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ALIDATION OF SIMPLE SEQUENCE REPEATS MARKERS FOR CHARCOAL ROT AND *RHIZOCTONIA* ROOT ROT IN SOYBEAN GENOTYPES

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ABSTRACT
Soybean is a significant legume in a world's oilseed cultivation scenario. Among biotic stresses Charcoal rot and *Rhizoctonia* root rot are the prime causes for an enormous loss in soybean production and still there is no prominent work has been carried out effectively to address this problem. The present investigation was carried out with the objective to characterize soybean genotypes for yield and its attributing characters and validation of gene-based SSR markers against charcoal rot and *Rhizoctonia* root rots diseases. On the basis of divergent traits, genotypes *viz.*, JS335, JS 20-69, JS 97-52, KDS980 and KDS992 were found to be the most divergent and promising genotypes and may be used as parents in future hybridization programme to develop resistance /tolerance against *Rhictonia* root rot and charcoal rot by using conventional and/or molecular breeding methods.

KEYWORDS: Molecular breeding, Hybridization, Fungal diseases, Oilseed, SSR markers.

INTRODUCTION

Soybean [*Glycine max* (L.) Merr] (2n=40) is an economically important dicot legume in a world's oilseed cultivation scenario, having a prominent position in terms of high productivity, profitability and maintaining soil fertility too (FICCI, 2014). On account of its multifarious uses and limitless benefits, soybean is rightly called as "golden bean", "miracle bean" or "wonder crop" of the 20th century (Orf, 2010). Soybean contributes to the economy and foreign earnings of our country as it contributes 42% and 25% to the national oilseeds and edible oil production, respectively. It contains essential amino acids particularly glycine, tryptophan and lysine, similar to cow's milk and animal proteins. India occupied 4th in terms of global soybean production area, 11 million ha and 5th in production (11 million metric tons) after United States, Brazil, Argentina and China (USDA, 2018-19). In India Madhya Pradesh, Maharashtra, Rajasthan, Karnataka, and Andhra Pradesh are major soybean-growing states that contributed 96% of production in decreasing order of production. 99% areas under soybean is rainfed (Sky Met Whether Services, 2017).

Broad spectrum biotic stress which reduces both yield and seed quality is Charcoal rot caused by the soil borne polyphagous fungus *Macrophomina phaseolina* (Tassi) Goid (Goidanish, 1947) this was first observed in the United States in 1949 (Young, 1949). Infection occurs in all plant parts of susceptible soybean. Infection initially starts in the roots which eventually spread to the whole plant. The yield loss can go up to 80% in severe cases (Yang and Navi, 2005). The second most destructive biotic stress is *Rhizoctonia* root and hypocotyl rot, caused by *Rhizoctonia solani* (Yang, 1996). Losses 35% is reported with *Rhizoctonia* root rot in epidemic conditions. Still there is no prominent work has been carried out to effectively encounters these problems. As such more concentrated efforts are required to direct towards to work upon this aspect using different approaches. Biotic stress can be controlled by using an integrated management approach either by cultural practices including crop rotation, tillage, irrigation, or chemical control like seed treatments could be used to minimize damage caused by fungal pathogen in soybeans; however, none of these of practices has been implemented for controlling the disease (Mengistu *et al*., 2007; Twizeyimana *et al*., 2012).So development of resistant varieties is an effective means of disease controlling on the basis of eco-friendly, cost benefit and easily available and use for farmer in production purpose. Still we are lacking of resistance varieties but moderately resistant cultivars are currently commercially available (Twizeyimana *et al*., 2012).

Screening is first step for selection of desired parents to make crosses and develop resistant variety with genetic background of popular variety. For development of resistant variety either conventional method or marker assisted selection approach can be applied but conventional method needs more time and highly affected by environment, generation after generation we have to fix the characters for a particular genotype and environment interaction and the whole process including efficient labors with more screening. Meanwhile marker assisted selection is an indirect selection process where a trait of interest is selected based on marker (morphological, biochemical or DNA/RNA variation) linked to a trait of

interest. Considering above facts in mind, the present investigation was carried out with the objective to characterize soybean genotypes for yield and attributing characters and validation of gene-based SSR markers against charcoal rot and *Rhizoctonia* root rots diseases.

MATERIALS AND METHODS

Plant material

A set of 53 genotypes of soybean (Table 1) were used for screening against charcoal rot and *Rhizoctonia* root rot diseases.

