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Research Article

Genotypic Performance and Assessment of Selection Criteria for Yield and Oil Content in Advanced Lines of *Brassica juncea*

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ABSTRACT

The current study was undertaken to assess genotypic performance and to develop a reliable criterion for the selection of improved seed yield and oil quality in advanced lines of mustard (*Brassica juncea* L.). Fourteen advanced breeding lines and a commercial check were evaluated in a field experiment using a randomized complete block design with three replications. Various phenological parameters, yield components, and oil quality parameters such as oil content, oleic acid and saturated fatty acids were measured and analyzed using analysis of variance, correlation, path analysis, principal component (PCA), cluster and path coefficient analysis to understand the relationships among the characters and genetic divergence. Among all genotypes, L13 (BR-13) performed better than all others, followed by L9 (BR-9) and L8 (BR-8), while L2 (BR-2) and L5 (BR5) were the poorest-performing genotypes. Thus, the current study revealed that the genotype L13 (BR-13) is the most promising line with early flowering, high seed yield, high oil content, high oleic acid and low saturated fatty acids. Seed yield was found to be significantly and positively associated with number of branches per plant, thousand seed weight, oil content and oleic acid, while saturated fatty acids were found to be negatively associated with yield and oil quality characters. PCA analysis indicated that the first two components accounted for the largest proportion of total variation, separating the genotypes into distinct groups based on yield-oil quality complexes and phenological characteristics. Cluster analysis grouped the genotypes into distinct classes based on early-flowering, high-yielding oil-rich lines and late-flowering, vegetatively vigorous but oil-inefficient types. Path analysis further confirmed the role of thousand seed weight and number of branches per plant as important indirect contributors to oil content through seed yield. The results provide clear selection criteria and demonstrate the value of integrating multivariate tools for accelerating genetic gains in mustard breeding programs.



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Keywords: Advance breeding lines, Cluster analysis, Multivariate analysis, Mustard, Oil contents, Principal component analysis, Seed yield, Selection criteria

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INTRODUCTION

Mustard (*Brassica juncea* L.) is one of the major oilseed crops that is grown on a large scale in South Asia and it plays a vital role in maintaining the livelihood of agricultural communities in the region (Panjabi *et al.*, 2019). Unlike canola oil, mustard oil is characterized by its distinct pungent taste, which is the basis for its

wide acceptance in culinary practices (Saleem et al., 2023). Its oil is preferred due to its nutritional and functional benefits. Mustard seeds are rich in appreciable amounts of unsaturated fatty acids, such as oleic and linoleic acids, which have been widely documented to display cardioprotective effects (Poddar et al., 2022). Its oil has also been used for its antimicrobial properties and medicinal uses (Akhtar and Khan, 2024). *B. juncea* has been found to have adaptability to varied agricultural practices due to its fast growth rate, adaptability and ability to thrive in marginal soil conditions. Its use in agricultural practices helps in enhancing soil structure and fertility, thereby ensuring sustainable agricultural practices (Ozturk et al., 2026). In Pakistan, mustard is the third most significant oilseed crop after cotton and sunflower and is mainly grown in the provinces of Punjab, Khyber Pakhtunkhwa and specific regions of Sindh (Khan et al., 2024; Vessar et al., 2025). However, the current production of domestic mustard is not enough to meet the domestic demand, resulting in a heavy reliance on imported edible oils (Hussain et al., 2023).

Improving the productivity of indigenous mustard in the country is a promising approach to minimize import costs, increase agricultural income and enhance nutritional status at the population level (Panjabi et al., 2019). However, the pace of improvement in productivity and quality of oil in Pakistan is constrained by the lack of genetic diversity in the locally grown mustard germplasm, which also shows low resistance to major biotic and abiotic stresses. The success of breeding programs relies greatly on the availability of enough genetic variation associated with both local and exotic germplasm material (Wang et al., 2017; Jordan et al., 2011). Unfortunately, the germplasm base of several elite mustard cultivars grown in Pakistan is narrow, thus limiting the development and growth of improvement of the potential of the yield, the quality of oil and the resistance to stresses.

