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RESEARCH ARTICLE

Screening of lentil genotypes during germination and early growth stages under PEG-induced drought stress

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ABSTRACT

The objective of this experiment was to determine drought tolerance exhibited by lentil lines developed by the International Center for Agricultural Research in the Dry Areas (ICARDA) in an artificial environment, and to investigate the relationships between traits with various methods. This experiment examined 15 traits of 21 lentil (*Lens culinaris* Medik.) accessions grown under artificial drought stress created with polyethylene glycol (PEG)-6000 (0%, 10%, 15% and 20%) concentrations during germination and early seedling stages. Germination characteristics, seedling developmental properties and root system architecture traits were investigated to observed the impacts of drought stress. The originality lies in enabling the identification of drought-tolerant and sensitive genotypes through a brief and practical research method, while shedding light on the key traits by principal component analysis. The first two PCs explained 22.9% and 31.7% (total 54.6%) under optimal conditions while they described 14% and 58.3% (total 72.3%) under PEG-induced drought conditions, respectively. Variation in PC1 was mostly contributed by positive coefficients of germination index, uniformity of germination and germination energy, and negative coefficients of mean germination time. Variation in PC2 was mostly contributed by positive coefficients of seedling vigor index, root fresh weight and root dry weight. 'Tigris', G3664 and G3840 exhibited higher performance in terms of germination characteristics, while G3710, G3829 and G3840 produced higher DM accumulation, total biomass and lateral roots. Overall, PC-biplot denoted that selection based on germination index and seedling vigor index at germination and seedling stages would improve drought tolerance. In conclusion, genotypes G3840 and G3664 were identified as drought-tolerant, whereas genotypes G35, G3659, G3759, G3837, and G3844 were classified as drought-sensitive. In addition, G3664, G3840 and G3710 exhibited the highest stress tolerance index (STI) under artificial drought conditions.

Key words: Biplot, drought tolerance, *Lens culinaris*, plant stress, principal component analysis, radar graph.

INTRODUCTION

Lentil (*Lens culinaris* Medik.) is an important food source due to high protein (20.6%-31.4%) content (Jarpa-Parra, 2017). It also contains important dietary components such as carbohydrates, fiber, antioxidants, vitamins, probiotics and amino acids (Kumar et al., 2018). Lentil has also an economically critical status with high nutritional content. It is the third most important legume plant produced in the world after chickpeas and peas (FAO, 2023). About 5.6 million tons of lentil are produced in a total area of 5.6 million hectares over the world in 2022. This is estimated to reach 8.4 million tons at the end of the

2024. Turkey is an important lentil producer country with an annual production of 445 000 t. Lentil production has increased by 3.4% from 430 000 to 445 000 t in the last 5 yr (2017-2022) in Turkey (FAO, 2023).

Crop yield and plant growth are affected by environmental conditions such as biotic and abiotic stresses. In particular, drought is one of the most destructive abiotic stresses affecting crop productivity and food security in the world (Kour and Yadav, 2022). Over the past decade, it has caused about 30 billion USD in lost productivity globally (Gupta et al., 2020). It has also been found that drought can lead to a 13%-94% loss in crop yield depending on its intensity and duration (Gupta et al., 2020). Drought stress is the most important factor that has serious harmful effects on the growth and development of lentil (Zeroual et al., 2022). It has been found that up to 50% yield losses occur in the production of lentil exposed to drought (Mart, 2022). Drought significantly inhibits lentil emergence, germination and seedling growth. In addition, it negatively affected plant-water relations, photosynthesis, root and shoot development (Zeroual et al., 2022). The negative effects of drought on the lentil crop have become a critical problem. Thus, the selection of appropriate lentil genotypes for drought stress is a critical step in successful production. Significant variations in stomatal conductivity, water availability, other functional properties, and yield components are observed as a result of drought exposure across different varieties (Sánchez-Gómez et al., 2019). According to these results, it can be said that the selection of suitable varieties is among the innovative and sustainable solutions to reduce the negative effects of drought. The areas where lentil is grown are generally agricultural lands that do not have irrigation facilities and are suitable for the dry farming system. For this reason, grain yield and quality in lentil are adversely affected by low precipitations.

In order to increase the water and nutrient uptake in plants, drought-tolerant genotypes have some advantages via effective root systems and physiological responses against drought stress. The primary objective of the experiment was to examine the germination characteristics, as well as the shoot and root development, of 21 different lentil genotypes under polyethylene glycol (PEG)-induced stress conditions. Thus, the originality lies in enabling the identification of drought-tolerant and sensitive genotypes through a brief and practical research method, while shedding light on the key traits that need to be examined in a similar experiment. Drought tolerance of the lines used in this study has not yet been investigated in an artificial environment. Furthermore, investigation of correlations between traits using radar and biplot charts facilitated to comment relationships and insight pivotal characteristics for drought tolerance.

