

Original Research Article

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Characterization of Interspecific Hybrids in Soybean (*Glycine max* L.)

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ABSTRACT

Successful interspecific hybrids were obtained when we crossed the cultivated soybean with wild annual *Glycine soja*. Although the success rate was low, whereas, the success rate was zero in case of wild perennial *G. tomentella*. Probably there is need to improve our crossability skills. Alternately, embryo rescue technique may be employed to recover the hybrids between cultivated soybean and wild perennial species. The hybridity between cultivated and wild annual soybean (*Glycine soja*) hybrids was confirmed at morphological and molecular level. Molecular markers are useful to confirm the hybridity of interspecific hybrids. SSR markers were used to test F₁ hybrid plant obtained from cross of cultivated (*Glycine max*) and wild species (*Glycine soja*). Sufficient variability was found for all the traits studied. Seed yield per plant showed significantly positive correlation with reproductive phase, pods per plant, pod length, petiole length, harvest index and 100-seed weight. Significantly negative correlation was observed with plant height and internode length.

Keywords

Soybean, F₁ hybrid,
SSR markers

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Introduction

Soybean (*Glycine max* [L.] Merr.) is one of the most economically important crops in the world, providing a source of high-quality proteins for feed and food as well as vegetable oil/fuels for human consumption and industrial use (Hartman *et al.*, 2011). It has been widely accepted that soybean was

domesticated from its annual wild relative *Glycine soja* in China approximately 6000–9000 years ago (Carter *et al.*, 2004; Kim *et al.*, 2012) resulting in dramatic morphological and physiological modifications often referred to as the “domestication syndrome” (Hammer 1984). This process was followed by varietal diversification, forming a multitude of soybean landraces adapted to diverse eco-

regions for cultivation in agricultural systems. This scenario, i.e., the single origin of cultivated soybeans, appears to be well supported by recent investigation of genome-wide diversity among cultivated and wild soybeans at population levels, in which all cultivated accessions were exclusively grouped together into a single clade interior to the *G. soja* clades (Han *et al.*, 2016; Song *et al.*, 2015; Zhou *et al.*, 2015). Crop relatives have been used for decades for breeding, in particular to transfer genes of resistance or tolerance to pests, diseases or abiotic stress to the cultivated species. Introgression breeding has been extensively used in the genetic improvement of some of the most important Leguminous crops, like soybean.

The genetic base of modern soybean cultivars is narrow since most of the parents used in crossing are from soybean gene pool 1 (GP 1). To guarantee future global food security and sustainable crop production, there exists a need to broaden the genetic base of soybean cultivars. The wild relatives of soybean are a potential reservoir of diversity for this purpose. The perennial *Glycine tomentella* (Hayata) and the annual *Glycine soja* (Sieb. and Zucc.) have been hybridized successfully with the domesticated soybean to produce breeding lines suitable for yield testing (Ma and Nelson, 2012; Kabelka *et al.*, 2004; Singh *et al.*, 1990). These wild species are an excellent source of genetic variability, agronomically useful genes, biotic and abiotic stresses. These invaluable traits could be exploited to broaden the genetic base of soybean (Chung and Singh 2008). These species also harbor some undesirable genetic traits, for example, vining, lodging susceptibility, lack of complete leaf abscission, seed shattering and small black coated seeds, however, desirable ones could be sorted out during the course of selection in successive segregating generations. Of the two, the wild progenitor of soybean, *G. soja*,

is the most easily accessible to breeders and has a wealth of diversity preserved in the USDA soybean collection (Carter *et al.*, 2004). Thus, it may be an excellent source of new agronomic genes and traits (Lee *et al.*, 2008). This wild soybean has the same chromosome number as the cultivated soybean, crosses freely *via* insect or manual hybridization, and progeny are usually completely fertile (Singh and Hymowitz 1988; Weber 1950). Keeping above in view, the present investigation was undertaken, to develop hybrids between *Glycine max* and *Glycine soja* and confirm their hybridity at morphological and molecular level.

