

EVALUATION OF F₁ HYBRID DEVELOPED THROUGH SELF-INCOMPATIBILITY MECHANISM IN RADISH

Abstract: The radish (*Raphanus raphanistrum* subsp. *sativus*) is an edible root vegetable of the Brassicaceae family with 57 (20 Parents and 37 F₁s) radish genotypes to study the agronomic characteristics, the genetic diversity of these genotypes and to investigate the heterotic effects of the F₁ hybrids which is developed through self-incompatibility mechanism and to know the relationship between the diversity among the parents and high heterosis expressed in the selected hybrids. The genetic divergence among 57 genotypes of radish (*Raphanus sativus* L.) was determined through PCO, PCA, Cluster analysis (CLSA) and Mohalanobis's D₂ analysis. Through multivariate analysis based on 16 characters, 57 genotypes were grouped into five distant clusters. All the genotypes were grouped into five clusters. The highest inter-cluster distance was observed between cluster II and III and the lowest between II and V. The maximum values of the inter-cluster distance indicated that the genotypes belonging to cluster II and cluster V have far diverged from those of cluster III. The relationship study between genetic diversity and heterosis revealed that genetic distance between cluster II and cluster V was lowest (1.823) but the cross between the parental genotypes showed better heterotic performance among 37 crosses. Significant positive correlation between genetic distance and heterosis was found in pod length. Considering heterotic performance and self-incompatibility status the crosses P₇Xp₈ (SIXSI), P₃₁Xp₂₆ (SIXSI), P₄Xp₂₂ (SCXSI), P₂₄Xp₂₆ (SIXSI), P₄Xp₃₁ (SCXSI), P₁₁Xp₃₁ (SCXSI), and P₂₂Xp₂₃ (SIXSI) appeared to be promising.

Key words: Radish, F₁ hybrid, Self-incompatibility and Heterosis

Introduction

Radish (*Raphanus sativus* L., 2n = 2x = 18) is one of the popular and widely consumed winter vegetables of Bangladesh belonging to the family cruciferae (Brassicaceae) or the mustard family. It ranks second in both in acreage and major vegetable crop in Bangladesh (Ivy, 2008). It is widely cultivated in the cool season of BD covering an area of 22.5 thousand hectares and producing 20.5 thousand M. tons. Its yield rate is 3.73 M.tons per acres (BBS, 2016).

Radish is cross-pollinated quick growing vegetable crop, helps in increasing cropping intensity. Its fleshy edible roots and tender leaves are rich in Ca, P and Vit-C, protein and vitamin C and supplies a variety of minerals (Crampton, 2016). Radishes are useful for liver and gallbladder and liver troubles. Although radish is one of the most widely grown winter vegetables in Bangladesh, the production of vegetable seeds in Bangladesh is below the national requirements. Hybrid varieties give higher yield and better quality than open-pollinated varieties. Both the hybrid and biennial types do not produce seeds under Bangladesh condition. So, huge quantities of seeds are being imported every year at the cost of valuable foreign currency. Again the imported seeds are not always available in time for sowing. Moreover, the seeds produced by hybrid varieties are not suitable for getting better production. In this situation, it is very important to develop hybrids with high yield potential with consistent performance over diverse environments.

Recently hybrid seed production using self-incompatibility (SI) in many vegetables has drawn the attention of breeders. SI system is widely used to produce hybrid seeds in radish (Kalloo, 2011). The level of SI varied considerably within the same species and even within a self-incompatible population and there are gradations in individual plants (Dalai and Ahamed, 2009). Discovery of self-incompatibility as an alternative source of male sterility in radish has opened up an avenue in the production of hybrid seed on a commercial scale. Utilization of SI for hybrid seed production is considered better than the use of CMS. As because in the former hybrid seeds can be obtained from both the parents (Hinata and Nishio, 2000). Therefore, research work on various aspects of self-incompatibility system needs to be explored for its utilization in breeding. For the production of hybrids, selection of parent is very important. Genetic diversity can be used as a major tool in parent selection for efficient hybridization program (Govindaraj et al. 2015) if it is positively correlated with heterosis. So for efficient exploitation of heterosis in radish, genetic diversity and its relationship with

the heterosis need to be known. There are two types of heterosis will be evaluated 1) Mid-Parent Heterosis:

