Theor Appl Genet (2006) 112: 752–759
DOI 10.1007/s00122-005-0180-3

ORIGINAL PAPER

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Comparison of phenotypic and molecular distances to predict heterosis and F_1 performance in Ethiopian mustard (*Brassica carinata* A. Braun)

Received: 14 July 2005 / Accepted: 30 November 2005

Abstract Predicting heterosis and F_1 performance from the parental generation could largely enhance the efficiency of breeding hybrid or synthetic cultivars. This study was undertaken to determine the relationship between parental distances estimated from phenotypic traits or molecular markers with heterosis, F_1 performance and general combining ability (GCA) in Ethiopian mustard (*Brassica carinata*). Nine inbred lines representing seven different geographic regions of Ethiopia were crossed in half-diallel. The nine parents along with their 36 F_1s were evaluated in a replicated field trail at three locations in Ethiopia. Distances among the parents were calculated from 14 phenotypic traits (Euclidean distance, ED) and 182 random amplified polymorphic DNA (RAPD) markers (Jaccard's distances, JD), and correlated with heterosis, F_1 performance and GCA sum of parents (GCA_{sum}). The correlation between phenotypic and molecular distances was low ($r = 0.34$, $P \le 0.05$). Parents with low molecular distance also had low phenotypic distance, but parents with high molecular distance had either high, intermediate or low phenotypic distance. Phenotypic distance was highly significantly correlated with mid-parent heterosis ($r = 0.53$), F_1 performance ($r = 0.61$) and GCA ($r = 0.79$) for seed yield. Phenotypic distance was also positively correlated with (1) heterosis, F_1 performance and GCA for plant height and seeds plant^{-1}, (2) heterosis for number of pods plant^{-1}, and (3) F_1 performance for 1,000 seed weight. Molecular distance was correlated with GCA_{sum} ($r = 0.36$, $P \le 0.05$) but not significantly with heterosis and F_1 performance for seed yield. For each parent a mean distance was calculated by averaging the distances to the eight other parents. Likewise, mean heterosis was estimated by averaging the heterosis obtained when each parent is crossed with the other eight. For seed yield, both mean ED and JD were significantly correlated with GCA ($r = 0.90$, $P \le 0.01$ for ED and $r = 0.68$, $P \le 0.05$ for JD) and mean heterosis ($r = 0.79$, $P \le 0.05$ for ED and $r = 0.77$, $P \le 0.05$ for JD). In conclusion, parental distances estimated from phenotypic traits better predicted heterosis, F_1 performance and GCA than distances estimated from RAPD markers.

Electronic Supplementary Material Supplementary material is available for this article at http://dx.doi.org/10.1007/s00122-005-0180-3 and is accessible for authorized users.

Communicated by A. Charcosset

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Introduction

Ethiopian mustard (*Brassica carinata* A. Braun) is among the oldest oil crops cultivated in Ethiopia (Simmonds 1979), however, hardly cultivated in other parts of the world. It is the third most important oil crop in the highlands of Ethiopia (CSA 2003). Owing to its drought and heat tolerance, the crop is now being considered as an alternative to *B. napus* and *B. juncea* in dryer areas of Canada (Rakow 1995) and as a potential oil crop in Spain (Velasco et al. 1995) and India (Singh 2003).

Different researchers reported substantial heterosis in the major oilseed *Brassicas* (for review see Leon and Becker 1995; McVetty 1995) that stimulated a worldwide interest for developing hybrid cultivars. In Canada, China and Europe, especially Germany, hybrids are becoming the major cultivar types in *B. napus* cultivation (Dianrong 1999; Frauen et al. 2003). In the absence of effective pollination control for hybrid seed production, heterosis could be exploited by developing synthetic cultivars. The development of synthetic varieties is easier and less expensive than hybrid varieties (Becker et al. 1999) but does not utilize all the available heterosis. Identifying parental combination with strong yield heterosis is the most important step in developing hybrids (Diers et al. 1996; Becker et al. 1999; Melchinger 1999). Thus, if heterosis could be predicted from data of the parental generation, costs associated with making crosses and field evaluation to select heterotic crosses could be largely reduced. The level of genetic diversity between parents has been proposed as a predictor of F_1 performance and heterosis (Moll et al. 1965; Falconer and Mackay 1996). In the practical application of hybrid breeding, however, a strong correlation between heterosis and genetic distance has been rarely observed (Melchinger 1999).

