



Research Article

Evaluation of the Effect of Different Levels of Drought Stress on the Germination of Canola (*Brassica napus* L.) Genotypes Using PEG-6000

Mani Amiri 

Young Researchers and Elite club, Tabriz Branch, Islamic Azad University, Tabriz, Iran

*Corresponding authors: drmaniamiri@gmail.com

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Abstract

The experiment was conducted to investigate drought stress simulated by polyethylene glycol (PEG) at four osmotic levels of -0.6, -0.8, -1.0, and -1.2 MPa on germination and early seedling growth of 15 genotypes of Canola (*Brassica napus* L.). Canola, a crucial oilseed crop, faces challenges in establishment under drought conditions, necessitating the identification of drought-resistant genotypes. Key parameters, including Germination Rate (GR), Germination Percentage (GP), Shoot Length (SL), Root Length (RL), Mean Germination Time (MGT), Shoot Elongation Rate (SER), Root-To-Shoot Ratio (RSR), Seedling Vigor Index (SVI) and Root Elongation rate (RER), investigated very precisely. The results indicated a significant interaction between the drought condition and genotype for all parameters studied and the interaction effect of genotype under drought conditions. The overall values of SVI, GR, SL, RL, GP, RER, and SER in the comprehensive analysis were reduced by 90%, 91%, 95.4%, 87%, 98%, 95.5%, and 91%, respectively. There was an increase of 94% for MGT and 51% for RSR compared to non-stressed conditions. Based on the results of PCA, RL, GP, and RER, the drought-tolerant genotypes were the most different, which shows that the studied genotypes reacted differently to four levels of water stress. Genotypes 3, 11, and 13 displayed superior drought tolerance, reflected in higher GP, RL, and RER, making them suitable candidates for cultivation in arid regions. These findings underscore the potential of drought-resilient Canola varieties to enhance crop productivity in water-limited environments, suggesting further field studies to confirm their applicability under real-world conditions.

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Keywords: Canola; Drought Stress; Germination; Germination Percentage; PEG-6000

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1. Introduction

Agriculture remains one of the mainstays of human civilization, which underpins food security, economic growth, and sustainable livelihood worldwide (FAO, 2023). Recent data indicate that more than 60% of the human population depends on agriculture for survival, with the Number increasing in developing regions (Ahluwalia et al., 2023). Meanwhile, with estimates of the

world population reaching 9.7 billion by 2050 (UN, 2023), agricultural productivity has to be raised by a minimum of 70% to meet the demand for food. On the other hand, agriculture has many significant challenges, which involve a change in climate, reduced water availability, and increasing degradation of soils (Challinor et al., 2016).

Nevertheless, drought remains the most important abiotic stress that affects agricultural productivity

worldwide. Recent estimates suggest drought stress if resilient varieties are not developed, could reduce crop yields by 20-30% in 2050 (Khan et al., 2023). Drought stress may exert many adverse effects on plants, from reduced water uptake nutrient availability is disturbed, to physiological functions related to impaired plant growth (Basal et al., 2023). This has made the areas dependent on agriculture suffer from food insecurity, especially in the arid zones where the rainfalls are scanty and unpredictable as well (Basal et al., 2023).

Canola is one of the important oilseed crops worldwide, scientifically known as *Brassica napus* L. With more than 13% of the world's vegetable oil production attributed to Canola, it plays a significant role in food production and biofuel generation (FAO, 2023; BiBi et al., 2024). It adapts to various environmental conditions, especially in temperate climates, making it an attractive crop for farmers and industries (Liu et al., 2024). The most abundant producing countries are Canada and China. However, active studies are still being conducted to extend the cultivation of Canola in arid and semi-arid areas due to its increasing demand (Liu et al., 2024). Despite its global importance, Canola is one of the most sensitive crops to environmental stresses, one of which is drought stress (Batoool et al., 2023). Water is one of the most critical germination factors that seeds of this crop need since it provides the basis for normal development. However, in the 2023 studies, it was established that within the population, this drought-tolerant potential may highly vary across different genotypes of Canola, some exhibiting remarkable resilience capacity against drought stress during its seedling growth stage (Jovanović et al., 2024).

Drought stress is most detrimental to Canola during germination and at the seedling stage. Drought reduces germination rate, delays seedlings' emergence, and suppresses root and shoot growth (Channaoui et al., 2023). Early drought stress during the development stages will make the plant suffer from long-lasting changes that can affect the aptitude for growth and reproduction during later stages in life (Li et al., 2024).

The application of polyethylene glycol (PEG) has now gained a standard position in agricultural research for simulating drought stress in laboratory conditions. PEG exerts osmotic stress similar to natural drought conditions; therefore, plant responses could be studied under controlled conditions (Luo et al., 2024). Recent studies using PEG treatments have identified that the selection of drought-resistant Canola genotypes is of prime importance and could contribute significantly to improving. Germination is the critical phase of a plant's life cycle and is particularly sensitive to available water. Successful germination gives a solid start to the growing season, while poor germination results in weak crop establishment and reduced yields (Nguyen et al., 2024). In

the case of Canola, water stress during the process of germination may impede radicle emergence, reduce the percentage of germinated plants, and inhibit seedling vigor—all the most critical factors for establishing a good healthy plant (Liew et al., 2024).

The practice in 2024 has approached, on the one hand, the physiological and biochemical response of Canola to drought stress at its germination stage. For instance, it has been observed that, on receipt of instructions for drought, the drought-tolerant genotype makes more efficient use of available water together with higher antioxidant defense by developing deeper roots to thrive when water is not available (Liew et al., 2024).

Therefore, developing drought-tolerant Canola genotypes is essential in food security against climate change. During the last few years, interest has grown in using genetic and biochemical markers to identify traits associated with drought tolerance in Canola. Such a trait can be manifested as a more extended root system, higher water-use efficiency, and better seedling vigor under stress conditions (Boter et al., 2023).

Among these, research conducted between 2023 and 2024 identified some promising genotypes of Canola, in which high resilience during drought stress was recorded. These genotypes performed well in laboratory-simulated drought conditions of germination percentage, length of root, and seedling vigor (Liu et al., 2024). Such traits would be incorporated into breeding programs with the hope of developing new varieties of Canola that could bear frequency events of drought (Batoool et al., 2023).

Drought stress imposed in growth chambers allows for the exact manipulation of the levels of stress imposed and tests the plant responses across diverse genotypes. As an osmotic agent to induce drought stress, PEG has been developed into a reliable method to screen plants for drought tolerance (Michel & Kaufmann, 2024). Such studies conducted in the laboratory can explain physiological and genetic mechanisms that enable some plants to tolerate water stress better than others (Reed et al., 2024).

This kind of research would mean taking those findings from the laboratory into the field and enabling drought-tolerant crops to be raised in areas of short water supply without a loss in their yield or quality (Channaoui et al., 2023).

This plant has faced many problems and difficulties in our country, Iran, over the last two decades. Therefore, studying and selecting resistant cultivars against drought stress is highly important. Understanding the attitude and all mechanisms related to drought resistance at the early stages of growth is very important. Consequently, the main objective of the present study was to investigate the effect of drought stress on germination and seedling growth and, finally, to select suitable and drought-tolerant Canola cultivars.

2. Materials and methods

2.1. Place of execution of the project

The experiment was conducted in the Ardabil region.

2.2. Plant materials

Fifteen domestic and foreign Canola genotypes were used (table 1). These genotypes were selected based on the different genetic structures and the phenotypic and agricultural performance they had shown in past experiments.

2.3. PEG treatment and experimental conditions

This experiment was conducted as a Complete Randomized Design (CRD) with three replications. Two factors were used in this experiment: the genotype and the drought. To reach osmotic potential and apply drought stress, polyethylene glycol 6000 (PEG-6000) solution was used for five levels of osmotic potential (drought stress), which includes osmotic potential 0 MP, control, -0.6 MP, -0.8 MP, -1 MP and -1.2. According to the method presented by Michel and Kaufman (1973) with the following relationship:

$$\psi = -(1.18 \times 10^{-2}) \times C - (1.18 \times 10^{-4}) + (2.67 \times 10^{-4}) \times CT + (8.39 \times 10^{-7}) \times C^2 \times C^2T \quad (1)$$

Distilled water was used for standard or control conditions. The results of previous research were used to apply the osmotic potential conditions. The success of seed root growth was considered based on their length reaching two millimeters (Batool et al., 2022). The seeds of each genotype, which were 50, were sterilized by soaking in the 5% sodium hypochlorite solution for 5 minutes, washed 5 times with distilled water, and dried with blotting paper to constant weight. Then, the Whatman paper was placed inside the Petri dishes, and the seeds were placed on them and placed in a dark environment with a stable temperature of 24 degrees Celsius with an error of ± 1 percent for germination. The present experiment was carried out on August 19, 2013, for eight days in the oil plant laboratory of the National Agricultural Research Institute of Iran.