The introgression of diverse genetic material through targeted hybridization helps to integrate desirable traits such as early maturity, strong plant type, increased seed size and improved fatty acid composition (Meena et al., 2025). Among the breeding targets, the improvement of oil quality has gained increasing importance, particularly through the enhancement of oleic acid and a reduction in saturated fatty acids, in response to the growing awareness and demand for healthier edible oils (Sagun et al., 2023). Simultaneously achieving high oil quality and high and stable seed yield is difficult due to the complex and interdependent nature of agronomic, phenological and biochemical traits (Sachan et al., 2024). Phenological traits, particularly flowering and maturity times, are critical in crop adaptation and productivity for *B. juncea*. Early-flowering lines may escape terminal drought and heat stress but are often linked with smaller seed size and lower oil content (Panjabi et al., 2019). Late-flowering lines may reach higher yield potential through longer growth duration, but they are often more vulnerable to terminal heat stress at the reproductive and grain filling phases, which can negatively impact both yield and oil quality (Prasad et al., 2008). Managing these phenological trade-offs is a critical challenge in mustard varietal development. Mustard yield and oil quality are complex quantitative traits controlled by multiple genes and are largely affected by environmental factors (Zandberg et al., 2022; Dey et al., 2024; Kumar et al., 2022). Branching per plant, plant height and thousand seed weight are direct contributors to final seed yield, while the ratio of oil content and fatty acid composition determines the economic value of the crop (Shelly, 2020; Sultana, 2017). Therefore, to develop effective and balanced breeding strategies that target yield stability along with oil quality improvement, it is necessary to understand the relationships among these traits.

Multivariate statistical methods including correlation analysis, principal-component analysis (PCA) and cluster analysis are equally useful in unravelling the complex interrelationships between phenotypic characteristics and in measuring genetic specialization between cultivars. Such methodological solutions contribute to identifying the most important attributes that cause phenotypic variability, enable the systematic categorization of genotypes and can be used as potent breeding program tools, which in turn leads to the formation of robust selection indices (Asadullah et al., 2024; Dash, 2021). In this regard, the current study was undertaken to compare the superior models of *B. juncea* at the field level employing a multivariate approach. The aims of the present study were to understand the relationships among the traits, measure the degree of genetic diversity and to detect the genotypes with an optimal index of yield-related and oil-quality characters for future mustard improvement.

MATERIALS AND METHODS

Experimental Site and Crop Season

The experiment was carried out during the Rabi season of 2024-2025 to evaluate the agronomic performance, yield potential and oil quality attributes of highly developed *Brassica juncea* lines. The experimental material, obtained from the Regional Agricultural Research Institute (RARI), Bahawalpur, included fifteen lines of *B. juncea*, which are highly developed breeding lines selected on the basis of diversity in phenology, plant type, yield attributes and oil quality traits. The experimental material comprised fourteen advanced breeding lines (L1-L14, namely BR-1 to BR-

14) and one locally adapted check variety (Cholistani Raya) widely cultivated under the prevailing agro-ecological conditions of the region.

Experimental Design and Crop Management

The experiment was laid out as a randomized complete block design (RCBD) with three replications to reduce variability due to environmental factors. Each experimental unit consisted of two rows for each entry, with a row length of 5.0 m. The distance between rows was maintained at 45 cm and the plant-to-plant distance within rows was 15 cm. The total plot size for each entry was 5.0 m × 0.90 m. The sowing of the experimental material was done by manual drill on October 15, 2024, using an equal seed rate and depth to ensure equal establishment of the crop. All plots were given equal agronomic management during the growing season. Fertilizer application, irrigation, weed control and plant protection practices were done as per the recommendations of the region for growing mustard. No special treatment was given to the experimental plots and the differences among the entries were measured without any treatment effect.

Data Recording and Trait Measurement

Phenological, morphological and yield-related characteristics were recorded using standardized methods regularly used in *Brassica juncea* assessment studies (Shyam *et al.*, 2022; Alam *et al.*, 2014). All parameters used in this study were recorded following standard procedures commonly adopted in *B. juncea* evaluation studies (Shyam *et al.*, 2022; Alam *et al.*, 2014). Phenological data were recorded on a plot-by-plot basis. Days to flowering (DF) were measured as the period from sowing to the point when about 50% of the plants in a plot had reached the stage of at least one open flower. Days to maturity (DM) were measured as the period from sowing to the point when about 80-90% of the siliques turned brown and seeds reached physiological maturity, as per the method described by Aslam and Qayyum (2025). For morphological and yield component traits, five representative plants were randomly identified from the central rows of each plot at physiological maturity. Plant height (PH) was measured in centimeters from the soil surface to the tip of the main inflorescence using a measuring rod, while branches per plant (BPP) were counted by identifying all the primary branches arising from the main stem, as per Margam *et al.* (2022). After harvest, plants from each plot were threshed separately to estimate yield parameters. Thousand-seed weight (TSW) was measured by counting and weighing 1000 clean, air-dried seeds using an analytical balance and expressed in grams. Seed yield per plot (SY, g) was recorded as the total seed yield from each plot and subsequently converted to seed yield per hectare (SY, kg ha⁻¹) using standard plot-to-area conversion factors, as per established mustard yield evaluation protocols (Aslam and Qayyum, 2025; Mohapatra, 2017).

