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RESEARCH ARTICLE

Evaluation of mustard genotypes [*Brassica juncea* (L.) Czern and Coss] for quantitative traits and character association of seed yield and yield components at sub Himalayan region of West Bengal (India)

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Abstract

Brassica juncea is an important industrial and commercial oilseed crop grown primarily in India. This study aimed to assess 56 genotypes of Indian mustard to quantify genetic diversity, which aids the breeder in identifying genetically divergent parents to evaluate the proportional contributions of various components towards overall divergence. All the 56 Indian mustard genotypes were tested in RBD with three replications for 2 consecutive years *i.e.* 2016-17 and 2017-18 during the *rabi* season. Observations were recorded for 11 yield and its attributing traits. The findings revealed that height up to first branching, aphid count, penetration force and seed yield per plant had maximum PCV and GCV signifying that genetic factors have a greater impact on the inflow of these traits. Height up to first branching, secondary branches per plant, primary branches per plant, siliquae per plant, aphid count and 1000 seed weight had strong heritability combined with GA as % of mean. These indicate that the traits were controlled by additive gene action. Seed yield per plant was significantly correlated with penetration force and siliquae per plant. As a result, it's reasonable to predict that improving these traits by selection, could lead to significant yield gains. Four of the eleven PCs had eigen values greater than 1.0, accounting for 69.94% of the variance. PC I, which explained 30.31% of the overall variance. Mahalanobis D^2 statistics revealed considerable genetic diversity among the genotypes. 56 genotypes were distributed into 7 clusters. This is anticipated that genotypes within a cluster are almost genetically related to one another. Cluster VII and II showed maximum inter-cluster divergence. From a breeding perspective, a divergence analysis revealed that genotypes like SKJM-05, RNWR-09-3, RW-351, B-85, DRMR-4001, RGN-386, TM52 276 and SKM-1313 can be selected as genetically divergent parents for hybridization to obtain desirable segregants.

Keywords

Brassica juncea, Genetic parameters, Path analysis, D2 statistics, Principal component analysis

Introduction

Brassica juncea (L.) Czern and Coss often known as Indian mustard, is a species of the Cruciferae of plants. It is an amphidiploid (2n = 36; AABB) species

that emerged from a cross between the monogenic diploid species *B. campestris* (L.) Koch (2n = 20, AA) and *B. nigra* (L.) (2n = 16, BB) (1). The plant species is thought to be one of the oldest cultivated species. Over the last few decades, these crops have risen to prominence as one of the world's major proponents of vegetable oil. Improvement in rapeseed-mustard technologies has created a nutritionally superior edible oil and meal that may be used as a protein source in livestock feed. Each year, *B. juncea* spreads across Asia, Africa, Australia, North America, and Europe as a wild and domesticated species (2). Despite the fact that it is grown throughout the country, seven states, namely Haryana, Rajasthan, Gujarat, Uttar Pradesh, Madhya Pradesh, West Bengal and Assam, account for more than 90% of its output and acreage. These crops are cultivated in West Bengal in the districts of Murshidabad, Nadia, Dinajpur, Malda, Birbhum South and North Parganas. Oil, vegetables, sauces, and fodder are all made from *B. juncea*. The seed oil content ranges from 38 to 46 % and is made up of unsaturated fatty acids (3). Pickles are made using seeds and oil and curries and vegetables are flavoured with it. Whole seeds, ground or crushed seeds, pastes, seasonings and oil are all used in cooking. Sulphur-containing glucosinolates give mustards their pungent fragrance and flavour. The protein-rich oil cake is primarily used as feedstuffs for animals (4). *B. juncea* and related species have around 50 insect pests, with the mustard aphid (*Lipaphis erysimi*) being one of the most damaging. Seed yields are reduced by 9 to 96 % and oil content is lowered by up to 10 % as a result of the insect (5).

Genetic variability is required for effective screening and breeding improvements in yield and its attributes. The level of phenotypic and genotypic variability in germplasm resources has an impact on the manifestation of economic features and even the responsiveness to selection (6). The genetic makeup, environmental factors, and genotype-by-environment relationships all contribute to the phenotypic variation of quantitative traits (7). As a result, the amount of heritability and sensitivity to the selection of a trait is impacted by the extent of phenotypic variance. Previous research has found more variability in seed yield and yield-related variables (8).

Heritability is calculated as the ratio of genetic variance (VG) to overall phenotypic variance (VP), and breeders use it to calculate the response to selection and measure the effect to which desired traits are passed down across different generations. Furthermore, the estimates alone are not adequate. Heritability does not show the likelihood of a phenotype being passed down, but rather what % of a phenotype can be attributable to genotype rather than the environment (9). As a result, heritability combined with genetic advancements is more reliable in predicting the genetic addition under selection (10). Simple correlation and path coefficient analyses have been frequently utilized to assess trait relationships and help genotype selection for desirable economic attributes (11). The direct and indirect effects of one or more causative variables on a response variable are differentiated using path coefficient analysis. The most significant predictor variable(s) on dependent variables can be determined via path analysis (12). Path analysis studies in Indian mustard reported that 1000 seed weight had positive direct effect on seed yield plant^{-1} indicating the importance of this trait for selection of high seed yielding genotypes (13).

Multivariate analysis is a valuable technique for determining the degree of genotypic difference between biological populations (14). The genetic diversity of plant populations is assessed using a variety of measures. Of these measures, multivariate analysis (15) gives the most accurate information of these metrics. The PCA analysis is a data reduction technique for analysing interdependence that seeks to minimize diverse and complex associations between collections of measured traits by revealing similar characteristics or elements that interconnect independent variables (16). Previously multivariate analysis in mustard was studied by (17). In their work, (18) used 33 genotypes to produce 5 groups and while evaluating 31 *Brassica juncea* genotypes for the purpose of choosing genetically diverse parents using Mahalanobis D^2 analysis.

Indian mustard is perhaps one of the most important oil crops in North Bengal. Nevertheless, its production efficiency in Bengal seems to have been low. As a result, understanding the scope and frequency of mustard yield performance assessment is crucial for future crop improvement. Therefore, the purpose of this study was to assess seed yield and its attributing traits in 56 genotypes of Indian mustard in order to delineate genotypes into different clusters for selection of genetically divergent parents for hybridization programme as well as to determine the proportional significance of several variables for crop improvement.

Materials and Methods

The experimental trial was designed at Instructional Farm, UBKV, Pundibari, Cooch Behar, West Bengal, for 2 consecutive years i.e. 2016-17 and 2017-18 during the *rabi* season. The farm was situated at 26^o 19’ 86” N latitude, 89^o 23’ 53” E longitude with an altitude of 43 m above the mean sea level. The research site's topsoil was sandy loam, which is representative of West Bengal's *Terai* region. The experimental site, which is situated in a humid subtropical climate, is just south of the cancer tropic. The seasons are divided into 3 categories: warm and dry (March-May), warm and humid (June-September), and cool and dry (November-February).

