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Identification and mapping of quantitative trait loci associated with soybean rust (*Phakopsora pachyrhizi*) resistance in genotype UG 5

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Soybean rust, *Phakopsora pachyrhizi,* is one of the most serious and widespread foliar diseases of soybean causing high yield losses world-wide. The objective of this study was to identify and map quantitative trait loci (QTLs) resistant to soybean rust in genotype UG 5. Ninety-seven F_2 mapping plants, obtained from a cross between Wondersoya and UG 5, were used for this study. Quantitative trait locus analysis using QTL IciMapping software identified three putative QTLs associated with soybean rust (SBR) on chromosomes 6, 9 and 18 with logarithms of odds (LOD) scores ranging from 3.47 to 8.23 and phenotypic variance explained by the QTLs ranging from 18.3 to 25.6%. The putative QTL detected on chromosome 9 is novel and has not been reported elsewhere. The putative QTLs identified in this study could help to facilitate SBR resistance breeding towards efficient marker-assisted selection approach and gene pyramiding leading to the development of durable resistance.

Key words: Linkage map, segregation, simple sequence repeat (SSR).

INTRODUCTION

Soybean (*Glycine max* [L.] Merrill) is one of the most important legume crops in the world providing a major source of high-quality protein and oil for human consumption and livestock feeds. However, soybean is attacked by a wide range of pathogens. Soybean rust (SBR), caused by *Phakopsora pachyrhizi*, is the most severe destructive foliar disease leading to high loss in yield and quality of soybean. Soybean rust was first reported in 1902 in Japan (Hennings, 1903) and subsequently spread from Asia to Africa, South America (Yorinori et al., 2005) and the United States of America (Schneider et al., 2005) through air-borne movement of urediniospores. In Africa, soybean rust was reported in Uganda, Kenya and Rwanda in 1996 (Tukamuhabwa et al., 2001), Zambia and Zimbabwe in 1998, Mozambique in 2000 and South Africa in 2001 (Levy et al., 2002) and

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further spread in the westward and central direction to Nigeria (Akinsanmi et al., 2001), Ghana (Bandyopadhyay et al., 2007), and Democratic Republic of Congo (Ojiambo et al., 2007). *P. pachyrhizi* has a unique ability to infect a broad range of legume species that contribute to a diverse and complex virulence pattern (Hartman et al., 2005). Considering the explosive nature of the disease and the high potential yield losses (up to 80%), soybean rust has long been viewed as a serious threat to soybean production worldwide. The development of resistant varieties to soybean rust could reduce the impact of the disease without the expensive, time-consuming and negative environmental impact of foliar fungicide applications.

So far, several major sources of soybean rust resistance genes (*Rpp1* to *Rpp7*) have been identified in soybean. However, the high virulence and variability of the pathogen isolates pose problems leading to breakdown of resistance. For example, resistance due to *Rpp1* to *Rpp4* have already been broken in China (Shan et al., 2012) and other three improved soybean rust resistant varieties (Namsoy 4M, Maksoy 1N, and Maksoy 2N) have succumbed to soybean rust in Uganda (Tukamuhabwa et al., 2009), suggesting that the SBR resistance genes are not durable. Therefore, discovering and mapping additional resistance genes in soybean is crucial to further improve the SBR resistance and develop durable SBR-resistant cultivars.

Soybean has a reasonably dense molecular marker linkage map (Song et al., 2004, 2010) where the association of markers to known genes has been studied by several groups. Molecular markers linked to *Rpp* genes in soybean have already been determined in different mapping populations, where specific genes (*Rpp1* to *Rpp7*) resistant to *P. pachyrhizi* have been identified and mapped to particular linkage groups (LGs). *Rpp1* from PI 200492 (Hyten et al., 2007), *Rpp1-b* from PI 594538A (Chakraborty et al., 2009), *Rpp1* allelic genes from PI 587886, PI 587880A (Ray et al., 2009), and PI 561356 (Kim et al., 2012) were mapped to the same region on soybean chromosome 18 (LG-G). *Rpp2* (Silva et al., 2008) was mapped on chromosome 16 (LG-J) and *Rpp3* (Hyten et al., 2009) and *Rpp* (Hyuuga) (Monteros et al., 2007) were mapped on chromosome 6 (LG-C2). *Rpp4* (Silva et al., 2008), *Rpp6* (Li et al., 2012) and *Rpp6907* (Chen et al., 2015) were mapped to different regions other than *Rpp1* on chromosome 18 (LG-G), and *Rpp5* (Garcia et al., 2008) was mapped on chromosome 3 (LG-N). More recently, *Rpp7* (Childs et al., 2016) has been mapped on chromosome 19 (LG-F).

