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ORIGINAL ARTICLE

Gb8, a new gene conferring resistance to economically important greenbug biotypes in wheat

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Abstract

Key message **A new greenbug resistance gene *Gb8* conferring broad resistance to US greenbug biotypes was identified in hard red winter wheat line PI 595379-1 and was mapped to the terminal region of chromosome 7DL.**

Abstract Greenbug [*Schizaphis graminum* (Rondani)] is a worldwide insect pest that poses a serious threat to wheat production. New greenbug resistance genes that can be readily used in wheat breeding are urgently needed. The objective of this study was to characterize a greenbug resistance gene in PI 595379-1, a single plant selection from PI 595379. Genetic analysis of response to greenbug biotype E in an $F_{2:3}$ population derived from a cross between PI 595379-1 and PI 243735 indicated that a single gene, designated *Gb8*, conditioned resistance. Linkage analysis placed *Gb8* in a 2.7-Mb interval in the terminal bin of chromosome 7DL (7DL3-082-1.0), spanning 595.6 to 598.3 Mb in the Chinese Spring IWGSC RefSeq version 1.0 reference sequence. *Gb8* co-segregated with a newly developed SSR marker *Xstars508*, positioned at 596.4 Mb in the reference sequence. Allelism tests showed that *Gb8* was different from three permanently named genes on the same chromosome arm and the estimated genetic distance between *Gb8* and *Gb3* was 15.35 ± 1.35 cM. *Gb8* can be directly used in wheat breeding to enhance greenbug resistance.

Introduction

Greenbug [*Schizaphis graminum* (Rondani)], a major insect pest of wheat on most continents, poses a threat to wheat production in the southern Great Plains of the USA, where annual losses caused by greenbug have been estimated at more than $100 million (Eddleman et al. 1999; LeClerg et al. 1965; Webster and Kenkel 1995). Greenbug uses its piercing and sucking mouthparts to remove plant assimilates from wheat leaves and stems, and feeding can inhibit plant growth to the point of death. Greenbug is also a vector of the virus disease commonly known as barley yellow dwarf (Johnson and Rochow 1972; Gray et al. 1998).

Pesticides were once widely used to control the frequent outbreaks of greenbug in the southern Great Plains, but host plant resistance has become a more economic, efficient and environment-friendly alternative to chemical control. To date, seven permanently designated greenbug resistance genes (*Gb1–Gb7*) have been documented in wheat. Of these, *Gb1*, a recessive gene conferring unique resistance to greenbug biotype F, was identified in Dickinson Selection 28 (CI 13833) (Curtis et al. 1960). *Gb2* and *Gb6* were identified in wheat accessions Amigo and GRS1201, respectively, with both genes located on a wheat–rye 1AL.1RS translocation chromosome (Hollenhorst and Joppa 1983; Porter et al. 1994). Deletion mapping revealed that *Gb2* and *Gb6* reside in the satellite region of chromosome 1RS, and the genetic distance between them was 15.8 cM with *Gb6* being distal to *Gb2* (Lu et al. 2010). Another gene, *Gb5*, was located on an interstitial chromosome segment transferred to wheat chromosome 7AL from *Ae. speltoides* (Dubcovsky et al. 1998), whereas *Gb3*, *Gb4* and *Gb7* from *Ae. tauschii* were located on the long arm of chromosome 7D (Weng and Lazar 2002; Weng et al. 2005; Martin et al. 1982). *Gb3* and *Gb4* were identified in Largo and CI 17959, respectively, both of which are synthetic wheat lines derived

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by amphiploidization of crosses between tetraploid durum wheat and *Ae. tauschii* (Joppa and Williams 1982; Martin et al. 1982; Tyler et al. 1987). *Gb7* was more recently identified in synthetic wheat breeding line W7984 and mapped to the proximal region of chromosome 7DL (Weng et al. 2005). Six temporarily named greenbug resistance genes, *Gba*, *Gbb*, *Gbc*, *Gbd*, *Gbx1* and *Gbz*, also originating from *Ae. tauschii*, were mapped to the long arm of chromosome 7D (Zhu et al. 2004, 2005), whereas *Gby*, another temporarily named gene identified in Sando's 4040, was located on chromosome 7A (Boyko et al. 2004).