MATERIALS AND METHODS

Experimental materials

The experiment was carried out under controlled conditions in the Field Crops Laboratory of Siirt University, Siirt, Turkey, in 2022. Two lentil (*Lens culinaris* Medik.) cultivars (Firat-87 and Tigris) and 19 lines developed by the International Center for Agricultural Research in the Dry Areas (ICARDA) for drought tolerance were used in the experiment. Lines were represented by their codes in the experiment. Codes of used lines are G35, G3658, G3662, G3844, G3653, G3659, G3837, G3759, G3673, G3726, G3819, G3928, G3710, G3664, G3713, G3829, G3840, G3678 and G3679. According to a study conducted by Günes et al. (2006), 'Tigris' and 'Fırat-87' are widely cultivated in the region known for their relative resistance to drought.

Experimental layout

The 21 lentil genotypes and four drought levels (0%, 10%, 15% and 20% PEG) were used in the experiment to observe the changes among genotypes. Osmotic potentials of 10%, 15% and 20% PEG solutions, which were set the formula of Michel and Kaufmann (1973), were -0.30, -0.51 and -0.80 MPa, respectively. The experiment was laid out according to completely randomized factorial design with three replicates. All petri dishes were sterilized in an autoclave at 121 °C for 20 min before experimenting. Seeds were kept in 10% sodium hypochlorite (NaClO) for 5 min for surface sterilization (Erman et al., 2022). Afterward, it was rinsed

three times with distilled water, and then completely free of surface water on coarse filter paper. The 25 homogeneous seeds were placed between two layers of filter paper placed in petri dishes (90 mm × 15 mm). Initially, 4 mL solution were applied to each petri dish (distilled water for the control group, PEG-6000 solutions at concentrations of 10%, 15% or 20% for drought stress) and an equal amount of solution was added following days. Petri dishes were kept in the dark at 4 ± 20 °C for 10 d. The number of germinated seeds was daily determined for 10 d and dead seeds were removed from the petri dishes.

Observations and calculations

Germination percentage (GP), mean germination time (MGT), germination index (GI), uniformity of germination (UG), germination energy (GE) and water content (WC) were calculated depending on counting daily germinated seeds. Germination characteristics were calculated with equations that were used by Özyazici and Acikbas (2021). The observations were taken from 10 plants randomly selected from the germinated seeds in each petri dish. Two millimeters root emergence were accepted as germination criteria. Before taking observations, seeds were cleared of excess water on the surface with filter paper and immediately weighted with a sensitive scale for determination of shoot fresh weight (SFW) and root fresh weight (RFW). The seedlings were carefully cut from the junction of the root and the stem and seeds were discarded. Randomly selected samples of radicles and pedicles were placed on a surface and scanned in color at 600 dpi resolution by hand scanner (EPSON L3050, Seiko Epson Corporation, Suwa, Japan). Scanned images were uploaded in the Image J software (Rasband, W.S., U.S. National Institutes of Health, Bethesda, Maryland, USA) and analyzed to determine shoot length (SL), root length (RL), number of lateral roots (NLR) and lateral root length (LRL) by the methods of Ceritoglu et al. (2020). After scanning of plants, they were placed in the oven at 68 °C up to losing humidity and weighed to determine shoot dry weight (SDW) and root dry weight (RDW). Stress tolerance index (STI) was calculated for each genotype to observe changes among genotypes depending on increasing PEG concentrations using the method of Fernandez (1992).

Statistical analysis

The ANOVA was subjected to data and Tukey HSD (honestly significant difference) test was used for grouping the means. In addition, the principal component analysis (PCA) was used to observe the relationships of properties with each other and their changes depending on drought stress in the R software (R Foundation for Statistical Computing, Vienna, Austria). Data was subjected to Tukey HSD test and PCA using "agricolae", "factoextra" and "FactoMineR" packages. The radar graph was created using Excel.

RESULTS

In the study, the responses of 21 lentil genotypes to drought were investigated using four different PEG levels during germination and early growth stages. Relationships between characters were observed and schematized PCA-biplot and radar chart. Genotypes (G), PEG levels (P), and G×P interaction caused significant differences ($p < 0.05$ or 0.01) in all investigated traits but RWC and SDW. Also, PEG levels and G×P interactions led to significant differences ($p < 0.01$ and $p < 0.05$, respectively) in LRL, whereas genotypes did not significantly affect it. The ANOVA results are given in Table 1.

The highest GP was obtained in 'Tigris' (99%) and the lowest one was determined in G35 (70.7%). Under different PEG levels, the control group demonstrated the highest GP, which gradually decreased with increasing PEG levels, reaching 65.2% at a 20% PEG concentration. The G×P interaction indicated that G3653, G3837, G3726, G3828, G3679 lines and 'Tigris' in the control group showed 100% germination. Also, G3653 and 'Tigris' exhibited 100% germination in 10% PEG. The lowest GP in terms of G×P interaction was obtained from G35 (16%) at 20% PEG. The highest MGT was obtained in G35 (4 d) whereas the lowest one was determined in 'Tigris' (2.61 d). The 20% PEG increased the MGT by 303.6% compared with control. The MGT varied between 1.00-6.36 d depending on G×P interaction. 'Tigris' had the highest GI (0.55) while G35 had the lowest one (0.30). The GI was reduced by 85.2%

when exposed to 20% PEG concentration compared to the control. The G3653, G3829 and 'Tigris' had the highest GI under control, whereas G35 exhibited the lowest one (0.02) at 20% PEG concentration.

Table 1. Tukey HSD values and significance degree of investigated traits according to ANOVA. $^{*}p < 0.05$; $^{**}p < 0.01$; ^{ns}: nonsignificant difference.

