Original Research Article

Genetic Diversity and Association Analysis for Agronomic and Yieldrelated Traits in Interspecific Progenies of Pigeonpea (*Cajanus cajan*)

ABSTRACT

The limited genetic diversity in existing cultivars emphasizes the need for incorporating potential exotics and wild relatives in breeding programs. This study focussed on assessing genetic variation and correlations among yield traits in F_8 and F_9 progenies resulting from nine interspecific crosses, involving three cultivated parental lines and three wild species. Results indicate significant genotypic variation for all traits studied. Phenotypic and genotypic coefficients of variation varied across agronomic parameters. Heritability estimates ranged from 22.61% (Primary branches ~~p~~plant^{-1}~~er plant~~) to 89.35% (Days to maturity) for different traits, emphasizing the potential for selection in breeding programs. Positive correlations were observed among various traits in both progeny generations, providing insights into the interplay of genetic factors influencing pigeonpea traits. The cluster analysis revealed that, 54 interspecific cross derived lines with three parents were grouped into four clusters, indicating the greater diversity among the lines for the selection of yield related traits for pigeonpea crop improvement.

Keywords: Cluster analysis, Correlation, Genetic diversity, Pigeonpea, Variability

1. INTRODUCTION

Pigeonpea (*Cajanus cajan* (L.) Millspaugh) holds significant economic importance as a grain legume crop, particularly in the developing countries situated in tropical and subtropical regions worldwide[1]. This crop is recognized for its nutritional value, containing approximately 20-22% protein, dietary fiber, as well as essential vitamins and micronutrients such as zinc (Zn) and iron (Fe). Consequently, pigeonpea plays a crucial role in ensuring food security and contributing to a balanced diet. Its multifaceted applications, including utilization as food, fodder, fuel, for soil conservation, integrated farming systems, and symbiotic nitrogen fixation, underscore its vital role in subsistence agriculture[2]

Globally, pigeonpea cultivation spans an area of 6.09 million hectares, yielding 5.01 million tonnes with a productivity rate of 822 kg~~/~~ha^{-1}[3]. Notably, India stands as the primary contributor, accounting for 90% of the global pigeonpea production. In India, pigeonpea is cultivated over 5.01 million hectares, resulting in a production of 3.89 million ~~tonnes~~ MTand a productivity of 859 kg~~/~~ha^{-1}. Despite these substantial production figures, the national harvest falls short of meeting the demands

Comment [AM1]: Please mention the Author name, Like thisVarshney et al. [1].

Comment [AM2]: Please give references of India's growing population, necessitating the import of approximately 1.2 million tonnes annually from African countries such as Mozambique, Tanzania, Uganda, and Kenya. This import practice incurs a cost of approximately USD 116.57 million [4].

The majority of currently available pigeonpea cultivars exhibit a limited genetic diversity, necessitating prompt remedial actions through the inclusion of potential exotics and wild relatives in breeding programs. Interspecific hybridization has proven effective in enhancing crop plants by facilitating the transfer of desirable genes and chromosomes from wild to cultivated species. Notably, *Cajanus scarabaeoides* has been successfully employed as a donor parent at the ICRISAT Centre to transfer high-protein genes to pigeonpea[5].

Cajanus cajanifolius, identified as the putative progenitor of pigeonpea, imparts resistance to bruchid and pod borer, as well as tolerance to drought, seed hardness, and elevated levels of both protein and methionine. Considering these factors, the present study aims to augment our understanding by investigating the F_8, and F_9 progenies resulting from nine inter-specific crosses, to assess the extent of genetic variation and correlations among yield traits in the F_8 and F_9 generation progenies derived from these inter-specific crosses.

2. MATERIAL AND METHODS

The experimental investigation was carried out at the Norman E Borlaug Crop Research Centre,Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand (29°N latitude; 79.30°E longitude; 243.84 meters above mean sea level).This study encompassed the examination of progenies resulting from nine inter-specific crosses originating from cultivated parental lines, namely, ICPL 84023, Pant A134, and UPAS120, and wild species, including *Cajanus scarabaeoides, Cajanus acutifolius, and Cajanus cajanifolius*. The crosses were executed earlier and advanced through pedigree method of selection. The lines were available at F_8 and F_9 were used for the study in the year 2009-10 and 2010-11 respectively~~.~~(Table 1).In the kharif seasons of 2009-10 and2010-11, seeds of F_8 and F_9 progenies were sowed, along with three cultivated parent lines, in a randomized complete block design, wherein total 57 progeny line (54 inter-specific progeny lines and the three cultivated parent lines) were sowed across three replications. Field data were recorded (90 cm x 30 cm, 3 m plot size) with on five randomly selected plants within each plot.