That most greenbug resistance gene was not originally identified in hexaploid wheat carries significance to cultivar development programs challenged by linkage drag when attempting introgression of some of the known resistance genes from distant or unimproved wheat relatives. Hence, additional years of pre-breeding might be needed before these genes emerge from cultivar development pipelines. On the other hand, *Gb3* has been introgressed into wheat cultivar TAM 110 (Lazar et al. 1997) and is widely used in wheat breeding programs in the southern Great Plains.

All available greenbug resistance genes confer resistance to a limited number of greenbug biotypes and new biotypes continue to be discovered (Berzonsky et al. 2002; Armstrong et al. 2015). For example, *Gb1* is the only gene conferring resistance to greenbug biotype F. But other economically important biotypes, such as B, C, E, G, H, I and K, are virulent to *Gb1* (Xu et al. unpublished). Therefore, identification of new greenbug resistance genes that can be readily used in wheat breeding is necessary for sustainable wheat production in the southern Great Plains.

We have recently screened germplasm available at the USDA-ARS National Small Grains Collection for resistance to greenbug biotype E and found that some plants of accession PI 595379 were biotype E-resistant. PI 595379 is a breeding line co-released by USDA-ARS and Kansas State University as KS95WGRC33 (Cox et al. 1996). A reselected line, PI 595379-1, was generated from a single greenbug-resistant plant. The objective of this study was to characterize the greenbug resistance gene in PI 595379-1 and to develop genomic tools to expedite its utilization in wheat breeding.

Materials and methods

Plant materials

PI 595379 is a hard red winter wheat breeding line selected from KS93U69*2/TA 2397, in which KS93U69 was derived from TAM107*3/TA 2460. TA 2397 and TA 2460 are *Ae. tauschii* accessions collected from Afghanistan and Iran, respectively (Cox et al. 1996). PI 595379-1 is a single plant selection with resistance to greenbug biotype E. An F_2 population and 193 $F_{2:3}$ lines derived from a cross between PI 595379-1 and PI 243735 were used to map the greenbug resistance gene in PI 595379-1. PI 243735, a wheat landrace collected from Iran, is highly susceptible to greenbug.

Evaluation of greenbug resistance

Greenbug biotype E was cultured on the susceptible wheat cultivar Custer. About 50 seeds were sown in 15-cm pots that were enclosed with cylindrical plastic cages vented on the side and top, and seedlings at the two-leaf stage were infested with about 50 aphids per pot. After 2–3 weeks, plants abundantly covered with aphids were used to infest test plants.

The two parents and 193 $F_{2:3}$ lines were evaluated for response to greenbug biotype E at the USDA-ARS Wheat, Peanut, and Other Field Crop Research Unit. A randomized complete block design with two replicates was used. For each replicate, 10 seeds per entry were planted in two cells of a 73-cell growing tray (Growing Systems, Inc., Milwaukee). Resistant (Largo) and susceptible (Custer) controls were planted in one and two cells, respectively, in each tray. The heavily infested culture plants were cut and placed close to test plantlets at 2 days post-emergence. The initial infestation intensity was 20–30 aphids per test seedling. The greenbug assay was conducted in a greenhouse with a supplemented photoperiod of 16 h at 22 ± 0.5 °C. The assay was terminated when the susceptible control had withered or died at 2 weeks post-infestation. Test plants that turned yellow, withered or died were classified as susceptible, and those remaining apparently normal were classified as resistant. The genotype of each F_2 plant was inferred from the corresponding $F_{2:3}$ responses.

An additional experiment aimed at evaluating the responses of PI 595379-1, Largo (*Gb3*), CI 17959 (*Gb4*), W7984 (Gb7) and Custer (susceptible control) to greenbug biotypes B, C, E, F, H, I and FL was also conducted. Five seeds from each line were planted into a 15-cm pot enclosed with a cylindrical plastic cage vented at the side and top. Plants in each pot were infested with a single greenbug biotype using the protocol described above, and there were three replicates for each treatment. All greenbug biotypes are maintained at the USDA-ARS Wheat, Peanut, and Other Field Crop Research Unit.

