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Inheritance and molecular mapping of new greenbug resistance genes in wheat germplasms derived from *Aegilops tauschii*

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Abstract Molecular mapping of genes for crop resistance to the greenbug, *Schizaphis graminum* Rondani, will facilitate selection of greenbug resistance in breeding through marker-assisted selection and provide information for map-based gene cloning. In the present study, microsatellite marker and deletion line analyses were used to map greenbug resistance genes in five newly identified wheat germplasms derived from *Aegilops tauschii*. Our results indicate that the *Gb* genes in these germplasms are inherited as single dominant traits. Microsatellite markers *Xwmc157* and *Xgdm150* flank *Gbx1* at 2.7 and 3.3 cM, respectively. *Xwmc671* is proximately linked to *Gba*, *Gbb*, *Gbc* and *Gbd* at 34.3, 5.4, 13.7, 7.9 cM, respectively. *Xbarc53* is linked distally to *Gba* and *Gbb* at 20.7 and 20.2 cM, respectively. *Xgdm150* is distal to *Gbc* at 17.9 cM, and *Xwmc157* is distal to *Gbd* at 1.9 cM. *Gbx1*, *Gba*, *Gbb*, *Gbc*, *Gbd* and the previously characterized *Gbz* are located in the distal 18% region of wheat chromosome 7DL. *Gbd* appears to be a new greenbug resistance gene different from *Gbx1* or *Gbz*. *Gbx1*, *Gbz Gba*, *Gbb*, *Gbc* and *Gbd* are either allelic or linked to *Gb3*.

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Introduction

The greenbug, *Schizaphis graminum* Rondani, is one of the major pests of wheat worldwide. In the United States, annual losses of wheat production from greenbug damage range from $60 million to more than $100 million dollars (USDA 1996; Webster et al. 2000). Efforts have been made to control greenbug damage using plant resistance. However, breeders are challenged by the occurrence of resistance-breaking biotypes and must continuously identify and incorporate new resistance genes into wheat cultivars.

One of the major sources for greenbug resistance in wheat is *Aegilops tauschii* ($2n = 2x = 14$, D genome). As the donor species of the wheat D genome (Kihara 1944; Cox 1998; Dvorak et al. 1998), *Ae. tauschii* shares complete homology with the D genome of wheat (*Triticum aestivum*, $2n = 6x = 42$, A, B and D genome) (Gill and Raupp 1987). This feature makes transferring of resistance genes from *Ae. tauschii* into wheat much easier than from other wheat-related wild species, such as *Triticum speltoides* (donor species of *Gb5*) (Dubcovsky et al. 1998) or rye (*Secale cereale*, donor species of *Gb2* and *Gb6*) (Hollenhorst and Joppa 1983; Porter et al. 1991). Therefore, *Ae. tauschii* has been preferably used by wheat breeders to integrate pest resistance traits into wheat cultivars. To date, wheat *Gb* genes originating from *Ae. tauschii* include *Gb3* in Largo (Hollenhorst and Joppa 1983), *Gb4* in CI17959 (Martin et al. 1982), *Gbz* in KSU97-85-3 (Zhu et al. 2004) and *Gbx* in KS89WGRC4 (Weng and Lazar 2002) and in W7984 (Weng et al. 2005). To avoid confusion, here we refer to the *Gbx* in KS89WGRC4 as *Gbx1*, and the *Gbx* in W7984 as *Gbx2*. *Gbz*, *Gb3* and *Gbx2* have been mapped to the distal region of wheat chromosome 7DL (Zhu et al. 2004; Weng et al. 2005). *Gb4* may be allelic or linked to *Gb3* (Fritz, unpublished data). The increase in number of *Gb* genes originating from *Ae. tauschii* with map locations on wheat chromosome 7DL suggests that a R gene cluster or family exists in wheat or *Ae. tauschii* chromosome 7DL.

Gbx1 in wheat germplasm KS89WGRC4 (Gill and Raupp 1987) is an uncharacterized greenbug resistance gene from an *Ae. tauschii* line TA1695. KS89WGRC4 is also resistant to the Hessian fly, *Mayetiola destructor* (Say), and soilborne mosaic virus (Gill and Raupp 1987; Gill et al. 1991). To date, a lack of information about the inheritance and chromosome location of *Gbx1* has limited the utilization of this gene in wheat breeding programs.