Materials and Methods

The experimental material for present investigation consisted of four cultivated soybean (*Glycine max* L.) varieties, viz., Bragg, SL-679, PS-1466 and PS-1469 and three wild soybean species (*Glycine soja*) lines, viz., *Glycine soja*, *Glycine soja* (PI 65549) and *Glycine soja* (PI 366121). The hybridization experiments were conducted at the Experimental Farm, Department of Crop Improvement, CSK HPKV, Palampur during *kharif* 2011 and 2012 using cultivated genotypes as female and wild species as male. Data were recorded with respect to; total number of soybean flowers pollinated, number of crossed pod set and Per cent pod set. F₁ hybrids were evaluated during 2012 and 2013. The F₁ hybrids along with their parents were raised in pots containing mixture of soil, sand and vermi-compost in 2:1:1 ratio in a completely randomized design (CRD) with unequal replications, as number of F₁ seeds varied for different crosses. For characterization of interspecific crosses involving cultivated and wild species data were recorded for each F₁ progeny and their parents. The agronomical data obtained from each cross combinations were analysed using the Statistical Analysis System (SAS

software). Hybridity of F₁ plants was confirmed by morphological and molecular markers.

Results and Discussion

Crossability of cultivated soybean with wild species

Data on number of buds pollinated, pod set and pod set percentage during *kharif* 2011 and 2012 at Palampur are presented in Table 1. In all, 1282 pollinations were attempted between *Glycine max* and *Glycine soja*. These pollinations resulted in the production of 53 F₁ pods. Similarly, 2271 pollinations were attempted between *Glycine max* and *Glycine tomentella*.

But the pod set percentage was zero. Difference among the soybean genotypes for crossability has also been reported by Nakayama and Yamaguchi (2002) indicating thereby the presence of genotypic differences for crossability within cultivated soybean.

Confirmation of hybridity of interspecific crosses

Establishing the true hybrid nature of crosses in the beginning of an experiment is important to develop reliable segregating populations for mapping of genes controlling desirable traits. The F₁ hybrids showed vigour which was established by their morphological expression.

Confirmation of hybridity at morphological level

True nature of crosses under study was confirmed in F₁'s for different traits at morphological level as given in Table 2. Leaf and plant morphology of parents and interspecific crosses are shown in Plate 1 (a and b), Plate 2 (a, b, c and d) and Plate 3 (a and b), respectively.

Confirmation of hybridity at molecular level

The hybridity of four interspecific crosses, viz., Bragg x *Glycine soja*, SL-679 x *Glycine soja* (PI-65549), PS-1466 x *Glycine soja* (PI-366121) and PS-1469 x *Glycine soja* was confirmed at molecular level through four SSR markers.

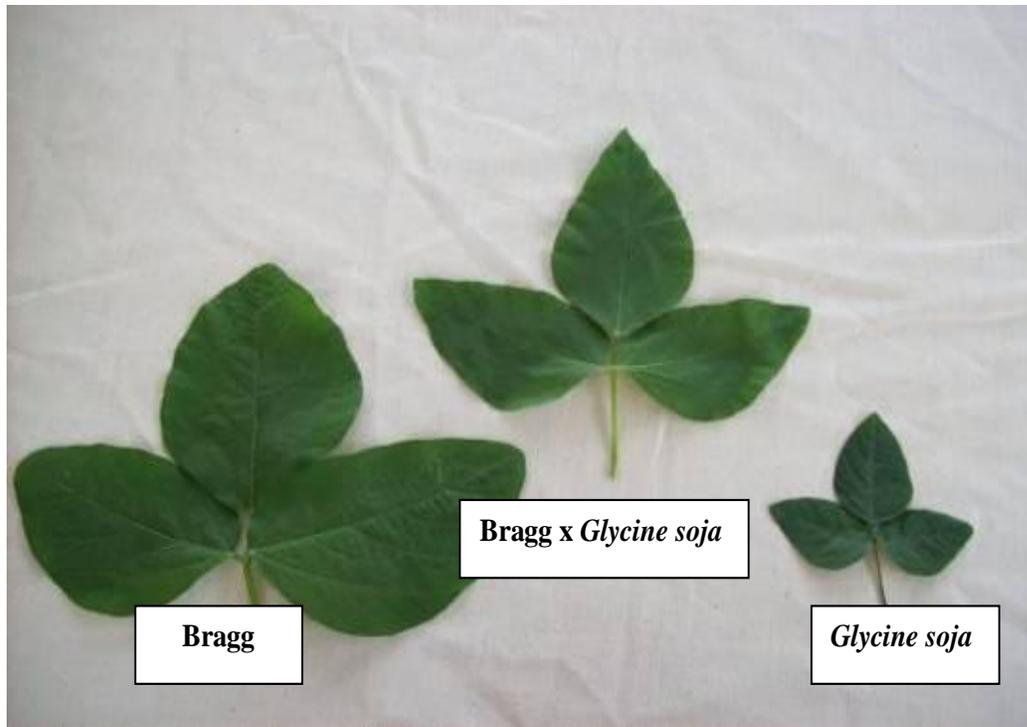
A total of 34 SSR markers were screened to confirm the hybridity of interspecific crosses. Of these only four markers, viz., Satt301, Salt77, Satt20 and Satt5 were found polymorphic between parents and hybrids and showed robust and reproducible bands as shown in Plate 4 (a, b and c).

