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Marker assisted *Rpp* gene introgression for Asian rust resistance in Soybean(*Glycine max* (L.) Merrill)

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ABSTRACT

The present work aimed to study marker-assisted Rpp gene introgression into widely adaptable and agronomical desirable soybean cultivar JS335 to enhance soybean Asian rust resistance against field isolates and broaden the genetic base by using ten microsatellite SSR markers. The four sources of Asiatic soybean rust resistance genes, for the present study were, PI200492 (Rpp1), PI 230971 (Rpp2), PI 462312 (Rpp3), and PI 459025 (Rpp4) The rust-resistant donor parents (Male) derived from a double cross of four parents PI 200492 (Komata), PI 230971, PI 462312 (Ankur) and PI 459025 (Bing Nan) were identified by field scoring and confirmed by molecular level for the presence of combination Rpp genes. These donor parents coded as SDP10, SDP18, SDP30, and SDP36 were simultaneously crossed separately with widely adaptable female JS335 viz; JS335 x SDP10, JS335 x SDP18, JS335 x SDP30, and JS335 x SDP36 during Kharif - 2017. Out of 10 SSR markers, 3 markers used for individual genotyping are Satt 191 amplified at 222 bp (Rpp1 gene), Satt 366-200 bp (Rpp2 gene), Satt 263-225 bp (Rpp3 gene) showed polymorphism into the original donors and derived donors. The marker-based analysis confirmed that a rust-resistant donor parent SDP10 had the Rpp1 and Rpp3 genes. It can be used with digenic donor males for improvement against rust in soybean. SDP18 with (Rpp2) gene, SDP30 (Rpp2), and SDP36 (Rpp3) can be used as monogenic rust donors for the development of resistant varieties in soybean. The derived F_1 of four crosses (JS335 x SDP10, JS335 x SDP18, JS335 x SDP30, and JS335 x SDP36) have both heterozygous banding patterns which indicate hybridism and further their inheritance-resistant gene of respective male parents.

Keywords: Soybean, SSR Marker, Asian Soybean rust, Gene incorporation

INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) is a wonder crop and economically important legume crop due to its high profitability, productivity, nutritional value, and its role in maintaining soil fertility. Soybean is cultivated in all types of soil over temperate and subtropical regions of the world as the major source of oil and proteins. The major constraint in soybean cultivation is biotic and abiotic stress, the estimated value of biotic stress loss is about 23 %. Out of this 11 % of loss is caused by plant pathogenic bacteria and fungi, whereas virus causes 1 % yield loss. Insect pests and nematodes cause an 11 % yield loss. Among the fungal disease of soybean, leaf rust (*Phakopsora pachyrhizi*) is one of the worldwide most serious foliar diseases of soybean. Under heavy infestation, losses of up to 75 % have been observed in unprotected fields (Yorinori *et al.,* 2005). Breeding for abiotic and biotic stress resistance is considered ideal for sustainable soybean production because it doesn't add to the costs of cultivation and is eco-friendly. To reduce the losses from biotic and abiotic stresses, it is necessary to discover and develop superior stress-tolerant soybean lines that can be used to develop genetically superior types. Genetic resistance is therefore an economic and strategically important means of controlling soybean rust disease (Arias *et al.,* 2008). Seven *Rpp* genes and three alleles for pathotype-specific resistance to soybean rust (*Rpp*) have been identified including *Rpp1* from PI 200492 (McLean and Byth, 1980), *Rpp2* from PI 230970 (Bromfield and Hartwig, 1980), *Rpp3* from PI 462312 (Ankur) (Bromfield and Melching, 1982; Hartwig and Bromfield, 1983; Hyten *et al.,* 2009), *Rpp4* from PI 459025B (Hartwig, 1986), *Rpp4b* from PI 423972 (King *et al.,* 2017); *Rpp5* from PI 200456 (Garcia *et al.* 2008) , *Rpp6* from PI 567102B (Li *et al.,* 2012), *Rpp1-b* (another allele at the *Rpp1* locus) from PI 594538A (Chakraborty *et al.,* 2009) and *Rpp3*(*Hyuuga*) (An allele at the *Rpp3* locus) from the Japanese cultivar *Hyuuga*, designated PI