The CIMMYT Elite 97 synthetic hexaploids and CIMMYT D-genome synthetic hexaploids have been created by crossing durum wheat (*T. turgidum*) ($2n = 4x = 28$, AB genomes) with *Ae. tauschii* to introduce new genetic variability for resistance or tolerance to abiotic and biotic stresses into wheat (Mujeeb-Kazi et al. 1996; Honrao et al. 2003). Smith and Starkey (2003) evaluated 149 synthetic hexaploids for resistance to greenbug biotype I, and found that approximately one-third of them expressed high levels of antibiosis (reduced population growth). Some of these lines are also resistant to several wheat diseases (Mujeeb-Kazi et al. 1998; Singh et al. 1998). In addition, our preliminary data suggested that TA4152L94, TA4152L24, and TA4064.2 express moderate levels of resistance to biotype 1 of the Russian wheat aphid (RWA), *Diuraphis noxia* (Mordvilko). To our knowledge, no other wheat germplasm has been identified as resistant to both greenbug and RWA. Therefore, the elucidation of the inheritance, chromosome location and molecular markers linked to *Gb* genes in the CIMMYT synthetic hexaploids will help breeders develop wheat cultivars resistant to greenbug and RWA.

The objectives of this study were to establish the inheritance mode of *Gbx1* and four *Gb* genes from a selected group of CIMMYT synthetic hexaploids, and to determine their chromosome locations based on linked molecular markers.

Materials and methods

Plant materials

Five populations were evaluated in this study (Table 1). The *Gb* genes in KS89WGRC4 and the synthetic hexaploids TA4152L94, TA4152L24, TA4063.1 and TA4064.2 were designated as *Gbx1*, *Gba*, *Gbb*, *Gbc* and *Gbd*, respectively. The mapping populations for each of these genes were designated as the *Gbx1* population, *Gba* population, *Gbb* population, *Gbc* population and *Gbd* population, respectively. These populations were derived from crosses between KS89WGRC4 and TX91V3308, or Stanton and the synthetic hexaploids (Table 1). TX91V3308 is a wheat breeding line containing the 1BL/1RS translocation. Stanton is a current wheat cultivar in Kansas. Both Stanton and TX91V3308 are highly susceptible to greenbug biotypes E, I and K. Seeds of TA4152L94 (*Gba*), TA4152L24 (*Gbb*), TA4063.1 (*Gbc*), TA4064.2 (*Gbd*), Chinese Spring (CS) wheat, the CS derived nulli-tetra line of wheat chromosome 7D (CS N7D-T7B), the 7DS ditelosomic line (CS Dt7DS) and six 7D long arm deletion lines (CS del7DL) were provided by the Wheat Genetic Resource Center at Kansas State University (KSU). Seeds of KS89WGRC4, TX91V3308 and Stanton were provided by the wheat-breeding project, Department of Agronomy at KSU.

Phenotypic assessment

Twelve to 16 seeds from each of the 92 families of the *Gbx1* $F_{4:5}$ population, and 73, 63, 87, 93 seeds from the *Gba*, *Gbb*, *Gbc* and *Gbd* F_2 populations were planted in 53.3×35.5 cm plastic flats filled with PRO-MIX 'BX' potting mix (Hummert Inc., Earth City, MO, USA) in the greenhouse. Both resistant and susceptible parents of each population were included with each planting of their progeny. Plants were grown in the greenhouse at 20–25°C with 55–70% relative humidity and 15L:9D photoperiod. At the two-leaf stage, each plant was infested with two greenbug biotype I adults (Smith and Starkey 2003). Twenty-one days after infestation, plants were rated using the 1–6 damage scale (Porter et al. 1982). Based on chlorosis and plant vigor, plants exhibiting no chlorosis were scored as 1; plants with ≤ 25% chlorosis were scored as 2; plants with > 25% but ≤ 50% chlorosis were scored as 3; plants with > 50% but ≤ 75% chlorosis were scored as 4; plants with > 75% but < 100% chlorosis were scored as 5; and plants that were entirely chlorotic or dead were scored as

Table 1 Gene designation, pedigree, *Ae. tauschii* source, and mapping populations of wheat germplasm resistant to greenbug biotype I

Gb gene	Resistance source	Pedigree	Donor source (*Ae. tauschii*)	Population
Gbx1	KS89WGRC4	Wichita/TA1695//2*Wichita	TA1695[c]	KS89WGRC4/TX91V3308 ($F_{4:5}$)
Gba	TA4152L94[a]	CETA/*Ae.tauschii*	WX1027[d]	Stanton/TA4152L94 (F_2)
Gbb	TA4152L24[a]	CROC 1/*Ae.tauschii*	WX224[d]	Stanton/TA4152L24 (F_2)
Gbc	TA4063.1[b]	68111/RUBGY//WARD/[TA2477]	TA2477[c]	Stanton/TA4063.1 (F_2)
Gbd	TA4064.2[b]	ALTAR 84/[2481]	TA2481[c]	Stanton/TA4064.2 (F_2)

