

SIMULTANEOUS SELECTION FOR EARLY-MATURITY AND HIGH SEED AND OIL YIELD IN ADVANCED SPRING CANOLA BREEDING LINES UNDER TERMINAL HEAT AND DROUGHT STRESS

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ABSTRACT

Developing climate-resilient canola cultivars is crucial for improving oilseed productivity in drought-prone regions. The present study aimed to identify the early-maturing and high-yielding canola lines suitable for the Moghan region in Northwest Iran, addressing challenges like terminal heat and drought stress. The study assessed 21 canola genotypes, comprising 18 promising open-pollinated breeding lines and three commercial check cultivars (RGS003, Dalgan, and Hyola 4815), over two consecutive cropping seasons of 2018-2019 and 2019-2020. The multi-trait genotype-ideotype distance index (MGIDI) was used to evaluate the performance of 21 canola lines. During the growing seasons, the data were recorded on phenological, physiological, and productive traits. Results showed significant variation across genotypes, particularly in phenological traits like days to maturity (DMA) and structural traits such as plant height (PH) and stem diameter (SD). Overall, the three canola genotypes (G9, G12 and G13) exhibited early maturity and outperformed others by avoiding terminal heat and drought stress, and improvements were observed in SY (4.8%) and OY (6.2%). Furthermore, these three promising genotypes exhibited superior performance in seed and oil yields, revealing their considerable adaptability to terminal heat and drought stress and related agronomic traits. This study highlighted the efficiency of multivariate selection indices, such as the MGIDI, in significantly enhancing crop yield and stress tolerance.

Keywords: canola breeding, MGIDI, stress tolerance, seed yield, phenological.

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INTRODUCTION

The Brassicaceae family is recognized as one of the ten most commercially important crop plant families (Warwick *et al.*, 2006). Canola (*Brassica napus* L.) has been utilized for oil production for millennia within this particular family (Wu *et al.*, 2018). According to the FAO (FAOSTAT, 2021), canola is one of the most significant oilseeds globally, with seeds containing over 40% oil and the remaining meal being rich in protein. After soybeans, it stands as the world's most major source of edible oil (Seymour *et al.*, 2012). Despite the steady increase in the amount of land dedicated to growing canola and the subsequent rise in production, there are still significant worries regarding the ability to meet the demand for edible oil, which is projected to reach 9.1 billion by 2050 (Bakhshi, 2021). In Iran, the area dedicated to canola cultivation in the 2022-2023 crop year exceeded 191,250 hectares, producing an average yield of 1636 kg per hectare and a total seed yield of 329,800 tons (Anonymous, 2023). However, this yield is below the global average. Increasing crop productivity

per unit area remains a primary goal of oilseed rape breeding programs.

The success of any breeding program hinges on understanding key traits, their genetic control, inheritance patterns, and the environmental factors affecting their expression (Chaghakaboodi *et al.*, 2012; Hill, 2012). Seed yield in oilseed rape is influenced by the genetic potential of the variety, climatic conditions, soil type, and crop management practices. Furthermore, the continuous occurrence of climate change and the vulnerability of canola to abiotic environmental factors pose a significant risk to canola cultivation in various regions across the globe (Lobell and Gourdji, 2012). Additionally, there have been reports indicating that canola and other Brassica species are particularly susceptible to drought due to their predominant cultivation in dry and semi-arid locations (Majidi *et al.*, 2015). Canola is particularly susceptible to increased occurrences of heat and drought stress (Jensen *et al.*, 1996). Exposing canola to heat or drought stress would likely result in decreased yield and yield components (Jensen *et al.*, 1996; Mirzaei *et al.*, 2013), reduced oil concentration (Jan *et al.*, 2017), and

alterations in fatty acid composition (Gharechaei *et al.*, 2019; Rezaeizadeh *et al.*, 2019; Li *et al.*, 2021) and phenological characteristics (Koscielny *et al.*, 2020; Farahani *et al.*, 2022). Consequently, many approaches have been employed to address the challenges posed by drought stress. These include the implementation of irrigation systems, enhancement of crop management techniques, and utilization of plant breeding technologies (Majidi *et al.*, 2015).

The Moghan region in northwest Iran boasts favorable environmental conditions for canola production, making it the leading area for this crop in the country. Despite this, the expansion of canola cultivation in the region faces limitations due to environmental stressors such as terminal heat and drought stress in late April and early June, overlapping with critical reproductive stages such as flowering and seed filling (Zeinalzadeh-Tabrizi *et al.*, 2022). To address these challenges, selecting early-maturing and high-yielding canola lines is crucial. In recent years, Iran's canola breeding program has focused on this challenge. Therefore, identifying suitable selection indices that can recognize high-yielding lines based on traits like early maturity and higher seed yield is of great importance. To aid in genotype selection using data from several characteristics, Olivoto and Nardino (2021) present a new multi-trait genotype-ideotype distance index (MGIDI). Monte Carlo simulations are used to test the suggested index's effectiveness. In these simulations, the number of genotypes, evaluated traits, and correlation structure between traits are varied across different situations, and the success of picking traits with intended gains is estimated. By integrating MGIDI into selection processes, breeders can optimize the selection of progenitors with the desired traits, ultimately improving crop varieties for farmers and end-users while addressing challenges such as abiotic stresses and quality limitations

(Adewumi *et al.*, 2023; Silva *et al.*, 2023; Singamsetti *et al.*, 2023; Ouattara *et al.*, 2024).

To address the challenges of terminal heat and drought stress in canola production, it was hypothesized that early-maturing canola genotypes, selected through the multi-trait genotype-ideotype distance index (MGIDI), would be identified as lines with high seed and oil yields, capable of mitigating the adverse effects of terminal heat and drought stress in Northwest Iran. By using the MGIDI, the study aims to enhance canola breeding programs, improve crop productivity, and overcome abiotic stresses, ultimately benefiting farmers and end-users.

