

Genetic Variability Studies among Yield and Its Contributing Traits in Mustard (*Brassica napus* L.)

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Abstract Mustard (*Brassica napus*) belongs to the Brassicaceae family and is the major edible oil producing crop of Bangladesh. The country spent a huge amount of foreign currency to import edible oil, which could be minimized through enhancing and improving the existing germplasms. To do this, the genetic variability and diversity of the existing germplasm should be evaluated. Thirty-five genotypes of mustard were evaluated based on randomized complete block design with three replications. The genotypes were found significantly variable for most of the characters. Comparatively phenotypic variances were higher than the genotypic variances for most of the characters studied. The high GCV value was observed for number of seed yield per plant. Seed yield per plant exhibited the highest value of heritability followed by number of siliquae per plant while plant height exhibited the lowest value of heritability. The significant positive correlation with seed yield per plant were found in number of primary branches, siliquae length, number of seed per plant, number of seed per siliquae and thousand seed weight. Path co-efficient analysis revealed that days to maturity, plant height, number of primary branch, siliquae length, number of siliqua per plant, number of seed per siliqua, and thousand seed weight, had direct positive effect on yield per plant. Whereas, days to 50% flowering, length of main raceme and number of secondary branch had direct negative effect on yield per plant. The genotypes were classified in four clusters. The genotypes of cluster IV showed better mean performance while cluster III showed lower mean performance for important component traits including yield per plant. Considering distance and agronomic performance genotypes G23, G5, G22 and G24 might be recommended for future hybridization program.

Keywords Genetic Variability, Heritability, Correlation, Genetic Diversity, Yield, Mustard

1. Introduction

Mustard (*Brassica napus*) belongs to the genus *Brassica* of the family Brassicaceae (former Crucifereae),

is mainly a self-pollinating crop, although on an average 7.5 to 30% out-crossing does occur under natural field conditions [1;2]. *Brassica napus*, *B. campestris* and *B. juncea* are the three species of mustard those produce edible oil. The major mustard producing regions are China, Indian subcontinent, Canada and Northern Europe [3]. The genus *Brassica* has generally been divided into three groups namely- rape seed, mustard and cole crops. The rapeseed group includes the diploid *Brassica napus* L., rape (AACC, $2n=38$) while the mustard groups include species like *Brassica juncea*; *Brassica nigra* and *Brassica carinata* [4]. Rapeseed is a relatively young species that originated in a limited geographic region through spontaneous hybridizations between turnip rape (*B. rapa* L., AA, $2n = 20$) and cabbage (*B. oleracea* L., CC, $2n = 18$) genotypes [5], resulting in an amphidiploid genome comprising the full chromosome complements of its two progenitors.

Globally, mustard is one of the most important oil crops of the world after soybean and groundnut [6]. Mustard oil meets the one third of edible oil requirement of the country [7]. It is not only a high energy food but also a carrier of fat soluble vitamins like A, D, E and K in the body and used as a condiment, salad, green manure, fodder crop, and a leaf and stem as vegetable in the various mustard growing countries.

In Bangladesh mustard occupying 2.88 million ha in 2013-14 and 3.25 million ha in 2014-15 of land and the total production was 2.81 million metric tons and 3.5 million metric tons, respectively [8]. Bangladesh has been facing acute shortage of edible oil for the last several decades. Therefore, we need to import oil and oilseeds to meet up the deficit. Our internal production can meet only about 21% of our consumption and the rest 79% is needed to import [9]. A huge amount of foreign exchange involving over 160 million US\$ is being spent every year for importing edible oils due to insufficient oil production in Bangladesh [10]. In Bangladesh the major mustard growing districts are Comilla, Tangail, Jessore, Faridpur, Pabna, Rajshahi, Dinajpur, Kushtia, Kishoregonj, Rangpur and Dhaka [11]. Although mustard is an

important crop but the cultivation area of this crop is declining due to late harvesting of high yielding Aman rice and increased cultivation of Boro rice and on an average we are losing in an area of 104,000 hectare and production 68,000 tons of mustard and rapeseed in last ten years [11]. Average per hectare yield of mustard in our country is alarmingly low compared to the advanced countries like Germany, France, UK and Canada which producing 6.67 t/ha, 5.07 t/ha, 3.26 t/ha, 3.08 t/ha, respectively [12].

