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RESEARCH ARTICLE

Genetic Divergence Studies in Doubled Haploids of Ethiopian Mustard (*Brassica carinata* A. Braun)

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The doubled haploids of Ethiopian mustard (*Brassica carinata* A. Braun) were evaluated along with mustard under two environments during *rabi,* 2010-11. Analysis of variance for different traits such as days to flower initiation, days to 50 per cent flowering, days to 75 per cent maturity, plant height, number of primary branches per plant, number of secondary branches per plant, siliquae per plant, length of main shoot, siliquae on main shoot, siliqua length, seeds per siliqua, 1000-seed weight, seed yield per plant, biological yield per plant, harvest index and per cent oil content revealed the presence of significant genetic variability for all characters except siliqua length and per cent oil content in Environment I (Env. I). On the other hand in Environment II (Env. II), the presence of significant genetic variability for most of the traits was observed. Pooled analysis over environments revealed the presence of g × e interactions for all characters except days to flower initiation and per cent oil content. All the 33 genotypes could be grouped into three clusters. Maximum genotypes were placed in cluster I. The cluster I exhibited maximum intra-cluster distance (1.12), while maximum inter-cluster distance was observed between cluster II and III (2.34). Maximum contribution towards genetic divergence was due to days to 75 per cent maturity.

Key Words: Cluster analysis, Ethiopian mustard, Genetic divergence.

Introduction

Oilseed crops are the backbone of Indian agricultural economy and occupy an important position in daily diet, being a rich source of fats and vitamins. India is the second largest rapeseed-mustard growing country and accounts for 21.7% area in the world after China. Among oilseeds, rapeseed-mustard is the second most important oilseed crop of the country after groundnut and plays a significant role in Indian oil economy by contributing about 28.6% to the total oilseed production (Shekhawat *et al*., 2014).

Rapeseed-mustard is the third important oilseed crop in the world after soybean (*Glycine max*) and palm (*Elaeis guineensis* Jacq.). The crop occupies an area of 33.58 million ha with a total annual production of 67.76 million tonnes and productivity 2018 kg/ha. In production**,** India (11.6%) ranks third after China (22.9%) and Canada (19.7%). The global production of rapeseed-mustard oil is around 12-14 million tonnes. In India, the crop occupies an area of 6.50 million ha with a total production of 8.02 million tonnes and productivity of 1262 kg/ha (Priyamedha *et al*., 2017).

Rapeseed-mustard in general, has shown a declining trend both in acreage and production largely due to lack of suitable cultivars for different ecosystems, fluctuations in weather conditions, cultivation in marginal and sub marginal lands and prevalence of various abiotic and biotic stresses. The present day varieties are more susceptible to *Alternaria* blight and white rust. Hence, the most suitable alternate way to increase productivity is by adoption of high yielding, input responsive genotypes having resistance against various biotic and abiotic stresses. The success of any breeding programme depends upon the nature and magnitude of variability present in the germplasm stock. The chances of initiating an effective breeding programme are greater if more genetic variability is available with the plant breeder. Thus, studies on genetic diversity are useful as a general guide for the choice of parents for future hybridization programme in order to obtain high heterotic response and superior transgressants. Estimation of degree of

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divergence within biological population and computation of relevant contribution of different components to the total divergence is done through Mahalanobis's generalized distance (D^2-statistic) method. Therefore, an attempt was made in the present study to estimate the nature and extent of genetic divergence and identify suitable donors on Ethiopian mustard.

Materials and Methods

The materials for the present investigation comprised 33 genotypes including 28 doubled haploids (DH) obtained through anther culture technique, one advanced breeding line (P-138) and four (3 mustard and 1 karan rai) check varieties *viz.,* Nav Gold, RCC-4, Pusa Jaikisan and Jayanti. The doubled haploids were obtained from the cross Jayanti × RCC-6-1 developed in the Department of Agricultural Biotechnology, CSK HPKV, Palampur. All the genotypes were raised at the experimental farm of Department of Crop Improvement, CSK HPKV, Palampur in randomized complete block design with three replications in the plot size of 3.0 × 0.60 m^2 on two different sowing dates *viz.*, 12th October, 2010 (Env. I) and 29th October, 2010 (Env. II). The row to row and plant to plant spacings were kept at 30 cm and 15 cm, respectively. The recommended cultural practices were followed to raise the crop. Data were recorded on various traits *viz.,* days to flower initiation, days to 50 per cent flowering, days to 75 per cent maturity, plant height (cm), number of primary branches per plant, number of secondary branches per plant, siliquae per plant, length of main shoot (cm), siliquae on main shoot, siliqua length (cm), seeds per siliqua, 1000-seed weight, seed yield per plant, biological yield per plant, harvest index (%) and percent oil content. The data were analysed statistically as per the method of Panse and Sukhatme (1985). Genetic diversity analysis was done as per D^2-analysis (Mahalanobis, 1936). Clustering was done by using Tocher's method (Rao, 1952).

