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RESPONSE OF BARLEY GENOTYPES TO PEG-INDUCED WATER STRESS AT SEEDLING STAGES

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ABSTRACT

Water stress negatively affects germination and seedling growth in barley, particularly during early developmental stages which are critical for crop establishment; however, divergent genotypes behave differently due to their divergent genetic makeup. To assess genotypic variations under water stress, thirty-five barley genotypes were evaluated under polyethylene glycol (PEG)-induced water stress conditions (0, 10, 20 and 30% PEG) at the Crop Sciences Institute, National Agricultural Research Center, Islamabad. Seeds were germinated in petri dishes, arranged in a completely randomized design, with three replications. Results revealed significant differences among genotypes and PEG levels for germination percentage, root, shoot, and coleoptile lengths (cm), and dry matter (mg). Increasing PEG concentration delayed germination and reduced all major growth attributes. Likewise, the seedling vigor index and coefficient of relative inhibition had different results for various genotypes. Pearson correlation analysis indicated strong positive associations among germination percentage, coleoptile length, seedling length, and vigor index, whereas the coefficient of relative inhibition was negatively correlated with these traits. Principal component analysis showed that the first two components explained 88.2% of total variance, with germination and seedling traits contributing maximum to PC1. Overall, genotypes NARC-01, NARC-04, NARC-06, NARC-07, NARC-10, NARC-13, NARC-14, NARC-16, NARC-17 and Sanobar-96 demonstrated superior water stress tolerance, suggesting their potential as parental lines for developing drought-resilient barley cultivars.

Keywords: Barley genotypes, polyethylene glycol, In-vitro conditions, drought stress, coleoptile length, seedling vigor index, coefficient of relative inhibition.

INTRODUCTION

Due to climate change, non-uniform changes are expected in precipitation patterns worldwide, which can increase the frequency of water stress that can result in yield depletion. One of the main factors restricting yield is water stress, which has a negative impact on global food availability (Hussain *et al.*, 2019). Barley (*Hordeum vulgare* L.) is an essential cereal crop, its grains are used as the staple food in poor regions (Reis *et al.*, 2020) and the straw serves as feed for their livestock (Biswas *et al.*, 2023). It is grown on broader environmental range where unfavorable climates may prevail. In such conditions, barley encounters water stress during seed germination and early growth stages. These stages are the most vulnerable to water stress, so presenting a challenge in barley production (Hellal *et al.*, 2018). During germination and early stages, water stress is experienced severely which decides whether a genotype is tolerant or susceptible to water stress (Marcek *et al.*, 2019). Consequently, water stress causes reduction in different physiological and biochemical markers of barley, which results in lower grain and straw productivity (Blum, 2017). Water stress lowers plant production by reducing growth and causes stomata closure which retards photosynthetic activity (Abdel-Moneam *et al.*, 2014). On the other hand, most traits do not exhibit the same behaviors under stressful conditions (Kumar *et al.*, 2018), so enhanced water stress tolerance is important in crop improvement (Luo *et al.*, 2019). Fawzy *et al.* (2014) stated that shoot length and seedling vigor index are the most important parameters for water stress evaluation beside root and coleoptile lengths. Furthermore, El-Denary and El-Shawy (2014) investigated that sensitive barley genotypes resulted in declined germination percentage, shoot length, and total dry matter, while the resilient genotypes recorded stable values for the studies traits.

Water stress in plants is studied through field conditions and in-vitro/lab-controlled conditions The limitations which field conditions can face are that it is almost impossible to develop and maintain a specific water potential for longer periods. So, to cope with this problem and maintain the desired water potential for assessing seedlings under water stress conditions, various chemicals are used for this purpose. One of the most widely used agents is polyethylene glycol. Polyethylene glycol (PEG-6000) is specifically used to evaluate seeds at in-vitro conditions because it induces water stress (Vanani *et al*., 2020). Due to its larger molecular weight, it may not cross the cell wall easily, so it induces osmotic stress to the seedlings. Since seedling survival is crucial for successful growth, development and higher production (Blum and Tuberosa, 2018), the significance of seedling research is highlighted by the fact that water stress can be induced by lowering osmotic potential (Ghanem and Al-Farouk, 2024).

Due to the increasing severity of water stress conditions, it is critically important to develop drought-tolerant varieties. Since barley is important as food and feed crop, but field-based evaluations of water stress are often limited by environmental variability. Therefore, this study was conducted using PEG-induced water stress to accurately evaluate and distinguish drought-tolerant barley genotypes from a large set of available genotypes.

MATERIALS AND METHODS

Experimental Details and Treatments: Thirty-five barley genotypes were evaluated for their germination and seedlings characteristics in October 2021 in Fodder Improvement Laboratory, Crop Sciences Institute (CSI), National Agricultural Research Center (NARC), Islamabad-Pakistan. The experiment was arranged in completely randomized design (CRD), replicated three times, consisted of two factors, i.e. 35 barley genotypes, and 4 water stress levels induced by polyethylene glycol-6000 (PEG-6000). Seeds of the 35 barley genotypes were collected from CSI, NARC, Islamabad-Pakistan (Table 1).

Preparation and Application of PEG Solutions: Polyethylene glycol (PEG-6000) solutions were prepared at 0%, 10%, 20%, and 30% on weight/volume basis in distilled water and stirred until fully dissolved. Sterilized 9 cm Petri dishes were taken, having two layers of Whatman filter paper, and seeds were surface-sterilized with 1% sodium hypochlorite for 2 minutes, followed by thorough rinsing. Ten seeds of each genotype were placed evenly on each petri dish, which then received 5 mL of the respective PEG solution, while the control petri dishes received only distilled water instead of PEG solution. The dishes were monitored on daily basis to maintain consistent moisture by applying the respective PEG solutions.

According to Jacomini *et al*. (1988), 0, 10, 20 and 30% PEG induce water stress by producing osmotic potentials, expressed in Mega Pascal (MPa) units. Control (0 % PEG) produced 0.00 MPa, 10 % PEG produced -0.45 MPa, 20 % PEG produced -0.90 MPa and 30 % PEG produced -1.35 MPa osmotic potential.

Data Recorded: Days to germination were counted from the date of sowing till maximum seeds germinated in each petri dish. The threshold level for considering a seed as germinated was 2 cm radicle length. Germination percentage (%) was determined as the ratio of germinated seeds to the total seeds, multiplied by 100, as clearly illustrated in the following formula.

$$\text{Germination percentage} = (\text{Germinated seeds} / \text{Total seeds sown}) \times 100$$

The root, shoot and coleoptile lengths (cm) were recorded 14 days after sowing (DAS) using a measuring tape, where three measurements were taken and averaged to be used for statistical analysis. For recording root and shoot dry weight (mg), the seedlings were separated into root and shoot with scissors, dried in electric oven at 65ºC for 6 hours, and weighed with the help of electric digital balance. To calculate root-shoot dry weight ratio, the following formula was used.

$$\text{Root-shoot dry weight ratio} = \text{Root dry weight} / \text{Shoot dry weight}$$

The seedling vigor index (SVI) was calculated by multiplying the seedling length (shoot length + root length) with germination percentage, and then dividing it by 100, the formula is given below.

$$\text{SVI} = (\text{Seedling length} \times \text{Germination percentage}) / 100$$

The coefficient of relative inhibition (CRI) was calculated according to the formula given by Mercado (1973) as following:

$$\text{CRI} = (\text{Biomass of non-stressed plants} - \text{Biomass of stressed plants}) / \text{Biomass of non-stressed plants}$$

Statistical Analyses: The data were analyzed for barley genotypes, water stress levels, and their interaction with two-way ANOVA using Statistix 8.1 software. Least significant difference (LSD) test was applied to compare means at 5% level of probability. Pearson Correlation and Principal Component Analysis (PCA) were performed with programming language in R-studio. The correlation was used to explore relationships between germination percentage, seedling growth traits, vigor index, and coefficient of relative inhibition, while PCA was conducted to summarize variability and identify traits contributing to drought tolerance.

