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Association of genetic distance and combining ability with heterosis in Indian mustard (*Brassica juncea* L)

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Abstract

Heterosis and combining ability were carried out by evaluation of thirty-two F_1s of Indian mustard (*Brassica juncea L*) generated in Line × Tester mating design involving eight lines and four testers, in randomized complete block design for thirteen quantitative traits. ANOVA showed significant differences among parents as well as among hybrids for almost all the traits studied. The coefficient of variation for different traits was found higher in parents in comparison to hybrids. The mean performance of hybrids was found higher than parents for all the traits except for days to 50% flowering, days to maturity and oil content. ANOVA for combining ability exhibited significant variance for most of the traits for both general and specific combining ability. The PCA analysis using quantitative traits explained that PC-I contributed maximum of 90.24% with Eigen value of 977.94, and positively correlated with number of primary branches, total number of siliquae on main shoot, total number of siliquae per plant, seed yield per plant, days to 50 % flowering, plant height, point to first siliqua and main shoot length. Thirteen F_1 combinations showed significant and positive heterosis over the mid parent. The cross NRCHB 101 × JN032 showed 109.45 percent better parent heterosis for seed yield per plant. The genetic distance among genotypes (parents and F_1s) was calculated by using 13 quantitate traits and SSR markers. The association of morphological genetic distance and molecular genetic distance with SCA and heterosis revealed that molecular genetic distance has significant positive correlation (0.43*), while, morphological genetic distance has significant negative correlation (-0.53**) with mid parent heterosis of seed yield per plant. Moreover, SCA for seed yield per plant showed significant positive association and regression with mid parent heterosis for seed yield per plant (0.57**) and better parent heterosis of seed yield per plant (0.67**). The study shows that SCA and molecular genetic distance are important determinant of heterosis in Indian mustard.

Keywords**:** *Combining ability*, *genetic distance, heterosis*, *SSR markers*

Introduction

Oilseed brassicas (rapeseed-mustard) are the second most important edible oilseed crop in India after groundnut, accounting nearly 30% of the total oilseeds produced in the country. Among all seven cultivated *Brassica* species [*B. juncea* (L.) Czern. & Coss.], *B. napus* L., *B. rapa* ssp. Yellow Sarson, *B. rapa* L. ssp. Brown Sarson and *B. rapa* ssp. Toria, *B. carinata* Braun. and *B. nigra* (L.) Koch.), *B. juncea* (Indian mustard) is an important crop in India contributing about 75-80 per cent of the area as well as production obtained from rapeseed-mustard (Anonymous, 2020). Indian mustard being an important source of edible oil in India, possesses the capacity to reduce the import of edible oil, which is currently 70 per cent of total edible oil demand. To increase yield potential of the crop and development of better genotypes, reshuffling of the genes through hybridization in suitable parents and exploitation of heterosis is an important one among all other breeding strategies. High level of heterosis has been utilized for development of hybrids in Indian mustard since the decade of 1980s (Banga and Labana, 1984; Dhillon *et al*., 1990). Identification and selection of appropriate parental combinations for production of superior F_1 hybrids, using combining ability has remained one of the most important stages in heterosis breeding (Labana *et al*., 1975; Pradhan *et al*., 1993; Mahto and Haider, 2004; Yadava *et al*., 2012). Further, heterotic response of F_1 is by and large associated with diversity of the parents, therefore, choosing right parents by gathering information on both genetic distance and combining ability is essential for prediction of heterosis. Genetic diversity in Indian mustard based on quantitative trait (Gupta *et al*., 1991; Alie *et al*., 2009) and molecular markers (Batley *et al*., 2003; Chen *et al*., 2011) have been done by several workers. Further, molecular markers especially, SSRs have been used for prediction

of best heterotic crosses in Indian mustard (Vinu *et al*., 2013). As studied in Indian mustard, specific combining ability and heterosis in a series of crosses also quantifies genetic distances among the genotypes (Shekhon and Gupta, 1995). In addition, study on correlation provides a better understanding of the association among different traits, which help breeders to formulate an appropriate breeding strategy to improve a number of traits simultaneously. Correlation between genetic distance, specific combining ability and heterosis has also been studied in Indian mustard (Bansal *et al*., 2012) and maize (Srdic *et al*., 2007). Above facts shows the importance of the study on genetic distance, combining ability and level of heterosis as well as the association among them. The present work is a very basic study to learn the role of quantitative traits and SSR based genetic distance and combining ability in prediction of heterosis in Indian mustard by calculating correlation, regression among them.

