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Original Research Article Genetic Diversity and Association Analysis for Agronomic and Yield Related Traits in Interspecific Progenies of Pigeonpea (Cajanus cajan)

Mahadeva Swamy HK ^{a,b*}, Amaresh ^a, Aswini Nunavath ^c, Bajpai GC ^b, Verma SK ^b, Gopalareddy K ^a, Maruthi RT ^a, Sreenivas V ^a and Chandana Behera ^d

^a ICAR- Sugarcane Breeding Institute, Coimbatore -641 007, India.
 ^b G. B. Pant University of Agriculture and Technology, Pantnagar -263 145, India.
 ^c ICAR- National Institute of Biotic Stress Management, Raipur -493 225, India.
 ^d Odisha University of Agriculture & Technology, Bhubaneswar -751 003, India.

Authors' contributions

This work was carried out in collaboration among all authors. Authors MSHK, BGC and VSK designed and implemented the experiment. Authors Amaresh and AN analysed the data and wrote the manuscript. Authors GK, MRT, SV and CB reviewed the manuscript. All authors read and approved the final manuscript.

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*Corresponding author: E-mail: hkmswamy44@gmail.com;

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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 plant⁻¹) 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 pigeon pea 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; pigeon pea; variability; wild species; interspecific cross.

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, Varshney et al. [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 [3]. Notably, India stands as the primary contributor, accounting for 90% of the global pigeonpea production. India was the world's top producer of pigeon peas, with 4.34 million tonnes produced on 5.05 million hectares of land and an 859 kg/ha vield [4]. Despite these substantial production figures, the national harvest falls short of meeting the demands 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 [5].

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 [6].

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 interspecific crosses.

2. MATERIALS 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 and 2010-11, seeds of F_8 and F_9 progenies were sowed, along with three cultivated parent lines, in a randomized complete block design, wherein 54 inter-specific progeny lines and the three cultivated parent lines were sowed across three replications. Standard package of practices were followed to raise the good crop. Field data were recorded from 171 plots (90 cm x 30 cm, 3 m plot size) with on five randomly selected plants within each plot.

The observations on the number of days to 50% flowering, number of days to maturity, Plant height, number of primary branches plant⁻¹, number of pods plant⁻¹ and seed yield plant⁻¹ 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 using software Statistical Tool for Agricultural Research (STAR) Version: 2.0.1. The procedures employed for the analysis of variance adhered to the framework outlined by [7]. 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 terms, following the methodology proposed by [8]. The calculation of heritability in the broad sense involved determining the ratio of genotypic variance to phenotypic variance, expressed as a percentage [9]. 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 [10].

3. RESULTS AND DISCUSSION

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. 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 (11-15].

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 plant⁻¹ (34.25%), succeeded by seed yield plant⁻¹ (31.64%), and primary branches plant⁻¹ (22.97%), while the minimum PCV % was observed for days to maturity (8.05%). Similar trends were observed in the subsequent kharif 2010, with the maximum PCV % recorded for pods plant⁻¹ (35.92%), followed by seed yield plant⁻¹ (33.74%) and primary branches plant⁻¹ (25.14%), and the minimum PCV % registered for days to maturity (7.35%) (Supplementary Table S1 and Supplementary Table S2). In parallel, the assessment of genetic coefficient of variation (GCV %) during the kharif season 2009 indicated the highest values for pods plant-1 (29.55%), seed yield plant⁻¹ (24.81%). branches plant⁻¹ and primary (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 plant⁻¹ (27.58%), seed yield plant⁻¹ (20.83%), and primary branches plant⁻¹ (11.95%), and the minimum GCV % observed for days to maturity (Supplementary Table (6.94%)S1 and Supplementary Table S2). Additionally, the evaluation of genotypic coefficient of variation (GCV %) in the kharif season 2009 revealed the highest values for seed yield plant⁻¹ (24.81%), pods plant⁻¹ (29.55%), and primary branches plant⁻¹ (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 plant⁻¹ (20.83%), followed by pods plant⁻¹ (27.58%), while the minimum GCV % was noted for days to maturity (6.94%) (Supplementary Table S1 and Supplementary

Table S2). Similar findings with high variability for different yield related traits was also reported in pigeonpea (11-18].