UG 5 is a locally available genotype showing good resistance to SBR in Uganda, which seems to have a unique gene controlling resistance to soybean rust. It was the only line found resistant to different isolates of SBR in Uganda since 1996 among the Ugandan germplasm collections (Maphosa et al., 2013; Kawuki et al., 2003). UG 5 was also found to be SBR resistant to different *P. pachyrhizi* isolates in Nigeria (Twizeyimana et al., 2009) and USA (Twizeyimana and Hartman, 2012). However, the genes in UG 5 controlling resistance to soybean rust are not yet identified and mapped.The objective of the present study was, therefore, to identify and map quantitative trait loci (QTLs) associated with resistance to soybean rust in UG 5 genotype.

MATERIALS AND METHODS

Study site

Phenotypic evaluation and molecular work were carried out at Makerere University Agricultural Research Institute-Kabanyolo (MUARIK), Uganda, from 2017 to 2018 in screen-house and biotech laboratory, respectively.

Development of mapping population and *P. pachyrhizi* isolate evaluation

The parental soybean genotypes used for the development of mapping population were soybean rust-susceptible genotype (Wondersoya) from Nigeria and Uganda local SBR resistant genotype UG 5. The susceptible genotype Wondersoya as a female parent was crossed to the resistant genotype UG 5 to develop a mapping population consisting of 97 segregating F_2 (Wondersoya x UG 5) plants for SBR. The F_2 mapping population and the parental genotypes were grown in plastic pots and scored for SBR resistance. Three plants were maintained in each pot filled with soil from the field in order to have adequate plants for leaf sampling and phenotypic evaluation. The *P. pachyrhizi* pressure was readily available in the screen-house which had favorable conditions for the pathogen and was maintained on SBR susceptible soybean genotypes (Wondersoya and Nam-II). Therefore, the parental genotypes and the F_2 progenies were evaluated against *P. pachyrhizi* urediniospores in a screen-house under natural infestation. The data was recorded when the plants reached R6 reproductive stage (full-seed stage). Plants were evaluated for soybean rust reactions by examining disease severity (DS) based on a 1 to 5 scale (Miles et al., 2008) and lesion types. Reddish brown (RB) lesion types are associated with resistance while TAN lesions are indicators of susceptibility. Plants with DS score of 1 to 3 were considered resistant, while those with DS score of 4 or 5 were considered susceptible (Souza et al., 2014).

DNA extraction and marker analysis

Genomic DNA was extracted from young leaves of the parental genotypes and 97 individual F_2 plants using cetyltrimethyl ammonium bromide (CTAB) method (Lemos et al., 2011). The concentration of DNA samples was determined using a nano-drop spectrophotometer from the absorbance data of DNA sample at 260 nm. The purity of the DNA sample was determined by A260/A280 ratio (1.8 to 2.0 of pure DNA). The integrity of the extracted DNA was estimated on 0.8% agarose gel electrophoresis. Subsequently, DNA was diluted to a final concentration of 50 ng/µl for polymerase chain reaction (PCR). Out of the 97 F_2 leaf samples taken, the DNA of 86 samples was with good quality and was used for genotyping.

For the linkage analysis, a total of 122 SSR markers were chosen based on their distribution throughout the integrated molecular linkage map of soybean (Song et al., 2004) including those markers flanking the previously mapped *Rpp* genes and were

Table 1. Marker names, forward and reverse primer sequences of the polymorphic SSR markers.

