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Optimization of Genetic divergence for the occurrence of Heterosis in Blackgram (*Vigna Mungo* L.)

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Abstract

A study was conducted with aim of relating genetic divergence among the parents with frequency and magnitude of heterosis in the F_1 generation. The divergence among the parents were classified into four classes *viz.,* DC1, DC2, DC3 and DC4. Heterosis was computed as per cent improvement over the better parent for ten important components of the yield. Genetic divergence was measured by D^2 statistic. If mean (m), standard deviation (s) of divergence values among them parents. It was postulated that two parents whose genetic divergence D^2 values lies between M-S and M+S, (DC2 or DC3) were heterotic than the parental divergence values exceeds the limits (M-S, M+S).

Key words : Genetic distance, Parental divergence, Heterosis. Divergent class.

Blackgram is an important pulse crop. It is cultivated as solo as well as an alley crop. It is a good protein supplement for the vegetarians. There is a great demand for blackgram dhal. At present the production is not sufficient to meet out the requirements. The production of blackgram is 22 lakh tonnes in India (GoI, 2023). Majority of the south Indians consumes blackgram in the form of idly and vada as their main breakfast as well as snacks. The recommended level of pulse crop for human being is pulses required in daily diet of 55 grams per head/day *i.e.,* 20 kg/ annum/ person. But the available quantity of pulses is 44.93g per head/day *i.e.,* 16kg/annum/ person[2]. There is a gap in the demand and supply chain. It is imperative to augment the production through increased productivity that is inherited.

Genetic diversity places a vital role in the choice of parents for hybridization programme. Genetic divergent parents are likely to give heterotic hybrids and throw some useful superior segregants in the later segregating generations[15]. However, practically all the crosses involving divergent parents are not yielding heterotic crosses. There may be optimum level of diversity for the observation of hybrid vigor. There is a limit to genetic divergence for the occurrence of heterosis, according to Arunachalam and Bandyopadhyay[3]. Boraiah *et al.*[4] have amply registered the importance of parental genetic divergence in the realization of heterotic hybrids. The present study unveils the optimum level of genetic divergence for

the occurrence of heterotic hybrids by evaluating 96 genotypes and their 24 hybrids evolved by crossing eight lines and three testers selected based on genetic divergence.

Ninety-six genotypes of blackgram (Table-1) were obtained from different sources (New Delhi, Vamban and Kanpur). The experiment was conducted at Plant Breeding Farm, Department of Genetics and Plant Breeding, Faculty of Agriculture, Annamalai University during June-August, 2021. The selfed seeds of the ninety-six genotypes were raised in Randomized Block Design (RBD) with three replications. Each genotype was grown in a single row of 3m length with a spacing of 30×10 cm. Five randomly selected competing plants were observed for ten quantitative traits *viz.,* days to fifty percent flowering, plant height, number of branches per plant, number of clusters per plant, number of pods per cluster, number of pods per plant, pod length, number of seeds per pod, hundred seed weight and seed yield per plant. The data were subjected to D^2 analysis as per the procedure outlined by Mahalanobis[13] and Rao[17].

Eight lines and three testers which were selected based on genetic diversity (Table-2) were crossed in line × tester mating design to evolve 24 hybrids. The crossing program was completed during January-March 2022. The twenty-four hybrids thus evolved by crossing 8 × 3 combinations were sown during June-August 2022. The parents as well as the hybrids were selfed. The hybrid seeds along with the seeds of the parents were sown in a single row of 3 m length, with a spacing of 30 × 10 cm in Randomized Block Design (RBD) with three replications. Five randomly selected competing plants were observed for the aforementioned. The data were subjected to line × tester analysis as suggested by Kempthrone[10]. Recommended agronomic practices as need based plant production measures were judiciously followed.

The divergence classification of the genotypes was conducted by utilizing the mean and standard deviation of all D^2 values, as provided by Arunachalam and Bandyopadhyay[3]. The technique was devised to categorize the parental divergence into four divergence classes (DC1, DC2, DC3 and DC4). After calculating the mean (m) and standard deviation (s) of the D^2 values, the following divergence classes were created.

