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ORIGINAL ARTICLE

Analysis of recombinant inbred line populations derived from wheat landraces to identify new genes for wheat stem sawfly resistance

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Abstract

Wheat landrace accessions were chosen from areas of the world with historical European wheat stem sawfly (*Cephus pygmaeus* L.) selection pressure to develop six recombinant inbred line (RIL) populations. Molecular maps were constructed, and resistance due to antibiosis and antixenosis was assessed at sites in Montana naturally infested by *Cephus cinctus* Norton, the wheat stem sawfly (WSS). Novel QTLs were identified along with QTL previously identified in elite germplasm. A newly identified QTL on chromosome 1B provided a new source for pith-filled solid stems. An allele for resistance on chromosome 4A unrelated to solid stems was identified in four of the six RIL populations. A landrace from Turkey, PI 166471, contained alleles at three QTLs causing high levels of larval mortality. None of the QTLs were related to stem solidness, but their combined effect provided resistance similar to that observed in a solid-stemmed check cultivar. These results show the utility of genetic populations derived from geographically targeted landrace accessions to identify new alleles for insect resistance. New PCR-based molecular markers were developed for introgression of novel alleles for WSS resistance into elite lines. Comparison of results with previous analysis of elite cultivars addresses changes in allele frequencies during the wheat breeding process.

Introduction

Variation in feeding habits impacts the potential host range of insect pests important in crop production. Some insect pests feed on multiple plant species and pose complex challenges in an agroecosystem by having many suitable hosts (Kennedy and Storer 2000). These species are most often managed with broad spectrum intervention strategies, such as pesticide application (Kogan 1998). In contrast, there are also a number of severe insect pests that are more constrained in their host range. These insect pests have co-evolved to specialize on a particular plant taxon, often representing a single crop species. These plant species are good targets for pest management via host plant resistance (Painter 1958). Reasons for the limited host range include mechanisms such as antixenosis, whereby the insect does not recognize or select the host, and antibiosis, where insect survival is reduced by plant defenses after infestation (Painter 1958).

Wild progenitors and landrace relatives of crop plants often harbor genes for insect resistance that have been lost in the genetic bottleneck resulting from domestication (Chen et al. 2015; Dávila-Flores et al. 2013; Rosenthal and

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Dirzo 1997). For this reason, landrace accessions have been a source of useful genes for insect resistance traits (Valdez et al. 2012; Arnason et al. 1994; Pelgrom et al. 2015; Laamari et al. 2008). A targeted geographical approach to screening landrace accessions has been successful, because geography underlies plant–insect interactions (El Bouhssini et al. 2011; Chen et al. 2015). Searching for new sources of insect resistance in geographical regions of the world where the plant and the insect pest shared the longest evolutionary history can increase the likelihood of success.

Several species of wheat stem sawflies infest wheat throughout the world. The WSS of the Northern Great Plains is *Cephus cinctus* Norton, which likely adapted to wheat from native grasses shortly after the tillage of the prairie began (Beres et al. 2011). A closely related species, *Cinctus pygmaeus* (L.), is distributed throughout Western Europe, North Africa and the Middle East (reviewed in Shanower and Hoelmer 2004). The life cycles of the two species and the damage caused by their interaction with wheat are indistinguishable. The known resistance mechanism for *C. cinctus* of solid stems also provides resistance to *C. pygmaeus* (Damania et al. 1997). Areas of the world infested by *C. pygmaeus* represent some of the earliest instances of wheat cultivation. Thus, landraces collected from these areas have been under pressure from the WSS since the dawn of wheat domestication. One area of focus for WSS researchers has been Turkey, primarily due to the prevalence of *Cephus* species that infest wheat (Korkmaz et al. 2010). Damania et al. (1997) found a high frequency of solid-stemmed lines in a collection of Turkish durum wheat landraces. Damania (1988) also found a high frequency of solid stem durum wheat landraces in Morocco. The WSS species *Cephus fumipennis* Eversmann is endemic to western China (Chen et al. 2004). Resistant wheat varieties with solid stems have been identified in heavily infested areas (Chen et al. 2004).

The biology of the wheat stem sawfly indicates several points in its life cycle where genetic resistance may occur. Key points of the WSS life cycle are that the short-lived adults emerge in late spring and early summer, and lay eggs (oviposit) inside newly developed wheat stems (Morrill et al. 1992). The larvae feed inside the stem after the eggs hatch. At the end of the growing season, the large larvae cut the stem at its base (Morrill et al. 1992). This stem cutting prior to harvest causes lodging. Lodging, coupled with reduced grain weight due to larval tunneling (Delaney et al. 2010), can result in severe loss of profitability (Beres et al. 2007; Knodel et al. 2009).

