Identification of rust resistant lines and their genetic variability and character association studies in soybean [*Glycine max* (L.) Merr.]

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Abstract

Segregating populations (F$_3$) of two crosses involving two high yielding varieties - JS335 and JS9305 (both susceptible to rust) and one germplasm line EC241780 (resistant to rust) were screened through artificial inoculation for resistance to soybean rust. Six among the 62 progeny lines were resistant or moderately resistant to rust. The selected lines had yield level at par with JS335 and/or JS9305. Significant genetic variations were observed in the progeny lines for yield and other yield attributing characters except pod length and number of seeds per pod. The differences between phenotypic and genotypic coefficient of variations for the characters were narrow. High estimates of heritability coupled with high genetic advances were recorded for a number of traits. Positive and significant phenotypic correlation of seed yield was observed with number of branches per plant, pods per plant, pod weight, number of seeds per plant, biomass and harvest index. The identified lines will be the primer for developing improved soybean genotypes with resistance to rust disease.

Key words: Soybean rust, artificial inoculation, resistant lines, variability, heritability

Introduction

Soybean [*Glycine max* (L.) Merr.], a high energy legume crop with 38-44 per cent protein and 18-22 per cent oil has occupied the number one position in area and production among the oilseed crops in India. To sustain its production and to have enhanced export of deoiled cake (DOC), the crop needs to be protected from the biotic and abiotic stresses that may cause catastrophic damage to it. Among the biotic stresses (diseases) of soybean, rust disease is the most devastating one. Soybean rust, also called as Asian soybean rust is caused by two species of fungus *Phakospora pachyrhizi and P. meibomiae*. The Asian soybean (old world) rust caused by *P. pachyrhizi* is more destructive than South American (new world) rust (*P. meibomiae*). In India, Asian soybean rust (ASR) is the prevalent one causing large scale damage to the crop. Losses caused by it is > 30 per cent which may go up to 100 per cent [1] in congenial environment, heavy load of inoculums or epidemic depending upon season and cultivars grown. At present, the only method used to control soybean rust is fungicide application, which is neither economic nor environment friendly. Similarly, the outcome of integrated management practices of rust is far from encouraging. In this context, the genetic resistance has proven to be the most viable, appropriate and environment friendly method for control of rust. Therefore identification of resistant sources and involving them in hybridization programme is one of the major breeding objectives for soybean rust management. However for genetic improvement of crop, information on variability, its nature and magnitude as well as character association is essential as it helps in formulating selection criteria for different traits in breeding programme. Hence, the present study was undertaken to screen two F$_3$ populations against rust resistance and, also to analyze the extent of genetic variability present in them. The populations were developed by crossing one rust resistant genotype (EC241780) with two susceptible genotypes (JS335 and JS9305), and the two segregating generations were screened under artificially inoculated conditions to identify rust resistant lines with better yield potential.

Material and methods

The experimental materials were comprised of two F_3 populations of crosses involving two popular but rust susceptible varieties namely, JS 335 and JS 9305 and one rust resistant donor genotype EC241780. Eighteen progenies from the cross JS 335 x EC241780 and 44 progenies from JS9305 x EC241780 were grown during 2007-08 at Main Agricultural Research Station, University of Agricultural Sciences, Dharwad. Screening of the plants was done in net house under controlled conditions. Standard package of practices were followed to ensure good crop growth. The observations on pustule density and sporulation intensities were recorded on middle leaves following the "three digit scientific notation system" formulated by international working group on soybean rust (IWGSR) [2]. As per this system, score 111 signifies that observations were taken on bottom third leaves which showed no rust pustules and hence reaction is immune. Similarly 123 denotes bottom third leaves infected with a few highly sporulating pustules indicating succeptible reaction, and so on.

For genetic analysis, observations for traits were recorded on fifteen randomly selected plants in each line with two replications. The targeted traits are days to flowering, plant height(cm), number of branches per plant, number of pods per plant, pod length(cm), pod weight(g), number of seeds per pod, number of seeds per plant, seed yield per plant(g), hundred seed weight(g), biomass(g) and harvest index. The genotypic and phenotypic coefficient of variation, heritability and genetic advance for each character was computed as per standard formulae [3-5].

