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RECOMBINANT BREEDING TO GENERATE TRANSGRESSIVE SEGREGANTS FOR GRAIN YIELD AND COMPONENT TRAITS IN SOYBEAN

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ABSTRACT

Yield and its component traits were studied in 181 F5 progenies, derived from a cross of NRCSL 3 x NRC 142, in an augmented RCBD, with 5 check genotypes to identify transgressive segregants for yield traits. Substantial variation for all the traits, except seeds per pod, was documented. High estimates of genotypic coefficient of variation, phenotypic coefficient of variation, heritability and genetic advance were recorded for pods per plant, biological yield, and economic yield. Transgressive segregants, surpassing the best checks, were observed for all the agronomic traits. Progenies 20-3-784-1 and 20-3-1557 outyielded the best check for biological yield, economic yield, harvest index and 100-seed weight. Around 80% of the progenies manifested resistance against Yellow Mosaic Disease (YMD). The results reinforce the utility of hybridization in generation of promising genotypes having high biological and economic yields, and improved genetic potential.

Keywords: Genetic variability, Hybridization, Transgressive segregants, Yellow mosaic disease (YMD)

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Soybean (*Glycine max* L. Merrill) is a miracle legume. It has rightfully been referred to as 'Golden bean', 'Cow of field', 'Gold from the soil' and 'Wonder crop', because of its various nutritional and therapeutical benefits. The soybean has higher protein content (~40%) than all other legumes with high digestibility and quality similar to animal proteins (Hughes *et al.,* 2011). Low levels of carbohydrates, high values of vitamins, minerals, essential fatty acids and isoflavones particularly enhance the value of soybean as food and feed (Messina, 2016). Soybean, therefore, is the crop of choice when it comes to tackling the protein and energy demands of an ever-growing human population.

Soybean holds the topmost position and importance amongst seed legumes cultivated across the globe, contributing approximately 66 per cent of animal feed and one-fourths of edible oil of the world production, thereby, accounting for one-tenths of the global trade in agriculture. India holds 5th rank in area and production after Brazil, USA, Argentina and China. India produced 11 million metric tonnes of soybean on an area of 13 million hectares in the year 2023. Although India holds 5th rank globally in terms of production, the productivity levels are merely 0.84 t/ha which is quite low when compared to the global average (2.84 t/ha) (USDA-FAS, 2024).

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Since its introduction as a crop in 1960s, productivity levels of soybean in India have lingered around 0.7-0.8 t/ha. The lower productivity can be attributed to many factors such as cultivation on marginal lands, rainfed cultivation, biotic stresses, limited mechanization and lower genetic potential (Aggarwal *et al.,* 2013). Under Punjab conditions Yellow Mosaic Disease (YMD) is a major constraint in soybean production. The disease can cause upto 100 per cent losses depending upon cultivar and the crop stage of incidence (Wrather *et al.,* 1997).

Hybridization is the tool of choice for breeders to combine and shuffle the genes of genotypes having desired traits. The recombinants generated are evaluated in subsequent segregating generations for desirable agronomic and morphological traits. However, hybridization itself becomes a futile exercise, unless some transgressive phenotypes are generated. Working in the direction to achieve increased genetical potential coupled with disease resistance a cross *viz*., NRCSL 3 x NRC 142 was made. NRCSL 3 also known as NRC 149 was notified in 2022 for North plain zone). NRCSL 3 has resistance to YMD, derived from PI 171443 (Singh *et al.,* 1974). NRC 142 is a food grade variety having stable yields across multiple environments in India and has been notified for Central zone and South zone. However, it is susceptible to YMD and, therefore, is not suitable for growing under North Indian conditions particularly

Punjab, where high incidence of YMD is reported. The present study was, therefore, undertaken with the objectives to assess genetic variation and to identify transgressive segregants having higher yield and other productivity traits from F_5 population of the cross NRCSL 3 x NRC 142.