TABLE 1: List of soybean genotypes with their parentage

S. No.	Genotypes	Source/Pedigree	S. No.	Genotypes	Source/Pedigree
1.	JS 20-29	JS 97-52 x JS 95-56	28.	RSC-10-52	NRC 37X JS335
2.	JS 20-69	JS 97-52 x SL 710	29.	SL -1123	Selection from AGS751
3.	JS 335	JS 78-77 x JS 71-05	30.	SL-1068	SL755XSL525
4.	JS 20-98	JS 97-52x JS SL710	31.	AGS 111	Germplasm accession
5.	JS 20-94	JS 97-52 x JS 20-02	32.	EC457286	Germplasm accession
6.	JS 93-05	Selection from PS 73-22	33.	MACS725	JS93-05X MAUS71
7.	JS 20-116	JS 97-52 x JSM 120 A	34.	SP 37	Not known selection
8.	JS 95-60	Selection from PS 73-22	35.	NRC -125	EC54688xps1044
9.	JS 97-52	PK 327 x L 129	36.	NRC-132	JS97-52X PI086023
10.	JS 20-84	JS 98-63 x PK 768	37.	NRC-134	NRC7XAGS191
11.	JS 20-34	JS 98-63 x PK 768	38.	NRC SL-1	JS335XSL525
12.	JS 20-71	JS 97-52 x JS 90-5-12-1	39.	PS 1092	PS1042 x MACS 450
13.	RVS 2007-6	JS 20-10 x MAUS162	40.	PS 1613	PS1225XPS1042
14.	RVS 2011-35	JS 335 X PK 1042	41.	AMS 2014-1	AMS99-33XH6P5
15.	RVS 2001-4	JS 93-01x EC 390981	42.	KDS 992	JS93-05XEC241780
16.	RVS -14	JS 93-05x EC 390981	43.	VLS -94	VL Soya59X VS2005-1
17.	RVS -24	J.P 120 x JS 335	44.	SKF-SPS -11	Not known selection
18.	RVS -18	JSM110XJSM66	45.	RVS 76	MAUS-162XJSM-66
19.	NRC- 76	NRC-37XL-27	46.	NRC127	JS97-52XPI542044
20.	NRC -86	RKS15XEC481309	47.	KDS980	JS93-05XAMS1
21.	NRC- 130	EC390977XEC538828	48.	G-29	Germplasm
22.	NRC -131	EC390977XEC538828	49.	RSC-10-70	JS335X Bragg
23.	NRC -147	Germplasm accessions C210	50.	RSC-10-71	Bragg XJS335
24.	AMSMBC -18	Mutant of Bragg	51.	NRC-2	Induced mutant of Bragg
25.	AMS-100-39	Mutant of JS93-05	52.	MACS-15-20	NRC37XMohetta
26.	MACS – 1520	EC241780XMACS330	53.	MACS-58	JS2 x Improve pelican
27.	MACSNRC-1575	PI542044XJS9305			

These genotypes were collected from Jawaharlal Nehru Agricultural University, Jabalpur and Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior. The genotypes having divergent reactions against both fungal diseases *via*: susceptible, tolerant and resistant reaction towards charcoal rot and *Rhizoctonia* root rot. The field experiments were conducted at Research Farm and molecular work was carried out at Department of Plant Molecular Biology & Biotechnology, College of Agriculture, RVSKVV, Gwalior (M.P.).

Morpho-physiological studies

Among morpho-physiological parameters plant height (cm), number(s) of primary branches per plant and leaf area (cm^2 / plant) with the help of Automatic Leaf Area Meter were measured.

Postharvest studies

Among post harvest parameters the number(s) of pods per plant, number(s) of seed per pod, yield per plant (g), biological yield/plant (g) and harvest index (%) were analyzed. Harvest index was worked out by the formula given by Donald and Hamblin (1976).

Molecular characterization

Genomic DNA isolation from young leaves of soybean genotypes was carried out by modified CTAB methods (Murray and Thompson, 1980). Extracted DNA was purified and quantified with the help of Nanodrop spectrophotometer. Quantified DNA samples were diluted upto the concentration of 25ng/ µl. Highly polymorphic linked SSR markers four for *Macrophomina phaseolina* (Table: 2) and five for *Rhizoctonia solani* (Table: 2) were used to screen out 53 soybean genotypes. The primers were synthesized by Integrated DNA Technologies. Polymerase chain reaction was performed in 10µl reaction mixture comprising of 10X dream *Taq* buffer (1µl), 5U/µl *Taq* DNA polymerase, 15µl dNTP (1mM), 6.75µl NFW, 0.5 µl of forward and reverse primers each (10 pM) and 50 ng/µl of genomic DNA in a Thermocycler (BIO-RAD). Following parameters were followed amplify the templates: Initial denaturation at 94°C for 1 min followed by 35 cycles of denaturation 94°C for 40sec (denaturation), annealing 52-55 °C for 90 sec, extension at 72°C for 60 sec. The final extension was performed at 72°C for 7 min.