Materials and Methods

Experimental materials and layout

Thirty-two F_1s were generated in line × tester mating design by crossing 8 lines (NRCHB 101, NRCDR 02, Pusa Bold, Shivani, Pusa Mustard-25, Kranti, BAUSM-92-1-1, BAUM 2007) and 4 testers (Heera, JN032, Pusa Mustard-21, RGN-73) of Indian mustard during *Rabi* 2013-14. These F_1s along with their parents were evaluated in randomized complete block design with two replications in three dates of sowing *viz*. 21st October, 6th November and 21st November (considered as environments) during *rabi* 2014-15 at Crop Research Farm of Birsa Agricultural University, Ranchi (Jharkhand), India (23°17'N 85 °19'E). Each genotype was sown in a single row plot of 3 m length with 30 cm row to row and 15 cm plant to plant spacing. All the recommended packages of practices were followed to raise the crop. The soil pH of the experimental region was acidic (5.2) with high organic carbon (0.81%), 448 kg/ha nitrogen, 42.9 kg/ha phosphorus and 160.74 kg/ha potassium content. The data were recorded on five randomly selected plants for 13 quantitative traits that include, plant height (cm) , number of primary branches, main shoot length (cm), point to first siliqua, total number of siliquae on main shoot, total number of siliquae per plant, siliqua length (cm), number of seed per siliqua, seed yield per plant (g), 1000-seed weight (g), days to 50 % flowering, days to maturity and oil content (%). Per cent oil content was determined by Soxhlet apparatus (AOAC, 1997).

Genomic DNA extraction and molecular marker analysis

Genomic DNA extraction and molecular marker analysis was done at ICAR-Directorate of Rapeseed-Mustard Research, Bharatpur during 2019-20. Genomic DNA from parents and F_1s were isolated using cotyledonary leaves of *in-vitro* germinated selfed seed of parents and F_1 seeds of hybrids following CTAB method (Doyle and Doyle, 1990) with slight modifications. The isolated DNA was quantified using spectrophotometer. A total of eleven polymorphic SSRs were used for genotyping of parents and hybrids. Amplification reactions were carried out in a volume of 20 µl containing 10 mM Tris HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 200 mM each dNTP, 0.4 µM forward and reverse primers, 1.0-unit *Taq* DNA polymerase (Genetics Biotech Asia Pvt. Ltd.) and 30 ng of genomic DNA. Amplifications were carried out in a Veriti® 96-well Fast Thermal Cycler (PE Applied Biosystems, USA). The PCR reaction condition consisted of an initial denaturation at 94 °C for 5 min followed by 35 cycles of denaturation at 94 °C for 45 s, annealing of the primer at 58 °C for 40 s and an extension at 72 °C for 1 min. Finally, 1 cycle of 7 min at 72°C was used for the final extension, followed by storing at 4 °C. The reproducibility of the amplification products was checked twice for each primer. SSR-amplified products were resolved on 2.5 % super fine resolution agarose gels in Tris–borate-EDTA buffer and stained with ethidium bromide (0.5ng/ml). The gels were viewed and photographed in gel documentation and image analysis system (Syngene, UK).

Data analysis

ANOVA, combining ability and level of heterosis were calculated by pooling the quantitative traits data of the three environments following the method given by Singh and Chaudhary, 1985 and Kempthorne, 1957. Morphological genetic distance using quantitative traits and Jaccard's molecular distance using binary matrices (0/1) generated through the particular SSR marker were estimated in DARwin 6 software (Perrier, 2006). The Polymorphic information content (PIC) and resolving power of the SSR markers were estimated using methods given by Anderson *et al.* (1993) and Prevost and Wilkinson (1999) respectively. UPGMA dendrogram based on quantitative traits and SSR markers was constructed using NTSYS pc 2.2 software. Spearman's correlation and Principal component analysis (PCA) was done by SPSS software.

