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Morphological and SSR Marker based Selection for an Elite YMV Resistant Breeding Line from a Segregating Population of Soybean [*Glycine max* (L.) Merrill]

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ABSTRACT

Background: JS335 is a leading variety of soybean in central India with high stability, but YMV susceptible. These leads to the incorporation of the YMV resistance in the elite genotypes of soybean variety JS335.

Methods: 45 breeding lines obtained from a cross between two parents SL525 (YMV resistant) and JS335 (YMV susceptible) along with two parents and a check SL958 were analysed for diversity using Mahalanobis D^2 and 19 polymorphic SSR markers. These lines were evaluated for yield and component traits and YMV, during 2020 at two locations *viz.* Ludhiana and Gurdaspur, India.

Result: Diversity analysis based on Mahalanobis D^2 statistics divided genotypes into five clusters. The maximum (288464.1) and minimum (15103.7) inter-cluster distance were observed between clusters 4 and 5 and clusters 2 and 5, respectively. SSR marker analysis grouped a total of 48 genotypes into three major clusters. The mean for agronomic traits of genotypes was categorised and SLJS 41-2 was found YMV resistant and exhibited a high yield (32.1 g/plant). The contribution of JS 335 towards SLJS 41-2 was found to be 62.5%. This line could further be tested at multilocation to evaluate adaptability and subjected to different yield trials for varietal release or used as a potential YMV donor.

Key words: Genetic diversity, Mahalanobis distance, Soybean, SSR markers, YMV.

INTRODUCTION

Soybean is a prominent grain legume and the second most important oilseed crop in the world based on total production and international trade (Kumar *et al*., 2022). Soybean is renowned by names *viz*. miracle bean, golden bean, wonder bean etc. because of its chemical composition (20% oil and 40% protein) and is cultivated in diverse agro-climatic zones of the world and India as human and animal food (Ibanda *et al*., 2018). In India, its demand is increasing tremendously over past two decades, which is forcing large-scale productivity. However, the productivity of soybean in India is hovering around 1.04 t/ha compared to 2.87 t/ha in the world due to infestation of YMV (FAOSTAT, 2023).

Soybean is damaged by viral, bacterial and fungal diseases around the world. However, Yellow mosaic virus (YMV) causes 15-75% yield loss in soybean (Mishra *et al*., 2022). YMV is damaging almost all major soybean varieties in the country making them susceptible (Nichal *et al*., 2018). To overcome this hurdle, hybridisation is used for imparting disease resistance and phenotypic selection in segregating population of soybean. Success depends on parent selection for hybridisation and selection efficiency in breeding population based on phenotypic evaluation. Genetic variability based on morphological parameters does not give a clear picture of genetic variability present in material as quantitative traits are highly influenced by the environment. Hence, a highly reliable and precise method is required for genetic variability assessment in the germplasm.

SSR markers are used in genomic studies, marker-assisted selection and cultivar identification owing to their co-dominance nature and high reproducibility. Molecular profiling of new varieties, breeding lines, can serve as supporting proof for the individuality of the variety, which has attained greater importance for the PPVFR' Act 2001 under GATT, where genotype characterisation for varietal identification and clear distinctness of variety is perquisite (Chauhan *et al*., 2015).

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Hence, in present investigation, diversity analysis of 48 soybean-derived breeding lines from, SL 525 × JS 335 using SSR markers and morphological traits were conducted to isolate an elite YMV resistant breeding line from a segregating population.

MATERIALS AND METHODS

Plant materials and experimental details

The investigation was conducted in 2020 at two locations.Pulses Research area, PAU, Ludhiana and Regional Research Station, Gurdaspur. The experimental material consisted of 48 breeding lines of $F_{2:7}$ generation of a cross between SL525 (YMV resistant) and JS335. The variety JS 335 is known to possess wide adaptability, non- shattering , early durated one of the high yielding varieties which makes it a leading variety of central India,but it is susceptible to YMV (Anonnymous, 2018). Furthermore, parents *viz*., SL 525 and JS 335 and one check,*i.e*. SL 958, were used. According to symptoms of disease severity in leaves and growth pattern, genotypes were scored on the scale of 0-9; Where 0=Highly resistant, 1=Resistant, 3=Moderately resistant, 5=Moderately susceptible, 7=Susceptible, 9=Highly susceptible, (Mayee and Datar, 1986).