TABLE 2 Molecular markers used for screening against *Rhizoctonia* root rot and Charcoal rot

Primer	Forward 5'-3'	Reverse 5'-3'
Satt281	AAG CTC CAC ATG CAG TTC AAA AC	TGC ATG GCA CGA GAA AGA AGT A
Satt177	CGT TTC ATT CCC ATG CCA ATA	CCC GCA TCT TTT TCA ACC AC
Satt245	AAC GGG AGT AGG ACA TTT TAT T	GCG CCT CCT GAA TTT CAA AGA ATG AAG A
Sat_232	GCG CGT CCT TTA TTT AAT TTA ATA TGA A	GCG TGG CTT TGC TAA TAA TGA ATG AT
Sct_028	TCGCCGGTACAAAAG	CGAATGAACAAACA
Satt512	AACGTCTTCAAGTCAAGTGCCTACA	GCCCACATAGTTTTCATTTTTCTCCA
S60211-TB	GAAGATCCTAACACGATGGCCG	TTCGTTGTTTCCTTCATTGCCG
Sat_117	TTTGGCAGTTTCTTGTAG	GCTGGATCGCAGTTA
S63880-CB	AGTCCTCCTCGCCAACAACAAC	TTCATTTCATTTCCAAGCGGT

Data analysis

The genetic profile of 53 soybean genotypes was scored on the basis of difference in allele size using SSR markers. The major allele frequency, polymorphism information content (PIC) and genetic distance based clustering was performed with Unweighted Pair Group Method for Arithmetic average (UPGMA) tree using Power Marker v3.25 software (Liu and Muse, 2005) and the dendrogram was constructed using MEGA 4.0 software (Tamura, Dudley, Nei and Kumar, 2007). SSR data was again subjected to cluster analysis followed by bootstrap analysis with 1000 permutations for all the genotypes using Mega 4.0 software. The population structure for 53 soybean genotypes comprising between genotype and susceptible and resistance one was inferred using Structure 2.3.4 software (Pritchard, Stephens & Donnelly, 2000). The structure outputs were visualized using Structure Harvester from which Evanno plots were constructed (Earl, & von Holdt, 2012). Duncan's Multiple Range Test (DMRT) (P =0.05) was used to evaluate differences among clusters for significance by using SPSS ver. 19.0 software.

Results and Discussion

The presence of significant variability and its mode of inheritance for high grain yielding and its attributing characters will be helpful to achieve target in crop improvement. This study was carried out to screen tolerant/resistant genotypes of soybean against *Rhictonia* root rot *and* charcoal rot for future improvements in soybean.

Morpho-physiological characterization

Plant height ranged between 43.47cm (NRC-2) to 111.01cm (NRC- 76) with average of 69.90 cm. Genotypes NRC-76 and RVS-76 were proved significantly taller over other genotypes. Days to flower initiation were varied between 35.50 days (JS 20-34) and 45.50 days (AMS-MS- 58) with mean value 38.21 days. Genotypes JS 93-05 followed by MACSNRC-1575, JS 20-94, NRC- 76 and SP 37 demonstrated significantly early for flowering initiation. Days to 50% flowering was ranged between 43.5 days (JS 20-98) and 54.0 days (AMS-MS-58) with mean value as 46.95 days. Genotype RVS 2007-6 intimately followed by genotypes SL -1123, JS 20-94, NRC- 76, NRC -131, SP 37, NRC SL-1, KDS 992 and NRC -125 significantly initiated early 50% flowering. Days to maturity was recorded in range of 65.5 days (SL-1068) to 92.5 days (JS-93-05) with mean value of 76.07 days. Genotypes JS 95-60, NRC -131and NRC -125 performed significantly early maturity. Number(s) of primary branches per plant was varied between 1.55 (MACSNRC-1575) and 8.0 (NRC-76) with mean value 5.22. Genotypes NRC- 76, NRC -131 and AMSMBC-18 produced significantly maximum numbers of primary branches. Leaf area was measured in range between 12.85 cm^2 (PS-1092) to 78.03 cm^2 (NRC-131) with mean value 35.93 cm^2.Genotype AMS-100-39 closely followed by genotypes MACSNRC-1575 and NRC-131 were occupied significantly more leaf area. Chlorophyll content was varied between 2.2350 mg/ml (NRC-76) to 14.4 mg/ml (MACS-725) with mean value 7.38 mg/ml. Genotypes RVS 2001-4 intimately followed by genotypes RVS -14 and MACS725 showed significantly higher chlorophyll a content. Chlorophyll b content was ranged between 1.03 mg/ml (VLS-94) and 25.25 mg/ml (JS-20-94) with mean value 10.39 mg/ml. Genotypes JS20-69 and RVS-14 synthesized significantly higher magnitude of chlorophyll b content. Numbers of seed per pod was varied in range of 1.65 (MAS-MS-58) to 3.8 (JS-95-60). Genotypes JS 93-05 and JS 95-60 showed significantly higher numbers of seed per pod. Numbers of pods per plant was ranged between 6.20 (NRC-2) to 45.23 (JS-335) with mean value 23.56. Genotypes JS20-29, AMS-MS-58 and JS 335 produced significantly higher numbers of pod per plant. Yield per plant was ranges from 14.51g (PS-1613) to 106.05g (JS20-29) with mean value 56.47. Genotypes KDS980, JS 335, JS 97-52 and JS 20-29 gave significantly higher yield. The average value of biological yield per plant was ranged between 68.0g (JS-20-71) to 210g (JS-20-29) with mean value 115.82g. Genotypes KDS980, JS335 and JS 20-29 produced significantly higher biological yield. The average value of harvest index was recorded as 48.31with extent of dispersion from 21.11 (SP-37) to 64.82(JS 97-52). Genotypes RSC-10-71 and JS 97-52 showed significantly higher harvest index.