MATERIALS AND METHODS

Experimental location: The study was carried out in the Moghan Agricultural Research Station in Parsabad, Iran (N39°39' E 47°48', sea level= 78 m) for the two cropping seasons of 2018-2019 and 2019-2020. Based on data from the Iran Meteorological Organization (IRIMO), this area experiences a semi-humid and moderately warm climate. Over the past 30 years, the average annual precipitation has been 251 mm, with most of it occurring in the autumn and early spring. The region experiences a range of precipitation from a minimum of 72.9 mm to a maximum of 523 mm every year. The average annual maximum temperature is 35°C, while the average annual minimum temperature is 8°C. The mean yearly relative humidity stands around 71%. Figure 1 shows the meteorological data for the location during the experimental years.

The soil texture of the experimental field was sandy-loam, and the soil type was cambisol, according to the World Reference Base for Soil Resources (WRB) (FAO, 1998). The physicochemical properties of soils in the experimental field are shown in Table 1.

Table 1. The physicochemical characteristics of the soils utilized in the experimental setting

| Soil depth | Salinity (Ds m ⁻¹) | pH | Organic Carbon (%) | N (%) | Available P (ppm) | Available K (ppm) | Zn (ppm) | Fe (ppm) |
|------------|--------------------------------|------|--------------------|-------|-------------------|-------------------|----------|----------|
| 0-30 cm | 0.702 | 7.72 | 2.760 | 0.2 | 1.85 | 469 | 7.14 | 3.94 |
| 30-60 cm | 0.736 | 7.75 | 0.781 | 0.7 | 1.09 | 267 | 10.46 | 4.27 |

Plant materials: This study utilized 21 plant genotypes, comprising 18 promising open-pollinated breeding lines and three commercial check cultivars, RGS003, Dalgan and Hyola 4815. The breeding lines are part of the spring canola breeding program in Iran. The experiment followed a randomized complete block design (RCBD) with three replicates, conducted over two consecutive crop years.

Field practice: Each experimental plot consisted of six rows, each 5 meters long and spaced 30 centimeters

apart, resulting in a plant density of 60 plants per plot. Fertilizer application was guided by soil analysis results. At planting, 150 kg/ha of phosphorus (triple superphosphate) was applied, along with 200 kg/ha of nitrogen (ammonium nitrate), split evenly between planting and the stem elongation stage. No diseases were observed during the growing period, and weeds were managed chemically. Before planting, trifluralin was applied at 2 liters per hectare. Planting occurred in late October each crop year. Gallant Super herbicide was

applied at 0.8 liters per hectare before the rosette stage. Irrigation was provided as necessary.

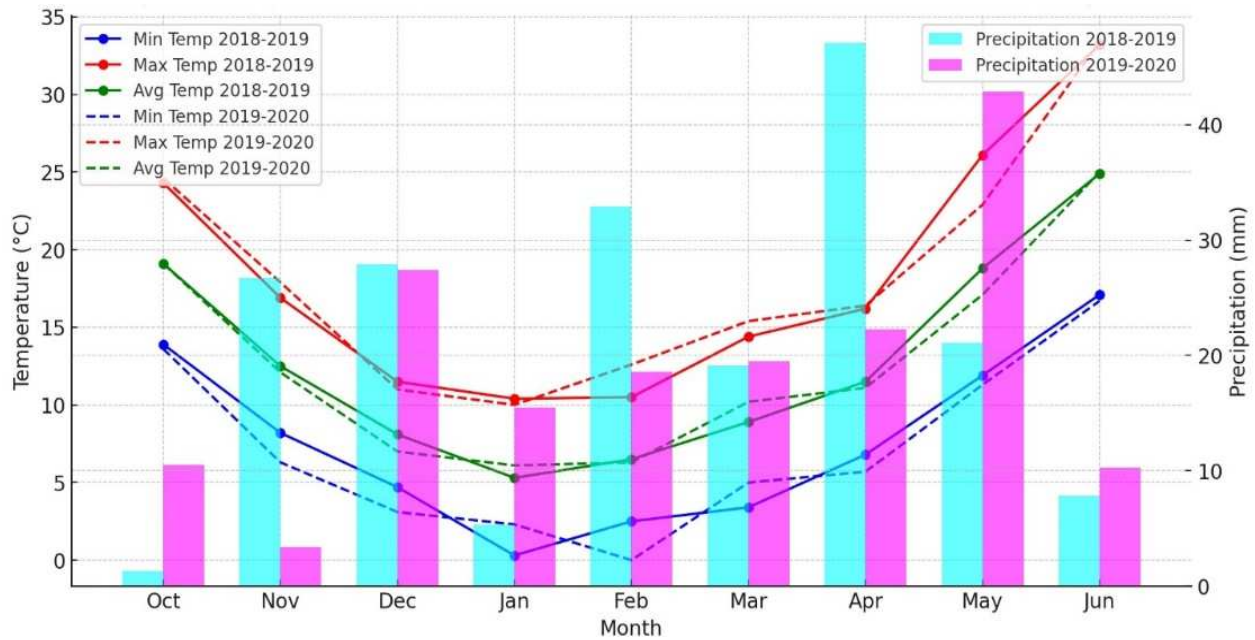


Figure 1. Meteorological data during the experimental years

Data collection: Throughout the growing season, various characteristics were recorded according to UPOV (2011). Days to flower initiation (DFI) and completion (DFC) were recorded as the number of days from sowing to when 50% of plants in a plot showed the first open flower and ceased producing new flowers, respectively, through daily visual inspections; the flowering period (FP) was calculated as DFC minus DFI. The seed filling period (SFP) was determined as the days from DFC to when 50% of pods reached physiological maturity, and days to maturity (DMA) were counted from sowing to when 95% of pods were mature, assessed by pod color and texture. Plant height (PH), first pod height (FPH), and main stem length (MSL) were measured at maturity using a ruler from the soil surface to the top of the main stem, lowest pod, and stem tip, respectively, averaging 10 plants per plot. Branch number (BN) and pod number per plant (PN) were counted on 10 plants per plot, while stem diameter (SD), pod length (PL), pod thickness (PT), and pod diameter (PD) were measured using a digital caliper, averaging 10 plants or pods per plot. Seed number per pod (SNP) was determined by counting seeds in 10 pods per plant across 10 plants. The 1000-seed weight (SW) was obtained by weighing 1000 dried seeds using a precision balance. Chlorophyll content (SPAD) was measured at flowering using a SPAD meter on 5 leaves per plant across 10 plants. Seed yield (SY) was calculated by weighing dried seeds harvested from a plot, expressed per unit area (kg/ha).

