# INTEGRATIVE MORPHO-PHYSIOLOGICAL AND GENETIC CHARACTERIZATION OF BARLEY GENOTYPES FOR CLIMATE RESILIENCE UNDER WATER DEFICIT CONDITIONS

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**Abstract.** Drought is a major global challenge that threatens crop productivity, quality, and sustainable agriculture. Water deficit conditions induce physiological and genetic impairments in field crops, negatively impacting seed germination, growth, and overall development. This study aimed to assess the morpho-physiological and genetic responses of diverse barley genotypes to water deficit conditions, providing insights into climate resilience. A total of 95 barley genotypes were evaluated under normal and water deficit-stressed environments using a completely randomized design with three replications. The results revealed significant genetic variation across all studied traits. Notably, root-shoot length exhibited the lowest reduction percentage (RP), while root length, fresh seed weight, dry seed weight, relative water content, relative injury, cell membrane thermo-stability, carotenoids, and chlorophyll b displayed strong positive correlations. Shoot length, however, showed a non-significant association with all attributes. High heritability estimates for root length suggest a strong additive genetic effect, indicating its potential for genetic improvement. The genotypes G1, G25, and G61 exhibited minimal RP values and were identified as water deficit-tolerant, whereas G5, G16, and G77 showed higher RP values, categorizing them as water deficit-susceptible. The biplot analyses further confirmed the similar classification of drought tolerant and susceptible genotypes consistency across environments. These findings highlight key selection traits for breeding water deficit-resilient barley cultivars, contributing to sustainable agricultural practices in water-limited environments.

**Keywords:** *drought, cereal, yield, heritability, environments*

## Introduction

Barley (*Hordeum vulgare* L.) is one of the most important cereal crops worldwide, valued for its adaptability to diverse environmental conditions, including drought-prone regions. As climate change intensifies water scarcity, improving barley's drought resilience has become crucial for sustainable agriculture and food security (Boudiar et al., 2020). Water deficit stress significantly affects barley growth, particularly during the seedling stage, by limiting physiological processes such as photosynthesis, stomatal regulation, and osmotic adjustment. Identifying and screening barley genotypes with superior morpho-physiological traits can help develop drought-tolerant varieties with enhanced survival and productivity under water-limited conditions (Sallam et al., 2019; Elakhdar et al., 2023). Understanding the genetic potential of seedling traits in response to water deficit stress provides valuable insights into breeding strategies for improving barley resilience and ensuring stable yields in challenging environments. Global crop production and food security are seriously threatened by water scarcity. The attention is given to the breeding program at the terminal stage of the barley for improving water deficit tolerance, due to the utmost importance of the grain yield rather than breeding at seedling stage for water deficit tolerance. The stress in both stages can be important for the crop that is the main reason why the water deficit stress during all the vegetative stages should be considered and studied (Abid et al., 2016; Moursi et al., 2020).

Barley plays a crucial role in enhancing food security and promoting sustainable agriculture globally, particularly in marginal and arid regions where it is often the only viable crop option for farmers. Its adaptability to various biotic and abiotic stresses allows it to thrive in challenging environments, making it a key resource for risk-averse farmers (Visioni et al., 2023). Barley exhibits several specific traits that enhance its suitability for cultivation in marginal and arid regions, primarily due to its inherent abiotic stress tolerance. Research indicates that barley can thrive in harsh environments with limited water resources, showing significant genetic variation in water deficit resistance among different genotypes (Kumar et al., 2022; Khalid et al., 2024). Breeding programs have successfully developed lines that demonstrate increased productivity and stability under water deficit conditions, with some varieties showing nearly double the yield during severe water deficit years (Abid et al., 2016; Moursi et al., 2020). Additionally, biochemical and molecular mechanisms, such as drought escape strategies and favorable morphophysiological traits, contribute to the resilience of barley (Elakhdar et al., 2023). Global crop production and food security are seriously threatened by water scarcity. Attention is given to the breeding program at the terminal stage of the barley for improving water deficit tolerance, due to the utmost importance of the grain yield rather than breeding at seedling stage for water deficit tolerance. The stress given to the barley at both stages can be important for the crop that is the main reason why the water deficit stress during all the vegetative stages should be considered and studied (Kumar et al., 2022; Afzal et al., 2024). In all the cereal crops, the barley is the most widely used crop due to its high nutritive values. The increase in the demand for food due to the increase in population creates new problems for the breeder to breed new barley cultivars that will produce higher grain yield, and also be resistant to different biotic and other environmental stresses (Boudiar et al., 2020; Alsamadany et al., 2024). Barley plants are very responsive to the water deficit at jointing, tillering, booting, filling and anthesis stage and suffer a great loss. The barley develops tillers, primordia of spikes, spikelet and florets at the tillering stage in the barley plant, the water deficit can cause the grain yield loss at this stage (Kumar et al., 2022). While drought stress is a direct abiotic factor causing physiological and developmental disruptions in barley, indirect agronomic factors—such as low-quality seeds, outdated or non-recommended practices, and poor soil and nutrient management—can exacerbate drought impacts. For instance, poor-quality seeds may exhibit weak germination and limited root development, reducing water uptake under drought. Similarly, inappropriate sowing time and suboptimal agronomic practices can impair root establishment and canopy development, making plants more susceptible to water stress. These interacting factors collectively contribute to a compounded reduction in yield under drought conditions (Song et al., 2024).

Genetic variation among barley genotypes significantly influences their growth under water deficit conditions, as evidenced by multiple studies. The remobilization of stored assimilates, crucial for yield under water deficit, varies among genotypes, with the Yousef genotype demonstrating superior remobilization efficiency, maintaining stable levels of key enzymes and metabolites like sucrose synthase and glucose 6-phosphate, which are vital for seed development and oxidative stress mitigation (Hajibarat et al., 2024). Research indicates that, genotype G15 was identified as superior under water-deficit conditions due to its higher chlorophyll content and carotenoids, which are essential for photosynthesis and stress tolerance (Saidi et al., 2024). The drought tolerance index further highlights genotype OB1878-ON-50 as a significant resource for breeding due to its superior drought tolerance during germination (Hajibarat et al., 2024; Pham-Khanh et al., 2024). Additionally, variations in stomatal density among genotypes have been linked to water-use efficiency (WUE), with low-stomatal-density varieties showing better biomass retention and wilting resistance under drought stress (Ashraf et al., 2022; Robertson et al., 2023). Furthermore, transcriptomic analyses reveal that root zone-specific responses to water deficit are crucial, highlighting the importance of genetic regulation in enhancing resilience against drought (Ashraf et al., 2022; Alsamadany et al., 2024).