Determination of Oil Content and Fatty Acid Composition

Oil percentage was estimated using Soxhlet extraction. Finely powdered seed samples (5 g) were oven-dried to a constant weight and extracted with petroleum ether (boiling point range 40-60 °C) for 6-8 hours. After removing the solvent, oil percentage was calculated on a dry weight basis and expressed as a percentage of seed weight, as described in (Latimer Jr, 2012) and in earlier Brassica-related studies (Kumar *et al.*, 2023; Liu, 2021). For fatty acid estimation, the extracted oil was transformed into fatty acid methyl esters (FAMES) following standard transesterification techniques and subjected to gas chromatography (GC) with a flame ionization detector. Oleic acid (OA, %) and saturated fatty acids (SFA, %) were estimated by matching peaks with authenticated standards and expressed as a percentage of total identified fatty acids. The analysis procedure followed established methods in mustard and oilseed studies (Hossain *et al.*, 2018; Hamad and Chakraborty, 2024; Chakraborty *et al.*, 2025).

Statistical Analysis

All experimental results were processed using the R-Studio software (version 4.5). Analysis of variance (ANOVA) was performed using the randomized complete block design (RCBD) to assess the differences among genotypes for all traits and mean separation was carried out using the least significant difference (LSD) test at $p \leq 0.05$. Pearson's correlation analysis was used to examine the associations between phenological, agronomic, yield and oil quality traits. Multivariate analysis, including principal component analysis (PCA) and cluster analysis, was carried out on standardized variables to detect the primary sources of variation and to group genotypes based on overall similarity in traits. Path analysis was performed using a structural equation modeling (SEM) approach to decompose the direct and indirect contributions of the primary traits to seed yield and oil quality. All graphical outputs for the Results section were saved in PNG format.

RESULTS

Genotypic Variability for Phenology, Plant Architecture, Yield and Oil Quality Traits

The tested mustard genotypes showed highly significant variation ($p < 0.05$) for all the evaluated agronomic, yield and oil quality parameters, as shown in Figure 1.

