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Full Length Research Paper

Marker-assisted backcrossing for associating nutritional quality to drought tolerance in barley

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Backcross breeding enhanced by marker assisted selection is currently the most powerful method used to transfer one or a few genes controlling specific traits from one line into another. This method was used to cross naked barley variety, "Tombari" known as A, having high nutritional quality with hulled variety, "Giza131" called B, highly tolerant to drought. The objective of this work is to transfer the hulless variety into the drought tolerant variety. Five generations were realized. In each one, the best performing lines were chosen based on their agronomic, nutritional and molecular features. In the last generation, five remarkable barley lines were selected (H32, H33, H34, H36 and H47). In order to highlight the allele linked to the nudity trait, molecular profile of sKT7 marker, which is tightly linked to the "nud" locus, revealed two specific alleles (I and II). The first one (51 bp) was found in the two parental lines and their progenies. However, the second one (67 bp) was found only in the naked lines (parent A and progenies). This specific allele found only in the naked barley is different from that found by other researchers in Asia and North Europe. This means that naked barley has undergone multiple mutations. Some of the selected lines subjected to physiological stress induced by PEG-6000 showed greater tolerance to stress than their presumed tolerant parents.

Key words: Barley, naked, backcross, nutritional quality, drought tolerance.

INTRODUCTION

Varietal selection has been in existence for a longtime where plants are selected according to some desirable traits with the best varieties or genotypes being crossed (Joshi and Witcombe, 1996). This selection has led to the diversification and creation of many improved varieties. To accomplish this objective, several breeding techniques were used differing in terms of efficiency and duration. The back cross method is one of these techniques. It is the most commonly used method to insert a trait into an elite cultivar (Bishwas et al., 2016). The variety that donates a gene is called "donor parent" while that which receives a gene is called "recipient parent". The two

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selected parents are crossed and hybrid is successively backcrossed to the recurrent parent. We introduce some interesting characteristics into the recurrent parent to improve one or two specific defects in it.

Backcrossing is used by many breeders for upgrading some inadequate traits within an existing elite cultivar. The characteristic could be a trait, a gene or even an anonymous locus or chromosome segment (Hospital, 2005). In each generation, progeny was chosen for the traits of interest and then backcrossed to the recurrent parent. In this scheme, the proportion of genome from the donor parent is reduced except for the part hosting the characteristic of interest. Usually, repeated backcrossing is mainly used to improve qualitatively inherited traits such as disease resistance, insect resistance and color, since the presence of target trait genes must be confirmed phenotypically at the individual level. Selection based on phenotypic level must clearly discriminate between segregating progeny. It has been used by breeders to distinguish between the recurrent parent and offspring (Yé et al., 2009; Hasan et al., 2015). Selection based on molecular marker alleles is mainly used when the effects of target alleles are difficult or expensive to measure phenotypically, and/or do not have a consistent phenotypic expression under certain specific selection conditions (Yé et al., 2009).

Several countries, particularly those in the North Africa, have their food ration based on barley. It is a species that adapts well to arid conditions, is rich in nutrients and some of its varieties are also rich in ß-glucan and proteins (Lahouar et al., 2017). Among the available barley varieties, some of them show great tolerance to drought. The use of these tolerant varieties in the production system would increase yield and yield stability in harsh environments while maintaining the same quality of harvested products. The combination of nutritional quality and drought tolerance represents one of the most complex challenges in the field of plant breeding. In recent years, incontestable research efforts have been made in genetics to identify the genes involved in nutritional quality (gene nud) and to understand the genetic determinism of tolerance to environmental stresses.

In this frame, the main objective of this work is to create new barley lines with tolerance to drought and having nutritional quality summarized in the traits coded by the gene (nud). In a previous work, we characterized sixteen North African barley genotypes based on their nutritional quality and drought tolerance levels. The quality characters are summed up in grain color, β-glucan and protein contents. Drought tolerance is based on yield traits under water deficit and some indices of drought tolerance. The results have led to the selection of a Tunisian naked genotype, with good nutritional quality (yellow grain color, high protein and ß-glucan content) called "Tombari" and an Egyptian drought tolerant genotype called "Giza131" (Ben Naceur et al., 2018, in press). Since the introgression of gene of interest is useful for genetic improvement in breeding programs, we have used these two genotypes in a scheme of back crossing process where “Tombari” genotype was used as donor parent and “Giza 131” as recurrent parent.

MATERIALS AND METHODS

Plant material

Two barley genotypes (*Hordium vulgare* L.), "Tombari and G131" , with contrasting characters were crossed at the National Agronomic Research Institute of Tunisia. «Tombari or parent A» is a 6-row Tunisian landrace collected in 2000 from the South Tunisian region. It is a naked grain genotype, matures late with a prostrate habit, susceptible to disease and is characterized by an exceptional high nutritional quality. «Giza 131 or parent B» is a 6-row improved Egyptian genotype. It is vigorous with erected habit, matures half-early, is drought tolerant, resists fungal diseases and highly productive under favorable conditions.

Molecular analysis

The resulting F1 progeny (Tombari x G131) was controlled with molecular markers to check the crossing result. Then some of them were crossed with the recurrent parent (G131) to produce F2 or the first backcross generation (BC1F1). After molecular screening, the third (F3) generation was obtained by self-pollination to generate the BC1F2, on which we evaluate the agronomical traits and nutritional quality. The selected BC1F2 progenies based on agronomical and nutritional quality were crossed with the recurrent parent to produce the BC2F1 (F4 generation) which was self-pollinated to obtain BC2F2 (F5 generation). Selected plants are expected to be very similar to the recurrent parent and also possess the target traits (nutritional quality, precocity to escape terminal drought and tolerance to drought). Backcross progenies with the target traits were also selected based on molecular markers tightly linked to the genes controlling these traits (Table 1).

sKT7 is a dominant Sequence Characterized Amplified Region (SCAR) marker associated with the “nud” allele of the naked barley parent called “Kobinkatagi” (Taketa et al., 2004; Gustafsson, 2013). EBmac624 is a microsatellite region located close to the centromere of the chromosome 6H associated to the $HvCO_2$ CO-like genes promoting flowering for barley (Wang et al., 2010).