DC1: $D^2 \geq (m+s)$,

DC 2: $m \geq D^2 \geq (m+s)$,

DC3: $(m-s) \geq D^2 < m$,

DC4: $D^2 > (m-s)$.

For each cross, the divergence class to which the D^2 value between their parents belonged was established. The total number of crosses (n), the proportion of crosses exhibiting positive heterosis values (p), and the mean (x) for each character across all of these crosses in the divergence class were calculated. Since, even extremely low positive heterosis values would be included in this approach, it was decided to establish a standard norm for heterosis and find frequencies of crosses that showed heterosis at or above the norm. The mean heterosis value of these crosses with a positive heterosis value for that character was chosen as the norm (k). Furthermore, the mean (y) for each character

Table-1. List of genotypes selected for D^2 analysis

S.No.	Genotypes	Source	S. No	Genotypes	Source
1	ADT3	NPRC, Vamban	49	IC-281995	NBPGR, New Delhi
2	ADT5	NPRC, Vamban	50	IC-470241	NBPGR, New Delhi
3	APK1	NPRC, Vamban	51	IC-519619	NBPGR, New Delhi
4	BGP247	NPRC, Vamban	52	IC-281981	NBPGR, New Delhi
5	CB-P.131	NPRC, Vamban	53	IC-519768	NBPGR, New Delhi
6	CB-P.133/18	NPRC, Vamban	54	IC-519685	NBPGR, New Delhi
7	CB-P.30/1	NPRC, Vamban	55	IC-519678	NBPGR, New Delhi
8	CO5	NPRC, Vamban	56	IC-519801	NBPGR, New Delhi
9	CO6	NPRC, Vamban	57	IPU99-12	IIPR, Kanpur
10	DDU-8	NBPGR, New Delhi	58	IPU99-232	IIPR, Kanpur
11	IC-261171	NBPGR, New Delhi	59	IPU99-43	IIPR, Kanpur
12	IC-261172	NBPGR, New Delhi	60	IPU99-6	IIPR, Kanpur
13	IC-261181	NBPGR, New Delhi	61	LBG17	IIPR, Kanpur
14	IC-261182	NBPGR, New Delhi	62	LBG623	IIPR, Kanpur
15	IC-281975	NBPGR, New Delhi	63	LBG648	IIPR, Kanpur
16	IC-519620	NBPGR, New Delhi	64	LBG752	IIPR, Kanpur
17	IC-281986	NBPGR, New Delhi	65	LBG787	IIPR, Kanpur
18	IC-281987	NBPGR, New Delhi	66	MDU1	NPRC, Vamban
19	IC-281989	NBPGR, New Delhi	67	NANDI	NRI Agritech Pvt. Ltd
20	PKGU 1	NBPGR, New Delhi	68	ADT6	NPRC, Vamban
21	IC-281991	NBPGR, New Delhi	69	NPU-180	NBPGR, New Delhi
22	IC-281993	NBPGR, New Delhi	70	NUL7	NBPGR, New Delhi
23	IC-281994	NBPGR, New Delhi	71	IC-281990	NBPGR, New Delhi
24	IC-436946	NBPGR, New Delhi	72	PLU703	NBPGR, New Delhi
25	IC-281996	NBPGR, New Delhi	73	PU31	NBPGR, New Delhi
26	IC-281998	NBPGR, New Delhi	74	SRI	NRI Agritech Pvt. Ltd
27	IC-282000	NBPGR, New Delhi	75	T9	NBPGR, New Delhi
28	IC-282002	NBPGR, New Delhi	76	TBG-104	NBPGR, New Delhi
29	VBN7	NPRC, Vamban	77	TMV1	NBPGR, New Delhi
30	IC-282004	NBPGR, New Delhi	78	TU-68	NBPGR, New Delhi
31	IC-282009	NBPGR, New Delhi	79	TU94-2	NBPGR, New Delhi
32	IC-398989	NBPGR, New Delhi	80	VBG-10.010	NPRC, Vamban
33	IC-413309	NBPGR, New Delhi	81	VBG-11.027	NPRC, Vamban
34	IC-426769	NBPGR, New Delhi	82	VBG-13.017	NPRC, Vamban
35	VBN 10	NPRC, Vamban	83	VBN1	NPRC, Vamban
36	IC-436612	NBPGR, New Delhi	84	VBN2	NPRC, Vamban

