Australian Journal of
Crop Science
AJCS

AJCS 9(6):494-503 (2015) ISSN:1835-2707

Relationship between heterosis and genetic diversity in Indian pigeonpea [Cajanus cajan (L.) Millspaugh] accessions using multivariate cluster analysis and heterotic grouping

Praveen Pandey[1,2]*,Vankat R. Pandey[1,2], Ashutosh Kumar[1,2],Sunil Yadav[3],Dinesh Tiwari[2] and Rajesh Kumar[2]

[1]Indian Institute of Pulses Research, Kanpur-208024 (U.P.) India
[2]Narendra Deva University of Agriculture &Technology, Kumarganj, Faizabad-224229 (U.P.), India
[3]Jawahar Lal Nehru Krishi Vishwavidyalaya, Rewa-486001 (M.P.) India

***Corresponding author: pandeypraveen1986@yahoo.com**

Abstract

Pigeonpea (*Cajanus cajan* (L.) Millspaugh) is one of the most important food legume crops in the semi-arid regions of the world. India is the largest pigeonpea growing country however; its productivity per unit area by world standard is sparingly low and stagnant owing to several biotic and abiotic stresses. In order to increase its productivity, high yielding and disease resistant varieties/hybrids should be developed. Hence, an attempt was made to assess relationship between heterosis and genetic diversity as well as forming heterotic groups for pigeonpea breeding. Three CMS lines were crossed with 20 elite genotypes/restorers in a line x tester mating system and the resultant 60 F_1 hybrids along with their parents were evaluated for various morphological traits to predict the genetic relationship among parents and heterosis in their crosses. The parental genotypes under study fell into five distinct non-overlapping clusters. Maximum intra-cluster distance was in cluster III (263.80) followed by cluster IV (253.62) and cluster I (244.81). The inter-cluster distances varied from 299.93 (between cluster I and II) to 727.79 (between cluster II and IV). Generally, the crosses derived from high diversity group showed high positive significant heterosis for seed yield. However, some crosses give very high negative heterosis for seed yield although their parents belong to a high diversity group. The reason for this possibly will be linkage of alleles for complex genetic traits as biomass and yield. Consequently, the precision of genetic distance can be obtained by estimating genetic distance through molecular techniques. Among the top fifteen heterotic hybrids for seed yield, ten crosses were resulted from crossing between parents of low diversity while, five from the high diversity group. Heterotic grouping evinced that usually, yield, heterosis and specific combining ability are higher in inter-group crosses than in intra-group crosses. It could be concluded that genetic diversity can be utilized as a reliable parameter for predicting heterosis in hybrids.

Keywords: *Cajanus cajan* (L.) Millspaugh, genetic diversity, heterosis, heterotic groups.
Abbreviations: CGMS_Cytoplasmic-genic male sterility, SCA_Specific combining ability, BPH_Better parent heterosis, SH_Standard heterosis.

Introduction

Pigeonpea [*Cajanus cajan* (L.) Millspaugh] is one of the major food legume crops in India as well as semi-arid regions of the world. It has been recognized as a good source of protein particularly in the developing countries where the majority of peoples depend on vegetarian foods. Pigeonpea has emerged as the most important pulse crop in India covering 4.04 m ha area and contributing 2.65 mt production with the productivity of 656 kg/ha. Globally, it is grown on about 5.60 million hectares area with production of 4.31 million tonnes and average yield of 770.8 kg per hectare. It contributes about 5.3 per cent share in global pulse production (FAOSTAT, 2012). The average yield of pigeonpea in India is sparingly low compared to the world average. The major constraints to productivity in pigeonpea are several abiotic stresses (drought, salinity and water-logging) and biotic stresses (*Fusarium wilt*, sterility mosaic disease, and pod borer insects). Researcher's emphasized the genetic improvement of pigeonpea for more than five decades including breeding for reducing crop duration; improving seed quality and overcoming the major biotic and abiotic constraints to enhance the yield potential and several cultivars were developed. However, the progress in the genetic enhancement of yield has been limited and the improved cultivars failed to boost productivity of the crop owing to various climatic, edaphic, and crop management factors (Varshney et al., 2007; Saxena 2008). Narrow genetic diversity in cultivated genotypes has further hampered the successful utilization of traditional breeding, consequently pigeonpea referred as an 'Orphan Crop Legume' (Varshney et al., 2010). Therefore, use of an alternative approach such as hybrid technology is crucial to augment the yield of pigeonpea for ensuring food and nutritional security.

Heterosis is the phenomenon of superiority of F_1 hybrids over its parents and it is the outcome of high degree heterozygosity in the genome. The hypothetical concerns also implied that genetic diversity among prospective parents is important for the success of a hybrid breeding program as it determines the magnitude of heterosis in F_1 hybrids to a large extent (Tecklewold and Becker 2006). However, a strong correlation between heterosis and parental genetic distance has been rarely observed (Melchinger 1999; Singh and Singh 2004). To get a proper picture of the genetic divergence, multivariate cluster analysis with the aid of D^2 statistics has been successfully used (Mahalanobis, 1936; Bhatt, 1970;

Table 1. Analysis of variance (ANOVA) for 11 quantitative traits of line x tester set of crosses and their parents in pigeonpea.

