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Assessment of genetic divergence in thirty-five genotypes of oilseed *Brassica* species

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Abstract
The experimental material comprised of 35 genotypes including 20 F_2s/F_3s and 15 parents of different *Brassica* species. The Mahalanobis's D^2 statistics of genetic divergence indicated the presence of wider genetic diversity among genotypes in both year 2015-16 and 2016-17. All 35 genotypes were grouped into six clusters in 2015-16 and five clusters in 2016-17. Highest number of genotypes were accommodated in C I followed by cluster II during 2015-16. While in 2016-17 cluster I was the largest with 31 genotypes. The inter and intra cluster distance of the 35 genotypes showed wide range of estimation in both years. The highest inter-cluster distance was observed between cluster II & IV followed by cluster II & V and cluster III & VI in 2015-16. In 2016-17 the cluster II & V showed maximum inter-cluster distance followed by cluster III & IV and cluster IV & V. The maximum intra-cluster distance was shown by cluster I. The relative contribution of each character towards divergence was maximum for oil yield per plant in 2015-16 followed by seeds per siliqua, oil content, test weight, plant height and days to maturity. While in 2016-17, test weight contributed maximum towards divergence followed by oil yield per plant, oil content, Seeds per siliqua, secondary branches and siliqua length.

Keywords: Genetic divergence, d^2 statistics, clusters, genotypes, *Brassica* species

Introduction
Rapeseed-mustard is an important edible oilseed crop in India after Soybean. It is grown over an area of 6.5 million ha with production and productivity of 7.28 million tons and 1128 kg/ha, respectively (Anonymous, 2015). Most of rapeseed-mustard cultivars grown in India have very narrow genetic base which limits their further crop improvement. Genetic variability in respect to genetic diversity is the prerequisite for the crop improvement. It plays an important role in plant breeding because hybrids between lines of diverse origin generally display a greater heterosis than those between closely related strains (Singh, 1986). Selection of parents based on genetic divergence has become successful in several crops (Ashana and Pandey, 1980; Ananda and Rawat, 1984) [2, 1]. The quantification of genetic diversity by D^2 analysis (Mahalanobis, 1936) can help in choosing diverse parents for a successful breeding programme. Thus, keeping this in view, the present research work was planned to determine genetic divergence among 35 genotypes of different oilseed *Brassica* species comprising 20 F_2s/F_3s populations (designated as V1 to V20) and 15 parents (designated as V21 to V35).

Material and Methods
The experimental material comprised of 20 segregating populations (F_2s / F_3s) and 15 parents (Nine *B. juncea* lines, two *B. napus* lines, one line each of *B. rapa* var toria, *B. rapa* var. yellow sarson, *B. carinata* and *B. nigra*) Table 1. These genotypes represented a very wide range of diversity available in the respective species. The segregating populations were derived through interspecific crossing during *Rabi* 2013-14. F_1s were planted during 2014-15 and Colchicine treatment was given to sterile interspecific F_1s. The F_1s were selfed to develop F_2 populations during *Rabi* 2014-15. Likewise, F_2s were selfed to develop F_3s population in subsequent year. Twenty F_2s/ F_3s population along with fifteen parents were evaluated for two consecutive years Rabi 2015-16 and 2016-17 at research field, College of Agriculture Gwalior (MP) India. The experiments were laid out in randomized block design with two replications at spacing of 45 X 15 cm in paired rows. Ten plants from parent and 40 plants from F_2s/ F_3s were selected randomly for recording of various observations. Data for different traits *viz*. days to 50% flowering (DF), plant height (PH), nos. of primary branches per plant (PB), nos. of secondary branches per plant (SB), main shoot length (MSL), siliquae on main shoot (SOMS), siliquae per plant (SPP), siliqua length (SL), seeds per siliqua (SPS), test weight (TW),

days to maturity (DM), seed yield per plant (SYPP), oil content (OC) and oil yield per plant (OYPP) were recorded from randomly selected plants.

