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EVALUATION OF BRASSICA GENOTYPES FOR MORPHOLOGICAL AND BIOCHEMICAL ATTRIBUTES

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SUMMARY

Brassica napus is an important oilseed crop, commonly grown worldwide for multiple purposes. A planned multi-locational experiment evaluated the morphophysiological and biochemical attributes of 80 *Brassica napus* genotypes. Data on various yield and oil-related parameters were evaluated for genetic variability, correlation (r), and broad-sense heritability (h^2BS). The analysis of variance revealed the presence of significant variations among the genotypes for all studied traits. Plant height showed a positive correlation with erucic acid (0.290) and glucosinolate (0.264), while secondary branches revealed a positive association with seed yield (2.90). Principal component analysis indicated the first five principal components (PCs) contributed up to 73% of total variation, with the first PC representing the highest percentage of variation. The biplot suggests the genotypes Faisal canola, Diamond canola, AS-333, and HMU-1621B can be beneficial in breeding programs to develop new accessions with improved seed yield and oil-related attributes of *Brassica*.

Keywords: Biplot, genetic diversity, heritability, oil contents, PCA

Key findings: Eighty accessions, screened for yield and related traits, indicated the genotypes Faisal canola, Diamond canola, AS-333, and HMU-1621B exhibited the highest yield and fatty acid content.

INTRODUCTION

Rapeseed and mustard are major oil-contributing crops globally, with their rich protein and oil content. They contain 40%–46% good-quality oil, and their meals consist of 38%–40% protein with a complete amino acid profile, including lysine, methionine, and cysteine. Almost 80% of the edible oil requirement comes from imports from other

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countries. Moreover, domestic production fulfills less than 20% of the country's demand (Aftab *et al*., 2019). Rapeseed-mustard cultivation occurs on 509,900 hectares, producing 0.251 million tons of oil and 0.785 million tons of seed (Pakistan Economic Survey, 2022-23). Its planting appears in more than 50 countries globally, ranking it as the third-largest source of vegetable oil. The main growing countries of *Brassica napus* are Canada, Central China, and Australia.

Edible oil is an essential part of the human diet and a chief source of fatty acids. However, its production is extremely low in Pakistan. Several multiple abiotic factors cause a lower oil production, i.e., rising temperature, deficiency of agricultural inputs, and water shortage, which primary impact the yield and quality of the *Brassica* genotype. Canola, as mainly grown on marginal lands, indicates its production acquires influences from inadequate agronomic practices, as well as, the use of low-yielding varieties (Ahmed *et al*., 2016; Sharma *et al*., 2016). The prime reason for low production is the researchers paying less attention to improving the genetic architecture of oilseed crops. During 2022–2023, Pakistan imported 2.681 million tons of edible oil valued at USD 3,562 million (Rs. 826,482 million). The estimated amount of locally produced edible oil is 0.496 million tons, while the expected amount of edible oil available from each source (rapeseed and mustard, sunflower, canola, and cottonseed) is 3.177 million tons (Pakistan Economic Survey, 2022–2023).

Gul *et al*. (2018) studied genetic variability in *Brassica napus* L. and reported genotypes showed highly significant differences in all traits, such as, seed yield, days for 50% flowering, pod length, pods per main raceme, and primary branches per plant. Ullah *et al*. (2017) examined the genetic variation, heritability, and association between various biochemical characteristics in *Brassica rapa* L. Significant variations were evident in parameters, such as, glucosinolate, oil contents, erucic acid, oleic acid, and linolenic acid. For almost all traits, genotypic variances were higher than the environmental variances, revealing high heritability. Khan *et al.* (2022) evaluated genetic variability, correlation (r), and broad-sense heritability (h^2BS) in *Brassica napus* accessions to identify yield-contributing traits. Significant percentages of PCV and GCV appeared for branches on the main stem, 1000-seed weight, and seeds per silique, showing the presence of considerable variability for these parameters. Yadav *et al*. (2022) performed principal component analysis (PCA) to estimate the variation among genotypes of *Brassica juncea* L. They reported PC1 contributed approximately 36.19% of total variation, while PC2, PC3, and PC4 contributed approximately 33.56%, 9.79%, and 6.90% of the overall variation, respectively.