In agreement with the classical theories of heterosis, Ali et al. (1995) in *B. napus*, Falk et al. (1994) in *B. rapa* and Pradhan et al. (1993) in *B. juncea* using morphological markers and geographic origin observed an increase in heterosis with increasing parental distance. But in predicting heterosis from molecular-based distance, contrasting results were reported in the oilseed *Brassicas*. Diers et al. (1996) and Knaak and Ecke (1995) using restriction fragment length polymorphism (RFLP) markers and Riaz et al. (2001) using sequence-related amplified polymorphism (SRAP) in *B. napus* reported a strong association of heterosis with marker heterozygosity and recommended their use for predicting heterosis. Becker and Engqvist (1995) indicated the usefulness of RAPD-based genetic distance estimates to predict heterosis for leaf dry matter production in *B. napus*. In contrast to the above reports, in resynthesized *B. napus*, Girke (2002) observed low correlations between RFLP-based genetic distance and heterosis. In *B. napus* Shen et al. (2003) found a positive association between hybrid seed yield and genetic distance estimated from amplified fragment length polymorphism (AFLP), but the coefficient of determination was low. In *B. juncea*, even though genetic distance was useful to group parents into heterotic groups, the absence of direct association of genetic distance with heterosis has been reported by Jain et al. (1994). Melchinger (1999) reviewed experiments designed to determine the association of genetic distance estimated from molecular markers with heterosis and made the following conclusions: there is a strong correlation in crosses among more or less related lines; there is a weak correlation in crosses among non-related lines within the same gene pool; and there is no correlation for crosses when the parents belong to different gene pools.

To our knowledge, there is no report on the association of genetic divergence and heterosis in *B. carinata*. Therefore, this study was conducted with the following objectives: (1) to asses genetic diversity among nine inbred lines of *B. carinata* obtained from different geographic regions of Ethiopia using molecular markers and phenotypic traits, (2) to estimate variation for heterosis, GCA and F_1 performance from a diallel set of crosses among the nine inbred parents, and (3) to compare the respective usefulness of the two kinds of genetic distance measures for the prediction of heterosis, GCA and F_1 performance.

Materials and methods

Parental lines and crosses

The nine parents were S_5 lines developed from a germplasm collection representing seven different *B. carinata* growing regions of Ethiopia (Table 1). Parents 6 and 9, having the same S_2 parent, were deliberately included in the crossing scheme. The inbred lines development and parental selection procedure is described by Teklewold (2005). During 2002–2003 off-seasons in Ethiopia, a diallel set of 36 crosses (without reciprocals) was produced. Seeds of the parental inbred lines were produced during the same period by growing 20 plants of each line under isolation.

DNA extraction and PCR amplification

Total genomic DNA was extracted from 0.1 g of young leaves taken from a single plant per genotype using Nucleon PhytoPure DNA extraction kit (Amersham International PLC 1997). Forty-two random primers from Operon Technologies (Alamada, CA, USA) were used for PCR amplification. The reactions were carried out in a 25 μl volume containing 1× reaction buffer [200 mM Tris–HCl, pH 8.55, 160 mM $(NH_4)_2SO_4$ 0.1% (v/v)], 3.0 mM $MgCl_2$, 0.4 mM of dNTPs (dATP, dCTP, dGTP and dTTP), 0.16 μM primer, 1.0 U of Taq DNA polymerase and 25 ng of genomic DNA template. DNA amplifications were performed in a Perkin Elmer 480 thermocycler programmed for a preliminary step of 1 min at 94°C, 45 cycles of 30 s at 92°C, 60 s at 35°C and 2 min at 72°C and a final step of 6 min at 72°C. Then samples were held at 4°C until the RAPD fragments were separated by electrophoresis using 1.8% agarose gel and visualized with ethidium bromide under UV light.