The root is the first sensing organ for stress perception, during a shortage of water. In water deficit condition, root development is encouraged by longer roots because they can draw moisture from soil at deeper level. The length of the root at seedling stage of the plant is the key genetic trait to increase the potential of the plant for the grain yield at water deficit conditions (Song et al., 2024; Kadege et al., 2024). The researcher reported that relative water content and root to shoot ratio were the basis for the selection criteria for the barley in the water deficit tolerance (Abdelrady et al., 2024).

As many Barley scientists mentioned, the growth of barley influence by the water deficit condition, but these changes also vary genotypically in varieties. The researcher concluded that the relative water content can help as the basis of selection of plant at early germinating stage in less water conditions (Alsamadany et al., 2024). The relationship of relative water content with the volume of the cell can correctly show the distribution of the water and the amount of the water absorbed (Kumar et al., 2022; Lavrukhina et al., 2024). Grow the barley plants under normal and water deficit conditions and conclude that the genotypes which were grown in the normal conditions perform better having optimum relative water content and root-to-shoot ratio, the selection of the genotypes in the seedling conditions is much easier and cheap method. These methods were also less laborious, the characteristics showed an additive gene effect with moderate to high variability in different conditions (Li et al., 2023; Alsamadany et al., 2024). There was a dire need of selection and breeding for the water deficit tolerant genotypes which was short of in the past. The selection for effective yield and yield traits, the study of the heritability and genetic advance is used which predicts the selection of the genotypes as heritability is highly helpful metric to evaluate the influence of the environment and heredity on the traits (Hajibarat et al., 2024; Shabbir et al., 2024).The plants with high genetic advance and high amount of heritability reveals that strong effect of the additive gene effects for the variation of the traits and selection is based upon the traits which can be of vital importance to increase the grain yield (Ashraf et al., 2022; Alsamadany et al., 2024). The present work executed and planned to estimate genetic variation, heritability and genetic advance. The breeders can choose the top genotypes from the diverse environment with the help of heritability estimations.

The main objective of the experiment is to estimate the genetic variations, heritability and genetic advance under the stressed and non-stressed conditions which can be used to improve the studied traits in future breeding program. The heritability and genetic advance along with the phenotypic and genotypic correlation coefficients had been used in the barley to study the variations in the breeding germplasm and to characterize the selection procedures and to improve the traits under study.

## Materials and methods

### *Experimental design*

The experiment was initiated with 15*15 cm sand-filled polyethylene bags at wire house of the Plant Breeding and Genetics department, The Islamia University of Bahawalpur, Pakistan. The experiment was conducted as completely randomized design (CRD) under factorial with three replications in well water (control) and water deficit conditions during growing seasons 2023-24. Although the number of replications was limited to three due to space and resource constraints, the use of uniform polyethylene bags, controlled environmental conditions, and consistent soil moisture monitoring helped reduce environmental variability. This, combined with the observed significant differences in ANOVA and moderate-to-high heritability for most traits, supports the statistical robustness of the design.

### *Experimental materials*

Total ninety-five (95) barley genotypes were considered as experimental materials. Seeds were placed in soil containing polyethylene (poly) bag for germination. Plant growths were maintained for 2-week. One seed per poly bag was selected for the experiment. One set of 95 genotypes has been regularly irrigated (100% of field capacity) while the other set of same barley genotypes was exposed to water deficit condition (at 50% field capacity). The genotypes were mentioned in *Table S1*. The soil field capacity was measured using pressure membrane chamber apparatus (Gugino et al., 2009).

### *Measurement of plant growth and other physiological parameters*

The root length (RL) and shoot length (SL) were measured using centimeter scale. Fresh seedling weight (FST) and dry seedling weight (DST) determined using electronic weight balance. The Relative water content (RWC) was measured using following *Equation 1*:

$$RWC = \frac{(FW - DW)}{TW - DW} \qquad \text{(Eq.1)}$$

where TW in the plant turgid weight obtained at the beginning of the dehydration process, FW is the sample fresh weight and DW is the plant dry weight obtained.

Cell membrane thermo-stability (CMT), carotenoid contents, chlorophyll a and b were measured from 3 weeks old barley seedlings in normal and water deficit environments. The cell membrane thermo-stability (CMT) was calculated by the procedures which were given by Blum and Ebercon (1981). To measure leaf osmotic potential (OP) a comparison of psychometric and pressure chamber techniques was used (Turner, 1981). The chlorophyll a, b and total carotenoid content were measured by using the following formulas (Röbbelen, 1957; Lichtenthaler and Wellburn, 1983):

$$Chl\ a\ (\frac{\mathrm{mg}}{\mathrm{g}}) = [12.7 \times (\mathrm{OD663}) - 2.69 \times (\mathrm{OD645})] \times \frac{\mathrm{V}}{1000} \times \mathrm{W} \qquad \text{(Eq.2)}$$

$$Chl\ b\ \left(\frac{\mathrm{mg}}{\mathrm{g}}\right) = [22.9 \times (\mathrm{OD645}) - 4.68 \times (\mathrm{OD663})]\ \times \mathrm{V}/1000 \times \mathrm{W} \qquad \text{(Eq.3)}$$

where V, volume of extract; W, weight of fresh leaves; OD, optimal density.

The total carotenoid content was calculated by using following formula:

$$\mathrm{Carotenoids} = \mathrm{Acar}/\mathrm{EM} \times 100 \qquad \text{(Eq.4)}$$

$$\mathrm{Acar}\ = [(\mathrm{OD480}) + 0.114 \times (\mathrm{OD663})] - 0.638 \times (\mathrm{OD645}), \mathrm{Em} = 2500 \qquad \text{(Eq.5)}$$

where the unit of the chlorophyll a, b and carotenoids is mg $g^{-1}$ fresh weight.

