Diversity Analysis in Brassica Species for Selection of Genotypes for Drought Tolerance Breeding Based on Biometrical Traits

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Abstract:

Thirteen genotypes belonging to five species *Brassica rapa, Brassica juncea, Brassica carinata, Brassica napus and Eureca sativa* were evaluated to study the diversity among the genotypes and to identify the desirable and potential parent in mustard breeding programme for drought tolerance during the year 2008-09 at College of Agriculture Nagpur.. The analysis of variance and analysis of dispersion revealed highly significant differences among the genotypes for the traits studied viz. days to 50% flowering, days to maturity, plant height, number of primary branches, number of silique per plant, 1000 seed weight and seed yield per plant. Thirteen genotypes grouped into five clusters by D^2 analysis. The maximum intercluster distance was recorded between cluster IV and cluster III. The canonical analysis indicated the importance of character like number of silique, days to maturity, days to 50% flowering and seed yield per plant towards the source of variation. Drought tolerant genotype RTM-314 and T-27 deviated from all other genotypes on the basis of genetic markers uniqueness for drought tolerance as compare to other genotypes.The results of the study lead to conclusion that the cultivated genotype ACN-9 and Pusa Bold of *Brassica juncea* can be further improved for earliness by crossing with Ragini and Bhavani (*Brassica rapa*) and by crossing with RTM-314 and T-27 (*Eureca sativa*) for drought tolerance.

Keywords: Mustard genotypes, biometrical traits, D^2 analysis

Introduction:

Indian mustard (*Brassica juncea* L Czern and Coss) is an important oil seed crop which has received attention of geneticist and breeders for it genetic improvement. The important species of Brassica that are extensively cultivated commercially are *Brassica juncea*, *Brassica campestris* and *Brasica napus* out of which *Brassica campestris* and *Brassica juncea* are largly cultivated in Asia (India, China, Pakistan, Bangladesh and Nepal) while *Brassica napus* in Sweden, Germany, France, Canada and Australia. Indian mustard is mostly grown on light textured soil as it conserves moisture from monsoon rains thus it invariably suffers from moisture stress during reproductive phase of growth. Genetic variability within a species offers valuable for studying the mechanism of drought tolerance. The analysis of genetic variation and relatedness in germplasm are of great value for genetic resources conservation and plant breeding programme over the years. Methods for assessing genetic diversity have ranged from classical strategies such as morphological analysis, biometrical analysis to biochemical strategies and molecular techniques (Demissie *et al*, 1998). The availability of genetic markers for identification of a superior expression of a trait will be useful to score the genotypes with least influence of the environment on the genetic make up of that particular character. Choice of parents influences the success of breeding programme in self pollinated crop like mustard and other crop also. The assessment for choice of parents were conducted by many workers in various crop species using different

types of marker system like biometrical traits. *Brassica juncea* is a cultivated species of Vidarbha region having high yielding capacity. However, as it is grown with the conserved moisture from monsoon rain it invariably suffers from moisture stress during different phases of growth. The unpredictable rainfall received also results in unfavorable residual moisture. This affects the yielding potential of the mustard varieties grown in the Vidarbha region. Therefore there is a need for development of high yielding and early maturing mustard varieties along with the drought tolerant trait. In an attempt to fulfill this need partly this experiment is taken up to screen the available mustard species based on biometrical traits and molecular markers with the objective to identify the parents for hybridization to develop drought tolerant varieties.

Material and Methods:

This experiment was conducted during 2008-2009, at the farm and laboratory of Botany section, College of Agriculture, Nagpur. The experimental material consisted of thirteen genotype of mustard belonging to five different species viz., *Brassica rapa* (toria) – Bhavani, PT-303, T-9, *Brassica rapa* (yellow sarsan) – YST-151 & Ragini, *Brassica juncea* – Pusa Bold & ACN-9, *Brassica rapa* (brown sarsan) – BSH-1 & KBS-3, *Eureca sativa* – RTM-314 & T-27, *Brassica carinata* – PC-5 & *Brassica napus* – GSL-1. These thirteen genotypes were evaluated in a randomized complete block design with two replications and spacing of 45 x 10cm^2. All recommended cultural practices were followed to raise a good crop. Observations were recorded on five randomly selected plants from each genotype on Days to 50 per cent flowering, Days to maturity, Plant height (cm), Number of primary branches, Number of siliquae plant^{-1}, Number of seeds siliqua^{-1}, 1000 seed weight (g) and Seed yield plant^{-1} (g). The biometrical data were subjected to the D^2 analysis as method given by Mahalonobis (1936). Grouping of genotypes into clusters was done as per the method described by Rao (1952) and indentifying of superior genotypes was done as per the method described by Bhatt (1970).

