Predicting Heterosis and F_1 Performance based on Combing Ability and Molecular Genetic Distance of Parental Lines in Ethiopian Mustard

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Abstract: Ethiopian mustard (*Brassica carinata* A. Braun) is one of the oldest oil crops cultivated and utilized by farmers for many purposes. However, it is one of the most neglected and least genetically studied crops. The improvement of the crop mainly depends on line breeding, but the crop is amenable to heterosis breeding. However, information regarding heterosis is scanty and the identification of parental lines from phenotypic observation is expensive and time-consuming. Therefore, this study was conducted to determine the association of genetic distances of seven *Brassica carinata* A. Braun lines measured by random amplified polymorphic DNA (RAPD) markers with heterosis, F_1 performance, and general combining ability (GCA). The study was aimed at comparing the effectiveness of parental GCA effects and genetic distance in predicting heterosis and F_1 performance. Seven *Brassica carinata* lines and their 21 F_1s generated in a half diallel fashion were evaluated in a replicated field trial for two years (2009/10 and 2010/11) at G.B. Pant University, India. Per se performances and GCA effects of the parents, heterosis and F_1 performance were calculated based on mean values from the two years for 13 traits. Correlations were computed among genetic distance, heterosis, GCA, and F_1 performances. Genetic distances among the parents were calculated from 95 random amplified polymorphic DNA (RAPD) markers and dendrogram was constructed using Unweighted Pair Group Method with Arithmetic Means (UPGMA) method, which effectively grouped the parental lines in to three major clusters. The measured genetic distance was significantly correlated with parental GCA sum only for plant height ($r = 0.6$) and percent oil content ($r = 0.55$). However, the correlations with mid and better parent heterosis and F_1 performance were non-significant for all traits except a negative and significant correlation observed between genetic distance and better parent heterosis for length of main shoot. The correlation between GCA and F_1 performance was positive and significant for most of the traits. Mid and better parent heterosis had positive and significant correlations with GCA for days to 90% maturity, length of main shoot, and number of secondary branches. In addition, better parent heterosis of number of seeds per pod was positive and significantly correlated with the GCA of the parents. Correlation of GCA effects and parental performance was positive for all traits and significant in most cases. It could be concluded that molecular marker based distances is not a reliable predictor of heterosis, combining ability, and F_1 performance whereas GCA is better in predicting heterosis, parental line, and F_1 performances for the crop species.

Keywords: *Brassica carinata* A. Braun; General Combing Ability; Genetic Distance; Heterosis; Random Amplified Polymorphic DNA (RAPD)

1. Introduction

Brassica carinata A. Braun evolved as a natural cross between *Brassica nigra* (BB) (n=8) and *Brassica oleracea* (CC)" (n=9), in the highlands of the Ethiopian plateau, the adjoining portion of East Africa and the Mediterranean coast with underwent further chromosomal doubling (2n = 34) (U, 1935 cited by Gomez-Campo and Prakash, 1999). Ethiopian mustard is one of the oldest oil crops cultivated in Ethiopia (Simmonds, 1979). Farmers grow the crop as a leafy vegetable in their gardens at altitudes between 1500 and 2600 m.a.s.l. Traditional utilization of this crop embraces quite an array of purposes including ground seeds are used to grease a bread-baking clay pan, cure certain ailments or stomach upsets, the leaves of young plants are good source of vegetable relish and to prepare beverages. It also plays a role as a break crop for the cultivation of cereals with comparable ecological amplitude (Nigussie *et.al.,* 1997). The crop is also the third most important oil crop next to niger seed (*Guizotia abyssinica* Cass.) and linseed (*Linum usitatissimum* L) (CSA, 2003). It is higher yielding, more resistant to diseases, insect pests, and resistance to seed shattering than *Brassica napus* with the additional agronomic advantages of better tolerance for semi-arid conditions (Knowles *et al.,* 1981; Malik, 1990). Hence, the crop can serve as an important source of genes, which are rare in other oilseed *Brassicas*. Because of its

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drought and heat tolerance, the crop is now considered as an alternative to *Brassica napus* and *Brassica juncea* in dryer areas of Canada (Rakow, 1995), Spain (Velasco *et al.,* 1995), Australia (Fletche, 1997), India (Singh, 2003), USA and Italy (Cardone *et al.*, 2003). Besides, the gene source to improve other *Brassicas*, it become the interest of European countries, Canada and Australia for biodiesel production (De Rougement *et.al.*, 1989; Bozzini *et.al.,* 2007; University of Western Australia, 2007).

Line breeding to some extent and mass selection are the dominant breeding methods used to improve Ethiopian mustard. However, development of synthetic or hybrid cultivars have been successful in other oilseed *Brassica* ssp. **(**Becker *et al.*, 1999; Miller, 1999). Ethiopian mustard (BBCC) sharing one of its genome with *Brassica juncea* (AABB) and the other with *Brassica napus* (AACC) (U, 1935 cited by Gomez-Campo and Prakash, 1999) could be amenable for heterosis breeding as to its close relatives. But, information regarding heterosis is scanty where only one published report is available so far (Adefris and Becker, 2005).

Heterosis has been exploited extensively in crop production and has been a powerful force in the evolution of plants. But, one of the most expensive steps in heterosis utilization is the identification of parental combinations that produce superior F_1 hybrids. In maize, heterosis has been extensively exploited and several methods have been developed to predict hybrid performance using genetic markers (Frisch *et al.*, 2010; Maenhout *et al.*, 2010; Schrag *et al.*, 2010; Steinfath *et al.*, 2010). Considering the cost and time required to evaluate hybrid heterosis in the field, the use of genetic markers to predict the best heterotic combinations is the best alternative. Because, DNA molecular markers, i) identify great polymorphism, ii) not influenced by environment, and iii) can be evaluated at any development stages of the crop (Williams *et al.*, 1990).

The prediction of heterosis from parental genetic distance has been of great interest to breeders. This increases the efficiency of hybrid breeding programs since the superior crosses could be predicted before field evaluations through parental line screening. Genetic diversity can be investigated with data from pedigree, morphology, isozymes, storage proteins or DNA markers.Estimated genetic distances can be compared with heterosis from field experiments. However, limitations in traditional methods made the prediction of heterosis difficult (Hinze and Lamkey, 2003). More recently, molecular markers have been used to detect the variation in the DNA sequence underlying the analysis of the existing genetic dissimilarity of the parents. Examples of DNA markers presently used in *Brassica* are restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), random amplified polymorphism DNA (RAPD), and simple sequence repeat (SSR) and single nucleotide polymorphisms (SNP). These markers have the advantage of simplifying the screening of parents, which can be done directly in the DNA evaluation (Liu *et al.*, 2002; Adefris and Becker 2005; Balestre *et al.*, 2008; Dandolini *et al.*, 2008; Silva *et al.*, 2009; Riaz *et al.* 2011).

There are reports indicating that random amplified polymorphic DNAs (RAPDs) have been successfully used to estimate genetic distance in *Brassica*. Many scientists reported that RAPD is effective in estimating genetic diversity in *Brassica* species (Divaret *et al.* 1999; Wang *et al.*, 2000; Adefris and Becker, 2005; Waqar *et al.*, 2007; Ghosh *et al.* 2009; Wisal *et al.*, 2011). It is believed that genetic differences between parents are the primary cause of heterosis. Therefore, it is important to estimate genetic distance of *Brassica carinata* lines using random amplified polymorphic DNAs and to test the correlation of parental distance with heterosis. To our knowledge, there is only one report on the association of genetic divergence and heterosis in *Brassica carinata* A. Braun (Adefris and Becker, 2005), which call for similar studies to establish genetic divergence as the predictor of heterosis in this crop. It is also necessary to test combining ability of parents as predictor of heterosis and F_1 performance as compared with genetic distance measured from RAPD molecular markers. Therefore, the objectives of this study were i) to asses genetic distances among seven *Brassica carinata* lines using random amplified polymorphic DNA (RAPD) markers, and ii)to determine associations among genetic distances, heterosis, F_1 performance and general combining ability (GCA) effects of parents in the crop species.