Total DNA was extracted from young leaves of a single plant per genotype as described in Chaabane et al., (2012). The PCR reactions were carried out in a 25 µl reaction volume containing 18.8 µl ddH_2O, 2 µl Taq Buffer 10x (containing $MgCl_2$, 1.5 mM final concentration), 0.4 µl dNTP (10 mM), 0.8 µl forward primer (10 µM), 0.8 µl reverse primer (10 µM), 0.2 µl Taq polymerase (5 U/µl) and 2 µl template DNA (50 ng/µl). Amplifications were performed in a DNA thermocycler programmed for one cycle of 95°C for 2 min and 35 consecutive cycles of 1 min denaturing at 94°C, 1 min annealing at 55°C and 1 min elongation at 72°C followed by 2 min of post-elongation at 72°C. Amplified PCR products were separated by the “Experion” automated electrophoresis system (Bio-Rad). A 100-bp DNA ladder (Promega) was used as the molecular size standard.

Morphological and nutritional assessment

After molecular analysis, the plants having naked grain (“nud” marker allele) were evaluated for some morphological traits (main

Table 1. Primers used in the study.

Primer	Sequence	Ta (°C)	Associated allele
EBmac624	F: AAAAGCATTCAACTTCATAAGA R: CAACGCCATCACGTAATA	55	"$HvCO_2$" CO-like genes located on 6H chromosome promoting flowering (Wang et al., 2010)
sKT7	F: CGATATGCTTGTCCGTGATG R: TGCTCGTACTGCATCGACTC	55	"nud" allele carrying traits of quality (Taketa et al., 2004; Gustafsson, 2013; Wirkijowska et al., 2016)

stem length, number of grains/spike, spike weight, 1000-kernel weight and grains yield) and nutritional quality (grain color, protein content and β-glucan content).

Root length assessment in stressful media

For each retained hybrid line and parent, 20 grains of barley were placed in 90-mm diameter Petri dishes lined with filter paper containing either distilled water (Control) or a polyethylene glycol solution (PEG-6000) at a concentration of 10% (Stress). Each treatment (stressed or none stressed) is repeated 4 times. The germination is carried out in a germination room (temperature: 25°C day/18°C night and 12 h light). After the incubation of the Petri dishes in the germination room for seven days, we measured the following parameters:

(i) The root length in the control and stressed conditions.
(ii) The stress tolerance index (STI) based on root length as defined by Fernandez (1992):

$$STI = [(Yp) \times (Ys) / \bar{Y}p^2]$$

Where, Ys and Yp are the root length, respectively under stressed and non-stressed conditions, $\bar{Y}p$ is the mean length of all retained lines including the parental ones under favorable conditions.

RESULTS AND DISCUSSION

Assessment of BC1 progeny (BC1F1)

Molecular analysis

To reduce the large number of donor chromosome, a minimum of six backcross generations is needed in the conventional backcrossing method. However, marker-assisted backcrossing may need two or three backcross generations only.

The crossing between A (Tombari) and B (Giza131) generated grains called F1. These grains were sown to verify if they were truly genetically different from the parental lines (A and B). Based on molecular test, only the F1 hybrid plants having both the marker alleles of the donor parent and recurrent parent were selected and backcrossed with the recurrent parent (Giza 131) to generate the first backcross generation (BC1F1). The molecular characterization by means of EBmac624 showed, in most cases, a new profile different from the parental lines (A or B). This means that the backcrossing between F1 and recurrent parent was successful. After elimination of the inbred lines, the rest of the offspring having the marker allele of the "nud" gene were labeled as H1, H2, H3, and H25. The barley seeds obtained during the BC1F1 were sown (auto crossed) to obtain BC1F2 (or F3 generation) for evaluation based on morphological and nutritional features.

Agro-morphological analysis

The BC1F1 progenies were sown for evaluation based on morphological and protein features and for implementing the second back-cross (BC2). At maturity stage, the main stems of each individual plant were measured along with the number of grain/spike, spike weight and 1000-kernel weight. Variance analysis of these parameters showed significant difference between the different progenies. The Newman-Keuls test allowed us to select the best hybrids (Table 2).

The hybrid plants having the longest stems (H3; H4 and H18), those having the highest main spike weight (H3; H8; H9 and H18), highest grain number/spike (H3; H4; H8; H9 and H18) and highest 1000-kernel weight (H3; H4; H8; H10; H16; H18; H22; H23) were selected. Among the analyzed hybrids, H3, H4, H8 and H18 seemed to be the most interesting plants.

Nutritional quality analysis

Protein analysis showed that H3, H4, H8, H9, H12, H17, H18 and H24 hybrids had the highest protein content (Table 2). The combination of those having the highest protein content and those having the best agronomic traits (H3, H4 H8 and H8 progenies) were selected and backcrossed with the recurrent parent (Giza 131) to obtain the BC2F1 progeny, which was auto crossed to obtain the BC2F2. The β-glucan content was not determined in this work, because we have shown in a previous work that the richness in β-glucan content correlated with the naked caryopsis (Ben Naceur, 2013). Therefore, from the beginning we have selected only the naked lines, and sure about their high β-glucan content.

Table 2. Agronomical traits and protein content of the BC1F2 progeny.

