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Research Article

Comprehensive analysis of phenotypic variation and selection strategies for yield-related traits in recombinant inbred lines of soybeans

Shivani Jawarkar[1], Manoj Shrivastava[1], Pawan K. Amrate[1], G. K. Satpute[2] and Vikrant Khare[3]*

[1]Department of Plant Breeding and Genetics, Jawaharlal Nehru Krishi Vishwavidyalaya, Jabalpur, Madhya Pradesh, 482004, India.
[2]Division of Crop Improvement, Indian Institute of Soybean Research, Indore, Madhya Pradesh, 462066, India
[3]Division of Nuclear Agriculture and Biotechnology, Bhabha Atomic Research Centre, Mumbai, Maharashtra, 400085, India.
***E-Mail**: vikrantkhare@jnkvv.org

Abstract
In the present study, genetic diversity was assessed in RIL population developed at Indian Institute of Soybean Research (IISR) Indore by crossing of released varieties JS 97-52 and NRC 37. The Analysis of variance suggested that all the studied traits differed significantly. High heritability with high genetic advance as percent of mean was observed for plant height, number of primary branches, number of pods/plants, number of seeds/plants, days to maturity, biological yield, 100 seed weight, harvest index and seed yield per plant. Significant positive association with grain yield per plant was observed for days to 50% flowering, days to maturity, plant height, number of primary branches per plant, number of pods per plant, number of seeds per plant, biological yield per plant, harvest index and 100 seed weight. The highest positive direct effect towards grain yield was exerted by biological yield per plant, followed by harvest index and number of pods per plant. Diversity analysis revealed that Cluster VI and XII had highest inter cluster distance.

Keywords: Recombinant inbred lines, soybean, and genetic divergence

INTRODUCTION

Soybean [*Glycine max* (L.) Merril] is called as the “Wonder Crop” of the 20th century because of its high protein content (40%) and oil content (20%). For its strength, qualities, and numerous uses, it has been designated the “Golden bean” (Adsul *et al.,* 2018). In Madhya Pradesh soybean occupies 5.57 million hectares area with production of 5.23 million tonnes and productivity 939 kg/ha (Anonymous, 2022). Madhya Pradesh has the largest area under soybean in the country and hence it is called “Soya State in India. Globally in soybean production the United States is the leading country followed by Brazil, China and Argentina, while India occupies the fifth rank (Anonymous, 2020). Recombination is created by crossing contrasting parent strains and then inbreeding them for isogenicity to produce recombinants, which are subsequently bred to produce an ongoing resource for trait mapping and analysis (Daniel, 2012). To exploit genetic variations based on phenotypic data genotypes are selected (Bisen *et al.,* 2015). The likelihood of receiving the ideal character in the selection process is increased by higher

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genetic variability (Sulistyo and Mejaya, 2018). The term “heritability” refers to the influence of genetic variables on phenotypic diversity (Akram *et al.,* 2016). High heritability indicates that genetic influences regulate the character in a way that makes it simple to pass on and be improved by selection (Ali *et al.,* 2016). An evaluation to determine the level of variability available for yield traits and their heritability values is of immense help to the breeders to choose the breeding approaches for improvement of yield attributing factors. The association between a plant’s characters is known as phenotypic correlation, whereas the connection between hereditary traits is known as genotypic correlation (Faot *et al.,* 2019). Path coefficient analysis provides information on the direct and indirect effects of each contributing characteristic on yield, as well as allowing breeders to rank genetic features rendering to their influence. To classify and detect parental diversity, the Mahalanobis’ D^2 statistics and multivariate statistical technique, such as principal component analysis was performed (PCA) (Venkataramana *et al.,* 2022). D^2 technique helps in selection of diverse parent to achieve maximum heterosis (Ghazy *et al.,* 2015; Kiprotich *et al.,* 2015; Sharma *et al.,* 2020; Kumar *et al.,* 2023). PCA is acknowledged as dimension reduction method that may be used to condense a larger number of variables into a smaller set that retains all the information (Massy, 1965). The study aims to find association among yield contributing traits in RIL population of soybean and also to assess the extent of genetic diversity among them.