Host plant resistance due to solid stems has been the major means of WSS control over the past 70 years. WSS larval development is impaired when feeding in solid, pith-filled, stems. Larvae often die before being able to cut the stems. Use of solid stem varieties to combat the WSS is common in the southern Prairie Provinces of Canada, Montana and western North Dakota (Beres et al. 2017). A single locus, termed *Qss.msub-3BL*, has been shown to control the majority of variation for stem solidness (Cook et al. 2004). The solid stem allele was initially discovered in landrace S-615 from Portugal and used to produce the first solid-stemmed cultivar, named Rescue (Platt et al. 1948). The majority of currently grown WSS-resistant spring and winter wheat varieties in the US and Canada descend from Rescue (Cook et al. 2017). Lines containing the S-615-derived solid stem allele maintain solid stems throughout their lifespan. This has made solid stems a convenient marker for resistance, as plant breeders can assess solidness by visual observation of stem cross sections. An allele at *Qss.msub-3BL* that provided a unique form of resistance was identified by Varella et al. (2016). This allele, referred to as the Conan-derived allele to denote its initial discovery, resulted in stem solidness only in early stem elongation. This growth stage coincides with WSS oviposition and early larval growth. Stem solidness due to the Conan allele is lost during stem maturation (Varella et al. 2016). Recombinant inbred lines containing the alternative stem solidness alleles at *Qss.msub-3BL* have shown that the Conan allele provides a higher level of resistance than the S-615 allele (Talbert et al. 2014).

Varella et al. (2017a) showed that allelic variation at several loci interacts with the WSS to cause resistance. A QTL on chromosome 4A impacted the relative attraction of plants to oviposition by WSS females leading to moderate resistance due to antixenosis. The favorable allele for this locus was found at a high frequency in elite North American wheat lines (Varella et al. 2015). This was true even for lines developed for areas without a history of WSS pressure. The alleles for solid stem at the QTL on chromosome 3B caused antibiosis in the form of larval mortality. The "Conan" allele for early solidness caused antibiosis and also caused female WSS to abort oviposition after insertion of the ovipositor for a unique form of antixenosis. The alleles conferring solid stems were only present in elite North American lines developed specifically for WSS resistance (Varella et al. 2015). Given the shared evolutionary history of wheat and the WSS, additional loci that impact their interaction to provide resistance may occur in a broader germplasm base.

Two complementary approaches facilitate the investigation of the causes of WSS resistance in landraces. First, the recent development of inexpensive SNP-based genotyping (Poland and Rife 2012; Wang et al. 2014) allows inexpensive development of dense genetic maps. Second, dissection of stems from infested sites allows determination of mechanisms of resistance. The amount of egg-laying, or infestation, measures antixenosis and the amount of larval mortality measures antibiosis (Peterson et al. 2011; Talbert et al. 2014; Buteler et al. 2015). Importantly, one cause of larval mortality unrelated to host plant resistance are parasitoids

that vary in number from site to site and year–year (Morrill 1997; Weaver et al. 2004, 2005). Parasitoid-induced mortality needs to be determined to avoid inflating estimates of mortality due to antibiosis caused by the plant.

Varella et al. (2017b) showed that 14% of the hexaploid wheat landrace accessions from historic WSS-infested areas showed some level of resistance due to either antibiosis or antixenosis. A subset of resistant landraces identified by Varella et al. (2017b) were used to develop recombinant inbred line populations for this report. The populations were genotyped based on single nucleotide polymorphism (SNP) variation and grown at WSS-infested sites. Stems were dissected to assess antixenosis and antibiosis. Quantitative trait locus (QTL) analysis was performed to determine the genetic basis of observed resistance. Results have implications regarding the biological and genetic interaction between WSS and wheat landraces from areas where both have been present for millennia. Additionally, comparison of resistance genes in current cultivars versus landraces provides insights into the impact of modern breeding on crop diversity.

Materials and methods

Recombinant inbred line populations

Varella et al. (2017b) described the screening of 1409 hexaploid wheat landrace accessions from regions of the world where WSS are endemic. Approximately 14% of the accessions showed some level of resistance due to either antibiosis or antixenosis. Almost half of the resistant accessions displayed the common characteristic of solid stems as traditionally measured near maturity. Four accessions with high levels of resistance, namely PI 166471, PI 565386, PI 576680 and PI 166331, were selected as parents to create RIL populations. The common parent for all crosses was the adapted semidwarf cultivar Hi-Line (Lanning et al. 1992). Populations were advanced to the F_5 generation by single seed descent beginning at the F_2 generation.

Two additional RIL populations were included in the WSS-phenotyping trials and QTL analyses. Landrace accessions PI 166333 from Turkey and PI 185715 from Portugal were used as parents in crosses to CIMMYT cultivar Berkut in a previous nested association mapping panel (Jordan et al. 2018; Blake et al. 2019). The European WSS is endemic to both Turkey and Portugal.

Phenotyping

Experiments were planted as hill plots in Amsterdam, MT, USA, in 2016 and 2017 and in a second location with a history of WSS infestation in Big Sandy, MT, USA, in 2017. Experiments were conducted in a randomized complete block design with two or three replications. Check varieties included parental lines as well as susceptible hollow-stemmed check Reeder (PI 613586) and resistant solid-stemmed check Choteau (Lanning et al. 2004). The amount of stem cutting at maturity was determined for all populations in all sites. In addition, all stems from hills were individually collected at maturity in late August of each year. At least two sites were analyzed by stem dissection for each population. Every stem was dissected to determine the percentage of stems with WSS larvae (infestation). The percentage of cut stems was determined. Stem dissection revealed varying levels of parasitism of the WSS larvae by endemic braconid parasitoids (Runyon et al. 2002; Sherman et al. 2010), which was recorded for each plot. Percent mortality was calculated by subtracting the number of parasitized larvae from the total dead larvae, divided by total number of infested stems.

