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Genetic diversity analysis of Indian mustard (*Brassica juncea* L.)

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Abstract
Genetic divergence assessed in twenty genotypes of Indian mustard [*Brassica juncea* (L.) Czern & Coss.] using D^2 statistics for eighteen characters enabled grouping of all the genotypes into five clusters. Seed yield/plant, biological yield/plant, 1000- seed weight, and harvest index were the major contributors for genetic diversity among the genotypes. Out of 5 clusters, cluster I and II was the largest comprising 8 genotypes followed by cluster V consisting of 2 genotypes, cluster III and IV with 1 genotype, respectively. The cluster I exhibited maximum intra- cluster distance (70.74), while maximum inter-cluster distance was observed between cluster V and IV (857.56).

Keywords: Indian mustard, genetic divergence, D^2 analysis, cluster analysis

Introduction
Rapeseed-mustard is one of the most important oilseed crops of India. Out of the total rapeseed-mustard production of India, Indian mustard accounts for 75-80% and contributes 24.2% of the total edible oil pool of the country. The major mustard growing states of India are Rajasthan, Uttar Pradesh, Gujarat, Madhya Pradesh, Assam, Bihar, Odissa, Haryana, Punjab and West Bengal. Genetic diversity in general is the total variability present among different genotypes of a species. In plant breeding, genetic diversity plays an important role because hybrids between lines of diverse origin, generally, display greater heterosis than those between closely related parents and may generate broad spectrum of genetic variability in segregating population. The availability of genetic variability present in the breeding material plays major role in planning breeding programme to develop superior cultivars or hybrids. In general, the genetically divergent parents are utilized to obtain the desirable recombinants in segregating generations. Thus, knowledge of genetic diversity in *Brassica juncea* could help breeders and geneticists to understand the organisation of genetic variability in the germplasm, predict combinations that would produce the best genotypes and facilitate to widen the genetic basis of breeding material for selection. Under these premises, the present study was carried out to measure the genetic divergence present among the available genotypes of Indian mustard and to find out the possible parents for use in hybridization programme for development of commercial hybrids or varieties. Therefore, the present investigation was carried out to determine the divergence among 20 different genotypes of rapeseed mustard.

Materials and Methods
Plant material comprises of twenty germplasm including one national and two zonal check cultivars of *B. juncea* L. for estimation of agro-morphological variation. The germplasm were provided by All India Co-ordinated Research Project- Rapeseed and Mustard centres: DRMR, Bharatpur, Rajasthan, CCSHAU, Hisar, Haryana, BARC, Trombay, Maharashtra, GBPUAT, Pantnagar, Uttarakhand, CSAUAT, Kanpur, U. P, IARI, New Delhi, Sriganganagar, Rajasthan, DR. RPCAU, Dholi, Bihar, was laid out in Randomized Complete Block Design (RCBD) with three replications during Rabi season (2017-18) and was planted on 13th October 2017 at the research research farm of TCA Dholi, DRPCAU, Pusa, Bihar (India) (25. 50 N, 85. 40E and 52. 12 m MSL) in Loam soil (8. 4 pH). The recommended package and practices were followed to raise good crop. The data was recorded on eighteen quantitative traits viz: plant height (cm), days to first flower open, days to 50 % flowering, number of primary branches, number of secondary branches, length of primary mother axis, days to physiological maturity, number of siliqua on primary branches,

silique on secondary branches, silique on primary mother axis, number of seeds per siliquae, siliquae length (cm), 1000 seeds weight (g), total biological yield (g) per plant, harvest index (%) and seed yield per plant (g), vegetative phase duration and post-anthesis phase duration. The recorded morphological traits data were averaged and analyzed for simple statistics i.e. mean, variance, range, frequency distribution, coefficient of variance and standard deviation using computer software. Cluster and principal component analysis (PCA) was performed on the recorded data for quantitative traits. Before to cluster and PCA, mean of each parameter was standardized so that to avoiding scaling differences effects. For all the pairs of accessions Euclidean distance co-efficient were calculated. Amongst various classificatory analyses, utilized to understand workable variability, D^2 - statistic using Tocher method (Tocher Rao, 1952) [5] and Euclidean method (Rao, 1952) [5] based on wards' minimum variance dendogram are successfully utilized by various crop breeders for clustering and quantitative measurement of divergence among the genotypes (varieties, strains, mutants, ECs, ICs, etc.) Mahalanobis (1936) [6] D^2 - statistic was used for assessing genetic divergence among the test entries. The clustering of D^2 values was formed by using generalized distance based- Tocher's method as described by Rao (1952) [5] and also by using Non-hierarchical Euclidean cluster analysis was conducted using computer package (Windostat version 8. 5) whereas the formula given by was utilized for the calculation of intra and inter – cluster distances.

