Integrative
Plant Biotechnology
ISSN: 3007-2735 (Online), 3007-2727 (Print)

Research Article

Multivariate Analysis Reveals Genetic Diversity and Superior Oil Quality Traits in Advanced Mustard (*Brassica juncea*) Genotypes for Future Breeding

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Article History
Received: June 02, 2025
Accepted: June 15, 2025
Published Online: June 17, 2025

Cite this article
Hussain, S., Bukhari, M. S. J., Akhtar, I., Akram, M. I., Saleem, S., Ghazali, H. M. Z. U., Hafeez, Z., Chaudary, A., Jahangir, M. & Ijaz, M. (2025). Multivariate analysis reveals genetic diversity and superior oil quality traits in advanced mustard (Brassica juncea) genotypes for future breeding. Integrative Plant Biotechnology, 3, 135–144.

ABSTRACT

Mustard (*Brassica juncea* L.) is a vital oilseed crop in Pakistan, valued for its nutritional oil and adaptability to diverse agroclimatic zones. However, limited genetic diversity and suboptimal varieties constrain yield and oil quality, posing a challenge to meet rising demand and reduce edible oil imports. This study evaluated twelve advanced mustard genotypes at the Regional Agricultural Research Institute, Bahawalpur, using a randomized complete block design with three replications to explore agronomic performance and oil quality traits. Key parameters including days to flowering and maturity, plant height, branching, 1000 seed weight, seed yield, oil content, and fatty acid composition were recorded. Multivariate analysis—principal component analysis (PCA) and cluster analysis—were employed to decipher genetic diversity and trait interrelationships. The results revealed significant variation among genotypes for all traits, with PCA and cluster analysis grouping genotypes into three distinct clusters. One cluster included genotypes characterized by taller plants and higher saturated fatty acid content but lower seed weight and oil content, indicating potential biomass producers with lower oil quality. Another cluster comprised genotypes with early maturity and moderate yield traits. The third cluster stood out with higher branching, seed weight, oil content, and oleic acid levels, representing the most promising group for breeding high-yielding, oil-rich mustard varieties. Strong correlations among oil content, oleic acid, and yield traits suggest simultaneous improvement is feasible, though a trade-off with saturated fatty acids exists. The study presents the importance of exploiting genetic diversity in mustard by using multivariate tools to devise breeding strategies to develop stress resilient, high-yielding, and oil superior mustard cultivars for agricultural sustainablility and food security in the counrty.

Keywords: Mustard, Principal component analysis, Cluster analysis, Multivariate analysis, Oil contents, Seed yield

INTRODUCTION

Mustard (Brassica juncea) is an important crop for the farming communities in South Asia. Mustard oil is different from canola oil because it has a stronger, more noticeable flavor. People like mustard oil not only for its taste, but also for its health benefits. Mustard seeds are rich in healthy unsaturated fatty acids like oleic acid and linoleic acid. Mustard oil has been used for better heart health and to fight against germs in the past (Kaur *et al.*, 2019).

https://doi.org/10.55627/pbiotech.003.02.1353

In addition to the utilization of mustard oil in food, it has been used for making pickles and other traditional medicine. Similarly, agronomic resilience and rapid growth make mustard a good option in different crop rotation systems that improve soil structure and fertility (Sachan *et al.*, 2024). In Pakistan, mustard is the third most important oilseed crop after cotton and sunflower. The main areas where this crop is grown are the provinces of Punjab, Khyber Pakhtunkhwa and in some parts of Sindh province. Mustard oil is consumed on daily basis in Pakistan, but the country does not produce enough domestic mustard to meet its own needs. Due to this fact, the country pays a lot of money to import the oil it needs. This dependency on imported edible oil puts a lot of pressure on the foreign exchange reserves of the country.

This shows an important opportunity to increase local mustard production to reduce spending on imports, increase agricultural incomes, and possibly improve the nutritional health of the population (Hussain *et al.*, 2023). However, the local mustard germplasm has lower genetic diversity and is not tolerant to biotic and absiotic stresses. To address this challenge, we need to focus on improving mustard genetic makeup by cross breeding and subsequence selection for high yielding mustard genoptypes with better oil quality stress tolrance attributes. The breeding of any crop depends on the amount of genetic diversity present in the local as well as exotic germplasm resources (Ali *et al.*, 2009; Ali *et al.*, 2011). Unfortunately, the genetic base of many commercial mustard varieties, especially in Pakistan, is relatively narrow that limits the chance to improve important traits like yield, oil quality, and resilience to stress. By crossing diverse lines and introducing new genes, breeders can combine the best traits including early maturity, sturdy plants, larger seeds, and healthier oil profiles (Cai *et al.*, 2016).