Traits	Tukey HSD/F prob.		
	Genotype	PEG	G×P
Germination percentage	8.58**	2.68**	20.43**
Mean germination time	0.385**	0.119**	0.912**
Germination index	0.077**	0.024**	0.181**
Uniformity of germination	7.069**	2.206**	16.826**
Germination energy	10.017**	3.114**	23.850**
Relative water content	5.175ns	1.616**	12.312ns
Seedling fresh weight	0.00621**	0.00195**	0.01482**
Root fresh weight	0.00512**	0.00158**	0.01214**
Seedling dry weight	0.00359ns	0.00112**	0.00851ns
Root dry weight	0.000544**	0.000167**	0.001166**
Seedling length	0.820**	0.552**	1.953**
Root length	0.141**	0.254**	1.937**
Number of lateral roots	0.820**	0.256**	1.953**
Lateral root	0.391ns	0.122**	0.931*
Seedling vigor index	140.8**	43.9**	335.2**

The highest SL (4.47 cm) was observed in G3710 while the shortest one (2.58 cm) was determined in G3837. Considering the mean of PEG concentrations, SL was measured as 5.71 cm under optimum conditions while it was determined as 0.91 cm under 20% PEG. The longest SL was found in G3659 in the control group with 7.12 cm and the shortest SL (0.06 cm) was obtained from G3837 under 20% PEG concentration. However, shoot formation was not observed in G35 and G3679 under 20% PEG concentration. The PEG levels caused significant differences ($p > 0.01$) in the SDW, however, not genotypes or G×P interaction. The highest SDW (0.004 g) was found in the control and 10% PEG-treated seedlings, while the lowest SDW was determined in 20% PEG-treated ones. The ANOVA indicated significant differences at a 1% level for RDW based on genotypes, PEG levels, and G×P interaction. Among the genotypes, G3658 had the highest RDW, while G3726 had the lowest. The mean RDW for the control group was 0.004 g, whereas for the 20% PEG concentration it was measured as 0.001 g. When examining the effect of the genotype-PEG interaction, the highest RDW was found in G3829 within the control group.

Among the genotypes, G3828 had the highest RL (4.97 cm) while G3726 had the shortest RL (2.54 cm). In terms of drought levels, the control group exhibited the highest RL, while the genotypes exposed to 20% PEG concentration had the shortest RL, ranging from 5.53 to 1.71 cm. The G3710 had the highest RL (7.17 cm) overall, while G35 had the shortest RL with 0.85 cm. Considering the mean of genotypes according to PEG levels, the highest NLR (2.48) was observed in G3653, and the lowest NLR (1.09) was observed in G3819. According to PEG levels, the highest NLR was obtained in the control group as 3.03. The NRL decreased with increasing PEG concentrations, therefore, it was found 0.07 under 20% PEG. In terms of G×P interaction, the highest NLR was observed in G3829 (4.93) under control. Considering the mean of the genotypes according to PEG levels, the LRL changed between 0.28-0.53 cm. Depending on the increasing PEG concentrations, LRL decreased and was recorded in the range of 0.67-0.03. The highest LRL was found in G3819 as 1.72. Since radicle was not observed in some of the genotypes treated with 20% PEG concentration, lateral root formation did not occur (Table 2).

Table 2. Germination and development performance of lentil accessions under drought stress. GP: Germination percentage; MGT: mean germination time; GI: germination index; UG: uniformity of germination; GE: germination energy; WC: water content; SFW: seedling fresh weight; RFW: root fresh weight; SDW: seedling dry weight; RDW: root dry weight; SL: seedling length; RL: root length; NLR: number of lateral roots; LRL: length of lateral roots; SVI: seedling vigor index.

