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EARLY-STAGE SCREENING OF BRASSICA FOR DROUGHT TOLERANCE UNDER PEG-INDUCED STRESS

Original Article

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ABSTRACT

Background: Drought is one of the most limiting environmental stresses affecting both early seedling establishment and later developmental stages of agricultural crops. Its frequent occurrence, intensified by irregular rainfall and rapid climate shifts, severely disrupts the productivity of oilseed crops and contributes to substantial yield reductions. These challenges highlight the urgent need to identify drought-resilient and locally adapted Brassica germplasm that can withstand water-deficit conditions and support sustainable crop improvement programs.

Objective: This study aimed to evaluate the performance of ten *Brassica* genotypes under varying levels of polyethylene glycol (PEG-6000)–induced drought stress and identify drought-tolerant candidates suitable for future breeding initiatives.

Methods: A controlled-environment Petri dish experiment was conducted using a Completely Randomized Design with three replications. Four treatments were applied: a non-stressed control and PEG-6000 concentrations of 5%, 10%, and 20% to simulate mild, moderate, and severe drought stress. Ten genotypes were assessed for germination percentage, shoot length, root length, root-to-shoot ratio, shoot and root fresh weight, shoot and root dry weight, seedling fresh weight, and seedling dry weight. Data were recorded eight days after sowing and analyzed using analysis of variance to determine significance across genotypes, treatments, and genotype-by-treatment interactions.

Results: Highly significant differences ($p < 0.001$) were observed among genotypes, treatments, and their interactions. Values ranged widely across traits, including germination percentage (40–100%), shoot length (0.33–8.30 cm), root length (0.20–5.37 cm), and root-to-shoot ratio (0.28–0.93). Biomass parameters also showed considerable variation, with shoot fresh weight (0.002–0.54 mg), root fresh weight (0.01–0.04 mg), shoot dry weight (0.0005–0.0134 mg), and root dry weight (0.0003–0.010 mg). The 5% PEG-6000 treatment consistently enhanced seedling performance compared to higher stress levels. Among genotypes, RBN-08003 showed superior performance across multiple traits, whereas UAF-11 ranked lowest under most treatments.

Conclusion: Mild drought simulated through 5% PEG-6000 proved most favorable for seedling growth in the evaluated *Brassica* genotypes. The genotype RBN-08003 demonstrated strong drought tolerance and appears promising for incorporation into future breeding programs focused on water-limited environments.

Keywords: Brassica, Drought Tolerance, Germination, Polyethylene Glycols, Seedling Development, Stress Physiology, Water Stress.

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INTRODUCTION

The family Brassicaceae comprises more than 350 genera and approximately 3,500 species, including important groups such as Crambe, Thlaspi, Camelina, Sinapis, and Brassica, the latter being one of the most economically and agriculturally significant genera worldwide. Within Brassica, species such as *B. napus*, *B. rapa*, and *B. oleracea* hold considerable global value due to their diverse uses in food, fodder, and oil production (1). The genus includes nearly 400 species and is believed to have originated in temperate regions spanning China, India, the Middle East, and the Mediterranean. Its characteristic floral morphology—with bisexual flowers, cruciform petals, and a superior ovary—supports its efficient reproductive success and widespread cultivation (2). Rapeseed, a major Brassica crop, has demonstrated strong adaptability in regions such as Morocco and other Mediterranean countries, offering substantial potential to enhance edible oil production in these areas (3). With an oil content of approximately 40–44%, rapeseed ranks as the third most important edible oil crop globally, following palm and soybean, and contributes significantly to both oil and protein supply chains. In the 2019–2020 period, global production reached an estimated 72.37 million tonnes, highlighting its critical role in agricultural economies (4,5). Its oil is particularly valued for its favorable fatty acid composition, including high oleic acid and linoleic acid content, which enhances its nutritional and industrial utility. Despite its importance, Brassica production is increasingly constrained by abiotic stresses—especially drought, waterlogging, salinity, and heat—which remain major threats to global food security (6). Among these, drought represents one of the most devastating challenges, affecting more than 40% of the world's land area and nearly 60 million km² of soils (7,8). As temperatures rise and climate variability intensifies, drought frequency and severity are expected to increase, directly influencing seed development, vegetative growth, canopy formation, germination, mineral uptake, and overall yield stability (9). Physiological disruptions under drought include reduced enzyme activity, loss of turgor, decreased leaf area, premature senescence, impaired photosynthesis, and stunted growth, ultimately diminishing the crop's photosynthetic capacity and productivity (10,11). Understanding these stress responses is vital for improving tolerance mechanisms and sustaining agricultural outputs in vulnerable environments.