The relative cell injury % was calculated with the formula:

$$\text{Relative Cell Injury \%}\ =\ 1\ -\ (1\ -\ \mathrm{T1}/\mathrm{T2})/\ (1\ -\ \mathrm{C1}/\mathrm{C2})\ \times\ 100 \qquad \text{(Eq.6)}$$

where T1 = conductivity reading at 49 °C of treated sample, T2 = conductivity reading at 120 °C of treated sample, C1 = conductivity reading at room temperature of control sample, and C2 = conductivity reading at 120 °C of control sample.

### *Statistical analysis*

All physiological data were exposed to analysis of variance (ANOVA) technique (Steel and Torrie, 1981) by using statistix 8.1. Post-hoc mean comparison test (Tukey’s HSD) following the ANOVA for each key traits were conducted to highlighting statistically significant differences among genotypes (Tukey, 1949). Reduction percentages were performed to conclude the performance of genotypes based on the seedling traits in normal and water deficit conditions. The reduction percentage (RP) for each trait was calculated using the formula:

$$\begin{aligned} &Reduction\ Perecntage \\ &= [\left(\begin{matrix} Values\ in\ normal\ conditions\ - \\ Values\ in\ drought\ conditions \end{matrix} \middle| Values\ in\ normal\ conditions\right)] \times 100 \end{aligned} \qquad \text{(Eq.7)}$$

which represents the relative decrease in trait performance due to water deficit stress. Those characters displayed significant differences between studied genotypes were further investigated for phenotypic and genotypic correlation analyses (Falconer, 1996). Highly significant effects were classified as α= 0.05, whereas significant effect was classified as α= 0.01. Principal component analysis biplot, heritability, and genetic advance (Jolliffe, 2002; Yadesa, 2022) calculations were performed using R studio 4.1.3 (Team, 2010) with the support of the metan and agricolae packages (Mendiburu, 2019; Olivoto and Lúcio, 2020).

## Results

### *Analysis of variance*

The results of ANOVA for various characters under stress and non-stress (normal) environments are presented in *Table 1*. The analysis of variance (ANOVA) revealed significant mean squares for genotypes, environment, and their interaction. Furthermore, the variance analysis of traits for the 95 genotypes demonstrated significant differences in the mean squares of genotypes for all studied attributes. This indicates the presence of genotypic variability among the studied genotypes for the measured traits, as shown in *Table 1*.

***Table 1.*** *Mean sum of square (MSS) of studied traits obtained from Analysis of variance of 95 genotypes under normal and water deficit environment*

| Sources of variations | ENV | GET | ENV*GET | Error | Total |
|---|---|---|---|---|---|
| **DF** | 1 | 94 | 94 | 378 | 569 |
| **CMT** | 800.50$^{**}$ | 91.30$^{**}$ | 201.00$^{**}$ | 3.52 | |
| **DST** | 1.92$^{**}$ | 1.08$^{**}$ | 0.85$^{**}$ | 0.76 | |
| **FST** | 16.81$^{**}$ | 26.13$^{**}$ | 21.45$^{**}$ | 1.34 | |
| **RI** | 4193.10$^{**}$ | 90.24$^{**}$ | 300.01$^{**}$ | 13.44 | |
| **RL** | 591.08$^{**}$ | 166.39$^{**}$ | 77.03$^{**}$ | 6.34 | |
| **SL** | 217.44$^{**}$ | 44.30$^{**}$ | 16.48$^{**}$ | 2.04 | |
| **RSR** | 0.30$^{**}$ | 0.14$^{**}$ | 0.02$^{**}$ | 0.01 | |
| **RWC** | 1321.80$^{**}$ | 408.48$^{**}$ | 312.09$^{**}$ | 15.13 | |
| **CT** | 0.98$^{**}$ | 0.73$^{**}$ | 0.74$^{**}$ | 0.19 | |
| **OP** | 75.11$^{**}$ | 45.11$^{**}$ | 23.87$^{**}$ | 1.98 | |
| **Chl a** | 0.10$^{**}$ | 0.06$^{**}$ | 0.07$^{**}$ | 0.02 | |
| **Chl b** | 0.69$^{**}$ | 0.52$^{**}$ | 0.49$^{**}$ | 0.21 | |

Abbreviation: DF=degree of freedom, ENV=environments, GET=genotypes, ENV*GET=environment into genotype interaction, RL=root length, SL=shoot length, RSR=root/shoot ratio, FST=fresh seedling weight, DST=dry seedling weight, RWC= relative water content, CMT=cell membrane thermo- stability, RI=Relative Injury, CT=carotenoids, OP=osmotic potential, chl a=chlorophyll a, chl b=chlorophyll b

### *Performance of genotypes in studied traits under normal and water deficit conditions*

By using reduction percentage, the performance of genotypes in normal and water deficit environments were screened. The genotypes had low value of reduction percentage showed their best performance in both environments while the genotypes that had maximum value of reduction percentage have worst performance in both the environments as showed in *Table S2* and *S3*. Percentage reduction in each yield variable for each environment was calculated under the intercepts of descriptive model. *Table S2* and *S3* presents the results of Tukey’s Honest Significant Difference (HSD) test, applied to the reduction percentages of various seedling traits across 95 barley genotypes under drought stress. The table assigns grouping letters (e.g., 'a', 'b', 'c', etc.) to each genotype for every trait based on the mean separation under normal and water deficit conditions and calculation reduction percentage. Genotypes sharing the same letter are not significantly different from one another at the 5% significance level, while those with different letters differ significantly in their response. For each trait, the genotypes are ranked from the lowest to highest reduction percentage, and Tukey groupings are provided accordingly. Genotypes assigned letters earlier in the alphabet (e.g., 'a', 'ab', 'abc') typically show lower reductions and considered as drought tolerant. While those grouped with letters later in the alphabet (e.g., 'd', 'e') exhibit higher reductions, depending on the trait and categorized as drought susceptible genotypes. We selected three drought-tolerant genotypes and three susceptible genotypes based on this analysis, as presented in *Table 2,* which is discussed later under the heading of each trait. This multiple comparison test allows for the clear identification of statistically distinct groups.