Trait	PEG (%)	Best genotypes (drought-tolerant)	Worst genotypes (drought-sensitive)
GP, %	0	G3653, G3837, G3726, G3828, G3679 and 'Tigris' (100.0) followed by G3662, G3844, G3664, G3829 and G3840 (98.7)	G3673 (88.0) followed by G3659 (89.3)
	10	G3653 and 'Tigris' (100.0) followed by G3664 and G3679 (98.7)	G3678 (78.7) followed by G3828 (85.3)
	15	G3658 (100.0) followed by G3662, G3673, G3664, G3829, G3679 and 'Tigris' (98.7)	G3678 (81.3) followed by G35, G3819 and G3828 (86.7)
	20	'Tigris' (97.3) followed by G3664 (86.7)	G35 (16.0) followed by G3837 and G3759 (36.0)
MGT, d	0	G3653 and 'Tigris' (1.00) followed by G3664, G3829 and 'Tigris' (1.03)	G3710 (1.87) followed by G3713 and G35 (1.77)
	10	'Tigris' (1.87) followed by G3659 and G3679 (2.03)	G3759 and G35 (3.33) followed by G3658 (3.07)
	15	G3664 (2.77) followed by G3840 (3.07)	G35 (4.56) followed by G3759 (4.00)
	20	'Tigris' (4.33) followed by G3710 and G3664 (5.00)	G35 (6.36) followed by G3837 (6.16)
GI	0	G3653 (0.99) followed by G3829 (0.97)	'Fırat-87' (0.65) followed by G3710 (0.67)
	10	'Tigris' (0.69) followed by G3679 (0.62)	G35 (0.30) followed by G3759 (0.32)
	15	G3664 (0.37) followed by G3710 (0.35)	G35 (0.19) followed by G3819 (0.23)
	20	'Tigris' (0.23) followed by G3664 (0.18)	G35 (0.02) followed by G3844, G3837 and G3759 (0.06)
UG	0	G3653 (97.5) followed by 'Tigris' (95.0)	G3710 (50.8) followed by G35 (53.2)
	10	'Tigris' (54.3) followed by G3679 (49.6)	G35 (26.1) followed by G3759 (28.8)
	15	G3664 (35.6) followed by G3840 (31.8)	G35 (19.0) followed by G3819 (22.4)
	20	'Tigris' (22.7) followed by G3664 (17.3)	G35 (2.5) followed by G3837 (5.9)
GE	0	G3653 (97.3) followed by G3837 and 'Tigris' (94.7)	'Fırat-87' (34.7) followed by G3844 (44.0)
	10	'Tigris' (49.3) followed by G3679 (40.0)	G3658, G3759, G35 and 'Fırat-87' (5.3) followed by G3844 and G3819 (8.0)
	15	No germination on 1st day	No germination on 1st day
	20	No germination on 1st day	No germination on 1st day
WC, %	0	G3840 (92.5) followed by G3713 (92.3)	'Tigris' (90.0) followed by G35 (90.6)
	10	G3653 (90.3) followed by 'Fırat-87' and G3829 (89.9)	G3673 (73.3) followed by G3819 (87.9)
	15	G3828 (91.4) followed by G3662 (88.5)	G3844 (85.8) followed by G3829 and G3837 (86.3)
	20	G3829 (84.8) followed by G3658 (84.6)	'Tigris' (78.9) followed by G3819 (80.5)
SFW, g	0	G3673 (0.0608) followed by 3667 (0.0605)	3828 (0.0401) followed by G3710 (0.0424)
	10	G3659 (0.0469) followed by 'Tigris' 0.0422)	G3837 (0.0269) followed by 3710 (0.0291)
	15	G3664 (0.0321) followed by G3662 (0.0305)	G3844 (0.0181) followed by G3713 (0.0198)
	20	G3840 (0.00111) followed by G3664 (0.0098)	G3713, G35 and G3679 (0.0000) followed by G3844 and G3759 (0.0014)
RFW, g	0	G3759 (0.0563) followed by G3829 (0.0557)	'Tigris' (0.0284) followed by nG3726 (0.0323)
	10	G3759 (0.0405) followed by G3658 (0.0389)	G3726 (0.0225) followed by G3828 (0.0241)
	15	G3658 (0.0225) followed by G3759 (0.0250)	G3844 (0.173) followed by G3678 (0.0177)
	20	G3840 (0.0107) followed by G3664 (0.0101)	G3659 (0.0048) followed by G3726 (0.0058)
SDW, g	0	G3759 (0.0065) followed by G3679 (0.0054)	'Tigris' (0.0031) followed by G3828 (0.0033)
	10	G3673 (0.0166) followed by G3659 (0.0048)	G3710 (0.0030) followed by G3828 (0.0033)
	15	G3659 (0.0036) followed by G3679 (0.0035)	G3828 (0.0020) followed by G3844 (0.0025)
	20	G3664 (0.0019) followed by G3819 and G3840 (0.0016)	G35, G3679 (0.0000) followed by G3713 (0.0001)
RDW, g	0	G3759, G3829 (0.0046) followed by G35, G3679, G3658 and G3837 (0.0045)	G3726 (0.0028) followed by G3710 (0.0029)
	10	G3844 (0.0046) followed by G3658 (0.0043)	G3726 (0.0025) followed by G3726, G3828 (0.0028)
	15	G3658 (0.0032) followed by G3759 (0.0031)	G3828 (0.0018) followed by G3673 (0.0020)
	20	G3664, G3819 (0.0017) followed by G3658 (0.0016)	G3659 (0.0009) followed by G3726, G3837 (0.0010)
SL, cm	0	G3659 (7.12) followed by G3678 (6.72)	'Tigris' (3.95) followed by G3844 (4.54)
	10	'Tigris' (6.35) followed by G3659 (6.10)	G3837 (2.81) followed by G3759 (3.89)
	15	G3664 (4.11) followed by G3710, G3828 (4.05)	G3844 (1.91) followed by G3837 (2.45)
	20	G3840 (2.04) followed by G3710 (1.79)	G35, G3679 (0.00) followed by G3837 (0.06)
RL, cm	0	G3710 (7.17) followed by G3829 (7.16)	G3726 (3.77) followed by 'Tigris' (3.81)
	10	G3828 (4.95) followed by G3658(4.59)	G3726 (2.40) followed by G3713 (2.94)
	15	G3828 (5.75) followed by G3759 (5.31)	'Fırat-87' (2.60) followed by G3673 (2.77)
	20	G3840 (2.80) followed by G3710 (2.73)	G35 (0.85) followed by G3713 (1.07)
NLR	0	G3829 (4.93) followed by G3653 (4.00)	G3840 (2.07) followed by 'Fırat-87' (2.20)
	10	G3713 (4.20) followed by 'Tigris' (3.83)	G3759 (0.87) followed by G3819 (1.50)
	15	'Tigris' (2.43) followed by G3653 (2.23)	G3844 (0.00) followed by G3819 (0.40)
	20	G3710 (0.77) followed by G3659, G3673 and G3829 (0.17)	G3658, G3662, G3844, G3837, G3759, G35, G3726, G3819, G3828, G3664, G3713, 'Fırat-87', G3840, G3679, G3678 and 'Tigris' (0.00) followed by G3653 (0.13)
LRL, cm	0	G3819 (1.72) followed by G3678 (0.90)	G3664 (0.43) followed by G3828 (0.45)
	10	G3713 (0.87) followed by G3653 (0.83)	G3759 (0.29) followed by G35 (0.38)
	15	'Tigris' (0.71) followed by G3840 (0.66)	G3844 (0.00) followed by G3819 (0.13)
	20	G3710 (0.25) followed by G3829 (0.09)	G3658, G3662, G3653, G3837, G3759, G35, G3726, G3819, G3710, G3664, G3713, 'Fırat-87', G3840, G3679, G3678 and 'Tigris' (0.00) followed by G3659 (0.07)
SVI	0	G3759 (1286) followed by G3829 (1267)	'Tigris' (776) followed by G3819 (887)
	10	'Tigris' (1062) followed by G3659 (953)	G3726 (608) followed by G3837 (648)
	15	G3828 (853) followed by G3759 (813)	G3844 (439) followed by 'Fırat-87' (525)
	20	G3840 (415) followed by G3710 (373)	G35 (14) followed by G3837 (41)

