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ORIGINAL RESEARCH

Genetic variation, character association and genetic divergence analysis among Mustard (*Brassica spp.* L.) in Bangladesh.

Most Morium BEGUM[1], Md. Ekhlas UDDIN[2], Sezanur RAHMAN[3,4], Md. Shahadat HOSSAIN[1], Rumana Ferdous BINT-A- RAHMAN[5], Md. Rezanur RAHMAN[3], Hossain Md. FARUQUEE[3]*

[1]Genetic Engineering and Biotechnology, University of Rajshahi, Bangladesh
[2]Biochemistry and Molecular Biology, Gono Bishwabidalay, Bangladesh
[3]Biotechnology and Genetic Engineering, Islamic University, Bangladesh
[4]Biochemistry and Microbiology, North South University, Bangladesh
[5]Bangladesh Sericulture Research and Training Institute, Bangladesh
*Corresponding Author email: faruquee@btge.iu.ac.bd

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ABSTRACT

The average availability of oils and fats per capita per annum is 3.8 kg against the requirement of 11 kg in Bangladesh. It is essential to exploitation of genetic diversity for increasing oilseeds production. In this study 25 mustard genotypes are subjected for variability, character association and genetic divergence experiment in field, during November to March. Randomized complete block design with three replications was followed for the study. The traits plant height, number of pods and pod length showed high heritability coupled with high genetic advance as percentage of mean. Correlation coefficient study indicated that plant height, number of primary branch and number of pods were positively and significantly correlated with seed yield. Path coefficient study show that number of pods, pod length and number of seed contributed maximum to seed yield in positive direction. The genetic divergence assessed through D^2-statistics. The genotypes were grouped into four clusters for yield and yield contributing traits. Significant differences were found among the genotypes for all the traits, where Rai-5 produced high seed yield which was the tallest one and BARI-12 was the earliest in maturity.

KEY WORDS: *Mustard genotype, Variability of oilseed, Character association, Genetic divergence*

INTRODUCTION

The average availability of oils and fats per capita per annum is 3.8 kg (10.55 g/head/day) against the requirement of 11 kg in Bangladesh, while most developed countries consume about 20 kg. Since 1972 Bangladesh have released high yield new varieties, however internal production can meet only about 29% of our consumption (8 g/day/head) and is imported (Alam, Begum, & Roy, 2014).

Yield is classified as biological yield (total biomass) and economic yield (the economically useful part of the plant) which is a result of various physiological processes occurring in plants among varieties which influences different phonotypical characters (Mandal & Sinha, 2004).

Diversity can be determined by three different levels, first one is genetic diversity, in which the variation observed at gene and germplasm level, the second one is diversity of species, in which richness of species at a specific location and the third one is the Eco-system diversity, in which crop species interact with their environment (Saleem et al., 2017). Diversity is potent for different crops to fulfill the gap between demand and production across the world. Various morpho-biochemical and molecular methods are used to study

genetic variability among local and exotic plant germplasm. The proper evaluation of important crop species helps in the identification and utilization of improved genotypes (Ghosh, Haque, Parvin, Akhter, & Rahim, 2009; M. S. Iqbal, Haque, Nath, & Hamim, 2014; S. Iqbal, Hamim, Haque, & Nath, 2015; S. Jan, Z. Shinwari, & M. Rabbani, 2016; S. A. Jan, Z. K. Shinwari, & M. A. Rabbani, 2016; Khan et al., 2016; Sanvicente, Rodríguez-Estrella, Lozano-Garza, & García-De-León, 2016).

So for the prosperity of the country, it is essential to increase the production of these crops and for maximum production, exploitation of genetic diversity of these crops is essential. Therefore, the present study was performed to estimate and characterize the genetic variation and relationships among Indian mustard genotypes through agro-morphological traits to identify and select promising germplasm for traits of economic significance.

