COMPARATIVE ASSESSMENT OF GENETIC DIVERSITY BETWEEN *BRASSICA NAPUS* AND *BRASSICA JUNCEA* BASED ON PHENOTYPIC TRAITS

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SUMMARY

Rapeseed and mustard crops' extensive promotion for crop diversification and their potential to favorably respond to breeding programs depends on their existing nature and the magnitude of genetic variability. Therefore, the progressive research sought to estimate the genetic diversity of 40 genotypes of *Brassica napus* (20) and *Brassica juncea* (20) based on phenotypic characters. The material, grown in randomized complete block design, had three replications during winter 2020–2021. The mean square from analysis of variance demonstrated significant differences for all the parameters among examined varieties, indicating that utilized material contained sufficient genetic variability. Regarding average performance, two mustard genotypes, Dhoom-I and Anmol Raya, performed better for seed yield plant^{-1} and can undergo assessment in upcoming breeding programs for enhancing seed yield. Meanwhile, high heritability occurred in oil content, silique length, 1000-seed index weight, and plant height. Diversity analysis, cluster mean, and total divergence contribution revealed maximum differences for various traits and possessed noteworthy sources for future breeding programs. The magnitude of cluster distance indicated that cluster VI comprised two mustard genotypes (Early Raya and Sindh Raya) and cluster IV consisted of three rapeseed genotypes (Rohi Sarson, hyola-401, and Kn-277), demonstrating that maximum distance is more diverse and helpful for upcoming heterotic recombination. Comparing genetic variation between rapeseed and mustard genotypes showed that rapeseed genotypes displayed more genetic variability in the first three components of PCA than its counterpart, yet expressing that mustard genotypes also have enormous valuable genetic resources. Similarly, genotypes Rohi Sarson, hyola-401, and Kn-277 resulted in the highest genotypic scores in three-dimensional graphs; hence, these genotypes are more diverse and can benefit future hybridization programs.

INTRODUCTION

Rapeseed (*Brassica napus* L.*)* and mustard (*Brassica juncea* L.) are major oil-contributing crops with rich protein and oil content globally, including Pakistan, ranking third in importing edible oil, after machinery and other petroleum products. Almost 80% of the edible oil requirement comes from importing from other countries, with a vast amount of foreign exchange spent yearly. Moreover, edible oil production is less than 20% of the country's requirement (Ahmad *et al*., 2013; Aftab *et al*., 2019).

A plant breeder's main aim is to enhance the seed yield, as it is the resultant product of different yield-contributing traits, which affect it directly or indirectly. Therefore, it is essential to monitor the contribution of each characteristic to pay attention to those traits having maximum influence on yield features (Tabassum *et al*., 2015). Moreover, identifying a more diverse parent from existing genotypes and selecting an appropriate parent is essential before starting any crop improvement program (Neeru *et al*., 2015).

Exploring the genetic diversity of oil seed crops at a large scale and using those genotypes in breeding programs is the best way to develop high-yielding oil seed genotypes to increase the production and reduce edible oil imports from different countries. The main component for developing high-yielding oil crop varieties is partitioning phenotypic variability into genotypic (heritable) and environmental (non-heritable) components. Plant breeders always expect the minimum influence of environmental factors from total variation among the genetic material, with the impact of the environment calculated through heritability. Moreover, the seed yield, the focal trait of interest, is very complex because of its dependence on the number of morphological characteristics controlled by genes and environmental factors. Therefore, dividing overall variability into heritable and its non-heritable components favors the breeders to adopt the appropriate and suitable breeding procedure to improve further the available genetic stocks (Choudhary *et al*., 2015; Prasad and Patil, 2018; Pradhan *et al.,* 2021; Yasin *et al*., 2023).

The study of genetic diversity in rapeseed and mustard germplasm is crucial for selecting suitable genotypes for breeding programs. Genetic diversity is an essential element of plant breeding for identifying the more diverse and choice breeding materials from the available germplasm. It is also helpful to get desirable segregants from the segregating population. In creating high-yielding genotypes with acceptable traits, it is imperative to evaluate the divergence in Indian mustard genotypes (Singh *et al*., 2014, 2016; Meena *et al*., 2017).

Usually, the genetic improvement of any crop heavily depends on the detailed information of the available genetic material. In addition, advanced methods of genomic description collected germplasm evaluation and utilization of plant genetic resources are simple steps to develop ideal improved oil seed genotypes (Saikia *et al*., 2018).

Besides, diversity analysis also considerably facilitates the plant breeders in identifying suitable parents concerning a specific breeding objective with great relevance in heterosis breeding programs to know the high genetic divergent parent (Joya *et al*., 2017). Considering the value of genetic diversity, the presented study sought to find more genetically diverse parents from the population of rapeseed and mustard genotypes.

MATERIALS AND METHODS

Experimental material and procedure

A total of 40 varieties, comprising 20 rapeseed and 20 mustard (Table 1) served as specimens grown for determining the genetic diversity among them. The experiment ran at the botanical garden, Department of Plant Breeding and Genetics, Sindh Agriculture University, Tandojam, Pakistan, during the winter of 2020–2021. The research was in a randomized complete block design with three replications.

Table 1. Rapeseed and mustard genotypes used in the study.