Table 1 Inbred lines of *B. carinata*, their parental accession code along with area of collection and altitude

Parental code	Parental accession code[a]	Regions of collection	Altitude (m) of collection sites
1	PGRC/E 21192	Welega	2,090
2	PGRC/E 21245	Gonder	1,860
3	PGRC/E 21068	Bale	2,500
4	PGRC/E 21224	Kefa	1,750
5	PGRC/E 21289	Wolo	2,570
6	PGRC/E 21071	Bale	2,640
7	PGRC/E 21007	Arssi	2,900
8	PGRC/E 21316	Shewa	2,430
9	PGRC/E 21071	Bale	2,640

[a] Accession code refers to the accession identification number of the Plant Genetic Resource Centre of Ethiopia

Field evaluation

During the main season (June to December 2003), the F_1s and their parents were evaluated in the field at three locations in Ethiopia (Holetta, Kulumssa and Debre-zeit), using a Randomized Complete Block Design with three replications. Plots consisted of three rows of 5 m length and 30 cm inter-row spacing with 10 cm inter-plant spacing. To provide uniform competition, the two outer rows of each plot were planted with genotype PGRC/E 2085 characterized by shorter plant height (112 cm) and early maturity (144 days to mature). Fourteen phenotypic traits were recorded: days to flowering, days to maturity, number of primary branches plant^{-1}, number of secondary branches plant^{-1}, number of pods plant^{-1}, pod length (cm), plant height (cm), stem diameter (cm), number of seeds pod^{-1}, number of seeds plant^{-1}, seed yield (g plant^{-1}), 1,000 seed weight (g), oil content (%) and oil yield plant^{-1}. Days to flowering and maturity were recorded on plot bases, while the other phenotypic traits were recorded on the same set of ten randomly chosen plants of the central row.

Data analysis

Total number of bands and number of polymorphic bands generated by each primer were determined. Jaccard's coefficient of similarity (JCS) was calculated from polymorphic RAPD bands as $\mathrm{JCS}_{jk} = \frac{N_{11}}{N_{11}+N_{10}+N_{01}}$ (Jaccard 1908), where JCS_{jk} is similarity between parents j and k; N_{11} is number of bands present in both parents; N_{10} number of bands present only in parent j; N_{01} number of bands present only in parent k. Jaccard's distance (JD) was calculated as 1-JCS. Euclidean distance (ED) was computed from the 14 phenotypic traits after standardization (subtracting the mean value and dividing it by the standard deviation) as $\mathrm{ED}_{jk} = \sqrt{\sum_{i=1}^{n}\left(x_{ij} - x_{ik}\right)^2}$ (Sneath and Sokal 1973), where ED_{jk} = distance between parents j and k; x_{ij} and x_{ik} = phenotypic value of the ith character for parents j and k, respectively; and n = number of phenotypic traits used to calculate the distance. Both distances were computed using NTSYS-pc version 2.1 (Rohlf 2001). The distance matrix from phenotypic traits and the similarity matrix from molecular markers were used to construct dendrograms based on the unweighted pair-group method with arithmetic means (UPGMA). Using the same NTSYS software, cophenetic value matrix was calculated to test the goodness of fit for the cluster analysis to the original distance matrix and correspondence between ED and JD was determined by the product moment correlation derived from Mantel Z test.

Variance analysis was performed to determine whether there were significant differences between the F_1s and their parents and among the F_1s using PLABSTAT (Utz 2001) software. Combining ability analysis was carried out using AGROBASE$^{\mathrm{TM}}$ software (Agronomix Software Inc., Canada) following Griffing's Method IV Model II. Absolute mid-parent (AMPH) and relative mid-parent (RMPH) heterosis were calculated as follows: $\mathrm{AMPH} = \mathrm{F}_1 - \mathrm{MP}$; $\mathrm{RMPH} = \left(\frac{\mathrm{F}_1-\mathrm{MP}}{\mathrm{MP}}\right) \times 100$, where $\mathrm{F}_1 = \mathrm{F}_1$ performance and $\mathrm{MP} = \left(\frac{P_1+P_2}{2}\right)$.