2. Materials and Methods

2.1. Estimates of General Combining Ability and Heterosis

Seven parental inbred lines namely; HCO-211, HCO-288, PBC-2005-1, Kiran (bold), Kiran (early), Jayanti and PBC-2006-4 were used in this study. Apart from the self-fertile nature of *Brassica carinata,* the parental lines were selfed before crossing, followed crossing with each other in a half-diallel fashion. A total of 21 F_1 crosses were generated at G. B. Pant University of Agriculture and Technology, Crop Research Center in 2008/09 and 2009/10 cropping seasons. The crossing was done by hand emasculation and bud pollination on 25 to 35 plants per line.

Then, an experiment consisting of the seven parental lines and 21 F_1 progenies was conducted for two consecutive cropping seasons (2009/10 and 2010/11) using a randomized complete block design with three replications. Each plot consisted of three rows of 5 m length and 30 cm inter-row and 10 cm intra-row spacing. All necessary crop management practices were applied as recommended for *Brassica* spp. in the study area.

In both seasons, except days to 50% flowering and 90% plant maturity that were recorded on plot basis, all other phenotypic traits were recorded from the same 10 randomly selected plants of the central row as follows: days to flowering (days from sowing until 50%

of the plants in a given plot produced flowers); days to maturity (days from sowing until about 90% of the pods matured); number of primary branches per plant (counted as the number of productive branches originating from the main stem); number of secondary branches per plant (productive branches developed from the primary branches); pod length (length of six randomly taken pods per plant; two each from bottom-middle-, and top-borne branches); plant height (the length of the main stem measured from the base to the tip of the main stem); length of main shoot (the length of the main shot measured from base of most top primary branch to the tip); number of seeds per pod (number of seeds obtained from the same pods used to estimate pod length were divided by the number of pods); seed yield per plant (the average weight of bulk of seeds obtained from all pods borne by a 10 sampled plant at the central row); 1000-seed weight (g); percentage of oil content (determined by nuclear magnetic resonance spectrometry at Center for National Oil Seed, India); biological yield (10 randomly selected plants harvested from the base, dried and weighted), harvest index (seed yield per plant/biological yield per plant x 100).

Absolute and relative mid and better parent heterosis as increase or decrease of F_1 hybrid over mid and better parent values were computed using Microsoft Excel program for each character with the formulae proposed by Gravois (1994), Fehr (1987), Falconer (1989) and others as follows: absolute mid parent heterosis (AMPH) = F_1–MP and relative mid parent heterosis $\text{MPH (\%)} = \frac{\text{F1}-MP}{\text{MP}}\ x\ 100$, where mid parent value is $\text{MP} = \frac{\text{P1+P2}}{2}$ and absolute better parent heterosis (ABPH) was calculated as F_1-BP and better parent heterosis (BPH%) or heterobeltiosis $= \text{BPH (\%)} = \frac{\text{F1}-\text{BP}}{\text{BP}}\ x\ 100$, where BP was the mean value of the higher performing parent of the hybrid. Significance of mid parent heterosis were tested as per the method proposed by Panse and Sukhatme (1961) where critical difference is calculated for mid parent heterosis as CD = $\left[\sqrt{3xEMS/2r}\right]$ x t value at error degree of freedom and CD for better parent heterosis= $\left[\sqrt{2xEMS/r}\right]$ x "t" value at error degree of freedom; r is number of replications; EMS is error mean square and t is table value of 't' at error degree of freedom at 5% and 1% probability level.

Combining ability analysis was performed according to Griffing's method II Model I (Griffing, 1956). Data analysis was conducted using MSTAT-C 1986 Michigan University statistical software. The data from the F_1 crosses and parents were subjected to analysis of variance for randomized complete block design. Analysis of variance was computed for each season and the error variance ratio of each trait was computed and the homogeneity of error variances was tested against table "F" value at 5% and 54 degree of freedom (Gomez and Gomez, 1984). All the error variance ratios computed for all traits were less than the F" value at 5% probability suggested the homogeneity of error variances. This allowed to calculate the mean values of the two seasons for each trait in each replication and it was used to compute analysis of variance and combining ability analysis on the basis of pooled mean. Further genetic analysis was performed for those parameters in which statistically significant differences existed among genotypes and GCA mean squares.

2.2. Diversity Study Based on RAPD Markers

DNA Extraction and PCR Amplification

Total genomic DNA was extracted from 0.2 g of young leaves. Leaves were taken from three weeks old seedlings from each line grown at G.B. Pant University of Agriculture and Technology Crop Research Center. Leaves were ground using a mortar and a pestle to fine powder and kept under liquid nitrogen in a 2 ml eppendorf tube. The powder was homogenized with 500 µL of DNA extraction buffer (4% SDS, 0.1 M Tris-Hcl, 10 mM EDTA, pH 8.0) and an equal volume of phenol: chloroform: isoamyl alcohol in the ratio of 25: 24: 1 respectively, was added to it. The whole mixture was vigorously shaken for 20-30 second and aqueous phase was recovered by centrifugation at 5000 rpm for 5 min. The supernatant was transferred to a fresh tube and the DNA was precipitated from it by adding $1/10^{th}$ volume of 3 M sodium acetate (pH 5.0) with an equal volume of isopropanol. The DNA was pelleted by centrifugation for 7 minutes, washed twice with ice cold 70% ethanol, dried at 37°C and dissolved in 40-45µg/ml of TE buffer (10 mMTris-Hcl, 1mM EDTA, pH 8.0) containing 40 µg/ml RNAse A. The concentration of DNA was estimated by comparing its intensity with that of the λDNA of known concentration on a 0.8% agarose gel ithTris borate EDTA (TBE) buffer. The DNA was diluted with double distilled, autoclaved and de-ionized water at the ratio of 1:5 concentrations for use in PCR.

Twelve random primers were used for PCR amplification (Table 2). The reactions were carried out in a 25 µl volume containing 1 x reaction buffer [200 mMTris–HCl, pH 8.55, 160 mM $(NH4)_2SO_4$ 0.1% (v/v)], 3.0 mM $MgCl_2$, 0.4 mM of dNTPs (dATP, dCTP, dGTP and dTTP), 0.16µM primer, 1.0 U of Taq DNA polymerase and 25 ng of genomic DNA template. DNA amplifications were performed in thermocycler programmed as indicated in the following table.

Table 1. DNA amplifications.

Cycle	Denaturation Temperature	Time	Annealing Temperature	Time	Extension Temperature	Time
First cycle	94°C		4 min.			
35 cycle	94°C	1 min.	36°C	2 min	72°C	2 min
Last cycle					72°C	10 min

Table 2. Detailed description and sequence information of primers.

Primer	Sequence	Size	TM^{0}C	GC content (%)	Mol.Wt (Da)
OPA-03	5'AGTCAGCCAC 3'	10bp	34.3	60	2997
OPA-04	5'AATCGGGCTG 3'	10bp	35.1	60	3068
OPA-07	5'GAAACGGGTG 3'	10bp	33.2	60	3117.1
OPA-10	5'GTGATCGCAG 3'	10bp	33.1	60	3068
OPA-11	5'CAATCGCCGT 3'	10bp	36.7	60	2988
OPA-18	5'AGGTGCCGTT 3'	10bp	38.1	60	3059
AC-11	5'CCTGGGTCAG 3'	10bp	35.1	70	3044
AC-20	5'ACGGAAGTGG 3'	10bp	34.3	60	3117.1
TIBMBB-17	5'ACGGAAGTGG 3'	10bp	41	60	2973
TIBMBB-02	5'TGCTCGGCTC 3'	10bp	40.1	60	2995
TIBMBB-13	5'CTTCGGTGTG 3'	10bp	32.7	60	3050
TIBMBB-16	5'CTGGTGCTCA 3'	10bp	34.3	60	3019

TM^{0}C = Melting temperature of primer, bp = base pair, Mol.Wt (Da) = Molecular weight of the primer.