Rapeseed genotypes	Source of collection	Mustard genotypes	Source of collection
Punjab Sarson	Oilseed Research Institute, Faisalabad,	Bard-I	National Agriculture Research Center, Islamabad
Rohi Sarson	Oilseed Research Institute, Faisalabad	D-Khanpur Raya	Oilseed Research Institute, Faisalabad
NARC Sarson	National Agriculture Research Center, Islamabad	D-Bahawalpur Raya	RARI, Bahawalpur
PARC canola hybrid	National Agriculture Research Center, Islamabad	Coral-432(C)	ICI, Pvt. Ltd
Faisal canola	Oilseed Research Institute, Faisalabad	MS-4	Pakistan Agriculture Research Council, Islamabad
Hyola-401	ICI, Pvt. Ltd	Dhoom-l	Pakistan Agriculture Research Council, Islamabad
Rainbow	ARI, Tarnab, Peshawar	Super Raya	ORI, Faisalabad
Pakola	National Agriculture Research Center, Islamabad	MS-2	Pakistan Agriculture Research Council, Islamabad
Con-I	National Agriculture Research Center, Islamabad	HUM-321 (A)	Agriculture Research Institute, Tandojam
Con-II	National Agriculture Research Center, Islamabad	HUM-321 (B)	Agriculture Research Institute, Tandojam
Con-III	National Agriculture Research Center, Islamabad	K, J-230	Pakistan Agriculture Research Council, Islamabad
Wester	National Agriculture Research Center, Islamabad	Galaxy	Pakistan Agriculture Research Council, Islamabad
Dunkeld	National Agriculture Research Center, Islamabad	BRJ-1104	Pakistan Agriculture Research Council, Islamabad
Abasin-95	Nuclear Institute for Food and Agriculture, Peshawar	K.J-221	Pakistan Agriculture Research Council, Islamabad
Hyola-42	ICI, Pakistan seeds Lahore	Anmol Raya	Oilseed Research Institute, Faisalabad
Hbo-555	Pakistan Agriculture Research Council, Islamabad	AARI-Canola	Ayoub Agriculture Research Institute, Faisalabad
Mun-1	Pakistan Agriculture Research Council, Islamabad	JS-13	Pakistan Agriculture Research Council, Islamabad
Kungola	Pakistan Agriculture Research Council, Islamabad	Early Raya	Pakistan Agriculture Research Council, Islamabad
Hbo-63	Pakistan Agriculture Research Council, Islamabad	Sindh Raya	Pakistan Agriculture Research Council, Islamabad

Data recorded and statistical analysis

Data recording ensued for different yield-associated traits, such as plant height (cm), branches plant[-1], silique plant[-1], seeds silique[-1], silique length (cm), seed yield silique[-1] (g), seed yield plant[-1] (g), 1000-seed weight (g), and oil content (%). Ten randomly selected plants of each genotype attained data recording, with the average of each parameter considered for data analysis. The obtained data underwent analysis of variance of all the parameters of 40 genotypes of rapeseed and mustard using the statistical computer package (Statistix vr.8.1). Moreover, employing the least significant difference (LSD) at a 5% level helped separate the various means. Genetic diversity estimates of *B. napus* and *B. juncea* based on phenotypic characters ran by cluster and principal component analyses to identify more divergent parents among 40 genotypes of rapeseed and mustard using Indostat ver. 2.0 software. The study also used Tocher's clustering method to detect the genetic distance and relationship among the parents for cluster analysis. Genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), their relative difference (RD), and heritability analysis in broad sense calculations, as suggested by Singh and Chaudhary (1985), Sivasubramanian and Madhava (1973), and Falconer and Mackay (1981), are as follows:

$$\delta^2 g = \frac{MSG - MSE}{r}$$

$$\delta^2 p = \delta^2 g + \delta^2 e$$

$$GCV = \frac{\sqrt{\delta^2 g}}{\overline{x}} \times 100$$

$$PCV = \frac{\sqrt{\delta^2 p}}{\overline{x}} \times 100$$

$$RD = \frac{PCV - GCV}{PCV} \times 100$$

$$hB_2(\%) = \frac{= \delta^2 g}{\delta^2 p} \times 100$$

Where:
$\delta^2 e$ = environmental variance
$\delta^2 g$ = genotypic variance
$\delta^2 p$ = phenotypic variance
$hB_2(\%)$ = heritability broad sense in percentage
GCV = genotypic coefficient of variation
PCV = phenotypic coefficient of variation
RD = Relative difference

RESULTS AND DISCUSSION

Analysis of variance

The analysis of variance for all the parameters is in Table 2. The acquired results are significantly different at $P \leq 0.01$ probability level for plant height (cm), the branches plant[-1], silique length (cm), seed yield plant[-1], seed index (1000-seed weight), and oil content (%) and at $P \leq 0.05$ probability level for silique plant[-1], seeds silique[-1], and seed yield silique[-1] for all the rapeseed and mustard genotypes, indicating a sufficient amount of genetic variability is present among their varieties. The presented results agree with Singh *et al.* (2020), who recorded maximum variation in different morphological characteristics. Similarly, Saikia *et al.* (2018) also observed substantial differences in yield-associated traits of mustard.

Mean performance of rapeseed and mustard genotypes

The mean performance recording for all studied traits appears in Tables 3a and 3b. Plant height is a chief parameter, which enhances seed yield by comprising maximum branches assisting the proper distribution of photosynthesis. The average range for plant height was 165.27 to 125.20 cm. The highest plant height measurement was in the PARC canola hybrid (165.27 cm), followed by NARC

Table 2. Analysis of variance for various phenotypic traits in 20 each genotypes of rapeseed and mustard.

Traits	Mean squares		
	Replication	Genotypes	Error
d.f.	2	39	78
Plant height (cm)	56.582	420.436**	128.647
Branches plant^{-1}	0.43233	1.03586**	0.47917
Silique plant^{-1}	1294.66	4122.40*	2444.46
Seeds silique^{-1}	0.04258	1.20195*	0.0601
Silique length (cm)	0.08914	6.98855**	0.15046
Seed yield silique^{-1}	0.000233	0.000773*	0.000453
Seed yield plant^{-1} (g)	3.363	22.8345**	6.9552
1000-seed weight (g)	0.01521	1.67613**	0.05500
Oil content (%)	0.0636	11.4604**	0.0214

d.f. = Degree of freedom, **, * = Significant at 1% and 5% level of probability, respectively.