Molecular characterisation

Genomic DNA was extracted from young leaves by modified CTAB method (Doyle and Doyle, 1987). PCR was performed using 108 SSR primers obtained from USDA-ARS Soybean Genome Database (Russel and Sambrook, 2000). The total number of alleles was detected in each genotype for each marker. The binary matrix was prepared with amplified bands, scoring 1 (band present) and 0 (band absent).

Statistical analysis

Diversity analysis

The average data of Ludhiana and Gurdaspur locations for each character was used in Mahalanobis D^2 statistics (Mahalanobis, 1936), explained by Rao (1952) for grouping genotypes into various clusters, using WINDOSTAT 8.1.

Estimation of PIC value and cluster analysis

To estimate the usefulness of markers, the number of amplicons/alleles/marker, allelic frequency and polymorphic information content (PIC), was computed.

$$PIC = 1 - \Sigma x_i^2$$

Where,

x_i = Relative frequency of the i^{th} allele of the SSR loci)

Genetic diversity was calculated by using DARwin software (Perrier and Jacquemond-Collect, 2006). The unweighted pair groups method with arithmetic mean (UPGMA) method was adopted to produce a dendrogram (Sneath, 1973).

Evaluation of agro-morphological characters

The data on twenty morphological parameters *viz*., fresh weight and dry weight of plants (30, 45 and 60 DAS), plant height (60 DAS), plant height (maturity), days to flowering, days to maturity, branches/plant, nodes/plant, pods/plant, pods/node, seeds/pod, 100-seed weight, harvest index, seed yield/plant, protein content, oil content were recorded from five random plants and meanvalues were recorded.

RESULTS AND DISCUSSION

Genetic diversity through mahalanobis D^2 statistics

Genetic diversity among 48 lines of soybean was conducted based on 20 characters and the Euclidian distance graph was obtained based on D^2 statistics (Fig 1).

Distribution of genotypes into different clusters

Lines were distributed into five clusters based on similarities and differences among the genotypes (Table 1). Cluster 1 had 15 genotypes, the highest among all clusters, cluster 2 contained 9 genotypes, including SL 525 and Cluster 3 had 14 genotypes including JS 335. Cluster 4 and 5 contained each five genotypes. The genotypes within the cluster indicated less diversity among genotypes and genotypes between clusters were more diversed. More number of genotypes were grouped with JS 335 compared to SL 525. The presence of different genotypes in different clusters indicated the presence of diversity among lines. These results were found similar with work conducted by Shinde *et al.* (2013), in which they used 40 soybean genotypes to estimate diversity by using Mahalanobis D^2 and genotypes were categorised into 12 clusters with D^2 value ranging 27.14-36.16. 20 genotypes were present in cluster 1, followed by two genotypes in each cluster 2 and 4, respectively. However, Upadhyay *et al.* (2022) grouped genotypes into clusters using Mahalanobis D^2 statistics.

Identification of desirable lines

The maximum (288464.1) and minimum (15103.7) inter-cluster distance were observed between clusters 4 and 5

Table 1: Clustering by mahalanobis D^2 analysis.