Genetic diversity in rapeseed and mustard germplasm is crucial for selecting suitable genotypes for breeding programs. Broad-sense heritability (h^2BS) determines the ability of parents to transfer the desirable characters to their progeny. Heritability and genetic advancement assist breeders in making efficient selections. PCA, a type of multivariate analysis that primarily assesses the genetic diversity among germplasm while studying numerous traits, plays a significant role in selecting and developing high-yielding cultivars (Jan *et al*., 2024). Understanding the extent of genetic variability and interrelationships among yield and oil-related traits is essential for developing a new climatic resilience *Brassica* genotype. In Pakistan, evaluating the extensive *Brassica* germplasm for genetic variability has been challenging. Therefore, the main objective of this study was to explore the genetic variability and association between plant morphology and quality attributes of various *Brassica* genotypes.

MATERIALS AND METHODS

Experimental layout

A multi-locational experiment commenced at the research area of the Plant Breeding and Genetics, Faculty of Agriculture Sciences and Technology, Bahauddin Zakariya University, Multan, Pakistan (30°25′N, 71°51′E, 122 masl) and Chak No. 133/10R (29°57′N, 71°37′E, 120 masl) District Khanewal during 2022–2023.

Collected seeds of *Brassica* accessions came from the National Agriculture Research Center (NARC) in Islamabad. Eighty lines of *Brassica napus* with diverse genetic backgrounds incurred evaluation for their agronomic characteristics under field conditions. Collected genotypes, grown in randomized complete block design (RCBD), had three replications. The experimental fields have clay loamy soil with a medium fertility level. The climate in this area is moist in the summer and dry in the winter. The meteorological data recorded during the study year are available in Figure 1.

Crop husbandry

A multi-location trial was conducted, with sowing on October 26 and November 2, 2022, at the respective sites.The manual planting of seeds of all genotypes used a hand drill, with approximately 45 cm row spacing at each site. The plant-to-plant spacing of 5 cm ensured uniform germination through the plant thinning. Before planting, the fertilizer application at 80:40 kg NP ha^{-1}, utilized urea and diammonium phosphate at each site. Standard agronomic practices remained uniformly throughout the crop-growing seasons at both sites. Two irrigations helped meet the moisture requirement of the crop in both sites. In 2022, higher precipitation was notable during the *Brassica* production season (Figure 1).

Morphological and yield traits

At maturity, morphological data collection occurred from five randomly selected plants in each replication. These included plant height (cm), the number of primary and secondary branches per plant, the number of silique per plant, seeds per silique, a thousand-seed weight (g), and seed yield per plant (g), used for biometrical analysis.

Oil quality traits

Assessing the erucic acid, glucosinolate, and oil content percentages, which came from cleaned dried samples of 5 g from each selected plant, used the SpectraAlyzer 2.0 device (ZEUTEC GmbH, Rendsburg, Germany; Serial No. 110457). The near-infrared (NIR) spectroscopy provides a rapid, non-destructive, efficient, and inexpensive method of analysis (Tesfaye *et al*., 2024).

