Trends in Biosciences 10(6), Print : ISSN 0974-8431, 1437-1445, 2017

Scoring of Four *RPP* Genes/Qtls Pyramided Generations for Rust Resistance in Soybean (*Glycine max* (L.) Merrill)

SACHIN D. PARHE*[1], VIVEK P. CHIMOTE[2], MILIND P. DESHMUKH[3]

[1]*Genetics & Plant Breeding, Department of Agricultural Botany, Mahatma Phule Agriculture University Rahuri, Maharashtra*
[2]*Agricultural Botany, Mahatma Phule Agriculture University Rahuri, Maharashtra*
[3]*Agriculture Research Station, Kasbe, Digraj, Sangli, Maharashtra*
email : parhe.sachin@gmail.com

ABSTRACT

Scoring for rust resistance was done as per the immune, reddish brown (RB) lesions or resistant reaction and TAN lesions or susceptible reaction as well as their rust grade. Out of four parental genotypes used *Rpp1* gene donor PI 200492 (Komata) gave immune reaction to rust with low disease grade of 0.92. Both *Rpp2* and *Rpp4* gene donors, PI 230971 and [PI 459025 (Bing Nan)] had RB lesion (incomplete resistance) with both having 1.28 disease grade. *Rpp3* gene donor PI 462312 (Ankur) showed localized TAN lesions with disease grade 1.83 only on the abaxial (lower) leaf surfaces. However, it was not as severely susceptible as susceptible check JS335 which had TAN lesions uniformly covering both the leaf surfaces. In other words *Rpp1* (from PI 200492), *Rpp2* (from PI230970 and PI230971) and *Rpp4* (from PI459025 B/F) genes worked, while *Rpp3* gene resistance (from Ankur) was broken down. In cross-A, *i.e.* PI200492 (*Rpp1*) × PI230971 (*Rpp2*) all the F_1s produced recorded rust resistance with 1.15 grade, while in the segregating F_2, 93.9% plants had either immune/RB reaction and rest 6.0% plants showed susceptible reaction to rust. In cross-B i.e. PI462312 (*Rpp3*) × PI459025 (*Rpp4*), 80.8 % F_1 plants had RB lesions with resistant reaction while rest plants (19.2%) recorded susceptible. The F_2 because of segregation 24.2% plants were rust susceptible. In cross-C, [PI200492 (*Rpp1*) × PI230971 (*Rpp2*)× PI462312 (*Rpp3*) × PI459025 (*Rpp4*)], *i.e.* double cross hybrid because of segregation 85.5% plant recorded immune/RB reaction to rust; rest 14.5% plant recorded susceptible reaction to rust disease with overall 1.44 grade.

Key words *Scoring, Phakopsora pachyrhizi, Rpp genes, soybean (Glycine max (L.) Merrill), lesion (Immune, RB, TAN) types.*

Soybean rust (*Phakopsora pachyrhizi* Syd. & P. Syd.,) is one of the most serious foliar diseases of soybean worldwide (Langenbach *et al.*, 2016). Under heavy infestation, losses of up to 75% have been observed in unprotected fields (Yorinori *et al.*, 2005). The rapid spread of soybean rust, together with the potential of causing severe yield losses, makes it a very important disease of soybean (Miles *et al.*, 2003). In India, Madhya Pradesh is the leading state in producing soybean followed by Maharashtra. On an average Madhya Pradesh and Maharashtra produces 52 and 34 per cent of countries total production respectively. There is progressive increase in area and production in the Maharashtra State up to 2014-15. However there are fluctuations in the productivity ranging between 728 kg/ha to 1582 kg/ha. This is mainly due to occurrence of soybean leaf rust in the high productive zone of Maharashtra (Satara, Sangli and Kolhapur, India) since 1994-95. It has also been detected in many Asian countries and reached South American countries such as Brazil, Paraguay, Argentina, Bolivia and Colombia in recent years (Rossi, 2003; Yorinori *et al*., 2005).

The "lesion type" classification, based on lesion color and the number of sporulating uredinia, has been used to identify the virulence of soybean rust isolates (Bromfield, 1984). However, lesion color can vary a lot and some resistant lesions that do not sporulate can be found in tan-colored lesions. In addition, continuous variation of lesion color among varieties makes it difficult to group all phenotypes into a limited number of lesion types, such as RB (Resistant) and TAN (Susceptible) (Kato and Yorinori, 2008). On the other hand, lesion color is known to be controlled by resistance genes. For example, quantitative trait locus (QTL) analysis of lesion color in 120 F_2 plants revealed that major resistance genes, *Rpp2* from PI230970 and *Rpp4* from PI459025, can genetically contribute to the darkening of lesion color under greenhouse conditions. Thus, lesion color should also be considered when selecting resistant genotypes. Bonde *et al*. (2006) suggested that the number and size of uredinia are desirable indexes for detecting resistance derived from major genes and also partial resistance to soybean rust. These indexes are represented by numerical values and are considered more suitable than the index based on lesion color for deciding resistance because of their objectivity. The objective of present study was to score rust from all four parents, their F_1s, F_2s derived from selfing and double cross hybrids

Table 1. Scoring of rust disease intensity based on grades in various disease categories by Mayee and Datar (1986)

Grade	Disease category	Disease intensity
0	Absolute resistance/ Immune	0 %
1	Highly resistance	1 %
3	Moderately resistance	1.1 to 10 %
5	Moderately susceptible	10.1 to 25 %
7	Susceptible	25.1 to 50 %
9	Highly susceptible	More than 50 %

Table 2. Distribution of rust grades across lesion types in cross-A [Komata (PI 200492) × PI 230971] to soybean rust