Characterization of genotypes using SSR markers

Biological approaches for the control of soil-borne diseases are not very successful due to diverse factors, such as variability in performance and poor efficacy under optimal conditions for disease development, stemming from the complex and dynamic host plant × pathogen × biocontrol agent × environment interactions (Nelson *et al*., 2004). Due to non-availability of root rot resistant cultivars has made the use of chemicals an inescapable necessity. As such, genetic improvement by breeding for resistance is the only tenable option. Genetic resistance is the most effective, cost-efficient and environment friendly method for disease control (Hogenboom, 1993). Plant breeders have mostly relied on germplasm resources and crop wild relatives for useful genetic variants to create

novel gene combinations in crop improvement programmes (Brown *et al*., 1989).

Modern breeding approaches such as marker-assisted selection (MAS) are more efficient and precise for targeted trait improvement. In the present study a set of five genes linked SSR molecular markers were used for validation of resistant gene linked with *Rhizoctonia* root rot across fifty-three soybean genotypes. Among five SSR primers Satt693 was found to be monomorphic and remaining four was polymorphic. Similar results have also been documented in a previous study of Zhao *et al*. (2005) with soybean genotypes where three SSR markers, *viz*., Satt281, Satt177 and Satt245 were found to be linked with *Rhizoctonia* root rot resistant gene in segregating population. The primer which showed highest gene diversity and PIC values was Satt232 while the lowest gene diversity and PIC values were observed for the primer Satt245. Gene diversity varied in range of 0.69 (Satt281) to 0.49 (Satt245). The major allele frequency ranged between 0.3 (Satt232) to 0.5 (Satt245) with a mean value of 0.4646. Zhao *et al*. (2005) reported parallel results with higher PIC and gene diversity values in three SSR markers *i.e.* Satt281, Satt177 and Satt245 (Table 5). They have reported that marker-assisted selection coupled with phenotypic selection in later generations, should help to facilitate the development of soybean cultivars resistant to *Rhizoctonia* root and hypocotyl rot.

