Published April 27, 2017

JOURNAL OF PLANT REGISTRATIONS

GERMPLASM

Registration of Two Soybean Germplasm Lines Containing Leaf-Chewing Insect Resistance QTLs from PI 229358 and PI 227687 Introgressed into 'Benning'

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Abstract

Two soybean [*Glycine max* (L.) Merr.] germplasm lines, Benning-ME (Reg. No. GP-405, PI 679958) and Benning-MGHE (Reg. No. GP-406, PI 679959), were developed by the University of Georgia Agricultural Experiment Stations. Control of insect pests is crucial in soybean production; host-plant resistance reduces the need for insecticide applications, thus diminishing production costs and pesticide concerns. In soybean, resistance to a broad range of leaf-chewing insects is found in the Japanese accessions PI 229358 and PI 227687. In PI 229358, resistance is conferred by quantitative trait loci (QTLs) M, H, and G. In PI 227687, resistance is conferred by QTL-E. To enhance soybean resistance to leaf-chewing insects, QTLs of PI 229358 and PI 227687 were pyramided in Benning-ME and Benning-MGHE, which are near-isogenic lines of 'Benning' obtained through marker-assisted backcrossing. Under field conditions, Benning-ME and Benning-MGHE sustain 67 and 57% less defoliation than Benning, respectively. To determine the QTL introgressions in each line, high-density single-nucleotide polymorphism (SNP) genotypes were obtained using the SoySNP50K iSelect BeadChip (Illumina). To facilitate selection of lines carrying a specific QTL pyramid, Kompetitive Allele Specific Polymerase Chain Reaction markers were developed for high-throughput genotyping. These lines are valuable genetic resources for breeding of host-plant resistance to insects in soybean. The combination of QTL-M and QTL-E provides agriculturally relevant levels of resistance, and with only two loci, the use of this pyramid is feasible in a breeding program.

Journal of Plant Registrations 11:185–191 (2017).
doi:10.3198/jpr2016.04.0019crg
Received 5 Apr. 2016.
Accepted 23 Aug. 2016.
Registration by CSSA.
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Insect pests affect soybean [*Glycine max* (L.) Merr.] production. Particularly, high levels of leaf damage by chewing insects indirectly affect seed yield and quality (Haile et al., 1998). Corn earworm [CEW, *Helicoverpa zea* (Boddie)], soybean looper [SBL, *Chrysodeixis includens* (Walker)], velvetbean caterpillar [VBC, *Anticarsia gemmatalis* (Hübner)], and bean leaf beetle [*Cerotoma trifurcata* (Forster)] are among the most economically important insects affecting US production (Boethel, 2004, Musser et al., 2014). Plant resistance to these pests reduces the need for insecticide applications, thus diminishing production costs and pesticide concerns. The Japanese soybean landraces PI 229358 and PI 227687 have been widely used as sources for resistance to leaf-chewing insects (USDA-ARS, 2015). Resistance in these PIs is conferred via antibiosis and antixenosis. Antibiosis encompasses the detrimental effects on insect physiology (Painter, 1951), and antixenosis refers to the discouragement of insect colonization or feeding (Hulburt, 2002, Kogan and Ortman, 1978). PI 229358's resistance is conferred by three quantitative trait loci (QTLs). Quantitative trait locus M confers antibiosis and antixenosis, QTL-G confers antibiosis, and QTL-H confers antixenosis (Rector et al., 1999, 2000). Quantitative trait loci G and H are minor QTLs that are only expressed if QTL-M is present (Zhu et al., 2006). PI 227687's resistance is conferred by QTL-E via antibiosis and antixenosis (Hulburt, 2002). 'Benning' (Boerma et al., 1997) near-isogenic lines (NILs) carrying each and all of PI 229358's QTLs were released by Zhu et al. (2007). G05-Ben229IR-MGH is the most resistant of these lines (Zhu et al., 2008). However, QTL-G is associated with yield drag (Warrington et al., 2008), which hinders the deployment of this pyramid.