MATERIALS AND METHODS

A cross between NRCSL 3 (♀) and NRC 142 (♂) was made during *kharif* 2020. Choice of NRC 142 as pollinator was made due to its susceptible nature to YMD and the problems associated in hybrid seed development. F_1s were grown in off-season nursery during winter 2020-21, F_2 population of 1573 plants was grown in *kharif* 2021 (Fig. 1a and b). The F_2 plants were labeled with the prefix '20-3' (Year of the cross – cross number). A total of 181 single plant selections were made from the F_3 sown as plant to progeny rows in *kharif* 2022 (Fig. 2). These were advanced in an offseason nursery (Fig. 3) and F_5 progenies were evaluated in *kharif* 2023 (Fig. 4). All the activities were carried out in experimental area of Pulses section (Soybean), Department of Plant Breeding and Genetics, Punjab Agricultural University (PAU), Ludhiana (Lat. 30.913571, Long. 75.785228).

The F_5 progenies were evaluated in an augmented randomized complete block design (ARCBD) (Federer, 1956) generated by PBTools software (PBTools, 2014). The experiment consisted of 181 progenies, 5 checks *viz.,* SL 958, SL 955, SL 1074, SL 979 and NRCSL 3 (parent). NRC 142 (parent) was included as a test treatment in the study. The experiment consisted of 210 treatment plots distributed in 5 blocks, with 42 experimental plots/block. The checks were assigned randomly and replicated 5 times. The entries were raised in paired rows of 1.75 m length with row to row spacing of 45 cm and plant to plant spacing of 0.05 m was maintained.



Fig. 1a. Juvenile stage of F_2 population, *Kharif* 2021



Fig. 1b. Disease reaction of F_2 population, *Kharif* 2021



Fig. 2: F_3 plant to progeny rows, *Kharif* 2022



Fig. 3. Offseason advancement of F_4 generation



Fig. 4. F_5 generation in field, *Kharif* 2023

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Table 1. Analysis of variance of morphological traits

ANOVA Treatment adjusted

Source	*Df*	Mean Sum of Squares								
		DF	DM	PH	PPP	SPP	BY	EY	HI	HSW
Block (ignoring Treatments)	*4*	48.52**	113.28**	639.89**	696.24**	0.04	735.11**	86.60**	73.52**	6.08**
Treatment (eliminating Blocks)	*189*	42.70**	63.50**	198.70**	549.20**	0.04	908.74**	127.81**	39.57**	2.55**
Treatment: Check	*4*	101.46**	29.34**	379.02**	770.45**	0.07	145.18**	82.68**	57.98**	15.91**
Treatment: Test and Test vs. Check	*185*	41.43**	64.24**	194.80**	544.42**	0.04	925.24**	128.78**	39.17**	2.26**
Residuals	*16*	1.66	1.44	67.76	36.54	0.04	22.70	3.40	5.35	0.56

ANOVA Block Adjusted

Source	*Df*	Mean Sum of Squares								
		DF	DM	PH	PPP	SPP	BY	EY	HI	HSW
Treatment (ignoring Blocks)	*189*	43.69**	65.65**	210.43**	563.37**	0.04	921.21**	129.51**	40.80**	2.66**
Treatment: Check	*4*	101.46**	29.34**	379.02**	770.45**	0.07	145.18**	82.68**	57.98**	15.91**
Treatment: Test	*184*	27.41**	58.80**	207.40**	559.98**	0.04	935.29**	129.73**	40.52**	1.89**
Treatment: Test vs. Check	*1*	2808.72**	1471.91**	93.78	357.49**	0.16	1435.39**	276.87**	23.51	90.09**
Block (eliminating Treatments)	*4*	1.66	11.54**	85.52	26.90	0.02	145.49**	6.18	15.71	0.78
Residuals	*16*	1.66	1.44	67.76	36.54	0.04	22.70	3.40	5.35	0.56

Significance codes: **P <= 0.05*; ***P <= 0.01*

Abbreviations used: DF: Days to flowering, DM: Days to maturity, PH: Plant height (cm), PPP: Pods per plant, SPP: Seeds per pod, BY: Biological yield (g), EY: Economic yield (g), HI: Harvest index (%), HSW: Hundred seed weight (g)

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The recommended package of practices was followed. Observations for days to flowering, days to maturity, plant height (cm), pods per plant, seeds per pod, biological yield, economic yield, harvest index and 100-seed weight were documented. The days to 50% flowering and maturity were taken on per plot basis while the rest of the data were recorded from 5 randomly taken plants. Seeds per pod were recorded as average of seeds from 20 pods.