Results and Discussion:

The analysis of variance was highly significant between the genotype for all the seven quantitative traits (table 1). This revealed the presence of considerable variation among the genotypes. The genotypes were genetically diverse than the existing varieties ie. Pusa Bold and ACN-9. The thirteen genotypes were grouped into five different clusters (Table 2). Cluster II was the largest comprising of 6 genotypes. The next largest cluster was cluster III with 3 genotypes followed by cluster I with 2 genotypes. Cluster IV and V included one genotype in each cluster. The genotypes of *Brassica juncea* and *Brassica carinata* were grouped in the same cluster. Similarly genotypes of the species *Eureca sativa* were grouped in a single cluster and divergent from other species. The genotypes belonging to *Brassica rapa* were observed to show divergence among themselves as they were distributed in three clusters. Hence there is a scope for selection of potential genotype for genetic improvement for yield and other yield contributing characters. Dhillon *et al.*, (1999) observed grouping of 55 genotypes into eight clusters and Srivastav and Singh (2000) also observed the grouping of 26 Indian mustard genotypes into six clusters

on the basis of D^2 analysis. The number of cluster into which the genotypes are grouped is often noticed to vary with set of genotypes used for cluster analysis depending on the extent of distance from one another. The clustering pattern have direct relationship with quantitative characters. Therefore the selection of genotypes for hybridization should be done separately for the aspect depending on the objectives of hybridization.

The relative importance of individual characters toward genetic divergence can be known on the basis of size of the coefficients of canonical vectors (Rao, 1952). The values of first three canonical vectors and canonical roots were presented in table 3 and 4 respectively. In the canonical analysis nearly 86.633% of the total variation for seven quantitative characters was found to be accounted by the first three canonical vectors. This suggested that differentiation for these traits among the 13 genotypes was nearly completed in three phases. Further the coefficients in the first three canonical vectors indicated that out of seven quantitative characters, plant height, days to maturity, primary branches plant^{-1}, siliquae plant^{-1} and seed yield plant^{-1} were important in the I vector which was the major axis of differentiation and accounted for 61.614 per cent of the total variation. Seed yield plant^{-1} and siliquae plant^{-1} were important characters in the secondary axis of differentiation which accounted for 16.622 percent of the variation. Important characters in the III vector were days to maturity, days to 50% flowering and siliquae plant^{-1} accounting to 8.397 percent of the variation. This suggested that parents selected on the basis of this eight traits may be expected to be genetically diverse. Thakur *et al.*, (1989) observed that the differentiation for ten characters among 44 genotypes was completed in four phases, of which the first two canonical roots accounted for 64.80% and 13.15% of the total variation they also observed that secondary branches plant^{-1}, pods plant^{-1}, 1000 seed weight ,oil content and seeds siliquae^{-1} constituted the major axis of differentiation. Days to maturity, plant height and seed yield plant^{-1} constituted the second axis of differentiation and appears to have contributed towards total genetic divergence through natural and human selection. This discrepancies' in the result might have been different sets of material studied in different investigation and also due to role of environmental variability. According to the contribution of individual characters towards genetic divergence, the relative importance of the character is judged by several workers in different crops. However, Singh (1981) stated that this method of determining the influence of characters on genetic divergence is incorrect. Because according to him the transformed uncorrelated characters (Yi) do not have any biological meaning. He further pointed out that the workers who did use this method have wrongly taken characters Yi to be uncorrelated Xi amplify a one to one correspondence between these two variables. In fact Yi is a linear function of several Xi values. Gupta *et al.*, (1991) used cluster means (multiple regression analysis) based on correlated data, to know the relative importance of characters in causing genetic divergence.