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506764 (Silva *et al.,* 2008). *Rpp7* was recently discovered in PI 605823 based on its resistance to *P. pachyrhizi* (Child *et al.,*2018).The threat of soybean rust in soybean production is only because of resistance breakdown due to the development of new races of the pathogen which break the resistance of a single gene. Therefore; attempts are being made to enhance the rust resistance against multiple isolates by bringing together multiple *Rpp* genes into a single background. Another aspect of soybean cultivation is its narrow genetic base. Most of the genotypes are agronomically suitable and have the capability of high-yield production but with the onset of rust, these genotypes became highly susceptible. However, some of lines/cultivars showed resistance against rust but poor yield and are not suitable agronomically. Introducing such resistant genes (Rpp genes) into well-adapted lines/ cultivars helps broaden the genetic base against rust. Gene introduction involves the incorporation of desirable genes into a single genotype (Agronomical desirable with wider adaptability) to overcome a narrow genetic base. The availability of saturated linkage maps in soybean with the help of molecular markers makes marker-assisted selection feasible for specific resistance genes in the early generations (Song *et al.,* 2004). Moreover, several SSR markers tightly linked to known sources of resistance have been mapped, making it possible to trace them during hybridization and facilitate their identification through marker-assisted selection. The success of marker-assisted gene introduction strategies is largely facilitated by the availability of molecular markers which are tightly linked to the gene of interest. The aim of the present study was to introduce rust resistance *Rpp* genes into agronomically desirable, wider adaptable but rust susceptible soybean variety JS335 background to enhance soybean rust resistance to pathogen isolates and broaden the genetic base for improvement in rust résistance, wider adaptability and higher yield potential in soybean cultivar JS335 background.

MATERIALS AND METHODS

Plant material and Crossing program

For the present investigation, four rust-resistant donor parents (male) were derived from the F6 generation of double cross hybrid (PI 200492-Komata × PI 230971) × (PI 462312-Ankur × PI 459025-Bing Nan) developed by crossing four different resistance source each having single gene *Rpp1, Rpp2, Rpp3, Rpp4* (Parhe, 2016; Parhe *et al.,* 2017a, b, c). The segregating generations having combinations of different *Rpp* genes were subsequently evaluated up to the F_5 generation under hotspot rust screening at Agriculture Research Station (ARS), Kasbe Digraj, Sangli. Furthermore, F_6 generation was sown in Kharif 2017; at the hotspot Agricultural Research Station, Kasbe Digraj, and evaluated for rust screening. Resistant lines were identified and coded as SDP10, SDP18, SDP30, and SDP36. These coded SDP lines (Male parent) crossed separately with common female JS335 during Kharif 2017. The presence of resistance *Rpp* genes was confirmed in resulting hybrids, male parents, and original Rpp donors. (Checks PI 200492- Komata *Rpp1*, PI 230971*(Rpp2),* PI 462312-Ankur (*Rpp3*), PI 459025-Bing Nan-*Rpp4*) by using linked molecular markers, Confirmed F_1 of four crosses were selfed as well as backcrossed with P_1 parent JS335 to generate F_2 and BC_1's of four crosses during the summer of 2018. For rust screening of P_1, P_2, and $F_1$of four crosses, 4 original *Rpp* gene donors (Checks),were carried out in randomized block design (RBD) with three replications however, segregating generation F_2 and BC_1's sown in Single block during *Kharif* 2018 at Agricultural Research Station, Kasbe Digraj, District-Sangli (field condition) a hot spot for rust occurrence allocated ideal conditions. The experiment was done on 25th July 2018 (Late sown) for maximum disease development. Sowing was conducted in rows of 3 m in length and having 45 x 10 cm distance in a row to row and plant to plant respectively. One row was assigned to P_1s, P_2s, and F_1s, while 10 rows were to F_2s and two rows to F_3s. This has permitted the raising of 30 plants in each of the P_1s, P_2s, and F_1s, 300 plants in each of the F_2s, and 60 plants in F_3s, in all four crosses. A fertilizer dose of 50:75:00 NPK kg/ha for the irrigated situation was applied at the time of sowing. For the even spread of the disease, an aqueous suspension of rust spores was sprayed on the experimental material. The disease first appeared in 1st week of September 2018. Initially, ash to TAN-colored pustules was