Characters	Sources of variation								
	Replications [2]	Treatments [82]	Parents [22]	Lines [2]	Testers [19]	Lines vs Testers [1]	Parents vs Crosses [1]	Crosses [59]	Error [132]
Days to 50 % flowering	2.24	251.21**	45.80**	5.44*	44.28**	155.36**	23.52**	331.66**	1.23
Days to maturity	2.37	140.02**	45.52**	18.11**	50.36**	8.18*	442.29**	170.13**	1.49
Plant height (cm)	0.45	935.35**	411.41**	179.31**	322.61**	2562.72**	1628.50**	1118.97**	8.38
Primary branches/ plant	1.58	4.19**	1.70**	0.72	1.88**	0.37	7.22**	5.06**	0.51
Secondary branches/ plant	29.93	140.93**	101.91**	178.42**	94.83**	83.42*	279.27**	153.13**	12.51
Pods/ plant	2.66	9361.31**	2245.57**	468.74**	2535.59**	288.69**	84484.09**	10741.37**	25.71
Seeds/ pod	0.04	0.31**	0.34**	1.39**	0.25**	0.02	3.61**	0.24**	0.02
100-seed weight (g)	0	6.02**	9.87**	15.91**	9.75**	0.12	1.01**	4.67**	0.07
Seed yield/ plant (g)	3.64	1421.23**	494.78**	433.14**	526.80**	9.66	3099.20**	1738.24**	4.6
Biological yield/ plant (g)	1.85	6233.61**	4732.80**	3696.26**	4991.04**	1899.26**	2284.02**	6860.17**	55.33
Harvest-index (%)	1.21	177.45**	17.09**	4.79**	18.24**	19.77**	1358.97**	217.22**	0.81

*,** significant at 5% and 1% probability levels, respectively, [ ] value in parenthesis represents degree of freedom

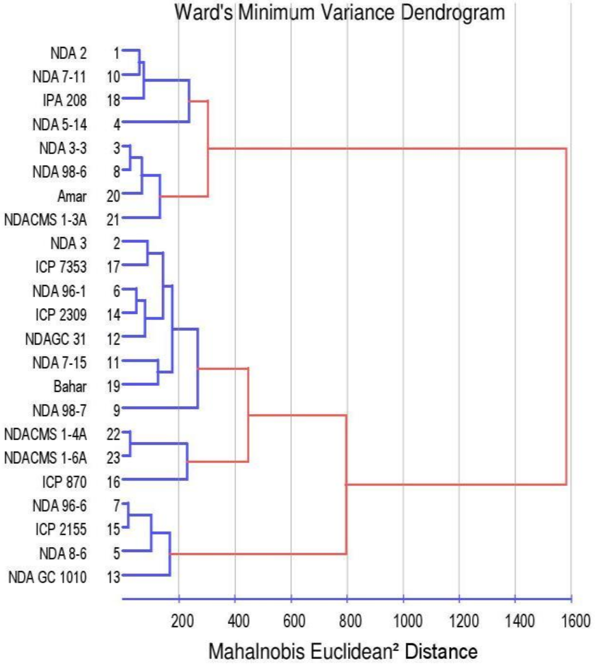

Fig 1. Dendrogram of 23 accessions based on morphological data using Euclidean distance matrix.

Table 2. Estimates of specific combining ability (SCA) effects of parents for 11 quantitative characters in pigeonpea.