Marker name	Primer sequence	
	Forward	**Reverse**
Sat_001	gcggatacgaccaaaaattgtt	gcgaactgcgaagatactaccc
Sat_064	tagctttataatgagtgtgatagat	gtatgcaagggattaattaag
Sat_112	tgtgacagtataccgacataata	ctacaaataacatgaaatataagaaata
Sat_263	gcggtcgatcgtttcaattagtatg	gcgctggcagccctttattatc
Sat_286	gcgttgcttgctaagtagtgtttttaatcct	gcgtctcccatcatgcaacttcaata
Sat_372	gcgtctcgaggtaattatctatttatctttt	gcgagtttggtaacatcgagtattgat
Satt100	Acctcattttggcataaa	ttggaaaacaagtaataataaca
Satt160	tcccacacagttttcatataatata	catcaaaagtttataacgtgtagat
Satt163	aatagcacgagaaaaggagaga	gtgtatgtgaaggggaaaaacta
Satt183	Taggtcccagaatttcattg	caccaaccagcacaaaa
Satt184	gcgctatgtagattatccaaattacgc	gccacttactgttactcat
Satt191	Cgcgatcatgtctctg	gggagttggtgttttcttgtg
Satt200	gcgataaatggttaatgtagataa	gcgaaaggacagatagaaagaga
Satt229	tggcagcacacctgctaagggaataaa	gcgaggtggtctaaaattattacctat
Satt245	Aacgggagtaggacattttatt	gcgcctcctgaatttcaaagaatgaaga
Satt264	Ccttttgacaattatggcatata	gcatagaagggcatcattcagat
Satt281	aagctccacatgcagttcaaaac	tgcatggcacgagaaagaagta
Satt288	gcggggtgatttagtgtttgacacct	gcgcttataattaagagcaaaagaag
Satt294	gcgggtcaaatgcaaattattttt	gcgctcagtgtgaaagttgtttctat
Satt308	gcgttaaggttggcagggtggaagtg	gcgcagctttatacaaaaatcaacaa
Satt309	Gcgccttcaaattggcgtctt	gcgccttaaataaaacccgaaact
Satt337	gcgtaaatctgatatatgttaccactga	ggccagatacccaagttgtacttgt
Satt346	ggagggaggaaagtgttgtgg	gcgcatgcttttcataagttt
Satt414	gcgtattcctagtcacatgctatttca	gcgtcataataatgcctagaacataaa
Satt440	Tgagaacgtttgaaaagagat	gaagagattaagcataaagaatactt
Satt442	Cctggacttgtttgctcatcaa	gcggttcaaggcttcaagtagtcac
Satt444	Tgcaaaaatacgggttcataat	agaggaagcgagactaatagaag
Satt460	gcgcgatgggctgttggtttttat	gcgcatacgatttggcatttttctattg
Satt522	Gcgaaactgcctaggttaaaa	ttaggcgaaatcaacaat
Satt597	Gctgcagcgtgtctgtagtat	cgaggcacaaccatcaccac
Satt632	gggctatgaagggaatggaaagga	cccatattgaagatttgaagtaat
Satt643	cgggataaatagaagtggaaca	ttggcaaatgtgaaatgtata
Satt649	ttactggccgtgtttacccgtgtaa	gcggacgttataagatttttttatcatg

used to analyze the polymorphisms between the parental genotypes. The SSR markers showing polymorphism between the two parents were used to genotype individual F_2 plants of the mapping population. Primer sequences of the SSR markers were obtained from SoyBase (http://soybase.agron.iastate.edu). The names and primer (forward and reverse) sequences of the polymorphic SSR markers used are shown in Table 1. The oligonucleotides used in this study were purchased from BiONEER C&D Center, South Korea.

PCR amplifications were performed in Thermo Cycler Block (96 universal gradient, Thermo Scientific®) in 10 µl final volume containing 5 µl premix (AccuPower® PCR Master Mix containing 100 mM dNTPs, 1.0 U *Taq* DNA polymerase; BiONEER C&D Center, South Korea), 0.25 µl of each primer (10 pM), 1 µl of template DNA (50 ng) and 3.5 µl of ddH_2O. The PCR thermo-cycler was programmed with an initial denaturation step at 95°C for 5 min (preheating) and 35 cycles each with 30 s DNA denaturation at 95°C, 30 s annealing at 55°C and 40 s extension at 72°C followed by a final extension step at 72°C for 5 min (to fill in the protruding ends of the newly formed PCR products) and a 4°C soak (for preservation till the products are taken out from the machine). The PCR products were finally separated on 3% (w/v) agarose gel for 2 h at 120 V in 1 X TAE buffer using a gel electrophoresis apparatus (Model V16.2 Gibco BRL, Gaithersburg, MD, USA). Gels were visualized under UV trans-illuminator (M-15 UVP Upland, CA 91786 USA) and photo-documented with a digital camera. DNA fragment sizes were determined based on a 100 bp DNA standard ladder (BiONEER C&D Center, South Korea) and marker alleles of SSRs were scored manually.

QTL mapping and statistical analysis

Chi-square (χ^2) analysis was used to test Goodness-of-fit between observed and expected segregation ratios of soybean rust phenotypes and genotypes of SSR markers in the F_2 population.

Table 2. Soybean rust lesion type and disease severity in Wondersoya x UG 5 population and their parents.

