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Genetic distance and heterosis in Indian mustard: developmental isozymes as indicators of genetic relationships

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Abstract The use of isozymes as indicators of genetic diversity and as markers for the selection of agronomic traits has been proposed in different crop species. The present investigation was conducted to study the use of isozyme-derived genetic distance between parents in predicting the F_1 heterosis in Indian mustard. In addition, the interaction of isozyme-based diversity with quantitative trait and pedigree-based diversity measures, and its role in predicting hybrid heterosis has also been examined. Sixteen Indian mustard lines and their 48 crosses (12×4, line × tester crossing) were evaluated over two environments for isozyme and quantitative morphological characters. The results from this study suggest that the heterotic response to isozymic changes is more responsive in crosses derived from morphologically and pedigree-wise related parents in comparison to crosses derived from unrelated parents. It was possible to improve heterosis predictions by partitioning the isozyme-based genetic distance into general genetic distance and specific genetic distance and correlating the latter with the specific combining ability of morphological traits. The possible reasons for these observations are discussed.

Key words Isozyme diversity · Heterosis · Phenotypic distance · Pedigree backgrounds · *Brassica juncea.* L

Introduction

The selection of suitable parents that give a high proportion of transgressive segregates, is an important prerequisite for the successful execution of a breeding programme. Genetic diversity and testcross data based on metric traits are the generally considered criteria for parental selection (Cox and Murphy 1990; Smith et al. 1990; Moser and Lee 1994). Apart from being costly and time consuming, these measures of parental selection suffer from the complexities of polygenic inheritance and high magnitudes of genotype × environment interaction.

In recent years biochemical and molecular markers have been advocated both as heterosis predictors and for describing the genetic relationships between cultivars (Frei et al. 1986; Geredes and Tracy 1994; Moser and Lee 1994). They provide a wide array of simply inherited neutral markers that can give the breeder a reliable blueprint of superior genotypes. Such information, along with performance data, can be used to predict F_1 heterosis, as well as for estimating the variance in segregating generations (Souza and Sorrells 1991). While molecular markers like RFLPs and RAPDs have an advantage over isozymes in providing a comparatively high frequency of polymorphic loci, the inexpensive nature of isozymes makes them preferable in preliminary studies (Suarez et al. 1991).

The main purpose of the present study was to evaluate the utility of developmental isozyme differences of parents in predicting hybrid heterosis in Indian mustard. Morphological differences between parents and their pedigree backgrounds are the other criteria that have been frequently used as distance indicators to predict F_1 heterosis. Therefore, the utility of isozymic distance in relation to two other distance measures was also examined.

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Materials and methods

Forty-eight single-cross hybrids of Indian mustard were produced by crossing 12 lines to four testers (see Table 1). These lines and testers were selected from the germplasm of 118 lines on the basis of a preliminary screening for agromorphological performance and isozyme diversity. The parents and hybrids were evaluated over a

period of 2 years (designated as environments 1 and 2 in Results and discussion) in the field. The parental lines were grown in replicated randomized, and the F_1s in replicated compact, family block design, with three replications in each design. Agronomic practices, data recording, methods for estimation of oil yield and crude protein content, and the determination of phenotypic diversity (PD) are described elsewhere (Sekhon and Gupta 1992). The morphological traits studied in the F_1s are given in Table 2.

The parents were analyzed for isozymes of peroxidase, esterase and acid phosphatase at six stages of plant development (20 and 45-day-old leaves and 20-, 40-, 56- and 72-day-old seeds). Sampling procedures and enzymatic assays were as described previously (Sekhon and Gupta 1992). Isozyme variants were inferred from (1) studies on the developmental expression of isozymes in Indian mustard cultivars and F_1s (Sekhon 1991), and (2) previous studies on the genus *Brassica* (Thorpe et al. 1987). Thirty-one isozyme variants belonging to nine zones of enzyme activity were scored independently in a 0, 1 matrix with 1 representing the presence and 0 representing the absence of a band. The 0–1 data was converted into a similarity, and subsequently a dissimilarity (1-similarity) or genetic-distance (GD), matrix using a simple matching coefficient (described in Sekhon and Gupta 1992).