At final harvest, two lateral rows were removed as marginal strips. To determine seed oil content, a 5-

gram seed sample from each plot was analyzed using Nuclear Magnetic Resonance (NMR) technology. The analysis utilized the German Bruker minispec mq20 Model, following International Standard ISO-5511 (1992) guidelines. The NMR device was calibrated daily with a reference sample and further calibrated with pre-prepared standard samples. For this experiment, 5 grams of rapeseed were used, although the minimum requirement is 3 grams. The sample was placed in the designated cell of the device, and oil content was measured in under one minute. Oil yield (OY) was calculated by multiplying the oil content by the seed yield.

Statistical analysis: Prior to data analysis, outliers were identified and the normality of the data was assessed using the Grubbs' test and the Shapiro-Wilk test. Next, the assumption of homogeneity of variance was assessed using Bartlett's test.

The MGIDI index idea revolves four primary steps. (i) Adjusting the traits to have a range of 0-100, (ii) Employing factor analysis to consider the correlation structure and reduce the dimensions of the data, (iii) Developing an ideotype based on desired or known trait values, and (iv) Calculating the distance between each genotype and the planned ideotype (Olivoto and Nardino, 2021).

After conducting a combined analysis of variance, a two-way table (X_{ij}) was generated with estimated means for each genotype in the rows and traits in the columns. Here, t represents the number of genotypes $t = 1, 2, \dots, 21$ and v denotes the number of

traits $v = 1, 2, \dots, 14$. To standardize the values, the table was rescaled to rX_{ij} as described by (Olivoto *et al.*, 2019):

$$rX_{ij} = \frac{\eta_{nj} - \varphi_{nj}}{\eta_{oj} - \varphi_{oj}} \times (\theta_{ij} - \eta_{oj}) + \eta_{nj}$$

where η_{nj} and φ_{nj} are the new maximum and minimum values for trait j after rescaling, respectively; η_{oj} and φ_{oj} are the original maximum and minimum values for trait j , respectively; and θ_{ij} is the original value for the j th trait of the i th genotype. For traits where higher values are preferable, $\eta_{nj} = 100$ and $\varphi_{nj} = 0$ were used. The rescaled table, rX_{ij} , thus has a range of 0-100 for all columns, with 0 being the least desired value and 100 the most desired. The ideal genotype would score 100 for all traits. Next, a factor analysis (FA) was performed to reduce data dimensionality and understand the relationships among traits. The FA model used was:

$$F = Z(A^T R^{-1})^T$$

where F is a $g \times f$ matrix containing the factorial scores; Z is a $g \times p$ matrix with rescaled means; A is a $p \times f$ matrix of canonical loadings; and R is a $p \times p$ correlation matrix of the indices. Here, g , f , and p denote the number of accessions, retained factors, and calculated indices, respectively. A $[1 \times p]$ vector served as the ideotype matrix. The final phase involved calculating the Euclidean distance between the scores of each accession and the ideal accession to derive the MGIDI index using the equation:

$$MGIDI_i = \sum_{j=1}^f [(\gamma_{ij} - \gamma_j)^2]^{0.5}$$

The variable γ_{ij} represents the score of the i th accession in the j th factor, where i ranges from 1 to t and j ranges from 1 to f . Here, t represents the number of accessions and f represents the number of factors. Additionally, γ_j represents the j th score of the ideal accession. The accession with the lowest MGIDI value is closest to the ideal, indicating desirable values for all indices. Selection differential for all traits was calculated with a selection intensity of approximately 10%. Data manipulation and index computation were performed using the 'metan' package in R (Olivoto and Lúcio, 2020).

RESULTS

Multivariate analysis and comprehensive trait evaluation: The multivariate analysis provided a holistic understanding of the performance of 21 canola genotypes over two consecutive cropping seasons. By employing the multi-trait genotype-ideotype distance index (MGIDI), we were able to distill a broad range of phenological, physiological, and productive traits into a unified index. This allowed for the selection of superior genotypes in canola breeding lines based on a wide array

of characteristics, ensuring a balanced approach to improving seed and oil yield alongside traits related to early maturity and stress tolerance. In particular, the phenotypic variability observed across genotypes, in terms of flowering period, seed weight, and pod-related characteristics, provided essential insights into the genetic and environmental factors influencing performance (Table 2). This variation, when analyzed using multivariate techniques, highlighted the complexity of canola breeding, emphasizing the importance of integrating multiple traits into selection decisions.

Phenological traits: early maturity and drought avoidance: The days to flower initiation (DFI) and days to maturity (DMA) emerged as critical traits for determining the suitability of genotypes for the Moghan region, which experiences terminal heat and drought stress. The observed range of DFI across genotypes (138-150.33 days) and DMA (216.33-222 days) reflected the diverse phenological responses of the genotypes (Table 2). Early-maturing genotypes demonstrated their capacity to escape terminal drought, a key adaptation trait for the semi-arid conditions of northwest Iran. Moreover, the flowering period (FP) spanned 40.33 to 53 days, with genotypes exhibiting shorter flowering periods being better suited for the region's environmental challenges (Table 2). The capacity of a genotype to reduce its reproductive phase and accelerate maturity while maintaining seed production is advantageous for managing environmental risks like heat stress, which can adversely impact pod formation and seed development. Early-flowering genotypes were not only able to avoid heat stress but also contributed to more stable yields across both years of the experiment, demonstrating their robustness under variable environmental conditions.

Plant architecture: height and pod formation as yield determinants: Plant height (PH) and first pod height (FPH) are structural traits that have a direct influence on the ability of plants to withstand abiotic stresses, such as soil moisture deficits and extreme temperatures. The variation in PH, which ranged from 124.10 cm to 186.93 cm (mean 164.45 cm), and FPH, with values ranging from 71.20 cm to 132.27 cm (mean 107.04 cm), was indicative of differing plant architectures across the 21 genotypes (Table 2).