In 3 replications, the experiment was performed using 56 genotypes of Indian mustard in a RCBD design. The list of genotype was mentioned in (Table 1). Genotypes were sown in 3 rows, each measuring 3 m, in each plot. The row-row spacing was kept at 30 cm, while the plant-to-plant gap was managed at 15 cm, by appropriate thinning. All inter-cultural practices required for a productive mustard crop were applied to achieve a robust and competitive crop stand. The field was treated to fine topsoil before sowing. The fertilizer was applied as a basal dose of 60: 40: 40 kg/ha of Nitrogen, Phosphorus and Potash with half of the N applied as a top dressing subse-

quently. Irrigation was supplied on a need-by basis and intercultural operations like thinning and weeding were carried out as required.

Table 1. List of mustard genotypes

Sl No.	Genotype	Denotation
1	B-85(Seeta)	G1
2	RW-351(Bhagarathi)	G2
3	RW-85-59 (Sarna)	G3
4	RW-4C-6-3 (Sanjukta Asech)	G4
5	NPJ-194	G5
6	TM-276	G6
7	Rohini (SC)	G7
8	KMR-15-4	G8
9	PR-2012-9	G9
10	Divya-88	G10
11	RL-JEB-52	G11
12	Kranti-NC	G12
13	DRMRIJ-15-85	G13
14	RH1202	G14
15	NPJ-196	G15
16	RMM-09-10	G16
17	JMM-927-RC	G17
18	RRN-871	G18
19	KM-126	G19
20	SKM-1313	G20
21	RB-77	G21
22	DRMR-15-5	G22
23	KMR-53-3	G23
24	RL-JEB-84	G24
25	Ganga	G25
26	RGN-73-JC	G26
27	RH-1209	G27
28	PR-2012-12	G28
29	RGN-385	G29
30.	NPJ-195	G30
31.	Maya-C	G31
32.	SKJM-05	G32
33.	SVJ-64	G33
34.	Sitara-Sreenagar	G34
35.	RH-0923	G35
36.	DRMR-15-16	G36
37.	NPJ-198	G37
38.	JMM-927-RC	G38
39.	DRMR-15-47	G39
40.	RGN-389	G40
41.	RAURD-214	G41
42.	DRMR-15-14	G42
43.	DRMR-4001	G43
44.	RGN-384	G44
45.	NPJ-197	G45
46.	RB-81	G46
47.	NPJ-200	G47
48.	DRMR-15-9	G48
49.	KMR-L-15-6	G49
50.	PRD-2013-9	G50
51.	DRMRIJ-15-66	G51
52.	RH-1368	G52
53.	RH-1325	G53
54.	RGN-386	G54
55.	RNWR-09-3	G55
56.	PRD-2013-2	G56

Source of germplasm is Pulses and Oilseed Research Station (PORS) and Banaras Hindu University (BHU)

Data collection was done by following the below mentioned steps

1. In each replication, 5 plants were chosen at random from 3 rows for each genotype.
2. For easy identification, plants were properly tagged, while border plants were left untagged.
3. Five tagged plants from each genotype in each replication were used to obtain data.

Days to 50% flowering [FLOW] was collected on a plot basis from each replication as the number of days from when the seeds were sown to when 50% of the plants flowered in each genotype. Plant height [PH] (cm) was measured at maturity in cm from the base to the tip of the plant. Height up to first branching [HFFB] (cm) is the main stem which was measured from the ground surface to the very first siliqua-yielding branch (in cm). Primary branches per plant [PBP] was counted, at maturity, the first order of branches sprouting from each selected plant's main stem and the aggregate was used to calculate the primary branches per plant. Secondary branches per plant [SBP] were noted at maturity, the secondary branches coming from the primary branches and the average of 5 plants was recorded as the secondary branches per plant. Siliquae per plant [SP] were counted, at maturity, in each of the 5 plants chosen and the mean of 5 plant siliquae was recorded as siliquae per plant. Five siliquae were picked from each plant and seeds were counted, with the average noted as seeds per siliqua [SD_SIL]. Aphid populations were counted on a 10-cm twig of the central stalk from 5 randomly selected plants in each replication at 7-day intervals at morning hours *i.e.* at 7-9 am and were recorded as aphid count [AC]. 1000 seed weight [SW] (g) from the bulk yield were counted at random from the bulk yield and weighed in gramme (g) using an electronic balance with 2 decimal places. Seed yield per plant [SY_P] (g) were measured from 5 matured plants' total siliquae, which were threshed, sun dried and cleaned seeds were weighed. The average of

these weights was used to compute the seed production per plant in grammes. Penetration force [PF] (Kpascal) was used to assess penetrating force. Six apical twigs of 10 cm were selected randomly out of each replication. The positive and negative pressures were measured using a Texture Analyser according to the methodology. The positive pressure was estimated and expressed in several graphs in the system connected to the Texture analyser. Positive pressure is the amount of force required to penetrate the resistant plant twig's tissue. The aphid applies pressure to the twig while puncturing it.

Statistical analysis

For statistical analysis, the average data from each replication was used. To establish the importance of variance among genotypes, the data was examined using a randomized block design for several genotypes (treatments). The methodology for evaluating the randomized complete block design had been developed (19). Analysis of variance (ANOVA) was estimated by agricolae package of R software (20). The genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) were computed following (21) method. Recommendations are on GCV and PCV range of high (>25%), moderate (10-25%) and low (10%) (22). Heritability in broad sense h^2 (B) was calculated as a ratio of genotypic variance to total of phenotypic variance (23). As per one study, heritability into three categories: low (<30%), medium (30 to 60%) and high (60%) (24). As proposed by (23), the predicted genetic advancement under selection for several traits was computed in (1960). Genetic parameters were computed for traits across years by using variability package of R software (25). The association between different attributes was assessed as correlation (between two variables). Association among all quantitative traits was estimated using the Pearson's correlation coefficients in R software using metan package (26). The association between traits was visualized using corrplot package (27). The path-coefficient study was carried out using (28) technique, which was later extended (29). It was calculated by using variability package of R software (25). Genetic diversity in 56 genotypes was assessed using Mahalanobis D^2 statistics following (30). The clustering pattern was proposed (31). The hierarchical clustering was performed by using the package dendextend in R software (32). Principal components with eigen values greater than one were investigated by using the package FactoMineR (33). The original collection of variables can be transformed into a new set of uncorrelated variables known as principle components. Factoextra (34) package in R programme was used to create biplots of quantitative and qualitative variables and individuals, as well as the dendrogram.

Computation of Rescaled index Value

According to one report, rescaling index approach, an overall ranking of 56 mustard genotypes was performed for eleven traits, of which ten are yield contributing traits and one was the aphid population (35). Two ways of rescaling were used to normalize index value depending on the relationship of characters with the main factor (seed yield). The standardization was performed using the formula when the observed values were favourably connected to the primary factor like yield.

$$y_{id} = (X_{id} - \min X_{id})/(\max X_{id} - \min X_{id})$$

and when the values of X_{id} are negatively related to the main factor seed yield, the standardized values would be computed by

$$y_{id} = (\max X_{id} - X_{id})/(\max X_{id} - \min X_{id})$$

where, Min X_{id} and Max X_{id} are the minimum and maximum of ($X_{i1}, X_{i2}, \ldots\ldots X_{in}$) respectively.