Total Plants (in 3 Replicas)								
Generations	**Immune**	**RB lesions**			**TAN lesions**			
		Disease grade			**Disease grade**			
	0	1	3	5	1	3	5	7
P_1	13	18	5	0	0	0	0	0
P_2	0	31	5	0	0	0	0	0
F_1-cross-A	18	45	15	0	0	0	0	0
F_2-cross-A	99	656	89	1	0	55	0	0
% of Total Plants in 3 Replicas								
Generations	**Immune**	**RB lesions**			**TAN lesions**			
		Disease grade			**Disease grade**			
	0	1	3	5	1	3	5	7
P_1	36.1	50	13.9	0	0	0	0	0
P_2	0	86.1	13.9	0	0	0	0	0
F_1-cross-A	23.1	57.7	19.2	0	0	0	0	0
F_2-cross-A	11	72.9	9.9	0.1	0	6.1	0	0

(DCH) as per Immune, Reddish Brown (RB), and TAN colored pustules and grading them 0 to 9 disease scale.

MATERIALS AND METHODS

The present investigation was conducted during the period from 2014-15 and 2015-16 at Post Graduate Institute Research Farm, Department of Agricultural Botany, Central Campus, Rahuri and Agriculture Research Station, Kasbe-Digraj, Sangli, India. On the basis of resistance to rust of soybean four genotypes were selected for present investigation. Out of four, a genotype PI200492 (Komata) was immune to rust and three genotypes (PI230971, PI462312 (Ankur) and PI459025 (Bing Nan) were resistant to rust. These four genotypes were crossed in pair wise manner to produce two combinations for rust resistance.

The experiment was laid out in Randomized Block Design with three replications. The experimental material consisted of 9 treatments consisting four parents, $2F_1$s, $2F_2$s and $DCHF_1$. The parents, F_1s, F_2s, and $DCHF_1$.were randomized separately in each of the three replications. Sowing was done in rows of 3m length and 30 cm apart

Table 3. Distribution of rust grades across lesion types in cross-B [PI 462312 (Ankur) × PI 459025 (Bing Nan)] to soybean rust

Total Plants (in 3 Replicas)								
Generations	**Immune**	**RB lesions**			**TAN lesions**			
		Disease grade			**Disease grade**			
	0	1	3	5	1	**3**	**5**	**7**
P_3	0	0	0	0	28	3	3	2
P_4	0	31	5	0	0	0	0	0
F_1-cross-B	0	62	1	0	0	8	3	4
F_2-cross-B	0	670	12	1	53	68	77	19
% of Total Plants in 3 Replicas								
Generations	**Immune**	**RB lesions**			**TAN lesions**			
		Disease grade			**Disease grade**			
	0	1	3	5	1	**3**	**5**	**7**
P_3	0	0	0	0	77.8	8.3	8.3	5.6
P_4	0	86.1	13.9	0	0	0	0	0
F_1-cross-B	0	79.5	1.3	0	0	10.3	3.8	5.1
F_2-cross-B	0	74.4	1.3	0.1	5.9	7.6	8.6	2.1

Table 4. Distribution of rust grades across lesion types in cross-C (F_1 of cross-A × F_1 of cross-B) to soybean rust

Total Plants (in 3 Replicas)								
Generation	**Immune**	**RB lesions**			**TAN lesions**			
		Disease grade			**Disease grade**			
	0	1	3	5	1	3	5	7
F_1-cross-A	18	45	15	0	0	0	0	0
F_1-cross-B	0	62	1	0	0	8	3	4
DCH	20	56	1	0	0	2	6	5
% of Total Plants in 3 Replicas								
Generation	**Immune**	**RB lesions**			**TAN lesions**			
		Disease grade			**Disease grade**			
	0	1	3	5	1	3	5	7
F_1-cross-A	23.1	57.7	19.2	0	0	0	0	0
F_1-cross-B	0	79.5	1.3	0	0	10.3	3.8	5.1
DCH	22.2	62.2	1.1	0	0	2.2	6.7	5.6

accommodating 30 plants at 10 cm distance in a row. An infector row of the rust susceptible check cultivar JS 335 was sown after each row of genotype to ensure development of sufficient inoculums. The various generations viz. P_1, P_2, P_3, P_4 and their single cross hybrid (F_1s), their F_2s and double cross hybrid ($DCHF_1$) were used to study the inheritance of rust resistance. One row was assigned to P_1, P_2, P_3, P_4, F_1s, and two rows for $DCHF_1$, while 30 rows to F_2s. This has permitted for raising of 30 plants in each of P_1, P_2, P_3, P_4, F_1s, 60 plants of $DCHF_1$ and 300 plants in each of the F_2s, in each of the three replication for each cross. Fertilizer dose of 50 kg N and 75 Kg P2O5/ha for irrigated situation was applied at the time of sowing. The operations like thinning, weeding, hoeing, irrigation and plant protection were carried out regularly as per need and stage of crop growth.

The pair wise crossing was undertaken between four parents i.e. PI200492 (*Rpp*1 gene donor) as a male parent; PI230971 (*Rpp*2 gene donor) as a female parent; PI462312 (*Rpp*3 gene donor) as a male parent; PI459025 (*Rpp*4 gene donor) as a female parent and derived F_1 (SCH) were be further inter crossed to get double cross hybrids $DCHF_1$ i.e. {(PI200492 × PI230971) × (PI462312 × PI459025)}. In which pollination takes place before opening of flower. Therefore healthy buds were emasculated in evening between 16.00 to 18.00 hrs. and pollinated on next day morning (Carlson and Lersten, 1987). The parental genotypes were collected for crossing programme from Soybean Breeder, Agriculture Research Station, Kasbe Digraj, Sangli, India.