Genotype	Lesion type	No. of plants‡	DS†
Parent 1 - Wondersoya (Susceptible)	TAN	-	4.0
Parent 2 - UG 5 (Resistant)	RB	-	1.8
F_2 progenies	RB	70	2.3
	TAN	27	4.0

TAN: Tan lesions; RB: reddish brown lesions; DS: disease severity. †Mean disease severity score on a scale of 1 to 5: 1 = no visible lesions, 2 = light infection with few lesions present, 3 = light to moderate infection, 4 = moderate to severe infection, and 5 = prolific lesions. ‡The number indicates the sum of the number of homozygous RB and segregating lines.

Analysis of variance and regression analysis were used to test the significance of the association between SBR phenotype and flanking markers and to estimate how much phenotypic variation could be explained by flanking markers, respectively.

QTL IciMapping version 4.1 software (http://www.isbreeding.net) was used for linkage and QTL mapping. IciMapping uses inclusive composite interval mapping (ICIM), which is a modified algorithm of composite interval mapping (CIM) (Li et al., 2007). The SSR marker orders and distances were calculated using the MAP functionality in QTL IciMapping with a LOD score of 3.0 as a linkage threshold and a recombination frequency value of 0.30. The linkage map was constructed with genetic distances (cM) calculated using the Kosambi function (Kosambi, 1943) and linkage groups were named based on the chromosome information of the genomic sequence (http://soybase.agron.iastate.edu). Genomic regions significantly associated with disease severity were detected as QTLs using BIP functionality in QTL IciMapping with a significance logarithms of odds (LOD) threshold of 3.0. The estimated order of markers determined by the QTL IciMapping software was used for QTL analysis. The QTL positions for the disease severity were defined as the peaks of maximum LOD score.

RESULTS

Reaction of parental lines and progenies to soybean rust

Phenotypic evaluation of soybean parental lines showed variation in their reaction to SBR (Table 2). The resistant parental genotype, UG 5 produced typical RB lesions with a mean disease severity (DS) score of 1.8, while the susceptible parental genotype, Wondersoya produced TAN lesions with a mean DS score of 4.0. The DS score ranged from 1.2 to 2.8 in genotype UG 5 and 3.0 to 5.0 in genotype Wondersoya. The disease severity range for the F_2 progenies was 1.3 to 5.0 with a population mean of 2.8. The F_2 plants with RB lesions had a mean disease severity score of 2.3 ranging from 1.3 to 2.8, while those with TAN lesions had a higher mean severity score of 4.0 ranging from 2.9 to 5.0.

Segregation of the F_2 mapping population is shown in Table 3. The F_2 mapping population showed segregation of 69 plants with resistant phenotype and 28 plants with susceptible phenotype based on disease severity score. Moreover, based on the lesion types, 70 plants showed RB lesions and 27 plants showed TAN lesions among the 97 F_2 plants of the mapping population. A chi-squared test in both cases revealed that the observed segregation fitted well with the expected segregation ratio of a single dominant resistance gene, 3:1 (resistant: susceptible) in the F_2 generation.

Marker screening and segregation distortion

A total of 122 SSR markers were tested for polymorphism between the resistant and susceptible parental genotypes, out of which 33 SSRs were found to be polymorphic showing 27.05% of polymorphism. The distribution of the different genotypes among the F_2 populations is shown in Table 4. The majority of markers showed an excess of the heterozygote genotypes with the exception of four markers (Satt294, Satt444, Satt288 and Satt440), which showed an excess of homozygote alleles for susceptibility to soybean rust and five markers (Satt309, Satt100, Satt229, Satt442 and Sat_263), which showed an excess of homozygote alleles for soybean rust resistance. The Chi-squared test analysis for the polymorphic markers showed 42.4% segregation distortion which deviated significantly (critical $\chi^2 = 5.99$; d.f. = 2; $P \leq 0.05$) from the 1:2:1 Mendelian segregation ratio in the progeny mapping population.

Genotyping and linkage mapping analysis

Linkage analysis was performed using QTL IciMapping 4.1 software with 86 F_2 individuals and 33 polymorphic SSR loci. This resulted in the formation of eight linkage groups (LGs) comprising 25 SSR loci (Figure 1). The number of SSR markers in an individual chromosome or linkage group (LG) varied from two (LGs-K, B1, J and L) to six (LG-G). The remaining 8 SSR markers were found to be unlinked.

QTLs associated with resistance to soybean rust in UG 5

The putative QTLs associated with soybean rust resistance detected on genotype UG 5, their respective

Table 3. Segregation of F_2 population to soybean rust resistance and lesion type in UG 5.

