Association Of Heterosis With Combining Ability And Genetic Divergence In Sesame (Sesamum Indicum L.)

Arna Das, Sarita Pandey, Tapash Dasgupta

Abstract: Parental diversity is considered desirable to exploit heterosis in any breeding program. The present investigation was undertaken to search out whether any relationship existed between heterosis of cross combinations with phenetic divergence, combining ability and genetic divergence of parents in sesame. Seven sesame genotypes and their 21 cross combinations developed through half diallel mating were assessed for morphological markers, microsatellite markers and seed storage protein polymorphism to estimate different parameters. The clustering patterns of parents differed for morphological, protein and simple sequence repeats (SSRs), though some concordance was observed between phenetic and genetic divergence of parents. Heterosis, both mid-parent heterosis% (MPH%) and better parent heterosis% (BPH%), was positively and significantly correlated with specific combining ability and hybrids per se, but no specific trend transpired between morphological, protein and SSR marker data. However, SSR based genetic diversity (GD) value of above 0.5 between parents, predicted heterotic crosses more reliably.

Index Terms: Sesamum, correlation, divergence, heterosis, microsatellite markers, seed storage protein polymorphism

1 INTRODUCTION

Sesame, an underutilized oilseed crop, is gaining popularity due to its multiple health benefits. The benefits, most notably include, reducing effect on blood plasma cholesterol and blood pressure [1], curing skin infections and restricting growth of cancerous cells [2], [3]. The huge deficit of oilseed production in India can be overcome by vertical expansion of sesame. Despite occupying highest area under cultivation in sesame, India (432.02 Kg/ha) lags behind Myanmar (543.92 Kg/ha) and China (1259.1 Kg/ha) on productivity aspect [4]. To reap the benefit of increasing world market demand of sesame and to reduce the deficit in oilseed production in India, sesame productivity needs to be augmented significantly. Commercial exploitation of heterosis is a fast and simple traditional breeding approach to achieve higher yield in any crop. The shining examples are maize and rice. But hybrid development is an expensive and laborious on-field process. Sesame has one advantage which favors the exercise of heterosis i.e. its high outcrossing rate [5], [6]. Other factor, like male sterility line development, is yet to be sorted out. But how heterosis can be predicted from parental performance dwindling myriad crossing program between genotypes needs to be investigated on.

- Arna Das is currently working as Research Associate in Transfer of Technology Division, National Institute of Research on Jute & Allied Fibre Technology, Indian Council of Agricultural Research, Kolkata, India, PH-0091 9433903523. E-mail: <u>arna das@hotmail.com</u>
- Sarita Pandey is currently pursuing PhD in Genetics & Plant Breeding, Institute of Agricultural Science, University of Calcutta, India, PH-0091 9433244413. E-mail: <u>isarita06@mail.com</u>
- Tapsh Dasgupta is currently the Director, Institute of Agricultural Science, University of Calcutta, India, PH-0091 9748699912.
- E-mail: <u>tapashdg@rediffmail.com</u>

In other words, a large number of parents can be assessed for heterosis without actual crossing if any predictive means exists for performance of future crosses. Phenetic or phenotypic divergence relies on the combined effect of genotype and environment and so is obviously environmental sensitive. On the contrary, divergence, when is assessed through molecular markers offers a precise and reproducible idea on actual genetic divergence of a population. In some cases, like in rice [7] and in maize, [8], [9] correlations were observed between molecular genetic divergence and heterosis; but weak or insignificant relationship between these two parameters was the general trend of outcome in crops, like brassica [10] sunflower [11] and sweet corn [12]. Reports on association of genetic diversity of parents with heterotic cross are still not available in sesame. The aim of the present study, was to examine, the relationship between parental divergence with heterosis, for yield and yield related traits in 21 cross combinations, through assessment of combining abilities, phenetic divergence and genetic divergence of seven parents. In genetic divergence study, two molecular methods, namely, soluble seed storage protein and SSR markers have been employed.

2 MATERIALS AND METHODS

2.1 Field Experimentation

Seven popular sesame varieties in the state of West Bengal, India (Table 1) were used as parents in a half diallel crossing program. Parents and their 21 F_1 s were planted in complete randomized block design with three replications in summer, 2011 at the Calcutta University Experimental Farm, Baruipur, in the district of South 24-Paraganas, (Latitude 22°22', Longitude 88°26') West Bengal, India. The soil texture was sandy loam type with pH 7.20. The area was characterized by an average rainfall of 16.2 mm and an average temperature of 36°C during February, 2011 to May, 2011. Each F_1 entry was grown in between the rows of corresponding parental lines. The spacing was maintained at 45 cm between rows and 10 cm between plants. Recommended cultural practices were followed.

Serial No.	Variety/ Advanced Breeding Lines	Place of Origin in India
1	Gujarat Til -2 (GT-2)	Gujarat
2	Tilottama	West Bengal (Selection from local germplasm)
3	B-14	West Bengal (Selection from local germplasm)
4	CUMS 3	Mutant Of Rama (Developed at Calcutta University)
5	CUMS 9	Mutant Of SI 1666 (Developed at Calcutta University)
6	CUMS 11	Mutant Of SI 1666 (Developed at Calcutta University)
7	CUMS17	Mutant Of IC 21706 (Developed at Calcutta University

TABLE 1 DETAILS OF SEVEN PARENTS

2.2 Recording of Morphological Data

The observations of nine morphological quantitative characters (traits), namely, plant height (cm), days to 50% days to maturity, number of primary flowering, branches/plant, capsule length (cm), number of capsules/plant, number of seeds/capsule, 1000 seed weight (gm) and seed yield/plant (gm) were recorded for both parents and F₁s, in ten randomly selected plants per replication. The data were then averaged.