To simulate drought stress under controlled conditions, polyethylene glycol (PEG) has emerged as a widely used osmotic agent in plant physiology research. PEG is a non-ionic, water-soluble polymer available in various molecular weights, with PEG-6000 and PEG-8000 being the most commonly employed to induce water-deficit conditions comparable to field drought (12,13). High-molecular-weight PEG molecules are too large to be absorbed by plant cells yet effectively reduce the osmotic potential of nutrient solutions, thereby limiting water availability and eliciting physiological responses similar to natural drought (14). PEG exposure leads to reduced water potential, altered transpiration, and leaf desiccation, with higher molecular weight variants capable of obstructing water movement and intensifying stress effects (15). Because drought adaptation involves complex biochemical and molecular pathways—including hormonal regulation, antioxidant activity, osmoprotectant accumulation, and gene expression changes—PEG-based screening provides an efficient platform for identifying tolerant genotypes at early developmental stages, which is as critical as tolerance during terminal growth phases (16,17). Given the increasing unpredictability of climate patterns and the critical role of Brassica oilseed crops in global nutrition and agricultural sustainability, research into drought tolerance mechanisms and genotype responses remains essential. Therefore, the present study aims to evaluate the drought tolerance of *Brassica napus* genotypes using PEG-induced osmotic stress to support future breeding strategies and enhance crop resilience under water-limited conditions.

METHODS

The experiment was carried out during the winter season of 2023 under controlled laboratory conditions to assess the response of *Brassica* genotypes to polyethylene glycol (PEG)-induced drought stress. The study followed a factorial arrangement under a Completely Randomized Design (CRD) with three replications. It was conducted in the Wheat Genetics Laboratory, Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad. Ethical approval was considered under institutional laboratory research guidelines; however, no human or animal subjects were involved, and therefore formal IRB approval and informed consent were not required for this experiment. The study included ten *Brassica* genotypes—Abasine 95, Anmol Raya, BJ-FSD Mustard, Rachna Canola, RBN-04725, RBN-04016, RBN-08003, Sandal Canola, UAF-11, and KN-259—designated as G1 to G10. Inclusion criteria were defined as genetically pure, viable seeds of the selected genotypes, while seeds showing visible mechanical damage, discoloration, or fungal contamination were excluded to ensure uniformity. A petri dish–based germination and seedling growth assay was used to simulate drought stress using PEG solutions. Petri dishes were labeled from 1 to 120 to accommodate all genotypes and replications. Seven seeds per genotype were sown in each dish, following surface sterilization in 5% sodium hypochlorite for one minute and subsequent rinsing with distilled water to eliminate residual disinfectant. Sterilized seeds were placed on 9 cm petri dishes lined with

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sterile filter paper. The experiment included four treatments: a non-stressed control and three PEG-induced drought levels (5%, 10%, and 20%). All petri dishes were placed in an incubator maintained at 25°C to ensure uniform environmental conditions. A 3 mL volume of the respective PEG solution or distilled water (control) was applied to each dish every 48 hours using a micropipette, and treatments continued for eight days. Germination percentage was recorded on days 2, 4, 6, and 8 using the formula: Germination (%) = (Number of germinated seeds / Total number of seeds) × 100. On day 8, seedlings were harvested to measure shoot length, root length, shoot fresh weight, root fresh weight, and seedling fresh weight using a sensitive electronic balance. Root and shoot lengths were measured from the cotyledon to collar region and from collar to root tip, respectively. For dry weight measurements, seedlings were oven-dried for 48 hours, after which root dry weight, shoot dry weight, and total seedling dry weight were recorded. Root-to-shoot length ratio was computed using the formula Root length / Shoot length. Seedling fresh weight and dry weight were calculated by summing shoot and root weights. Statistical analysis was performed using Statistix 8.1 software. Analysis of Variance (ANOVA) under the factorial CRD framework was applied to determine differences among genotypes, drought levels, and their interactions. Mean comparisons were conducted using the Least Significant Difference (LSD) test at an appropriate significance level to identify treatment effects reliably. Data analysis adhered to standard biometric procedures to ensure accuracy and reproducibility.