Variety plays an important role in producing high yield of mustard because different varieties varied differently for their genotypic characters. Improved variety is the first and foremost requirement for initiation and accelerated crop production program. High yielding varieties (HYVs) can contribute to get optimum yield as well as the highest percentage of oil. The yields of these cultivars range between 1.4 to 2.1 t/ha [13].

Development of an effective breeding program depends on the existence of genetic variability for various economic characters in the gene pool. Selection is effective only when there is enough magnitude of variability in the breeding population. An understanding of precise magnitude of variability present in a population is important in formulating the most appropriate breeding technique for improvement of various characters. The estimation of character associations helps to identify the relative importance of independent characters contributing to dependent ones and suggest upon the character(s) that may be useful as indicator for one or more of other characters. On the other hands, character associations between yield components can be used as the best guide for successful yield improvement by indirect selection.

The development of an intensive breeding and improvement program needs detailed biological information and an understanding of genetic variation for yield and its components.

There must be a thorough knowledge of the existence of genetic variability, the mode of inheritance of economic characters, heritability, the kind of gene action and the relative magnitude of additive, dominance and total genotypic and phenotypic variances of the population. So in the context of the above mentioned situation, the present piece of work was undertaken to select elite genotypes to utilize as parents for future hybridization programs.

2. Material and Methods

2.1. Plant Materials and Experimental Design

Thirty-five mustard genotypes were used in this study (Table 1). The seeds were collected from the Department of Genetics and Plant Breeding, Sher-e-Bangla Agricultural University (SAU), Dhaka-1207, Bangladesh. The experiment was carried out at the SAU experimental farm following a randomized complete block design (RCBD) with 3 replications. The unit plot was 10 m with 10 rows and lines to line and plant to plant distances were 30 cm and 10 cm, respectively. The recommended fertilizers and cultural practices were done to maintain a healthy crop growth. The harvesting was done separately for each genotype when 80% of plants were mature.

Table 1. List of mustard genotypes used in this study

Genotype	Accession no.	Source	Genotype	Accession no.	Source
G1	NAP 1005	SAU	G19	BARI-13	BARI
G2	NAP 0762	SAU	G20	NAP 0717-2	SAU
G3	NAP 2057	SAU	G21	NAP 1005	SAU
G4	NAP 10012	SAU	G22	NAP 2037	SAU
G5	NAP 9904	SAU	G23	NAP 206	SAU
G6	NAP 10019	SAU	G24	NAP 2037	SAU
G7	NAP 2022	SAU	G25	NAP 0869	SAU
G8	NAP 0733-1	SAU	G26	NAP 10009	SAU
G9	NAP 10020	SAU	G27	NAP 0876	SAU
G10	NAP 08-04	SAU	G28	NAP 2001	SAU
G11	NAP 248	SAU	G29	NAP 2013	SAU
G12	NAP 0865	SAU	G30	NAP 0837	SAU
G13	NAP 9901	SAU	G31	NAP 2066	SAU
G14	NAP 108	SAU	G32	NAP 1007	SAU
G15	NAP 205	SAU	G33	NAP 9905	SAU
G16	NAP 9906	SAU	G34	NAP 179	SAU
G17	NAP 0885	SAU	G35	NAP 10017	SAU
G18	NAP 10014	SAU			

2.2. Data Collection

The data were recorded on 15 randomly selected plants for days to 50% flowering, days to 80% maturity, plant height (cm), length of main raceme (cm), number of primary branches per plant, number of secondary branches per plant, number of siliquae per plant, siliquae length (cm), number of seeds per siliqua, 1000-seed weight (g) and seed yield per plant (g).