When the heterosis is estimated over the mid-parent i.e. mean value or average of the two parents is known as mid-parent heterosis. Where F₁ is mean of F₁ and MP is mean of two parents. Whereas the heterosis is estimated over the better parent is known as better parent heterosis. It is also known as heterobeltiosis and Where BP is mean of better parents (Srivnives, 2005). Successful exploitation of heterosis through the economic production of hybrid seed in a large scale is dependent on various essential prerequisites and information in this regard has not yet been fully explored on radish in Bangladesh. Therefore, conceiving the above idea the present investigation was undertaken with the following objectives:

- To study the agronomic characteristics of parents and F₁ hybrids of Radish.
- To know the genetic diversity among the genotypes (parents and hybrids).
- To investigate the heterotic effects of the F₁ hybrids for some desirable yield contributing characters.
- To study the relationship between the diversity among the parents and high heterosis expressed in the selected hybrids.

MATERIALS AND METHODS

The experiment was conducted at the department of Plant Breeding the Bangladesh Agricultural University, Mymensingh, Bangladesh, during the winter season of 2008-2009. The location of the site is 24.00 N latitude and 90.00 E longitudes with an elevation of 8.4 m above sea level, where humidity (70-80%) average and temperature (20-13°C) during the vegetative season (Nov.-Jan.) for Radish. In several species of radish reproductive behavior shows older, unpollinated flowers are more likely to self. If flowers are receiving pollen, staying open longer may increase the number of mates. In wild radish, for which there is considerable information on seed paternity, older flowers produce fewer seeds and appear to discriminate less among pollen donors. Pollen donor performance can also be linked to maternal plant age. Different pollinators and mates are available across the season. Also in wild radish, maternal plants appear to exert the most control over paternity when they are of intermediate age (Weiss, 1995).

Pollen longevity and/or viability may change based upon environmental conditions and thus will change as the environment changes over a season. Changes in the cell membrane, carbohydrate levels, temperature, relative humidity and pollen morphology all appear to affect how long a pollen grain remains viable (Dafni and Firmage, 2000) and most of these variables are in some way related to how much water the pollen grain needs to maintain viability (Pacini et al., 2006).

The experiment with 37 hybrids and 20 parents were grown in tri-replicated Randomized Complete Block (RCB) design. Two rows of each constituted the experimental unit. The row spacing was 60 cm having plant spacing of 30 cm within the row. Data were recorded at edible root stage and seed maturing stages. At both the time ten randomly selected competitive plants from each plot were taken for recording the following characters:

1. Plant height
2. No of leaf /plant
3. Leaf length
4. Leaf breadth
5. Root length
6. Root breadth
7. Root weight
8. Booting stage
9. 1st flowering stage
10. No of primary branches /plant
11. No of secondary branches /plat
12. Pod /plant
13. pod length
14. Pod breadth
15. Seed /pod
16. 100 seed weight

All the collected data of the present study were statistically analyzed. The statistical analyses for various characters under investigation were done and the analysis of variance for each of the characters was performed by F test and mean values were separated by DMRT (Steel and Torrie, 1980). The analysis of variance was done according to Gouliden's methods (1998). Simple correlation

coefficient characters at phenotypic and genotypic levels were analyzed following Hayes *et al.* (2004) and Rashid (2003). Correlation coefficients were further partitioned into direct and indirect effects by path coefficient analysis described by Dewey and Lu (1999). The principal coordinate analysis is equivalent to PCA but it is used to calculate inter-unit distances. Heterosis can be analysis through quantitative trait locus (QTL) analysis using a recombinant inbred line (RIL). Through the use of all dimensions of it gives the minimum distance between each pair of n points using similarity matrix (Rashid,2003). Inter-distance between genotypes was studied by PCO.

Genotypes were divided into groups on the basis of data set into some number of mutually exclusive groups. The clustering was done using hierarchical classification. In Genstat, the algorithm is used for searching. The optimal values of the criteria followed by some initial classification of the genotypes into required number of groups, the algorithm repeatedly transfers genotypes from one group to another so long as such transfer improved the value of the criterion. When no further transfer can be found to improve the criterion, the algorithm switches to a second stage which examines the effect of swapping two genotypes of different classes. The canonical vector analysis is based on the roots and vectors of $W-1B$, where w is the pooled within-groups covariance matrix and B is the among groups covariance matrix. It provides two-dimensional plots, which helped in separating different populations involved.

Once the clusters were formed, the average intra-cluster distance for each cluster was calculated by taking all possible D^2 values within the numbers of cluster obtained from PCO. The formula used was D^2/n , where D^2 is the sum of distances between all possible combinations (n) of the genotypes included in a cluster. The square root of the average D^2 values represent the distances (D^2) within the cluster.