2.4. Data collection methods

All the germinated seeds inside the Petri dishes were counted and recorded for eight days. In the end, GP, GR, GK, and MGT values were calculated using the following parameters (abbreviation table).

$$GP = \frac{N8}{50} \times 100 \quad (2)$$

N8 is the Number of germinated seeds after the eighth day.

$$GR = \sum [(Gi - Gi - 1)/i] \quad (3)$$

Where i and G_{i-1} are the germination percentages on day $i-1$ (Ullah et al., 2021; Mubeen et al., 2021).

$$MGT = 1/GR \quad (4)$$

A germination test was done 8 days after the beginning of the experiment, measuring the length of the stem and the root using a vernier caliper. The root length was measured from the collar to the tip root, and the stem length was measured from the cotyledon to the collar. Then, the root-to-stem ratio was calculated by dividing the root length by that of the stem. The seedling germination index (SVI) was used for the evaluation of seed germination according to Abdul-Baki et al. (1973):

$$SVI = [\text{seedling length (m)} \times \text{germination percentage}] \quad (5)$$

The leaf elongation rate (LER) was evaluated using the method of Channaoui et al. (2019), and the stem elongation rate (SER, in cm per day) was evaluated. The calculation for SER used the subsequent formula:

$$SER = GP = \frac{SLE - SLS}{TE - TS} \quad (6)$$

Considering that the TE-TS relationship is used to show the beginning of flowering to the conclusion, the rate of root length increase in centimeters per day was calculated using the following formula:

$$SER = GP = \frac{RLE - RLS}{TE - TS} \quad (7)$$

TE - TS indicates the duration of the measurement period in terms of days, RLS and RLE indicate the root length at the end and beginning of the measurement period, respectively.

2.5. Statistical analyses

Variance in the significant difference between genotypes and osmotic levels produced was analyzed using ANOVA with interaction effects at a significant level ($p < 0.05$).

Table 1. the genotypes used in the study

NO.ber	Variety	NO.ber	Variety
1	Modena	9	Julius
2	Anatol	10	ES Saffhir
3	Baraka	11	Nap10
4	Neptune	12	GKH1103
5	Iyonit	13	Lioness
6	RPC2023	14	SLM046
7	NKFair	15	Bily
8	Eldo		

Table 2. Results of Variance analysis for studied genotypes and traits

SOV	DF	Mean of Squares								
		GP	GR	MGT	SL	RL	R-SR	SVI	RER	SER
Replication	2	1.703	399.8**	0.001**	3.307**	39.97**	4.34	335**	0.11	0.001
Genotypes (G)	14	859.3**	447.97**	0.052**	0.509**	7.71**	9.16**	4111.7**	0.84**	0.034**
drought stress (D)	4	63909.9**	20362.98**	2.176**	172.27**	644.8**	77.32**	1662911**	6.22**	3.28**
G × D	56	141.21**	69.43**	0.049**	0.093**	0.976**	6.88**	531.2**	0.21**	0.028**
Residuals	148	3.003	0.331	0.005	0.0004	0.015	2.72	0.863	0.05	0.014
CV%		3.11	1.78	1.11	4.28	1.9	15.58	3.57	2.28	1.28

** and * Significant at 0.001 and 0.05 probability levels

PCA was accomplished after treatment, and genotype averages were compared and categorized using DMRT. Cultivars were assessed according to such characteristics and grouped into categories. This analysis used the Minitab software for Windows, version 22; SPSS for Windows, V 23; and the MSTATC software.

3. Results

The results of the analysis of variance (Table 2) demonstrated that all germination and early seedling growth parameters were significantly impacted by genotype (G), drought stress (D), and their interaction (G × D), except for MGT, which was exclusively affected by drought. The potential to find drought-tolerant and drought-sensitive genotypes is shown by the 15 genotypes' variable responses to different levels of drought and their expression of genetic difference across all studied parameters, except for MGT (Table 2). Osmotic potential and genotype significantly impacted the percentage of seed germination (GP). However, this measure was not significantly affected by the genotype-stress level interaction. The ability to germinate was reduced considerably due to the drop in water potential (Channaoui et al., 2019).

3.1. Impact of drought stress on characteristics of germination

A decrease in water absorption rate through the seed coat under stress conditions might be responsible for the decline in seed germination percentage. Seedlings developed to germinate seeds in the water-deficit environment exhibit reduced vigor and a lower germination index. Soil water deficit stress increases the average time required for seed germination (Queiroz et al., 2019). Water stress could significantly negatively affect the GP for the lVITY studied in Fig. 1. With no drought stress, at 0 MPa, GP for Genotypes 5, 6, and 14 was 94%, 93%, and 83%, respectively. In comparison, that for the rest of the genotypes was 100%. Thus, the overall average was 97.41%. Under severe drought stress, at -1.2 MPa, seeds of Genotypes 2, 4, 6, 9, and 14 could not germinate; however, the germination percent for the remaining

genotypes varied from 6%, in the case of Genotype 5, up to 23%, in the case of Genotype 11 (Fig. 1). Under the intermediate stress of -0.8 MPa, 'Genotype NO. 11' exhibited the highest germination percentage (67%), followed by 'Genotype NO. 1' (64%), 'Genotype NO. 9' (63%), and 'Genotype NO. 13' (62%), but 'Genotype NO. 5' recorded the lowest mean value (36.55%). Conversely, GP had a minor effect from mild drought stress (-0.6 MPa), ranging from 70% in 'Genotype NO. 5' to 100% in 'Genotype NO. 3, 9, and 11' (Fig. 1). The current research indicates that, irrespective of genotypes, heightened dryness during the germination stage diminished germination ability via decreasing water potential. This aligns with the results of other investigations on *Brassica napus* (Channaoui et al., 2019; Toosi et al., 2014). Genotypes 2, 11, and 13 without water stress achieved peak germination percentage on the fourth day of imbibition (Fig. 2). Genotypes 5, 6, 10, and 14 attained their growth performance peak on the eighth day. Under extreme drought circumstances (-1.2 MPa), germination commenced on the fifth day for 'Genotypes NO. 3, 8, 11, and 12' and on the seventh day for 'Genotypes NO. 1, 7, 9, 10, 13, and 15'. Nonetheless, the seeds of 'Genotypes NO. 2, 6, 9, and 14' could not germinate even after 9 days under these severe stress circumstances (Fig. 2). Genotypes 3 and 11 have superior germination ability under acute drought stress compared to other genotypes while Genotype 14 is the most vulnerable. The amount of drought affected the GR and showed considerable heterogeneity across genotypes. Without drought, growth rates varied from 38.17% for 'Genotype NO. 5' to 74.42% for 'Genotype NO. 11', yielding an overall average of 58.18% (Fig. 3).

At -1.2 MPa of significant stress, a considerable reduction in GR was seen in all examined genotypes, with a mean value of 2.76%. 'Genotype NO. 11' exhibited exceptional resilience, achieving the most excellent mean growth rate (GR) of 8%, whereas 'Genotype NO. 3' recorded an average GR of 5% (Fig. 3). In contrast, the lowest mean GR values were seen in 'Genotypes NO. 4 and 14' (0%). At an intermediate stress level of -0.8 MPa, the highest GR value was observed in 'Genotype NO. 11' (56%), followed by 'Genotype NO. 13' (48%) and 'Genotype NO. 12' (40%).