The reason behind utilizing rescaled index value is to rank the genotype on the basis of the positive and negative traits. The positive traits are those in which higher value is desirable like in the present study PH, HFFB, FLOW etc. The negative traits those for which the lowest value is desirable like lower aphid population is more desirable than higher population. To include both positive and negative traits in the overall ranking of genotypes required the transformation of data to rescaled index value. In case of Positive traits, Rescaled index value would be 1 for the best genotype with highest performance and it would be 0 for the poorest performing genotype for any given positive traits. The genotype with superior performance would always show higher index value. However, in case of negative trait like aphid population, in the present study, the genotype with lowest population would exhibit rescaled index value of 1 and genotype with highest aphid population exhibit a rescaled index value of 0. Two different formulas are used to make the rescaled index value independent of the kind of traits i.e. whether it is positive or negative. Finally, the addition of given genotype for all the traits under study, the genotype with maximum index value would be ranked 1 depending on its rescaled index value for individual traits.

Results and Discussion

Individual years were analysed using the analysis of variance, as well as a pooled analysis by combining both the years (Table 2). Individual genotypes vary considerably for all the yield contributing traits in the both the years. PH, HFFB, FLOW, PBP, SW and PF were all significantly differing in the pooled analysis. SBP, SP, SD_SIL, AC and SY_P did not differ substantially, despite these parameters differing significantly in individual years. Except for SW, the interaction of years and genotypes differed significantly for other traits.

General mean, range and genetic parameters for the 11 traits are presented in the (Table 3) and violin plot showing the distribution for 11 yield attributing traits in Indian mustard are shown in (Fig. 1). The highest and lowest ranges for PH were 207.25 cm and 132.37 cm respectively, with a grand mean value of (179.06); 91.67 cm and 25.77 cm for HFFB respectively, with a grand mean value of

Table 2. ANOVA for 11 traits in Indian mustard genotypes over two years

Year	Sources of variation	d.f.	Mean Sum of Square										
			PH	HFFB	FLOW	PBP	SBP	SP	SPS	SW	AC	PF	SY_P
First Year	Replication	2	46.11	357.54	0.00	0.27*	0.16	5105.66*	0.56	0.16	36.08	137.02	18.81*
	Genotypes	55	814.39**	1257.05**	29.61**	0.39**	0.61**	2833.58**	7.67**	1.19**	258.16**	641.50**	15.90**
	Error	110	72.07	195.67	0.94	0.051	0.25	758.44	1.59	0.25	15.25	265.61	3.15
	Total	167	316.24	547.17	10.37	0.164	0.37	1493.93	3.58	0.56	95.50	387.86	7.54
Second Year	Replication	2	3.32	14.87	0.08	1.06*	6.33*	1478.10*	0.31	1.05**	7.77	413.40	80.00**
	Genotypes	55	687.34**	589.49**	55.43**	1.55**	9.03**	4137.98**	1.54*	0.74**	35.54*	1978.07**	12.94**
	Error	110	2.89	16.55	0.86	0.20	0.88	286.68	0.83	0.02	19.56	432.48	6.17
	Total	167	228.31	205.22	18.82	0.65	3.63	1569.34	1.06	0.27	24.68	941.28	9.29
Pooled	Replication	4	24.72	186.21	0.04	0.66**	3.24**	3291.88**	0.43	0.61*	21.93	275.21	49.41**
	Years (Y)	1	24293.00**	22544.28**	379.31**	330.01**	3248.52**	274588.08**	117.62**	0.13	4315.34**	70039.49**	874.65**
	Genotypes	55	1300.82**	1399.14**	59.02*	1.74**	4.99	2966.37	3.56	1.65**	153.50	1687.21*	13.11
	Y × G	55	200.91	447.41	26.02	0.19	4.65	4005.19	5.65	0.27	140.21	932.36	15.72

PH= Plant height (cm), **HFFB**=Height up to first fruiting branch (cm), **FLOW**=Days to 50% flowering, **PBP**=Primary branches per plant, **SBP**= Secondary branches per plant, **SP**= Siliqua per plant, **SPS**=Seeds per siliqua, **SW**=1000 seed weight (g), **AC**=Aphid count, **PF**=Penetration force and **SY_P**=Seed yield per plant (g)

*, ** Significant at 5% and 1% levels of probability, respectively

Table 3. Mean, range and genetic parameters for 11traits in Indian mustard

S. No.	Parameters/Trait	Mean	Range		GCV (%)	PCV (%)	Heritability % (broad sense)	Genetic advance as percent of
			Lowest	Highest				
1	PH	179.06	132.37	207.25	8.09	8.46	91.53	15.96
2	HFFB	65.22	25.77	91.67	22.44	25.23	79.12	41.13
3	FLOW	46.88	39.00	54.00	6.59	6.88	91.84	13.01
4	PBP	2.97	1.75	4.20	16.87	20.33	68.85	28.85
5	SBP	6.85	4.17	9.17	12.54	14.74	72.44	21.99
6	SP	146.22	89.83	194.80	13.77	17.72	60.48	22.08
7	SPS	12.36	10.40	14.10	5.13	7.98	41.42	6.81
8	SW	4.60	3.20	5.73	10.93	12.28	79.25	20.05
9	AC	12.99	4.76	23.58	36.72	42.97	73.02	64.64
10	PF	73.36	43.63	129.88	20.30	27.26	55.46	31.14
11	SY_P	8.19	5.32	14.22	14.47	23.61	37.61	18.29

PH= Plant height (cm), **HFFB**=Height up to first fruiting branch (cm), **FLOW**=Days to 50% flowering, **PBP**=Primary branches per plant, **SBP**= Secondary branches per plant, **SP**= Siliqua per plant, **SPS**=Seeds per siliqua, **SW**=1000 seed weight (g), **AC**=Aphid count, **PF**=Penetration force and **SY_P**=Seed yield per plant (g)

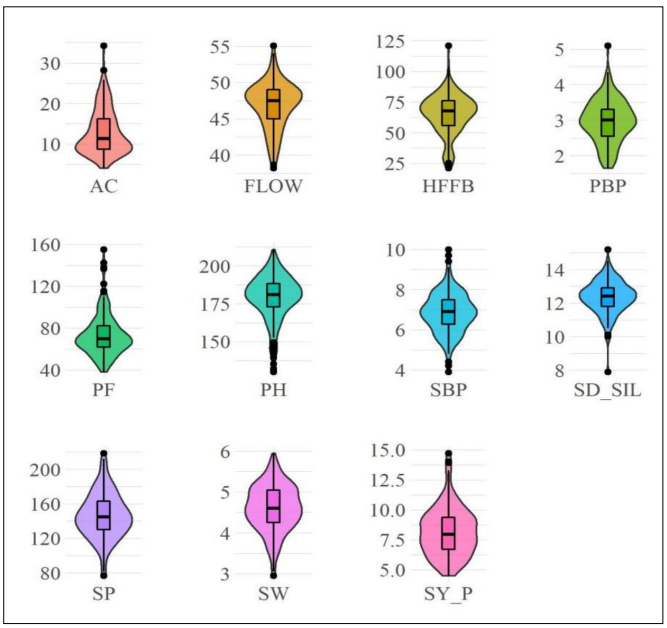

Fig. 1. Violin plot for eleven yield attributing traits in Indian mustard.