In addition to analysis of WSS parameters, the RIL were also planted at a non-WSS-infested site in Bozeman MT over a 2-year period. This trial allowed assessment of both early and late stem solidness as described by Varella et al. (2016). Early stem solidness was assessed by collecting three plants of each plot at approximately 35 days after planting when plants were at Zadok 32 (two internodes detectable). Solidness of the main stem was assessed by longitudinal dissection. Late stem solidness was assessed by collecting the main stems of three plants of each plot at maturity in late August. Stems were dissected, and each internode was rated for stem solidness as described above. The scale was 1–5, with 1 being completely hollow internodes and 5 being completely solid internodes (Cook et al. 2004).

Statistical analysis

Analysis of variance was conducted for each response variable for each of the six populations for the hill plot experiments for each site and then using a model for a randomized complete block combined over sites using PROC GLM in SAS (SAS Institute, Inc. 2010). Least-squares means were obtained for the RIL entries for each environment and combined over environments. A combined analysis of variance using RIL means over locations was used to conduct single-marker analysis with marker genotypes for QTL identification. Stem solidness data were obtained for each population as a single replication over years. Analysis of variance to determine genotype effects was conducted using PROC GLM using years as replications.

Genotyping

90K iSelect genotyping

The RIL populations were genotyped using the Illumina 90K iSelect assay. Genomic DNA samples were sent to the

USDA ARS Genotyping Laboratory at Fargo, North Dakota, for genotyping. Data analysis was conducted using Illumina's GenomeStudio 2011 v1 software (Illumina, Inc., San Diego CA, USA). Allele calls for each SNP was inspected manually. Markers with more than 10% missing genotypes, monomorphic, or highly distorted were discarded. The genotyping procedure for RIL populations derived from PI 166333 and PI 185715 has been previously described by Jordan et al. (2018).

Sequence-based SNP genotyping

Genomic DNA from RIL was quantified using PicoGreen (Life Technologies) and normalized to ~ 50 ng μL^{-1} of DNA per line. DNA samples were sent to the USDA ARS Small Grains Genotyping Laboratory located in Fargo, ND. Libraries for sequencing were prepared according to Saintenac et al. (2013) using the PstI/MseI combination of enzymes. Sequencing was performed on an Illumina NextSeq 500 platform with single read lengths of 150 base pairs. The analysis pipeline was conducted using TASSEL software version 4.0 (Glaubitz et al. 2014). Briefly, tag counts were generated and merged using default parameters with the FastqToTagCountPlugin and MergeMultipleTagCountPlugin, respectively. Bowtie 2 version 2.2.9 was used to align tags to the wheat pseudo-reference genome (ftp://ensemblgenomes.org/pub/plants/release-31/fasta/triticum_aestivum/dna/) (IWGSC 2018). The output of the alignment was converted to a "Tags On Physical Map" (TOPM) file by the SAMConverterPlugin. The SeqToTBTHDF5Plugin and ModifyTBTHDF5Plugin were used to generate a "Tags by Taxa" (TBT) file containing sorted and demultiplexed reads. SNPs were called using the DiscoverySNPCallerPlugin with the following non-default parameters: minimum value of F (inbreeding coefficient = 1-Ho/He) [mnF]: 0.8, minimum minor allele frequency (default: 0.01) [mnMAF]: 0.02, and minimum minor allele count (default: 10) [mnMAC]: 100,000. Duplicate sites were merged with the MergeDuplicateSNPsPlugin. Finally, SNPs with low taxon coverage and low or high minor allele frequency were filtered out with the GBSHapMapFiltersPlugin and the non-default parameters: minimum site coverage (default: no filter) [mnScov]: 0.2, minimum minor allele frequency (default: 0.0) [mnMAF]: 0.01, and maximum minor allele frequency (default: 1) [mxMAF]: 0.5.

Genetic linkage map construction and QTL analysis

Linkage map construction for RIL populations was conducted using R/qtl (Broman et al. 2003) and R/ASMap (Taylor and Butler 2014) packages in R. Polymorphic markers that had more than 25% missing data or showed significant Mendelian segregation distortion (Chi-square test, $p < 1.0e^{-7}$, $df = 1$) were excluded. Co-segregating markers were also discarded. The *mstmap* function (Wu et al. 2008) from R/ASMap package was used to group and order markers. Map distances (cM) were calculated using the Kosambi function with a significant threshold of p value = $1e^{-7}$ for linkage group formation. A heat map of estimated recombinant fractions and LOD scores was used for checking marker order on each linkage group. Standard interval mapping (Broman and Sen 2009) was conducted using the *scanone* function and the Haley–Knott regression method. Significance thresholds ($p < 0.05$) for LOD scores were determined using permutations with 1000 replications. Genetic linkage map construction for RIL populations derived from PI 166333 and PI 185715 has been previously described by Jordan et al. (2018).