The ultimate objective of mustard breeders is to improve oil quality by boosting oleic acid levels and reducing saturated fats, because people are becoming more health conscious and markets demand the oils with better quality (Sachan *et al.*, 2024). Nonetheless, it is difficult to improve these traits while still getting good yields and plants that can thrive under stressed conditions. Mustard plants have many interconnected traits, when the breeders try to improve one, another might change unexpectedly. The statistical tools like principal component analysis (PCA) and cluster analysis help breeders to see patterns and relationships among traits and group the genotypes with similar characteristics (Ali *et al.*, 2015). The phenological plasticity of *Brassica juncea*, particularly concerning flowering time, presents significant trade-offs for varietal development. Early-flowering genotypes may confer mechanisms that facilitate drought escape which mostly comes at the cost of compromised seed size and oil contents (Nanda *et al.*, 1996). In contrast, late-flowering genotypes frequently exhibit improved yield potential. Although, this advantage is offset by higher susceptibility to terminal heat stress during the reproductive phases of the crop where grain filling takes place. The optimization of this temporal balance is therefore important in the breeding priorities for cultivar improvement in the future.

Biotic and abiotic stresses significantly affect both quantitative yield parameters and qualitative oil attributes in oilseed crops (Yadav *et al.*, 2022). Consequently, breeding programs must address these interconnected challenges to develop genotypes with superior stress resilience and phenotypic stability. Cutting-edge techniques like as genomic prediction and marker-assisted selection (MAS) present viable strategies to accelerate genetic gains by early detection of offspring with desired allelic combinations. However, in natural production systems, a thorough evaluation of germplasm accession for several agaronomic and oil quality features is required (Veerala *et al.*, 2021). In order to explain the correlated responses and identify genotypes displaying superior agronomic and oil quality attributes, the current study was designed to characterize twelve advanced mustard genotypes using multivariate analysis such as principal component analysis (PCA), correlation, and cluster analysis.

MATERIALS AND METHODS

Experimental Site and Design

The field trial of the present study was conducted during the cropping season of 2024-2025 at the Regional Agricultural Research Institute (RARI), Bahawalpur, Pakistan (29°23'52.044" N latitude and 71°40'30.72" E longitude). The soil type of the the experimental site was sandy loam, which is ideal for growing mustard because of its adequate drainage and moderate fertility. For this experiement, a triplicate randomized complete block design (RCBD) was used to assess twelve advanced mustard genotypes including check varieties. Four equal rows of length 5 meters of each genotyoe with row-to-row and plant-to-plant distances at 45 cm and 15 cm, respectively, while the plot area was 9 m^2. The plots received optimized fertilizers, regular irrigations, and proper weed management.

Data recoding for yield and morphological traits

The data for different morphological and yield attributes were recorded from five gaurded plants that were randomely selected from the plot mentioned above. The data for days to flowering (DTF) were recorded as the number of days from sowing until 50% of plants per plot showed the flowering (Zhang *et al.*, 2017). Days to maturity (DTM) were determined as the duration from sowing to physiological maturity, identified by pod desiccation and characteristic pigmentation changes in >80% of pods. Similarly, plant height (PH) was assessed at physiological maturity from soil surface to apical meristem (Ali *et al.*, 2009). Number of primary branches was counted to assess architectural plasticity. For the determination of 1000-seed weight (TSW), cleaned, 200 dried seeds were counted and weighed and then multiplied with 5. Seed yields in g were determined from each plot under experimentation and then kg/ha was calculated on the basis of this data.

Measurement of oil quality parameters

Oil content was quantified via Soxhlet extraction apparatus utilizing petroleum ether (40–60°C boiling range) following ISO 659:1998 principles, expressed as percentage of seed dry mass (Saini & Keum, 2018). However, oleic acid and saturated fatty acids were determined according to Raza *et al.*, 2009.

Statistical analysis

The data from different morphological and oil parameters were subjected to Analysis of Variance (ANOVA) under RCBD scheme. For the comparison of means, least signifcant different (LSD) test with 95% level of confidence (α=0.05). Multivariate relationships were determined through Principal Component Analysis (PCA), while genetic deviations were assessed via cluster analysis based on the PC analysis using R studio v4.3.2.

RESULTS

Performance of mustard genotypes for different parameters

The figure presents boxplots comparing 12 advanced mustard genotypes on 10 agronomic and oil quality traits: days to flowering, days to maturity, plant height, branches per plant, 1000 seed weight, seed yield (g/plot and kg/ha), saturated fatty acids, oleic acid content, and total oil content. Letters above boxplots denote statistically significant differences (LSD test) between genotypes at $p < 0.05$. LSD values provide thresholds for significance.