Then samples were stored at 4^{0}C until the RAPD fragments were separated by electrophoresis using 1.8% agarose gel and visualized with ethidium bromide under UV light.

For data analysis, total number of bands and number of polymorphic bands generated by each primer were determined. For statistical analysis, all the scorable bands were considered as single locus/allele. The loci were scored as present (1) or absent (0). Bivariate 1-0 data matrix was generated. Jaccard's coefficient of similarity (JS) was calculated from polymorphic RAPD bands as JSjk = $\left[\frac{N11}{N11+N10+N01}\right]$ (Jaccard, 1908) as cited by Adefris and Becker (2005) and others where; JS_{jk} = is similarity between parents j and k;N11 is number of bands present in both parents; N10 is number of bands present only in parent j; N01 is number of bands present in parent k.

Similarities were computed using NTSYS-pc version 2.1 (Rohlf, 2001). The distance matrix from molecular markers was used to construct dendrograms based on the Unweighted Pair Group Method with Arithmetic Means (UPGMA) (Nei and Li,1979)using the same NTSYS software. Distances of lines were calculated from JS as Jaccard distance/genetic distance (JD/GD) = 1- JS, where Jaccard's coefficient of similarity was computed as indicated above.

2.3. Correlation of Heterosis, General Combining Ability, and Genetic Distance

Correlations of parental genetic distances with absolute mid parent heterosis (AMPH), absolute better parent heterosis (ABPH), GCA sum of parents and F_1 performance were computed. GCA sum is calculated as the sum of two parents' GCA effects involved in producing the hybrids under consideration. Correlation coefficients were also computed to detect associations of GCA sum of parents with mid parent heterosis and F_1 performance for each trait. In addition, correlation was calculated for parental performance and their GCA effects. Correlation was computed using STATISTICA 7 basic statistical analysis software (STATISTICA Software, 2002).

3. Results

3.1. Analysis of Variance and Mean Performance of Genotypes

Analysis of variance for data from each cropping season as well as for pooled means over the two years showed significant genotypic differences for all yield and yield related traits studied (Tables 3 and 4). GCA mean squares were highly significant for all traits except for number of pods per plant (Table3).

Table 3. Mean squares for yield and yield related traits from the pooled mean analysis of variance in a 7x7 diallel cross of Ethiopian mustard (*Brassica carinata* A. Braun), 2009/10 and 2010/11.

Trait	Genotype (27)	GCA (6)	Error (54)
Days to 50% flowering	606.01**	1465.10**	18.69
Days to 90% plants maturity	147.09**	468.53**	18.93
Plant height (cm)	367.98**	631.10**	150.19
Length of main shoot (cm)	373.14**	1151.45**	69.90
Number of primary branches	10.63**	23.99**	1.95
Number of secondary branches	151.51**	277.31**	34.67
Number of pods per plant	24837.14**	9006.99	6698.91
Pod length (cm)	0.11**	0.192**	0.03
Number of seeds per pod	2.76**	4.22*	1.51
Seed yield per plant (g)	40.01**	52.15*	21.55
Thousand seeds weight (g)	0.18*	0.433**	0.12
Biological yield (g)	1118.38**	1161.6*	466.75
Harvesting index (%)	9.85**	7.604*	3.08
Percent oil content (%)	5.52**	15.59**	0.96

** & **, significant $P < 0.05$ and $P < 0.01$, respectively. Numbers in parenthesis indicates degree of freedom.*

The mean values of F_1 hybrids were higher than the values for parents for all traits except for seed oil content, for which early flowering and early maturing are considered as desirable traits. Moreover, five best performing genotypes out of the 28 (21 hybrids & 7 parental lines) were identified of which all or four were hybrids. Among the parents, P1 for early flowering and maturing, P2 for number of seeds per pod and harvest index, P3 for seed yield per plant and harvest index, P5 for pod length and seed oil content, P6 for plant height and number of primary branches and P7 for harvest index were selected among the five best performing genotypes (Table 5).

Table 4. Mean squares from analysis of variance of separate years (2009/10 & 2010/11) and error ratio variance in a 7x7 diallel cross of Ethiopian mustard (*Brassica carinata* Braun).

	2009/10 cropping season			2010/11 cropping season			Error variance ratio
Trait	Replication (2)	Genotype (27)	Error (54)	Replication (2)	Genotype (27)	Error (54)	
Days to 50% flowering	83.61	606.01**	18.69	190.23	583.68**	25.29	1.35
Days to 90% plants maturity	44.3	147.09**	18.93	214.1	175.07**	23.75	1.25
Plant height (cm)	428.57	367.98**	150.19	4878.45	775.67**	206.35	1.37
Length of main shoot (cm)	57.07	373.14**	69.9	130.82	402.81**	61.83	1.13
Number of primary branches	9.08	10.63**	1.95	12.95	11.11**	2.35	1.21
Number of secondary branches	74.01	151.51**	34.67	359.52	99.15**	36.3	0.96
Number of pods per plant	37853.01	24837.14**	6698.91	122677.18	16062.77**	5698.02	1.18
Pod length (cm)	0.01	0.11**	0.03	0.01	0.19**	0.03	1.00
Number of seeds per pod	0.01	2.76**	1.51	0.87	2.56**	1.23	1.23
Seed yield per plant (g)	64.89	40.01**	21.55	243.29	50.93**	18.77	1.15
Thousand seeds weight (g)	0.43	0.18*	0.12	0.28	0.18**	0.11	1.09
Biological yield (g)	1653.77	1118.38**	466.75	6052.04	1245.15**	399.54	1.17
Harvesting index (%)	12.36	9.85**	3.08	53.31	5.87**	3.89	1.26
Percent oil content (%)	0.87	5.52**	0.96	0.004	4.40**	0.703	1.37

** & **, significant at P < 0.05 and P < 0.01, respectively. Numbers in parenthesis indicates degrees of freedom.*

Table 5. Summary of mean values of genotypes and five best performing genotypes for 14 yield and yield related traits in desired direction in a 7x7 diallel cross of Ethiopian mustard (*Brassica carinata* Braun).

DAF (50%)		DAM (90%)		PLH (cm)		LMS (cm)		NPB		NSB		NPP	
Geno.	Perf.	Geno.	Perf.	Geno.	Perf.	Geno.	Perf.	Geno.	Perf.	Geno.	Perf.	Geno.	Perf.
P2 x P3	67	P1 x P3	144	P5 x P7	236.9	P1 x P4	75.33	P5 x P7	16	P5 x P7	39	P4 x P6	484
P2 x P4	67	P1 x P4	144	P2 x P5	225.2	P1 x P7	74.93	P4 x P6	14	P4 x P6	39	P4 x P5	436
P2 x P6	67	P1 x P5	144	P6	224.8	P1 x P2	72.87	P6	13	P2 x P6	35	P5 x P7	431
P1	69	P1	146	P5 x P6	224.2	P2 x P6	72.7	P5 x P6	13	P2 x P5	35	P2 x P4	395
P1 x P4	69	P1 x P2	146	P3 x P6	222.4	P1 x P3	71.47	P3 x P6	13	P4 x P5	32	P2 x P5	377
Mean parents	92		155		203.7		51.34		10		18		182
Mean F_1s'	79		153		211.3		58.27		11		27		318
Grand mean	83		153		209.4		56.53		11		24		284
CD (5%)	8.65		8.7		24.51		16.72		2.8		12		164
CD (1%)	11.5		11.57		33.39		22.2		3.71		16		218
CV (%)	5.24		2.84		5.85		14.79		12.6		24.1		28.9

DAF (50%) = days to 50% flowering, DAM (90%) = days to 90% plants maturity, PLH (cm) =plant height, LMS (cm) = length of main shoot, NPB = number of primary branches, NSB = number of secondary branches per plant, NPP = number of pods per plant, Geno = genotype, Perf = performance, P1=HCO-211, P2 = HCO-288, P3 = PBC-2005-1, P4 = Kiran (Bold), P5 = Kiran (Early), P6 = Jayanti, P7 = PBC-2006-4, CD (5%) = critical difference at 5% probability, CD (1%) = critical difference at 1% probability and CV (%) = coefficient of variation.