RESULTS

Variability and genetic parameters: The analysis of variance for days to maturity, plant height, number of primary branches per plant, number of pods per plant, pod length, number of seeds per pod, 1000 seed weight and seed yield per plant of 25 mustard genotypes were highly significant (Table 1), and it is observed that different genotypes were better for different traits. Phenotypic, genotypic and environmental coefficient of variations, heritability in broad sense and genetic advance for yield and different yield contributing traits in mustard are presented in the Table 2. Correlation coefficients between seed yield and its different contributing traits of 25 mustard genotypes are presented diagrammatically in Figure 1, which indicated that there was strong inherent relationship among the traits. Residual effect was 0.42 for path coefficient analysis, which is denoted as 'R' in the diagram. This

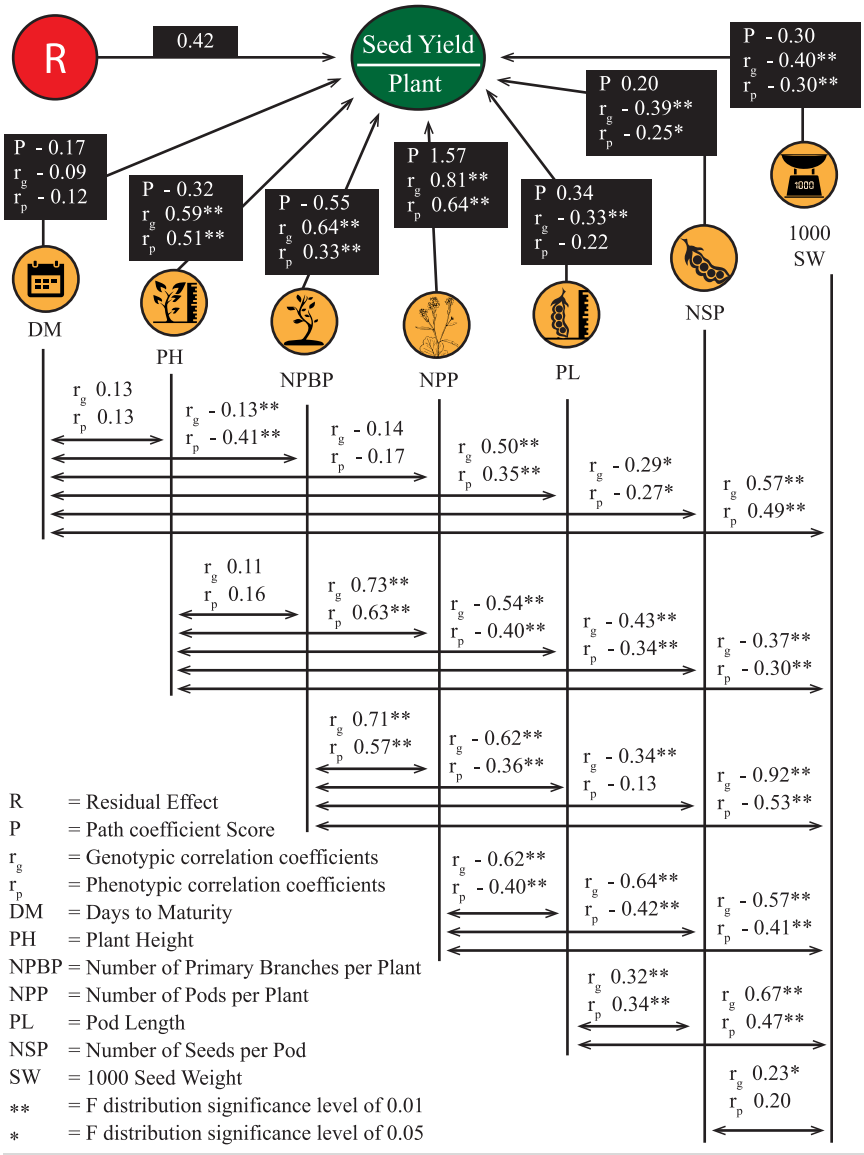

Figure 1: Genotypic path diagram of seed yield and yield contributing traits on seed yield per plant. Single arrowed lines indicate direct effects and double arrowed lines indicate correlation coefficients and R indicates the residual effect.

diagram also present cause and effect relationship of seed yield and yield contributing traits.

