



Relationship of molecular and phenotypic divergence with hybrid performance in Indian mustard (*Brassica juncea*)

VINU V¹, NAVEEN SINGH², SUJATA VASUDEV³, S C GIRI⁴, RAJENDRA SINGH⁵, B DASS⁶ and D K YADAVA⁷

ICAR–Indian Agriculture Research Institute, New Delhi 110 012

Received: 7 May 2015; Accepted: 9 May 2016

ABSTRACT

Predicting hybrid performance from the parental generation could largely enhance the efficiency of hybrid breeding programmes. To determine the relationship of parental distances estimated from phenotypic traits and SSR markers with F₁ performance, average heterosis and heterobeltiosis in 44 indigenous and exotic genotypes of Indian mustard [*Brassica juncea* (L.) Czern. and Coss.], were studied. Jaccard's genetic distances (JD) and Manhattan genetic distances (MD) were taken as criteria to classify the genotypic pairs into four diversity groups, viz. high, intermediate high, intermediate low and low. Seventy crosses representing the four diversity groups each for JD and MD were evaluated. Placement of higher number of significantly better hybrids was in extreme diversity groups created using JD, while, it was higher in intermediate diversity groups generated through MD. Low regression values were observed between JD among genotypic pairs and mean performance ($R^2 = 0.02$), average heterosis ($R^2 = 0.046$) and heterobeltiosis ($R^2 = 0.15$). Similarly, low regression values were observed between MD among genotypic pairs and mean performance ($R^2 = 0.033$), average heterosis ($R^2 = 0.046$) and heterobeltiosis ($R^2 = 0.009$). The slope of linear regression curve, placement of hybrids on the plot and low regression values in all the cases revealed that there is no significant association between genetic distance and hybrid performance. Therefore, desirable genetic diversity, in form of heterotic pools, needs to be identified from indigenous and exotic germplasm for expression of heterosis.

Key words: *Brassica juncea*, Genetic diversity, Heterosis, Hybrid performance

The total global oil demand is escalating with the increasing population and improving life standards. To meet out this demand, major jump in the oilseed productivity is needed through genetic interventions. Exploitation of heterosis has become a priority for increasing crops productivity. Identifying parental combination with significant yield heterosis is the most important step in developing hybrids. Researchers have reported substantial amount of heterosis in rapeseed-mustard (Gupta *et al.* 2011, Yadava *et al.* 2012, Singh *et al.* 2015) which encouraged the breeders to put concerted efforts in making large number of crosses and their extensive testing for identification of heterotic combinations. However, this exercise is cost and time intensive. It is, therefore, imperative to find a simple and reliable method that could predict heterosis from information on parents.

The level of genetic diversity between parents has been proposed as a predictor of heterosis. In agreement with this

theory, positive association between morphological distances or geographic origin with heterosis was observed in *B. napus* (Ali *et al.* 1995), *B. juncea* (Pradhan *et al.* 1993) and *B. rapa* (Falk *et al.* 1994). However, the identification of heterotic combinations based on morphological characters may get influenced by environmental factors. Molecular markers, independent of environmental effect, provide a new technological base for selection of parental lines and prediction of hybrid performance. Scattered efforts have been made in *B. napus* and some other species to assess the genetic distances using molecular markers for prediction of heterosis. The molecular markers, such as Random Amplified Polymorphic DNA (RAPD), were used in calculating genetic distances (Becker and Engqvist 1995, Riaz *et al.* 2001, Qian *et al.* 2009). The outcomes from these studies are by and large conflicting (Shen *et al.* 2004, Yu *et al.* 2005), moreover, most of these studies involved small number of hybrids. Comprehensive account on assessment of genetic distance using highly abundant molecular markers, such as Simple Sequence Repeats (SSRs), for prediction of heterosis in Indian mustard [*Brassica juncea* (L.) Czern. and Coss.] is very limited. It would be interesting to calculate the genetic distances involving both SSR markers and morphological yield contributing parameters, compare them and to work out the relationship of these genetic distances