(65.22); 54 days and 39 days for FLOW respectively, with a grand mean value of (46.88); 4.20 and 1.75 for PBP respectively, with a grand mean value of (2.97); 9.17 and 4.17 for SBP respectively, with grand mean of (6.85); 194.80 and 89.83 for SP with grand mean of (146.22);14.10 and 10.40 for SPS respectively, with grand mean of (12.36); 5.73 and 3.20 for SW respectively, with grand mean of(23.58); 4.76 and 12.99 for AC respectively, with a grand mean value of (12.99); 129.88 and 43.63 for PF respectively, with a grand mean value of (73.36) and 14.22 g and 5.32 g for SY_P, respectively, with a grand mean value of (8.19) respectively. The significance of genotypic variance determines whether there is enough variability among mustard genotypes for certain qualities to exist. As a result, selecting superior parental types has a significant possibility of enhancing seed yield and its attributing traits.

For PH (8.46 and 8.09), FLOW (6.88 and 6.59) and SD_SIL (7.98 and 5.13) the PCV and GCV were both low

(less than 10%). Moderate PCV and GCV (10-25%) were observed for PBP (20.33 and 16.87), SBP (14.74 and 12.54), SP (17.72 and 13.77) and SW (12.28 and 10.93). High PCV and GCV (>25%) were observed for HFFB (25.23 and 22.44), AC (42.97 and 36.72), PF (27.26 and 20.30) whereas SY_P showed high PCV (23.61) and moderate GCV (14.47). The genetic parameters for the 11 variables evaluated in this study indicated that HFFB, AC, PF and SY_P had maximum PCV and GCV (>25%). This is in agreement with (36) for HFFB and (37) reports for AC and PF. The PCV and GCV understudies for the various traits didn't vary significantly, signifying that genetic factors have a greater impact on the inflow of these traits than environmental variables.

The broad sense heritability for the 11 traits under investigation as displayed in (Table 3). PH (91.53), HFFB (79.12), FLOW (91.84), PBP (68.85), SBP (72.44) and SP (60.48), SW (79.25) and AC (73.02) all have high heritability. SD_SIL (41.42), PF (55.46) and SY_P (37.61) was the attributes that exhibiting moderate heritability. Heritability is used in crop improvement to determine the worth of selection for specific economic features. PH, HFFB, FLOW, PBP, SBP and SP have high heritability, indicating that the environment has little to no impact on the expression among these traits. This means that a high degree of connection between genotypic and phenotypic variation, and hence greater susceptibility to selection, is associated with high heritability. While evaluating 21 different genotypes of yellow sarson, (38) found high heritability for all traits, which include DF, PH, NPB, NSB, LMR, NSMR, LS, NSS, 1000 -SW and SYP; (39) observed high heritability for HFFB.

The estimations of genetic advance were divided into three categories: strong genetic advance greater than 20%, moderate genetic advance between 10-20% and low genetic advance less than 10 %. Except for PH (15.96%), FLOW (13.01%) and SY_P (18.29), which had modest genetic advance as a % of mean and SD_SIL (6.81%), which had the least genetic advance as a % of mean. Any trait with a high heritability (h^2) and a high genetic advancement as % of the mean is under additive gene control and improving such attributes is advantageous. The traits HFFB, PBP, SBP, SP, SW and AC exhibited high heritability and genetic advance as a % of mean in the current investigation (40) for SW and (41) for primary branches per plant in Indian mustard germplasm collection showed comparable outcomes.

Evaluations of genotypic correlation coefficient among the 11 yield attributing traits in Indian mustard were presented in (Table 4) and (Fig. 2). In the genotypic correlation study, PH was found to have a positive association with HFFB (0.906), FLOW (0.725) and SW (0.406). FLOW (0.762) and SW (0.453) demonstrated a favourable relationship with HFFB. Only one attribute SW was positively correlated with FLOW (0.438). PBP was positively associated with SBP (0.436) and AC (0.367) which meant that there was greater infestation on plant which had higher PBP. SBP had positive association with only SP (0.432) and on the other hand SP was positively associated with PF (0.354) and SY_P (0.259). The PF was positively linked with SY_P (0.312). Overall, the trait association study revealed

Fig. 2. Genotypic correlation coefficient among the eleven yield attributing traits in Indian mustard.

that 2 traits, SP (0.259) and PF (0.312), showed a significant correlation with SY_P. In this study, genetic correlation analysis found that two variables, SP and PF, had a positive relationship with SY_P, implying that improving these two features will result in a higher SY_P. A significant and positive correlation between seed yield and the siliquae per plant was reported (42). In Indian mustard genotypes, the attributes like PH (-0.275), HFFB (-0.381) and FLOW (-0.351) were shown to be negatively linked with SY_P, indicating that reducing these traits will directly lead to an increase in SY_P.

The path analysis was estimated among the 11 traits in the present research (Table 5). Using path analysis and treating seed yield as an effect and the other variables as causes, the correlation coefficients from the association study were partitioned into direct and indirect effects of yield attributing variables towards seed yield. In the path coefficient analysis, PH and SP had a strong direct effect on SY_P, but they also exhibited a positive association with SY_P.PH (-0.275) had a negative significant relationship with SY_P, with a positive direct effect (0.378). The direct effect was greater than the correlation, implying a negative indirect effect of PH via other traits such as HFFB (-0.588), FLOW (-0.149), PBP (-0.003), SD SIL (-0.003), and PF (-0.027). Correlation of SP (0.259) with SY_P was positive and its direct effect was also positive (0.208) but direct effect was lesser than coefficient of correlation. The indirect positive effect of SY_P was observed via PH (0.005), HFFB (0.087), FLOW (0.036), AC (0.020) and PF (0.018). The HFFB (-0.381) had a negative correlation with SY_P, and it had a negative direct influence (-0.650). The correlation was greater than the direct effect. This was due to the trait's indirect negative impact on FLOW (-0.156), SP (-0.038), SD_SIL (-0.001) and PF (-0.023). Similarly, FLOW (-0.351) had negative association with SY_P and its direct effect is also negative (-0.204) although its direct effect is

Table 4. Evaluations of genotypic correlation coefficient among the 11 yield attributing traits in Indian mustard

Trait	HFFB	FLOW	PBP	SBP	SP	SPS	SW	AC	PF	SY_P
PH	0.906**	0.725**	0.034	-0.172	0.015	-0.038	0.406**	-0.326***	-0.538**	-0.275**
HFFB		0.762**	-0.009	-0.281**	-0.133	-0.020	0.453**	-0.278**	-0.450**	-0.381**
FLOW			-0.158	-0.268*	-0.177	-0.045	0.438**	-0.348**	-0.641**	-0.351**
PBP				0.436**	0.177	-0.355**	-0.242*	0.397**	-0.175	-0.167
SBP					0.432**	-0.421**	0.043	0.044	0.034	0.039
SP						-0.055	-0.221	-0.264*	0.354**	0.259*
SPS							0.028	-0.030	0.058	0.186
SW								-0.147	-0.294**	-0.090
AC									0.010	-0.069
PF										0.312**

* = Significant at 5% probability level, ** = Significant at 1% probability level

PH= Plant height (cm), **HFFB**=Height up to first fruiting branch (cm), **FLOW**=Days to 50% flowering, **PBP**=Primary branches per plant, **SBP**= Secondary branches per plant, **SP**= Siliqua per plant, **SPS**=Seeds per siliqua, **SW**=1000 seed weight (g), **AC**=Aphid count, **PF**=Penetration force and **SY_P**=Seed yield per plant (g)