Morphological and Biochemical Characterization of Germplasms

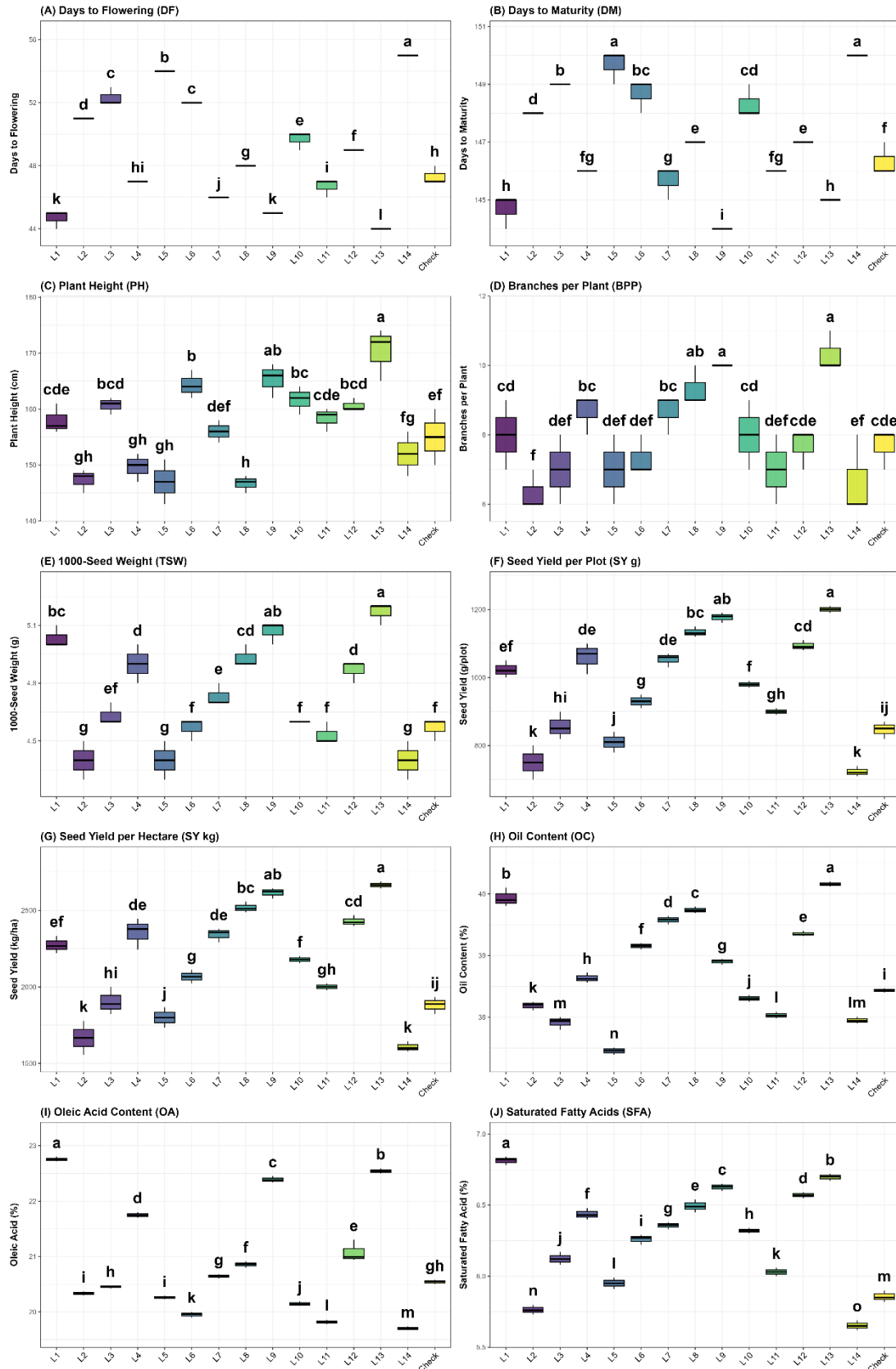
Boxplots represent variation across 3 replications; letters indicate LSD test ($p < 0.05$) groups.

Figure 1. Phenotypic data of superior mustard genotypes for agronomic and oil quality characters. Box-and-whisker plots show the interquartile range, with horizontal lines indicating median values and whiskers showing variability in each replication. Different letters above the box plots indicate statistically significant differences among genotypes according to the LSD test at $p < 0.05$; genotypes sharing the same letter are not significantly different.

The high degree of variation among the genotypes indicates a significant level of genetic diversity in the test material and the ability to distinguish between better and poorer genotypes. Days to flowering (DF) ranged from 44–46 days in early-flowering genotypes (L1, L7, L13) to 52–54 days in late-flowering genotypes such as L5 and L14 (Figure 1A). A similar trend was observed for days to maturity (DM), which varied from about 144 days in early-maturing lines to nearly 150 days in late-maturing genotypes (Figure 1B). Plant height (PH) also differed considerably among genotypes, ranging from about 145 cm to over 170 cm (Figure 1C). Branches per plant (BPP) showed clear variability, with higher branching observed in genotypes such as L9, L13 and L8 compared with lower branching in L2 and L5 (Figure 1D). Thousand seed weight (TSW) varied from about 4.3 g to 5.3 g, with heavier seeds recorded in L13, L9 and L10 (Figure 1E). Seed yield also differed markedly among genotypes, ranging from about 700 g per plot in low-yielding genotypes to more than 1100 g per plot in superior lines such as L13 and L9 (Figure 1F), corresponding to approximately 1800-2700 kg ha⁻¹ (Figure 1G). Oil quality traits also exhibited clear genotypic differences. Oil content (OC) ranged from about 37.5% to above 40%, with higher values observed in L13, L1 and L7 (Figure 1H). Oleic acid (OA) varied between approximately 18.5% and 23%, while saturated fatty acids (SFA) showed an opposite trend, with lower levels in high-performing genotypes and higher levels in poorer lines such as L5 and L14 (Figure 1I-J).

Correlation matrix among agronomic and oil quality traits

Correlation analysis (Figure 2) revealed strong and meaningful correlations among the traits studied. DF and DM showed an almost perfect correlation ($r = 0.97$), thus supporting their strong developmental link. Both traits showed significant negative correlations with SYg, SYkg, TSW, OC and OA (r values ranging from -0.65 to -0.85), thus implying that advanced phenological development was advantageous to reproductive efficiency and oil accumulation under the given environment. Seed yield-related traits (SYg and SYkg) showed high correlations ($r > 0.90$) and strong positive correlations with TSW ($r = 0.93$), BPP ($r = 0.88$), OC ($r = 0.79$) and OA ($r = 0.73$). These correlations highlighted the importance of sink size and assimilate partitioning in yield determination. Oil content and oleic acid content showed strong positive correlations ($r = 0.81$) and both traits showed strong negative correlations with SFA ($r = -0.81$). Plant height showed weak and nonsignificant correlations with yield and oil-related traits, thus implying that overgrowth is not directly beneficial to economic productivity.