Table 1: List of F_2s/ F_3s population and parents used in research experiment

Genotype	Pedigree	Genomic constitution
V1	NGM-43 X PT-303	*B. juncea* x *B. rapa var toria*
V2	NGM-17 X PT-303	*B. juncea* x *B. rapa var toria*
V3	KM-11 X T-42	*B. juncea* x *B. rapa var yellow sarson*
V4	NGM-6 X T-42	*B. juncea* x *B. rapa var yellow sarson*
V5	NGM-17 X T-42	*B. juncea* x *B. rapa var yellow sarson*
V6	PL-58 X PT-303	*B. juncea* x *B. rapa var toria*
V7	PT-303 X GPM-O-5	*B. rapa var toria x B. juncea*
V8	(PT-303XGPM-O-5) X GPM-O-5	(*B. rapa var toria x B. juncea*) x *B. juncea*
V9	PT303 X GPM-O-5	*B. rapa var toria x B. juncea*
V10	T-42 X GPM-O-58	*B. rapa var yellow sarson x B. juncea*
V11	T-42 X NGM-17	*B. rapa var yellow sarson x B. juncea*
V12	PT-303 X *B. nigra*	*B. rapa var toria x B. nigra*
V13	PL-6 X BN-11	*B. juncea* x *B. napus*
V14	PL-6 X BN-10	*B. juncea* x *B. napus*
V15	PL-58 X BN-10	*B. juncea* x *B. napus*
V16	PL-58 X BN-11	*B. juncea* x *B. napus*
V17	BN-11 X PL-6	*B. napus x B. juncea*
V18	KM-11 X CRP-09	*B. juncea x B. carinata*
V19	T-42 X PL-58	*B. rapa var yellow sarson x B. juncea*
V20	GPM-O-1 X PT-303	*B. juncea* x *B. rapa var toria*
V21	NGM-43	*B. juncea*
V22	NGM-17	*B. juncea*
V23	KM-11	*B. juncea*
V24	NGM-6	*B. juncea*
V25	PL-58	*B. juncea*
V26	GPM-O-5	*B. juncea*
V27	GPM-O-58	*B. juncea*
V28	PL-6	*B. juncea*
V29	GPM-O-1	*B. juncea*
V30	BN-10	*B. napus*
V31	BN-11	*B. napus*
V32	PT-303	*B. rapa var toria*
V33	T-42	*B. rapa var yellow sarson*
V34	CRP-09	*B. carinata*
V35	Banarasi Rai	*B. nigra*

Statistical analysis

The D^2 statistical analysis suggested by Mahalanobis (1936) was used for assessing the genetic divergence among 35 genotypes. The analysis was carried using the observations recorded during the *Rabi* 2015-16 and 2016-17. A method suggested by Tocher (Rao, 1952) was used for grouping of genotypes into different clusters based on the D^2 values. The intra and inter cluster distance was calculated by the formula given by Singh and Chaudhary (1977).

Results

Genetic divergence along with genetic variability are of greatest interest for a breeder as these play a vital role in framing a successful breeding programme. Analysis of genetic divergence has been used to quantify (a) the genetic distance between the genotypes (b) identify promising types to initiate crossing program and (c) To relate clustering pattern to a geographical origin.

Mahalanobis generalized distance (D^2) analysis

The D^2 analysis for year 2015-16 and 2016-17 was carried out using all the fourteen characters. The generalized distance (D^2) was also calculated for all 35 genotypes. All 35 genotypes (20 F2s/F3s populations and 15 parents) were grouped into six clusters in 2015-16. Among the clusters, cluster I was the largest with 27 genotypes followed by cluster II with 4 genotypes. Cluster III, IV, V and VI had one genotype, respectively, Table 2. Similarly, in 2016-17 all 35 genotypes were grouped into five clusters. Among them, cluster I was the largest with 31 genotypes. Cluster II, III, IV, V each had one genotype, respectively, Table 3.