Table 5. Continued

POL (cm)		NSP		SYP (g)		TSW (g)		BIOY (g)		HI (%)		% Oil	
Geno.	Perf.	Geno.	Perf.	Geno.	Perf.	Geno.	Perf.	Geno.	Perf.	Geno.	Perf.	Geno.	Perf.
P1 x P5	4.15	P2 x P5	16	P4 x P6	24.05	P2 x P3	4.35	P2 x P4	135	P7	20.43	P5	43.15
P2 x P7	4.14	P2	16	P2 x P4	23.48	P2 x P7	4.28	P2 x P7	127.7	P4 x P6	19.44	P5 x P6	42.88
P5	4.13	P4 x P7	15	P5 x P7	22.42	P2 x P4	4.13	P5 x P7	125	P1 x P3	19.24	P5 x P7	42.34
P1 x P2	4.12	P3 x P4	15	P2 x P5	21.13	P3 x P5	4.11	P4 x P6	125	P3	19.14	P3 x P6	42.14
P2 x P6	4.11	P1 x P6	15	P3	20.68	P3 x P6	4.1	P2 x P6	115	P2	19.04	P3 x P5	41.81
Mean parents	3.8		14		16.64		3.69		83.79		18.68		41.01
Mean F_1s'	3.9		14		16.79		3.82		97.79		17.14		40.72
Grand mean	3.9		14		16.75		3.79		94.3		17.53		40.79
CD (5%)	0.3		2. 5		9.28		0.69		43.21		3.51		1.25
CD (1%)	0.4		3.3		12.35		0.92		57.47		5.06		1.70
CV (%)	4.22		8.79		27.87		9.11		22.89		10.01		2.41

POL (cm) = pod length, NSP = number of seed per pod, SYP (g) = seed yield per plant, TSW (g) = thousand seeds weight, BIOY (g) = biological yield, HI (%) = harvest index, % Oil = percent oil content, Geno = genotype, Perf = performance, P1 = HCO-211, P2 = HCO-288, P3 = PBC-2005-1, P4 = Kiran (Bold), P5 = Kiran (Early), P6 = Jayanti, P7 = PBC-2006-4, CD (5%) = critical difference at 5% probability, CD (1%) = critical difference at 1% probability and CV (%) = coefficient of variation.

3.2. Estimates of General Combining Ability and Heterosis

Estimates of general combining ability (GCA) effects showed that parental lines had either positive or negative significant GCA effects for all traits except for seed yield per plant, biological yield, and harvest index (Table 6). Among the parents, P5 (Kiran early) and and P6 (Jayanti) had positive and significant general combining ability (GCA) effects for 7 and 6 out of 13 traits, respectively, including seed oil content. These parents could be considered as good combining parents whereas other five parents had positive GCA effects only for three and two traits and negative significant GCA effects at least for two and three traits. Particularly, P1 (HCO-211) showed negative and significant GCA effects for six traits that can be considered as poor combiner parent.

The magnitude of mid parent heterosis varied for the different traits and cross combinations (Table 7). Mid parent heterosis ranged from -42.18 for harvest index to 100% for number of secondary branches. Hybrid mean MPH ranged from -13.23% for days to 50 flowering to 48.13% for number of secondary branches. All hybrids showed negative mean MPH for four traits namely; days to 50% flowering, days to 90% maturity, harvest index and percent oil content. On the other hand, all hybrids displayed positive MPH (%) for number of secondary branches, which ranged from 11.76 to 100%. More than half of the hybrids (11 and above) exhibited MPH for 10 traits. Nine and seven hybrids displayed positive MPH for number of seeds per pod and percent oil content, respectively. Minimum number of hybrids displaying positive MPH was for harvest index. Three heterotic hybrids, P1 x P3, P2 x P4 and P2 x P5 recorded positive and significant MPH (%) for seven traits followed by P5 x P7 which registered positive and significant MPH (%) for six traits. Other seven hybrids (P1 x P2, P1 x P7, P2 x P7, P3 x P6, P4 x P5, P4 x P7 and P6 x P7) also displayed highest in magnitude and positive significant MPH (%) for five traits.

Varied number of F_1 hybrids displayed better parent heterosis (BPH%) in both direction ranging from -43.96 (harvest index) to 137.95% (number of pods per plant). Among the 21 F_1 hybrids, 15 for number of secondary branches, eight for pod length and thousand seeds weight, six for number of primary branches and seed yield per plant displayed positive and significant better parent heterosis. Among the five heterotic hybrids that registered the highest BPH (%) in the desired direction; P2 x P4 for seven traits, P5 x P6, P2 x P7, P2 x P5 and P2 x P7 for six and P2 x P6 for five traits displayed the highest and significant BPH (%). Three hybrids, P6 x P7, P1 x P3 and P4 x P5 for four traits exhibited the highest and significant BPH (%) (Table 8).

Table 6. Estimates of general combining ability (GCA) effects for yield and yield related traits of seven parental lines in a diallel cross of *Brassica carinata* A. Braun evaluated in 2009/10 and 2010/11.

Parent	DAF (50%)	DAM (90%)	PLH (cm)	LMS (cm)	NPB	NSB	POL (cm)	NSP	SYP (g)	TSW (g)	BIOY (g)	HI (%)	% Oil
P1	-9.33**	-6.95**	-5.54**	11.99**	-1.52**	-4.69**	0.07*	0.12	-1.82	-0.11	-11.6	0.35	-0.81**
P2	-11.1**	-4.95**	-1.38	1.74	-0.48	2.20*	0.08*	0.75**	0.23	0.19**	5.34	-0.4	-0.89**
P3	7.22**	2.09**	0.75	-2.79	-0.63*	-3.47**	0.03	-0.18	-0.55	0.15*	-7.03	0.50	-0.13
P4	3.63**	1.94*	-5.74**	-4.89*	-0.04	0.87	-0.1*	0.08	0.37	-0.14	2.27	-0.11	0.02
P5	5.70**	3.90**	5.63**	-8.25**	0.89**	2.20*	0.07*	0.04	0.79	0.02	3.83	0.01	1.26**
P6	4.22**	2.09**	6.50**	2.97*	1.00**	2.42*	-0.1*	-0.44	-0.10	-0.07	3.05	-0.61	0.64**
P7	-0.37	1.90*	-0.22	-0.77	0.79**	0.46	-0.02	-0.37	1.08	-0.05	4.12	0.27	-0.09
SE (gi)	0.77	0.78	2.18	1.49	0.25	1.05	0.03	0.22	0.83	0.06	3.85	0.31	0.18
SE (gi-gj)	1.18	1.18	3.34	2.28	0.38	1.60	0.05	0.33	1.26	0.09	5.88	0.48	0.27

** & **, significant P < 0.05 and P < 0.01, respectively. DAF (50%) = days to 50% flowering, DAM (90%) = days to 90% plants maturity, PLH (cm) = plant height, LMS (cm) = length of main shoot, NPB = number of primary branches, NSB = number of secondary branches, POL (cm) = pod length, NSP = number of seed per pod, SYP (g) = seed yield per plant, TSW (g) = thousand seeds weight, BIOY (g) = biological yield, HI (%) = harvesting index, % Oil = percent oil content. P1 = HCO-211, P2 = HCO-288, P3 = PBC-2005-1, P4 = Kiran (Bold), P5 = Kiran (Early), P6 = Jayanti, P7 = PBC-2006-4.*

Table 7. Mid parent heterosis for yield and yield attributes in 7x7 diallel cross of *Brassica carinata* evaluated in 2009/10 and 2010/11.