2.3. Statistical Analysis

The data were analyzed for different components. Phenotypic and genotypic variance was estimated by the formula used by Johnson et al. [14]. Heritability and genetic advance were measured using the formula given by Singh and Chaudhury [15]. Genotypic and phenotypic co-efficient of variation were calculated by the formula of Burton [16]. Correlation coefficient analysis was done by the formula proposed by Miller et al. [17]. Path coefficient analysis was performed according to the method described by Dewey and Lu [18]. The principal component analysis (PCA), was done by using GENESTAT software program [19]. The heatmaps were created using online heatmap tool (www2.heatmapper.ca).

3. Results and Discussion

3.1. Genetic Variability

The analysis of variance (ANOVA) revealed the existence of significant variations for days to 50% flowering, days to 80% maturity, plant height, siliqua length, number of seeds per siliqua and seed yield per plant among 35 mustard genotypes (Table 2). This suggests that there is an opportunity for the genetic improvement of the mustard for these traits. The higher phenotypic variance

(σ^2_p) compared to the genotypic variance (σ^2_g) for the studied traits indicates the prevalence of environmental effect for the phenotypic expression of these traits (Table 2). The higher σ^2_g was found for plant height, siliqua length, and number of seeds per siliqua (Table 2). The results indicate the presence of high genetic variability for these traits. The high heritability was observed for number of primary branches per plant, number of secondary branches per plant, siliqua length, and number of siliqua per plant, number of seeds per siliqua, 1000-seed weight and seed yield per plant. However, siliqua length, number of siliqua per plant, number of seeds per siliqua, 1000-seed weight and seed yield per plant showed high heritability with high genetic advance in percentage of mean. Thus suggests that these traits under additive genetic control and selection might be effective for these traits.

The heritability estimates together with genetic advance are more authentic for selecting the leading genotype (Jonson et al., 1955). Afrin et al. [20] found low heritability with high genetic advance in percentage of mean for plant height. Khan et al. [21] found high heritability coupled with high genetic advance for number of secondary branches per plant. High heritability was reported by Aytac and Kinaci [22] while Zare and Sharafzadeh [23] found low broad sense heritability for siliqua length in rapeseed. Sadat et al. [24] reported high heritability with high genetic advance for number of siliquae per plant. High heritability for number of seeds per siliqua was observed by Yadava et al. [25]. High heritability along with considerable genetic advance in percentage of mean was reported by Afrin et al. [20] for thousand seed weight. Aytac and Kinaci [22] mentioned the high heritability and genetic advance for seed yield selection for this character would be effective. Rameeh [26] also found high heritability with high genetic advance for seed yield in *B. napus*. L. Rahim et al. [27] reported similar results for number of pods per plant and seed yield per plant in mungbean.

Table 2. Estimation of genetic parameters for 11 characters among 35 genotypes in mustard

Traits	GMS	CV(%)	σ^2_g	σ^2_p	GCV	PCV	h ² b	GA	GA (%)
50F	10.1681**	5.63	1.650	6.867	3.17	6.46	24.033	1.297	3.198
DM	28.6381**	2.41	1.414	5.552	1.41	2.79	25.464	1.236	1.461
PH	79.5323**	7.84	10.478	58.577	3.66	8.65	17.887	2.820	3.188
LMR	1.1775	10.20	0.253	0.672	7.91	12.91	37.573	0.635	9.994
NPB	1.0015	11.87	0.288	0.426	17.08	20.80	67.446	0.907	28.892
NSB	1.0860	18.82	0.303	0.480	24.57	30.94	63.046	0.900	40.184
NSP	0.5371	3.60	0.154	0.229	5.15	6.28	67.185	0.663	8.695
SL	788.1780**	6.60	253.695	280.789	20.20	21.25	90.351	31.188	39.559
NSS	54.4734**	7.37	17.488	19.497	21.75	22.96	89.698	8.159	42.428
TSW	1.2403	1.79	0.412	0.416	19.70	19.78	89.698	8.159	42.428
SYP	9.7191**	2.05	3.234	3.251	28.28	28.35	99.474	3.695	58.094