***Table 2.*** *Performance of genotypes on the basis of Reduction Percentage*

| **Traits** | **Best Performing Genotypes** | **Worst Performing Genotypes** |
|---|---|---|
| Root length | G61 (-63.64), G1 (-61.21) and G25 (-55.34) | G5 (53.16), G16 (49.35) and G77 (46.00) |
| Shoot length | G61(-10.04), G1(8.34) and G25(10.09) | G77(50.69), G5(48.28) and G16(46.35) |
| Root/shoot ratio | G61(-98.35), G25(-92.68) and G1(-97.78) | G05(34.01), G16(35.60) and G77(44.18) |
| Fresh seedling weight | G1(14.73), G61(25.0) and G25(26.25) | G77(43.63), G5(42.27) and G16(44.16) |
| Dry seedling weight | G61(4.23), G25(9.77) and G1(10.51) | G77(62.19), G5(59.38) and G16(61.51) |
| Relative water content | G1(11.60), G25(10.13) and G61(10.21) | G5(15.66), G16 (15.24) and G77(17.87) |
| Cell membrane thermostability | G1(14.23), G25(14.42) and G61(14.42) | G77(13.15), G5(18.47) and G16(18.80) |
| Relative injury | G61(13.61), G1(13.32) and G25(13.32) | G5 (17.54), G16 (17.20) and G77 (16.91) |
| Osmotic potential | G61(-64.49), G1(-61.92) and G25(-57.37) | G5(-8.68), G16(-22.18) and G77(-11.96) |
| Carotenoids | G1(15.41), G25(15.63) and G61(15.85) | G77 (23.44), G5(23.44) and G16(22.96) |
| Chlorophyll a | G61(0.85), G1(0.86) and G25(0.86) | G5(2.81), G16(1.00) and G77(1.02) |
| Chlorophyll b | G1(10.71), G25(10.82) and G61(10.82) | G77(14.76), G5(14.76) and G16(14.66) |

### *Root length*

The genotypes G61 (-63.64), G1 (-61.21) and G25 (-55.34) had low value of reduction percentage in RL trait. So, the genotypes G61, G1 and G25 had desirable root length in normal and water deficit conditions. The genotypes G5 (53.16), G16 (49.35) and G77 (46.00) had high value of reduction percentage in RL trait, so it means that these genotypes had poor performance for root length in the stress condition as represented in *Table 2*. The standard deviation (SD) of this trait was observed as ±4.51 under normal conditions while in water deficit stress conditions the SD ±2.64 value was recorded. The mean value for the root length ranging from 3.67 to 25 cm with grand mean 11.74 cm under the non-stress condition. The mean value ranging from 0.98 cm to 17.98 cm under the stress condition with grand mean 8.38 cm as mentioned in the *Table 3*.

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***Table 3.*** *Estimation of heritability and genetic advance among barley seedling traits*

| Traits | Maximum | Minimum | Average | SD | Heritability | GA | GA% |
|---|---|---|---|---|---|---|---|
| **RL(N)** | 25 | 3.67 | 11.7474 | 4.51 | 0.786 | 7.3137 | 62.2581 |
| **RL(WD)** | 17.98 | 0.98 | 8.38 | 2.64 | 0.3783 | 2.2764 | 27.1605 |
| **SL(N)** | 30 | 13 | 22.56 | 3.1 | 0.8518 | 5.566 | 24.6719 |
| **SL(WD)** | 26.02 | 9.185 | 17.7873 | 2.8 | 0.5197 | 3.335 | 18.7493 |
| **RSR(N)** | 0.95 | 0.16 | 0.5231 | 0.19 | 0.75 | 0.3043 | 58.1695 |
| **RSR(WD)** | 0.9423 | 0.0524 | 0.4767 | 0.13 | 0.3361 | 0.1068 | 22.4041 |
| **FST(N)** | 1.11 | 0.72 | 0.9412 | 0.05 | 0.8 | 0.0903 | 9.5937 |
| **FST(WD)** | 1.15 | 0.43 | 0.5968 | 0.06 | 0.5962 | 0.0886 | 14.847 |
| **DST(N)** | 0.505 | 0.105 | 0.2645 | 0.07 | 0.8372 | 0.1131 | 42.767 |
| **DST(WD)** | 0.29 | 0.085 | 0.1516 | 0.02 | 0.3333 | 0.0238 | 15.6996 |
| **RWC(N)** | 85 | 60 | 71.2637 | 4.62 | 0.7606 | 7.268 | 10.1987 |
| **RWC(WD)** | 75.45 | 50.45 | 61.64 | 4.25 | 0.4548 | 4.339 | 7.0393 |
| **CMT(N)** | 77 | 54 | 64.2386 | 4.18 | 0.7986 | 6.8969 | 10.7364 |
| **CMT(D)** | 66.47 | 43.47 | 53.6454 | 3.9 | 0.5021 | 4.3243 | 8.0609 |
| **RI(N)** | 78.909 | 55.909 | 66.2096 | 4.16 | 0.7965 | 6.8436 | 10.3363 |
| **RI(WD)** | 68.929 | 45.929 | 56.1668 | 3.87 | 0.4969 | 4.2573 | 7.5797 |
| **OP(N)** | 2.18 | 1.6 | 1.8787 | 0.12 | 0.6144 | 0.1565 | 8.3301 |
| **OP(WD)** | 2.92 | 2.14 | 2.6042 | 0.13 | 0.4125 | 0.1362 | 5.2301 |
| **CT(N)** | 0.49 | 0.3 | 0.4078 | 0.04 | 0.6875 | 0.0566 | 13.8809 |
| **CT(WD)** | 0.415 | 0.225 | 0.3323 | 0.03 | 0.375 | 0.0309 | 9.2999 |
| **Chl a (N)** | 1.66 | 1.35 | 1.4877 | 0.07 | 0.75 | 0.1156 | 7.7706 |
| **Chl a (WD)** | 1.646 | 1.336 | 1.4723 | 0.06 | 0.5185 | 0.0785 | 5.3319 |
| **Chl b (N)** | 0.66 | 0.45 | 0.5417 | 0.04 | 0.6875 | 0.0566 | 10.4479 |
| **Chl b (WD)** | 0.5911 | 0.3811 | 0.4724 | 0.03 | 8.1989 | 0.4 | 6.753 |