Results and Discussion

The analysis of variance and dispersion were highly significant among all the genotypes for all eighteen characters studied which revealed the presence of considerable genetic variability among the genotypes. All 20 genotypes were grouped into 5 clusters using the Tocher's method (Table 1). Out of 5 clusters, cluster I was the largest comprising 15 genotypes followed by cluster IV is oligo-genotypic and cluster II, III and V is mono-genotypic respectively, although these were solitary in regard to multivariate composition. The clustering pattern indicated that there was a considerable diversity among the genotypes, and there was no relationship between the genetic and geographical diversity of the genotypes, but the distribution of the genotypes was random and independent. This could be due to genetic drift, selection pressure and environmental effect, which create morphological diversity rather than actual genetic distances. Similarly, the strains developed at one station were also 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. Similar results have also been reported earlier by Singh *et al.* (2010) in Indian mustard

The characters viz. test weight (50.00), seed yield per plant (13.16), biological yield per plant (7.37), days to first flower open (6.32), physiological maturity (6.32), post anthesis phase duration (6.32) harvest index (3.16) and vegetative phase duration (3.16) contributed more than 85 % towards the total divergence (Table-5). Parallel to the present results, maximum contribution towards the divergence for number of secondary branches/ plant was previously reported by Doddabhimappa *et al.,* (2010) [2], and number of siliquae on main shoot by Somu (2001) [9]

The cluster means for different characters are presented in Table 7. Cluster IV possessed high mean values for plant height (206.76), number of primary branches (6.24), number of secondary branches (28.57), length of primary mother axis (81.00), siliqua on primary branches (142.00), siliqua on secondary branches (408.8), siliqua on primary mother axis (63.10), biological yield per plant (225.55), seed yield per plant (52.78), physiological maturity (119.00) and days to 50% flowering (65.66). Cluster II for days to first flower open (50.41). Cluster I for siliqua length (4.91) and harvest index (25.28). Cluster III for post anthesis phase duration (83.33) and cluster V for test weight (7.40) and seeds per siliqua (12.52).However, genotypes of clusters I and III were early in flowering, minimum value for days to physiological maturity and higher in seed yield. This further indicated that good parents could be selected for hybridization on mean basis. These results are supported by Goswami and Behl (2006) [3], Kumar *et al.* (2007) [4], and Yu-cheng *et al.* (2007) [10].

The magnitude of inter- cluster distances was greater than intra-cluster distances suggesting the presence of considerable diversity among the clusters (Table 6). The intra-cluster D2 values varied from 0.00 to 124.41 within cluster, and 86.34 to 857.56 between clusters. This indicated that clusters were homogenous within themselves and heterogeneous between themselves. The cluster V exhibited maximum intra-cluster distance (124.41) which indicates that genotypes in this cluster are more diverse than the other clusters. Maximum inter-cluster distance was observed between cluster V and IV (857.56) followed by cluster V and I (662.37); cluster IV and III (579.80); cluster IV and II (461.81); cluster V and II (446.32); cluster VI and I (442.41); cluster V and III (234.73), and cluster III and I (226.82) indicating wider genetic diversity between the genotypes in these clusters. Large inter-cluster distance signifies that genotypes grouped in these clusters were different from the genotype of other clusters for one or more characters, which made them so divergent from other. 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 when crossed.