The objective of this research is to enhance soybean resistance to leaf-chewing insects by combining the QTLs from PI 229358 and PI 227687. The new NILs Benning-ME (Reg. No. GP-405, PI 679958; QTL-M and QTL-E) and Benning-MGHE (Reg. No. GP-406, PI 679959; QTL-M, QTL-G, QTL-H, and QTL-E) are highly resistant to CEW, SBL, VBC, and fall

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Abbreviations: CEW, corn earworm; EDTA, ethylenediaminetetraacetic acid; FAW, fall armyworm; KASP, Kompetitive Allele Specific Polymerase Chain Reaction; MG, maturity group; NIL, near-isogenic line; QTL, quantitative trait locus; SBL, soybean looper; SNP, single-nucleotide polymorphism; SSR, simple sequence repeat; VBC, velvetbean caterpillar.

armyworm [FAW, *Spodoptera frugiperda* (J.E. Smith)] (Ortega et al., 2016). Pyramiding two insect resistance loci (i.e., QTL-M and QTL-E) is feasible in a breeding program. To characterize the QTL introgressions in Benning-ME and Benning-MGH, high-density single-nucleotide polymorphism (SNP) genotypes were obtained using the SoySNP50K iSelect BeadChip (Illumina). Kompetitive Allele Specific Polymerase Chain Reaction (KASP) assays were designed to detect SNP alleles flanking each QTL; these assays facilitate high-throughput genotyping and selection of breeding lines carrying a specific QTL combination.

Methods

Development of the Insect-Resistant Germplasm Lines

The germplasm lines Benning-ME and Benning-MGHE are BC_6F_2–derived NILs developed from [Benning (7) × PI 229358] × [Benning (7) × PI 227687]. Benning is a Maturity Group (MG) VII cultivar derived from an F_4 plant descended from the cross 'Hutcheson' × 'Coker 6738' (Boerma et al., 1997). The insect resistance sources PI 229358 (MG VII) and PI 227687 (MG VIII) are Japanese cultivars known as 'Soden-daizu' and 'Miyako White', respectively (USDA-ARS, 2015). Simple sequence repeat (SSR) markers were used during backcross and selfing generations to select lines carrying a particular QTL combination: Sat_258 and Satt702 for QTL-M (Zhu et al., 2009), Sct_199 and Satt191, for QTL-G (Zhu et al., 2008), Sat_334 and Sat_122, for QTL-H (Zhu et al., 2008), and Sat_112 and Satt411 for QTL-E (Hulburt, 2002). Primer sequences for the SSR markers were obtained from SoyBase (http://www.soybase.org) (Grant et al., 2010). Genomic DNA isolation, polymerase chain reaction, and electrophoresis protocols for SSRs were performed as described by Zhu et al. (2008). In brief, the breeding scheme was as follows: Benning-MGH, the $BC_6F_{2:3}$ Benning NIL carrying QTL-M, QTL-G, and QTL-H, was developed using a marker-assisted backcross approach from a cross between Benning and PI 229358; Benning-E, the $BC_6F_{2:3}$ Benning NIL carrying QTL-E, was developed in a marker-assisted backcross approach from a cross between Benning and PI 227687; Benning-MGH was crossed to Benning-E; and finally, the $F_{2:3}$ plants carrying the QTL pyramids QTL-M and QTL-E and QTL-M, QTL-G, QTL-H, and QTL-E were identified through marker-assisted selection. Seed increased from the $F_{2:3}$ lines were used for the insect resistance bioassays (Ortega et al., 2016).

Graphical Genotypes

The SoySNP50K iSelect BeadChip (Illumina) (Song et al., 2013), which contains 52,041 SNPs distributed throughout the soybean genome, was used to genotype Benning, PI 229358, PI 227687, Benning-MGH, Benning-E, Benning-ME, and Benning-MGHE. The SoySNP50K assays were performed at Michigan State University, East Lansing, using an Illumina iScan platform. The SNP genotype calling was done in GenomeStudio 2011.1 software (Illumina, 2011).

