Theor Appl Genet (2004) 109: 289–293
DOI 10.1007/s00122-004-1632-x

ORIGINAL PAPER

L. C. Zhu · C. M. Smith · A. Fritz · E. V. Boyko · M. B. Flinn

Genetic analysis and molecular mapping of a wheat gene conferring tolerance to the greenbug (*Schizaphis graminum* Rondani)

Received: 1 September 2003 / Accepted: 9 February 2004 / Published online: 5 May 2004

Abstract The greenbug, *Schizaphis graminum* (Rondani), is one of the major pests of wheat worldwide. The efficient utilization of wheat genes expressing resistance to greenbug infestation is highly dependent on a clear understanding of their relationships. The use of such genes will be further facilitated through the use of molecular markers linked to resistance genes. The present study involved several F_2 wheat populations derived from crosses between susceptible cultivars and resistant germplasm carrying different greenbug resistance genes. These populations were used to characterize the inheritance of a wheat gene (*Gbz*) conferring tolerance to greenbug biotype I, to identify molecular markers linked to *Gbz*, and to investigate the relationship between *Gbz* and *Gb3*, a previously identified greenbug resistance gene. Our results indicated that *Gbz* is inherited as a single dominant gene. Microsatellite marker *Xwmc157* is completely linked to *Gbz*, and *Xbarc53* and *Xgdm46* flank *Gbz* at distances of 5.1 and 9.5 cM, respectively. Selection of *Gbz* using marker *Xwmc157* alone gives breeders 100% selection accuracy. *Gbz* may be placed in the distal region of the long arm of the wheat chromosome 7D. The results of allelism tests indicated that *Gbz* is either allelic or tightly linked to *Gb3*.

Communicated by D.A. Hoisington

L. C. Zhu · C. M. Smith (✉) · E. V. Boyko
Department of Entomology, Kansas State University, Manhattan, KS 66506, USA
e-mail: cmsmith@ksu.edu
Fax: +1-785-5326232

A. Fritz
Department of Agronomy, Kansas State University, Manhattan, KS 66506, USA

M. B. Flinn
Department of Zoology, Southern Illinois University, Carbondale, IL 62901, USA

Introduction

The greenbug, *Schizaphis graminum* (Rondani), is one of the major pests of wheat worldwide. In the United States, the greatest greenbug-related losses have been reported in the Southern Great Plains areas of Texas, Oklahoma and Kansas (Hollenhorst and Joppa 1983). Annual losses of United States wheat production from greenbug damage are as great as $250 million, including the cost of pesticides and yield loss (Suszkiw 2002).

The utilization of host plant resistance is an environmentally safe, cost effective way to manage greenbug damage. Greenbug resistance of plants is classified into three categories as antibiosis, antixenosis or tolerance (Painter 1951; Horber 1980). Antibiosis describes the adverse effect of a plant on the biology of the pest insect while antixenosis explains the inability of plant to serve as a host when an alternative host exists. Tolerance, however, refers to the ability of plants to withstand or recover from insect damage (Smith 1989). Greenbug-resistant wheat lines often express one or more of these categories to the infestation of different greenbug biotypes (Webster and Porter 2000; Flinn et al. 2001; Smith and Starkey 2003).

Six greenbug resistance genes have been designated, as well as wheat germplasm carrying resistance genes (Table 1). Among these, *Gb3* is the only gene to have been incorporated into a wheat cultivar (TAM 110) and to convey resistance against currently prevalent greenbug biotypes (Lazar et al. 1997). However, since *Gb3* expresses antibiosis to greenbugs, which often leads to the development of new virulent greenbug biotypes, the discovery and deployment of new greenbug resistance genes is urgently needed. Studies to characterize greenbug resistance genes, tag them with molecular markers and determine their allelic relationships provide an opportunity for wheat breeders to use them in molecular marker assisted selection (MAS) schemes to deploy these genes against greenbug.

Among the non-designated genes, *Gbx* is either allelic or tightly linked to *Gb3* (Weng and Lazar 2002). *Gby* has been mapped on wheat chromosome 7A (Boyko et al.