RL=root length, SL=shoot length, RSR=root/shoot ratio, FST=fresh seedling weight, DST=dry seedling weight, RWC= relative water content, CMT=cell membrane thermo- stability, RI=Relative Injury, CT=carotenoids, OP=osmotic potential, chl a=chlorophyll a, chl b=chlorophyll b, SD= standard deviation, GA= genetic advance, N=normal, WD=water deficit

### *Shoot length*

The genotypes G61 (-10.04), G1(8.34) and G25(10.09) had less value of reduction percentage as compared to all other genotypes in SL trait while the genotypes G77(50.69), G5(48.28) and G16(46.35) had maximum value of reduction percentage. The standard deviation (SD) of this trait was observed as ±3.1 under normal conditions while in water deficit stress conditions the SD ±2.80 value was recorded. The genotypes G61, G1 and G25 performed best and genotypes G5, G16 and G77 had worst performing ability in SL trait as seen in *Table 2*. The trait shoot length having minimum value 13 cm and maximum value 30 cm with the mean 22.56 cm under the normal environment while shoot length ranging from 9.18 cm to 26.02 cm with the mean 17. 78 cm under the water deficit environment as shown in *Table 3*.

### *Root/shoot ratio*

The standard deviation (SD) of this trait was observed as ±0.19 under normal conditions while in water deficit stress conditions the SD ±0.13 value was recorded as shown in *Table 3*. The minimum value of RSR was 0.16 and maximum value was 0.95. The root shoot length had grand mean 0.52 under the normal condition. Under the stress condition, the minimum value was 0.05 and maximum value was 0.94 with grand mean 0.47 as shown in *Table 3*. The genotypes G61 (-98.35), G25(-92.68) and G1(-97.78) had low value of reduction percentage in R/S trait and had the ability to perform better. The genotypes G05(34.01), G16(35.60) and G77(44.18) had high value of reduction percentage in R/S traits so these genotypes did not perform best under the stress condition.

### *Fresh seedling weight*

The genotypes G1(14.73), G61(25.0) and G25(26.25) had minimum value of reduction percentage in FST trait. These genotypes had best performing ability in this trait, while the genotypes G77(43.63), G5(42.27) and G16(44.16) had maximum value of reduction percentage and having low ability to perform in water deficit environment as mentioned in *Table 2*. The standard deviation (SD) of this trait was observed as ±0.05 under normal conditions while in water deficit stress conditions the SD ±0.06 value was recorded. For fresh seed weight, the minimum and maximum value recorded was 0.72 and 1.11 having grand mean value 0.94 under the normal condition while under water deficit condition, the recorded values were 0.43 and 1.15 with grand mean 0.59 as displayed in *Table 3*.

### *Dry seedling weight*

The genotypes G61(4.23), G25(9.77) and G1(10.51) had low value of reduction percentage in DST trait. These genotypes had best performing ability as compared to other genotypes. The standard deviation (SD) of this trait was observed as ±0.07 under normal conditions while in water deficit stress conditions the SD ±0.02 value was recorded as displayed in *Table 3*. The genotypes G77(62.19), G5(59.38) and G16(61.51) had maximum value of reduction percentage and performed worst in this trait. The mean value observed for dry seed weight ranging from 0.10 to 0.50 and grand mean was 0.26 under the non-stress condition. The mean values ranging from 0.08 to 0.29 having grand mean value 0.15 under the water deficit condition as observed in *Table 3*.

### *Relative water content*

The standard deviation (SD) of this trait was observed as ±4.62 under normal conditions while in water deficit stress conditions the SD ±4.25 value was recorded. In RWC trait, the genotypes G1(11.60), G25(10.13) and G61(10.21) had lowest value of reduction percentage and performed best in both the environments. In comparison with the best performing genotypes, the genotypes G5(15.66), G16 (15.24) and G77(17.87) had high value of reduction percentage and had low ability to performed in water deficit environment as showed in *Table 2*. Relative water content had minimum value 60 and maximum value 85 and grand mean value was 71.26 under the normal environment while under stress condition, it is ranging from 50.45 to 75.45 and having grand mean 61.64 as shown in *Table 3*.

### *Cell-membrane thermostability*

The genotypes G1(14.23), G25(14.42) and G61(14.42) had lowest reduction percentage and genotypes G77(13.15), G5(18.47) and G16(18.80) had highest reduction percentage in CMT trait as mentioned *Table 2*. So, the genotypes G01, G25 and G61 had best performing ability and genotypes G5, G16 and G77 had low ability to perform. The standard deviation (SD) of this trait was observed as ±3.90 under normal conditions while in water deficit stress conditions the SD ±4.25 value was recorded. The minimum and maximum value observed for this trait was 54 and 77 with grand mean 64.23 under the normal condition while it ranges from 43.47 to 66.47 having grand mean 53.64 as mentioned in *Table3*.

### *Relative injury*

The genotypes G61(13.61), G1(13.32) and G25(13.32) showed minimum reduction percentage as compared to the other genotypes while the genotypes G5 (17.54), G16 (17.20) and G77 (16.91) had maximum value for the reduction percentage as shown in *Table 2*. The standard deviation (SD) of this trait was observed as ±4.61under normal conditions while in water deficit stress conditions the SD ±3.87 value was recorded as mentioned in *Table 3*. Therefore, the genotypes G61, G1 and G25 were considered as water deficit tolerant while genotypes G5, G16 and G77 were included in the water deficit susceptible genotypes. The grand mean value recorded for this trait was 66.20 and minimum & maximum value was 55.90 and 78.90 in the normal condition while in stress condition, the values recorded were 45.92 and 68.98 with grand mean 56.16 as expressed in *Table 3*.