RESULTS

Germination Traits: By analyzing the data, it was revealed that germination timings and germination percentages were significantly affected by stress levels as well as barley genotypes. Meanwhile, the interactive effect was significant for germination percentage, while it remained non-significant for days to germination. From the Table 2, it is evident that overall, the germination process was slower in seedlings which received denser PEG solutions. Conversely, the seedlings which received no or less PEG treatment emerged earlier and took less time to germinate. The data presents that maximum days to germination were taken by 30% PEG (7.92 days), followed by 20% PEG (5.73) and 10% PEG (3.54) respectively, while minimum days to germination (3.08) were taken by control (non-stress) seedlings. Out of genotypes, the duration taken to germination varies among genotypes. Maximum days to germination (5.42) were taken by G35 which was statistically similar to G11, G15, and G18 up to G33. On the other hand, G7 germinated in minimum days (4.75). Table 3 explains data for impact of water stress levels, genotypes, and their interaction on germination percentage. On an average basis of all the studied genotypes, the control treatments showed maximum germination (86.57%), while water stress levels of 10, 20 and 30% PEG resulted in 79.14, 63.52 and 42.29% germination respectively. In genotypes, maximum germination percentage (82.50) was noted for G7 which was statistically not different from G1, G4, G6, G10, G13, G14, G16 and G17. The minimum germination rate (58.37%) was noted in G18. The interaction between water stress levels and genotypes was also significant for germination percentages, where maximum seedlings germinated in G1 × Control treatment, while minimum germination was recorded for G31 × 30% PEG.

Morphological Traits: Data for root, shoot, and coleoptile lengths are presented in Table 4, Table 5 and Table 6 respectively. On averaged basis, these parameters demonstrated less productivity as much as the water stress level increased, while genotypes had different values depending on the genetic makeup of each genotype. From the analyzed data (Table 4), it is evident that the longest roots (10.43cm) were recorded under the control condition, i.e. the seedlings which received 100% water instead of PEG solutions produced longer roots compared to those which received PEG solutions in various concentrations. With respect to root length, the 10%PEG solution recorded relatively shorter roots (9.08cm) with 12.60 % relative decrease when compared to pure water treatment. Similarly, 20%PEG produced (7.87cm) long roots, while the shortest roots (6.80cm) were produced by 30%PEG, showing a huge relative decrease of 34.90%. In genotypes, the longest roots (10.03cm) were recorded for G7 which was statistically similar to G1, 6, 7, 10, 13, 14 and 16. The G24 was recorded to produce the shortest root length (7.43cm), which was statistically at par with G8, 20, 27, 28 and 31. The interactive effect of barley genotypes and water stress levels was also significant on root length. The data analyzed for shoot length are presented in Table 5, which shows that shoot length was maximum (12.16cm) under the control treatments, followed by 10% PEG (11.08cm) and 20% PEG (9.86cm). Minimum shoot length (7.97cm) was noted in 30% PEG showing a 34.55% relative decrease compared to control treatments. The G7 produced maximum shoot length (12.03cm), while minimum shoot length (8.84) was recorded by G19. A significant difference was noted among various combinations of barley genotypes and water stress levels on the shoot length. Data for coleoptile length is illustrated in Table 6. The table reveals that maximum coleoptile length (7.68cm) was recorded under control conditions (no water stress), followed by 10% PEG (6.88cm). The 20 and 30% PEG resulted in smaller coleoptiles having average values of (4.55cm) and (3.82cm) respectively. Regarding genotypic variation, G13 exhibited the longest coleoptiles (6.64cm), and in contrast, G31 had the shortest coleoptile length (5.09cm).

Dry Matter Contents, Seedling Vigor Index and Coefficient of Relative Inhibition: There was significant effect of water stress levels, barley genotypes and their interaction on the dry matter contents, seedling vigor index as well as coefficient of relative inhibition. Generally, an increase in water stress levels had adverse impact on the traits mentioned. The statistically analyzed data for root dry weight, shoot dry weight, root/shoot dry weight ratio, seedling vigor index, and coefficient of relative inhibition are given in Table 7, Table 8, Table 9, Table 10, and Table 11 respectively. It is decoded from the Table 7 that root dry matter was significantly influenced by water stress levels, barley genotypes and their interaction. As the water stress increased, the root dry weight of all genotypes showed a general decline. Under control conditions (non-stress), the average root dry weight was 4.92mg. By applying 10% PEG solution, the root dry weight decreased to 4.20mg showing 14.82% reduction compared to control. At 20% PEG, it dropped further to 3.59mg (27.03% reduction), and 30% PEG resulted in the minimum root dry weight of 2.98mg, revealing 39.63% relative decrease compared to non-stress seedlings. The G1 retained higher root dry weight, whereas G13 showed lower root dry weight, while G8 had lowest root dry weight under control conditions (3.28mg) and experienced sharp decrease under water stress conditions. Similarly, G11 showed a significant reduction in root dry weight, with 52.81% relative decrease at 30% PEG. Table 8 describes that under control conditions, the average shoot dry weight across all genotypes was maximum (6.11mg). As water stress increased, the average shoot dry weight decreased to 5.41mg at 10% PEG (11.39% relative decrease), 4.82mg at 20% PEG (21.06% relative decrease), and even 4.45mg at 30% PEG (26.99% relative decrease). Shoot dry weight was maximum (6.10mg) in G14, statistically at par with G13 (6.07mg) and G1 (6.05mg). The minimum shoot dry weight (3.92mg) was noted in G8. It is evident from the Table 9 that without giving any stress to seedlings, the average root/shoot dry weight ratio was 0.81, while it decreased with an increase of stress levels. We recorded 0.78 root/shoot dry weight ratio in 10% PEG, 0.75 in 20% PEG and 0.68 in 30% PEG. Out of genotypes, maximum root/shoot ratio (0.86) was shown by G25, while minimum (0.54) by G11. The data in Table 10 reveals the significant impact of barley genotypes, water stress levels, and their interaction on seedling vigor index (SVI). With respect to water stress levels, maximum seedling vigor index (19.62) was exhibited by control treatments, which were reduced with increasing water stress, viz. 10% PEG resulted in (16.01) SVI and 20% PEG showed (11.36) SVI. The lowest SVI (6.45) was calculated for the highest water stress level. Maximum SVI (18.40) was recorded for G7, which is statistically in line with genotype 1 and 13, while SVI was minimum (10.79) in G24, which was statistically not different form G3, 11, 20, 27, 29, and 35. The analyzed data in Table 11 demonstrates that the impact of barley genotypes, water stress levels as well as their interaction was significant on coefficient or relative inhibition (CRI). The CRI was maximum (0.326) in the highest water stress level of 30% PEG-induced stress level, followed by 20% PEG (0.237), while minimum CRI (0.129) was recorded for the seedlings treated with 10% PEG-induced stress. The CRI was maximum (0.229) in genotype 26, which was not statistically different from genotypes 8, 11, 12, 15, 20 and 35. Moreover, CRI was recorded to be minimum in genotype 13, statistically similar to genotypes 6, 14, 16, 21, 22, 24 and 34.

Correlation: The Pearson correlation analysis revealed significant positive relationships among most of the parameters studied (Figure 1). Germination percentage (G%), coleoptile length (CL), shoot length (SL), root length (RL), and seedling vigor index (SVI) showed strong positive correlations with each other (r = 0.80–0.97; $p < 0.001$) which means that an increase in one trait was closely linked with betterment in others. Similarly, shoot and root dry weights (SDW and RDW) were highly correlated with seedling growth traits. In contrast, the coefficient of relative inhibition (CRI) presented a significant negative correlation with all other growth and germination parameters, while days to germination (DTG) was also negatively associated, suggesting that higher stress inhibition and delayed-germination reduced seedlings performance. These results highlight the interdependence of early growth parameters and confirm that tolerant seedlings have better germination efficiency and stress tolerance.