MATERIALS AND METHODS

The experimental material consists of 96 RILs population two parents and two checks. The RIL population was developed at Indian Institute of Soybean Research through cross between JS-97-52 and NRC37. The line JS-97-52 is characterized by strong antibiosis against major pest and suitable for excessive moisture, while NRC37, primarily known as Ahilya 4, is an old drought sensitive variety and having good agronomic characteristics. The F$_9$ generation of Recombinant Inbred Lines (RILs) was employed as the experimental material in this study. The experiment was carried out in the year of *kharif* season during 2021 with two replications in a randomized complete block design (RCBD) at seed breeding farm, JNKVV, Jabalpur. Geographically JNKVV, Jabalpur is located at 23.90^0 north latitude and 79.58^0 east longitudes at a 411.87 meters elevation of above mean sea level. For each of RILs two rows were planted with the 1.5-meter row lengths. All the standard package and practices were adopted to grow a good crop. The quantitative traits like days to 50% flowering, days to maturity, plant height (cm), number of primary branches per plant, number of pods per plant, number of seeds per plant, biological yield per plant (g), 100 seed weight (g), harvest index (%), and seed yield per plant (g) were recorded manually. All the statistical analyses were carried out using INDOSTAT software.

RESULTS AND DISCUSSION

The analysis of variance, indicated that the mean sum of squares due to genotypes were highly significant for all the traits in the current study (**Table 1**). This clearly suggests that the RIL population have a significant amount of exploitable genetic variability. These conclusions mirrored with the findings of Piankra *et al.* (2018), Kumari *et al.* (2019), Kuswantoro *et al.* (2021). The genetic variability was studied at genotypic and phenotypic level. For all of the traits, PCV was higher than GCV. The presence of environmental factors and interactions between genes and environment tends to increase the observed phenotypic variability, making PCV higher than GCV in many cases (Malek *et al.* 2014). The higher value of PCV and GCV were obtained for harvest index (27.71 % and 26.23 %), followed by seed yield/ plant (27.69 % and 26.84 %), biological yield (27.40 % and 26.44 %), number of pods/plant (21.78 % and 20.86 %), number of seeds/ plant (21.48 % and 20.95 %) respectively. Traits influenced by multiple genes and their interactions, known as polygenic traits, can exhibit higher GCV and PCV (Nawaz *et al.* 2019). Moderate PCV and GCV were observed for plant height (19.09 % and 18.02 %), number of primary branches per plant (16.46 % and 15.84 %), 100 seed weight (16.42 % and 15.21 %) (**Table 2**). The outcomes were consistent with Berhanu *et al.* (2019); Dubey (2015); Bairwa *et al.* (2020). High heritability with high genetic advance percent of mean is thought to aid in the effective selection of desired features for future advancement (Khare, 2022). The character with high heritability need not have strong genetic progress. High or moderate heritability combined

Table 1. Analysis of variance for quantitative traits

S. No	Source of variation	DF	Days to 50% Flowering	Days to maturity	Plant Height	No. of primary branches per plant	No. of pods per plant	No. of seeds per plant	100 seed wight	Biological yield per plant	Harvest Index	Seed Yield per plant
1	Replications	1	7.6**	0.6	34.2**	0.01	1.5	78.9	6.7***	4.8	0	0.8
2	Treatments	99	5.3***	31.5***	82.7***	1.2***	143.1***	897.1***	5.6***	64.2***	366.6***	15.4***
3	Error	99	1.1	1.2	4.8	0.1	6.2	22.4	0.4	2.3	20.1	0.5

**, ***significant difference at p<0.01, p<0.001 respectively

Table 2. Components of genetic variability

S. No.	Character	Mean	Range		Coefficient of variance		h^2b (%)	GA as % of mean 5%
			Min.	Max.	PCV (%)	GCV (%)		
1	Days to 50% flowering	46.44	42	49.5	3.82	3.18	69	5.47
2	Days to maturity	105.1	98.5	112	3.84	3.7	93	7.36
3	Plant height (cm)	34.62	23	51.33	19.09	18.02	89.1	35.03
4	No. of Primary branches	4.86	3.05	6.7	16.46	15.84	92	31.42
5	No. of pods per plant	39.63	19.12	67.66	21.78	20.86	91	41.16
6	No. of seeds per plant	99.78	47.81	169.16	21.48	20.95	95	42.1
7	100 seed weight(g)	10.57	6.7	14.35	16.42	15.21	85	29.04
8	Biological Yield (g)	21.04	11.66	43.92	27.4	26.44	93	52.55
9	Harvest index (%)	50.17	25.68	95.85	27.71	26.23	89.6	51.14
10	Seed yield/ plant (g)	10.19	5.75	23.95	27.69	26.84	94	53.59