Table 2: Distribution of 35 genotypes (20 F2s/F3s populations designated as V1-V20 and 15 Parents designated as (V21-V35) into different clusters during 2015-16

Cluster	Nos of genotypes	Name of genotypes
Cluster-1	27	V8 ((PT-303XGPM-O-5) X GPM-O-5), V26 (GPM-O-5), V21 (NGM-43), V14 (PL-6XBN-10), V29 (PL-6), V13 (PL-6XBN-11), V24 (NGM-6), V25 (PL-58), V1 (NGM-43XPT-3-03), V2 (NGM-17XPT-303), V22 (NGM-17), V4 (NGM-6XT-42), V31 (GPM-O-1), V6 (PL-58XPT-303), V15 (PL-58XBN-10), V5 (NGM-17XT-42), V27 (GPM-O-58), V7 (PT-303XGPM-O-5), V16 (PL-58XBN-11), V9 (PT-303XGPM-O-5), V17 (BN-11XPL-6), V12 (PT-303 X *B. nigra*), V23 (KM-11), V3 (KM-11XT-42), V20 (GPM-O-1-1XPT-303), V30 (BN-11), V28 (BN-10)
Cluster-2	4	V10 (T-42XGPM-O-58), V11 (T-42XNGM-17), V19 (T-42XPL-58), V33 (T-42)
Cluster-3	1	V32 (PT-303)
Cluster-4	1	V18 (KM-11 X *B. carinata*)
Cluster-5	1	V35 (*B. nigra*)
Cluster-6	1	V34 (*B. carinata*)

Table 3: Distribution of 35 genotypes (20 F2s/F3s populations designated as V1-V20 and 15 parents designated as (V21-V35) into different clusters during 2016-17

Cluster	Nos of genotypes	Name of genotypes
Cluster-1	31	V21 (NGM-43), V22 (NGM-17), V2 (NGM-17XPT-303), V31 (GPM-O-1), V26 (GPM-O-5), V13 (PL-6XBN-11), V29 (PL-6), V24 (NGM-6), V4 (NGM-6XT-42), V1 (NGM-43XPT-3-03), V25 (PL-58), V6 (PL-58XPT-303), V8 ((PT-303XGPM-O-5) X GPM-O-5), V15 (PL-58XBN-10), V7 (PT-303XGPM-O-5), V27 (GPM-O-58), V5 (NGM-17XT-42), V14 (PL-6XBN-10), V3 (KM-11XT-42), V16 (PL-58XBN-11), V17 (BN-11XPL-6), V12 (PT-303 X *B. nigra*), V23 (KM-11), V30 (BN-11), V28 (BN-10), V9 (PT-303XGPM-O-5), V20 (GPM-O-1-1XPT-303), V10 (T-42XGPM-O-58), V33 (T-42), V19 (T-42XPL-58), V18 (KM-11X *B. carinata*).
Cluster-2	1	V11 (T-42XNGM-17)
Cluster-3	1	V32 (PT-303)
Cluster-4	1	V34 (*B. carinata*)
Cluster-5	1	V35 (*B. nigra*)

Intra and Inter cluster D^2 values

Maximum differences among the genotypes within the same cluster (intra-cluster) was shown by cluster I (155.95) followed by cluster II (126.05) during 2015-16. Rest other cluster III, IV, V and VI showed zero intra cluster distances, table 4. During 2016-17 the maximum differences among the

genotypes within the same cluster (intra-cluster) was shown by cluster I (449.06). Rest all clusters II, III, IV and V showed zero intra cluster distances, table 5.

During 2015-16 diversity among the clusters varied with inter cluster distances of 325.44 to 1387.21. The cluster II & VI showed maximum inter-cluster distance (1387.21) followed by cluster II & V (1243.93) and cluster III & VI (1193.17). The lowest inter-cluster distance was observed between cluster IV and V (325.44) followed by cluster I and IV (363.48), Figure 1. In 2016-17 diversity among the clusters varied with inter cluster distances of 945.63 to 2825.49. The cluster II & V showed maximum inter-cluster distance (2825.49) followed by cluster III & IV (2089.59) and cluster IV & V (1972.96). The lowest inter-cluster distance was observed between cluster I and II (945.63) followed by cluster I and III (986.61), Figure 2.