Table 1 Name, source and chromosome location of wheat genes expressing resistance to greenbug

Gene	Germplasm Source	Origin	Chromosome Location	References
*Gb*1	DS28A	*T. durum*	?	–
*Gb*2	Amigo	*S. cereale*	1AL/1RS	Hollenhorst and Joppa 1983
*Gb*3	Largo (CI17895)	*Ae. tauschii*	7D	Hollenhorst and Joppa 1983; Weng and Lazar 2002
*Gb*4	CI17959	*Ae. tauschii*	7D	Martin et al. 1982; Fritz unpublished
*Gb*5	CI17882	*T. spel-toides*	7AL	Dubcovsky et al. 1998
*Gb*6	GRS1201	*S. cereale*	1AL/1RS	Porter et al. 1991
Gba	TA4152L94	*Ae. tauschii*	?	Smith and Starkey 2003
Gbx	KS89WGRC4	*Ae. tauschii*	7D	Weng and Lazar 2002

2004), and *Gba*, carried by the CYMMYT synthetic wheat line TA4152L94, expresses a high level of resistance to greenbug biotype I (Smith and Starkey 2003). Molecular characterization of *Gba* is underway.

Gbz is a greenbug resistance gene carried by *Aegilops tauschii* line TA1675, and was transferred into the bread wheat cultivar 'Wichita' by backcrossing to develop the resistant wheat germplasm KSU97-85-3 (Gill and Raupp 1987). Flinn et al. (2001) observed that *Gbz* confers tolerance resistance to greenbug biotype I, the most prevalent greenbug biotype in Kansas and in the U.S. Tolerance places no pressure for the selection of new biotypes (Smith 1989). Therefore, the use of *Gbz* and other greenbug tolerance genes can provide durable greenbug resistance for wheat and should be preferred for use in the integrated management of greenbug.

The objectives of this study were to determine the inheritance mode and chromosome location of *Gbz*, to identify molecular markers linked to *Gbz*, and to establish the genetic relationship between *Gbz* and *Gb3*.

Materials and methods

Plant materials

Three F_2 populations were created in this study from the crosses Jagger × KSU97-85-3, Stanton × KSU 97-85-3, and KSU97-85-3 × Largo. The seeds of wheat germplasm KSU97-85-3 were provided by Dr. Bikram Gill in the Wheat Genetics Resource Center, Kansas State University. Jagger and Stanton are current winter wheat cultivars in Kansas with excellent agronomic traits, but both are susceptible to greenbug infestation. Largo is a greenbug-resistant germplasm carrying *Gb3* (Table 1).

Phenotypic assessment

One hundred and twenty six plants of the F_2 population derived from the cross Jagger × KSU97-85-3, 72 plants of the F_2 population derived from the cross Stanton × KSU 97-85-3, and 158 plants of the F_2 population derived from the cross KSU97-85-3 (*Gbz*) × Largo (*Gb3*) were planted in plastic 53.3×35.5 cm flats filled with Jiffy potting mix in a greenhouse, and the plants of the appropriate resistant parent (KSU97-85-3 or Largo) and susceptible parent (Jagger or Stanton) were included with each planting of their progenies. The plants were grown under 20–25°C, 55–70% relative humidity and a 15L: 9D photoperiod. At the two-leaf stage, each of the individual plants were infested with three greenbug biotype I adults using methods described by Smith and Starkey (2003). Three weeks after infestation, all susceptible control plants in each flat were dead or dying. Plants were rated using the 1–6 damage rating scale developed by Porter et al. (1982). Based on chlorosis and plant vigor, plants exhibiting very little to no chlorosis were rated as 1; plants with ≤25% chlorosis were rated as 2; plants with >25% but ≤50% chlorosis were rated as 3; plants with >50% but ≤75% chlorosis were rated as 4; plants with >75% but <100% chlorosis were rated as 5; and plants that were entirely chlorotic or dead were rated as 6. No intermediate level of resistance or susceptibility (ratings of 3 and 4) was expressed in the populations. Plants rated from 1 to 2 were scored as resistant (R), and plants rated from 5 to 6 were scored as susceptible (S).

DNA isolation

A single leaf from each F_2 plant of the Jagger × KSU97-85-3 population was harvested at the two-leaf stage of plant development 3 days before infestation. DNA was isolated from the collected leaf tissue using the modified CTAB/phenol extraction and ethanol-precipitation method described by Gill et al. (1991).

PCR amplification

PCR amplification was carried out using 2× PCR master mix from Promega (Madison, Wis.) 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. The PCR conditions were as described by Liu et al. (2002). Amplified products were separated in 3% agarose gels at 6 V/cm in 1× TBE buffer. Gels were stained with eithidium bromide with a final concentration of 0.5 μg/ml. DNA fragments were visualized under UV light and recorded by an AMBIS Radioanalytic Imaging System (Digital Imagers, Madison, Wis.).