Table 5. Genotypic path coefficient analysis showing the direct (diagonal) and indirect (off-diagonal) effects of ten yield attributing traits on the seed yield in mustard

Traits	PH	HFFB	FLOW	PBP	SBP	SP	SPS	SW	AC	PF	Correlation with SY_P
PH	**0.378**	-0.588	-0.149	-0.003	0.024	0.003	-0.003	0.066	0.025	-0.027	-0.275**
HFFB	0.342	**-0.650**	-0.156	0.001	0.039	-0.038	-0.001	0.073	0.021	-0.023	-0.381**
FLOW	0.274	-0.495	**-0.204**	0.013	0.037	-0.037	-0.004	0.070	0.027	-0.033	-0.351**
PBP	0.013	0.006	0.032	**-0.083**	-0.061	0.037	-0.032	-0.039	-0.031	-0.009	-0.167
SBP	-0.065	0.183	0.055	-0.036	**-0.140**	0.090	-0.039	-0.007	-0.003	0.002	0.039
SP	0.005	0.087	0.036	-0.015	-0.060	**0.208**	-0.005	-0.036	0.020	0.018	0.259*
SPS	-0.014	0.013	0.009	0.029	0.059	-0.012	**0.092**	0.005	0.002	0.003	0.186
SW	0.154	-0.294	-0.090	0.020	-0.006	-0.046	0.003	**0.162**	0.011	-0.015	-0.090
AC	-0.123	0.180	0.071	-0.033	-0.006	-0.055	-0.003	-0.024	**-0.077**	0.001	-0.069
PF	-0.203	0.292	0.131	0.014	-0.005	0.074	0.005	-0.048	-0.001	**0.051**	0.312**

PH= Plant height (cm), **HFFB**=Height up to first fruiting branch (cm), **FLOW**=Days to 50% flowering, **PBP**=Primary branches per plant, **SBP**= Secondary branches per plant, **SP**= Siliqua per plant, **SPS**=Seeds per siliqua, **SW**=1000 seed weight (g), **AC**=Aphid count, **PF**=Penetration force and **SY_P**=Seed yield per plant (g)
* = Significant at 5% probability level, ** = Significant at 1% probability level, Residual effect= 0.69

greater than correlation value. The association between PF (0.312) and SY_P was positive, as was the direct effect (0.051), however, the correlation coefficient was greater than the direct effect. This means that the positive indirect effect of PF is supplemented by HFFB (0.292), FLOW (0.131), PBP (0.014), SP (0.074) and SD_SIL (0.005), with the HFFB having the greatest via effect. So, any improvement in HFFB would support the PF which has a positive association with SY_P. The highest direct negative effect was of AC followed by PBP. The AC (-0.069) had no association with seed yield per plant but however the positive association of penetration force (0.312) with SY_P proved that genotypes with stronger twigs required greater PF. Aphid infestation alone was not a detrimental factor for reducing SY_P. Hence the association with the other attributing traits of SY_P played a significant influence in the expression of this trait. The high residual effect of 0.69 indicated that the 10 yield attributing traits included in this research were unable to explain the total variation in seed yield. Perhaps more yield attributing traits need to be faber up to account for the most of the variation in seed yield. Unlike the current findings (43) stated positive and maximum direct effects on PH and SP on seed yield.

All 56 mustard genotypes were divided into seven clusters based on D^2 analyses. (Table 6) shows the clustering pattern and the distribution of genotypes into distinct clusters. Eighteen genotypes of Indian mustard fall under cluster VI [JMM-927-RC, RRN-871, KM-126, SKM-1313, RB-77, DRMR-15-5, KMR-53-3, RL-JEB-84, Ganga, RGN-73-JC, RH-1209, PR-2012-12, RGN-385, NPJ-195, Maya-C, SVJ-64, JMM-927-RC and DRMRIJ-15-66] followed by 12 genotypes in cluster IV [TM-276, KMR-15-4, RL-JEB-52, Kranti-NC, DRMRIJ-15-85, RH1202, NPJ-196, RMM-09-10,DRMR-15-47, RGN-389, RAURD-214 and DRMR-15-14]; 8 genotypes in cluster V [PR-2012-9, Sitara-Sreenagar, RH-0923, RGN-384,

Table 6. Distribution of 56 Indian mustard genotypes into different clusters

Cluster No.	Total no. of germplasm accessions	Source	Name of germplasm accessions
I	6	Pulses and Oilseed Research Station (PORS) and Banaras Hindu University (BHU)	B-85(Seeta), RW-4C-6-3(SanjuktaAsech), Divya-88, NPJ-200, DRMR-15-9 and RH-1368
II	3		RW-351(Bhagarathi), DRMR-15-16 and PRD-2013-2
III	7		RW-85-59(Sarna), NPJ-194,Rohini(SC), NPJ-198, DRMR-4001, RB-81 and KMR-L-15-6
IV	12		TM-276, KMR-15-4, RL-JEB-52, Kranti-NC, DRMRIJ-15-85, RH1202, NPJ-196, RMM-09-10,DRMR-15-47, RGN-389, RAURD-214 and DRMR-15-14
V	8		PR-2012-9, Sitara-Sreenagar, RH-0923, RGN-384, NPJ-197, PRD-2013-9, RH-1325 and RGN-386
VI	18		JMM-927-RC, RRN-871, KM-126, SKM-1313, RB-77, DRMR-15-5, KMR-53-3, RL -JEB-84, Ganga, RGN-73-JC, RH-1209, PR-2012-12, RGN-385, NPJ-195, Maya -C, SVJ-64, JMM-927-RC and DRMRIJ-15-66
VII	2		SKJM-05 and RNWR-09-3

NPJ-197, PRD-2013-9, RH-1325 and RGN-386]; 7 genotypes in cluster III [RW-85-59 (Sarna), NPJ-194, Rohini (SC), NPJ-198, DRMR-4001, RB-81 and KMR-L-15-6]. Six genotypes were found in cluster I [B-85 (Seeta), RW-4C-6-3, Divya-88, NPJ-200, DRMR-15-9, and RH-1368]; 3 genotypes were observed in cluster II [RW-351 (Bhagarathi), DRMR-15-16 and PRD-2013-2]; and two genotypes were seen in cluster VII [SKJM-05 and RNWR-09-3]. All 56 mustard genotypes were divided into 7 clusters based on D^2 analysis indicating that the clustering pattern of these genotypes discovered that germplasm accrued from the same source can be classified into diverse clusters. As genotypes from the same geographical area were assembled into multiple clusters, it was revealed that geographic distribution is not the only criterion to associate with genetic divergence. This is more in agreement with previous reports when examining 46 Indian mustard germplasm accessions, (44) reported seven clusters, and (18) identified five clusters in their experiment using 33 genotypes. A dendrogram depicting the genetic distance between the 56 Indian mustard genotypes is shown in (Fig. 3).