Results

RAPD band polymorphism and parental relatedness

In total, 366 RAPD bands were scored across the nine parental lines. Of these, 182 (49.7%) were polymorphic. Forty-two primers generated between 3 and 15 bands with an average of 8.7 bands per primer. Primer OPAH-17, OPAG-18 and OPAH-9, generated 13, 14 and 15 bands, respectively. The number of polymorphic bands ranged from one to eleven with an average of 4.3 per primer. The primers OPAG-18 with eleven and OPAH-17 with eight and three others (OPAG-11, OPAJ-8 and OPAH-9) each with seven polymorphic bands had the highest number of polymorphic bands (see S1 in the electronic supplementary material).

As all except two of the parental lines had no known pedigree relationship and were derived from accessions collected from different geographic region, the ranges of estimates of ED and JD were fairly large. JD among pairs of the nine parental lines ranged from 0.18 to 0.73 with a mean of 0.61. Euclidean distance varied from 0.62 to 2.20 with a mean of 1.37. For both ED and JD, parents 6 and 9 that are known to be related by pedigree, showed the smallest distance. The parental combination with the highest distance index differed when identified by phenotypic traits or by molecular markers. In case of ED, parents 1 and 3 had the highest values (2.20)

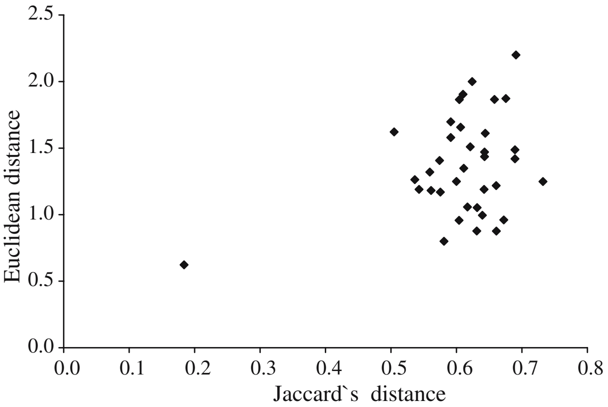

Fig. 1 Relationship between the 36 phenotypic (ED) and molecular (JD) distances calculated from all pair-wise combinations of the 9 parental lines

followed by parents 1 and 7 and parents 3 and 4 with ED values of 2.00 and 1.91, respectively. In case of JD, parents 3 and 6 (0.73) and parents 1 and 3 (0.69) had the highest and second highest distance values. Two parental combinations, 3 and 7, and 3 and 9, each with a JD of 0.69, were the third most distant parents. The JD was plotted against the ED (Fig. 1). The relationship between the ED and JD seemed to be dependent on the pedigree background of the parents. Association between ED and JD was high for the two closely related parents. In case of unrelated parents, large values for JD were sometimes associated with large values for ED, but sometimes with small values. This resulted in a lack of consistent association between ED and JD and the fit between them as tested by two-way Mantel test was low with $r=0.34$, but still significant ($P \leq 0.05$). Excluding the distance value calculated from the two most related parental lines resulted in a large reduction of the correlation coefficient ($r=0.11$, not significant).

Cluster analysis based on phenotypic traits and molecular data

The dendrograms from UPGMA cluster analyses based on ED and JD matrixes are presented in Figs. 2 and 3, respectively. The cophenetic correlation between the dendrograms and the original data matrixes showed a good fit with matrix correlation of $r=0.84$ for the phenotypic data and $r=0.92$ for the molecular data. Except for the two related parents (parents 6 and 9) the two distance estimates did not produce equivalent groupings. The JD based dendrogram failed to divide the parents into clearly distinct groups. Clustering based on ED resulted in the formation of three major groups of which two clusters were comprised by a single parental line while the rest of the seven parental lines formed one big cluster. Within this big cluster, three sub-groups could be differentiated.