Progeny	Plant height	Grain number/spike	Spike weight	1000-kernel weight	Protein content
H1	56^{G}	25.23 ^{J}	0.90 ^{K}	35.67 ^{DE}	10.10^{D}
H2	76.6 ^{CDEF}	25.25 ^{J}	0.90 ^{K}	35.64 ^{DE}	11.48^{CD}
H3	93.2^{A}	46.85 ^{AB}	2.26 ^{B}	48.24 ^{C}	13.53^{B}
H4	92.7^{A}	46.10 ^{B}	2.07 ^{BCDE}	44.90 ^{D}	13.58^{B}
H5	77.4^{CDEF}	37.00 ^{FGH}	1.79 ^{EFG}	48.38 ^{C}	11.09^{CD}
H6	88.7^{AB}	37.95 ^{DEFG}	1.46 ^{HI}	38.47 ^{DE}	10.05^{DE}
H7	88.7^{AB}	39.15 ^{CDEFG}	1.44 ^{HI}	36.78 ^{DE}	10.82^{CD}
H8	88.7^{AB}	43.30 ^{BC}	2.19 ^{BC}	50.58 ^{BC}	13.29^{BC}
H9	82. ^{BCD}	42.40 ^{BCDE}	2.17 ^{BC}	51.18 ^{BC}	13.62^{B}
H10	76.3^{CDEF}	37.40 ^{FGH}	1.95 ^{CDEFG}	52.14 ^{BC}	11.88^{C}
H11	74.7^{F}	40.50 C^{DEF}	1.80 ^{EFG}	44.44 ^{D}	9.04^{DE}
H12	92.3^{A}	29.75 ^{IJ}	1.24 ^{IJ}	41.68 ^{D}	13.16^{BC}
H13	75.5^{EF}	42.70 ^{BCD}	1.84 ^{DEFG}	43.09 ^{D}	12.35^{BC}
H14	78^{CDEF}	32.50 ^{HI}	1.12 ^{JK}	34.46 ^{DE}	11.80^{CD}
H15	78^{CDEF}	32.50 ^{HI}	1.12 ^{JK}	34.46 ^{DE}	11.80^{Cd}
H16	82.6^{BCD}	29.80 ^{IJ}	2.04 ^{BCDEF}	68.46 ^{A}	11.53^{CD}
H17	94.8 ^{A}	40.60 ^{CDEF}	1.76 ^{FG}	43.35 ^{D}	13.12^{BC}
H18	76.2^{DEF}	41.05 ^{CDEF}	2.84 ^{A}	69.18 ^{A}	13.50^{BC}
H19	89.7A	51.40 ^{A}	1.84 ^{DEFG}	35.80 ^{DE}	12.37^{BC}
H20	77.5^{CDEF}	36.40 ^{FGH}	1.68 ^{GH}	46.15 ^{C}	11.11^{CD}
H21	81.3^{CDE}	37.55 ^{EFG}	2.12 ^{BCD}	56.46^{B}	11.46^{CD}
H22	82^{CD}	40.25 ^{CDEF}	2.06 ^{BCDE}	51.18 ^{BC}	11.99^{C}
H23	75.2^{EF}	40.80 ^{CDEF}	1.75 ^{FG}	42.89 ^{D}	12.63^{BC}
H24	73.3 ^{F}	34.55 ^{GHI}	0.92 ^{K}	26.63 ^{g}	15.13^{A}
H25	56 ^{G}	32.50 ^{HI}	1.09 ^{JK}	33.54 ^{DEF}	11.11^{CD}

Assessment of BC2F2 progeny

Agro-morphological characterization

The BC2F2 seeds were sown and labeled as H26, H27, H28, H50. The morphological characterization is reported in Table 3. Analysis of variance revealed significant differences for all characters among the different BC2F2 progenies. The Newman-Keuls classification showed different separate classes for each characteristic. The class of longest plant height was composed of H30, H32, H33, H34, H36, H37, H38, H41, H43, H47 and H49, among which H32, H33, H34, H36 and H47 were the tallest. The class of highest number of grains/spike was composed of H32, H33, H34, H36 and H47. The class of highest spike weight was composed of H32, H34, H36, H47 and H48. Finally, the 1000-kernel weight showed the same trend as that of the spike weight and pointed out H32, H33, H34, H35, H36, H40, H42, H43, H45, H47 and H48 as the most important progenies, occupying the first class. Based on these characteristics, H32, H33, H34, H36 and H47 were the most interesting hybrids.

Nutritional quality analysis (protein content)

Analysis of variance revealed significant differences ($p<0.05$) for protein content (Kjeldahl method) among the different BC2F2 progenies. The highest protein content was determined in H47; H32; H34; H36; H41 and H44 (Figure 1). In this group, the protein content varied between 13.87% (H44) and 15.11% (H47). The lowest content varied between 11% (H45) and 11.42% (H26). Such difference could be attributed to the interaction between the genetic background of the hybrid plants and the environment. The interaction (genotype x environment) affected barley protein content and was highly dependent on the cultivar as reported by several authors (Qi et al., 2006; Arendt and Zannini, 2013). Genotype–environment interactions for protein content in barley may also involve pleiotropic or linkage interactions with genes responsible for variation in these traits

Table 3. Morphological characteristics of the BC2F2 progeny (F5 generation).