Rust lesion/pustules type and scoring in soybean

To study the resistance to leaf rust of soybean observations on intensity of rust were recorded on 10 randomly selected plants in P_1, P_2, P_3, P_4 and their single

Table 5. Per cent distribution of rust lesion types in all 3 soybean crosses studied

Name of cross	Generations	% Immune	% RB	Resistant (% Immune + RB)	Susceptible (% TAN)	Average Disease Grade
	P_1	36.1	63.9	100.0	0.0	0.92
	P_2	0.0	100.0	100.0	0.0	1.28
Cross-A	F_1	23.1	76.9	100.0	0.0	1.15
	F_2	11.0	82.9	93.9	6.1	1.21
	Ratio			15.02	0.98	
	P_3	0.0	0	0	100	1.83
	P_4	0.0	100.0	100.0	0.0	1.28
Cross-B	F_1	0.0	80.8	80.8	19.2	1.69
	F_2	0.0	75.8	75.8	24.2	1.65
	Ratio			12.13	3.87	
Cross-C	DCH	22.2	63.3	85.5	14.5	1.44

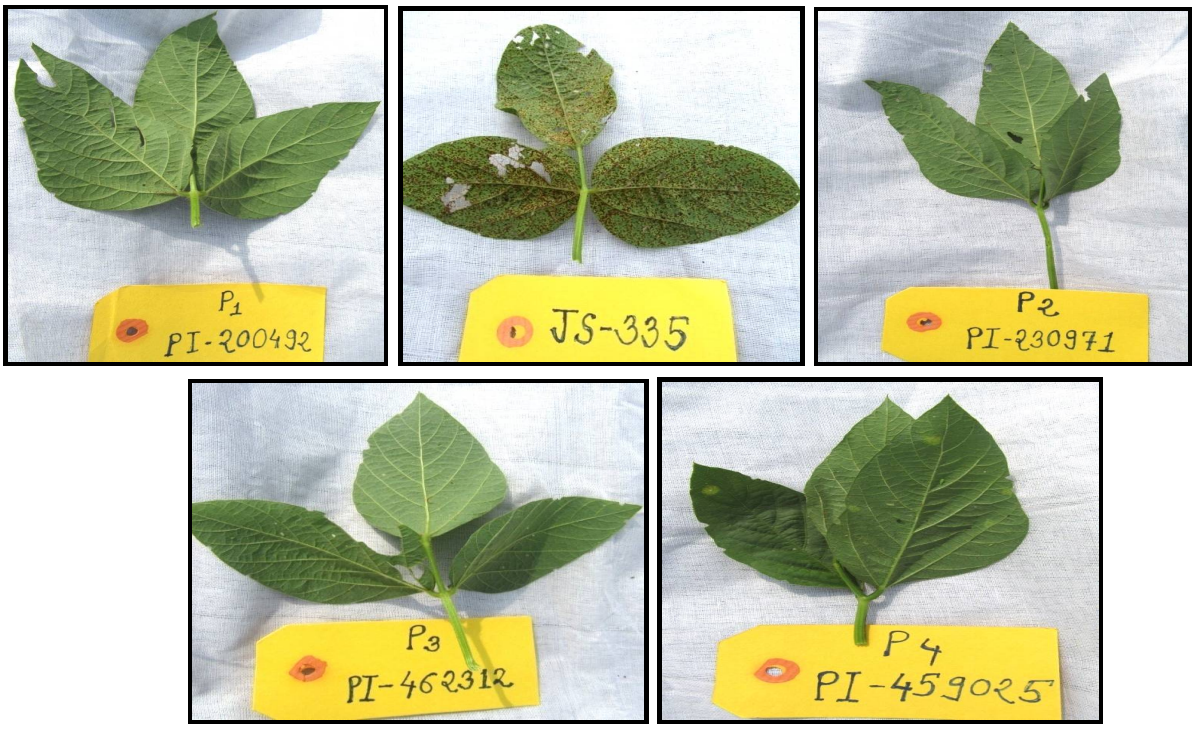

Fig. 1. Parental responses to soybean rust across lesion types as compare to control (susceptible cultivar JS-335).

cross hybrids (F_{1s}), 276-281 F_{2s} plants and 25- 30 plants in double cross hybrid generation ($DCHF_1$). Plants were classified in to resistant (R) and susceptible (S) on the basis of colour of rust pustules as immune, non-sporulating Reddish Brown (RB) Lesions (incomplete resistance) and profusely sporulating TAN lesions (susceptible) as well as in their coverage intensity on 0-9 scale by Mayee and Datar (1986) in table 1.

RESULTS AND DISCUSSION

Scoring for rust resistance was done as per the immune, reddish brown (RB) lesions or resistant reaction and TAN lesions or susceptible reaction to rust. Out of four parent genotypes used *Rpp1* gene donor PI 200492 (Komata) was found to give immune reaction with leaf rust. *Rpp2* and *Rpp4* gene donor genotypes PI 230971, and [PI 459025 (Bing Nan)] were resistant to leaf rust with RB lesion (incomplete resistance). *Rpp3* gene donor PI 462312 (Ankur) gave a few, highly localized patches of TAN lesions (hereafter referred to as TAN-localized) that were detected only on the abaxial (lower) leaf surface localized TAN lesions (Figure 1). But it was not as severely susceptible as susceptible check JS335 which had TAN-colored lesions uniformly covering both the leaf surfaces.