Development of near-isogenic lines (NILs)

The Hi-Line/PI 166471 RIL population was used to derive NILs for two resistance QTLs on chromosomes 1B and 4A following the heterogeneous inbred family method described by Barrero et al. (2015). Briefly, F_5 plants were genotyped to identify heterozygous individuals, which were then allowed to self-pollinate. $F_{5:6}$ plants were genotyped, and homozygous lines for each of the alleles were identified. A pair of resistant and susceptible NIL derived from heterozygous F_5 RIL are expected to be approximately 97% identical at loci not linked to the target QTL. KASP markers for resistance QTLs included Kukri_c47679_85 and IAAV3960 on chromosomes 1B and 4A, respectively (Wang et al. 2014; http://polymarker.tgac.ac.uk/Markdown?md=DesignedPrimers). Six additional KASP markers (Online Resource 1) used to verify NIL genotypes were designed based on GBS SNP flanking sequences using the PolyMarker automated pipeline (Ramirez-Gonzalez et al. 2015).

Results

A total of six RIL populations developed from crosses of landrace populations to elite cultivars were evaluated in at least two WSS-infested sites for resistance (Table 1). Four of the landraces were selected as parents based on screening of 1409 accessions over 2 years in Montana (Varella et al. 2017b). Two of the landrace parents had been used previously as parents in development of a nested association mapping panel involving diverse accessions (Jordan et al. 2018). These two landraces were chosen due to their origins in Turkey and Portugal, where a closely related species of WSS is endemic.

Populations were not all grown in the same sites and years, which impacted relative levels of WSS damage. The percentage infestation, indicating the number of WSS

Table 1 Parental lines used for the development of recombinant inbred line populations

Landrace parent			Elite parent			Number of RIL	Number of markers
PI number	Local identifier	Origin	PI number	Local identifier	Origin		
PI 166471	SW 86	Turkey	PI 549275	Hi-Line	Montana	115	2616
PI 565386	SW216-2	Turkmenistan	PI 549275	Hi-Line	Montana	98	2655
PI 576680	SW171-3	Turkey	PI 549275	Hi-Line	Montana	90	2425
PI 166331	SW81-4	Turkey	PI 549275	Hi-Line	Montana	91	1277
PI 166333	LR33	Turkey		Berkut	Mexico	75	6426
PI 185715	LR37	Portugal		Berkut	Mexico	75	5018

present, varied significantly among the sites (Table 2). Cutting of infested stems may not occur due to either parasitoid attack or to plant resistance mechanisms. Percent mortality not due to parasitoids is adjusted for parasitoid-caused mortality (Table 2) and thus represents mortality due to plant resistance. The solid-stemmed cultivar Choteau had lower infestation and caused greater larval mortality than hollow-stemmed Reeder at all three sites (Table 2). This resulted in lower levels of stem cutting at all three sites.

Phenotypic assessment of populations

Berkut/PI 185715

This cross was one of 32 included in a previous nested association mapping panel (Jordan et al. 2018; Blake et al. 2019). PI 185715 from Portugal had significantly lower stem cutting, lower percentage infestation and greater larval mortality than Berkut (Table 3). Percent stem cutting and larval mortality were not significantly different between PI 185715 and the solid-stemmed resistant cultivar Choteau. PI 185715 did not differ from Berkut for either early or late stem solidness. The RIL varied significantly for all WSS measurements. In this population and subsequent populations, there were significant location by genotype interactions for many traits (data not shown), though the magnitude of the interaction was lower than the genotype effect.

Hi-Line/PI 166471

PI 166471 from Turkey was selected as showing a high level of resistance from a landrace screening experiment (Varella et al. 2017b). Table 4 shows that PI 166471 and Hi-Line did not differ for stem cutting by WSS, WSS infestation or larval mortality in the sites employed for this study. Both parents had greater stem cutting and lower larval mortality than the resistant cultivar Choteau and did not differ significantly from the susceptible cultivar Reeder. PI 166471 had significantly greater values for both early and late stem solidness. The RIL showed significant variation for all WSS resistance measurements.

Hi-Line/PI 576680

PI 576680 from Turkey was selected as showing resistance based on a landrace screening study at two locations (Varella et al. 2017b). No significant differences were observed between the parents for percent stem cutting, infestation or larval mortality (Table 5). The parents did not differ for stem solidness. Choteau had greater stem solidness, lower cutting, lower infestation and greater larval mortality than either PI 576680 or Hi-Line. The RIL showed significant variation for all WSS resistance measurements.

Hi-Line/PI 565386

PI 565386 from Turkmenistan was selected based on resistance observed in a landrace screening trial (Varella et al. 2017b). PI 565386 had significantly lower stem cutting and greater larval mortality than Hi-Line (Table 6). PI 565386 did not differ significantly from Choteau for percent cutting or for larval mortality. The parents did not differ for stem solidness. The RIL showed significant variation for all WSS resistance measurements.

Hi-Line/PI 166331

PI 166331 from Turkey was selected as showing resistance in a screening nursery (Varella et al. 2017b). No significant differences were detected between the parents for cutting, infestation, or larval mortality in the trials summarized in Table 7.

Berkut/PI 166333

This population was initially constructed as part of a previously described nested association population (Jordan et al. 2018; Blake et al. 2019). This trial was only tested in one location due to lack of differences shown by the parents and heavy cutting in the RIL population (Table 8).

