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Genetic assortment of Indian mustard (*Brassica juncea*) germplasm for rainfed conditions

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Abstract

Indian mustard (*Brassica juncea* L) grown under rainfed conditions is usually affected by drought stress at different stages resulting in negative effect on yield. The assessment and quantification of morphological diversity for the traits contributing towards drought tolerance studied on 442 germplasm linesin an alpha lattice design for 10 morphological characters during Rabi 2018-19. Significant differences were observed among the genotypes for most of the characters studied under rainfed conditions. The hierarchical cluster analysis using R-software grouped all the genotypes into 4 clusters comprising of 127, 134, 100 and 81 germplasm lines; respectively. The highest distance was observed between cluster I and II (1290.51) followed by cluster I and III (1112.21). However, minimum intra-cluster distance was observed among cluster II indicating similarity of germplasm lines in the cluster. Maximum intra-cluster distance was observed among the genotypes of cluster III indicating diversity among the genotypes. Dendrogram demonstrated sufficient diversity among the germplasm lines for various traits and association between different clusters. The results concluded that morphological diversity in the studied material structured by germplasm lines could be utilized in breeding program to develop transgressives egregants with a wide range of adaptability to drought conditions in semi-arid regions.

Keywords*: Cluster, drought tolerance, Indian mustard*

Introduction

Brassica crops are important contributors to total oilseed production and occupy a third place for source of vegetable oil in the world. The genus Brassica belongs to the family *Brassicaceae*, which comprises around 100 species, including rapeseed (*Brassica napus* L), Indian mustard (*B. juncea* L), cabbage (*B. oleracea* L) and turnip rape (*B. rapa* L), mainly grown for oil, condiments, vegetables or fodder (Ashraf and McNeilly, 2004). There are also many wild relatives possessing useful agronomic traits, such as tolerance for cold, salinity and drought conditions (Warwick, 2000).

Rapeseed-mustard, growing over an area of 6.02 mha, ranks second most important oilseed crop in India (after soybean).Indian mustard is a major oilseed crop of Indian subcontinent, northern China and East European countries and also an option for stressed ecologies of Canada and Australia. *B. juncea* (AABB; 2n = 36), is a natural allotetraploid from diploid parents *B. rapa* (AA; 2n = 20) and *B. nigra* (BB; 2n = 16); originated in the areas encompassing Mediterranean, Irano-Turanian and Saharo-Sindian geographies (NagaharuU, 1935, Kaur*et al.,* 2014). With the development of canola quality, *B. juncea* further expanded to drier regions in Australia and Canada (Parker, 1999) and developed as a complimentary oilseed crop to canola (*B. napus*) for cultivation in hot and low-rainfall areas (Oram and Kirk, 1995), where canola did not perform well. Drought is the most serious problem for global agriculture approximately affecting 40% of the world's land area. Even worse, global climate change is predicted to lead to extreme temperatures and severer prolonged drought in some parts of the world, which will have a dramatic impact on crop growth and productivity (Trenberth *et al.,* 2014).

Indian mustard crop is well adapted to low moisture cropping system due to its low water requirement (80-240 mm). However, various abiotic stresses such as drought, salinity and cold spells lead to low productivity of this crop in India. Nearly, 30.7% area (1.81 mha) of Indian mustard is under rainfed farming in India. About 30 % (12.3 thousand ha) area of Indian mustard of Punjab state is confined to Western arid zone of state with average rainfall <200mm. In spite of good farm practices, the productivity of Indian mustard in Punjab state is 14.98 quintal per ha (2017-18). Moisture stress tolerance in plants is a very complex trait and acrucial aspect is its correct assessment using reliable parameters (Chauhan

et al., 2007). Development of moisture stress resilient Indian mustard varieties will enhance the economic viability of this crop. The reproductive phase of the plant is more susceptible to drought stress (Hall, 1992). Nasri *et al.* (2008) observed a considerable decrease in siliquae number per plant, seeds number per siliquae, weight of 1000 seeds and amount of oil in five canola varieties under drought condition. Decrease in photosynthetic reaction leads to pod termination, subsequently reduction in pods number (Dicpenbrock, 2002). Thus, there is an urgent need to develop varieties that can maintain optimum yield levels under abiotic stresses. However, due to the multigenic and quantitative nature of stress tolerance in plants, efforts to improve crop performance under drought and salinity have been elusive. This challenge has given plant scientists an impetus to improve drought and salt tolerance. There is great interest in breeding stress-tolerant varieties, since significant inter and intra-specific variation for drought and salinity tolerance exists within Brassica, which needs to be exploited through selection and breeding.