Productive traits: seed and oil yield, pod number: Seed yield (SY) and oil yield (OY) served as the principal criteria for evaluating the agronomic performance of the canola genotypes across both growing seasons. Seed yield demonstrated substantial variation among the genotypes, ranging from 1950.67 to 4041.33 kg/ha, with an overall mean of 3481.16 kg/ha. This broad variability reflects significant genetic diversity and highlights opportunities for yield enhancement through selective breeding. Oil yield also exhibited considerable genotypic variation, spanning from 831.33 to 1776.33 kg/ha, with a

mean value of 1431.10 kg/ha. Although oil yield was closely associated with seed yield, it was also influenced by other yield components, particularly 1000-seed weight

(SW), pod number per plant (PN), and seed number per pod (SNP).

Table 2. Descriptive statistics calculated for studied agro-morphological traits across two years experiment

| Variable | Mean | SE Mean | StDev | Variance | CoefVar | Minimum | Maximum | Range |
|----------|---------|---------|--------|-----------|---------|---------|---------|---------|
| DFI | 144.08 | 0.42 | 2.72 | 7.38 | 1.89 | 138.00 | 150.33 | 12.33 |
| DFC | 190.40 | 0.57 | 3.71 | 13.74 | 1.95 | 181.00 | 198.00 | 17.00 |
| FP | 46.33 | 0.41 | 2.64 | 6.97 | 5.70 | 40.33 | 53.00 | 12.67 |
| SFP | 29.41 | 0.48 | 3.13 | 9.83 | 10.66 | 23.67 | 37.00 | 13.33 |
| DMA | 219.82 | 0.25 | 1.65 | 2.73 | 0.75 | 216.33 | 222.00 | 5.67 |
| PH | 164.45 | 2.27 | 14.68 | 215.58 | 8.93 | 124.10 | 186.93 | 62.83 |
| FPH | 107.04 | 2.10 | 13.60 | 184.85 | 12.70 | 71.20 | 132.27 | 61.07 |
| MSL | 107.05 | 1.41 | 9.13 | 83.31 | 8.53 | 87.00 | 127.53 | 40.53 |
| BN | 6.04 | 0.09 | 0.61 | 0.38 | 10.16 | 4.57 | 7.67 | 3.10 |
| SD | 11.98 | 0.20 | 1.27 | 1.61 | 10.60 | 9.00 | 14.00 | 5.00 |
| PN | 295.03 | 7.01 | 45.43 | 2063.59 | 15.40 | 168.20 | 405.47 | 237.27 |
| PL | 6.05 | 0.09 | 0.60 | 0.36 | 9.92 | 5.07 | 7.83 | 2.77 |
| PT | 4.99 | 0.05 | 0.33 | 0.11 | 6.60 | 4.10 | 5.65 | 1.55 |
| SNP | 27.83 | 0.45 | 2.93 | 8.56 | 10.52 | 18.27 | 32.40 | 14.13 |
| SW | 3.76 | 0.06 | 0.38 | 0.14 | 10.06 | 2.97 | 4.98 | 2.01 |
| SPAD | 45.80 | 0.39 | 2.51 | 6.31 | 5.48 | 37.30 | 49.03 | 11.73 |
| SY | 3481.16 | 65.51 | 424.55 | 180239.28 | 12.20 | 1950.67 | 4041.33 | 2090.67 |
| OY | 1431.10 | 28.58 | 185.25 | 34316.77 | 12.94 | 831.33 | 1776.33 | 945.00 |

Variables measured including days to flower initiation (DFI), days to flower completion (DFC), flowering period (FP), seed filling period (SFP), days to maturity (DMA), plant height (PH), first pod height (FPH), main stem length (MSL), branch number (BN), stem diameter (SD), pod number per plant (PN), pod length (PL), pod thickness (PT), seed number per pod (SNP), pod diameter (PD), 1000-seed weight (SW), chlorophyll content (SPAD), seed yield (SY), and oil yield (OY).

Among these traits, pod number per plant (PN) and seed number per pod (SNP) played critical roles in determining overall productivity. The number of pods per plant ranged from 168.20 to 405.47, with an average of 295.03, while SNP varied between 18.27 and 32.40, averaging 27.83. Genotypes producing a higher number of pods and more seeds per pod demonstrated greater yield potential, suggesting that reproductive efficiency was a key determinant of final seed and oil output under the prevailing environmental conditions. The observed ranges in these traits underscore their potential utility in breeding programs aimed at improving canola productivity through the enhancement of reproductive traits.

Factor analysis: dimensionality reduction and trait grouping: Factor analysis was employed to reduce the complexity of the dataset, grouping the various agro-morphological traits into five primary factors. These factors cumulatively explained 85.5% of the total variance, allowing for a more targeted approach to selection (Table 3). The first factor (FA1), which explained 32.4% of the variance, was dominated by yield-related traits such as seed yield (SY), oil yield (OY), branch number (BN), and chlorophyll content (SPAD). This factor clearly highlighted the primary contributors to overall productivity, reinforcing the

importance of selecting genotypes with high reproductive capacity and photosynthetic efficiency (Table 3).

The second principal factor (FA2) accounted for 16.8% of the total phenotypic variance and was predominantly defined by structural and reproductive traits, including main stem length (MSL), pod number per plant (PN), plant height (PH), and stem diameter (SD) (Table 3). Among these, MSL and PN exhibited the highest factor loadings, indicating their central role in this component. These traits collectively influence the plant's architecture and reproductive potential, both of which are critical for achieving high yield performance.

Main stem length contributes to overall plant stature and the spatial arrangement of reproductive organs, facilitating better light interception and potentially enhancing pod development along the stem. A higher pod number per plant directly increases yield potential, as it reflects greater reproductive capacity. Plant height and stem diameter, although secondary within this factor, remain important for maintaining structural integrity, particularly under environmental pressures such as wind or drought. Robust stems help support increased pod loads and reduce lodging risk, thereby ensuring stable productivity under field conditions.