Table 2 Wheat stem sawfly parameters for solid-stemmed Choteau and hollow-stemmed Reeder from three locations

Location	No. popns	Percent infestation			Percent mortality due to parasitism			Percent mortality not due to parasitism			Percent cutting		
		Choteau	Reeder	*p* value	Choteau	Reeder	*p* value	Choteau	Reeder	*p* value	Choteau	Reeder	*p* value
Amsterdam 2016	2	18	30.7	0.06	0	0	1	69.4	39.4	0.008	7.3	19.3	0.05
Amsterdam 2017	5	35.7	63.6	< 0.0001	11.2	9.1	0.47	62.1	26	0.0002	9.5	40.7	< 0.0001
Big Sandy 2017	5	48.2	61.5	0.01	5.2	9	0.18	52.4	30.6	0.0125	17	37.9	0.0001
Means across locations		34	51.9	< 0.0001	5.5	6	0.79	61.3	32	< 0.0001	11.3	32.6	< 0.0001
p value for locations		< 0.0001			0.008			0.16			0.003		

Identification of QTL

Quantitative trait loci controlling variation for WSS resistance traits were identified in four of the six RIL populations. Two distinct QTLs were identified in a population of 75 RIL from the Berkut/PI 185715 cross based on a genetic map with 5018 markers (Table 9). Landrace PI 185715 contributed an allele for low infestation and low cutting for a QTL on chromosome 4B. Landrace PI 185715 contributed an allele for greater larval mortality on chromosome 4A.

Two separate QTLs were also identified from the Hi-Line/PI 166471 cross based on a population of 115 RIL and a genetic map with 2616 markers (Table 9). The allele from PI 166471 on chromosome 1B contributed greater early and late stem solidness and resulted in lower stem cutting, lower infestation and greater mortality. The Hi-Line allele for a QTL on chromosome 4A resulted in lower stem cutting and infestation and greater larval mortality.

Two QTLs were identified for a population of 90 individuals for the Hi-Line/PI 576680 cross based on a map with 2425 markers (Table 9). The allele from PI 576680 at a QTL on chromosome 4A caused less stem cutting and decreased infestation. An allele from Hi-Line for a QTL on chromosome 3B caused greater larval mortality but had no impact on stem solidness. The closest marker for the QTL on 3B was 40 Mb from a candidate gene for stem solidness (data not shown) based on nucleotide BLASTN search against the *Triticum aestivum* genomic sequence (IWGSC 2018).

Three QTLs were identified for Hi-Line/PI 565386 RIL population of 98 individuals based on a map with 2655 markers (Table 9). The landrace PI 565386 contributed an allele for less stem cutting and greater larval mortality for QTLs on chromosomes 1B and 4A. The landrace PI 565386 also contributed an allele for greater WSS mortality on chromosome 6A. No QTLs for WSS resistance were identified from the Hi-Line/PI 166331 cross (91 RIL, 1277 markers) or the Berkut/PI 166333 cross (75 RIL, 6426 markers).

Detailed genetic maps constructed for five populations are shown in Online Resources 2–6. The genetic map for Berkut/PI 166333 is found in Jordan et al. (2018). The locations of QTL are shown in Online Resources 7–10.

Confirmation of QTL from Hi-Line/PI 166471

Two QTLs for resistance were identified in this RIL population. PI 166471 contributed an allele at a QTL on chromosome 1B that conferred early and late stem solidness, as well as resistance to the WSS. Hi-Line contributed an allele at a QTL on chromosome 4A that conferred WSS resistance and had no measurable impact on stem solidness. KASP markers were developed from SNPs underlying the QTL peaks for both loci. Near-isogenic lines were developed using the heterogeneous inbred family method

Table 3 Phenotypic means for wheat stem sawfly resistance for parents, checks and recombinant inbred lines (RIL) derived from Berkut/PI 185715 cross

Genotype	Early solidness (1–5)	Late solidness (1–5)	Infestation (%)	Mortality (%)	Cutting (%)
Locations	2	3	3	3	3
Replications	2	3	7	7	7
Berkut	3.2	1.8	57.0	17.1	43.3
PI 185715	2.5	1.8	38.1	46.2	21.0
Choteau	4.2	3.2	30.1	59.0	12.1
Reeder	1.9	1.5	50.1	26.2	35.4
LSD	1.2	0.4	13.1	22.9	12.1
RIL mean	3.0	1.8	46.6	34.4	29.7
RIL range	1.9–3.9	1.4–2.4	32.7–64.6	14.2–65.1	12.1–47.4
RIL *F* value	1.3	2.4***	1.6 ***	1.3	2.5***

*, **, ***Significant at $p < 0.05$, 0.01 and 0.001, respectively

Table 4 Phenotypic means for wheat stem sawfly resistance for parents, checks and recombinant inbred lines (RIL) derived from Hi-Line/PI 166471

Genotype	Early solidness (1–5)	Late solidness (1–5)	Infestation (%)	Mortality (%)	Cutting (%)
Locations	2	2	2	2	2
Replications	2	2	4	4	4
Hi-Line	2.0*	1.3	70.1	34.5	42.2
PI 166471	3.4	1.9	63.6	27.5	40.5
Choteau	ND	ND	53.8	65.8	17.5
Reeder	ND	ND	61.2	22.4	41.1
LSD	0.7	0.6	17.5	17.8	14.7
RIL mean	2.6	1.5	67.0	28.8	41
RIL range	2.0–4.9	1.0–3.7	38.7–86.1	11.5–71.5	9.3–58.0
RIL *F* value (*p*)	13***	7.7***	2.6 ***	2.5***	3.9***