S. No.	Crosses	Days to 50 % flowering	Days to maturity	Plant height (cm)	Primary branches/ plant	Secondary branches/ plant	Pods/ plant	Seeds/ pod	100-seed weight (g)	Seed yield/ plant (g)	Biological yield/ plant (g)	Harvest-index (%)
1	NDACMS1-3A X NDA 2	1.41*	-2.11**	5.95**	0.27	7.59**	50.54**	0.17	-0.57**	19.78**	26.76**	5.21**
2	NDACMS1-3A X NDA 3	4.63**	0.67	5.57**	0.28	8.25**	32.58**	0.12	0.99**	18.86**	17.54**	6.02**
3	NDACMS1-3A X NDA 3-3	3.97**	2.01**	2.22	2.34**	1.32	39.92**	0.62**	-0.71**	18.49**	14.26**	8.86**
4	NDACMS1-3A X NDA 5-14	-6.81**	-3.99**	-11.06**	0.26	-3.18	-28.35**	-0.37**	-0.45**	-11.93**	36.00**	-6.10**
5	NDACMS1-3A X NDA 8-6	-5.92**	-1.66*	-0.69	-0.40	1.94	-5.90*	-0.32**	0.32*	-8.74**	-1.37	-4.11**
6	NDACMS1-3A X NDA 96-1	-2.92**	-0.44	25.54**	1.55**	2.54	7.19*	-0.34**	-1.40**	-11.02**	68.67**	-8.79**
7	NDACMS1-3A X NDA 96-6	-4.48**	-5.33**	0.81	-0.42	0.59	-3.77	0.08	1.47**	10.91**	3.59	4.30**
8	NDACMS1-3A X NDA 98-6	-16.59**	-10.55**	-0.68	3.03**	10.02**	67.60**	0.25**	0.68**	28.72**	56.12**	8.25**
9	NDACMS1-3A X NDA 98-7	-3.48**	-2.11**	6.18**	-0.48	10.33**	37.46**	0.11	-0.49**	12.77**	1.39	5.56**
10	NDACMS1-3A X NDA 7-11	-7.14**	-3.66**	1.52	0.45	0.83	-5.39	0.01	-2.21**	-4.52*	-19.93**	-0.54
11	NDACMS1-3A X NDA 7-15	-0.92	1.56*	-2.68	0.42	2.59	33.79**	0.02	-0.13	16.71**	-32.91**	9.17**
12	NDACMS1-3A X NDAGC 31	-5.59**	-1.44*	9.62**	0.56	1.78	31.17**	-0.10	0.77**	-8.19**	2.78	-4.29**
13	NDACMS1-3A X NDAGC 1010	0.30	3.01**	-2.40	-1.13**	-3.01	-62.71**	-0.19*	0.65**	-19.67**	32.52**	-11.04**
14	NDACMS1-3A X ICP 2309	-3.26**	-2.66**	-12.32**	-1.04*	-6.72**	-42.06**	0.08	0.73**	-7.01**	-28.22**	0.31
15	NDACMS1-3A X ICP 2155	15.97**	11.78**	-12.03**	-1.64**	-6.55**	11.16**	0.18*	0.93**	11.80**	13.35**	3.54**
16	NDACMS1-3A X ICP 870	20.19**	11.89**	-8.98**	-0.26	-7.48**	-22.29**	-0.10	-0.02	-10.03**	-42.35**	0.15
17	NDACMS1-3A X ICP 7353	11.19**	3.45**	-3.15	-0.67	-6.09**	22.71**	0.08	0.46**	10.27**	1.86	4.24**
18	NDACMS1-3A X IPA 208	11.08**	5.45**	-19.29**	-0.96*	-5.73**	-47.60**	0.10	-0.57**	-18.22**	-60.37**	-3.30**
19	NDACMS1-3A X BAHAR	-5.37**	-2.77**	15.56**	-0.54	-3.24	-73.68**	-0.21*	0.13	-24.93**	-43.72**	-9.07
20	NDACMS1-3A X AMAR	-6.26**	-3.11**	0.31	-1.62**	-5.78**	-42.38**	-0.19*	-0.58**	-24.05**	-45.98**	-8.38**
21	NDACMS1-4A X NDA 2	-2.94**	0.48	-9.85**	-0.72	-7.95**	-11.92**	0.02	-0.41**	-8.96**	-17.25**	-0.70
22	NDACMS1-4A X NDA 3	-4.38**	-2.74**	-17.13**	0.07	-3.00	3.96	0.09	-1.60**	-7.97**	-7.30**	-1.60**
23	NDACMS1-4A X NDA 3-3	-2.38**	-0.41	-13.60**	-2.03**	-4.26*	-63.31**	-0.47**	-1.10**	-30.15**	-39.50**	-12.76**
24	NDACMS1-4A X NDA 5-14	1.17	1.26	1.34	-0.95*	-5.86**	-49.46**	0.23*	-0.68**	-22.69**	-77.69**	-5.17**
25	NDACMS1-4A X NDA 8-6	15.06**	8.26**	6.56**	0.52	0.85	-64.80**	0.25**	-1.26**	-22.85**	-56.41**	-6.34**
26	NDACMS1-4A X NDA 96-1	16.73**	13.14**	-12.91**	-1.23**	-4.05*	-50.60**	0.24**	0.07	-15.32**	-91.62**	1.91**
27	NDACMS1-4A X NDA 96-6	8.84**	7.26**	4.07*	0.77	-7.10**	-14.72**	0.02	1.06**	-0.54	11.42**	-0.67
28	NDACMS1-4A X NDA 98-6	12.06**	6.70**	1.38	-2.18**	-7.00**	-109.34**	-0.32**	-1.70**	-44.14**	-68.88**	-14.57**
29	NDACMS1-4A X NDA 98-7	3.84**	-0.52	-1.90	-0.39	-6.59**	-32.60**	-0.21*	2.00**	-5.67**	2.35	-1.99**
30	NDACMS1-4A X NDA 7-11	11.17**	7.59**	-12.55**	-0.44	0.76	-12.99**	0.21*	0.88**	-4.27**	-0.85	-0.14
31	NDACMS1-4A X NDA 7-15	3.73**	3.48**	-13.86**	-0.49	-6.95**	48.23**	-0.28**	0.68**	12.56**	-5.64	5.82**
32	NDACMS1-4A X NDAGC 31	1.39*	-1.52*	-2.97	-1.27**	-3.58	50.42**	0.38**	0.70**	39.55**	37.97**	14.42**
33	NDACMS1-4A X NDAGC 1010	-0.05	-2.08**	-5.68**	0.63	1.22	16.22**	-0.20*	0.18	1.34	-36.25**	5.16**
34	NDACMS1-4A X ICP 2309	-1.61*	-0.08	4.53**	0.47	3.81	34.52**	0.00	-0.18	9.14**	33.47**	0.88
35	NDACMS1-4A X ICP 2155	-15.05**	-6.63**	20.20**	2.33**	9.51**	23.66**	0.04	-0.50**	6.85**	37.13**	-0.55
36	NDACMS1-4A X ICP 870	-22.83**	-14.86**	9.89**	-0.19	1.80	15.50**	0.15	-0.45**	4.02**	28.28**	-0.29
37	NDACMS1-4A X ICP 7353	-3.49**	0.03	4.66**	1.07*	10.84**	17.19**	-0.08	-0.23	4.32**	31.16**	-0.93
38	NDACMS1-4A X IPA 208	-11.94**	-9.63**	14.82**	1.36**	10.57**	64.47**	0.02	1.53**	31.57**	87.53**	5.17**
39	NDACMS1-4A X BAHAR	-7.72**	-8.52**	5.18**	0.32	9.53**	78.48**	0.04	0.28	28.48**	72.65**	6.24**
40	NDACMS1-4A X AMAR	-1.61*	-1.19	17.82**	2.37**	7.45**	57.10**	-0.11	0.74**	24.73**	59.44**	6.11**
41	NDACMS1-6A X NDA 2	1.53*	1.63*	3.90*	0.45	0.37	-38.62**	-0.20*	0.98**	-10.82**	-9.51**	-4.51**
42	NDACMS1-6A X NDA 3	-0.25	2.07**	11.57**	-0.35	-5.25*	-36.53**	-0.21*	0.61**	-10.89**	-10.24**	-4.42**
43	NDACMS1-6A X NDA 3-3	-1.58*	-1.59*	11.38**	-0.32	2.94	23.39**	-0.15	1.81**	11.66**	25.24**	3.90**
44	NDACMS1-6A X NDA 5-14	5.64**	2.74**	9.72**	0.70	9.04**	77.81**	0.13	1.13**	34.63**	41.69**	11.28**
45	NDACMS1-6A X NDA 8-6	-9.14**	-6.59**	-5.87**	-0.12	-2.79	70.70**	0.08	0.94**	31.59**	57.79**	10.44**
46	NDACMS1-6A X NDA 96-1	-13.81**	-12.71**	-12.64**	-0.32	1.51	43.41**	0.10	1.34**	26.34**	22.95**	6.88**
47	NDACMS1-6A X NDA 96-6	-4.36**	-1.93**	-4.88**	-0.34	6.50**	18.49**	-0.10	-2.53**	-10.36**	-15.01**	-3.63**
48	NDACMS1-6A X NDA 98-6	4.53**	3.85**	-0.70	-0.85*	-3.02	41.74**	0.08	1.02**	15.42**	12.76**	6.32**