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The observations on the number of days to 50% flowering, number of days to maturity, ~~Plant~~ plant height, number of primary branches ~~per~~ plant^{-1}, number of pods plant^{-1} ~~p~~plant^{-1} ~~er plant~~and ~~Seed~~ seed yield plant^{-1} ~~p~~plant^{-1} ~~er plant~~were recorded at appropriate growth stages.In the analysis of variance within the experimental traits, the coefficients of variability, heritability~~,~~ and simple correlation were determined through the utilization of mean data encompassing all parameters. Statistical analysis was conducted to assess the significance of disparities among the means of diverse characters. The procedures employed for the analysis of variance adhered to the framework outlined by [6].Phenotypic, genotypic, and environmental coefficients of variation were computed by expressing the ratio of the respective standard deviation to the overall mean of the characters in percentage

Comment [AM4]: Which software was used for the analysis terms, following the methodology proposed by [7].The calculation of heritability in the broad sense involved determining the ratio of genotypic variance to phenotypic variance, expressed as a percentage [8]. The anticipated genetic advance under selection was computed.Correlation coefficients at both the phenotypic and genotypic levels, relating to all conceivable pairs of characters, were estimated using the methodology presented by Searle [9].

3. RESULTS AND DISCUSSIONS

Analysis of Variance: Analysis of variance was conducted for progenies of nine interspecific crosses and their three cultivated parents, revealing that the mean square attributable to genotypes was profoundly significant for all examined traits at a 5% level of significance. This observation signifies the existence of substantial variability within the studied material. The mean squares associated with genotypes, replications, as well as the general mean, standard error of the mean, critical difference at a 5% level (CD at 5%), and coefficient of variation (CV) are comprehensively presented in Table 2 and Table 3.

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Phenotypic and Genotypic coefficient of variation: During the kharif seasons of 2009-10 and 2010-11, various agronomic traits were assessed to determine the phenotypic and genotypic variations within the studied crop. In the kharif season 2009, the coefficient of phenotypic variation (PCV %) reached its highest value for pods ~~p~~plant^{-1}~~er plant~~ (34.25%), succeeded by seed yield ~~p~~plant^{-1}~~er plant~~ (31.64%), and primary branches ~~p~~plant^{-1}~~er plant~~ (22.97%), while the minimum PCV % was observed for days to maturity (8.05%). Similar trends were observed in the subsequent kharif2010, with the maximum PCV % recorded for pods ~~p~~plant^{-1}~~er plant~~ (35.92%), followed by seed yield ~~p~~plant^{-1}~~er plant~~ (33.74%) and primary branches ~~p~~plant^{-1}~~er plant~~ (25.14%), and the minimum PCV % registered for days to maturity (7.35%) (Supplementary Table S1and SupplementaryTable S2).

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In parallel, the assessment of genetic coefficient of variation (GCV %) during the kharif season2009indicated the highest values for pods ~~p~~plant^{-1}~~er plant~~ (29.55%), seed yield ~~p~~plant^{-1}~~er plant~~ (24.81%), and primary branches ~~p~~plant^{-1}~~er plant~~ (17.06%), while the lowest GCV % was observed for days to maturity (6.26%). Corresponding observations were made in the kharif 2010-11, with maximum GCV % values noted for pods ~~p~~plant^{-1}~~er plant~~ (27.58%), seed yield ~~p~~plant^{-1}~~er plant~~ (20.83%), and primary branches ~~p~~plant^{-1}~~er plant~~ (11.95%), and the minimum GCV % observed for days to maturity (6.94%) (Supplementary Table S1 and Supplementary Table S2).

Additionally, the evaluation of genotypic coefficient of variation (GCV %) in the kharif season2009 revealed the highest values for seed yield ~~p~~plant^{-1}~~er plant~~ (24.81%), pods ~~p~~plant^{-1}~~er plant~~ (29.55%), and primary branches ~~p~~plant^{-1}~~er plant~~ (17.06%), with the lowest GCV % observed for days to maturity (6.26%). Analogous results were obtained in the kharif season 2010, where the maximum GCV % was recorded for seed yield ~~p~~plant^{-1}~~er plant~~ (20.83%), followed by pods ~~p~~plant^{-1}~~er plant~~ (27.58%), while the minimum GCV % was noted for days to maturity (6.94%) (Supplementary Table S1 and Supplementary Table S2).