Bulked segregant analysis (BSA)

Genomic DNA was extracted from seedling leaves using a method described by Dubcovsky et al. (1994). Biotype E-resistant and E-susceptible bulks were constructed by pooling DNA from 10 F_2 plants showing a homogeneous

resistant response and 10 F_2 plants exhibiting homogeneous susceptibility, respectively. A set of over 1000 SSR markers were used to genotype the contrasting bulks and parents. Molecular markers distinguishing the two bulks and parents were further used to genotype the entire F_2 population.

An SSR assay protocol using a LiCor DNA analyzer was followed (Xu et al. 2005). In brief, the 19-base M13 primer, which was labeled with an infrared fluorescence dye, was used in PCR reactions, and the forward primer of each SSR marker was modified by ligation to the M13 sequence at the 5′ end. For SSR assays, 50–100 ng DNA was used in 10 µl reaction mixtures containing 0.5 µM each of the forward, reverse and M13 primers, 2 mM MgCl2, 0.2 mM of each dNTP, 0.2 U Taq DNA polymerase and 1 × PCR buffer. The reaction mixture was denatured at 95 °C for 5 min, followed by five cycles of 95 °C for 45 s, 68 °C for 5 min and 72 °C for 1 min, with a decrease of 2 °C in annealing temperature in each cycle. A second touchdown program began with five cycles of 95 °C for 45 s, 58 °C for 2 min and 72 °C for 1 min with a reduction in annealing temperature of 2 °C per cycle. PCR continued with an additional 25 cycles of 95 °C for 45 s, 50 °C for 2 min and 72 °C for 1 min, and a 5-min final extension step at 72 °C was executed. PCR products were denatured and separated in 6.5% polyacrylamide gels in a LiCor IR-4300 DNA Analyzer.

Development of SSR markers in the genomic region harboring the target gene

BSA allowed the identification of SSR markers closely linked to the greenbug resistance gene in PI 595379-1. Based on genomic locations of these markers, all SSR loci in the target region of the Chinese Spring reference sequence were identified using GMATA software (Wang and Wang 2016). Together with the annotated Chinese Spring reference sequence (Appels et al. 2018), the identified SSR loci were visualized in Jbrowse and examined manually. A total of 28 SSR loci in non-transposon regions were chosen to develop SSR markers. The newly developed SSR markers were tested for polymorphism between PI 595379-1 and PI 243735, and polymorphic markers were used to genotype the F_2 population.

Allelism tests

To determine the allelic relationship between *Gb3* and *Gb8*, 771 and 1989 F_2 plants derived from PI 595379-1 × TAM112 were evaluated for responses to greenbug biotype E in the spring and fall of 2019, respectively. Greenbug biotype E assays were also conducted for 160 F_2 plants derived from W7984 × PI 595379-1 to confirm the assumption that the greenbug resistance gene in PI 595379-1 was independent of *Gb7*. In addition, 488 F_2 plants from Largo × TA4125L94 were evaluated for responses to greenbug biotype E to confirm the allelic relationship between *Gb3* and *Gba*. Both TAM112 and Largo carry *Gb3*, whereas W7984 and TA4125L94 carry *Gb7* and *Gba*, respectively. Five plants were seeded in each cell of 73-cell growing trays. Largo and Custer were planted in each tray as the resistant and susceptible controls, respectively. The same protocol used to evaluate the $F_{2:3}$ population, described above, was used for allelism tests.

Data analysis

Mapmaker 3.0b (Lincoln et al. 1993) was employed in linkage analysis with a logarithm of the odds score of 3.0 as the threshold to declare linkage, and the Kosambi function was used to convert recombination values to genetic distance (Kosambi 1944). MapChart was used to draw the linkage map (Voorrips 2002).