Table 1: Clustering pattern of 20 genotypes of Indian mustard genotypes on the basis of Tocher method

Cluster No	Intra-cluster distance	No. of genotypes	Genotypes
I	20.21	15	KMR (E) 16-1,PRE-2013-19,NPJ- 197, Varuna, RH0555, Urvashi, NPJ -201, RGN-368, RAURD14- 11, BPR-541-4, DRMR15-9, RAURD-14-18, Rajendra Sufalam, DRMR4001, Pusa mustard
II	0.00	1	RGN-13
III	0.00	1	Pusa Mahak
IV	41.47	2	RAURD-212,TPM-1
V	0.00	1	RAURD-78

Table 2: Clustering pattern of 20 genotypes of Indian mustard genotypes on the basis of non – hierarchical Euclidean method

Cluster no	Intra-cluster distance	No of genotypes	Genotypes in cluster
I	70.74	8	DRMR15-9, NPJ- 201, DRMR4001, Pusa Mustard, RAURD14- 11, BPR-541-4, RGN- 368, RGN-13
II	42.07	8	RAURD 14-18, Rajendra sufalam, KMR (E)16- 1, PRE-2013-19, NPJ- 197, Varuna, RH0555, Urvashi
III	0.00	1	Pusa Mahak
IV	0.00	1	RAURD-78
V	124.41	2	RAURD-212, TPM-1

Table 3: Suitable divergent genotypes based on inter cluster distances in Tochers method

S.No	Inter-cluster	Clusters	Divergent	No. of crosses
	distance		genotypes	
1	293.82	II X IV	RGN-13 X RAURD-212	2
2	285.85	IV X V	RAURD-212 X RAURD-78	2
3	193.27	III X V	Pusa Mahak X RAURD 78	1
4	177.51	I X IV	KMR (E) 16-1 X RAURD 212	6
5	153.08	I X V	PRE2013-19 X RAURD 78	6
Total crosses =				17

Table 4: Suitable divergent genotypes based on inter cluster distances in Euclidean method

S. No	Inter-cluster	Clusters	Divergent	No. of crosses
	distance		genotypes	
1	857.56	IV X V	RAURD 78 X RAURD 21	2
2	662.37	I X V	DRMR4001 X RAURD 212	16
3	579.81	III X IV	Pusa Mahak XRAURD 78	1
4	461.81	II X IV	RAURD 14-18 X RAURD 78	8
5	446.3	II X V	NPJ 197 X RAURD 212	16
Total crosses =				43

Table 5: Contribution of different characters towards genetic divergence in Brassica juncea

Characters	% contribution
Plant height	0
Number of primary branches	0
Number of secondary branches	0
Length of primary mother axis	00.53%
Silique on primary branches	1.05%
Silique on secondary branches	0
Silique on primary mother axis	0
Silique length	1.05%
Seeds per silique	1.05%
Biological yield per plant	7.37%
Harvest index	3.16%
Seed yield per plant	13.16%
Days to first flower open	6.32%
Vegetative phase duration	3.16%
Physiological maturity	6.32%
Post anthesis phase duration	6.32%
Days to 50% flowering	0.53%
Test weight	50.00%

Table 6: Average Intra (diagonal) and inter- cluster distances distance D2 value in Indian mustard

	1	2	3	4	5
	Cluster	Cluster	Cluster	Cluster	Cluster
1. Cluster	70.736	86.344	226.82	442.417	662.370
2. Cluster		42.067	156.056	461.814	446.328
3. Cluster			0	579.809	234.737
4. Cluster				0	857.561
5. Cluster					124.414

Table 7: Cluster mean values for seed yield and its components in Indian mustard

	Plant Height cm	Primary Branches (nos.)	Secondary Branches	Length of Primary Mother Axis	Siliqua On Primary Branches	Siliqua On Secondary Branches	Siliqua On Primary Mother Axis	Siliqua Length (cm)	Seeds Per Siliqua (no.)	Biological Yield (GMS)	harvest Index	Seed Yield /Plant	Days to First Flower Open	Vegetative Phase Duration	Physiological Maturity	Post Anthesis Phase Duration	Days to 50 % Flowering	Test Weight
1 Cluster	183.597	3.916	15.813	67.695	108.708	243.779	44.055	4.971	11.733	86.584	25.289	19.361	45.833	36.250	116.875	79.000	58.792	3.586
2 Cluster	185.639	3.928	15.056	70.375	103.083	211.972	44.555	4.807	11.527	67.499	22.351	14.351	50.417	37.042	116.708	74.583	60.292	4.454
3 Cluster	162.333	3.667	14.220	51.667	88.000	244.223	42.110	4.643	11.333	62.780	21.110	13.277	41.667	35.333	116.000	83.333	56.667	5.113
4 Cluster	206.767	6.243	28.527	81.000	142.000	408.890	63.107	4.617	10.993	225.557	20.917	52.787	47.000	35.000	119.000	73.333	65.667	4.777
5 Cluster	168.993	3.800	15.278	72.612	110.278	256.805	48.110	4.750	12.528	77.167	20.777	14.678	48.333	37.167	116.000	76.167	59.000	7.407