Polymorphic SNPs between Benning and PI 229358, Benning and PI 227687, and Benning versus PI 229358 and PI 227687 were identified using FlapJack (Milne et al., 2010). A graphical genotype (Young and Tanksley, 1989) of Benning-MGH, Benning-E, Benning-ME, and Benning-MGHE was created using Graphical Genotypes GGT 2.0 (van Berloo, 2008). The introgressions at QTL-M (chromosome 7), QTL-G (chromosome 18), QTL-H (chromosome 12), and QTL-E (chromosome 15) were estimated using the graphical genotypes.

Development of KASP Genotyping for Insect Resistance QTLs

Genomic DNA isolated from seeds of Benning NILs was used for the SNP-genotyping assays. The DNA extraction protocol was modified from Kamiya and Kiguchi (2003). Briefly, cotyledonary tissue was harvested with a scalpel and placed into a 2-mL tube, and 600 μL of digestion buffer (10 mM tris hydrochloride [Tris-HCL, pH 7.8], 5 mM ethylenediaminetetraacetic acid [EDTA], 0.5% sodium dodecyl sulfate [SDS], 0.5% nonyl phenoxypolyethoxylethanol [NP-40], and 0.5% polyethylene glycol sorbitan monolaurate [Tween-20]) and 2 μL of proteinase K (20 mg mL^{-1}) were added. The tube was vortexed for 10 min, incubated at 55°C for 45 min, and left at room temperature for 15 min. Six hundred microliters of phenol:chloroform:isoamyl-alcohol (25:24:1) were added to the tube; the sample was mixed by inversion to form an emulsion and centrifuged at 13,200 rpm for 6 min. The supernatant was transferred to a 1.5-mL tube and mixed with 500 μL of chloroform:isoamyl-alcohol (24:1); the tube was centrifuged at 13,200 rpm for 6 min, and the supernatant was transferred to a 1.5-mL tube. The chloroform:isoamyl-alcohol extraction was repeated until a clear supernatant was obtained. DNA was precipitated by adding 1 volume of isopropanol; the tube was centrifuged at 13,200 rpm for 6 min and the supernatant was discarded. Finally, the DNA pellet was washed with 70% ethanol, dried, and resuspended in 50 μL of TE/RNase buffer (10 mM Tris-HCL [pH 8.0], 1 mM EDTA, and 100 μg mL^{-1} ribonuclease A).

The KASP genotyping system (KBioscience) was used to develop a high-throughput genotyping assay of insect-resistant NILs. The KASP assay included QTL-flanking SNPs for QTLs G, H, and E, which were identified from the graphical genotypes of Benning-ME and Benning-MGHE. The assay also included the functional SNP for the insect resistance gene in QTL-M. Each 4-μL KASP reaction consisted of 2 μL of 2× KASP mastermix, 2 μL of 10 to 20 ng $μL^{-1}$ genomic DNA, and 0.106 μL of primer mix (allele-specific primers at 1.4 μM, and common primer at 3 μM). Polymerase chain reactions were performed in a T100 Thermal Cycler (Bio-Rad Laboratories) using a *Taq* polymerase activation period (94°C for 15 min), followed by a touchdown amplification step consisting of 10 cycles of 94°C for 20 s and 65°C for 1 min (decreasing 0.8°C $cycle^{-1}$), then 29 cycles of 94°C for 20 s and 57°C for 1 min. The KASP assay was read in a LightCycler480 (Roche Diagnostics); a single fluorescence acquisition was recorded after incubating the samples at 37°C for 1 min.

Characteristics

Similar to Benning, Benning-ME and Benning-MGHE have determinate growth habit and belong to MG VII. Both lines have purple flowers, tawny pubescence, yellow seed coats, and sharp trichomes. Benning-ME has a tan pod wall color, while Benning-MGHE has a brown pod wall color. Benning-ME has a brown hilum of varying intensity, and Benning-MGHE has a brown

Fig. 1. Graphical genotype of (a) Benning-MGH, built with single-nucleotide polymorphisms (SNP) markers between Benning and PI 229358; (b) Benning-E, built SNPs with between Benning and PI 227687; (c) Benning-ME and Benning MGHE, built with polymorphic SNPs between Benning and both PI 229358 and PI 227687. Red indicates Benning alleles, dark blue indicates PI alleles, and light blue indicates heterozygous loci. QTL, quantitative trait locus.