¹Ph D Scholar (e mail: vinu.kathu@gmail.com), ²Senior Scientist (e mail: n.singhk@rediffmail.com), ^{3&7}Principal Scientist (e mail: sujatavasudev@gmail.com, dkygenet@gmail.com), ⁴Senior Technical Officer (e mail: satishgirigenet@gmail.com), ^{5&6}Chief Technical Officer (e mail: rajendraslakra@gmail.com, bhagwandass391@gmail.com), Division of Genetics.

with hybrid performance in *B. juncea* genotypes. Involving genotypes from different geographical regions of India and those with exotic origin could harbour different set of alleles due to differential selection pressure and thus would represent larger genetic variability. Furthermore, generating large number of hybrids involving genotypic pairs with variable genetic diversity in this self pollinated species would help in presenting a better understanding of relationships.

MATERIALS AND METHODS

The experimental materials for the present study comprised of 44 Indian mustard genotypes, including commercial varieties and pure lines of indigenous and exotic origin, representing different geographical regions. These also include conventional, ‘0’ (low erucic acid varieties) and ‘00’ (low erucic and glucosinolates) quality genotypes. Standard package of practices were followed to raise a good crop.

These genotypes were raised in randomized block design with three replications for studying morphological diversity at IARI, New Delhi during *rabi* season. Each plot comprised of four rows of 5 m length. The row to row distance was kept at 30 cm, whereas plant to plant distance was kept at 10-15 cm. Data on 12 yield and yield contributing traits, viz. plant height (cm), days to maturity, point to first branch (cm), number of primary branches, number of secondary branches, number of siliquae on main shoot, main shoot length (cm), point to first siliqua (cm), siliqua length (cm), number of seeds/siliqua, seed yield/plant (g) and 1 000 seed weight (g) was recorded on five competitive plants in each plot, except for maturity where it was taken on plot basis.

Leaf tissues for DNA isolation were collected from young expanding leaves of plants at 5-7 leaf stage. DNA was isolated from all the 44 genotypes using the modified CTAB method (Doyle and Doyle 1990) and diluted to a final concentration of 20 ng/μl using TE (10 mM Tris-HCl and 1 mM EDTA) buffer. A total of 143 A and B genome derived SSR primers, known to express polymorphism among Indian mustard genotypes or in diploid *Brassica* species (Vinu *et al.* 2013), were used for studying the polymorphism. The amplification reaction was carried out in 10 μl reaction volume containing 10 × Taq buffer, 1 mM MgCl₂, 10 mM dNTPs, 200 pmol primer, 1 units Taq DNA polymerase and 20 ng template DNA. PCR amplification was programmed for 35 cycles after an initial denaturation cycle for 5 min at 94°C. Each cycle consisted of a denaturation step at 94°C for 1 min, an annealing step at 58°C for 1 min, and an extension step at 72°C for 2 min, followed by extension cycle for 7 min at 72°C in the final cycle. The amplified fragments were resolved on 2% agarose gel. Bands were scored as 0 for absence and 1 for presence in each genotype.

The phenotypic data recorded on 12 yield and yield related traits were subjected to analysis of variance (ANOVA). Manhattan dissimilarity coefficients (Sokal and Michener 1958) were calculated by pair-wise comparisons

of varieties using NTSYS-pc 2.02 programme (Rohlf 1998) involving phenotypic data recorded on yield and yield contributing traits. From the binary data generated by SSR primers similarity matrix was calculated using Jaccard’s coefficient (Sneath and Sokal 1973), pooling together alleles across all loci, using NTSYS-pc 2.02 software (Rohlf 1998). From the similarity coefficients matrix, thus generated, the dissimilarity coefficients (Genetic distance = 1- similarity coefficient) were calculated. Clustering of genotypes was done by using both phenotypic and molecular data, based on an average linkage algorithm (UPGMA, unweighted pair group method with an arithmetic average) with the help of DARwin 5.0 programme (Perrier and Jacquemoud-Collet 2006).

Mean and standard deviation of both the genetic distance matrices were calculated and were used independently for classifying the diversity groups (Fig 1).