49	NDACMS1-6A X NDA 98-7	-0.36	2.63**	-4.28*	0.87*	-3.74	-4.86	0.10	-1.51**	-7.09**	-3.74	-3.57**
50	NDACMS1-6A X NDA 7-11	-4.03**	-3.93**	11.03**	-0.01	-1.59	18.38**	-0.22*	1.33**	8.79**	20.78**	0.68
51	NDACMS1-6A X NDA 7-15	-2.81**	-5.04**	16.54**	0.08	4.36*	-82.02**	0.27**	-0.55**	-29.27**	38.56**	-14.99**
52	NDACMS1-6A X NDAGC 31	4.19**	2.96**	-6.65**	0.71	1.79	-81.59**	-0.27**	-1.47**	-31.36**	-40.75**	-10.13**
53	NDACMS1-6A X NDAGC 1010	-0.25	-0.93	8.07**	0.50	1.79	46.49**	0.39**	-0.83**	18.33**	3.73	5.88**
54	NDACMS1-6A X ICP 2309	4.86**	2.74**	7.78**	0.57	2.91	7.54*	-0.07	-0.55**	-2.13	-5.25	-1.19*
55	NDACMS1-6A X ICP 2155	-0.92	-5.15**	-8.16**	-0.69	-2.96	-34.82**	-0.22*	-0.42**	-18.65**	-50.48**	-2.99**
56	NDACMS1-6A X ICP 870	2.64**	2.96**	-0.91	0.46	5.68**	6.78*	-0.05	0.47**	6.00**	14.07**	0.14
57	NDACMS1-6A X ICP 7353	-7.69**	-3.48**	-1.51	-0.41	-4.75*	-39.90**	0.00	-0.23	-14.59**	-33.02**	-3.31**
58	NDACMS1-6A X IPA 208	0.86	4.18**	4.47**	-0.41	-4.84*	-16.87**	-0.12	-0.96**	-13.35**	-27.17**	-1.87**
59	NDACMS1-6A X BAHAR	13.08**	11.29**	-20.74**	0.23	-6.29**	-4.80	0.17	-0.42**	-3.55	-28.93**	2.83**
60	NDACMS1-6A X AMAR	7.86**	4.29**	-18.13**	-0.75	-1.67	-14.73**	0.30**	-0.16	-0.68	-13.46**	2.27**
	SE(Sij)	0.41	0.45	1.06	0.26	1.29	1.85	0.06	0.10	0.78	2.72	0.33
	SE(Sij-Ski)	0.91	1.00	2.36	0.59	2.89	4.14	0.13	0.21	1.75	6.07	0.74

*,** significant at 5% and 1% probability levels, respectively

Table 3. Heterotic groups based on yield-specific combining ability data (SCA).

NDACMS 1-3A	NDACMS 1-4A	NDACMS 1-6A
Bahar	NDA 98-6	NDAGC 31
Amar	NDA 3-3	NDA 7-15
NDAGC 1010	NDA 8-6	ICP 2155
IPA 208	NDA 5-14	ICP 7353
ICP 870	NDA96-1	NDA 3
ICP 2309		NDA 2
NDA 7-11		NDA 96-6
		NDA 98-7

Table 4. Estimates of average intra- and inter-cluster distances in pigeonpea.

Clusters	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V
Cluster I	244.81	299.93	558.94	690.94	524.58
Cluster II		149.7	535.38	727.79	689.3
Cluster III			263.8	404.74	474.86
Cluster IV				253.62	533
Cluster V					191.39

*Bold figure represent intra-cluster distance

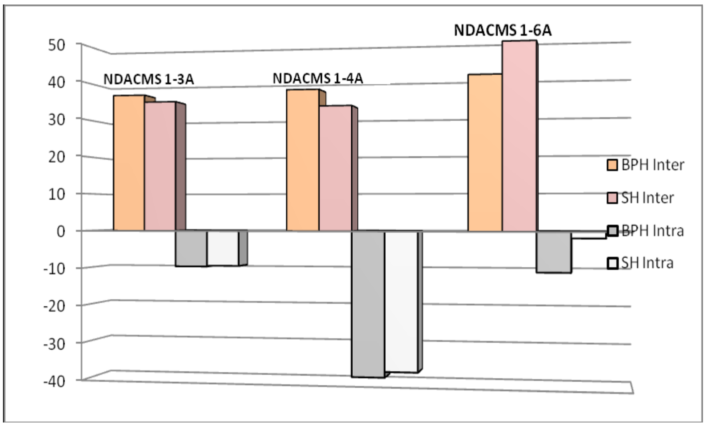

Fig 2. Percentage of intra-group and inter-group heterosis for each tester.