Results and Discussion

Analysis of variance indicated the presence of sufficient genetic variability for all characters except siliqua length and per cent oil content in Env. I (Table 1). In Env. II, the presence of sufficient genetic variability for days to flower initiation, days to 50 per cent flowering, days to 75 percent maturity, plant height, number of primary branches per plant, number of secondary branches per plant, siliquae per plant, 1000-seed weight, seed yield per plant and harvest index was observed. Pooled analysis over environments revealed the presence of g × e interactions for all characters except days to flower initiation and per cent oil content (Table 2). The presence of g × e interaction has greatly influenced the variation due to genotypes to the extent that genotypic differences recorded in individual environments have vanished for these characters. Zehra and Gukan (2009) observed significant differences for plant height, number of branches per plant, number of pods per plant, pods

Table 1. Analysis of variance for different characters of *Brassica carinata* in Env. I and Env. II

S. No.	Characters	Env. I		Env. II	
		Mean Squares			
		Genotypes	Error	Genotypes	Error
		32	64	32	64
1	Days to flower initiation	153.08**	10.87	203.82**	37.03
2	Days to 50% flowering	68.91**	8.33	198.81**	8.43
3	Days to 75% maturity	189.79**	8.94	119.96**	44.47
4	Plant height (cm)	2318.26**	207.41	226.03*	117.46
5	Number of primary branches /plant	2.60**	0.57	2.19**	1.08
6	Number of secondary branches /plant	7.44**	0.97	7.8**	3.92
7	Siliquae/ plant	3703.66**	666.21	8929.05**	2579.37
8	Length of main shoot (cm)	127.24**	23.73	48.36	37.67
9	Siliquae on main shoot	180.78**	35.26	45.55	41.95
10	Siliqua length (cm)	0.263	0.217	0.23	0.160
11	Seeds /siliqua	2.36*	0.87	2.43	2.08
12	1000-seed weight (g)	1.15**	0.10	0.20*	0.11
13	Seed yield /plant (g)	7.36**	1.42	3.54**	1.47
14	Biological yield /plant (g)	140.18**	34.49	68.41	64.92
15	Harvest index (%)	44.47**	19.81	71.77*	39.51
16	Oil content (%)	11.54	12.56	12.75	15.50

* and **indicate significance at P ≤ 0.05 and 0.001, respectively

Table 2. Analysis of variance for different characters of *Brassica carinata* in pooled over the environments

S. No.	Characters	Mean Squares			
		Genotypes	Environments	Genotype × Environment (g × e)	Pooled error
		32	1	32	128
1	Days to flower initiation	337.18**	255.68**	17.72	23.95
2	Days to 50% flowering	235.09**	147.68**	32.63**	8.38
3	Days to 75% maturity	264.37**	6.55	45.38**	10.21
4	Plant height (cm)	1322.76	15254.73**	1221.53**	162.43
5	Number of primary branches / plant	1.52	20.97**	3.27**	0.82
6	Number of secondary branches / plant	7.57	250.26**	7.67**	2.44
7	Siliquae /plant	7940.97	1426.19	4691.74**	1622.49
8	Length of main shoot (cm)	90.82	955.68**	84.79**	30.70
9	Siliquae on main shoot	93.19	3224.25**	133.14**	38.60
10	Siliqua length (cm)	0.23	0.44	0.323*	0.20
11	Seeds /siliqua	2.21	52.08**	2.58**	1.47
12	1000-seed weight (g)	0.86*	0.19	0.48**	0.11
13	Seed yield /plant (g)	3.64	0.05	7.25**	1.44
14	Biological yield /plant (g)	105.19	251.16	103.41**	46.70
15	Harvest index (%)	47.92	199.20	68.29**	29.70
16	Oil content (%)	18.31**	81.08**	6.10	13.62

* and ** indicate significance at $P \leq 0.05$ and 0.01, respectively

per main stem, pod length, 1000-seed weight, seed yield per plant and percent oil content in two environments. Yared *et al.* (2012) also observed highly significant differences for days to flower initiation and days to maturity in Ethiopian mustard.