Cross	DAF (50%)	DAM (90%)	PLH (cm)	LMS (cm)	NPB	NSB	POL (cm)	NSP	SYP (g)	TSW (g)	BIOY (g)	HI (%)	% Oil
P1 x P2	15.94**	-1.02	3.27	23.02**	25**	12.5**	7.15**	0.00	-3.37	1.08**	-1.22	-1.14	-2.59**
P1 x P3	-17.16**	-4.95	7.49	22.31**	12.5**	70.37**	1.4**	0.00	13.22**	4.69**	26.55	1.45	1.46**
P1 x P4	-17.37**	-4.95	5.2	39.08**	0.00	22.58**	-1.82**	-7.14**	3.73	4**	14.43	-7.6**	-0.83**
P1 x P5	-17.65**	-6.19*	0.14	5.47	11.11**	33.33**	3.23**	0.00	3.14	1.2**	17.39	-14.86**	-0.59**
P1 x P6	-12.94**	-2.97	3.38	14.21*	10.00**	31.43**	8.14**	15.38**	34.44**	3.55**	46.78**	-5.56**	0.82**
P1 x P7	-14.45**	-4.26	5.27	32.15**	5.26**	37.50**	3.05**	7.69**	3.83	-1.67**	16.81	-7.55**	-1.67**
P2 x P3	-20.71**	-3.27	6.38	2.89	22.22**	69.70**	0.52**	-13.3**	-13.6**	11.68**	7.77	-9.53**	0.38
P2 x P4	-19.76**	-3.27	10.62	19.33**	0.00	67.57**	2.51**	-6.67**	59.84**	12.84**	70.16**	-5.04**	-3.9**
P2 x P5	-15.29**	-4.52	11.82	0.41	30.00**	79.49**	0.63**	6.67**	30.92**	-5.01**	34.5*	-2.55*	-1.88**
P2 x P6	-21.18**	-3.27	1.54	33.55**	-9.09**	70.73**	15.45**	0.00	-23.39**	10.45**	36.9	-42.18**	-1.26**
P2 x P7	-20.23**	-3.9	7.12	30.91**	14.29**	63.16**	11.89**	0.00	16.44**	13.83**	48.17**	-17.46**	-1.71**
P3 x P4	2.02	0.00	-1.89	2.8	0.00	25**	-4.26**	7.14**	-31.34**	0.14	-19.54	-4.64**	-0.89**
P3 x P5	-1.49	1.26	1.25	-7.5	0.00	11.76**	0.87**	0.00	-22.75**	4.85**	-9.97	-4.31**	-0.64**
P3 x P6	-2.49	2.55	3.29	6.27	18.18**	38.89**	5.75**	0.00	-9.36**	10.51**	4.93	-1.00	4.01**
P3 x P7	-11.76**	1.9	4.65	17.66**	14.29**	27.27**	1.32**	0.00	-29.39**	-2.51**	-5.54	-17.46**	1.95**
P4 x P5	-3.52	1.89	2.96	8.1	9.09**	68.42**	-10.1**	-7.14**	24.73**	-3.93**	21.06	4.62**	-2.72**
P4 x P6	-10.55**	3.82	0.89	6.82	16.67**	95**	2.95**	7.69**	52.02**	-1.01**	44.78**	10.64**	-1.38**
P4 x P7	-26.73**	0.00	-3.13	11.92**	-4.35**	40.54**	2.83**	15.38**	-15.33**	4.79**	9.23	-18.14**	-3.91**
P5 x P6	-0.99	2.52	3.2	-17.25**	8.33**	33.33**	-0.67**	7.69**	-26.11**	2.96**	-10.27	-13.8**	1.16**
P5 x P7	-28.78**	1.88	14.46	-9.65	39.13**	100**	0.39**	7.69**	18.00**	-0.92**	32.04*	-7.4**	0.74**
P6 x P7	-32.68**	-5.06	-7.85	29.34**	-20**	12.2**	15.8**	16.67**	-32.82**	1.54**	-21.64	-7.52**	-23.67**
Mean	-13.23	-1.52	3.81	12.94	9.65	48.13	3.19	2.75	2.52	3.48	17.30	-8.14	-1.77
CD (5%)	6.11	6.15	17.33	11.82	1.97	8.33	0.25	1.74	6.57	0.49	30.55	2.48	0.38
CD (1%)	8.13	8.18	23.05	15.73	2.63	11.08	0.33	2.31	8.73	0.65	40.64	3.30	0.51

** & **, significant at P < 0.05 and P < 0.01, respectively. DAF (50%) = days to 50% flowering, DAM (90%) = days to 90% plants maturity, PLH (cm) = plant height, LMS (cm) = length of main shoot, NPB = number of primary branches, NSB = number of secondary branches, POL (cm) = pod length, NSP = number of seed per pod, SYP (g) = seed yield per plant, TSW (g) = thousand seeds weight, BIOY (g) = biological yield, HI (%) = harvesting index, % Oil = percent oil content. P1 = HC)-211, P2 = HCO-288, P3 = PBC-2005-1, P4 = Kiran (Bold), P5 = Kiran (Early), P6 = Jayanti, P7 = PBC-2006-4.*

Table 8. Better parent heterosis for yield and yield attributes in 7x7 diallel cross of *Brassica carinata* evaluated in 2009/10 & 2010/11.

Cross	DAF (50%)	DAM (90%)	PLH (cm)	LMS (cm)	NPB	NSB	POL (cm)	NSP	SYP (g)	TSW (g)	BIOY (g)	HI (%)	% Oil
P1 x P2	15.94**	-2.01	2.94	8.44	11.11**	-5.26	5.37**	-6.25**	-15.23**	-3.10**	-12.56	-1.79	-3.22**
P1 x P3	-30.00**	-7.01	3.85	6.35	0	64.29**	0.76**	0	-12.62**	-0.26	5.85	0.52	1.03
P1 x P4	-29.59**	-7.01	4.08	12.1	-18.18**	5.56	-3.32**	-7.14**	-7.87	2.54**	-1.22	-11.34**	-3.26**
P1 x P5	-30.69**	-9.32*	-4.11	-10.91	-9.09**	10.00*	0.48**	0	-15.07**	-3.57**	-4.26	-16.13**	-4.43**
P1 x P6	-26.73**	-7.01	-4.19	6.06	-15.38**	4.55	0.26	7.14**	11.25**	2.82**	21.24	-7.88**	-1.37
P1 x P7	-28.85**	-8.18*	2.06	11.5	-16.67**	15.79**	-0.77**	0	-19.79**	-3.01**	-5.24	-11.26**	-2.98**
P2 x P3	-33.00**	-5.1	3.11	1.29	22.22**	47.37**	-1.77**	-18.75**	-25.68**	10.97**	0.94	-9.77**	-0.79
P2 x P4	-31.63**	-5.1	9.79	7.53	-9.09**	63.16**	2.37**	-12.50**	57.58**	6.72**	65.3	-9.45**	-5.65**
P2 x P5	-28.71**	-7.45*	7.41	-4.43	18.18**	75.00**	-3.63**	0	21.58**	-5.61**	22.34	-4.62**	-5.08**
P2 x P6	-33.66**	-5.1	-5.62	26.22**	-23.08**	59.09**	8.73**	-12.50**	-28.44**	5.17**	26.37	-43.96**	-2.79**
P2 x P7	-33.65**	-6.29	4.18	24.44**	0	63.16*8	9.52**	-12.50**	0.29	10.59**	33.92	-20.26**	-2.54**
P3 x P4	1.00	0	-4.21	-6.04	-9.09**	11.11*	-6.31**	7.14**	-41.63**	-5.87**	-22.5	-9.30**	-3.81**
P3 x P5	-1.98	-2.48	0.31	-10.61	-9.09**	-5	-1.21**	0	-28.92**	4.85**	-12.77	-6.58**	-3.11**
P3 x P6	-2.97	0	-1.08	-1.04	0	13.64**	-2.53**	-7.14**	-17.07**	4.59**	3.3	-4.28**	1.23
P3 x P7	-13.46**	-1.26	4.28	13.55	0	10.53*	-3.03**	-7.14**	-29.50**	-5.87**	-9.08	-20.07**	0.07
P4 x P5	-4.95	-2.48	-0.38	2.03	9.09**	60.00**	-13.80**	-7.14**	14.33**	-9.69**	13.12	1.87	-4.36**
P4 x P6	-11.88**	0	-5.56	-8.45	7.69**	77.27**	-3.17**	0	40.15**	-1.71**	37.36*	8.79**	-1.65*
P4 x P7	-28.85**	-1.26	-5.09	5.78	-8.33**	36.84**	0.53**	7.14**	-27.93**	1.92**	1.41	-24.47**	-5.00**
P5 x P6	-0.99	0	-0.28	-25.35**	0	27.27**	-10.17**	0	-26.58**	-2.55**	-11.7	-14.65**	-0.63
P5 x P7	-29.81**	0	13	-9.78	33.33**	95.00**	-5.81**	0	8.73*	-4.34**	31.12	-12.38**	-1.88*
P6 x P7	-33.65**	-1.26	-12.05	16.55*	-23.08**	4.55	11.33**	16.67**	-38.46**	-0.55	-23.42	-13.31**	-1.47
Mean	-19.91	-0.04	0.59	3.11	-1.88	34.95	-0.77	-2.52	-8.61	0.19	7.6	-10.97	-2.46
CD (5%)	7.06	7.1	20.01	13.65	2.28	9.62	0.28	2.01	7.58	0.57	35.28	2.87	1.57
CD (1%)	9.39	9.45	26.62	18.15	3.03	12.79	0.38	2.67	10.08	0.75	46.92	3.81	2.05