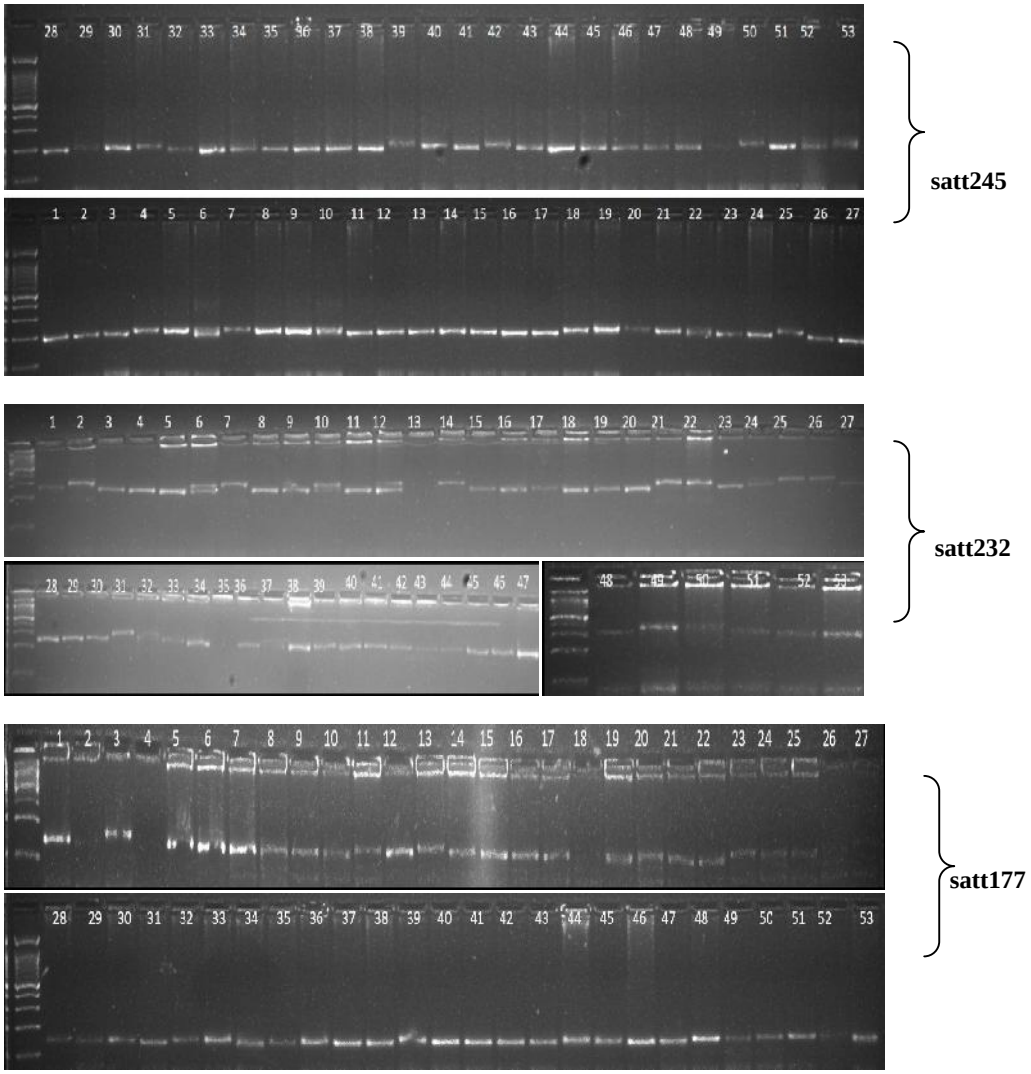

FIGURE 1 Gel image of soybean germplasm representing polymorphic SSR Primer

Note: 1. JS 20-29, 2. JS 20-69, 3. JS 335,4. JS 20-98,5. JS 20-94,6. JS 93-05,7. JS 20-116 8. JS 95-60,9. JS 97-52,10.JS 20-84,11. JS 20-34,12. JS 20-71,13. RVS 2007-6,14. RVS 2011-35,15. RVS 2001-4,16. RVS-14,17. RVS-24,18.RVS-18,19.NRC-76, 20.NRC-86, 21.NRC-130, 22.NRC-131,23.NRC-147, 24.AMSMBC -18, 25.AMS-100-39, 26.MACS–1520,27.MACSNRC-1575, 28. RSC-10-52, 29.SL -1123, 30.SL-1068, 31.AGS111,32.EC457286,33.MACS725, 34.SP37, 35.NRC-125, 36. NRC-132, 37. NRC-134, 38. NRC SL-1, 39.PS1092, 40.PS1613, 41.AMS2014-1, 42.KDS992**,**43.VLS-94,44.SKF-SPS -11,45. RVS76, 46.NRC127, 47.KDS980, 48.G-29, 49.RSC-10-70, 50.RSC-10-71, 51.NRC-2,52.MAUCS-1520 and 53.AMS-MS-58.

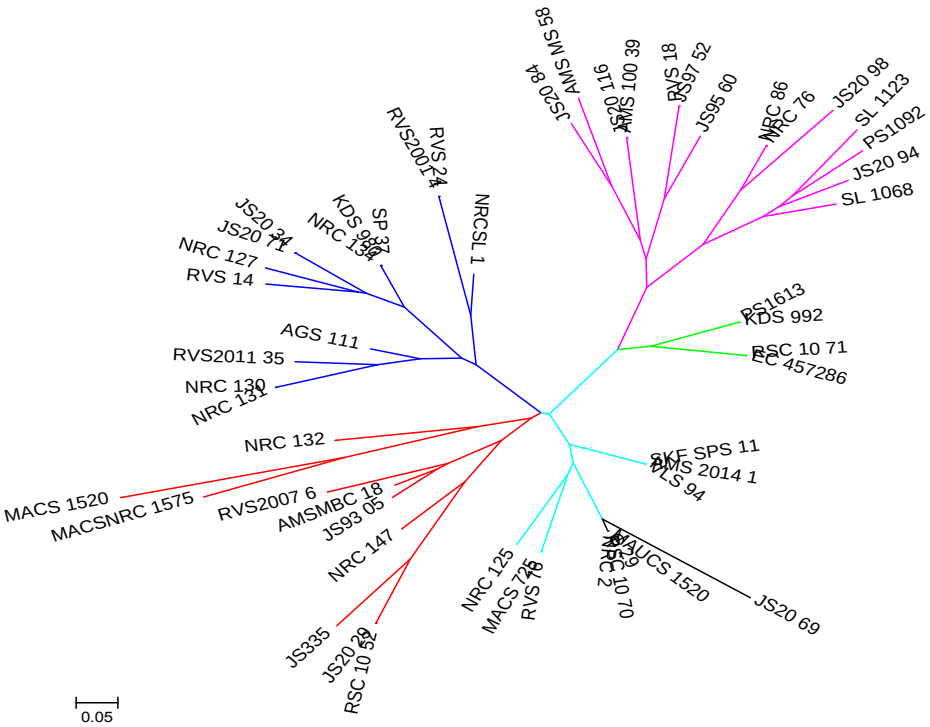

FIGURE 2: UPGMA tree (rotational form) based on similarity index of 4 SSR markers for 53 soybean genotypes using banding pattern analysis