Results

Inheritance of greenbug resistance in PI 595379-1

PI 595379-1 was highly resistant to greenbug biotype E; no symptoms of tissue damage were observed after 2 weeks of infestation. Yellow chlorotic spots with necrotic lesions were observed on PI 243735, the susceptible parent used in this study, after 3–4 days of infestation, and these plants gradually turned yellow, withered and died after 2 weeks of infestation.

Of 193 $F_{2:3}$ lines from PI 595379-1 × PI 243735, 48 and 56 lines showed homogeneous resistant and homogeneous susceptible responses, respectively, and the remaining 89 lines segregated. The Chi-squared test indicated segregation at a single locus ($\chi^2_{1:2:1} = 1.82$, $df = 2$, $p = 0.403$).

Molecular mapping of the greenbug resistance gene in PI 595379-1

Tests of the two parents and contrasting bulks with more than 1000 SSR primer pairs evenly distributed across all wheat chromosomes identified three SSR markers distinguishing the resistant and susceptible bulks, namely *Xbarc111*, *Xwmc824* and *Xgwm428*. These markers, previously mapped to chromosome 7DL, were used to genotype the F_2 population derived from PI 595379-1 × PI 243735. Linkage analysis indicated that all three were associated with the greenbug resistance gene in PI 595379-1, designated *Gb8*. *Xbarc111* was 10.4 cM proximal to *Gb8*, whereas *Xwmc824* and *Xgwm428* were 7.4 and 12 cM distal to *Gb8*, respectively (Fig. 1).