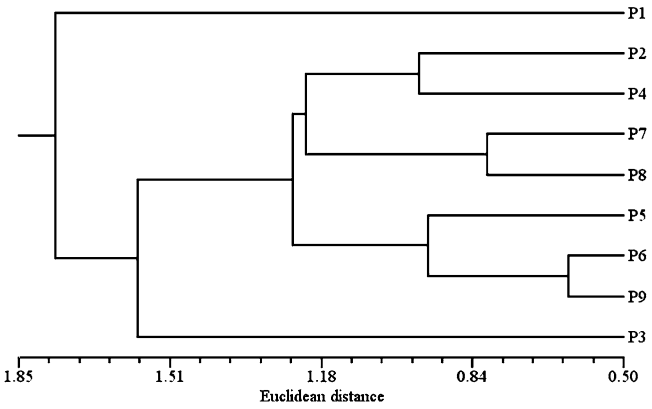

Fig. 2 Similarity among nine *B. carinata* inbred lines revealed by UPGMA cluster analysis of Euclidean distance based on 14 phenotypic traits

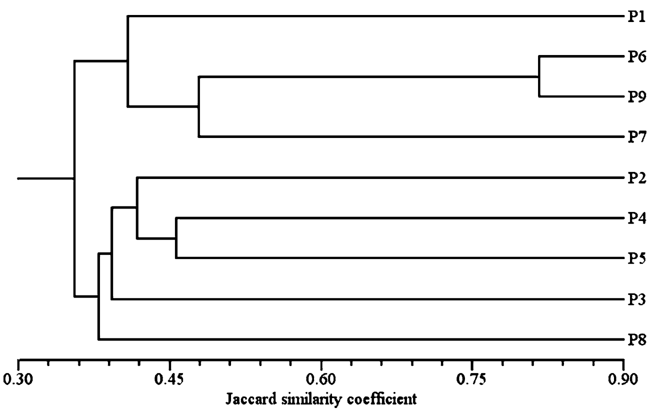

Fig. 3 Similarity among nine *B. carinata* inbred lines revealed by UPGMA cluster analysis of the Jaccard's coefficient of similarity calculated from 182 polymorphic RAPD bands

Parental and F_1 performance and heterosis

In all phenotypic traits, a combined analysis of variance over the three locations showed the presence of highly significant ($P \leq 0.01$) genotype effect among the nine parental inbreds and the 36 F_1 (data not presented). The comparison of parents versus F_1, which indicates the presence of heterosis, was also highly significant ($P \leq 0.01$). General combining ability effect was significant for all traits except number of secondary branches and pods plant^{-1} (Teklewold and Becker 2005a). Variance due to SCA was significant only for number of secondary branches and pods plant^{-1} and 1,000 seed weight. All traits had significant GCA and SCA (location interaction effects. Mean heterosis for seed yield and six other important traits is presented in Table 2. The degree of heterosis varied considerably from trait to trait. Seed yield exhibited the highest RMPH, 67.0% followed by number of seeds plant^{-1}, numbers of pods plant^{-1}, number of secondary branches, plant height, number of primary branches and 1,000 seed weight. RMPH for seed yield varied from 25.1 (parents 1×6) to 145.4 (parents 3×8). Among the 36 crosses, four crosses gave more than 100% RMPH (data not presented).

Correlation of parental distance with heterosis, GCA_{sum} and F_1 performance

The correlation of ED and JD with heterosis, GCA_{sum} and F_1 performance is given in Table 3. Euclidean distance was significantly correlated with AMPH for numbers of pods plant^{-1}, plant height, seed yield and number of seeds plant^{-1}. Euclidean distance was also significantly correlated with parental GCA_{sum} for plant height, seed yield and number of seeds plant^{-1}. Correlation between ED and the F_1 performance was significant for plant height, seed yield (Fig. 4), 1,000 seed weight and number of seeds plant^{-1}.

