658
genotype	SSM0016	SSM0317	-0.3131	0.2102	174	-1.49	0.1381
genotype	SSM0017	SSM0317	-0.1715	0.2123	174	-0.81	0.4203
genotype	SSM0018	SSM0317	-0.2261	0.2066	174	-1.09	0.2753
genotype	SSM0019	SSM0317	-0.3390	0.2050	174	-1.65	0.1001
genotype	SSM0020	SSM0317	-0.1931	0.2050	174	-0.94	0.3475
genotype	SSM0021	SSM0317	-0.2973	0.2050	174	-1.45	0.1488
genotype	SSM0022	SSM0317	-0.09869	0.2066	174	-0.48	0.6334
genotype	SSM0023	SSM0317	-0.09909	0.2066	174	-0.48	0.6320
genotype	SSM0024	SSM0317	-0.1515	0.2050	174	-0.74	0.4610
genotype	SSM0027	SSM0317	-0.1666	0.2119	174	-0.79	0.4328
genotype	SSM0028	SSM0317	-0.2892	0.2095	174	-1.38	0.1692
genotype	SSM0029	SSM0317	0.2515	0.2115	174	1.19	0.2360

(CONTINUED)

Table A6. Differences of Least Squares Means (LSmeans) comparing the average score of the euplasmic lines compare to the alloplasmic lines inoculated with isolate Pti2 of tan spot (Continued)

Effect	Alloplasmic	Euplasmic	Estimate	Standard Error	DF	t Value	Pr > |t|
genotype	SSM0036	SSM0317	0.06746	0.2105	174	0.32	0.7490
genotype	SSM0039	SSM0317	-0.2099	0.2081	174	-1.01	0.3144
genotype	SSM0045	SSM0317	-0.00122	0.2377	174	-0.01	0.9959
genotype	SSM0049	SSM0317	0.000616	0.2057	174	0.00	0.9976
genotype	SSM0050	SSM0317	-0.05439	0.2066	174	-0.26	0.7926
genotype	SSM0054	SSM0317	0.3309	0.2057	174	1.61	0.1096
genotype	SSM0069	SSM0318	-0.8686	0.1822	174	-4.77	<.0001
genotype	SSM0076	SSM0318	-0.8069	0.1931	174	-4.18	<.0001
genotype	SSM0085	SSM0318	-0.7953	0.2003	174	-3.97	0.0001
genotype	SSM0107	SSM0318	-0.5992	0.1929	174	-3.11	0.0022
genotype	SSM0187	SSM0258	-0.5261	0.1835	174	-2.87	0.0046
genotype	SSM0191	SSM0258	0.1844	0.1872	174	0.98	0.3260
genotype	SSM0192	SSM0258	-0.00597	0.1807	174	-0.03	0.9737
genotype	SSM0193	SSM0258	0.1875	0.1782	174	1.05	0.2942
genotype	SSM0194	SSM0258	-0.1014	0.1807	174	-0.56	0.5755
genotype	SSM0195	SSM0258	-0.08830	0.1767	174	-0.50	0.6179
genotype	SSM0196	SSM0258	-0.2519	0.1953	174	-1.29	0.1990
genotype	SSM0197	SSM0258	-0.2079	0.1844	174	-1.13	0.2610
genotype	SSM0199	SSM0258	-0.1169	0.1767	174	-0.66	0.5090
genotype	SSM0202	SSM0258	-0.1420	0.1828	174	-0.78	0.4384
genotype	SSM0206	SSM0258	-0.5089	0.1789	174	-2.84	0.0050
genotype	SSM0207	SSM0258	0.03567	0.1815	174	0.20	0.8444
genotype	SSM0208	SSM0258	-0.1595	0.1975	174	-0.81	0.4205
genotype	SSM0210	SSM0258	0.05324	0.1767	174	0.30	0.7635
genotype	SSM0229	SSM0258	-0.01431	0.1807	174	-0.08	0.9369

(CONTINUED)

Table A6. Differences of Least Squares Means (LSmeans) comparing the average score of the euplasmic lines compare to the alloplasmic lines inoculated with isolate Pti2 of tan spot (Continued)

Effect	Alloplasmic	Euplasmic	Estimate	Standard Error	DF	t Value	Pr > |t|
genotype	SSM0232	SSM0258	-0.1123	0.2109	174	-0.53	0.5952
genotype	SSM0237	SSM0258	-0.3106	0.1856	174	-1.67	0.0960
genotype	SSM0240	SSM0258	-0.4787	0.1893	174	-2.53	0.0123
genotype	SSM0253	SSM0258	0.05187	0.1767	174	0.29	0.7694
genotype	SSM0254	SSM0258	0.1144	0.1828	174	0.63	0.5323
genotype	SSM0258	SSM0317	0.4262	0.1430	174	2.98	0.0033
genotype	SSM0258	SSM0318	-1.0675	0.1367	174	-7.81	<.0001
genotype	SSM0317	SSM0318	-1.4938	0.1501	174	-9.95	<.0001

Mixed model procedures using a general linear model (GLM) compare average score estimation between euplasmic and alloplasmic scores.