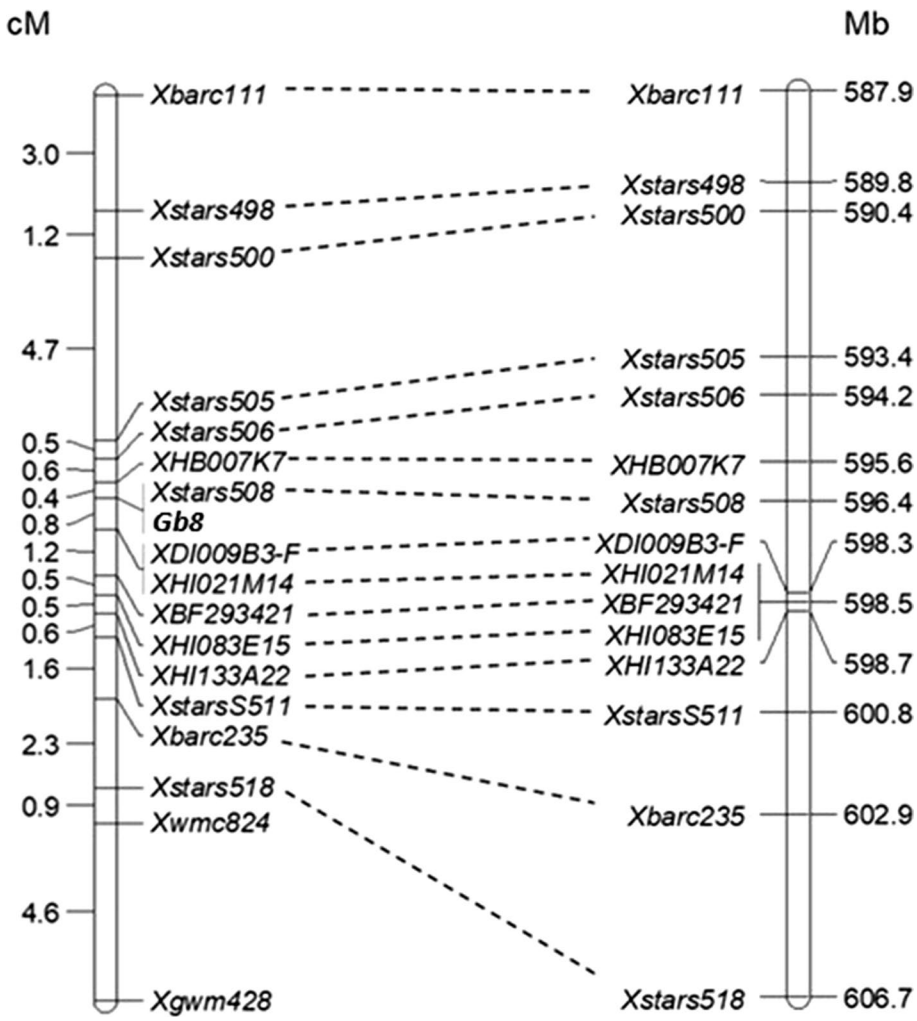

Fig. 1 Linkage (left) and physical (right) map of *Gb8*. Marker names are shown at the right of the linkage map and the left of the physical map. Genetic distances in cM are given on the left of the linkage map, and the physical positions of some markers on the Chinese Spring IWGSC RefSeq version 1.0 reference assembly are provided on the right of the physical map

We mapped the genomic sequence flanking *Xbarc111* (https://wheat.pw.usda.gov/cgi-bin/GG3/report.cgi?class=sequence;name=BV211495) to approximately 587.92 Mb in the Chinese Spring reference sequence IWGSC RefSeq version 1.0 (Appels et al. 2018). We searched the genomic region spanning 588–612.3 Mb where *Gb8* might reside, and identified 2900 SSR loci. Based on genomic locations and motifs, 28 were selected to develop molecular markers (Table 1). These newly developed markers were subsequently tested for polymorphism between the parents, leading to the identification of 7 polymorphic SSR markers. These markers, as well as another 7 polymorphic CAPS (cleaved amplified polymorphic sequence) markers previously mapped to this region (Azhaguvel et al. 2012), were used to genotype the F_2 population.

Gb8 co-segregated with SSR marker *Xstars508*, positioned at 596.4 Mb in the Chinese Spring reference sequence IWGSC RefSeq version 1.0 (Fig. 1). *Gb8* and *Xstars508* were assigned to an interval of about 2.7 Mb, flanked by CAPS markers *XHB007K7* (595.6 Mb) and *XDI009B3-F* (598.3 Mb).

All makers flanking *Gb8* except the newly developed SSR makers, including *Xbarc111*, *XHB007K7*, *XDI009B3-F*, *XHI021M14*, *XBF293421*, *XHI083E15*, *XHI133A22*, *Xbarc235* and *Xwmc824*, were previously mapped to the terminal bin 7DL3-0.82-1.0 (Azhaguvel et al. 2012). Given that the order of molecular markers on our linkage map was consistent with that in the Chinese Spring reference sequence, the SSR markers developed in this study, *Xstars498*, *Xstars500*, *Xstars505*, *Xstars506*, *Xstars508*, *Xstars511* and *Xstars518*, as well as *Gb8*, should be in the same bin.

Responses of PI 595379-1, Largo, CI 17959 and W7984 to different greenbug biotypes

Gb8, *Gb3*, *Gb4* and *Gb7* were mapped to chromosome 7DL. Therefore, we compared the responses of PI 595379-1 (*Gb8*), Largo (*Gb3*), CI 17959 (*Gb4*) and W7984 (*Gb7*) to greenbug biotypes B, C, E, F, H, I and FL. All lines were resistant to biotypes C, E and I, but were susceptible to

Table 1 Primer sequences, SSR motifs, genomic locations and PCR product sizes of newly developed SSR markers in the terminal region of wheat chromosome 7DL