[a]CIMMYT elite 97 synthetic hexaploid
[b]CIMMYT D-genome synthetic hexaploid
[c]KSU WGRC *Ae. tauschii* accession number
[d]'WX' (wild cross) identification number used at CIMMYT for *Ae. tauschii* accession

6. Because no intermediate degree of resistance or susceptibility (3 and 4) was expressed in plants of these populations, plants with a rating of 1 or 2 were rated as resistant (R), and plants with a rating of 5 or 6 were rated as susceptible (S). The $F_{4:5}$ families composed of entirely resistant plants were rated as resistant (R), the $F_{4:5}$ families composed of entirely susceptible plants were rated as susceptible (S), and the $F_{4:5}$ families composed of both resistant and susceptible plants were rated as heterogeneous (H).

DNA isolation

At the two-leaf stage, a single leaf from each F_2 plant of the mapping populations, the parental lines, the Chinese Spring wheat (CS), the CS-derived aneuploids and deletion lines was harvested for DNA isolation. For the *Gbx1* $F_{4:5}$ population, a single leaf from each plant of each family was harvested and combined for extraction. DNA was isolated using the modified CTAB/phenol extraction and ethanol-precipitation method (Gill et al. 1991).

PCR amplification

PCR amplification was carried out using 2X PCR master mix (Promega, Madison, WI, USA) following the manufacturer's instructions. Each PCR reaction mixture contained 12.5 μl master mix, 50 ng template DNA, and 150 ng each of left and right flanking primers in a total volume of 25 μl. PCR conditions were as described by Roder et al. (1995). Amplified products were separated in 3% agarose gels at 4 V/cm in 1X TBE buffer. Gels were stained with ethidium bromide. DNA fragments were visualized under UV light and recorded using an AMBIS Radioanalytic Imaging System (Digital Imagers, Madison, WI, USA).

Parental polymorphism screening

Since most of the currently identified *Gb* genes originating from *Ae. tauschii* have been mapped to wheat chromosome 7D (Weng and Lazar 2002; Zhu et al. 2004; Weng et al. 2005), we screened markers in chromosome 7D for linkage to the new *Gb* genes. Sixty-five wheat chromosome 7D-specific microsatellite markers and sequence tagged site markers were screened for polymorphism between KS89WGRC4 and TX91V3308. Thirty-six wheat chromosome 7DL-specific microsatellite markers were screened for polymorphism between the resistant and susceptible parents of each mapping population derived from the four synthetic hexaploids. Information on primer sequences was obtained from Roder et al. (1998), Pestsova et al. (2000), and Gupta et al. (2002), http://www.scabusa.org and http://wheat.pw.usda.gov.

Bulk segregant analyses and marker linkage

A modified method for bulk segregant analysis was used to screen molecular markers putatively linked to the different *Gb* genes (Zhu et al. 2004). Briefly, primers amplifying polymorphic fragments between resistant and susceptible parents were used to amplify DNA samples from five individual resistant F_2 plants or $F_{4:5}$ families, and from five individual susceptible F_2 plants or $F_{4:5}$ families. Primers amplifying fragments associated with greenbug resistance were then used to amplify DNA from entire mapping populations. MAPMAKER 3.0 (Lander et al. 1987) was used to construct genetic linkage maps with a LOD >3.0 threshold using the Kosambi map function (Kosambi 1944).

Aneuploid and deletion analysis

The chromosome locations of molecular markers linked to *Gbx1*, *Gbz*, *Gba*, *Gbb*, *Gbc* and *Gbd* were established based on the presence or absence of the amplified PCR products of expected sizes in DNA of the CS genetic stocks. Microsatellite markers were assigned to a specific chromosome arm using the nulli-tetrasomic and ditelosomic lines and to specific chromosome regions based on the absence of expected fragments in the deletion lines.

Allelism test between *Gbd* and *Gbz*

In order to reveal their genetic relationships, we attempted to cross KSU97-85-3 (*Gbz*) with TA4152L94 (*Gba*), TA4152L24 (*Gbb*), TA4063.1 (*Gbc*), or TA4064.2 (*Gbd*) to develop F_2 populations for allelism tests. However, because of unsynchronized flowering times, disease and insect damage, we obtained F_2 seeds only from the cross KSU97-85-3 (*Gbz*) x TA4064.2 (*Gbd*). 205 plants from this F_2 population were evaluated in the greenhouse for resistance to greenbug biotype I. Growth conditions, method of greenbug infestation and phenotypic scoring were the same as described previously in the "Phenotypic assessment" section. The recombination rate between *Gbd* and *Gbz* was calculated using the formula: $r^2 = 4\times$(number of susceptible plants)/total number of plants (Allard 1956), where r is the recombination rate between *Gbd* and *Gbz*.

