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DISEASE AND INSECT RESISTANCE AND QUALITY CHARACTERIZATION OF  
SIX CIMMYT SYNTHETIC HEXAPLOID WHEATS

BY

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A THESIS

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The Graduate College at the University of Nebraska  
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Major: Agronomy

Under the Supervision of Professor P. S. Baenziger

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DISEASE AND INSECT RESISTANCE AND QUALITY CHARACTERIZATION OF  
SIX CIMMYT SYNTHETIC HEXAPLOID WHEATS

Kayse Marie Onweller, M.S.

University of Nebraska, 2011

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The germplasm sources of common wheat (*Triticum aestivum* L.) are eroded by selection pressures applied by plant breeders and the disappearance of landraces. The erosion causes a loss of potentially useful resistance genes, among other agronomic and quality genes. Continuously changing pathogen races and insect biotypes affecting the Great Plains requires identification of new sources of resistance. Synthetic hexaploid wheats (SHWs), *T. turgidum* (BBAA) x *T. tauschii* (DD) hybridizations, offer ways to utilize resistances trapped in the diploid and tetraploid ancestors of common wheat. Six SHWs were assayed for resistance to a variety of fungal diseases, viruses, and aphids to determine their spectrum of resistance. The six SHWs possessed a combination of resistance to races TPMK, TTTT, and the Ug99 family; races of the causal agent stem rust (*Puccinia graminis* f. sp. *tritici*); the causal agent of stripe rust (*P. striiformis* f. sp. *tritici*) race Pst-100, and the greenbug aphid [*Schizaphis graminum* Rondani (Homoptera: Aphididae)] biotypes E, I and K. Additionally, the high molecular weight glutenin (HMW) alleles were studied. The HMW allele combinations were different from those commonly found in current Great Plains wheats. The allele combinations included the

*Glu-B* alleles 14+15, 20x+20y, 6\*+8\*, 6+8 and 7+8. *Glu-D* alleles present included 2+12 and 2+T2.

Upon discovering NSGC 9711 and PI 648810 were resistant to greenbug aphid, plant populations previously developed were employed to uncover the inheritance of the resistance. A series of tests were conducted using F<sub>1</sub>s, F<sub>2</sub>s, F<sub>2:3</sub>s and BC<sub>1</sub>F<sub>2</sub>s. A single dominant gene hypothesis was rejected due to an overabundance of susceptible individuals in the F<sub>2</sub> and F<sub>2:3</sub> populations. However, the F<sub>2:3</sub> and BC<sub>1</sub>F<sub>2</sub> families indicated resistance in both synthetics was from a single dominant gene, thus NSGC 9711 and PI 648810 is reported to carry a single dominant gene for resistance to greenbug biotype E.

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**FORWARD**

This thesis is written as two manuscripts in the format required for publication in Crop Science journal.



**LIST OF ABBREVIATIONS**

GBE – greenbug biotype E  
GBI – greenbug biotype I  
GBK – greenbug biotype K  
GS166 – the cross Goodstreak/NSGC 9711  
GS356 – the cross Goodstreak/PI 648810  
HMW – high molecular weight glutenin  
IT – infection type  
SHW – synthetic hexaploid wheat

**TABLES LIST****CHAPTER I**

Table 1 Plant introduction number, CIMMYT identifier, and pedigree of six synthetic hexaploid wheats. Also listed are identifiers for *T. tauschii* donors at CIMMYT, KSU=Kansas State University, AUS= Australian Winter Cereals collection, and CPI= Commonwealth Plant Introduction collection.

Table 2. Reaction based on Stakman et al. (1962) of synthetic material to stem rust isolates.

Table 3. Reaction of synthetic material to various diseases and insect pests of the Great Plains.

Table 4. HMW glutenin subunits contained by synthetic lines and checks.

**CHAPTER II**

Table 1. Plant introduction number and pedigree of six synthetic hexaploid wheats. Also listed are identifiers for *T. tauschii* donors as given by CIMMYT during the wide cross program, Kansas State University, Australian Winter Cereals collection, and the Commonwealth Plant Introduction collection.

Table 2. Resistant and susceptible observed values, expected one gene ratio, the expected observation values and the resulting  $\chi^2$  value for individuals, families, and the individuals within the segregating families.

**FIGURE LIST**

**CHAPTER I**

Figure 1. Migration pattern of the high molecular weight glutenin subunits found in 6 synthetic wheats

**APPENDIX LIST**

Appendix 1. Listed are the germplasm sources resistance genes were identified in, the designated gene name, the genome origin, and reaction type to various greenbug biotypes. Listed in parenthesis is the *Triticum tauschii* accession donor.

## CHAPTER I

IDENTIFICATION OF COMMON GREAT PLAINS DISEASE AND INSECT  
RESISTANCES IN SIX SYNTHETIC HEXAPLOID WHEATS AND AN  
ASSESSMENT OF THEIR HIGH MOLECULAR WEIGHT GLUTENINS

## ABSTRACT

The germplasm resources of common wheat (*Triticum aestivum* L.) are eroded by selection pressures applied by plant breeders and the disappearance of landraces. With erosion comes the loss of potentially useful resistance genes, among other beneficial genes for agronomic and quality traits. The continual change in races of Great Plains pathogens and insects requires identification of new sources of resistance. Synthetic hexaploid wheats (SHWs), *T. turgidum* (BBAA) x *T. tauschii* (DD) hybridizations, offer ways to utilize resistances identified in the diploid and tetraploid ancestors of common wheat. In this study six SHWs were assayed for resistance to a variety of fungal diseases, viruses, and aphids to determine their resistance spectrum. Resistance to stem rust (causal agent *Puccinia graminis* f. sp. *tritici*) races TPMK, TTTT, and the Ug99 family; stripe rust (causal agent *P. striiformis* f. sp. *tritici*) race Pst-100, and the greenbug aphid (*Schizaphis graminum*) biotypes E, I and K. Additionally, the high molecular weight glutenin alleles were studied. The allele combinations were different from those commonly found in current Great Plains wheats and included the *Glu-B* alleles 14+15, 20x+20y, 6\*+8\*, 6+8 and 7+8. *Glu-D* alleles represented included 2+12 and 2+T2.