Cluster	Frequency	Genotypes
1	15	SLJS 4-2, SLJS 25-1, SLJS 3-2-1, SLJS 43-3-2,SLJS 3-3, SLJS 25-2, SLJS 13-1, SLJS 3-2-2,SLJS 9-2, SLJS 14-3, SLJS 43-2, SLJS 25-4, SLJS 37-1, SLJS 1-2,SL958
2	9	SLJS 3-1, SLJS 14-2, SLJS 23-2, SLJS 29-1, SLJS 43-1, SL 525, SLJS 37-1-1, SLJS 23-3, SLJS 26-2
3	14	SLJS 29-3, SLJS 43-8, SLJS 9-3, SLJS 25-3, SLJS 11-2, SLJS 33-1, SLJS 9-1,SLJS 1-1, SLJS 2-1, JS 335, SLJS 1-3, SLJS 29-4, SLJS 43-5, SLJS 43-7
4	5	SLJS 29-2, SLJS 4-3, SLJS 33-2, SLJS 14-1, SLJS 4-1
5	5	SLJS 23-4, SLJS 34-1, SLJS 41-1, SLJS 41-2, SLJS 43-6

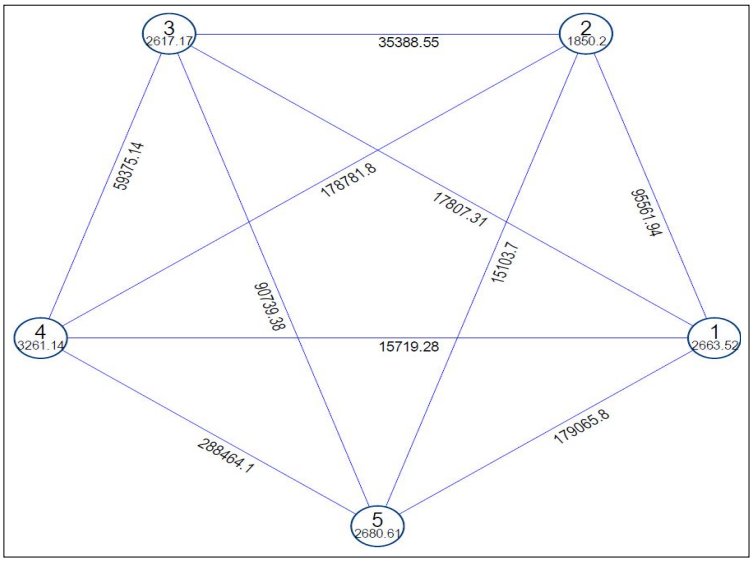

Fig 1: Average intra and inter-cluster distance based on morphological traits of soybean genotypes.

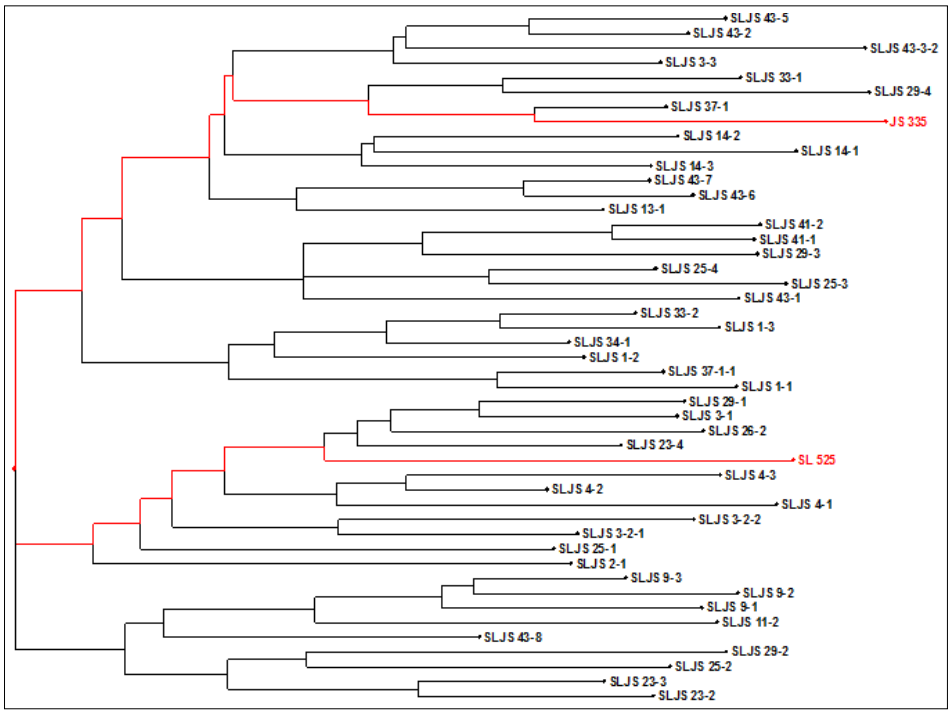

Fig 2: Dendrogram obtained by SSR marker analysis using DARwin 5.