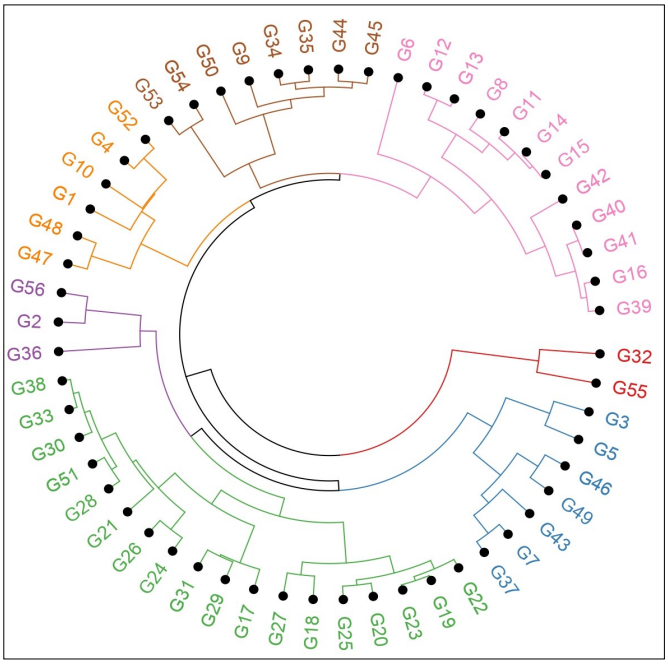

Fig. 3. Dendrogram showing the clustering of 56 Indian mustard genotype.

The average intra-cluster and inter-cluster distance values (D^2 values) were calculated and are presented in the (Table 7). Cluster VII (13.85) had the highest average intra-cluster divergence value, followed by cluster II (12.96), cluster III (9.69), cluster I (8.79), cluster V (7.17), cluster IV (6.85), and cluster VI (6.85). Cluster VII and II (49.50) recorded the highest inter-cluster D^2 value followed by cluster VII and I (44.69), cluster VII and III (38.97), cluster VII and V (38.85), cluster VII and cluster IV (38.23), cluster VII and VI (36.93), cluster II and I (32.27), cluster III and cluster II (30.58), cluster V and II (28.91), cluster IV and II (28.49), cluster III and I (27.14), cluster VI and II (26.31), cluster V and I (24.59), cluster V and III (24.52), cluster IV and III (23.19), cluster VI and I (23.04), cluster IV and I (22.80), cluster IV and III (22.07), cluster V and IV (19.65), cluster VI and V (19.44) and cluster VI and IV (17.97).

Maximum inter-cluster D^2 value was recorded among cluster VII and II (49. 50) followed by cluster VII and I. Comparable confirming results were previously achieved by (45) in *Brassica rapa* and (46) in *Brassica juncea*. The genotypes belonging to the highest inter clusters may be selected for a breeding programme. Hybrids between genotypes from these clusters will express high heterosis and produce more valuable segregants.

The cluster mean values for 11 traits were calculated and displayed in (Table 9). Based on cluster mean analysis, the highest cluster mean value for SP (160.59), SD_SIL (13.13), PF (88.29) and SY_P (11.22) were recorded in cluster II, whereas lowest mean value for SP (132.38) and SD_SIL (11.55) was observed in cluster III. The highest PH and HFFB genotypes were represented in cluster IV with recorded mean of (193.83) and (77.12) respectively. The shortest mean PH of (160.20) was observed in cluster II. Early flowering (49.81) and PBP (3.63) was recorded highest in cluster V, whereas late flowering (42.00) and short HFFB (43.58) was observed in cluster I. Lowermost number of AC was observed in cluster VI (10.08), whereas maximum AC was seen in cluster III having mean value of (18.91). SW showed highest mean value (8.87) in cluster VII. Similar results obtained by (47) for most of the traits mentioned above.

In this paper, the contribution and expression of

Table 7. Average intra and inter-cluster D^2 values of Indian mustard genotypes

Cluster	I	II	III	IV	V	VI	VII
I	**8.79**	32.27	27.14	22.80	24.59	23.04	44.69
II		**12.96**	30.58	28.49	28.91	26.31	49.50
III			**9.69**	23.19	24.52	22.07	38.97
IV				**6.85**	19.65	17.97	38.23
V					**7.17**	19.44	38.85
VI						**6.76**	36.93
VII							**13.85**

Table 8. Description of the genetically divergent clusters and distance (D^2 value) between the genotypes selected

Cluster combination	Inter cluster distance (D^2 value)	Genotype selected from the cluster	Distance between the genotype selected (D^2 value)
Cluster VII and cluster II	49.50	SKJM-05 in cluster VII andRW-351 in cluster II	54.55
Cluster VII and cluster I	44.69	RNWR-09-3 in cluster VII and B-85 in cluster I	56.09
Cluster VII and cluster III	38.97	SKJM-05 in cluster VII and DRMR-4001 in cluster III	51.24
Cluster VII and cluster V	38.85	SKJM-05 in cluster VII and RGN-386 in cluster V	66.63
Cluster VII and cluster IV	38.23	SKJM-05 in cluster VII and TM-276 in cluster IV	67.16
Cluster VII and cluster VI	36.93	SKJM-05 in cluster VII and SKM-1313 in cluster VI	54.44

Table 9. Cluster means for 11traits of Indian mustard genotypes and their contribution towards divergence

Cluster	PH	HFFB	FLOW	PBP	SBP	SP	SPS	SW	AC	PF	SY_P
I	169.60	43.58	42.00	3.34	7.77	156.01	12.79	4.14	13.60	83.68	8.86
II	160.20	50.26	43.83	2.95	6.43	160.59	13.13	3.95	18.86	88.29	11.22
III	166.34	58.31	43.64	3.11	7.09	132.38	11.55	4.78	18.91	74.44	7.78
IV	193.83	77.12	48.13	3.01	6.69	157.45	12.03	4.78	11.06	79.68	8.46
V	186.23	72.90	49.81	3.63	7.21	135.43	12.10	4.67	14.42	56.48	7.62
VI	178.31	66.68	47.89	2.47	6.45	142.74	12.74	4.66	10.08	72.01	7.71
VII	169.80	61.62	49.25	3.13	6.97	150.88	12.50	8.87	13.80	58.04	8.18
Percent Contribution	22.30	8.60	21.80	6.80	6.60	5.10	3.40	10.40	8.20	3.50	3.40

PH= Plant height (cm), **HFFB**=Height up to first fruiting branch (cm), **FLOW**=Days to 50% flowering, **PBP**=Primary branches per plant, **SBP**= Secondary branches per plant, **SP**= Siliqua per plant, **SPS**=Seeds per siliqua, **SW**=1000 seed weight (g), **AC**=Aphid count, **PF**=Penetration force and **SY_P**=Seed yield per plant (g)

various traits studied in relation to genetic divergence are described in (Table 9). The table showed that PH contributed the most to divergence (22.30%), followed by FLOW (21.80%), SW (10.40%), HFFB (8.60%), AC (8.20 %), PBP (6.80 %), SBP (6.60%), SP (5.10%), PF (3.50%), SD_SIL (3.40%), and SY_P (3.40%). As a result, in addition to picking genotypes for hybridization from clusters with a greater inter-cluster distance, one may consider selecting parents based on the degree of divergence.