Results and Discussion

Descriptive statistics and PCA

Analysis of variance revealed significant differences for all 13 quantitative traits among 12 parents and their 32 F_1 combinations in all the three dates of sowing (environments) depicting wide range of variability among

Table 1: Statistical parameters of quantitative traits in Indian mustard scored in parents and hybrids

	PH	PB	MSL	PFS	SMS	SPP	SL	SS	SY	TSW	D50F	DM	OC
Parents (*N*=12)													
Mean	122.8	3.7	57.9	64.2	34.8	129.3	3.9	11.9	5.4	3.6	53.0	113.4	41.2
Min	109.3	3.0	49.9	53.3	27.7	95.1	3.0	10.7	3.2	2.3	44.7	107.8	39.3
Max	134.4	4.7	68.0	75.0	44.3	165.1	4.4	13.3	6.9	5.0	56.8	117.5	42.4
SE (±)	2.2	0.1	1.3	1.8	1.4	5.8	0.1	0.2	0.3	0.2	0.9	1.0	0.2
CV (%)	6.3	11.6	7.8	9.6	13.6	15.6	10.7	6.4	16.4	22.4	6.0	3.1	2.1
Hybrid (*N*=32)													
Mean	134.8	4.3	63.9	68.2	40.9	165.0	3.8	12.2	6.1	3.3	53.6	114.5	41.1
Min	116.5	3.5	54.9	55.2	31.2	124.4	3.5	11.6	5.0	2.4	49.0	112.3	39.8
Max	151.3	4.9	73.1	79.0	54.9	239.1	4.2	13.1	7.6	4.0	57.7	116.7	42.5
SE (±)	1.67	0.06	0.94	1.07	0.99	4.62	0.04	0.06	0.1	0.08	0.36	0.18	0.1
CV (%)	7.0	8.3	8.3	8.8	13.7	15.9	5.8	2.8	10.1	13.9	3.8	0.9	1.4

PH: plant height; PB: number of primary branches; MSL: main shoot length; PFS: point to first siliqua; SMS: total number of siliquae on main shoot; SPP: total number of siliquae per plant; SL: siliqua length; SS: number of seed per siliqua; SY: seed yield per plant; TWT: 1000-seed weight; D50F: days to 50 % flowering; DM: days to maturity; OC: oil content

parents and hybrids. In general, high coefficient of variation for different traits was exhibited by parents as compared to hybrids. The mean performance of hybrids was found higher than parents for all the traits except for days to 50% flowering, days to maturity and oil content, however not in significant manner (Table 1). Among hybrids the mean seed yield per plant was higher (6.08g) in comparison to parents (5.41g). Furthermore, among hybrids highest seed yield per plant (7.63g) was recorded for the cross NRCHB-101 × JN032 whereas among parents Pusa Bold was the highest yielder (6.93g).

PCA was carried out based on 13 quantitative traits to identify important traits contributing towards variation. First three components contributed a total of 99.0% of variation in which PC-I contributed maximum of 90.2% with Eigen value of 977.9. PC-I showed significant positive correlation with number of primary branches, total number of siliquae on main shoot, total number of siliquae per plant, seed yield per plant, days to 50 % flowering, plant height, point to first siliqua and main shoot length while PC-II was found associated positively with days to 50 % flowering, plant height and point to first siliqua (Fig. 1).