Sarson (164.07 cm), whereas the minimum plant height resulted in Con-III, with the height of 125.20 cm. The higher the branches plant^{-1}, plays a vital role in obtaining the final seed yield consisting of maximum number of pods. The maximum number of branches plant^{-1} came from Early Raya (6.80), followed by PARC canola hybrid (6.20); however, hbo-63 produced the minimum number of branches (4.06). The number of siliques also has a sizable contribution to influencing the final yield of the plants. Relating to the silique plant^{-1}, the maximum number of silique plant^{-1} was with genotype B.K.J-1104 (263.43), followed by B-J-D, BARD-I (255.40), whereas Con-I resulted in minimum silique plant^{-1} (116.07).

Moreover, seeds silique^{-1} is an essential parameter to obtain more yield. The variety Punjab Sarson possessed the maximum seeds silique^{-1} (13.00), followed by Rohi Sarson (12.80), whereas the minimum seeds silique^{-1} resulted in MS-4 (10.46). The supreme silique length is crucial for setting a vast number of seeds, and results for the silique length ranged from 2.82 to 7.14 cm. The highest value for silique length showed in Rohi Sarson (7.14 cm), followed by Punjab Sarson at 6.98 cm. However, the minimum stretch occurred in J.S-13 (2.82 cm). The supreme seed yield silique^{-1} is the main breeding objective for improving seed yield per plant in genotypes. Punjab Sarson weighed a maximum seed yield silique^{-1} (0.117 g), followed by Dhoom-I (0.0587 g). Conversely, the minimum seed yield silique^{-1} came from B-K-J-1104 (0.020 g).

Yield is the central target for every breeding strategy; hence, seed yield plant^{-1} is critical. Seed yield plant^{-1} ranged from 5.05 to 15.04 g, resulting in genotype Dhoom-I exhibiting the maximum weight (15.040 g), followed by Anmol Raya (14.847 g). In contrast, the minimum weight for seed yield plant^{-1} was with Con-I (5.05 g). The genotype Dhoom-I also produced the maximum 1000 seed index weight (5.56 g), followed by Early Raya (5.17 g), but the minimum emerged with genotype K.J-230 (2.21 g). Concerning oil content, KN-277 contained the highest amount (38.99%), followed by Hyola-401 (38.87%), with the minimum amount carried by the variety Kungola (31.13%). Among all genotypes, including rapeseed and mustard, PARC canola hybrid, NARC Sarson, Early Raya, B-J-D, BARD-I, Punjab Sarson, Rohi Sarson, Dhoom-I, and KN-277 performed better for various traits and possessed potency for genetic resources.

Components of variance and heritability (bs)

The amount of the total variance in the gene pool improves qualitative and quantitative parameters. If traits possessed high heritability, it is more significant for the selection. (Bhagasara *et al.*, 2017). Heritability, with genotypic (GCV) and phenotypic coefficient of variations (PCV), is also helpful for variability. The magnitude of variability on PCV and GCV for all the

Table 3(a). Mean performance of rapeseed and mustard genotypes for plant height (cm), number of branches/ plant, number of silique/plant, number of seeds/silique, and silique length(cm).

Genotypes	Plant height (cm)	Number of branches plant[-1]	Number of siliques plant[-1]	Number of seeds silique[-1]	Silique length (cm)
Punjab sarson	154.07	5.4000	161.73	13.000	6.9800
Rohi sarson	160.00	6.1333	166.67	12.800	7.1467
NARC sarson	164.07	5.5333	168.27	12.067	5.7333
PARC canola hybrid	165.27	6.2000	157.47	12.933	6.0467
Faisal canola	151.00	5.0000	170.47	12.233	6.3533
hyola-401	147.87	5.6000	169.20	11.667	6.4267
Rainbow	161.07	4.8667	158.07	12.233	6.1733
Pakola	156.13	4.8667	146.60	12.233	6.5267
Con-I	126.00	4.0667	116.07	12.267	6.0667
C0n-II	128.53	5.0000	137.67	11.367	6.1800
Con-III	125.20	4.7333	166.53	12.600	6.2200
Westar	118.93	4.3333	144.53	12.467	5.6467
Dunkeld	127.40	5.0000	159.40	12.200	5.1733
Abasin-95	128.67	4.4000	127.07	11.267	5.7067
Hyola-42	132.07	4.8000	150.67	11.467	5.2967
Hbo-555	145.33	4.8667	182.87	11.333	5.9467
Mun-I	151.40	4.6667	148.13	10.500	6.7933
SKungola	138.47	4.2000	134.93	11.667	5.7067
Hbo-63	137.00	4.0667	126.73	11.367	6.3600
Kn-277	125.47	4.2667	139.20	11.833	6.6067
AARI-Canola	148.80	5.9333	243.47	12.200	3.2000
J.S-13	156.80	5.2667	195.33	10.533	2.8200
B-J-D,BARD-I	139.00	5.4667	255.40	11.667	3.0600
B-J-D, Khanpur Raya	144.53	4.8000	210.00	12.333	3.0267
Early Raya	146.87	6.8000	185.33	12.367	3.2400
Sindh Raya	147.47	5.2667	193.73	11.267	3.5200
MS-4	144.73	5.6000	202.33	10.467	3.3333
Anmol Raya	153.87	5.2000	219.13	11.733	3.2800
Dhoom-I	147.40	5.1333	187.80	11.500	3.2333
Super Raya	146.93	5.2667	203.67	11.533	3.1600
MS-2	147.27	5.2000	169.40	10.667	3.1067
HMU-321	147.40	5.1333	213.73	11.300	3.2933
157.53	150.07	5.7333	184.00	11.467	3.3133
HMU-322	141.00	4.9333	129.33	11.567	3.3133
B-J-D, Bhawalpur Raya	157.53	5.4667	161.00	11.367	3.2267
K-J-230	148.80	4.8000	144.13	11.333	3.1333
Galaxy	151.20	4.4667	242.27	11.400	3.0867
B-K-J-1104	160.47	4.8667	263.33	11.200	3.0933
K-J-221	156.93	5.2000	223.53	11.467	3.6933
Coral-432-C	153.93	5.3333	155.20	11.567	3.7133
LSD (5%)	18.437	1.1252	80.368	1.4475	0.6305
Mean	145.8733	5.0967	175.3600	11.7108	4.6974
S.E	6.5485	0.3997	28.5450	0.5141	0.2240
C.V	7.7754	13.5819	28.1943	7.6042	8.2559