*, **, ***Significant at $p < 0.05$, 0.01 and 0.001, respectively

Table 5 Phenotypic means for wheat stem sawfly resistance for parents, checks and recombinant inbred lines (RIL) derived from Hi-Line/PI 576680

Genotype	Early solidness (1–5)	Late solidness (1–5)	Infestation (%)	Mortality (%)	Cutting (%)
Locations	2	2	2	2	2
Replications	2	2	4	4	4
Hi-Line	1.9	1.6	51.2	31.3	28.2
PI 576680	3	1.7	57.6	33.2	29.1
Choteau	4.3	3.9	32.2	71.2	6.6
Reeder	1.9	1.1	68.1	29.7	43.7
LSD	1.3	0.6	19.5	19.5	14.2
RIL mean	2.6	1.7	54.3	35.9	30.5
RIL range	1.6–3.6	1.3–2.5	28.9–76.7	15.9–61.4	10.9–52.4
RIL *F* value (*p*)	1.1	1.8**	2.0***	1.9***	2.7***

*, **, ***Significant at $p < 0.05$, 0.01 and 0.001, respectively

(Barrero et al. 2015; Varella et al. 2017a). Table 10 shows that the NIL pairs for the 1B QTL differed significantly for both early and late stem solidness. The NIL pairs for the chromosome 4A QTL showed no difference in either early or late stem solidness.

Discussion

Prior to the coverage of the wheat-producing areas of the world with intensively bred wheat varieties, several

Table 6 Phenotypic means for wheat stem sawfly resistance for parents, checks and recombinant inbred lines (RIL) derived from Hi-Line/PI 565386

Genotype	Early solidness (1–5)	Late solidness (1–5)	Infestation (%)	Mortality (%)	Cutting (%)
Locations	2	2	2	2	2
Replications	2	2	4	4	4
Hi-Line	2.7	1.2	66.8	43.3	35.6
PI 565386	2.8	1.4	66.4	68.5	14.2
Choteau	3.2	3.6	56.5	68.0	13.3
Reeder	2.1	1.2	64.8	34.4	35.0
LSD	0.9	0.4	18.6	17.9	15.0
RIL mean	2.4	1.3	63.3	39.7	30.9
RIL range	1.6–3.5	1.0–1.7	26.5–87.7	14.2–65.7	9.0–53.0
RIL *F* value (*p*)	1.6**	1.5*	2.5***	2.9***	3.4***

*, **, ***Significant at $p < 0.05$, 0.01 and 0.001, respectively

Table 7 Phenotypic means for wheat stem sawfly resistance for parents, checks and recombinant inbred lines (RIL) derived from Hi-Line/PI 166331

Genotype	Early solidness (1–5)	Late solidness (1–5)	Infestation (%)	Mortality (%)	Cutting (%)
Locations	1	1	2	2	2
Replications	1	1	4	4	4
Hi-Line	2	2.1	61.7	30.5	33.9
PI 166331	3.7	2.0	62.4	47.3	28.5
Choteau	4.0	4.8	30.5	28.1	14.8
Reeder	2.0	1.9	50.8	33.5	28.7
LSD	ND	ND	20.1	20.5	15.5
RIL mean	3.1	2.7	51.8	37.4	26.8
RIL range	1.8–4.7	1.7–3.9	21.8–76.1	19.3–73.9	6.7–49.2
RIL *F* value (*p*)	ND	ND	2.4***	3.0***	3.0***

No QTLs for sawfly resistance were identified in this population

*, **, *** Significant at $p < 0.05$, 0.01 and 0.001, respectively

Table 8 Phenotypic means for wheat stem sawfly resistance for parents, checks and recombinant inbred lines (RIL) derived from Berkut/PI 166333

Genotype	Early solidness (1–5)	Late solidness (1–5)	Infestation (%)	Mortality (%)	Cutting (%)
Locations	1	2	1	1	1
Replications	1	2	3	3	3
Berkut	2.9	2.1	30.7	18.1	24.6
PI 166333	3.3	2.1	26.7	37.9	13.4
Choteau	4	ND	14.7	72.2	4.9
Reeder	2.1	ND	34.7	46.2	18.0
LSD	ND	0.5	17.8	39.0	14.5
RIL mean	2.5	2.0	26.0	44.6	14.9
RIL range	1.7–4.0	1.7–2.48	7.8–49.3	0.0–98.9	0.0–40.5
RIL *F* value (*p*)	ND	1.5*	2.2***	1.7**	1.8**

No QTLs for resistance were identified in this population

collections of traditional varieties were conducted and the seed was deposited in storage facilities (Zeven 1998). These landrace collections serve as a resource for plant breeding programs (Lopes et al. 2015) and also provide a basis for genetic dissection of the historical processes that separate landraces from modern wheat varieties. Analysis of SNP variation has shown that modern wheat varieties as a whole have retained much of the variation present in

Table 9 Quantitative trait loci identified for wheat stem sawfly resistance traits in six recombinant inbred line populations