Genotype	Total No. of plants	Observed No. of plants		Expected ratio	χ^2	P value
Disease severity						
Parent 1 (Wondersoya)	10	0(R)	10(S)	-	-	-
Parent 2 (UG 5)	10	10(R)	0(S)	-	-	-
Wondersoya x UG 5	97	69(R)	28(S)	3(R): 1(S)	0.77	0.379
Lesion type (LT)						
Parent 1 (Wondersoya)	10	0(RB)	10(TAN)	-	-	-
Parent 2 (UG 5)	10	10(RB)	0(TAN)	-	-	-
Wondersoya × UG 5	97	70(RB)	27(TAN)	3(R): 1(S)	0.42	0.519

χ^2 = Chi-squared value at 1 d.f. (Critical χ^2 = 3.84); R: Resistant; S: susceptible; RB: reddish brown lesion; TAN: tan lesions.

Table 4. Chi-squared analysis of 33 polymorphic SSR markers in the F_2 population.

Marker	[a]Progeny segregation	χ^2 (d.f.=2)	[b]P-value	Marker	Progeny segregation	χ^2 (d.f.=2)	P-value
Satt337	21:44:20	0.13	0.937	Sat_064	29:43:14	5.23	0.073
Satt643	21:42:23	0.14	0.933	Sat_001	27:32:27	5.63	0.060
Satt183	23:42:21	0.14	0.933	Sat_286	16:54:16	5.63	0.060
Satt632	24:43:19	0.58	0.748	Satt160	32:39:15	7.46	0.024
Satt649	23:38:25	1.26	0.534	Satt264	22:53:11	7.47	0.024
Satt597	26:38:22	1.53	0.464	Satt440	23:29:34	11.93	0.003
Satt245	17:43:26	1.88	0.390	Sat_112	16:59:11	12.49	0.002
Satt346	17:43:26	1.88	0.390	Sat_263	33:27:26	13.05	0.001
Satt522	22:37:27	2.26	0.324	Satt200	7:47:32	15.28	0.000
Satt308	28:39:19	2.63	0.269	Satt442	38:32:16	16.88	0.000
Satt191	27:45:14	4.12	0.128	Satt163	20:60:6	18.00	0.000
Satt414	29:42:15	4.60	0.100	Satt229	41:33:12	24.21	0.000
Satt281	25:33:28	4.86	0.088	Satt288	24:8:53	75.80	0.000
Satt460	30:40:16	4.98	0.083	Satt444	14:14:57	81.73	0.000
Satt184	29:33:24	5.23	0.073	Satt100	42:33:10	85.00	0.000
Sat_372	21:39:25	0.95	0.621	Satt309	53:6:27	86.00	0.000
-	-	-	-	Satt294	12:12:62	102.84	0.000

[a]Homozygous for 'UG 5' allele : Heterozygous : Homozygous for 'Wondersoya' allele; χ^2 = calculated Chi-squared value according to the expected Mendelian genotypic segregation ratio of 1:2:1 (Critical χ^2 = 5.99); d.f.: degree of freedom; [b]Significance level of 5%.

positions and effects are shown in Table 5. Three QTLs, explaining 63.13% of the total phenotypic variation in the population, were detected on three different linkage groups associated with SBR resistance by QTL IciMapping with a genome-wide LOD threshold of 3.0 (Table 5 and Figure 2). The QTL with the highest peak was located on chromosome 18 (LG-G) at a LOD score of 8.18 and accounted for 25.71% of the phenotypic variation in the population. This QTL was flanked by markers Sat_064 at a distance of 6.52 cM and Sat_372 at a distance of 30.31 cM with additive and dominance effects of -0.7336 and -0.5066, respectively. Two other QTLs were detected on chromosome 6 (LG-C2) and chromosome 9 (LG-K) at a LOD score of 3.47 and 7.36, respectively. The phenotypic variance explained by these two QTLs was 18.27 and 19.15%, respectively. The QTL on chromosome 6 was located at distance of 21.5 cM from Satt643 and 39.0 cM from Satt281 with additive and dominance effects of -0.626 and -0.2639, respectively. On chromosome 9, the QTL was flanked by SSR markers Satt264 and Satt337 at a distance of 4.0 and 3.99 cM, respectively, with additive effect of -0.4293 and dominance effect of -0.7738.