Table 1. Distribution of SoySNP50K single-nucleotide polymorphisms used to draw the graphical genotypes. Benning and PI alleles are listed for each chromosome (Chr.).

Chr.	LG†	Benning-MGH			Benning-E			Benning-ME			Benning-MGHE		
		Total	Benning	PI 229358	Total	Benning	PI 227687	Total	Benning	PI 229358 + PI 227687	Total	Benning	PI 229358 + PI 27687
1	D1a	522	517	5	445	443	2	173	171	2	173	171	2
2	D1b	830	577	253	963	924	39	453	450	3	453	441	12
3	N	505	504	1	617	597	20	260	233	27	260	208	52
4	C1	660	655	5	775	772	3	441	439	2	441	439	2
5	A1	732	731	1	842	839	3	468	467	1	468	437	31
6	C2	504	502	2	697	696	1	249	249	0	249	249	0
7	M	620	410	210	851	843	8	366	194	172	366	196	170
8	A2	662	657	5	952	947	5	377	376	1	377	369	8
9	K	509	502	7	482	475	7	198	195	3	198	195	3
10	O	547	545	2	637	631	6	330	327	3	330	327	3
11	B1	523	468	55	516	481	35	253	249	4	253	210	43
12	H	523	327	196	583	580	3	350	350	0	350	265	85
13	F	670	636	34	817	813	4	386	384	2	386	363	23
14	B2	699	694	5	723	712	11	340	339	1	340	339	1
15	E	913	854	59	660	641	19	388	331	57	388	375	13
16	J	529	523	6	695	684	11	312	296	16	312	307	5
17	D2	607	572	35	681	678	3	368	367	1	368	367	1
18	G	1,024	873	151	1,300	1,268	32	431	391	40	431	347	84
19	L	402	401	1	931	928	3	257	257	0	257	257	0
20	I	386	371	15	420	336	84	245	203	42	245	202	43
Total		12,367	11,319	1,048	14,587	14,288	299	6,645	6,268	377	6,645	6,064	581

† LG, linkage group.