2.3 Molecular Marker Assay

2.3.1 Seed Storage Protein Marker Assay

Soluble seed storage protein was first extracted from seed samples following modified Lowry's method [13]. Sodium Dodoecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) (12% separating gel and 4% percent stacking gel) was then carried out following modified method of Laemmli [14] in a regular mini (10 cm × 10 cm) vertical gel system (Biotech Laboratories, India). Standard marker protein namely, Dalton Mark VI (Sigma, USA) was used for estimation of molecular weights of sample protein bands through Gel Documentation Unit (UVP, USA).

2.3.2 DNA Marker Assay Primers

Microsatellite or SSR markers were used in the study and it included both genomic and expressed sequence tagged -SSR markers (EST-SSR). DNA was extracted from leaves of 10-12 day old seedlings following modified CTAB (Hexadecyl trimethyl ammonium bromide) method [15]. 10 microsatellite primers (Table 5), as described by Dixit et al. [16] were attempted for PCR amplification. Furthermore, 30 EST-SSR primers were developed utilizing database of NCBI [17]. PCR amplification was carried out in 25 µl reaction mixture, in 0.1 ml PCR tubes, consisting of 50 ng sesame DNA, 10x PCR buffer, 50 mM MgCl₂, 2 mM dNTPs, 0.1 units Taq DNA polymerase (5 unit/ µl), 50 mM of each primer pair in Eppendorf gradient PCR (Eppendorf Pro, AG 6321). The program in the thermocycler was carried out as follows: preheating for 5 min at 95 °C, 30 cycles of 30 seconds denaturation at 95 °C, 30 seconds at the annealing temperature of particular primer pairs, 30 seconds at 72 °C (extension), Final extension at 72 °C for 5 minutes and hold at 4 °C. The PCR products were separated in a 2% Agarose Gel (Sigma USA). Ethidium bromide (10 mg/ml) (Biorad, USA), 6x Loading dye and 50 base pairs (bp) DNA ladder (Fermentas Life Sciences, Gene Ruler[™]) were used.

2.4 Statistical Analyses

2.4.1 Morphological Data Analyses

Analysis of variance (ANOVA) and combining ability analyses following Griffing's model I, method 2 [18] were carried out with modified DIALLEL-SAS method [19]. Average general combining ability (GCA AVG) of each hybrid combination was estimated from GCA estimates of respective parental pairs for each character. BPH% and MPH% was calculated using Microsoft Excel Ver.7.0 [20].

2.4.2 Analyses of Molecular Data

The protein fragments and DNA fragments were given a score of '1' or '0' as presence or absence for all genotypes. The grouping of molecular data based on their genetic similarity (GS) was carried out through the software NTSYS pc ver 2.20 [21]. At first, the values were standardized using the program STAND. The morphological dissimilarity matrix was computed through program SIMINT, whereas, molecular similarity data were generated by the program, SIMQUAL. Lastly, SAHN program was used for generating data for tree. Finally tree plot was used for creating dendrogram. GD was estimated as 1 - GS. Simple correlation coefficients among different parameters were calculated using Microsoft Excel Ver.7.0 (Microsoft. Inc., Redmond, WA). Mantel Test [22] for correlation matrices obtained from morphological, protein and SSR data was also carried out through NTSYS pc Ver 2.20. Morphological, protein and DNA diversity estimates were denoted as genetic diversity for morphology (GDM), genetic diversity for protein (GDP) and genetic diversity for SSR (GDS), respectively.

3 RESULTS

3.1 Evaluation of Hybrids and Parents in Field Trial

It is evident from ANOVA (Table 2) that all the parents and 21 F_1 hybrids differed significantly (p = 0.01) from each other in field trial for the nine recorded traits. Hence a wide range of hybrids in different trait combination offered a unique opportunity for selection.

TABLE 2MEAN SUM OF SQUARES FOR ANALYSIS OFVARIANCE

d.f.	PH ⁵ (cm)	PrBr	DF	DM	CL (cm)	CP	SC	SW (gm)	SY (gm)
2	2	16.447	0.102	0.143	0.036	0.005	1.816	7.937	0.005
27	27	453.246**	1.803**	9.479**	5.517**	0.106**	152.045**	73.489**	0.185**

**Significant at 1% level of significance at error d.f.

^a d.f.= Degree of freedom

^b PH=Plant height; PrBr =Number of primary branches/plant; DF=Days to 50% flowering

DM=Days to maturity; CL= Capsule Length; CP= number of capsules/plant; SC=Number of seeds/capsule

SW=1000 seed weight; SY= Seed yield/plant

The estimates of MPH% and BPH% revealed (Table 3) that both the heterosis% dispersed significantly over negative to positive values for all the nine traits in 21 F_1 hybrids. It was observed that primary branches/plant exhibited highest, as well as lowest estimates of MPH% and BPH% over all traits. Seed yield and main yield component, namely, capsules/plant, exhibited positive mean BPH% averaged over all crosses. On the contrary, all characters exhibited positive average value for MPH%, except plant height, days to 50% flowering and 1000 seed weight.