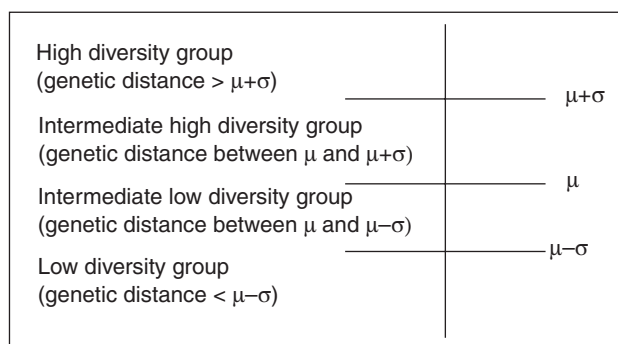


Fig 1 Grouping based on genetic distances among genotypic pairs

Genetic distance, each from Jaccard’s similarity coefficients and Manhattan dissimilarity coefficients, between genotypic pairs was taken as a criterion for generating crosses from all diversity groups at IARI Regional Station, Wellington in the offseason. To represent all the four diversity groups a total of 70 F₁s were generated. These hybrids along with their parents were grown in a randomized block design with three replications at the IARI experimental farm. Each plot comprised of single row of 3 m length. Observations were recorded in each replication on 12 yield and yield contributing traits as per standard methods on five competitive plants, except for maturity where it was taken on plot basis. These data were then subjected to analysis of variance and average heterosis and heterobeltiosis were calculated. Critical Differences (CD) were used for testing the significance of hybrids.

Linear regression equation was developed by taking genetic distances among parental genotypic pairs used in generating each of the 70 hybrids as independent variable and yield in terms of mean performance of hybrid, average heterosis or heterobeltiosis as dependent variable.

RESULTS AND DISCUSSION

Prediction of hybrid performance is of primary interest to all hybrid crop breeding programmes and has attracted

Table 1 Analysis of variance of the 70 F₁ hybrids and their parents and mean performance of hybrids

Source	Replications	Genotypes	ESS	SEM	CD (P=0.05)	Mean	Range
df	2	111	222				
Plant height	248.86*	1798.26*	117.93	6.68	17.60	232.00	193-264
Days to maturity	0.79*	26.63*	0.58	0.44	1.23	149.59	140-154
Point to first branch	174.91*	1549.41*	89.50	5.46	15.33	72.16	15-132
No. of primary branches/plant	0.16	5.52*	0.64	0.46	1.30	6.27	4.33-9.17
Secondary branches/plant	5.67	84.06*	6.34	1.45	4.08	16.26	5.17-28.0
Main shoot length	30.5	390.43*	47.91	3.99	11.22	68.23	38.5-101.8
Point to first siliqua	19.94*	62.99*	13.09	2.08	5.86	9.25	4.5-33.5
No. of siliquae on main shoot	0.58	209.05*	24.01	2.83	7.94	50.68	36-70.67
Siliqua length	-0.01	0.54*	0.07	0.15	0.42	3.52	2.4-4.24
No. of seeds/siliqua	0.34	9.53*	1.88	0.79	2.22	14.93	10.23-19.23
Seed yield/plant	2.25	70.24*	5.05	1.29	3.64	18.53	10.5-31.6
1000 seed weight	0.03	2.86*	0.14	0.22	0.61	4.00	1.83-5.98

enormous efforts. If heterosis could be predicted from data of the parental generation, costs involved in making large number of crosses and field evaluation to select heterotic combinations could be reduced drastically. However, even with the discovery of molecular markers, the prediction of F₁ performance based on genetic distance is far from direct practical application (Burstin and Charcosset 1997, Melchinger 1999). Therefore, through this study an attempt has made to understand the diversity among the genotypes and to see the relationship between genetic distance and hybrid performance.