Principal Component Analysis (PCA): The principal component analysis (PCA) revealed that the first two components explained 88.2% of the total variance, with PC1 accounting for 78.1% and PC2 for 10.1% (Figure 2). Most of the germination and seedling parameters, including germination percentage, coleoptile length, shoot length, root length, shoot dry weight, root dry weight, and seedling vigor index (SVI), were strongly and positively associated with PC1, indicating their close relation and collective contribution to seedling performance. Conversely, days to germination and coefficient of relative inhibition were negatively associated with PC1, reflecting their negative association with growth and vigor traits. Root/shoot dry weight ratio exhibited a moderate association with the PC2, suggesting its partial independence from the major growth-related component. Overall, PCA clearly separated the traits contributing positively to vigor from those linked to stress and delayed germination.

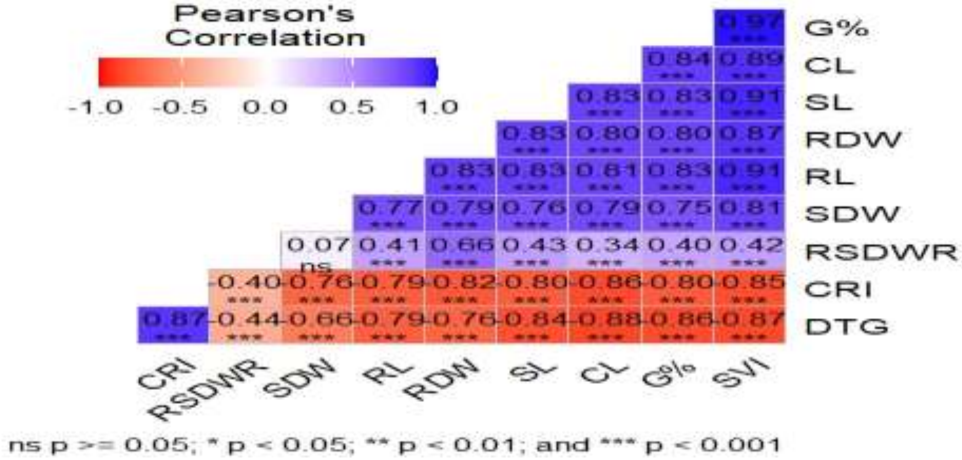

Figure 1. Correlation analysis by Pearson method among various variables. G% = germination percentage, CL = coleoptile length, SL = shoot length, RDW = root dry weight, RL = root length, SDW = shoot dry weight, RSDWR = root/shoot dry weight ratio, CRI = coefficient of relative inhibition, DTG = days to germination, SVI = seedling vigor index.

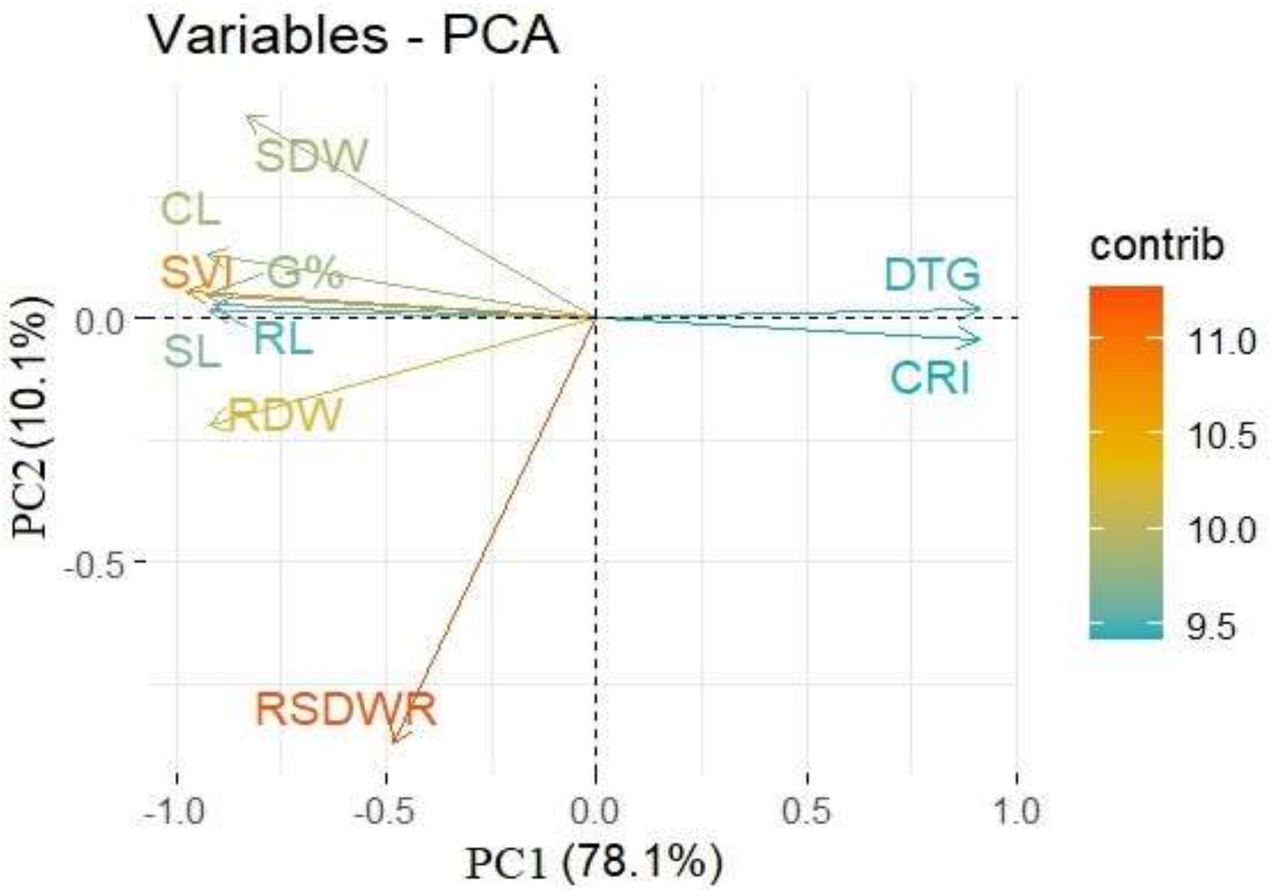

Figure 2. Principal Component Analysis (PCA) among various variables. G% = germination percentage, DTG = days to germination, RL = root length, SL = shoot length, CL = coleoptile length, RDW = root dry weight, SDW = shoot dry weight, RSDWR = root/shoot dry weight ratio, SVI = seedling vigor index, CRI = coefficient of relative inhibition.

Table 1: Codes and names of the genotypes used in the experiment

S. No.	Code	Name of Genotypes	S. No.	Code	Name of Genotypes
01	G1	NARC-01	19	G19	NARC-19
02	G2	NARC-02	20	G20	NARC-20
03	G3	NARC-03	21	G21	NARC-21
04	G4	NARC-04	22	G22	NARC-22
05	G5	NARC-05	23	G23	NARC-23
06	G6	NARC-06	24	G24	NARC-24
07	G7	NARC-07	25	G25	NARC-25
08	G8	NARC-08	26	G26	NARC-26
09	G9	NARC-09	27	G27	NARC-27
10	G10	NARC-10	28	G28	NARC-28
11	G11	NARC-11	29	G29	NARC-29
12	G12	NARC-12	30	G30	NARC-30
13	G13	NARC-13	31	G31	NARC-31
14	G14	NARC-14	32	G32	NARC-32
15	G15	NARC-15	33	G33	Sultan-Talagang
16	G16	NARC-16	34	G34	Sanobar-96
17	G17	NARC-17	35	G35	Haider-93
18	G18	NARC-18			

Table 2: Data regarding days to germination of barley genotypes as affected by water stress levels