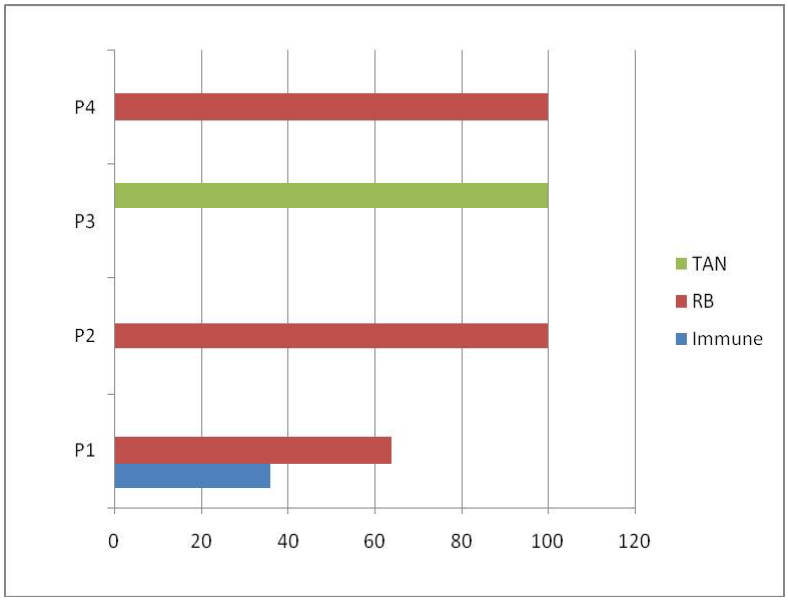

Fig. 5. Percent distribution of rust lesion types in parents.

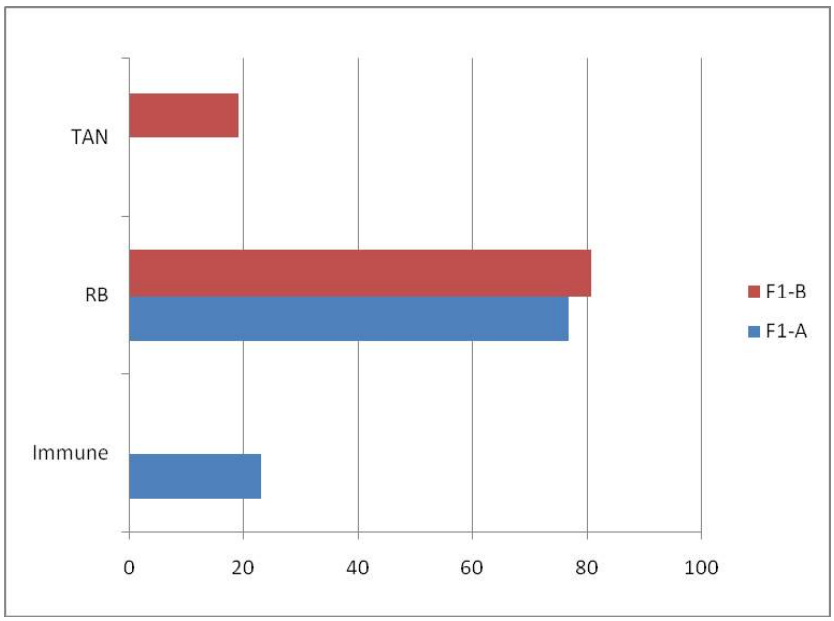

Fig. 6. Percent distribution of rust lesion types in both F_1s.

Lesion types with disease grades in Cross-A: [Komata (PI 200492) × PI 230971]

Out of the total 36 P_1 plants, 13 plants (36.1 %) recorded 0 grade showing immune reaction. Rest 63.9% showed reddish brown lesions with 18 plants recording 1 rust grade and 5 plants recording 3 rust grade resistant reaction to rust. The average disease grade of parent Komata was 0.92. All 36 P_2 (PI 230971), recorded RB lesions with resistant reaction of 1.28 average grade to rust. Out of 36 plants, 31 and 5 plants recorded 1 and 3 disease grade, respectively. Out of 78 F_1 of cross-A plants, 18 plants (23.1%) were immune (0 grade) and rest 60 (76.9 %) were RB lesioned. The F_1 with 1.15 average disease grade recorded 45 plants of 1 grade and 15 plants of 3 grade resistant reaction to soybean rust. F_2 generation had average disease grade 1.21 with 82.9% plants being RB lesioned. 656, 89 and 1 plant recorded 1, 3 and 5 grade soybean rust resistance, respectively. Ninety-nine F_2 plants (11 %) out of 900 plants were immune to rust. Rest 55 F_2 plants (6.1 %) were TAN lesioned recording 3 disease grade susceptible reaction to soybean rust (Table 2 and Table 5).

Lesion types with disease grades in Cross-B: [PI 462312 (Ankur) × PI 459025 (Bing Nan)]

All plants of parent P_3, recorded localized TAN lesion

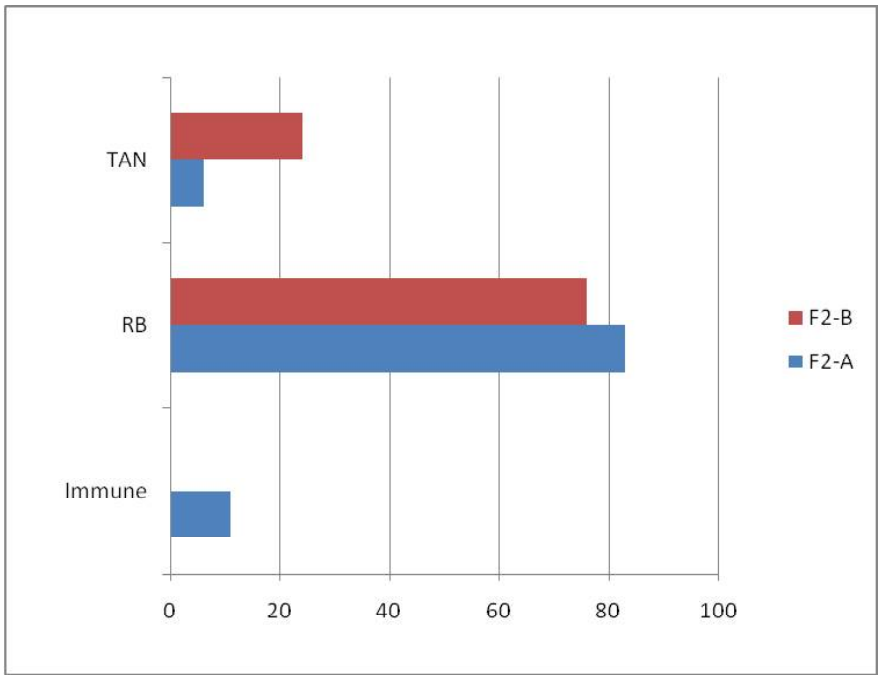

Fig. 7. Percent distribution of rust lesion types in both F_2s.