The method of Kempthorne (1957) was used for a combining ability analysis on F_1 agronomic data to calculate general combining ability and specific combining ability effects. The same analysis was performed on isozyme genetic distance (GD) values to partition them into general genetic distance (GGD) and specific genetic distance (SGD). Mid-parent heterosis (MPH) was determined by subtracting the respective mid-parent mean from the F_1 mean in each environment. Simple correlation coefficients were calculated for PD, GD and SGD values with the F_1 agromorphological performance (FIP), specific combining ability (SCA), and mid-parent heterosis values (MPH).

To study the interaction of isozymic distance measure with morphological and pedigree distances, cross combinations between similar (SPD) and dissimilar phenotypic diversity (DPD), and similar (SP) and dissimilar pedigree background (DP) lines, were identified. Only two testers, RLM 198 and RLM 514, for which both pedigree-related and unrelated lines were available were used to study isozyme-pedigree interactions. Correlations were determined between GD and F1P in SPD, DPD, SP and DP types of crosses.

Results and discussion

GD estimates between the parental lines of 48 crosses are shown in Table 1. GD values were computed from isozymic variants expressed over six developmental stages. GD values ranged from a low of 0.11, between RLC 701 and Prakash, to 0.47, between RLM 185 and Kranti. The four testers showed more variation in mean GD values with 12 lines (coefficient of variation 24.13) than the lines showed with testers (coefficient of variation 8.29).

To check if the GD estimates based on isozymes reflect true associations among the lines, these were subjected to external validation criteria (Mumm et al. 1994). For this, the matrix of 48 isozymic GD values was compared to a matrix (data not shown) based on morphological relationships (PD). The correlation coefficient between GD and PD was moderately high (0.46**), indicating a good agreement between the two data sets. A perfect correspondence between the two is not expected as each data set represents a somewhat different part of the genome and is subject to different sources of error. While the number of assayed isozyme loci and the size and variability of the reference population are the strongest factors affecting GD, phenotypic diversity (PD) is likely to be affected by environment, genotype × environment interaction, non-additive genetic effects, and the selection criteria used for the development of cultivars (Moser and Lee 1994). To address some of the factors influencing GD, we took subsets of isozyme loci to see if the correlation coefficients between GD and PD remain valid. The correlations between PD and GD estimates based on seed and leaf developmental isozymes were 0.40** and 0.41** respectively. Nevertheless, an increase in the assayed isozyme loci is likely to give a better picture of genetic diversity. Also, PD does not represent the true genetic distance as most of the phenotypic characters used to compute PD are characters of economic importance and thus have been subject to considerable selection pressure for the development of these cultivars. As a result, many of these lines are culminations of artificial selection for the economically important morphological attributes and hence the associations on the basis of such attributes are likely to give a distorted picture of genetic variation. The inclusion of morphological

Table 1 Genetic distance (GD), general genetic distance (GGD), and specific genetic distance (SGD) values between 12 lines and four testers of Indian mustard

Tester	Line												GGD
	RLM 185	RLM 612	RLM 629	RLC 701	RLC 1017	RLC 1101	RH 765	RH 8304	RS 61	Prai 1118	RAUR 1	KRV Tall	
GD													
Kranti	0.47	0.42	0.40	0.40	0.42	0.19	0.27	0.38	0.43	0.44	0.36	0.28	
Prakash	0.17	0.30	0.28	0.11	0.17	0.32	0.32	0.21	0.17	0.19	0.15	0.27	
RLM 198	0.27	0.23	0.13	0.25	0.21	0.28	0.30	0.23	0.19	0.30	0.21	0.25	
RLM 514	0.38	0.30	0.32	0.32	0.30	0.28	0.23	0.30	0.38	0.27	0.23	0.23	
SGD													
Kranti	0.12	0.08	0.07	0.08	0.10	−0.13	−0.05	0.06	0.10	0.10	0.06	−0.04	0.09
Prkash	−0.10	0.03	0.03	−0.14	−0.08	0.08	0.07	−0.04	−0.09	−0.07	−0.08	0.03	−0.06
RLM 198	−0.01	−0.04	−0.13	0.00	−0.05	0.03	0.04	−0.03	−0.08	0.03	−0.03	0.00	−0.04
RLM 514	0.07	0.00	0.03	0.04	0.02	0.00	−0.06	0.01	0.09	−0.03	−0.04	−0.05	0.01
GGD	0.04	0.03	0.00	−0.01	−0.01	−0.01	0.00	0.00	0.01	0.02	−0.04	−0.03	