GMS = Genotypic mean sum of Square, CV (%) = Coefficient of variation, σ^2_g = Genotypic variance, σ^2_p = Phenotypic variance, 50F = Days to 50% flowering, DM = Days to 80% maturity, PH = Plant height (cm), LMR = Length of main raceme, NPB = Number of primary branches per plant, NSB = Number of secondary branches per plant, NSP = Number of siliqua per plant, NSS = Number of seed per siliqua, SL = Siliqua length, TSW = Thousand seed weight (g), SYP = Seed yield per plant, GCV = Genotypic coefficient of variation and PCV = Phenotypic coefficient of variation.

3.2. Association Studies

Yield is a complex trait and controlled by polygene and very often influenced by environment. Therefore, phenotypic selection based only on yield is not effective. Correlation and path co-efficient helps the way to select plant for breeding purpose by the plant breeders.

Genotypic and phenotypic correlation coefficients among 11 characters are presented in Table 3 and Figure 1. In most instances, there was a close agreement between genetic correlations and phenotypic correlations. The genotypic correlations were higher than phenotypic correlations for the studied traits. Seed yield had a highly significant positive correlation with number of primary branches per plant, siliqua length, number of siliqua per plant, number of seeds per siliqua, 1000-seed weight and seed yield per plant at both the genotypic and phenotypic level. Among these, number of siliqua per plant, 1000-seed weight and seed yield per plant highly significant strong positive correlation with seed yield at both levels suggests the high degree of association between these traits. Nasim et al. [28] reported positive correlation between pod length and 1000- seed weight. Malik et al. [29] and Jeromela et al. [30] reported positive correlation between siliqua per plant and seed yield. Rameeh [26] reported that number of seeds per siliqua had positively correlated with seed yield per plant. Hakim [31] mentioned that yield had significant positive correlation with number of pods per plant in mungbean. As genetic correlation coefficient was higher than phenotypic correlation coefficient, it reveals that the

apparent association of two characters is mainly due to genetic effects.

The direct and indirect effects of yield contributing traits on seed yield were analyzed by path analysis. Seed yield per plant was considered as effect (dependent variable) while remaining traits were treated as causes (independent variables) and shown in Table 4. The results showed that number of siliqua per plant had maximum direct effect followed by number of seeds per siliqua on seed yield. Number of siliqua per plant had a positive indirect effect to all the traits and finally this trait had a highly significant positive genotypic correlation with yield per plant. Similarly, number of seeds per siliqua had a positive indirect via most of the traits except for days to 50% flowering, showed a positive correlation with seed yield per plant. These results indicate the correlation is mainly due to the direct effect of a character and it was realized via indirect positive and negative effects. It reveals the true relationship between them and direct selection for this trait will be rewarding for the genetic improvement of yield. Sharafi et al. [32] found the number of siliqua per plant had the highest direct effect on seed yield. They also reported that number of seeds per siliqua had direct positive effect on yield per plant. On the contrary, a negative direct effect for seeds per siliqua on seed yield per plant has been reported by Basalma [33]. The value of residual effect was 0.211. It indicated that beside the component characters, there was an influence of some other attributes (approx. 21.1%) on seed yield.

Table 3. Genotypic (r_g) and phenotype (r_p) correlation coefficients among different pairs of yield and yield contributing characters for different genotype of mustard