37	IC-436610	NBPGR, New Delhi	85	VBN3	NPRC, Vamban
38	IC-436678	NBPGR, New Delhi	86	VBN4	NPRC, Vamban
39	IC-436715	NBPGR, New Delhi	87	VBN5	NPRC, Vamban
40	IC-436717	NBPGR, New Delhi	88	VBN6	NPRC, Vamban
41	IC-436720	NBPGR, New Delhi	89	IC- 282003	NBPGR, New Delhi
42	IC-436736	NBPGR, New Delhi	90	IC-436750	NBPGR, New Delhi
43	IC-436747	NBPGR, New Delhi	91	VBN11	NPRC, Vamban
44	VBN 9	NPRC, Vamban	92	IC 436647	NPRC, Vamban
45	IC-436753	NBPGR, New Delhi	93	VBN8	NPRC, Vamban
46	IC-436765	NBPGR, New Delhi	94	NIRMAL	NPRC, Vamban
47	IC-436882	NBPGR, New Delhi	95	Paiyur 1	KVK, Paiyur
48	IC-436922	NBPGR, New Delhi	96	U23	NBPGR, New Delhi

Table-2. Parents selected for line into tester analysis

Parent Codes	Name of genotypes	Cluster	Source
L_1	IC- 282003	IV	NBPGR, New Delhi
L_2	IC-436750	IX	NBPGR, New Delhi
L_3	IC -519619	II	NBPGR, New Delhi
L_4	IC-281990	V	NBPGR, New Delhi
L_5	IC-281995	III	NBPGR, New Delhi
L_6	IC-436647	X	NBPGR, New Delhi
L_7	IC-281981	VI	NBPGR, New Delhi
L_8	IC-519678	VII	NBPGR, New Delhi
T_1	ADT 6	VIII	NPRC, Vamban
T_2	VBN8	XI	NPRC, Vamban
T_3	VBN 10	I	NPRC, Vamban

across such crosses and the proportion of crosses (q) with heterosis values larger than or equal to k were calculated. Additionally, the maximum heterosis value (t) observed in each divergence class for each trait was recorded.

The relative significance of the divergence classes was assessed by taking into account the values of p, x, q, and y. In order to establish a consensus on the ranking, a scoring system was implemented, which encompassed the aforementioned variables. A score of 1 was assigned to the divergent class with the highest p value, while the subsequent class received a score of 2, and so on. Whenever there was a tie, the classes involved in the tie received the same score. The scores over p and x were added across the ten characters to obtain a final score for each divergence class. Similar procedure was adopted for q and y. According

Table-3. Analysis of variance for 96 blackgram genotypes for various characters

Source	df	MSS									
		DFF (Days)	PH (cm)	NBP	NCP	NPC	NPP	PL (cm)	NSP	HSW (g)	SYP (g)
Replication	2	0.8438	2.1901	0.3035	0.2926	0.0976	4.1935	0.0815	0.1677	0.0174	0.0667
Treatment	95	30.5891**	404.5662**	0.9482**	8.8450**	1.6678**	136.1768**	0.5390**	2.0110**	2.3176**	25.5792**
Error	190	0.7069	0.8217	0.1106	0.1228	0.1489	1.6286	0.1026	0.1616	0.0080	0.0408

**Significant at 1 per cent level

DFF : Days to fifty percent flowering (days)
PH : Plant height (cm)
NBP : Number of branches per plant
NCP : Number of cluster per plant
NPC : Number of pods per cluster
NPP : Number of pods per plant
PL : Pod length (cm)
NSP : Number of seeds per pod
HSW : Hundred seed weight (g)
SYP : Seed yield per plant (g)