Chi-square analyses

Chi-square analysis was used to determine whether the segregation ratio among the numbers of homogeneous resistant families, heterogeneous families and susceptible families in the *Gbx1* population fit a 7:2:7 ratio expected for a single dominant gene in the F_4 generation, whether ratios between the number of resistant plants and susceptible plants in the F_2 mapping populations fit a 3:1 ratio expected for the inheritance of a single dominant

gene, and whether the resistant and susceptible plants in the F_2 population derived from KSU97-85-3 (*Gbz*) × TA4064.2 (*Gbd*) segregated in a 15:1 ratio indicative of two independent dominant genes. *P* values were determined from Chi-square tables (Rao 1998).

Results

Inheritance of *Gb* genes

Of the 92 families in the *Gbx1* population, 37 were resistant (R), 12 were heterogeneous (H), and 43 were susceptible (S), which fits the expected segregation ratio of R:H:S = 7:2:7 for a single dominant gene in the F_4 generation (Table 2). Of the 73 plants in the *Gba* population, 48 were resistant and 25 were susceptible. Among the 63 plants in the *Gbb* population, 46 were resistant and 17 were susceptible. In the *Gbc* population, 59 of 87 plants were resistant and 28 were susceptible. In the *Gbd* population, 70 of 93 plants were resistant and 23 were susceptible. The segregation of each of these *Gb* genes fits the expected ratio of R:S = 3:1 for a single dominant gene in a F_2 population (Table 2). Therefore, *Gbx1, Gba*, *Gbb*, *Gbc* and *Gbd* are all inherited as single dominant traits.

Molecular markers linked to *Gb* genes

Twenty of the 65 microsatellite markers used to amplify KS89WGRC4 and TX91V3308 detected polymorphic fragments. Of the 36 markers tested with the four synthetic hexaploids and the susceptible parent Stanton, 17 detected polymorphism between Stanton and TA4152L94, 15 detected polymorphism between Stanton and TA4152L24, 18 detected polymorphism between Stanton and TA4063.1, and 18 detected polymorphism between Stanton and TA4064.2. Linkage analyses revealed that 11 wheat chromosome 7DL-specific microsatellite markers were associated with the five *Gb* genes (see examples in Fig. 1). *Xwmc157* and *Xgdm150* flanked *Gbx1* at 2.7 and 3.3 cM, respectively. *Xwmc671* was proximately linked to *Gba, Gbb, Gbc* and *Gbd* at 34.3, 5.4, 13.7 and 7.9 cM, respectively. *Xbarc53* was distally linked to *Gba* and *Gbb* at 20.7 and 20.2 cM, respectively, *Xgdm150* was distally linked to *Gbc* at 17.9 cM, and *Xwmc157* was distally linked to *Gbd* at 1.9 cM (Fig. 2).

Chromosome location of microsatellite markers linked to *Gb* genes

The primers WMC671, BARC53, GDM150 and WMC157 amplified fragments of expected sizes from DNA of CS but failed to amplify fragments of the expected sizes from DNA of CS N7D-T7B and CS Dt7DS and all the 7DL deletion lines. These results indicated that these markers are located in the missing fragment adjacent to deletion point 7Del-3 (FL = 0.82) (Fig. 2f).

Allelic analysis of *Gbd* and *Gbz*

Among the 205 F_2 plants from the cross KSU97-85-3×TA4064.2, 204 were resistant and 1 was susceptible, which did not fit the segregation ratio of 15R:1S for two independent genes (Table 2). The recombination rate (*r*) between *Gbd* and *Gbz* was 7.0%, indicating that *Gbd* is in a locus independent of *Gbz*.

Discussion

Chromosome location of *Gb* genes

In the current study, we determined that *Gbx1*, *Gba*, *Gbb*, *Gbc* and *Gbd* were all linked to microsatellite markers on the long-arm of wheat chromosome 7D (Fig. 2). The results of aneuploid and deletion line analyses clearly demonstrate that the markers flanking these genes (*Xwmc671*, *Xbarc53*, *Xgdm150* and *Xwmc157*) are situated within the distal 18% region of wheat chromosome 7DL. Therefore, *Gbx1*, *Gba*, *Gbb*, *Gbc* and *Gbd* are located in this same region. Since

Table 2 Segregation for resistance to greenbug biotype I in different wheat populations