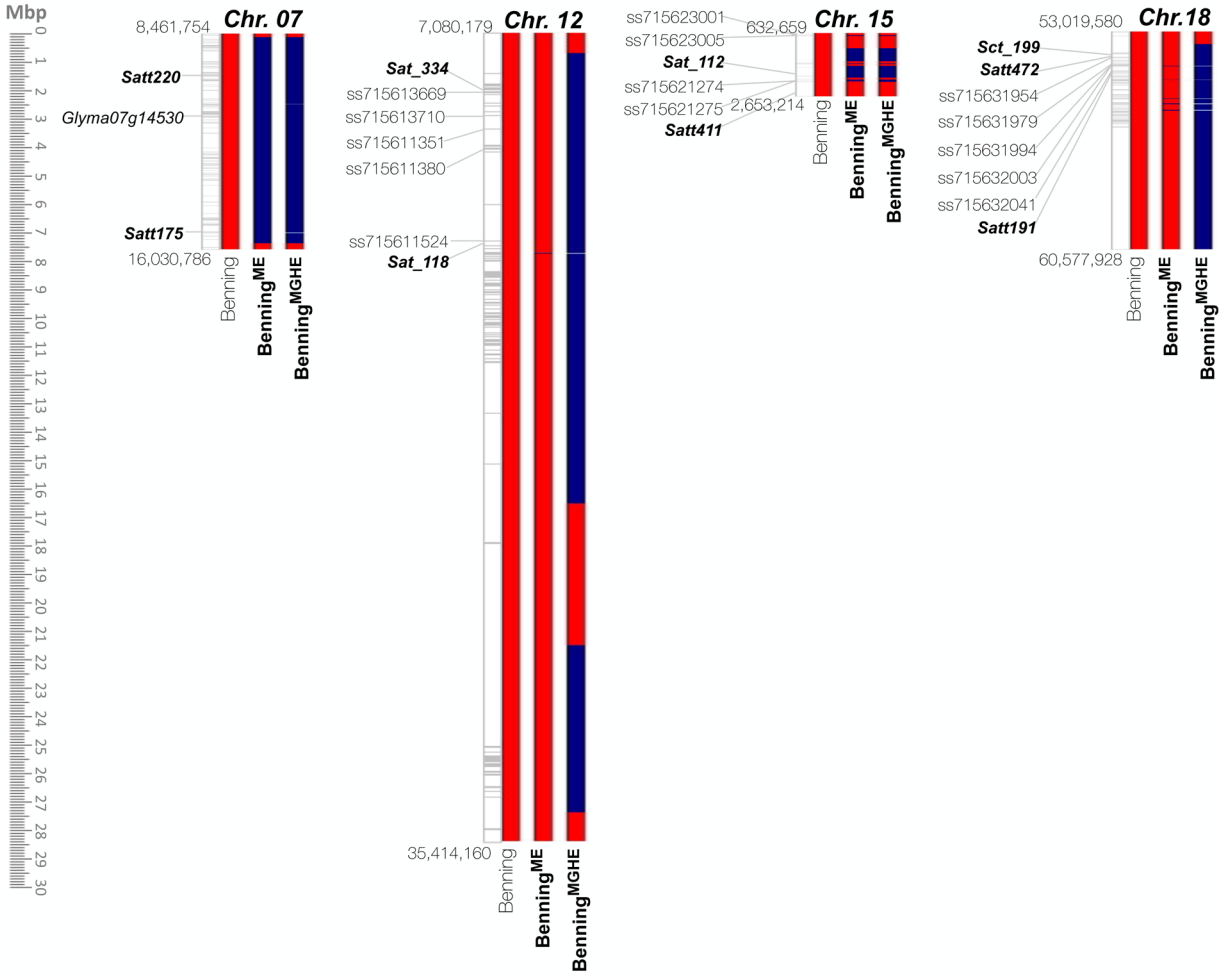

Fig. 2. Insect resistance quantitative trait loci showing PI introgressions in Benning-ME and Benning-MGHE. Red indicates Benning alleles, dark blue indicates PI alleles, and light blue indicates heterozygous loci. Single sequence repeat markers used for line development, and new Kompetitive allele-specific polymerase chain reaction markers are indicated by the arrows. Chr., chromosome.

Table 2. Kompetitive allele-specific polymerase chain reaction markers for selection of insect resistance quantitative trait loci.