Population	Trait	QTL designation[a]	Peak marker	Chromosome	QTL position (cM)	CI Low (cM)	CI High (cM)	LOD	Source of favorable allele	Percent effect
Berkut/PI 185715	Infestation (%)	1	Tdurum_contig54559_211	4B	81	70	89.8	3.6	PI 185715	20
	Mortality (%)	2	BobWhite_c10583_352	4A	146	142.5	154	5.5	PI 185715	28.7
	Cutting (%)	1	BobWhite_c5694_1201	4B	86.7	67	92	4.5	PI 185715	24.1
Hi-Line/PI 166471	Early Solidness	3	S2_2000757	1B	100	96	102	28.6	PI 166471	69.5
	Late Solidness	3	S2_2000757	1B	99	94	102	23	PI 166471	60.2
	Infestation (%)	3	S2_8770685	1B	89	86	127	7.41	PI 166471	20.2
	Infestation (%)	4	S10_202549960	4A	64	50	73	4.2	Hi-Line	10
	Mortality (%)	3	S3_597719	1B	96	86	112	7.3	PI 166471	21.8
	Mortality (%)	4	S10_204391758	4A	62	42	73	4.8	Hi-Line	13.8
	Cutting (%)	3	S2_8770685	1B	89	74	115	6	PI 166471	16.5
	Cutting (%)	4	S10_204391758	4A	62	58	66	5.28	Hi-Line	14.1
Hi-Line/PI 576680	Infestation (%)	5	S19_6244559	4A	16	0	29	4.47	PI 576680	20.5
	Mortality (%)	6	S8_747588527	3B	241	224	248	4.44	Hi-Line	20.3
	Cutting (%)	5	S10_208503596	4A	10.9	0	32	4.12	PI 576680	19
Hi-Line/PI 565386	Mortality (%)	7	S5_64258044	1B	123	98	130	4.7	PI 565386	14.1
	Mortality (%)	8	S10_211171863	4A	25	0	39	3.6	PI 565386	9.5
	Mortality (%)	9	S16_103975173	6A	94.9	89	102	4.4	PI 565386	11.1
	Cutting (%)	7	S5_64258044	1B	123	100	129	4.2	PI 565386	14.6
	Cutting (%)	8	S10_200076579	4A	26	1	40	4.5	PI 565386	15.8
Hi-Line/PI 166331			No QTL identified							
Berkut/PI 166333			No QTL identified							

[a]QTL designation refers to QTL identified on maps shown in Online Resources 7–10

Table 10 Single factor analysis of variance for near-isogenic lines (NIL) developed for 1B and 4A QTL found in Hi-Line/PI 166471 grown at Post Farm in 2017

Trait	4A QTL			1B QTL		
	Hi-Line allele	PI 166471 allele	*p* value	Hi-Line allele	PI 166471 allele	*p* value
Early solidness	2.3	2.4	Ns	2.2	3.3	< 0.0001
Late solidness	2.6	2.3	Ns	2.3	3.3	< 0.0001

landraces (Cavanagh et al. 2013). However, the variation is highly apportioned among subsets of varieties representing different growth habits and geographical areas. Several genomic regions show signatures of selection during the breeding and domestication process, though alternative alleles at selected loci were often present in elite breeding populations (Cavanagh et al. 2013).

The development of varieties resistant to the wheat stem sawfly provides an example of the successful utilization of wheat landraces. The solid stem characteristic identified in a Portuguese landrace, S-615, was used to develop the first commercial solid-stemmed cultivar for North America (Platt et al. 1948). Stem solidness causes resistance primarily due to antibiosis. The allele conferring solid stems at *Qss.msub-3BL* is now found in most solid-stemmed varieties cultivated in North America (Cook et al. 2017). Varella et al. (2015) found that the allele for stem solidness at *Qss.msub-3BL* was absent from a set of 234 elite spring lines from North America, except for varieties intentionally bred for WSS resistance. Sherman et al. (2010) identified a second QTL on chromosome 4A that controlled the relative amount of oviposition by female WSS, a form of antixenosis. The favorable allele for the 4A locus was present in a high frequency in elite North American lines independent of previous selection for WSS resistance (Varella et al. 2015).

A challenge in screening for resistance to the wheat stem sawfly is the need for an immature stem for egg-laying and the need to allow the plant to mature to determine the fate of the WSS larva developing in the stem. This makes field trials necessary for large-scale screening. The inability to easily grow WSS on artificial media (Macedo et al. 2005) dictates that trials must be planted in areas expected to have large WSS populations. Table 2 illustrates the variability in WSS infestation that may occur despite choosing sites based on previous high WSS levels. Reasons for variable WSS damage may include weather patterns that impact either the WSS or the plant and parasitoids that kill WSS larvae in the stem prior to cutting. Controlling for the impact of parasitoids is accomplished by dissecting stems to determine whether lack of cutting is due to plant-induced mechanisms or parasitoids. However, weather patterns that may impact WSS infestation and cutting are not controllable.

The inherent difficulties in field-based screening for WSS resistance have made the use of markers for resistance a high priority. The most widely used marker for decades has been the presence of solid stems conferred by the allele at *Qss.msub-3BL* introduced from the Portuguese landrace (McNeal 1959). Assessment of stem solidity near plant maturity by visual scoring of cross-sectioned internodes allows screening of thousands of genotypes in a short period of time. The recent discovery of a second allele at *Qss.msub-3BL*, which conferred solidness only early in stem elongation (Varella et al. 2016), shows that stem solidity assessed late in development is not a perfect marker for resistance. In addition, other genetically controlled mechanisms of resistance may exist that are unrelated to solid stems (Varella et al. 2017a). Molecular markers for resistance traits not controlled by morphological differences would be useful for plant breeding efforts.