S. No.	Cultivated Parents	Feature	Remarks
1	ICPL84023 (G55)	Determinate, early maturing line, resistant to SMD and Wilt	Derived from the cross ICP 69997 X ICP 7220
2	PA134 (G56)	Indeterminate, early maturing line	Developed by Pantnagar University
3	UPAS120 (G57)	Indeterminate, early variety	UPAS 120' was developed at Pantnagar University from a spontaneous mutant found in a germplasm line 'P-4758'
S. No.	Interspecific crosses		
1	ICPL 84023 x <i>C. scarabaeoides</i> (G1-G6)		<i>C. scarabaeoides</i> is a species native to Asia. Source for A2 CMS line.
2	ICPL 84023 x C. acutifolius (G7-G12)		<i>C. acutifolius</i> is native of North Australia
3	ICPL 84023 x <i>C. cajanifolius</i> (G13-G18)		<i>C. cajanifolius</i> native of Indian subcontinenet. Source for A4 CMS line.
4	PA134 x C. scarabaeoides (G19- G24)		
5	PA134 x C. acutifolius (G25-G30)		
5 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 <i>C. cajanifolius</i> (G49- G54)		

Table 1. List of interspecific crosses and their cultivated parents

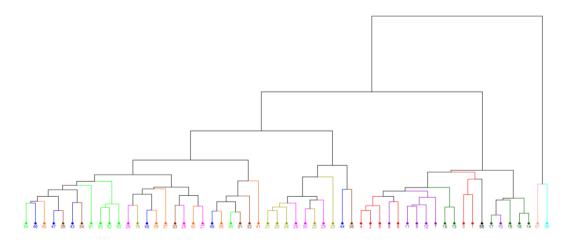


Fig. 1. Cluster analysis for 54 lines derived from 9 interspecific cross progenies of pigeonpea along with the three cultivated parents (ICPL 84023, PA 143, UPAS 120) and wild species (*C. scarabaeoides, C. acutifolius,* and *C. cajanifolius*)

Source of variation	d.f.	Days to 50% flowering	Days to maturity	Plant height (cm)	Primary branches/ plant	Pods plant ⁻¹	Seed yield plant ⁻¹
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

Table 2. Analysis of variance for agronomic and yield related traits in F₈ progenies of interspecific crosses and cultivated parents

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

Table 3. Analysis of variance for agronomic and yield related traits in F₉ progenies of interspecific crosses and cultivated parents

Source of variation	d.f.	Days to 50% flowering	Days to maturity	Plant height (cm)	Primary branches/ plant	Pods plant ⁻¹	Seed yield plant ⁻¹
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 plant⁻¹ (NP), Number of Pods plant⁻¹ (NP), Days to 50% flowering (DF), Days to maturity (DM) and Seed yield plant⁻¹ (SY)

Cluster	PH	NB	NP	DF	DM	SY	Genotypes
	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
	151.19	13.53	157.04	78.47	174.50	34.55	G20, G21, G22, G23, G24,
							G28, G29, G30, G36, G44
	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

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 plant⁻¹ (74.44%), followed by seed yield plant⁻¹ (61.49%), and day to maturity (60.51%). Conversely, plant height exhibited the lowest heritability at 38.76%. In the subsequent kharif season of 2010-11, heritability values ranged from 22.61% for primary branches plant⁻¹ to 89.35% for days to flowering. Moderate heritability was observed for seed yield plant⁻¹ (38.13%), pods plant⁻¹ (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 mean highest value was recorded for pods plant⁻¹ (52.53%), followed by seed yield plant⁻¹ (40.08%) and primary branches plant⁻¹ (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 plant⁻¹ (43.62%), followed by days to 50% flowering (28.03%), seed yield plant⁻¹ (26.68%), with the lowest value observed for days to maturity (13.54%) (Supplementary Table S1 and Supplementary Table S2). Heritability estimates of yield traits shows moderate to high heritability for pods plant⁻¹, seed yield and days to maturity in both F_8 and including day to maturity, days to 50% flowering in F₉. High heritability for different characters were reported in pigeonpea by different studies [19-25]. Yield contributing characters had moderate heritability with moderate to high genetic advance as per cent mean were exhibited by pods plant⁻¹, seed yield plant⁻¹ in F₈ progenies and in F₉ it was found only plant⁻¹. seed yield This indicates in 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 F₈ progeny generation, a robust and positive correlation was evident for all examined traits. except in the case of plant height and seed yield plant⁻¹.

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 plant⁻¹, and the number of primary branches plant⁻¹. However, there was no statistically significant correlation observed between plant height and seed yield plant⁻¹. 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 Supplementary Table S3 and Supplementary Table S4. Notably, a highly significant and positive correlation was identified for all traits in F₉ progeny generation. Similar findings were also reported by different studies [26-31] in past.

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 plant⁻¹ 11.85, number of pods plant⁻¹ 122.32, days to flowering 85.24, days to maturity 176.85, grain yield plant⁻¹ 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 plant⁻¹ was 13.53, number of pods plant⁻¹ was 157.04, days to flowering was 78.47, days to maturity about 174.50, seed yield plant⁻¹ was 34.55 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 plant⁻¹ 8.92, number of pods plant⁻¹ was 86.66, days to flowering was 70.53, days to maturity about 156.65, seed yield plant⁻¹ 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 of primary branches plant⁻¹ was 10.29, number of pods plant⁻¹ was 185.91, days to flowering was 74, days to maturity about 174.83, seed yield plant⁻¹ was 20.12 g (Table 4). Considering the higher yield plant⁻¹ in the genotypes of second cluster were best for further utilization in crop improvement. The cluster analysis among the pegionpea hybrids [32], land races [33], germplam lines [34,35] 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.