Genotypes (G)	Water Stress (WS)				Means
	Control	**10% PEG**	**20% PEG**	**30% PEG**	
G1	3.00 f	3.33 f	5.33 e	7.33 cd	4.75 c-d
G2	3.00 f	3.67 f	5.67 e	8.00 a-c	5.08 a-d
G3	3.00 f	3.33 f	5.67 e	8.00 a-c	5.00 a-d
G4	3.00 f	3.33 f	5.33 e	7.67 b-d	4.83 b-d
G5	3.00 f	3.67 f	5.33 e	8.00 a-c	5.00 a-d
G6	3.00 f	3.33 f	5.33 e	7.67 b-d	4.83 b-d
G7	3.00 f	3.33 f	5.33 e	7.33 cd	4.75 d
G8	3.00 f	3.33 f	6.00 e	8.00 a-c	5.08 a-d
G9	3.00 f	3.67 f	5.67 e	8.00 a-c	5.08 a-d
G10	3.00 f	3.33 f	5.33 e	7.67 b-d	4.83 b-d
G11	3.00 f	3.67 f	5.67 e	8.33 ab	5.17 a-c
G12	3.00 f	3.33 f	5.67 e	8.17 a-c	5.04 a-d
G13	3.00 f	3.33 f	5.33 e	7.00 d	4.67 d
G14	3.00 f	3.33 f	5.33 e	7.67 b-d	4.83 b-d
G15	3.00 f	3.67 f	5.67 e	8.33 ab	5.17 a-c
G16	3.00 f	3.33 f	5.33 e	7.33 cd	4.75 d
G17	3.00 f	3.33 f	5.33 e	7.67 b-d	4.83 b-d
G18	3.00 f	3.67 f	6.00 e	8.33 ab	5.25 a-b
G19	3.33 f	3.67 f	6.00 e	8.00 a-c	5.25 a-b
G20	3.33 f	3.67 f	6.00 e	8.00 a-c	5.25 a-b
G21	3.00 f	3.67 f	6.00 e	8.33 ab	5.25 a-b
G22	3.00 f	3.67 f	6.00 e	8.33 ab	5.25 a-b
G23	3.33 f	3.67 f	6.00 e	8.00 a-c	5.25 a-b
G24	3.00 f	3.67 f	6.00 e	8.33 ab	5.25 a-b
G25	3.00 f	3.67 f	6.00 e	8.33 ab	5.25 a-b
G26	3.33 f	3.67 f	6.00 e	8.00 a-c	5.25 a-b
G27	3.00 f	3.67 f	6.00 e	8.00 a-c	5.17 a-c
G28	3.00 f	3.67 f	6.00 e	8.00 a-c	5.17 a-c
G29	3.33 f	3.67 f	6.00 e	7.83 a-d	5.21 a-b
G30	3.00 f	3.67 f	6.00 e	8.00 a-c	5.17 a-c
G31	3.00 f	3.67 f	6.00 e	8.00 a-c	5.17 a-c
G32	3.33 f	3.67 f	6.00 e	8.00 a-c	5.25 a-b
G33	3.33 f	3.67 f	6.00 e	8.00 a-c	5.25 a-b
G34	3.00 f	3.33 f	5.33 e	7.00 d	4.67 d
G35	3.33 f	3.67 f	6.00 e	8.67 a	5.42 a
Means	3.08 d	3.54 c	5.73 b	7.92 a	
LSD ($p \leq 0.05$)	WS = 0.15; G = 0.46; WS × G = ns				

Means having same letter(s) in row or column are statistically similar.
PEG = polyethylene glycol, ns = non-significant.

Table 3: Data regarding germination percentage of barley genotypes as affected by water stress levels

Genotypes (G)	Water Stress (WS)			
	Control	**10% PEG**	**20% PEG**	**30% PEG**
G1	96.67 a	90.00 a-c	80.00 b-f	60.00 h-l
G2	83.33 a-e	76.67 c-g	53.33 j-n	43.33 m-q
G3	86.67 a-d	76.67 c-g	56.67 i-m	40.00 n-r
G4	96.67 a	90.00 a-c	73.33 d-h	60.00 h-l
G5	90.00 a-c	83.33 a-e	56.67 i-m	43.33 m-q
G6	93.33 ab	80.00 b-f	76.67 c-g	56.67 i-m
G7	96.67 a	90.00 a-c	83.33 a-e	60.00 h-l
G8	83.33 a-e	76.67 c-g	46.67 l-p	36.67 o-s

G9	86.67 a-d	73.33 d-h	53.33 j-n	40.00 n-r
G10	86.67 a-d	83.33 a-e	73.33 d-h	56.67 i-m
G11	86.67 a-d	76.67 c-g	46.67 l-p	36.67 o-s
G12	86.67 a-d	76.67 c-g	53.33 j-n	40.00 n-r
G13	96.67 a	90.00 a-c	76.67 c-g	63.33 g-k
G14	93.33 ab	86.67 a-d	73.33 d-h	60.00 h-l
G15	86.67 a-d	76.67 c-g	50.00 k-o	36.67 o-s
G16	93.33 ab	86.67 a-d	80.00 b-f	56.67 i-m
G17	96.67 a	90.00 a-c	73.33 d-h	63.33 g-k
G18	83.33 a-e	73.33 d-h	46.67 l-p	30.00 q-s
G19	93.33 ab	86.67 a-d	76.67 c-g	53.33 j-n
G20	86.67 a-d	76.67 c-g	53.33 j-n	36.67 o-s
G21	80.00 b-f	76.67 c-g	63.33 g-k	40.00 n-r
G22	76.67 c-g	76.67 c-g	63.33 g-k	30.00 q-s
G23	80.00 b-f	73.33 d-h	66.67 f-j	30.00 q-s
G24	80.00 b-f	73.33 d-h	60.00 h-l	33.33 p-s
G25	80.00 b-f	73.33 d-h	60.00 h-l	30.00 q-s
G26	83.33 a-e	76.67 c-g	66.67 f-j	33.33 p-s
G27	80.00 b-f	76.67 c-g	70.00 e-i	36.67 o-s
G28	83.33 a-e	73.33 d-h	60.00 h-l	30.00 q-s
G29	83.33 a-e	73.33 d-h	60.00 h-l	30.00 q-s
G30	83.33 a-e	76.67 c-g	60.00 h-l	33.33 p-s
G31	83.33 a-e	76.67 c-g	66.67 f-j	23.33 s
G32	83.33 a-e	76.67 c-g	60.00 h-l	26.67 r-s
G33	83.33 a-e	73.33 d-h	56.67 i-m	40.00 n-r
G34	83.33 a-e	76.67 c-g	63.33 g-k	56.67 i-m
G35	83.33 a-e	76.67 c-g	63.33 g-k	33.33 p-s
LSD ($p \le 0.05$)	WS × G = 15.01			

Means having same letter(s) in row or column are statistically similar.
PEG = polyethylene glycol

Table 4: Data regarding root length (cm) of barley genotypes as affected by water stress levels

Genotypes (G)	**Water Stress (WS)**			
	Control	**10% PEG**	**20% PEG**	**30% PEG**
G1	11.17 b-g	10.23 h-m	8.77 s-e	8.40 x-j
G2	9.43 m-t	8.77 s-e	7.50 m-r	6.07 v-a
G3	9.33 n-u	8.67 t-g	7.70 i-p	6.07 v-a
G4	10.77 d-j	8.77 s-e	8.83 q-d	8.00 e-n
G5	10.07 j-o	9.17 p-y	7.00 p-u	6.07 v-a
G6	11.13 b-g	10.13 i-n	9.57 m-s	8.30 z-m
G7	11.63 a-c	9.60 l-r	9.63 l-q	8.60 u-h
G8	8.83 q-d	8.10 d-n	7.13 o-t	6.00 w-a
G9	9.57 m-s	8.67 t-g	8.10 d-n	6.27 u-y
G10	10.70 e-j	10.13 i-n	9.63 l-q	8.23 a-m
G11	10.07 j-o	8.77 s-e	8.17 b-m	6.10 v-a
G12	10.40 g-l	9.23 p-w	7.53 l-r	6.33 t-y
G13	11.77 ab	11.27 b-f	7.53 l-r	9.07 p-z
G14	11.47 a-e	10.20 h-m	8.50 v-i	8.47 w-i
G15	9.57 m-s	8.83 q-d	6.83 q-v	6.13 v-z
G16	11.57 a-d	10.63 f-k	8.80 r-e	8.83 q-d
G17	12.27 a	9.27 o-w	8.97 q-b	9.63 l-q
G18	10.87 c-j	8.47 w-i	7.83 h-o	6.63 s-x
G19	10.40 g-l	8.97 q-b	7.87 g-o	7.63 j-q
G20	8.83 q-d	8.80 r-e	6.77 r-w	6.23 u-y
G21	9.53 m-s	8.33 z-l	7.90 f-o	5.87x-a