Min = Minimum, Max = Maximum, PCV = phenotypic coefficient of variance, GCV = genotypic coefficient of variance, h^2b = Heritability in broad sense, GA = Genetic advance

with high or moderate genetic advance implies additive gene action, which is efficient tool for selection, whereas high heritability associated with low genetic advance, or *vice versa*, shows non-additive gene action, which is ineffective in selection. Additive gene action, associated with high or moderate heritability and genetic advance, allows for more reliable selection (Taneva *et al.* 2019). Understanding these relationships helps plant breeders tailor their selection strategies for different traits and populations. The results of correlation analysis (**Table 3**) revealed that days to 50% flowering, days to maturity, plant height, number of primary branches per plant, number of pods per plant, number of seeds per plant, biological yield per plant, harvest index and hundred seed weight were found to have a significant positive correlation with seed yield per plant. Harvest index was found to have a strong negative correlation with biological yield/plant using the phenotypic correlation coefficient. Chavan *et al.* (2016); Mahbub and Shirazy, (2016); Balla and Ibrahim, (2017); Lyimo *et al.* (2017) found similar results. Days to flowering, plant height, number of branches, number of productive nodes, number of pods, and hundred seed weight were observed to have a positive phenotypic association with grain yield. Similar findings were observed by Sulistyo and Purwantoro., 2018; Kuswantoro *et al.* (2020). The path coefficient is a partial regression coefficient that is directional, has no unit, and is easy to understand. The greatest positive direct effect toward grain yield was observed by biological yield per plant (0.8732), followed by harvest index (0.8108), number of pods per plant (0.6626) (**Table 4**). The days to maturity (0.075), 100 seed weight (0.0509), days to 50%

Table 3. Phenotypic correlation for yield attributing traits

Character	Days to maturity	Plant height	Number of primary branches per plant	Number of pods per plant	Number of seeds per plant	100 seed weight	Biological yield per plant	Harvest index	Seed yield per plant
Days to 50% flowering	0.21 **	0.24 **	0.23 **	0.18 *	0.17 *	-0.15 *	0.23 **	-0.16 *	0.1
Days to maturity		0.20**	0.22**	0.07	0.07	-0.29 **	0.27**	-0.14*	0.16*
Plant height			0.41**	0.27**	0.26**	-0.1	0.16*	0.09	0.22**
Number of primary branches/plant				0.45**	0.46**	-0.01	0.22**	0.05	0.24**
Number of pods/plant					0.99**	0.02	0.37**	0.31**	0.69**
Number of seeds/ plant						0.01	0.37**	0.32**	0.69**
100 seed weight							0.01	-0.04	-0.01
Biological yield/plant								-0.46 **	0.54**
Harvest index									0.45**

*, **significant difference at p<0.05, p<0.01 respectively

Table 4. Genotypic path coefficient for yield component traits

Characters	Days to 50% flowering	Days to maturity	Plant height	Number of primary branch per plant	Number of pods per plant	Number of seeds per plant	100 seed weight	Biological yield per plant	Harvest index
Days to 50% flowering	**0.0047**	0.0013	0.0017	0.0012	0.0011	0.0011	-0.0008	0.0012	-0.0008
Days to maturity	0.0202	**0.075**	0.0162	0.0178	0.0046	0.0053	-0.0256	0.0219	-0.0124
Plant height	-0.0046	-0.0028	**-0.013**	-0.0058	-0.0034	-0.0033	0.0018	-0.002	-0.0013
Number of primary branches/plant	-0.0187	-0.0179	-0.0337	**-0.0754**	-0.0355	-0.0361	0.0002	-0.0169	-0.0041
Number of pods/plant	0.1608	0.041	0.1705	0.3118	**0.6626**	0.6614	-0.0106	0.247	0.2138
Number of seeds/ plant	-0.119	-0.0367	-0.1318	-0.2495	-0.5204	**-0.5213**	0.0085	-0.1965	-0.166
100 seed weight	-0.009	-0.0173	-0.0072	-0.0002	-0.0008	-0.0008	**0.0509**	-0.0005	-0.0035
Biological yield/plant	0.2301	0.2553	0.1363	0.1957	0.3255	0.3292	-0.009	**0.8732**	-0.3938
Harvest index	-0.1362	-0.1341	0.0791	0.0437	0.2616	0.2582	-0.0562	-0.3656	**0.8108**
Seed yield/ plant	0.1282	0.1638	0.218	0.2392	0.6954	0.6935	-0.0407	0.5617	0.4427