Figure 1: Association of first three principal components (PCs) with different traits of Indian mustard

Combining ability and heterosis

ANOVA for combining ability exhibited significant variance for plant height, number of primary branches, total number of siliquae per plant, silique length, seed yield per plant and 1000-seed weight for both general combining ability (GCA) and specific combining ability (SCA) along with highly significant SCA × environment interaction for number of primary branches, total number of siliquae per plant, seed yield per plant, 1000-seed weight and days to maturity (Table 2). Estimation of gene action for quantitative traits showed non additive gene action for seed yield per plant, number of seed per siliqua, days to 50 % flowering and days to maturity while additive gene action for all other traits. Similar results were also

Table 2: ANOVA for combining ability of parents and hybrids of Indian mustard

Sources of variation	*df*	PH	PB	MSL	PFS	SMS	SPP	SL	SS	SY	TSW	D50F	DM
GCA (Line)	7.0	460.5 *	1.7**	54.9	170.3	98.1	5229.2**	0.59**	0.52	5.45*	1.87**	21.57	5.54
GCA (Tester)	4.0	3642.1**	1.8*	929.5**	1092.2**	1175.2**	19333.1**	1.80**	1.44	2.01	8.11**	67.64**	8.51
SCA	28.0	171.9 *	0.5**	78.4 *	73.0	51.9	1485.6**	0.07	0.53*	2.20**	0.25**	14.97**	4.95**
Environments	2.0	736.6**	5.6**	136.2	1155.7**	1071.3**	18047.9**	0.09	14.4**	61.8**	6.21**	491.8**	5210.7**
Env x GCA (Line)	14.0	145.5	0.3	88.2	54.7	61.9	2211.8	0.09	0.54	4.14	0.24	13.96**	9.43*
Env x GCA (Tester)	8.0	317.1*	0.5	67.0	150.3**	76.5	1040.0	0.07	0.36	2.98	0.43	11.23	6.54
Env x SCA	56.0	117.8	0.4**	51.6	51.8	38.4	1682.2**	0.07	0.50*	2.26**	0.22**	5.64	4.24**

PH: plant height; PB: number of primary branches; MSL: main shoot length; PFS: point to first siliqua; SMS: total number of siliquae on main shoot; SPP: total number of siliquae per plant; SL: siliqua length; SS: number of seed per siliqua; SY: seed yield per plant; TWT: 1000-seed weight; D50F: days to 50 % flowering; DM: days to maturity; OC: oil content

observed by Singh *et al.* (2015) in Indian mustard. Further, combining ability effects were calculated for parents and crosses, as the nature and magnitude of GCA and SCA effects help in identifying superior parents as well as crosses and their utilization in further breeding programme. The lines *viz.* NRCHB 101, Pusa Bold and tester *viz.* RGN-73 were found to be good general combiner for the traits like seed yield per plant, 1000-seed weight, plant height, point to first siliqua, point to first branching. The cross combination, NRCHB 101 × JN032, was identified to be a good specific combiner with the highest seed yield per plant. Other crosses like Pusa Mustard-25 × JN032, Pusa Bold × Pusa Mustard-21, Kranti × Heera, Pusa Bold × Pusa Mustard-21, BAUM 2007 × JN032 and BAUSM 92-1-1 × RGN-73 were also found to be prominent (Data not shown). Cross combinations with high SCA effects were also been reported earlier using line × tester analysis in Indian mustard by Yadava *et al.* (2012) and Singh *et al.* (2013).

Heterosis was calculated over mid parent and better parent for the traits under study. The mid parent heterosis for seed yield per plant ranged from "6.52% (NRCDR 02 × Pusa Mustard-21) to 74.26% (Pusa Bold × Pusa Mustard-21) with an average of 19 per cent (Fig. 2). Thirteen F_1 combinations showed significant and positive heterosis over the mid parent. For better parent heterosis, the cross NRCHB 101 × JN032 was the best combination showing 109.45 percent heterosis for seed yield per plant. Better parent heterosis up to 107.21 percent was reported earlier by Singh *et al.* (2015), while, Vaghela *et al.* (2011) and Verma *et al.* (2011) reported 44.8% and 80.97% heterosis for seed yield in crosses *of Indian mustard.*

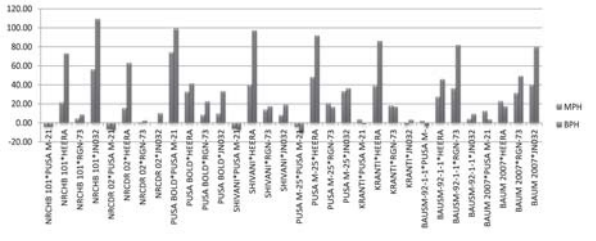

Figure 2**:** Per cent mid parent heterosis (MPH) and better parent heterosis (BPH) of hybrids of Indian mustard