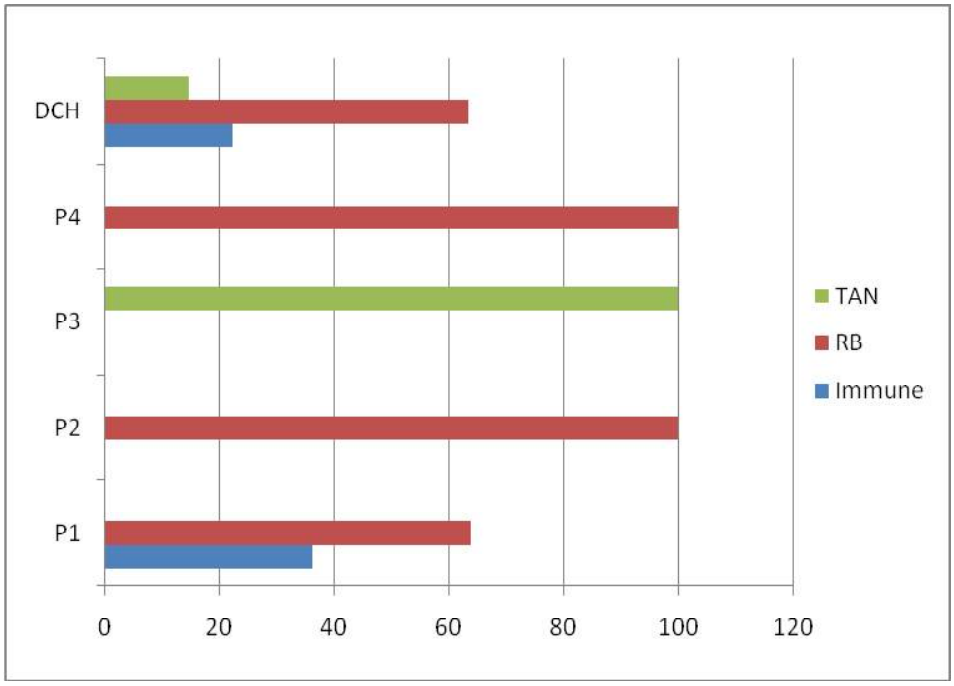

Fig. 8. Percent distribution of rust lesion types in DCH and parents.

with an average disease grade of 1.83. Out of its total 36 plants, 28, 3, 3 and 2 plants recorded disease grade of 1, 3, 5 and 7, respectively. Unlike JS 335 which had TAN lesions covering almost entire plant with 9 score, PI 462312 (Ankur) had TAN lesions with lower grade of 1.83 indicating it as moderately susceptible with some additional defense mechanism. Parent P_4 had 100 % RB lesioned resistant plants with an average grade of 1.28. Out of 36 plants, 31 and 5 plants recorded 1 and 3 rust grade, respectively. In F_1, out of 78 plants, 62 and 1 plants showing reddish brown lesions recorded disease grade of 1 and 3, respectively. Among rest 15 TAN lesion plants, 8 plants recorded 3 grade localized TAN lesions. 3 plants recorded 5 grade, 4 plants recorded 7 grade susceptible reactions to Asiatic soybean rust. Thus these F_1s recorded an average grade of 1.69 with 80.8 % plants being RB lesioned resistant while rest 19.2 % being TAN lesioned susceptible. In F_2 generation with a low disease grade of 1.65, 75.9 % plants recorded reddish brown reaction and 24.1 % plants recorded TAN lesioned susceptible reaction to rust. Out of 900 F_2 plants, 683 plants were RB lesioned with 670, 12 and 1 plants recording 1, 3 and 5 rust grade respectively. Among rest 217 TAN lesioned plants, 53, 68, 77 and 19 plants had disease grade of 1, 3, 5 and 7, respectively (Table 3 and Table 5).