observed on the susceptible female JS335, and later on, the disease was spread to the entire field, for the rust screening, observations on rust intensity and sporulation were recorded on 40 plants from parents and F_1s and original *Rpp* source (Checks) in each replication 200 to 300 plants from F_2s and 20 to 30 plants from B_1s generations (Segregating) of all the four crosses was in the single block without replication.

Screening for rust resistance (lesion/pustules type) in original sources, male donors, and five generations of four crosses

Plants of five generations along with checks under hotspot rust screening were classified as resistant (R) or susceptible (S), in accordance with rust pustules color (sporulation) as follows Immune (no sporulation) that is complete resistance, Reddish-brown (RB) lesions (incomplete resistance), and profusely sporulation TAN lesions (Susceptible). The rust-pustule intensity was recorded by using a 0–9 scale given by Mayee and Datar 1986, where 0 = absolute resistant reaction with 0% disease intensity, 1 = highly resistant reaction with 1% disease intensity, 3 = moderately resistant with 1.1–10% disease intensity, 5 = moderately susceptible reaction with 10.1–25% disease intensity, 7 = susceptible reaction with 25.1–50% disease intensity, and 9 = highly susceptible reaction with more than 50% disease intensity.

DNA extraction, polymerase chain reaction amplification and agarose gel electrophoresis

The first unifoliate leaves were collected from individual plants of five generations of four crosses for DNA extraction. The leaves were stored in a cryo can having liquid nitrogen the transportation from ARS, Kasbe Digraj, Sangli, to the central campus, Rahuri. Leaf samples were kept in a −80°C freezer until used. The genomic DNA of four original *Rpp*s gene sources (checks), rust-resistant donor parents, common female JS335, and twelve plants of each F_1s of four crosses were extracted by using a modified CTAB protocol (Keim *et* al. 1988). The polymerase chain reaction (PCR) for SSR markers was conducted.(Röder et al. 1998). Agarose gel electrophoresis was carried out in all the generations for genomic DNA study. The concentration of DNA was optimized according to the intensity of electrophoresed DNA as compared with the known control, lambda phase DNA (50 ng/µl). Initially, four rust-resistance gene-donor parents were surveyed for polymorphism with 10 SSR primers (Table 1). Earlier reported primer sequences for the soybean rust-resistance genes *Rpp1, Rpp2, Rpp3*, and *Rpp4* are available at https://soybase.org/ (Grant et al. 2010) these are used in the study. This information was further used to confirm polymorphic markers in the original *Rpp* source (checks),derived donor male, and their respective hybrid plants used for analysis and conditions for the amplification of SSR primers.

PCR and condition for amplification of SSR primers

For amplification of the microsatellite markers, PCR was carried out in a thermal cycler (Bio-Rad C1000, Life science (Research, Education, Process Separations, Food Science), Gurgaon, India) prepared solutions used in the reaction were first thawed and kept on ice throughout the procedure. The PCR cocktail (20 µl) was prepared and mixed using various components, such as ultrapure H_2O (11 µl), PCR buffer B (100mM Tris (pH 9.0), 500 mM KCl, and 0.1% Gelatin) of 10X conc. (2.0 µl), 2 mM $MgCl_2$ (1.7 µl), 1 mM deoxyribonucleotide triphosphate (dNTPs) mix (2.0 µl), 5 mM forward and reverse SSR primers (1 µl each), 3 unit/µl Taq DNA polymerase (0.3 µl), and 30 ng/µl concentrated template DNA (1 µl).