Average intra and inter cluster distance among eight characters is presented in table 5. The intra cluster variation is ranged from 0 (cluster IV and V) to 128.863 (cluster III). Cluster III recorded the highest intra cluster distance (D^2 =128.863)

followed by cluster II (D^2 = 62.249) and cluster I (18.063). The average inter cluster distance was maximum between cluster IV and cluster III (D^2 =822.636) followed by cluster V and cluster III (D^2 =671.370) cluster IV and cluster II (D^2 =609.253). Cluster V and cluster II (D^2 =498.722), cluster III and cluster I (D^2 =369.563). The minimum average inter cluster distance was observed between cluster I and cluster I (D^2 =18.063).

The mean of cluster mean (Table 6) for all characters indicated that maximum variation was accounted by plant height (109.6154) followed by days to maturity (98.4615), number of siliquae plant^{-1} (77.6539). The mean was lowest for 1000 seed weight (2.4962).The coefficient of variance of cluster mean for all the characters indicated that maximum variation was accounted by number of siliquae plant^{-1} (28.0304) followed by 1000 seed weight (19.6889), branches plant^{-1} (11.3442) and seed yield plant^{-1} (9.1534). The variance was lowest for plant height (4.310g).

According to Anand and Rawat (1984), Gupta *et al*,(1991), Sachan and Verma (2000), the pattern of grouping clusters proved that geographic diversity need not necessarily be related to genetic diversity. This means that geographic diversity though important may not be the only factor in determining genetic divergence. According to Bhatt (1970) the mean statistical distance may be considered arbitrarily as a guide line and crosses between parents belonging to different clusters having the same or higher inter-cluster distance may be attempted in the mean average of inter-cluster distance (D = 8.401) was computed and the cross combinations above this mean are arranged in the descending order and presented in table 6.

The maximum inter cluster distance (D^2 = 822.636) existed between cluster IV and cluster III. Therefore, crosses between genotypes belonging to the cluster having highest inter cluster distance will yields better segregates. The clusters containing either I or II genotypes seen to be genetically diverse from each other and also from other genotypes under study. Hence, genotypes Ragini, Bhavani, RTM-314, T-27 etc, showed significance for their use in hybridization programme to obtain desirable transgrates in subsequent generation.

The crosses may be chosen from widely separated cluster but as it is observed in the present study, that there are several genotypes in the widely separated clusters. The final selection of the parents to be used may be carried out in the light of suggestions made by Allard (1960). The genotypes to be used may be selected almost without exception or its proven performance in the areas of intended used including quantitative characters. Hence, as observed in this study the proven performance of the genotype included in the widely separated clusters for highly variable characters like days to 50 % flowering, days to maturity, plant height, number of siliquae plant^{-1}, seed yield plant^{-1} were considered as the criterion for selecting genotypes for hybridization to obtain desirable transgrates in subsequent generation and are listed in table 7. Improvement in mustard for fulfilling the breeding objectives can be brought about by further improving the existing varieties i.e. Pusa Bold and ACN-9 (*Brassica juncea*) for earliness by hybridization with genotypes Ragini and Bhavani (*Brassica rapa*). Similarly, Pusa

Bold and ACN-9 can also be improved for drought tolerance by hybridization with RTM-314 and T-27 (*Eureca sativa*).

Table. 1- Analysis of variance for seven characters in 13 genotypes of *Brassica spp.*

Source of variation	d.f	Mean sum of square						
		Days to 50% Flowering	Days to maturity	Plant height (cm)	Branches plant^{-1}	No.of siliquae plant^{-1}	1000 seed weight (g)	Seed yield plant^{-1} (g)
Replication	1	3.1154	591.3846	96.1538	0.3462	0.0465	408.0385	0.0104
Genotypes	12	128.5513**	208.0385**	1828.679**	1.6538**	0.9254**	5690.5322**	7.5714**
Error	12	13.1154	46.2179	22.3205	0.1795	0.2415	473.7885	0.0586

** Significance of 1 % Level

Table. 2- Distribution of thirteen genotypes of mustard in different clusters based on seven quantitative characters