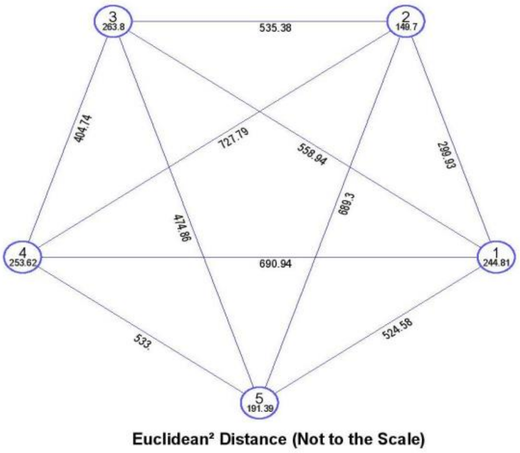

Fig 3. Cluster diagram showing inter and intra-cluster distances.

Arunachalam, 1981; Katiyar et al., 2004; Gupta et al. 2008; Pratap et al., 2011). To exploit maximum heterosis using Cytoplasmic-Genic Male Sterility (CGMS) we have must know the combining ability of different male sterile and restorer lines. Restoration of male fertility is also critical and important as it governs the viability of hybrids. Parents with a higher general combining ability and a large genetic distance produce a hybrid with better yield performance (Cox and Murphy 1990, Diers et al., 1996). Heterotic grouping refers categorizing germplasm groups that are genetically diverse and that produce superior hybrids when crossed. Crossing representatives of different heterotic groups will maximize heterozygosity, hybrid vigour and yield stability of the new cultivars (Fan et al., 2009; Esposito et al., 2013). The enhanced yield stability attributed to enhanced capacity for individual buffering in heterozygous genotypes. For improving breeding efficiency breeders ought to classify all the parental lines into as few heterotic groups as possible because more heterotic group requires more inter-group crosses, consequently breeding efficiency will be reduced. Heterotic grouping based on genetic distances and yield-specific combining ability, is useful for recognition of parents that would produce superior hybrids without making all possible crosses among the prospective parents (Riday et al., 2003; Bhatnagar et al., 2004; Teklewold and Becker, 2006; Fan et al., 2004 & 2009). Although, the information on combining ability and heterosis in pigeonpea is available but, reports concerning pattern of heterotic groups by yield-specific combining ability not published yet. Keeping in view the above perspectives an experiment was undertaken with following major objectives: (i) to construct relationship between heterosis and parental genetic distance. (ii) to form heterotic groups by yield-specific combining ability, and (iii) to identify appropriate parents for heterosis breeding for achieving a quantum jump in yield of pigeonpea.

Results and Discussion

Analysis of variance (ANOVA)

The analysis of variance (Table 1) revealed that for treatments, lines, testers, lines x tester interactions, parents, crosses and parents vs. crosses are highly significant for all the characters studied indicating that the parents included in this investigation exhibit sufficient variability. The presence of large amount of variability might be due to diverse source of materials taken as well as environmental influence affecting the genotypes.

Specific combining ability and heterotic grouping

Combining ability illustrates the breeding value of parental lines to generate hybrids, and thus aids in the recognition of parents with high GCA and parental combinations with high SCA (Sprague and Tatum 1942; Griffings 1956). It also exposes the nature of gene action involved in the inheritance of a trait. The specific combining ability that are supposed to be manifestation of non-additive gene action, are very important for discrimination of crosses for their genetic worth as breeding materials. The sixty crosses studied (Table 2) twenty-six crosses showed significant positive SCA effect for seed yield among them only two crosses had significant and desirable SCA effects for all the eleven characters. The hybrids NDACMS 1-4A x NDAGC 31, NDACMS 1-6A x NDA 5-14, NDACMS 1-6A x NDA 8-6, NDACMS 1-4A x IPA 208, NDACMS 1-3A x NDA 98-6, NDACMS 1-4A x Bahar, NDACMS 1-6A x NDA 96-1, NDACMS 1-4A x Amar and NDACMS 1-3A x NDA 2 identified best specific combiner for seed yield per plant and its major attributes. The high SCA value of these hybrids indicates that the expression of these traits is determined by dominance, epistatic and various other gene interactions (Griffings 1956; Baker 1978; Girase and Deshmukh 2000). Generally, the crosses showing significant and desirable SCA effects were associated with high per se performance for respective traits but it is not always true. Hence, for appraising the superiority of a cross both *per se* performance and SCA effects should be considered. These findings are in agreement with previous workers (Khorgade et al., 2000; Pandey and Singh, 2002; Jahagirdar, 2003; Sekhar et al., 2004; Banu et al., 2006; Baskaran and Muthiah, 2007; Sarode et al., 2009; Kumar et al., 2009; Vaghela et al., 2009; Shoba and Balan, 2010; Gupta et al., 2011; Kumar et al., 2012). Heterotic grouping helps in sorting germplasm lacking which breeding programs might rely on crossing and testing parents in a more disorganized manner. Based on yield-specific combining ability data (SCA), the parental genotypes were classified into three heterotic groups (Table 3). Positive values for SCA between lines indicate that the lines are in different heterotic groups while negative SCA values indicate that the lines are in the same heterotic group. Generally, yield, heterosis and specific combining ability (SCA) are higher in inter-groups crosses than in intra-group crosses (Fig. 1). This is steady with hypothetical prospect that SCA and heterosis would be lower in crosses within a heterotic group than in crosses between groups. No information about heterotic groups in pigeonpea has been published to date.