DISCUSSION

Host-plant resistance and/or tolerant is one of the best strategies for soybean improvement to soybean rust. Importance of introgression of resistance genes into

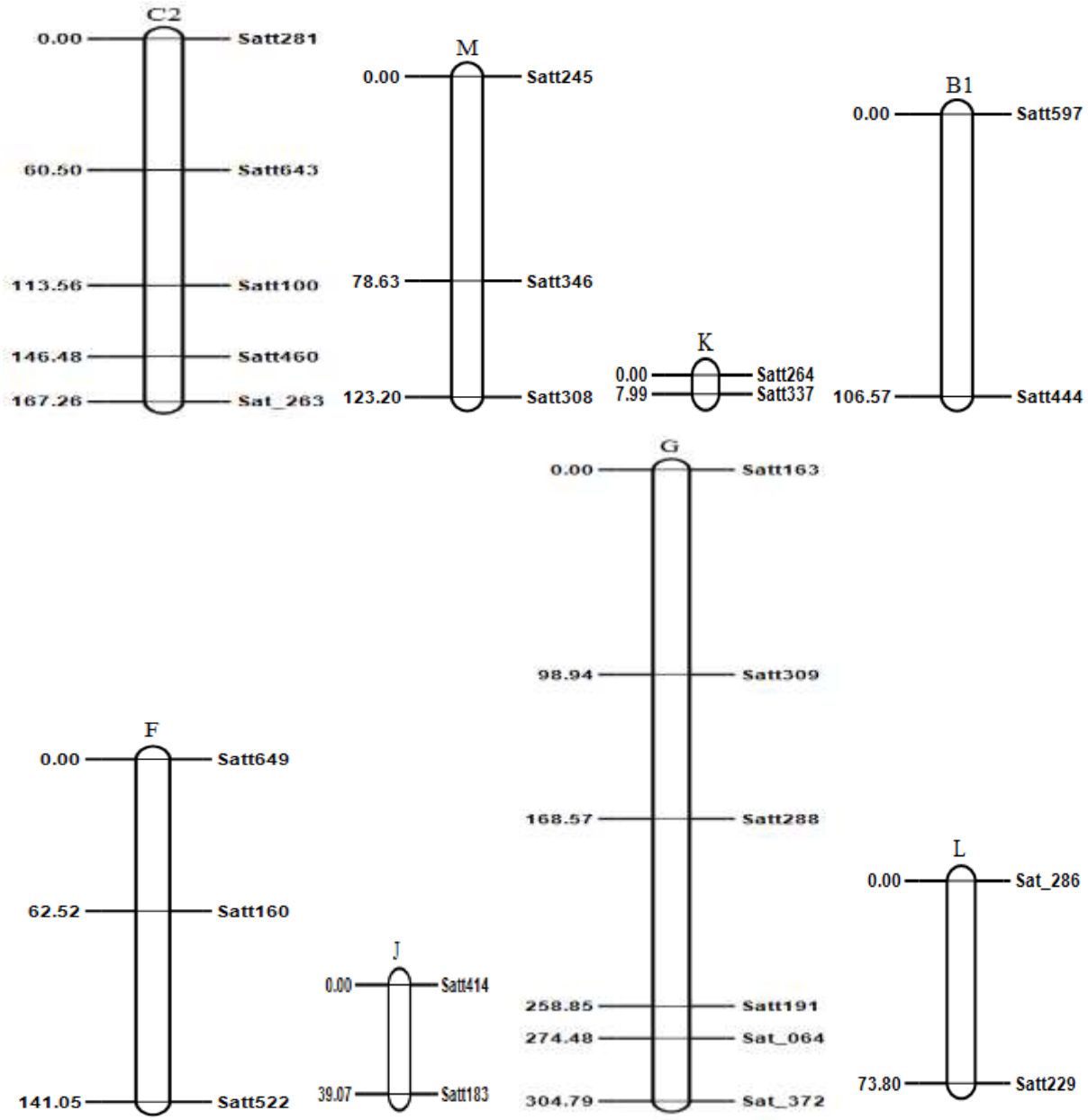

Figure 1. Linkage map of 25 SSR markers. C2, M, K, B1, F, J, G and L are the linkage groups formed; the numbers to the left-side of the map are positions of the SSR markers in cM.

soybean crops is increasing as fungicides lose efficacy due to adaptation of the pathogen as well as the concern for environmental pollution causing human health problems and increased production costs of chemicals. In many cases, pyramiding genes into elite cultivars is required for sustained resistance to soybean rust which requires identification and mapping of additional genes resistance to soybean rust.

In the current study, the skewed distribution towards the resistance parent for soybean rust severity score suggested dominance over susceptible parent. Rust-infected lines in majority of the F_2 plants of this study developed the type of RB lesion associated with resistance (Table 3). In previous genetic studies of resistance to soybean rust, dominant (*Rpp*), recessive (*rpp*), and incompletely dominant resistance genes have been reported in crosses with various sources of resistance (Li et al., 2012; Ray et al., 2011; Chakraborty et al., 2009; Calvo et al., 2008; Garcia et al., 2008; Monteros et al., 2007).