The G3664 exhibited the highest STI with 0.27 in the GI, followed by G3829, G3679, G3840 with 0.23, 0.21 and 0.20, respectively. The genotypes showing the lowest STI were G3759 and G3844 with 0.07. The G3664 showed the highest STI with 0.92, following G3662 and G3840 with 0.90. Genotypes showing the lowest STI_{GP} (STI for GP) is G35 with 0.16. The genotypes showing the highest STI_{SL} were G3840, G3664 and G3828 genotypes with 0.38, 0.33 and 0.29, respectively. The lowest STI was in G35 with a value of 0.01, followed by G3844 and G3837 genotypes with 0.04. The G3664 exhibited the highest STI_{SDW} with 0.46, whereas G3837 showed the lowest one with 0.13. While the STI_{SL} was 0.48 in G3710, the lowest STI_{SL} was 0.07 in G35. The G3664 and G3840 have the highest STI in terms of all traits, while G3659 and G3837 are with the lowest stress tolerance (Table 3).

Table 3. Drought stress tolerance index (STI) values of genotypes based on some selected traits. GP: Germination percentage; GI: germination index; SVI: seedling vigor index; DW: dry weight; SL: seedling length.

Genotype	STI_{GI}	STI_{GP}	STI_{SVI}	$STIS_{DW}$	STI_{SL}
G3658	0.15	0.72	0.16	0.27	0.22
G3662	0.19	0.90	0.18	0.28	0.20
G3844	0.07	0.40	0.04	0.16	0.09
G3653	0.19	0.77	0.20	0.23	0.26
G3659	0.10	0.43	0.09	0.17	0.17
G3837	0.08	0.39	0.04	0.13	0.10
G3759	0.07	0.38	0.06	0.27	0.16
G35	0.03	0.16	0.01	0.15	0.07
G3673	0.13	0.58	0.11	0.24	0.19
G3726	0.16	0.70	0.12	0.18	0.17
G3819	0.15	0.58	0.11	0.39	0.20
G3828	0.19	0.80	0.29	0.22	0.36
G3710	0.18	0.80	0.16	0.25	0.48
G3664	0.27	0.92	0.33	0.46	0.36
G3713	0.16	0.85	0.16	0.15	0.19
'Fırat-87'	0.14	0.82	0.16	0.21	0.19
G3829	0.23	0.88	0.27	0.31	0.30
G3840	0.20	0.90	0.38	0.30	0.42
G3679	0.21	0.75	0.11	0.20	0.14
G3678	0.11	0.51	0.18	0.29	0.35
'Tigris'	0.34	1.04	0.21	0.29	0.11
Main	0.16	0.68	0.16	0.25	0.23

Two-way principal component biplot (PC-biplot) for genotypes × morphological attribute responses with 21 lentil accessions during early growth stage under control and drought (20% of PEG) conditions. The first two PCs (PC1 and PC2) explained 22.9% and 31.7% (total 54.6%) under control conditions while they described 14% and 58.3% (total 72.3%) under PEG-induced drought conditions, respectively. Arrows point out the intensity of morphological attribute impacts in PC1 and PC2. Direction and distance of arrows exhibit the extent of characters affecting PCs. Parallel vectors indicate a significant positive relationship among related characters while perpendicular or opposing ones suggest no association or negative relationship, respectively. The data matrix including 15 traits for 21 genotypes was generated and tabulated by R to get all 15 PCs and their loadings for all related characters (Figure 1).