Genetic distances and grouping of parents: The analysis of variance for 12 yield and yield contributing traits revealed that 44 genotypes taken for this investigation had significant genetic variation (data not given). Metric data on 12 phenotypic traits were used to calculate the Manhattan dissimilarity coefficients (MD). Manhattan dissimilarity coefficients ranged from 0.07 - 0.47 with an average of 0.23 (Table 1). In the molecular analysis out of 143 SSR primers used 134 were reported to express polymorphism and out of 355 amplified products, 33 loci were reported monomorphic. Using the binary data generated by 134 polymorphic SSR primers Jaccard's similarity coefficients were calculated and converted to genetic distances (JD). JD ranged from 0.17 to 0.68 with an average of 0.42 (Table 1). It seems that inclusion of five exotic collections from Poland, Australia

and East Europe, and 39 Indian genotypes developed from different national breeding programmes located in different regions contributed significantly to this variation in our study. Such significant genetic variation was also been reported by Vaishnavi *et al.* (2006), Alie *et al.* (2009), Singh *et al.* (2010) and Yadava *et al.* (2009) on metric traits in *B. juncea*.

Genotypic pairs were classified into highly diverse, intermediate high diverse, intermediate low diverse and low diverse groups independently based on both the genetic distances. In general, same genotypic pair fall in different diversity group delineated using morphological and molecular data.

Hybrid evaluation

To identify heterotic combinations and to know suitability of parents for generating good segregants, 70 hybrids developed from diverse parents were evaluated. The analysis of variances for all the 12 quantitative traits including hybrids and parents was done (Table 1). The variation due to treatments was significant for all the 12 yield contributing traits. The mean performance of hybrids for each of the 12 yield related traits are presented in the same table. The range of mean performance (10.51 to 31.61g), average heterosis (-43.34 to 104.66 %) and heterobeltiosis (-47.26 to 81.29 %) for seed yield per plant

Table 2 Mean and range of heterosis estimated for 12 phenotypic traits on 70 crosses of *B. juncea*

Parameter	Plant height (cm)	Days to maturity	Point to first branch (cm)	No. of primary branches	No. of secondary branches	Main shoot length (cm)	Point to first siliqua (cm)	No. of siliquae on main shoot	Siliqua length (cm)	No. of Seeds/siliqua	Seed yield/plant (g)	1000 seed weight (g)
Average heterosis	10.94 (-8.8 to 37.7)	0.15 (-4.52 to 6.62)	20.25 (-79.45 to 88.14)	11.12 (-42.85 to 75.86)	31.08 (-45.76 to 145.75)	0.24 (-34.33 to -33.33)	-1.44 (-61.18 to 300)	6.48 (-24.69 to 46.88)	3.60 (-17.67 to 41.93)	2.98 (-28.50 to 34.34)	15.89 (-43.34 to 104.66)	-1.45 (-63.38 to 36.02)
Hetero-beltiosis	18.92 (1.07 to 65.75)	1.31 (-4.09 to 9.73)	49.17 (-78.1 to 277.54)	-1.75 (-54.39 to 64.52)	9.72 (-58.26 to 137.97)	-8.64 (-40.58 to 19.70)	43.96 (-50.59 to 547.06)	-2.36 (-39.27 to 31.25)	-3.93 (-30.31 to 27.85)	-4.38 (-31.02 to 32.04)	4.80 (-47.26 to 81.29)	-14.37 (-67.33 to 21.66)

Table 3 Comparison of diversity groups generated by morphological and molecular genetic distances in explaining mean performance, average heterosis and heterobeltiosis for seed yield

Parameters	Diversity group							
	Molecular data based genetic distances				Phenotypic data based genetic distances			
	High	Intermediate high	Intermediate low	Low	High	Intermediate high	Intermediate low	Low
No. of genotypic pairs used for generating hybrids	21	12	18	19	16	13	33	8
Mean Genetic distance of the group	0.51	0.45	0.40	0.29	0.37	0.27	0.18	0.12
Mean yield performance of the hybrids@	20.56 (10)	17.59(2)	15.38(1)	19.86 (6)	17.84 (3)	16.92(1)	19.02(10)	20.49 (5)
Average heterosis (%) #	25.7 (10)	20.9(6)	-1.6(2)	18.5(7)	28.5 (7)	18.6(4)	9.4(10)	13.1(4)
Mean heterobeltiosis (%) [§]	13.99 (11)	6.62(5)	-11.73(4)	9.15 (5)	9.05 (8)	10.70(10)	0.65(5)	3.78 (4)