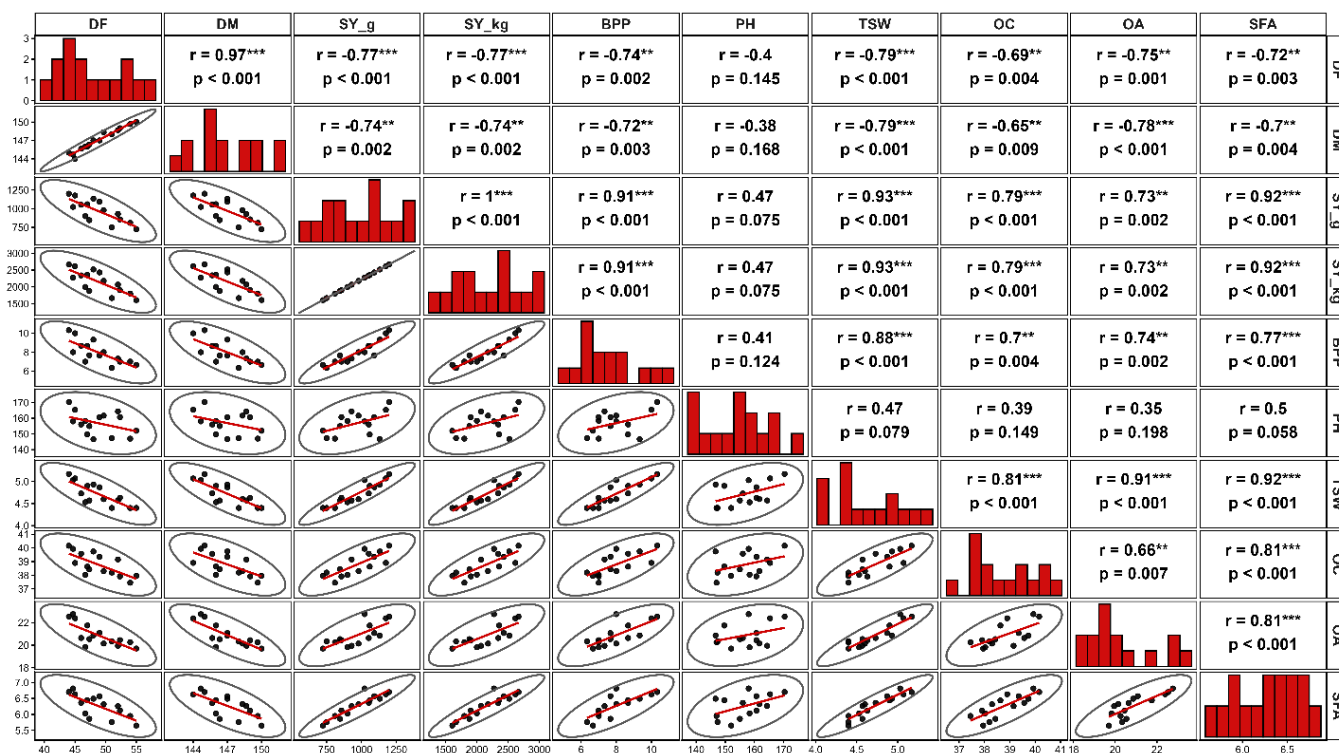


Figure 2. Correlation matrix showing the relationships between phenological, morphological, yield and oil quality traits in elite mustard lines. The upper panels show the Pearson correlation coefficients (r) and significance levels, whereas the lower panels show the bivariate scatter plots along with the trend lines. Abbreviations: DF = days to flowering; DM = days to maturity; PH = plant height; BPP = branches per plant; TSW = thousand seed weight; SYg = seed yield per plot; SYkg = seed yield per hectare; OC = oil content; OA = oleic acid; SFA = saturated fatty acids.