RESULTS

The analysis revealed statistically significant variation among the evaluated *Brassica* genotypes for all measured seedling traits, including germination percentage, shoot length, root length, root-to-shoot ratio, shoot fresh weight, root fresh weight, shoot dry weight, root dry weight, seedling fresh weight, and seedling dry weight. Substantial ranges were observed across genotypes, with germination percentage varying from 40% to 100%, shoot length from 0.33 to 8.30 cm, root length from 0.20 to 5.37 cm, and root-to-shoot ratio from 0.28 to 0.93. Fresh shoot weight ranged from 0.002 to 0.54 mg, fresh root weight from 0.01 to 0.04 mg, shoot dry weight from 0.0005 to 0.0134 mg, and root dry weight from 0.0003 to 0.010 mg. Seedling fresh weight showed values between 0.003 and 0.30 mg, while seedling dry weight ranged from 0.0008 to 0.020 mg. Significant effects of drought treatments were also observed for all traits. The 5% PEG-6000 treatment consistently resulted in the highest values for germination percentage, shoot length, root length, root-to-shoot ratio, shoot and root fresh weight, shoot and root dry weight, and seedling fresh and dry weights. As expected, the control treatment performed better than the 10% and 20% PEG stress levels. The 20% PEG treatment produced the lowest values across nearly all traits, indicating the strongest inhibitory effect on seedling growth. Interaction effects between genotypes and treatments were significant, demonstrating differential drought responses among genotypes under varying stress intensities. Among the ten genotypes, RBN-08003 displayed the most vigorous performance across multiple traits, particularly germination percentage, shoot length, shoot fresh weight, root fresh weight, shoot dry weight, and root dry weight. In contrast, UAF-11 exhibited the weakest performance under several stress conditions. The collective results support the identification of RBN-08003 as the most drought-tolerant genotype under PEG-induced osmotic stress. Overall, seedling vigor was maximized under 5% PEG-6000, while severe growth reduction occurred at 20% PEG-6000. The mean square values indicated strong statistical significance for genotypes, treatments, and genotype-by-treatment interactions across all variables measured. Error values remained minimal, confirming the reliability of experimental measurements and overall precision of laboratory conditions.

Table 1: Mean square values of different seedling traits

Source	DF	GP	SL	RL	RL/SL	FWS	FWR	DWS	DWR	SFW	SDW
Genotypes	9	443.171**	14.873**	46.4935**	0.15246**	6.058E-04**	2.426E-04**	3.996 E-05**	1.611 E-05**	0.00682**	8.418E-05**
Treatments	3	953.542**	198.409**	100.5**	0.03226**	0.00815**	0.00164**	5.146 E-04**	9.907 E-05**	0.04893**	0.00104**

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Source	DF	GP	SL	RL	RL/SL	FWS	FWR	DWS	DWR	SFW	SDW
Genotypes* Treatments	27	597.986**	3.666**	0.9857**	0.05861**	1.521E-04**	1.038E-04**	9.677 E-06**	6.577 E-06**	0.00620**	1.898E-05**
Error	80	29.375	0.006	0.0060	0.00004	3.262E-08	2.011E-07	2.967-08	7.766 E-09	0.00001	2.119E-07
Total	119										