Value in parenthesis represents: @number of hybrids having significantly higher mean performance; #number of hybrids having significant superiority over mean value of the parents; [§]number of hybrids having significant superiority over respective better parents.

revealed genetic variation among the hybrids for this trait (Table 1 and 2). The degree of heterosis varied considerably among traits. Significant heterosis for plant height, number of primary and secondary branches, length of siliqua, seed yield per plant and 1 000-seed weight was also reported by Ram *et al.* (1976). Several workers have reported reasonable degree of heterosis in Indian mustard for number of branches/plant (Singh *et al.* 1985, Varshnay *et al.* 1985, Ali *et al.* 2000).

Associating genetic distances with hybrid performance

Mean JD of the four diversity groups, viz. high, intermediate high, intermediate low and low diverse were 0.51, 0.45, 0.40 and 0.29, whereas mean MD were observed as 0.37, 0.27, 0.18 and 0.12 for respective groups in the same order (Table 3). Mean yield performance was observed highest (20.56 g) for high diversity group using JD between genotypic pairs, whereas, based on MD it was highest (20.49 g) for low diversity groups. Number of hybrids having significant mean yield performance, average heterosis and heterobeltiosis, placed in different diversity groups created using JD and MD values, are presented in Table 3. When molecular data were used to create diversity group, larger number of significantly better hybrids were observed from parental pairs having high and low diversity. When basis of delineating the diversity group was morphological data, larger number of superior hybrids were from parents having intermediate genetic distances (Table 3).

The level of genetic diversity between parents has been considered as a predictor of heterosis. In agreement with this theory, positive association between morphological distances or geographic origin with heterosis was observed in *B. napus* (Lefort-Buson *et al.* 1986, Ali *et al.* 1995), *B. juncea* (Gupta *et al.* 1991, Pradhan *et al.* 1993) and *B. rapa* (Falk *et al.* 1994). On the other hand, contradictory reports were available when molecular markers were used in making such predictions. In majority of previous studies on this aspect in *B. juncea*, small number of hybrids were used to

establish such relationships. Therefore, including larger number of hybrids, as taken in this study, were required for thorough understanding of these associations. In this study, placement of higher number of significantly better hybrids was in extreme diversity groups (highly diverse + low diverse) created using JD, while, it was higher in intermediate diversity groups generated (intermediate high diverse + intermediate low diverse) using MD. The two approaches used for establishing diversity groups are thus not corresponding in explaining the placement of these superior hybrids with respect to mean performance, average heterosis and heterobeltiosis for seed yield. Teklewold and Becker (2006) observed similar results in *B. carinata* using the molecular and phenotypic distances for their ability to predict heterosis and F₁ performance. Li *et al.* (1998) suggested that parents with moderate extent of genotypic divergence could produce applicable heterotic hybrids which may be due to harmonized genome in the F₁ hybrid. Similar results were obtained from our study using MD based grouping of genotypic pairs.

To observe the relationship of genetic distances (both JD and MD) with mean performance, average heterosis and heterobeltiosis regression analysis was done. Low regression values were observed between JD among genotypic pairs and mean performance ($R^2 = 0.02$), average heterosis ($R^2 = 0.046$) and heterobeltiosis ($R^2 = 0.15$). Similarly, low regression values were observed between MD among genotypic pairs and mean performance ($R^2 = 0.033$), average heterosis ($R^2 = 0.046$) and heterobeltiosis ($R^2 = 0.009$). The slope of linear regression curve, placement of hybrids on the plot and low regression values in all the cases revealed that there is no strong association between genetic distances and hybrid performance.