Progeny	Plant height	Grain number/spike	Spike weight	1000-kernel weight
H25	63.75^{CDEFGHIJ}	27.50^{G}	1.12^{HI}	40.73^{CDE}
H26	63.72^{CDEFGHIJ}	27.40^{G}	1.11^{HI}	40.51^{CDE}
H27	57.25^{J}	36.50^{ABCDEFG}	1.48^{DEFGHI}	40.68^{CDE}
H28	60.75^{GHIJ}	36.50^{ABCDEFG}	1.56C^{DEFGH}	42.37^{BCD}
H29	63.75^{CDEFGHIJ}	32.67^{CDEFG}	1.27^{FGHI}	38.99^{CDE}
H30	69.75^{ABCDEF}	40.00^{ABCD}	1.69BC^{DEF}	42.17^{BCD}
H31	62.50^{DEFGHIJ}	38.50^{ABCDE}	1.60C^{DEFG}	41.56^{BCD}
H32	70.00^{ABCDE}	42.17^{AB}	1.80^{ABCD}	42.65^{BCD}
H33	70.50^{ABCD}	40.50^{ABC}	1.74^{ABCDE}	43.04^{ABC}
H34	71.45^{ABC}	41.67^{AB}	1.94^{ABC}	46.68^{AB}
H35	63.50^{CDEFGHIJ}	31.33^{DEFG}	1.37^{EFGHI}	43.72^{ABC}
H36	73.25^{AB}	45.33^{A}	2.12^{A}	46.80^{AB}
H37	67.75^{ABCDEFG}	37.17^{ABCDEF}	1.46^{DEFGHI}	39.28^{CDE}
H38	67.75^{ABCDEFG}	37.17^{ABCDEF}	1.46D^{EFGHI}	39.28^{CDE}
H39	64.75^{BCDEFGHIJ}	34.50B^{CDEFG}	1.42^{DEFGHI}	41.25^{BCD}
H40	56.25^{J}	36.33B^{CDEFG}	1.55C^{DEFGH}	42.70^{ABC}
H41	68.50^{ABCDEFG}	28.83^{FG}	1.08^{I}	37.34^{DE}
H42	58.50^{HIJ}	34.33^{BCDEFG}	1.63^{CDEFG}	47.43^{A}
H43	66.75^{ABCDEFGH}	34.50^{BCDEFG}	1.46D^{EFGHI}	42.46^{ABC}
H44	61.25^{FGHIJ}	33.33B^{CDEFG}	1.37^{DEFGHI}	41.20^{BCD}
H45	58.00^{IJ}	31.00^{EFG}	1.42D^{EFGHI}	45.81^{AB}
H46	66.00B^{CDEFGHI}	27.67^{G}	1.13H^{I}	40.72^{CDE}
H47	74.00^{A}	42.50^{AB}	2.08^{AB}	48.94^{A}
H48	61.50^{EFGHIJ}	39.67A^{BCDE}	1.77AB^{CDE}	44.58^{ABC}
H49	68.25^{ABCDEFG}	31.17^{DEFG}	1.25^{GHI}	40.21^{CDE}
H50	63.00^{CDEFGHIJ}	35.33^{BCDEFG}	1.39^{DEFGHI}	39.34^{CDE}

(Emebiri et al., 2005). Based on these two characterizations, the plants with the best agronomic and nutritional performance were H32, H33, H34, H36 and H47.

Molecular analysis

Electrophoresis profiles showing results of PCR products generated by EBmac624 primer are illustrated in Figure 2. Each peak materializes the band obtained during the separation of PCR products. Although some of them seem to be similar, all profiles are almost different from each other. Table 4 representing the band size confirms the difference among them. Marker assisted introgression is not always what can be guessed. Some crossing over might occur to generate new profiles different from the expected ones. According to Hospital (2005), many experiments have reported that genomic profiles of the produced progenies are relatively close to what is expected but not exactly identical to what is really obtained. In this case, the progenies carrying 151±2pb band are those having incorporated the genetic background of the recurrent parent (Giza131).

The result showed some progenies contain 131 bp bands (H26; H27; H28; H29; H30; H32; H33; H34; H35; H36; H39; H40; H41). Some others contain 151 bp bands (H26; H28; H29; H30; H32; H33; H34; H35; H36; H41; H43; H44; H45and H47) and some others contain either 131 or 151±2 bp band or both at the same time. Those containing the two bands are H26; H28; H29; H30; H32; H33; H34; H35; H36 and H41. According to this study, the progenies gathering the best agronomic performance and nutritional quality (naked), in addition to the genetic background of the parent B, are H32, H33, H34, H36 and H47 which would be evaluated as promoting lines or used in a third back-cross.

Confirmation of the "nud" gene presence in the selected hybrids

sKT is a sequence-characterized amplified region (SCAR) marker. It is the result of cross between two barley varieties: Kobinkatagi (naked barley) and Triumph (hulled barley). This marker co-segregates with the locus

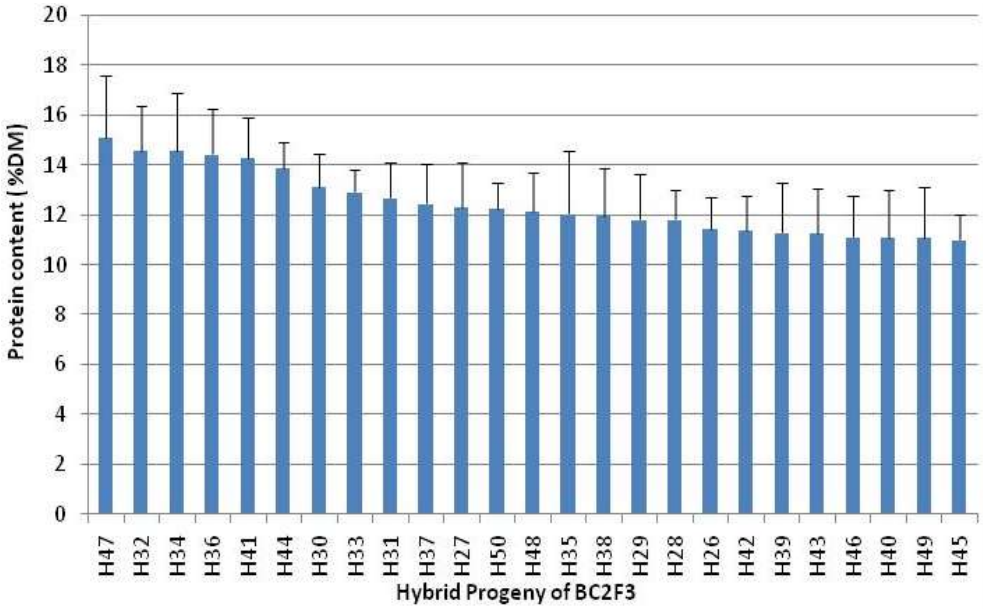

Figure 1. Protein content variation in the BC2F2 progeny.