Table 3(b). Mean performance of rapeseed and mustard genotypes for seed yield silique^{-1}, seed yield plant^{-1} (g), number of silique plant, seed index (1000 seed in g), and oil content (%).

Genotypes	Seed yield silique^{-1}	Seed yield plant^{-1} (g)	Seed index (1000 seed in g)	Oil content (%)
Punjab sarson	0.1173	11.260	3.7333	34.140
Rohi sarson	0.0347	9.137	3.2900	38.703
NARC sarson	0.0267	8.227	3.7667	33.250
PARC canola hybrid	0.0353	8.680	3.9067	34.177
Faisal canola	0.0360	8.840	3.5833	34.950
hyola-401	0.0433	8.203	3.0833	38.870
Rainbow	0.0260	7.627	3.5833	33.360
Pakola	0.0460	8.077	3.2400	34.150
Con-I	0.0333	5.053	3.4600	32.237
C0n-II	0.0287	6.653	2.9400	31.733
Con-III	0.0273	6.003	3.5500	32.073
Westar	0.0273	7.857	2.8933	31.427
Dunkeld	0.0247	6.043	3.8433	32.963
Abasin-95	0.0253	5.090	3.3267	35.300
Hyola-42	0.0273	5.873	3.1367	36.287
Hbo-555	0.0427	10.340	2.9667	34.770
Mun-I	0.0393	8.050	3.7167	32.043
SKungola	0.0313	6.567	3.0967	31.133
Hbo-63	0.0333	5.557	3.0767	34.033
Kn-277	0.0400	8.240	3.0100	38.997
AARI-Canola	0.0393	11.707	3.2000	32.240
J.S-13	0.0230	7.823	3.6333	34.230
B-J-D,BARD-I	0.0267	10.750	3.4000	34.850
B-J-D,Khanpur Raya	0.0473	12.720	4.9000	36.170
Early Raya	0.0560	11.440	5.1700	32.317
Sindh Raya	0.0487	12.563	5.1233	31.910
MS-4	0.0447	13.077	4.6433	32.943
Anmol Raya	0.0500	14.847	4.0567	33.063
Dhoom-I	0.0587	15.040	5.5667	34.453
Super Raya	0.0400	12.123	4.9267	34.077
MS-2	0.0567	13.170	4.5500	34.130
HMU-321	0.0307	9.387	2.6533	31.757
S-9	0.0440	12.073	3.6633	33.137
HMU-322	0.0373	7.923	4.2900	34.407
B-J-D, Bhawalpur Raya	0.0307	7.567	2.9600	32.340
K-J-230	0.0313	6.257	2.2133	31.510
Galaxy	0.0267	8.547	3.2767	34.187
B-K-J-1104	0.0200	8.827	3.2067	33.217
K-J-221	0.0393	13.827	3.2500	32.460
Coral-432-C	0.0367	8.050	3.4833	35.470
LSD$_{0.05}$	0.0346	19.431	0.3812	0.2380
Mean	0.0383	9.2273	3.6342	33.8366
S.E	0.0123	1.5226	0.1354	0.0845
C.V.	55.5159	28.58	6.4530	0.4326

Table 4. Components of variance and heritability for phenotypic traits in 20 each genotypes of *Brassica napu*s and *Brassica juncea.*

Traits	GV	PV	EV	GCV%	PCV%	ECV%	RD%	h^2 (bs) %
Plant height (cm)	97.26	140.14	42.88	6.76	8.11	7.77	16.68	69.40
Branches plant[-1]	0.18	0.34	0.16	8.45	11.52	13.58	26.68	53.70
Silique plant[-1]	559.31	1374.13	814.81	13.48	21.14	28.19	36.20	40.70
Seeds silique[-1]	0.13	0.40	0.26	3.15	5.40	7.60	41.66	34.0
Silique length (cm)	2.28	2.33	0.05	32.13	32.48	8.25	1.08	97.8
Seed yield silique[-1]	0.02	0.03	0.02	26.94	41.87	55.51	35.65	41.40
Seed yield plant[-1]	5.29	7.61	2.32	24.93	29.89	28.58	16.60	43.21
1000-seed weight (g)	0.54	0.55	0.018	20.22	20.56	6.45	1.65	96.70
Oil content (%)	3.81	3.82	0.07	5.77	5.77	0.43	0.08	99.8

GV = Genotypic variance, PV = Phenotypic variance, GCV = Genotypic coefficient of variation, PCV = Phenotypic coefficient of variation, RD = Relative difference, h^2 (bs) = heritability in broad sense.

parameters is in Table 4, with the components of variance distributed to genotypic and phenotypic variances. In the study findings, GCV ranged from 3.153% to 32.133%. The maximum GCV recorded (>20%) was for silique length (cm) (32.133%), followed by seed yield silique[-1] (26.94%), whereas the lowest was in seeds silique[-1] (3.15%). It refers to those parameters with the recorded highest value for GCV that can further improve through hybridization. Similarly, high PCV appeared in seed yield silique[-1] (41.87%), followed by seed yield plant[-1] (37.92%). However, the lowest value for PCV came from seeds silique[-1] (5.40%). The maximum difference between GCV and PCV resulted in seeds silique[-1] (41.66%), followed by silique plant[-1] (36.20%), seed yield silique[-1] (35.56%), and branches plant[-1] (26.68%). Likewise, the minimum difference occurred in oil content (%) (0.086%), followed by silique length (1.08%) and 1000 seed index weight (1.65%). The more the difference indicates that the environment influences the characteristics.