Evaluation of interspecific crosses

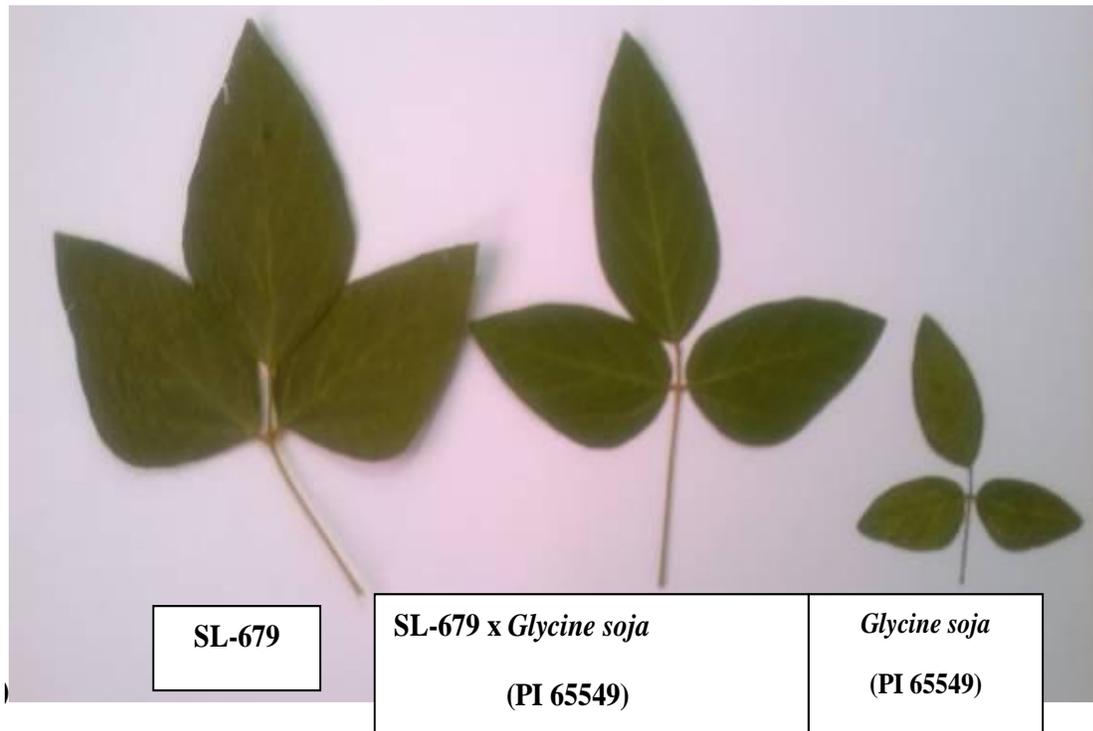
Four interspecific crosses along with their parents were evaluated under field conditions to investigate their actual performance for different agronomic traits. Analysis of variance (Table 3) revealed significant differences among genotypes for all the traits studied, viz., days to 50% flowering, days to 75% maturity, reproductive phase, plant height, branches per plant, internode length, nodes per main stem, petiole length, pods per plant, seeds per pod, pod length, biological yield per plant, seed yield per plant, harvest index and 100-seed weight indicating thereby presence of sufficient variability and scope of selection for these traits. Range and mean values of parents and their interspecific crosses for different characters are presented in Table 4.