TABLE 3 DETAILS OF MID-PARENT AND BETTER PARENT HETEROSIS

	Mid-Pa	Mid-Parent Heterosis (%) Better Parent He							
		MPH%		BPH%					
Characters	Mnimum	Maximum	Mean	Mnimum	Maximum	Mean			
Plant Height (cm)	-23.13**	18.81**	-2.78	-30.75 ⁸⁸	12.17**	-9.29			
Number of Primary Branches/Plant	-73.58**	858.33**	40.16	-86.79 ^{××}	79.16**	-8.46			
Days to 30% Flowering	-5.49**	5.01**	-0.89	43.71**	5.63 ^{xx}	4.04			
Days To Maturity	-3.17**	4.00**	0.65	-3.36**	1.88 **	-2.76			
Capsule Length (cm)	-14.03**	10.73**	0.08	-23.67**	7.58**	-9.43			
Number of Capsules/ Plant	41.25**	48.43**	18.39	-23.51**	36.62**	6.36			
Number of Seeds/ Capsule	-20.61**	17.25**	0.82	-23.38**	12.18**	-11.20			
1000 Seed Weight (gm)	-22.29 ⁸⁸	20.80**	-0.79	-27.87 ⁸⁸	19.20 ^{××}	4.34			
Seed Yield/Plant (gm)	-18.42 ⁸⁸	69.44**	23.96	-20.26 ⁸⁸	46.06 ^{××}	12.90			

** t value significant at 1% level of significance

3.2 Diversity Assessment

Parental diversity based on nine morphological characters (Fig.1) revealed that GT-2, CUMS 3 and B-14 were significantly divergent than rest of six parents. Highest GDM (1.926) was observed between GT-2 and CUMS 3, while, a lowest GDM value of 0.871 was noticed between the genotype CUMS 9 and CUMS 11 (Table 4). GDM gave widest divergence range, as well as highest average among the 3 types of GD estimates. But only 38% of the 21 cross combinations surpassed average GDM value (1.377) (Table 4).

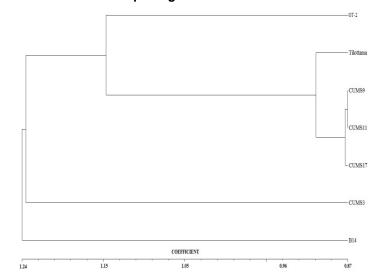


Fig.1. Dendrogram of seven parents based on

morphological characters

TABLE 4 GENETIC DISTANCE VALUES BASED ON MORPHOLOGICAL, PROTEIN AND DNA MARKERS

Parents	GDEstimates	GT-2	Tilottama	8-14	CUMS3	CUMS9	CUMS11	CUMS 17
GT-2	GDM	0.00	1.143	1.749	1.926	1.292	1.764	1.852
	GDP	0.00	0.333	0.222	0.444	0.222	0.556	0.333
	GDS	0.00						
Tilottama	GDM	0.00 1.885 1.527 0.907 1.667 1.343 0.00 0.333 0.356 0.356 0.222 0.667 0.462 0.00 0 0 0.00 0.222 0.667 0.462 0.00 1.238 1.372 1.305 1.286 0.00 0.444 0.444 0.556 0.333 0.769 0.308 0.00 0 0.00 1.234 0.00 1.234 1.384 1.274 0.333	1.343					
	GDP		0.00	0.333	0.556	0.556	0.222	0.667
	GDS	0.462	0.00					
8-14	GDM			0.00	1.238	1.372	1.305	1.286
	GDP			0.00	0.444	0.444	0.556	0.333
	GDS	0.769	0.308	0.00				
CU MS 3	GDM				0.00	1.234	1.384	1.274
	GDP				0.00	0.444	0.778	0.333
	GDS	0.308	0.154	0.462	0.00			
CU MS 9	GDM					0.00	0.871	0.874
	GDP					0.00	0.778	0.333
	GDS	0.692	0.385	0.385	0.538	0.00		
CUMS 11	GDM						0.00	1.030
	GDP						0.00	0.667
	GDS	0.538	0.231	0.231	0.385	0.154	0.00	
CUMS 17	GDM							0.00
	GDP							0.00
	GDS	0.462	0.308	0.615	0.308	0.385	0.385	0.00

Upper Two Diagonal: GDM = Genetic Divergence for Morphological Marker

GDP= Genetic Divergence for Protein Marker

Lower Diagonal: GDS = Genetic Divergence for SSR Marker; GDM Avg = 1.377 GDP Avg = 0.455 GDS Avg = 0.403

Dendrogram of seven parents based on morphological traits clearly revealed (Fig.1) presence of two clusters. The

smaller one had only two genotypes, namely, CUMS 3 and B-14. The other cluster comprised of remaining five. Among which, the parents CUMS 9 and CUMS 11 were very close. These two genotypes, together with Tilottama and CUMS 17, clearly, were close enough to form a sub-cluster separating out GT-2 alone. SDS-PAGE exhibited a total of 22 protein bands (Fig.2) in the seven parental genotypes, of which 13 bands were polymorphic. Average number of bands per parental genotype was found to be 16.43. The molecular weights of polymorphic bands ranged from 14 KD to 126 KD. GDP revealed an average of 0.455 (Table 4). More than 38% of the 21 hybrid combinations had genetic distance more than average (0.455). Lowest GDP (0.222) was observed between the parents GT-2 and B-14, GT-2 and CUMS 9 and also between Tilottama and CUMS 11. On the contrary, highest GDP (0.778) was noticed between the parents CUMS 3 and CUMS 11 and between CUMS 9 and CUMS 11 (Table 4).