Traits		DM	PH	LMR	NPB	NSB	SL	NSP	NSS	TSW	SYP
50F	r_g	0.9086**	-0.699**	0.2882	0.2196	-0.2006	0.1297	-0.1106	0.0765	-0.1296	-0.2101
	r_p	0.899**	-0.2857	0.1186	0.1022	-0.1382	0.0364	-0.0645	0.065	-0.0925	-0.153
DM	r_g		-0.4506**	0.3861*	0.1357	-0.3238	0.0968	-0.1666	0.1561	-0.0563	-0.1599
	r_p		-0.2672	0.1288	0.0669	-0.2195	0.0303	-0.0998	0.1249	-0.0404	-0.1213
PH	r_g			0.241	-0.0604	-0.035	0.2399	0.1057	0.0762	0.22	0.2484
	r_p			0.2209	-0.0708	-0.007	0.1443	0.0689	0.0241	0.1348	0.1545
LMR	r_g				0.1072	-0.3068	0.3697*	0.0288	0.0473	0.0142	-0.0592
	r_p				0.0641	-0.2041	0.2454	0.0058	0.0194	0.0035	-0.0465
NPB	r_g					-0.3373*	0.5153**	0.7626**	0.491**	0.7858**	0.7067**
	r_p					-0.2479	0.4473**	0.6941**	0.478**	0.7296**	0.6579**
NSB	r_g						-0.5432**	-0.3322	-0.1738	-0.1249	-0.1871
	r_p						-0.4734**	-0.2895	-0.1441	-0.1183	-0.1711
SL	r_g							0.5144**	0.3202	0.3477*	0.3791*
	r_p							0.464**	0.2859	0.3262	0.3551*
NSP	r_g								0.6719**	0.8481**	0.8643**
	r_p								0.6512**	0.8324**	0.8493**
NSS	r_g									0.6898**	0.7705**
	r_p									0.6771**	0.7568**
TSW	r_g										0.9448**
	r_p										0.9426**

** = Significant at 1%; * = Significant at 5%.

50F= Days to 50% flowering, DM= Days to 80% maturity, PH= Plant height (cm), LMR= Length of main raceme, NPB= Number of primary branches per plant, NSB= Number of secondary branches per plant, NSP= Number of siliqua per plant, NSS= Number of seed per siliqua, SL= Siliqua length, TSW= Thousand seed weight (g) and SYP= Seed yield per plant.