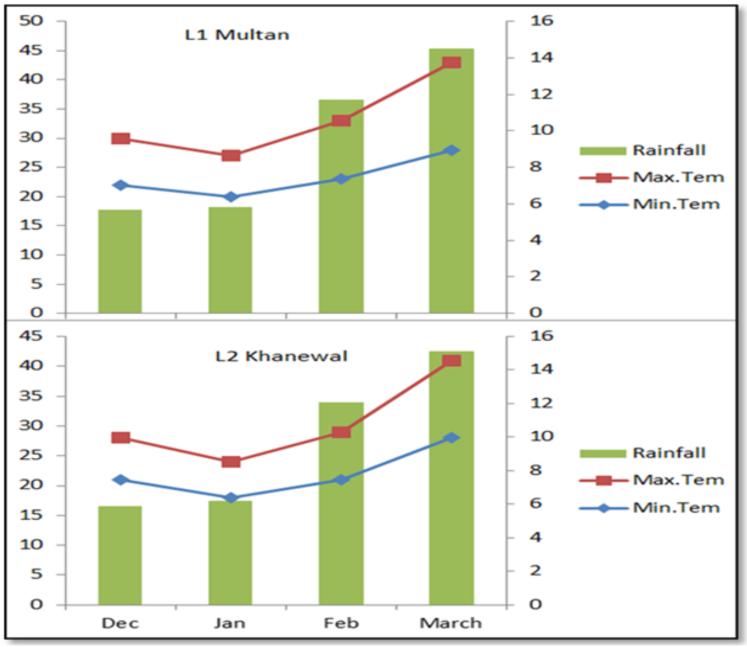

Figure 1. Weather data of the experimental site.

Data analysis

The collected data underwent analysis of variance (ANOVA) presented by Steel *et al*. (1997), using the randomized complete block design. Ogunbayo *et al*. (2005) utilized the average data to determine the best genotype for yield-contributing traits through principal component analysis (PCA) performed by XLSTAT software version 25.03 (2023). The variance components, including genotypic variance (σ^2g), phenotypic variance (σ^2p), and error variance (σ^2e) reached calculation according to the formula provided by Panse and Sukhatme (1967).

RESULTS AND DISCUSSION

Analysis of variance

Analysis of variance (ANOVA) showed highly significant differences among genotypes for the studied traits, except for primary branches per plant. The location had a significant effect for thousand-seed weight and oil contents (%) but was nonsignificant for plant height, number of silique/plant, number of secondary and primary branches/plant, number of seeds/silique, yield/plant, erucic acid, and glucosinolate contents. The morphological and oil quality traits had different ranges, as follows: plant height (93.33–185.00 cm), primary branches per plant (5.33–10.67), silique per plant (202.33–364.33), number of secondary branches per plant (10.33–29.33), seeds per silique (12.33–29.67), thousand-seed weight (1.47–2.66 g), seed yield per plant (15.91–41.46 g), oil contents (34.36%–42.70%), erucic acid (0.26%–51.00%), and glucosinolate (10.16%–103.38%) (Table 1).

Cowling *et al*. (2023) reported significant variations among all genotypes for plant height, silique/plant, secondary branches/plant, number of primary branches/plant, number of seeds/silique, thousand-seed weight, yield/plant, oil content, erucic acid, and glucosinolate content percentages. The presented study reported nonsignificant differences for the number of primary branches/plant, but other studies reported substantial differences for this trait. The contradiction in the results may be due to the variation in genotypes, different locations, and the effect of crop nutrition (Li and Wu, 2023). Oil contents and seed yield revealed considerable effects from temperature and soil moisture. Higher oil content in dry areas could refer to the influence of temperature on biochemical processes that contribute to oil production. Furthermore, Cheema *et al*. (2012) stated higher canola oil contents in dry areas and observed the performance of canola cultivars in various geographical locations based on temperature. Different cultivars of *Brassica* have varied potential for oil content and seed yield (Cheema *et al*., 2012; Ahmed *et al*., 2016). In the current study, the genotypes differ significantly based on oil contents (%) and seed yield.

Correlation analysis

All the traits have a positive association with each other, except for oil contents, which showed a negative correlation with all other traits. A maximum correlation (-0.36) was evident between glucosinolate and oil contents (%). A strong positive correlation (0.84) between erucic acid and glucosinolate contents existed (Table 2). A high correlation was also noticeable between yield-related traits, secondary branches, and seed yield (0.29). Additionally, higher values appeared in oil-related traits, such as, erucic acid (0.35%) and glucosinolate (0.45%). Gupta *et al*. (2018) supported our findings, reporting a positive correlation between secondary branches and seed yield. Lodhi *et al*. (2016) determined the oil content range (36.8%–43.2%) and disclosed a negative association between seed yield and oil content percentage, which is approximately similar to the presented studies. Positive correlation depicts both traits can undergo further improvement together, but care is necessary in selecting negatively correlated attributes for study objectives to be uncompromised.