Cluster analysis

Cluster analysis was done using SSR markers as well as quantitative traits as morphological markers to assess genetic diversity among parents and F_1s. A total of 70 Brassica specific SSR markers were screened, from them 11 polymorphic and reproducible markers were used to amplify 31 alleles. PIC (Polymorphic Information Content)

Table 3: SSR marker details and their polymorphism parameters

Markers	Primer details (5'-3')	TB	PIC	PI	Rp
B07	F: AGAGATTCAAACCGAGTGCCR: GGGGCTAGCTTCATCATCC	3	0.44	1.32	2.95
D10	F: GATGCCCCAAATCTGTTACGR: CAATTCGTGAAAAATAGCCG	2	0.32	0.64	2.18
A10	F: TTTTGTTGCGATCTTGAAGCR: ACACTTCCCAATGTCAAACG	2	0.31	0.61	1.77
A09	F: CGCGAGTAAATCAATGTGAATCR: CGACCCACCAACTCACTAAC	2	0.38	0.77	2.68
Ni3H07	F: GCTGTGATTTTAGTGCACCGR: AGCCGTTGATGGAATTTTTG	4	0.35	1.39	1.82
Ni3 F01	F: AGCCGCTAAAGAGAAGGTCCR: CGCTTTCAAGCTCTCTCCC	2	0.29	0.58	3.18
C08	F: CCCTAACACGGTGTCAACAGR: GGCAGAATCATCGAGAGGTC	4	0.27	1.08	3.95
E05	F: CTCGTCTCAGGGATTATGTCGR: CAGACAGAGGATAGACCGAACC	2	0.23	0.46	3.41
F01	F:CGTATGTAGAGAGAGAGAGAGAGAGAGR: AGAACCGTTGAGGTGCTGTC	2	0.19	0.38	3.55
G09	F: CTCGAGGCGCGTTTTACCR: CTCAATCGCATGCATAATCG	3	0.29	0.87	3.32
BRMS-034	F:GATCAAATAACGAACGGAGAGA R:GAGCCAAGAAAGGACCTAAGAT	5	0.37	1.83	3.05
Mean/total		31	0.31	0.90	2.90

TB: total band, PIC: polymorphic information content, PI: primer index, Rp: resolving power

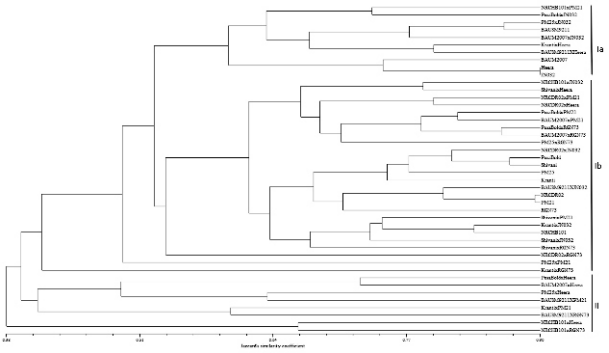

Figure 3(a): UPGMA dendrogram of parents and hybrid based on SSR markers

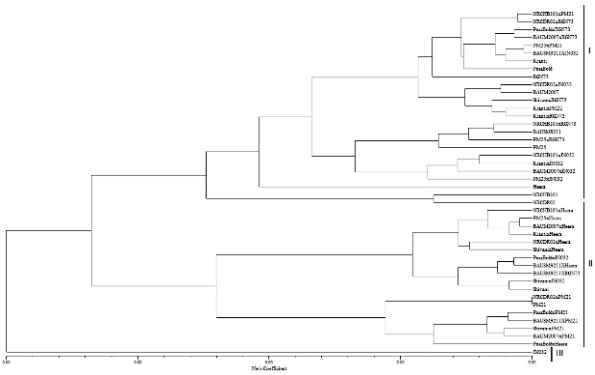

Figure 3(b): Nei's coefficient dendrogram of parents and hybrid based on quantitative traits.