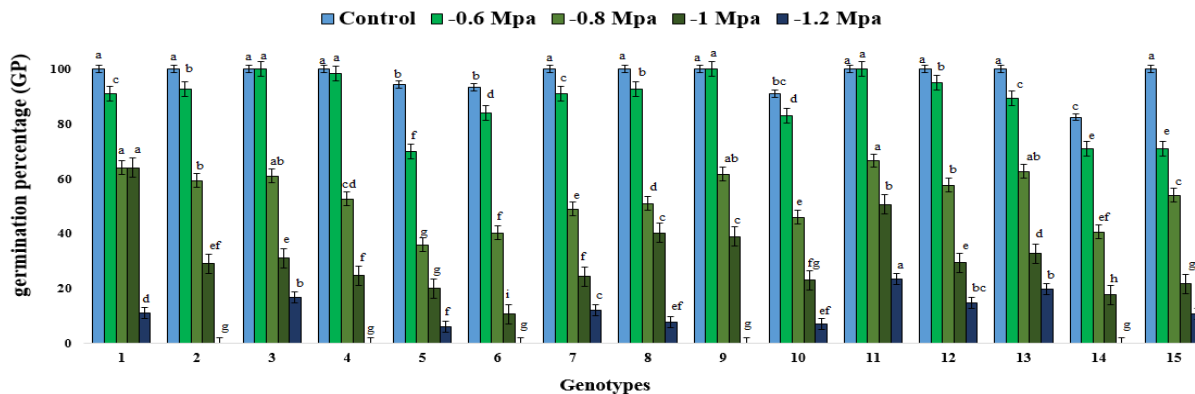


Figure 1. Comparison of the average effect of osmotic stress applied on the studied seeds. Error bars represent \pm standard deviation (SD). Items with the same alphabet do not show significant differences based on Duncan's test ($p < 0.05$)

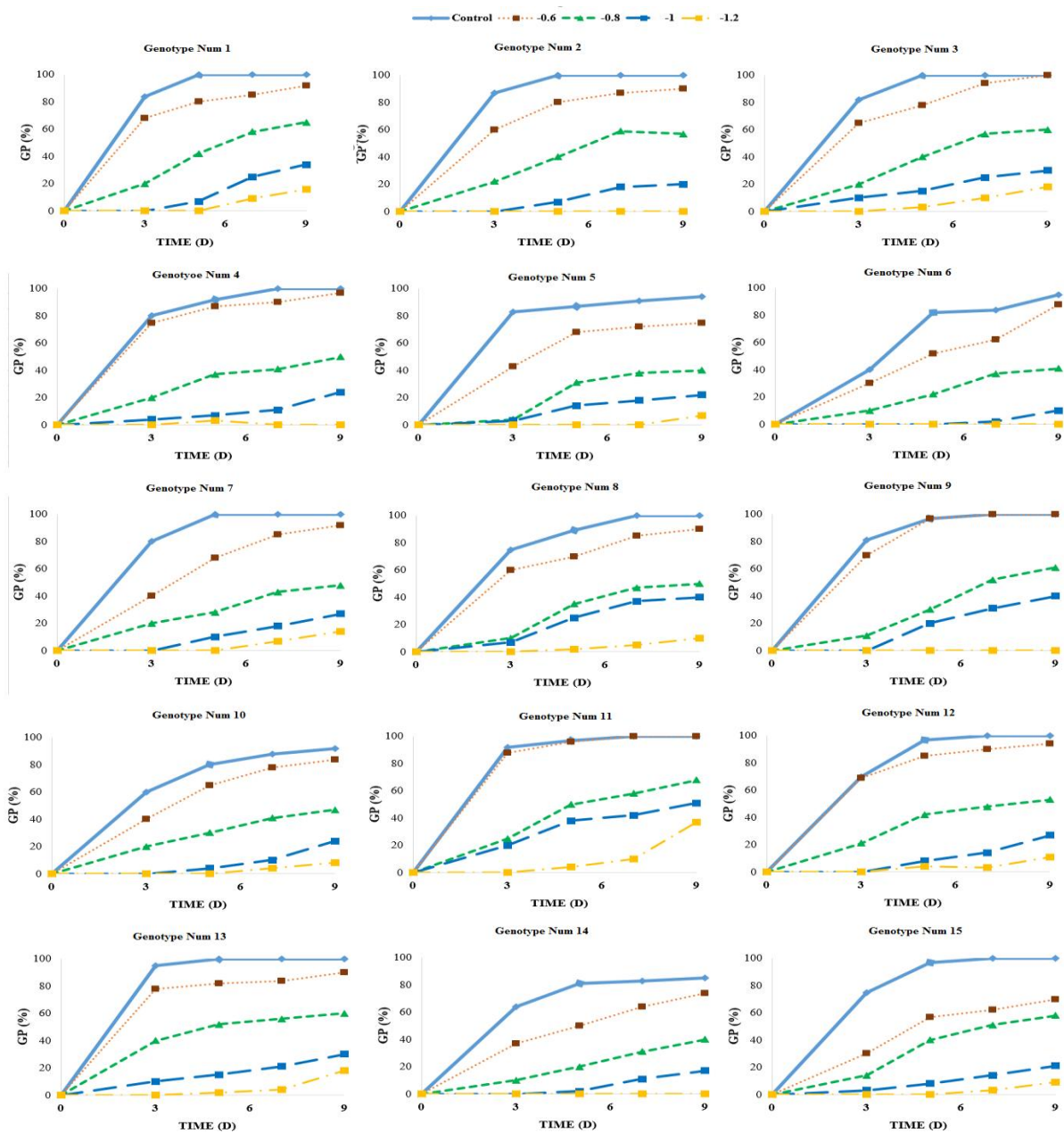


Figure 2. Germination diagram of different seeds used in the experiment focusing on their sensitivity to applied osmotic stress

Conversely, the lowest GR values were recorded in 'Genotype NO. 7' at 28.46%, with an overall average of

36.11% (Fig. 3). Under mild stress conditions (-0.6 MPa), 'Genotype NO. 11' demonstrated the highest germination

rate at 65%, but 'Genotype NO. 5' and 'Genotype NO. 6' recorded the lowest rates at 36.21% and 35.34%, respectively (Fig. 3). The MGT was strongly impacted by drought, increasing with higher drought levels (Fig. 4). In control settings (Control), MGT varied from 0.013 d in 'Genotype NO. 11' to 0.031 d in 'Genotype NO. 5', yielding an overall average of 0.02 d. In severe drought conditions (T1), 'Genotype NO. 11' exhibited the lowest mean germination time (MGT) of 0.021 days, followed by 'Genotype NO. 6' and 'Genotype NO. 8', which had MGT values of 0.039 days and 0.038 days, respectively (Fig. 4). Under situations of severe and moderate stress, 'Genotypes NO. 7, 11, and 14' had the least impact, demonstrating the lowest average values for MGT, namely (0.029, 0.021, and 0.034 d) at -0.8 MPa and (0.043, 0.22, and 0 d) at -1.2 MPa (Fig. 4). The varieties 'Genotypes NO. 3 and 4' have shown tolerance to these circumstances since their MGT values are statistically similar to those of 'Genotypes NO. 7, 11, and 14'.

3.2. Effects of drought stress on the traits of seedling development

In non-stressful situations (Control), the mean shoot length (SL) was 5.24 cm. The genotypes 'NO. 11' and 'NO. 13' had the most excellent mean values, measuring 5.99

cm and 5.89 cm, respectively (Fig. 5). Conversely, 'Genotype NO. 6' had the st petite shoot length, averaging 4.73 cm. Under mild drought circumstances (-0.6 MPa), all genotypes exhibited a significant reduction in shoot length, with an overall average of 2.09 cm. Nonetheless, 'Genotype NO. 11' and 'Genotype NO. 13' exhibited the most significant average values (2.46 cm and 2.39 cm, respectively), followed by 'Genotype NO. 3' (2.45 cm), although 'Genotype NO. 12' had the lowest shoot length (1.68 cm) (Fig. 5). A significant decrease in SL was seen under moderate drought circumstances (-0.8 MPa), with a mean value of 1.65 cm. The cultivars 'Genotype NO. 3' and 'Genotype NO. 11' had the most excellent SL values, measuring 2.06 cm and 2.13 cm, respectively, proving their superior tolerance to various drought conditions (Fig. 5). Conversely, 'Genotype NO. 12' and 'Genotype NO. 15' had the lowest SL values (1.32 and 1.29 cm, respectively), indicating their susceptibility to water stress. Significant reduction in SL was seen in all genotypes under extreme drought stress conditions (-1.2 Mpa) (Fig. 5). Only 'Genotype NO. 3' and 'Genotype NO. 11' successfully generated shoots, measuring 0.43 cm and 0.39 cm in length, respectively. The findings highlight the exceptional early seedling development potential and stress resilience of the cultivars 'Genotype NO. 3' and 'Genotype NO. 11' under severe drought conditions.