Table 4: Mean of intra and inter cluster D² values of 35 *Brassica* genotypes during 2015-16

Cluster	I	II	III	IV	V	VI
I	155.95	701.89	434.75	363.48	472.26	545.65
II		126.05	513.70	613.61	1243.93	1387.21
III			0.0	371.10	737.03	1193.17
IV				0.0	325.44	635.58
V					0.0	695.75
VI						0.0

Diagonal values indicate intra-cluster D² values.

Table 5: Mean of intra and inter cluster D² values of 35 *Brassica* genotypes during 2016-17

Cluster	I	II	III	IV	V
I	449.06	945.63	986.61	1288.93	1947.99
II		0.0	1282.04	1542.06	2825.49
III			0.0	2089.59	1416.58
IV				0.0	1972.96
V					0.0

Diagonal values indicate intra-cluster D² values.

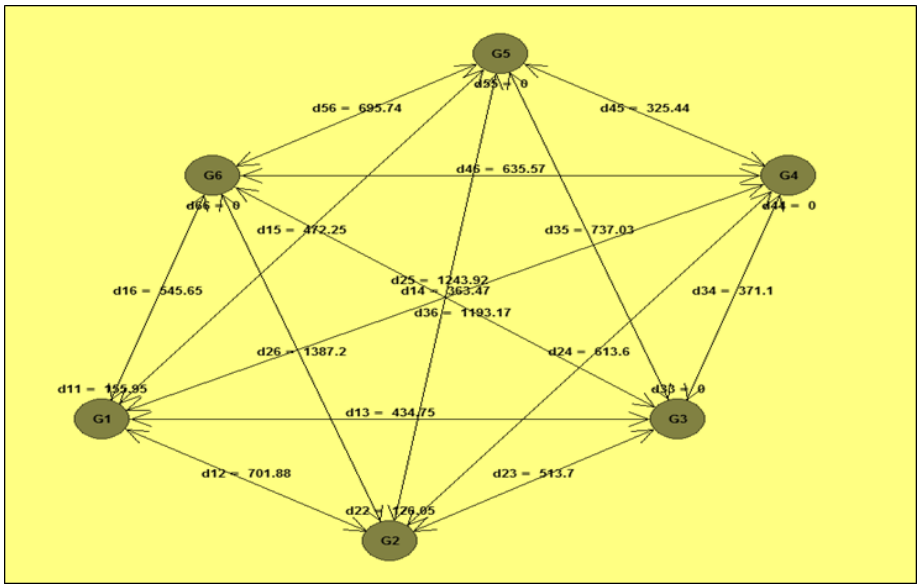

Fig 1: Average intra and inter cluster D² values of 35 *Brassica* genotypes during 2015-16