Girke (2002), Shen *et al.* (2003) and Yu *et al.* (2005) in *B. napus* and Jain *et al.* (1994) in *B. juncea* reported a low association of DNA markers based diversity with heterosis. However, contrasting results have been reported by Plieske and Struss (2001) using SSR markers and Shen *et al.* (2003)

using AFLP markers in *B. napus*, where they observed positive association between hybrid seed yield and genetic distance. Knaak and Ecke (1995), Diers *et al.* (1996) and Riaz *et al.* (2001) independently reported the usefulness of molecular markers based parental distance to predict heterosis, especially when the parents are genetically related. 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.

Reason for conflicting report about the association of genomic diversity with hybrid performance may be due to i) recognition of genomic differences in non-coding regions ii) presence of genomic variation for traits unrelated to yield and iii) cancellation effect due to presence of epistasis or coexistence of negative alleles, along with the positive ones, for different QTLs responsible for trait expression. Heterosis, therefore, seems to be due to presence of parental differences for yield related QTLs and their complementation in F₁ generation (Gaikwad *et al.* 2014) with minimum cancellation effect by alleles of such QTLs in the expression of yield. It is therefore, suggested that for improving yield heterosis its related heterotic QTLs need to be identified and utilized, rather than considering total genomic diversity as a basis for improving the magnitude of heterosis. Furthermore, heterotic pools need to be identified from indigenous and exotic germplasm so that desirable genetic variation can be exploited for expression of heterosis.