The comparative analysis of 12 advanced mustard genotypes reveals significant variation across key agronomic and oil quality traits. Genotype BR_L2-409 consistently showed early flowering and maturity, with the shortest duration to flowering (~44 days) and maturity (~145 days), compared to later genotypes such as BR_L2-404 and BR_L2-405 which flowered and matured significantly later (Figures A and B). In terms of plant architecture, BR_L2-404 was the tallest (~165 cm), whereas BR_L2-407 was among the shortest, indicating diversity in plant height within these genotypes (Figure C). Branching per plant, an important yield component, was highest in BR_L2-409, reflecting its potential for more pod sites, while BR_L2-402 had the least branches (Figure D). Seed traits further highlighted BR_L2-409's superiority, producing the heaviest 1000 seed weight (~5.2 g) and achieving the highest seed yields both per plot (~2200 g) and per hectare (~2400 kg), markedly outperforming lower-yielding genotypes like BR_L2-406 (Figures E, F, and G). Regarding oil composition, BR_L2-409 had a favorable fatty acid profile with relatively low saturated fatty acids (~5%) and the highest oleic acid content (~23%), beneficial for oil stability and nutritional quality, while genotypes such as BR_L2-404 showed higher saturated fatty acid levels (~6%) (Figures H and I). Additionally, BR_L2-409 and BR_L2-408 demonstrated the highest total oil content (~40%), an economically important trait, whereas BR_L2-402 recorded the lowest oil content (~36%) (Figure J). Overall, BR_L2-409 emerged as a promising genotype combining early maturity, high yield, and superior oil quality traits, making it a strong candidate for breeding programs focused on improving both agronomic performance and oil characteristics. In contrast, genotypes like BR_L2-404 and BR_L2-405, although taller and later maturing, exhibited higher saturated fatty acids and delayed phenology, which may limit their adaptability or oil quality for certain applications. These findings emphasize the trade-offs between phenological traits, yield components, and oil quality, highlighting the importance of integrated selection in mustard improvement.

Among the 12 advanced mustard lines, BR_L2-409 demonstrates superior performance in key agronomic (early flowering/maturity, high seed weight, branches) and oil quality parameters (high oleic acid and oil content), making it a promising candidate for breeding programs aiming at high yield and oil quality. Conversely, BR_L2-404 and BR_L2-405 show traits indicative of later maturity and higher saturated fat, which may limit their desirability depending on target environments and market needs.

Correlated response of different parameters in mustard genotypes

The correlation matrix in Figure 2 illustrates the pairwise relationships among key agronomic and oil quality traits in 12 advanced mustard genotypes, using colored pie charts where red indicates positive correlation and blue indicates negative correlation, with intensity reflecting correlation strength. Phenological traits, days to flowering (DTF) and days to maturity (DTM), exhibited a very strong positive correlation with each other (close to 1), confirming that genotypes which flower late also mature late. Both DTF and DTM showed strong positive correlations with seed yield components