Cluster No.	Total no of genotypes	Genotypes
I	2	RTM-314 (*Eureca Sativa*), T-27 (*Eureca Sativa*)
II	6	T-9(*Brassica Rapa*-toria), BSH-1(*Brassica rapa*-Brown Sarson), GSL-1(*Brassica napus*), PT-303(*Brassica rapa*-Toria), KBS-3 (*Brassica rapa*-Brown sarson), YST-151 (*Brassica rapa*-Yellow Sarson)
III	3	PUSA BOLD(*Brassica Juncea*), ACN-9(*Brassica Juncea*), PC-5 (*Brassica carinata)*
IV	1	RAGINI (*Brassica rapa*-Yellow Sarsan)
V	1	BHAWANI (Brassica *rapa*-Toria)

Table. 3- The value of first three canonical vectors and canonical root

Vector	Plant height (cm)	Branches plant^{-1}	Days to 50% flowering	Days to maturity	1000 seed weight	Silique plant^{-1}	Seed yield plant^{-1}
I	0.445	0.414	-0.282	0.443	-0.429	0.145	0.139
II	0.024	-0.020	0.183	-0.027	0.081	0.653	0.683
III	0.171	-0.148	0.795	0.126	-0.374	0.198	-0.349

Table. 4- Values of three canonical roots and their contribution expressed as per cent of the total variation

Root	Value	Contribution in percentage
λ_1	468.3	61.614
λ_2	144.2	16.622
λ_3	77.6	8.397
Total	690.1	86.633
Sum of all canonical roots	796.57	100.000
Residual	106.47	13.367

Table. 5- Average Intra And Inter-Cluster Distance(D^2) BY Toucher's Method

	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V
Cluster I	18.063	314.820	369.563	149.844	168.885
Cluster II		62.249	274.781	609.253	498.722
Cluster III			128.863	822.636	671.370
Cluster IV				0.000	161.382
Cluster V					0.000

Table. 6- Cluster means for eight quantitative characters in mustard

Clusters	Plant height (cm)	Branches/ plant	Days to 50% flowering	Days to maturity	1000 seed weight	Siliquae / plant	Seed yield/ plant(g)	Total protein (%)
Cluster I	85.500	4.250	44.500	103.750	3.100	61.250	2.295	20.823
Cluster II	123.000	3.667	48.500	100.667	2.150	67.500	1.680	27.970
Cluster III	134.833	3.667	58.667	103.167	3.167	146.833	5.820	27.557
Cluster IV	64.500	1.000	45.000	81.000	2.300	12.000	0.710	20.430
Cluster V	47.000	4.000	35.500	78.000	1.500	29.500	1.550	31.500
Mean	109.615	3.576	47.730	98.461	2.496	77.653	2.645	26.466
C.V	4.310	11.84	7.587	6.904	19.68	28.030	9.153	3.5166

Table. 7- Selected genotypes for hybridization

Sr.no	Cluster combination	Average inter cluster distance	Desirable cross combination
1	IV X III	822.636	Ragini(earliness) X Pusa Bold and ACN-9 (number of siliquae per plant and seed yield per plant
2	V X III	671.370	Bhavani (earliness) X Pusa Bold and ACN-9 (number of siliquae per plant and seed yield per plant)
3	IV X II	609.253	Ragini(Days To Maturity) X BSH-1 (Seed Yield Per Plant.
4	V X II	498.722	Bhavani (earliness) X BSH-1 (Seed Yield Per Plant)
5	III X I	369.563	Pusa Bold and ACN-9 (number of siliquae per plant and seed yield per plant) X RTM-314 and T-27 (drought tolerance)*
6	II X I	314.820	BSH-1 (Seed Yield Per Plant) X RTM-314 and T-27 (drought tolerance)*
7	III X II	274.781	Pusa Bold and ACN-9 (number of siliquae per plant and seed yield per plant) X BSH-1 (Seed Yield Per Plant)
8	V X I	168.885	Bhavani (earliness) X RTM-314 and T-27 (drought tolerance)*
9	V X IV	161.382	Bhavani (earliness) X Ragini (Days To Maturity)
10	IV X I	149.844	Ragini (Days To Maturity) X RTM-314 and T-27 (drought tolerance)*

* Identified as source of drought tolerance

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