MARKER CUMS 3 CUMS 9 CUMS 11 CUMS 17 GT-2 TILOTTAMA B-14 MARKER

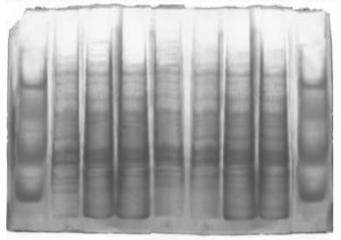
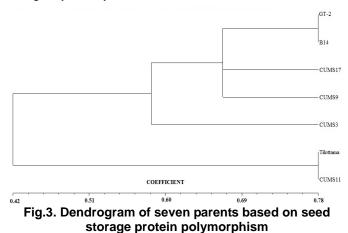


Fig. 2. SDS-PAGE banding pattern of seven parents

Grouping of seven parents based on genetic similarity of seed storage soluble protein polymorphism (Fig.3), produced two clusters in a dendrogram. The parents, Tilottama and CUMS 11 formed a cluster. The other five parents constituted a separate cluster with GT-2 and B-14 being very nearby in that cluster.



Among the 30 SSR primers only four markers gave polymorphic bands (Fig.4) among seven parents and

revealed a total of 15 alleles ranging from 173 bp to 300 bp (Table 5). GDS showed an average of 0.403 (Table 4). Around 38% of the hybrids had genetic distance more than average (0.403) (Table 4). Lowest GDS (0.154) was observed between Tilottama and CUMS 3 and also between parents CUMS 9 and CUMS 11. On the other hand, the highest GDS (0.769) was recorded between parents GT-2 and B-14 (Table 4). The average no. of alleles per locus was found to be 3.75 (Table 5).

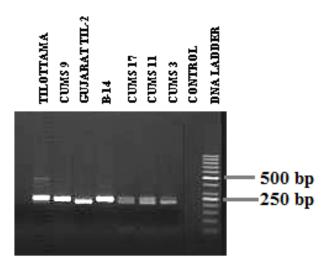


Fig.4. Banding pattern of seven parents for SSR marker SE-SSR 12

TABLE 5

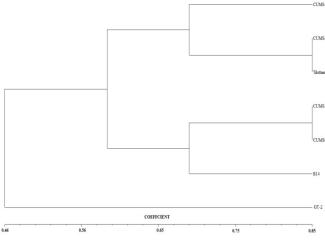
DESCRIPTION OF P	RIM	ER PAIR	S
	7.4	GC Content	

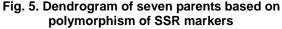
Primer Name		Sequence 5 ⁷ -3 ⁷	Ta" (℃)	GC Content (%)	OS (bp) ^b	No. of Alleles				
SE-SSR 05	F	GCAAACACATGCATCCCT	58	50	09 (hn)*	4				
ac-aan Up	R	GCCCTGAT GATAAAGCCA	20	50						
SE-SSR 06	F	CCATTGAAAACTGCACACAA	58	40	200-213	2				
at-aak Ub	R	TCCACACACAGAGAGCCC	50	61						
SE-SSR 07	F	TCTTGCAATGGGGATCAG	58	50	173-196	3				
ac-aan 07	R	CGAACTATAGATAATCACTTGGAA	20	33						
CE CCD 10	F	GCTGAGGAGTCTT GAAGCAGA	60	52.3	223-290	6				
at-aak 12	R	CAAAATCCCCCAACTCGATA	60	45						
	F GCTGAGGAGTCTTGAAGCAGA 50 52.3 223-290									

^a = Annealing temperature; ^b=Observed size (base pairs)

Clustering pattern from SSR data exhibited two clusters (Fig.5). It is conspicuous from the clustering pattern, that the parent GT-2, developed in Gujarat (A state in Western India), formed a separate cluster and the genotype was distinctly diverse from rest of parents belonging to West Bengal. In the other cluster, with remaining six genotypes, distance between CUMS 9 and CUMS 11 were very narrow and both of these congregated together along with genotype B-14 forming a sub-cluster. Similarly, genotype

CUMS 3, Tilottama and CUMS 17 constituted another subcluster.





Association of Different Parameters with 3.3 Heterosis and Specific Combining Ability Estimates Interestingly, BPH%, MPH%, specific combining ability (SCA) estimates and F₁ per se performance were significantly and positively correlated with each other for all traits (Table 6). Association of both BPH% and MPH% with other parameters, namely, GCA AVG, GDM, GDP and GDS were rarely significant (Table 6). MPH% for primary branch exhibited a significant negative association with GCA AVG. No other significant association of either of the heterosis% with GCA AVG was noticed for any other character. On the other hand, significant associations of BPH% and MPH% with any of the three GD estimates (GDM, GDP and GDS) were found in three traits only, namely, days to 50% flowering, days to maturity and capsule length (Table 6). GD estimates were significantly related to SCA estimates for days to maturity and for capsule length only (Table 6). Despite negative association between GDM and GDP, between GDP and GDS and positive association between GDM and GDS (Table 6), the matrix correlation coefficient (r) values, for these pair of parameters, derived from Mantel Test, was 0.34, 0.008 and -0.17 respectively. The values were significant at p = 0.92, 0.51 and 0.25 with t = 1.41, 0.03 and -0.68 respectively; this indicated that phenetic divergence is a vague indicator of actual genetic divergence and was not reliable enough to draw any conclusion on their mutual association. To epitomize the relationship of heterosis and parental divergence, grouping of 21 cross combinations were carried out based on values of GDM, GDP, GDS for seed yield and four component traits, namely, capsules/plant, primary branches/plant, days to 50% flowering and days to maturity. Correlation coefficients of BPH% and MPH% with GDM, GDP and GDS were determined for each and every group separately (Table 7).