value of these SSR markers ranged from 0.19 (F01) to 0.44 (B07) (Table 3). Jaccard's similarity coefficient ranged from 0.166 (NRCHB 101 × Heera; BAUM 2007 × JN032) to 0.905 (Heera; JN032). The dendrogram thus generated using the Jaccard's similarity coefficient was divided into two major clusters (Fig. 3a). Cluster-I was further divided into two sub-clusters [Ia (10 genotypes) and Ib (26 genotypes)] containing both parents and F_1s. It was quite visible from cluster-Ia that the testers Heera (selection from exotic line) and JN032 (exotic line) showed highest similarity. Cluster-II contained eight crosses with Heera, Pusa Mustard-21 and RGN-73 as tester parents. A Nei's coefficient dendrogram (Fig. 3b) constructed using 13 quantitative traits divided parents and hybrids into three major clusters. Cluster-I was the largest cluster harboring 25 genotypes followed by cluster-II (18 genotypes). Cluster-III consisted only one genotype i.e. JN032 (exotic genotype), differentiating it from other indigenous genotypes. In order to check concordance between dendrogram based on SSRs and quantitative traits, Mantel test was conducted which showed non-significant association (r = -0.0056, t = -0.075, p = 0.47) between two dendrograms. The non-significant correlation between SSRs based and quantitative traits-based matrix was also reported earlier by Vinu *et al*. (2013) in Indian mustard. The non-significant and negative correlation between genetic distances calculated from the two approaches can be explained because of influence of environmental factors on quantitative traits contrary to the molecular markers. Further, molecular markers report genetic variation in both coding and non-coding regions, while non-coding regions do not affect the phenotype.

Association studies

The association of molecular and morphological genetic distance with SCA and heterosis was observed using Spearman's rank correlation coefficient (Table 4). SSR based molecular genetic distance showed significant positive correlation with mid parent heterosis of seed yield per plant (0.43*), mid parent heterosis of total siliquae per plant (0.46**), better parent heterosis of total siliquae per plant (0.47**) and hybrid mean of total

Table 4: Spearman's rank correlation coefficient between different parameter recorded in parents and hybrids of Indian mustard

Traits	PH_ MPH	SPP_ MPH	SY_ MPH	TSW_ MPH	PH_ BPH	SPP_ BPH	SY_ BPH	TSW_ BPH	OC_ BPH	SPP_ SCA	PH_ SCA	SY_ SCA	TSW_ SCA	OC_ SCA	PM_ SY	F1_ PH	F1_ SPP	F1_ SY	F1_ TSW
MD	0.307	0.46**	0.432*	-0.184	0.404*	0.469**	0.240	-0.196	0.276	0.213	0.268	0.169	0.059	0.106	-0.306	0.395*	0.489**	0.270	-0.365*
GD	-0.352*	-0.621**	-0.534**	0.384*	-0.506**	-0.776**	-0.366*	0.265	-0.523**	-0.008	0.006	-0.132	-0.090	-0.135	0.468**	-0.375*	-0.575**	-0.203	0.655**
PH_MPH	1	0.596**	0.438*	-0.470**	0.946**	0.592**	-0.023	-0.502**	-0.037	0.227	0.431*	0.285	-0.114	-0.211	-0.397*	0.933**	0.637**	0.036	-0.500**
SPP_MPH		1	0.823**	-0.566**	0.676**	0.957**	0.452**	-0.643**	0.313	0.377*	0.261	0.329	-0.079	-0.148	-0.56**	0.615**	0.921**	0.429*	-0.649**
SY_MPH			1	-0.285	0.476**	0.830**	0.760	-0.419*	0.301	0.369*	0.240	0.573**	0.031	0.005	-0.702**	0.411*	0.785**	0.576**	-0.539**
TSW_MPH				1	-0.603**	-0.541**	-0.033	0.852**	-0.408*	0.077	-0.236	0.053	0.486**	-0.020	0.058	-0.576**	-0.468**	-0.117	0.605**
PH_BPH					1	0.684**	0.052	-0.603**	0.140	0.171	0.486**	0.232	-0.140	-0.111	-0.382*	0.963**	0.698**	0.113	-0.594**
SPP_BPH						1	0.482**	-0.562**	0.423*	0.329	0.203	0.328	-0.043	-0.066	-0.602**	0.597**	0.910**	0.425*	-0.714**
SY_BPH							1	-0.027	0.257	0.45**	0.311	0.667**	-0.089	0.076	-0.404*	-0.009	0.461**	0.707**	-0.292
TSW_BPH								1	-0.321	0.131	-0.197	0.107	0.423*	0.037	0.200	-0.577**	-0.519**	-0.158	0.530**
OC_BPH									1	-0.181	-0.065	-0.100	0.076	0.580**	-0.022	0.074	0.209	0.346	-0.190
SPP_SCA										1	-0.35	0.08	.026	0.021	0.031	0.052	0.561	0.021	0.214
PH_SCA											1	0.338	-0.350	-0.201	0.014	0.512**	0.306	0.335	-0.156
SY_SCA												1	0.047	-0.030	-0.219	0.249	0.371*	0.600**	-0.119
TSW_SCA													1	0.015	-0.030	-0.177	-0.140	-0.005	0.457**
OC_SCA														1	-0.058	-0.077	-0.128	-0.043	-0.093
PM_SY															1	-0.346	-0.624**	0.069	0.691**
F1_PH																1	0.681**	0.065	-0.561**
F1_SPP																	1	0.274	-0.394*
F1_SY																		1	0.060
F1_TSW																			1