REFERENCES

- Ali A, Shah S A and Hassan S. 2000. Mutant heterosis in oilseed rape. *Cruciferae Newsletter* **22**: 67–8.
- Ali M, Copeland L O, Elias S G and Kelly J D. 1995. Relationship between genetic distance and heterosis for yield and morphological traits in winter canola (*Brassica napus*). *Theoretical and Applied Genetics* **91**: 118–21.
- Alie F A, Singh T, Tariq and Sharma P K. 2009. Genetic diversity analysis in Indian mustard [*Brassica juncea* (L.) Czern & Coss]. *Progressive Agriculture - An International Journal* **9**(1): 50–3.
- Becker H C and Engqvist G M. 1995. The potential of resynthesized rapeseed for hybrid breeding. (In) *Proceedings of 9th International Rapeseed Conference: Rapeseed Today and Tomorrow*. Murphy D J (Ed.). Cambridge, UK, pp 113–5.
- Burstin J and Charcosset A. 1997. Relationship between phenotypic and marker distances: theoretical and experimental investigations. *Heredity* **79**: 477–83.
- Diers B W, McVetty P B E and Osborn T C. 1996. Relationship between heterosis and genetic distance based on Restriction Fragment Length Polymorphism markers in oilseed rape (*Brassica napus* L.). *Annals of Botany* **36**: 79–83.
- Doyle J J and Doyle J L. 1990. Isolation of Plant DNA from fresh tissue. *Focus* **12**: 13–5.
- Falk K, Rakow G, Downey R and Spurr D. 1994. Performance of inter-cultivar summer turnip rape hybrids in Saskatchewan. *Canadian Journal of Plant Science* **74**: 441–5.
- Gaikwad K B, Singh N, Bhatia D, Kaur R, Bains N S, Bharaj T S and Singh K. 2014. Yield-enhancing heterotic QTL transferred from wild into cultivated species of rice (*Oryza sativa* L.). *PLoS ONE* **9**(6): e96939. doi:10.1371/journal.pone.0096939.
- Girke A. 2002. Neue Genpools aus resynthetisiertem Raps (*Brassic napus* L.) für die Hybridzucht. Dissertation, Universität Göttingen: <http://www.webdoc.sub.gwdg.de/diss/2002/girke/girke.pdf>.
- Gupta P, Chaudhary and Lal S K. 2011. Heterosis and combining ability analysis for yield and its components in Indian mustard (*Brassica juncea* L. Czern & Coss). *Academic Journal of Plant Science* **4**(2): 45–52.
- Gupta V P, Sekhon M S and Satija D R. 1991. Studies on genetic diversity, heterosis and combining ability in Indian mustard [*Brassica juncea* L. (Czern and Coss)]. *Indian Journal of Genetics* **51**: 448–53.
- Jain A, Bhatia S, Banga S S, Prakash S and Laxmimurukan M. 1994. Potential use of Random Amplified Polymorphic DNA (RAPD) technique to study genetic diversity in Indian mustard (*Brassica juncea*) and its relatedness to heterosis. *Theoretical and Applied Genetics* **88**: 116–22.
- Knaak C and Ecke W. 1995. Genetic diversity and F₁ performance in European winter oilseed rape (*Brassica napus* L.). (In) *Proceedings of 9th International Rapeseed Conference: Rapeseed Today and Tomorrow*. Murphy D J (Ed.). Cambridge, UK, pp 110–2.
- Lefort-Buson M, Guillot-Lemoine B and Dattee Y. 1986. Heterosis and genetic distance in rapeseed (*Brassica napus* L.): Use of different indicators of genetic divergence in a 7 × 7 diallel. *Agronomie* **6**: 839–44.
- Li R H, Xue C G, Yuan L P, He Y Q, Sun C Q, Yu S B, Li H X and Wang X K. 1998. Differentiation and classification of parental lines and favorable genic interactions affecting F₁ fertility in distant crosses of rice (*O. sativa* L.). *Theoretical and Applied Genetics* **96**: 526–38.
- Mahmood T, Ali M, Anwar M and Iqbal S. 2003. Heterosis for some quantitative characters in *Brassica juncea* L. *Asian Journal of Plant Science* **2**: 71–3.
- Mahto J L and Haider Z A. 2004. Short communication heterosis in Indian mustard (*Brassica juncea* L. Czern & Coss). *Journal of Tropical Agriculture* **42**(1-2): 39–41.
- Melchinger A E. 1999. Genetic diversity and heterosis. (In) *The Genetics and Exploitation of Heterosis in Crops*, pp 99–118. Coors J G and Pandey S (Eds). American Society of Agronomy Inc. and Crop Science Society of America Inc., Madison, USA.
- Perrier X and Jacquemoud-Collet J P. 2006. *Dissimilarity Analysis and Representation for Windows*. Version 5. Available at <http://darwin.cirad.fr/>.
- Plieske J and Struss D. 2001. Microsatellite markers for genome analysis in *Brassica*. I. Development in *Brassica napus* and abundance in Brassicaceae species. *Theoretical and Applied Genetics* **102**: 689–94.
- Pradhan A K, Sodhi Y S, Mukeropandhyay and Pental D. 1993. Heterosis breeding in Indian mustard (*Brassica juncea* L.): Analysis of component characters contributing to heterosis for yield. *Euphytica* **69**: 219–29.
- Qian W, Li Q, Noack J, Sass O, Meng J, Frauen M and Jung C. 2009. Heterotic patterns in rapeseed (*Brassica napus* L.): II. Crosses between European winter and Chinese semi-winter lines. *Plant Breeding* **128**: 466–70.
- Qian W, Sass O, Meng J, Li M, Frauen M and Jung C. 2007. I.