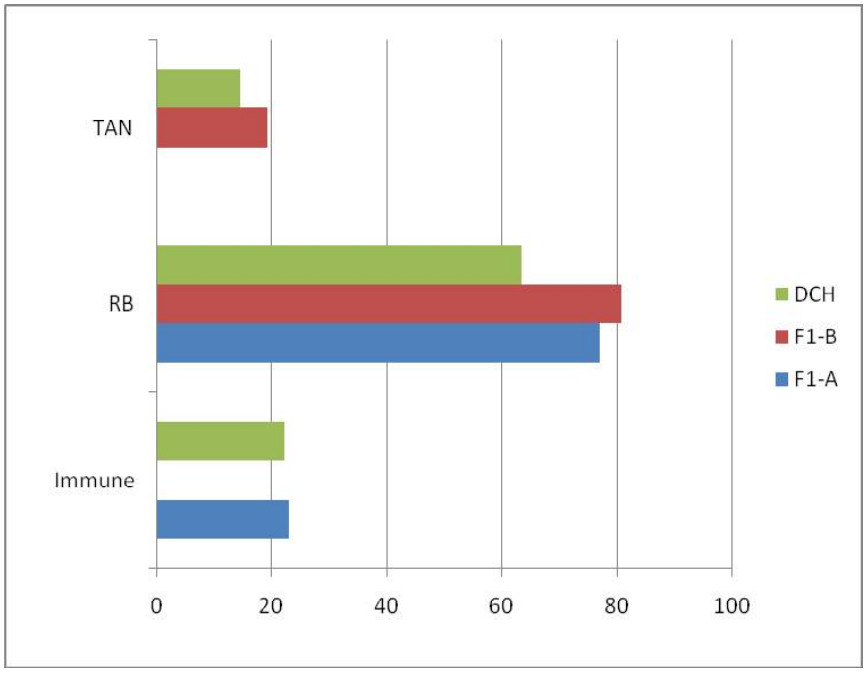

Fig. 9. Percent distribution of rust lesion types in DCH and both F_1s.

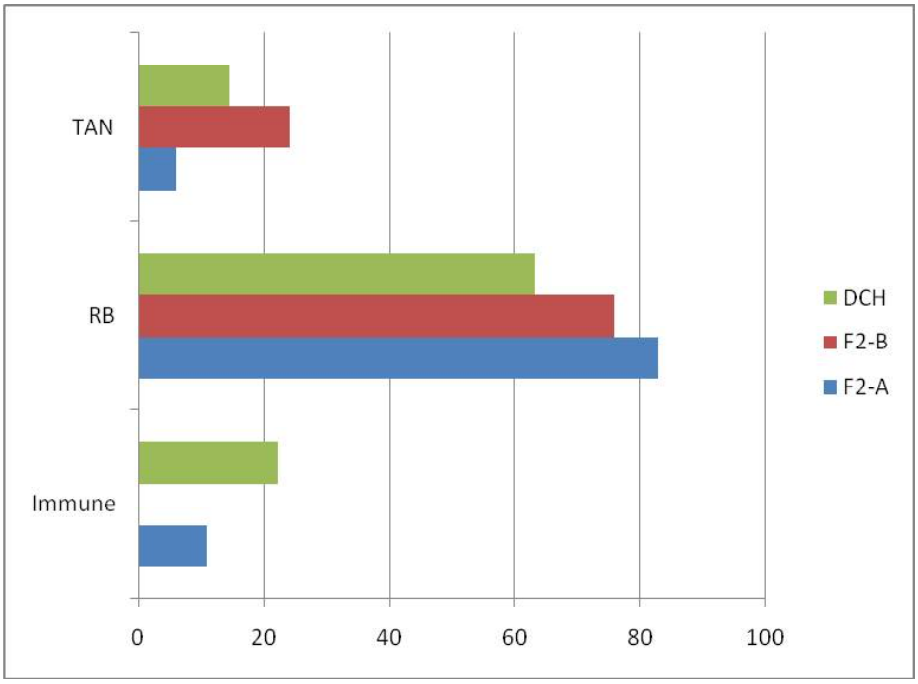

Fig. 10. Per cent distribution of rust lesion types in DCH and both F_2s.

Lesion types with disease grades in cross-C: (F_1 of cross-A × F_1 of cross-B)

In F_1 of cross-A out of 78 plants studied, 18 plants recorded no infection (0 disease grade) showing immune reaction to rust. 45 plants recorded 1 disease grade and 15 plants recorded 3 disease grade showing reddish brown lesions and recorded resistant reaction to rust. In F_1 of cross-B, out of 78 plants, 62 and 1 plants recorded grade of 1 and 3, respectively with reddish brown lesions indicating resistance to rust. The remaining 15 plants were TAN lesioned, out of which 8, 3 and 4 plants showed rust grade of 3, 5 and 7, respectively and therefore were recorded susceptible to rust. In double cross hybrid (DCH) out of 90 plants, 20 plants recorded 0 disease grade (immune reaction to rust), 56 and 1 plants recorded 1 and 3 disease grade (reddish brown lesioned and resistant to rust). The remaining plants showed TAN lesions and susceptible reaction to rust of soybean of which 2, 6 and 5 plants recorded disease grade of 3, 5 and 7, respectively (Table 4) being TAN lesioned susceptible. Thus in DCH, 22.2 % plants had no infection (immune), 63.3 % plants recorded RB lesions, while 14.5 % plants recorded TAN lesions with 1.44 average disease grade (Table 4 and Table 5).

Genetic resistance is an economically and strategically important means of controlling soybean rust disease (Arias *et al.*, 2008). Eight *Rpp* genes have been identified including *Rpp1* (McLean and Byth, 1980), *Rpp2* (Bromfield and Hartwig, 1980), *Rpp3* (Ankur) (Bromfield and Melching, 1982; Hartwig and Bromfield, 1983), *Rpp4* (Hartwig, 1986); *Rpp5* (Garcia *et al.,* 2008) , *Rpp6* (Li *et al.,* 2012), *Rpp1-b* (another allele at the *Rpp1* locus) (Chakraborty *et al*. 2009), and *Rpp* (*Hyuuga*) (An allele at the *Rpp3* locus) (Silva *et al.,* 2008). However, the threat posed by soybean rust to soybean production is worsened by resistance breakdown associated with single gene resistance present in most cultivars. Therefore attempts are being made to enhance the rust resistance against multiple isolates by bringing together multiple *Rpp* genes into a single background. Gene pyramiding involves assembling multiple desirable genes into a single genotype to overcome narrow range resistance /instability conferred by single gene resistance to soybean rust (Lemos *et al*., 2011).