PCR thermal cycling condition

The PCR thermal cycling consists of initial denaturation at 94°C for 5 min. for a single cycle, followed by denaturation at 94°C for 30 sec. annealing according to primer temperature (42–56°C) for 30 sec. for 35 cycles, and final extension at 72°C for 5 min. for a single cycle. After amplification, PCR products were stored at 4°C until gel electrophoresis was conducted. After PCR amplification, the PCR products (SSR analysis) were separated via 2 % metaphor agarose gel electrophoresis.

Table 1: The detail of the SSR primers used in the present study

Sr. No.	Primer (s)	Sequence	Location	Gene
1.	Satt66	F:AGATTGGGTGAGAACATAAG R:GGAGAGCGTAAAAGAAATTC	Chr. 18	Rpp1/ Rpp1b
2.	Satt191	F: CGCGATCATGTCTCTG R: GGGAGTTGGTGTTTTCTTGTG		
3.	Satt366	F: GCGGCACAAGAACAGAGGAAACTATT R:GCGGACATGGTACATCTATATTACGAGTATT	Chr. 16	Rpp2
4.	Satt460	F: GCGCGATGGGCTGTTGGTTTTTAT R: GCGCATACGATTTGGCATTTTTCTATTG		
5.	Satt263	F: CACCCAATCATGATAGCATTTTAT R: CTCATGGAATTGTCTTTCAGTTTC	Chr. 6	Rpp3 Hyuuga
6.	SSR1788-1	F: TGAAATTGGAAACGATCGCAACG R: TGCTTCTTTCTTTCTTTATCCGCTCC		
7.	Satt288	F: GCGGGGTGATTTAGTGTTTGACACCT R: GCGCTTATAATTAAGAGCAAAAGAAG		
8.	Satt143	F: GTGCCACAAATTTAAAATTACTCA R: TCCCTCCCTTTTGATTTACAC	Chr. 18	Rpp4 –
9.	Satt612	F: GTCATACTGGGTGTTTCATTTATGAC R: GCGCCTTTTAGTCTCTGAAAGTATTT		
10.	Rpp4TM	F: GTTTGCTTCAAGGGGTCCACA R: AACATCCCGCACAATGTCATGC		

RESULTS

The result obtained from marker-assisted *Rpp* gene introgression for Asian rust resistance in Soybean (*Glycine max* (L.) Merrill) are mentioned clearly in Tables 2 to 4 having details of rust and molecular data.

Screening of various generations for rust resistance

Out of four original *Rpp* gene donors (Checks) used, *Rpp1* gene donor PI200492 (Komata) was an immune reaction with disease grade 0.92. *Rpp2* gene donor PI230971 had a disease grade of 1.10. *Rpp3* gene donor PI 462312 (Ankur) showed RB reaction and TAN reaction to rust with disease grade 2.38. *Rpp4* gene donor PI 459025 (Bing Nan) with 1.35 disease grade had RB lesions (incomplete resistance). However, female parent JS335 was severely susceptible (100% susceptibility) with TAN-colored lesions uniformly covering both the leaf surfaces with disease grade 8.79. All the donor male parents such as SDP10, SDP18, SDP30, and SDP36 had 100% resistant plants with RB response against rust, while SDP10 had 35.90% plants with immune reaction against rust. In cross JS335 x SDP10, all the F_1 plants were rust resistant; 45.84 %of plants among them showed an immune reaction, while in the F_2, segregation was observed with 8.01 % of plants showing a susceptible reaction. In the B_1 generation, 35.72%of plants were rust susceptible. In cross JS335 x SDP18, all the F_1 plants recorded resistant response; while in the F_2 generation, segregation was observed with 25.53 %of plants being rust susceptible. In the B_1 generation, 41.67% of plants were rust susceptible. In cross JS335 x SDP30, all the F_1 plants were rust resistant, while in the F_2, 28.47% of plants showed susceptible reactions to rust because of segregation. In the B_1 generation, 42.85% of plants were susceptible. In cross JS335 x SDP36, all the F_1 plants were rust resistant, while in the F_2 generation, segregation was observed with 26.42 %of plants showing susceptible reaction to rust. In the B_1 generation, 54.17% of plants were susceptible. (Table 2)