### *Osmotic potential*

The genotypes G61(-64.49), G1(-61.92) and G25(-57.37) had minimum value of reduction percentage in OP trait and showed the best performance. On the other hand, the genotypes G5(-8.68), G16(-22.18) and G77(-11.96) had the maximum value of reduction percentage and their performance were worst in water deficit environment as mentioned in *Table 2*. The standard deviation (SD) of this trait was observed as ±0.12 under normal conditions while in water deficit stress conditions the SD ±0.13 value was recorded. The minimum, maximum and grand mean values exhibited for osmotic potential was 1.6, 2.18 and 1.87 under the normal condition while in stress condition, the values were 2.14, 2.92 and 2.60 as showed in *Table 3*.

### *Carotenoid*

The standard deviation (SD) of this trait was observed as ±0.04 under normal conditions while in water deficit stress conditions the SD ±0.03 value was recorded as shown in *Table 3*. In carotenoid trait, the genotypes G1(15.41), G25(15.63) and G61(15.85) had lowest values of reduction percentage and performed best in the normal and water deficit conditions. The genotypes G77 (23.44), G5(23.44) and G16(22.96) had highest value of reduction percentage and didn’t perform best in water deficit environment. The mean value for this attribute was ranging from 0.3 to 0.49 and grand mean value was 0.40 under the non-stress condition while it is ranging from 0.22 to 0.41 and having grand mean 0.33 as mentioned in *Table 3*.

### *Chlorophyll A*

The genotypes G61(0.85), G1(0.86) and G25(0.86) had low reduction percentage in chl a trait as compared to other genotypes. The genotypes G61, G1 and G25 had the ability to perform best in both normal and stresses condition. The genotypes G5(2.81), G16(1.00) and G77(1.02) had highest value of reduction percentage in chl a and performed worst as shown in *Table 2*. The standard deviation (SD) of this trait was observed as ±0.07 under normal conditions while in water deficit stress conditions the SD ±0.06 value was recorded. Under the normal environment, the minimum value 1.35, maximum value 1.66 and grand mean value 1.48 was recorded. Under water deficit condition, the values were 1.33, 1.64 and 1.48 as shown in *Table 3*.

### *Chlorophyll b*

The standard deviation (SD) of this trait was observed as ±0.04 under normal conditions while in water deficit stress conditions the SD ±0.03 value was recorded as shown in *Table 3*. The genotypes G1(10.71), G25(10.82) and G61(10.82) had minimum value of reduction percentage in chl b trait, and these genotypes performed best in both normal and water deficit conditions, while the genotypes G77(14.76), G5(14.76) and G16(14.66) had maximum value of reduction percentage and these performed worst in water deficit environment as shown in *Table 2*. The chlorophyll B had the minimum value 0.45, maximum value 0.66 and gram mean value 0.54 under the normal condition while in water deficit condition, the values for this was 0.3, 0.5 and 0.47 as displayed in *Table 3*.

## ***Heritability and genetic advance***

In plant breeding, heritability is the estimation of phenotypic variability. It indicates the transfer of attributes from parents to its progeny. In particular traits expression, the information provided by heritability about the degree of genetic control and breeding value is predicted by the phenotypic reliability. When there is sufficient variability that is present in the population it shows only the broad sense heritability. The broad sense heritability for root length in both normal and water deficit condition was (70%) which is high heritability value and (37%) which is moderate value of heritability respectively. The broad sense heritability exhibited for the shoot length is high (85%) and moderate (51%) in normal and water deficit condition respectively as showed in *Table 3*. The heritability for RSR had revealed the result in normal and water deficit condition was (75%) which was high heritability value and (30%) which was moderate value of heritability. The broad sense heritability for FST exhibited the results under normal and water deficit condition was (80%) which was high value of heritability and (60%) moderate value of heritability. The broad sense heritability exhibited for DST was high (83%) and (33%) which is moderate revealed in normal and water deficit condition respectively. The heritability indices in RWC were high (76%) in normal condition and moderate in water deficit condition (45%) followed by 95 genotypes. The cell membrane thermo-stability (CMT) and relative injury (RI) had heritability value (80%) and (79%) which was high heritability value under normal condition while in water deficit condition (50%) and (49%) was moderate heritability as reported in Table 3. The result observed in the study that the heritability was high for OP and CT was (61%) and 68% under normal condition while in water deficit condition, heritability was moderate (41%) and (37%). The heritability for chl a and chl b was high in normal condition was (75%) and (68%) while in water deficit condition heritability value was (51%) and (81%).

The important selection parameter through which the degree of target traits improved is genetic advance. The genetic advance % exhibited in root length was high in RL was 62% followed by the 95 genotypes under normal condition and under stress condition RL 27% was high. The genetic advance percentage was high (24%) in SL (shoot length) and 58% in RSR (root shoot ratio) under normal condition while under stress condition, the result had shown that genetic advance percentage in SL (18%) was moderate and in RSR (22%) was high. The FST (fresh seedling weight) and DST (dry seeding weight) observed the genetic advance mean percentage was 9.59% which was low and 42.7% was high in normal condition while in water deficit condition FST and DST revealed the results of genetic advance mean percentage was moderate followed 14.84% and 15.69%. The RWC (relative water content) exhibited the results of genetic advance mean percentage was 10.19% moderate under non stress condition while RWC observed (7.03%) which was low under stress condition. The genetic advance mean percentage exhibited for CMT was moderate (10.73%) in normal condition while in water deficit its percentage was low (8.06%). The OP exhibited genetic advance mean percentage was low (8.33%) and (5.23%) both in normal and water deficit condition. The genetic advance mean percentage was moderate for CT (13%) in normal condition while under stress condition its percentage was low (9%). The genetic advance mean % of chl b was low (7%) and (5.3%) under stress and non- stress condition. The chl b had moderate genetic advance percentage in normal condition while its percentage was low (6.7%) under water deficit condition. The highest genetic advance mean percentage in all the traits was found in RL 62% under normal condition and the lowest genetic advance mean percentage was found in OP 5.23% under water deficit condition as showed in *Table 3*.