"nud" (Taketa et al., 2004). The PCR conducted on the hybrid DNA (H32, H33, H34, H36 and H47) and their parents (A and B) using the sKT7 primer generated the following profile (Figure 3) and the molecular weights associated with it (Table 5). The PCR product (Table 5) revealed two alleles: I and II. A 50bp allele was found in both parents and hybrids (except for H34) and a second allele of 67 bp was found only in naked barley (parent A and the naked progenies H32, H33, H34, H36 and H47). This allele (67 bp) could be associated with the “nud” gene in North African barley.

Taketa et al. (2004) worked on several accessions of Asiatic barley (wild, domesticated hulled and naked), using the same sKT7 primer; they identified a band of 470bp in their naked varieties. They concluded that the naked barley originated from a single mutation that occurred on the hulled barley in the Asian Region. In our opinion, the Taketa’s allele (470 bp) cannot be used as an indicator of all naked barley in the world. It would be specific for Asian barley only.

Furthermore, another study conducted by Saisho and Purugganan (2007) on naked barley from eastern and western Asia identified two closely related loci with different allelic frequencies. This suggests two independent origins of naked barley and contradicting the hypothesis of Taketa al. (2004) for which barley is controlled by a single locus, meaning a single origin. Archeological remains of naked barley have been found in the Nordic European countries (Finland), suggesting independent Finnish origin in naked barley. More recent work conducted by Gustafsson (2013) on Nordic barley (Northern Europe) using the same primer (sKT7) showed different profiles from what Taketa et al. (2004) found. This means that Nordic naked barley did not carry the same mutation reported by Taketa and suggests that naked barley is the result of several mutations occurring independently in Asia, North Europe and North Africa.

Effect of physiological stress induced by PEG-6000 on seedling growth

The ability of the plants to extend their root length in stressful conditions is a reliable indicator of the their tolerance to water deficit. This allows them to absorb water and nutrients in deep layers. That way we used the root elongation in stressful conditions to assess the selected barley lines’ tolerance to drought. Table 6 reveals the variability in root length both under favorable and stressful conditions induced by PEG-6000. Variance analysis of this parameter showed a significant difference ($p<0.05$) in both cases of water regime, indicating that the selected lines reacted differently to physiological stress. Under favorable conditions, the ANOVA and the Newman-Keuls classification showed 4 significantly different groups of which H36, H34 and H30 occupied the first group while H32 occupied the last group with an average varying between 3.2 and 7.7 cm. This result, which related to the genetic variability of the barley lines, is in agreement with those observed by Soltani et al. (2006) on wheat.

Under stress induced by PEG-6000, root length (RL) is greatly affected, but the response intensity and harmful effects of such stress depend on the genetic background of the lines. The highest average (RL) varied between 3.85 and 4.64 cm and was observed for the first group