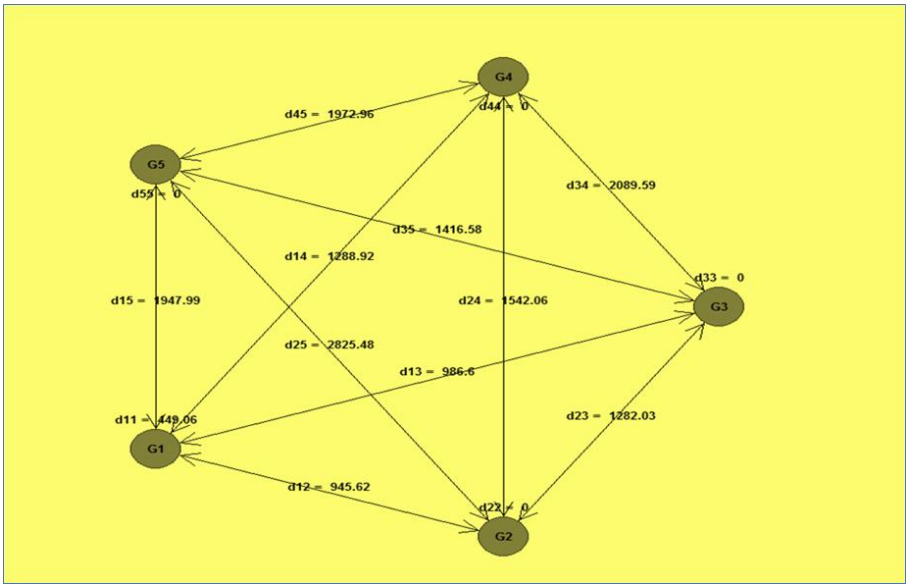

Fig 2: Average intra and inter cluster D² values of 35 *Brassica* genotypes during 2016- 17

Per cent contribution of characters towards divergence

The per cent contribution of each character towards divergence has been estimated in 2015-16, table 6. It was observed that oil yield per plant contributed maximum (34.10) towards divergence followed by seeds per siliqua (31.24), oil content (14.66), test weight (10.00), plant height (8.87) and

days to maturity (7.24). The remaining characters *viz*., days to 50% flowering, primary branches, secondary branches, main shoot length, siliquae on main shoot, siliquae per plant and siliqua length did not contribute significantly to the total divergence. Similarly, per cent contribution of each character towards divergence has been estimated during 2016-17, Table 7. It was observed that test weight contributed maximum (24.54) towards divergence followed by oil yield per plant (20.84), oil content (11.86), seeds per siliqua (9.14), secondary branches (8.38) and siliqua length (7.89). The remaining characters *viz*., days to 50% flowering, plant height, primary branch, main shot length, siliquae on main shoot, siliquae per plant and days to maturity did not contribute significantly to the total divergence.

Table 6: Relative contribution of characters towards divergence in 35 *Brassica* germplasm during 2015-16

S. No.	Characters	Percent contribution
1	Days to 50% flowering	3.19
2	Plant height	8.87
3	Nos of primary branches	0.87
4	Nos secondary branches	5.33
5	Main shoot length	2.01
6	Siliquae on main shoot	2.43
7	Siliquae per plant	-1.32
8	Siliqua length	5.35
9	Seeds per siliqua	31.24
10	Test weight	10.00
11	Days to maturity	7.24
12	Seed yield per plant	-23.98
13	Oil content	14.66
14	Oil yield per plant	34.10

Table 7: Relative contribution of characters towards divergence in 35 *Brassica* germplasm during 2016-17

S. No.	Characters	Percent contribution
1	Days to 50% flowering	5.79
2	Plant height	5.66
3	Nos of primary branches	2.18
4	Nos secondary branches	8.38
5	Main shoot length	3.53
6	Siliquae on main shoot	2.29
7	Siliquae per plant	6.48
8	Siliqua length	7.89
9	Seeds per siliqua	9.14
10	Test weight	24.54
11	Days to maturity	6.06
12	Seed yield per plant	-14.64
13	Oil content	11.86
14	Oil yield per plant	20.84

Discussion

All 35 genotypes (20 F_{2s}/F_3s populations and 15 parents) were grouped into six and five different clusters in 2015-16 and 2016-17, respectively. Highest number of genotypes were accommodated in C I followed by cluster II. Cluster III, IV, V and VI had one genotype during 2015-16. In 2016-17 cluster I was the largest with 31 genotypes. Cluster II, III, IV, V each had one genotype, respectively.