Table 2: % distribution of rust lesion types in all 4 soybean crosses

Name of cross	Generations	% Immune	% RB	Resistant (% Immune + RB)	Susceptible (% TAN)	Average Disease Grade
1.	PI 200492	48.72	51.28	100.0	0.0	0.92
2.	PI 230971	0.0	100.0	0.0	0.0	1.10
3.	PI 462312	0.0	69.24	69.24	30.76	2.38
4.	PI 459025	0.0	100.0	100.0	0.0	1.35
	P_1	0.0	0.0	0.0	100.0	8.79
	P_2	35.90	64.10	100.0	0.0	1.12
Cross	F_1	45.84	54.16	100.0	0.0	1.20
JS335 x	F_2	6.18	85.81	91.99	8.01	3.17
SDP10	B_1	3.57	60.71	64.28	35.72	4.60
	Ratios F_2			14.52	1.28	
	Ratio of B_1			2.58	1.42	
	P_1	0.0	0.0	0.0	100	8.79
	P_2	0.0	100.0	100.0	0.0	1.76
Cross	F_1	0.0	100.0	100.0	0.0	1.96
JS335 x	F_2	0.0	74.47	74.47	25.53	5.03
SDP18	B_1	0.0	58.33	58.33	41.67	5.83
	Ratio of F_2			2.98	1.02	
	Ratio of B_1			1.17	0.83	
	P_1	0.0	0.0	0.0	100	8.79
	P_2	0.0	100.0	100.0	0.0	1.46
Cross	F_1	0.0	100.0	100.0	0.0	1.34
JS335 x	F_2	0.0	71.53	71.53	28.47	4.81
SDP30	B_1	0.0	57.15	57.15	42.85	5.07
	Ratio of F_2			3.14	0.86	
	Ratio of B_1			1.15	0.85	
	P_1	0.0	0.0	0.0	100	8.79
	P_2	0.0	100.0	100.0	0.0	1.97
Cross	F_1	0.0	100.0	100.0	0.0	2.08
JS335 x	F_2	0.0	73.58	73.58	26.42	5.12
SDP36	B_1	0.0	45.83	45.83	54.17	6.08
	Ratio of F_2			2.95	1.05	
	Ratio of B_1			1.08	0.92	

Confirmation of molecular markers associated with rust resistance in donor parents

The rust-resistant donor parents SDP10, SDP18, SDP30, and SDP36 derived from a double cross of four parents PI 200492 (Komata), PI 230971, PI 462312 (Ankur), and PI 459025 (Bing Nan) having four rust-resistant genes *(Rpp1, Rpp2, Rpp3, Rpp4)* were identified by field scoring and confirmed by molecular level. Genomic DNA from leaf samples of four original donors. The DNA quality and integrity was confirmed which showed a distinct DNA band without any smear, confirming that it is not degraded in any sample. Comparative band intensity with that of lambda phage DNA was used for further diluting samples to uniform concentrations (50 ng/µl) for PCR reactions. Out of four rust resistance genes, only the Rpp1 gene was confirmed in SDP10 by using Rpp1 specific Satt191-222 bp marker, while the Rpp2 gene was confirmed in SDP18, SDP30 and SDP36 by using Rpp2 gene specific Satt366-200 bp marker. The Rpp3 gene was also confirmed in SDP10 based on the Rpp3 specific Satt263-225 bp marker. However, the presence of the Rpp4 gene could not be confirmed due to the monomorphic banding pattern in all parents and original donors (Checks) (Table 3).