Genetic Diversity: Principal Component Analysis (PCA) also helps in assessment of diversity in multivariate scale, which showed that the first component axes largely accounted for the variation among the genotypes (81.23%) followed by the second component axes (12.93%) and thus first and second components accounted for (94.16%), which covered the major part of the total variation. Based on the values of principal component scores 2 and 1, a two dimensional scatter diagram was constructed, which apparently show 4 cluster (Figure 2(a)). The pattern of distribution show that largest cluster (VI) contain 13 genotypes (BARI-7, BARI-8, BARI-13, BARI-14, BARI-15, GPB-1, GPB-2, GPB-3, GPB-5, GPB-6, GPB-7, GPB-8 and GPB-10) followed by cluster (III) 5 genotypes (BARI-6, GPB-4, GPB-9, SS-75 and Ts-72) cluster (II) contain 4 (BARI-10, BARI-11, Daulat and Rai-5) and cluster (I) contain 3 (BARI-9, BARI-12 and Tori-7). The highest intra cluster distance was computed for cluster (IV) (3.05) followed by the cluster III (1.26). The intra cluster distance of cluster I and cluster II were 0.22 and 0.81 respectively. Canonical variate analysis was performed to obtain the inter cluster distance which was maximum between II and IV (10.02) followed by the distance between cluster II and III (9.01) and between the cluster I and IV (8.32), while the distance was minimum between cluster III and IV (3.30) followed by the distance between cluster I and III (5.15), which is diagrammatically presented in Figure 2(b).

Inter genotypic distances (D^2) were obtained from principal coordinate analysis between the pairs of 25 mustard genotypes and are presented in Table 3. The highest inter genotypic distance was 2.05, which was observed between the genotypes GPB-2 and BARI-11, followed by the distance 2.04 observed between the genotypes BARI-14 and BARI-11. The lowest inter genotypic distance was 0.18, which was observed between the genotypes Tori-7 and BARI-12 which was followed by 0.23 between genotypes GPB-9 and GPB-6.

Intra cluster mean for eight seed yield and yield contributing traits, and divergence obtained form CVA is presented in Table 4. This result indicated that this trait had the highest contribution towards the divergence among 25 mustard genotypes. In vector-I, the other important traits responsible for genetic divergence in the major axis of differentiation were plant height, number of pods per plant and seed yield per plant. Again, in vector-II, number of seeds per pod was important for divergence. The negative values in both the vectors for days to maturity, pod length and 1000 seed weight indicated that these three traits had the lowest contribution to the total divergence among the mustard genotypes.

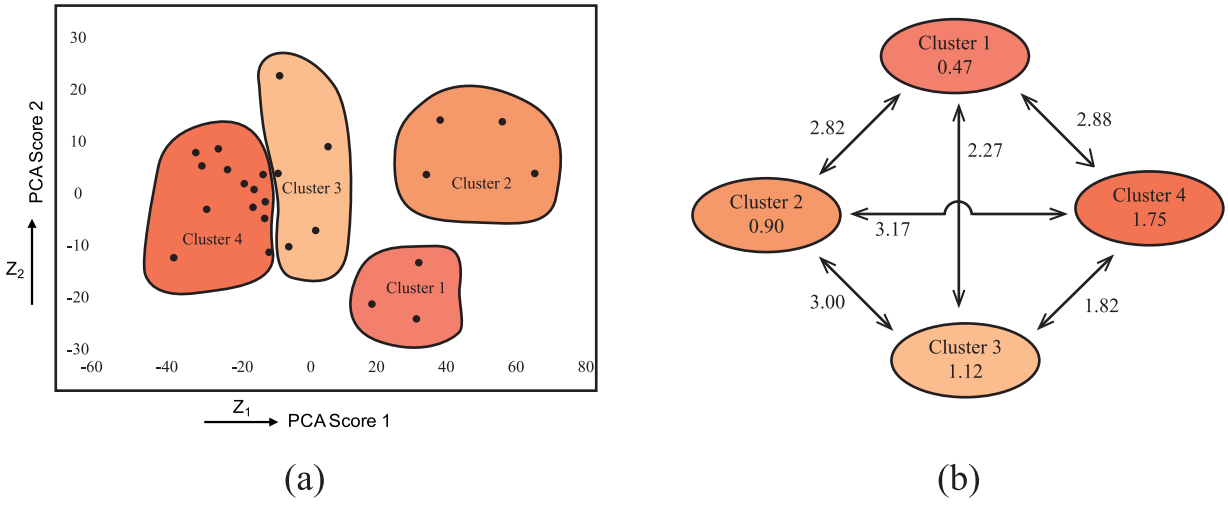

Figure 2: (a) Scatter diagram of 25 mustard genotypes based on their principal component scores for seed yield and yield contributing traits. **(b)** Cluster diagram (arbitrary) showing the average intra and inter cluster distances (D) for seed yield and yield contributing traits of 25 mustard genotypes.