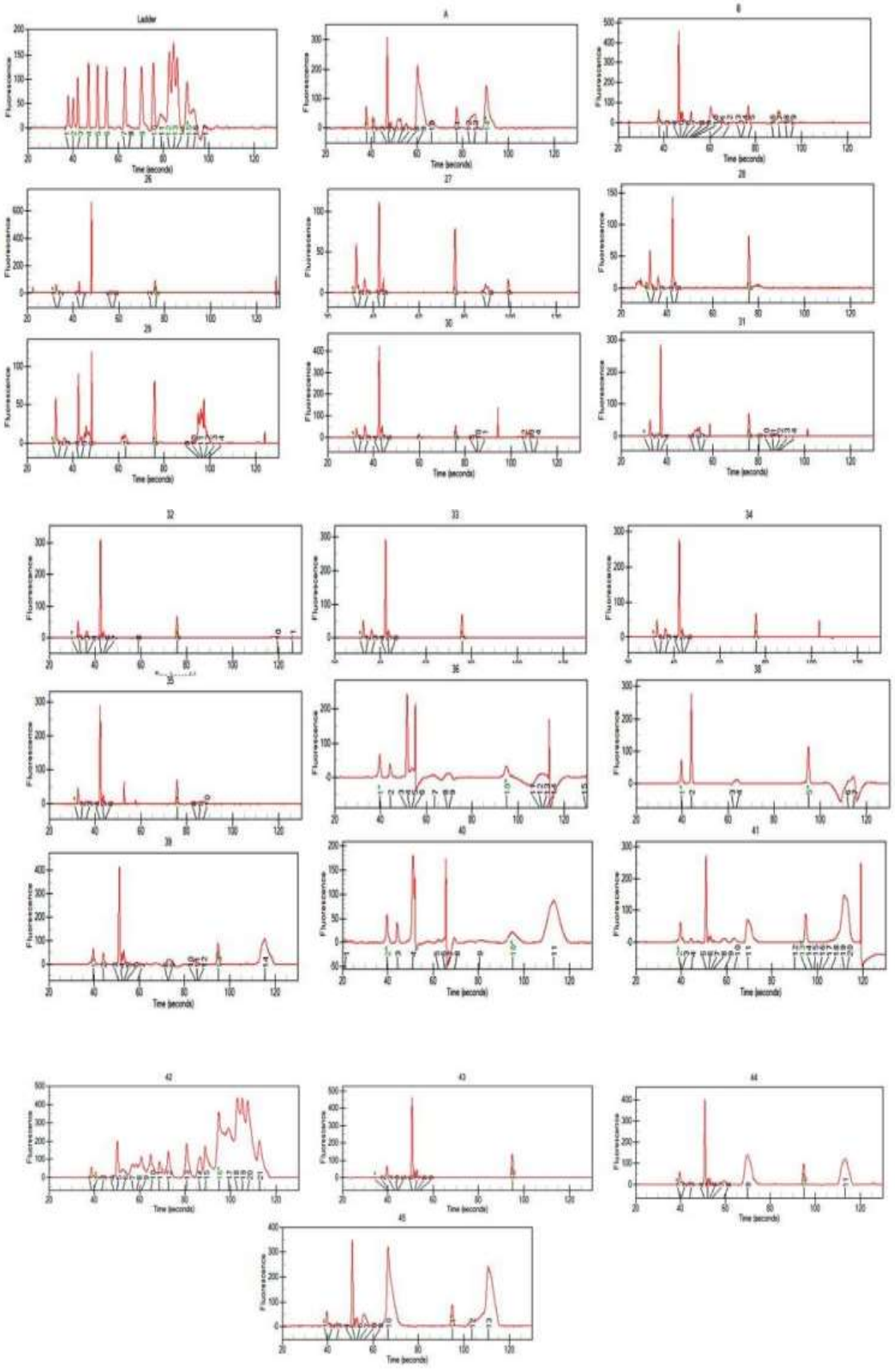

Figure 2. Electrophoretic profiles of the PCR products of BC2F4 progenies generated using EBmac624 primer.

Table 4. Size of bands obtained by the primer EBmac 624 during the backCross-2 (BC-2).

Parent A	Parent B	Progenies									
		H26 (pb)	H27 (pb)	H28 (pb)	H29 (pb)	H30 (pb)	H31 (pb)	H32 (pb)	H33 (pb)	H34 (pb)	H35 (pb)
39	38	19	20	20	21	21	21	21	22	22	22
102	106	50	50	50	50	48	50	50	51	50	50
113	115	**135**	**133**	**132**	**131**	132	270	130	130	130	129
121	126	153	160	151	151	151	**309**	150	149	149	149
162	153	344			**198**	160	**319**	160	160	160	159
175	162	**358**			471	**398**		**378**	175		
207	174										
268	269										
345	335										
553	457										

Parent A	Parent B	H36 (pb)	H38 (pb)	H39 (pb)	H40 (pb)	H41 (pb)	H42 (pb)	H43 (pb)	H44 (pb)	H45 (pb)	H46 (pb)	H47 (pb)
39	38	52	50	51	52	19	41	19	19	19	22	19
102	106	137	271	131	131	129	100	126	128	128	163	134
113	115	153	285	146	271	145	119	142	143	143		153
121	126	163		158	304	156	148	153	155	155		
162	153	178		171	309	169	206	167	169	169		
175	162	285		228	353	228	223		233	193		
207	174	354		400	358	281	250		363	244		
268	269	365		405		356	300			322		
345	335			673			349					
553	457						398					

Legend: common bands typical bands for parent A typical bands for parent B or typical band for hybrids.

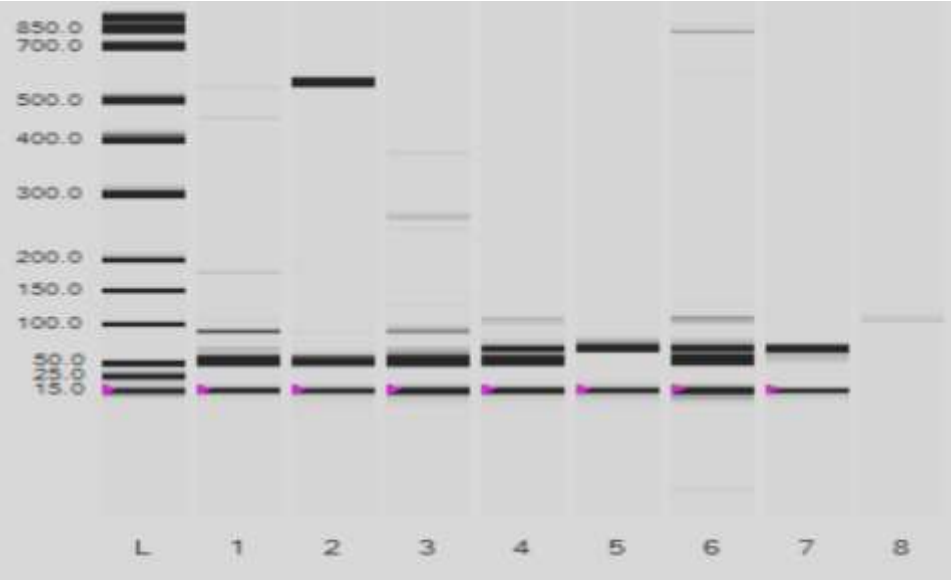

Figure 3. Electrophoretic profiles of parental lines and their selected progeny obtained by sKT7 primer.L= 1kb marker; Well 1: parent A; Well 2: parent B; Wll 3: H32; Well 4: H33; Well 5: H34; Well 6: H36; Well 7: H46; Well 8: H47.

Table 5. Molecular weight (pb) of bands obtained by sKT7 primer on parental and their selected progeny lines.

Marker	A (Naked)	B (Hulled)	H32 (Naked)	H33 (Naked)	H34 (Naked)	H36 (Naked)	H47 (Naked)
50	50	51	51	51		52	51
100	67		66	67	67	68	67
150	90		90	109		110	105
200	179					829	
300							

Table 6. Root length of selected hybrid lines and their parents, under physiological stress induced by PEG-6000.