Table-4. Divergence classification of blackgram genotypes based on mean and standard deviation of D^2 values

Divergence class	Range of D^2 values	No. of crosses	L×T pairs
DC 1	≥ 2171.567	7	L_1T_1, L_3T_1, L_4T_1, L_5T_1, L_7T_2, L_7T_3, L_8T_2.
DC 2	$1210.21 \geq D^2 \geq 2171.567$	6	L_1T_2, L_1T_3, L_2T_1, L_3T_3, L_8T_1, L_8T_3.
DC 3	$248.853 \geq D^2 < 1210.21$	10	L_2T_2, L_3T_2, L_4T_2, L_4T_3, L_5T_2, L_5T_3, L_6T_1, L_6T_3, L_7T_1
DC 4	> 248.853	1	L_2T_3

Table-5. Proportion of crosses with positive heterosis and their average magnitude for four divergent classes in blackgram

Divergent class / Characters		DC 1	DC 2	DC 3	DC 4
	(n)	**7**	**6**	**10**	**1**
DFF	(p)	4	5	7	1
	(x)	4.32	8.80	9.02	1
	(t)	-5.17	-6.96	-4.13	7.76
PH	(p)	5	5	6	0
	(x)	20.96	16.99	26.88	0
	(t)	-47.73	-20.83	-46.19	0
NBP	(p)	2	2	5	1
	(x)	20.60	10.32	29.44	22.92
	(t)	22.86	12.07	53.23	22.92
NCP	(p)	1	2	4	1
	(x)	31.85	31.85	29.71	15.29
	(t)	31.85	46.43	40.13	15.29
NPC	(p)	0	2	5	1
	(x)	0	17.60	19.73	7.59
	(t)	0	26.39	25.00	7.59
NPP	(p)	2	5	6	1
	(x)	29.07	31.30	47.73	30.48
	(t)	44.62	60.90	62.39	30.48
PL	(p)	1	0	3	1
	(x)	11.63	0	18.54	18.50
	(t)	11.62	0	27.50	18.50
NSP	(p)	0	0	0	0
	(x)	0	0	0	0
	(t)	0	0	0	0
HSW	(p)	0	3	1	1
	(x)	0	21.84	13.74	45.05
	(t)	0	28.02	13.74	45.05
SYP	(p)	0	1	2	0
	(x)	0	19.71	25.34	0
	(t)	0	19.71	39.08	0
Score	52	40	22	52	

Table 6. Proportion of crosses showing more than overall average heterosis and average magnitude given by those crosses of 10 characters in 4 divergence classes in blackgram

Divergent class / Characters		DC 1	DC 2	DC 3	DC 4
	(n)	**7**	**6**	**10**	**1**
DFF	(q)	2	3	5	0
	(y)	3.38	7.36	6.70	0
PH	(q)	4	3	2	0
	(y)	14.27	18.52	45.36	0
NBP	(q)	1	1	2	1
	(y)	22.86	12.07	45.16	22.92
NCP	(q)	1	1	3	1
	(y)	31.85	41.43	36.23	15.29
NPC	(q)	0	1	3	1
	(y)	0	17.60	24.07	1
NPP	(q)	1	2	4	1
	(y)	44.62	47.82	57.20	30.48
PL	(q)	1	0	2	1
	(y)	11.63	0	22.06	18.50
NSP	(q)	0	0	0	0
	(y)	0	0	0	0
HSW	(q)	0	1	1	1
	(y)	0	28.02	13.74	45.05
SYP	(q)	0	1	1	0
	(y)	0	19.71	39.08	0
Score		49	37	24	49

to Arunachalam and Bandyopadhyay[3], the divergence class with the lowest overall score would have the highest average heterosis magnitude and the highest frequency of heterotic crosses.