Using the values of intra-and inter-cluster distance diagram. It gives a brief idea of the pattern of diversity among the genotypes included in a cluster.

RESULTS AND DISCUSSION

The results on the basis of agronomic characteristics, analysis of variance, diversity and heterosis and the relationship between diversity and heterosis based on different characters of 57 radish genotypes (20 parents and 37 F_1 s) are presented in Table 1 to Table 6. Analysis of variance revealed significant differences for all the characters among the genotypes (Table 4), indicating considerable genetic variability in the material.

Table 1: Total variation among the 16 characters describing 57 lines of radish

Principal component axis	Eigen values	% of total variation accounted for	Cumulative percent
Plant height	41.914	61.69	61.69
Root length	10.723	15.78	77.47
Root breath	4.705	6.93	84.4
Root weight	3.245	4.78	89.18
No of leaf	1.796	2.64	91.82
Leaf length	1.253	1.84	93.66
Leaf breath	1.075	1.58	95.24
Booting stage	0.895	1.32	96.56
1 st flowering	0.778	1.14	97.7
No of P B/plant	0.536	0.79	98.49
No of S B/plant	0.413	0.61	99.1

Pod per plant	0.297	0.44	99.54
Pod length	0.195	0.29	99.83
Pod breath	0.074	0.11	99.94
Seed per pod	0.034	0.05	99.99
100 seed wt	0.007	0.01	100

Genetic diversity of the radish genotypes were analyzed by three multivariate techniques, results of which are discussed as follows:

Principal component analysis: The principal component analysis yielded Eigenvalues of each principal component axes of the ordination of genotypes with the first axes totally accounted for the variation among the genotypes, while five of these with Eigenvalues above unity accounted for 91.82 %. (Table 1) The first ten principal axes accounted for 98.49 % of the total variation among the 16 characters describing 57 lines of radish.

Construction of scatter diagram

Based on the values of principal component scores 1 and 2 obtained from the principal component analysis, a two- dimensional scatter diagram (Z_1 - Z_2) using component score 1 as X-axis and component score 2 as Y-axis was constructed, which has been presented in Fig- 1. The position of the genotypes in the scatter diagram was apparently distributed into five groups, which indicated that there exists considerable diversity among the genotypes of radish. The scattered diagram for the genotypes of five clusters revealed that the cluster-II and cluster-IV were distantly located which suggesting more diverged from rest of the clusters.

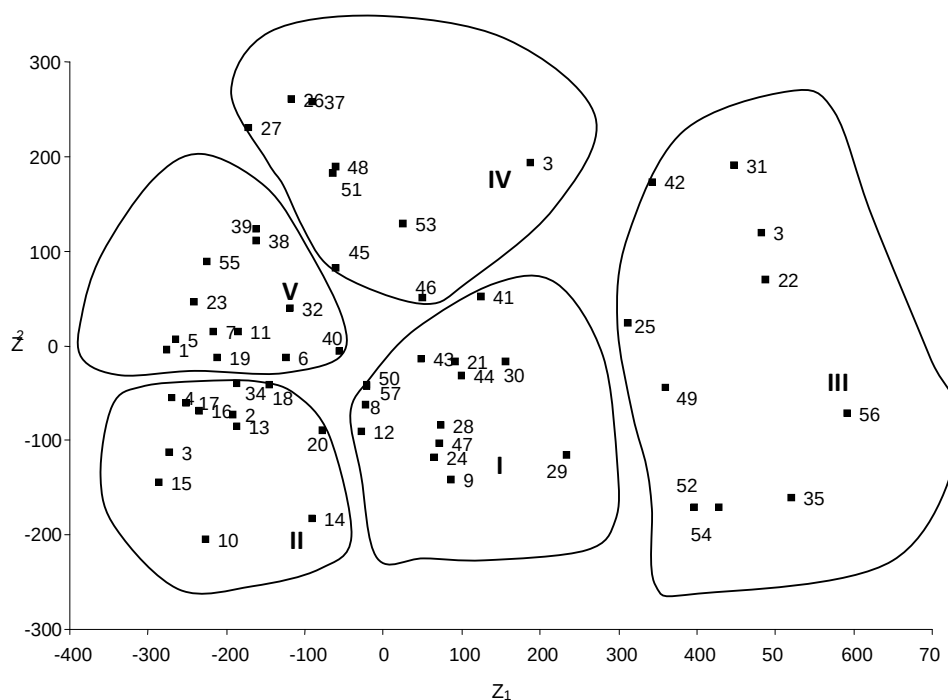


Figure 1: Relative position of 57 genotypes of radish in Z_1 - Z_2 graph using their principal component score based on quantitative characters superimposed with clustering.