Table 2: Mahalanobis D^2 cluster distance matrix.

Cluster	1	2	3	4	5
1	2663.52	95561.94	17807.31	15719.28	179065.8
2		1850.2	35388.55	178781.8	15103.7
3			2617.17	59375.14	90739.38
4				3261.14	288464.1
5					2680.61

and clusters 2 and 5, revealing genotypic diversity between clusters (Table 2). Maximum (3261) and minimum (1850.2) intra-cluster distances were recorded for cluster 4 and cluster 2, respectively. The more intra-cluster distance, the more diverse the genotypes within cluster. The cluster means for 20 characters (Table 3) showed considerable differences for all characters between the clusters. No difference in mean cluster was observed for fresh weight (30 DAS), dry weight (30 DAS), plant height (60 DAS), number of nodes/plant, number of pods/node, seeds/pod, 100-seed weight, harvest index, protein content and oil content. The minimum mean for fresh weight (45 DAS) and dry weight (45 DAS) were observed in cluster 1 where as high means were detected for seed yield/plant, plant height at maturity and fresh weight (60 DAS) in cluster 1. Seed yield and plant height at maturity had minimum mean in cluster 5. Fresh weight (45 DAS) and branches/plant had high mean in cluster 2. Genotypes can be selected from specific cluster using mean of different clusters. Genotypes for seed yield may be selected from cluster (1, 4), for pods/plant (1 and 5), branches/plant (2 and 5). Similar results were obtained by Naik *et al.* (2016).

Genetic diversity analysis through molecular markers

Out of a total 108 SSR markers, 19 were found polymorphic. The high levels of polymorphism found in SSR markers among soybean accessions highlighted the usefulness of markers in genetic variation identification. Kumar *et al*. (2022) successfully employed SSR markers in soybean genetic diversity analysis.

Cluster analysis using SSR data

The results of cluster analysis are represented in Table 4 and Fig 2. Total 48 genotypes were grouped into three major clusters. Cluster A had 26 genotypes, including JS 335 and cluster B contained 12 genotypes including SL 525 and cluster C of 9 genotypes. Cluster A was categorised into two sub groups *viz*. A1 and A2. A1 contained 20 genotypes and A2 had 6 genotypes. Cluster B had 12 genotypes and 9 genotypes were included in cluster C. Similar results were obtained by Mulato *et al.* (2010).

Evaluation of soybean genotypes present in clusters for different observed traits

The mean for traits was classified based on genotypes present in each cluster to isolate lines with agronomic superiority, high yielding and YMV resistance along with high contribution of JS 335. SLJS 41-2 was resistant to YMV and high yielding (32.1 g/plant). The contribution of JS 335 towards SLJS 41-2 was 62.5%. Comparison of mean seed yield for different clusters revealed that SLJS 41-2 (32.1 g/ plant) and SLJS 43-7 (32.4 g/plant) had 62.5 and 52.94 per cent contribution, from JS 335 and were better yielder than SL 525 (30.0 g/plant) and YMV resistant. These two lines should be further evaluated over locations to test adaptability and yield potential. Similar results were obtained by Kumar *et al*. (2022).

Table 3: Mean D^2 values of clusters for agronomic traits of soybean.