Data for disease incidence was recorded as per Singh and Singh (2000). Briefly, per cent disease incidence (PDI) was calculated taking percentage of diseased/total plants; severity grade (SG) and corresponding response values (RS) were given based on cumulative disease scores of the plot. Coefficient of infection was calculated as product of DI x RS, and was used thereon to assign disease reaction (DR) to the genotypes (Table 2).

The data were subjected to analysis of variance as per Federer (1956), by using R software package 'augmented RCBD' (Aravind *et al.,* 2020). The same package was used to compute adjusted means, as well as various genetic parameters such as genotypic and phenotypic coefficients of variation, heritability (broad sense) and genetic advance (Burton and Devane, 1953; Allard, 1960; and Miller *et al.,* 1958). Standard error of difference between check and a treatment were calculated as per Federer (1956). Least significant interval (LSI) was calculated using two tailed t-df, (alpha 0.05) values for days to flowering and days to maturity, while for rest of the parameters one tailed t-df (alpha 0.05) values were used. LSI was then used for post-hoc comparisons. Correlations amongst the traits were calculated and plotted by using R package 'Performance Analytics' (Peterson *et al.,* 2018).

RESULTS AND DISCUSSION

Evaluation for morphological and agronomical traits and identification of transgressive segregants

Data were recorded for nine traits for all the entries. As per analysis of variance (ANOVA), all the traits, except seeds per pod, were found to be significantly different amongst the entries (Table 1). The coefficient of variation (CV) for all the traits was less than 10 % (data not shown); values < 20 % are considered reliable for field trails (Poehlman, 2013). The mean sum of squares was significantly different at 1% level of significance for Treatments, Checks and Treatment vs Checks for all the traits except seeds per pod. Out of the two parents, *viz.,* NRCSL 3 and NRC 142, only NRCSL 3 was included as a check. Since, NRC 142 is susceptible to YMD, all

Table 2. Disease reaction of F_5 progenies (PDI, SG, RV and CI are given as observed range)

Sr. No.	No. of F_5 progenies (Percentage)	Percent Disease Incidence (PDI)	Severity Grade (SG)	Response Value (RV)	Coefficient of Infection (CI)	Disease Reaction (DR)
1	115 (63.53 %)	0-9.52	0-2	0.00-0.50	0.00-4.00	Highly Resistant (HR)
2	31 (17.12 %)	8.11-12.00	1-4	0.25-1.00	4.05-9.00	Resistant (R)
3	16 (8.83 %)	11.11-18.52	1-4	0.25-1.00	10.34-16.67	Moderately Resistant (MR)
4	9 (4.97 %)	20.51-27.27	4	1.00	20.51-27.27	Moderately Susceptible (MS)
5	1 (0.55 %)	55.56	4	1.00	55.56	Susceptible (S)
6	9 (4.97 %)	100	4	1.00	100	Highly Susceptible (HS)

the yield traits are expected to be underestimated. Therefore, it was not considered to identify desirable segregants. Post-hoc comparisons, using adjusted means, were made with best checks for all the traits except seeds per pod and have been discussed below.

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Days to flowering

SL 958 was the earliest check to exhibit flowering, while SL 1074 was the last check to flower with mean 59.6 and 69.4 days to flowering, respectively. The parent NRCSL 3 was at par with the best check (60.2 days), while pollen parent NRC 142 was significantly early in flowering (50.42 days). A total of 130 F_5 progenies were significantly early in flowering than SL 958, while 12 progenies were significantly late to flower. However, none of these were late in flowering than the last flowering check, SL 1074. Six F_5 progenies (20-3-35, 20-3-780, 20-3-1067, 20-3-1215, 20-3-1278, and 20-3-1482) were the earliest with mean of 45.52 days, while the latest entry to flower, 20-3-148 took 66.52 days.