### *Genotypic/phenotypic correlation*

In the present investigation, correlation analyses were conducted among seedling traits under both normal and water-deficit conditions across 95 barley genotypes, as presented in *Table 4*. Phenotypic correlation coefficients are indicated above the diagonal, while genotypic correlation coefficients are provided below the diagonal. The root and shoot length showed the positive and highly significant correlation at phenotypic level while positive and non- significant association at genotypic level. The root length was associated positive and highly significant with RSR, FST, DST, RWC, CMT, RI, OP, CT, chl a and chl b at both phenotypic and genotypic level in the non- stressed condition as seen in Table 4, but the root length association with OP was non-significant under water deficit condition. The genotypic correlation revealed that root length was positive and highly significantly correlated with all the traits under the water deficit environment.

The shoot length reported negative relationship with RSR but highly significant and negative correlation with chl a while showed non-significant association in the normal condition at phenotypic level. In the stressed condition, the SL trait showed negative correlation with the RSR trait and show non-significant association at phenotypic level. The other traits were positively and highly significant in non-stressed condition at phenotypic level. The shoot length mentioned negative correlation with RSR and chl a and non-significant results in the normal environment at genotypic level. The shoot length was significant and negatively correlated with RSR trait under water deficit environment at genotypic level. The SL relationship with FST, RWC, CMT, RI, OP, CT and chl a were highly significant while non-significant with RSR and chl b at genotypic and phenotypic level in stress environment as mentioned *Table 4*.

The observed study showed that the RSR trait was highly significant and positive correlate with all the studied traits in both normal and water deficit environments at phenotypic and genotypic level, but only one trait OP showed negative and non-significant value with RSR in water deficit condition at phenotypic level as shown in *Table 4*.

***Table 4.*** *Correlation coefficient among the seedling attributes under normal and water deficit condition*

| Traits | Envi | RL | SL | RSR | FST | DST | RWC | CMT | RI | OP | CT | Chl a | Chl b |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **RL** | N | | $0.19^{**}$ | $0.93^{**}$ | $0.64^{**}$ | $0.71^{**}$ | $0.62^{**}$ | $0.58^{**}$ | $0.58^{**}$ | $0.55^{**}$ | $0.56^{**}$ | $0.50^{**}$ | $0.59^{**}$ |
| | WD | | $0.29^{**}$ | $0.86^{**}$ | $0.59^{**}$ | $0.44^{**}$ | $0.52^{**}$ | $0.55^{**}$ | $0.55^{**}$ | $0.08^{ns}$ | $0.44^{**}$ | $0.45^{**}$ | $0.56^{**}$ |
| **SL** | N | $0.19^{ns}$ | | $-0.15^{**}$ | $0.06^{ns}$ | $0.04^{ns}$ | $0.10^{ns}$ | $0.07^{ns}$ | $0.07^{ns}$ | $0.06^{ns}$ | $0.07^{ns}$ | $-0.06^{ns}$ | $0.06^{ns}$ |
| | WD | $0.39^{**}$ | | $-0.21^{ns}$ | $0.20^{**}$ | $0.11^{ns}$ | $0.18^{**}$ | $0.19^{**}$ | $0.18^{**}$ | $0.18^{**}$ | $0.22^{**}$ | $0.12^{ns}$ | $0.17^{**}$ |
| **RSR** | N | $0.93^{**}$ | $-0.14^{ns}$ | | $0.59^{**}$ | $0.67^{**}$ | $0.55^{**}$ | $0.53^{**}$ | $0.53^{**}$ | $0.52^{**}$ | $0.52^{**}$ | $0.51^{**}$ | $0.54^{**}$ |
| | WD | $0.78^{**}$ | $-0.26^{*}$ | | $0.49^{**}$ | $0.39^{**}$ | $0.42^{**}$ | $0.46^{**}$ | $0.49^{**}$ | $-0.02^{ns}$ | $0.33^{**}$ | $0.40^{**}$ | $0.48^{**}$ |
| **FST** | N | $0.82^{**}$ | $0.08^{ns}$ | $0.77^{**}$ | | $0.93^{**}$ | $0.68^{**}$ | $0.64^{**}$ | $0.64^{**}$ | $0.66^{**}$ | $0.62^{**}$ | $0.62^{**}$ | $0.68^{**}$ |
| | WD | $0.85^{**}$ | $0.35^{**}$ | $0.66^{**}$ | | $0.60^{**}$ | $0.66^{**}$ | $0.64^{**}$ | $0.64^{*}*$ | $0.10^{ns}$ | $0.61^{**}$ | $0.61^{**}$ | $0.67^{**}$ |
| **DST** | N | $0.88^{**}$ | $0.07^{ns}$ | $0.83^{**}$ | $0.95^{**}$ | | $0.69^{**}$ | $0.64^{**}$ | $0.64^{**}$ | $0.68^{**}$ | $0.60^{**}$ | $0.61^{**}$ | $0.69^{**}$ |
| | WD | $0.63^{**}$ | $0.26^{*}$ | $0.47^{**}$ | $1.02^{**}$ | | $0.53^{**}$ | $0.56^{**}$ | $0.55^{**}$ | $0.03^{ns}$ | $0.46^{**}$ | $0.51^{**}$ | $0.58^{**}$ |
| **RWC** | N | $0.77^{**}$ | $0.14^{ns}$ | $0.69^{**}$ | $0.86^{**}$ | $0.84^{**}$ | | $0.55^{**}$ | $0.55^{**}$ | $0.59^{**}$ | $0.52^{**}$ | $0.47^{**}$ | $0.60^{**}$ |
| | WD | $0.71^{**}$ | $0.39^{**}$ | $0.48^{**}$ | $0.88^{**}$ | $0.93^{**}$ | | $0.55^{**}$ | $0.55^{**}$ | $-0.02^{ns}$ | $0.52^{**}$ | $0.45^{**}$ | $0.59^{**}$ |
| **CMT** | N | $0.76^{**}$ | $0.08^{ns}$ | $0.71^{**}$ | $0.82^{**}$ | $0.81^{**}$ | $0.73^{**}$ | | $0.99^{**}$ | $0.46^{**}$ | $0.47^{**}$ | $0.56^{**}$ | $0.59^{**}$ |
| | WD | $0.74^{**}$ | $0.39^{**}$ | $0.53^{**}$ | $0.85^{**}$ | $0.74^{**}$ | $0.71^{**}$ | | $0.99^{**}$ | $0.12^{*}$ | $0.47^{**}$ | $0.55^{**}$ | $0.59^{**}$ |
| **RI** | N | $0.76^{**}$ | $0.08^{ns}$ | $0.71^{**}$ | $0.81^{**}$ | $0.80^{**}$ | $0.74^{**}$ | $0.10^{**}$ | | $0.46^{**}$ | $0.47^{**}$ | $0.56^{**}$ | $0.59^{**}$ |
| | WD | $0.74^{**}$ | $0.40^{**}$ | $0.53^{**}$ | $0.85^{**}$ | $0.74^{**}$ | $0.72^{**}$ | $0.99^{**}$ | | $0.11^{*}$ | $0.47^{**}$ | $0.55^{**}$ | $0.59^{**}$ |
| **OP** | N | $0.79^{**}$ | $0.10^{ns}$ | $0.75^{**}$ | $0.93^{**}$ | $0.93^{**}$ | $0.83^{**}$ | $0.71^{**}$ | $0.71^{**}$ | | $0.58^{**}$ | $0.56^{**}$ | $0.62^{**}$ |
| | WD | $0.40^{**}$ | $0.27^{**}$ | $0.29^{**}$ | $0.26^{*}$ | $0.16^{ns}$ | $0.11^{ns}$ | $0.41^{**}$ | $0.40^{**}$ | | $0.11^{*}$ | $0.10^{ns}$ | $0.02^{ns}$ |
| **CT** | N | $0.72^{**}$ | $0.1^{ns}$ | $0.67^{**}$ | $0.87^{**}$ | $0.81^{**}$ | $0.77^{**}$ | $0.66^{**}$ | $0.66^{**}$ | $0.85^{**}$ | | $0.48^{**}$ | $0.51^{**}$ |
| | WD | $0.59^{**}$ | $0.47^{**}$ | $0.33^{**}$ | $0.82^{**}$ | $0.71^{**}$ | $0.74^{**}$ | $0.72^{**}$ | $0.72^{**}$ | $0.28^{**}$ | | $0.48^{**}$ | $0.50^{**}$ |
| **Chl a** | N | $0.66^{**}$ | $-0.09^{ns}$ | $0.69^{**}$ | $0.79^{**}$ | $0.76^{**}$ | $0.63^{**}$ | $0.73^{**}$ | $0.73^{**}$ | $0.81^{**}$ | $0.68^{**}$ | | $0.59^{**}$ |
| | WD | $0.53^{**}$ | $0.24^{*}$ | $0.42^{**}$ | $0.76^{**}$ | $0.72^{**}$ | $0.58^{**}$ | $0.72^{**}$ | $0.72^{**}$ | $0.25^{*}$ | $0.59^{**}$ | | $0.57^{**}$ |
| **Chl b** | N | $0.76^{**}$ | $0.07^{ns}$ | $0.71^{**}$ | $0.92^{**}$ | $0.90^{**}$ | $0.81^{**}$ | $0.77^{**}$ | $0.77^{**}$ | $0.95^{**}$ | $0.75^{**}$ | $0.79^{**}$ | |
| | WD | $0.65^{**}$ | $0.41^{**}$ | $0.39^{**}$ | $0.93^{**}$ | $0.90^{**}$ | $0.80^{**}$ | $0.77^{**}$ | $0.78^{**}$ | $0.24^{*}$ | $0.71^{**}$ | $0.75^{**}$ | |