## INTRODUCTION

Rigorous selective breeding, and dwindling production of landraces all contribute to genetic erosion and narrowing of crop germplasm (Gepts, 2006). Common wheat (*Triticum aestivum* L.) is an allohexaploid having undergone all of these occurrences. The first hybridization occurred ~500,000 years ago between an unknown diploid species and the diploid *Triticum urartu* [Tum. ex Gandil; (Huang et al., 2002)]. The resulting tetraploid *Triticum turgidum* (L.) subsp. *dicoccoides* (Körn. ex Asch. & Graebn.), evolved to *T. turgidum* (L.) subsp. *dicoccum* (Schrank ex Schüb.). About 8,000 years ago the next hybridization occurred between *T. turgidum* and *Triticum tauschii* (Coss.). The primitive hexaploid later evolved to common wheat (Dreisigacker et al., 2008). The number of individuals involved in these historical hybridization events is unknown. Arguably there were few (Ladizinsky, 1985), resulting in only a small portion of the A, B and D genomes' total variation being available for breeding improvement in common wheat. Hence, modern wheat has undergone a genetic bottleneck. Further compounding this bottleneck are the selection intensities imposed for cultivar development and growers adoption of modern cultivars that replace and eventually eliminate landraces.

Considering the continual erosion of wheat diversity (e.g. Fu et al., 2006), incorporating new variation into wheat germplasm is valuable. Furthermore, due to the rate at which pathogens evolve new virulent pathotypes and the consequent breakdown of deployed resistant genes, the continual identification of new sources of resistance is required to genetically protect crops. Though others report that common wheat diversity is increasing (Christiansen et al., 2002; Warburton et al., 2006), the increased



characterization and use of wild progenitors and their derived progeny clearly support their value as genetic resources. “Synthetic” hexaploid wheat (SHW) provides a seamless way of introducing genes into modern common wheat that are locked within diploid and tetraploid *Triticum* species. Some of the first *T. turgidum* by *T. tauschii* hybridizations were generated by McFadden and Sears (1944). Since the first SHWs were developed in the 1940s, more than 1,000 spring and 180 winter synthetic wheat lines have been generated at CIMMYT alone (van Ginkel and Ogonnaya, 2007). As would be expected using wild progenitor germplasm, much of the genetic variation introduced by SHWs is of low value. Thus, knowing which SHWs have the plant breeder’s trait of interest will reduce unnecessary time and resources spent on evaluations. Such knowledge is obtained by evaluating wild germplasm, a necessary and fundamental undertaking because their use is inhibited by what little descriptive information is available (Nass and Paterniani, 2000).

One effort to characterize germplasm was by Ma et al. (1995a), who while searching for stripe rust [incited by *Puccinia striiformis* f. sp. *tritici* (Westend.)] resistance screened a sample of 74 SHWs. They found 18 lines with adult resistance and 5 lines with independent seedling resistance. Ogonnaya (2008) screened 253 SHWs for resistance to cereal cyst nematode [*Heterodera avenae* (Wollenweber)], root lesion nematode [*Pratylenchus thornei* (Sher and Allen)], tan spot [incited by *Pyrenophora tritici-repentis* (Died.)], leaf rust [incited by *Puccinia triticina* (Erikss.)], stem rust [incited by *Puccinia graminis* f. sp. *tritici* (Erikss. & E. Henn.)], and stripe rust. A number of synthetics were resistant to each disease studied. A promising result was more

than 50 of the 243 SHWs expressed resistance to 4 diseases simultaneously. After identifying novel alleles, the genes can be transferred to improved cultivars and the loci can be mapped or cloned. For example, a wheat soilborne mosaic virus resistance QTL allele carried by the synthetic TA 4152-4 was mapped (Narasimhamoorthy et al., 2006), and attempts to clone the *Gb3* gene from the amphiploid ‘Largo’ (Joppa and Williams, 1982) are underway (Rudd and Menz, 2008).

Those using synthetic wheat characterized for a particular trait of interest should ask: What other genes are gained in addition to those for which the synthetic was originally selected? Knowing the answer to this question may help prioritize which parents should be used for crossing and which populations should be considered for multiple trait selection.

In this study six synthetic lines were investigated for their response to the diseases: stem rust, leaf rust, stripe rust, powdery mildew [incited by *Blumeria graminis* (DC.)E.O. Speer f. sp. *tritici* Em. Marchal], Wheat streak mosaic virus and Triticum mosaic virus and the insects: Hessian fly [*Mayetiola destructor* (Say) (Diptera: Cecidomyiidae)], greenbug [*Schizaphis graminum* (Rondani) (Homoptera: Aphididae)], and Russian wheat aphid [*Diuraphis noxia* (Mordvilko) (Homoptera: Aphididae)]. These pests have and continue to cause economic losses in the Great Plains wheats. Also investigated was the composition of high molecular glutenins to understand the diversity of their quality alleles. The six synthetics were initially selected based on their tolerance to drought and modern wheat architecture from a collection of 400 SHWs developed at the International Maize and Wheat Improvement Center (CIMMYT). Because these

synthetic lines were selected mostly for their drought potential, little was known about their disease, insect or quality characteristics.

## MATERIALS & METHODS

Six synthetic hexaploid wheats previously identified for drought tolerance were evaluated for response to common pathogens and insect pests of the Great Plains.

Additionally, high molecular weight (HMW) glutenin subunits were assessed. Pedigree information on the six synthetic hexaploids evaluated in this study is presented in Table 1. Additionally, the *T. tauschii* parents of the synthetic lines have been used by other researchers and their identifiers are crosslisted in Table 1.

### Disease Evaluation

#### Stem Rust

Evaluation of synthetic lines to *P. graminis* f. sp. *tritici*, was conducted at the USDA-ARS Cereal Disease Laboratory in St. Paul, Minnesota. The U.S. races TPMK and TTTT and the Ug99 family TTKSK, TRTT, TTKST, and TTTSK were tested (Jin et al., 2007).

#### Leaf Rust

Reaction to *P. triticinia* was tested at the University of Nebraska-Lincoln. A Nebraska field-collected race was used. The protocol, briefly, is as follows: Synthetic lines plus checks ‘Arapahoe’ and ‘Cheyenne’ were planted in a 1 part topsoil/ ½ part sand/ ½ part vermiculite/ 1 part peat moss media. Inoculation occurred after the first and second leaves had fully expanded. *P. triticinia* spores, collected originally from a Nebraska field infection, were increased on ‘Thatcher’ suspended in a Tween 20 (40ul per L of ddH<sub>2</sub>O) solution and dispensed from a pressurized spray bottle until leaves were

uniformly wet. Inoculated seedlings were placed in a chamber with 100% humidity at 20°C for 12 hours. The seedlings were then moved to a greenhouse chamber held at 27°C day/23°C night with photoperiod of 15h:9h (day:night). Infection was first observed approximately 7-10 days post-inoculation and infection type was scored following the 0-4 scale described by McIntosh et al. (1995) 14 days post-inoculation.