Molecular marker screening and linkage analysis

The F_2 population derived from the cross Jagger × KSU97-5-3 was used for molecular marker analysis. Based on the fact that all the greenbug-resistant genes originating from *Ae. tauschii* are located on wheat chromosome 7D, 55 chromosome 7D-specific microsatellite and sequence tagged site (STS) primer sets were screened for polymorphisms between KSU 97-85-3 and Jagger. Primers or primer sequence information were obtained from the John Innes Center (Norwich, UK), Roder et al. (1998), Pestsova et al. (2000), Gupta et al. (2002), http://www.scabusa.org, http://wheat.pw.usda.gov and Sourdille (unpublished). Primers amplifying polymorphic

fragments between resistant and susceptible parents were used to amplify DNA samples from five individual resistant F_2 plants and DNA samples from five individual susceptible F_2 plants. The primers amplifying fragments associated with *Gbz* were used to amplify DNA of all F_2 plants of the Jagger × KSU97-85-3 population for linkage analysis. MapMaker 2.0 (Lander et al. 1987) was used to perform linkage analysis and to construct a genetic linkage map (LOD >3.0) using the Kosambi mapping function (Kosambi 1944).

Chi-square analyses

Chi-square analysis was used to determine whether the ratio between the number of resistant and susceptible plants in the F_2 populations fit the 3:1 ratio expected for the inheritance mode of a single dominant gene, and whether the molecular markers segregated co-dominantly to fit the 1:2:1 ratio expected for plants possessing fragments of the resistant parent (R), both fragments of resistant and susceptible parents (H), or fragments of the susceptible parent (S). *P* values were determined from chi-square tables (Rao 1998).

Results

Inheritance of greenbug resistance

Of 126 F_2 plants derived from the cross Jagger × KSU97-85-3, 94 plants were resistant and 32 plants were susceptible. Of 72 F_2 plants derived from cross Stanton × KSU97-85-3, 54 plants were resistant and 18 plants were susceptible. The segregation ratio in both populations fits the 3:1 (resistant: susceptible) inheritance mode expected for a single dominant gene (Table 2). These results indicated that greenbug resistance in KSU97-85-3 is controlled by a single dominant gene.

Molecular markers linked to *Gbz*

Of 55 primer pairs evaluated, 20 amplified polymorphic fragments between the resistant parent KSU97-85-3 and the susceptible parent Jagger. All 20 primer pairs were then used to amplify the small sets of DNA samples from resistant and susceptible plants, and five of the 20 microsatellite primers amplified DNA fragments showing putative linkage to *Gbz*. Microsatellite primer BARC53 amplified a 315-bp fragment from DNA of the resistant parent KSU97-85-3 and each of the five resistant F_2 plants and a 295-bp fragment from DNA of the susceptible parent Jagger and each of the five susceptible F_2 plants (Fig. 1a). Microsatellite primer GDM46 amplified a 135-bp fragment from DNA of KSU97-85-3 and each of the five resistant F_2 plants and a 150-bp fragment from DNA of Jagger and each of the five susceptible F_2 plants (Fig. 1b). Microsatellite primer GWM428 amplified a 135-bp fragment from DNA of KSU97-85-3 and each of the five resistant F_2 plants and a 150-bp fragment from DNA of Jagger and each of the five susceptible F_2 plants (Fig. 1c). Microsatellite primer GWM437 amplified a 120-bp fragment from DNA of KSU97-85-3 and each of the five resistant F_2plants and a 90-bp fragment from DNA of Jagger and each of the five susceptible F_2 plants (Fig. 1d). Microsatellite primer WMC157 amplified a 150-bp fragment from DNA of KSU97-85-3 and each of the five