Table 1. Analysis of variance and genotypic and phenotypic coefficient of variation in *Brassica* accessions.

Traits	GMS	G × L	Mean(±)SE	Range	h^2BS	GCV	PCV
PH	2491.92**	208.46	143.49±1.67	93.33-185.00	0.84	19.46	21.28
NSP	10945.6**	67.8	300.63±3.37	202.33-364.33	0.96	19.96	20.35
NSB	84.358**	9.32	18.763±0.31	10.33-29.33	0.68	26.24	31.92
NPB	16.8062	3.05	8.0188±0.09	5.33-10.67	0.38	23.72	38.57
NSS	86.606**	7.50	20.29±0.31	12.33-29.67	0.71	24.84	29.49
TSW	0.29621**	0.094**	1.9568±0.02	1.47-2.66	0.84	15.56	17.00
YP	163.395**	42.47	31.168±0.46	15.91-41.46	0.41	19.53	30.32
OC	16.2500**	7.92**	39.244±0.16	34.36-42.70	0.46	5.04	7.40
EAC	1585.52*	13.03	15.187±1.28	0.26-51.00	0.96	150.38	153.34
GSL	4390.72**	32.09	54.132±2.14	10.16-103.38	0.98	70.41	71.20

PH = Plant Height, NSP = Number of Silique/Plant, NSB = Number of Secondary Branches/Plant, NPB = Number of Primary Branches/Plant, NSS = Number of Seeds/Silique, TSW = Thousand Seed Weight, YP = Yield/Plant, OC = Oil Contents (%), EAC = Erucic Acid (%), and GSL = Glucosinolate (%).

Table 2. Phenotypic correlation among yield causative characters of *Brassica* accessions.

Variables	PH	NSP	NSB	NPB	NSS	TSW	YP	OC	EAC	GSL
PH	1									
NSP	0.018	1								
NSB	0.122	0.220	1							
NPB	0.013	-0.001	0.149	1						
NSS	0.086	0.168	0.354	0.159	1					
TSW	-0.032	0.032	0.034	0.091	-0.071	1				
YP	0.061	0.102	0.290	-0.001	0.049	0.026	1			
OC	-0.043	-0.018	-0.301	0.088	-0.119	0.002	-0.037	1		
EAC	0.290	0.124	0.353	0.178	-0.021	0.019	0.152	-0.272	1	
GSL	0.264	0.135	0.459	0.194	0.167	-0.042	0.112	-0.368	0.848	1

PH = Plant Height, NSP = Number of Silique/Plant, NSB = Number of Secondary Branches/Plant, NPB = Number of Primary Branches/Plant, NSS = Number of Seeds/Silique, TSW = Thousand Seed Weight, YP = Yield/Plant, OC = Oil Contents (%), EAC = Erucic Acid (%), and GSL = Glucosinolate (%).

Coefficients of variation

The phenotypic coefficient of variation (PCV) is high for traits, such as, yield per plant, number of primary branches, secondary branches, erucic acid, and glucosinolate contents. Conversely, traits, such as plant height, number of silique/plant, and seeds/silique, have a moderate value of PCV. The heritability of the studied traits appears in Table 1. Maximum broad sense heritability emerged for glucosinolate (0.98), with the minimum observed for the number of primary branches (0.38). A higher estimate of broad-sense heritability indicates a higher chance of transferring the observed variation to the next generation, which is also applicable for early selection. The number of silique per plant, erucic acid (%), and glucosinolate (%) had the additive type of gene controlling them, as these traits show high heritability. Similar results previously came from Shaukat *et al*. (2015). Low heritability is often due to environmental effects on genotype, which can cause challenges for selection (Muhammad *et al*., 2014). However, broad-sense heritability estimates can be misleading because of significant environmental impacts, which requires explaining by evaluating narrow-sense heritability.