Results and discussion

In the present study, two soybean varieties *viz.*, JS 335 and JS 9305 which is occupying more than 50% soybean area in India were crossed with EC241780 to incorporate rust resistance into it. The F_1s of both the crosses were advanced to F_2 during *Kharif,* 2007. However, due to poor rust incidence in the field, the populations could not be tested for resistance. During *rabi* season (2007-08), all the 62 F_3 families (18 families from JS335 x EC241780 and, 44 from JS9305 x EC241780) including three parents were screened under artificial condition. The rust incidence was recorded on 30 days old plants from each progeny lines (Table 1). The donor EC241780 exhibited high level of resistance (score 221), whereas recipients JS335 and JS9305 were susceptible (score 243). Out of 62 F_3

Table 1. Reaction of progeny lines to soybean rust under artificially inoculated condition

Genotypes/line nos.	Score as per 3 digit system	Rust reaction
Parents		
JS 335	243	S
JS 9305	243	S
EC 241780	221	**R**
Cross 1 (JS 335 x EC 241780)		
Line Nos. 1,2,5,7,8 (5 lines)	243	S
Line Nos. 3,6,9,10,11,12,13,14, 15,18 (10 lines)	233	S
Line Nos. 4,17 (2 lines)	232	MR
Line No. 16(one line)	221	**R**
Cross 2 (JS 9305 x EC 241780)		
Line Nos. 1,3,7,21,23(5 lines)	221	**R**
Line Nos. 4,5,10,12,14,17,22,24, 25,35, 40, 41,42,44 (14 lines)	232	MR
Line Nos. 6,8,9,11,13,15,16,18, 19,20,26, 27,29,30,31,32,33,34, 36,37,38,39,43 (23 lines)	233	S
Line Nos. 2,28 (2 lines)	243	S

R = Resistant, MR = Moderately resistant, S=Susceptible

progenies, only six (one progeny line from the cross JS335 x EC241780 and five from JS9305 x EC241780) exhibited resistance (Fig. 1), 16 progenies (two from JS335 x EC241780 and 14 from JS9305 x EC241780) exhibited moderately resistance, and rests showed susceptible to highly susceptible reactions. In soybean, four genes have so far been reported that confers resistance against ASR. These four genes (*Rpp1, Rpp2, Rpp3* and *Rpp4*) alone or in various combinations confer resistance in various degrees, i.e. from moderately resistance to immune [6]. Even though single gene can offer good level of resistance however, such resistance is often broken down by rust fungus through evolution of new races or non-compatible host-pathogen reaction. Therefore, pyramiding of different resistance genes is required to overcome historical failure of monogenic resistance [7]. The genetic make-up of the resistant or moderately resistant progenies identified in the current study could not be established due to absence of disease reactions in F_1 and F_2 generations. However, molecular markers which are linked these resistance genes may be employed for this purpose. Once established, these genotypes will be fit to use as improved donor for rust resistance. The unique

advantage of such lines is that these have been developed on the genetic background of improved varieties, and hence there should not be any fear for linkage drag. Secondly, the identified lines found to possess a number of desirable traits including higher yield (Table 2). Line nos. 17 and 4 with moderate resistance yielded (24.9g and 18.93g/plant, respectively) higher than JS335 (17.36 g/plant) and JS9305 (17.36 g/plant). Days to flowering and other traits were also comparable to the recipient parents (Table 2). Similarly, the resistant line no.3 possessed yield and other traits similar to the recipient parent. Other lines with resistance/moderately resistance also had trait values in the desirable range. Hence, these lines deserve further evaluation and testing at several locations to identify improved soybean lines with resistance to rust.

To examine breeding utilities, genetic parameters were studied for the lines developed. It was observed that the progenie exhibited significant variability for yield and yield-attributing traits. The estimates depicting the genetic variability including mean, range, phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability (h^2), genetic advance (GA) and genetic advance over percent mean ((GAM) of both the crosses are present in Table 3. Baring a few traits like no. of seeds per pod and pod length, all other traits exhibited wide range of variations. This was substantiated by the fact that seed yield per plant, biomass, pods per plant and pod weight showed highest PCV and GCV indicating presence of extensive variability rendering selection effective. The estimates were more pronounced in the progenies of JS335 x EC241780, indicating more scope for the trait improvement in its progenies through selection. Moderate estimates of PCV and GCV were recorded for branches per plant and 100-seed weight while it was rather low for seeds per pod and days to flowering. As it has been seen here, these traits exhibited moderate to low PCV and GCV in a number of similar other studies [8, 9]. The narrow differences between PCV and GCV