MD: SSR based genetic distance, GD: Quantitative trait based genetic distance, PH_MPH: Mid parent heterosis of plant height, SPP_MPH: Mid parent heterosis of total siliquae per plant, SY_MPH: Mid parent heterosis of seed yield per plant, TSW_MPH: Mid parent heterosis of 1000-seed weight, OC_MPH: Mid parent heterosis of oil content, , PH_BPH: Better parent heterosis of plant height, SPP_BPH: Better parent heterosis of total siliquae per plant, SY_BPH: Better parent heterosis of seed yield per plant, TSW_BPH: Better parent heterosis of 1000-seed weight, OC_BPH: Better parent heterosis of oil content, PH_SCA: Specific combining ability for plant height, SPP_ SCA: Specific combining ability for total siliquae per plant, SY_ SCA: Specific combining ability for seed yield per plant, TSW_ SCA: Specific combining ability for 1000-seed weight, OC_ SCA: Specific combining ability for oil content, PM_SY: Parental mean of seed yield per plant, F1_PH: Hybrid mean of plant height, F1_SPP: Hybrid mean of total siliquae per plant, F1_SY: Hybrid mean of seed yield per plant, F1_TSW: Hybrid mean of 1000-seed weight.

siliquae per plant (0.49**). Further, the correlation between molecular genetic distance and parental mean of seed yield per plant was found non-significant and negative (-0.31) while, it was non-significant and positive with hybrid mean of seed yield per plant. Molecular genetic distance showed significant linear regression on mid parent heterosis of seed yield per plant (Fig. 4), indicated that molecular genetic distance could be good predictor of mid parent heterosis for seed yield per plant. However, it was found non-associated with better parent heterosis of seed yield per plant, that might be due to less genome coverage of the used SSR markers.

Quantitative trait based morphological genetic distance showed significant positive correlation with mid parent heterosis of 1000-seed weight (0.38*), parental mean of seed yield per plant (0.47**) and hybrid mean of 1000-seed weight. It also showed significant linear regression on mid parent heterosis of 1000-seed weight revealing that it could be used as predictor for estimation of heterosis for 1000 seed weight. However, morphological genetic distance showed significant negative correlation (-0.53**) and regression with mid parent heterosis for seed yield per plant and better parent heterosis of seed yield per plant (-0.37*) (Fig. 5). In a similar way, Bansal *et al.* (2012) also reported non-significant correlation between morphological genetic distance and heterosis for seed yield in *B. nigra*. The correlation between morphological genetic distance and hybrid mean of seed yield per plant (-0.20) was also found negative and non-significant, which was reported earlier also by Girke *et al.* (2011) and Jesske *et al.* (2013) in *B. napus*. The study indicates that the quantitative traits based morphological genetic distance is less effective in prediction of heterosis and hybrid performance for seed yield, however molecular marker based genetic distance can effectively predict the heterosis provided the markers covering whole genome to be deployed.