The correlation of JD with AMPH, GCA_{sum} and F_1 performance was mostly non-significant. Jaccard's

Table 2 Parental and F_1 mean along with mean absolute mid-parent (AMPH) and relative mid-parent heterosis (RMPH) in seven traits of *B. carinata* grown at three locations in Ethiopia

Phenotypic traits	Parental mean	F_1 performance	Mean AMPH	Mean RMPH (%)
Number of primary branches	9.89	10.48	0.58	6.2
Number of secondary branches	17.62	22.30	4.68	27.4
Number of pods plant^{-1}	197.51	268.60	71.07	37.2
Plant height (cm)	167.43	188.30	20.91	12.6
Seed yield (g plant^{-1})	8.44	14.01	5.57	67.0
1,000 seed weight (g)	3.58	3.73	0.15	4.7
Number of seeds plant^{-1}	2397.60	3815.40	1417.82	60.7

distance was significantly correlated with AMPH for number of secondary branches and pods plant^{-1}; and with GCA_{sum} for seed yield and number of seeds plant^{-1}. In none of the phenotypic traits, the correlation between JD and F_1 performance was significantly different from zero.

Correlation of mean parental distances with mean heterosis and GCA

Mean ED and JD were calculated for each parent by averaging the distance of a particular parent to the eight other parents and were correlated with their respective GCA and mean heterosis. The mean ED showed parent 1 followed by parent 3 as the most distant and parent 6 followed by parent 8 as the most closest parents compared to all others (Table 4). In case of mean JD, parent 3 followed by parent 1 were the most distant while parent 9 followed by parent 6 were the most closest parents to all others. Parent 3 showed the highest mean JD, GCA, mean AMPH and F_1 yield but the second highest mean ED, whereas parent 6 showed the lowest ED, GCA, mean AMPH and F_1 yield but the second lowest JD. Generally, the variation among parents was much larger for ED than for JD.

Mean ED and JD of each parent were significantly correlated with mean heterosis and GCA for seed yield and number of seeds plant^{-1}, and with mean heterosis for number of pods plant^{-1} (Table 5). Mean JD was also significantly correlated with heterosis for number of secondary branches.

Discussion

Developing a guideline helpful to select the most heterotic parents is important but remains a problem in heterosis breeding. Theoretical considerations (Cress 1966; Falconer and Mackay 1996) and experimental results (Moll et al. 1965) suggest the dependence of heterosis on genetic diversity. However, even with the discovery of molecular markers, the prediction of F_1 performance based on genetic distance is far from direct practical application (Burstin and Charcosset 1997; Melchinger 1999). This study, the first of its kind for *B. carinata*, was an attempt to study the association of parental divergence (estimated from molecular marker and phenotypic traits) to heterosis, F_1 performance and GCA.

Random amplified polymorphic DNA markers were applied for cultivar identification and estimation of genetic relatedness in 21 *B. carinata* inbred lines, containing the 9 parents used in this study, (Teklewold 2005) and their 43 progenitor accessions (Teklewold and Becker 2005b). These studies showed the absence of clear correspondence between geographic origin and genetic diversity within the Ethiopian gene pool. For the inbred lines, the reproducibility of RAPD bands was also tested and in the same laboratory under a stringent reaction condition, the average genetic similarity of the

Table 3 Phenotypic correlation coefficients of Euclidean and Jaccard's distances with absolute mid-parent heterosis (AMPH), sum of parental GCA and F_1 performance in seven phenotypic traits of *B. carinata* grown at three locations in Ethiopia

Phenotypic traits	Euclidean distance			Jaccard's distance		
	AMPH	F_1 performance	Parental GCA_{sum}	AMPH	F_1 performance	Parental GCA_{sum}
Number of primary branches	0.12	0.18	−0.21	0.09	0.07	−0.07
Number of secondary branches	0.29	0.02	0.02	0.47**	0.27	0.07
Number of pods plant^{-1}	0.38*	0.12	0.33	0.41*	0.28	0.21
Plant height (cm)	0.50**	0.49**	0.47**	0.33	0.25	0.19
Seed yield (g plant^{-1})	0.53**	0.61**	0.79**	0.28	0.24	0.36*
1,000 seed weight (g)	0.16	0.34*	0.32	0.02	0.13	0.04
Number of seeds plant^{-1}	0.48**	0.47*	0.72**	0.22	0.16	0.39*