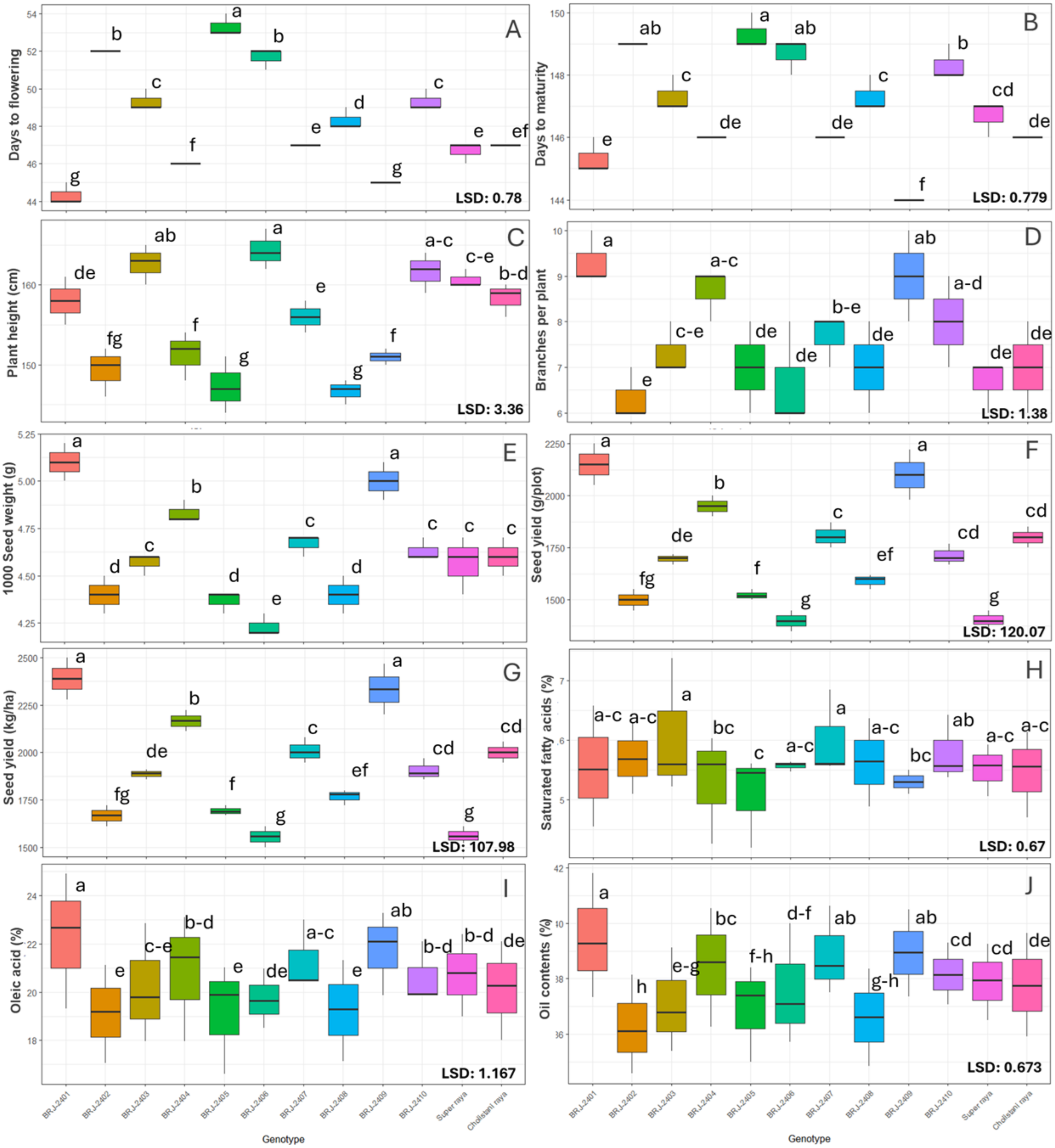

Figure 1. Performance of different advanced mustard genotypes for yield and oil attributes. The box plots demonstrate the means of different agronomic and oil quality attributes. The means were compared using the least significant difference (LSD) test. Different letters on different means demonstrated that they are significantly different from each other.

such as 1000 seed weight (TSW), oil content (OC), and oleic acid (OA), indicating that delayed flowering and maturity tend to be associated with higher seed size, oil concentration, and favorable fatty acid composition. In contrast, DTF and DTM were strongly negatively correlated with saturated fatty acids (SFA), suggesting that earlier flowering genotypes tend to have higher saturated fat content in seeds. This inverse relationship could reflect biochemical trade-offs affecting oil composition depending on phenology. Plant architectural traits, including branches per plant (BPP) and plant height (PH), also showed significant positive correlations with TSW, OC, and OA, highlighting the contribution of plant vigor to oil-related traits.

https://doi.org/10.55627/pbiotech.003.02.1353

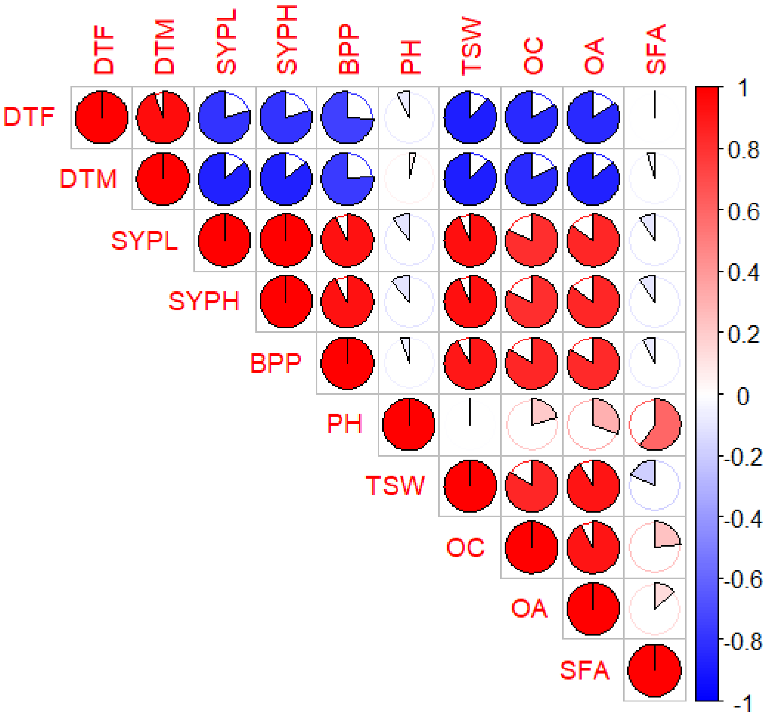

Figure 2: Correlation analysis of mustard agronomic and oil traits. The red color shows a significant positive correlation while the blue color demonstrates the negative significant correlation. All other colors display non-significant correlations.