TABLE 6 CORRELATION COEFFICIENTS BETWEEN DIFFERENT PARAMETERS

	Correlation			GCA			
Characters	Between	SCA	Per Se F ₁	AVG	GDM	GDP	GDS
	врн	0.831*	0.773*	0.120	-0.036	-0.079	-0.053
Plant Height	MPH	0.857*	0.856*	0.222	-0.121	-0.137	-0.061
	SCA		0.772*	0.024	-0.039	-0.180	-0.226
No. of Primary	врн	0.660*	0.704*	-0.078	-0.288	0.175	-0.167
Branches/Plant	МРН	0.385*	0.133	-0.459*	-0.174	-0.089	-0.002
	SCA		0.667*	0.009	-0.289	0.498	-0.162
Days to 50%	BPH	0.753 [×]	0.485*	0.194	-0.373*	0.445*	-0.653*
	МРН	0.768 [×]	0.418*	-0.005	-0.155	0.251	-0.429*
Flowering	SCA		0.463*	0.089	-0.122	0.286	-0.254
	BPH	0.737 ^x	0.682*	-0.239	0.327	-0.703*	0.599 [×]
Days to Maturity	MPH	0.715 [*]	0.745*	-0.213	0.290	-0.626*	0.712*
	SCA		0.643*	-0.125	0.167	-0.816*	0.344*
	врн	0.450 [×]	0.452 [×]	-0.129	-0.360*	0.558*	-0.599 [×]
Capsule Length	MPH	0.767*	0.719*	-0.153	-0.241	0.526*	-0.334 [×]
	SCA		0.682*	-0.063	-0.085	0.385*	-0.255
Number of	BPH	0.783*	0.800*	-0.09	-0.165	0.085	-0.099
Capsules/Plant	МРН	0.672*	0.723*	-0.283	0.033	0.002	-0.127
	SCA		0.569*	-0.079	-0.151	0.096	-0.195
Number of	врн	0.869*	0.811*	-0.180	-0.120	0.244	-0.082
Seeds/Cap sule	MPH	0.903*	0.918*	-0.020	-0.118	0.263	-0.117
	SCA		0.883*	0.021	-0.147	0.329	-0.046
1000 Seed	BPH	0.636 [×]	0.744*	0.075	-0.026	0.058	-0.284
Weight	MPH	0.767 [×]	0.770 [×]	0.014	0.058	0.167	-0.278
	SCA		0.682*	-0.130	-0.005	0.338	-0.026
	BPH	0.766 [×]	0.742 [×]	0.069	-0.027	0.221	-0.186
Seed Yield/Plant	МРН	0.721×	0.809*	-0.0003	-0.011	0.202	-0.274
	SCA		0.713*	0.045	-0.189	0.348	-0.173

GDM Vs GDP = -0.343 GDM Vs GDS = 0.109 GDP Vs GDS = -0.481^* r value significant at 5% level of significance

The GDM grouping disclosed significant but inconsistent association of both the heterosis (MPH% and BPH%) with GDM, GDP and GDS for all five traits. Significant association occurred rarely in GDP grouping, except for days to maturity (Table 7). Hence, prediction of heterosis based on GDM or GDP is not adequate enough to avoid laborious field experimentation. Grouping based on GDS represented a different scenario. The most important fact was presence of significant positive association of heterosis with GDS in group III (GDS > 0.50), for yield (Table 7). From the above results, it is observed that three types of markers, showed three different type of clustering patterns with no specific trend between them. But from the correlation table (Table 7) it appeared that parental grouping based on molecular divergence offered more conclusive result in general, than other grouping. It consolidates the perception of divergence study to obtain heterotic hybrid.