*Significant at $p \le 0.05$

**Significant at $p \le 0.01$

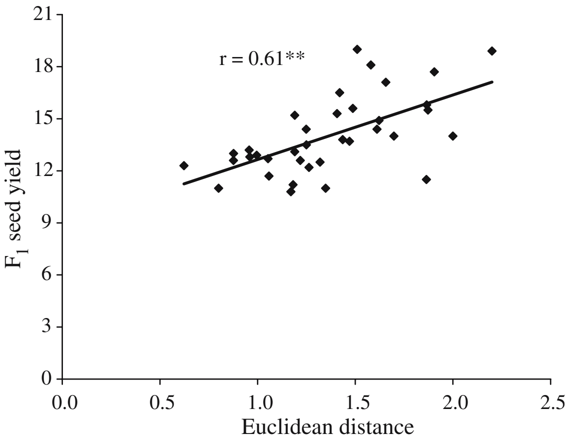

Fig. 4 Relationship between Euclidean distance and F_1 performance for seed yield (g plant^{-1})

Table 4 Mean Euclidean (ED) and Jaccard's (JD) distances, GCA, mean absolute mid-parent heterosis (AMPH) and F_1 performance for seed yield (g plant^{-1}) of the nine parents of *B. carinata* grown at three locations in Ethiopia

Parents	Distance		GCA	Mean AMPH	F_1 performance
	ED	JD			
Parent 1	1.77	0.55	1.59	5.97	15.40
Parent 2	1.32	0.52	1.14	5.15	13.01
Parent 3	1.66	0.56	2.63	7.87	16.32
Parent 4	1.36	0.53	0.50	5.76	14.45
Parent 5	1.22	0.53	0.63	5.02	13.47
Parent 6	1.06	0.49	1.69	3.70	12.53
Parent 7	1.42	0.53	0.20	6.22	13.83
Parent 8	1.21	0.54	0.68	5.59	13.41
Parent 9	1.26	0.48	0.37	4.85	13.70

same two inbred lines was incorrectly estimated in only 2.75% of the cases. Indeed, the consequences of errors due to limited reproducibility of RAPD markers are reported to be less serious in completely homozygous genotypes (Hallden et al. 1996). In other *Brassica* crops, RAPD markers were considered to be as efficient as RFLP markers for estimating intra-specific genetic relationships among genotypes (Demeke et al. 1992; Dos Santos et al. 1994; Thormann et al. 1994). Hence, the information documented so far in the oilseed *Brassicas* suggests that RAPD assay can be used to determine genetic relatedness of inbred parents with a reasonable precision. The precision of distance estimates could be tested by a cross validation approach. In this study we preferred to include all markers in the calculation of JD because this yields the best estimator.

Burstin et al. (1995) and Burstin and Charcosset (1997) illustrated theoretically and experimentally that molecular marker distance does not necessarily correspond directly to phenotypic trait distances but may be related in a triangular-shaped manner. In this experiment too, low JD was associated with low ED, whereas higher JD was associated with either high or medium or low ED (Fig. 1). According to Burstin and Charcosset (1997) polygenic inheritance and linkage disequilibrium could cause such relationship. In polygenic inheritance, a given quantitative value can be obtained with different gene combinations, hence similar distance measurement can be obtained from both related and distant genotypes. The non-linear relationship between the two types of distances exacerbated as the number of loci that contribute to the variation of the traits used to estimate phenotype distances increases. The relationship is further complicated by linkage disequilibrium. With no linkage disequilibrium between DNA marker and QTL for phenotypic traits, the two types of distances vary independently and both high and low molecular distances can correspond to similar morphological distances.

The molecular and phenotypic distance measurements differed in their ability to predict GCA, heterosis and F_1 performance. Euclidean distance showed a higher correlation with heterosis, parental GCA and F_1 performance than JD. Similar to our results, in two subspecies of *Medicago sativa*, Riday et al. (2003) found a significant correlation of heterosis with morphological distance but not with molecular distance (microsatellite and AFLP markers). Association of morphological distances with heterosis was also observed in *B. napus* (Lefort-Buson et al. 1986; Ali et al. 1995) and *B. juncea*

Table 5 Phenotypic correlation coefficients of mean Euclidean (ED) and Jaccard's (JD) distances with GCA and mean absolute mid-parent heterosis (AMPH) for seven phenotypic traits in the nine parents of *B. carinata* grown at three locations in Ethiopia