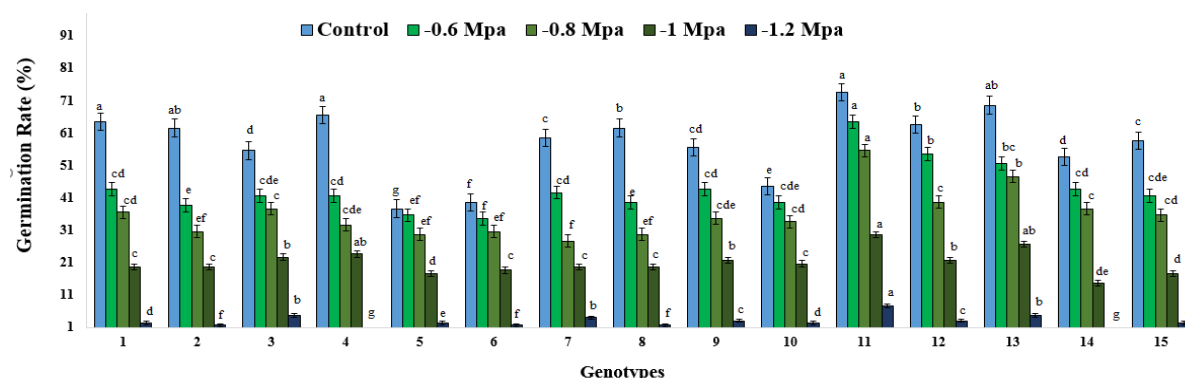


Figure 3. Impact of drought stress on the germination rate (GR) in 15 Canola genotypes. The error bar represents ± standard deviation (SD). Items with the same alphabet do not show significant differences based on Duncan's test (p<0.05)

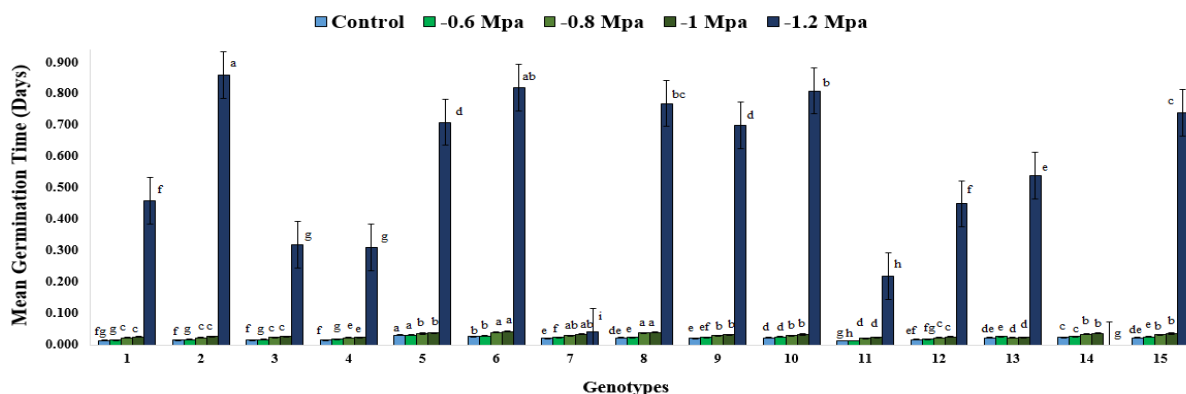


Figure 4. Mean Germination Time (MGT) in 15 Canola genotypes due to drought stress. The error bar shows the standard deviation (SD) of ±. Items with the same alphabet do not show significant differences based on Duncan's test (p<0.05)

Applying PEG-induced drought stress significantly affected the examined genotypes' shoot elongation rate (SER). Without stress, on Day Five (d5), 'Genotype NO. 11' shows the greatest SER at 0.59 cm/d, followed by 'Genotype NO. 10' at 0.53 cm/d, whilst 'Genotype NO. 14' presented the lowest SER at 0.24 cm/d (Fig. 6). On day seven (d7), 'Genotype NO. 10 and 11' exhibited the greatest SER, with mean values of 0.75 cm/d correspondingly (Fig. 6).

In contrast, the lowest SER values were observed for 'Genotype NO. 8, 14, and 15' (0.31, 0.34, and 0.34 cm/d). Over the nine days, 'Genotypes NO. 11 and 13' showed a marginal rise with SER values of 1.12 and 0.99 cm/d,

respectively, whereas 'Genotype NO. 10' had a comparatively lower SER of 0.64 cm/d. Conversely, 'Genotype NO. 14' had a markedly reduced SER, averaging 0.47 cm/d (Fig. 6). Under drought stress circumstances, SER was substantially decreased in all genotypes.

Under mild stress at -0.8 MPa, 'Genotype NO. 11' exhibited the highest shoot elongation rate of 0.54 cm/d, closely followed by 'Genotype NO. 15' with an average shoot elongation rate of 0.34 cm/d. Conversely, 'Genotypes NO. 8 and 12' had the lowest SER of 0.19 cm/d. During intermediate stress at d5, 'Genotype NO. 11' had the highest SER, both recorded at 0.25 cm/d (Fig. 6).

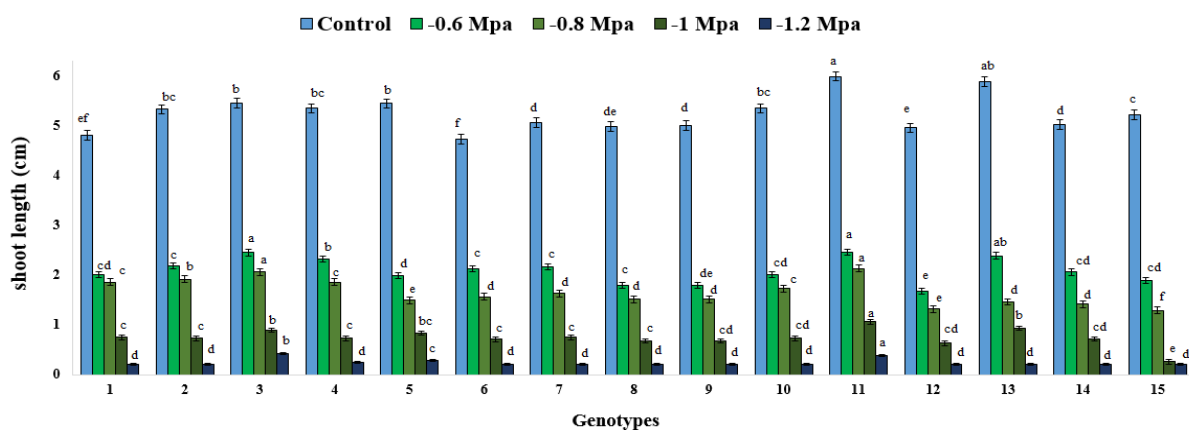


Figure 5. Impact of drought stress on shoot length (SL) in 15 Canola genotypes. The error bar represents ± standard deviation (SD). Items with the same alphabet do not show significant differences based on Duncan's test (p<0.05)

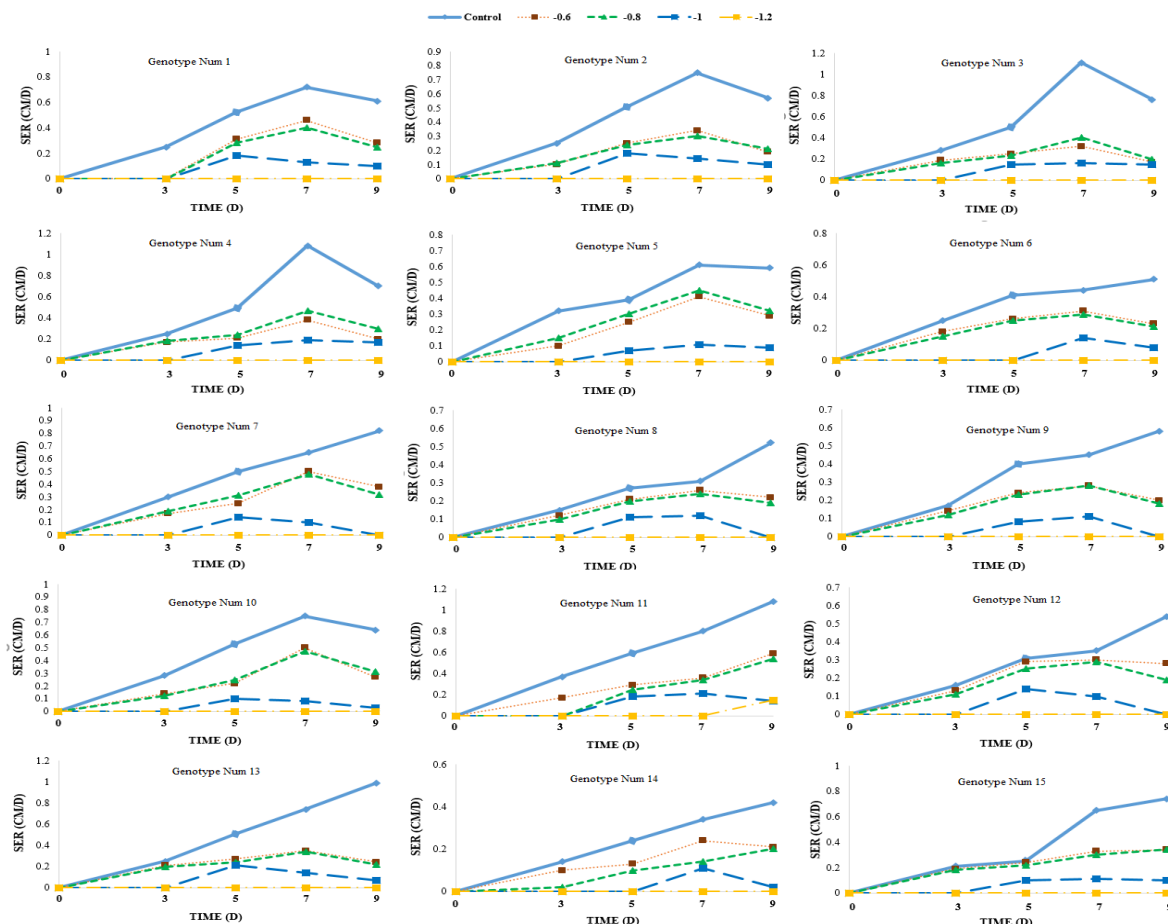


Figure 6. Impact of drought stress on the shoot elongation rate (SER) over time in 15 Canola genotypes