Comment [AM5]: Need some citation and discussion about GCV and PCV

Estimation of heritability and Genetic advance for various agronomic and yield traits: Results from the kharif seasons of 2009-10 and 2010-11 indicate varying heritability estimates for different agronomic traits. In the kharif 2009-10 dataset, the trait with the highest heritability was pods ~~p~~plant^{-1}~~er plant~~ (74.44%), followed by seed yield ~~p~~plant^{-1}~~er plant~~ (61.49%), and day to maturity (60.51%). Conversely, plant height exhibited the lowest heritability at 38.76%. In the subsequent kharif season of2010-11, heritability values ranged from 22.61% for primary branches ~~p~~plant^{-1}~~er plant~~ to 89.35% for days to flowering. Moderate heritability was observed for seed yield ~~p~~plant^{-1}~~er plant~~ (38.13%), pods ~~p~~plant^{-1}~~er plant~~ (58.94%), and plant height (45.20%) during this period (Supplementary Table S1 and Supplementary Table S2).Specifically, during kharif 2009-10, the Genetic advance as % of meanhighest value was recorded for pods ~~p~~plant^{-1}~~er plant~~ (52.53%), followed by seed yield ~~p~~plant^{-1}~~er plant~~ (40.08%) and primary branches ~~p~~plant^{-1}~~er plant~~ (26.07%). Conversely, days to maturity exhibited the lowest value at 16.94%. In the subsequent kharif season of 2010-11, the observed results demonstrated the highest genetic advance mean for pods ~~p~~plant^{-1}~~er plant~~ (43.62%), followed by days to 50% flowering (28.03%), seed yield ~~p~~plant^{-1}~~er plant~~ (26.68%), with the lowest value observed for days to maturity (13.54%) (Supplementary Table S1 and Supplementary Table S2).

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Comment [AM6]: Need some citation and discussion about heritability and genetic advance

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Genotypic and phenotypic correlation coefficients: In the F_8 progeny generation, a significant and positive correlation was observed between the duration of flowering and variables such as plant height, pods ~~p~~plant^{-1}~~er plant~~, and the number of primary branches ~~p~~plant^{-1}~~er plant~~. However, there was no statistically significant correlation observed between plant height and seed yield ~~p~~plant^{-1}~~er plant~~. Conversely, for the remaining traits, highly significant and positive correlations were identified. In the subsequent F_{10} progeny generation, all studied characteristics exhibited a highly significant and positive correlation, as detailed in SupplementaryTableS3andSupplementaryTableS4.

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Cluster analysis for interspecific cross progenies: The cluster analysis revealed that, 54 interspecific progenies and three parents were grouped into four clusters (Fig. 1). The first cluster (I) consists of 26 lines, for the cluster average plant height about 159.58 cm, number of primary branches ~~p~~plant^{-1}~~er plant~~11.85,number of pods ~~p~~plant^{-1}~~er plant~~ 122.32, days to flowering 85.24, days to maturity 176.85, grain yield ~~p~~plant^{-1}~~er plant~~ 26.91 g. This cluster was mainly formed from the lines derived from, UPAS 120 with all three wild species and few of PA 134 with all three wild species. In the second cluster (II), 10 lines were grouped together with average plant height of 151.19 cm, number of primary branches ~~p~~plant^{-1}~~er plant~~was 13.53, number of pods ~~p~~plant^{-1}~~er plant~~ was 157.04, days to flowering was 78.47, days to maturity about 174.50, seed yield ~~p~~plant^{-1}~~er plant~~ was34.55 g. This cluster mainly contains the genotype from PA 134 with all three wild species except G44 which is derived from UPAS 120 crossed with C. *acutifolius*. The third cluster (III) consists of 18 lines with one parental line (ICPL 84023), having average plant height of 148.76 cm, number of primary branches ~~p~~plant^{-1}~~er plant~~ 8.92, number of pods ~~p~~plant^{-1}~~er plant~~ was 86.66, days to flowering was 70.53, days to maturity about 156.65, seed yield ~~p~~plant^{-1}~~er plant~~ was 18.79 g. The III cluster consists of lines only from ICPL 84034 with all three wield species along with the parent ICPL 84023. The last cluster (IV) consist of two parental lines, PA 134 and UPAS 120, with average plant height of 179.55 cm, number

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of primary branches ~~p~~plant^{-1}~~er plant~~was 10.29, number of pods ~~p~~plant^{-1}~~er plant~~ was 185.91, days to flowering was 74, days to maturity about 174.83, seed yield ~~p~~plant^{-1}~~er plant~~ was 20.12 g (Table 4).