The Anova for ten traits for ninety-six genotypes is presented in Table-3. It indicated the presence of high genetic differences among the genotypes utilized in the presented study. Hence, further analysis is appropriate.

The divergence classes were formed based on the mean and standard deviation of the D^2 values of parents (Table-3). Four intervals were defined to represent the four divergence classes, which were constituted using the mean of D^2 values (1210.21) and the standard deviation (961.36).

Heterosis holds significant implications for the development of hybrids in self-pollinated crops as well. Due to the impracticality of evaluating large F_2 populations resulting from

each cross studied in F_1 for subsequent breeding purposes, breeders are often limited to selecting a small number of crosses in F_1. In this regard, heterosis can serve as a crucial parameter for selection studies. For instance, Pungle[16] demonstrated that heterotic F_1 hybrids yield a greater proportion of productive progenies in F_5 and subsequent generations compared to non-heterotic F_1 hybrids.

According to East and Hayes[7], crosses between dissimilar parents typically result in more substantial heterosis than those between parents who are closely related. In practical scenarios, heterosis arises due to the divergence between parents. However, it has been observed that heterosis does not always occur when divergent parents are crossed (Crees[5]). Hence, it becomes imperative to investigate the potential boundaries of parental divergence within which there exist reasonably high probabilities for the manifestation of heterosis[3].

The present study is an attempt in this regard using the experimental results (Tables 4 and 5). The experimental evidence provided in this study has suggested a definite relationship between parental divergence and F_1 heterosis.

Arunachalam and Bandyopadhyay[3] used the percentage of heterotic crosses p (proportion of crosses showing positive values of heterosis) and q (the portion of crosses showing a heterosis value greater than or equal to k - the mean heterosis value of these crosses with positive value of heterosis for that character) as parameters in their decision-making process to account for unequal numbers of crosses and heterotic ones falling in different divergence classes. In addition, both the general level of heterosis given by average heterosis value of those crosses showing positive heterosis) and a selected level (over a norm defined by overall mean heterosis) provided by the values x and y were considered in conjunction (Arunachalam and Bandyopadhyay[3]) while the former would take into account the magnitude of F_1 improvement over mid parent value, however slight it might be, the latter would give weightage to those F_1's showing substantial improvement whose F_2's a breeder would like to search on priority for desirable transgressive segregants[3,17].

The divergence class DC3 showed the lowest total score closely followed by DC1. According to Arunachalam and Bandyopadhyay[3] DC3 will be the most desirable one with high frequency of heterotic crosses and high average magnitude of heterosis. Even though DC2 also showed lower score next to DC3, it consisted only a very few number of crosses and the average heterosis was less than DC3 or DC2. Next to DC3, the divergence class DC2 showed higher number of heterotic crosses and high average magnitude of heterosis. Thus, the present results have brought out the definite superiority of DC3 and DC2 over DC1 as far as occurrence of high preparation of heterotic crosses or of high value of heterosis was concerned (Table-5 and 6). This argument is consistent with findings of Dharawad *et al.*[6] in brinjal, Laxuman *et al.*[12], Krishnamoorthy *et al.*[11] in chilli, Rao *et al.*[18] in sunflower, Keerthi *et al.*[9] in dolichos beans, Anilkumar[1] in maize, Boraiah *et al.*[4] in blackgram. The

concept that parental divergence has limitations for the ideal manifestation of heterosis was similarly established by previous investigations on the interbreeding of divergent geographical populations in maize (Moll *et al*[14], Thirugnanakumar[19] in sesame.

The present study provides sufficient ground for conceiving those limits and for the hypothesis in general that _ if 'm' and 's' are the mean and standard deviation of the values of the divergence (given by D^2) among parents, the chances for the existence of a higher frequency of heterotic crosses and with high values of heterosis are more when the parents are chosen to have their divergence in the interval (m-s, m+s) compared to the crosses between parents whose divergence fails outside that interval. This is in agreement with Arunachalam and Bandyopadhyay[3], Anilkumar[1] in maize, Boraiah *et al*.[4] in blackgram.

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