Root length of hybrid lines and their parents under favorable conditions		Root length of hybrid lines and their parents under stress induced by PEG-6000 at 10%	
Genotypes	**Means**	**Genotypes**	**Means**
H36	7.70^{A}	H36	4.64^{A}
H34	7.03^{A}	H47	4.42^{AB}
V30	6.73^{AB}	H34	4.23^{AB}
V10	5.64^{BC}	H32	4.20^{AB}
H33	5.53^{BC}	H33	3.85^{AB}
H47	5.06^{C}	V30	3.56^{B}
H32	3.20D	V10	1.87^{C}

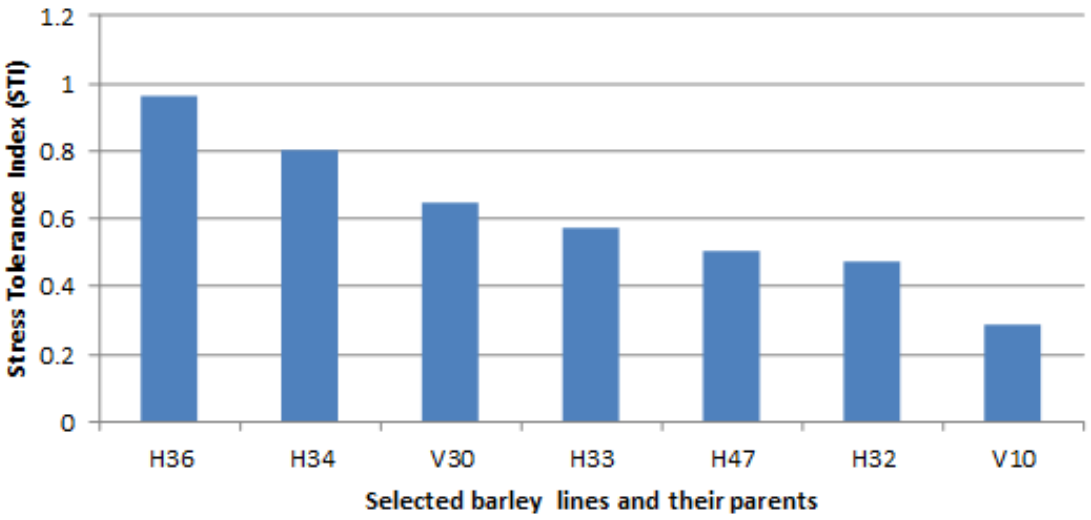

Figure 4. Variation of the (STI) according to the selected barley lines, based on root length.

including H36, H47, H34, H32 and H33. On the other hand, the lowest (RL) was found at V10 line (1.82 cm). The ability of the first group, especially H36 line to develop their roots under water deficit conditions suggests the induction of certain genes involved in root elongation by stress or the structure modification allowing roots to sustain their development, in accordance with what was reported by Badiow et al. (2004).

The stress tolerance index (STI), based on root length, showed diversity among the lines in response to physiological drought (Figure 4). Assessing lines as per this selection index leads to the identification of few lines (H36 and H34) as drought tolerant ones. It showed also, the superiority of H36 compared to all other genotypes while V10 (parental line) appeared as the most sensitive. V30 (recurrent parental line) which is supposedly the

most tolerant to drought appeared intermediate.

Conclusion

This study was carried out through five generations using assisted molecular markers; which led to the identification of at least two remarkable lines based on their agronomic, nutritional and molecular traits. The selected lines H36 and H34 subjected to physiological stress have performed better than the remainders and their parental lines. Molecular analyses of the sKT7 profile have shown 67bp allele in the naked lines suggesting a putative domestication different from that reported in other geographic sites. This indicates that barley was domesticated more than twice.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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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