### **Stripe Rust**

Reaction to *P. striiformis* f. sp. *tritici*, causal agent of stripe rust, was tested at the USDA-ARS hard winter wheat genetics research unit in Manhattan, KS. The protocol using race PST-100 is as follows (Robert Bowden, personal communication). Seedlings were grown with replication in 20 x 20 x 5 cm aluminum pans in Metromix 360 (Hummert International, Earth City, MO). The six synthetic lines plus check cultivars were planted in clumps of four to six seedlings. Plants were grown in a growth chamber at  $20 \pm 1^\circ\text{C}$  and photoperiod 15h:9h (day:night) at a light intensity of  $300\text{-}500 \mu\text{mol m}^{-2}\text{sec}^{-1}$ . Desiccated urediospores of stripe rust race PST-100 were suspended in Soltrol 170 light oil (Chevron Phillips Chemical Company, The Woodlands, TX) and sprayed onto seedlings at the two leaf stage until seedlings were uniformly wet. The oil was allowed to evaporate for 10 minutes and then plants were placed in a dark dew chamber at  $12 \pm 2^\circ\text{C}$  with 100% relative humidity for approximately 16 h. Plants were moved back to the initial growth chamber for symptom development. Infection types were recorded for the first and second leaf using a 0 to 9 scale (Line and Qayoum, 1992) when the susceptible controls showed fully developed symptoms. Four to six seedlings per line were scored and a consensus rating was determined. The adult plant assay was

conducted similarly except entries were grown in 1 liter plastic pots (2-3 plants per pot) in the greenhouse at 18-24°C and photoperiod 15h:9h (day:night) until anthesis. Flag leaves were scored for stripe rust IT (Line and Qayoum, 1992) and percent disease severity.

### **Powdery Mildew**

Thirty isolates of *B. graminis* f. sp. *tritici* were tested (unreplicated) at the USDA-ARS unit located at North Carolina State University in Raleigh, NC. Tested isolates were collected from Nebraska, Oklahoma, Kentucky and the Eastern U.S. A confirmation test was performed (with four replicates) on synthetics NSGC 9711, PI 648733, PI 648810, and PI 648823 using 22 of the 30 initial isolates (Parks et al., 2008). The eight omitted isolates were virulent on all synthetic lines in the unreplicated study.

### **Wheat streak mosaic virus and Triticum mosaic virus**

Reactions to wheat streak mosaic virus and triticum mosaic virus were assessed at the USDA-ARS unit located at University of Nebraska-Lincoln in Lincoln, NE using a protocol outlined in Tatineni et al. (2010).

### **Insect Evaluation**

#### **Hessian Fly**

Reaction to a *M. destructor* mixture was assessed at the USDA-ARS hard winter wheat genetics research unit in Manhattan, KS using the protocol outlined in Chen et al. (2009).

## Greenbug and Russian Wheat Aphid

Reaction to *S. graminum* biotype E, I and K as well as *Diuraphis noxia* biotypes 1 and 2 was assessed at the USDA-ARS lab located in Stillwater, OK. The protocol for both aphid screens is as follows. Seeds were treated with Captan fungicide [N-trichloromethylthio-4-cyclohexene-1,2-dicarboximide (Southern Agricultural Insecticides, Palmetto, FL)] at planting to control any seed-borne fungal diseases. Cone flats, filled with Redi-earth (Sun Gro Horticulture, Vancouver, BC Canada) were planted with five seed per cone. The check cultivars 'Amigo' [greenbug biotype E (GBE) susceptible], 'Largo' (GBE resistant), 'OK101' (Russian wheat aphid (RWA) 1& 2 susceptible), 'Halt' (RWA 1 resistant), and 'Stars 0601' (RWA 1 & 2 resistant) were planted as a continuous middle row in the flat as well as randomly between entries to be tested against the respective aphid. Planted flats were contained in a mesh cage to prevent aphid spread or contamination. Greenhouses were maintained between 20-23°C ambient temperature under a 14:10 (day:night) photoperiod. When plants were at the two leaf stage, aphid-infested leaves were placed between the seedling rows. Aphids were free to move to living material as their transplant leaf died. The seedlings were sprayed with Isotox [Gamma isomer of 1,2,3,4,5,6-hexachloro cyclohexane (The Scotts Company, Marysville, OH)] when the susceptible checks were completely chlorotic. Rating was qualitative using the following guide: individual plants that had little or no damage were scored resistant, individual plants manifesting chlorosis and some minor necrosis were given a moderate resistance rating, and those individual plants that were severely chlorotic or necrotic were scored as susceptible.

**Quality Analysis**

The high molecular weight (HMW) glutenin subunits were extracted using a modified protocol of Graybosch and Morris (1990).



## RESULTS

The results from the Triticum mosaic virus and Wheat streak mosaic virus assays indicated that the six SHWs were susceptible. Similarly, susceptible reactions to Hessian fly mixtures and Russian wheat aphid biotypes 1 and 2 were found. Lastly, the causal agent of a Nebraska leaf rust isolate was virulent on all lines tested. However, some form of resistance was found to the following: powdery mildew, stem rust, stripe rust and the greenbug aphid. Differences were also found among the glutenin alleles.

### **Powdery Mildew**

The unreplicated detached leaf assay of 30 *B. graminis* f. sp. *tritici* isolates suggested synthetics PI 648646, PI 648733, PI 648758, PI 648823 were resistant to at least one isolate. Synthetics NSGC 9711 and PI 648810 were moderately susceptible to 15 isolates collectively and susceptible to the rest. To confirm the resistance in PI 648733 and PI 648823 and the susceptibility NSGC 9711 and PI 648810, a four replicate assay was performed involving 22 of the 30 original isolates. Synthetic PI 648733 exhibited resistance to seven isolates in the unreplicated assay and three in the replicated assay, one of which was the same isolate (NEB 8-1). Synthetic PI 648823 was the most susceptible line in the replicated assay, being susceptible to 20 isolates and moderately susceptible to 2 isolates. The replicated averages for NSGC 9711 and PI 648810 suggested resistant reactions to three isolates (OKL 9-2, OKL 3-2, and NEB 3-2). Both NSGC 9711 and PI 648810 were resistant to isolate OKL 9-2. PI 648810 was resistant to isolate OKL 3-2 and NSGC 9711 was resistant to isolate NEB 3-2. In the unreplicated assay, NSGC 9711 and PI 648810 were moderately susceptible to these isolates.