Out of 15 PCs of eigenvalues, the first five PCs found with eigenvalues were selected due to higher than 1 under optimum conditions. The other nine PCs were considered as nonsignificant due to eigenvalues less than 1. The first five PCs exhibited 83.4% of the total variation under normal conditions. Similarly, the first five PCs found with eigenvalues were selected due to higher than 1 under PEG-induced stress conditions and they showed 81.68% of total variation. Variation in PC1 was mostly contributed to

by positive coefficients of GI, UG and GE whereas it was mostly contributed to by negative coefficients of MGT. Thus, strong negative correlation between some traits such as MGT and GI can be observed via coefficients of PCs. Variation in PC2 was mostly contributed to by positive coefficients of SVI, RFW and RDW (Table 4).

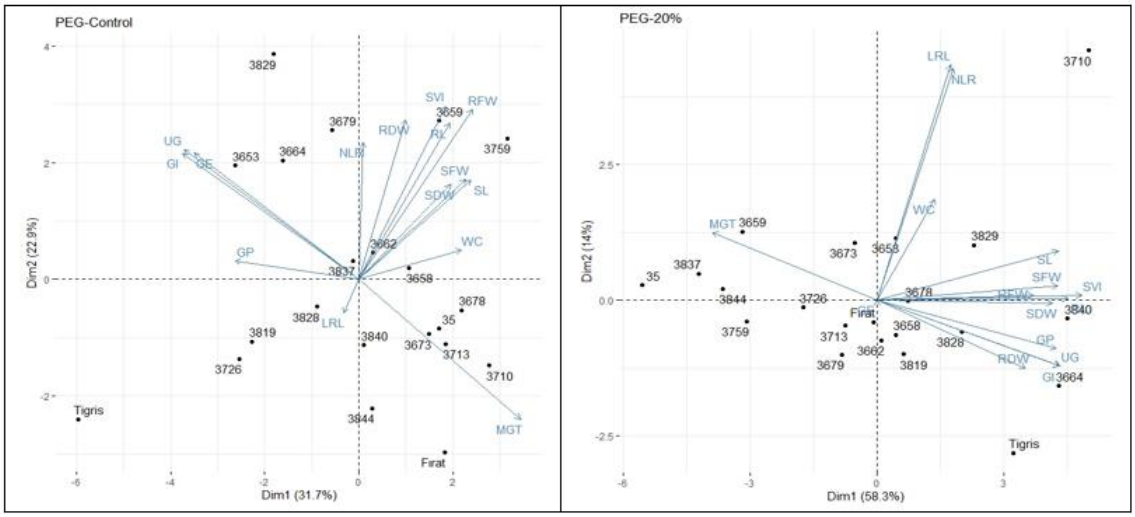

Figure 1. Biplot of germination and seedling growth characteristics of 21 lentil genotypes under optimum and 20% PEG-induced drought stress conditions. Arrows show investigated traits while points indicate lentil genotypes. GP: Germination percentage; MGT: mean germination time; GI: germination index; UG: uniformity of germination; GE: germination energy; WC: relative water content; SFW: seedling fresh weight; RFW: root fresh weight; SDW: seedling dry weight; RDW: root dry weight; SL: seedling length; RL: root length; NLR: number of lateral roots; LRL: length of lateral roots; SVI: seedling vigor index (SVI).

Table 4. Coefficients of first five principal components (PC) for 15 traits of lentil genotypes under 20% PEG-induced conditions.

Traits	PC1	PC2	PC3	PC4	PC5
Germination percentage	0.1951612	0.1937346	-0.1528661	-0.43096160	-0.14919372
Mean germination time	-0.4334495	0.1085085	-0.0333606	0.02589529	0.04981446
Germination index	0.4443855	-0.0841820	-0.0156348	-0.13124310	-0.12541218
Uniformity of germination	0.4379285	-0.0272908	-0.0342680	-0.18795823	-0.14879510
Germination energy	0.4173706	-0.1486917	0.0076086	-0.07283666	-0.11746512
Seedling vigor index	0.2001304	0.4485413	-0.0388266	0.20288429	-0.18160470
Relative water content	0.0572530	-0.0490901	-0.6324371	0.25820256	0.03848014
Shoot fresh weight	0.1089405	0.3172546	0.2373499	0.30476370	-0.10684461
Root fresh weight	-0.0770169	0.4422329	-0.1592139	-0.33223144	0.08279717
Shoot dry weight	-0.0373567	0.1029020	0.6505884	-0.20339207	-0.06519126
Root dry weight	-0.0961602	0.4269426	-0.1035443	-0.33574459	0.16603975
Shoot length	0.2075844	0.2822759	0.16632831	0.51984821	0.03643878
Root length	-0.0483569	0.3654943	-0.1662498	0.10814037	-0.29493764
Number of lateral roots	0.2250670	0.0877220	0.0349737	0.08229237	0.61109750
Length of lateral roots	0.2066846	0.0842775	0.0189748	-0.04304382	0.61423619
Eigenvalue	4.3768	3.5907	1.9601	1.5521	1.0193
Variability, %	33.63	17.84	15.25	8.29	6.67