Our aim for pyramiding of four rust resistance genes (*Rpp*1, *Rpp*2, *Rpp*3, *Rpp*4) in this study was to enhance soybean rust resistance to pathogen isolates and broaden the genetic base for rust resistance in the available soybean cultivar. In the present study *Rpp1* gene donor PI 200492 (Komata) was found immune, *Rpp2* and *Rpp4* gene donor, PI 230971 and PI 459025 (Bing Nan) respectively were resistant with RB lesion (incomplete resistance); while *Rpp3* gene donor PI 462312 (Ankur) produced highly localized TAN lesions on the lower leaf surface. But Ankur was not as susceptible as check JS335 which had TAN-colored lesions uniformly covering both the leaf surfaces resulting in almost complete leaf drop. PI 200492 has been identified as donor of *Rpp1* gene conferring immunity (McLean and Byth, 1980), while PI 230970, PI 462312 (Ankur) and PI 459025B have been reported as donors of *Rpp2* (Bromfield and Hartwig, 1980), *Rpp3* (Bromfield and Melching, 1982; Hartwig and Bromfield, 1983) and *Rpp4* (Hartwig, 1986) genes that conferred hypersensitive response. Mahajan (2015) had also reported that under controlled glass house conditions resistance by *Rpp2* (from PI230970 and PI230971) and *Rpp4* (from PI459025 B/F) genes worked against isolates from different conditions with *Rpp1* gene resistance (from Komata) was broken only in western Maharashtra state, while *Rpp3* gene resistance (from Ankur) was broken in entire Maharashtra state.

Scoring for rust resistance was done with distribution of rust grade as per the lesion type *viz.* immune, reddish

brown (RB) lesions or resistant reaction and TAN lesions or susceptible reaction to rust. Out of four parent genotypes used *Rpp1* gene donor PI 200492 (Komata) was found to give immune reaction to rust and the average disease grade of parent Komata was 0.92.

In cross-A (*Rpp1* × *Rpp2*), F_1 with average 1.15 rust grade had quarter of plants showing no infection, while rest three quarters showed RB lesions typical of partial rust resistance. In its F_2, most of the population showed either RB or immune reaction. Because of segregation 6.1 per cent plants showing susceptible reaction to rust were also recorded. In cross-B (*Rpp3* × *Rpp4*) with most of F_1 plants (80.8 %) recorded RB lesions with almost all of them having 1 grade, however few exceptional RB plants recorded 3 to 5 rust grades. Few plants recorded as susceptible TAN reaction with grade range from 3 to 7. In the F_2s, almost three quarter of them had RB lesions with 1 rust grade, with few exceptional RB plants having 3 to 5 rust score. Rest quarter of F_2 plants had TAN lesions with range of 1 to 7 rust grade. In cross-C i.e. double cross hybrid [(*Rpp1*× *Rpp2*) × (*Rpp3* × *Rpp4*)] majority of them (62.2 %) recorded 1grade RB lesions and some plants (22.2 %) had no infection. Just 1.1% of DCH plants had 3 rust grade. Rest 14.5 % plants recorded susceptible TAN reaction with 3 to 7 rust grades. In soybean limited references such as Maphosa *et al.* (2012), Yamanaka *et al*. (2015) are available and they suggested marker assisted gene pyramiding for broaden the genetic resistance in soybean. Maphosa *et al.* (2012) observed complementary epistatic gene action with substantially increase resistance to soybean rust through reduced severity and reduced sporulating lesions in the soybean plants with two gene combinations. Yamanaka *et al.* (2015) observed significantly higher resistance in pyramided lines carrying multiple *Rpp* genes. Although the resistance donor sources exhibited susceptible phenotypes, highly resistant phenotypes with almost no sporulation were observed in the three *Rpp*-pyramided lines. Therefore, pyramided lines carrying *Rpp* gene combinations are useful in soybean breeding for conferring broad spectrum, high resistance to ASR isolates that are virulent to the varieties carrying monogenic resistance. Bhor *et al*. (2014) demonstrated that markers linked to both *Rpp1b* like and *Rpp4* genes were present in resistant segregating F_2 plants of soybean thereby contributing complementarily to rust resistance. They further observed that the parent EC 241780 used by them is gene pyramided and thereby gives more durable resistance.

CONCLUSION

Out of four parent genotypes used *Rpp1* gene donor PI 200492 (Komata) was immune to rust with disease grade 0.92. *Rpp2* and *Rpp4* gene donors PI 230971, and [PI 459025 (Bing Nan)] with both 1.28 disease grade had RB lesions (incomplete resistance). *Rpp3* gene donor PI 462312 (Ankur) gave a few, highly localized patches of TAN lesions with disease grade 1.83 (Plate 5), however it was not as severely susceptible as susceptible check JS335. In cross-A, all the F_1s produced recorded rust resistance with 1.15 grade, while in the F_2, 93.9 % plants were either immune or RB reaction and rest 6.1% plants were susceptible reaction. In cross-B, 80.8 % F_1 plants recorded RB lesions and resistant reaction to rust and while few plants (19.2 per cent) recorded susceptible. In the F_2 population with overall 1.69 grade, 24.2 per cent of plants recorded rust susceptible because of segregation. In cross-C, i.e. among double cross hybrids with 1.44 grade because of segregation 85.5 % plants recorded either immune or RB reaction to rust while rest 14.5 per cent plant recorded susceptible reaction.

ACKNOWLEDGMENT

The first author is grateful to the Post Graduate Institute Research Farm, Department of Agricultural Botany, Mahatma Phule Krushi Vidyapeeth, Rahuri, Ahmednagar, Maharashtra and Agriculture Research Station (ARS), Kasbe-Digraj, Sangli (Maharashtra), India for providing valuable facilities for the present investigation.