### **Stem Rust**

PI 648758 was the most resistant line in the seedling assay of *P. graminis* f. sp. *tritici* (Table 2). The highest infection type (IT) PI 648758 expressed to the six isolates was a 2+ on the 1 to 4 scale described by Stakman et al. (1962). PI 648823 demonstrated moderate resistance to 4 isolates (IT 2+) and was susceptible to isolates TRTT and TTTT (IT 3). Overall, PI 648733 was the most susceptible, exhibiting an IT 4 reaction to 4 of the 6 assayed isolates. PI 648733 was the only line that was susceptible to isolate TPMK, a once problematic isolate found in the U.S (Kolmer et al., 2007). PI 648646 and PI 648810 exhibited a range of ITs to all isolates tested (Table 2).

### **Stripe Rust**

Seedlings of PI 648646, PI 648758 and PI 648823 were resistant in the *P. striiformis* f. sp. *tritici* assay, with average infection types 2.75, 2.75, and 2.67, respectively on the 0 to 9 scale described by Line and Qayoum (1992). PI 648733 was susceptible at the seedling stage (IT 7.25), yet resistant as an adult (IT 2). NSGC 9711 and PI 648810 were moderately resistant at the seedling and adult stage. PI 648646, PI 648733, PI 648758 and PI 648823 demonstrated good adult plant resistance to *P. striiformis* (Table 3).

### **Greenbug aphid**

Synthetics NSGC 9711 and PI 648810 were resistant to greenbug biotypes E, I, and K; all of which are members of the greenbug agricultural clade (Shufran et al., 2000). To date, no resistance genes in wheat are able to distinguish between GBE, GBI and

GBK. Synthetic PI 648646 was susceptible to GBE, GBI and GBK and synthetics PI 648733, PI 648758, and PI 648823 were susceptible to GBI and GBK. The latter three synthetics exhibited a heterogeneous response to GBE in preliminary testing. Upon further testing it was determined that synthetics PI 648733, PI 648758, and PI 648823 were also susceptible to GBE.

### **HMW glutenins**

The six synthetics possess glutenin combinations different from those of the Great Plains wheats based on the SDS-PAGE migration patterns of their glutenin alleles. Three synthetic lines possess the *Glu-D* allele 2+12 and three possess the *Glu-D* allele 2+T2 (Table 4 and Figure 1). Greater variability was noted in the subunit pairs encoded by the *Glu-B* allele. The *Glu-B* subunit pair 14+15 was identified in NSGC 9711 and PI 648810. We would also expect PI 648823 to carry the 14+15 allele because its pedigree suggests it shares the same durum parent as PI 648810 (Table 1). This was not the case, since the remaining 4 synthetic lines each had a different allele (Table 4 and Figure 1). *Glu-A* alleles were null in these synthetic lines.

## DISCUSSION

The resistance profile of the six synthetic lines varied to the diseases and insects under investigation. Resistance was not found to Triticum mosaic virus, Wheat streak mosaic virus, leaf rust, a Hessian fly mixture, or Russian wheat aphid biotypes 1 and 2. However, at least one synthetic line possessed some level of resistance to powdery mildew, stem rust, stripe rust, and the greenbug aphid. Additionally, assessment of quality parameters via HMW glutenins revealed allele combinations different than those commonly found in Great Plains wheat varieties.

During this study, heterogeneous responses to *P. graminis* f. sp. *tritici* and the greenbug aphid were found. Because synthetic wheats involve chromosome doubling, homozygosity was expected, thus the heterogeneous response was surprising. However, heterogeneous reactions have been reported previously. Assefa and Fehrman (2004) experienced similar responses to *P. graminis* f. sp. *tritici* in a collection of *Triticum tauschii* accessions. They explained their results by non-uniform inoculations, segregating genes within a heterogeneous accession, or seed mishandling. Moreover, studies on individual tetraploid and diploid progenitors have reported resistance that is reduced or nullified upon hybridization (Assefa and Fehrman, 2004; Lage et al., 2003; Ma et al., 1995b). Genes active in trait suppression have been found in all three genomes (Lutz et al., 1994). Synthetics generated by hybridization of heterogeneous *T. durum* and *T. tauschii* accessions would lead to the heterogeneous responses experienced in the stem rust and greenbug assays. Furthermore, random chance would allow us to grow a susceptible synthetic to use in crosses for the development of populations for inheritance

studies. Further investigation of synthetic PI 648823 response to greenbug biotypes E, would aid in determining the homozygosity of the line. This line showed no resistance to GBI and GBK and a mixed response to GBE. Identification of true GBE resistance and susceptibility to GBI and GBK, would be the first time in wheat that a gene(s) was able to discern between these biotypes.

High molecular weight glutenin diversity should be considered when incorporating new variation. Through years of selection, the HMW glutenin composition of Great Plains wheats has been narrowed mainly to the combination A- null, B- 7+8, D- 5+10 (Shan et al., 2007). This combination produces end-use quality fitting of the U.S. milling requirements. End-use quality can suffer greatly if glutenin combinations differ from that previously mentioned. Further research is necessary to determine what effect the subunit combinations of the 6 SHWs studied has on quality.

These synthetic lines, selected for drought tolerance, exemplify the abundance of valuable genetic variation contained in SHWs. Our findings support previous identification of new sources of disease resistance in wild germplasm (Cox et al., 1992; Lutz et al., 1994; Ma et al., 1995b; Rizwan et al., 2007). Because we sampled only six SHWs, failure to identify resistance to the viruses, leaf rust, Hessian fly or Russian wheat aphid was not surprising. Increasing the sample size of studied synthetics would aid in discovering one or more accessions harboring resistance to the insects and diseases studied here. For example, Hessian fly resistance has been incorporated in common wheat by direct crossing with *T. tauschii* accessions (Miranda et al., 2010) and Russian wheat aphid resistance was identified in durum donors (Beyer et al., 2011). The

information presented in this study is encouraging to those considering the use of SHWs in their programs. Finally, trying to understand the genetic basis of the resistance genes identified in the present research leads to future research opportunities.