Table 3: SSR markers which were used for confirmation of Rpp genes in original donors and SDP parents

Sr. No.	Name of primer	Size (bp)	Chromosome Number	Confirmed Gene	Original Donor	SDP parents
1.	Satt191	222	18	*Rpp1*	PI 200492	SDP10 SDP18
2.	Sat_366	200	16	*Rpp2*	PI 230971	SDP30 SDP36
3.	Satt263	225	6	*Rpp3*	PI 462312	SDP10

Confirmation of hybridism with molecular markers associated with rust resistance in F_1 hybrids

F_1 plants of cross JS335 x SDP10 had twin fragments inherited from both the parents (P_1 and P_2) with three primers used *i.e*. 195 bp and 222 bp with Satt191primer for *Rpp1* gene while 231 bp and 225 bp were amplified by Satt263 primer for *Rpp3* gene which confirmed the resistance governed by *Rpp1* and *Rpp3* genes. They had either immune or reddish-brown reactions. However, Satt366 amplified at 198 bp and 210 bp markers which did not match with original donor's band PI230971 for *Rpp2* gene; thereby indicating an absence of *Rpp2* gene in SDP10 and derived hybrid plants. All these three gene-specific markers confirmed hybridism and heterozygosis for both parents which had immune and reddish brown (RB) responses. F_1 of JS335 x SDP18 had twin fragments inherited from both the parents (JS335 and SDP18) with three primers used *i.e.* 198 bp and 200 bp amplified by Satt366 primer for *Rpp2* gene which conclude the presence of *Rpp2* gene. While other two primers, Satt191 and Satt263 both amplified bands of varying size of 195 bp and 207 bp for the *Rpp1* gene, whereas 231 bp and 201 bp for the *Rpp3* gene which concluded the absence of these two genes in SDP18 and their two hybrids. These three primers confirmed the hybridism and heterozygosis of F_1s which had incompatible reddish brown rust reaction (RB). All the plants of F_1 of JS335 x SDP30 had twin fragments inherited from both the parents (JS335 and SDP30) with three primers used. *i.e.* 198 bp and 200 bp were amplified by Satt366 primer for the *Rpp2* gene which concluded the presence of the *Rpp2* gene. However, the other two primers Satt191primer and Satt263 primer both had varying sizes 195 bp and 207 bp for *Rpp1* gene whereas 231 bp and 201 bp for *Rpp3* gene, thereby suggesting the absence of these two genes in SDP18 and their two hybrids. These three primers confirmed their hybridism and heterozygosis which had incompatible reddish brown rust reaction (RB). All the plants of F_1 of JS335 x SDP36 had twin fragments inherited from both the parents (JS335 and SDP36) with three primers used *i.e.* 198 bp and 200 bp were amplified by Sat_366 primers for *Rpp2* gene which concludes the presence of *Rpp2* gene. However, other two primers Satt191primer and Satt263 primer both had varying size of 195 bp and 228 bp for *Rpp1* gene whereas 231 bp and 213 bp for *Rpp3* gene, which suggests the absence of these two genes in SDP36 and their two hybrids. These three primers confirmed their hybridism and heterozygosis which had incompatible reddish brown rust reaction (RB).The amplified products of all four primers for *Rpp4* gene were monomorphic, hence it was difficult to conclude the presence or absence of *Rpp4* gene. All these three gene-specific markers confirmed hybridism and heterozygosis for both parents. (Table no.4)

DISCUSSION

Host plant resistance is an economic and important strategy for control of soybean rust disease (Arias et al. 2008). There are several different monogenic resistant sources for soybean rust but they are poor yielding and linked with undesirable trait lead to problem of linkage drag. Our aim was identification of digenic *Rpp* gene combination donor and incorporation of either of four rust-resistance genes (*Rpp1, Rpp2, Rpp3*, and *Rpp4*) into agronomically desirable but rust-susceptible soybean cultivar JS335, by using such donor. The study was conducted to enhance soybean rust resistance to pathogen isolates and broaden