LITERATURE CITED

Arias CAA, Toledo JFF, Almeida LA, Pipolo AE, Carneiro GES, Abdelnoor RV, Ribeiro AS 2008. Asian rust in Brazil: Varietal resistance. *JIRCAS Working Report*, **58**:29-30.

Bhor TJ 2014. Genetic analysis of yield components and rust resistance in soybean [*Glycine max* (L.) Merrill]. A Ph.D. thesis submitted to Mahatma Phule Krishi Vidyapeeth, Rahuri.

Bonde MR, Nester SE, Austin CN, Stone CL, Frederick RD, Hartman GL, Miles MR (2006). Evaluation of virulence of *Phakopsora pachyrhizi* and *P. meibomiae* isolates. *Plant Dis*. **90**:708–716.

Bromfi eld, K.R. 1984. Soybean rust. Monogr. 11. Am. Phytopathological Soc., St. Paul, MN.

Bromfield KR, Hartwig EE 1980. Resistance to soybean rust and mode of Inheritance. *Crop Sci*. **20**: 254-255.

Bromfield KR, Melching JS 1982. Sources of specific resistance to soybean rust. *Phytopathol*. **72**: 706.

Carlson JB, Lersten NR 1987. Reproductive morphology. In: Wilcox JR, ed. Soybeans: improvement, production and uses. 2nd edn. Madison: American Society of Agronomy and Academic Press, pp. 95–133.

Chakraborty, N., Curley, J., Frederick, R. D., Hyten, D. L., Nelson, R. L., Hartman, G. L., & Diers, B. W. 2009. Mapping and Confirmation of a New Allele at from Soybean PI 594538A Conferring RB Lesion–Type Resistance to Soybean Rust. *Crop science*, **49** (3), 783-790.

Garcia, A., Calvo, É. S., de Souza Kiihl, R. A., Harada, A., Hiromoto, D. M., & Vieira, L. G. E. 2008. Molecular mapping of soybean rust (Phakopsora pachyrhizi) resistance genes: discovery of a novel locus and alleles. *Theoretical and Applied Genetics*, **117** (4), 545.

Hartwig EE, Bromfield KR 1983. Relationship among three genes conferring specific resistance to rust in soybeans. *Crop Sci*. **23**: 237- 239.

Hartwig EE 1986. Identification of a fourth major gene conferring resistance to soybean rust. *Crop Sci.* **26**: 1135-1136.

Kato M, Yorinori JT 2008. A study on race composition of *Phakopsora pachyrhizi* in Brazil: a difficulty of race identification. In: Kudo H et al (eds) JIRCAS working rep no. 58: facing the challenge of soybean rust in South America. Japan International Research Center for Agricultural Sciences (JIRCAS), Tsukuba, pp 94–98.

Langenbach C, Campe R, Beyer SF, Mueller AN, Conrath U 2016.

Fighting Asian soybean rust. *Front. Plant Sci.* **7** (236):797. DOI:10.3389/fpls.2016.00797.

Lemos, N. G., e Braccini, A. D. L., Abdelnoor, R. V., de Oliveira, M. C. N., Suenaga, K., & Yamanaka, N. 2011. Characterization of genes Rpp2, Rpp4, and Rpp5 for resistance to soybean rust. *Euphytica*, **182** (1):53.

Li, S., Smith, J. R., Ray, J. D., & Frederick, R. D. 2012. Identification of a new soybean rust resistance gene in PI 567102B. *Theoretical and Applied Genetics*, **125** (1), 133-142.

Mahajan SB 2015. Marker assisted selection for rust resistance in soybean. A Ph.D. thesis submitted to Dept. of Plant Pathology. and Agricultural Microbiology, Mahatma Phule Krishi Vidyapeeth, Rahuri.

Maphosa M, Talwana H, Tukamuhabwa P 2012. Enhancing soybean rust resistance through *Rpp2*, *Rpp3* and *Rpp4* pair wise gene pyramiding. *Afr. J. Agric. Res.* **7** (30): 4271-4277.

McLean, R. J., & Byth, D. E. 1980. Inheritance of resistance to rust (*Phakopsora pachyrhizi*) in soybeans. *Crop and Pasture Science*, **31** (5), 951-956.

Miles MR, Frederick RD and Hartman G 2003. Soybean rust: is the U.S. soybean crop at risk? In APSNET Feature. American Phytopathological Society. Available on http://www.apsnet.org/online/feature/rust/ Access on 11 July 2007.

Mayee CD, Datar VV 1986. Phytopathometry. Technical Bulletin-1(Special Bulletin-3) Marathwada Agricultural University, Parbhani pp. 95.

Rossi RL 2003. First report of *Phakopsora pachyrhizi*, the causal organism of soybean rust in the province of Misiones, Argentina. *Plant Disease* **87:** 102.

Silva, D. C., Yamanaka, N., Brogin, R. L., Arias, C. A., Nepomuceno, A. L., Di Mauro, A. O. & Abdelnoor, R. V. 2008. Molecular mapping of two loci that confer resistance to Asian rust in soybean. *Theoretical and Applied Genetics*, **117** (1), 57-63.

Yamanaka N, Morishita M, Mori T, Lemos NG, Hossain Md. M, Akamatsu H, Kato M, Yamaoka Y 2015. Multiple *Rpp*-gene pyramiding confers resistance to Asian soybean rust isolates that are virulent on each of the pyramided genes. *Trop. Plant Pathol.* **40** (5):283-290.

Yorinori JT, Paiva WM, Frederick RD, Costamilan LM, Bertagnolli PF, Hartman GL, Godoy CV, Nunes JJ 2005. Epidemics of soybean rust (Phakopsora pachyrhizi) in Brazil and Paraguay from 2001 to 2003. *Plant Dis.* **89**:675-677.

Received on 03-02-2017 *Accepted on 09-02-2017*