Table 2. Rust resistant lines and their salient features for yield and yield attributing traits

S.No.	Cross	Progeny line no.	Salient features of progenies						
			Days to flowering	Plant height (cm)	No. of branches	No. of pod/ plant	100-seed weight (g)	Seed yield (g)	Rust reaction*
1.	JS 335 x EC 241780	16	37.00	29.00	2.70	27.83	16.60	11.45	R
		4	38.00	58.59	4.37	55.83	13.98	18.93	MR
		17	40.50	59.03	4.17	63.90	14.79	24.97	MR
2.	JS 9305 x EC 241780	1	36.50	55.67	3.10	37.07	12.16	12.37	R
		3	39.00	51.70	3.70	42.43	13.72	16.54	R
		7	39.00	51.93	3.53	39.26	9.13	13.20	R
		21	39.00	43.36	3.82	45.78	10.76	14.19	R
		23	36.50	44.30	3.16	30.67	12.32	14.23	R
		4	36.50	54.10	3.30	41.73	12.85	15.98	MR
		5	38.50	41.63	3.40	42.26	11.84	16.77	MR
		10	37.50	49.00	3.80	39.80	10.87	11.32	MR
		12	44.50	38.47	3.16	35.27	11.55	13.75	MR
		14	37.50	49.10	4.23	45.17	9.96	14.52	MR
		17	36.00	39.20	2.40	32.80	13.19	14.54	MR
		22	45.50	37.93	3.17	38.77	14.84	15.14	MR
		24	39.00	34.03	2.36	19.36	15.39	12.51	MR
		25	38.00	37.10	3.47	31.03	13.22	16.29	MR
		35	37.00	51.26	3.58	34.97	11.43	15.17	MR
		40	45.00	56.63	4.16	47.86	11.07	19.35	MR
		41	37.00	39.83	3.27	32.77	10.48	14.01	MR
		42	38.00	35.37	2.96	27.73	11.58	10.61	MR
		44	37.00	53.90	3.83	44.46	11.46	13.00	MR
3.	Recipient parent	JS 335	37.00	42.74	4.57	52.06	14.43	18.69	S
4.	Recipient parent	JS 9305	36.00	41.93	3.62	44.90	14.05	17.36	S
5.	Donor parent	EC241780	52.00	60.07	3.53	45.50	14.32	14.41	R

* R – Resistant, MR – Moderately resistant and S – Susceptible.

Table 3. Estimates of variability parameters for different quantitative traits in F_3 population of JS335 x EC241780 (C-1) and JS9305 x EC241780 (C-2)

S.No.	Traits	Cross	Mean	Range	PCV	GCV	h^2 (%)	GA	GAM
1	Days to flowering	C-1	39.62	37.00-52.00	9.02	8.78	94.90	6.98	17.61
		C-2	39.29	35.50-52.00	9.36	9.12	95.00	7.20	18.32
2	Plant height (cm)	C-1	54.33	29.00-65.51	16.69	13.04	61.00	11.40	20.98
		C-2	45.45	25.50-64.63	20.80	18.53	79.30	15.45	33.99
3	Number of branches per plant	C-1	3.88	2.70-4.76	18.46	16.89	83.70	1.24	31.95
		C-2	3.48	2.36-4.57	15.08	18.65	70.40	0.76	21.83
4	Pod length (cm)	C-1	3.41	2.50-4.10	15.58	14.58	87.60	0.96	28.15
		C-2	3.52	2.92-3.99	9.99	7.26	52.90	0.38	10.79
5	Pods per plant	C-1	49.68	27.83-63.90	21.13	19.54	85.50	18.49	37.21
		C-2	38.45	19.36-53.60	20.50	18.74	83.60	13.57	35.29
6	Pod weight (g)	C-1	27.07	14.18-34.66	23.75	22.46	89.40	11.84	43.73
		C-2	20.69	13.04-30.72	18.11	16.49	83.00	6.41	30.98
7	Number of seeds per pod	C-1	2.65	2.40-2.85	5.39	4.59	72.70	0.21	7.92
		C-2	3.46	2.55-3.65	5.34	5.07	90.00	0.34	9.82
8	Number of seeds per plant	C-1	131.32	69.93-172.09	22.80	21.49	88.80	54.78	41.71
		C-2	123.44	78.20-175.40	18.82	18.33	94.80	45.40	36.77
9	100-seed weight(g)	C-1	14.21	11.86-17.16	12.57	8.87	49.80	1.83	12.87
		C-2	12.38	8.85-15.92	14.07	11.35	65.10	8.34	18.90
10	Biomass per plant(g)	C-1	34.06	23.58-43.55	22.56	21.85	93.80	14.85	43.59
		C-2	28.73	20.45-42.17	17.34	15.50	79.90	8.20	28.54
11	Harvest index	C-1	52.18	34.14-57.81	12.37	10.21	68.20	9.06	17.36
		C-2	51.10	34.14-59.85	11.15	8.74	61.50	7.22	14.12
12	Seed yield per plant(g)	C-1	17.86	11.28-24.97	26.16	25.08	91.90	8.85	49.55
		C-2	14.81	7.64-20.32	20.73	19.54	88.80	5.62	37.94