Residual value = 0.34

flowering (0.0047), had a lower direct influence, whereas number of seeds per plant (-0.5213), had a very strong negative direct effect on seed yield per plant, followed by number of primary branches /plant (-0.0754), and plant height (-0.0130) (**Table 4**). Days to 50 percent flowering had the largest positive indirect effect on plant height (0.0017), while 100 seed weight (-0.0008), harvest index (-0.0008) had the greatest negative indirect effect. These findings resembled with Nagarajan *et al.* (2015); Akram *et al.* (2016); Amogne *et al.* (2020); Mishra *et al.* (2015). Path analysis helps breeders to identify which traits have the most direct and significant impact on the grain yield (Abdel *et al.* 2022). This information is crucial for prioritizing traits in breeding programs. Following Mahalanobis D^2 statistics, the RILs were categorised into 12 groups (T**able 5)** and the proportion of contribution of all the characteristics to genetic divergence is presented in Table 6. Cluster I had large number of genotypes while cluster III had three genotypes. However, other clusters had only one genotype. Similar results were reported by Thakur *et al.* (2015); Kumar *et al.* (2018); Mishra *et al.* (2018); Soniasabanam *et al.* (2018); Bijarania, (2020); Shilpashree *et al.* (2021). The average intra cluster D^2 values revealed that Cluster I had the highest intra-cluster D^2 value (85.56), followed by cluster III (52.14), while the rest of the clusters were mono-genotypic and had no intra-cluster divergence. Clusters VI and XII had the highest inter cluster divergence (537.96), while cluster XIII and X had the lowest inter cluster divergence (59.00). Inter cluster distance optimize hybridization strategies by selecting parents from different clusters to maximizing genetic diversity in the breeding population (Upadhyay *et al.* 2022). Biological yield was observed to contribute the most to genetic difference, followed by days to maturity, harvest index, number of primary branches, number of pods/plants, plant height,100 seed weight, number of seeds/ plants, seed yield/ plant and days to 50% flowering. Similar findings were observed by Kumari *et al.* (2019), Mishra *et al.* (2018) and Bijarania, (2020). Clusters XII and III had the highest cluster mean values for most yield attributing factors, while cluster V had the highest cluster mean values for phenological attributes. By the help of cluster mean genotypes may identify with desirable trait to facilitating the development of cultivars with superior multi-trait performance (Azam *et al.* 2023). PCA gives a roadmap for reducing a complex data set to a smaller dimension with minimal effort (Khare, 2022). **Table 7** shows the eigen values, percent variance, and cumulative percentage of principal components analysis. Only four principal components (PCs) out of ten showed a value greater than 1.00, indicating 76.2 percent variability among the variables evaluated. As a result, the relevance of these four PCs was given appropriate consideration for further clarification. For the traits in this study, PC1 had the largest variability (34.9%), followed by PC2 (1.8%), PC3 (12.96%), and PC4 (10.37%). PC1 was linked with number of primary branches per plant, number of pods per plant, number of seeds per plant, and the biological yield per plant, which accounted for the most variability. Harvest index was dominant in the second PC, 100 seed weight was dominant in the third PC and number of primary branches was dominant in the fourth PC. Dubey *et al.* (2018) and Kumar *et al*. (2015) reported similar results. In PC1, 27 genotypes observed to have high values for variables such as number of primary branches per plant, number of pods per plant, number of seeds per plant, biological yield per plant, and seed yield per plant. In PC2, 23 genotypes had high values for Harvest index (%). In PC3, 22 genotype had high value for 100 seed weight and biological yield. In PC 4, 14 lines had higher values