However, PH had a slight negative correlation with SFA, implying taller plants tend to have lower saturated fatty acid content. Seed quality traits were tightly interrelated: TSW, OC, and OA were all highly positively correlated, reflecting that larger seeds often contain higher oil content and oleic acid percentage. Meanwhile, SFA was negatively correlated with TSW, OC, and OA, emphasizing the inverse relationship between saturated fat content and desirable oil quality parameters. Interestingly, seed yield per plot (SYPL) and seed yield per hectare (SYPH) showed strong positive correlations with each other and moderate positive correlations with oil content and oleic acid but had negative correlations with phenological traits, indicating that genotypes with longer and more siliques tend to mature earlier and possess better oil quality profiles. Altogether, the correlation analysis reveals that later-maturing genotypes generally produce larger seeds with higher oil and oleic acid content but lower saturated fatty acids. Plant vigor traits also positively influence oil yield and quality. These interdependencies are critical for selecting mustard genotypes with an optimal balance of growth duration, yield potential, and oil quality.

Principal Component Analysis

The Principal Component Analysis (PCA) biplot (Figure 3) provides a visual summary of the relationships among mustard genotypes and their measured traits, complementing the correlation analysis findings. The first two principal components together explain a substantial 88.2% of the total variation (PC1 = 70.5%, PC2 = 17.7%), highlighting the major axes along which genotypes and traits differ.

The first principal component (PC1), which captures the largest share of variability, clearly separates genotypes based on traits related to seed quality and yield, such as 1000 seed weight (TSW), oil content (OC), oleic acid (OA), and branching per plant (BPP). These traits cluster together and point towards the negative side of PC1, indicating they are positively correlated with one another, as also demonstrated by the strong positive correlations observed in Figure 2. This cluster represents genotypes characterized by larger seeds, higher oil concentration, and better fatty acid profiles, reflecting favorable agronomic performance. On the opposite side along PC1 lie saturated fatty acids (SFA) and plant height (PH), traits that contrast with seed and oil quality. The negative association of SFA with oil content and oleic acid seen in the correlation matrix is also echoed here by the opposing directions of their vectors in the biplot. Taller plants with higher saturated fatty acid content tend to cluster apart from high-oil, high-oleic acid genotypes, suggesting a trade-off between plant vigor, seed quality, and oil composition. The second principal component (PC2) mainly separates genotypes based on their phenological characteristics, particularly days to flowering (DTF) and days to maturity (DTM).