The Chi-squared (χ^2) test for disease severity scores and lesion type was 0.569 and 2.832; P = 0.451 and

Table 5. Summary of putative QTLs for the resistance character detected in UG 5.

QTL	Flanking marker1	Position (cM)	Flanking marker 2	Position (cM)	QTL position (cM)	LOD	Additive effect	Dominance effect	LG	PVE (%)
QTL1	Satt281	0.0	Satt643‡	60.5	39.0	3.47	-0.6260	-0.2639	C2	18.27
QTL2	Satt264	0.0	Satt337‡	7.99	4.0	7.36	-0.4293	-0.7738	K	19.15
QTL3	Sat_064‡	274.48	Sat_372	304.79	281.0	8.18	-0.7336	-0.5066	G	25.71

PVE: Phenotypic variation explained by each QTL; LG: linkage group; LOD: logarithms of odds; cM: centi-Morgan. ‡Nearest flanking marker.

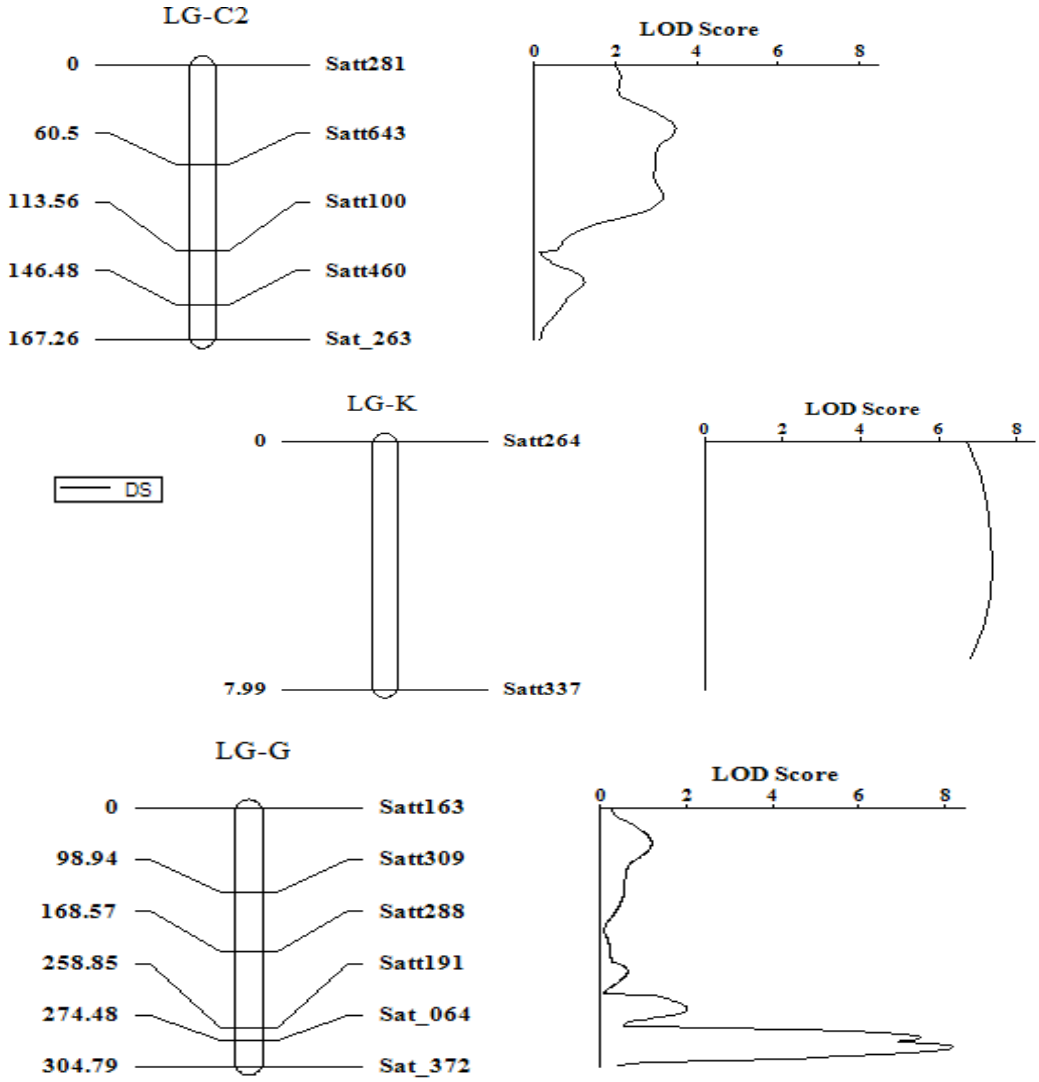

Figure 2. Genomic regions and SSR markers significantly associated with resistance to SBR detected using QTL IciMapping software in Wondersoya × UG 5.