SCA for seed yield per plant showed significant positive association and regression with mid parent heterosis for seed yield per plant (0.57**) and better parent heterosis of seed yield per plant (0.67**) (Fig. 6). Furthermore, significant positive association of SCA of total siliquae per plant, plant height, 1000-seed weight and oil content was observed with their respective mid parent heterosis and better parent heterosis indicating that SCA is good predictor of heterosis and can be used reliably. Hybrid mean of seed yield per plant exhibited significant positive association and linear regression with mid parent heterosis for seed yield per plant (0.58**), better parent heterosis of seed yield per plant (0.71**) and SCA for seed yield per plant (0.60**). Hybrid mean of 1000-seed

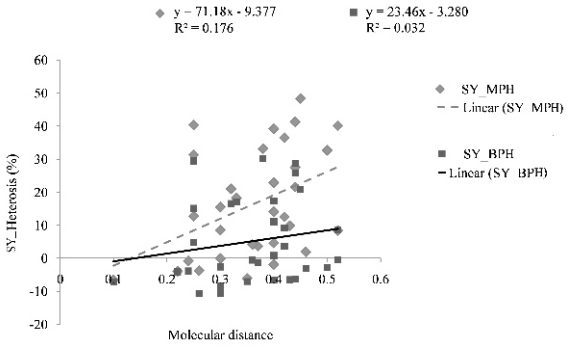

Figure 4: Regression plot of association of molecular genetic distance with seed yield heterosis in Indian mustard.

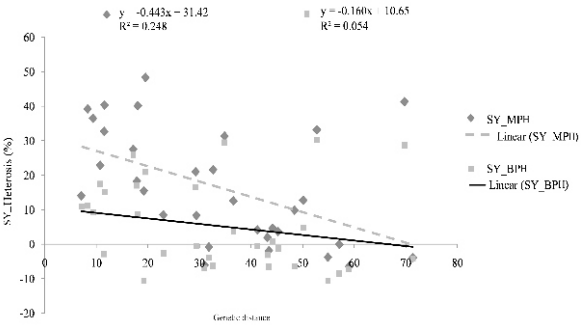

Figure 5: Regression plot of association of morphological genetic distance with seed yield heterosis in Indian mustard

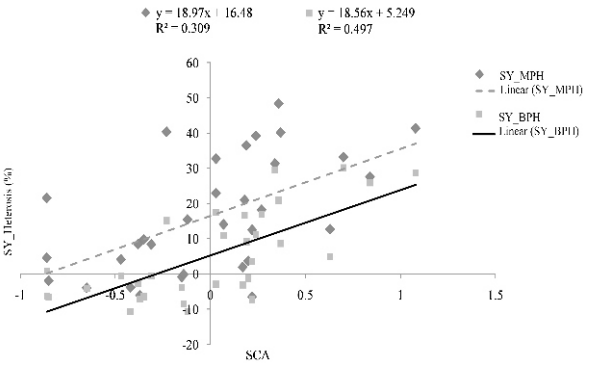

Figure 6: Regression plot of association of specific combining ability (SCA) with seed yield heterosis in Indian mustard

weight was also found significantly associated with mid parent heterosis for 1000-seed weight (0.61**), better parent heterosis of 1000-seed weight (0.53**) and SCA for 1000-seed weight (0.46**). Moreover, parental mean for seed yield per plant did not show positive correlation with molecular genetic distance, heterosis, SCA and *per se* performance of hybrids reflecting its non-significant contribution in prediction of hybrid performance for this

trait. Devi and Singh (2011) while working on maize also reported that the parental mean cannot be used for prediction of hybrid performance as well as selection of parents. The present study shows that SCA is an important determinant of heterosis in Indian mustard. Also the molecular marker based genetic distance is more effective than quantitative traits based genetic distance in prediction of heterosis.

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