(i) Non-hierarchical clustering:

Non- hierarchical clustering using covariance matrix grouped 57 genotypes of radish into five different clusters. These results confirmed the clustering pattern of the genotypes obtained through principal component analysis. The pattern of distribution of genotypes into five clusters is described in Table 2.

Table 2: Distribution of 57 genotypes of radish in five clusters

Cluster no.	Member number	Genotypes
I	15	P ₁₃ , P ₁₅ , P ₂₂ , P ₄ XP ₁₆ , P ₄ XP ₂₆ , P ₅ XP ₂₆ , P ₇ XP ₈ , P ₈ XP ₇ , P ₁₇ XP ₁₂ , P ₁₇ XP ₁₅ , P ₂₂ XP ₄ , P ₂₂ XP ₂₃ , P ₂₆ XP ₄ , P ₃₁ XP ₄ , P ₃₅ XP ₃₁
II	12	P ₅ , P ₇ , P ₈ , P ₁₆ , P ₂₃ , P ₂₄ , P ₂₆ , P ₃₁ , P ₃₂ , P ₃₃ , P ₃₅ , P ₁₂ XP ₇ ,
III	10	P ₄ XP ₂₂ , P ₄ XP ₃₁ , P ₁₀ XP ₂₆ , P ₁₃ XP ₅ , P ₁₃ XP ₃₅ , P ₁₇ XP ₃₂ , P ₂₆ XP ₁₀ , P ₃₁ XP ₂₆ , P ₃₂ XP ₃₃ , P ₃₅ XP ₁₃
IV	9	P ₅ XP ₁₁ , P ₅ XP ₂₄ , P ₁₁ XP ₃₁ , P ₁₅ XP ₁₇ , P ₂₃ XP ₂₂ , P ₂₄ XP ₂₆ , P ₂₆ XP ₅ , P ₃₁ XP ₁₁ , P ₃₁ XP ₃₅
V	11	P ₄ , P ₁₀ , P ₁₁ , P ₁₂ , P ₁₇ , P ₃₄ , P ₄ XP ₂₄ , P ₁₁ XP ₅ , P ₁₆ XP ₅ , P ₁₆ XP ₂₄ , P ₃₂ XP ₃₄ ,

(ii) Canonical Vector Analysis (CVA):

Canonical vector analysis was performed to obtain the inter-cluster distances (Mahalanobis's D^2 values). The intra-cluster distances were computed by the values of inter- genotypic distance matrix of PCO according to Rashid (2003). These values of inter-cluster distance (D^2) are presented in Table 3. Statistical distances represent the index of genetic diversity among the clusters.

Table 3: Average intra and inter cluster distances (D^2) for 57 lines of radish

	Cluster- I	Cluster- II	Cluster- III	Cluster- IV	Cluster- V
Cluster-I	1.013				
Cluster-II	3.776	0.947			
Cluster-III	4.468	8.240	1.471		
Cluster- IV	3.845	4.487	6.561	1.241	
Cluster- V	3.795	1.823	7.950	2.879	0.852

The intercluster distance was maximum between cluster II and III (8.240).The inter-cluster distance was minimum between cluster II and V (1.823). The maximum values of inter-cluster distance indicated that the genotypes belonging to cluster II have far diverged from those of cluster III and cluster V. The genotypes belonging to the distant clusters could be used in hybridization program for obtaining a wide spectrum of variation among the segregates. Similar reports were also made by Bansal et al. (2012), Mokate et al. (2007).

Heterosis

The mean sum of a square from analysis of variance for different leaf and root characters are presented in Table 6. The analysis of variance revealed highly significant differences for all the characters among the entries. The mean sum of square due to parent differed significantly, indicating a great deal of diversity among them. The estimates of heterosis over better parent are presented in Table 6.