The extent of variability and diversity available decides the success of crop improvement programme making essential to know the spectrum of diversity in any crop species and parents based on genetic divergence (Ashana and Pandey, 1980; Ananda and Rawat, 1984). For successful utilization of genetic variability, crop breeders emphatically search for the traits of importance and subsequently to incorporate it genetically into usable variety. There is limited information on documentation for variation for moisture stress tolerance in *B. juncea.* Punjab Agricultural University (PAU), Ludhiana has an enormous reservoir of *B. juncea* germplasm stock comprising introgression lines derived from wild relatives/ species, land races, old cultivars and advance breeding lines.Evaluation of genetic diversity is important to know the source of genes for a particular trait within the available germplasm (Tomooka, 1991). Therefore present investigation was carried out to determine the divergence among 442 lines of *B. juncea* for yield and its component traits under moisture stress (rainfed) conditions.

Materials and Methods

A fixed diverse germplasm stock of 442 Indian mustard genotypes, comprising introgression lines, land races, old cultivars and advance breeding lines was used to document the variability for seed yield and its component traits under rainfed conditions (RF) at PAU Regional Research Station, Abohar, Fazilka (semi-arid plains with scattered sand dunes; located at 30.15° N, 74.19° E) during 2018-19. Experiment was conducted in an alpha lattice design in two replications with each genotype sown in a plot of two rows of 2m at a row to row and plant to plant spacing of 30 cm and of 10 cm, respectively. All the recommended agronomic and protection measures were taken to raise healthy crop. At maturity, the crop was harvested manually. Data were recorded at physiological maturity on yield and itscomponent traits and were analysed statistically. Based on five randomly taken plants of each genotype of each replication, plant height was determined on standing crop by measuring the height of plant from its base up to the end of main shoot. Main shoot length was recorded by measuring the length of main raceme. Number of primary branches (NPB) recorded by counting the siliquae bearing branches emerging from main shoot of the plant, number of secondary branches (NSB) was recorded by counting siliquae bearing branches emerging from primary branches and number of siliquae on the main shoot (SPMS) was recorded by counting total siliquae attached to main shoot. Seed yield (SY, gm) was recorded on plot basis after manual thrashing and drying of seed of each genotype. Seed yield/plant (gm) was calculated by dividing seed yield per plot by number of plants harvested per plot. Statistical software META-Rver 6.0 (Multi-EnvironmentTrial Analysis using R) developed by CIMMYT was used for analysis of data (Alvarado *et al.*, 2016). Statistical analysis included calculus of BLUPs (Best Linear Unbiased Predictors) and Herarchical Cluster analysis was done using R software (Version 20). UPGMA (Unweighted pair group method with arithmetic mean) with City Block distance was used for clustering the genotypes.

Results and Discussion

Genotypic valuesof variables *viz*., flowering traits: Days to 50% flowering (FW50); days to 100% flowering (FW100) and Flowering duration (FD); yield and its component traits *viz.,* plant height (PH), primary branch number (NPB), secondary branch number (NSB), main shoot length (MSL), number of siliquae per main shoot (SPMS) and siliqua length (SL) were determined under rainfed conditions. Table 1 depicted the variance components under rainfed condition.

Heritability, an important predictor of the degree to which a population can respond to artificial or natural selection was highest for NPB followed by MSL, NSB, SL, SY, FW100, FW50, PH and was minimum for FD. The genotypic variance was significant for all the traits under study, which revealed the presence ofconsiderable variability among the genotypes. This suggested that adequate scope is available for selection of superior genotypes aimed at enhancing genetic yield potential of *B. juncea*. This suggested that adequate scope is available for selection of superior genotypes aimed at enhancing genetic yield potential of *B. juncea*.