TABLE 7 CORREALATION TABLE BASED ON GROUPING OVER GD ESTIMATES

				GDM 0	rouping			GDP Grouping GDS Grouping											
Trais	Association	Group I		Group I	I	Group I	I	Group I		Group	Π	Group	Π	Group I	I	Group I	I	Group	Л
	with	0.80-1.3	0	1.30-1.8	0	>1.80		⊲0.25		0.25-0.	50	>0.50		⊲0.25		0.25-0.5	50	×0.50	
		BPH	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH	MPH
	GDM	-0.33	-0.45	-0.03	-0.18	0.41	-0.05	-0.93	-1.00	0.15	0.15	-0.70	-0.79	-0.22	0.01	0.09	0.03	0.34	0.17
Seed Yield/Plant	GDP	0.63*	0.62*	-0.21	-0.14	-0.10	-0.54	-0.98	-1.00	0.14	-0.18	0.93	0.74	-0.13	-0.28	0.59*	0.57*	-0.72	-0.80
	GD8	-0.35	-0.29	0.02	-0.24	-0.81	-0.46	-0.45	-0.70	0.00	0.02	-0.44	-0.43	-0.09	-0.13	0.10	-0.03	0.86*	0.87*
Number of	GDM	-0.56*	-0.65*	0.06	0.07	-0.10	-0.33	-0.67	-0.84	0.46	0.65	-0.73	-0.76	-0.37	0.11	-0.24	-0.03	0.22	0.27
Capsoles/	GDP	0.38	0.36	-0.26	-0.29	0.41	0.19	-0.78	-0.92	-0.35	-0.40	0.78	0.52	0.25	-0.19	0.21	0.27	-0.62	-0.69
Plant	GD8	-0.38	-0.27	0.09	-0.08	0.58	0.76	-0.01	-0.27	0.25	0.22	-0.61	-0.59	-0.64	-0.44	0.37	0.18	0.71	0.81*
Primary	GDM	0.14	0.03	-0.70*	-0.54	-0.37	-0.47	-0.97	-0.96	0.21	0.40	-0.62	-0.57	0.02	0.65	-0.29	-0.21	-0.22	0.04
Branches/	GDP	-0.12	-0.23	0.50	0.34	0.14	0.04	-0.93	-0.90	-0.20	-0.27	0.73	0.44	-0.42	-0.79	0.18	-0.16	-0.11	-0.18
Plant	GD8	-0.16	0.05	-0.41	-0.53	0.79	0.85	-0.88	-0.90	0.25	0.15	-0.17	-0.11	0.26	0.21	0.39	0.44	0.00	0.19
Days to 50%	GEM	-0.34	-0.41	-0.17	0.14	-0.47	-1.00*	-0.59	-0.73	-0.05	-0.08	-0.37	0.03	0.67	0.95	-0.65	-0.53	-0.40	0.11
Flowering	GDP	0.57*	0.42	0.28	0.05	0.03	-0.90	-0.45	-0.61	-0.26	-0.33	-0.35	-0.42	-0.52	-0.90*	0.50	0.46	0.74	0.76
	GD8	-0.65*	-0.32	-0.89*	-0.72*	0.85	0.83	-0.98	-1.00	-0.12	-0.19	-0.41	0.27	0.68	0.34	-0.06	0.07	-0.89*	-0.69
	GDM	0.39	0.42	0.90*	0.79*	-0.25	0.04	0.99*	0.75	0.43	0.58	0.79	0.82	0.93	0.86	0.07	0.03	0.48	0.79
Days to Maturity	GDP	-0.85*	-0.88*	-0.67*	-0.55	0.26	0.53	0.95	0.64	-0.15	-0.24	-0.80	-0.61	-0.90*	-0.90*	-0.67*	-0.62*	-0.16	-0.19
	GDS	0.73*	0.79*	0.52	0.72*	0.70	0.47	0.85	1.00*	0.22	0.28	0.52	0.63	0.74	0.86	0.26	0.18	0.35	0.51

*r value significant at 5% level of significance

4 DISCUSSION

Significant amount of dominance (non additive) variance for any character is generally a prerequisite for exploitation of heterosis. SCA estimates measure dominance component. In the present study, strong association between heterosis (%), hybrids per se and SCA estimates (Table 6) satisfies the proposition about non-additive genetic effect. On the contrary, GCA effects reflect additive gene action; poor correlation between heterosis and GCA AVG, observed in the present study, is expected, as heterosis is, mainly a function of non additive gene effect. The present finding, that SCA effects did not correlate significantly with GD estimates corroborated with the observation of [23], [24]. GDS and Cluster analysis based on GDS are in agreement with the geographic origin of the genotypes, but GDM corresponded poorly with the origin, whereas GDP did not correspond at all. There may be two major reasons for not obtaining good association between heterosis and diversity indices. Firstly, different indices may not be involved with expression of heterosis. Similarly, parents selected for the study perhaps did not represent true picture of the total

gene pool in sesame. It is difficult to conclude about the above disputes due to unavailability of insufficient data. Still among diversity indices, diversity through SSR markers would go a long way, as SSR markers circumvent environmental variation, unlike morphological and protein markers which are environmental sensitive. The common tradition of detecting GD estimates is to select copious number of DNA markers with wide coverage over genomes [25]. Insufficient genome coverage due to fewer SSR markers may be one of the causes for not achieving good association of heterosis with GDS for all traits. [26] opined that use of large number of markers would result in positive correlation between parental diversity and F₁ performance. In sesame, though work on DNA markers like RAPDs [27] and AFLPs [28] was found, but only recently, reports are coming on sesame genome sequences [29], [30] and on SSR markers including ESTs in sesame [31], [16], [32], [33], [34], [35]. QTL mapping for heterosis in sesame is still unexplored and augmenting DNA markers throughout the genome, with markers at the target QTL, seemed to be not feasible as only limited information about sesame genome sequence is available. So, it will be imprudent to conclude about the poor correlation of heterosis for yield with low GD, mainly for failure of marker technology in prediction of heterosis. In other words, if markers are in linkage disequilibrium with quantitative trait loci (QTL), then surely it will lead toward a predictive value. [36] were of the belief that prediction of heterosis through marker loci would foster meaningful results if the selected markers are specifically linked to the target traits considered for heterosis. Murty [37] and Banerjee & Kole [38] earlier, reported relationship between parental phenotypic diversity and heterosis in sesame, but no such report is available where molecular diversity is involved. In the present study, significant association between GDS and heterosis, transpired in GDS grouping for seed yield and capsules/plant, and proportional increase of heterosis with parental diversity, explicated that DNA markers reflect true divergence between genotypes. Feeble or week positive correlation between GDM and GDS estimates, envisages need of further confirmation, to figure out whether only GDS between parents would be enough to predict the strength of heterosis or not.