SNP ssID†	Chr.‡	bp	FAM primer§	VIC primer¶	Common Primer	Benning allele	PI 229358 allele
Glyma07g14530	*Gm07*	11,281,192	GAAGGTGACCAAGTTCATGCT-TGGGTGTGAATGTTTATTGTGA	GAAGGTCGGAGTCAACGGATT-TGGGTGTGAATGTTTATTGTGG	CTGCTCTTGGCAGAGTGTGCCACC	A	G
ss715613669	*Gm12*	9,189,112	GAAGGTGACCAAGTTCATGCT-CAACACCTAGTTTTTTACCACAACA	GAAGGTCGGAGTCAACGGATT-AACACCTAGTTTTTTACCACAACG	TCTGTTTAAAAGGTCAACCTCTCC	A	G
ss715613710	*Gm12*	9,972,984	GAAGGTGACCAAGTTCATGCT-AACCTCATGTAAATGTTGTCA	GAAGGTCGGAGTCAACGGATT-AACCTCATGTAAATGTTGTCG	GACGATTGACGACCCTTGTT	T	C
ss715611351	*Gm12*	10,445,762	GAAGGTGACCAAGTTCATGCT-TAAGCCTCTCCTCGCTTTTGCT	GAAGGTCGGAGTCAACGGATT-AGCCTCTCCTCGCTTTTGCC	ATGCAATGATTGGGTGCTAAG	T	C
ss715611380	*Gm12*	11,157,215	GAAGGTGACCAAGTTCATGCT-GTGGTGAAGATGGTGGGCA	GAAGGTCGGAGTCAACGGATT-GTGGTGAAGATGGTGGGCG	CCAAGCGACATCGTTTCTTT	A	G
ss715611524	*Gm12*	14,384,675	GAAGGTGACCAAGTTCATGCT-ATGACACCTAGATCTGGTGCA	GAAGGTCGGAGTCAACGGATT-ATGACACCTAGATCTGGTGCG	AGAGCGTGAGCAGGATTCTG	C	T
ss715631954	*Gm18*	53,905,333	GAAGGTGACCAAGTTCATGCT-GAGGATGCAACGGCTGTGGTA	GAAGGTCGGAGTCAACGGATT-GAGGATGCAACGGCTGTGGTG	CCACGGTCTACGCCTCACCC	G	A
ss715631979	*Gm18*	54,061,528	GAAGGTGACCAAGTTCATGCT-CAGGCAAGGGCTAAGATGC	GAAGGTCGGAGTCAACGGATT-TCAGGCAAGGGCTAAGATGT	TTTCAAAAGTATCCATTTGTTGC	G	A
ss715631994	*Gm18*	54,137,764	GAAGGTGACCAAGTTCATGCT-CTAGCTCCTGTTCATCAGAAATCTG	GAAGGTCGGAGTCAACGGATT-CTAGCTCCTGTTCATCAGAAATCTT	AAAATTTCCTGGCTGGGTTT	T	G
ss715632003	*Gm18*	54,191,166	GAAGGTGACCAAGTTCATGCT-GAAGGATTAAATAAAAAACACTCACA	GAAGGTCGGAGTCAACGGATT-GAAGGATTAAATAAAAAACACTCACC	CCAGAAGTTCACCATCACCA	C	A
ss715632041	*Gm18*	54,391,672	GAAGGTGACCAAGTTCATGCT-CAATTCGATTTTTGGATAATGC	GAAGGTCGGAGTCAACGGATT-CCAATTCGATTTTTGGATAATGT	TCCACTTGGCAATTTACGTG	C	T
							PI 227687 allele
ss715623001	*Gm15*	714,829	GAAGGTGACCAAGTTCATGCT-TCTGTTCAAACTCATGCAGAAGA	GAAGGTCGGAGTCAACGGATT-CTGTTCAAACTCATGCAGAAGG	CAAATTCCGCGAGGTAAGTC	A	G
ss715623005	*Gm15*	718,311	GAAGGTGACCAAGTTCATGCT-TCGCGTCTCTTGGTGTCAG	GAAGGTCGGAGTCAACGGATT-CGCGTCTCTTGGTGTCAA	CTAAAGGCACAGGCCTCCAT	T	C
ss715621274	*Gm15*	2,152,272	GAAGGTGACCAAGTTCATGCT-TGGAGGGTGGTTATAGGTCTTGT	GAAGGTCGGAGTCAACGGATT-GGAGGGTGGTTATAGGTCTTGC	GTAAAAATCAACCACAGATGAGC	G	A
ss715621275	*Gm15*	2,154,788	GAAGGTGACCAAGTTCATGCT-GACACCCGATCAAGATTCAAG	GAAGGTCGGAGTCAACGGATT-GACACCCGATCAAGATTCAAA	CGAGGTCCTTGTATGGGTTG	T	C

† Single-nucleotide polymorphism ss identification number.