According to PC-biplot, the observed traits displayed different behaviors under control and stressful conditions with distinct relationships between genotypic responses. The WC, SL, SDW, SFW, RFW, RL, RDW, and NRL traits appeared to exhibit positive correlations with each other, while these traits showed a negative relationship with LRL under control conditions. Additionally, UG, GI, GE, and GP traits were strongly positively correlated with each other, while they exhibited a strong negative correlation with MGT. Among the 15 genotypes, G3653, G3664, G3679, G3659 and G3759 demonstrated superior characteristics under control conditions. However, the scenario changed when genotypes were exposed to artificial drought stress. In this case, LRL, NRL, and WC traits showed a strong positive correlation; however, they did not have any significant correlation and separated from other traits. On the other hand, all traits exhibited a strong negative correlation with MGT. The G3840 and G3664 exhibited the highest performance under 20% PEG conditions, thereby stood out as being tolerant to drought. Also, G35, G3659, G3759, G3837 and G3844 genotypes were determined as drought-sensitive genotypes (Figure 1).

Genotypes were classified using a radar graph based on investigated characters across three PEG levels and control (Figure 2). The use of a radar graph clearly illustrates the inhibition of the examined traits, especially in response to increasing PEG concentrations. The 'Tigris', i.e., reference genotype, had a high GP value even at the maximum PEG concentration, but it exhibits low tolerance in terms of seedling development. Previously, through the PC-biplot, it was noticeable that the drought-tolerant G3664 and G3840 were less affected by increasing PEG concentrations, as also evidenced within the radar graph visualization. Similarly, Evamoni et al. (2023) indicated that artificial drought stress via PEG-6000 can provide an effective and fast tool to identification of drought tolerance during germination and early growth stage in rice.

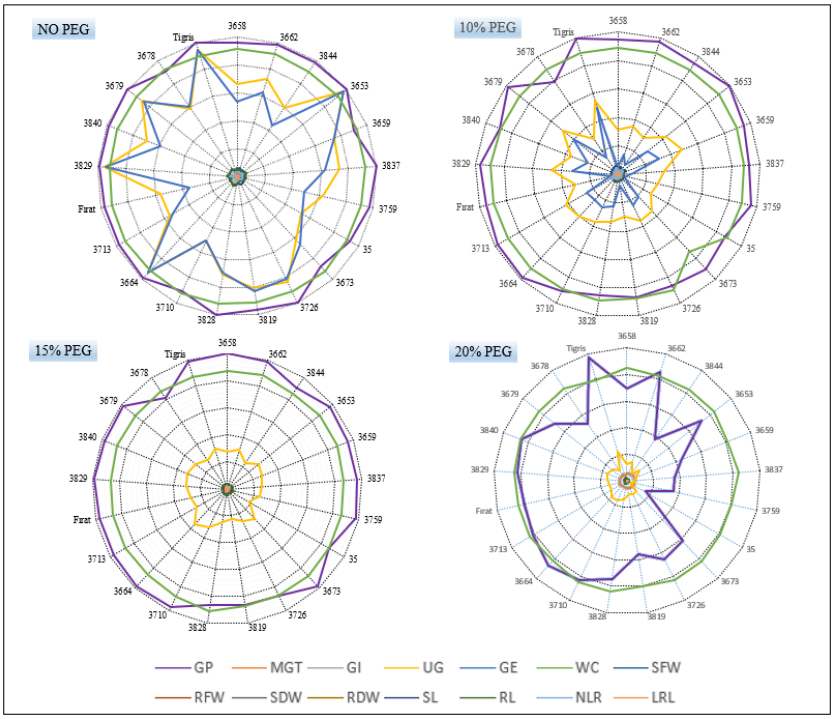

Figure 2. Radar graph between investigated traits under optimum and PEG-induced drought stress (10%, 15% and 20%) conditions. GP: Germination percentage; MGT: mean germination time; GI: germination index; UG: uniformity of germination; GE: germination energy; WC: water content; SFW: seedling fresh weight; RFW: root fresh weight; SDW: seedling dry weight; RDW: root dry weight; SL: seedling length; RL: root length; NLR: number of lateral roots; LRL: length of lateral roots; SVI: seedling vigor index.

DISCUSSION

Increasing PEG levels reduced the germination characteristics of lentil by a higher concentration of osmolytes (proline, glycine betaine and soluble sugars), reactive oxygen species (hydrogen peroxide and superoxide anion) and lipid peroxides in the plants (Majid et al., 2020). Germination and seedling development stages are vital for the healthy growth of the plant (Biju et al., 2017). However, drought during the germination process significantly hinders the formation of healthy seedlings (Lin et al., 2017). The PEG treatment during the germination stage causes osmotic stress due to increased viscosity; therefore, it is used as a drought simulator in studies (Muscolo et al., 2014). Vus et al. (2021) reported that over 85% and 60% regression occurred in the shoots and roots of many varieties under 19.5% of PEG-6000 solution respectively. In addition, the correlation coefficient indicated that shoots were more sensitive to the osmotic effect compared to roots. The results obtained from our research indicate that the germination process is negatively affected due to increasing PEG concentrations. Thus, they are compatible with the results of previous studies.