G22	10.47 f-k	8.10 d-n	8.03 d-n	6.27 u-y
G23	10.90 c-i	8.27 z-m	7.13 o-t	6.27 u-y
G24	8.37 y-k	8.17 b-m	7.80 h-p	5.37 za
G25	9.47 m-t	8.70 t-f	7.93 f-o	6.00 w-a
G26	10.20 h-m	9.30 o-v	7.53 l-r	5.97 w-a
G27	9.47 m-t	8.03 d-n	6.87 q-v	6.00 w-a
G28	9.87 k-p	8.20 a-m	7.17 o-s	5.90 x-a
G29	11.17 b-g	8.20 a-m	7.83 h-o	6.23 u-y
G30	11.07 b-g	9.30 o-v	6.33 t-y	5.73y-a
G31	9.20 p-x	8.20 a-m	7.87 g-o	5.30 zb
G32	11.13 b-g	9.00 q-a	7.57 k-r	6.23 u-y
G33	11.73 ab	10.67 e-k	7.33 n-s	6.17 v-z
G34	11.00 b-h	10.67 e-k	8.93 q-c	7.70 i-p
G35	11.63 a-c	8.13 c-n	6.53 s-y	6.07 v-a
LSD (p≤0.05)	WS × G = 0.80			

Means having same letter(s) in row or column are statistically similar.
PEG = polyethylene glycol

Table 5: Data regarding shoot length (cm) of barley genotypes as affected by water stress levels

Genotypes (G)	**Water Stress (WS)**			
	Control	**10% PEG**	**20% PEG**	**30% PEG**
G1	13.07 b-g	12.10 h-p	11.37 o-x	9.60 k-t
G2	12.37 f-k	10.47 y-j	9.83 g-r	8.07 z-f
G3	11.27 p-y	10.27 c-n	9.10 q-x	7.57 e-k
G4	13.80 ab	12.17 h-o	11.40 n-x	9.63 j-t
G5	12.23 g-n	11.50 l-v	9.77 i-s	7.93 a-g
G6	13.33 b-d	11.77 j-s	11.27 p-y	9.50 m-v
G7	14.47 a	12.27 g-m	11.57 k-t	9.80 h-s
G8	12.23 g-n	11.17 r-a	8.70 v-b	6.93 j-l
G9	12.50 d-j	11.53 k-u	10.67 v-g	8.90 t-z
G10	12.83 c-h	11.43 m-w	11.73 j-s	9.97 e-p
G11	11.90 i-r	10.77 t-e	9.57 l-u	7.80 c-i
G12	12.23 g-n	10.70 u-f	10.13 d-o	8.13 y-e
G13	13.30 b-e	12.63 c-i	10.33 a-m	8.57 w-c
G14	13.13 b-f	12.30 f-l	11.43 m-w	9.67 j-t
G15	11.53 k-u	10.57 x-i	9.47 n-v	7.70 d-j
G16	13.33 b-d	12.50 d-j	10.77 t-e	9.00 r-x
G17	13.27 b-e	12.63 c-i	9.73 i-t	7.97 a-g
G18	12.67 c-i	12.33 f-l	8.97 s-y	7.20 g-l
G19	10.20 c-o	9.40 o-w	8.73 u-a	7.03 h-l
G20	12.07 h-q	11.03 s-c	9.57 l-u	7.80 c-i
G21	11.90 i-r	11.17 r-a	10.70 u-f	7.87 b-h
G22	12.47 e-j	10.03 e-o	9.70 j-t	9.10 q-x
G23	10.77 t-e	9.90 f-q	9.13 p-x	6.97 i-l
G24	11.13 r-b	9.97 e-p	9.13 p-x	6.90 j-l
G25	11.37 o-x	10.70 u-f	9.60 k-t	7.40 e-l
G26	10.57 x-i	10.03 e-o	8.47 x-d	6.70 l
G27	11.17 r-a	10.43 y-k	9.03 r-x	6.67 l
G28	12.30 f-l	10.70 u-f	9.53 m-v	7.30 e-l
G29	12.03 h-q	10.70 u-f	9.00 r-x	7.23 e-l
G30	10.73 t-f	10.30 b-n	9.03 r-x	6.83 k-l
G31	12.17 h-o	11.00 s-c	9.60 k-t	7.83 c-h
G32	11.50 l-v	10.40 z-l	9.83 g-r	6.93 j-l
G33	11.23 q-z	10.13 d-o	8.60 w-c	6.83 k-l
G34	13.43 bc	12.63 c-i	10.63 w-h	8.50 x-d

G35	10.97 s-d	10.13 d-o	8.90 t-z	7.13 g-l
LSD ($p \leq 0.05$)	WS × G = 0.89			

Means having same letter(s) in row or column are statistically similar.
PEG = polyethylene glycol

Table 6: Data regarding coleoptile length (cm) of barley genotypes as affected by water stress levels

Genotypes (G)	**Water Stress (WS)**			
	Control	**10% PEG**	**20% PEG**	**30% PEG**
G1	8.81 a	7.71 f-j	5.41 w-y	4.81 z-d
G2	7.11 k-n	6.38 q-t	4.29 d-i	3.69 j-n
G3	7.21 j-m	6.53 o-s	4.21 e-j	3.61 k-o
G4	8.61 a-c	7.61 g-k	4.81 z-d	4.21 e-j
G5	7.31 i-l	6.91 l-p	4.21 e-j	3.61 k-o
G6	8.11 c-g	7.71 f-j	5.51 v-x	4.91 y-c
G7	8.71 ab	7.81 e-i	4.91 y-c	4.31 d-i
G8	7.13 k-n	6.13 r-u	3.94 h-l	2.91 p-r
G9	8.01 d-h	6.71 m-q	3.99 g-l	3.21 n-p
G10	8.31 a-e	7.71 f-j	5.01 x-b	4.41 c-h
G11	6.61 n-r	5.97 t-v	3.81 i-m	2.61 q-r
G12	7.71 f-j	7.15 k-m	4.01 g-l	3.31 m-p
G13	8.41 a-d	8.01 d-h	5.41 w-y	4.81 z-d
G14	8.41 a-d	8.01 d-h	4.81 z-d	4.21 e-j
G15	7.81 e-i	7.00 l-o	4.31 d-i	3.21 n-p
G16	8.41 a-d	7.91 d-h	5.41 w-y	4.81 z-d
G17	8.61 a-c	7.61 g-k	4.71 a-e	4.11 f-k
G18	7.81 e-i	6.71 m-q	3.91 h-l	3.31 m-p
G19	7.80 f-i	6.40 q-t	5.26 x-z	4.43 c-h
G20	7.71 f-j	6.91 l-p	4.04 f-k	3.31 m-p
G21	6.13 r-u	6.07 s-u	4.74 a-e	4.14 f-k
G22	7.10 k-n	6.17 r-u	4.14 f-k	3.54 l-o
G23	6.13 r-u	5.80 u-w	5.27 x-z	4.23 e-j
G24	7.20 j-m	6.95 l-p	4.84 z-d	4.24 e-j
G25	8.00 d-h	7.60 g-k	3.79 j-m	2.84 p-r
G26	8.60 a-c	6.84 l-q	3.94 h-l	3.14 o-q
G27	7.80 f-i	6.03 s-v	4.94 y-c	4.34 d-i
G28	7.90 d-h	6.20 r-u	3.99 h-l	2.54 r
G29	8.20 b-f	7.90 d-h	3.84 i-m	3.24 n-p
G30	6.50 o-s	5.80 u-w	5.34 w-z	4.74 a-e
G31	6.10 s-u	5.83 u-w	4.50 c-g	3.91 i-l
G32	8.30 a-e	7.50 h-k	3.74 j-n	3.14 o-q
G33	6.20 r-u	5.83 u-w	5.13 x-a	3.93 h-l
G34	8.80 a	6.87 l-q	5.14 x-a	4.57 b-f
G35	7.00 l-o	6.45 p-t	3.93 h-l	3.24 n-p
LSD ($p \leq 0.05$)	WS × G = 0.51			