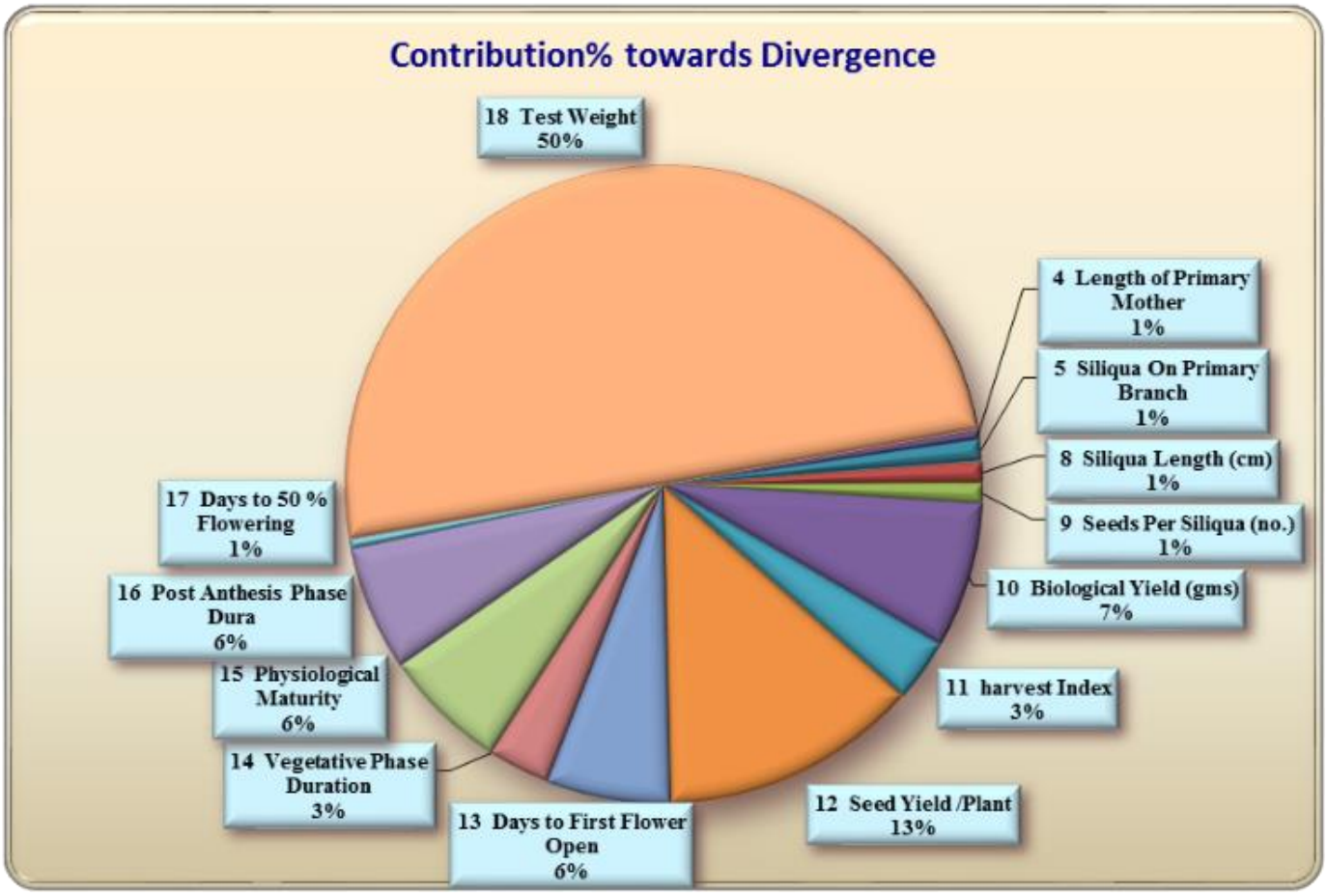

Fig 1: Contribution of different characters towards genetic divergence in *Brassica juncea*

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