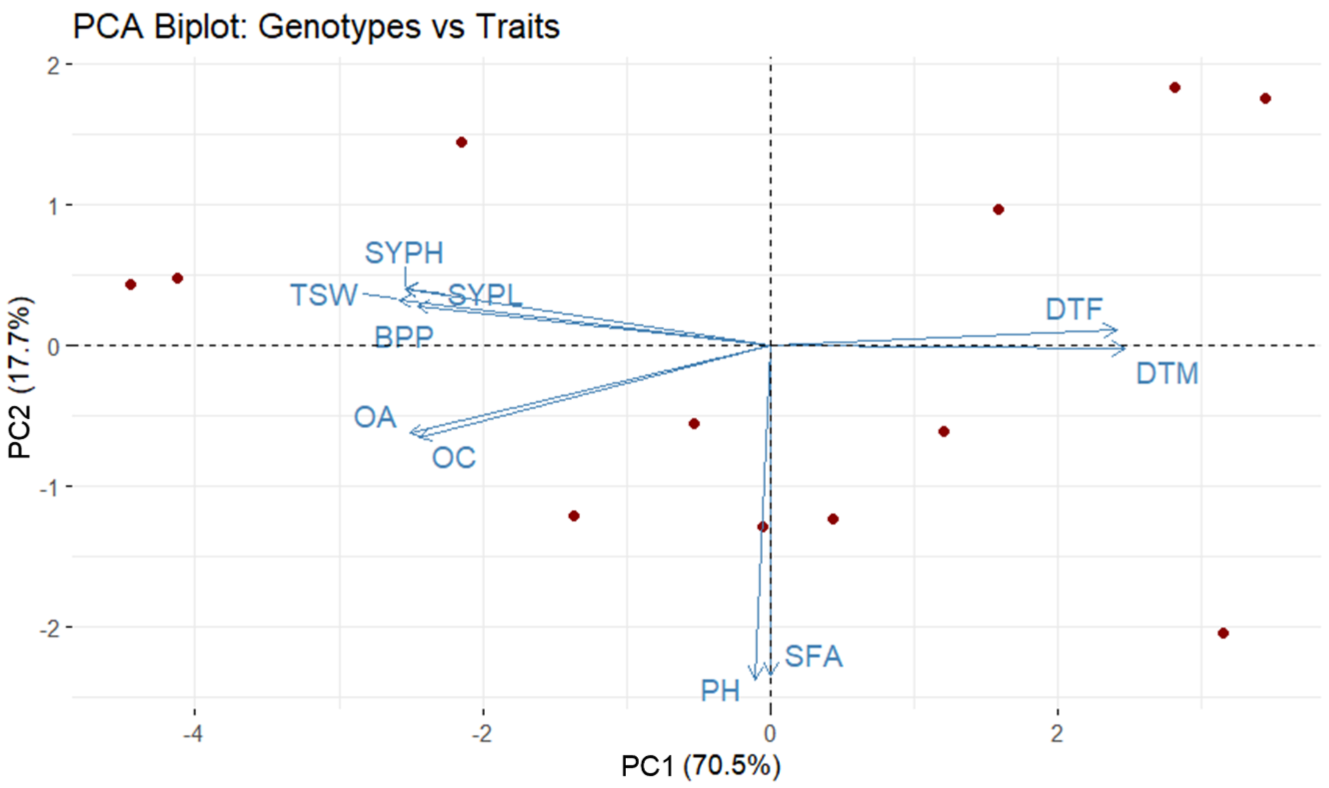

Figure 3. Principal Component Analysis (PCA) biplot shows relationships among mustard genotypes and their measured traits. PC1 contributed maximum (70.5%) to the total diversity followed by PC2 (17.7%) and were plotted to display character associations.

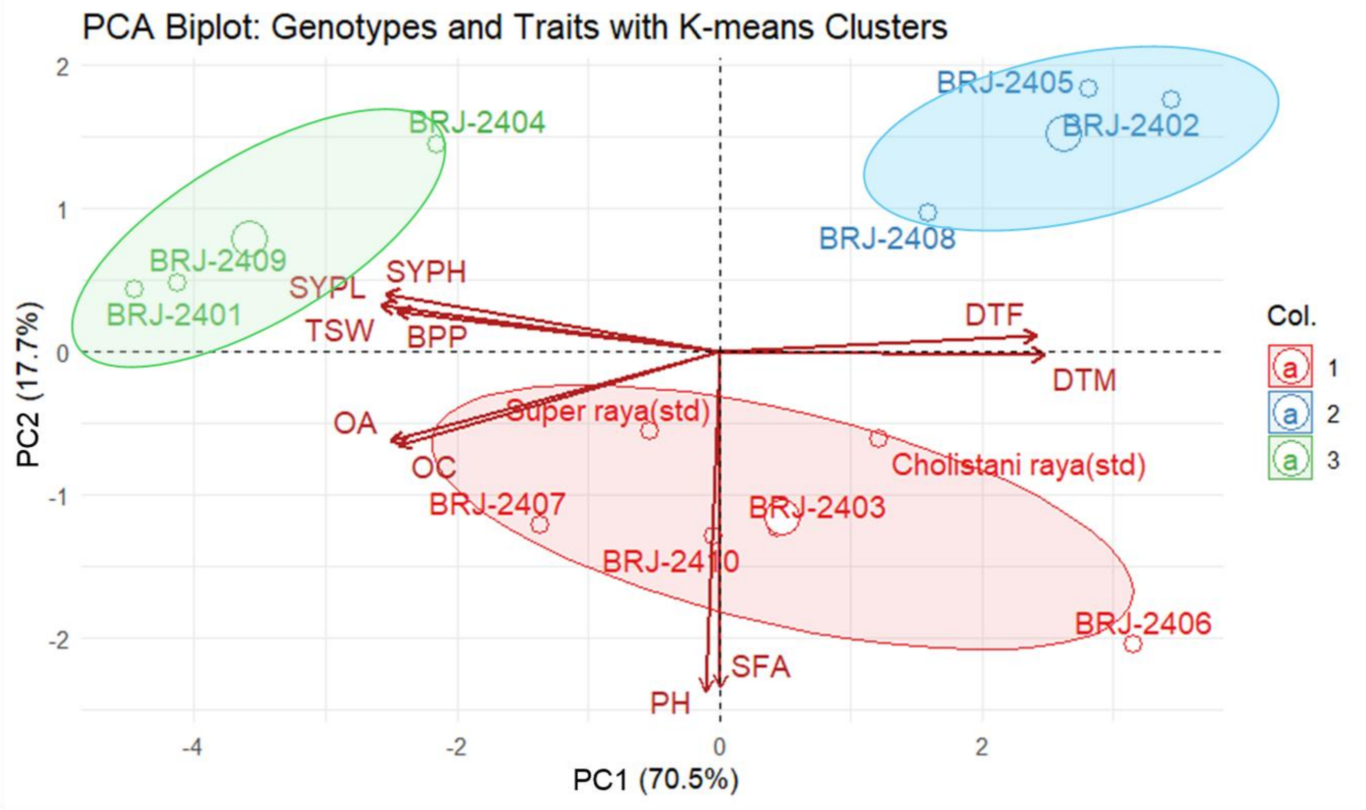

Figure 4. Clustering of genotypes based on the PC coordinates of the first two PCs conditioning the highest diversity. Different clusters show different colors.