The heritability in a broad sense for all the phenotypic traits is also in Table 4. The recorded high heritability was for the attribute of oil content (99.8%), followed by silique length (cm) (97.8%), 1000 seed index weight (96.8%), and plant height (69.4%). Although, the branches plant[-1], seed yield plant[-1], and seed yield silique[-1] resulted in heritability of 53.7%, 43.21%, and 41.66%, respectively. A low heritability was notable in silique plant[-1] (40.7%) and seeds silique[-1] (34%). High heritability for these parameters showed that

traits had less influence from the environment and more effect from genetic governance by the action of additive genes. Hence, these parameters can attain improvement through the direct phenotypic selection. Aktar *et al.* (2019) reported similar results related with these findings, as they recorded high heritability with maximum GCV and PCV for most yield-associated traits in brassica. Furthermore, Begum *et al.* (2018) observed moderate and high heritability in various phenotypic characteristics of mustard.

Cluster analysis

Cluster and principal component analysis is a suitable technology for the distribution of various biological populations at the genotypic level and helped assess comparison effects in different components evaluating the aggregate amount of the diversity at inter and intra-cluster levels (Zahan *et al.*, 2008). Cluster analysis is more significant in identifying divergent genotypes. Performing the cluster analysis ran Tocher's method based on D^2 values. The examined result demonstrated that all 40 genotypes could categorize into six clusters, with their grouping obtained from a dendrogram by Tocher's method, as presented in Table 5. Clusters II and III comprised more number of the genotypes (11), including Con-I, Con-III, Mun-I, Con-II, Wester, Kungola, HMU-321, K-J-221, AARI-Canola, B-J-D, Bhawalpur Raya, and K-J-230. Meanwhile, Cluster III consisted of Super Raya, MS-2, HMU-322, Dhoom-1, JS-13, Galaxy, B-J-D, BARD-I, S-9,

Table 5. Clustering composition with distribution of 20 each genotypes of *Brassica napus* and *Brassica juncea* by Tocher's method.

Cluster number	Number of genotypes	Genotypes
I	(8) 3,7,13,19,8,4,1,16	NARC Sarson, Rainbow, Dunkeld, Hbo-63, Pakola, PARC canola hybrid, Punjab Sarson, Hbo-555.
II	(11) 9,11,17,10,12,18,32,39,21,35,36	Con-I, Con-III, Mun-I, Con-II, Wester, Kungola, HMU-321, K-J-221, AARI-Canola, B-J-D, Bhawalpur Raya, K-J-230
III	(11) 30,31,34,29,22,37,23,33,28,27,38	Super Raya, MS-2, HMU-322, Dhoom-1, JS-13, Galaxy, B-J-D, BARD-I, S-9, Anmol Raya, MS-4,B-K-J-1104
IV	(3) 2,6,20	Rohi Sarson, hyola-401, Kn-277
V	(5) 5,14,40,15,24	Faisal Canola, Abasin-95, Coral-432-C, hyola-42, B-J-D, Khanpur Raya
VI	(2) 25,26	Early Raya, Sindh Raya

Table 6. Estimation of mean values of six clusters for nine phenotypic traits by Tocher's method for 20 each genotypes of *Brassica napus* and *Brassica juncea.*

Clusters	Plant height (cm)	Branches plant^{-1}	Silique plant^{-1}	Seeds silique^{-1}	Silique length (cm)	Seed yield silique^{-1}	Seed yield plant^{-1} (g)	1000-seed weight (g)	Oil content (%)
I cluster	151.29	5.10	157.64	12.17	6.12	0.04	8.23	3.51	33.86
II cluster	140.73	4.87	166.70	11.68	4.83	0.03	8.08	3.08	31.90
III cluster	148.98	5.19	204.73	11.25	3.16	0.04	11.29	4.11	33.88
IV cluster	144.44	5.33	158.36	12.10	6.73	0.04	8.53	3.13	38.86
V cluster	142.04	4.87	162.68	11.77	4.82	0.03	8.11	3.69	35.64
VI cluster	147.17	6.03	189.53	11.82	3.38	0.05	12.00	5.15	32.11

Anmol Raya, MS-4, and B-K-J-1104, followed by first cluster (8), including genotypes NARC Sarson, Rainbow, Dunkeld, Hbo-63, Pakola, PARC canola hybrid, Punjab Sarson, and Hbo-555. The three clusters, V, IV, and VI, consisted of five, three, and two genotypes, respectively. Cluster V had genotypes Faisal Canola, Abasin-95, Coral-432-C, hyola-42 and B-J-D, and Khanpur Raya. The three genotypes, Rohi Sarson, hyola-401, and Kn-277, were within Cluster IV. Cluster VI contained Early Raya and Sindh Raya. The distribution of the genotypes in the number of clusters indicates that the studied material possesses more genetic diversity. Similar results came from Singh *et al.* (2020), who categorized 28 genotypes of mustard into five clusters based on D^2 value. Ali *et al.* (2017) grouped *Brassica rapa* genotypes in various clusters and reported maximum diversity among them.