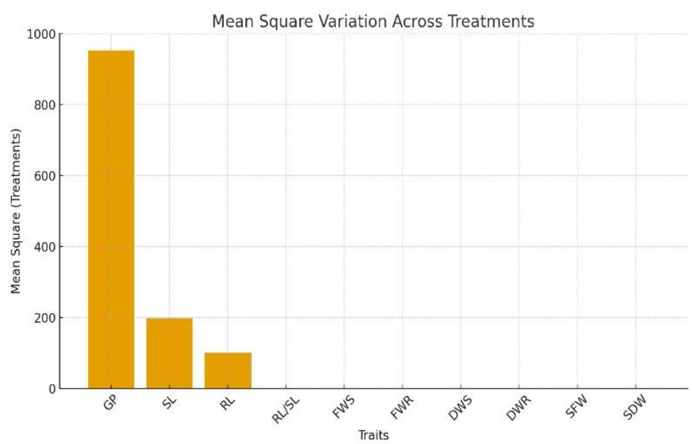


Figure 1 Mean Square Variation Across Treatments

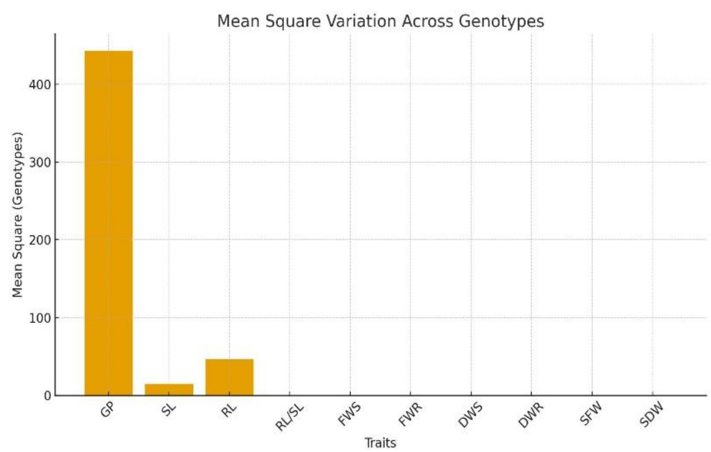
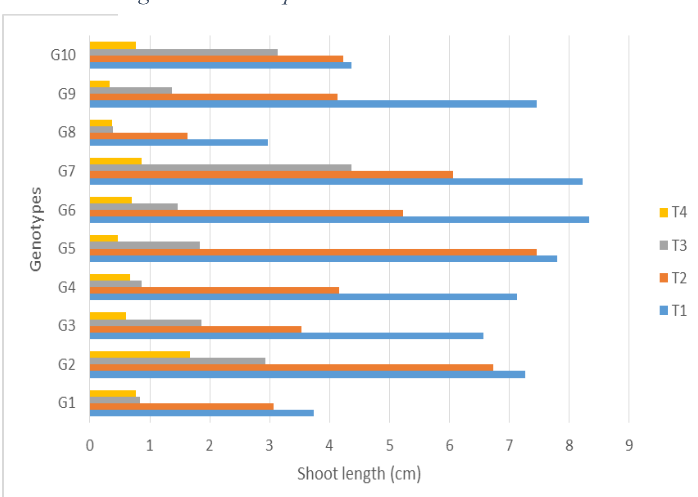


Figure 1 Mean Square variations Across Genotypes



Effect of drought treatments on shoot length (cm)

Figure 3 Effect of Drought Treatments on Shoot length (cm)

Effect of drought treatments on root length (cm)

Figure 4 Effect of Drought Treatments on Root Length (cm)

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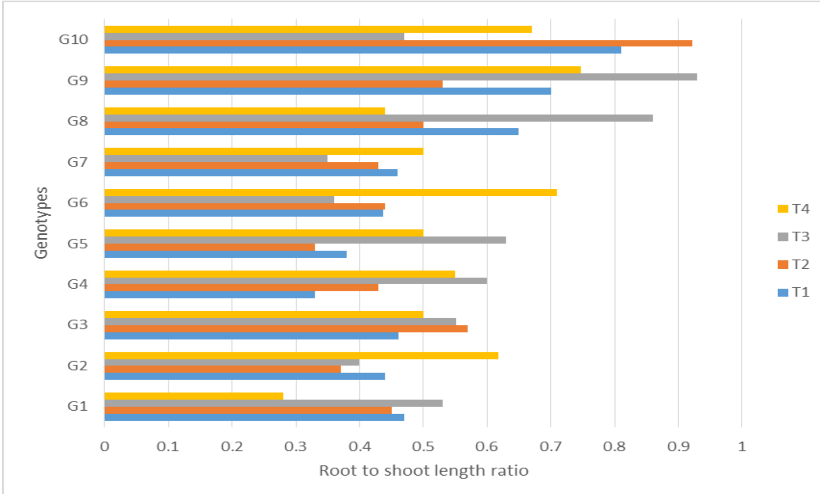