Table 2: Distribution of mustard germplasm in different clusters under rainfed conditions

Cluster group no.	Genotypes	No.
I	B-272 , B-316, B-326, B-351, B-366, Bio-179, Bio-2, Bio-209, Bio-467, Bio-559, Bio-QM-1, BLAZE, PBR 97, CM 101-213, CM 101-2-88, CM 2-1, CM 21-13, CM 21-9-39, CM 60-44-3, CM 6-3, CM-10-6, CM-11, CM-11-51, CM-11-8, CM-2, CM-20-2, CM-21-1, CM-21-8, CM-21-9, CM-21-9-35, CM-4, CM-9-2, CRL 1359, BC4-4-1, CRL 1359-A-2-1-4, CRL-1359-18-21, CRL-1359-18-21-22, CRL-1359-18-47, CRL-1359-19-23-52, CRL-1359-60-1-2-4, CROSSER 7-1, CROSSER 7-5, CSR-1012, CSR-103, CSR-1034, CSR-1037, CSR-1053, CSR-1175, CSR-1202, CSR-171, CSR-18, CSR-218, CSR-225, CSR-238, CSR-253, CSR-255, CSR-317, CSR-403, CSR-58, CSR-59, CSR-60, CSR-713, CSR-717, CSR-764, CSR-78, CSR-79, CSR-816, CSR-841, CSR-943, DAR-111, DAR-155, DAR-55, DAR-8, DHR-1, DHR-9907, DNA(WF)-8-10, ELM 31-20, ELM-079, ELM-103, ELM-105, ELM-38, ELM-39, ELM-5, ELM-85, GLM 31-10, GLM 31-13, GLM-3, GLM-4A, GM-16, GM-1B, GM-2, GMCN-10, GMCN-12, GMCN-146-13, GMCN-3, GMCN-69, GMCN-7, GMCN-76, GMCN-8, HES-17, HLM 37-1, HLM 39-21, HLM 39-5, HLM 41-13-2, HLM 9906, HUJM-05-03, HYT-33, IC-331818, IC-331819, ISB-101, ISB-12, ISB-2, ISB-66, ISB-76, ISB-89, ISB-92, ISB-93, JGM-19, JMG-50, JMG-59, JMG-9901, JMG-993, JS-29, JWMR 946-1-3, JWMR-13, K-230-27, K-31-273, KH-2099, KM-555, L-171-7, L-299-64, L-341-22, L-65, LM-114-6, LM-127-27, MCP-12-211N-1, NDR-2017-2, NHO-2-2, NHO-2-30, NPJ-134, NR-1, NR-106-1, NR-4, NRCDR-2, NRCDR-507, ORH-2-2, PBR-320, PBR-378, PF-8, PNB-PL-68, PRG-2001-65, PRG-8903, PRG-906, PRG-920, PRG-939, RH 0644, RH 0749, RK-08-1, RRN-624, RRN-671, SKM-0939, SKM-508	127
II	B-312, B-410, Bio-169-95, BLM-2-5, BR-17, CM 21-10, CM 21-11, CM-11-12, CM-21-7, CM-38, CM-8, CN-10-7719, CRL 1359-18-19, CRL 1359-19-75-4-5, CRL-1359-19, CSR-158, CSR-879, CSR-901, DAR-1, Divya 33, Divya-22, Divya, DNA (WF)-3, DNA (WF)-4, DNA (WF)-6, DRMR 675-39, DRMR-08-293, DRMRIJ-13-3, GLM 4-1, GLM 4-2, GLM-21-1, GM-2 (LR), GMCN 