Lack of genetic variability in plant varieties results in naturally low yielding potential; inefficient plant types also have low yielding potential; there are no suitable ideotypes for various cropping systems; poor harvest index; low crop management; increased weed competition; and susceptibility to biotic and abiotic stresses are the main obstacles to achieving higher yields. As like in our study, high variability for different yield characters was also reported in pigeonpea from other studies ~~(~~[10-14]. Heritability estimates of yield traits shows moderate to high heritability for pods ~~p~~plant^{-1}~~er plant~~, seed yield and days to maturity in both F_8 and including day to maturity, days to 50% flowering in F_9. High heritability for different characters were reported in pigeonpeaby different studies [15-18]. Yield contributing characters had moderate heritability with moderate to high genetic advance as per cent mean were exhibited by pods ~~p~~plant^{-1}~~er plant~~, seed yield ~~p~~plant^{-1}~~er plant~~ in F_8 progenies and in F_9 it was found only in seed yield ~~p~~plant^{-1}~~er plant~~. This indicates preponderance of additive gene action for these traits. In rest of the characters, in both the generations the estimates of low heritability with low genetic advance as % mean indicates the preponderance of non-additive gene action and hence selection may not be effective for these characters. The disagreement of the previous reports with the present investigation on heritability for some characters could be ascribed to the population and the environment in which the estimates of heritability for some characters could be ascribed to the population and the environment in which the estimates of heritability were taken as the heritability estimates are subjected to these conditions.In the F_8 progeny generation, a robust and positive correlation was evident for all examined traits, except in the case of plant height and seed yield ~~p~~plant^{-1}~~er plant~~. Notably, a highly significant and positive correlation was identified for all traits in F_9 progeny generation. Similar findings were also reported by different studies [19-24] in past. The cluster analysis among the pegionpea hybrids [25], land races [26],germplam lines [28] were reported earlier, however the clustering patterns of interspecific pre-bred lines carried out in this study is unique. The lines clustered were distinguishable based on the modern cultivars involved in the cross. Further studies using molecular tools will be helpful and through a light on combining ability and gene introgression patterns in the interspecific crosses of pegionpea.

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4. CONCLUSION

Consequently, the findings suggest that the presence of considerable genetic diversity for the grain yield and its related traits, including seed yield ~~p~~plant^{-1}~~er plant~~and the number of pods ~~p~~plant^{-1}~~er plant~~, demonstrating substantial genetic advancement. Notably, a noteworthy and positive correlation was observed between seed yield ~~p~~plant^{-1}~~er plant~~ and the absence of primary branches, as well as the number of pods ~~p~~plant^{-1}~~er plant~~. Enhancements in these traits are anticipated to positively influence seed yield. The results of the cluster analysis showed that four clusters comprising 54 interspecific cross-derived lines with three parents were formed, suggesting a higher degree of diversity among the lines for the selection of yield-related traits for the improvement of the pigeonpea crop.

Comment [AM9]: In all section per plant or per pod ect should be replace plant^{-1}

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Table 1.List of interspecific crosses and their cultivated parents

S. No.	Cultivated Parents	
1	ICPL84023	Determinate, early maturing line (G55)
2	PA134	Indeterminate, early maturing line (G56)
3	UPAS120	Indeterminate, early variety (G57)
S. No.	Interspecific crosses	
1	ICPL 84023 x *C. scarabaeoides*(G1-G6)	
2	ICPL 84023 x *C. acutifolius*(G7-G12)	
3	ICPL 84023 x *C. cajanifolius*(G13-G18)	
4	PA134 x *C. scarabaeoides*(G19-G24)	
5	PA134 x *C. acutifolius*(G25-G30)	
6	PA134 x *C. cajanifolius*(G31-G36)	
7	UPAS 120 x *C. scarabaeoides*(G37-G42)	
8	UPAS 120 x *C. acutifolius*(G43-G48)	
9	UPAS 120 x *C. cajanifolius*(G49-G54)	

Table 2. Analysis of variance for agronomic and yield related traits in F_8 progenies of inter-specific crosses and cultivated parents.