Cross combination (S×R)	Generation	Gene	# Plants (families) observed[a]			Ratio observed	Ratio expected	χ^2	P^c
			R	H	S	R:S	R:S		
Stanton×TA4152L94	F_2	*Gba*	48	–[b]	25	1.9:1	3:1	3.33	0.068
Stanton×TA4152L24	F_2	*Gbb*	46	–	17	2.7:1	3:1	0.13	0.716
Stanton×TA4063.1	F_2	*Gbc*	59	–	28	2.1:1	3:1	2.40	0.122
Stanton×TA4064.2	F_2	*Gbd*	70	–	23	3.0:1	3:1	0.004	0.952
KSU97-85-3×TA4064.2	F_2	*Gbz*×*Gbd*	205	–	1	204:1	15:1	11.6	0.0007
KS89WGRC4×TX91V3308	$F_{4:5}$	*Gbx1*	37	12	43	6.2R:2H:7.1S	7R:2H:7S	0.472	0.79

[a] *R* resistant, *H* heterogeneous, *S* susceptible
[b] Not applicable
[c] $P > 0.05$ = fits the expected segregation ratio

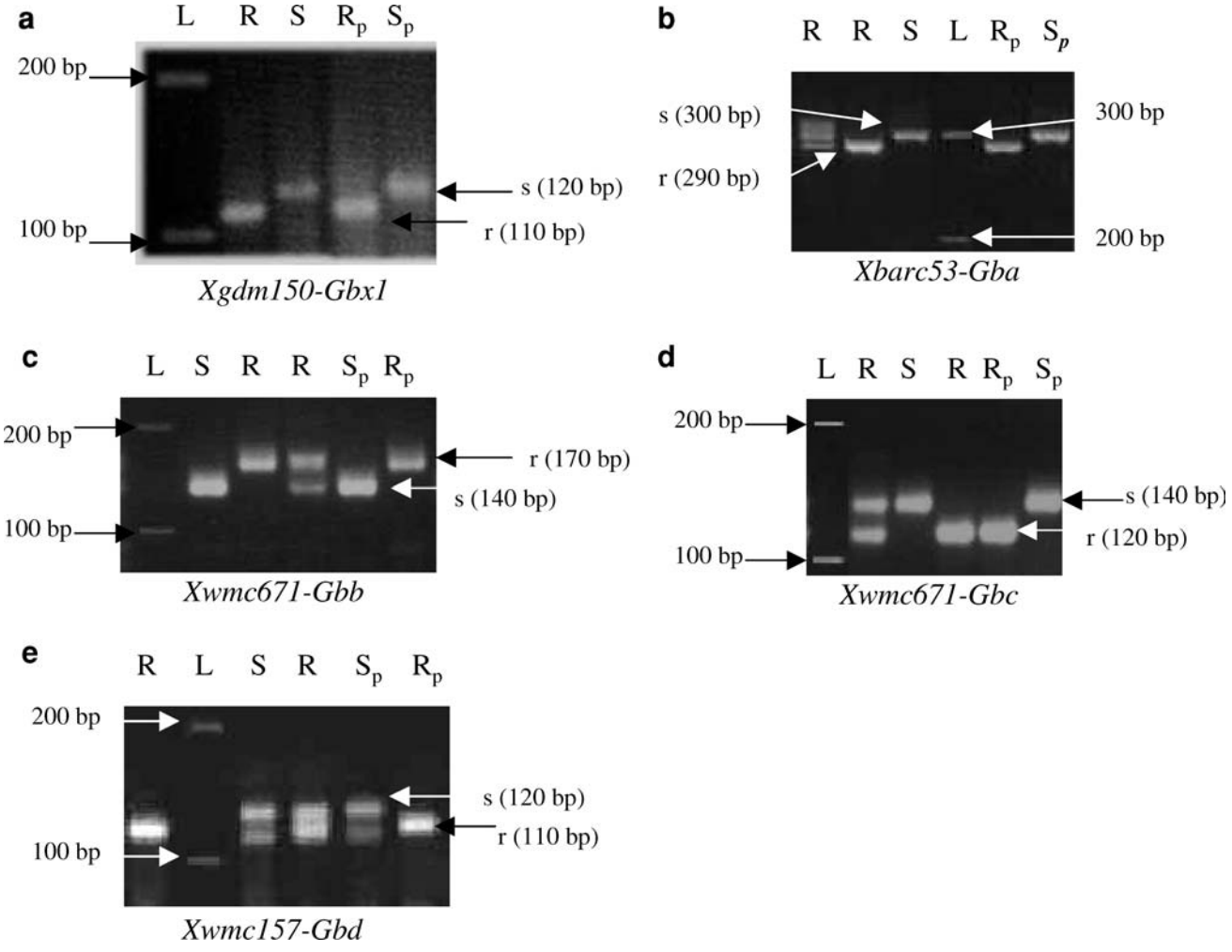

Fig. 1 Polymorphic fragments amplified from DNA of the segregating populations containing *Gbx1* (**a**), *Gba* (**b**), *Gbb* (**c**), *Gbc* (**d**), *Gbd* (**e**) and each of their resistant and susceptible parents. The amplification products were electrophoresed in 3% agarose gels. R_p resistant parent, S_p susceptible parent. *R* resistant phenotype, *S* susceptible phenotype, *L* 100-bp ladder, *r* PCR fragment amplified from resistance parent, *s* PCR fragment amplified from susceptible parent