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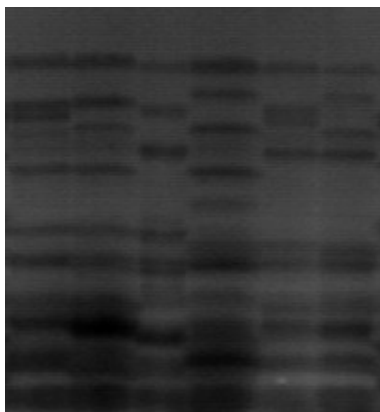
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**FIGURES**

**Figure 1.** Migration pattern of the high molecular weight glutenin subunits found in 6 synthetic wheats. The following subunits were determined (L to R) Lane 1- NSGC 9711: A- null B- 14+15 D- 2+T2; Lane 2- PI 648646: A- null B- 7+8 D- 2+T2; Lane 3- PI 648733: A- null B- 20x + 20y D- 2+12; Lane 4- PI 648758: A- null B- 6\*+8\* D- 2+T2; Lane 5- PI 648810: A- null B- 14+15 D- 2+12; Lane 6- PI 648823: A- null B- 6+8 D- 2+12



## TABLES

**Table 1.** Plant Introduction number, CIMMYT identifier, and pedigree of six synthetic hexaploid wheats. Also listed are identifiers for *T. tauschii* donors at CIMMYT, KSU=Kansas State University, AUS= Australian Winter Cereals collection, CPI= Commonwealth Plant

		<u>Synthetic Hexaploid</u>				<u><i>T. tauschii</i></u>		
PI #	CIMMYT cross identifier (wide cross #)	Pedigree	CIMMYT wide cross			KSU #	AUS #	CPI #
			#					
NSGC 9711	CIGM90.682 (172)	Snipe/Yavaros 79//Dackiye/Teal/3/Ae. Squarrosa (904)	904			2477	24154	110812
648646	CIGM88.1313-1B	68112/Ward//Ae. Squarrosa (369)	369			2528	unk	unk
648733	CIGM92.1631 (290)	Garza/Boyerol//Ae. Squarrosa (241)	241			3282	24061	110719
648758	CIGM92.1678 (320)	Laru/ Ae. Squarrosa (333)	333			2482	24159	110817
648810	CIGM93.205 (380)	Decoy 1/Ae. Squarrosa (256)	256			2400	24078	110737
648823	CIGM93.225 (396)	Decoy 1/Ae. Squarrosa (322)	322			2471	24148	11086

**Table 2.** Reaction based on Stakman et al. (1962) of synthetic material to stem rust isolates.

CSU/UNL						
identifier	TPMK	TTTT	TTKSK	TRTT	TTKST	TTTSK
SYN 166	2	2	2+	2	3+	4
SYN 194	2-	4	4	2	n/a	n/a
SYN 274	4	2+	4/2	4	4	4
SYN 303	2	2	2+	2	2+	2+
SYN 356	2	3	4	2	n/a	n/a
SYN 370	2	2+3	2+	3	2+	2+

**Table 3.** Reaction of synthetic material to various diseases and insect pests of the Great Plains.

CSU/UNL designation	Powdery Mildew <sup>†</sup>	Stripe Rust (seedling) <sup>‡</sup> (IT 0-9)	Stripe Rust (adult) <sup>‡</sup> (IT 0-9)	Greenbug E <sup>§</sup>	Greenbug I <sup>§</sup>	Greenbug K <sup>§</sup>
SYN 166	13	4.5	4	R	R	R
SYN 194	N/A	2.75	1	S	S	S
SYN 274	14	7.25	2	S	S	S
SYN 303	N/A	2.75	2	S	S	S
SYN 356	11	3.25	4	R	R	R
SYN 370	2	2.67	2	S	S	S

<sup>†</sup> Listed are the number of isolates (out of 22) to which at least a moderate resistant based on Parks et al. (2008) reaction occurred in a replicated assay .

<sup>‡</sup> Scores based on Line and Qayoum (1992).

<sup>§</sup> Scores described in Materials and Methods.

**Table 4.** HMW glutenin subunits contained by synthetic lines and checks.

CSU/UNL Identifier	Glutenin Subunit		
	A	B	D
SYN 166	Null	14+15	2+T2
SYN 194	Null	7+8	2+T2
SYN 274	Null	20x+20y	2+12
SYN 303	Null	6*+8*	2+T2
SYN 356	Null	14+15	2+12
SYN 370	Null	6+8	2+12
CHECKS			
Ben	Null	6+8	N/A
Endurance	2*	6*+8*	5+10
NuPlains	2*	20x+20y	5+10
PI 471075	1Ax+1Ay	unknown	Null
TAM107	2*	7+8	2+12
TA 2450	N/A	N/A	43+44
Sappo	Null	14+15	N/A



**CHAPTER II**

GENETIC ANALYSIS OF GREENBUG BIOTYPE E RESISTANCE INHERITANCE  
IN TWO SYNTHETIC HEXAPLOID WHEATS

**ABSTRACT**

Greenbug aphids [*Schizaphis graminum* Rondani (Hemiptera: Aphididae)] cause economic losses in common wheat (*Triticum aestivum* (L.)) and sorghum (*Sorghum bicolor* (L.)). Three biotypes, E, I and K, are the most prevalent in agriculture and currently only sorghum cultivars are able to differentiate these biotypes. To avoid break down of resistance in common wheat, it is necessary to find new sources of resistance. Synthetic hexaploid wheats (SHWs), *T. turgidum* (BBAA) x *T. tauschii* (DD) hybridizations, have been an excellent source of greenbug resistance. Previously, we tested six SHWs for reaction to biotypes E, I and K. Resistance was found in two synthetic lines and a genetic analysis was conducted using F<sub>1</sub>S, F<sub>2</sub>S, F<sub>2:3</sub>S and BC<sub>1</sub>F<sub>2</sub>S. A single dominant gene hypothesis was rejected due to an overabundance of susceptible individual plants in F<sub>2</sub>S and F<sub>2:3</sub>S. However, the F<sub>2:3</sub> and BC<sub>1</sub>F<sub>2</sub> family data indicated resistance in both synthetics was from a single dominant gene, hence the results are best explained by each SHW having a single dominant gene controlling resistance to greenbug biotype.