Character	Fresh weight 30 at DAS (g)	Dry weight at 30 DAS (g)	Fresh weight at 45 DAS (g)	Dry weight at 45 DAS (g)	Fresh weight at 60 DAS (g)	Dry weight at 60 DAS (g)	Plant height at 60 DAS (cm)	Plant height at maturity (cm)	Days to flowering	Days to maturity	No. of branches/ plant	No. of nodes/ plant	No. of pods/ plant	No. of pods/ node	No. of seed/ pod	100-seed weight (g)	Harvest index (%)	Seed yield/ plant (g)	Protein content (%)	Oil content (%)
1	10.7	2.59	28.85	8.45	46.8	14.34	66.14	70.21	4.15	19.13	86.51	9.78	26.56	2.69	4.88	59.99	119.83	33.47	36.65	20.27
2	10.08	2.47	35.72	9.84	61.33	17.14	65.44	66.09	3.96	18.49	91.06	9.83	25.94	2.69	5.49	59.87	119.89	32.65	36.53	20.44
3	10.05	2.40	30.99	8.69	52.16	14.98	66.08	68.32	4.02	18.99	93.57	9.6	26.48	2.69	5.47	59.99	120.57	32.97	36.69	20.55
4	10.73	2.81	26.02	8.31	41.01	13.85	69.47	71.62	4.23	19.42	79.49	9.63	25.43	2.71	4.53	60.37	121.10	34.37	36.45	20.49
5	10.46	2.29	38.83	10.54	67.20	18.61	64.86	64.59	3.76	17.93	89.41	9.72	27.08	2.62	5.49	59.22	118.75	30.60	36.18	20.34

Table 4: Clustering by SSR data.

Cluster	Subcluster	Frequency	Genotypes
A	A1	20	SLJS 43-5, SLJS 43-2, SLJS 43-3-2, SLJS3-3, SLJS 33-1, SLJS 29-4, SLJS 37-1, JS335, SLJS 4-2, SLJS 14-1, SLJS 14-3, SLJS 43-7, SLJS 43-6, SLJS 13-1, SLJS 41-2, SLJS 41-1, SLJS 29-3, SLJS 25-4, SLJS 25-3, SLJS 43-1
	A2	6	SLJS 33-2, SLJS 1-3, SLJS 34-1, SLJS 1-2, SLJS 37-1-1, SLJS 1-1
	B	12	SLJS 29-1, SLJS 3-1, SLJS 26-2, SLJS 23-4, SL525, SLJS 4-3, SLJS 4-2, SLJS 4-1, SLJS 3-2-2, SLJS 3-2-1, SLJS 25-1, SLJS 2-1
	C	9	SLJS 9-3, SLJS 9-2, SLJS 9-1, SLJS 11-2, SLJS 43-8, SLJS 29-2, SLJS 25-2, SLJS 23-3, SLJS 23-2

Table 5: Per cent contribution of parents to each line.