Table 3. Eigenvalues, explained variance, factorial loadings after varimax rotation, and communalities obtained in the factor analysis.

| VAR | FA1 | FA2 | FA3 | FA4 | FA5 | Communality | Uniquenesses |
|----------------|--------------|--------------|--------------|-------------|--------------|-------------|--------------|
| DFI | -0.01 | 0.04 | -0.98 | 0.03 | 0.12 | 0.97 | 0.03 |
| DFC | 0.10 | 0.08 | -0.75 | -0.09 | -0.64 | 1.00 | 0.00 |
| FP | 0.13 | 0.04 | 0.28 | -0.14 | -0.92 | 0.96 | 0.04 |
| SFP | -0.08 | -0.07 | 0.37 | 0.15 | 0.86 | 0.91 | 0.09 |
| DMA | 0.08 | 0.04 | -0.93 | 0.03 | -0.06 | 0.87 | 0.13 |
| PH | -0.50 | -0.52 | -0.03 | 0.58 | 0.15 | 0.88 | 0.12 |
| FPH | -0.58 | -0.37 | -0.11 | 0.57 | 0.19 | 0.85 | 0.15 |
| MSL | 0.15 | -0.89 | 0.15 | 0.06 | -0.05 | 0.84 | 0.16 |
| BN | 0.73 | -0.56 | 0.05 | -0.06 | -0.10 | 0.87 | 0.13 |
| SD | -0.26 | -0.56 | 0.03 | 0.52 | 0.12 | 0.67 | 0.33 |
| PN | 0.10 | -0.84 | -0.03 | -0.20 | 0.12 | 0.78 | 0.22 |
| PL | 0.10 | 0.24 | -0.05 | 0.77 | 0.35 | 0.78 | 0.22 |
| PT | -0.29 | -0.05 | 0.06 | 0.83 | -0.01 | 0.79 | 0.21 |
| SNP | -0.45 | -0.10 | -0.01 | 0.68 | 0.32 | 0.78 | 0.22 |
| SW | 0.24 | 0.07 | -0.02 | 0.85 | -0.04 | 0.78 | 0.22 |
| SPAD | -0.84 | 0.06 | 0.07 | 0.42 | 0.00 | 0.89 | 0.11 |
| SY | -0.90 | -0.04 | 0.11 | 0.07 | 0.23 | 0.89 | 0.11 |
| OY | -0.91 | 0.10 | 0.06 | -0.19 | -0.05 | 0.89 | 0.11 |
| Eigenvalues | 5.84 | 3.01 | 2.78 | 2.09 | 1.67 | | |
| Variance (%) | 32.4 | 16.8 | 15.4 | 11.6 | 9.27 | | |
| Cumulative (%) | 32.4 | 49.2 | 64.6 | 76.2 | 85.5 | | |

Variables measured including days to flower initiation (DFI), days to flower completion (DFC), flowering period (FP), seed filling period (SFP), days to maturity (DMA), plant height (PH), first pod height (FPH), main stem length (MSL), branch number (BN), stem diameter (SD), pod number per plant (PN), pod length (PL), pod thickness (PT), seed number per pod (SNP), pod diameter (PD), 1000-seed weight (SW), chlorophyll content (SPAD), seed yield (SY), and oil yield (OY).

The third principal factor (FA3) accounted for 15.4% of the total variance and was primarily defined by phenological traits, most notably days to flower initiation (DFI), days to maturity (DMA), and days to flower completion (DFC), all of which exhibited strong negative loadings (Table 3). These traits are central to defining the crop's developmental timing and adaptation strategy, particularly in environments like the Moghan region, where terminal heat and drought stress are common during the late growing season.

Early flowering and maturity enable genotypes to complete critical reproductive stages before the onset of stress, effectively functioning as a drought and heat escape mechanism. The strong association of DFI and DMA with this factor indicates that FA3 captures genotypic differences in phenological development, which are crucial for breeding canola varieties adapted to regions with short or stress-prone growing seasons. Genotypes with lower values for DFI and DMA are better suited for such conditions, as they minimize exposure to yield-limiting environmental extremes during flowering and grain filling.

The fourth factor (FA4) accounted for 11.6% of the total variance and was primarily associated with pod- and seed-related traits, including pod length (PL), pod thickness (PT), seed number per pod (SNP), and 1000-

seed weight (SW) (Table 3). These traits directly influence the plant's reproductive efficiency by determining the physical capacity of each pod to carry seeds and the size and weight of the seeds produced. Higher values in SNP and SW contribute positively to total seed yield by increasing both the number and mass of seeds per unit area.

The strong loadings of these traits in FA4 highlight their collective contribution to yield formation. Genotypes that exhibit longer pods with thicker walls and higher seed numbers per pod are better equipped to maximize seed output. Likewise, greater seed weight indicates better seed filling and potentially higher oil content, adding value beyond simple yield. Therefore, selection based on FA4 can enhance both the quantity and quality of the harvestable product in canola breeding programs.

The fifth factor (FA5) explained 9.27% of the total variance and was defined by traits related to the duration of reproductive development, particularly flowering period (FP) and seed filling period (SFP), both of which showed high loadings (Table 3). These traits influence how long a plant remains in critical reproductive stages and therefore affect its exposure to environmental stress, especially under terminal drought and heat conditions.

Shorter FP and SFP may be advantageous in regions like Moghan, where terminal stresses occur from late April onward. By shortening these phases, genotypes can complete flowering and seed development before severe environmental conditions set in. The clustering of FP and SFP within FA5 emphasizes the role of temporal adaptation in stress-resilient genotype selection. Therefore, breeding programs targeting harsh or short-season environments should consider these duration traits as key components for achieving stability and resilience in yield performance.

Selection differential: multi-trait improvement via MGIDI: The MGIDI index was used to rank the genotypes based on their overall performance across all traits, with a focus on both yield and stress tolerance. The top three genotypes exhibited substantial improvements in both seed and oil yield, while also demonstrating favorable phenological traits like early maturity (Figure 2). These genotypes were identified as having the lowest MGIDI scores, indicating their proximity to the ideal ideotype.