Phenotypic traits	GCA		Mean AMPH	
	ED	JD	ED	JD
Number of primary branches	−0.24	−0.07	0.16	0.21
Number of secondary branches	0.02	0.17	0.63	0.93**
Number of pods plant^{-1}	0.38	0.42	0.83**	0.78*
Plant height (cm)	0.54	0.42	0.67	0.41
Seed yield (g plant^{-1})	0.90**	0.68*	0.79*	0.77*
1,000 seed weight (g)	0.37	0.02	0.31	−0.19
Number of seeds plant^{-1}	0.82**	0.76*	0.78*	0.81**

*Significant at $p \leq 0.05$
**Significant at $p \leq 0.01$

(Pradhan et al. 1993). Heterotic response for yield and some of its components could be associated with agronomic trait differences between the parents because the genes controlling the variation in phenotypic traits from which the parental distances were computed are at least partially responsible for the expression of heterosis, too. However, the efficiency of phenotypic traits may be limited by time required, cost (especially in developed nations where labour cost is high) and their interaction with environment.

Euclidean distance was correlated best with GCA-sum followed by F1 performance and AMPH. The higher correlation coefficient between ED and GCA_{sum} could be explained by the low error variance associated to GCA estimates as more observations are involved in calculating GCA than AMPH and F_1 performance. The higher association of ED to F_1 performance than to heterosis has a practical advantage since F_1 performance is the main objective of cultivar development.

As in many other crops, correlation coefficients calculated between JD and RMPH, and F_1 performance for seed yield were positive but not significant. The correlation between JD and GCA_{sum} was significant ($P \leq 0.05$) for seed yield, but the magnitude of this correlation was too low (0.36) to be of predictive power ($R^2 = 0.110$). Similarly, in *B. napus* Girke (2002), Shen et al. (2003) and Yu et al. (2005) and in *B. juncea* Jain et al. (1994) reported a low association of DNA markers with heterosis. However, in contrast to our observation, Knaak and Ecke (1995), Diers et al. (1996) and Riaz et al. (2001) reported the usefulness of molecular markers based parental distance to predict heterosis especially when the parents are genetically related. According to Bernardo (1992) inadequate genome coverage, random dispersion of molecular markers (unlinked to QTL) and different levels of dominance could be the reasons for low correlation between molecular distances and heterosis and/or F_1 performance. The existence of multiple allelism (Cress 1966) and epistasis (Moll et al. 1965) could also cause the low correlation of JD heterosis and F_1 performance.

The magnitude of correlation between the two types of distance indices and GCA or heterosis was increased by calculating mean ED, JD and AMPH for each parent. For seed yield all correlations were significant but for the other traits some relatively high correlations did not reach statistical significance due to the low number of degrees of freedom.

The experimental evidence presented here indicates the presence of substantial heterosis and a method that helps in the selection of parents that depict heterotic response. Therefore, the development of hybrids and/or inbred line-based synthetic cultivars should increase seed yield of *B. carinata* parallel to what is observed in *B. napus*. As high as 39% cross-fertilization (Teklewold, unpublished data, 2005) has been observed in *B. carinata* that allows synthetic variety development. The highly significant correlation of ED with GCA for seed yield should be a useful indicator in selecting parents with high GCA.

In conclusion, this study indicates that molecular marker based distances are not suitable for a reliable prediction of heterosis, combining ability or hybrid yield in *B. carinata*. However, distance calculated from phenotypic traits was able to predict heterosis, F_1 performance and parental GCA for seed yield and some other important agronomic traits. It would be particularly rational and profitable to use phenotypic distance estimates to select those parents having a high GCA before making extensive testcrosses. Once the distances among prospective parents are ascertained, only the more promising crosses need to be produced and evaluated.

Acknowledgements The first author was a recipient of Ph.D. fellowship from Catholic Academic Exchange Service (KAAD), Germany. The authors are grateful for the technical help provided by Tadesse Debele, Kassahun Kumssa, Tadesse Deme, Wondemagne W/semyat and the staff of Kulumssa Research Centre for Oil Crops.

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