Cluster means for various traits

Cluster mean analysis is valuable for the selection of parameters. Their mean values are available in Table 6. Cluster I exhibited the highest mean value for plant height (151.29), with the lowest recorded in Cluster II (140.73). Likewise, Cluster III demonstrated a maximum value for silique plant^{-1} (204.73) and the minimum resulted in Cluster I. Silique length and oil content gave maximum mean values in Cluster IV (6.73) and (38.86), with their minimum values surfacing in Clusters III and II. Cluster VI showed the supreme mean value for seed yield silique^{-1}, seed yield plant^{-1}, and 1000-seed index at 0.05, 12.00, and 5.15, respectively, whereas Cluster IV displayed their lowest mean values. This result proposed that varieties in different clusters recorded with a high cluster mean value for the above characteristics may be effective in the

Table 7. Intra-, inter-cluster distance among 20 each genotypes of *Brassica napus* and *Brassica juncea.*

Clusters	I cluster	II cluster	III cluster	IV cluster	V cluster	VI cluster
I cluster	7.86	17.25	11.86	37.35	15.62	17.63
II cluster		8.36	18.05	52.21	29.01	12.55
III cluster			9.35	39.11	16.57	15.17
IV cluster				3.61	25.44	51.31
V cluster					9.62	27.64
VI cluster						5.00

hybridization for trait improvement. Singh *et al.* (2020) noted high cluster means for different morphological parameters in *Brassica juncea*. Sultana *et al.* (2021) stated that high cluster mean values contained by various aggregates could be valuable in future hybridization. They observed almost similar mean values among 62 genotypes of *Brassica napus* based on 10 morphological parameters.

Cluster distance

The cluster distance recording in 40 genotypes included 20 *Brassica napus* and 20 *Brassica juncea*. Categorizing genotypes resulted in six clusters based on D^2 values using Tocher's method (Table 7) and diagrammatically represented in a dendrogram of intra- and inter-cluster distance among the 40 genotypes (Figure 1). They gave considerably different ranges. The maximum cluster distance occurred between Cluster IV and Cluster VI. Cluster VI accounted for two mustard genotypes, Early Raya and Sindh Raya, which manifested the highest inter-cluster gap from Cluster IV (51.31). Next to having a high inter-cluster distance is between Clusters II and IV (52.21), comprising three rapeseed varieties, Rohi Sarson, hyola-401, and Kn-277. The high inter-cluster space was also between Clusters II and V (29.01), followed by Clusters IV and V (25.44) and Clusters I and IV (37.35). The maximum distance between the two clusters showed that those genotypes presented in clusters possessed more genetic diversity. These outcomes indicate the highest inter-cluster distance in different aggregates and genotypes exhibited may benefit upcoming hybridization programs. Also, the genotypes are genetically divergent and suitable for

heterotic combinations, i.e., Cluster VI (Early Raya and Sindh Raya) with Cluster IV (Rohi Sarson, hyola-401, and Kn-277). A similar manner can refer to Cluster IV with the prime group of the genotype's Clusters II, V, and I. Rohi Sarson, hyola-401, and Kn-277 rapeseed varieties are genetically divergent and demonstrate distance from various groups of the clusters. Contrastingly, the smallest inter-cluster distance (11.86) was with Clusters I and III, followed by Clusters II and VI (12.55), Clusters III and VI (15.17), and Clusters I and V (15.62). The lowest cluster distance consequences that these presented genotypes in clusters possess the shortest distance are closely related and finding the similarity. The intra-cluster distance ranged from 3.61 to 9.62. Maximum intra-cluster distance D^2 value showed in Cluster V (9.62), followed by Cluster III (9.35) and Cluster II (8.36). Meanwhile, Clusters I, VI, and IV exhibited 7.86, 5.00, and 3.61 intra-cluster distances, respectively. Almost all the study results agreed with Meena *et al.* (2020) and Ravi *et al.* (2018), verifying maximum inter- and intra-cluster distances among 36 genotypes of mustard they classified into seven cluster groups based on Tocher's method.