Parental genetic diversity and its relationship with heterosis

The cluster analysis grouped all genotypes into five distinct non-overlapping clusters (Fig. 2) to reveal presence of substantial genetic diversity among the genotypes and indicated that this material may serve as good source for selecting the diverse parents for hybridization programme aimed at isolating desirable segregants for seed yield and other important characters. Earlier workers have also reported substantial genetic divergence in the pigeonpea (Katiyar et al., 2004; Gupta et. al., 2008; Sawant et al., 2009; Bhadru, 2011; Pratap et al., 2011). Genetically diverse parents are preferred for use in hybridization programme because crosses involving divergent parents have been found to provide greater possibility for obtaining desirable segregants in segregating generations. Cluster analysis (Table 4 & Fig 3) revealed that maximum intra-cluster distance was in cluster III (263.80) followed by cluster IV (253.62) and cluster I (244.81). The inter-cluster distances varied from 299.93 (between cluster I and II) to 727.79 (between cluster II and IV). The importance of genetic diversity for selecting parents for recombination breeding in crops including pigeonpea to recover transgressive segregants has also been repeatedly emphasized (Moll et al., 1962; Arunachalam, 1981; Sawant et al., 2009; Bhadru, 2011; Pratap et al., 2011). A wide range of variation in the estimates of heterosis over better parent (heterobeltiosis) and standard variety (standard heterosis) in both positive and negative direction was observed for seed yield per plant, indicating the existence of substantial heterosis in the hybrids (Table 5). The heterobeltiosis varied from -85.06 (NDACMS 1-4A x NDA 8-6) to 33.74% (NDACMS 1-4A x IPA 208) and standard heterosis varied from -82.57 (NDACMS 1-4A x NDA 8-6) to 26.28% (NDACMS 1-6A x NDA 98-6). For commercial exploitation of heterosis, yield advantage of 20-30% over best available standard variety is necessary to encourage farmers to take up hybrid cultivation. In our experiment, four combinations viz., NDACMS 1-6AxNDA 98-6, NDACMS 1-6AxNDA 5-14, NDACMS 1-4AxIPA 208, NDACMS 1-6AxICP 870 exhibited standard heterosis more than 20% increased seed yield. A perusal of Table 5 brings forth relationship of genetic diversity with heterosis. The cross combinations NDACMS1-4A x IPA 208, NDACMS1-6A x NDA 96-1, NDACMS1-6A x NDA 8-6, NDACMS1-6A x NDA 7-11 and NDACMS1-4A x AMAR resulted from crossing between parents belonging to high diversity group showed high positive significant heterosis for seed yield. However, the crosses viz., NDACMS1-4A X NDA 3-3, NDACMS1-3A X NDAGC 1010, NDACMS1-3A X NDA 8-6, NDACMS1-4A X NDA 98-6, NDACMS1-4A X NDA 7-11, NDACMS1-6A X NDAGC 31, NDACMS1-3A X ICP 2155, NDACMS1-3A X ICP 870, NDACMS1-4A X NDA 2 and NDACMS1-3A X NDA 96-6 gives sparingly high negative heterosis for seed yield although their parents belong to high diversity group. The reason for this could have been linkage of alleles for biomass and yield. Frequency of heterotic crosses and magnitude of heterosis for yield and its components were found to be higher in crosses between parents with intermediate divergence than the extreme ones (Arunachalam et al., 1983; Datta et al., 2004). In contrary with most of the previous results in the present investigation among the top fifteen heterotic hybrids for seed yield, ten crosses were resulted from crossing between parents belonging to low diversity group while five from high diversity group. Heterosis expressions could be related to morphological differentiation between the parental genotypes. These morphological traits are more likely controlled by a subset of loci, which could explain why random markers, some of which unrelated to these traits, fail to show an association between genetic distance and heterosis. Thus, heterosis probably also exists due to different allelic combinations at particular loci in each parent that when brought together in hybrid combination, complement each other resulting heterosis expressions (Bingham et al., 1994). However, it is also noticed that ICP 870 members of cluster IV in combination with the CMS of the same cluster produced highly significant heterosis due to highest intra-cluster distance. Singh et al. (1981) also reported that parents of the same cluster gave the highest heterosis suggesting the absence of relationship between genetic diversity and heterosis.

Materials and Methods

Plant material and experimental design

Three cytoplasmic male sterile lines (females) were crossed with twenty elite genotypes as testers (males) in a line x tester mating system(Table 1).The entire crosses were made during 2011-12 and enough hand pollinated seeds was produced. The resultant 60 F_1 hybrids along with their 23 parents and standard check variety NDA-2 were evaluated at Research Farm of Genetics and Plant Breeding, Narendra Deva University of Agriculture & Technology, Faizabad

Table 5. Extent of per cent heterosis over better parent (BPH) and standard varieties (SH) for seed yield in pigeonpea.