Path coefficient analysis of yield determination

Path analysis helped to understand the direct and indirect effects of phenological, morphological and seed-related variables on SY kg⁻¹ (Figure 3). TSW showed strongest positive direct effect on seed yield (0.62), thus confirming seed size as the key factor governing seed yield. This was further supported by the strong positive direct effect of OC on seed yield (0.73). BPP had a positive direct effect on seed yield (0.24). However, DF showed a negative association with BPP (−0.77) and seed yield, suggesting that delayed flowering may be associated with reduced branching and lower yield. DM had a weak negative direct effect on seed yield (−0.08) but had an indirect positive effect through its positive correlation with BPP (0.30). Plant height (PH) had a negligible direct effect on seed yield (0.07) and overall effects on seed yield were realized through indirect effects via TSW (0.14). DF and DM had a strong positive correlation (0.96), thus indicating that they had synchronized phenological development. However, their overall indirect effects on seed yield were negative due to negative effects on TSW and BPP. DF also had negative indirect effects on PH (−0.38) and TSW (−0.39), thus further reducing seed yield potential, as shown in Figure 3. The path analysis suggests that TSW, OC and BPP are strongly associated with seed yield, while prolonged phenological development negatively affects seed yield due to reduced branching and seed development.

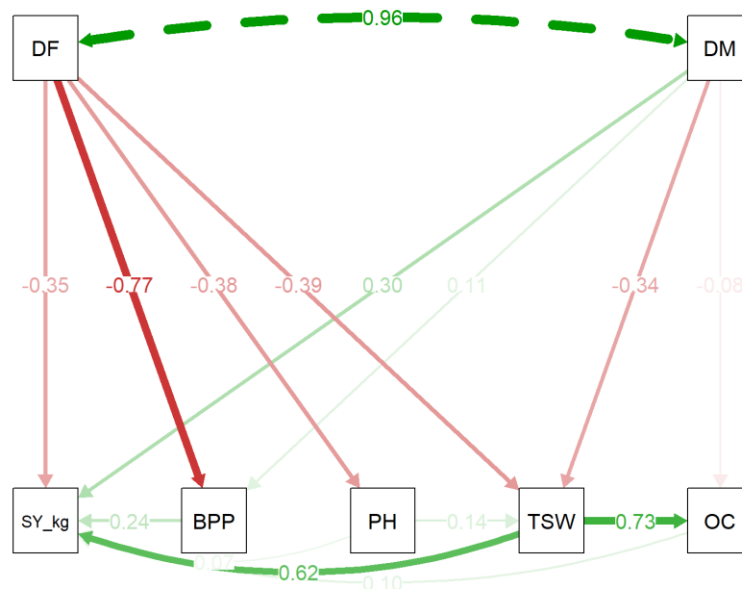


Figure 3. Path coefficient analysis showing direct and indirect effects of phenological, morphological and yield components on seed yield and oil content in mustard. Green arrows indicate positive effects and red arrows indicate negative effects, with arrow thickness proportional to effect magnitude. Values along paths represent standardized path coefficients. Abbreviations: DF = days to flowering; DM = days to maturity; PH = plant height; BPP = branches per plant; TSW = thousand seed weight; SY_g = seed yield per plot; SY_{kg} = seed yield per hectare; OC = oil content.

Principal component analysis and trait structure

Principal component analysis revealed a large degree of multivariate genetic divergence among the tested mustard genotypes. The first two principal components together accounted for 84.7% of the total phenotypic variation, with PC1 explaining 76.8% and PC2 explaining 7.9% of the variation (Figure 4A). The sharp cut-off in eigenvalues after PC2 indicates that most of the informative variation was captured in the first two dimensions (Figure 4B).

The loading values of PC1 were primarily determined by yield and oil quality-related traits such as seed yield (SY g and SY kg), thousand seed weight (TSW), oil content (OC), oleic acid (OA) and branches per plant (BPP), which all showed positive loadings and clustered together in the first dimension (Figure 4C). Saturated fatty acids (SFA) showed negative loadings, thus confirming their strong negative association with yield and desirable oil quality traits. This negative association clearly indicates a strong biochemical and agronomic trade-off between oil quality improvement and saturated lipid accumulation. The second principal component (PC2) was mainly characterized by phenological characters, specifically days to flowering (DF) and days to maturity (DM), which had strong positive loadings on this component (Figure 4C).