** & **, significant at P < 0.05 and P < 0.01, respectively. DAF (50%) = days to 50% flowering, DAM (90%) = days to 90% plants maturity, PLH (cm) = plant height, LMS (cm) = length of main shoot, NPB = number of primary branches, NSB = number of secondary branches, POL (cm) = pod length, NSP = number of seed per pod, SYP (g) = seed yield per plant, TSW (g) = thousand seeds weight, BIOY (g) = biological yield, HI (%) = harvesting index, % Oil = percent oil content,. P1 = HCO-211, P2 = HCO-288, P3 = PBC-2005-1, P4 = Kiran (Bold), P5 = Kiran (Early), P6= Jayanti, P7 = PBC-2006-4.*

3.3. RAPD Band Polymorphism and Parental Genetic Distance

Molecular sizes of amplified fragments (bands) ranged approximately between 150 to 2000 bp. In total, 95 RAPD bands were scored across the seven lines. Of these, 58 (61.05%) were polymorphic. Twelve primers generated between 5 and 12 bands with an average of 7.92 bands per primer. Primer OPA-04, OPA-07 and OPA-11, generated 12, 11 and 10 bands, respectively. The primers OPA-04 and OPA- 07 and TIBMBB-16 each with eight had also the highest number of bands. Two others namely; OPA-11 and TIBMBB-13 each with five exhibited relatively higher number of bands as compared to the other seven primers. The number of polymorphic bands ranged from one to ten with an average of 4.83 per primer. Primer OPA-04, OPA-07 and TIBMBB-16 had highest number of polymorphic bands of 10 (83%), 8 (73%) and 7 (88%), respectively (Table 9). Sample of PCR amplification profile of seven *Brassica carinata* lines from 12 RAPD primers are presented in Figures 1 to 2.

Table 9.Polymorphism exhibited by 12 RAPD primers in seven lines of *Brassica carinata* A. Braun.

Sr.No.	Primer Name	Sequence	Number of bands amplified	Number of polymorphic bands	% polymorphism
1	OPA-03	5'AGTCAGCCAC 3'	6	4	67
2	OPA-04	5'AATCGGGCTG 3'	12	10	83
3	OPA-07	5'GAAACGGGTG 3'	11	8	73
4	OPA-10	5'GTGATCGCAG 3'	9	4	44
5	OPA-11	5'CAATCGCCGT 3'	10	5	50
6	OPA-18	5'AGGTGCCGTT 3'	5	3	60
7	AC-11	5'CCTGGGTCAG 3'	6	3	50
8	AC-20	5'ACGGAAGTGG 3'	8	4	50
9	TIBMBB-17	5'ACGGAAGTGG 3'	7	4	57
10	TIBMBB-02	5'TGCTCGGCTC 3'	5	1	20
11	TIBMBB-13	5'CTTCGGTGTG 3'	8	5	63
12	TIBMBB-16	5'CTGGTGCTCA 3'	8	7	88
Average			7.92	4.83	58.75

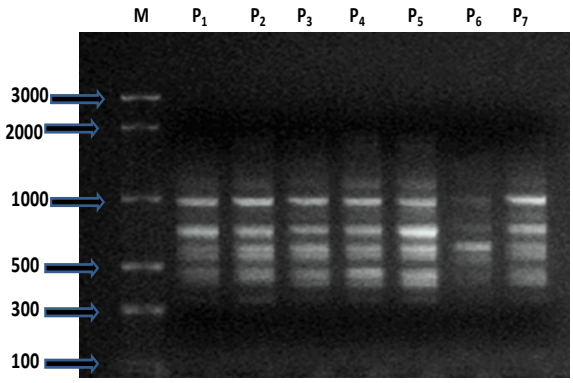

Figure 1. PCR amplification profile of seven lines of *Brassica carinata* using RAPD primer OPA-03.

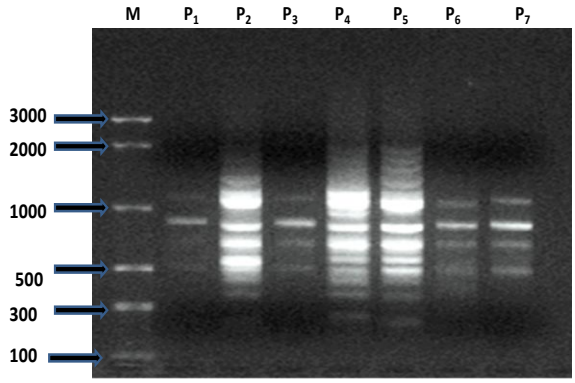

Figure 2. PCR amplification profile of seven lines of *Brassica carinata* using RAPD primer OPA-04P1 = HCO-211, P2 = HCO-288, P3 = PBC-2005-1, P4 = Kiran (Bold), P5 = Kiran (Early), P6 = Jayanti, P7= PBC-2006-4.

On the basis of the data obtained from 12 RAPD primers, a dendrogram was constructed using Unweighted Pair Group of Arithmetic Means (UPGMA) (Nei and Li, 1979). Clustering based on JS (Jaccard's similarity) resulted in the formation of three clusters (Figure 3) of which one cluster consisted only one line i.e. Kiran (early) while the four *Brassica carinata* lines namely; HCO-288, Kiran (bold), PBC-2005-1 and PBC-2006-4 formed the second cluster and the other two lines, namely; HCO-211 and Jayanti formed the third cluster. Within the second cluster two sub groups were observed. The first sub-group comprised HCO-288 and Kiran (bold), while the other sub-group consisted of PBC-2005-1 and PBC-2006-4.