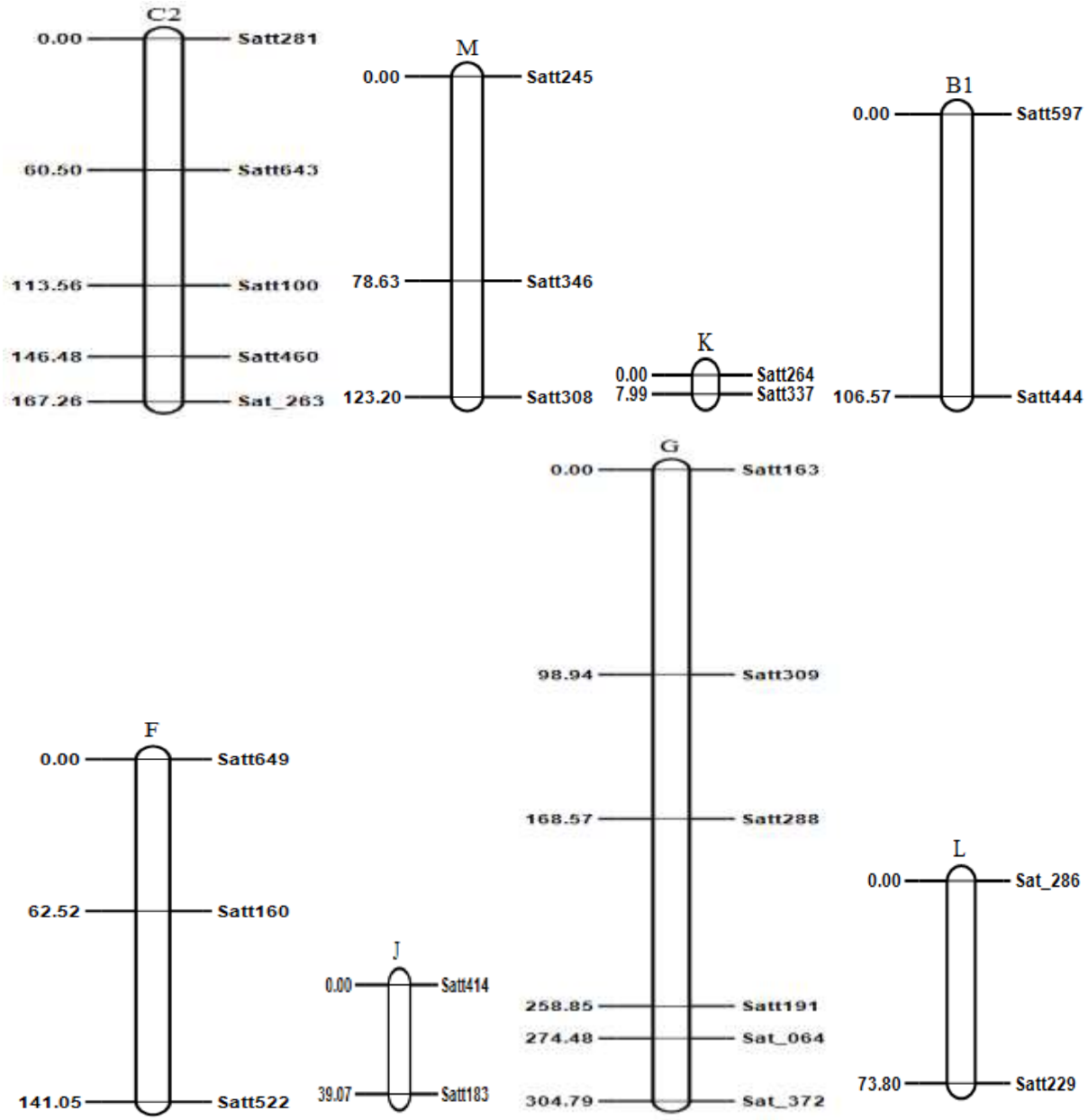

Figure 1. Linkage map of 25 SSR markers. C2, M, K, B1, F, J, G and L are the linkage groups formed; the numbers to the left-side of the map are positions of the SSR markers in cM.

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.

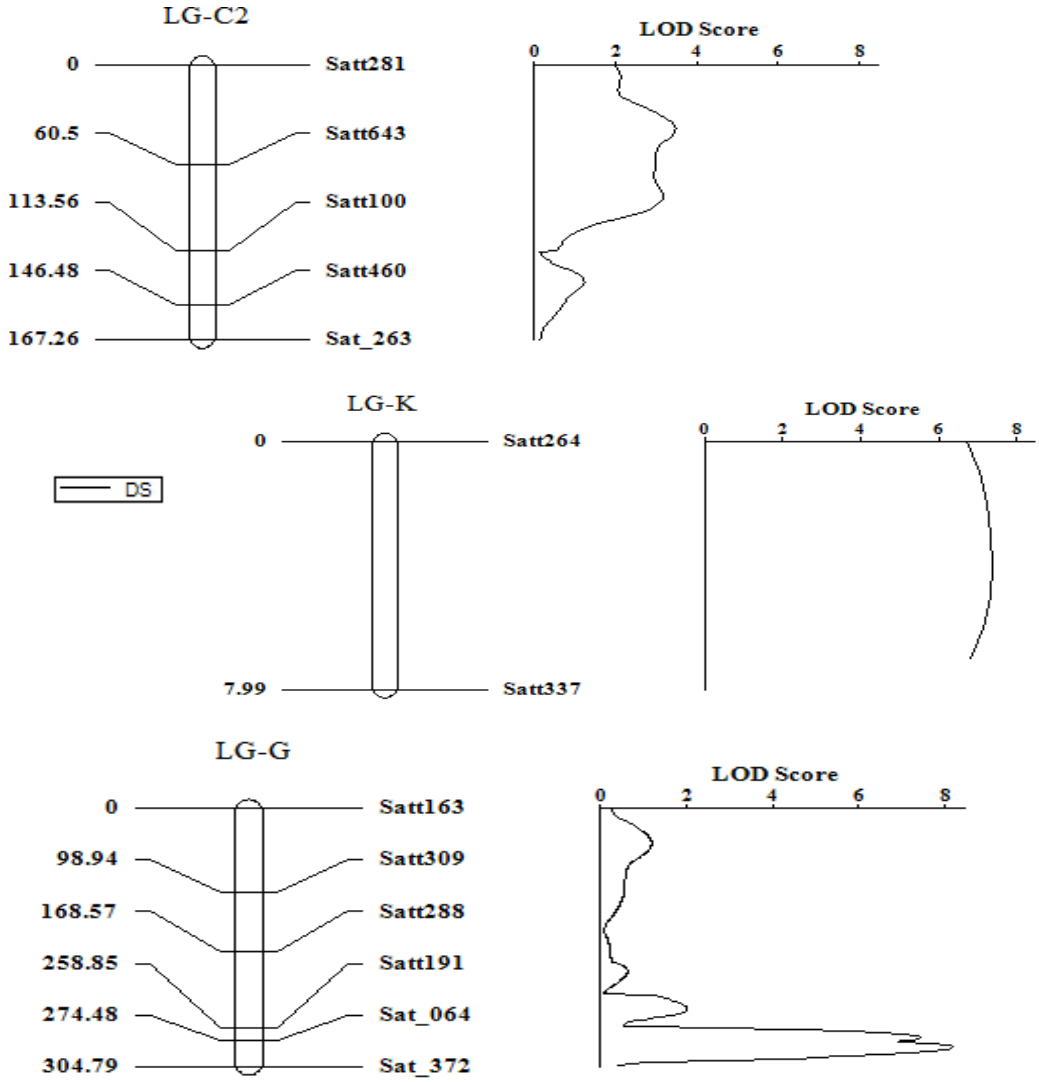

Figure 2. Genomic regions and SSR markers significantly associated with resistance to SBR detected using QTL IciMapping software in Wondersoya × UG 5.

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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