DISCUSSION

Seed yield is a quantitative character whose expression depends on the interaction of their component traits, components of seed yield may be varied with genotypes and their phenotypic expression may also be influenced by the environment. In our study, all the yield and yield contributing traits are significantly different among the genotypes.

Table 1: Mean performances of yield and yield contributing traits in mustard

Varities / Lines	Day to maturity (DM)	Plant height (cm)	Number of primary branch (NPBP)	Number of pods per plant (NPP)	Pod length (PL)	Number of seeds per pod (NSP)	1000 Seed weight (g)	Seed yield per plant (g) (SYP)
BARI-12	74.33	113.7	4.33	86.20	5.10	15.90	1.85	7.12
BARI-13	93.00	101.1	2.60	29.27	6.62	16.40	2.37	6.20
Rai-5	88.00	147.0	4.00	95.40	4.30	13.17	2.12	9.39
TS-72	75.00	98.40	4.00	53.07	5.20	16.97	2.40	5.66
Tori-7	78.67	98.87	4.67	80.00	5.18	16.20	1.95	6.96
BARI-10	88.67	126.6	3.82	81.97	4.03	11.99	2.07	5.74
BARI-7	88.33	99.60	2.67	45.80	5.50	18.47	2.75	3.85
BARI-15	77.33	93.40	3.00	29.67	4.79	19.13	2.41	4.27
BARI-11	93.33	140.5	2.87	109.5	4.17	7.90	2.76	8.61
Daulat	89.33	138.1	3.25	80.10	4.53	13.81	1.93	5.80
BARI-9	76.67	103.0	4.53	92.07	6.23	17.13	2.12	6.52
BARI-6	77.33	121.1	2.33	53.20	5.48	16.73	2.25	5.83
BARI-14	74.67	80.87	3.80	26.87	4.65	26.97	2.32	3.65
BARI-8	93.67	102.7	2.60	43.53	7.13	21.27	2.72	2.94
SS-75	85.00	125.3	1.80	36.60	6.13	29.67	2.55	5.56
GBP-1	87.33	100.4	1.93	32.73	6.67	17.57	2.51	3.19
GBP-2	91.00	96.40	1.93	26.47	6.20	13.97	2.61	1.64
GBP-3	93.00	98.80	2.40	42.47	6.50	16.60	2.66	2.35
GBP-4	91.67	100.5	2.73	61.40	7.70	19.83	2.65	8.35
GBP-5	92.67	95.40	3.40	43.20	6.07	11.40	2.30	5.98
GBP-6	93.33	95.47	2.80	48.07	7.28	16.97	2.66	5.93
GBP-7	85.67	92.07	2.53	51.40	5.63	18.07	2.82	5.83
GBP-8	90.67	99.20	1.93	38.80	6.78	18.13	3.10	4.48
GBP-9	88.00	106.4	3.37	45.40	7.52	18.47	2.83	5.46
GBP-10	93.67	97.27	1.40	24.63	7.10	18.60	2.90	4.56

Standard error of mean of all the traits was found within the judicial range in the present study indicating the precision of recording the experimental data. The degree of coefficient of variability (CV%) was indicated by the range of variation. However, for all the genotypes of a particular character CV% varied from genotype to genotype. But it was observed that days to maturity and plant height showed almost equal phenotypic and genotypic coefficient of variation. High values of phenotypic and genotypic coefficient of variation were found in number of primary branches per plant, number of pods per plant and seed yield per plant. It suggested good scope for improvement of characters through selection. We find, plant height, number of pods per plant, pod length, number of seeds per pod, 1000 seed weight and seed yield per plant exhibited high heritability coupled with high genetic advance as percentage of mean. Therefore, phenotypic selection for these traits would give positive response for improving seed yield.