Simple correlation for yield and related traits

Yield is a complex character and a function of several component characters. Direct selection based on yield alone will not be very effective in crop improvement programmes.



(a)



(b)

Plate 1 (a and b) Leaf morphology of parents and interspecific crosses



Bragg



Bragg x *Glycine soja*



Glycine soja

(a)



SL-679

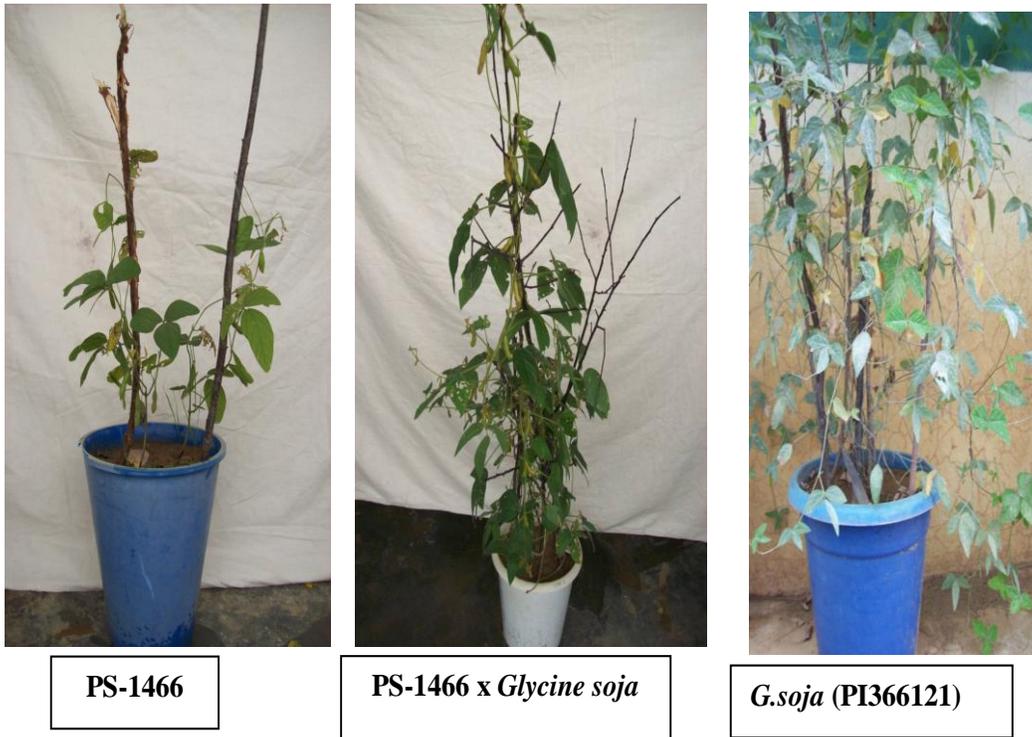


SL-679 x *Glycine soja*

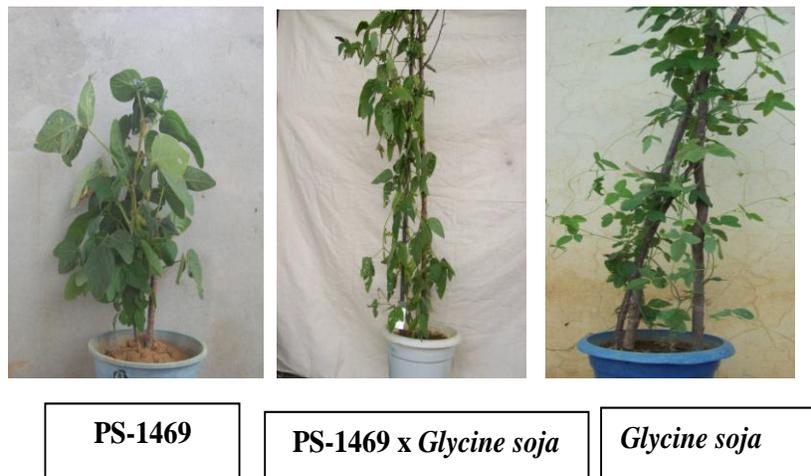


Glycine soja

(b)