Population size for the RIL populations varied from a 75 to 116 lines (Table 1). These population sizes, coupled with inherent challenges of field-based screening for WSS resistance, suggest that only QTLs with relatively large effects were likely to be detected. This is indicated by the observation that the detected QTLs all had percent effect of greater than 10% (Table 9). Four of the six RIL populations evaluated in this study revealed a QTL on chromosome 4A that impacted WSS cutting through both decreased infestation and greater larval mortality in the stem (Table 9). This QTL was originally identified in a cross between elite spring wheat cultivars Reeder and Conan (Sherman et al. 2010). This QTL was also detected as impacting WSS resistance in an association mapping panel of elite North American spring wheat lines (Varella et al. 2015). Thus, variation at this region appears to be high both in modern wheat lines and in primitive landrace accessions. Cavanagh et al. (2013) found that sequence variation in landrace accessions was often well represented in modern cultivars. Variation for alleles at the 4A QTL indicates an example of functional variation also conserved in elite germplasm. This result shows an example of a gene for resistance to the WSS that is not related to solid stems, and could only be identified by field-based screening. The confirmation sets of NIL confirm that the 4A QTL is not associated with stem solidity.

An unexpected result from the present analysis was that three populations developed from crosses of landraces to Hi-Line showed a significant QTL on chromosome 4A (Table 9). In two of the RIL populations with Hi-Line, the landrace contributed an allele for resistance. For one population, Hi-Line contributed the allele for resistance. A

consensus map constructed for chromosome 4A showed that the QTL in the four populations were all within a 14 cM region of the chromosome (data not shown), suggesting a single QTL or two closely linked QTLs. The original discovery of the QTL in the Reeder/Conan RIL population (Sherman et al. 2010) showed two QTL peaks in close proximity linked in repulsion. The difference in direction of impact for the Hi-Line alleles at this QTL in the present study may be explained by the presence of linked QTLs that vary in allelic state in the landrace accessions. Alternatively, the possibility of multiple alleles at a single QTL cannot be dismissed. Multiple alleles for resistance have been identified at the major locus for solid stems *Qss.msub-3BL*. The standard allele from Portuguese landrace S-615 shows greater WSS resistance than the allele for hollow stems (Sherman et al. 2015), but less resistance than the allele for early solidness derived from Conan (Talbert et al. 2014).

An important QTL conferring both stem solidness and WSS resistance was also identified on chromosome 1B (Table 9). The allele conferring stem solidness and resistance was contributed by PI 166471 from Turkey. This QTL was not identified in the five other populations because neither parent of these RIL populations contained the allele for solid stems. The QTL had a large impact on stem solidity as indicated by a LOD score of greater than 20 and an percent effect on variation among the RIL of close to 70%. By comparison, the percent variation controlled by *Qss.msub-3BL* in a population segregating for the S-615 allele and the allele for hollow stems was 76% (Cook et al. 2004). The confirmation sets of NIL developed for this locus confirmed its impact on stem solidity (Table 10). The KASP markers used for development of the NIL will be useful for introgression of this new allele for solid stems into elite wheat germplasm to provide a new source of WSS resistance.

Landrace PI 565386 was notable in that the level of cutting and the amount of larval mortality were similar to that seen for the solid-stemmed resistance check Choteau (Table 2). However, PI 565386 did not have solid stems. Alleles for resistance were identified in this line at three different loci (Table 9). These included alleles for high WSS mortality at QTL on chromosomes 4A, 1B and 6A. The 4A allele is in the same chromosome region as that observed in three other RIL populations. The QTL on chromosome 1B is within 40 Mb of the QTL for solid stems identified in Hi-Line/PI 166471 RIL population based on a BLASTN search against the wheat genome sequence (IWGSC 2018). However, the 1B QTL from Hi-Line/PI 565386 had no impact on stem solidness (Table 10). This result may indicate that stacking several loci impacting resistance may be necessary to achieve the same level of resistance conferred by a high degree of stem solidness.

Two additional QTLs were identified in individual RIL populations. Landrace PI 185715 contributed an allele for low infestation and low cutting for a QTL on chromosome 4B. Hi-Line contributed an allele for high larval mortality for a QTL on chromosome 3B in the PI 576680/Hi-Line RIL population.

Conclusion

Six RIL populations were developed from landrace accessions selected from areas of the world with historic pressure from endemic species of WSS over many centuries. Novel QTLs for resistance included a previously unknown locus controlling stem solidness. Other QTLs unrelated to solid stems impacted infestation and larval mortality in the North American native species of WSS. A resistance QTL prevalent in elite breeding lines was also identified in the landraces. The importance of stem solidness and resulting variation in mortality of larval WSS is reinforced by several instances of QTL on different chromosomes, suggesting these arose independently. Our results provide practical tools for plant improvement and also address the maintenance of genetic diversity in the genetic progression from primitive landraces to elite modern cultivars.

Author contribution statement ACV, DKW and LET designed the study. ACV, NKB and JPC constructed genetic maps and conducted QTL analysis. ACV, MLH and DKW performed entomological investigation. NKB, PFL and H-YH designed and conducted field trials. KWJ, EA and SC conducted molecular genotyping experiments. ACV and LET prepared initial draft of the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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