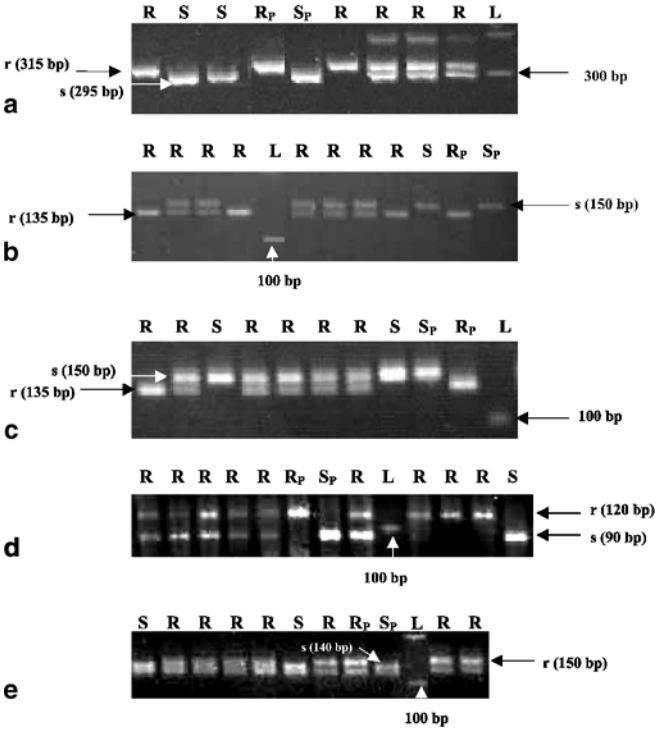

Fig. 1a–e DNA fragments amplified from F_2 progeny of the cross Jagger × KSU97-85-3 using microsatellite primers. **a** BARC53, **b** GDM46, **c** GWM428, **d** GWM437, **e** WMC157. *R* Resistant phenotype, *S* susceptible phenotype. R_P resistant parent, S_P susceptible parent. *L* 100 bp ladder

Table 2 Segregation for resistance to greenbug feeding in wheat F_2 populations derived from the crosses Jagger × KSU97-85-3, Stanton × KSU97-85-3 and KSU97-85-3 × Largo. *S* Susceptible, *R* resistant. $P>0.05$ = fit to the expected segregation ratio

Cross combination (S×R)	No. of plants		Ratio observed	Ratio expected		
	Observed					
	R	S	R:S	R:S	X^2	*P*
Jagger × KSU97-85-3	94	32	2.9:1	3:1	0.011	0.92
Stanton × KSU97-85-3	54	18	3:1	3:1	0	1
KSU97-85-3 × Largo	158	0	158:0	158:0	0	1

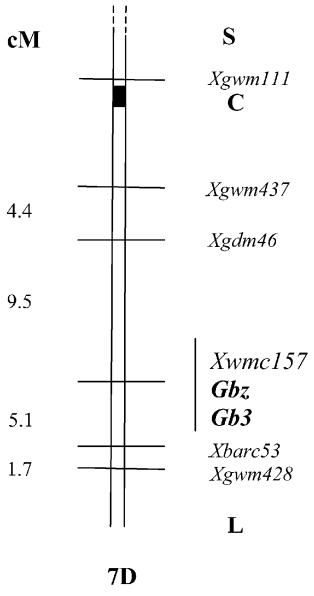

Fig. 2 Genetic linkage map of wheat chromosome 7DL consisting of a greenbug resistance gene *Gbz* and the linked microsatellite markers. *S*, *L* Short or long chromosome arm, *C* centromere position

resistant F_2 plants and a 140-bp fragment from DNA of Jagger and each of the five susceptible F_2 plants (Fig. 1e).

The results of linkage analyses indicated that microsatellite marker *Xwmc157* co-segregated with *Gbz*. Markers *XBarc53*, *Xgwm428, Xgdm46* and *Xgwm437* were linked to *Gbz* at 5.1 cM, 6.8 cM, 9.5 cM and 13.9 cM, respectively (Fig. 2). Based on the established map locations of the microsatellites used, the linkage group around the *Gbz* locus was determined to be located on the long arm of chromosome 7D. The five microsatellites identified in the linkage group associated with *Gbz* segregated according to a 1:2:1 ratio in the 114–117 F_2 plants tested (data not shown).

Allelic analysis of *Gbz* and *Gb3*

All 158 individual plants from the F_2 population KSU97-85-3 × Largo were resistant to greenbug biotype I with no segregation for susceptibility. This result indicates that *Gbz* is tightly linked or allelic to *Gb3* (Table 2).

Discussion

Chromosome location of *Gbz*

Our results indicated that *Gbz* is linked to five microsatellite markers located on the long arm of wheat chromosome 7D (Roder et al. 1998; Pestsova et al. 2000; Gupta et al. 2002; http://www.scabusa.org), among which, *Xwmc157* co-segregated with *Gbz*, and *Xbarc53* was linked to *Gbz* at a distance of 5.1 cM (Fig. 2). Based on the physical map of wheat chromosome 7D (http://www.scabusa.org), the microsatellite marker *Xbarc53* is located in the distal region of the long arm of the chromosome. Given the extremely high rate of recombination in the distal region of wheat chromosome 7DL (Boyko et al. 2002) and the tight linkage between *Gbz* and the marker *Xbarc53*, *Gbz* may be placed in the distal region of the long arm of the wheat chromosome 7D. In addition, the allellism test results indicated that *Gbz* is either allelic or tightly linked to *Gb3*. As a result, *Gb3* can also be placed in the same chromosome region as *Gbz*. *Gb3*, a greenbug resistance gene also from *Ae. tauschi* (PI 268210), was first placed on wheat chromosome 7D by Hollenhorst and Joppa (1983) using monosomic analysis, and was further localized by Weng and Lazar (2002) to a 55.6 cM region between microsatellite markers *Xgwm111* and *Xgwm428* on wheat chromosome 7D.