1-2, GMCN-182, GMCN-186, GMCN-19218, GMCN-73, HD-2, HEB-3, HLM-31-22, HUJM-07-01, IC-248736, ISB-95, JMG-001, JMG-02-01, JMG-244, JMG-9005, JMG-903, JMG-951, JMG-959, JMM-08-2, KM-26, KMR-11-1, KMR-11-2, KMR-13-3, Kranti, KRISHNA, MCN-05-4, MCN-14, MCN-17, MCN-20, MCN-21, MCN-26, MCN-29, MCN-36, MCP-12-624, MNC-05-12, MSC-5, NDR-05-1, NDR-190, NDRC-190-8-16, NDRC-190-8-31, NDRC-190-8-5, NDRS-2001, NDRS-2011, NPJ-17, NPJ-180, NRCDR-601, NRCDR-701, NRCHB-101-DT-2, Parasmani-2-10, PBR-300, PBR-368, PBR-375, PBR-422, PHJ-02-402, PR-2009-11, PR-2009-6, PR-8905, PRB-2004-3-4, PRL-2007-7, PTJ-2-85, PTJ-3-5, PUSA BOLD DT, PUSA BOLD DT-1, RB-50, RB-55, RB-73, RGN-197, RGN-329, RGN-332, RH 0761, RH-03-42, RH-0406, RH-0555, RK-05-1, RL-1359 , RL-2106, RMM-09-2, RMM-09-4, RRN-598, RRN-604, RRN-673, RRN-772, RRN-778, Sahib-36, SKM-425, TERIWRBJ-32-1, TM-151	134
III	B-378, BPR-55, CCJJ-1,CM-10, CONSULT-1, CRL-1359-19-12-24, CRL-1359-19-47-21, CRL-1359-19-75-11, CRL-1359-6-11-13-5, CSR-1031, CSR-157, DHR-9601, DHR-9701, DHR-9901, DNA (WF) 8-22, Giriraj, EC-392021, ELM-2, ELM-21, GMCN-139, GMCN-167, JMG-143, JMG-210, JMG-305, JMG-927, JMWR-941-1-2, JS-21, MRJ-15-6, NF-1, PBG-1188, PCR-10-3, PCR-3, PCR-7, PRB-2004-06, PRG-2006, PRO-9907, PTJ-3-100, PTJ-3-20, PTJ-3-65, PTJ-3-69, PTJ-3-72, PTJ-3-79, PTJ-3-94, RC-214, RH 0904, RKM 5, RMM-09-1-1, RRN-631, TM-106-1, TM-204, TNM-17	100
IV	B-384, Bio-197, Bio-440, CCBJ-1, CM 21-16, CM-10-1, CM-10-5, CM-10-7, CM-10-9, CM-11-6, CM-11-7, CM-11-9, CM-6-2, CM-9, CRL 1359-13-6-2, CRL 1359-175-13-19, CRL-13-18-11, CRL-1359-13-15, CRL-1359-175-1, CRL-1359-175-13, CRL-1359-18-19-17, CRL-1359-60, CRL-1359-60-75, CRL-1359-64-175-13B, CROSSER 7-4, CSR-1110, CSR-209, CSR-352, CSR-392, CSR-740, DAR-3, DAR-5, DAR-6, DAR-7, DNA(WF) -8, ELM-48, ELM-7, ELM-9, GLM 12, GLM 1-2, GLM 5-1, GMCN-100, GMCN-187, GMCN-8-1, HLM 31-25-3, HLM 40-33-11, HLM-36-3-2, HUJM-02-01, ISB-80, JLM-96, JM-0600-4, JMG-128, JMG-278, JMM-05-6, JMM-08-1, KLM-119, KMR-13-4, L-171-7-65, MCN-11-19 DT-2, MCN-11-19 DT-3, MCN-31, MCN-34, MSC-1, MSC-3, NDR-05-2, NFJ-2, NHO-2-1, NHO-3-11, NHO-3-13, NHO-3-2, NJHO-3-21, NJHO-7-20, NML-64, NPJ-2-9, NRCHB-06-5912, NRCHB-101-DT-3, NRCQR-837, Pankhonali, PANTNAGAR-1, PBG-1007, PBG-93-5716, PBR-423, PCR-9604-2, PRG-1291, PRG-14, PRG-151, PRG-168, PRG-2001-62, PRG-210, PRG-225, PRG-7860, PRG-905, PRG-909, PRG-9901, PTJ-3-102, PTJ-3-64, PTJ-3-90, Purvi Raya-1, PUSA BOLD DT-2, RGN-163, RGN-228, RGN-282, RH 0704, RH-02-02, RH-0512, RK-06-1, RK-06-2, RMM-09-1, RRN-608, SKM-740	81