S.No	GEN	PH	NB	NP	DF	DM	GY
1	G44	170.1	14.05	170.34	87	175	37.47
2	G23	144.7	14.25	170.09	74.33	171	37.42
3	G36	156	12.33	166.84	86	177.67	36.7
4	G22	149.9	14.38	158.91	76.33	175.33	34.96
5	G30	152.4	13.03	156.07	80.67	177.67	34.34
6	G29	142.6	12.88	154.67	81	173.67	34.03
7	G21	146.2	13.87	152.9	75	172	33.64
8	G20	149.2	16.17	149.57	75	174.33	32.91
9	G28	153.5	11.5	145.88	74.33	176.33	32.09
10	G24	147.3	12.8	145.16	75	172	31.93
11	ICPL 84023 (G55)	144.6	7.59	81.6	51.67	142	12.63
12	PA 134 (G56)	178.5	9.88	185.77	66.33	171.33	19.02
13	UPAS 120 (G57)	180.6	10.7	186.05	81.67	178.33	21.21

Table 5. List of top 10 grain yielding progenies along with checks

Note: GEN- Genotype; PH-Plant height; NB- Primary branches plant¹; NP-Number of pods plant¹; DF Days to 50% flowering; DM- Days to maturity; GY-Grain yield.

4. CONCLUSION

Consequently, the findings suggest the presence of considerable genetic diversity for the grain yield and its related traits, including seed yield plant⁻¹ and the number of pods plant⁻¹, demonstrating substantial genetic advancement. Notably, a noteworthy and positive correlation was observed between seed yield plant⁻¹ and the absence of primary branches, as well as the number of pods plant⁻¹. Enhancements in these traits are anticipated to positively influence seed vield. 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. Considering the higher yield plant⁻¹ in the genotypes of second cluster were best for further utilization in crop improvement. 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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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Character	Range	General mean	PCV%	GCV%	ECV%	Heritability (%)	Genetic advance	Genetic advance as % of mean
Days to 50% flowering	51.66-96.33	76.72±4.94	16.04	11.52	11.16	51.61	13.08	17.05
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 plant ⁻¹	6.93-14.38	10.78±0.95	22.97	17.06	15.38	55.16	2.81	26.07
Pods plant ⁻¹	72.82-215.82	126.46±12.64	34.25	29.55	17.31	74.44	66.43	52.53
Seed yield plant ⁻¹ (g)	11.55-37.47	23.80±2.69	31.64	24.81	19.63	61.49	9.54	40.08

Table S1. Range, general mean, heritability, genetic advance PCV and GCV in F₈ progenies and cultivated parents for yield and yield related traits

Table S2. Range, general mean, heritability, genetic advance PCV and GCV in F₉ 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 branches plant-1	7.39-15.46	11.92±1.52	25.14	11.95	22.12	22.61	1.39	11.66
Pods plant ⁻¹	80.05-212.31	141.56±18.81	35.92	27.58	23.01	58.94	61.75	43.62
Seed yield plant ⁻¹ (g)	12.29-40.003	25.34±3.96	33.74	20.83	26.53	38.13	6.76	26.68

	Days to 50% flowering	Day to maturity	Plant height (cm)	Primary branches plant ⁻¹	Pods plant ⁻¹	Seed yield plant ⁻¹
Days to 50%	r _p	0.604**	0.211*	0.450**	0.270*	0.473**
flowering	r _g	0.990**	0.470**	0.887**	0.398**	0.810**
Day to	r _p		0.325**	0.533**	0.512**	0.525**
maturity	r _g		0.656**	0.909**	0.720**	0.806**
Plant height	r _p			0.255*	0.534**	0.066
(cm)	r _g			0.253*	0.964**	0.102
Primary	r _p				0.509**	0.775**
branches	r _g				0.508**	0.929**
plant ⁻¹						
Pods plant ⁻¹	r _p					0.577**
	r _g					0.393**

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

* 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 plant ⁻¹	Pods plant ⁻¹	Seed yield plant ⁻¹
Days to 50%	r _p	0.757**	0.549**	0.566**	0.279**	0.625**
flowering	r _g	0.797**	0.790**	0.999**	0.285**	0.919**
Day to	r _p		0.558**	0.422**	0.466**	0.454**
maturity	r _g		0.858**	0.831**	0.567**	0.674**
Plant height	r _p			0.551**	0.443**	0.448**
(cm)	r _g			0.999**	0.640**	0.751**
Primary	r p				0.519**	0.707**
branches plant ⁻¹	rg				0.363**	0.808**
Pods plant ⁻¹	r _p					0.649**
·	rg					0.317**

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