Genetic diversity among the genotypes is important for effective breeding program as the genetically diverse parents are known to produce high heterotic effects and wide segregants for developing high yielding varieties. With the development of advanced biometrical methods, such as, multivariate analysis (Rao, 1952) based on Mahanobis D2-statistic and Principal Component Analysis (PCA), it has become

Table 2. Phenotypic variance (σ^2p), genotypic variance (σ^2g) Environ mental variance (σ^2e). Phenotypic coefficient of variation (PCV%). Genotypic coefficient of variation (GCV%), Environmental coefficient of variation (ECV%), Habitability in broad sense (h^2_b%), Genetic advance (GA) and Genetic advance as % of mean (GA%) for yield and yield contributing traits in mustard.

Genetic parameters	Yield and Yield components							
	Days to maturity	Plant height cm	Number of primary branches / plant	Number of pods / plant	Pod length cm	Number of seeds / pod	1000 seed weight (g)	Seed Yield / plant (g)
σ^2p	55.266	308.044	1.344	754.122	1.533	23.788	0.144	4.522
σ^2g	44.466	264.288	0.555	532.855	1.055	17.766	0.101	3.133
σ^2e	10.801	43.755	0.788	221.277	0.499	6.022	0.044	1.399
PCV%	8.601	16.422	38.701	50.566	21.133	28.277	14.988	39.111
GCV%	7.722	15.211	24.911	42.501	17.455	24.433	12.755	32.566
ECV%	3.801	6.199	29.611	27.399	11.911	14.233	7.877	21.677
h^2_b%	80.455	85.801	41.455	70.666	68.211	74.677	72.399	69.301
GA	12.322	31.022	0.999	39.977	1.744	7.501	0.555	3.033
GA %	14.266	29.022	33.044	73.601	29.699	43.488	22.344	55.833

Table 3: Ten of each lower and higher inter genotypic distance between pairs of genotypes for yield and yield contributing traits in mustard.

10 lower D^2 values	Genotypic combination	10 higher D^2 values	Genotypic combination
0.183	Tori-7 and BARI-12	2.050	GPB-2 and BARI-11
0.227	GPB-9 and GPB-6	2.037	BARI-14 and BARI-11
0.228	Daulat and BARI-10	2.008	GPB-2 and Rai-5
0.236	BARI-9 and Tori-7	1.902	GPB-10 and BARI-11
0.253	BARI-9 and Tori-12	1.786	GPB-10 and Raj-5
0.270	GPB-7 and GPB-6	1.766	GPB-2 and BARI-12
0.344	GPB and GPB-6	1.754	GPB-2 and BARI-9
0.347	GPB-7 and GPB-6	1.752	SS-75 and BARI-11
0.364	GPB-8 and GPB-7	1.742	BARI-14 and Raj-5
0.373	GPB-7 and GPB-7	1.729	GPB-1 and BARI-11

possible to quantify the magnitude of genetic diversity among the germplasm for their evaluation in respect of breeding program. Both analyses are important for assessment of genetic divergence among the parents and the relative contribution of different traits to the total divergence (Jombart, Devillard, & Balloux, 2010; Price et al., 2006). In this study, PCA revealed that eight principal component axes for yield contributing traits accounted for 100% variability among 25 mustard genotypes of which first and second components covered the major part of the total variations. Although, the genotypes were apparently distributed in four groups in the scatter diagram for seed yield and yield contributing traits indicated that there exists considerable diversity among the genotypes.

By using of nonhierarchical clustering applying covariance matrix 25 mustard genotypes for seed yield and yield contributing traits were grouped into four distinct clusters. The intra cluster distance in all the three clusters were more or less low indicated the genotypes within the same cluster were closely related. The inter cluster distance were larger than the

Table 4: Intra cluster mean for eight seed yield and yield contributing traits, and divergence obtained from CVA.

Characters	Clusters				Relative contributions	
	I	II	III	IV	Vector-I	Vector-II
Days to maturity	76.566	89.833	83.401	88.801	-0.202	-0.628
Plant height (cm)	105.201	138.033	110.355	96.366	+0.328	-0.388
Number of primary branches per plant	4.511	3.488	2.855	2.544	+0.394	+0.303
Number of pods per plant	86.099	91.744	49.933	37.155	+0.450	-2.202
Pod length	5.501	4.266	6.411	6.222	-0.376	-0.119
Number of seeds per pod	16.411	11.722	20.333	17.977	-0.255	+0.428
1000 seed weight (g)	1.977	2.222	2.544	2.633	-0.391	-0.284
Seed yield per plant (g)	6.877	7.399	6.177	4.222	+0.363	-0.205

intra cluster distances, which suggested that there were wide genetic diversity among the genotypes of different clusters. On the basis of inter cluster distance it is suggested that crossing among the genotypes between the cluster I & III and II & III would be more useful in creating maximum genetic variability and will provide opportunity to obtain seed yield recombinants.