5 CONCLUSION

It may be concluded from the above experiment that parental diversity, based on morphological and seed storage protein polymorphism did not corroborate well with heterotic expression of characters in hybrids. Study on microsatellite markers depicted a different picture, where heterosis seemed to be explained by parental diversity to some extent.

6 ACKNOWLEDGMENT

The first author thanks the Department of Genetics and Plant Breeding, Institute of Agricultural Science, University of Calcutta, Kolkata, India where this research work was carried out as the part of PhD dissertation work of the first author prior her joining to the current institute she is affiliated to.

7 REFERENCES

- [1]. D. Sankar, M.R.Rao, G.Sambandam and K.V. Pugalendi, "Effect of Sesame Oil on Diuretics or Beta-Blockers in The Modulation of Blood Pressure, Anthropometry, Lipid Profile, and Redox Status," Yale Journal of Boilogy and Medicine Vol.79, no.1, pp. 19-26, 2006.
- [2]. H. Hibasami, T. Fujikawa, H.Takeda, S. Nishibe, T. Satoh, T. Fujisawa and K. Nakashima, "Induction of Apoptosis by *Acanthopanax Senticosus* HARMS and Its Component, Sesamin in Human Stomach Cancer KATO III Cells," *Oncology Reports*, Vol. 7, no. 6, pp. 1213-1216, 2000.
- [3]. Y. Miyahara , H. Hibasami, H. Katsuzaki, K. Imai and T. Komiya," Sesamolin from Sesame Seed Inhibits Proliferation by Inducing Apoptosis in Human Lymphoid Leukemia Molt 4B Cells," *International Journal of Molecular Medicine*, Vol. 7, no. 4, pp. 369–371, 2001.
- [4]. Food and Agriculture Organization of the United Nations, http://faostat.fao.org/site/339/default.aspx [accessed 13 March 2013].
- [5]. M.O. Khidir, "Natural Cross-Fertilization in Sesame under Sudan Conditions," *Experimental Agriculture*, Vol.8, no. 1, pp. 55-59, 1972.
- [6]. R. Pathirana, "Natural Cross-Pollination in Sesame {Sesamum Indicum L.)," Plant Breeding, Vol. 12, no. 2, pp. 167-170, 1994.
- [7]. P. Xangsayasane, X. Fangming, E.H. Jose and H.B. Teresita, "Hybrid Rice Heterosis and Genetic Diversity of IRRI And Lao Rice," *Field Crops Research*, Vol. 117, no. 1, pp. 18-23, 2010.
- [8]. M.S. Drinic´, S. Trifunovic´, G. Drinic´ and K. Konstantinov, "Genetic Divergence and Its Correlation to Heterosis in Maize as Revealed by SSR-Based Markers," *Maydica*, Vol. 47, pp. 1-8, 2002.
- [9]. J. Srdic´, M.S. Drinic´, Z. Pajic´ and M. Filipovic´, "Characterization of Maize Inbred Lines Based on Molecular Markers, Heterosis and Pedigree Data," *Genetika*, Vol. 39, pp. 355-363, 2007.
- [10]. P. Bansal, S. Banga and S.S. Banga, "Heterosis as Investigated in Terms of Polyploidy and Genetic Diversity Using Designed *Brassica Juncea* Amphiploid and Its Progenitor Diploid Species," *PLOS ONE* Vol. 7, pp. e29607, 2012.
- [11]. S. Gvozdenovic´, S.P. Dejana, S. Jocic´ and V. Radic´, "Correlation between Heterosis and Genetic Distance Based on SSR Markers in Sunflower (*Helianthus Annus* L.)," *Journal of Agricultural Science*, Vol. 54, no. 1, pp. 1-10, 2009.
- [12]. K.F. Solomon, Z. Aldo and S.D. Mulugeta,

"Combining Ability, Genetic Diversity and heterosis in Relation to F1 Performance of Tropically Adapted Shrunken (Sh2) Sweet Corn Lines," *Plant Breeding*, Vol. 131, no. 3, pp. 430-436, 2012.

- [13]. O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, "Protein Measurement with The Folin-Phenols Reagent," *Journal of Biological Chemistry*, Vol.193, pp. 265-275, 1953.
- [14]. U.K. Laemmli, "Cleavage of structural Proteins During The Assembly of The Head of Bacteriophage $T_{4.}$ ". *Nature*, Vol. 227, doi:10.1038/227680a0, pp. 680-685, 1970.
- [15]. J.J. Doyle and J.L. Doyle, "Isolation of plant DNA from fresh tissue," *Focus*, Vol. 12, no. 1, pp. 13-15, 1990.
- [16]. A. Dixit, M.H. Jin, J.W. Chung, J.W. YU, H.K. Chung, K.H. Ma, Y.J. Park and E.G. Cho, "Development of Polymorphic Microsatellite Markers in Sesame (Sesamum indicum L.)," *Molecular Ecology*, Vol. 5, no. 4, pp. 736-738, 2005.
- [17]. National Centre for Biotechnology Information, http://www.ncbi.nlm.nih.gov/nucest/JK085557.1 [accessed 12 September 2011].
- [18]. B. Griffing, "Concept of General and Specific Combining Ability in Relation To Diallel Crossing System," *Australian Journal of Biological Sciences*, Vol. 9, pp. 463-493, 1956.
- [19]. Y. Zhang and M.S. Kang, "DIALLEL-SAS: A Program for Griffing's Diallel Methods. In: Kang MS ed. Handbook of Formulas and Software for Plant Geneticists and Breeders.1st edition. New York, USA: Food Products Press, an imprint of The Haworth Press, Inc. Pp.1-19, 2003.
- [20]. Microsoft Excel Ver.7.0 (Microsoft. Inc., Redmond. WA)
- [21]. F.J. Rohlf, NTSYS-pc: Numerical Taxonomy and Multivariate Analysis System, Version 2.20, Applied Biostatistics, New York, 2005.
- [22]. N.A. Mantel, "The Detection of Disease Clustering and A Generalized Regression Approach," *Cancer Research*, Vol.27, no. 2, pp. 209-220, 1967.
- [23]. A.E. Melchingar, J. Boppenmaier, B.S. Dhillon, W.G Pollmer and R.G. Hermann, "Genetic Diversity for RFLPs in European Maize Inbreds: II. Relation to Performance of Hybrids within Versus Between Heterotic Groups for Forage Traits," *Theoretical and Applied Genetics*, Vol. 84, no.5-6, pp. 672-681, 1992.
- [24]. A.E. Melchingar, M. Lee, K.R. Lamkey and W.W. Woodman, "Genetic Diversity for Restriction