The clustering pattern indicated that there was a lot of diversity among the genotypes. This could be due to recombination of genomes of different species in segregating population (F_2/F_3) and genetic drift, selection pressure and environmental effect in parents. Similarly, the genotypes developed at one geographical location were grouped in different clusters which suggested that there might have been introgression of genes among the genotypes of various origins and operation of similar forces of selection. The findings were in close agreement with those obtained by Yadav *et al*., (1985) [17], Thakur *et al*., (1989) [14], Verma and Sachin (2000) [15], Chaudhary and Joshi (2001) [3], Gangapur *et al*., (2010) [5], Goyat *et al*., (2012) [6], Singh *et al*., (2013) [12] and Singh *et al* (2018) [13]. Similar grouping of rapeseed-mustard genotypes in to different clusters was reported by Shathi *et al* (2012) [11], Chauhan *et al*. (2008) [4] and Singh *et al*. (2007) [16].

Intra and Inter cluster D^2 values

The inter and intra cluster distance of the 35 genotypes showed wide range of estimation during both years 2015-16 and 2016-17. The highest inter-cluster distance was observed between cluster II and IV followed by cluster II and V and cluster III with VI in 2015-16. It was noted that the genotypes grouped into these clusters were highly divergent from each other. Parent selection from highly divergent cluster is expected to manifest high heterosis in hybridization. The lowest inter cluster distance was observed between cluster IV and V & cluster I and IV suggesting close relationship among these clusters. The maximum intra cluster distance among the genotypes was observed in cluster I followed by cluster II.

The cluster III, IV, V and VI showed zero intra cluster distances under both conditions (2015-16 and 2016-17). In 2016-17 the cluster II & V showed maximum inter-cluster distance followed by cluster III & IV and cluster IV & V. Selection of diverse parents having most of the desirable characters from such clusters and using them in breeding programs is likely to produce more transgressive segregants and heterotic F1's. The lowest inter-cluster distance was observed between cluster I and II & cluster I and III. The maximum intra-cluster distance was shown by cluster I. These results were similar with previous findings by Khan *et al* (2013) [7], Singh *et al* (2013) [12], Shathi *et al* (2012) [11] and Yared (2011) [18]. Singh *et al* (2018) [13] also reported that selection of diverse parents with most of the desirable characters from such clusters were likely to produce more transgressive segregants and heterotic F_1s when crossed.

Per cent contribution of characters towards divergence

The relative contribution of each character towards divergence was maximum for oil yield per plant in 2015-16 followed by seeds per siliqua, oil content, test weight, plant height and days to maturity. The characters *viz*., days to 50% flowering, primary branches, secondary branches, main shoot length, siliquae on main shoot, siliquae per plant and siliqua length did not contribute significantly to the total divergence. While in 2016-17, test weight contributed maximum towards divergence followed by oil yield per plant, oil content, seeds per siliqua, secondary branches and siliqua length. The characters *viz*., days to 50% flowering, plant height, primary branch, main shot length, siliquae on main shoot, siliquae per plant and days to maturity did not contribute significantly to the total divergence.

Perusal of results showed that characters oil yield per plant, test weight, oil content, secondary branches, siliqua length and seeds per siliqua were contributed considerably towards divergence. Similar results were reported by Verma and Sachan (2000) [15]; Patel *et al*., (2006) [9], Singh *et al* (2018) [13]. Similarly, Gangapur *et al*. (2010) [5] also indicated that number of secondary branches per plant attributed maximum per cent towards divergence. On the other hand, Shathi *et al*.,

(2012) [11] indicated that oil content, 1000 seed weight and yield per plant contributed lowest to the total divergence.

Conclusion

The clustering pattern indicated substantial diversity among the genotypes. The inter and intra cluster distance of the 35 genotypes showed wide range of estimation during both years 2015-16 and 2016-17. The genotypes grouped into these clusters were highly divergent from each other. Parent selection from highly divergent cluster is expected to manifest high heterosis in hybridization. Selection of diverse parents having most of the desirable characters from such clusters and using them in breeding programs is likely to produce more transgressive segregants and heterotic F1's the characters having great contribution to total divergence were responsible for genetic diversity. The characters test weight, oil content, siliqua length and seeds per siliqua were among the phenotypic traits contributing towards seed & oil yield per plant and can be used as indices for future breeding.

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