Means having same letter(s) in row or column are statistically similar.
PEG = polyethylene glycol

Table 7: Data regarding root dry weight (g) of barley genotypes as affected by water stress levels

Genotypes (G)	**Water Stress (WS)**			
	Control	**10% PEG**	**20% PEG**	**30% PEG**
G1	5.77 a	5.23 cd	4.28 m-r	3.82 v-d
G2	4.82 e-k	4.06 q-z	3.60 b-h	3.14 i-o
G3	4.91 d-j	4.10 p-y	3.58 c-h	3.12 i-p

G4	5.77 a	5.01 c-i	4.25 n-s	3.79 x-d
G5	4.95 d-i	4.20 n-v	3.68 z-f	3.22 h-n
G6	5.27 cd	4.97 d-i	4.20 n-w	3.03 j-q
G7	5.90 a	5.07 c-h	4.28 m-r	3.82 v-d
G8	3.28 g-l	3.00 k-q	2.51 st	2.05 uv
G9	4.91 d-j	4.47 k-p	3.30 f-l	2.84 n-s
G10	5.37 bc	4.96 d-i	4.05 q-z	3.59 c-h
G11	3.92 r-c	3.30 f-l	2.31 tu	1.85 vw
G12	4.91 d-j	4.15 o-x	3.28 g-l	2.82 o-s
G13	5.73 ab	5.12 c-g	4.47 k-p	4.01 r-z
G14	5.67 ab	5.16 c-f	4.18 n-w	3.72 y-e
G15	4.70 h-l	3.88 s-c	3.02 j-q	2.56 st
G16	5.19 c-e	4.56 j-n	4.12 o-x	3.00 k-q
G17	6.01 a	5.00 c-i	4.30 m-r	3.84 u-d
G18	5.13 c-f	4.30 m-r	3.34 e-l	2.88 m-s
G19	4.97 d-i	4.10 p-y	3.95 r-c	3.07 j-p
G20	5.14 c-f	4.24 n-t	3.27 g-l	2.81 o-s
G21	4.69 h-l	4.03 q-z	4.00 r-a	3.07 j-p
G22	4.30 m-r	4.07 q-y	3.83 u-d	3.25 g-m
G23	4.73 h-l	4.54 j-n	3.28 g-l	1.82vw
G24	3.98 r-b	2.74 p-s	3.07 j-p	2.61 r-t
G25	5.12 c-g	4.21 n-u	3.59 c-h	3.36 e-k
G26	4.41 l-q	3.86 t-d	3.22 h-n	1.62 w
G27	4.49 k-o	3.04 j-p	3.05 j-p	2.59 q-t
G28	4.65 i-m	3.89 s-c	3.24 g-m	2.97 l-r
G29	4.80 f-k	4.05 q-z	3.95 r-c	3.49 d-i
G30	4.69 h-l	4.10 p-y	3.39 e-j	2.33 tu
G31	4.74 g-l	3.62 a-g	3.02 j-q	2.56 st
G32	4.83 e-k	3.81 w-d	3.92 r-c	3.28 g-l
G33	4.81 e-k	4.04 q-z	3.11 i-p	2.65 q-t
G34	4.69 h-l	4.04 q-z	4.02 r-z	3.22 h-n
G35	4.97 d-i	4.02 r-z	3.04 j-p	2.58 st
LSD (p≤0.05)	WS × G = 0.38			

Means having same letter(s) in row or column are statistically similar.
PEG = polyethylene glycol

Table 8: Data regarding shoot dry weight (g) of barley genotypes as affected by water stress levels

Genotypes (G)	Water Stress (WS)				Means
	Control	10% PEG	20% PEG	30% PEG	
G1	7.25 a	6.48 a-h	5.41 m-H	5.04 u-S	6.05 a
G2	6.33 b-l	5.27 o-M	4.88 w-T	4.51 H-X	5.25 d-g
G3	6.14 c-p	5.35 n-L	4.60 C-X	4.23 Q-X	5.08 e-h
G4	6.77 a-d	5.95 d-t	5.40 m-I	5.03 v-S	5.79 a-b
G5	6.53 a-g	5.04 u-S	4.57 E-X	4.10 T-Y	5.06 e-i
G6	6.81 a-d	6.48 a-h	5.63 g-z	5.26 p-M	6.05 a
G7	7.13 ab	6.58 a-f	5.57 i-B	5.20 q-O	6.12 a
G8	5.17 r-P	4.29 P-X	3.32 Y-Z	2.88 Z	3.92 L
G9	6.12 c-p	5.64 g-z	4.48 K-X	4.11 T-Y	5.09 e-h
G10	6.83 a-d	6.17 c-o	5.20 q-O	4.83 x-U	5.76 a-b
G11	6.17 c-o	5.24 p-N	4.87 x-T	4.50 I-X	5.20 e-h
G12	6.24 b-n	5.21 q-O	4.68 B-W	4.31 O-X	5.11 e-h
G13	6.67 a-e	6.40 a-j	5.78 e-w	5.41 m-H	6.07 a
G14	6.87 a-c	6.45 a-j	5.72 f-x	5.35 n-L	6.10 a
G15	5.98 c-t	5.03 v-S	4.28 P-X	3.91 V-Y	4.80 g-k
G16	6.45 a-j	5.94 d-u	5.59 h-A	4.78 z-W	5.69 a-d

G17	6.59 a-f	6.19 c-n	5.34 n-L	4.97 v-T	5.77 a-b
G18	6.13 c-p	5.41 m-G	4.92 v-T	4.55 F-X	5.25 c-f
G19	6.08 c-q	5.56 j-B	5.29 o-M	4.45 L-X	5.34 b-e
G20	6.38 a-k	5.48 k-D	4.49 J-X	4.12 T-Y	5.12 e-h
G21	5.48 k-C	4.88 x-T	4.64 C-X	4.70 A-W	4.93 e-k
G22	5.70 f-y	5.39 m-J	4.51 I-X	3.94 U-Y	4.88 f-k
G23	5.58 i-B	4.80 y-U	4.60 C-X	4.22 R-Y	4.80 g-k
G24	4.95 v-T	4.64 C-X	4.33 O-X	4.27 P-X	4.55 j-k
G25	5.80 e-v	5.08 t-R	4.24 Q-X	3.88 W-Y	4.75 h-k
G26	6.05 c-s	4.74 z-W	4.32 O-X	3.95 U-Y	4.77 h-k
G27	5.45 l-E	5.17 s-P	4.46 L-X	4.40 M-X	4.87 f-k
G28	5.42 m-F	4.95 v-T	4.58 D-X	4.24 Q-X	4.80 g-k
G29	6.28 b-m	5.10 t-R	4.62 C-X	4.25 Q-X	5.06 e-i
G30	5.27 p-M	4.73 A-W	4.34 N-X	4.12 T-Y	4.61 i-k
G31	5.44 l-F	5.13 t-Q	4.61 C-X	4.86 x-T	5.01 e-i
G32	5.82 e-v	5.21 q-O	4.58 D-X	4.15 S-Y	4.94 e-j
G33	6.07 c-r	4.89 w-T	5.10 t-R	4.73 A-W	5.20 e-h
G34	6.46 a-i	6.13 c-p	5.40 m-I	4.81 y-U	5.70 a-c
G35	5.36 n-K	4.52 G-X	4.29 P-X	3.77 X-Z	4.49 k
Means	6.11 a	5.41 b	4.82 c	4.45 d	
LSD ($p \leq 0.05$)	WS = 0.15; G = 0.45; WS × G= ns				

Means having same letter(s) in row or column are statistically similar.
PEG = polyethylene glycol, ns = non-significant.