The cluster analysis revealed that all the genotypes could be grouped into three clusters (Table 3 and Fig. 1). Different clustering pattern in rapeseed-mustard was also reported by earlier workers (Yadav *et al.,* 1985; Verma and Sachan, 2000; Thul *et al.,* 2004; Patel and Patel, 2006; Mukesh *et al.,* 2007; Singh *et al.,* 2007; Mahmuda *et al.,* 2008). Different clustering pattern in different environments has also been reported by Goswami and Behl (2006).

Among all clusters, cluster I was found to be largest one. Verma and Sachan (2000), Patel and Patel (2006), Mukesh *et al.* (2007) and Singh *et al.* (2007) also grouped genotypes into different clusters and reported that cluster I was the largest one. Cluster I contained 29 genotypes such as P-43, P-45, P-51, P-103, P-122, P-96, P-62, P-26, P-74, P-101, P-89, Jayanti, P-33, P-133, P-24, P-63, P-77, P- 56, P-137, P-39, P-34, P-75, P-138, P-64, P- 117, P-17, P-92, P-31 and P-23. Cluster II had three genotypes *viz.,* RCC-4, Nav Gold and Pusa Jaikisan while cluster III had only one genotype *i.e.* P-12. The clustering pattern indicated that all the mustard checks formed separate clusters while all doubled haploids appeared in separate clusters. This further supported that Indian mustard (AABB, 2n = 36) has been originated in china by hybridization between *Brassica campestris* (AA) and *B. nigra* (BB) in nature while Ethiopian mustard (BBCC, 2n = 34) has been originated in Ethiopia by hybridization between *B. oleracea* (CC) and *B. nigra* (BB), though, one genome is common in both species. These results therefore, emphasized that the parents should be selected on the basis of total divergence for the traits used for an overall improvement in the yield.

The intra-cluster distances were comparable for clusters I (1.12) and II (1.00) (Table 4). Since the intra-cluster distance was low, the chances of developing

Table 3. Cluster composition in *Brassica carinata* following multivariate analysis in pooled over the environments

Cluster number	Number of genotypes	Genotypes
I	29	P-43, P-45, P-51, P-103, P-122, P-96, P-62, P-26, P-74, P-101, P-89, Jayanti, P-33, P-133, P-24, P-63, P-77, P- 56, P-137, P-39, P-34, P-75, P-138, P-64, P- 117, P-17, P-92, P-31 and P-23
II	3	RCC-4, Nav Gold and Pusa Jaikisan
III	1	P-12