Conversely, 'Genotype NO. 14' exhibited no shoot elongation (SER = 0.1) over this period. At d9, 'Genotype NO. 11' exhibited the greatest SER, with average values of 0.15 cm/d (Fig. 6). Under extreme drought stress (-1.2 MPa), only 'Genotypes NO. 11 and 13' demonstrated shoot growth. Under drought stress, a significant and profound reduction of the RL was observed for all studied genotypes compared to that induced in the complete absence of stress. The mean TRL (Total Root Length) recorded from all the lots without stress was 10.63 cm, while 'Genotype NO. 11' gave the highest value of 13.2 cm, and 'Genotype NO. 9' gave the minimum of 9.20 cm among the various studied genotypes. Through all levels of drought stress, 'Genotype NO. 11' had the most extended average root length: 10.75 cm at -0.6 MPa, 9.88 cm at -0.8 MPa, 4.25 cm at -1 MPa, and 1.75 cm at -1.2 MPa. 'Genotype NO. 13' had average root lengths of 7.22 cm at -0.8 MPa, 4.1 cm at -1 MPa, and 1.21 cm at -1.2 MPa. In contrast, 'Genotypes NO. 2 and 14' demonstrated the most petite root lengths of 2.1 cm and 2.17 cm at -1 MPa osmotic potential, while both did not germinate under -1.2 MPa stress conditions. PSC also demonstrated average values for RL in the rest of the genotypes. These results emphasize that 'Genotype NO. 11' and, up to some extent, 'Genotype NO. 13' has a well-established root system in drought and non-stress conditions.

The root-to-shoot ratio (RSR) varied from 1.76 in 'Genotype NO. 15' to 2.28 in 'Genotype NO. 1' under optimal hydration circumstances. As the osmotic potential

decreased from 0 to -1 MPa, signifying an escalation in drought severity, all genotypes tended to augment their RSR (Fig. 8). At light stress (-0.6 MPa), the most excellent RSR values were observed for 'Genotypes NO. 10, 11, and 12' (4.75, 4.68, and 5.09, respectively), while 'Genotype NO. 4' had the lowest RSR value (3.25) (Fig. 8). At an intermediate stress of -0.8 MPa, 'Genotype NO. 15' exhibited the most excellent RSR value of 6.14, while 'Genotype NO. 3' recorded the lowest at 3.62 (Fig. 8). Under severe drought stress (-1.2 MPa), 'Genotype NO. 11' had the most excellent RSR value (7.18), signifying its exceptional tolerance to extreme water scarcity. Irrespective of genotypes, seedling vigor index (SVI) values were significantly elevated in the absence of drought stress (control) compared to their presence. The mean value was 482, ranging from 457.4 in 'Genotype NO. 1' to 524.3 in 'Genotype NO. 13' (Fig. 9). Under moderate stress, SVI dropped markedly to an average of 123. The peak values were seen in 'Genotype NO. 11' (185) and 'Genotype NO. 3' (175) (Fig. 9). A significant decline was seen at the elevated drought level of -1MPa, with 'Genotype NO. 11' exhibiting the most excellent SVI value of 47.5, followed by 'Genotype NO. 3' at 45, and 'Genotype NO. 14' registered the lowest value of 20 (Fig. 9). Under significant stress (-1.2MPa), 'Genotype NO. 11 and 3' exhibited the highest SVI values of 11 and 10, respectively, confirming their exceptional resistance to varying drought conditions during germination and early seedling development.

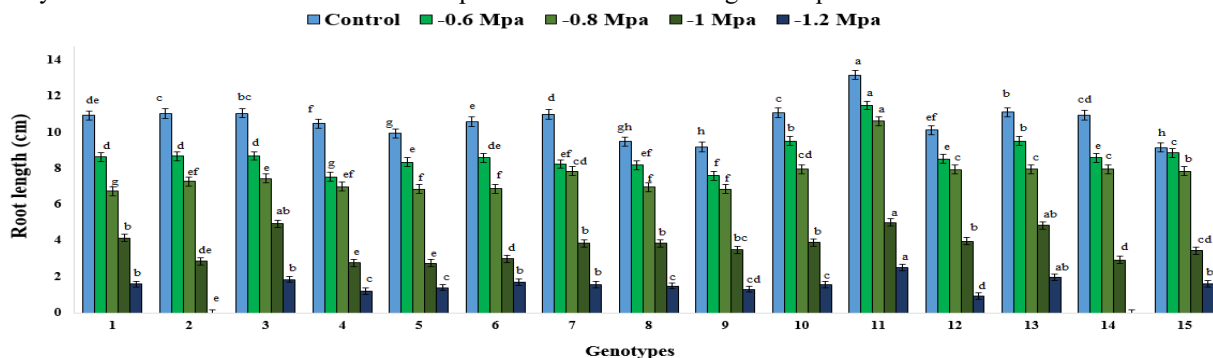


Figure 7. Effects of drought stress on root length (RL) in 15 Canola genotypes. The error bar denotes ± standard deviation (SD). According to Duncan's test at (p<0.05), bars with similar alphabets are not significantly different

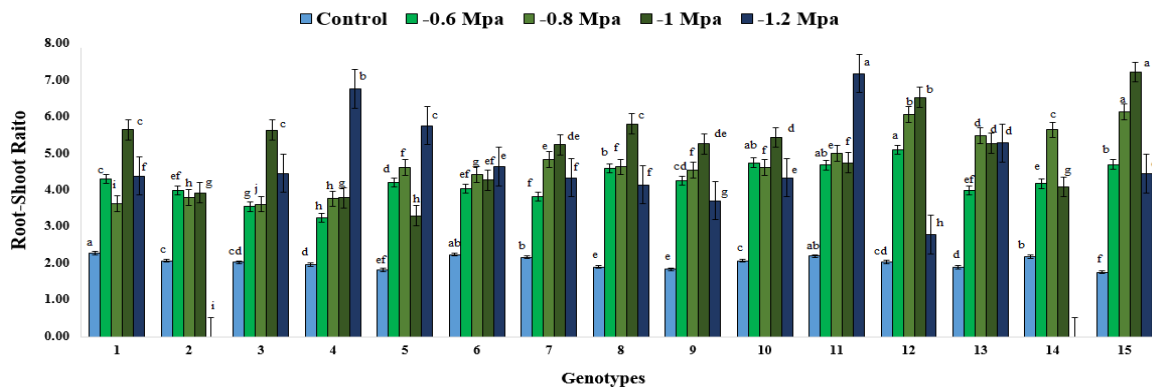


Figure 8. Effects of drought stress on RSR in 15 Canola genotypes. The error bar shows ± SD. According to Duncan's test at (p<0.05), bars with similar alphabets are not significantly different

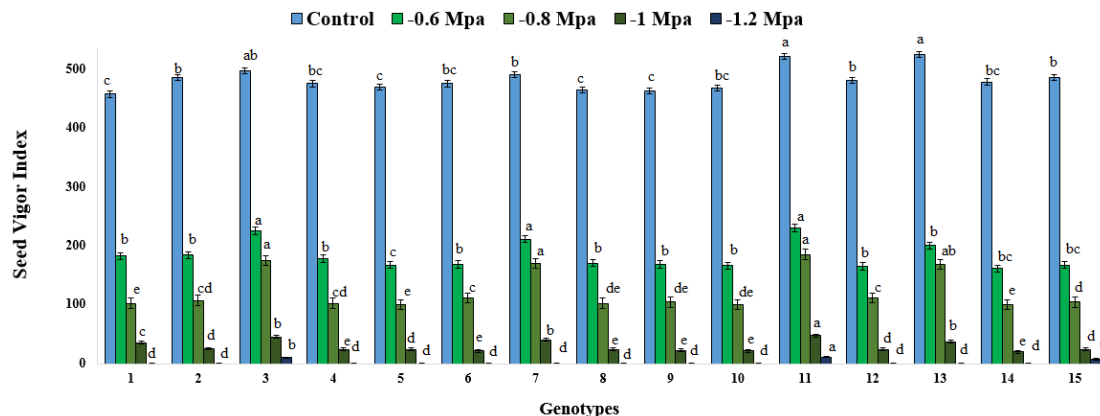


Figure 9. Impact of drought stress on seedling vigor index (SVI) in 15 Canola genotypes. The error bar represents ± standard deviation (SD). According to Duncan's test at (p<0.05), bars with similar alphabets are not significantly different