Table 4: Molecular confirmation of F_1 in JS335xSDP10, JS335 x SDP18, JS335 x SDP30 and JS335 x SDP36

Markers	Parents and number of F_1 plants studied (bp sized band observed)															
	Base pair	JS335xSDP10				JS335 x SDP18				JS335 x SDP30				JS335 x SDP36			
		JS335	SDP10	1	2	JS335	SDP18	1	2	JS335	SDP30	1	2	JS335	SDP36	1	2
Satt191 *(Rpp1)*	222	195	222	222, 195	222, 195	195	207	207 195	207 195	195	207	207 195	207 195	195	228	228, 195	228 195
Sat_366 *(Rpp2)*	200	198	210–	210, 198	210, 198	198	200	200, 198	-	198	200	200 198	-	198	200	200, 198	200 198
Satt263 *(Rpp3)*	225	231	225	225, 231	225, 231	231	201	201, 231	201 231	231	201	201 231	201 231	231	213	213, 231	213 231
Rpp genes present in plant	*Rpp 1,2 & 3*	-	*Rpp 1 & 3*	*Rpp 1 & 3*	*Rpp 1 & 3*	-	*Rpp2*	*Rpp2*	*Rpp2*	-	*Rpp2*	*Rpp2*	-	-	*Rpp 2*	*Rpp2*	*Rpp2*
Reaction to rust disease	Immune & Reddish brown	Suscep-tible	Immune & Reddish brown	Immune & Reddish brown	Immune	Suscept ible (s)	Reddi sh brown	Reddi sh brown	Reddi sh brown	(S)	Reddi sh brown	Reddi sh brown	Reddi sh brown	(S)	Redd ish brow n	Reddi sh brown	Reddi sh brown

Note:
1 *= Satt 191-222 bp (Rpp1),*
2 *= Sat_366-200bp (Rpp2)*
3 *= Satt263-225bp (Rpp3)*