Days to maturity

Female parent NRCSL 3 exhibited longest maturity duration (140.6 days) as compared to NRC 142 (112.84 days). SL 958 was again the best check maturing in 136.4 days. Similar to flowering, 113 progenies were significantly early maturing than SL 958. Progeny no. 20-3-1222 was the earliest entry to mature, taking 110.84 days. Twenty-four progenies were late maturing than the early check SL 958, and 4 of these progenies namely 20-3-26, 20-3-29, 20-3-115-2 and 20-3-1495 were significantly late maturing than NRCSL 3. 145 progenies were statistically early maturing than NRCSL 3.

Plant height (cm)

The parents NRCSL 3 and NRC 142 had mean height of 103.96 and 63.42 cm, respectively, while SL 979 was the shortest amongst the checks (86.52 cm). Only 9 of the progenies were significantly shorter than SL 979. Most of the progenies were taller than SL 979, with 151.41 cm being recorded as the maximum height. The shortest progeny 20-3-1375 was recorded to be 53.41 cm in height. 39 progenies were significantly shorter than NRCSL 3, indicating that genes for short plant height were contributed by NRC 142. The progenies 20-3-274 and 20-3-1335 were significantly taller than NRCSL 3, with height of 151.41 and 128.72 cm, respectively.

Pods per plant

NRCSL 3 had significantly higher pods per plant (83.72) *vis-à-vis* NRC 142 (31.34). SL 955 outperformed all other checks having highest 86.48 pods per plant. Forty F_5 lines were superior than SL 955 for pods per plant. Maximum number of pods per plant were recorded for 20-3-761 (148.31) followed by 20-3-131 (138.71).

Biological yield (g)/plant

NRCSL 3 had significantly higher biological yield (80.08 g/plant) than NRC 142 (30.47 g/plant) and was also the superior most check. Approximately 40 per cent of the F_5 entries exhibited transgressive segregation for this trait, and had significantly higher biological yields than NRCSL 3. Two of the entries, *i.e.,* 20-3-38 and 20-3-1335 had more than double the biological yield than NRCSL 3, with 165.3 g (yield of 20-3-1335) being the maximum value for biological yield.

Economic yield (g)/plant

NRCSL 3 was the best check for economic yield, same as biological yield, with mean yield of 26.45 g/plant. However, modest values of biological yield were recorded for NRC 142 (5.98 g/plant). Out of 181 F_5 progenies, 55 transgressive segregants were observed that significantly out-yielded the best

check. Three progenies, 20-3-140, 20-3-627 and 20-3-937, yielded more than double of the best check with respective economic yields of 53.97, 57.17, and 54.57 g/plant.

Harvest index (%)

The pollen parent, NRC 142 had a harvest index of 16.08 %, the lowest value recorded in the trial. NRCSL 3 was the check having highest harvest index (33.08 %). Around 16 % of the tested lines had statistically higher values of harvest index than the check, with 54.19 % (20-3-481) being the maximum harvest index value. Eleven lines had outstandingly high harvest index values (>40 %).

100-Seed weight (g)

The best check, NRCSL3 had mean 100-seed weight of 11.5 g, 1.5 times more than NRC 142 (7.8 g). Adjusted 100-seed weight of 14 F_5 progenies was significantly more than NRCSL 3, with four genotypes; 20-3-16, 20-3-165, 20-3-430 and 20-3-1374, having remarkably high values (>14 g). Thus, these 14 lines manifested transgressive segregation for 100-seed weight.

Progeny number 20-3-784-1 and 20-3-1557 were highly desirable segregants as these genotypes outperformed best check NRCSL 3 for biological yield, economic yield, harvest index and 100-seed weight. Genotypes 20-3-39-1, 20-3-115-2, 20-3-140, 20-3-627, 20-3-644, 20-3-657-1, 20-3-657-2, 20-3-821, 20-3-937, 20-3-1098, 20-3-1495, and 20-3-1537 were statistically superior than the best check for three traits *viz.,* biological yield, economic yield and harvest index. Similarly, five F_5 lines (20-3-274, 20-3-430, 20-3-468, 20-3-1212 and 20-3-1415) were better performing than NRCSL 3 for biological yield, economic yield and 100-seed weight.