S. No.	Crosses	BPH	SH	Cluster information	Parental genetic distance	Parental diversity class
1	NDACMS1-3A X NDA 2	4.85	7.89**	Cluster II x Cluster I	299.93	Low
2	NDACMS1-3A X NDA 3	29.15**	4.98	Cluster II x Cluster III	535.38	Medium
3	NDACMS1-3A X NDA 3-3	-7.72*	-26.35**	Cluster II x Cluster II	149.7	Low
4	NDACMS1-3A X NDA 5-14	-84.63**	-81.24**	Cluster II x Cluster I	299.93	Low
5	NDACMS1-3A X NDA 8-6	-81.76**	-78.72**	Cluster II x Cluster V	689.3	High
6	NDACMS1-3A X NDA 96-1	-68.42**	-73.61**	Cluster II x Cluster III	535.38	Medium
7	NDACMS1-3A X NDA 96-6	-21.77**	-10.67**	Cluster II x Cluster V	689.3	High
8	NDACMS1-3A X NDA 98-6	31.12**	13.13**	Cluster II x Cluster II	149.7	Low
9	NDACMS1-3A X NDA 98-7	19.50**	-15.11**	Cluster II x Cluster III	535.38	Medium
10	NDACMS1-3A X NDA 7-11	-35.71**	-45.01**	Cluster II x Cluster I	299.93	Low
11	NDACMS1-3A X NDA 7-15	-24.63**	-46.46**	Cluster II x Cluster III	535.38	Medium
12	NDACMS1-3A X NDAGC 31	-80.15**	-79.15**	Cluster II x Cluster III	535.38	Medium
13	NDACMS1-3A X NDAGC 1010	-79.32**	-80.22**	Cluster II x Cluster V	689.3	High
14	NDACMS1-3A X ICP 2309	-5.05	-26.56**	Cluster II x Cluster III	535.38	Medium
15	NDACMS1-3A X ICP 2155	-20.69**	-12.31**	Cluster II x Cluster V	689.3	High
16	NDACMS1-3A X ICP 870	-14.25**	-39.09**	Cluster II x Cluster IV	727.79	High
17	NDACMS1-3A X ICP 7353	15.63**	-17.86**	Cluster II x Cluster III	535.38	Medium
18	NDACMS1-3A X IPA 208	-63.90**	-66.43**	Cluster II x Cluster I	299.3	Low
19	NDACMS1-3A X BAHAR	-76.57**	-81.12**	Cluster II x Cluster III	535.38	Medium
20	NDACMS1-3A X AMAR	-72.31**	-78.98**	Cluster II x Cluster II	149.7	Low
21	NDACMS1-4A X NDA 2	-19.96**	-17.64**	Cluster IV x Cluster I	690.94	High
22	NDACMS1-4A X NDA 3	-9.27**	-17.72**	Cluster IV x Cluster III	404.74	Low
23	NDACMS1-4A X NDA 3-3	-79.44**	-81.36**	Cluster IV x Cluster II	727.79	High
24	NDACMS1-4A X NDA 5-14	-83.72**	-80.13**	Cluster IV x Cluster I	690.94	High
25	NDACMS1-4A X NDA 8-6	-85.06**	-82.57**	Cluster IV x Cluster V	533	Medium
26	NDACMS1-4A X NDA 96-1	-59.13**	-62.93**	Cluster IV x Cluster III	404.74	Low
27	NDACMS1-4A X NDA 96-6	-21.70**	-10.59**	Cluster IV x Cluster V	533	Medium
28	NDACMS1-4A X NDA 98-6	-75.49**	-77.77**	Cluster IV x Cluster II	727.79	High
29	NDACMS1-4A X NDA 98-7	-17.72**	-25.38**	Cluster IV x Cluster III	404.74	Low
30	NDACMS1-4A X NDA 7-11	-20.16**	-27.60**	Cluster IV x Cluster I	690.94	High
31	NDACMS1-4A X NDA 7-15	-28.95**	-35.57**	Cluster IV x Cluster III	404.74	Low
32	NDACMS1-4A X NDAGC 31	3.45	8.63**	Cluster IV x Cluster III	404.74	Low
33	NDACMS1-4A X NDAGC 1010	-28.97**	-32.04**	Cluster IV x Cluster V	533	Medium
34	NDACMS1-4A X ICP 2309	26.15**	14.41**	Cluster IV x Cluster III	404.74	Low
35	NDACMS1-4A X ICP 2155	-11.91**	-2.6	Cluster IV x Cluster V	533	Medium
36	NDACMS1-4A X ICP 870	8.91**	-1.22	Cluster IV x Cluster IV	253.62	Low
37	NDACMS1-4A X ICP 7353	-0.35	-9.62**	Cluster IV x Cluster III	404.74	Low
38	NDACMS1-4A X IPA 208	33.74**	24.38**	Cluster IV x Cluster I	690.94	High
39	NDACMS1-4A X BAHAR	26.87**	15.06**	Cluster IV x Cluster III	404.74	Low
40	NDACMS1-4A X AMAR	21.66**	10.33**	Cluster IV x Cluster II	727.79	High
41	NDACMS1-6A X NDA 2	-6.71*	-0.58	Cluster IV x Cluster I	690.94	High
42	NDACMS1-6A X NDA 3	-8.26**	-2.23	Cluster IV x Cluster III	404.74	Low
43	NDACMS1-6A X NDA 3-3	-5.81*	0.39	Cluster IV x Cluster I	690.94	High
44	NDACMS1-6A X NDA 5-14	2.08	24.60**	Cluster IV x Cluster III	404.74	Low
45	NDACMS1-6A X NDA 8-6	1.02	17.89**	Cluster IV x Cluster II	727.79	High
46	NDACMS1-6A X NDA 96-1	11.28**	18.60**	Cluster IV x Cluster I	690.94	High
47	NDACMS1-6A X NDA 96-6	-17.09**	-5.33	Cluster IV x Cluster V	533	Medium
48	NDACMS1-6A X NDA 98-6	22.24**	26.28**	Cluster IV x Cluster III	404.74	Low
49	NDACMS1-6A X NDA 98-7	-13.37**	-7.67**	Cluster IV x Cluster V	533	Medium