the genetic base for rust resistance. In the present study, double cross hybrid (PI 200492-Komata × PI 230971) × (PI 462312-Ankur × PI 459025-Bing Nan) were developed, by crossing four different resistance source each having single gene *Rpp*1, *Rpp*2, *Rpp*3, *Rpp*4 (Parhe, 2016; Parhe *et al.,* 2017a, b, c). The segregating generations having combinations of different *Rpp* genes were subsequently evaluated for field rust screening up to the F_5 generation then resistant lines were coded as SDP10, SDP18, SDP30 and SDP36. These derived SDP rust line crossed with common female JS335 like JS335 x SDP10, JS335 x SDP18, JS335 x SDP30 and JS335 x SDP36.The original *Rpp1* gene source PI200492 (Komata) was found to be immune, the *Rpp2* gene source PI230971 and *Rpp4* gene source PI459025 (Bing Nan) were resistant, showing RB lesions (incomplete resistance), whereas Rpp3 gene source PI462312 (Ankur) produced highly localized tan lesions (susceptible) on the lower leaf surface. However, Ankur was not as susceptible as female JS335, which had tan-colored lesions uniformly covering both the leaf surfaces, resulting in almost complete leaf drop. PI200492 has been identified as the source of *Rpp1* gene that confers immunity (McLean and Byth, 1980), whereas PI230970, PI462312 (Ankur), and PI459025B have been reported to be donors of *Rpp2* (Yu et al. 2015; Bromfield and Hartwig 1980), *Rpp3* (Bromfield and Melching 1982; Hartwig and Bromfield 1983), and *Rpp4* (Hartwig1986) genes, respectively, and produced hypersensitive response. Mahajan (2015) had also reported that under controlled glasshouse conditions, resistance conferred by *Rpp2* (from PI230970 and PI230971) and *Rpp4* from PI459025 B/F) genes acts against different rust isolates from different geographical conditions; the *Rpp1* gene-conferred resistance (from Komata) was breached only in western Maharashtra state, whereas *Rpp3* gene-conferred resistance (from Ankur) was breached in the entire state of Maharashtra. Out of four resistant SDP parents derived from double cross of four different sources (*Rpp1* × *Rpp2)* x *(Rpp3* × *Rpp4*), SDP10 had showing 35.90% plants showing immune and 64.10 % plants showed RB lesions. Other three donor SDP18, SDP30 and SDP36 having 100% RB lesion (incomplete resistance). The common female 100% TAN lesion covering on both side of leaves. The F_1 plants of cross JS335 x SDP10 having immune(45.84%) and RB lesion (54.16%), However F_1 plants of cross JS335 x SDP18, JS335 x SDP30 and JS335 x SDP36 had 100% RB lesion that are typical of partial rust resistance. In the F_2 and BC_1 generations of cross JS335 x SDP10, 91.99% plant population of F_2 and 64.28 % plants of BC_1 generations showed resistant either RB or immune reaction; and 8.01%(F_2) and 35.72% (BC_1) plants were found to be susceptible to rust. In JS335 x SDP18, 74.47% (F_2) and 58.33 % (BC_1) of the population showed RB reaction; and 25.53% (F_2) and 41.67% (BC_1) plants were found to be susceptible to rust. In JS335 x SDP30, 71.53% (F_2) and 57.15 % (BC_1) of the population showed RB reaction; and 28.47% (F_2) and 42.85% (BC_1) plants were found to be susceptible to rust. In JS335 x SDP36, 73.58% (F_2) and 45.83 % (BC_1) of the population showed RB reaction; and 26.42% (F_2) and 54.17% (BC_1) plants were found to be susceptible to rust. Now day in soybean, there is availability of a saturated linkage map with the help of molecular markers makes marker-assisted selection for specific resistance genes and their identification in the early generations is easy and feasible (Song et al. 2004). The SSR markers are valuable genetic markers because they are co-dominant, detect high levels of allelic diversity, and are assayed efficiently via the PCR, the 10 microsatellite primer pairs, linked with the four loci of rust resistant genes*(Rpp1, Rpp2, Rpp3, and Rpp4*), used to detect polymorphism among the original source, derived SDP parents and to confirm the inheritance in F_1. The ten 10 SSR microsatellite yielded PCR amplification. Out of ten used primers three showed 100% polymorphismSatt191–222bp (*Rpp1*), Satt 366–200 bp (*Rpp2*), and Satt263–225 bp (*Rpp3*),showed clearly distinct polymorphic bands in PCR amplification. These markers were short-listed and used for identification and confirmation of rust-resistance genes among the individual F_1 plants of four crosses and used primer for *Rpp4* gene can show polymorphism so unable to conclude presence or absence of *Rpp4* gene. It was observed that these *Rpp*-gene combinations had improved rust resistance relative to individual donor parents. The marker based analysis for rust resistance confirmed that resistant donor parent SDP10 had *Rpp1* and

Rpp3 gene. It can be used with digenic donor instead of PI 200492 and PI 462312 male for improvement against rust in soybean. SDP18 with (*Rpp2*) gene, SDP30 *(Rpp2)* and SDP36 *(Rpp2)* can be used as monogenic rust donor for development of resistant variety in soybean. The derived F_1 of four crosses (JS335 x SDP10, JS335 x SDP18, JS335 x SDP30 and JS335 x SDP36) having both heterozygous banding pattern which indicates hybridism and further their inheritance resistant gene of respective male parents.

Conclusions

It is concluded that a rust-resistant donor parent SDP10 had an *Rpp1* and *Rpp3* gene combination so it can be used with digenic donor males for improvement against rust in soybean. SDP10 with (*Rpp2*) gene, SDP30 *(Rpp2),* and SDP36 *(Rpp2)* can be used as monogenic rust donors instead of PI230971 for the development of a resistant variety in soybean.

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