Source of variation	**d.f.**	**Days to 50% flowering**	**Days to maturity**	**Plant height (cm)**	**Primary branches/ plant**	**Pods ~~p~~plant^{-1}~~er plant~~**	**Seed yield ~~p~~plant^{-1}~~er plant~~**
Replication	2	100.32	66.27	1590.55	24.45	2373.95	107.19
Treatment	56	307.96**	408.38**	732.45**	12.90**	4670.46**	126.54**
Error	112	73.32	72.95	252.81	2.75	479.53	21.85
GM±		76.72	168.79	158.74	10.78	126.46	23.80
SEM		4.94	4.93	9.17	0.95	12.64	2.69
CD at 5%		13.82	13.78	25.66	2.67	35.34	7.54
CV (%)		11.16	5.06	10.01	15.38	17.31	19.63

* Significant at 5% level of probability ** Significant at 1% level of probability

Table 3. Analysis of variance for agronomic and yield related traits in F_9 progenies of inter-specific crosses and cultivated parents.

Source of variation	**d.f.**	**Days to 50% flowering**	**Days to maturity**	**Plant height (cm)**	**Primary branches/ plant**	**Pods ~~p~~plant^{-1}~~er plant~~**	**Seed yield ~~p~~plant^{-1}~~er plant~~**
Replication	2	3.03	650.91	3948.5	52.19	8326.38	382.60
Treatment	56	409.27**	507.87**	1596.74**	13.06**	5635.59**	130.51**
Error	112	63.99	63.98	509.07	6.95	1061.92	47.18
GM±		73.95	170.35	194.34	11.92	141.56	25.34
SEM		4.61	4.62	13.02	1.52	18.81	3.96
CD at 5%		12.91	12.92	36.41	4.25	52.59	11.08
CV (%)		10.81	4.69	11.60	22.12	23.01	27.10

* Significant at 5% level of probability ** Significant at 1% level of probability

Table 4. Details of five clusters with traits, Plant height (PH), Number of primary branches ~~p~~plant^{-1}~~er plant~~ (NP), Number of Pods ~~p~~plant^{-1}~~er plant~~ (NP), Days to 50% flowering (DF), Days to maturity (DM) and Seed yield ~~p~~plant^{-1}~~er plant~~ (SY).

Cluster	PH	NB	NP	DF	DM	SY	Genotypes
I	159.58	11.85	122.32	85.24	176.85	26.91	G54, G46, G40, G47, G35, G43, G34, G51, G53, G52, G50, G19, G26, G39, G48, G37, G33, G25, G42, G27, G45, G38, G49, G31, G32, G41
II	151.19	13.53	157.04	78.47	174.50	34.55	G20, G21, G22, G23, G24, G28, G29, G30, G36, G44
III	148.76	8.92	86.66	70.53	156.65	18.79	G1, G2, G3, G4, G5, G6, G7, G8, G9, G10, G11, G12, G13, G14, G15, G16, G17, G18, G55
IV	179.55	10.29	185.91	74	174.83	20.12	G56, G57

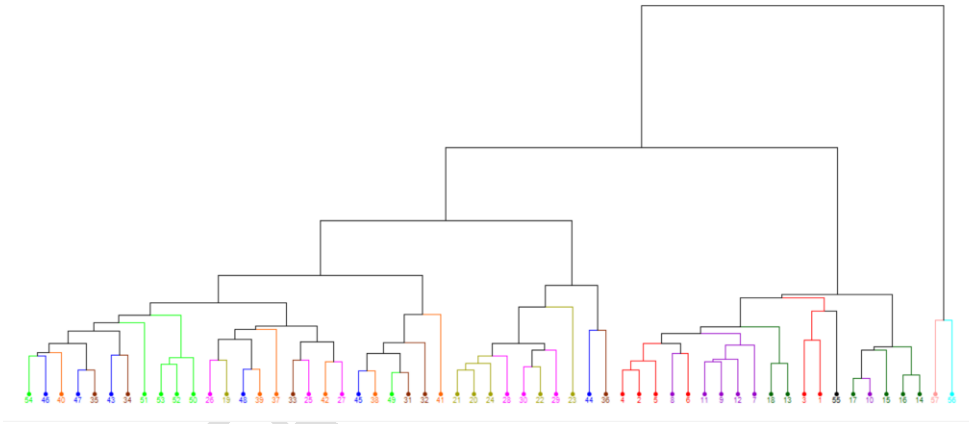

Fig. 1.Cluster analysis for 54 lines derived from 9 interspecific cross progenies of pigeonpea along with the parents

Table S1. Range, general mean, heritability, genetic advance PCV and GCV in F_8 progenies and cultivated parents for yield and yield related traits.