These traits point strongly toward the positive side of PC2, highlighting differences in the timing of crop development. Essentially, genotypes that flower and mature later cluster apart from those that develop earlier. This is visually supported by the biplot, where phenological traits are nearly at right angles (orthogonal) to seed yield and oil quality traits, matching the slight negative correlations seen in the earlier analysis. Together, the principal component analysis (PCA) and correlation results provide a clear understanding: genotypes with delayed flowering and maturity often produce larger seeds with higher oil content, including increased oleic acid, and show greater branching. On the other hand, early flowering genotypes tend to have higher saturated fat content and follow different growth patterns. These insights are invaluable for breeding programs that aim to strike the right balance between yield, oil quality, and environmental adaptation by selecting genotypes that fit well within these trait groupings.

Multivariate characterization of genotype clusters

Principal component analysis (PCA) coupled with K-means clustering revealed three phenotypically discrete clusters among the 12 advanced mustard genotypes (Figure 4), delineated by differential expression of agronomic and oil quality traits. This integrated analytical approach facilitates the identification of genotypic cohorts with convergent phenotypic architectures, thereby informing strategic breeding selections. Cluster 1 comprised of 5 genotypes (BRJ-2403, BRJ-2406, BRJ-2410, Super raya, Cholistani raya) and was the biggest of them all. The genotypes within this cluster exhibited pronounced vegetative vigor, manifesting as significantly greater plant stature (PH; $p<0.01$). However, this cluster demonstrated compromised oil quality parameters, characterized by elevated saturated fatty acid (SFA) concentrations concomitant with reduced 1000-seed weight (TSW), oleic acid (OA) content, and total oil content (OC). The inverse relationship between vegetative biomass accumulation and seed/oil quality metrics suggests potential metabolic trade-offs limiting their suitability for premium oil production systems.

Similarly, cluster 2 (BRJ-2402, BRJ-2405, BRJ-2408) was characterized by extended phenological phases, evidenced by significantly delayed flowering (DTF) and physiological maturity (DTM) relative to other clusters. Such prolonged vegetative and reproductive durations engender context-dependent adaptability, potentially advantageous in environments with protracted growing seasons but disadvantageous under terminal heat stress. Seed yield components and oil quality traits exhibited intermediate expression, positioning this cluster phenotypically between early and late developmental extremes. The genotypes in this cluster 3 (BRJ-2401, BRJ-2404, BRJ-2409) demonstrated superior reproductive architecture, including enhanced branching capacity (BPP), seed yield per plot (SYPL) and seed yield per hectare (SYPH), and significantly greater TSW ($p<0.01$). Altogether, PC analysis facilitated clustering exceeds univariate analysis by resolving complex trait associations. This multivariate study will enable breeders to identify genotypes with specific production objectives, thereby optimizing selection efficiency within targeted breeding pipelines.

DISCUSSION

Performance of mustard genotypes for different parameters

The evaluation of 12 advanced mustard genotypes showed clear differences in important agronomic and oil quality traits, highlighting the rich genetic diversity available for mustard improvement. Among the 12 genotypes under study, BR_L2-409 performed best for flowering and maturity as compared to other advanced lines. This earliness trait could be helpful in avoiding late season heat stress, that is now a days, considered as a major challenge in many mustard growing regions (Devi and Sharma, 2017). Similarly, early maturity shortens the crop cycle and could be helpful for the farmers to secure better yields in a shorter period. Plant height showed significant variation among the genotypes where BR_L2-404 was the tallest, while BR_L2-407 showed minimum plant height. This variation for plant height is vital because plant branching and height can affect the ability of crops to intercept more sunlight and, ultimately, more production (Rathore *et al.*, 2023). BR_L2-409 exhibited a greater number of branches and obviously more yield per plant that was due to higher 1000 grain weight in this genotype. This confirms earlier research showing that higher seed size frequently corresponds to higher total mustard yield (Sur *et al.*, 2023). Numerous factors affect seed yield; thus, it is especially beneficial to have a genotype that strikes a balance between early maturity, strong branching, and seed size (Ali *et al.*, 2011).

Given the economic and nutritional significance of mustard, oil quality is one of the most important factors. With a fatty acid composition that featured more oleic acid and less saturated fat, characteristics that enhance oil stability and provide health advantages, BR_L2-409 once more demonstrated promise (Sur *et al.*, 2023). Its commercial value was further enhanced by having one of the highest total oil contents. However, even though they were taller and matured later, genotypes like BR_L2-404 and BR_L2-405 had slower development and greater levels of saturated fat, which may make them less suitable for areas where oil quality and speed of turnaround are important considerations.