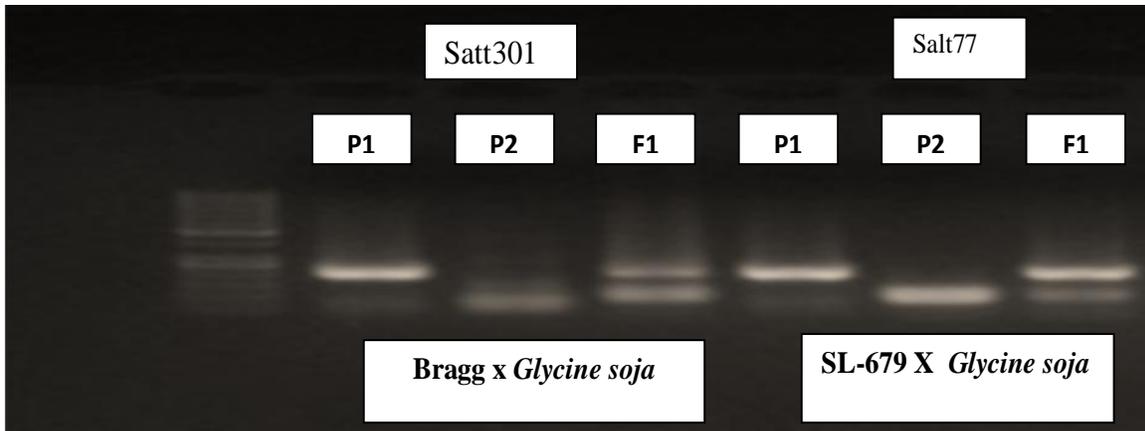


(c)

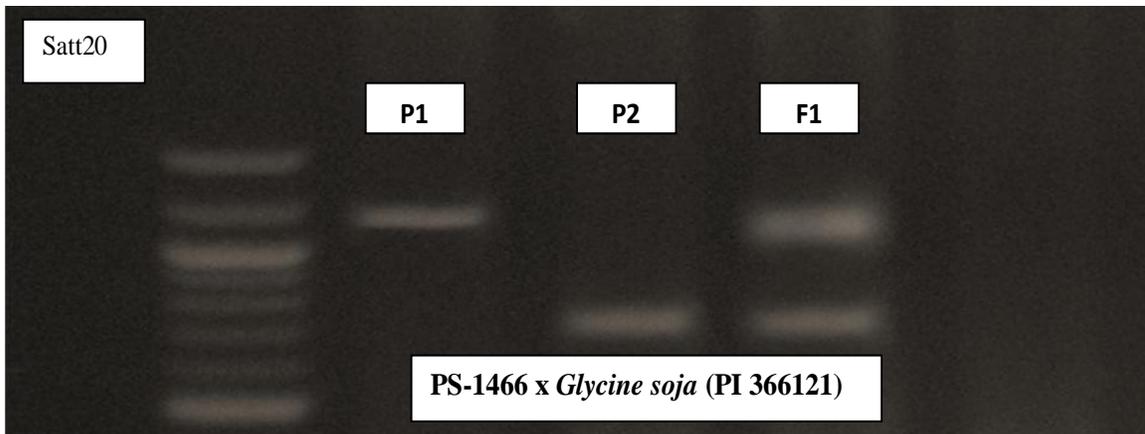


(d)