Table 3. Person's correlation analysis of all characteristics examined for 15 Canola genotypes under varying drought conditions

	GP	GR	MGT	SL	RL	RSR	SVI	RER
GP	1							
GR	0.773**	1						
MGT	-0.2	-0.44	1					
SL	0.43	0.545*	-0.335	1				
RL	0.518*	0.756**	-0.354	0.715**	1			
RSR	0.156	0.277	0.108	-0.163	0.285	1		
SVI	0.556*	0.680**	-0.501	0.781**	0.797**	0.139	1	
RER	0.538*	0.542*	-0.284	0.318	0.601*	0.185	0.68*	1
SER	0.562*	0.572*	-0.102	0.218	0.560*	0.133	0.157	0.56*

* Significant at 0.05 probability level. ** Significant at 0.001 probability level

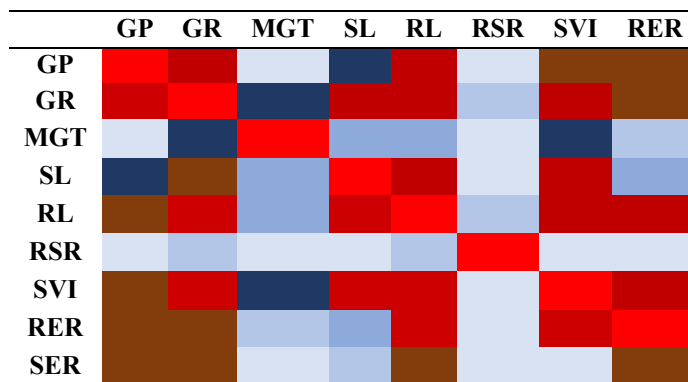


Figure 10. Correlation map to assess the correlation strength of all variables examined for 15 Canola genotypes under varying drought levels (Red indicates the most significant correlation, brown indicates a medium correlation, blue indicates a low correlation, and white indicates no connection)

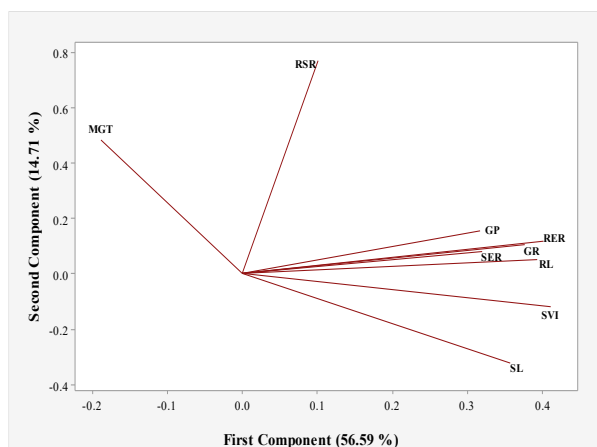


Figure 11. Correlation diagram between all traits studied

3.3. Correlations analysis

The Pearson correlation showed that most of the tested traits in Table 3 were significantly and strongly interrelated. The GP was highly positively correlated with GR ($r = 0.773^{**}$), RL ($r = 0.518^*$), SVI ($r = 0.556^*$), RER ($r = 0.538^*$), and SER ($r = 0.562^*$). GR was positively and significantly related to SL ($r = 0.545^*$), RL ($r = 0.756^{**}$), RER ($r = 0.542^*$), and RSR ($r = 0.572^*$). Similarly, the lengthening of RL showed a strong positive relation with RER ($r = 0.601^*$) and GR ($r = 0.756^{**}$) (Table 3). The SVI showed a very highly positive significant association with GP ($r = 0.556^*$), GR ($r = 0.680^{**}$), RL ($r = 0.797^{**}$), and SL ($r = 0.781^{**}$). The highest association was obtained by SVI for RL ($r = 0.797^{**}$), as represented in Table 3. These strong and substantial associations give ample knowledge about the interaction of the studied traits. Where the color map in Fig.10 illustrates red as the highest positive correlation, brown indicates moderate positive connections, blue indicates low positive correlations, and white indicates no correlation. Surprisingly, GP correlated to GR at $r = 0.773^{**}$. A correlation diagram was used to represent the trait associations, and a biplot diagram was used- Fig. 11. In a biplot diagram, the cosine of the angle between the trait vectors shows the strength of the correlation between characteristics. If the angle between the vectors is less than 90 degrees, the correlation is +1; an angle of 90 degrees means no correlation, and an angle of 180 degrees means a -1 correlation. In the correlations performed here, GR with RL correlates at $r = 0.756^{**}$, SVI with RL correlates at $r = 0.797^{**}$, and SL with RL correlates at $r = 0.781^{**}$. The strongest relation is still between SVI versus RL: $r = 0.797^{**}$. Correlation maps have been used in various plant studies to explore the relationship between various characteristics of plants, as can be seen from rice research upstairs (Semeskandi et al., 2023), maize (Ahmady and Mazloom, 2023), and Canola (Sadeghizadeh, 2023).

3.4. Principal component analysis (PCA)

Initially, it is essential to verify the adequacy of the data about the sample size and the interrelationship among the variables to assess the principal components. The KMO test statistic was used to verify data sufficiency, while the Bartlett test assessed data suitability for factor analysis. If the index value approaches one, the sample size is deemed appropriate for factor analysis; conversely, values typically below 0.5 suggest that the principal component analysis results are inadequate for the data. An index value ranging from 0.5 to 0.69 signifies average data quality, warranting cautious extraction, while values exceeding 0.7 indicate a suitable sample size. The KMO value for the whole dataset is 0.746, above 0.7. Consequently,

principal component analysis may be conducted on any data, and the conclusions obtained are legitimate. The KMO value obtained was 0.746 (Table 4), indicating the requisite correlation among the input variables for principal component analysis.

By the Eigenvalue analysis in Table 5, the two first components explained about 71.30% of the variance in the dataset in the PCA carried out on the test data. The red axis in the Eigenvalue chart stands for the cumulative variance. In contrast, the blue bar charts represent the Eigenvalues for the individuals, confirming that the variance explained by the first two components exceeds 71.30%. To this effect, the first component contributed to variance at 56.59%, while the contribution by the second component was 14.713%, as shown in Fig. 12. The first component accounting for more than 56.59% of the variance, the traits Germination Percentage, Germination Rate, Root Length and Shoot Length, Seedling Vigor Index, Root Elongation Rate, and Shoot Elongation Rate all contributed positively to this component. Of most importance, the Seedling Vigor Index (0.906) and Shoot Elongation Rate showed the most positive impact (0.919). The second component explained about 14.713% of the variation. It also indicated that Mean Germination Time and Root-Shoot Ratio positively contributed to this component. Also, the most profound influence was from Root-Shoot Ratio, 0.84. (Table 5). We analyzed the genotype distribution based on positive and negative coefficients of the first and second principal components. The first two components captured 86.41% of the total variance, hence the more significant part of the observed variation – Fig. 12.