SSR	Forward primer	Reverse primer	Motif	Location	Size
Xstars497	CATCTGTGTTTTGCCCCTTT	ATGCCTCCAAGGTATCCACA	(TC)22	588416075–588416457	383
Xstars498	CTCCTCTGAAATGTGCGTCA	AACCGGCAAGCCTACAAATA	(CT)24	589858880–589859137	258
Xstars499	TAGATCGCAGCCCTCCTAAA	TGCAGCCTCCTTTCTGATCT	(TC)31	590218280–590218430	151
Xstars500	ACCGGGTACAGCGTACTGTC	CCGATCTACCTCGATCTCCA	(CA)20 + (GA)22	590433055–590433400	346
Xstars501	AGTCTCCGGGTCAAAACCTT	GCACGGGTGTTAGCAGAATAA	(TA)23	590521295–590521644	350
Xstars502	CTCATGGTGGCGAGTGATTA	TGTTGATTAGTGAGCGAAGTACC	(AG)27	590669494–590669892	399
Xstars503	CGTTTCGGGGTCACTAGAAG	TGAGTTGGACTCCTCCCCTA	(TA)30	591748841–591749113	273
Xstars504	GCACACACTCCCACTCTCCT	TGGTTGGCTAAGCTTGCTCT	(CT)29	592816158–592816339	182
Xstars505	CAACGCCATTGTGGAAATTA	TGCTAGGGTTTCAGATTGTGTC	(ATC)5 + (TCA)21	593416902–593417301	400
Xstars506	AGCAAGCCATAAGCCCATAA	TAAGATCAGTGCTGCCTTGC	(TA)26	594148759–594149115	357
Xstars507	CCGTTGCAATAGCACACATC	CAACAACCAGCATCCATCAC	(TA)35	595937407–595937725	319
Xstars508	ATTGGGGTTTACAGGTGTGG	GTCCCAAAACTCCACCAAAA	(CT)26	596432029–596432262	234
Xstars509	AAGCGGGAAAGAGATGATGA	TGAGCTTGCCTATCCACAAA	(CT)23	597696777–597697149	373
Xstars510	TGGACCTCCATTCAGGTTTT	GGGAGAGGACATGAGCTGAC	(TC)24	598248104–598248433	330
Xstars511	ACTAGTCAACGGCGGTTTTG	ACGAAGAGGAGGAGGAGGAC	(TC)24	600766514–600766882	369
Xstars512	CTTTCACTCGTGGGGATGAC	ACCCTGCCTCTCTCCTTCTC	(GA)24 + (AG)6	602173059–602173210	152
Xstars513	CTGCTCCAGAGTCCATGGTT	GAAGATTGCCCTTGTTGGAA	(TA)29	602399661–602399921	261
Xstars514	ACAGAAGACGTGGCCTCAAG	GTTTACGCCATTGCAAGGTT	(TA)27	603864793–603865099	307
Xstars515	TATAATCCGGCAACGACACA	CCCCATCTCCCTCTCTCTCT	(GA)18	605181629–605181972	344
Xstars516	CACCATTGCCTTTTGTGATG	ACATGTTCGTGAGGTGCAAT	(AG)20	605228283–605228663	381
Xstars517	TGGCGGCAGTAAGTCTATCA	TAAGGAGCCGGTTGGTAATG	(TA)24	605742910–605743217	308
Xstars518	TCTCCATACACCCGCCTATC	CAGCTTTCCTGTGCTCTCCT	(CT)25	606647679–606647808	130
Xstars519	CCTGGTCTAGGCGGTTCATA	CCATTGGTGGAGAAACGAGT	(TC)26	608887966–608888108	143
Xstars520	ACGCAAGTTCCCTTGAAATG	ATATCAGGATCCGGGACACA	(CT)19 + (TC)5	609274383–609274756	374
Xstars521	CACGATGATGATGGAAGACG	GTTAGGGTTTGGTGGGGATT	(AT)46	610665653–610665895	243
Xstars522	CTTGGTACCCACACAAAGAAAA	TGCACCCATATGCAACAACT	(AG)29 + (GAGG)5	610800215–610800352	138
Xstars523	ATACGCGGCATTGTCCTATT	GGGGACGTGTATCTTGGTCA	(TC)24	611481368–611481724	357
Xstars524	GCAGTTGGTGTCGCAATAGA	AACCGACTATGCAGGTTGCT	(TC)26	612290536–612290796	261

Table 2 Responses of PI 595379-1, Largo, CI 17959, W7984 and Custer to greenbug biotypes B, C, E, F, H, I and FL

Biotype	PI 595379-1	Largo	CI 17959	W7984	Custer
B	R	S	S	S	S
C	R	R	R	S	S
E	R	R	R	R	S
F	S	S	S	S	S
H	R	R	S	S	S
I	R	R	R	R	S
FL	R	S	S	S	S

R resistant; *S* susceptible

biotype F. Biotype H was virulent to CI 17959 and W7984, but avirulent to PI 595379-1 and Largo. PI 595379-1 differed from Largo, CI 17959 and W7984 by conferring resistance to biotypes B and FL (Table 2).