Description of the genetically divergent clusters and distance (D^2 value) between the genotypes selected were presented in (Table 8). On minute assessment of distance it was exhibited that SKJM-05 in cluster VII and RW-351 in cluster II had a very high genotypic distance (D^2 = 54.55). Similar findings with large genetic distance between genotypes were found in other clusters, such as RNWR-09-3 in cluster VII and B-85 in cluster I (D^2 =56.09). SKJM-05in cluster VII and DRMR-4001 in cluster III had considerable genotypic distance (D^2 =51.24). SKJM-05 in cluster VII and RGN-386 in cluster V both had a high genotypic distance (D^2 = 66.63), followed by SKJM-05 in cluster VII andTM-276 in cluster IV (D^2 = 67.16). Cluster VII's SKJM-05 and Cluster VI's SKM-1313 have a high genotypic distance (D^2 = 54.44). Hence, on the basis of the higher inter cluster distance value, the crosses could be made among the genotypes of cluster VII and II (SKJM-05 and RW-351), cluster VII and I (RNWR-09-3 and B-85), cluster VII and III(SKJM-05 and DRMR-4001), cluster VII and V (SKJM-05 and RGN-386), cluster VII and IV (SKJM-05 and TM-276) and Cluster VII and VI (SKJM-05 and SKM-1313) as per their D^2 values for expecting better segregants. This clearly shows that the genotypes found in these clusters have a wide range of genetic diversity and might be exploited in a hybridization programme to improve seed yield. As a result, it would make sense to try crosses between genotypes from the aforementioned groupings. Furthermore, one or more genotypes from these clusters can be chosen for advanced genetic investigations employing diallel or line × tester analysis.

PCA was done on yield and its attributing traits in 56 genotypes of Indian mustard in this study. Table 10 shows the proportion of variance, cumulative proportion and

Table 10. Proportion of variance, cumulative proportion and eigen values of Indian mustard genotypes

	Eigen Value	Proportion of Variance (%)	Cumulative proportion of Variance (%)
PC1	3.33	30.31	30.31
PC2	1.79	16.31	46.62
PC3	1.50	13.67	60.28
PC4	1.06	9.65	69.94
PC5	0.90	8.18	78.11

Table 11. Eleven principal components along with their factor's loadings

Traits	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7	PC 8	PC 9	PC 10	PC11
PH	0.462	-0.118	0.201	-0.202	0.267	-0.075	0.102	-0.135	0.170	0.302	-0.682
HFFB	0.480	-0.056	0.067	-0.200	0.192	0.051	0.253	0.068	0.367	0.164	0.672
FLOW	0.493	-0.020	0.073	0.087	-0.061	-0.025	0.041	-0.169	-0.117	-0.832	-0.047
PBP	-0.075	-0.584	-0.148	-0.032	0.438	0.060	-0.008	0.615	-0.141	-0.183	-0.058
SBP	-0.194	-0.513	0.256	0.183	-0.137	0.113	-0.358	-0.250	0.605	-0.107	0.012
SP	-0.152	-0.203	0.631	0.005	0.217	0.266	0.176	-0.313	-0.505	0.081	0.170
SPS	0.006	0.472	-0.004	0.162	0.585	0.457	-0.417	-0.006	0.138	-0.086	-0.014
SW	0.193	-0.027	-0.021	0.810	-0.152	0.284	0.359	0.174	0.034	0.184	-0.092
AC	-0.185	-0.185	-0.573	0.081	0.362	-0.018	0.344	-0.585	0.041	-0.044	0.014
PF	-0.361	0.203	0.165	-0.293	-0.065	0.335	0.567	0.173	0.358	-0.287	-0.193
SY_P	-0.211	0.195	0.328	0.321	0.361	-0.708	0.148	0.082	0.178	-0.109	0.046

PH= Plant height (cm), **HFFB**=Height up to first fruiting branch (cm), **FLOW**=Days to 50% flowering, **PBP**=Primary branches per plant, **SBP**= Secondary branches per plant, **SP**= Siliqua per plant, **SPS**=Seeds per siliqua, **SW**=1000 seed weight (g), **AC**=Aphid count, **PF**=Penetration force and **SY_P**=Seed yield per plant (g)

eigen values. Four of the eleven PCs had eigen values greater than 1.0 and total variability of 69.94 % across the examined variables. PC1 was responsible for 30.31% of the overall variation, whereas PC2, PC3 and PC4 were responsible for 16.31%, 13.67% and 9.65% of the total variance respectively. PCA reduces data dimensionality by altering original variables into a new compact collection of variables while retaining the vital information of the original variables. PCA is a potent tool in current data analysis since it is a well-known multivariate technique for determining the lowest number of components that may describe the most variability out of the total, as well as ranking genotypes based on PC scores. Four of the 11 PCs had more than 1.0 eigen values and PC1 contributed maximum percent of the total variation. For the selection of the diverse parents, the principal components with more than one eigen value exhibited increased variability among the Indian mustard genotypes. It was found that 4 of the nine PCs studied in 67 aromatic rice germplasm accessions had more than 1.0 eigen values and had 70.14 % overall variability among the characters studied (48).

A scree plot (Fig. 4), which was created by constructing a graph between eigen values and PC numbers, was used to explain the % of variation connected with each PC. In the current study, PC1 revealed 30.31% variability with an eigen value of 3.33, which rapidly fell after that. A semi-curve line was created after PC4 which tended to become straight, with little fluctuation in each PC. In comparison to the other PCs, the scree plot graph clearly shows that PC1 would have the maximum variation. As a result, identifying the lines from this PC for genetic improvement programmes would be favourable. These findings are in accordance with the earlier findings of (49) representing the distribution of variance among the principle components.

(Table 11) shows 11 principal components along with their factor's loadings. The results showed that HFFB had maximum positive value (0.493) followed by FLOW (0.480) in PC1. SD_SIL recorded maximum positive value (0.472) followed by PF (0.203) in PC2 whereas SP showed high value (0.631) followed by SY_P (0.328). Both negative and positive loading values of distinct characteristics revealed the presence of positive and negative correlation patterns between the components and variables. As a result, the characters that loaded favourably contributed the most to diversity, and they were the ones that distinguished the clusters the most.

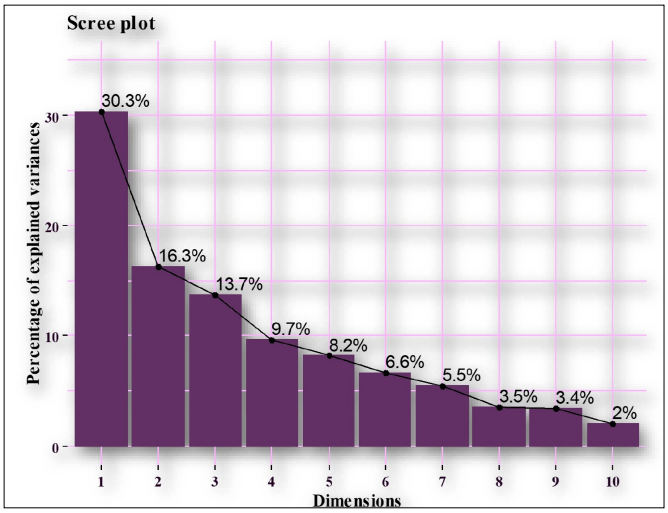

Fig. 4. Scree plot showing percentage of variance connected with various PC.