Principal component analysis

The use of principal component analysis (PCA) helped select the best-performing groups of accessions, ideal for all yield-related traits.

Principal component analysis (PCA) resulted in five PCs with eigenvalues of more than one or close to 1. The variability and eigenvalues of the principal components are available in Table 3. PC1 had the highest eigenvalue (2.88), which represented 28.86% of the total variation. The remaining components, PC2, PC3, PC4, and PC5, contributed approximately 12.65%, 11.71%, 10.37%, and 9.69% of total variation, respectively. Table 4 displays the association coefficients between characteristics and principal components (PCs). PC1 depended on factors, such as, erucic acid (0.797%), glucosinolate (0.877%), and the number of secondary branches per plant (0.744). PC2 has large loadings of two variables, the number of siliques per plant (0.469) and the number of seeds per silique (0.620). It also had high negative loadings for plant height (-0.322) and erucic acid (-0.453%).

PCA helps breeders efficiently select high-performing parents and overcome limitations in the selection process. In the past, various researchers have used PCA to study the relationship between morphological and biochemical characteristics (Vanlalneihi *et al*., 2020). All five main components represented 73.31% of the total variation. Yadav *et al*. (2022) identified 11 principal components (PCs) that explained around 75% of the variability. Pankaj *et al.* (2017) reported nine principal components (PCs) represented approximately 77% variability. According to Chahal and Gosal (2002), traits with higher absolute values in the first principal component, closer to unity, have a more significant impact on clustering than traits with lower absolute values, closest to zero. In the biplot, most *Brassica* accessions, as separated in the right-side quadrants, indicated higher

Table 3. Percentage and total variance for principal components.

Principal Components	Eigenvalue	Variability (%)	Cumulative- Eigenvalue	Cumulative %
PC1	2.886	28.863	2.886	28.863
PC2	1.266	12.657	4.152	41.52
PC3	1.171	11.712	5.323	53.233
PC4	1.038	10.379	6.361	63.612
PC5	0.97	9.698	7.331	73.31
PC6	0.859	8.587	8.19	81.897
PC7	0.847	8.47	9.037	90.367
PC8	0.436	4.359	9.473	94.726
PC9	0.416	4.161	9.889	98.887
PC10	0.111	1.113	10	100

Table 4. Correlation coefficients of traits with respect to principal components (PCs).

Variables	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10
PH	0.370	-0.322	0.076	-0.256	0.459	-0.515	-0.435	-0.140	0.056	0.007
NSP	0.276	0.469	-0.090	0.208	0.571	0.494	-0.229	-0.169	-0.036	0.006
NSB	0.744	0.365	-0.082	0.074	-0.113	-0.106	0.038	0.115	0.512	-0.017
NPB	0.309	-0.127	0.719	0.260	0.112	-0.049	0.455	-0.282	0.042	0.007
NSS	0.374	0.620	0.498	-0.059	-0.089	-0.234	-0.160	0.241	-0.275	-0.042
TSW	0.000	-0.151	-0.171	0.916	0.012	-0.266	-0.149	0.109	-0.069	0.016
YP	0.306	0.296	-0.556	-0.089	0.240	-0.353	0.533	-0.066	-0.176	0.018
OC	-0.621	-0.082	0.200	-0.034	0.575	0.005	0.234	0.405	0.125	0.026
EAC	0.797	-0.453	-0.084	-0.010	0.097	0.215	0.101	0.163	-0.100	-0.224
GSL	0.877	-0.291	0.012	-0.070	-0.043	0.200	0.019	0.173	-0.103	0.240

PH = Plant Height, NSP = Number of Silique/Plant, NSB = Number of Secondary Branches/Plant, NPB = Number of Primary Branches/Plant, NSS = Number of Seeds/Silique, TSW = Thousand Seed Weight, YP = Yield/Plant, OC = Oil Contents (%), EAC = Erucic Acid (%), and GSL = Glucosinolate (%).

values for plant height, number of silique/plant, number of secondary and primary branches/plant, number of seeds/silique, thousand-seed weight, yield/plant, erucic acid (%), and glucosinolate (%). On the other hand, the left-side quadrants of the biplot showed a higher oil content percentage (Figure 2).