observed here indicated the lesser influence of environment in expression of these traits. This is the other indication of genetic improvement for these traits to be effective through selection.

Genetic contribution to phenotypic expression of a trait is better reflected by the estimates of heritability. A higher estimate of heritability indicates presence of more fixable variability. In this study, high heritability was recorded for days to flowering, no. of branches per plant, harvest index and pod weight. It thus indicated that better expressions of these traits are primarily due to the genetic factors and hence fixable. Traits with moderate heritability estimate *viz.*, 100-seed weight and pod length indicates moderate influence of the environment in its expression. Hence selection for such traits should be made based on over-the-year's

performance in different locations. Success of such approaches has already been reported elsewhere [8].

Prediction of successful selection becomes more accurate if it is based on estimates of heritability coupled with genetic advance, because it gives estimates not only of genetic contribution but of expected genetic gain out of selection as well. In this study high heritability coupled with high genetic advance was recorded for plant height, no. of branches per plant, pods per plant, pod weight and seeds per plant. These traits are thus controlled by additive gene action and hence selection based on phenotypic observations would be effective. However, high heritability with moderate genetic advance was recorded for days to flowering, no. of seeds per pod, and harvest index indicating involvement of both additive and non additive gene action and hence

selection for these traits based on phenotypic observation alone may not be effective.

Depending upon the positive or negative effect of each interacting traits, the yield may either be high or low. Hence it is imperative to study the correlation among the yield attributing traits. In the present study it was found that no. of braches per plant, pods per plant, pod weight and harvest index showed positive and significant correlation with yield (Table 4). These traits are also found to have high heritability and high genetic advance. The same trend has been observed in the progenies from both the crosses. Thus selection based on these traits is expected to contribute towards yield enhancement. While estimating the associations among the yield attributing traits, it was observed that no. of branches per plant had significantly positive association with pods per plant, biomass, plant height and harvest index. Similarly, pods per plant had highly significant and positive association with no. of braches per plant, pod weight, seeds per plant, biomass and harvest index. Also, pod weight exhibited highly significant and positive association with branches per plant, no. of pods per plant and harvest index. Seeds per plant were found to have significant and positive correlation with branches per plant, pods per plant, pod weight and biomass. However it showed negative association with 100-seed weight. Other traits *viz*., pod weight, pods per plant and branches per plant also showed negative association with 100-seed weight. Trend of character

Table 4. Phenotypic correlation coefficients for seed yield and yield components in F_3 population of JS335 x EC241780 (C-1) and JS 9305 x EC241780 (C-2)