Table 5. Distribution of soybean genotypes in different clusters

Cluster	Number of genotypes	Genotypes
I	87	RIL-107-92,JS 2029, RIL-107-91, RIL-107-62, JS 335, RIL-107-97, RIL-107-116,RIL-107-100, RIL-107-178, RIL-107-135, RIL-107-88, RIL-107-29, RIL-107-18, RIL-107-56, RIL-107-67, RIL-107-85, RIL-107-60, RIL-107-73, RIL-107-49, RIL-107-137, RIL-107-133, RIL-107-70, RIL-107-157, RIL-107-55, RIL-107-84, RIL-107-57, RIL-107-48, RIL-107-143, RIL-107-181, RIL-107-51, RIL-107-41, RIL-107-140, RIL-107-151, RIL-107-95, RIL-107-141, RIL-107-118, RIL-107-131, RIL-107-58, RIL-107-124, RIL-107-96, RIL-107-134, RIL-107-172, RIL-107-65, RIL-107-126, RIL-107-177, RIL-107-76, RIL-107-83, RIL-107-159, RIL-107-21, RIL-107-19, RIL-107-12, RIL-107-28, RIL-107-71, RIL-107-68, RIL-107-1, RIL-107-168, JS-97-52, RIL-107-11, RIL-107-161, RIL-107-106, RIL-107-132, RIL-107-59, RIL-107-111, RIL-107-79, RIL-107-138, RIL-107-98, RIL-107-32, RIL-107-64, RIL-107-174, RIL-107-36, RIL-107-105, RIL-107-103, RIL-107-31, RIL-107-46, RIL-107-35, RIL-107-154, RIL-107-136, RIL-107-110, RIL-107-109, NRC 37,RIL-107-27, RIL-107-80, RIL-107-114, RIL-107-9, RIL-107-121, RIL-107-23, RIL-107-7
II	1	RIL-107-123
III	3	RIL-107-3, RIL-107-5, RIL-107-6
IV	1	RIL-107-125
V	1	RIL-107-102
VI	1	RIL-107-8
VII	1	RIL-107-24
VIII	1	RIL-107-127
IX	1	RIL-107-52
X	1	RIL-107-115
XI	1	RIL-107-173
XII	1	RIL-107-101

Table 6. Intra and Inter-cluster divergence (D^2) for seed yield and associated traits

Cluster Number	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
I	85.56	172.19	168.43	144	176.33	136.88	193.1	177.6	150.15	177.98	157.48	360.9
II		0	255.89	143.21	164.38	189.92	287.29	79.03	309.4	155.01	167.78	297.57
III			52.14	224.98	293.49	197.08	221.87	320.18	283.17	300.81	360.95	473.45
IV				0	247.11	149.25	363.52	271.05	195.43	343.42	231.35	490.14
V					0	220.99	255.75	93.92	368.34	211.77	131.82	169.3
VI						0	165.8	288.71	344.79	369.04	353.64	537.96
VII							0	328.71	405.8	303.15	363.66	491.6
VIII								0	286.08	59	66.29	176.97
IX									0	193.85	154.67	417.15
X										0	69.21	232.16
XI											0	227.37
XII												0

Table 7. Summery of principal component analysis

Principal component (PC)	Eigen value	Variability (%)	Cumulative %
PC 1	3.489	34.888	34.888
PC 2	1.801	18.013	52.901
PC3	1.296	12.960	65.861
PC4	1.037	10.373	76.234

for number of primary branches and 100 seed weight (g). These findings identical with Kumar *et al.* (2015); Dubey *et al.* (2018); Berhanu *et al.* (2019); Sileshi *et al.* (2019); Singh *et al.* (2020); Manav and Kumar, (2017).

The traits having significant positive association with grain yield and having high heritability along with high genetic advance percent of means were identified to be plant height, number of primary branches per plant, number of pods per plant, number of seeds per plant, biological yield and 100 seed weight. Whereas, highest positive direct effect toward grain yield were observed by biological yield per plant, followed by harvest index and number of pods per plant. Therefore, these traits may be used in the further selection programme. Due to higher genetic divergence between cluster XII and VI, genotypes might be used in future hybridization programme to exploit maximum heterosis. The PC1 show highest variability. Moreover, genotypes may be selected for number of primary branches per plant, number of pods per plant and number of seeds per plant from PC1.

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