Based on the divergence, 442 mustard genotypesinvolved in the present study were grouped into 4 clusters on the basis of Hierarchical cluster analysis (Fig. 1).Clustering pattern revealed the presence of considerable amount of genetic diversity in this material. In general, intercluster distances were relatively greater than intracluster distances showing that genotypes included indifferent clusters were genetically more diverse than the genotypes included within a cluster. Highest number of genotype were grouped in clusters-II having 134 genotypes, followed by cluster-I with 127, clusters III and IV containing 100 and 81 genotype respectively as seen from Table 2.

Table 1: Best linear unbiased prediction (BLUP) based genetic components of test traits of *B. juncea* germplasm stock under RF conditions

Particulars	FW50	FW100	FD	PH	NPB	NSB	MSL	SPMS	SL	SY
Heritability	0.822	0.860	0.632	0.720	0.948	0.942	0.947	0.851	0.917	0.864
Genotype Variance	18.194	24.237	18.195	158.099	0.502	4.683	122.175	40.673	0.182	1.592
Residual Variance	7.860	7.849	15.680	122.938	0.055	0.569	13.551	14.220	0.032	0.499
Grand Mean	58.791	66.773	124.995	187.572	3.926	10.268	63.974	49.350	4.292	7.648
LSD	4.997	5.108	6.837	18.493	0.449	1.440	7.043	6.838	0.341	1.406
CV	4.768	4.195	3.412	5.911	5.977	7.351	5.754	7.641	4.223	9.239

Where; FW 50: Days to 50% flowering. FW 100: Days to 100% flowering,
FD: Total flowering duration, PH: Plant ht (cm), NPB: No. of primary branches, NSB: No. of secondary branches, MSL: Main shoot length (cm), SPMS: No. of siliquae on main shoot, SL: Siliqua length (cm), and SY: Seed yield per plant (g)

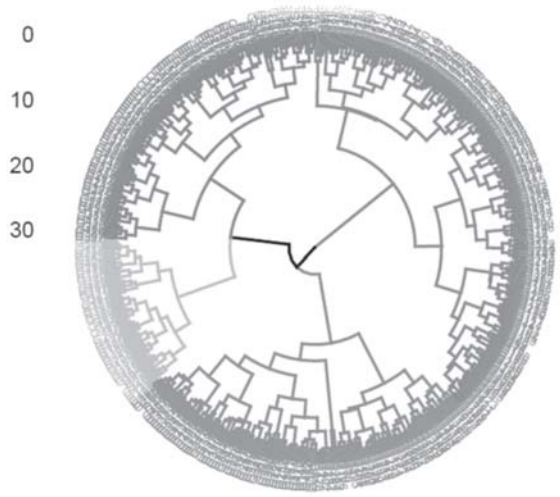

Fig. 1: Circular Dendrogram showing clustering pattern of 442 genotypes

Pattern of distribution of genotypes among various clusters reflected the considerable genetic variability present in the genotypes under study.The grouping of genotypes indicated that geographical distribution need not necessarily be the indicator of genetic divergence reported by Reddy *et al.* (2012); Kumari and Sheoran (2012); Verma and Sachan (2000) and Jeena and Sheikh (2003). Bansal *et al.* (1990) reported that clustering pattern was influenced by the pedigree of breeding lines. Similar results were found in case of Brassica juncea (Singh *et al.,* 2010).