Table 9: Data regarding root-shoot dry weight ratio of barley genotypes as affected by water stress levels

Genotypes (G)	**Water Stress (WS)**			
	Control	**10% PEG**	**20% PEG**	**30% PEG**
G1	0.80 a-t	0.81 a-r	0.80 a-t	0.76 b-v
G2	0.77 b-v	0.78 a-v	0.74 c-x	0.70 h-z
G3	0.80 a-s	0.77 b-v	0.79 a-u	0.75 c-x
G4	0.86 a-l	0.84 a-n	0.79 a-t	0.76 b-w
G5	0.76 b-w	0.84 a-n	0.81 a-r	0.79 a-t
G6	0.78 a-v	0.77 b-v	0.75 c-x	0.58 x-d
G7	0.83 a-o	0.77 b-v	0.77 b-v	0.74 d-y
G8	0.64 r-B	0.71 f-z	0.76 b-v	0.73 d-z
G9	0.81 a-s	0.80 a-t	0.74 c-x	0.70 i-z
G10	0.79 a-t	0.81 a-s	0.78 a-u	0.75 c-x
G11	0.64 s-B	0.63 s-B	0.48 B-D	0.42 D
G12	0.79 a-t	0.80 a-t	0.71 g-z	0.66 o-a
G13	0.86 a-k	0.80 a-s	0.78 a-v	0.75 c-x
G14	0.83 a-o	0.80 a-s	0.74 d-y	0.70 i-z
G15	0.79 a-t	0.78 a-v	0.71 f-z	0.66 o-a
G16	0.81 a-s	0.77 b-v	0.74 d-y	0.63 t-b
G17	0.91 a-c	0.81 a-r	0.81 a-r	0.78 a-v
G18	0.84 a-n	0.80 a-t	0.68 l-a	0.64 r-b
G19	0.82 a-q	0.74 c-x	0.75 c-x	0.69 j-a
G20	0.81 a-s	0.78 a-v	0.74 d-y	0.69 j-a
G21	0.86 a-k	0.83 a-p	0.86 a-j	0.65 q-a
G22	0.75 c-w	0.76 b-w	0.85 a-m	0.83 a-o
G23	0.85 a-n	0.95 a	0.72 e-z	0.43 cd
G24	0.81 a-s	0.59 w-c	0.71 f-z	0.61 u-b
G25	0.88 a-f	0.84 a-n	0.85 a-m	0.87 a-i
G26	0.73 d-z	0.82 a-q	0.76 c-w	0.42 D
G27	0.83 a-p	0.59 w-c	0.68 m-a	0.59 w-c
G28	0.86 a-k	0.79 a-t	0.71 g-z	0.70 h-z

G29	0.77 b-v	0.80 a-t	0.86 a-k	0.83 a-p
G30	0.89 a-d	0.88 a-g	0.78 a-u	0.57 y-D
G31	0.87 a-h	0.72 e-z	0.66 p-a	0.53 a-d
G32	0.83 a-o	0.73 d-z	0.86 a-l	0.79 a-t
G33	0.79 a-t	0.83 a-o	0.61 v-b	0.56 z-d
G34	0.73 d-z	0.67 o-a	0.75 c-x	0.68 n-a
G35	0.93 ab	0.89 a-e	0.71 g-z	0.69 k-a
LSD (p≤0.05)	WS × G = 0.17			

Means having same letter(s) in row or column are statistically similar.
PEG = polyethylene glycol

Table 10: Data regarding seedling vigor index of barley genotypes as affected by water stress levels

Genotypes (G)	**Water Stress (WS)**			
	Control	**10% PEG**	**20% PEG**	**30% PEG**
G1	23.43 a-c	20.10 d-f	16.11 n-x	10.80 g-m
G2	18.17 f-n	14.74 v-b	9.23 k-p	6.11 q-t
G3	17.85 h-p	14.51w-c	9.50 j-p	5.45 s-v
G4	23.76 ab	18.84 e-l	14.85 v-b	10.58 h-m
G5	20.07 d-f	17.24 k-t	9.49j-p	6.05 q-u
G6	22.84 bc	17.52 i-r	15.97 o-y	10.05 i-n
G7	25.23 a	19.66 d-i	17.67 h-r	11.04 g-l
G8	17.55 i-r	14.77 v-b	7.39 p-s	4.70 t-w
G9	19.12 e-l	14.80 v-b	10.01 i-n	6.07 q-u
G10	20.40 de	17.92 g-p	15.67 q-z	10.27 h-n
G11	19.01 e-l	14.95 u-a	8.29 n-p	5.12 t-w
G12	19.62 d-i	15.29 s-a	9.41 k-p	5.80 r-u
G13	24.22 ab	21.51 cd	13.69 z-e	11.17 g-l
G14	22.97 bc	19.50 d-j	14.62 v-b	10.86 g-l
G15	18.28 e-m	14.88 v-b	8.15 n-q	5.06 t-w
G16	23.24 a-c	20.04 d-g	15.65 r-z	10.11 i-n
G17	24.67 ab	19.71 d-h	13.71 z-e	11.09 g-l
G18	19.65 d-i	15.25 s-a	7.84 o-r	4.02 t-w
G19	19.20 e-l	15.88 p-y	12.75 b-g	7.82 o-r
G20	18.11 f-o	15.21 t-a	8.71 m-p	5.11 t-w
G21	17.07 l-u	14.95 u-a	11.78 d-i	5.51 s-v
G22	17.59 h-r	13.92 y-d	11.24 f-k	4.63 t-w
G23	17.38 j-s	13.32 a-f	10.86 g-l	3.98 t-w
G24	15.60 r-z	13.31 a-f	10.16 i-n	4.09 t-w
G25	16.67 m-v	14.21 x-c	10.52 h-m	4.00 t-w
G26	17.31 k-t	14.81 v-b	10.67 g-m	4.24 t-w
G27	16.51 m-w	14.17 x-c	11.13 g-l	4.63 t-w
G28	18.48 e-m	13.86 y-d	10.02 i-n	3.96 u-w
G29	19.33 e-k	13.86 y-d	10.10 i-n	4.04 t-w
G30	18.16 f-n	15.03 u-a	9.22 k-p	4.18 t-w
G31	17.80 h-q	14.71 v-b	11.62 e-j	3.08 w
G32	18.87 e-l	14.89 v-a	10.45 h-m	3.53 v-w
G33	19.13 e-l	15.26 s-a	9.05 l-p	5.20 t-w
G34	20.38 de	17.91 g-p	12.37 c-h	9.13 k-p
G35	18.84 e-l	14.01 x-c	9.77 i-o	4.39 t-w
LSD (p≤0.05)	WS × G = 2.82			

Means having same letter(s) in row or column are statistically similar.
PEG = polyethylene glycol

Table 11: Data regarding coefficient of relative inhibition of barley genotypes as affected by water stress levels.

Genotypes (G)	Water Stress (WS)		
	10% PEG	20% PEG	30% PEG
G1	0.10 h-m	0.26 m-u	0.32 f-k
G2	0.16 z-g	0.24 o-v	0.31 f-l
G3	0.14 b-j	0.26 l-t	0.34 d-j
G4	0.13 d-l	0.23 r-x	0.30 h-n
G5	0.20 u-b	0.28 i-s	0.36 b-g
G6	0.05 m-n	0.19 v-c	0.31 f-l
G7	0.11 g-m	0.24 n-v	0.31 g-m
G8	0.14 c-k	0.31 f-l	0.42 ab
G9	0.08 k-m	0.29 i-p	0.37 b-f
G10	0.09 j-m	0.24 n-v	0.31 g-m
G11	0.15 b-i	0.29 i-q	0.37 b-f
G12	0.16 z-g	0.29 i-r	0.36 b-g
G13	0.07 l-m	0.17 x-d	0.24 o-v
G14	0.07 l-m	0.21 t-a	0.28 j-s
G15	0.17 z-g	0.32 f-k	0.39 b-d
G16	0.10 i-m	0.17 z-g	0.33 e-j
G17	0.11 e-l	0.23 p-v	0.30 h-n
G18	0.14 c-k	0.27 k-t	0.34 d-i
G19	0.13 d-l	0.16 a-g	0.32 f-k
G20	0.16 a-h	0.33 f-j	0.40 bc
G21	0.12 d-l	0.15 b-j	0.24 p-v
G22	0.05 mn	0.17 z-f	0.28 j-s
G23	0.09 j-m	0.23 q-w	0.41 ab
G24	0.17 x-d	0.17 y-e	0.23 r-x
G25	0.15 b-i	0.28 i-s	0.34 e-j
G26	0.17 x-d	0.28 j-s	0.47 a
G27	0.17 x-d	0.24 n-u	0.30 h-o
G28	0.12 d-l	0.22 t-z	0.28 i-s
G29	0.17 w-d	0.23 s-y	0.30 h-n
G30	0.11 f-l	0.22 t-z	0.35 c-h
G31	0.14 c-k	0.24 n-v	0.27 k-t
G32	0.15 b-j	0.20 u-b	0.30 h-n
G33	0.18 w-d	0.24 n-v	0.32 f-k
G34	0.09 k-m	0.15 a-h	0.28 j-s
G35	0.17 w-d	0.29 i-q	0.39 b-d
LSD ($p \leq 0.05$)	WS × G = 0.08		