The soybean genotype showing the genetic relationships are showed by SSR based UPGMA tree. Total 53 genotypes were grouped into 5 clusters (Table: 6; Fig.2). Cluster 1 is grouped with two resistance genotypes *i.e*., JS-20 69 and NRC-2 with other 9 genotypes and cluster 2 having JS 93-05 resistance genotype and 2 moderately susceptible that are JS335 and JS-20-29 followed by 6 other genotypes. In cluster 3 forming group with genotypes JS20-34 and JS20-71 that are resistance and one susceptible genotypes NRSCL-1 followed by other 11 genotypes. Whereas cluster 4 formed groups with moderately susceptible (JS20-98, JS, 97-52 and NRC-86), tolerant (PS1092) and resistive genotypes (JS 95-60, JS20-116 and JS 20-94) with 8 other genotypes and cluster 5 having four resistant genotypes *i.e*., KDS-992, PS-1613, EC-457286 and one susceptible genotype *namely*: RSC-1071. Susceptible parents contributing resistance alleles to fungal pathogen as well so all susceptible genotype(s) under grouped of resistance genotype(s) may will have the resistance source of gene. This has already been reported in several other crops by Young *et al*. (1994), Mysteries *et al*. (1998) and Silva *et al*. (2019). The report emphasized that genomic regions harboring resistance to charcoal rot in soybean and it may facilitate breeding and molecular engineering progress to combat charcoal rot disease in the future. Grouping of resistant genotypes along with sensitive genotypes is occurring due to less number of primers used in the study. Other reason behind this could be, with time of evolution gene fragment has lost its effect of resistance and although at genomic level, genotype is resistant but disease symptoms appear under field conditions. There is an urgent requirement of more number(s) of primers to be used for getting more appropriate and reliable result to screen genotypes against these two diseases.

Second most destroying fungal disease of soybean is charcoal rot. Its management strategies in soybean include cultural methods, seed-applied fungicide and biological control, but these have not been effective or widely adopted and have provided limited control (Mengistu *et al*., 2015). In this scenario, genetic resistance may be the most feasible and sustainable method to manage charcoal rot (Mengistu *et al*., 2007). Complete resistance to *M. phaseolina* is not reported in any plant species, but identification of partial resistance has been reported in soybean, including moderately resistant cultivars, such as DT97-4290, used as a disease check standard (Pawlowski *et al*., 2015). However, investigations into commercially available germplasm and their general response to the fungus have not been widely performed. In present investigation a set of five gene linked SSR molecular markers to charcoal rot namely: Sct_028, Satt512, S60211-TB, Sat_177 and S63880-CB linked with charcoal rot were tested with soybean genotypes and only one primer *viz*: S60211 showed polymorphism among them.

The highest percent contribution towards genetic divergence was recorded by biological yield per plant. On the basis of divergent traits genotype *viz.*, JS335, JS 20-69, JS 97-52, KDS980 and KDS 992were found to be most divergent and promising genotypes and they may be used as parents in future hybridization programme. Genotype(s) that obtained resistance /tolerance against *Rhictonia* root rot and charcoal rot may be used further in molecular breeding programmes to develop resistant/tolerant varieties.

TABLE 3: Analysis of variance for yield traits in genotypes of soybean

Source of variation	D.f	Plant height	Days to flower initiation	Days to 50% flowering	Days to maturity	Numbers of primary branches /plant	Leaf Area (cm^2)	Chlorophyll a (mg/ml)	Chlorophyll b (mg/ml)	Numbers of seeds / pod	Numbers of pods / plant	Grain yield / plant (g)	Biological yield / plant (g)	Harvest index
Replications	1	20.65	0.01	42.35	9.66	13.62	25.44	100.51	46.21	0.38	35.59	40.33	70.95	161.11
Genotypes	52	459.08**	30.59**	26.12**	110.47**	4.60**	468.72**	16.73**	106.98**	0.33**	224.09**	1118.48**	3691.20**	86.79**
Error	52	16.38	1.07	2.54	2.76	1.02	2.81	1.51	1.93	0.04	3.87	3.40	4.42	13.71

Note: *, ** significant at 1 & 5% level of significance

TABLE 4: Mean observations on Grains yields and its components of different Genotype in soybean