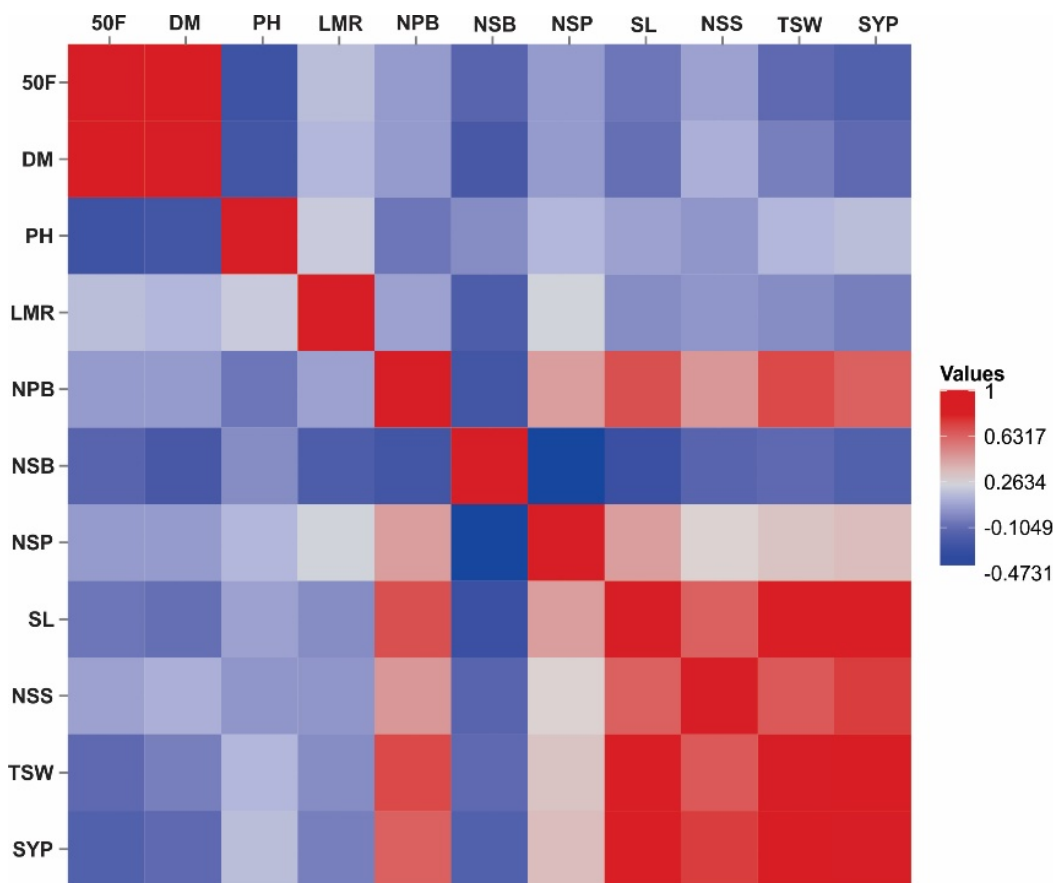


Figure 1. Correlation matrix showing correlation between different traits of mustard. The red and blues colors in the legend indicates highest and lowest values, respectively. 50F= Days to 50% flowering, DM= Days to 80% maturity, PH= Plant height (cm), LMR= Length of main

Table 4. Path coefficient analysis showing direct and indirect effects of different characters on yield of mustard

Traits	Direct effect	Indirect effect										r _g with Yield
		50F	DM	PH	LMR	NPB	NSB	SL	NSP	NSS	TSW	
50F	-0.171		0.021	-0.014	-0.010	-0.010	-0.010	-0.010	-0.015	0.016	-0.008	-0.210
DM	0.028	-0.152		-0.017	-0.005	-0.007	-0.006	-0.007	-0.022	0.033	-0.004	-0.160
PH	0.102	0.023	0.011		0.008	0.005	0.015	0.015	0.046	0.011	0.014	0.248
LMR	-0.040	-0.001	-0.005	0.014		-0.001	-0.002	-0.003	-0.013	-0.010	0.003	-0.059
NPB	0.124	0.013	0.013	0.005	0.012		0.013	0.012	0.317	0.181	0.018	0.707**
NSB	-0.008	0.012	-0.009	-0.004	0.000	-0.018		-0.003	-0.122	-0.040	0.004	-0.187
SL	0.003	0.012	0.005	0.014	0.001	0.045	0.008		0.207	0.090	-0.006	0.379*
NSP	0.520	0.006	0.003	0.010	0.005	0.070	0.005	0.003		0.227	0.014	0.864**
NSS	0.369	-0.007	0.008	0.003	0.005	0.055	0.004	0.003	0.319		0.011	0.771**
TSW	0.050	0.119	0.111	0.114	0.116	0.102	0.113	0.112	0.035	0.072		0.945**

Residual Effect = 0.211

**, * indicates significant at the 0.01 and 0.05 level, respectively. 50F = Days to 50% flowering, DM = Days to 80% maturity, PH= Plant height (cm), LMR= Length of main raceme, NPB= Number of primary branches per plant, NSB= Number of secondary branches per plant, NSP= Number of siliqua per plant, NSS= Number of seed per siliqua, SL= Siliqua length and TSW= Thousand seed weight (g).

3.3. Selection of Elite Genotypes

We further performed principal component analysis with 35 mustard genotypes for 11 yield and its component traits. The results showed eigen values of principal component axes of coordination of genotypes with the first axes totally accounted for the variation among the genotypes (38.75). These three principal components account for 71.84% of the total variation (Table 5). Zaman et al. [34] reported that first three axes accounted for 94.00% of the total variation whereas the first principal components accounted for 81.94%. We performed a cluster analysis using 35 mustard genotypes in respect of 11 traits using online tool (<http://www2.heatmapper.ca>). The result showed that 35 mustard genotypes were into four clusters (Table 5 and Figure 2). The maximum 12 genotypes were included in cluster III while only four for cluster IV (Table 6). The genotypes belong to cluster IV (G10, G24, G30 and G33) showed better performance for number of primary branches per plant, number of siliqua per plant, siliqua length, number of seeds per siliqua, 1000-seed weight and seed yield per plant (Figure 2). Among the genotypes of cluster IV, G24 had highest mean values for number of primary branches per plant, number of siliqua per plant, siliqua length, and seed yield per plant among all 35 mustard genotypes (Figure 2). Furthermore, the genotypes of cluster IV were distantly related among other clusters (Figure 3). The genetically diverse parent selection is one

of the key activities for a successful hybridization program [35].