MATERIALS AND METHODS

Sample Size: Total 25 genotypes of mustard were subjected for this experiment, where 15 genotypes (BARI-6, BARI-7, BARI-8, BARI-9, BARI-10, BARI-11, BARI-12, BARI-13, BARI-14, BARI-15, Daullat, Rai-5, SS-75, Tori-7 and TS-72) were collected from Bangladesh Agricultural Research Institute (BARI) Joydebpur, Gazipur and rest of 10 (GPB-1, GPB-2, GPB-3, GPB-4, GPB-5, GPB-6, GPB-7, GPB-8, GPB-9 and GPB-10) were from and Bangladesh Agricultural University (BAU), Mymensingh.

Experiment condition: The experiment was carried out in the land, which was medium high belonging to the Gangetic alluvial soil tract of AEZ-11 having calcareous dark grey flood plain soil (Rahman, 2010) with pH 8.3 in the time of November to March when average temperature was 21.5°C (Where highest temperature was recorded in March 33.4°C and lowest was in January 12.2°C) and total rainfall recorded in crop growing season was 35.9 mm. Average monthly humidity was recorded between 62.7% to 84.6%.
Urea, triple superphosphate (TSP), murate of potash (MP), gypsum, borax and Manure (cow dung) were used as fertilizer at doses of 30kg/ha, 140 kg/ha, 50 kg/ha, 200 kg/ha, 10 kg/ha and 1000 kg/ha respectively. All fertilizer and 50% urea were applied during final land preparation and remaining 50% urea was applied as top dressing at 25 days after sowing (DAS). The seeds were hand sown in rows on 21st November, about 3-4 cm depth from the soil surface.
Experimental Design: The experiment was laid out in a randomized complete block design (RCBD) with three replications. Each replication was consisted of 25 plots and each of the plots was 2.0 m long with five rows. The spacing was 40 cm between rows and 5 cm between plants in a row. The space maintained between the plots was 80 cm and between the replication was one meter.

Intercultural operations: Three irrigations at 21st, 50th and 75th day were applied during the growing seasons and Hand weeding was done at 15th and 30th days after sowing of seeds. Yellow mustard varieties mature in 80 to 85 days while brown and oriental types require about 90 to 95 days attaining maturity. The crop was harvested when more than 80% siliqua were ripped on 7th February. For collection of data 10 harvested crops were separated and siliqua were dried in sunlight, and then shelled and the seed were cleaned properly. Straw weight was recorded after drying. Seed weight was recorded after 3 days sun drying.

Collection of experimental data: Data were recorded on individual plant basis from 10 randomly selected plants of each genotype from the five rows per plot in each replication. Among the characters studied, days to maturity (when 80% of the plant populations showed complete emergence) were recorded on plot basis and plant height was recorded in the field and the remaining characters (Number of primary branch, Number of pods per plant, Pod length, Number of seeds per pod, Seed weight for 1000 Seeds) were recorded in the laboratory after harvesting. Total seed of 10 sample plants were weighted in gram and average was taken as seed yield per plant.

Data analysis: Standard error of mean, coefficient of variation and Analysis of variance was done for all

characters under study using the mean values. Formula for data analysis are given here according to (Lawal, 2014)

a. $Genotypic\ Variance = \frac{\text{Mean square due to variety} - \text{Mean square due to error}}{\text{Number of replication}}$

b. $Phenotypic\ variance = Genotypic\ variance + Mean\ square\ due\ to\ error$

c. $Genotypic\ \text{coefficient}\ of\ variation\ (GCV\ \%) = \sqrt{\frac{\text{Genotypic variance} \times 100}{\text{Population mean}}}$

d. $Phenotypic\ \text{coefficient}\ of\ variation\ (PCV\ \%) = \sqrt{\frac{\text{Phenotypic variance} \times 100}{\text{Population mean}}}$