Xwmc671 and *Xbarc53* also flank *Gbz* (Fig. 2e), *Gbz* can now also be located in the same region of wheat 7DL, where *Gb3* has been localized (Weng et al. 2005). *Gb4* is either allelic or linked to *Gb3* (Fritz, unpublished data), and *Gbx2* is 8.75 cM from *Gb3* (Weng et al. 2005), suggesting that *Gb4* and *Gbx2* are also located in the distal 18% region of wheat chromosome 7DL.

Genetic relationships among *Gb* genes

Two lines of evidence suggest that *Gbd* is a new gene different from *Gbz*. First, *Gbd* and *Gbz* reside at different loci. Second, these two genes express different categories of resistance to greenbug biotype I. *Gbz* expresses only tolerance (Flinn et al. 2001), but *Gbd* expresses antibiosis (Smith and Starkey 2003) to greenbug biotype I. These results suggest that different gene products are associated with each gene. Therefore, *Gbd* is most likely a new gene different from *Gbz*.

Gbx1 is located between *Xwmc157* and *Xgdm150* (Fig. 2g), but *Gbd* is proximal to both *Xwmc157* and *Xgwm150* (Fig. 2d), implying that *Gbx1* is distal to *Gbd*. The categories of resistance expressed by *Gbd* and *Gbx1* also differ, as *Gbx1* confers only tolerance (Boina et al. 2004) to greenbug biotype I, but *Gbd* confers antibiosis (Smith and Starkey 2003). Thus, *Gbd* is likely different from *Gbx1*.

Our current and previous studies (Zhu et al. 2004) indicate that *Gba*, *Gbb*, *Gbc*, *Gbd*, *Gbx1* and *Gbz* are linked to wheat microsatellites in the same chromosome region as *Gb3*. Therefore, these genes could be either allelic or linked to *Gb3*. The exact relationships between these genes and *Gb3* will remain unknown until allelism tests are conducted.

Gba and *Gbb* are flanked by *Xwmc671* and *Xbarc53*, while *Gbc* and *Gbd* are flanked by *Xwmc671* and *Xgdm150*. The PCR fragment sizes of *Xwmc671* and *Xbarc53* are the same in TA4152L94 (*Gba*) and TA4152L24 (*Gbb*), respectively, and the PCR fragment