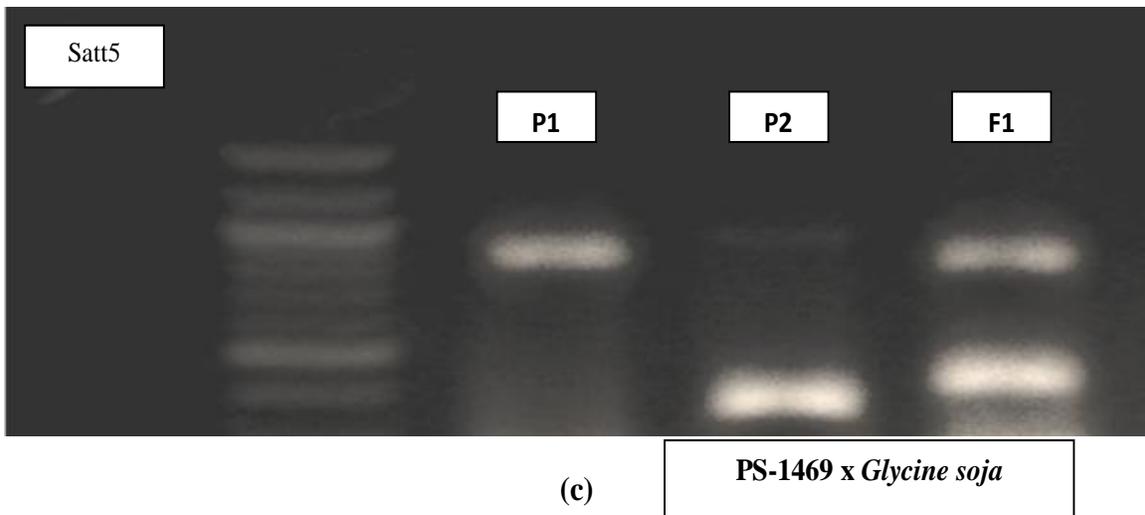
Plate 2 (a, b, c and d) Plant morphology of parents and interspecific crosses at morphological level



(a)



(b)



(c)

Plate 3 (a, b and c) Hybridity of parents and interspecific crosses at molecular level

Table.1 Number of buds pollinated and pod set (%) during *kharif* 2011 and 2012 in *Glycine max* and *Glycine soja* crosses

Cross combinations	Number of buds pollinated		Number of pod set		Pod set (%)	
	2011	2012	2011	2012	2011	2012
Bragg x <i>G. soja</i>	298	132	12	7	4.02	5.30
PS 1466 x <i>G. soja</i> (PI 366121)	223	104	9	3	4.03	2.88
SL-679 x <i>G. soja</i> (PI 65549)	153	110	8	2	5.22	1.81
PS 1469 x <i>G. soja</i>	184	78	8	4	4.34	5.12
Total	858	424	37	16	4.31	3.77

Table.2 Morphological characterization of parents and their F₁s

Traits	Cross Combinations											
	Bragg X <i>Glycine soja</i>			SL-679 X <i>Glycine soja</i> (PI 65549)			PS-1469 X <i>Glycine soja</i>			PS-1466 X <i>Glycine soja</i> (PI 366121)		
	P ₁	P ₂	F ₁	P ₁	P ₂	F ₁	P ₁	P ₂	F ₁	P ₁	P ₂	F ₁
Days to 50% flowering	Late	Medium	Late	Late	Medium	Late	Late	Medium	Late	Late	Medium	Late
Plant Growth type	Determinate	Indeterminate	Indeterminate	Determinate	Indeterminate	Indeterminate	Determinate	Indeterminate	Indeterminate	Determinate	Indeterminate	Indeterminate
Plant Growth habit	Erect	Semi-erect	Semi-erect	Erect	Semi-erect	Semi-erect	Erect	Semi-erect	Semi-erect	Erect	Semi-erect	Semi-erect
Plant height	Medium	Tall	Tall	Medium	Tall	Tall	Medium	Tall	Tall	Medium	Tall	Tall
Leaf Colour	Green	Dark green	Green	Green	Dark green	Green	Green	Dark green	Green	Green	Dark green	Green
Flower Colour	White	Purple	Purple	White	Purple	Purple	White	Purple	Purple	White	Purple	Purple
Pod Pubescence	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present
Pod Pubescence colour	Tawny (Brown)	Tawny (Brown)	Tawny (Brown)	Tawny (Brown)	Tawny (Brown)	Tawny (Brown)	Tawny (Brown)	Tawny (Brown)	Tawny (Brown)	Tawny (Brown)	Tawny (Brown)	Tawny (Brown)
Pod Colour	Yellow	Black	Brown	Yellow	Black	Brown	Yellow	Black	Brown	Yellow	Black	Brown
Seed Shape	Spherical	Elliptical	Spherical	Spherical	Elliptical	Spherical	Spherical	Elliptical	Spherical	Spherical	Elliptical	Spherical
Seed Colour	Yellow	Black	Yellow	Yellow	Black	Yellow	Yellow	Black	Yellow	Yellow	Black	Yellow
Seed Lustre	Shiny	Dull	Dull	Shiny	Dull	Dull	Shiny	Dull	Dull	Shiny	Dull	Dull
Seed Hilum colour	Black	Grey	Brown	Black	Grey	Brown	Black	Grey	Brown	Black	Grey	Brown
Seed Cotyledon colour	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow

Table.3 Analysis of variance for different traits in soybean

S. No.	Traits	Mean sum of squares		
		df	Genotypes	Error
1	Days to 50% flowering		26.39*	5.25
2	Days to 75% maturity		73.63*	9.05
3	Reproductive phase		0.01*	.001
4	Plant height		3511.53*	49.90
5	Branches/plant		8.23*	0.92
6	Nodes/main stem		4.55*	1.53
7	Internode length		31.60*	1.24
8	Petiole length		69.13*	0.43
9	Pods/plant		1556.29*	20.74
10	Seeds/pod		0.06*	0.03
11	Pod length		0.92*	0.03
12	Biological yield/plant		35.95*	12.12
13	Seed yield/plant		18.32 *	0.74
14	Harvest index		170.35*	20.14
15	100-seed wt		64.01*	1.50

* Significant at $P \leq 0.05$

Table.4 Overall range and mean performance of parents and their interspecific crosses for different traits in soybean

Traits	P ₁		P ₂		F ₁		BC ₁	
	Range	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE
Days to 50% flowering	55.67-64.67	60.58±0.94	56.67-61.67	58.83±1.81	61.67-64.67	62.87±1.13	63.00-66.00	64.67± 0.88
Days to 75% maturity	122.00-129.67	124.58±2.03	115.33-131.67	126.42±1.87	127.00-132.33	128.80 ±1.48	127.00-132.00	129.33± 1.45
Reproductive phase	0.62-0.66	0.64±0.02	0.45-0.60	0.52±0.01	0.57-0.59	0.58 ± 0.009	0.57-0.59	0.58± 0.01
Plant height	43.27-54.67	49.12±5.64	83.33-122.33	101.75±1.76	111.67-132.00	123.47± 3.62	120.00-127.00	123.33± 2.03
Branches/plant	3.93-5.67	5.21±0.42	6.00-9.67	7.50±0.68	5.33-7.33	6.53±0.61	7.00-8.00	7.33± 0.33
Nodes/main stem	8.67-11.13	10.30±0.58	9.33-13.33	11.00± 0.64	9.67-11.67	10.73±0.88	11.00-13.00	11.67± 0.67
Internode length	4.15-5.29	4.78±0.48	7.64-12.02	9.49± 0.67	10.65-13.32	11.68±0.82	9.23-11.55	10.65± 0.72
Petiole length	12.00-12.67	12.33±0.63	1.83-3.27	2.27±0.11	2.77-3.27	3.06 ±0.13	2.80-3.00	2.90± 0.06
Pods/plant	54.53-80.73	67.38±4.05	17.00-24.33	22.17±2.98	23.00-25.00	23.67±1.92	21.00-27.00	23.33± 1.86
Seeds/pod	1.91-2.23	2.08±0.06	1.93-2.37	2.06± 0.16	1.87-2.37	2.06±0.15	2.00-2.12	2.04± 0.04
Pod length	3.50-3.67	3.56±0.06	2.13-3.00	2.46±0.12	2.63-3.47	3.13±0.11	3.00-3.60	3.37± 0.19
Biological yield/plant	28.80-32.93	30.38±2.33	25.00-31.00	27.08±2.18	31.00-36.33	33.13±1.46	35.00-38.00	36.33± 0.88
Seed yield/plant	10.00-15.87	12.68±0.60	6.65-9.23	8.15±0.41	8.52-9.85	9.11±0.51	6.89-7.60	7.33± 0.43
Harvest index	35.24-49.00	41.92±3.03	26.37-34.85	30.59±2.97	23.46-30.24	27.72±2.09	21.33-25.03	23.46± 1.11
100 seed weight	11.47-18.50	14.78±1.07	3.75-6.18	5.13±0.52	6.18-8.07	7.15 ±0.36	7.68-9.13	8.96± 0.22