Allelism tests

Azhaguvel et al. (2012) mapped *Gb3* to the same terminal bin 7DL3-0.82-1.0 that carries *Gb8*. *Gb4* was either allelic or closely linked to *Gb3* (Zhu et al. 2005; Liu et al. 2015). Allelism tests were performed to determine whether *Gb8* and *Gb3* were allelic. We tested 771 and 1989 F_2 plants from PI 595379-1 × TAM112 in the spring and fall of 2019, respectively. TAM112 carries *Gb3*. In total, 10 of the 771 plants were susceptible in the spring experiment, whereas 17 of the 1989 plants were susceptible in the fall experiment, indicating that *Gb8* is not allelic to *Gb3*. The estimated recombination frequency and genetic distance between *Gb8* and *Gb3* were 0.149 ± 0.025 and 15.35 ± 1.35 cM, respectively.

Gb7 was mapped to the proximal region of 7DL (Weng et al. 2005). We evaluated 160 F_2 plants derived from W7984 × PI 595379-1 for reaction to greenbug biotype E; 150 and 10 plants were resistant and susceptible,

respectively, indicating independent inheritance ($\chi^2_{15:1}=0$, $df=1$, $p=1$).

To confirm the assumption that *Gb3* is either allelic to *Gba*, one of the six temporarily named genes on the long arm of chromosome 7DL (Zhu et al. 2005), 488 F_2 plants from Largo × TA4125L94 were also evaluated for resistance to greenbug biotype E. TA4125L94 carries greenbug resistance gene *Gba*, which was previously mapped to a genomic region near *Gb3*. We found no susceptible plant, indicating that *Gba* might be allelic with *Gb3*.

Discussion

Gb8 is a new greenbug resistance gene

Gb8 was identified in PI 595379-1, a progeny of *Ae. tauschii* accessions TA 2460 and TA 2379. *Ae. tauschii* is an important reservoir of greenbug resistance, from which *Gb3*, *Gb4*, *Gb7*, *Gba*, *Gbb*, *Gbc*, *Gbd*, *Gbx1* and *GbZ* have been sourced (Joppa and Williams 1982; Martin et al. 1982; Weng et al. 2005; Zhu et al. 2005). In this study, we mapped *Gb8* to a genomic region near *Gb3*; SSR marker *Xstars508* co-segregated with *Gb8*. Six markers closely flanking *Gb8* were dominant, indicating significant sequence difference between the parents in the target region. The target bands of *Xstars508* and *XDI021M14* were amplified from the susceptible Iranian landrace parent (PI 243735), but not from the resistant parent (PI 595379-1), and vice versa for the other four markers: *Xstars506*, *XDI009B3-F*, *XHB007K7* and *XBF293421*. Wheat (*Xstars506*, *Xstars508* and *XBF293421*) and *Ae. tauschii* (*XDI009B3-F*, *XHB007K7* and *XBF293421*) genomic sequences were used to design primers for these markers (Azhaguvel et al. 2012). Likely, *Gb8* resides on an *Ae. tauschii* chromosome segment inherited from either TA 2460 or TA 2379, and recombination events occurred in this region during the development of PI 595379-1, leading to the presence of the target bands in one parent (PI 595379-1 or PI 243735) and absence in the other (PI 243735 or PI 595379-1).

Of the permanently designated genes, *Gb3*, *Gb4* and *Gb7* were previously mapped to chromosome 7DL; of those, *Gb3* and *Gb7* were mapped to the terminal bin 7DL3-082-1.0 and the proximal region of 7DL (Weng et al. 2005), respectively, and *Gb4* was allelic or closely linked to *Gb3* (Zhu et al. 2005; Liu et al. 2015). *Gb8* was mapped to a genomic region near *Gb3*. Allelism tests indicated that *Gb8* was inherited independently of *Gb7*, and the genetic distance between *Gb8* and *Gb3* was estimated to be 15.35 ± 1.35 cM. Therefore, we conclude *Gb8* is a new greenbug resistance gene.