Fragment Length Polymorphisms: Relation to Genetic Effects in Maize Inbreds," *Crop Science*, Vol. 30, no. 5, pp. 1033-1040, 1990.

- [25]. M.S. Drinic´ and K. Konstantinov, "Genetic Diversity and Its Relationship to Heterosis in Maize as Revealed by PCR-Based Markers," *Genetika*, Vol. 30, no. 3, pp. 227-237, 1998.
- [26]. O.S. Smith, J.S.C. Smith, S.L. Bowen, R.A. Tegborg and S.J. Wall, "Similarities among A Group of Elite Maize Inbreds as Measured by Pedigree, F1 Heterosis and RFLPs," *Theoretical and Applied Genetics*, Vol. 80, no. 6, pp. 833-840, 1990.
- [27]. K.V. Bhat, P.P. Babrekar and S. Lakhanpaul, "Study of Genetic Diversity in Indian and Exotic Sesame (Sesamum Indicum L.) Germplasm using Random Amplified Polymorphic DNA (RAPD) Markers," *Euphytica*, Vol. 110, no. 1, pp.21–33, 1999.
- [28]. H.E. Laurentin and P. Karlovsky, "Genetic Relationship and Diversity in A Sesame (Sesamum Indicum L.) Germplasm Collection using Amplified Fragment Length Polymorphism (AFLP)," BMC Genetics, Vol. 7, no.10, doi:10.1186/1471-2156-7-10, 2006.
- [29]. D.K. Yi and K.J. Kim, "Complete Chloroplast Genome Sequences Oof Important Oilseed Crop Sesamum indicum L.," PLOS ONE, Vol. 7, pp.e35872, 2011.
- [30]. H. Zhang, H. Miao, L. Wang and M. Yue, "Genome Sequencing of The Important Oilseed Crop Sesamum Indicum L.," Genome Biology, Vol.14, no. 041, doi:10.1186/1471-2164-12-451, 2013.
- [31]. D. Kim, G. Zur, Y. Danin-Poleg Y, S. Lee, K. Shim, C. Kang and Y. Kashi, "Genetic Relationships of Sesame Germplasm Collection as Revealed by Inter-Simple Sequence Repeats," *Plant Breeding*, Vol. 121, pp. 259-262, 2002.
- [32]. L. Wei, H. Zhang, Y. Zheng, W. Guo and T. Zhang, "Development and Utilization of EST-Derived Microstellites in Sesame (*Sesamum indicum* L.)," *Acta Agronomica Sinica*, Vol. 34, pp. 2077- 2084, 2008.
- [33]. S.N. Sharma, V. Kumar and S. Mathur, "Comparative Analysis of RAPD and ISSR Markers for Characterization of Sesame (Sesamum indicum L.) Genotypes," Journal of Plant Biochemistry and Biotechnology, Vol.18, pp.37-43, 2009.
- [34]. B. Uzun and M.I. Cagirgan, "Identification of Molecular Markers Linked to Determinate Growth Habit in Sesame," *Euphytica*, Vol. 166, pp. 379-384, 2009.
- [35]. W. Wei, X. Qi, L. Wang, Y. Zhang, W. Hua, D. Li, H. Lv, X. Zhang, "Characterization of The Sesame

(Sesamum Indicum L.) Global Transcriptome Using Illumina Paired-End Sequencing and Development of EST-SSR Markers," *BMC Genomics,* Vol.12, no. 451, doi:10.1186/1471-2164-12-451, 2011.

- [36]. A. Charcosset and L. Essioux, "The Effect of Population Structure on The Relationship Between Heterosis and Heterozygosity at Marker Loci," *Theoretical and Applied Genetics*, Vol. 89, pp. 336-343, 1994.
- [37]. D.S. Murthy, "Heterosis, Combining Ability and Reciprocal Effects for Agronomic and Chemical Characters in Sesame," *Theoretical and Applied Genetics*, Vol. 45, pp. 394-299, 1975.
- [38]. P.P. Banerjee and P.C. Kole, "Heterosis, Inbreeding Depression and Their Relationship with Genetic Divergence in Sesame (*Sesamum Indicum* L.)," *Acta Agronomica Hungarica*, Vol. 58, pp. 313-321, 2010.