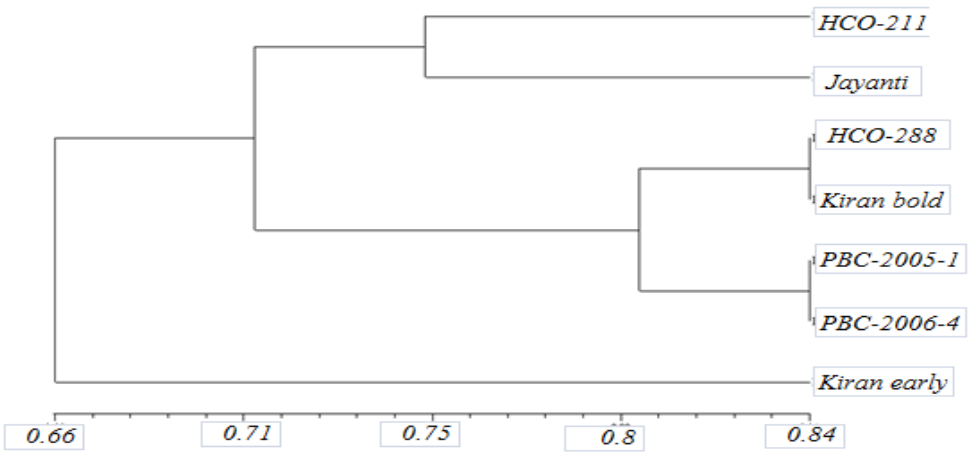

Figure. 3. Dendrogram constructed for seven lines of *Brassica carinata* A. Braun using 12 RAPD primers.

Jaccard's distance (JD) was calculated as 1-JS and JS (Jaccard's similarity) are presented in Table 7. Jaccard's distance (JD) ranged from 0.156 to 0.385 with the mean distance of 0.273 value. Among pairs of the seven lines, HCO-211 and Kiran (early) showed highest distance (0.385) followed by Kiran (bold)) and Jayanti with JD value of 0.375. Kiran (early) and PBC-2006-4, Kiran (early) and Jayanti, HCO-288 and Kiran (early) had JD values of 0.375, 0.365, 0.344 and 0.333, respectively. PBC-2005-1 and PBC-2006-4 and HCO-288 and Kiran (bold) had the lowest JD value (0.156). Other line combinations; PBC-2005-1 and Kiran (bold), Kiran (bold) and PBC-2006-4, HCO-211 and HCO-288 also had the lower distance values of 0.177, 0.187 and 0.198, respectively.

Table 10. Average estimate of Jaccard's distance (above diagonal) and Jaccard coefficients of similarity (below diagonal) among seven lines of *Brassica carinata* A. Braun using 12 RAPD primers.

	P_1	P_2	P_3	P_4	P_5	P_6	P_7
P_1		0.198	0.281	0.292	0.385	0.250	0.229
P_2	0.802		0.208	0.156	0.333	0.323	0.219
P_3	0.719	0.792		0.177	0.312	0.323	0.156
P_4	0.708	0.844	0.823		0.302	0.375	0.187
P_5	0.615	0.667	0.688	0.698		0.344	0.365
P_6	0.750	0.677	0.677	0.625	0.656		0.312
P_7	0.771	0.781	0.844	0.813	0.635	0.688	

P1 = *HCO-211, P2 = HCO-288, P3 = PBC-2005-1, P4 = Kiran (Bold), P5 = Kiran (Early), P6 = Jayanti and P7 = PBC-2006-4.*

3.4. Correlation among Heterosis, Genetic Distances, GCA of Parental Lines and F_1 Hybrids

Parental genetic distance was significantly correlated with parental GCA sum only for plant height (r=0.6) and percent oil content (r=0.55). However, the correlation coefficients between the parental genetic distances and GCA sums of parents were not significant for other traits. In addition, the correlation of parental genetic distance with absolute mid parent heterosis (AMPH) and F_1 performance was not significant for all traits. The correlation between parental genetic distance and absolute better parent heterosis (ABPH) was positive more than half of the traits (7 out of 13), but it was not strong and significant. On the other hand, the correlation between parental genetic distance and ABPH for length of main shoot was negative and significant (Table 11). The correlations between parental GCA sum and AMPH were significant for days to 90% maturity (r = 0.68), length of main shoot (r = 0.64), number of secondary branches (r = 0.41) and number of seeds per pod (r = -0.57). The correlation between GCA sum and ABPH was strong/significant for days to 90% plants maturity (r = 0.69), length of main

shoot (r = 0.46), number of secondary branches (r = 0.49) and biological yield (r = 0.43). Both AMPH and ABPH showed negative and significant correlations with GCA sum for number of seeds per pod. In general, both AMPH and ABPH showed positive correlations with GCA sum for 8 and 11 out of 13 traits, respectively.

Parental GCA sum was significantly correlated with F_1 performances for all traits except for number of seeds per pod (r = 0.26), seed yield per plant (r = 0.27) and harvest index (r = 0.35). This result was supported by the superiority of the hybrids obtained from the crossing of the two parents (Kiran early and Jayanti) that were identified as good combiner. Among the hybrids selected as best five performing genotypes for all traits, at least one hybrid at most all hybrids (plant height, percent seed oil content, number of primary and secondary branches) had one or both parents of these good combiners (Table 5).

Highly significant correlation was observed between parents' GCA effect with their *per se* performance for most of the traits. The correlation between parents' GCA effect with their *per se* performance was non significant but for pod length, seed yield per plant, biological yield, harvest index and percent oil content. The parents for which the trait that had higher performance also showed significant GCA effects. For instance, P1 had negative and significant GCA effects for days to 50% plants flowering and days to 90% plants maturity also identified as one of the five early flowering and maturing genotypes. The other parents, P6 for plant height and number of primary branches, P5 for seed oil content, P2 for number of seeds per pod and P7 for harvest index had positive and significant GCA effects that were also identified as one of the best performing genotypes for the same traits (Table 5 and Table 6).

The correlation between F_1 performance and mean and better parent mean values were positive for most of the traits. However, the correlation between F_1 performance and mean of the parental lines was positive and significant for days to flowering and maturity, length of main shoot, number of primary and secondary branches, thousand seeds weight and percent seed oil content. But better parent mean values showed positive and significant correlation with F_1 performance only for length of main shoot, thousand seeds weight and percent seed oil content. Both mid and better parent heterosis exhibited positive and significant correlations with F_1 performance for all traits except the correlation between better parent heterosis and F_1 performance for percent seed oil content was positive but significant correlation. The two heterosis estimates (mid and better parent heterosis) also showed positive and highly significant correlation for all traits (Table 11).

Table 11. Correlation coefficients of parental lines distances and GCA sum with heterosis and F_1 performance in 7 x 7 diallel crosses of *B. carinata* A. Braun for 13 traits.

	Jaccard's distance with			Parents' GCA sum with			F_1 performance with					
Trait	AMPH	ABPH	F_1 mean	AMPH	ABPH	F_1 mean	PM	BP	AMPH	ABPH	AMPH & ABPH	Parents' GCA & mean
Days to 50% flowering	-0.11	-0.05	0.08	0.08	0.33	0.92**	0.58*	-0.02	0.68**	0.87**	0.87**	0.92**
Days to 90% plants maturity	0.09	0.01	0.19	0.68**	0.69**	0.99**	0.84**	0.36	0.93**	0.93**	0.97**	0.99**
Plant height (cm)	-0.01	-0.18	0.37	-0.08	-0.2	0.89**	0.36	0.29	0.77**	0.60**	0.93**	0.89**
Length of main shoot (cm)	-0.34	-0.45*	-0.15	0.64**	0.46*	0.91**	0.77**	0.79**	0.91**	0.74**	0.90**	0.91**
Number of primary branches	0.16	0.13	0.39	-0.10	0.10	0.95**	0.58*	0.41	0.72**	0.72**	0.89**	0.95**
Number of secondary branches	0.28	0.23	0.39	0.41*	0.49*	0.96**	0.69**	0.34	0.96**	0.96**	0.98**	0.96**
Pod length (cm)	0.03	-0.18	0.05	-0.10	0.07	0.72	0.04	-0.15	0.71**	0.79**	0.91**	0.72
Number of seeds per pod	0.15	0.21	-0.06	-0.57*	-0.59*	0.95**	0.17	0.20	0.62**	0.49*	0.86**	0.95**
Seed yield per plant (g)	0.11	0.11	0.15	-0.03	0.13	0.57	-0.06	-0.09	0.89**	0.88**	0.96**	0.57
Thousand seeds weight (g)	-0.25	-0.21	-0.2	0.38	0.37	0.87**	0.57*	0.53*	0.87**	0.81**	0.93**	0.87**
Biological yield (g)	0.04	0.04	0.15	0.23	0.43*	0.64	0.15	0.18	0.93**	0.97**	0.96**	0.64
Harvest index (%)	0.11	0.14	0.01	0.18	0.17	0.49	-0.03	-0.1	0.95**	0.92**	0.98**	0.49
Percent oil content (%)	-0.01	-0.08	0.2	0.28	0.13	0.64	0.59*	0.68**	0.65**	0.36	0.83**	0.64