Six temporarily named genes conferring either tolerance or antibiosis resistance to greenbug biotype I, *Gba*, *Gbb*, *Gbc*, *Gbd*, *Gbx1* and *GbZ*, were also mapped in the terminal bin; these genes were either allelic or linked to *Gb3* (Zhu et al. 2004, 2005). Of these, *Gbx1* and *Gbz* encode tolerance to greenbug biotype I, whereas *Gbd* confers antibiosis resistance to biotype I; allelism test indicated *Gbd* was not allelic to *Gbz* (Zhu et al. 2005), suggesting that this genomic region may harbor multiple greenbug resistance genes. We confirmed that *Gba* was possibly allelic to *Gb3* and less likely allelic to *Gb8*. At present, we cannot determine whether *Gb8* is allelic to the other five genes because of the unavailability of related genetic stocks or mapping populations. More studies are needed to understand their relationship.

Use of *Gb8* in wheat breeding

About one-third of crop production is lost to insect pests, pathogens and weeds (Riegler 2018). Deutsch et al. (2018) estimated that insect pests could cause additional losses in grain yield of 10 to 25% per 1 °C of global temperature increase, which leads to higher metabolic rates and larger insect populations. Given that global average surface temperatures are projected to increase by 2–5 °C this century (Riegler 2018), breeding pest-resistant crops is essential for global food security.

Greenbug is one of the most important small grain pests in the southern Great Plains. Although a few genes for resistance to greenbug have been identified in wheat, only *Gb3* has been deployed in wheat production (Rudd et al. 2014) and novel greenbug resistance genes that can be readily used in wheat breeding are urgently required. *Gb8* was identified in PI 595379-1, a reselection of PI 595379 that is phenotypically similar to wheat cultivar TAM 107 (Cox et al. 1996). Except for greenbug resistance, no obvious difference was observed in other traits between PI 595379 and PI 595379-1. Therefore, *Gb8* can be directly used in wheat breeding with molecular markers closely linked to it, such as *Xstars505*, *Xstars506*, *Xstars508* and *Xstars511* having the potential to tag the resistance allele.

PI 595379-1 confers resistance to greenbug biotypes B and FL as well as C, E, I and H. Among the known greenbug resistance genes, only *Gb2* and *Gb6* are effective against biotype B (Xu et al. unpublished). Greenbug biotype FL, originally identified in seashore paspalum turfgrass (*Paspalum vaginatum*) in 2003, is virulent to *Gb3*, *Gb4*, *Gb5*, *Gb6* and *Gb7*, but avirulent to *Gb1* and *Gb2* (Nuessly et al. 2008). Given that PI 595379-1 was derived from TAM107 carrying *Gb2* (Porter et al. 1987), it is possible that PI 595379-1 also carry *Gb2*. We were not able to map *Gb2* in this study because *Gb2* does not confer resistance to biotype E (Xu et al. unpublished). More studies are needed to confirm this assumption.

Notably, greenbug biotype F collected from Canada bluegrass is virulent to all known greenbug resistance genes except for *Gb1* (Xu et al. unpublished). Although biotype F

is rarely found in field populations resident to the southern Great Plains, a prudent future breeding strategy would be to transfer *Gb1* from Dickinson Selection 28 to modern hexaploid wheat lines or identify new genes conferring resistance to biotype F.

PI 595379 also carries genes conferring resistance to leaf rust (*Lr41*), Septoria tritici blotch, glume blotch, Septoria nodorum blotch, tan spot and wheat curl mite (Cox et al. 1996). It is expected that PI 595379-1 also carries these genes. Successful characterization of these genes in PI 595379-1 will make it feasible to simultaneously transfer multiple biotic stress resistance genes from this source to elite cultivars.

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Author Contribution statement XX and GL designed and performed the research; BFC and JSA provided overall supports; XX wrote the paper. All authors read, revised and approved the manuscript.

Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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