Ali *et al*. (2018) found a 27.3% variation in the first two PCs in *Brassica napus* due to morphophysiological characters, while Ali *et al*. (2017) reported a 38.78% of the variation in the first two PCs. In the current study, the first two PCs contributed up to 41.52% of the total variation due to yield-contributing traits. Genotype HMU-1622C in PC1 showed a maximum plant height (184.83 cm), while genotype AS-333 showed a higher seed yield (40.10 g), and Diamond canola had the maximum oil content (42.53%). The range of plant height, seed yield, and oil contents lay between 93.33–185.00 cm, 15.91–41.46 g, and 34.36%–42.70%, respectively. Asrami *et al*. (2014) declared that 87.5% of the total variation in agronomic traits included plant height and pod length. The first PC1 exhibited maximum variation, indicating the selection of genotypes from this PC would be beneficial for initiating any breeding program.

CONCLUSIONS

The study showed significant variations among all morphological, yield, and oil-related attributes. Some improvements on traits, like the number of siliques per plant, erucic acid (%), and glucosinolate (%), can proceed through selection as they have high heritability. Breeders should select the best combinations of traits for breeding and high-yielding canola varieties or hybrids with resilience in different climatic conditions. The biplot suggests Faisal canola, Diamond canola, AS-333, and HMU-1621B genotypes can be favorable in future breeding programs to develop a canola variety with improved seed yield and oil-related attributes suitable for dry regions, such as most of Punjab, Pakistan.

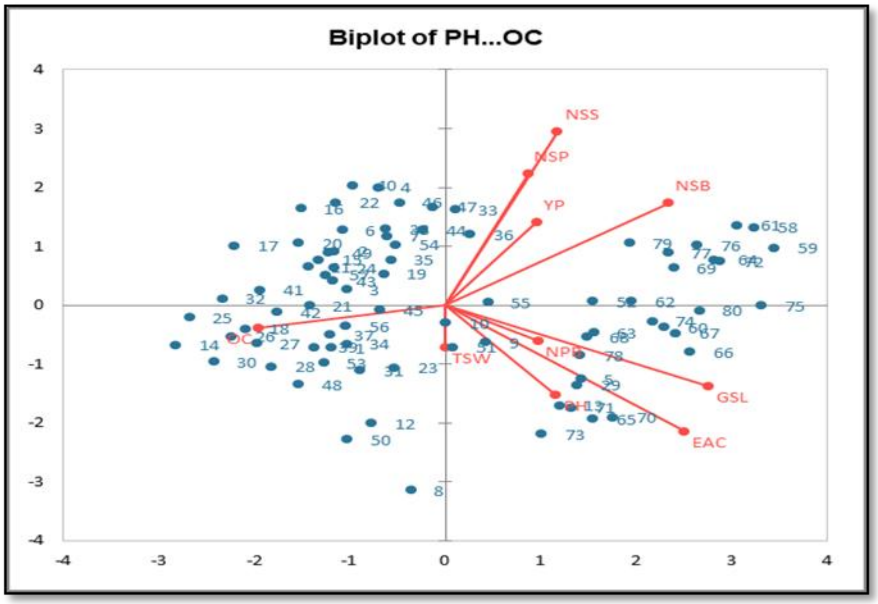

Figure 2. Biplot of PC1 and PC2 for all yield causative characters of *Brassica* accessions.

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