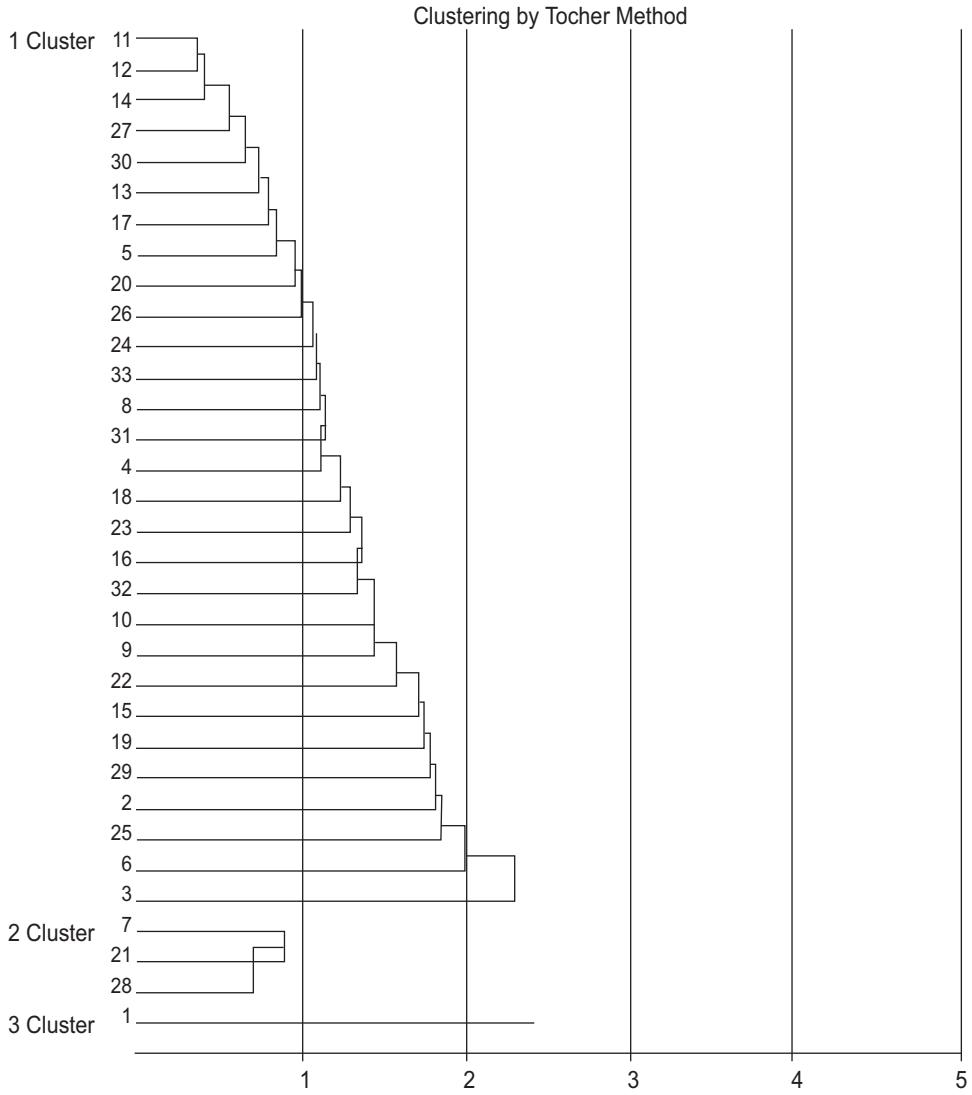

Fig.1. Dendrogram showing grouping of 33 *Brassica carinata* genotypes generated using D^2 multivariate analysis (Tocher's method) in pooled over the environment

good segregants by hybridization among parents within clusters would be low, therefore, it is logical to attempt crosses between the genotypes falling in different clusters based on inter-cluster distances. Patel and Patel (2006) reported highest intra-cluster distance in cluster II in Indian mustard.

The analysis of genetic divergence indicated the highest inter-cluster distance between clusters II and III (2.34) followed by distance between clusters I and II (2.21). The lowest inter-cluster distance was observed between clusters I and III (1.42). This clearly indicates that the genotypes included in these clusters are having sufficient genetic diversity and parents from diverse clusters could be used in hybridization programme for improving seed yield. Further, the clustering pattern suggested the parallelism between the genetic divergence and species-wise geographical distribution. However, Anand and Rawat (1984) and Gupta *et al.* (1991) suggested that geographical diversity of line does not necessarily reflect on index of its genetic diversity. No parallelism between geographical diversity and genetic diversity was reported by Verma and Sachan (2000) in Indian mustard. Geographical distribution of the cultivars did not significantly contribute to genetic divergence (Singh *et al.,* 2007).

Crosses involving parents belonging to most divergent clusters would be expected to manifest

Table 4. Average intra- and inter-cluster distance in pooled over the environments

Clusters	I	II	III
I	1.26 (1.12)	4.90 (2.21)	2.03 (1.42)
II		1.01 (1.00)	5.48 (2.34)
III			0.00 (0.00)

Values in bold letters are intra-cluster distances
Values in parenthesis are √ D2 = D values

maximum heterosis and release of desirable recombinants in segregating generations. Therefore, the parents should be selected from cluster combination between clusters II and III.