Traits	Cross	Days to flower	Branches/plant (no.)	Pods/plant (no)	Pod weight (g)	No. of seeds/plant (no)	100-seed weight (g)	Biomass/plant (g)	Harvest index (%)	Seed yield/plant (g)
Days to flower	C-1	1.0000	-0.154	-0.061	-0.180	-0.039	-0.188	0.099	-0.400	-0.170
	C-2	1.0000	-0.121	0.025	-0.130	-0.098	0.071	0.041	-0.318*	-0.197
Branches/plant (no)	C-1		1.0000	0.910**	0.919**	0.880**	-0.190	0.713**	0.361	0.803**
	C-2		1.0000	0.750**	0.757**	0.765**	-0.272	0.436**	0.356*	0.589**
Pods/plant (no)	C-1			1.0000	0.963**	0.932**	-0.247	0.751**	0.385	0.844**
	C-2			1.0000	0.846**	0.878**	-0.102	0.622**	0.350*	0.707**
Pod weight (g)	C-1				1.0000	0.925**	-0.197	0.759**	0.388	0.862**
	C-2				1.0000	0.846**	-0.119	0.717**	0.297*	0.751**
No. of seeds/plant (no.)	C-1					1.0000	-0.166	0.766**	0.421	0.873**
	C-2					1.0000	-0.207	0.626**	0.428**	0.772**
100-seed weight (g)	C-1						1.0000	0.210	-0.121	0.151
	C-2						1.0000	0.207	0.013	0.221
Biomass/plant (g)	C-1							1.0000	-0.044	0.837**
	C-2							1.0000	0.014	0.766**
Harvest index	C-1								1.0000	0.494*
	C-2								1.0000	0.592**
Seed yield/ plant (g)	C-1									1.0000
	C-2									1.0000

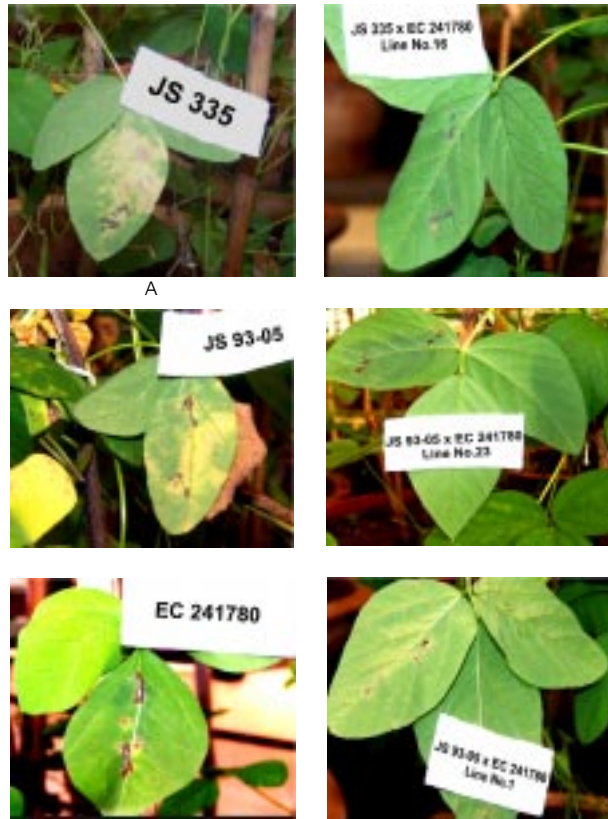*, ** Significant at 5% level and 1% level, respectively

Fig. 1. Soybean genotypes showing susceptible - (A) JS-335, (B) JS-9305, and resistant (C) EC-241780 reactions. Progenies (D, E, F) showing resistance to rust.

association was same in both the crosses except in one or two. It indicates that yield improvement would be achieved through the improvement in positively associated traits. Biomass as well as harvest index found to be positively associated with no. of branches per plant, pods per plant, pod weight, and seeds per plant. High harvest index coupled with high biomass indicates efficient conversion of photosynthates to economic yield. In this study it was found that biomass and harvest index is positively correlated. Biomass is also positively associated with other yield attributing traits. It thus showed that increase in yield in these rust resistant/moderately resistant lines is possible through increase in biomass and harvest index.

In this study, 3 lines have been identified which are resistant or moderately resistant to rust and have yield level similar to or more than JS335 and JS9305. There are some other potential lines with resistant or moderately resistant to rust. Line nos. 4, 17, 3, 5, and 40 are resistant/moderately resistant and have more no. of branches, pods, and seed yield per plant. Therefore evaluation, testing and further selection in the progenies of these identified lines will be practiced for final improvement in yield with rust resistance in soybean.

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