0.092, respectively and suggested a single dominant resistant gene associated with resistance to soybean rust. This was reflected in the χ^2 value that fitted the Mendelian segregation ratio of 3 (Resistance):1 (Susceptible) (Table 3). However, the marker analysis indicated that UG 5 carries more than one putative soybean rust resistance loci (Table 5 and Figure 2). This difference could be likely due to the smaller size of the F_2 mapping population used in the study and the number of F_2 plants used to assess both phenotypic and genotypic evaluation. This observation calls for further studies with increased number of markers and mapping population to

confirm the number of genes associated with resistance to soybean rust in UG 5.

The inclusive composite interval mapping (QTL IciMapping 4.1) showed three QTLs in association with DS on three different LGs (Figure 2). The maps created from this population were in good agreement with the consensus map created by Song et al. (2004) regarding markers' order but differed with regard to the distances between each marker. Probably, the small size of the population used in this study could be the cause for this discrepancy.

The putative QTL with the highest peak (LOD = 8.18) and highest phenotypic variance which accounted for PVE = 25.71% in association with SBR resistance was mapped to the genomic location of *Rpp1-b* locus (Chakraborty et al., 2009) flanked by the same markers (Sat_064 and Sat_372). This could be the dominant QTL controlling resistance to soybean rust in genotype UG 5. This most probably indicated that UG 5 carries the same allele as PI594538A, the source of the original *Rpp1-b*, on this locus. Allelism tests, however, will be required to confirm whether this locus is identical with the *Rpp1-b* gene located on chromosome 18 or not.

The second putative QTL detected in association with SBR resistance (LOD = 7.36 and PVE = 19.15%; Table 5) on chromosome 9 (LG-K; Figure 2) could carry a novel *Rpp* gene as no other *Rpp* gene was previously reported on this chromosome. The third putative QTL, with a LOD score of 3.47 and PVE of 18.27%, detected in association with SBR resistance was located on chromosome 6 (LG-C2; Figure 2) where two dominant (*Rpp (Hyuuga)* and *Rpp3*) and one recessive (*rpp3*) genes were previously reported from three different sources of resistance (Ray et al., 2011; Hyten et al., 2009; Monteros et al., 2007). The SSR markers flanking the previously reported genes were included in this study, for which none of them was found to be linked to the current putative QTL suggesting that this putative QTL in UG 5 could be a different allele as compared to the previously reported *Rpp* genes. To verify this, the relationship between UG 5 and the PIs containing the known *Rpp* genes will require allelism tests. The high phenotypic variance and negative effects (additive and dominance) of the QTLs indicated their involvement in resistance to SBR. The negative values for the additive and dominance effects of the QTLs (Table 5) were also evidences that both additive and dominance effects are important in the inheritance of resistance to SBR (Bassi et al., 2017).

UG 5 was found to be resistant to different isolates of *P. pachyrhizi* in different countries (Maphosa et al., 2013; Twizeyimana and Hartman, 2012; Twizeyimana et al., 2009; Kawuki et al., 2003). For instance, the genotype expressed an RB reaction when inoculated with field isolates from Nigeria and Uganda (Hailay et al., 2018; Maphosa et al., 2013; Twizeyimana et al., 2009), whereas, it showed an immune (no visible reaction) for 72 *P. pachyrhizi* isolates in USA as compared to the other six soybean genotypes with the known resistance genes (*Rpp1*, *Rpp2*, *Rpp3*, *Rpp-Hyuuga*, *Rpp4* and *Rpp5* (Twizeyimana and Hartman, 2012). The resistance of UG 5 to diverse isolates of SBR across wider agro-ecologies could, therefore, be due to the presence of more than one SBR resistance gene.

CONCLUSION AND RECOMMENDATION

This research provides evidence for the presence of three putative loci on chromosomes 6, 9 and 18 for soybean rust resistance in genotype UG 5. The QTL on chromosome 9 was novel for which no soybean rust resistance genes were previously reported. The putative QTLs identified in this study will help to facilitate SBR resistance breeding toward a more efficient marker-assisted selection approach and gene pyramiding leading to the development of durable resistance. The identified loci on this genotype need to be further screened on larger population size and increased number of markers from each linkage group to precisely locate and identify the putative genes. The structural and functional roles of the putative genes need to be determined.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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