Hierarchical clustering based on PCA scores further resolved the genotypes into four distinct clusters, each characterized by contrasting trait assemblages (Figure 4B; Figure 5). Cluster I, represented by elite genotypes such as L9 and L13, comprised high-performing entries with heavier seeds, greater branching intensity, elevated oil content and higher oleic acid concentration, coupled with relatively early maturity. Cluster II grouped late-flowering and late-maturing genotypes, which exhibited moderate yield potential but accumulated higher levels of saturated fatty acids, limiting their suitability for premium edible oil production. Cluster III, including the check variety, consisted of intermediate genotypes displaying balanced performance across traits but lacking pronounced superiority. Cluster IV comprised low-yielding genotypes with inferior seed weight and oil quality attributes.

The heatmap results (Figure 5) also support these multivariate trends, showing the standardized expression of traits in the clusters. The high-performing clusters are characterized by high values of seed yield, thousand seed weight (TSW), oil content and oleic acid, while the clusters with late phenology have lower expression of these traits along with higher saturated fatty acid (SFA) content. The strong agreement among PCA (Figure 4A-C), hierarchical clustering (Figure 4B; Figure 4C) and heatmap visualization (Figure 5) underscores the robustness of the observed genotypic structure and confirms the presence of distinct, biologically meaningful genetic groups within the evaluated mustard germplasm.

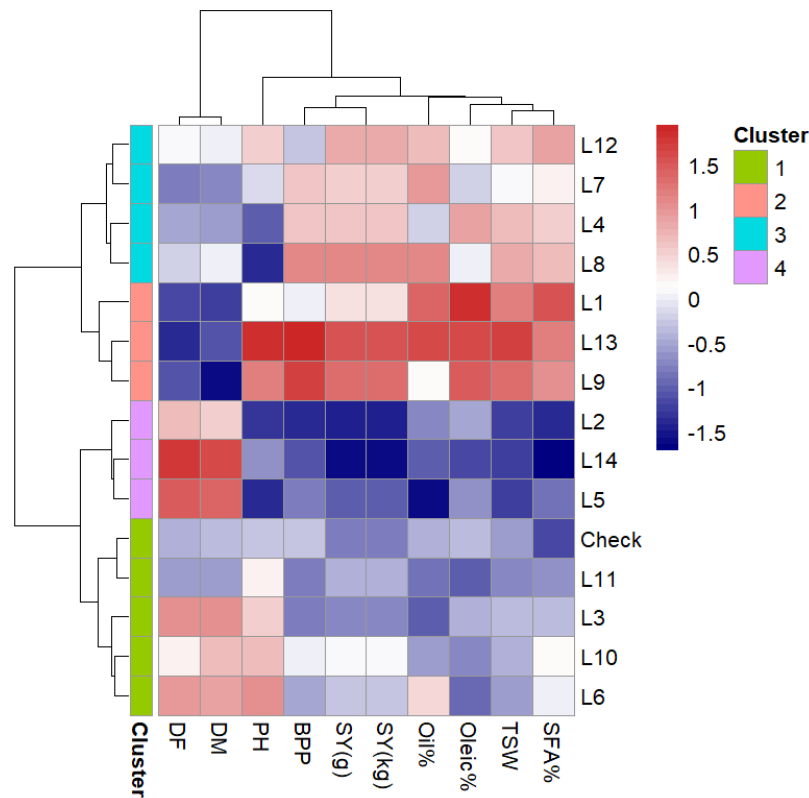


Figure 5. Heatmap of standardized expression of traits across mustard genotypes and clusters. The rows represent genotypes and the columns represent agronomic, yield and oil quality traits. Color bars show the expression of traits, with red showing higher standardized values and blue showing lower standardized values. Dendrograms show the similarity between genotypes and traits based on hierarchical clustering.

DISCUSSION

The present investigation revealed genotypic variation among the evaluated *B. juncea* advanced lines for phenological, morphological, yield and oil quality traits. This variation is a fundamental requirement for successful selection and improvement in oilseed crops, as emphasized by Choudhary *et al.* (2025), Aragi *et al.* (2023) and Perween *et al.* (2024). The unique genotype discrimination for traits further strengthens the effectiveness of the experimental material for the detection of superior breeding lines. The observed variation in days to flowering and maturity indicates differences in adaptation strategies of genotypes under the prevailing agro-climatic conditions. Early phenology was generally associated with better yield performance, which may be advantageous under terminal heat stress conditions commonly experienced in mustard-growing regions.