** & **, significant at P<0.05 and P<0.01, respectively. PM = parents of the hybrids mean value, BP = better parent mean value of F_1s', F_1 mean = F_1 mean performance, AMPH = absolute mid-parent heterosis, ABPH = absolute better parent heterosis, GCA sum = sum of general combining ability effects of the two parents' involved in producing hybrids.*

4. Discussions

The analysis of variance revealed significant differences among the genotypes (parental lines and F_1 crosses obtained from them) for all the traits. In addition, most or all of the high performing genotypes for all traits were F_1 hybrids. This suggestsa higher chance of creating variations through crossing of elite parental lines to improve the crop. The presence of variation is critical in any crop improvement which may be found in natural populations or induction created through crossing (Lewontiny and Birch, 1966; Stebbins, 1973; Eric *et al*., 2001).

Considerable number of hybrids exhibited positive and significant mid and better parent heterosis. The observed heterosis included the most economically important traits, namely, seed yield and seed oil content where 8 and 6 F_1s' displayed positive and significant MPH and BPH (%), respectively, though none of the hybrids exceeded their higher performing parents for seed oil content. This showed that hybrid production is important for the improvement of most of the traits including seed yield and early maturity in Ethiopian mustard. Negative or absence of heterosis for oil content is a common phenomenon in oil seed *Brassicas* (Banga and Labana, 1984; Brandle and McVetty, 1990;Schuler *et al*., 1992; Falk *et al*., 1994; Adefris and Becker**,** 2005). Heterosis for oil content could be much appealing, but the available experience in *Brassica napus* indicates that it is not an essential prerequisite for the success of hybrids as far as oil yield per plant could be maximized through higher yield. Early maturing might be considered as an advantage of hybrids for seed production in areas where growing seasons are short (short rainy seasons not supplemented with irrigation). But this might not be considered as a disadvantage in areas where the crop is used as a leafy vegetable where late (delayed) flowering is desired. The magnitude of heterosis observed in the present study was lesser than the magnitude of heterosis reported by Adefris and Becker (2005) for *Brassica carinata* which bMPH (%) for seed yield varied from 25.1 to 145.4 with a mean of 67%. However, the magnitude of MPH (%) observed in this study was higher than 15% (Leon, 1991) and 42% (Diers *et al*., 1996) in *Brassica napus* and 19% (Banga and Labana, 1984) in *Brassica juncea*. The magnitude of BPH (%) observed in this study across the traits was in the range of 50% (Pradhan *et al*., 1993) in *Brassica juncea*, and 69% (Brandle and McVetty, 1989) and 67% (Riazbb *et al.,* 2001) in *Brassica napus*.

In this study, RAPD was efficient in estimating the genetic distances of seven parental lines by grouping in three major clusters. The effectiveness of RAPD to estimate genetic distances was reported in *Brassica* species (Divaret *et al*., 1999; Wang *et al*., 2000; Adefris and Becker, 2005; Waqar *et al*., 2007; Ghosh *et al*., 2009 and Wisal *el al*., 2011). However, the estimated genetic distances of parents showed low correlation coefficients with heterosis and/or F_1 performance which could be attributed mainly to inadequate genome coverage, random dispersion of molecular markers and different levels of dominance. There are various reports on the extent of correlation between genetic distance and heterosis for various traits. Qian *et al*. (2007) observed a weak correlation between genetic distance and heterosis for interspecific crosses of European spring and Chinese semi winter lines. Kaur *et al*. (2007) observed a negative correlation between genetic diversity and hybrid performance in diverse morpho types of *Brassica rapa*.

For *Brassica carinata,* Adefris and Becker (2005) reported absence of correlation between the observed heterosis and genetic distance measured from RAPD markers. Riaz *et al*. (2011) also reported non-significant correlations between the genetic distance (GD) using SRAP molecular markers and oil content, plant height and maturity in *Brassica napus*. Similar to the results of other researchers, the results of this study confirmed that even though PCR based assays of RAPD estimate genetic distances of parents effectively, the estimated genetic distance could not precisely predict heterosis.

General combining ability effect of a population is an indicator of the relative value of the population in terms of frequency of favorable genes and of its divergence as compared to the other populations (Viana *et al*.,1999). The positive and significant correlations observed between GCA sum and F_1 performance for most traits and the relatively significant correlations of GCA sum with AMPH and ABPH for more traits, compared genetic distance measured from RAPD, indicates the importance of selecting parents on the basis of their GCA effect in producing hybrids with high performance. Similar to the current finding, Adefris and Becker (2005) reported positive and significant correlation of GCA sum with AMPH in *Brassica carinata*. This implies the importance of selecting parental lines on the basis of their combining ability to produce heterotic hybrids.

The observed strong correlations between F_1 performances and parents GCA effects for most of the traits and highly significant correlations of F_1 performances with both mid and better parent heterosis except in the case of better parent heterosis for seed oil content indicate the importance of additive gene action. However, the correlation coefficient did not attain unity (one) or drops to zero for any one of the traits. This indicates the involvement of epistasis other than dominance interactions in expression of F_1 performances and of heterosis. In a line crossing, the correlation of breeding values will be one if additive system is functioning, but if epistasis is functioning it drops below one and further falls to zero if dominance is involving in epistatic interactions (Pray and Goodnight, 1995; Goodnight, 1999). Poorer average performance of recombinant is explained by loss of favourable epistatic interaction present in the parents (Engquist and Becker, 1991). On the basis of the observed results and as suggested by Singh and Singh (1981), hybridization followed by selection could be suggested as a breeding procedure to develop pure-line

cultivars by taking the advantage of additive type epistasis (additive x additive) in all traits.

The observed positive and strong correlations of GCA sum of parents with parents and hybrid performances suggested that the parental lines included in this study performed well as a line as well as a parent in producing hybrids. This may be a good indicator for breeders to select their materials initially on the basis of parents' performance per se that can predict combining ability of parents and consequently the performance of crosses. Genetically, GCA is a consequence of additive gene action (Henderson, 1952; Welsh, 1981 and Falconer, 1989). If additive gene action is predominant in self-pollinated species, then the breeder can effectively select at various levels of inbreeding, because additive effects are readily transmissible from one generation to another (Gravois and Mc new, 1993).

5. Summary and Conclusion

The findings of this study indicated the possibility of predicting hybrid performances based on the general combining ability of parents. The strong and positive correlation between parents GCA effect and their performance also suggested that i) parental lines included in this study performed well as lines per se and also in hybrid combinations, ii) parents with desirable per se performances showed better combining ability and consequently hybrids performance, and iii) this may support the usual practice of breeders in selecting their materials on the basis of parental performance to include in crossing programs either to obtain high performing hybrids or to develop pure line cultivars through selection after hybridization followed by repeated self-pollination. This study also revealed that RAPD markers are effective in estimating genetic distances of few parental line of *Brassica carinata*, but parental distance computed from markers had no strong correlations with the observed heterosis, F_1 performance and parental GCA sum. This may be the consequence of using few number of primers that resulted inadequate genome coverage, random dispersion of molecular markers and different levels of dominance. Therefore, the future research should have to be directed towards the use primers as many as possible for adequate coverage of the genome. In addition, it is necessary to estimate distances of parental lines from phenotypic traits to predict heterosis in comparison to distances from molecular markers. The study generally suggested the importance of selecting materials initially on the basis of performance per se that can predict combining ability of parents and consequently the performance of crosses.

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