## INTRODUCTION

Devastating loss in U.S. wheat [*Triticum aestivum* L.] and sorghum [*Sorghum bicolor* L.] production can result when greenbug aphid [*Schizaphis graminum* (Rondani) (Hemiptera: Aphididae)] populations exceed economic thresholds. Kindler et al. (2003) has suggested a single feeding aphid on wheat in optimal growing conditions can cause 14.5 kg\*ha<sup>-1</sup> in yield loss. Their estimate increased to 34.5 kg\*ha<sup>-1</sup> in years with poor growing conditions. These production losses translate to economic loss. For example, Oklahoma has reported losses up to \$135 million (Kindler et al., 2002). Because such losses can result, the presence of aphids should be monitored, with an emphasis on monitoring greenbug susceptible varieties. In wheat, spring time pest management is advised when twelve aphids colonize on a single plant tiller. Aphid feeding symptoms appear as chlorosis and necrosis. Hence, when aphid populations reach the economic threshold, photosynthetic capacity is reduced and yield losses ensue. Additionally, greenbug aphids are a vector for Barley Yellow Dwarf virus, leading also to chlorotic symptoms (Blackman et al., 2000) and economic losses.

The well documented greenbug biotypes are designated A-K (Porter et al., 1997). Biotypes E, I and K are currently most problematic in agricultural crops. These biotypes along with older biotypes C and J form the agricultural clade. The clade was one of three proposed by Shufran et al. (2000) who used mitochondrial DNA polymorphisms to group eight of the eleven biotypes. A 2002 greenbug collection from agricultural crops and non-cultivated grass species grown in Nebraska, Oklahoma, and Texas found 13 previously unreported biotypes based on response to known resistance differentials (Burd

and Porter, 2006). Biotype E has been the prevalent wheat and sorghum pest. However, the prevalence of biotype I is increasing. Consequently, deployed host plant resistance to biotype I is increasing. Zhu et al. (2005) suggested a rise in biotype K populations will follow as a result of increased biotype I plant resistance. Since fewer sorghum biotype K resistance genes are known, this potential shift is a great concern to sorghum producers. The majority of currently used wheat greenbug resistance genes protect against biotypes E, I and K.

Tyler et al. (1987) summarized the known greenbug resistance genes in wheat identified before 1987. The genes *gb1*, *Gb2*, *Gb3*, *Gb4*, and *Gb5* were included. Thereafter, new genes including *Gbx* (Gill and Raupp, 1987), *Gb6* (Porter et al., 1997), *Gby* (Boyko et al., 2004), *Gbz* (Zhu et al., 2004), *Gb7* (Weng et al., 2005), and *Gba*, *Gbb*, *Gbc*, *Gbd* (Zhu et al., 2005) were discovered. Of all the reported genes in wheat, only *gb1*, originating from durum (*T. durum* L.) germplasm ‘Dickinson no. 485’ [CI 3707 (Curtis et al., 1960)], is recessive. The remaining resistant genes are dominant. All genes to-date are summarized in Appendix 1. The genes confer resistance by antibiosis, antixenosis or tolerance. In some cases, the resistance mode(s) is the only distinguishing factor among identified greenbug aphid resistance genes (Boina et al., 2005).

Examination of biotype appearance and respective resistant gene deployment suggested the release of resistant sorghum cultivars, not wheat cultivars, aided the appearance of new biotypes (Porter et al., 1997). The fall growth of southern grown winter wheats facilitates aphid overwintering and changes in photoperiod trigger the production of sexual aphids (Michaud, 2010). The identification of new biotypes (Burd

and Porter, 2006) is a probable outcome of genetic recombination. Release of new sources of resistance can thwart potential economic losses imposed by new virulent biotypes. Additionally, using multiple sources of resistance can delay the breakdown of currently useful resistance genes. For these reasons, discovery of new resistance alleles is needed. An excellent source to discover new resistance alleles is synthetic wheats. They have been the source of many greenbug resistance genes, as well as genes ameliorating biotic and abiotic stresses (Ogbonnaya et al., 2008).

In a previous study of six synthetic spring wheat lines, we identified two lines [NSGC 9711 (NSGC 9711) and PI 648810 (PI 648810)] with resistance to greenbug biotypes E, I and K. In the present study, we investigated the genetic basis of greenbug biotype E resistance contained in these two lines.

## MATERIALS & METHODS

Genetic analysis of Goodstreak/NSGC 9711 (herein referred to as GS166) and Goodstreak/PI 648810 (herein referred to as GS356) included F<sub>1</sub> plants, F<sub>2</sub> plants and BC<sub>1</sub>F<sub>2</sub> families (the recurrent parent was Goodstreak). The genetic analysis of GS356 also included F<sub>2,3</sub> families. Five F<sub>1</sub> GS166 seeds and 10 GS356 seeds were evaluated to determine if the resistance gene(s) were dominant or recessive. Additionally, 200 F<sub>2</sub> individuals and 40 BC<sub>1</sub>F<sub>2</sub> families of approximately 30 individuals per family were sent to the USDA-ARS lab located in Stillwater, OK from both populations. The F<sub>2,3</sub> families of GS356 consisted of approximately 30 individuals per family. Finally, 100 seeds of the PI 648810/NSGC 9711 F<sub>2</sub> population were included to test allelism of the respective genes.

All GS166 seeds were planted in the greenhouse in February of 2010. For GS356, seeds were planted on three dates in December of 2010. Seeds of both studies were planted in cone flats. Five seeds were planted in a cone and the center column was planted with check varieties ‘Largo’ (greenbug biotype E resistant), ‘OK 101’ (greenbug biotype E susceptible), and ‘Amigo’ (greenbug biotype E susceptible), each in a separate cone. Checks were also planted among the test material. Greenhouse conditions, greenbug biotype E infestation methods, and scoring criterion are described in Onweller et al. (in preparation).

A modification in the GS356 material rating was the additional classification of moderate resistance (MR) and moderate susceptibility (MS). The individuals rated MR and MS were grouped with the R individuals for statistical testing. Chi-square analysis

was performed on these data to test various genetic ratios (primarily one and two gene segregation ratios).