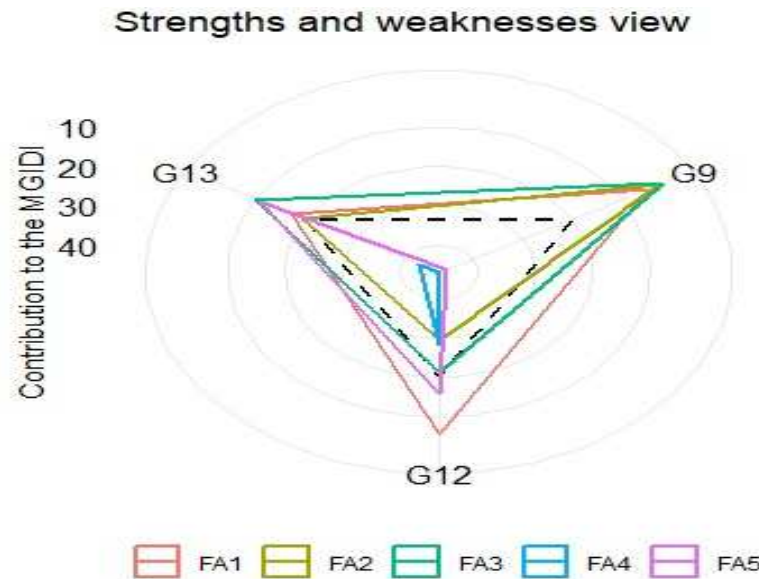


Figure 2. illustrates the strengths and weaknesses perspective of genotypes, represented by the proportion of each factor in the computed multitrait genotype-ideotype distance index (MGIDI). The closer a factor's proportion is to the external edge, the closer the genotypes within that factor align with the ideotype. The dashed line represents the theoretical value if all factors had equal contributions.

The selection differential for seed yield showed a 4.8% improvement, while oil yield increased by 6.2%, indicating the effectiveness of the multi-trait selection approach (Table 4). Additional gains were observed in traits like stem diameter (SD) and pod number per plant (PN), which increased by 5.81% and 14.1%, respectively. The selection differential for phenological traits like days

to maturity (DMA) and days to flower initiation (DFI) was also significant, with a reduction in DFI by 1.23%, contributing to earlier flowering and faster maturation times. These results highlight the effectiveness of the MGIDI index in identifying genotypes that combine high reproductive capacity with superior yield performance.

Table 4. Selection differentials for measured agro-morphological characters of canola. Bold values indicate traits with undesired selection deferential.

| VAR ^a | Factor ^b | Xo ^c | Xs ^d | SD | SD ^e (%) | sense | Goal |
|------------------|---------------------|-----------------|-----------------|-------|---------------------|----------|------|
| FPH | FA1 | 90.2 | 97 | 6.84 | 7.590 | increase | 100 |
| BN | FA1 | 5.56 | 5.87 | 0.312 | 5.610 | increase | 100 |
| SPAD | FA1 | 46 | 47.2 | 1.28 | 2.790 | increase | 100 |
| SY | FA1 | 3269 | 3426 | 157 | 4.800 | increase | 100 |
| OY | FA1 | 1344 | 1427 | 83.3 | 6.200 | increase | 100 |
| MSL | FA2 | 105 | 107 | 1.92 | 1.830 | increase | 100 |
| SD | FA2 | 10.9 | 11.6 | 0.635 | 5.810 | increase | 100 |
| PN | FA2 | 246 | 281 | 34.8 | 14.100 | increase | 100 |
| DFI | FA3 | 147 | 145 | -1.82 | -1.230 | decrease | 100 |

| | | | | | | | |
|------------|------------|-------------|-------------|----------------|---------------|-----------------|----------|
| DFC | FA3 | 194 | 192 | -1.61 | -0.832 | decrease | 100 |
| DMA | FA3 | 219 | 217 | -1.5 | -0.686 | decrease | 100 |
| PH | FA4 | 146 | 154 | 7.23 | 4.940 | increase | 100 |
| PL | FA4 | 6.04 | 5.99 | -0.0484 | -0.802 | increase | 0 |
| PT | FA4 | 4.93 | 5.02 | 0.0925 | 1.880 | increase | 100 |
| SNP | FA4 | 27.5 | 27.9 | 0.394 | 1.430 | increase | 100 |
| SW | FA4 | 3.9 | 4.12 | 0.215 | 5.500 | increase | 100 |
| FP | FA5 | 46.5 | 46.7 | 0.206 | 0.444 | decrease | 0 |
| SFP | FA5 | 24.9 | 25 | 0.111 | 0.446 | decrease | 0 |

^a Variables measured including days to flower initiation (DFI), days to flower completion (DFC), flowering period (FP), seed filling period (SFP), days to maturity (DMA), plant height (PH), first pod height (FPH), main stem length (MSL), branch number (BN), stem diameter (SD), pod number per plant (PN), pod length (PL), pod thickness (PT), seed number per pod (SNP), pod diameter (PD), 1000-seed weight (SW), chlorophyll content (SPAD), seed yield (SY), and oil yield (OY); ^bFcators produced by factor analysis named as FA; ^cXo and ^dXs are population mean before and after selection; ^eSD is selection differential.

Interestingly, the selection differential for phenological traits, such as days to flower initiation (DFI) and days to maturity (DMA), was negative, with reductions of 1.23% and 0.69%, respectively. This indicates that genotypes with earlier flowering and maturity were selected, which aligns with the study’s objective of identifying early-maturing lines that can escape terminal heat and drought stress.

The MGIDI index provided desired selection differential (SD) for 15 of the 18 studied traits, which represents a success rate of 83.33% in selecting traits with desired values. The three traits with undesired SD were PL (SD = -0.802%), FP (SD = 0.444%) and SFP (SD = 0.446%). The SD for traits in which high values are desired ranged from -0.802% (PL) to 14.1% (PN) with mean SD of 4.74%. For traits in which low values are desired, the SD ranged from -1.23% (DFI) to 0.446% (SFP) with a mean SD of -0.37%. However, not all traits responded as strongly to selection. For instance, pod length (PL) showed a negative selection differential of -

0.802%, suggesting that this trait was less responsive to the selection pressure applied. While pod length is an important determinant of seed size, its negative selection differential may indicate a trade-off with other traits, such as pod number per plant, which showed a significant positive response.