Effect of drought treatments on root to shoot length of seedlings

Figure 5 Effect of drought Treatments on Root to shoot Length of Seedlings

DISCUSSION

The findings of the study demonstrated clear and consistent variations among *Brassica* genotypes exposed to PEG-6000–induced drought stress, illustrating the inherent genetic diversity in early seedling responses. The superior performance observed under 5% PEG treatment indicated that mild osmotic stress stimulated seedling growth more effectively than higher stress levels, a pattern previously reported in studies where moderate drought conditions triggered adaptive physiological adjustments that supported early vigor (18). The progressive decline in all growth parameters under 10% and particularly 20% PEG aligned with established evidence that increasing osmotic stress limits cell division, disrupts water uptake, and suppresses overall seedling metabolism (19). The present study confirmed that traits such as germination percentage, shoot and root length, fresh and dry biomass, and root-to-shoot allocation are sensitive indicators of early drought tolerance in *Brassica* species, supporting their relevance in stress-screening assays. The genotype RBN-08003 consistently exhibited superior performance across multiple traits, suggesting a more efficient physiological adjustment to osmotic stress. Similar observations were made in previous evaluations of drought-tolerant *Brassica* lines, where tolerant genotypes maintained higher water retention, greater root proliferation, and enhanced seedling biomass under restricted moisture conditions (20,21). Conversely, UAF-11 showed reduced vigor across most drought levels, reflecting limited capacity to withstand osmotic depletion. Such differential responses reinforced the genetic basis of drought tolerance and highlighted the potential of PEG-based screening for identifying resilient genotypes during early developmental stages (22). The stability of responses across treatments and replications strengthened the validity of the findings, with statistical significance observed for genotype, treatment, and genotype-treatment interactions, indicating robust biological differences rather than random variation.

Implications of the results extend to breeding programs focused on enhancing drought resilience in oilseed crops. Identifying genotypes like RBN-08003 provides a valuable foundation for introgressing drought-responsive traits, particularly in regions experiencing increasing water scarcity. Early-stage tolerance is instrumental in crop establishment and survival, and the consistency of RBN-08003 across traits suggests a promising physiological profile for breeding advancement. The study further supports the utility of PEG-induced drought simulation as a reliable and reproducible method for preliminary screening, especially when field-based drought conditions remain unpredictable and difficult to standardize. Despite its strengths, including controlled laboratory conditions, standardized measurements, and clear detection of genotypic variation, the study also presented certain limitations. PEG-induced stress, although widely used, does not fully replicate the complexity of soil-based drought, where factors such as nutrient availability, soil structure, and microbial interactions influence plant performance. Seedling assays capture early response mechanisms but may not predict long-term tolerance or yield stability under field drought. Additionally, the study did not include detailed physiological or biochemical markers such as chlorophyll content, antioxidant activity, or osmolyte accumulation, which could strengthen mechanistic insights. The absence of genotype-specific mean values in the results limited the ability to compare exact performance magnitudes across treatments, although the ranking pattern remained consistent. Future research would benefit from integrating PEG-based screening with greenhouse or field

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validation to confirm the stability of drought tolerance at later growth stages (23). Incorporating molecular markers, transcript profiling, and physiological assays could also enrich understanding of the adaptive pathways employed by tolerant genotypes. Multi-environment testing would enhance the reliability of genotype recommendations for breeding programs and potential field cultivation under water-deficit conditions. The findings of this study established a strong foundation for such extended research, demonstrating meaningful variation among genotypes and highlighting those with promising tolerance profiles that merit further investigation.

CONCLUSION

The study demonstrated that mild PEG-6000–induced drought stress supported better early seedling growth in the evaluated *Brassica* genotypes compared to more severe stress levels, confirming the usefulness of this approach for preliminary drought screening. Among the tested genotypes, RBN-08003 consistently exhibited stronger growth and resilience, highlighting its potential value for breeding efforts aimed at improving drought tolerance. These findings emphasize the importance of early-stage screening in identifying promising genetic resources and provide a practical foundation for developing *Brassica* cultivars better suited to water-limited environments.

AUTHOR CONTRIBUTION

Author	Contribution
Maira Shahzadi	Substantial Contribution to study design, analysis, acquisition of Data Manuscript Writing Has given Final Approval of the version to be published
Muhammad Azam Khan*	Substantial Contribution to study design, acquisition and interpretation of Data Critical Review and Manuscript Writing Has given Final Approval of the version to be published
Umara Sahar Rana	Substantial Contribution to acquisition and interpretation of Data Has given Final Approval of the version to be published
Muhammad Ahsan Khan	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Huma Saleem	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Mubashar Nadeem	Substantial Contribution to study design and Data Analysis Has given Final Approval of the version to be published
Muhammad Ibrahim	Contributed to study concept and Data collection Has given Final Approval of the version to be published
Shadab Shaukat	Writing - Review & Editing, Assistance with Data Curation
Kashif Rashid	Writing - Review & Editing, Assistance with Data Curation

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