Parameters such as MGT, GE, UG and GI depending on PEG levels are important indicators of stress tolerance resulting from the genotypic characteristics of the lines. The primary role in starting the seed germination process is the presence of water in the environment and its uptake by the seed. The presence of water in the environment plays an important role as a reactant in the dissolution of metabolites, course and transport of enzymatic reactions, and the hydrolytic degradation of proteins, lipids and carbohydrates in the storage tissues of germinating seeds (Bialecka and Kepczynski, 2010). Amylase enzymes coordinate the germination process by converting endosperm starch into metabolizable sugars that provide energy for the growth of roots and shoots. However, if there is not enough water in the environment, both the hydrolysis process of carbohydrates and amylase enzyme activities are damaged and thus the germination process is delayed or completely stop (Zeid and Shedeed, 2006).

Investigated traits showed significant variations among genotypes under control and PEG conditions. In addition, characters belonging to the germination process were negatively affected in all genotypes. The MGT was prolonged, and the characters investigated as seedling development indicators were inhibited by artificial drought. In terms of the GP, MGT, GI, UG and GE, G3664, G3840, G3679, G3829, G3828, G3710 and G3653 were superior to 'Tigris', which is referenced as drought tolerant. However, the superior properties of these lines were better in terms of the average of the entire PEG concentration. The G3664 and G3840 started up in terms of STI (i.e., less inhibition despite increasing drought). Various studies have reported that with increasing drought levels or due to decreasing water levels in the environment. Germination characteristics of lentil are negatively affected, radicle emergence is delayed, and seedling development characteristics are slowed down compared to the control environment (Muscolo et al., 2014; Hojjat, 2016). Inhibition of seed germination is directly related to reserve mobilization, energy production through respiration, enzyme and hormonal activity, and dilution of protoplasm to increase metabolism for successful embryonic growth (Haouari et al., 2013). Since insufficient water in the environment blocks the activities of hydrolytic enzymes, the stored reserve cannot be broken down and the energy cannot be used sufficiently for radicle emergence, thus indirectly negatively affecting the seedling strength and development. Subrahmanyam et al. (2006) reported that as the severity and duration of drought increase, the oxygen level needed in photosystem II decreases and the photosynthesis process is damaged.

Although some genotypes developed better than others under control conditions (such as G3759, G3819 and G3829), they could not survive under severe drought or could develop at a very low level, while drought-tolerant genotypes (such as G3664, G3710 and G3840) were more susceptible to low drought levels. It has been determined that they can show significant development even in high droughts where they are less affected. This suggests that mitochondrial respiration may play an important role in providing ATP to the chloroplast, thereby supporting chloroplast functions and ultimately plant survival (Vercellino and Sazanov, 2022). Additionally, soluble sugar and proline content in plant tissues exposed to drought stress is an important indicator of STI. It was reported in the experiment where physiological

and biochemical responses to drought stress were investigated in lentil that drought-resistant genotypes produce more soluble sugar and proline but consume the available starch reserve faster. Therefore, among genotypes under drought stress, those with high proline production potential gain an advantage and can develop tolerance to stress (Mishra, 2014). Due to these and similar physicochemical properties, responses and tolerance thresholds of each genotype differ at various drought levels. Previous studies support the results obtained from the experiment (Muscolo et al., 2014; Mishra et al., 2016; Zeroual et al., 2022).

It is seen in the study results that both sensitive and tolerant lines are negatively affected during the germination process and seedling development period due to increasing drought stress. Various studies show that all genotypes are exposed to various inhibitions due to increased drought stress, but sensitive ones are more affected (Zeroual et al., 2022). In our research, it was determined that the G3664 and G3840, which were found to have high drought tolerance, were less affected by water shortage compared to the sensitive G3837 and G3759, in terms of many characteristics. In addition, Chapae et al. (2020) reported that PEG-6000 can be used to form artificial drought stress to observe the response of seedlings to water scarcity and provides an alternative tool under control conditions.

CONCLUSIONS

Twenty-one lentil genotypes were examined under artificial drought by exposing them to different PEG concentrations under laboratory conditions. As a result, different genotypes had various tolerances to drought by examining different germination and seedling characteristics. In summary, the behavior of these traits and genotypic responses differed between optimal and stressful conditions, with certain traits and genotypes demonstrating distinctive relationships and responses in each condition. The prominent traits observed during germination and early seedling stages of genotypes were visualized using the PC-biplot and radar chart methods. Briefly, 'Tigris', G3664 and G3840 exhibited higher performance in terms of germination characteristics, while G3710, G3829 and G3840 produced higher dry matter accumulation, total biomass and lateral roots. The PC-biplot indicated that selection based on germination index and seedling vigor index at germination and seedling stages would improve drought tolerance. In conclusion, G3840 and G3664 provided higher tolerance to drought among 21 lentil accessions. These methods allowed for the creation of a prediction for genotypes that could be considered valuable within breeding programs from the existing genetic pool.

Author contribution

Conceptualization: F.C., M.E. Methodology: F.C., M.E., M.C. Formal analysis: F.C. Research: M.C., S.S., Ö.U., R.K., R.Ö. Resources: S.S., Ö.U., R.K., R.Ö. Data curation: M.C. Writing-original draft: M.C., R.Ö. Writing-revising and editing: F.C., M.E., Ö.U. Visualization: M.C., F.C. Supervision: F.C., M.E. Project administration: F.C., M.E. Funding acquisition: F.C. All coauthors reviewed the final version and approved the manuscript before submission.

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