- Heterotic patterns in rapeseed (*Brassica napus* L.): Crosses between spring and Chinese semi-winter lines. *Theoretical and Applied Genetics* **115**: 27–34.
- Ram, Kashi, Ram K, Chauhan Y S and Katiyar R P. 1976. Partial diallel analysis in F₂ generation in Indian mustard. *Indian Journal of Agriculture Science* **46**(5): 229–32.
- Riaz A, Li G, Quresh Z, Swati M S and Quiros C F. 2001. Genetic diversity of oilseed *Brassica napus* inbred lines based on Sequence-Related Amplified Polymorphism and its relation to hybrid performance. *Plant Breeding* **120**: 411–5.
- Rohlf F J. 1998. *NTSYS-pc Numerical taxonomy and multivariate analysis system*. version 2.02. Available at www.exetersoftware.com/cat/ntsyspc/ntsyspc.html. Accessed on November 11, 2011.
- Shen J, Lu G, Fu T and Yang G. 2003. Relationship between heterosis and genetic distance based on AFLPs in *Brassica napus*. (In) *Proceedings of 11th International Rapeseed Conference*, Copenhagen, Denmark, pp 343–6.
- Singh D, Arya R K, Chandra N, Niwas R and Salisbury P. 2010. Genetic diversity studies in relation to seed yield and its component traits in Indian mustard (*Brassica juncea* L. Czern & Coss.). *Journal of Oilseed Brassica* **1**(1): 19–22.
- Singh H, Lather V S and Singh D. 1985. Extent of heterosis in relation to genotypic diversity in Indian x Exotic cross of Indian mustard (*Brassica juncea* L.). *Genet Iberica* **37**: 97–105.
- Singh N, Yadava D K, Vasudev S, Singh R, Giri S C, Dass B, Barun S and Prabhu K V. 2015. Combining ability and heterobeltiosis for yield and yield combining traits in high quality oil in Indian mustard (*Brassica juncea*) genotypes. *Indian Journal of Agricultural Sciences* **85**(4): 498–503.
- Sneath P H A and Sokal R R. 1973. *Numerical Taxonomy: The Principles and Practice of Numerical Classification*, p 573. Freeman and Company, San Francisco.
- Sokal R R and Michener C D. 1958. A statistical method for evaluating systematic relationships. *The University of Kansas Science Bulletin* **38**: 1 409–38.
- Teklewold A and Becker H C. 2006. Geographic pattern of genetic diversity among 43 Ethiopian mustard (*Brassica carinata* A. Braun) accessions as revealed by RAPD analysis. *Genetic Resources and Crop Evolution* **53**: 1 173–85.
- Turi N A, Raziuddin, Shah S S and Sardar A. 2006. Estimation of heterosis for some important traits in mustard (*Brassica juncea* L.). *Journal of Agricultural and Biological Science* **1**(4): 1–5.
- USDA. 2012. www.usda.gov/oce/forum/
- Vaishnava A, Sachan J N and Tewari S K. 2006. Genetic divergence for important quantitative traits in Indian mustard (*Brassica juncea* (L.) Czern and Coss). *Agricultural Science Digest* **26**(4): 269–72.
- Varshney S K. 1985. Heterosis in crosses between parents selected for harvest index and plant height in Indian rapeseed. *Indian Journal of Agricultural Sciences* **55**: 398–402.
- Verma O P, Singh H P and Singh V P. 1998. Heterosis and inbreeding depression in Indian mustard. *Cruciferae Newsletter* **20**: 75–6.
- Vinu V, Singh N, Sujata V, Yadava D K, Kumar S, Sugandh N, Bhat S R and Prabhu K V. 2013. Assessment of genetic diversity in *Brassica juncea* (*Brassicaceae*) genotypes using phenotypic differences and SSR markers. *Revista de Biologia Tropical* **61**(4): 1 919–34.
- Yadava D K, Sapra R L, Sujata V, Dass B and Prabhu K V. 2009. Selection of high diversity with a minimal set of accessions from Indian mustard (*Brassica juncea* (L.) Czern & Coss.) germplasm collection. *Indian Journal of Agricultural Sciences* **79**(7): 552–4.
- Yadava D K, Singh N, Sujata V, Singh R, Singh S, Giri S C, Dwivedi V K and Prabhu K V. 2012. Combining ability and heterobeltiosis for yield and yield contributing traits in Indian mustard (*Brassica juncea*). *Indian Journal of Agricultural Sciences* **82**(7): 573–7.
- Yadava T P, Singh H, Gupta V P and Rana R K. 1974. Heterosis and combining ability in raya for yield and its components. *Indian Journal of Genetics and Plant Breeding* **34**: 684–95.
- Yu C Y, Hu S W, Zhao H X and Guo A G. 2005. Genetic distances revealed by morphological characters, isozymes, protein and RAPD markers and their relationships with hybrid performance in oilseed rape (*Brassica napus* L.). *Theoretical and Applied Genetics* **110**: 511–8.