Contribution of total genetic diversity

Estimating the magnitude of total divergence at the genetic level used the ranking method in different phenotypic traits of the rapeseed and mustard genotypes. Their contribution (%) and times ranked first are in Table 8. The oil content parameter contributed the highest genetic divergence (73.46%) by holding 573-times ranking first, followed by silique length (cm) (15.64%) at 122 times, 1000 seed index
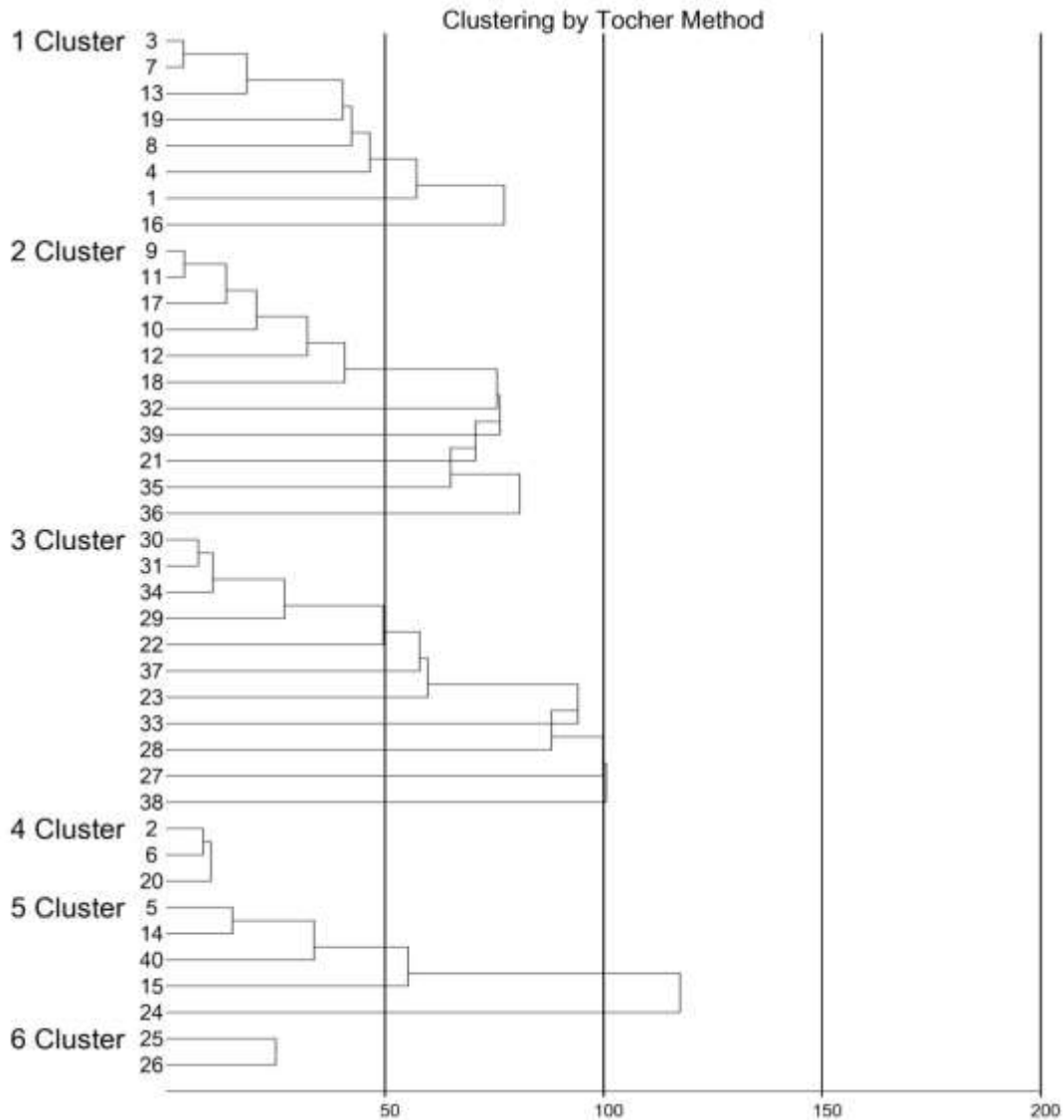
Figure 1. Dendrogram by Tocher's method among 40 genotypes of *Brassica napus* and *Brassica juncea.*

Table 8. Contribution of total diversity based on various traits in 20 each genotypes of rapeseed and mustard.

No.	Source	Times ranked 1st	Contribution (%)
1	Plant height (cm)	5	0.64%
2	Branches plant[-1]	1	0.13%
3	Silique plant[-1]	2	0.26%
4	Seeds silique[-1]	1	0.13%
5	Silique length (cm)	122	15.64%
6	Seed yield silique[-1]	1	0.13%
7	Seed yield plant[-1]	4	0.51%
8	1000-seed weight (g)	71	9.10%
9	Oil content (%)	573	73.46%

(9.10%) at 71 times. The others, such as branches plant[-1], seeds silique[-1], seed yield silique[-1], and seed yield plant[-1], contained a low amount of the total genetic divergence contribution. Hence, oil content (%), silique length (cm), and 1000 seed index are potential contributors to the genetic diversity and may serve well in future breeding programs. Hyder *et al.* (2021) contributed total genetic divergence percent for individual agronomic characteristics in Indian mustard. However, Gupta *et al.* (2021) examined various seed quality parameters of *Brassica juncea* toward the total genetic divergence contribution.

Principal component analysis

Principal component analysis (PCA) is a crucial technique to evaluate total genetic variation and help detect the relative genetic differences among the various traits of different crop species (Pankaj *et al.*, 2017). The presented study, PCA assessment ran based on the correlation matrix for all studied parameters (Table 9). The first three components contributed variation for rapeseed genotypes were 72.7% and for mustard genotypes at 64.8% variation, and the contribution of first three components for both rapeseed and mustard genotypes was consistent at 68.3%. On rapeseed genotypes regarding association, first PCA showed highly associated with all nine characters, for the second PCA with silique length, oil content, and seed yield silique[-1]. The traits, oil content, silique plant[-1], and branches plant[-1] remained with a superior score in the third PCA. For mustard cultivars, the first PCA correlated with seed yield silique[-1] (g), 1000-seed weight, seed yield plant[-1] (g), seeds silique[-1], silique length, and oil content. Meanwhile, the variables oil content, 1000-seed weight, and silique plant[-1] were important in PCA 2. However, the silique plant[-1] comprised the maximum score in the third PCA. On combined PCs of rapeseed and mustard germplasm, the first PCA had association with silique length and seeds silique[-1], second PCA associated with silique plant[-1], and third PCA correlated with 1000-seed weight and seed yield silique[-1] (g). Based on principal factor scores, all 40 genotypes aided the construction of a precise 3D Plot. The genotypic representation is in Figure 2. All the genotypes plotted in PC1, PC2, and PC3, fully accounted for differences at 68.3%. Variability for all the parameters came from the three rapeseed genotypes, Rohi Sarson, hyola-401, and Kn-277, exhibiting maximum genotypic scores for all the traits. These genotypes have a better value mean for all the parameters and can benefit further experiments. Almost all findings relate to Parvin *et al.* (2019), wherein they recorded maximum variation and high genotypic scores through PCA analysis in rapeseed genotypes.