Table.5 Simple correlation coefficients among different agro-morphological traits in soybean

	Days to 50% flowering	Days to 75% maturity	Reproductive phase	Plant height	Branches/plant	Pods/plant	Biological yield/plant	Harvest index	100-seed weight	Seeds/pod	Nodes/main stem	Internode length	Petiole length	Pod length
Seed yield/plant	0.1679	0.0596	0.7144*	-0.6720*	-0.3453	0.7841*	0.2809	0.8205*	0.6863*	-0.0020	-0.2160	-0.5919*	0.7975*	0.5259*
Days to 50% flowering		0.4436	0.3926	0.1984	-0.0678	0.0856	0.5678*	-0.1421	-0.0963	0.0298	0.0910	0.1597	0.0178	0.2729
Days to 75% maturity			0.1278	0.2312	0.4578	-0.2261	0.3014	-0.0825	-0.2583	0.1541	-0.1911	0.3139	-0.2750	-0.2319
Reproductive phase				-0.4558	-0.3837	0.5994*	0.4351	0.4864	0.6554*	0.1950	-0.1338	-0.4226	0.6672*	0.6820*
Plant height					0.3355	-0.8334*	0.2281	-0.7893*	-0.7089*	-0.0536	0.2217	0.9232*	-0.8698*	-0.3188
Branches/plant						-0.4936	-0.1607	-0.2439	-0.5324*	0.3575	0.1188	0.3201	-0.5660*	-0.5731*
Pods/plant							0.0837	0.7189*	0.7761*	0.0874	-0.1028	-0.7889*	0.9494*	0.6072*
Biological yield/plant								-0.3023	0.1584	0.1498	0.1471	0.1596	0.0704	0.4888
Harvest index									0.5928*	-0.0597	-0.3081	-0.6734*	0.7485*	0.2638
100 seed weight										0.1598	-0.2188	-0.6430*	0.8880*	0.7135*
Seeds/pod											-0.0040	-0.0933	0.1752	0.2750
Nodes/main stem												-0.1521	-0.1962	-0.0542
Internode length													-0.8062*	-0.3237
Petiole length														0.6823*

* Significant at $P \leq 0.05$

Grafius (1956) had also opined that the improvement of complex characters such as seed yield might be accomplished better through component breeding. Therefore, it is also important to gather information on association of yield with other characters and among themselves so as to form the basis to identify characters for increasing the efficiency of both direct and indirect selection and thereby defining an ideal plant type. Based on the estimates of correlation, the breeder will be able to decide the method of breeding to be followed to exploit the useful correlation.

In order to understand the nature and magnitude of correlations among seed yield per plant and other traits, estimates of simple correlation coefficients were computed for parents (cultivated and wild) and their interspecific crosses (Table 5).

Seed yield per plant exhibited significant and positive correlation with reproductive phase, pods per plant, harvest index, 100-seed weight, pod length and petiole length. Significant and positive correlation of seed yield with number of pods per plant and harvest index was observed by Barh *et al.*, (2014) and for 100-seed weight by Tomar *et al.*, (2014). It was significantly negatively correlated with plant height and internode length.

Significant positive correlation was observed for days to 50% flowering and biological yield per plant. Reproductive phase has significant positive correlation with pods per plant, 100-seed weight, petiole length and pod length. Plant height has positive correlation with internode length, whereas, negative correlation with pods per plant, harvest index, 100-seed weight and petiole length.

Branches per plant have negative correlation with 100-seed weight, petiole length and pod length. Pods per plant have positive correlation with harvest index, 100-seed weight, petiole length and pod length and negative correlation with internode length. Harvest index has positive correlation with 100-seed weight and

petiole length, whereas negative correlation with internode length. 100-seed weight has positive correlation with petiole length and pod length, whereas negative correlation with internode length.

Based on simple correlation studies, it can be concluded that seed yield per plant is positively correlated with reproductive phase, pods per plant, harvest index, 100-seed weight, pod length and petiole length and selection through these traits would be effective.

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