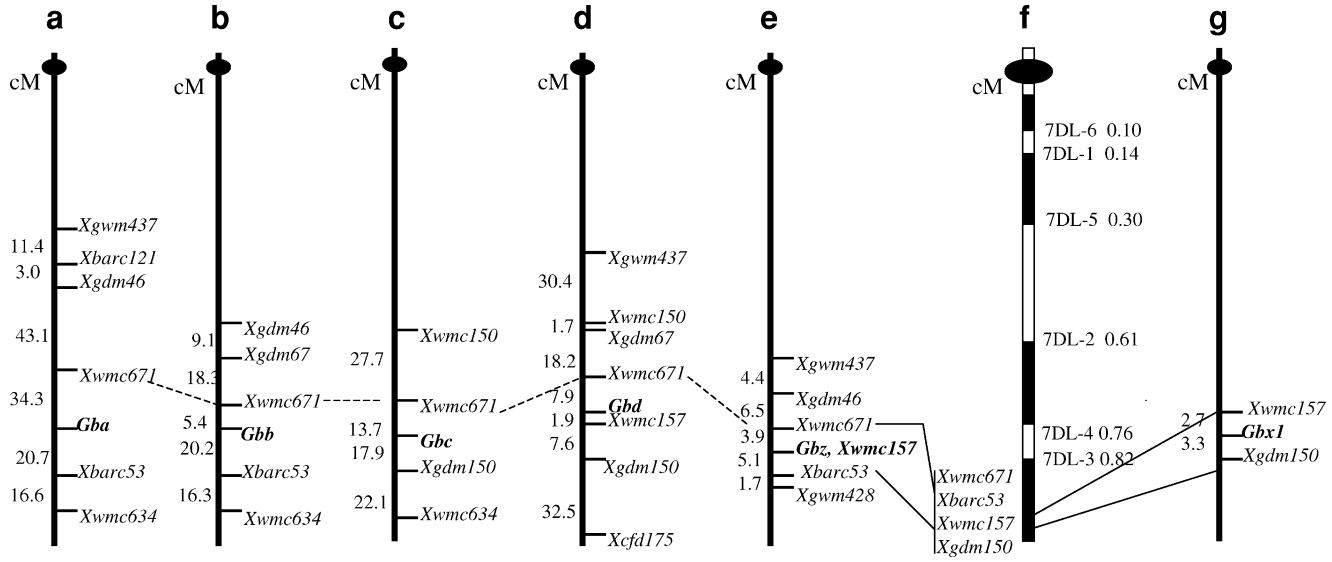

Fig. 2 Genetic and deletion maps of the *Gb* genes on wheat chromosome 7DL. **a**, **b**, **c**, **d**, **e** and **g** are genetic maps containing *Gba*, *Gbb, Gbc*, *Gbd*, *Gbz* and *Gbx1*, respectively. *Numbers to the left* of each genetic map are genetic distances between adjacent markers in centiMorgans. **f** is a deletion map for selected microsatellite markers. *Numbers to the right* of deletion-line symbols indicate fraction length (FL) of each corresponding breaking point. *Dashed lines* connect marker *Xwmc671* among different genetic maps, and *solid lines* connect markers between genetic maps and the deletion map

sizes of *Xwmc671* and *Xgdm150* are the same in TA4063.1 (*Gbc*) and TA4064.2 (*Gbd*), respectively (data not shown), suggesting that *Gba* and *Gbb* are closely related, while *Gbc* and *Gbd* are closely related. Because *Gba*, *Gbb*, *Gbc* and *Gbd* are proximal to marker *Xwmc671*, they are either allelic or linked. Further allelic and plant differential experiments will be required to clarify the relationships among these *Gb* genes.

Conclusion

Wheat germplasms KS89WGRC4, TA4152L94, TA4152L24, TA4063.1 and TA4064.2 represent new genetic resources for use in developing wheat cultivars resistant to greenbug and other arthropod pests. In the current study, we determined that the *Gb* gene carried by each of these wheat germplasms was inherited as a single dominant trait. Molecular markers linked to each gene were identified, and the chromosome location of these genes was narrowed to the 18% distal region of wheat chromosome 7DL. Some of these markers can be used directly for marker-assisted selection in wheat breeding. The information on the chromosome location of these genes indicates that the map-based cloning of these *Gb* genes is feasible. Our data also suggest that *Gbd* is a new gene different from *Gbz* or *Gbx1*. *Gba*, *Gbb*, *Gbc, Gbd* and *Gbx1* are either allelic or linked to *Gb3*. The information on the genetic relationships among these *Gb* genes will help breeders selectively use these genes to develop greenbug resistant germplasms, and to aid researchers to avoid redundancy in further mapping and cloning of these genes. Several mapping populations used in the current research were derived from Stanton, a RWA resistant wheat cultivar currently planted in Kansas. We anticipate that molecular markers identified in this research will be used by wheat breeders to transfer *Gb* genes into Stanton to develop an improved wheat cultivar with dual insect resistance.

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References

U.S. Department of Agriculture (1996) Agricultural statistics 1995–1996. Wheat, National Agricultural Statistics Service, US Department of Agriculture, Washington, DC

Allard RW (1956) Formulas and tables to facilitate the calculation of recombination values in heredity. Hilgardia 24:235–278

Boina D, Prabhakar S, Smith CM, Starkey S, Zhu L, Boyko E, Reese JC (2005) Categories of resistance to biotype I greenbug (Homoptera: Aphididae) in wheat lines containing the genes *Gbx* and *Gby*. J Kan Entomology Soc 78:(in press)

Cox TS (1998) Deepening the wheat gene pool. J Crop Prod 1:1–25

Dubcovsky J, Lukaszewski AJ, Echaide M, Antonelli EF, Porter DR (1998) Molecular characterization of two *Triticum speltoides* interstitial translocations carrying leaf rust and greenbug resistance genes. Crop Sci 38:1655–1660

Dvorak J, Luo MC, Yang ZL and Zhang HB (1998) The structure of the *Aegilops tauschii* genepool and the evolution of hexaploid wheat. Theor Appl Genet 97:657–670

Flinn MB, Smith CM, Reese JC, Gill B (2001) Categories of resistance to greenbug (Homoptera: Aphididae) biotype I in *Aegilops tauschii* germplasm. J Econ Entomol 94:558–563