Table 5. Eigen values and yield percent contribution of 11 characters of 35 genotypes of mustard

Traits	Eigen values	Percent variation	Cumulative % of percent variation
50F	4.262	38.75	38.75
DM	2.207	20.06	58.81
PH	1.434	13.03	71.84
LMR	0.959	8.71	80.55
NPB	0.669	6.08	86.63
NSB	0.514	4.68	91.31
SL	0.445	4.04	95.35
NSP	0.236	2.15	97.50
NSS	0.172	1.57	99.07
TSW	0.072	0.66	99.73
SYP	0.029	0.27	100.00

Table 6. Distribution of genotypes in different clusters

Cluster	Number of population	Genotypes
I	9	G1, G3, G7, G9, G11, G13, G14, G16 and G29
II	10	G2, G4, G15, G17, 18, G20, G26, G32, G34 and G35
III	12	G5, G6, G8, G12, G19, G21, G22, G23, G25, G27, G28 and G31
IV	4	G10, G24, G30 and G33

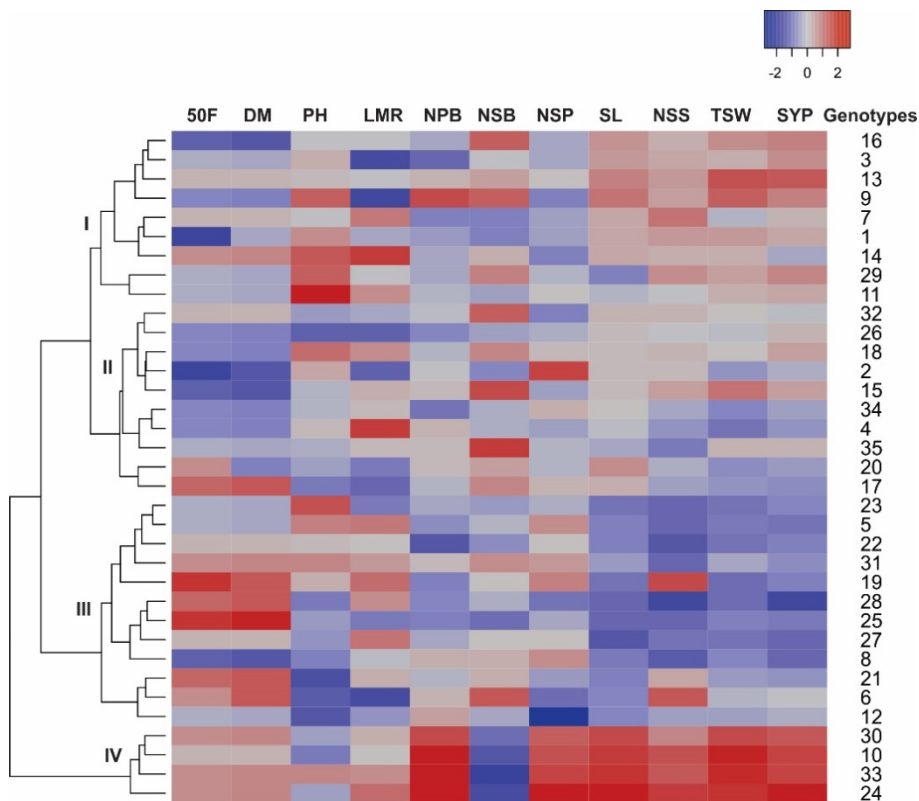


Figure 2. Heatmap representation of 35 mustard genotypes into four clusters in respect of 11 traits. The red and blue colors in the legend indicates highest and lowest values, respectively. 50F= Days to 50% flowering, DM= Days to 80% maturity, PH= Plant height (cm), LMR= Length of main raceme, NPB= Number of primary branches per plant, NSB= Number of secondary branches per plant, NSP= Number of siliqua per plant, NSS= Number of seed per siliqua, SL= Siliqua length, TSW= Thousand seed weight (g) and SYP= Seed yield per plant