Table 4: Inter parental distance

	P ₂	P ₃	P ₄	P ₅	P ₆	P ₇	P ₈	P ₉	P ₁₀	P ₁₁	P ₁₂	P ₁₃	P ₁₄	P ₁₅	P ₁₆	P ₁₇	P ₁₈	P ₁₉	P ₂₀
P ₁	1.65	1.5	1.1	0.4	1.7	1.5	2.0	2.3	2.1	1.5	2.1	1.6	2.2	1.8	1.7	0.8	2.0	1.4	2.1
P ₂		0.7	1.1	1.4	0.6	0.8	0.8	1.5	1.0	0.6	1.0	0.6	0.9	1.1	0.9	1.2	0.8	0.7	0.8
		4	0	9	8	4	0	9	1	3	8	8	5	6	5	9	5	2	9
		5	6	6	9	7	6	8	1	4	5	3	9	7	1	6	2	5	3

P ₃			1.0 9	1.3 3	1.2 0	0.8 0	1.4 6	2.1 2	0.9 1	0.8 6	1.6 1	1.0 0	1.3 0	0.8 1	0.7 7	1.3 1	1.0 8	1.0 2	1.2 9
P ₄				1.0 5	1.5 6	1.2 0	1.7 5	2.0 5	1.5 0	1.1 3	1.6 0	1.1 7	1.6 7	1.4 5	1.5 5	0.9 4	1.6 9	1.0 7	1.6 4
P ₅					1.5 7	1.2 7	1.8 2	2.1 9	1.9 5	1.2 8	1.9 9	1.5 2	2.0 2	1.7 8	1.5 0	0.8 0	1.7 7	1.2 3	2.0 2
P ₆						1.0 5	0.5 1	1.4 0	1.3 9	0.7 4	1.0 4	0.7 4	1.1 0	1.4 8	0.9 8	1.2 8	0.8 8	0.8 9	0.9 9
P ₇							1.4 1	2.0 4	1.3 0	0.4 1	1.4 9	1.0 7	1.3 7	1.0 6	0.7 0	1.2 4	0.8 1	0.8 8	1.2 5
P ₈								1.0 8	1.4 6	1.0 7	0.8 6	0.9 3	1.0 3	1.7 7	1.3 6	1.4 8	1.0 3	0.9 9	0.8 8
P ₉									1.9 4	1.7 0	0.8 3	1.4 4	1.2 4	2.4 6	2.1 4	1.7 2	1.7 6	1.4 1	1.4 3
P ₁₀										1.2 6	1.4 3	1.1 9	0.9 2	1.0 2	1.2 5	1.6 9	1.2 7	1.1 7	1.0 0
P ₁₁											1.1 5	0.7 7	1.1 8	1.1 9	0.8 1	1.0 9	0.7 0	0.7 0	1.0 4
P ₁₂												0.9 1	0.8 0	1.8 5	1.6 8	1.5 2	1.2 4	0.9 5	0.8 3
P ₁₃													0.9 9	1.2 6	1.1 6	1.1 1	1.1 0	0.8 4	1.0 7
P ₁₄														1.4 8	1.3 5	1.6 2	1.1 2	0.9 7	0.7 6
P ₁₅															0.9 1	1.6 1	1.3 7	1.3 1	1.4 8
P ₁₆																1.4 3	0.9 1	1.1 3	1.3 5
P ₁₇																	1.6 1	0.9 7	1.7 1
P ₁₈																		1.0 2	0.9 3
P ₁₉																			0.8 8

Table 5. Analysis of variance for different characters in radish

Source of variation	d.f	Plant height	No of leaf	Leaf length	Leaf breadth	Root length	Root breadth	Root volume	Root weight	Booting stage
Replication	2	0.012	0.995	1.524	4.472	2.545	0.893	348.108	2163.667	8.515
Genotypes	56	0.027**	39.732**	70.266**	12.175**	392.654**	87.784**	139158.008**	49302.627**	2.667**
Parents	19	0.024**	20.534**	83.933**	19.533*	47.363**	91.256**	19933.343**	10939.931**	2.312**
Hybrid	36	0.029**	46.555**	61.089**	8.234*	207.148**	80.706**	157321.728**	29554.726**	1.200**
Error	112	0.008	0.873	1.79	1.458	7.967	2.956	2628.418	1737.178	1.271

*P=0.05, *P=0.01

Source of variation	d.f	1 st flowering	50% flowering	No of primary b/plant	No of secondary b/plant	Pod/plant	Pod length	Pod breadth	Seed /pod	100 seed weight
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Replication	2	7.702	2.041	3.388	85.901	2814.9 88	4.543	0.162	0.292	0.004
Genotypes	56	24.71 4**	2.440 **	19.551 **	2117.417 **	286886 .16**	212.1 35**	1.964* *	4.377 **	0.164* *
Parents	19	4.929* *	2.330 **	36.446 **	1954.150 **	30364. 632**	149.3 91**	1.779* *	2.414 **	0.125* *
Hybrid	36	8.045* *	1.538 **	9.989**	1717.312 **	358579 .515**	249.5 28**	2.074* *	5.504 **	0.172* *
Error	11 2	2.833	2.874	1.273	29.252	1377.4 41	4.599	0.689	0.394	0.006