Means having same letter(s) in row or column are statistically similar.
PEG = polyethylene glycol

DISCUSSION

It is evident from the results that there were significant effects of stress levels and genotypic factors on germination traits, morphological parameters, dry matter contents, seedling vigor index and coefficient of relative inhibition. In this section of the paper, we have discussed the variations with their respective mechanisms, scientific reasoning, technical evidence, and in reference to previous research studies.

In this study, we recorded that increasing the concentrations of polyethylene glycol (PEG) resulted in longer germination times across all genotypes. This delayed germination reflects the physiological barrier imposed by osmotic stress. When the external water potential is lowered by PEG, seeds have higher internal water-potential gradient for water imbibition, which in turn delays the subsequent activation of metabolic machinery (Kylyshbayeva *et al*., 2024). Moreover, PEG-6000 is known to restrict water-transport by increasing solute concentration and effectively reducing the hydraulic conductivity at seedcoat (Davidson-Willis *et al*., 2024). Among the genotypes, those that germinated faster under stress likely possessed more seed-stored food, more reserved-mobilization, and higher membrane integrity which enabled a rapid uptake of water even under unfavorable conditions of lower water potential (Lateef *et al*., 2021). Likewise, faster radicle development is closely linked with higher enzymatic activity in drought-tolerant cereals (Li *et al*., 2025). Therefore, the observed variability in germination time reflects underlying genotypic differences in seed physiology rather than just by-chance. With respect to germination percentage, the decline with increasing PEG confirms such findings in barley and other cereals. As previously reported, while screening Iraqi barley accessions under PEG, germination percentage dropped significantly as osmotic potential became more negative. Lateef *et al*. (2021) observed reductions of up to 85% in root length and also severe declines in germination under PEG-6000 in barley genotypes. These declines reflect combined effects of insufficient water uptake, increased abscisic acid accumulation and suppressed gibberellic acid, which combinedly have caused to inhibit rapid emergence of radicles (Ramappa *et al*., 2023). In field drought conditions, the same physiological constraints (limited soil water, higher vapor pressure deficit, increased abscisic acid) are likely to happen, so the lower germination under PEG is an indicator of poor crop establishment at field level as well (Niu *et al*., 2025).

With respect to seedling growth, our data showed that root, shoot and coleoptile lengths decreased under stress conditions, as the genotypes resulted in the greatest reduction at 30% PEG, then 20% PEG, and the minimum reduction was noted at 10% PEG. This is consistent with the role of turgor in cell elongation, i.e. under low water potential, cell expansion slows down and the growth zones shrink (Muthusamy *et al*., 2020). Recent work shows that PEG treatment reduced root elongation of barley by 60-85% compared to control (Susilawati *et al*., 2022). The genotypic variation in the extent of reduction indicates that some genotypes maintain better osmotic adjustment which supports continued growth even under severe stress conditions. Our findings are in line with Cabeza *et al* (2024), who reported similar conclusions earlier. Importantly, from an actual field perspective, longer coleoptiles and deeper roots at the seedling stage enhance early access to sub-soil moisture and therefore improve establishment under drought (Mehmandar *et al*., 2023).

Root and shoot dry weights are the crucial physiological indicators of a genotype to maintain water uptake and sustain metabolism under water stress. In the present study, increasing PEG concentrations progressively reduced both root and shoot dry weights, revealing genotype-dependent differences in biomass allocation. Genotypes 19, 22, and 34 maintained relatively higher dry matter accumulation even at 30% PEG, whereas 8, 26, and 27 showed up to 40% reductions, reflecting significantly different drought-tolerance among our studied genotypes. These variations result from differential osmotic adjustment capacity, maintenance of root hydraulic conductance, and activation of antioxidative defense mechanisms, all of which are vital for sustaining cellular turgor and metabolic function under osmotic stress (Rizvi *et al*., 2022). Interestingly, the root/shoot dry weight ratio decreased from 0.78 at 10% PEG to 0.68 at 30% PEG, indicating a stronger inhibition effect on root elongation and biomass accumulation compared to shoots. Under PEG-induced osmotic stress, root growth can be suppressed due to reduced cell expansion and inhibited activity, leading to limited water flow for shoots (Ramappa *et al*., (2023). Genotypes capable of maintaining higher root/shoot ratio under such stress conditions often exhibit better recovery potential and yield stability under field drought conditions (Lotfi *et al*., 2022). Therefore, in our experiment, genotypes that retained relatively higher root biomass and sustained root/shoot ratios under increasing PEG concentrations represent promising genotypes for breeding programs and at improving drought resilience in barley.

The Seedling Vigor Index (SVI) declined sharply with increasing PEG concentration, reflecting the integrated effect of delayed germination, shorter growth and reduced biomass accumulation. Recent research on early screening of barley found that under PEG-induced water stress, SVI expressed significant variations in subsequent drought tolerance indexes (Habtegebriel, 2025). Since seedling vigor under stress is a key determinant of a good crop establishment, so these findings reinforce the use of PEG screening for early selection, even while acknowledging that field drought involves some additional stressors as well (Peršić *et al*., 2022). Finally, the Coefficient of Relative Inhibition (CRI) increased with increasing PEG concentration, suggesting severe inhibition in growth. From breeding perspectives, genotypes with lower CRI under high osmotic stress are preferable because they maintain higher growth-potential even under adverse conditions (Özkan & Levent, 2025; Mahpara *et al*., 2022). Therefore, genotypes highlighted in this study with higher germination percentages, longer roots and shoots, lower CRI and higher SVI can be tested in field trials under rain-fed or controlled water stress conditions. It can be concluded that early-stage assessment via PEG-induced osmotic stress reveals meaningful genotypic variation in germination, seedling growth and biomass allocation among barley genotypes. When aligned with physiological concepts (water uptake, turgor, hormone signaling, osmotic adjustment) and compared to recent literature, our findings support the selection of promising genotypes for drought-resilient programs. We recommend that genotypes having better performance in this study should be tested in field-based drought trials to validate them under real water-limited conditions as well.

Conclusion: This study revealed significant genotypic variation in barley genotypes to PEG-induced water stress. The genotypes NARC-01, NARC-07, NARC-13, NARC-16 and Sanobar-96 germinated earlier and had higher germination percentages, indicating better vigor and early establishment potential. These genotypes, along with NARC-04, NARC-06, NARC-10, NARC-14, and NARC-17 showed higher root, shoot and coleoptile lengths, as well as higher dry weights and root-shoot dry weight ratios. On the other hand, genotypes NARC-05, NARC-08, NARC-11, NARC-12, NARC-15, NARC-20 and NARC-26 showed higher coefficients of relative inhibition, reflecting water stress susceptibility. Increasing PEG concentration, particularly at 30%, significantly suppressed all growth parameters. Overall, genotypes NARC-01, NARC-04, NARC-06, NARC-07, NARC-10, NARC-13, NARC-14, NARC-16, NARC-17 and Sanobar-96 demonstrated strong water stress tolerance and are recommended for further field evaluation under natural drought conditions. The most resilient genotypes may serve as potential parent lines aimed at enhancing drought tolerance in barley.

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