https://doi.org/10.55627/pbiotech.003.02.1353

Correlation analysis

Study of character associations is essential for breeding strategies aimed at crop improvement (Ali *et al.*, 2009; Anwar *et al.*, 2009). Several important correlations were found in the present study. For instance, an expected strong correlation was found between days to flowering (DTF) and days to maturity (DTM) which suggested that genotypes that flower later also likely to mature later. This correlation is consistent with earlier results demonstrated that the phenological features in Brassica species are genetically linked to each other (Saini *et al.*, 2023). Similarly, seed yield and oil quality attributes like 1000 seed weight (TSW), oil content (OC), and oleic acid (OA) content showed favorable correlations with both DTF and DTM. This suggests that longer growing periods cause genotypes to allocate more resources to seed development, resulting in larger seeds with higher oleic acid and oil content. Similar correlations have been documented in mustard breeding efforts, highlighting the importance of phenological characteristics in evaluating seed quality (Saini *et al.*, 2023). On the other hand, phenological parameters (i.e., DTF and DTM) and saturated fatty acid (SFA) levels were negatively correlated, with earlier genotypes being linked to higher SFA content. One possible reason for this negative connection could be biochemical trade-offs in oil production pathways. In these pathways, quick development may restrict the buildup of unsaturated fatty acids. These results are similar to those of other studies that found oils with more saturated fat are often made by genotypes that mature early (Hemingway *et al.*, 2014; Sudarić and Vratarić, 2002). TSW, OC, and OA were positively correlated with branches per plant (BPP) and plant height (PH), indicating that more vigorous plants produce higher-quality seeds. This idea is supported by evidence that shows that growing plants well can help make the oil components we want to collect (Rathore *et al.*, 2023). Higher seed yields are not always linked to lower oil quality. There is a positive relationship between seed yield per plot (SYPL) and seed yield per hectare (SYPH). There is also a relationship between oil content and oleic acid. However, there were negative relationships between these yield features and phenological traits. This indicates that longer silique genotypes have superior oil quality profiles and tend to mature earlier. This observation shows how important it is to consider how different traits interact, and to use selection techniques that work together when breeding mustard (Saini *et al.*, 2023).

Principal component analysis and cluster analysis

The Principal Component Analysis (PCA) explained the connections between the important agronomic and oil quality traits in the 12 advanced mustard genotypes. When we look at the whole picture, the first two principal components explained 88.2% of the variation. PC1 explained 70.5% and PC2 explained 17.7%. These qualities are important for distinguishing genotypes because they explain a lot of the variation. PC1 mainly separated genotypes based on characteristics related to how much they produced and the quality of their seeds. These characteristics included the weight of 1000 seeds, the amount of oil and oleic acid, and the number of branches per plant. There was a clear connection between these qualities, as they appeared to be grouped together. On the other hand, plant height and saturated fatty acids were found on the other side of PC1. This indicates a negative relationship with the previously listed characteristics. This result is similar to other research that found a trade-off between plant vigor (how well the plant grows) and seed quality in breeding programs for mustard (Verma *et al.*, 2016). Using their phenological parameters (i.e., DTF and DTM), PC2 was able to tell the difference between the genotypes. Early flowering genotypes were on the negative side of PC2, while later flowering and maturity durations were on the positive side. According to Avtar *et al.* (2014), this difference shows that the timing of features has a big impact on the overall diversity among the genotypes. The PCA data showed three different genotype groups. Group 1 included genotypes with lower seed quality, but higher plant height and saturated fatty acid content. Genotypes in Cluster 2 had longer phases of development and showed attributes related to oil quality and seed yield that were in the middle. In addition to having better qualities, plants in Cluster 3 had better reproductive architecture, as seen by increased branching and higher seed output. In mustard breeding, these groups show the complex connection between oil quality and how well the crop performs, as Saleem *et al.* (2017) pointed out.

CONCLUSION

The study found that the performance and quality of the oil from 12 advanced mustard types varied a lot. Genotype BR_L2-409 stood out as a good plant to breed from because it has special qualities, produces a lot of seeds, and matures early. Important connections were revealed by correlation and PCA analyses: early genotypes contain more saturated fats, while later-maturing genotypes typically produce larger seeds with higher oil and oleic acid content. By studying how certain traits interact, the researchers were able to group genotypes into categories and identify the balance between oil quality and plant vigor. For breeders looking to balance how much is produced, the quality of the oil, and how well the plants adapt when improving mustard, these findings offer helpful direction.

AUTHOR CONTRIBUTIONS

Hussain, Akhtar, Akram, Chaudhary; Writing – original draft; Bukhari, Hafeez, Ghazali, Saleem, Shah, Ijaz; Writing – review & editing, Hussain, Akhtar, Bukhari, Ghazali, Hafeez; Formal analysis, Conceptualization, Validation, Supervision, Resources, Funding acquisition; Akram, Chaudhary, Ijaz, Saleem; Statistical Analysis, Graphic improvement. Shah; Oil quality analysis.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ACKNOWLEDGEMENTS

The authors express their deepest gratitude to Mr. Muhammad Irfan Yousaf, Scientific Officer, Cotton Research Station, Bahawalpur and Muhammad Zubair, Senior Scientist, Regional Agricultural Research Institute, Bahawalpur for their help in reviewing and improving the manuscript.

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