The first 2 PCs (PC1 and PC2) were strategized against each other in a biplot to examine the relationship between the Indian mustard genotypes based on yield and its attributing trait in the current research (Fig. 5). B-8 (Seeta), PR-2012-9, Kranti-NC, DRMRIJ-15-85, RH1202, NPJ -196, Ganga, Sitara-Sreenagar, RH-0923, RGN-389, RGN-384, NPJ-200, RB-81, PRD-2013-9, RH-1325, RGN-386 and

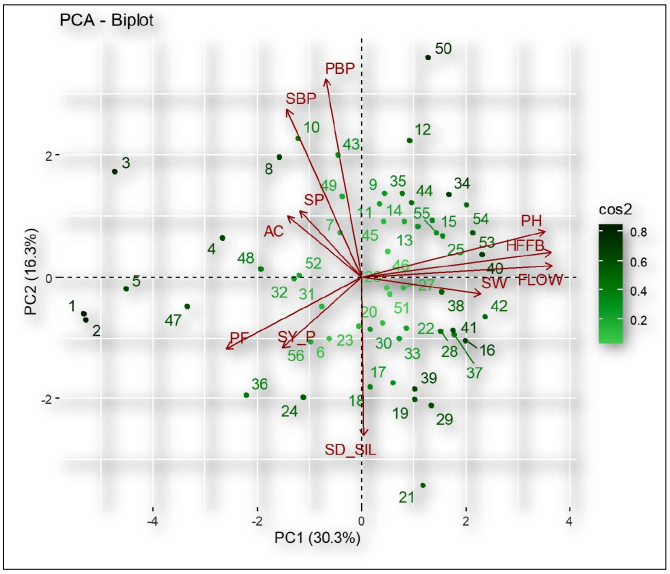

Fig. 5. Biplot among 11 traits and 56 genotypes in Indian mustard.

RNWR-09-3 designed a group in the right upper corner of the biplot with positive values for both the PCs and the traits PH, HFFB and FLOW were all placed in the same quadrant and influenced the SY_P. Contribution of different yield attributing traits to significant PC was showed in corrplot (Fig. 6). PH, HFFB, FLOW and PF were all associated with PC I, which explained 30.31% of the overall variance. PC II accounted for 16.31% of the overall variance and was primarily associated with the attributes PBP and SBP. On the other side, PC III explained 13.67% of the overall variation and was primarily driven by the traits SP and AC. PC IV was shown to be linked with variables such as SD_SIL and explained 9.65% of the total variation. This means that any improvement in these traits will lead to a significant improvement in seed yield. According to [51], the PCA-I is responsible for 23.35% of overall variability. The first principal component was highly influenced by silique length (0.073), silique length/width ratio (0.224), and shattering % (0.506). In *Helianthus annuus* genotypes, (50) found that factors like seed yield, days to maturity, plant height, leaf number, reproductive phase and total biomass contributed more to variation in the first principal component.

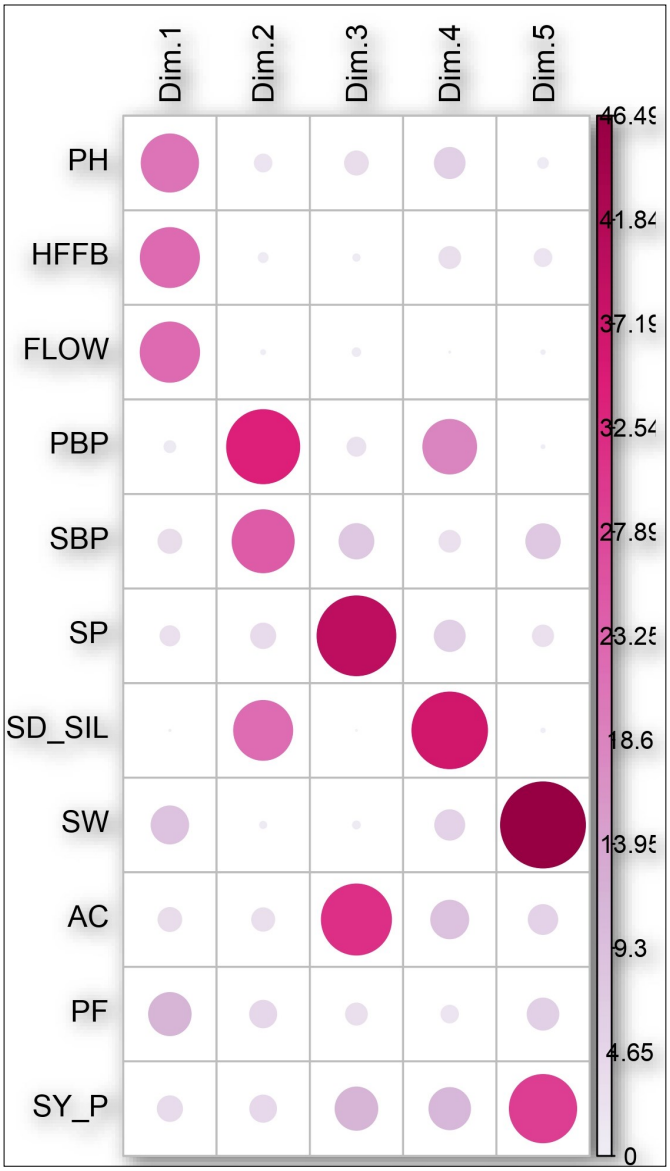

Fig. 6. Contribution of different yield attributing traits to significant PC.

According to rescaling index approach, an aggregate ranking of 56 mustard genotypes was carried out for 11traits (Supplementary Table 1), nine of these were yield contributing traits and the other 2 were AC and PF. From the RIV the ranking was done for 56 genotypes. It confirmed that based on all the 11traits including AC, the genotype RH1202 was the best and had the maximum RIV of 7.03 and ranked first followed by the genotypes NPJ-196 having RIV of 6.09 (rank-2) which was closely followed by another genotype with RIV of JMM-927-RC (rank 3). In this method, all 56 genotypes could be ranked based on their RIV, and the best of them might be chosen. Similarly (48) ranked 71 genotypes of Indian mustard and observed genotype PRD-2013-9 ranked first was among all the genotype studied.

Conclusion

A high variability was evident among the 56 genotypes of Indian mustard as the interaction between years and genotypes varies significantly. High heritability and genetic advance as a % of mean was observed for HFFB, PBP, SBP, SP, SW and AC implied that they are under additive gene control and improved through appropriate selection. Improvement in traits like SP and PF may result in an increase in SY_P as they showed favorable association with SY_P. PH, SP, SD_SIL, SW and PF had a positive direct effect on yield, revealing that direct selection for these traits can enhance the productivity. The PC1 component contributed the maximum % of overall variation, while another 3 principal components, PC2, PC3 and PC4, contributed less. If a suitable crossing programme is carried out using the eight most genetically divergent mustard genotypes identified on the basis of the inter cluster distance, intra cluster distance and D2 distance between the individual genotypes, namely SKJM-05, RNWR-09-3, RW-351, B-85, DRMR-4001, RGN-386, TM-276 and SKM-1313, a proper follow-up of this experiment would be justified.

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Authors contributions

The research was conceptualized and designed by SKR and MC; BS conducted the field experiments; MKD and SS did formal analysis; SR and RM drafted the manuscript; SN, BM, SS and LH edited the manuscript. The findings were discussed and the manuscript was written by all of the authors. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

Supplementary data

Supplementary Table 1.

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