## RESULTS

Evaluation of synthetic parents, NSGC 9711 and PI 648810, and Goodstreak revealed that NSGC 9711 and PI 648810 are resistant and Goodstreak is susceptible to greenbug biotype E. The five GS166 F<sub>1</sub>s and ten GS356 F<sub>1</sub>s tested were all resistant, which indicated each synthetic contained at least one dominant gene. The F<sub>2</sub> seeds of GS166 and GS356 populations were generated from untested F<sub>1</sub>s, because at the time of F<sub>2</sub> development the goal was to have large F<sub>2</sub> population sizes and F<sub>1</sub> seed was limited. These F<sub>2</sub> populations, tested for a single gene, segregated such that there was an overabundance of susceptible individuals (Table 2). Consequently, these populations failed to fit a single dominant gene ratio (GS166  $\chi^2=12.03^{**}$ ; GS356  $\chi^2=12.97^{**}$ ). The segregation of both F<sub>2</sub> populations statistically fit a 9:7 ratio (GS166  $\chi^2=3.07$ n.s. GS356  $\chi^2=3.55$ n.s.), which would indicate each synthetic parent carried two dominant epistatic genes or the segregating progeny involved a dominant resistance gene and a recessive suppressor gene (most likely coming from Goodstreak). To further resolve the inheritance of resistance, 200 F<sub>2:3</sub> GS356 families were developed from untested F<sub>2</sub> seeds. Of the 200 F<sub>2:3</sub> families, 40 were resistant (all individuals were R, MR, or MS), 107 segregated, and 53 were susceptible. The data fit the 1:2:1 ratio expected for single gene inheritance ( $\chi^2=2.16$ n.s.). Interestingly, when considered without the family structure, the F<sub>3</sub> individuals segregated 4345 R: 2853 S; which failed to fit the hypothesized single gene ratio ( $\chi^2_{5:3}=14.01^{**}$ ). However, unlike the F<sub>2</sub> populations the F<sub>3</sub> population did not fit a two dominant epistatic gene segregation ( $\chi^2_{25:39}=1372$ ), and thus could not confirm our F<sub>2</sub> observation. GS166 F<sub>3</sub> families and individuals were not tested.



Also tested were 40 BC<sub>1</sub>F<sub>2</sub> families of both GS166 and GS356. The segregation of these families indicated resistance can be explained by a single dominant gene (Table 2). When analyzed as individuals without family structure, both GS166 and GS356 exhibited more susceptible individuals than expected; thus a single gene ratio would be rejected (GS166  $\chi^2_{3:5}=9.23^{**}$ , GS356  $\chi^2_{3:5}=5.75^*$ ). Because BC<sub>1</sub>F<sub>2</sub> families segregate 1 segregating family to 1 susceptible family, we were able to analyze the segregation pattern in the segregating families. The expected segregation for a single gene within segregating BC<sub>1</sub>F<sub>2</sub> families is 3 R: 1 S. Upon looking at GS166 and GS356 segregating family individuals, we found GS166 supported a 3:1 while GS356 did not (Table 2).

The results from GS166 and GS356 F<sub>1</sub>s and their families suggested each synthetic contains a single dominant gene for resistance. To determine whether these two genes are allelic or at different loci, the F<sub>2</sub> progeny of the PI 648810/NSGC 9711 cross were evaluated. The F<sub>1</sub> response was not tested. Seventy-five F<sub>2</sub> individuals were tested and segregated 40R:35S. If the genes are allelic, no segregation should occur. If the genes are at unlinked loci and both dominant, a 15:1 ratio should occur. Because both genes were dominant in their respective Goodstreak/Synthetic F<sub>1</sub> study, we expected the data to fit either a 1 R:0 S or a 15 R:1 S ratio. However, the data failed to fit either ratio with more susceptible plants observed than expected for either ratio.

## DISCUSSION

Results from BC<sub>1</sub>F<sub>2</sub> and F<sub>2:3</sub> families led us to conclude a single dominant gene is responsible for greenbug biotype E resistance in NSGC 9711 and PI 648810. The robust nature of family inheritance studies provided insight to our individual plant data. Data surrounding the allelism of these two genes was inconclusive; however it is possible they are linked in repulsion or are independent genes. While the greenbug assay is generally able to distinguish between resistant and susceptible plants (Cheryl Baker, personal communication), our data consistently demonstrated an overabundance of susceptible plants. Given the large greenbug populations that developed in these assays, it is possible that some resistant plants were overwhelmed. The fact that an overabundance of susceptible plants remained even after MS individuals were considered resistant supports this hypothesis.

The *T. tauschii* donor to NSGC 9711, TA2477, has been previously described as greenbug resistant (Lage et al., 2003; Smith and Starkey, 2003, see Table 2 for crosslistings), therefore this resistance was not surprising. TA2477 is also the carrier of *Gbc* (Zhu et al. 2005). Assuming a homogeneous, homozygous accession sample was used to create the synthetics, it is likely the resistance in the present study is the same single gene as previously described. Zhu et al. (2005) studied F<sub>2</sub> populations carrying *Gbc* and found them to fit a 3 R :1 S ratio. However, there were 8% more susceptible observations than expected. Similarly, we observed 11% more susceptible individuals in both GS166 and GS356 F<sub>2</sub> evaluations. The overabundance of susceptible plants in our GS166 population may be a result of preferential transmission of the Goodstreak allele,

gene suppression, or a consequence of compatibility issues between modern and synthetic wheat. No similar information was available on the *T. tauschii* donor of PI 648810.

Susceptible overabundance has been reported during genetic analysis of greenbug and Russian wheat aphid *Diuraphis noxia* (Mordvilko) resistance (Beyer et al., 2011; Lazar et al., 1995). The anomalies have been explained by the segregation of multiple genes, small genome deletions in amphiploids, preferential gene transmission, or misclassification due to resistance genes being overwhelmed in the assays. Another anomaly is the observed suppression of greenbug resistance after the hybridization of susceptible *T. dicoccum* accessions with resistant *T. tauschii* accessions (Lage et al., 2003). Noteworthy is the *T. tauschii* donor (409 in Lage et al., 2003) to NSGC 9711 was reported to have reduced or completely suppressed resistance upon hybridization. Suppression of resistance has also been found upon pyramiding greenbug resistance gene *Gb6* with *Gb2* or *Gb3* (Porter et al., 2000). The combination of suppression with an assay that could overwhelm resistance may explain the PI 648810/NSGC 9711 F<sub>2</sub> progeny not fitting either the expected 1 R:0 S or 15 R:1 S ratios. Additional testing will be required to determine if these genes are independent or allelic.