Screening for Yellow Mosaic Disease (YMD) Resistance

The genotypes evaluated were classified into six categories based upon the Coefficient of Infection (CI) (Table 2). The checks (including parent NRCSL 3 (Fig. 5a) were all recorded to be Highly Resistant (HR) against the virus. However, the pollen parent, NRC 142 (Fig. 5b) was Highly Susceptible (HS) to the disease. Coefficient of infection (CI) was recorded to be 91.66 for NRC 142. A total of 115 lines out of the 181 (63.5%) F_5 progenies screened, exhibited HR reaction with CI ranging from 0 to 4. Thirty-one progenies were recorded to be Resistant (R) with CI values of 4.05 to 9. Seventeen and nine of the



Fig. 5a. Disease reaction (HR) of NRCSL 3



Fig. 5b. Disease reaction (HS) of NRC 142

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Table 3. Adjusted Mean, Range and Genetic variability parameters for different traits

Sr. No.	Trait	Mean	S.E.	Range	PV	GV	GCV	PCV	hBS	GA	GAM	GAM category
1	DF	53.72	0.40	45.52 - 69.40	27.41	22.95	8.92	9.74	83.73	9.04	16.83	Medium
2	DM	130.28	0.57	110.84 - 144.84	58.80	57.36	5.81	5.89	97.55	15.43	11.85	Medium
3	PH	95.46	1.02	53.41 - 151.41	207.40	139.64	12.38	15.09	67.33	20.00	20.95	High
4	PPP	81.19	1.71	14.75 - 148.31	559.98	523.45	28.18	29.15	93.48	45.63	56.20	High
5	SPP	2.26	0.02	1.66 - 3.05	Values not computed as P-value for 'Treatment: Test' is > *0.05.*							
6	BY	82.02	2.21	10.16 - 165.30	935.29	912.59	36.83	37.29	97.57	61.56	75.06	High
7	EY	25.59	0.82	1.37 - 57.17	129.73	126.33	43.93	44.51	97.38	22.88	89.43	High
8	HI	30.78	0.49	11.55 - 54.19	40.52	35.16	19.26	20.68	86.78	11.40	37.02	High
9	HSW	11.07	0.10	7.24 - 14.99	1.89	1.33	10.43	12.43	70.38	2.00	18.05	Medium

Abbreviations used: SE = Standard error, PV = Phenotypic variance, GV = Genotypic variance, PCV = Phenotypic coefficient of variation, GCV = Genotypic coefficient of variation, hBS = heritability (broad sense), GA = Genetic advance, GAM = Genetic advance as percentage of mean

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progenies were found to be Moderately Resistant (MR) and Moderately Susceptible (MS), respectively. Nine of the progenies were classified as HS to the disease.

Genetic variability parameters

Assessment of the nature as well as the magnitude of variation is important for plant breeding. The genetic variability parameters have thus been estimated and presented (Table 3). The values of genotypic coefficient of variation (GCV) were smaller than respective phenotypic coefficient of variation (PCV) for the traits under consideration. However, the difference between GCV and the respective PCV for all the traits was narrow, indicating that the impact of environment was not profound and genotypic component was the major source. The widespread range of PCV as well as GCV illustrate the variation present in the material. PCV values for days to flowering (9.74) and maturity (5.89) were towards the lower side, while moderate estimates were observed for plant height (15.09) and 100-seed weight (12.43). High estimates of PCV were recorded for pods per plant (29.15), biological yield (37.29), economic yield (44.51) and harvest index (20.68). GCV category for all the traits was same except harvest index (19.26) which lay in medium category. Variability analysis was not conducted for seeds per pod due to non-significant mean squares. Moderate to high estimates of coefficients of variation suggest that ample variation is present for all the traits except days to maturity for which low estimates were obtained. Similar estimates for GCV and PCV were reported by Joshi *et al.* (2018) and Chawan *et al.* (2023).