e. $Heritability\ (\%) = \frac{\text{Genotypic Variance}}{\text{Phenotypic Variance}} \times 100$

f. $Genetic\ advance\ (GA) = \frac{\text{Genotypic Variance}}{\text{Phenotypic Variance}} \times Selection\ intensity \times Phenotypic\ standard\ deviation$

g. $Genetic\ advance\ as\ percent\ of\ mean\ (GA\ \%) = \frac{\text{Genetic advance}}{\text{Population mean}} \times 100$

h. $Genotypic\ correlation\ \text{coefficient}\ (GCC) = \frac{\text{Genotypic covariance between the variables X1 and X2}}{\sqrt{\text{Genotypic variance of the variable X1} \times \text{Genotypic variance of the variable X2}}}$

i. $Phenotypic\ correlation\ \text{coefficient}\ (GCC) = \frac{\text{Phenotypic covariance between the variables X1 and X2}}{\sqrt{\text{Phenotypic variance of the variable X1} \times \text{Phenotypic variance of the variable X2}}}$

Path coefficient analysis: The components of correlation of different yield contributing traits with seed yield per plant were partitioned into components of direct and indirect effects by path coefficient analysis. In this study, seed yield per plant was considered as dependent character and yield contributing traits were considered as the causal factors. The following sets of simultaneous equations were used depending upon the cause and effect relationship: $r_{xy}=P_{xy}+r_{x2}P_{2y}+r_{x3}P_{3y}+r_{x4}P_{4y}$ .....$+r_{xn}P_{ny}$

Where,

r_{xy} = Correlation coefficient between one component character or causal factor (independent variable-x) to the seed yield (dependent variable-y).

n = 1.2.3.....n

P_{xy} = Path coefficient between the same character and seed yield.

r_{x2} = Correlation coefficient between the same character and one of the remaining yield components in turn.

After calculating the direct and indirect effects of the traits residual effect (R) was calculated as $R^2=1-\sum P_{iy}\ r_{iy}$ (Singh & Chaudhary, 1979) .

Where,

P_{iy} = Direct effect of the traits on seed yield

r_{iy} = Correlation co-efficient of the traits with seed yield.

Genetic Diversity Analysis: Univariate analysis of the individual trait was done for analysis of variance. Significant mean sum squares due to genotypes were estimated for all the seed yield and yield contributing traits by univariate analysis (Sharma, 2006), which would justify for further diversity analysis of mustard germplasm. Data on seed yield per plant and different yield contributing traits recorded in the present investigation was used for diversity analysis. Mean data of all the traits studied were subjected to multivariate analysis by using GENSTAT and Microsoft Excel software through four technique viz., principal component analysis, principal coordinate analysis, cluster analysis and canonical vector analysis did multivariate analysis.

Principal Component Analysis (PCA) is a multivariate technique, used to investigate the interrelationships among several characters and can be done from the sum of squares and products matrix for the characters. The PCA finds out the linear combinations of a set of variate that maximize the variation contained within a group of genotypes. Principal Coordinating Analysis (PCO) is used to calculate inter unit distances which equivalent to the PCA. Cluster Analysis was performed by D^2 analysis, which divides the genotypes based on the data set into more or less homogeneous groups. D^2 is the sum of squares of difference between any two populations for each of the uncorrelated variables which is defined by $D^2_x=\sum^P_i\sum^P_j(\lambda^{ij})d_id_j$

Where,

X = Number of metric traits in point.

P = Number of populations or genotypes.

λ^{ij} = The matrix reciprocal to the common dispersion matrix.

d_id_j = The different between the mean values of the two genotypes for the ith and jth characters respectively.

Canonical Vector Analysis (CVA) is complementary to D^2-statistics is a sort of multivariate analysis where canonical vectors and roots representing different axes of differentiation and the amount of variation accounted for by each of such axes respectively are derived. The average intra cluster distance for each cluster was calculated by taking all possible D^2 values within the members of a cluster obtained from PCO. The formula used to measure the average intra cluster distance was: Intra cluster distance= $\sum D^2/n$; Where, D^2 is the sum of distance between all possible combinations (n) of the genotypes included in a cluster. This cluster diagram represented the pattern of diversity among the genotypes and relationship between different genotypes included in the clusters.

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