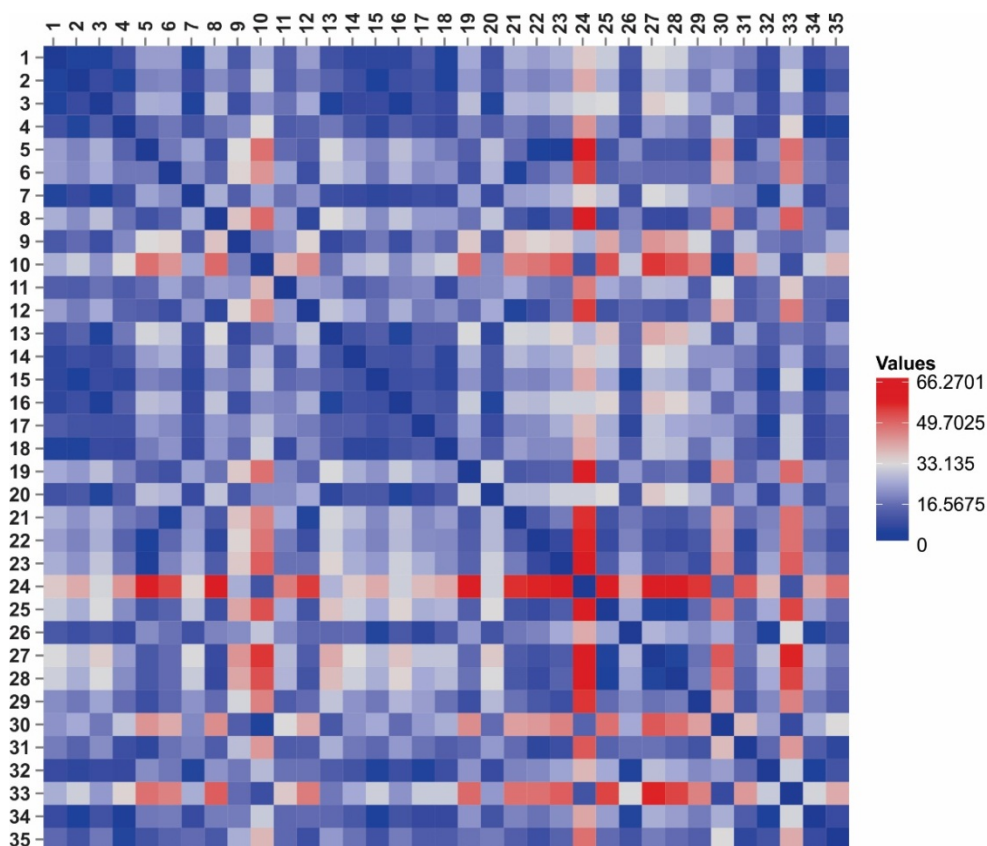


Figure 3. Distance matrix represents the pairwise comparisons between mustard genotypes in respect of 11 traits. The red and blues colors in the legend indicates highest and lowest values, respectively. 50F= Days to 50% flowering, DM= Days to 80% maturity, PH= Plant height (cm), LMR= Length of main raceme, NPB= Number of primary branches per plant, NSB= Number of secondary branches per plant, NSP= Number of siliqua per plant, NSS= Number of seed per siliqua, SL= Siliqua length, TSW= Thousand seed weight (g) and SYP= Seed yield per plant

4. Conclusions

Considering genetic parameters, interrelationships among major yield contributing traits, agronomic performance, genotypic distance, it can be concluded that the genotype belong to the cluster II and IV might product high heterosis. Therefore, genotypes G23, G5, G22 and G24 could be recommended for future hybridization program to develop new cultivars with higher yield and other agronomic performance.

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