Table 4. KMO value and Bartlett's test

Kaiser-Meyer-Olkin Measure of Sampling Adequacy.		0.746
Bartlett Test of Sphericity	Approx. Chi-Square	82.353
	df	36
	Sig.	0.000

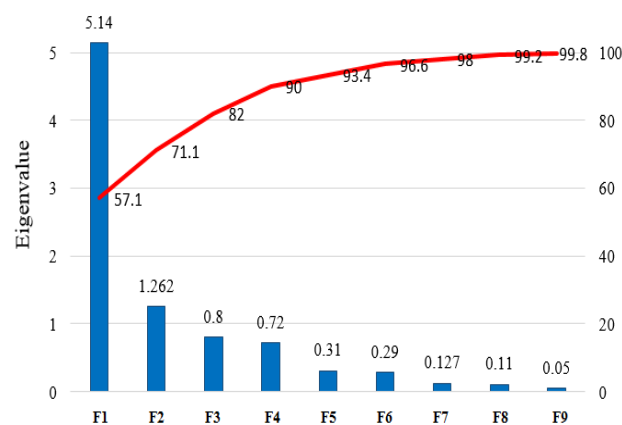


Figure 12. Eigenvalue diagram traits of 15 Canola genotypes in PC1 and PC9 biplot

Table 5. Principal components analysis of studied traits in Canola genotypes

studied traits in Canola genotypes	Principle component 1	Principle component 2	Extraction
GP	0.735	0.072	0.546
GR	0.861	0.007	0.742
MGT	-0.375	0.57	0.466
SL	0.753	-0.472	0.79
RL	0.89	-0.048	0.795
RSR	0.339	0.84	0.82
SVI	0.906	-0.251	0.884
RER	0.727	-0.017	0.529
SER	0.919	0.012	0.845
Total	5.093	1.324	-
% of Variance	56.59	14.713	-
Cumulative %	56.59	71.30	-

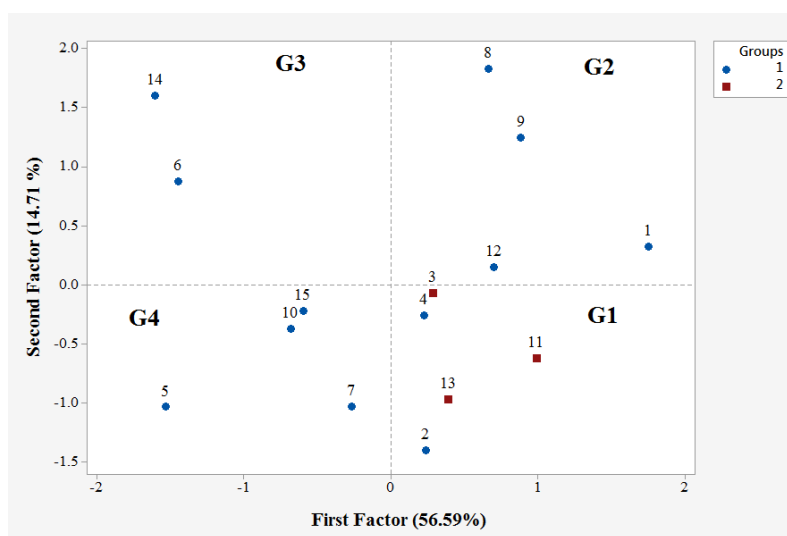


Figure 13. Results of decomposition into factors for quantitative traits of 15 canola genotypes in biplot

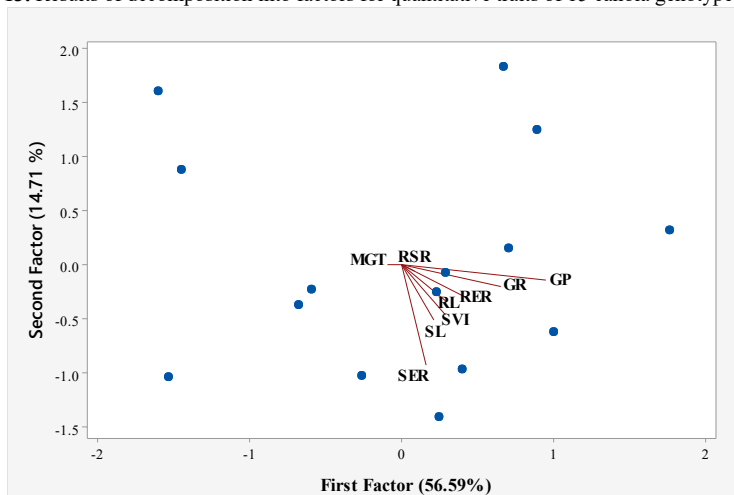


Figure 14. Distribution of studied genotypes in biplot based on decomposition into factors

The first component accounted for 71.30% of the total variation, with high values for germination percentage (0.735), germination speed (0.861), root length (0.89), and stem length (0.753). Multivariate statistics determined seedling strength. Index (0.906), root elongation rate (0.727), and stem elongation rate (0.919). This component

mainly represents seed germination and development of the root system. In contrast, the second component accounted for 14.713% of the variance among all the variables. It showed only a significant correlation with average germination time (0.57) and root-to-shoot ratio (0.84) (Table 5 and Fig. 13).

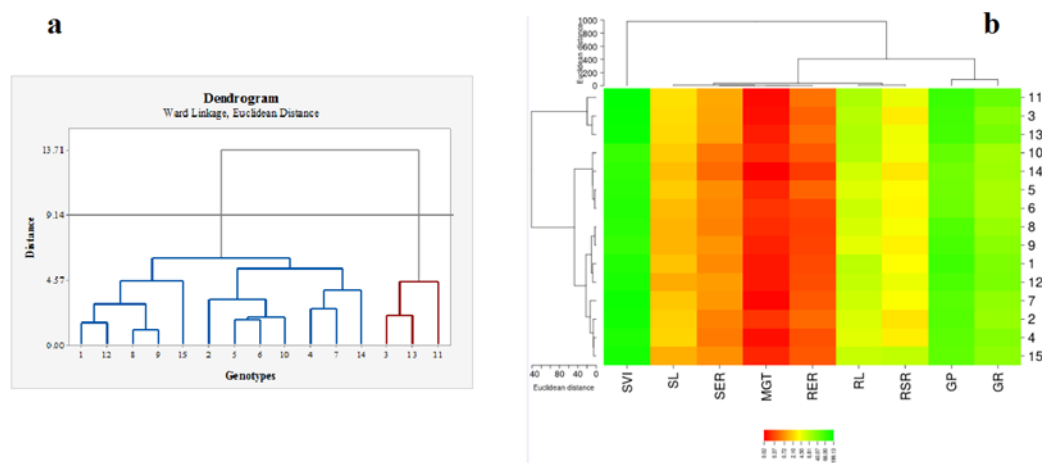


Figure 15. The cluster analysis and heat map results are based on the characteristics evaluated in the experiment: a) cluster analysis. b) Thermal map

Thus, component 2 reflects the development of aerial organs in the seedlings. In developing better and more accurate identification of drought-tolerant genotypes, a biplot diagram was performed based on two main components, which aim to focus on selection through multivariate approaches rather than using individual variables. A formed biplot in the north is divided into four groups respectively: G1 includes tolerant genotypes, G2 comprises relatively tolerant genotypes, G3 consists of relatively sensitive genotypes, and G4 is sensitive genotypes. Because of the NO diagram of selected genotypes 3, 11, and 13, all had high values for GP, GR, RL, SL, SVI, RER, and SER, and low values for MGT and RSR corresponding to superior drought tolerance at germination as well as early seedling growth.

In contrast, the genotypes in G2, namely 'Genotypes No. 1, 8, 9, and 12', showed favorable correlations with both PC1 and PC2, with significantly higher levels of GP, RER, and RSR. Thus, they can be considered relatively drought-tolerant during germination and the initial phases of seedling development. On the other hand, group G3, which includes 'Genotypes 6 and 14', has a positive correlation with PC2 and a negative correlation with PC1. These genotypes exhibit high scores for MGT and low scores for GP, GR, RL, SL, SVI, RER, and SER, suggesting they are comparatively drought-resistant. Group G4, comprising genotypes 5, 7, 10, and 15, displayed negative correlations with PC1 and PC2, indicating a greater susceptibility to drought than the other groups. Based on these findings, 'Genotypes NO. 3, 11, and 13' can be identified as the most effective and resilient to varying drought conditions during the germination and early seedling development phases (Figs. 13 and 14).

3.5. Cluster Analysis

Using the WARD method, the current study employed cluster analysis to classify 15 Canola genotypes. The heat map generated from the analyzed traits divided the genotypes into two main categories (Fig. 15 a,b). This

analysis categorized the genotypes into two primary groups. The first group was further divided into two subgroups: the first subgroup contained Genotype Number 11, while the second subgroup included Genotypes Number 3 and 13. The second leading group was also divided into two subgroups: the first subgroup comprised Genotypes 1, 8, 9, 12, and 15, and the second subgroup consisted of Genotypes 2, 4, 5, 6, 7, 10, and 14. The cluster analysis demonstrated that the genotypes were organized into two main groups and three subcategories (Fig. 15 a,b).