characters of no, or of indirect, economic value is likely to bring PD closer to the true genetic distance.

To study the relationships between GD and F_1 performance, simple correlations were worked out between GD values and six F_1 agronomic traits. Genotypic differences among 48 crosses for all the six traits were highly significant over the two environments (combined values over the 2 years are given in Table 2). Estimates of genotype × environment interaction variance (σ^2 ge) were highly significant but always smaller than the corresponding genotypic variances (σ^2 g). Broad-sense heritability ranged from 0.68 for SY to 0.97 for PH. The correlations between F_1 means and GD or PD values are also given in Table 2. Except for GD–DF and PD–OP, other correlations were either insignificant or else significant but low.

Identification of divergent lines which can be used in making superior hybrids, or which have the ability to give transgressive segregates in a hybridisation programme, is a costly and time-consuming task. A major part of breeding resources is devoted to evaluate lines in hybrid combinations. The term "molecular breeding" has been used to describe breeding programs where biochemical/molecular markers are used as diagnostics to speed up and to make the breeding programs more efficient and cost effective. However, for a marker to qualify as a "diagnostic marker" it needs to be strongly linked to the QTLs of interest. Regarding the use of correlations observed in the present study (Table 2) most of them are too low or too inconsistent (over two distance measures) to be used in breeding programs.

In an effort to improve the correlations between GD and hybrid heterosis, we partitioned the GD into general genetic distance, GGD, and specific genetic distance, SGD (see Table 1). This subdivision of GD indicated group effects. A significant portion (46%) of total variation in GD values was attributable to SGD. In the light of these results we decided to compare the correlations between SGD and SCA for different F_1 traits. Simple correlations of GD and SGD values with F1P, MPH and SCA values are given in Table 3. Considering these correlations, there was an invariable increase in the magnitude of the correlation from GD to SGD for SY, OY and OP. This was consistent with the result (data not presented) that, for OY and SY, SCA contributed 83% and 78%, respectively, to the total genotypic variation. For other traits, however, there was a general decrease in these correlations from GD to SGD. SCA will show a high correlation with SGD if all QTLs affecting a trait have equal dominance effects and epistasis is absent. Any deviation from this leads to a reduction in the correlation.

Predicting crosses which will give a high frequency of desirable pure lines is of prime importance in an autogamous crop breeding programme. Such a cross will have a high mean and high genetic variance. While the first can be determined from a high mid-parent value, the second is predicted from a high parental divergence. The second prediction is a relatively difficult process and is complicated by a number of factors. Different levels of dominance among hybrids, the pedigree relationship of parents, and the inclusion of randomly dispersed markers (not linked to QTLs) in heterozygosity computations, have been cited as some of the factors responsible for low correlations between parental divergence and F_1 heterosis. Using computer simulations, Charcoset et al. (1991) have shown that the correlation coefficient between heterosis and distance based on a marker 5 cM away from the marked allele is 0.611. The inclusion in heterozygosity computations of a second marker, which is 20 cM away from the first and 15 cM away from the marked allele, will lead to a drop in the correlation to 0.485.