Intra cluster distance was maximum for cluster III (175.98), followed by clusters I (154.15) and IV (146.98) and

Table 3: Analysis for Inter and Intra cluster distances for 442 genotypes of mustard

	C1	C2	C3	C4
C1	154.15			
C2	1290.51	145.02		
C3	1112.21	206.72	175.98	
C4	938.93	375.14	208.39	146.98

*Diagonal values are intra cluster distances

minimum forcluster II (145.02) which indicates the existence of maximum variability within cluster III. Inter cluster distance was maximum in case of Cluster I and II (1290.51) and minimumin case of clusters II and III (206.72) as shown in Table3. The higher intercluster distances exhibited the presence of more diversity among the genotypes involved in these clusters. The genotypes grouped into same cluster displayed the lowest degree of divergence from one another and when crosses are made among the genotypes of the same cluster, no transgressive segregant is expected from such combinations. The crosses between the genotypes belonging to distantly located clusters are likely to produce better transgressives egregants. So, it is desirable to select accessions from the clusters having high inter-cluster distance in there combination breeding programmes.

The genotypes for hybridization may be chosen from widely separated clusters (Fig. 1), as it is observed that there are several genotypes included in the crossing programme from widely separated clusters.Although, for

final selection of the parents for breeding programme, the genotypes to be used may be selected almost without exception or its proven performance in the areas of intended use including quantitative characters and include in crossing with the existing varieties for their further improvement (Allard, 1960).

To get more heterotic F_1's and large number of desirable transgressives egergants, selection of parents for hybridization should be properly based on genetic diversity rather than geographic diversity. An effective hybridization programme may be initiated involving the genotypes belonging to diverse clusters with high mean for almost all component characters. The cluster means for seed yield and its component characters revealed considerable differences among all the clusters for most of the characters studied.

In the present study, days to 50% flowering had the highest mean value in cluster IV and lowest mean value in cluster I as shown in Table 4. For days to 100% flowering, cluster IV exhibited the highest mean value and cluster I showed the lowest mean value. Cluster II had highest mean value for flowering duration while cluster I had the lowest one. Cluster II revealed the highest mean value for plant height, where as cluster I had the lowest mean value. No. of primary and secondary branches means were highest in Cluster III and minimum in cluster IV. MSL mean was highest in cluster II while SPMS mean maximum in cluster II and minimum in cluster IV. Seed yield was highest in cluster III and minimum in cluster IV. Comparative evaluation of cluster means suggested that for improving specific characters; select the genotypes from the cluster having high mean value fort hat character. It is assumed that maximum amount of heterosis will be

Table 4: Mean values of different clusters for 10 characters in Indian mustard

Cluster	FW50	FW100	FD	PH	NPB	NSB	MSL	SPMS	SL	SY
I	55.21	62.88	121.82	181.49	3.53	9.11	64.13	47.09	4.41	7.63
II	60.64	68.70	126.62	192.95	3.93	10.03	67.99	52.67	4.18	7.51
III	57.16	64.63	125.04	190.06	4.66	12.74	67.89	51.48	4.29	8.13
IV	61.73	70.55	125.78	182.12	3.47	9.03	51.78	43.47	4.33	7.31

manifested in cross combinations involving the parents belonging to most divergent clusters. But for a plant breeder, the objective is not only high heterosis but also reduction of duration. The greater distance between two clusters indicates the wider genetic diversity between genotypes. Cluster III has high yield and yield contributing traits. While cluster I has short flowering duration which could help to develop short duration varieties. So C I and C III could be used for developing high yielding and early flowering genotypes. Similar results were obtained by Verma and Sachan (2000); Goswami and Behal (2006); Kumar *et al.* (20070 and Yu-cheng*et al.* (2007).

Conclusion

The present study has led to improve the understanding of many interrelated processes involved in the genetic control of variation in the seed yield. Based on genetic divergence and per seperformance/genotypic worth forvarious traits some sort of inter-mating among diverseand promising genotypes was found important to further expand genetic variation for important yield attributing traits to build either improved populations or draw improved inbredlines for developing heterotic hybrids. Also the improved mustard populations must combine high seed yield. For that matter, selection in segregating populations as evident from present studies can be based seed yield along with its component traits. The results, thus, obtained in the present study would provide some guidelines in selection of parents and in theprediction of possible merits for genetic recombination and would also be of value in formulating model plant type for selection in segregating generations.

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