Table 9. Variables of phenotypic traits with their contribution to the first three components of rapeseed, mustard, and overall genotypes.

| Variables	Rapeseed genotypes			Mustard genotypes			Rapeseed and Mustard genotypes		
	PC1	PC2	PC3	PC1	PC2	PC3	PC1	PC2	PC3
Plant height (cm)	0.406	-0.117	0.087	-0.307	-0.279	0.162	-0.327	-0.096	-0.533
Branches plant[-1]	0.421	-0.164	0.319	0.260	-0.435	0.157	-0.379	-0.235	-0.340
Silique plant[-1]	0.368	-0.102	0.398	-0.065	0.133	0.825	-0.404	0.247	-0.259
Seeds silique[-1]	0.265	-0.283	-0.198	0.238	-0.084	0.295	0.072	-0.557	-0.112
Silique length (cm)	0.289	0.436	-0.324	0.143	-0.511	-0.212	0.377	-0.443	-0.075
Seed yield silique[-1] (g)	0.302	0.185	-0.599	0.539	-0.072	-0.124	-0.239	-0.475	0.383
Seed yield plant[-1] (g)	0.433	0.202	-0.080	0.450	-0.020	0.319	-0.499	-0.057	0.192
1000-seed weight (g)	0.225	-0.599	-0.167	0.495	0.199	-0.082	-0.359	-0.148	0.490
Oil content (%)	0.191	0.493	0.438	0.125	0.632	-0.104	0.026	-0.340	-0.299
Percent of variance	43.3	16.4	13.0	32.9	17.5	14.4	35.8	20.0	12.6
Cumulative % of variance	43.3	59.7	72.7	32.9	50.4	64.8	35.8	55.7	68.3
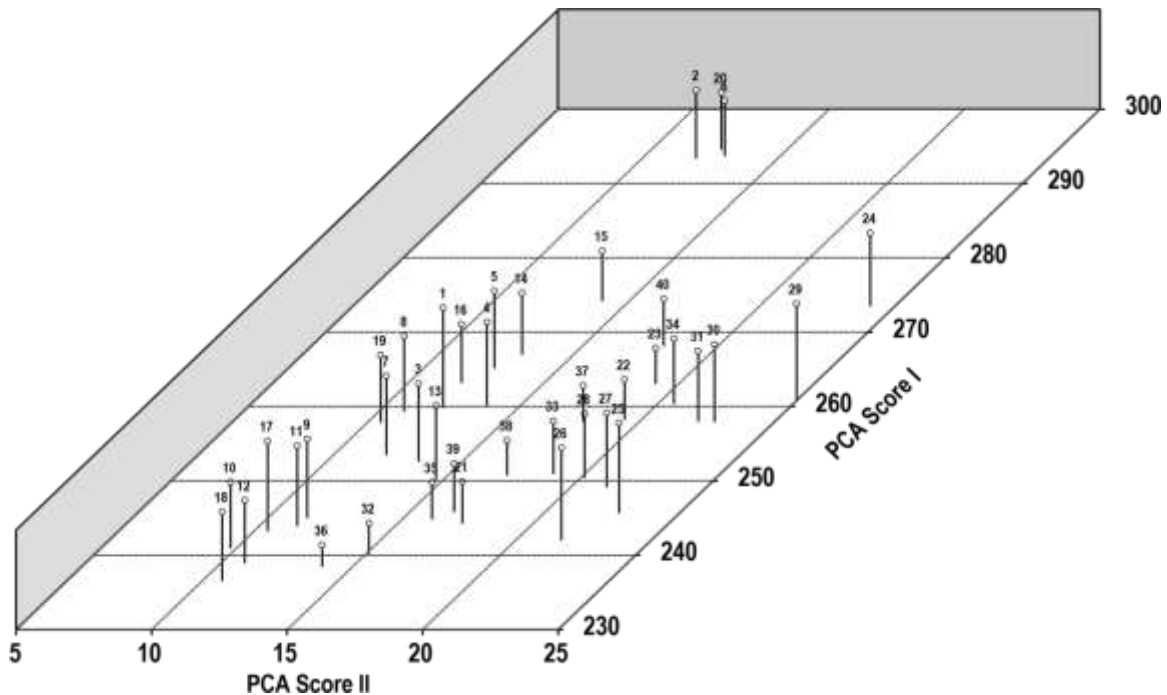
Figure 2. Three-dimensional plot based on the principal factor score for 20 each genotype of *Brassica napus* and *Brassica juncea.*

CONCLUSIONS

Based on the above-summarized results, the varieties of both species need inclusion in hybridization programs according to the magnitude of genetic distance, the contribution of different characteristics toward the total divergence, and the extent of cluster means for various traits' performance having maximum heterosis. Genotypes of various cluster categories were indicative to benefit crossing programs for obtaining a broad spectrum of variability. The latest research reports that among the genotypes, Early Raya and Sindh Raya of mustard, and Rohi Sarson, hyola-401, and Kn-277 of rapeseed revealed more diversity among studied materials and could be good combiners for interspecific hybridization. The principal component analysis provided a contrasting genetic variation between rapeseed and mustard genotypes, indicating that rapeseed varieties possessed more genetic variability than mustard. Although, PCA expressed that mustard cultivars also have good genetic resources. Genotypes, such as Rohi Sarson, hyola-401, and Kn-277 also

resulted in the highest genotypic scores in three-dimensional graphs; hence, these genotypes are more diverse and can better serve upcoming heterotic recombination. Based on the average performance, mustard genotypes, Dhoom-I and Anmol Raya, produced maximum seed yield plant^{-1}, demonstrating their significance in yield improvement. High GCV and PCV were notable in seed yield silique^{-1}, silique length, and seed yield plant^{-1}. It concludes that these traits are tractable for further betterment. High heritability (h^2.b.s) in most traits indicates they possess additive genes and can be effective for the upcoming selection.

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