The selection of markers that are linked specifically to marked alleles of interest, seems to be important for using molecular markers to accurately predict heterosis. Smith et al. (1990) argued that if QTLs are located only in certain regions of the genome, an arbitrarily selected set of markers that cover the entire genome will not

Table 2 Estimates of genetic parameters and correlation coefficients (r) between hybrid performance (F1P) and parental distance parameters (GD and PD) in 48 crosses

Trait[a]	Genetic parameter[b]			r between F1P and:	
	$\sigma^2 g$	$\sigma^2 ge$	$h^2_{(b,s)}$	GH	PD
SY	1.68**	1.51**	0.68	0.29*	0.30*
OY	0.38**	0.30**	0.71	0.33*	0.37**
OP	1.94**	0.84**	0.82	0.38**	0.50**
CPC	2.68**	0.23**	0.96	−0.39**	−0.23
PH	346.95**	21.01**	0.97	−0.34*	−0.11
DF	60.01**	7.49**	0.94	−0.49**	0.04

* $P \leqslant 0.05$; ** $P \leqslant 0.01$

[a] SY = seed yiedld (g/plant); OY = oil yield (g/plant); OP = oil percent; CPC = crude protein content (%); PH = plant height (cm); DF = days to 50% flowering

[b] $\sigma^2 g$, $\sigma^2 ge$, and $h^2_{(b,s)}$ represent genotypic variance, genotype × environment interaction variance, and broad-sense heritability respectively

[c,d] GD and PD are isozymic and phenotypic distances between parents

Table 3 Correlation coefficients of isozyme-based parental genetic distance (GD) and specific genetic distance (SGD) values with F_1 means (F1P), mid parent heterosis (MPH) and specific combinng ability (SCA) of hybrid traits

Trait[a]	Genetic distance			Specific genetic distance		
	F_1P	MPH	SCA	F_1P	MPH	SCA
SY	0.29*	0.12	0.27	0.32*	0.18	0.32*
OY	0.33*	0.16	0.30*	0.36*	0.21	0.36*
OP	0.38**	0.37**	0.41**	0.42**	0.38**	0.49**
CPC	−0.39**	−0.48**	−0.32*	−0.35*	−0.38**	−0.32*
PH	−0.34*	−0.30*	−0.27	−0.26	−0.25	−0.23
DF	−0.49**	−0.46**	−0.38**	−0.35*	−0.33*	−0.30*

* $P \leqslant 0.05$; ** $P \leqslant 0.01$

[a] For explanation see Table 2

accurately predict heterosis. A precise approach will be to pre-select markers based on linkage relationship with QTLs of interest. The observed low correlations between GD and heterosis in the present study are presumably due to the randomly selected set of isozyme markers, some of which are not in linkage disequilibrium with heterosis for the studied traits.

Frei et al. (1986) demonstrated a fundamental difference in the extent of correlation between GD and F1P, depending upon whether or not the parents are related to each other. Several studies in maize indicate that a close relationship between marker heterozygosity and heterosis is observed only if the parents are related by pedigree. Correlations drop significantly when unrelated parents are used to generate hybrids. Cox and Murphy (1990) compared distances based on pedigrees and quantitative morphological characters to predict F_2 heterosis in winter wheat. They noted a significant interaction between the two distance measures, and the classification by either enhanced the predictive value of the other. Such a system of "double classification" has been advocated to predict specific combining ability from pedigree and morphological data in oats (Souza and Sorrells 1991).

To compare the usefulness of different distance measures in terms of their interaction with each other and its effect on hybrid heterosis prediction, a system of "double classification" similar to that of Frei et al. (1986) was adopted. Crosses were divided into similar and dissimilar phenotypic diversity classes (SPD and DPD respectively) and similar (SP) and dissimilar (DP) pedigree classes, based on whether the parents used to generate them belong to related or distant morphological or pedigree goups. A comparison of associations between GD and F1P in the two morphological diversity classes (SPD and DPD), as well as in the two pedigree classes (SP and DP), revealed significant differences between them (Table 4). In general, the hybrid performance was more sensitive to GD changes in SPD and SP classes than in the corresponding DPD and DP classes. Correlations between GD and F1P were significantly smaller in DPD and DP classes in comparison to their counterparts. The exceptions were OP, CPC and DF for which GD-F1p associations remained relatively unchanged over two morphological diversity classes.