Gill BS, Raupp WJ (1987) Direct genetic transfers from *Aegilops squarrosa* L. to hexaploid wheat. Crop Sci 27:445–450

Gill KS, Lubbers EL, Gill BS, Raupp WJ, Cox TS (1991) A genetic linkage map of *Triticum tauschii* (DD) and its relationship to the D genome of bread wheat (AABBDD). Genome 34:362–374

Gupta PK, Balyan HS, Edwards KJ, Isaac P, Korzun V, Roder M, Gautier MF, Joudrier P, Schlatter AR, Dubcovsky J, De la Pena RC, Khairallah M, Penner G, Hayden MJ, Sharp P, Keller B, Wang RCC, Hardouin JP, Jack P, Leroy P (2002) Genetic mapping of 66 new microsatellite (SSR) loci in bread wheat. Theor Appl Genet 105:413–422

Hollenhorst MM, Joppa LR (1983) Chromosomal location of genes for resistance to greenbug in 'Largo' and 'Amigo' wheats. Crop Sci 23:91–93

Honrao BK, Misra SC, Bhagwat MD, Dixit RN, Surve VD, Khade VM, Rao VS (2003) Potential of synthetic wheats as new sources of leaf rust resistance. Cereal Rusts and Powdery Mildews Bulletin (http://www.crpmb.org/) 2003/0317honrao

Kihara H (1944) Die Entdeckung des DD-Analysators beim Weizen. Agric Hortic 19:889–890

Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugen 12:172–175

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newberg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Martin TJ, Harvey TL, Hatchett JH (1982) Registration of greenbug and Hessian fly resistant wheat grermplasm. Crop Sci 22:1089

Mujeeb-Kazi A, Rosas V, Roldan S (1996) Conservation of the genetic variation of *Triticum tauschii* (Coss.) Schmalh. (*Aegilops squarrosa* auct. Non. L.) in synthetic hexaploid wheats (*T. turgidum* L. s. lat. X *T. tauschii*; $2n = 6x = 42$, AABBDD) and its potential utilization for wheat improvement. Genet Res Crop Evol 43:129–134

Mujeeb-Kazi A, Gilchrist LI, Fuentes-Davila G, Velazquez C, Delgado R (1998) *Fusarium graminearum* resistance in alien germplasm and in a bread wheat/alien species derivative with multiple biotic stress resistances. Annu Wheat Newslett 45

Pestsova E, Ganal MW, Roder MS (2000) Isolation and mapping of microsatellite markers specific for the D genome of bread wheat. Genome 43:689–697

Porter KB, Peterson GL, Vise O (1982) A new greenbug biotype. Crop Sci 22:847–850

Porter DR, Webster JA, Burton RL, Puterka GL, Smith EL (1991) New sources of resistance to greenbug in wheat. Crop Sci 31:1502–1504

Rao PV (1998) Statistical research methods in the life sciences. Duxbury Press, New York, pp 889

Roder MS, Plaschke J, Konig SU, Borner A, Sorrells ME, Tanksley SD, Ganal MW(1995) Abundance, variability and chromosomal location of microsatellites in wheat. Mol Gen Genet 246:327–333

Roder MS, Korzun V, Wendehake K, Plaschke J, Tixier MH, Leroy P, Ganal MW (1998) A microsatellite map of wheat. Genetics 149:2007–2023

Singh SS, Sharma DN, Mehta H (1998) Resistance to *Puccinia recandita tritici* in synthetic hexaploid wheats. Indian J Genet 58:263–269

Smith CM, Starkey S (2003) Resistance to greenbug (Homoptera: Aphididae) biotype I in *Aegilops tauschii* synthetic wheats. J Econ Entomol 96:1571–1576

Webster J, Treat R, Morgan L, Elliot N (2000) Economic impacts of the Russian wheat aphid and greenbug in the western United States 1993–94, 1994–95, and 1997–98. US Department of Agriculture. ARS Service Report PSWCRL Rep 00–001

Weng Y, Lazar MD (2002) Amplified fragment length polymorphism- and simple sequence repeat-based molecular tagging and mapping of greenbug resistance gene *Gb3* in wheat. Plant Breed 121:218–223

Weng Y, Li W, Devkota RN, Rudd JC (2005) Microsatellite markers associated with two *Aegilops tauschii*-derived greenbug resistance loci in wheat. Theor Appl Genet 110:462–469

Zhu LC, Smith CM, Fritz A, Boyko EV, Flinn MB (2004) Genetic analysis and molecular mapping of a wheat gene conferring tolerance to the greenbug (*Schizaphis graminum* Rondani). Theor Appl Genet 109:289–293