*P=0.05, **P=0.01

Correlation between genetic divergence and heterosis

The Correlation coefficient between inter-genotypic distances and their heterotic effects in 37 hybrids were computed to quantify the relationship between them. A Significant and positive relationship was found for pod length and the negatively significant correlation coefficient was found for root breath, No. of leaf/plant, leaf breath, No of S.B/plant against better parents (Table 6). Only No. of S B/plant produced negatively correlation against both mid- parent and a better parent.

Table 6. Correlation coefficients (r) between mid-parent and better parent heterosis of 37 F₁'s for 16 characters in radish

Characters	Correlations	
	MP	BP
Plant height	0.049	0.046
Root length	-0.035	0.029
Root breath	-0.112	-0.394*
Root weight	0.019	-0.029
No of leaf	-0.290	-0.340*
Leaf length	0.102	-0.006
Leaf breath	-0.068	-0.316*
Booting stage	0.037	-0.118
1st flowering	0.260	0.301
No of P B / plant	0.159	0.110
No of S B / plant	-0.415*	-0.566**
Pod per plant	0.254	0.180
Pod length	0.384*	0.422**
Pod breadth	0.016	-0.012
Seed per pod	0.075	0.037
100 seed wt	-0.200	-0.244

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Characters	Cluster Mean						
	I	II	III	IV	V	Vector -1	Vector-2
Plant height (cm)	21.5	20.3	22.5	21.5	18.1	0.049	0.046
Root length(mm)	13.1	12.5	8.7	5.3	9.1	-0.035	0.029
Root breath(mm)	3.1	4.3	1.8	3.6	2.9	-0.112	-0.394*
Root weight(gm)	10.6	11.9	18.2	12.5	13.5	0.019	-0.029
No of leaf	7	6	8	5	9	-0.290	-0.340*
Leaf length(mm)	16.2	20.3	13.4	15.4	12.1	0.102	-0.006
Leaf breath(mm)	5.1	4.6	5.2	7.3	6.6	-0.068	-0.316*
Booting stage	45	30	23	25	27	0.037	-0.118
1st flowering(days)	27	26	28	32	25	0.260	0.301
No of P B / plant	13	12	9	11	15	0.159	0.110
No of S B / plant	6	9	10	11	17	-0.415*	-0.566**
Pod per plant	13.1	12.3	10.4	11.9	15.1	0.254	0.180
Pod length(mm)	8.0	7.3	6.1	5.0	9.4	0.384*	0.422**
Pod breadth(mm)	1.3	1.6	1.4	1.1	17	0.016	-0.012
Seed per pod(gm)	2.2	1.4	2.5	2.1	1.7	0.075	0.037
100 seed wt(gm)	18.3	23.3	24.1	19.2	20.2	-0.200	-0.244

204 Table 7. Cluster means and Relative contributions of 16 characters to the total divergence in Radish.

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CONCLUSIONS

Analysis of variance revealed significant differences among the genotypes for all the characters indicating considerable genetic variability in the materials. And all the genotypes were grouped into five clusters. Where, a scattered diagram for the genotypes of five clusters and the highest inter-cluster distance was observed between cluster II and III (8.240) and the lowest between II and V (1.823). Considering heterotic performance and self-incompatibility status the crosses $P_7 \times P_8$ (SIXSI), $P_{31} \times P_{26}$ (SIXSI), $P_4 \times P_{22}$ (SCXSI), $P_{24} \times P_{26}$ (SIXSI), $P_4 \times P_{31}$ (SCXSI), $P_{11} \times P_{31}$ (SCXSI), $P_{22} \times P_{23}$ (SIXSI) appeared to be promising. Significant a positive correlation between genetic distance and heterosis was found in pod length but positive non-significant correlation was observed in plant height, 1st flowering, no of p b/plant, pod/plant, seed/pod against both for mid-parent and a better parent. Then the crosses between the genotypes P_7 , P_8 , P_{23} , P_{24} , P_{26} , and P_{31} of cluster II and P_4 , P_{11} of cluster V produced higher heterosis.

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