These results are in agreement with earlier findings of Saroj *et al.* (2021), Sandhu *et al.* (2020), Kaur *et al.* (2023) and Chugh *et al.* (2022), which highlighted the yield superiority of early phenology in Brassica species. Branches per plant showed a strong association with seed yield, suggesting that increased branching may enhance reproductive sink capacity through a higher number of siliques among the morphological traits. Similar trends have been observed by Perween *et al.* (2024), Maurya *et al.* (2018), Gadi *et al.* (2020), Lodhi *et al.* (2014), Chakraborty *et al.* (2021) and Thanmichon *et al.* (2018) between branching ability and yield. Plant height had weak and irregular associations with SY, thus suggesting that higher plant height does not necessarily result in higher productivity. This result is in agreement with the previous findings of Saroj *et al.* (2021), who reported that higher plant height may result in reduced assimilate transport to the reproductive organs. Thousand seed weight had a strong positive association with SY and proved to be the most important yield-contributing factor. Thousand seed weight exhibited a strong positive relationship with yield, highlighting the importance of seed development and assimilate allocation in determining yield potential. Similar findings have been documented by Ahmad *et al.* (2021), Lakra *et al.* (2020) and Patel *et al.* (2026).

Oil quality traits exhibited clear genotypic differentiation and strong associations with yield components, suggesting that genotypes with efficient carbon partitioning to seeds also have higher accumulation of oil. This result is in agreement with previous studies by Sharma *et al.* (2021) and Ahmad *et al.* (2021), who found that yield improvement in mustard oil does not always result in a reduction in oil content. Oleic acid showed favorable associations with oil content and yield, whereas saturated fatty acids exhibited negative correlations with these traits. The negative correlation is in agreement with previous studies by Srivastava *et al.* (2024), Chakraborty *et al.* (2021) and Basak *et al.* (2024), who highlighted the nutritional and oxidative benefits of high-oleic mustard oil.

Multivariate analyses further clarified the genetic divergence and interrelationships among traits. Principal component analysis indicated that yield, seed weight, oil content, oleic acid and branching traits contributed substantially to the overall genetic variability among genotypes. Similar applications of PCA and cluster analysis for assessing genetic diversity in mustard have been reported by Bibi *et al.* (2017), ZOHORA (2022) and Saini *et al.* (2025). Heatmap analysis further supported the results by clearly indicating the synchronized expression of yield and oil quality traits in superior genotypes. Path coefficient analysis further suggested that thousand seed weight and branches per plant were strongly associated with seed yield, while phenological traits showed comparatively weaker or indirect relationships. The low residual effect indicated that the selected traits explained a substantial proportion of the variation in seed yield. Similar results have been obtained by Kumar (2022), Srivastava *et al.* (2024), Anjali *et al.* (2023) and Ahmad *et al.* (2021) in quantitative genetic analysis of oilseed crops.

The high quality of L13 in terms of phenological, yield and the quality of oil has led to its being a promising genotype in the development of varieties as well as in breeding programmes. Conversely, poor breeding value in variable conditions is indicated by the poor performance of L2 in various traits. This paper shows that early DF, higher BPP, increased TSW, increased OC and increased OA combined can be an effective method of making improvements in yield and oil quality in mustard. Such a combination of univariate and multivariate viewpoints offers a complete and biologically significant framework of the fast-tracking of genetic benefits in the breeding of *B. juncea*.

CONCLUSION

This current research revealed that there is a high genetic variation in the assessed genotypes of *B. juncea* traits of phenology, yield components and oil quality. L13 was superior to any other genotype showing high seed production, 1000 seed weight, high branching and improved oil quality as indicated by a high level of oleic acid and the low proportion of saturated fatty acids. L9 and L8 possessed good yield potential and good agronomic performances that were closely followed by L13, whilst L2 and L5 had the least yields in most of the measured characteristics. As a whole, L13 has the greatest potential in direct development and use in breeding schemes with the objective of high production and better oil quality. The low breeding value in the identified conditions of the investigated conditions was further validated by its poor position in PCA and cluster analysis. The check variety exhibited intermediate performance, underscoring the genetic gain achievable through the identified elite lines. These results indicate that simultaneous selection for early maturity, higher branching, greater seed weight and improved oil quality is an effective strategy for mustard improvement.

AUTHOR'S CONTRIBUTIONS

SH, SS, MZ, MSJB, NK; Writing – original draft; MI, MIA, HMZUG, MK, AC; Writing – review & editing, SH, MIA, MI, HMZUG, MZ, SS; Formal analysis, Conceptualization, Validation, Supervision, Resources, Funding acquisition; MK, NK, AC; Statistical Analysis, Graphic improvement; MI, MIA; Soil analysis; MSJB; Oil quality analysis.

CONFLICT OF INTEREST

Authors declare that they have no conflict of interest.

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