Individuals	Contribution of markers towards JS335	Contribution of markers towards SL525	Total no of markers	(%) Contribution of JS335	(%) Contribution of SL525
SLJS 1-1	9	7	16	56.25	43.75
SLJS 1-2	7	9	16	43.75	56.25
SLJS 1-3	11	5	16	68.75	31.25
SLJS 2-1	8	8	16	50.00	50.00
SLJS 3-1	6	12	18	33.33	66.66
SLJS 3-2-1	4	12	16	25.00	75.00
SLJS 3-2-2	5	11	16	31.25	68.75
SLJS 3-3	10	6	16	62.5	37.5
SLJS 4-1	9	8	17	52.94	47.05
SLJS 4-2	6	10	16	37.52	62.54
SLJS 4-3	5	11	16	31.25	68.75
SLJS 9-1	4	13	17	23.52	76.47
SLJS 9-2	5	10	15	33.33	66.66
SLJS 9-3	4	14	18	22.22	77.77
SLJS 11-2	7	12	19	36.84	63.15
SLJS 13-1	9	8	17	52.94	47.05
SLJS 14-1	8	9	17	47.05	52.94
SLJS 14-2	6	10	16	37.50	62.50
SLJS 14-3	9	7	16	56.25	43.75
SLJS 23-2	9	7	16	56.25	43.75
SLJS 23-3	6	10	16	37.50	62.50
SLJS 23-4	5	11	16	31.25	68.75
SLJS 25-1	7	9	16	43.75	56.25
SLJS 25-2	9	7	16	56.25	43.75
SLJS 25-3	11	7	18	61.11	38.88
SLJS 25-4	10	6	16	62.50	37.50
SLJS 26-2	8	9	17	47.05	52.94
SLJS 29-1	13	3	16	81.25	18.75
SLJS 29-2	8	7	15	53.33	46.66
SLJS 29-3	9	7	16	56.25	43.75
SLJS 29-4	12	4	16	75.00	25.00
SLJS 33-1	13	2	15	86.66	13.34
SLJS 33-2	10	5	15	66.66	33.33
SLJS 34-1	11	5	16	68.75	31.25
SLJS 37-1	11	4	15	73.33	26.66
SLJS 37-1-1	9	7	16	56.25	43.75
SLJS 41-1	10	5	15	66.66	33.33
SLJS 41-2	10	6	16	62.50	37.50
SLJS 43-1	7	11	18	38.88	61.11
SLJS 43-2	8	8	16	50.00	50.00
SLJS 43-3-2	8	10	18	44.44	55.55
SLJS 43-5	11	7	18	61.11	38.88
SLJS 43-6	8	8	16	50.00	50.00
SLJS 43-7	9	8	17	52.94	47.05
SLJS 43-8	7	10	17	41.17	58.82

YMV screening of the segregating population

The segregating population along with the two parents SL525 and JS335 were screened for YMV. JS335 showed high susceptibility (disease score: 7.3). SL525 displayed high degree of resistance (disease score: 0.5). The segregating population showed resistance (disease score: 0.5-1.3). The mean value for YMV resistance for genotypes grouped with JS 335 is 0.9 and genotypes grouped with SL 525 is 0.7 and the genotypes which were not clubbed with any of the parents expressed the mean value of 0.69. It is clear from the result that high degree of resistance for YMV was observed among the segregants and segregants clubbed with susceptible parents expressed more disease symptoms as compared to segregants clubbed with resistance parents. Similar results were obtained by Kujane *et al.* (2019).

Per cent contribution of parents to each line

Based on similarities and dissimilarities, the genotypes were grouped into 3 clusters by using DARwin 5.0. The parental contribution of each line was estimated by using behaviour of each marker to each parent (Table 5). The dendrogram was compared with parental contribution value. The genotypes in which the contribution of JS 335 was high, were grouped with JS 335 and genotypes in which the contribution of SL 525 was more, were grouped with SL 525 and the lines which had almost equal contribution of both parents, were grouped in third category. Qin *et al.* (2014) conducted an experiment to study contribution of parental lines (Hobbit and Zao5241) on sibling lines Jiduo 17 and Ji nf58. They found large segment of linkage group C1 and J were passed from maternal line Hobbit and segment from linkage group A1 was passed down from parental line Zao 5241. More number of genotypes had high contribution of JS 335.

CONCLUSION

Using Mahalanobis D^2 statistics, segregating population were grouped into 5 clusters. Genotypes from a specific cluster can be chosen based on the mean value of the different clusters. Genotypes for seed yield can be selected from the clusters (1, 4), pods/plant (1, 5) and branches/ plant (2, 5), respectively. The selected genotypes will be useful for identifying transgressive segregants and employed in hybridisation programmes. After comparing the dendrogram with parental contribution value, genotypes were grouped into three ategories according to the contribution of different parent. It was concluded that SLJS 41-2 and SLJS 43-7, had more percent contribution from JS 335 and were greater yielders than SL 525 and YMV resistant. These lines should be further tested at multilocation to evaluate their adaptability and subjected to yield trials to be released as a variety or used as a potential YMVdonor.

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Conflict of interest

All authors declared that there is no conflict of interest.

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