S.N	Genotype	Plant height (cm)	Days to flower initiation	Days to 50% flowering	Days to maturity	No. of primary branches /plant	Leaf Area (cm^2)	Chloro-phyll a (mg/ml)	Chloro-phyll b (mg/ml)	No. of seeds / pod	No. of pods / plant	Grain yield / plant (g)	Bio. yield / plant (g)	Harvest index
1	JS 20-29	71.5	38.5	48.5	87.0	4.0	15.2	8.0	17.5	2.5	42.5	106.1	210.7	50.4
2	JS 20-69	8s4.8	35.5	43.0	85.0	5.0	33.8	7.7	21.8	2.8	31.7	89.7	173.8	50.7
3	JS 335	76.1	38.5	48.0	89.5	6.2	13.4	5.6	17.5	1.8	45.2	94.2	187.9	50.1
4	JS 20-98	70.1	35.0	43.5	94.0	5.6	32.9	8.3	21.4	2.7	16.8	44.7	88.0	50.9
5	JS 20-94	83.3	36.5	47.0	86.5	5.1	36.8	7.7	25.3	2.5	19.4	48.5	94.9	51.2
6	JS 93-05	71.8	36.0	46.0	92.5	3.8	36.2	10.7	2.9	3.6	19.4	69.7	137.7	50.6
7	JS 20-116	86.0	34.5	44.5	76.5	4.0	35.0	8.1	14.0	2.8	32.5	90.3	180.0	50.2
8	JS 95-60	55.7	35.0	44.0	69.0	2.2	31.0	4.1	7.0	3.8	12.4	47.0	94.1	49.9
9	JS 97-52	73.2	35.5	44.5	81.0	6.6	20.7	10.4	16.8	2.7	35.4	95.5	155.6	64.8
10	JS 20-84	43.2	33.5	44.5	66.5	2.4	21.8	4.6	6.9	2.5	29.0	71.3	141.1	50.5
11	JS 20-34	46.4	34.5	44.5	66.5	2.9	20.6	9.5	16.4	2.7	16.6	44.9	87.1	51.5
12	JS 20-71	60.2	33.5	40.5	68.5	5.3	20.3	11.4	19.4	2.5	7.6	18.9	38.7	48.9
13	RVS 2007-6	89.3	35.5	46.5	67.0	2.9	22.0	11.1	14.5	2.4	20.7	49.3	97.8	50.4
14	RVS 2011-35	83.7	34.5	42.0	72.0	5.3	35.0	7.1	13.7	2.6	26.2	68.2	135.6	50.3
15	RVS 2001-4	73.5	33.5	44.5	81.5	6.3	34.1	12.7	21.6	2.3	21.6	49.7	97.3	51.1
16	RVS -14	78.7	34.5	44.0	70.5	4.3	53.4	13.7	23.0	2.3	35.8	82.1	163.5	50.2
17	RVS -24	86.3	35.0	44.5	81.0	4.5	41.2	11.3	12.7	2.7	24.9	67.1	132.5	50.6
18	RVS -18	78.6	34.5	44.5	85.0	2.0	44.2	6.5	20.2	2.7	22.5	60.8	120.9	50.3
19	NRC- 76	111.0	36.5	47.0	71.5	6.0	52.3	2.2	15.4	2.6	7.9	20.3	42.3	48.0
20	NRC -86	66.3	34.5	45.5	76.0	6.4	51.3	3.6	9.4	2.8	20.4	57.2	113.5	50.4
21	NRC- 130	67.2	33.5	41.0	67.0	2.7	30.8	5.5	16.4	2.4	26.6	63.9	126.0	50.7
22	NRC -131	80.8	34.5	47.0	69.0	6.1	78.0	10.3	7.3	2.2	32.5	71.0	140.5	50.5
23	NRC -147	84.0	35.5	43.5	70.5	5.4	44.4	4.6	12.8	2.1	25.3	53.1	104.6	50.8
24	AMSMBC -18	83.2	34.5	41.5	89.0	6.1	52.8	11.2	7.1	2.0	32.4	64.0	126.7	50.5
25	AMS-100-39	75.8	35.5	43.5	87.0	5.0	73.3	2.5	10.6	2.2	39.2	86.2	171.3	50.3
26	MACS – 1520	76.0	38.5	49.0	87.5	7.8	62.5	9.6	4.3	2.6	32.5	84.6	166.7	50.7
27	MACSNRC-1575	74.0	36.0	46.0	86.0	1.6	75.5	6.1	9.5	2.5	31.0	77.2	152.5	50.6
28	RSC-10-52	82.7	39.5	50.0	74.0	6.7	30.4	4.4	9.4	2.0	30.9	61.1	121.0	50.5
29	SL -1123	66.3	39.0	46.5	68.0	5.1	48.3	5.4	21.2	2.8	12.4	34.7	96.2	36.1
30	SL-1068	56.9	35.5	44.0	65.5	6.9	56.2	6.5	21.5	2.0	22.3	44.5	110.3	40.3
31	AGS 111	42.8	39.0	48.0	71.0	3.9	27.9	9.3	5.9	2.3	23.9	54.7	107.5	51.0
32	EC457286	57.5	39.5	48.5	71.0	5.3	26.4	7.4	8.0	1.9	23.4	44.5	90.8	49.0
33	MACS725	79.2	35.0	42.5	74.5	3.2	40.9	14.4	11.4	2.8	12.5	34.9	87.4	39.9
34	SP 37	73.7	37.5	47.0	82.0	6.3	38.4	6.6	6.8	2.8	6.8	19.0	89.9	21.1
35	NRC -125	66.7	41.0	47.5	69.5	5.9	30.7	9.7	8.8	2.7	15.8	42.6	104.4	40.8
36	NRC-132	68.0	44.0	50.0	74.0	6.5	14.0	10.6	2.4	2.0	21.3	42.1	104.1	40.5
37	NRC-134	53.7	42.0	49.0	75.0	5.5	16.1	3.7	14.5	2.7	6.9	18.6	42.3	43.8
38	NRC SL-1	49.8	38.5	47.0	70.0	6.3	31.1	9.8	19.4	2.5	20.2	50.3	99.2	50.7
39	PS 1092	42.2	40.0	49.0	72.5	4.1	12.9	5.8	3.7	2.4	17.4	41.7	82.1	50.9