Genotype ranking and strengths/weaknesses assessment: Figure 3 illustrates the strengths and weaknesses of the selected genotypes based on their MGIDI scores. The genotypes closest to the ideal ideotype exhibited strong performance in yield-related traits like seed yield, oil yield, and branch number, while also maintaining early maturity and structural integrity. The top three genotypes demonstrated superior performance in key traits such as seed yield (SY) and oil yield (OY), indicating their strong adaptability to the heat and drought stresses typical of terminal growing conditions.

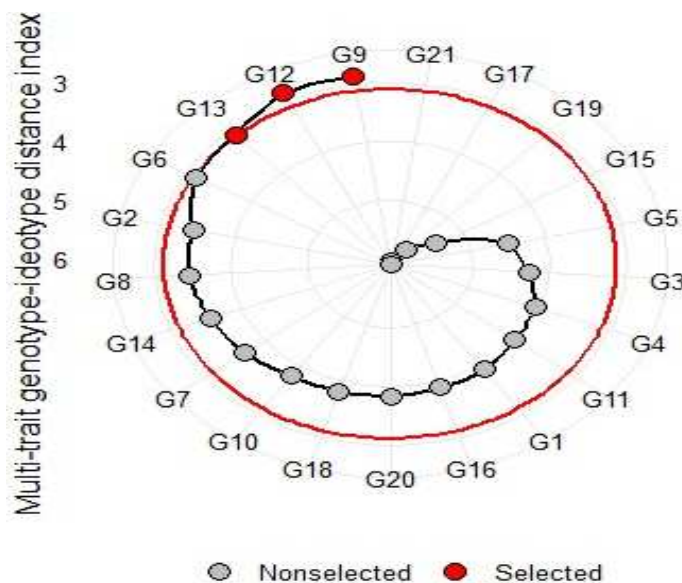


Figure 3. displays the ranking of genotypes determined by the MGIDI index. The top three genotypes from this ranking were utilized to calculate the selection differentials presented in Table 4.

DISCUSSION

The study identified early-maturing, high-yielding canola genotypes (G9, G12, G13) suitable for Northwest Iran's Moghan region, adaptable to terminal heat and drought stress, using the multi-trait genotype-ideotype distance index (MGIDI). MGIDI enabled comprehensive multivariate analysis of phenological, physiological, and productive traits, including days to maturity, plant height, stem diameter, seed yield, and oil yield (Olivoto *et al.*, 2022; Jyothsna *et al.*, 2024). Significant variation among 21 *Brassica napus* L. genotypes highlighted rich genetic diversity, crucial for breeding programs targeting yield and stress tolerance, consistent with Raman *et al.* (2022) on *Brassica rapa* L. MGIDI's integration of multiple traits into a single index facilitated efficient selection of superior genotypes for semi-arid environments like Moghan.

Early-maturing canola genotypes G9, G12, and G13 performed superiorly in Moghan, Iran, by completing flowering and seed filling before terminal heat and drought stress occurring between late April and June. This phenological strategy mitigates yield and quality losses during sensitive reproductive phases, aligning with findings that early-flowering genotypes in Iran achieve higher yields by avoiding drought (Bakhshi *et al.*, 2021; Shafiqhi *et al.*, 2021; Shafiqhi *et al.*, 2022). Similarly, Chakraborty *et al.* (2025) showed early maturity in *Brassica rapa* L. enhances yield stability under stress, and Liang *et al.* (2024) found early-maturing rice varieties increase yields in semi-arid regions, indicating phenological adaptation as a key strategy for crop resilience.

The early-flowering trait in canola genotypes G9, G12, and G13 mitigates heat stress by aligning reproduction with cooler periods, reducing sterility risks, as seen in rice with early morning flowering (Ayyenar *et al.*, 2023). This trait also enhances seed development, increasing SY by 4.8% and OY by 6.2%. Early flowering is vital in semi-arid Moghan, where late reproduction under water scarcity and high temperatures reduces yield (Liang *et al.*, 2024). MGIDI's focus on early maturity and balanced traits ensured these genotypes suited Moghan's constraints, providing a model for breeding in similar climates.

The MGIDI's multivariate approach effectively identified canola genotypes with balanced performance, improving SY and OY, aligning with Ghosh *et al.* (2023)'s PCA-based method for *Brassica juncea* L. MGIDI's strength lies in weighing multiple traits simultaneously, avoiding bias (Olivoto *et al.*, 2022). Positive correlations between PH, FPH, PN, SNP, and SY/OY emphasize structural and reproductive traits' role in yield potential. Cowling *et al.* (2023) noted taller plants with higher pod heights enhance productivity via better light interception and resource efficiency. Raman

et al. (2020) and Raman *et al.* (2022) identified a chromosome A09 locus linked to these traits, supporting optimized selection for genetic gains (Abdelsatar *et al.*, 2021; Menendez *et al.*, 2021; Shadan *et al.*, 2022).

The superior performance of canola genotypes G9, G12, and G13 may stem from regulatory modules for stress response and yield traits, as Yasin *et al.* (2023) identified genes linked to shattering resistance, crucial for yield retention under stress (Mustafa *et al.*, 2022). Their genetic stability likely sustained high SY and OY under Moghan's heat and drought. Genetic diversity among 21 genotypes aligns with Raman *et al.* (2022), who linked variation to fatty acid composition in *Brassica rapa* L., enhancing yield and quality. Integrating MGIDI with genomic tools, as suggested by Yasin *et al.* (2023), could refine trait selection for stress tolerance and productivity.