Based on the comparison of cluster means of different characters, it was observed that substantial differences existed among the cluster means for each character. Genotypes in cluster I had moderate values for all the characters studied (Table 5). Cluster II revealed maximum cluster means for siliqua length (4.06 cm), seeds per siliqua (10.79), 1000-seed weight (3.79 g) and biological yield per plant (37.89 g) and the genotypes recorded minimum days to flower initiation (65.94 days), days to 50 percent flowering (109.44 days), days to 75 percent maturity (148.50 days) and plant height (108.01 cm). Likewise, cluster III was characterized by maximum number of primary branches per plant (5.72), number of secondary branches per plant (11.70), siliquae per plant (329.37), length of main shoot (52.33 cm), siliquae on main shoot (33.00), seed yield per plant (6.91 g), harvest index (20.12%) and per cent oil content (40.00%). Based upon the inter-cluster distances and cluster means, the crosses among the genotypes belonging to clusters II and III, may give transgressants for higher seed yield, dwarf plant type, earliness in flowering and maturity, high biological yield and harvest index. Earlier workers have also attempted interspecific hybridization between *Brassica napus* × *B. carinata* (Sheikh *et al*., 2010b), *B. carinata* × *B. rapa* (Choudhary *et al.,* 2000), *B. juncea* × *B. carinata* (Sheikh *et al*., 2010a) and derived desirable transgressive segregants in the progeny by introgressing the desirable genes in Ethiopian mustard. Singh *et al.* (2010) also reported significant increase in shoot length, 1000-seed weight and significant decrease in maturity duration through interspecific hybridization in *B. carinata.*

The contribution of individual characters to divergence has been worked out in terms of number of times it appeared first (Table 6). Days to 75 per cent maturity contributed maximum (17.99%) towards total genetic divergence followed by plant height (17.42%) among 33 genotypes studied. Monalisa *et al*. (2005) reported that the number of siliquae per plant contributed maximum towards total genetic divergence followed by days to maturity and plant height.

The results from present study indicated that, selection of genotypes as superior and diverse parents for hybridization programme should be based on diverse clusters viz., II (Nav Gold, Pusa Jaikisan and RCC-4)

Table 5. Cluster means for different characters in pooled over the environments

S. No.	Characters	Clusters					
		I	II	III	Mean	Minimum	Maximum
1	Days to flower initiation	90.67	65.94	91.83	82.81	65.94	91.83
2	Days to 50% flowering	129.67	109.44	130.00	123.04	109.44	130.00
3	Days to 75% maturity	169.68	148.50	167.00	161.73	148.50	169.68
4	Plant height (cm)	113.73	108.01	118.80	113.51	108.01	118.80
5	No. of primary branches/ plant	4.91	4.00	5.72	4.88	4.00	5.72
6	No. of secondary branches/ plant	7.99	7.96	11.70	9.22	7.96	11.70
7	Siliquae / plant	172.09	156.41	329.37	219.29	156.41	329.37
8	Length of main shoot (cm)	42.43	40.33	52.33	45.03	40.33	52.33
9	Siliquae on main shoot	31.21	27.56	33.00	30.59	27.56	33.00
10	Siliqua length (cm)	3.64	4.06	3.83	3.84	3.64	4.06
11	Seeds/ siliqua	10.49	10.79	10.45	10.58	10.45	10.79
12	1000- seed weight (g)	2.56	3.79	2.57	2.97	2.56	3.79
13	Seed yield / plant	6.13	6.39	6.91	6.48	6.13	6.91
14	Biological yield / plant (g)	34.94	37.89	36.17	36.33	34.94	37.89
15	Harvest index (%)	18.45	12.28	20.12	16.95	12.28	20.12
16	Oil content (%)	36.67	37.36	40.00	38.01	36.67	40.00

Table 6. Contribution of individual characters to the divergence among 33 genotypes of *Brassica carinata*

S. No.	Characters	Times ranked Ist	Contribution (%)
1	Days to flower initiation	9	1.70*
2	Days to 50% flowering	67	12.69
3	Days to 75% maturity	95	17.99**
4	Plant height	92	17.42
5	Number of primary branches / plant	11	2.08
6	Number of secondary branches/ plant	24	4.55
7	Siliquae / plant	38	7.20
8	Length of main shoot	31	5.87
9	Siliquae on main shoot	27	5.11
10	Siliqua length	15	2.84
11	Seeds/ siliqua	20	3.79
12	1000-seed weight	12	2.27
13	Seed yield / plant	25	4.73
14	Biological yield / plant	19	3.60
15	Harvest index	17	3.22
16	Oil content	26	4.92

** Maximum contribution; * Minimum contribution

and III (P-12) to get heterotic crosses for getting superior recombinants in early segregating generations.

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