Character	Range	General mean	PCV%	GCV%	ECV%	Heritability (%)	Genetic advance	Genetic advance as % of mean
Days to 50%	51.66-96.33	76.72±4.94	16.04	11.52	11.16	51.61	13.08	17.05

flowering								
Days to maturity	142.0-190.3	168.79±4.93	8.05	6.26	5.06	60.51	16.94	10.04
Plant height (cm)	137.0-199.5	158.74±9.17	12.79	7.96	10.01	38.76	16.21	10.21
Primary branches ~~p~~plant^{-1}~~er~~ ~~plant~~	6.93-14.38	10.78±0.95	22.97	17.06	15.38	55.16	2.81	26.07
Pods ~~p~~plant^{-1}~~er~~ ~~plant~~	72.82-215.82	126.46±12.64	34.25	29.55	17.31	74.44	66.43	52.53
Seed yield ~~p~~**plant**$^{-1}$~~er~~ ~~plant~~ (g)	11.55-37.47	23.80±2.69	31.64	24.81	19.63	61.49	9.54	40.08

Table S2. Range, general mean, heritability, genetic advance PCV and GCV in F$_9$ progenies and cultivated for yield and yield related traits.

Character	**Range**	**General mean**	**PCV%**	**GCV%**	**ECV%**	**Heritability (%)**	**Genetic advance**	**Genetic advance as % of mean**
Days to 50% flowering	50.0-96.0	73.95±4.61	13.49	12.72	44.76	88.99	20.73	28.03
Days to maturity	145.0-187.66	170.35±4.62	7.35	6.94	2.40	89.35	23.06	13.54
Plant height (cm)	130.7-223.1	194.34±13.02	14.55	9.79	10.77	45.20	26.65	13.71
Primary	7.39-	11.92±1.52	25.14	11.95	22.12	22.61	1.39	11.66

branches ~~p~~plant^{-1}~~er plant~~	15.46							
Pods ~~p~~plant^{-1}~~er plant~~	80.05-212.31	141.56±18.81	35.92	27.58	23.01	58.94	61.75	43.62
Seed yield ~~p~~plant^{-1}~~er plant~~ (g)	12.29-40.003	25.34±3.96	33.74	20.83	26.53	38.13	6.76	26.68

Table S3. Phenotypic and genotypic correlation coefficients for yield and yield contributing traits in F_8.

	Days to 50% flowering	Day to maturity	Plant height (cm)	Primary branches ~~p~~plant^{-1}~~er plant~~	Pods ~~p~~plant^{-1}~~er plant~~	Seed yield ~~p~~plant^{-1}~~er plant~~
Days to 50% flowering	r_p	0.604**	0.211*	0.450**	0.270*	0.473**
	r_g	0.990**	0.470**	0.887**	0.398**	0.810**
Day to maturity	r_p		0.325**	0.533**	0.512**	0.525**
	r_g		0.656**	0.909**	0.720**	0.806**
Plant height (cm)	r_p			0.255*	0.534**	0.066
	r_g			0.253*	0.964**	0.102
Primary branches ~~p~~plant^{-1}~~er plant~~	r_p				0.509**	0.775**
	r_g				0.508**	0.929**
Pods ~~p~~plant^{-1}~~er plant~~	r_p					0.577**
	r_g					0.393**

* Significant at 5% level of probability ** Significant at 1% level of probability

Table S4. Phenotypic and genotypic correlation coefficients for yield and yield contributing traits in F_9.

	Days to 50% flowering	Day to maturity	Plant height (cm)	Primary branches ~~p~~plant^{-1}~~er plant~~	Pods ~~p~~plant^{-1}~~er plant~~	Seed yield ~~p~~plant^{-1}~~er plant~~
Days to 50% flowering	r_p	0.757**	0.549**	0.566**	0.279**	0.625**
	r_g	0.797**	0.790**	0.999**	0.285**	0.919**

Day to maturity	r_p		0.558**	0.422**	0.466**	0.454**
	r_g		0.858**	0.831**	0.567**	0.674**
Plant height (cm)	r_p			0.551**	0.443**	0.448**
	r_g			0.999**	0.640**	0.751**
Primary branches ~~p~~plant^{-1}~~er plant~~	r_p				0.519**	0.707**
	r_g				0.363**	0.808**
Pods ~~p~~plant^{-1}~~er plant~~	r_p					0.649**
	r_g					0.317**

* Significant at 5% level of probability ** Significant at 1% level of probability