The strong correlation between PN and SY highlights reproductive resilience's role in yield stability, with high-PN genotypes maintaining productivity under stress. However, trade-offs occurred, as increased PN or SNP sometimes reduced (Knoch *et al.*, 2021; Xin *et al.*, 2021; Norouzi *et al.*, 2023) pod or seed size (Xin *et al.*, 2021), underscoring complex yield component interactions. Breeding strategies must optimize both pod number and size for maximum yield. MGIDI's evaluation of these trade-offs ensured G9, G12, and G13 were selected for balanced performance, minimizing negative impacts on productivity.

Genotypes with higher FPH showed reduced susceptibility to soil-borne pathogens and mechanical stress, preserving reproductive structures (Kuzbakova *et al.*, 2022). Taller plants with greater FPH improved light interception and resource efficiency, boosting yields. These results align with Muleke *et al.* (2022), who noted optimized structural traits enhance yield stability in dry environments. Including PN and SNP in MGIDI's factor analysis (FA4) emphasizes prioritizing reproductive traits for stress-resilient canola genotypes in abiotic stress-prone regions.

Physiological traits like SD (5.81%) and SPAD (2.79%) were key to the superior performance of canola genotypes G9, G12, and G13. Thicker stems improved water storage and reduced lodging, enhancing stability under drought and heat (Raman *et al.*, 2020). Zubkova *et al.* (Zubkova *et al.*, 2022a; Zubkova *et al.*, 2022b) showed zeolite application boosts morphometric and biochemical traits in *Brassica napus* L., suggesting agronomic practices could enhance these genotypes. Muslimah *et al.* (2024) noted nutrient management improves physiological traits in *Brassica rapa* L., supporting genetic selection for stress tolerance. Higher SPAD values indicated better photosynthetic efficiency, aiding growth under stress and contributing to increased SY and OY through efficient resource allocation (Shafiqhi *et al.*, 2022).

The selection differential for SD underscores the role of structural traits in stress-prone environments. Thicker stems provide mechanical support and act as water and nutrient reservoirs, enhancing drought resilience. Raman *et al.* (2020) noted that taller, wider-stemmed plants better withstand abiotic stress by optimizing resource use. Integrating these traits into the MGIDI framework ensured G9, G12, and G13 were selected for comprehensive adaptability, making them ideal for Moghan's conditions.

Early flowering significantly boosted oil yield in canola genotypes G9, G12, and G13, producing oil with favorable traits (Shafiqhi *et al.*, 2022). Optimal flowering periods enhance oil quality, supporting early maturity's benefits for yield and quality (Bakhshi *et al.*, 2021). MGIDI's inclusion of OY balanced seed and oil yields, meeting global demand for high-quality edible oils (Adewumi *et al.*, 2023). The superior oil quality of these genotypes is vital for Moghan, where canola supports local and export markets.

MGIDI analysis identified weaknesses in secondary traits of G9, G12, and G13. Reduced SD in some genotypes may increase lodging risk under extreme weather (Raman *et al.*, 2020). Longer flowering durations could prolong stress exposure, lowering yield potential (Shafiqhi *et al.*, 2022). These limitations, noted by Muleke *et al.* (2022), suggest targeted cross-breeding to enhance SD and shorten flowering periods. Crossing G9, G12, or G13 with high-SD genotypes could improve stability while maintaining early maturity and yield, and selecting for shorter flowering could boost resilience.

MGIDI's holistic approach prioritized genotypes with strong yield and stress resilience but highlighted weaknesses needing attention for long-term success. Targeted breeding using marker-assisted selection or genomic tools, as proposed by Yasin *et al.* (2023), and agronomic interventions like those from Zubkova *et al.* (2022a) and Muslimah *et al.* (2024), could enhance SD and optimize field performance. The superior genotypes G9, G12, and G13 support sustainable canola production in Moghan by aligning early maturity with favorable conditions, reducing yield losses from heat and drought stress (Bakhshi *et al.*, 2021). This enhances resource efficiency and productivity in water-scarce regions (Liang *et al.*, 2024).

Agronomic practices can boost G9, G12, and G13 performance. Zeolite application improves morphometric and biochemical traits in *Brassica napus* L. (Zubkova *et al.*, 2022a; Zubkova *et al.*, 2022b), while foliar ethephon enhances production under stress (Altobiani and Al-Freeh, 2024). Nutrient management further improves physiological traits (Muslimah *et al.*, 2024), complementing genetic selection for yield and stress tolerance in Moghan. The success of these genotypes via MGIDI highlights its efficacy for stress-resilient canola. Future breeding should address

weaknesses like reduced SD and longer flowering durations using targeted cross-breeding or genomic selection (Yasin *et al.*, 2023). Marker-assisted selection for shattering resistance (Mustafa *et al.*, 2022) could enhance yield retention, supporting early maturity.

Field trials integrating G9, G12, and G13 with practices like those from Altobiani and Al-Freeh (2024) and Zubkova *et al.* (2022a), alongside precision agriculture techniques such as optimized irrigation and nutrient application, could enhance adaptability to Moghan's climate. Collaboration among breeders, agronomists, and farmers is crucial to ensure sustainable, productive canola cultivation in semi-arid regions. MGIDI's multi-trait assessment prioritizes balanced, high-yielding, resilient genotypes, making G9, G12, and G13 ideal for Moghan, boosting food security and economic growth. Future research should scale these findings to other semi-arid regions, combining MGIDI with genomic and agronomic strategies for next-generation canola varieties suited to changing climates.

Conclusion: This study validated the multi-trait genotype-ideotype distance index (MGIDI) in selecting early-maturing, high-yielding canola genotypes (G9, G12, G13) with strong adaptability to terminal heat and drought stress in Moghan, Iran. These genotypes achieved 4.8% and 6.2% increases in seed and oil yield, respectively, offering a solution to mitigate stress during reproductive stages. MGIDI's multivariate approach balanced yield and resilience traits, streamlining breeding for semi-arid regions. The selected genotypes promise enhanced canola production, supporting food security. However, reduced stem diameter and longer flowering durations in some genotypes may increase lodging and stress exposure. Future research should refine these traits via cross-breeding, validate findings across diverse environments, and integrate genomic and agronomic strategies to develop resilient canola varieties.

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