4. Discussion

Based on the results, it was found that in the current study, the simulated drought stress affected all the measured germination parameters, including MGT, GR, and GP (Figs. 1, 3, and 4). As expected, the negative impact on sensitive genotypes was much higher than on tolerant genotypes. The results showed that drought stress significantly decreased GR GP and MGT, consistent with the results of Kaya et al. (2020). Considering that seed germination is harmful to the growth and establishment of seeds and plant seedlings, and this process starts with the absorption of water and the presence of water for the activation of hydrolytic enzymes, which are used to break down starch, dissolve, and transport carbohydrates, is very necessary. Therefore, lack of water at this stage negatively affects successful seed germination (Khayatnezhad et al, 2010). For Seed priming is one of the methods to improve the efficiency of germination and initial growth of plants, which accelerates and enhances the physiological and biochemical processes of seeds by preparing seeds before planting. This process involves controlled pre-watering of the seeds, which leads to the activation of enzymes, regulation of hormones, and stimulation of seed metabolism without complete germination (Marthandan et al., 2020). During priming, essential enzymes such as amylase, lipase, and xylanase are activated, which causes the breakdown and transfer of seed food reserves to the

plant embryo. This ultimately contributes to faster and more uniform seed germination and improves seedling establishment (Rouhi and Sepehri, 2020). One of the effective methods of priming is using plant hormones such as gibberellin (GA3), which enhances germination and reduces the effects of seed aging. This hormone reduces oxidative damage by balancing the amounts of metabolites such as malondialdehyde (MDA) and reactive oxygen species (ROS). It contributes to more uniform germination and resistant seedlings (Gholamin and Khayatnezhad, 2021). These potential germination characteristics resulted in a rapid increase in seed germination and seedling growth.

In the study that focused on the impact of drought stress on seed germination and seedling growth, it was indicated that through drought stress, the reduction of germination and production of reactive oxygen species, which in turn damage cellular components and performance of the seedlings, is reduced. A 2024 study researched drought stress and physiological responses among canola plants, especially in their early stage. It has been identified that drought stress enhances ROS production, which negatively impacts the membranes of the cells, reducing metabolic power and reducing growth (Marthandan et al., 2020). Another recent 2023 study investigated the use of boric acid and gibberellic acid to enhance drought tolerance in Canola and found that ROS effects could be reduced and germination promoted through priming. These results demonstrated the role of these compounds in increasing seedling vigor against environmental stresses (Marthandan et al., 2020). Under severe drought conditions (osmotic potential of -1.2 MPa), the germination was considerably reduced in all the genotypes, while genotype NO. 3 and 11 did not germinate ultimately (Fig. 1). However, genotype NO. 11 exhibited less sensitivity to severe drought stress with the lowest mean reduction in germination percentage (<77%), proving its high tolerance to osmotic stress. Therefore, it may be considered a rich and promising source of germplasm for drought tolerance at germination. Our findings in this study showed that drought stress imposed at an early stage of growth was not good for the development, and it significantly reduced the length of roots and stems (Figs. 5 and 7). This result is consistent with previous research findings from other oilseed crops (Bouchyoua et al., 2024).

The reduction in meristematic growth and cell division within the plant root meristems is primarily responsible for reduced stem and root length in plants under water stress conditions. Our findings indicated that genotypes 3, 11, and 13 effectively developed more comprehensive root systems under mild, moderate, and severe levels of water stress, respectively Fig. 7. In addition, genotype NO.11 consistently ranks first in root length and root elongation under non-stress conditions and all three levels of drought

stress, indicating its higher resistance to drought at the seedling stage compared with other genotypes. Also, the root-to-stem ratio increased with the enhancement of drought severity, which stated that roots under drought stress tend to grow more longitudinally while the stem grows less.

This may be due to the redistribution of nutrients from the endosperm into the roots at the expense of reduced shoot growth. In the field, a well-developed root apparatus adaptive to soil moisture limitation can provide plants access to water from deeper soil layers, mitigating drought's adverse effects, particularly during the early seedling growth stages. For this reason, root length represents one of the main bases for selecting drought-resistant cultivars, as Rouhi and Sepehri (2020) reported. Because of the high correlation of this feature with germination percent, using this feature as an index is recommended to enhance the level of drought tolerance in rapeseed. As shown in Fig. 5, under mild and moderate stress conditions, more favorable shoot growth for genotypes No. 11 and 3 was observed than in the other studied genotypes. These results indicate that they resist drought stress more during the early stages of seedling growth than the corresponding other genotypes.

5. Conclusion

This research provides valuable insights into the drought tolerance of various rapeseed (*Brassica napus* L.) genotypes during germination and early seedling development, particularly under PEG-induced drought stress. Through systematic evaluation across multiple levels of water stress (-0.6, -0.8, -1.0, and -1.2 MPa), key traits such as germination percentage (GP), germination rate (GR), mean germination time (MGT), root and shoot lengths, root-to-shoot ratio (RSR), seedling vigor index (SVI), shoot elongation rate (SER), and root elongation rate (RER) were assessed. The results underscore the differential responses of rapeseed genotypes to drought, highlighting the potential for selecting drought-tolerant cultivars that can thrive in water-limited conditions. The significant decline observed in GP, GR, SL, RL, RER, and SER at high drought levels aligns with existing literature indicating that water deficit severely hinders the germination and growth of rapeseed by reducing water uptake and cellular expansion. A noteworthy increase in MGT and RSR across drought conditions suggests adaptive mechanisms in some genotypes, allowing them to reallocate resources to root growth in response to water stress. Specifically, genotypes 3, 11, and 13 consistently performed better in maintaining GP, RL, and RER under drought conditions, suggesting robust resilience to water stress. These genotypes exhibited notable root elongation and higher SVI scores, critical for early-stage seedling establishment under suboptimal moisture conditions. The

PCA and cluster analysis identified RL, GP, and RER as significant traits contributing to drought tolerance. The root length, a primary factor influencing water uptake, was particularly enhanced in drought-tolerant genotypes, underscoring the importance of root architecture in breeding programs. Genotypes that exhibited higher RSR also suggest an adaptive advantage, with roots becoming more prominent under stress at the expense of shoot development. This trait is vital for enhancing drought resilience in arid environments by enabling plants to access deeper soil moisture and maintain hydration, supporting their survival during early growth stages. In addition, the findings indicate that drought-tolerant genotypes managed better root growth and maintained a steady SVI, implying that these genotypes could sustain vigor under stress. The significant correlations observed between SVI, GP, and RL underscore their interdependence in ensuring robust germination and seedling establishment. These results contribute to the growing understanding that root system architecture, particularly root length and elongation rate, are key indicators of drought resilience. Moreover, the strong correlation between GP and drought tolerance further suggests that germination percentage can serve as a valuable screening criterion for identifying drought-resistant rapeseed genotypes. Importantly, the study reveals that some genotypes, specifically NO. Three and NO. 11 exhibited relatively high drought tolerance in mild and intermediate stress conditions. These genotypes managed to sustain both root and shoot growth better than others, highlighting their potential as candidates for further development and breeding. The observed resilience in these genotypes may be attributed to inherent physiological adaptations, such as efficient water use and maintaining cell turgor under low water availability. This aligns with prior research on PEG-induced drought stress, where resilient cultivars showed improved water retention, robust root growth, and reduced reactive oxygen species (ROS) accumulation in stress conditions. The findings of this study have significant implications for breeding programs focused on enhancing drought tolerance in rapeseed. Identifying drought-tolerant genotypes provides a practical foundation for developing cultivars tailored for cultivation in arid and semi-arid regions, where water scarcity is a primary constraint. This is particularly relevant in the context of climate change, which is expected to exacerbate drought conditions globally. By selecting genotypes with stronger root systems and high SVI, breeders can develop varieties that are not only drought-tolerant but also capable of sustaining yield in challenging environments. Future research should extend these findings through field trials to validate the laboratory-based drought responses observed in this study. While PEG-induced drought provides a controlled method to simulate water stress,

field conditions incorporate additional environmental variables that influence plant behavior. Understanding the performance of these genotypes across varied environmental conditions will enhance their potential for real-world application. Additionally, investigating the genetic and molecular mechanisms underlying drought tolerance in these genotypes could provide insights into specific gene expressions and pathways contributing to drought resilience. This would pave the way for more targeted breeding strategies, potentially utilizing molecular markers associated with root length, SVI, and other drought-tolerance traits. In conclusion, this research underscores the critical role of root system development, particularly root length and RSR, in facilitating drought tolerance during rapeseed germination and early seedling stages. The genotypes identified, notably NO. 3, NO. 11 and NO. 13 represent promising candidates for breeding programs to improve drought resilience. By leveraging these findings, agronomists and breeders can contribute to sustainable agricultural practices and enhance food security in drought-prone regions, thereby addressing the global challenge of water scarcity in agriculture.

Authors Contribution

All the authors have participated sufficiently in the intellectual content, conception, and design of this work or the analysis and interpretation of the data (when applicable), as well as the writing of the manuscript.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of interest

The author states that there is no conflict of interest.

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Abbreviations

GP	Germination percentage
GR	Germination rate
GK	Germination kinetics
MGT	Mean germination time
SL	Shoot length
RL	Root length
RSR	Root-Shoot Ratio
SVI	Seedling Vigor Index
RER	Root Elongation Rate
SER	Shoot Elongation Rate