40	PS 1613	69.9	33.5	42.5	70.5	4.6	29.5	8.8	2.1	1.7	8.5	14.5	46.0	31.5
41	AMS 2014-1	57.3	43.0	46.0	75.0	5.9	53.8	6.1	2.6	1.9	33.2	63.0	123.4	51.0
42	KDS 992	53.0	43.5	47.0	77.0	6.3	43.2	6.6	3.3	2.4	14.2	33.7	66.8	50.6
43	VLS -94	53.8	44.5	53.0	70.5	5.1	26.7	6.5	1.0	2.6	20.8	54.0	105.9	51.0
44	SKF-SPS -11	56.6	45.5	54.0	70.5	5.6	27.5	5.8	1.5	2.6	23.3	60.2	118.4	50.9
45	RVS 76	109.3	43.5	53.5	73.5	4.6	29.3	5.5	2.8	2.1	10.2	21.3	54.0	39.4
46	NRC127	81.8	43.0	49.5	77.0	5.4	39.6	6.8	1.1	2.4	30.0	72.1	142.2	50.7
47	KDS980	79.5	43.5	54.0	80.0	5.5	36.2	3.5	1.3	2.5	37.7	94.1	187.0	50.3
48	G-29	71.5	42.0	53.0	74.0	2.2	30.9	5.5	3.1	2.7	16.2	43.7	85.4	51.3
49	RSC-10-70	59.9	44.0	52.5	72.5	5.2	35.7	5.5	1.1	2.7	32.0	84.0	166.2	50.6
50	RSC-10-71	62.1	43.0	50.0	76.0	4.0	23.0	5.5	1.8	3.2	7.4	23.3	44.2	53.2
51	NRC-2	43.5	43.0	51.5	77.0	4.0	23.3	2.5	4.8	2.6	6.2	15.8	51.1	31.0
52	MACS-1520	63.4	44.5	50.5	73.0	5.5	39.2	8.3	2.9	2.5	34.0	84.8	167.6	50.6
53	AMS-MS-58	73.3	45.5	54.0	75.5	5.0	24.3	6.3	3.3	1.7	40.7	66.3	131.2	50.5
Mean		69.9	38.2	47.0	76.1	5.2	35.9	7.4	10.4	2.5	23.6	56.5	115.8	48.3
SE(m)		2.9	0.7	1.1	1.2	0.7	1.2	0.9	1.0	0.1	1.4	1.3	1.5	2.6
CD5%		8.1	2.1	3.2	3.3	2.0	3.4	2.5	2.8	0.4	0.9	3.7	4.2	7.4
CV%		5.8	2.7	3.4	2.2	19.3	5.0	16.6	13.4	7.6	8.4	3.3	1.8	7.7

TABLE 5: Summary of polymorphic SSR markers representing PIC value and other analysis data

Marker	Major Allele Frequency	Allele No	Gene Diversity	PIC
satt281	0.3962	4	0.6921	0.6359
satt177	0.4340	4	0.6401	0.5671
satt245	0.6226	3	0.4956	0.4047
satt232	0.4057	4	0.6769	0.6127
Mean	0.4646	3.75	0.6262	0.5551

TABLE 6: Genotype in different clusters based on UPGMA tree

Cluster	Number of genotypes	Genotypes
1	11	JS 20-69, MAUCS-1520,RSC-10-17, VLS-94, , G-29, NRC-2, RVS-16, NRC-125, MACS-725, SKF SPS-11 and AMS-2014-1
2	10	RSC-10-52, JS-20-29, JS335, NRC-147,JS-93-05, AMAMBC-18, RVS-2007-6, MACSNRC-1575, MACS-1520 and NRC-132
3	14	NRC-131, NRC-130, RVS-2011-35, AGS-111, RVS-14, NRC-127, JS-20-71, JS-20-34, NRC-134, KDS-980, SP-37, RVS-2001-4, RVS-24 and NRCSL-1
4	14	JS-20-84, AMS MS-58, JS-20-116, AMS100-39, RVS-18, JS-97-52, JS-95-60, NRC-86, NRC-76, JS-20-98, SL-1123, PS-1092, JS-20-98, SL-1123, PS-1092, JS-20-94 and SL-1068
5	4	KDS 992, PS 1613, RSC-10-71 and EC457286,

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