The poor heterotic response of F_1s to genetic diversity (GD in the present case) in crosses between unrelated lines has been frequently reported in the literature and several explanations have been put forward to explain the phenomenon. Souza and Sorrels (1991), on the basis of their studies on oats, proposed that autogamous species exhibit a reduction in agronomic fitness in highly heterozygous crosses due to an important form of interallelic interaction. In general, the increased hybrid vigour has been attributed to the masking effect of deleterious alleles in heterozygous combinations (Dobzhansky 1952). The correlation structure observed in the present study seems to fit well with this proposition. The crosses of related parents showed a linear heterotic response to the changes in parental diversity, according to the classical "masking of deleterious alleles" explanation of Dobzhansky. This explains the high GD-F1P correlations for SPD/SP crosses. However, a saturation is attained for such a "masking effect" after the parental diversity reaches a certain level. At this juncture, any further increase in hybrid heterozygosity due to an increased parental divergence does not seem to lead to a corresponding "masking effect". As a consequence, heterotic response does not remain a linear function of parental divergence and this leads to low GD-F1P correlations in DPD/DP crosses.

Different levels of dominance in crosses of related and unrelated lines may be another cause for the observations in the present study. High levels of dominance, which are expected to be more important in crosses of unrelated parents in comparison to related parent crosses, may mask the variability in the former. The consequence of such masking is reflected in decreased linearity between parental divergence and heterosis. Yet another causative factor may be the lack of co-adaptation between parental alleles in unrelated parent crosses. According to Souza and Sorrells (1991) morphological characters are measures of differences in parental adaptation. The lack of co-adaptation leads to an unpredictable heterotic response in DPD/DP crosses. Finally, the high level of "background similarity" according to Cox et al. (1985) may be responsible for low GD-F1P correlations in DP crosses. All those lines for which no pedigree records were available, were considered as unrelated. However, this seems to be an artificial assemblage, because no significant differences were noted for isozymic GD and morphological PD values between SP and DP classes (see Table 4). Instead, the DP class might be a blend of lines with varying degrees of relatedness.

In summary, the results of our study indicate that: (1) the low associations between GD values and F_1 heterosis will not be of much use in breeding programs when

Table 4 Simple correlation coefficients between GD and hybrid means in similar (SPD) and dissimilar (DPD) phenotypic diversty and similar (SP) and dissimilar (DP) pedigree classes of F_1s

Trait[a]	Phenotypic diversity		Pedigree background	
	SPD[b]	DPD	SP	DP
SY	0.51*	0.02	0.87**	−0.07
OY	0.48**	0.10	0.89**	0.00
OP	0.24	0.39*	0.90**	0.42
CPC	−0.36	−0.42	−0.68	−0.33
PH	−0.49*	0.04	0.51	−0.21
DF	−0.56**	−0.53**	−0.38	−0.07
GD value[c]	0.25	0.31	0.27	0.26
PD value[d]	19.99	41.41	34.87	36.88

* $P \leqslant 0.05$; ** $P \leqslant 0.01$

[a] For explanation see Table 2

[b] Number of crosses in SPD, DPD, SP and DP classes was 22, 26, 7 & 17 respectively

[c,d] GD and PD values are parental distance values

crosses between both related and unrelated parents are considered; (2) it is possible to improve heterosis predictions for at least some characters, by partitioning GD into GGD and SGD and correlating the latter with SCA; and (3) heterotic response is more sensitive to parental isozymic differences in morphologically or pedigree-related cross combinations in comparison to crosses between unrelated parents.

Regarding the last point, it is worth mentioning that usually the high-yielding hybrids in crop species are between unrelated parents. However, due to bad coadaptation of alleles in divergent crosses, their production potential may never be realised in new environments. A compromise between adaptation and diversity might be a worthwhile idea to consider when making a selection of suitable parents for a breeding programme. A prior survey for the identification of markers which show tight linkages with QTLs of interest is likely to result in a more reliable usage of markers in breeding programmes.

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