Estimates of heritability (broad sense) include both additive and non-additive effects and hence, help in determining the heritable portion of variation (Hanson *et al.,* 1956). Heritability (broad sense) of all the traits under study was recorded to be high. Heritability estimates ranged from 67.33 % for plant height to 97.57 % for biological yield. Similar values were observed for days to maturity (97.55 %) and economic yield (97.38 %), closely followed by days to flowering (93.94 %) and pods per plant (93.48 %). Heritability estimates for harvest index and hundred seed weight were 86.78 % and 70.38 %, respectively. Heritability estimated for all the traits was high, thus additive gene action was predominant for the characters under study. Chawan *et al.* (2023) and Chauhan *et al.* (2023) also found high heritability for yield and its component traits in soybean germplasm lines. Genetic advance expressed as percentage of mean (GAM) is another important parameter for selection. GAM was found to be in high category for plant height (20.95 %), pods per plant (56.20 %), biological yield (75.06 %), economic yield (89.43 %) and harvest index (37.02 %). GAM values for days to flowering, days to maturity and 100-seed weight were 18.89 %, 11.85 % and 18.05 %, respectively, and were all categorized as moderate. The highest and lowest values for GAM were observed for economic yield and days to maturity, respectively. Joshi *et al.* (2018) and Chauhan *et al.* (2023) documented similar findings in soybean. The narrow difference between GCV and PCV coupled with high heritability and genetic advance has important implications for breeders. Selection based on phenotypic values for these traits would be effective and the gains will be

realized in subsequent generations.

Correlation coefficient

Correlation was estimated and plotted as per Pearson's coefficient of correlation (Fig. 6). Strong and positive correlation was found between biological and economic yield (r = 0.89**). Similarly, pods per plant were positively and significantly correlated with biological (r = 0.82**) and economic yields (r = 0.77**). Days to flowering and days to maturity were moderately and significantly correlated (r = 0.57**). Plant height and biological yield (r = 0.57**); and economic yield and harvest index were also significantly correlated (r = 0.57**). Positive and significant correlations were also observed between days to maturity - economic yield; days to maturity - harvest index; plant height - pods per plant; and plant height - economic yield. Ullah *et al.* (2024) and Karyawati *et al.* (2021) also observed significantly positive correlation between economic yield and plant height, biological yield and hundred seed weight.

The gap between Indian (0.84t/ha) and global productivity (2.84 t/ha) is quite large. Under the current scenario of climate change and depleting water tables in rice-wheat based cropping systems, soybean crop can be a good alternative. However, adoption by farmers is primarily driven by remuneration, particularly in north-western plains zones, where rice is grown as a cash crop. Current levels of productivity of soybean are lower and cannot compete with paddy. Scaling up of productivity can be achieved by developing suitable varieties, mechanization technologies and agronomic practices. Therefore, a hybridization-based breeding effort was planned to scale up the productivity by increasing genetic potential of the crop. NRCSL 3 (SL 958 x NRC 94) was crossed with NRC 142 [Pedigree: (JS 97-52 x PI 596540) x PI542044] to combine desirable alleles from both the genotypes. The hybridization programme was successful in generating genetic variation as is evident from the analysis of data. This was probably due to different origin and descent of the parents as female parent was developed at PAU, Ludhiana, while pollen parent has been developed by