Our current results indicate the tight linkage or allelic relationship between *Gbz* and *Gb3*, which localizes the chromosome location of *Gb3* to the same region as *Gbz*. However, *Gbz* and *Gb3* may not be the same gene because they exhibit different categories of resistance to greenbug biotype I infestation. *Gb3* conveys antibiosis to greenbug biotype I, but not tolerance (Smith and Starkey 2003), while *Gbz* expresses only tolerance and no antibiosis (Flinn et al. 2001). Since the category of resistance may be influenced by the genetic background of a particular germplam carrying a resistance gene, studies of both genes in the same genetic background will be necessary to clarify whether *Gbz* and *Gb3* are the same gene or two different genes that are allelic or tightly linked. The exact identity of the two genes will likely remain unresolved until they are cloned and sequenced.

The utilization of *Gbz* in breeding

Gene pyramiding is regarded as a possible approach to provide durable plant resistance to insects (Yencho et al. 2000). However, gene pyramiding is prohibitively time-consuming (Porter et al. 2000). Since tolerance exerts no selection pressure on pest populations (Smith 1989), and the transfer of a single dominant gene is much less laborious and time-consuming than gene pyramiding, the incorporation of a single gene conferring tolerance resistance into currently used wheat cultivars will assure the durability of resistance and efficient gene utilization. Flinn et al. (2001) has established that tolerance is the only component of *Gbz* resistance to greenbug damage, and our current studies demonstrated that *Gbz* is inherited as a single dominant trait. Therefore, the utilization of *Gbz* is of great value in wheat breeding against greenbug infestation. The molecular markers identified in this study can be applied in MAS to accelerate the breeding process and improve selection precision for greenbug resistance traits.

Acknowledgements This work was financially supported by the Kansas Crop Improvement Association, the Kansas Agricultural Experiment Station, the Kansas Wheat Commission, and a KSU-CSREES IPM minigrant to L.C. Zhu. We thank Dr. Tom Harvey for the kind supply of greenbug biotype I, and Dr. Xuming Liu and Sharon Starkey for all their suggestions and help. Kansas Agricultural Experimental Station Contribution No. 04-045-J.

References

Boyko EV, Kalendar R, Korzun V, Fellers J, Korol A, Schulman AH, Gill BS (2002) A high-density cytogenetic map of the *Aegilops tauschii* genome incorporating retrotransposons and defense-related genes: insights into cereal chromosome structure and function. Plant Mol Biol 48:767–90

Boyko EV, Starkey SR, Smith CM (2004) Molecular genetic mapping of *Gby*, a new greenbug resistance gene in bread wheat. Theor Appl Genet (in press)

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 38:1655–1600

Flinn MB, Smith CM, Reese JC, Gill BS (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 relation 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, DelaPena 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

Horber E (1980) Types and classification of resistance. In: Maxwell TG, Jennings PT (eds) Breeding plants resistant to insects. Wiley, New York, pp 15–21

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

Lazar MD, Worral WD, Peterson GL, Porter KB, Rooney LW, Tuleen NA, Marshall DS, McDaniel ME, Nelson LR (1997) Registration of TAM110. Crop Sci 37:1978–1979

Liu, XM, Smith CM, Gill BS (2002) Identification of microsatellite markers linked to Russian wheat aphid resistance genes *Dn4* and *Dn6*. Theor Appl Genet 104:1042–1048

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

Painter RH (1951) Insect resistance in crop plants. University of Kansas Press, Lawrence, pp 520

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

Porter DR, Burd JD, Shufran KV, Webster JA (2000) Efficacy of pyramiding greenbug (Homoptera: Aphididae) resistance genes in wheat. J Econ Entomol 93:1315–1318

Rao PV (1998) Statistical research methods in the life sciences. Duxbury, New York, p 286

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

Smith CM (1989) Plant resistance to insects — a fundamental approach. Wiley, New York; pp 286

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

Suszkiw J (2002) Greenbug-resistant wheat. http://www.nps.ars.usda.gov/menu.htm?newsid=1971

Webster JA, Porter DR (2000) Plant resistance components of two greenbug (Homoptera: Aphididae) resistant wheats. J Econ Entomol 93:1000–1004

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

Yencho GC, Cohen MB, Byrne PE (2000) Applications of tagging and mapping insect resistance loci in plants. Annu Rev Entomol 45:393–422