‡ Chromosome number.

hilum. They are also similar to Benning in plant height, lodging score, and seed quality score. Benning-ME and Benning-MGH were evaluated for resistance to SBL, CEW, FAW, and VBC in antibiosis, antixenosis, and field-cage assays (Ortega et al., 2016). For SBL in the antibiosis assay, the average weight of SBL caterpillars feeding on Benning-ME, Benning-MGHE, and Benning was 58.7, 44.4, and 85.8 mg, respectively. In the SBL antixenosis assay, defoliation in Benning-ME, Benning-MGHE, and Benning was 24, 29, and 49%, respectively. In the SBL field-cage assay, which measures the effect of antibiosis and antixenosis, defoliation in Benning-ME, Benning-MGHE, and Benning was 21, 27, and 63%, respectively. The data for the antibiosis and antixenosis assays against CEW, FAW, and VBC were reported by Ortega et al. (2016).

Graphical Genotyping of Benning NILs

From the 52,041 SNPs in the SoySNP50K chip, 12,367 SNPs were polymorphic between Benning and PI 229358. In the Benning-MGH genome, 91.5% of the polymorphic SNP loci carried Benning alleles, while 8.5% carried PI 229358 alleles (Fig. 1a). 14,587 SNPs were polymorphic between Benning and PI 227687. In Benning-E, 98.0% SNP loci carried Benning alleles and 2.0% carried PI 227687 alleles (Fig. 1b). A total of 6645 SNPs were polymorphic between Benning and both PIs. In Benning-ME, 94.3% SNP loci carried the Benning allele and 5.7% carried either PI 229358 or PI 227687 (Fig. 1c), whereas in Benning-MGHE, 91.3% SNP loci carried the Benning allele and 8.7% carried either PI 229358 or PI 227687 (Fig. 1d). Plant introduction introgressions were also detected in other regions of the NILs' genome (Table 1).

SNP for Marker-Assisted Selection of Insect Resistance QTLs

The introgression of PI-derived DNA for each QTL, SSR markers used for selection of the NILs, and new SNP markers are shown in Fig. 2. Five SNP loci from the SoySNP50K chip were converted to KASP markers for QTL-H; five and four SNPs were also converted to KASP markers for QTL-G and QTL-E, respectively. Primer sequences for the KASP markers are listed in Table 2. Each KASP marker effectively distinguished the Benning allele from the PI allele when they were validated using the insect-resistant NILs genotyped with the SoySNP50K chip. These KASP markers still need to be validated for marker-assisted selection in an insect-susceptible genetic background other than Benning. Nonetheless, the Glyma07g14530 marker in QTL-M is the functional SNP; hence, the PI 229358 allele is unique to insect-resistant lines carrying QTL-M.

Conclusions

Breeding high-yielding soybean cultivars with agriculturally relevant levels of resistance to leaf-chewing insects has been a long-term goal. This is the first time that QTLs from PI 229358 and PI 227687 have been pyramided to enhance soybean resistance to insects. The germplasm lines Benning-ME and Benning-MGHE would be useful to soybean breeders for simultaneous selection of QTL-M and QTL-E and QTL-M, QTL-G, QTL-H, and QTL-E, respectively. Both NILs exhibit similar levels of resistance; therefore, Benning-ME is useful if breeders prefer to introgress only QTL-M and QTL-E and/or exclude QTL-G because of the yield penalty. Additionally, the KASP genotyping assays would assist in the selection of lines carrying a specific QTL combination. Furthermore, the graphical genotypes for the QTL introgression provide a reference for fine mapping and cloning of candidate genes responsible for insect resistance.

Availability

Seeds of Benning-ME and Benning-MGHE will be maintained by the Georgia Agricultural Experiment Stations at the University of Georgia, Athens. A small sample of seed may be requested from the corresponding author for research purposes. Seed of Benning-ME and Benning-MGHE has been deposited in the National Plant Germplasm System (Urbana, IL), where it will be available for distribution 20 yr after publication.

Acknowledgments

This research was supported by the United Soybean Board, the USDA Grant GEO-2011-04373, and by State and Federal monies allocated to the Georgia Agricultural Experiment Stations. The authors give special thanks to the iSCAN facility at the Michigan State University for running the SoySNP50K genotyping.

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