The present study aimed to understand the genetic basis of greenbug resistance in two synthetic hexaploid wheats. Results of the analyzed families (F<sub>1</sub>, F<sub>2,3</sub>, BC<sub>1</sub>F<sub>2</sub>) suggest both NSGC 9711 and PI 648810 carry a single dominant gene for greenbug biotype E resistance. We, most likely, confirmed the single gene donated by *T. tauschii* in NSGC 9711 is resistant to biotype I (Zhu et al., 2005) and report for the first time its effectiveness against biotype E. Furthermore, we report for the first time PI 648810

carries a single dominant gene for resistance to biotype E. These two parental lines, first selected for their tolerance to drought, provide evidence of the multitude of benefits to be gained from wild ancestors. The overabundance of susceptible individuals within various family structures could be the result of gene suppression; a phenomenon encountered by others, or an assay capable of overwhelming some plants carrying resistance. Therefore, the present study identified two new parental sources of greenbug biotype E resistance. Due to the possible suppression of resistance, breeders should consider this a possibility when working with these genes.

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## TABLES

**Table 1.** Plant introduction and pedigree of six synthetic hexaploid wheats. Also listed are identifiers for *T. tauschii* donors as given by CIMMYT during the wide cross program, Kansas State University, Australian Winter Cereals collection, and the Commonwealth Plant Introduction collection.

PI #	Pedigree	<u><i>T. tauschii</i></u>			
		CIMMYT wide cross #	KSU #	AUS #	CPI #
NSGC 9711	Snipe/Yavaros 79//Dackiye/Teal/3/Ae. Squarrosa (904)	904	2477	24154	110812
648646	68112/Ward//Ae. Squarrosa (369)	369	2528	unknown	unknown
648733	Garza/Boyeros//Ae. Squarrosa (241)	241	3282	24061	110719
648758	Laru/ Ae. Squarrosa (333)	333	2482	24159	110817
648810	Decoy 1/Ae. Squarrosa (256)	256	2400	24078	110737
648823	Decoy 1/Ae. Squarrosa (322)	322	2471	24148	11086

**Table 2.** Resistant and susceptible observed values, expected one gene ratio, the expected observation values and the resulting  $\chi^2$  value for individuals, families, and the individuals within the segregating families.

		<b>Individual</b>						
<b>Cross</b>	<b>Model</b>	Observed			Expected			
		<b>N</b>	<b>R</b>	<b>S</b>	<b>Ratio</b>	<b>R</b>	<b>S</b>	
GS166	F2	160	101	59	3:1	120	40	12.03**
GS166	BC1F2	1214	404	810	3:5	455	759	9.23**
GS356	F2	177	112	65	3:1	133	44	12.97**
GS356	F2:3	7198	4345	2853	5:3	4499	2699	14.01**
GS356	BC1F2	1892	659	1233	3:5	709	1182	5.75*
356/166	F2	75	40	35	1:0	75	0	n/a
					15:1	70	5	209.09**

		<b>Families</b>						
<b>Cross</b>	<b>Model</b>	Observed			Expected			
		<b>R</b>	<b>Seg</b>	<b>S</b>	<b>Ratio</b>	<b>R</b>	<b>Seg</b>	
GS166	BC1F2	18	18	22	1:1	20	20	0.4
GS356	F2:3	44	103	52	1:2:1	50	100	0.79
GS356	BC1F2	25	25	15	1:1	20	20	2.5

		<b>Individuals within Segregating Families</b>						
<b>Cross</b>	<b>Model</b>	Observed			Expected			
		<b>N</b>	<b>R</b>	<b>S</b>	<b>Ratio</b>	<b>R</b>	<b>S</b>	
GS166	BC1F2	551	404	147	3:1	413	138	0.83
GS356	F2:3	3860	2801	1059	3:1	2895	965	12.21**
GS356	BC1F2	1168	659	509	3:1	876	292	215.02**

\* Significant at the 0.05 level

\*\* Significant at the 0.01 level

## APPENDIX

**Appendix 1.** Listed are the germplasm sources the resistance genes were identified in, the designated gene name, the genome origin, and reaction type to various greenbug biotypes. Listed in parenthesis is the *Triticum tauschii* ascension donor.

Germplasm	Gene designation	Origin of resistance	Greenbug biotype							
			B	C	E	F	G	H	I	K
			Reaction to biotype							
DS 28A	<i>Gb1</i>	<i>T. turgidum</i> (L.)	S	S	S	R	S	S	S	S
Amigo	<i>Gb2</i>	<i>S. cereale</i> (L.)	R	R	S	S	S	S	S	S
Largo	<i>Gb3</i>	<i>T. tauschii</i> (Coss.)	S	R	R	S	S	R	R	R
CI 17959	<i>Gb4</i>	<i>T. tauschii</i> (Coss.)	S	R	R	S	S	S	R	R
CI 17882	<i>Gb5</i>	<i>T. speltoides</i> (Tausch)	S	R	R	S	S	S	R	R
GRS 1201	<i>Gb6</i>	<i>S. cereale</i> (L.)	R	R	R	S	R	S	R	R
W7984 (TA1651)	<i>Gb7</i>	<i>T. tauschii</i> (Coss.)	S	R	R	-	S	-	R	R
TA4152L94 (WX1027)	<i>Gba</i>	<i>T. tauschii</i> (Coss.)	-	-	-	-	-	-	R	-
TA4152L24 (WX224)	<i>Gbb</i>	<i>T. tauschii</i> (Coss.)	-	-	-	-	-	-	R	-
TA4063.1 (TA2477)	<i>Gbc</i>	<i>T. tauschii</i> (Coss.)	-	-	-	-	-	-	R	-
TA4064.2 (TA2481)	<i>Gbd</i>	<i>T. tauschii</i> (Coss.)	-	-	-	-	-	-	R	-
KS89WGRC4 (TA1695)	<i>Gbx</i>	<i>T. tauschii</i> (Coss.)	-	-	R	-	-	-	R	R
Sando's 4040	<i>Gby</i>	Unknown	-	-	R	-	-	-	R	-
KSU97-85-3 (TA1675)	<i>Gbz</i>	<i>T. tauschii</i> (Coss.)	-	-	-	-	-	-	R	R

Note: '-' denotes that greenbug response is unknown to identified gene. More testing is required.