50	NDACMS1-6A X NDA 7-11	4.68	11.56**	Cluster IV x Cluster II	727.79	High
51	NDACMS1-6A X NDA 7-15	-79.10**	-77.73**	Cluster IV x Cluster III	404.74	Low
52	NDACMS1-6A X NDAGC 31	-78.05**	-76.61**	Cluster IV x Cluster I	690.94	High
53	NDACMS1-6A X NDAGC 1010	5.98*	12.94**	Cluster IV x Cluster III	404.74	Low
54	NDACMS1-6A X ICP 2309	10.29**	17.54**	Cluster IV x Cluster III	404.74	Low
55	NDACMS1-6A X ICP 2155	-28.16**	-20.57**	Cluster IV x Cluster V	533	Medium
56	NDACMS1-6A X ICP 870	14.03**	21.52**	Cluster IV x Cluster III	404.74	Low
57	NDACMS1-6A X ICP 7353	-22.90**	-17.83**	Cluster IV x Cluster V	533	Medium
58	NDACMS1-6A X IPA 208	-27.13**	-22.35**	Cluster IV x Cluster IV	253.62	Low
59	NDACMS1-6A X BAHAR	-17.98**	-12.59**	Cluster IV x Cluster III	404.74	Low
60	NDACMS1-6A X AMAR	-13.20**	-7.49**	Cluster IV x Cluster I	690.94	High

Table 6. Top fifteen crosses showing significant positive heterosis for seed yield, their SCA effects and genetic distance.

S. No.	Crosses	SH	SCA	Genetic distance	Parental diversity class
1	NDACMS1-6A x NDA 98-6	26.28**	15.42**	404.74	Low
2	NDACMS1-6A x NDA 5-14	24.60**	34.63**	404.74	Low
3	NDACMS1-4A x IPA 208	24.38**	31.57**	690.94	High
4	NDACMS1-6A x ICP 870	21.52**	6.00**	404.74	Low
5	NDACMS1-6A x NDA 96-1	18.60**	26.34**	690.94	High
6	NDACMS1-6A x NDA 8-6	17.89**	31.59**	727.79	High
7	NDACMS1-6A x ICP 2309	17.54**	-2.13	404.74	Low
8	NDACMS1-4A x BAHAR	15.06**	28.48**	404.74	Low
9	NDACMS1-4A x ICP 2309	14.41**	9.14**	404.74	Low
10	NDACMS1-3A x NDA 98-6	13.13**	28.72**	149.7	Low
11	NDACMS1-6A x NDAGC 1010	12.94**	18.33**	404.74	Low
12	NDACMS1-6A x NDA 7-11	11.56**	8.79**	727.79	High
13	NDACMS1-4A x AMAR	10.33**	24.73**	727.79	High
14	NDACMS1-4A x NDAGC 31	8.63**	39.55**	404.74	Low
15	NDACMS1-3A x NDA 2	7.89**	19.78**	299.93	Low

during 2012-13. Geographically this experimental site is situated between 26.47 ^{0}N Latitude, 82.12 ^{0}E Longitude and at an altitude of 113 m above the mean sea level. Each genotype were grown in a randomized block design with three replications in separate plots of 4 m length of single row with intra- and inter-row spacing of 25 cm and 75 cm, respectively. Only one plant was maintained after thinning at each hill. To reduce competition between the crop and weeds for nutrient uptake, water absorption, and photosynthesis, two weeding were done at the early vegetative growth. The other recommended agronomic practices were done timely to raise good crop stand.

Traits measurement

The data collected on five randomly selected competitive plants in each genotype for eleven quantitative traits viz., days to 50% flowering, days to maturity, number of primary branches per plant, number of secondary branches per plant, plant height (cm), pods per plant, seeds per pod, 100-seed weight, seed yield per plant (g), biological yield per plant (g) and harvest index (%).

Divergent analysis

The genetic divergence was estimated by Mahalanobis' D^2 statistic as per Rao (1952) and cluster analysis as described by Anderberg (1993).The parents were classified into three categories namely; high, medium and low divergent groups based on Mahalanobis generalized distance (D^2). The mean (m) and standard deviation (sd) of the inter-cluster distances were calculated, 543.95 and 133.78 respectively. If the genetic distance between the parents was higher than 677.70 (m+sd), the cross were considered in the high divergent group. The parental combination was classified in the low divergent group if the genetic distance between the parents was lower than 410.19 (m-sd), while remaining crosses were in the medium divergent group.

Heterosis and heterotic grouping

The percent increase or decrease of F_1 hybrids over better parent as well as standard check variety was calculated to estimate possible heterotic effects (Fonseca and Patterson, 1968). The basic steps of heterotic grouping using yield-specific combining ability information are (i) find crosses with lower seed yield; if they have significant negative SCA, the two lines involved in the cross should be assigned to the same heterotic group; and then (ii) check the crosses with high yield and make sure no two lines with top high yield crosses are assigned to the same heterotic group (Fan et al., 2014). Specific combining ability was computed according to Kempthorne, (1957).

Conclusions

It could be concluded that in general, yield, heterosis and specific combining ability (SCA) are higher in inter-groups crosses than in intra-group crosses. When heterosis is compared on the basis of inter-cluster distances it is noticed that the chance of obtaining highly heterotic hybrid resulted from parental combination of low diversity and high diversity group. It may be possible that some of the estimates may be bias fed due to the inclusion of complex character such as yield. Besides, many of the genetic traits are highly influenced by environment and genotype x environment as well. Therefore, precision of the genetic distance can be obtained by estimating genetic distance through molecular markers.

Acknowledgements

The authors gratefully acknowledge the suggestions received from Dr. K. Kumar, Associate Professor, NDUA&T, Faizabad and Dr. Dibendu Datta, Principal Scientist, Indian Institute of Pulses Research to improve this manuscript.

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