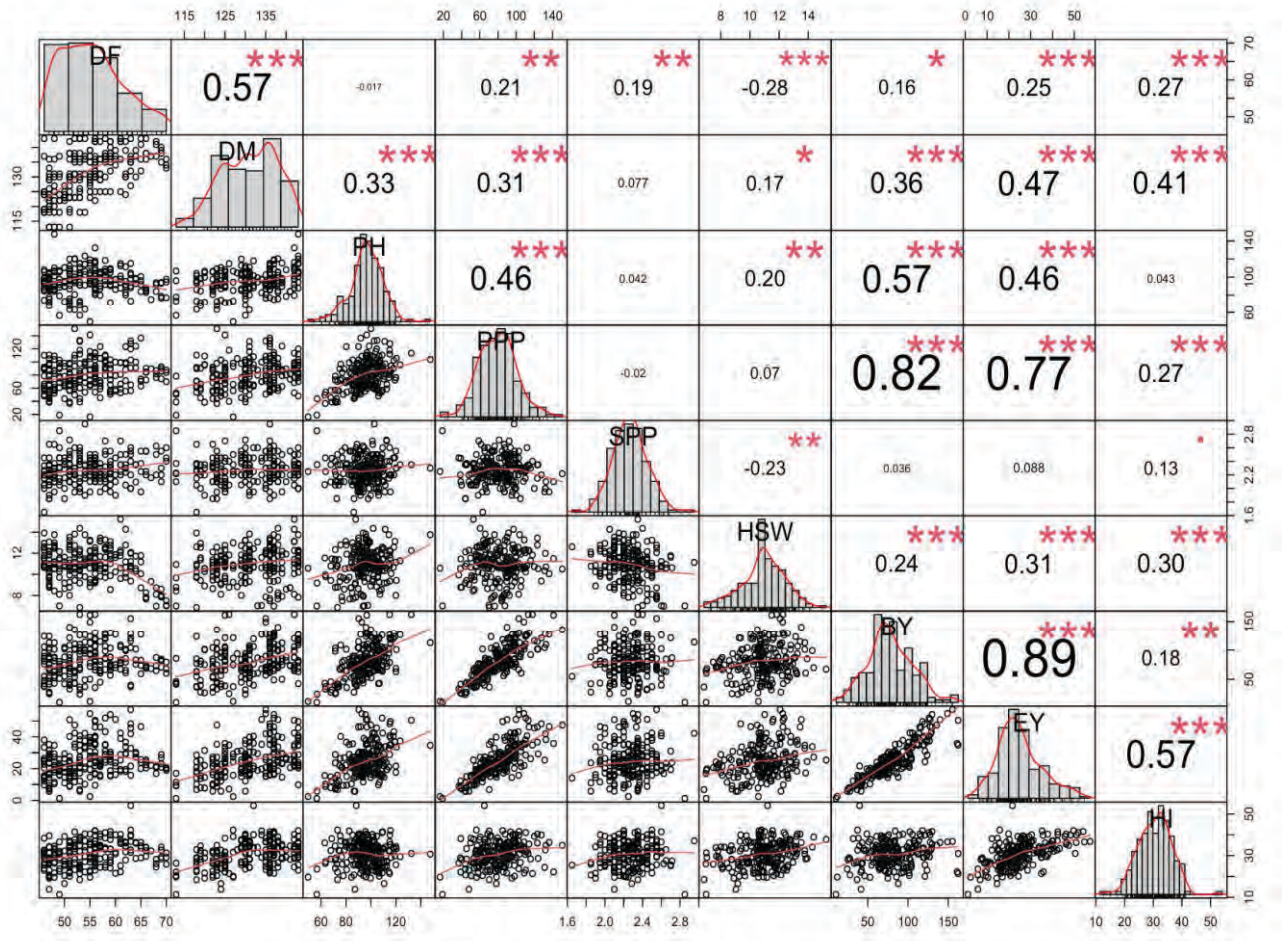


Fig. 6. Correlation coefficient (r) values for morphological traits

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IISR at Indore. The F_5 progenies were compared with best checks and parents of the cross to determine which entries out-did the checks and parents. The female parent NRCSL 3, was the best check for biological yield, economic yield, harvest index as well as hundred seed weight, thereby justifying its use as one of the parents.

NRC 142, the pollen parent was released in the central and southern zone for cultivation. The central zone is main region of soybean production; however, it generally escapes the attack of YMD. PAU can be regarded as the hotspot for YMD as the conditions during *kharif* season are congenial for vector (whitefly) survival and multiplication. The poor performance for NRC 142 for all the agronomic and yield contributing traits can be attributed to its susceptibility to YMD. Agronomic data of NRC 142 for traits is testament for the fact that disease resistance is one of the key factors to ensure sustained production. NRCSL 3, the female parent, and all the other check genotypes were highly resistant against YMD. Among the tested progenies, 80.65 % of the F_5 progenies were resistant against YMD, which was due to the resistance genes coming from NRCSL 3 and single plant selections performed in F_3 generation.

CONCLUSION

Thus, it can be concluded that hybridization between sensibly chosen parents was successful in generating ample and useful variation in form of transgressive segregants, for all the characters, except seeds per pod. The promising lines identified from present evaluation can be further tested for variety release.

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Authors' contribution

Conceptualization and designing of the work (BSG, IS, KSM); Execution of field/lab experiments and data collection (KSM, LK); Analysis of data and interpretation (PS, KSM); Preparation of manuscript (BSG, IS, NKD, KSM).

Conflicts of interest

The authors declare that they have no conflict of interests.

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