Abbreviation: RL=root length, SL=shoot length, RSR=root/shoot ratio, FST=fresh seedling weight, DST=dry seedling weight, RWC= relative water content, CMT=cell membrane thermo- stability, RI=Relative Injury, CT=carotenoids, OP=osmotic potential, chl a=chlorophyll a, chl b=chlorophyll b, N=normal, WD=water deficit. Above diagonal phenotypic correlations while, below diagonal is genotypic correlation

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The fresh seed weight was positive and highly significant associated with all the studied traits in the stressed and non-stressed conditions at both phenotypic and genotypic correlation, but the association between the FST and OP was negative and non-significant in the stressed condition at phenotypic level. The dry seed weight results showed that all the traits were positively and highly significant in both the normal and water deficit environments at both genotypic and phenotypic levels. The traits OP showed non-significant value with DST in the water deficit condition at both genotypic and phenotypic levels as displayed in *Table 4*.

RWC reported the positive and highly significant correlation with all studied attributes in both normal and water deficit conditions at both phenotypic and genotypic levels. The trait RWC had negative correlation with OP and had non-significant association at phenotypic level while positive and non-significant association at genotypic level in the water deficit environment. CMT had the positive correlation with all traits like RI, CT, OP, chl a, chl b, DST, FST, RWC, RSR and RL and all these traits were highly significant in both normal and water deficit condition at the phenotypic and genotypic level as mentioned in *Table 4*. The correlation between the SL and CMT was positive, but the SL had non-significant association in the normal condition while highly significant in the water deficit condition at both phenotypic and genotypic correlation as expressed in *Table 4*. The same results as CMT has also been observed in the trait RI. All studied indices were highly significant and showed positive correlation with RL except SL and OP in both stressed and non-stressed environments at genotypic and phenotypic environments. The RI correlation was positive and highly significant in the water deficit stress level but in normal condition the association was positive and non-significant at both phenotypic and genotypic environments.

The RSR and RWC had negative and non-significant association in the water deficit condition at the phenotypic level. The trait DST and RWC were non-significant and had positive correlation in the water deficit condition at genotypic level. The chl a and chl b had positive and significant association in the water deficit condition at genotypic level as shown in *Table 4*. The association of carotenoids and OP trait was significant in the water deficit condition at phenotypic level. But SL trait had positive and non-significant association in the normal condition at both phenotypic and genotypic levels. Carotenoids had the positive and highly significant correlation with all studied traits in normal and water deficit conditions at both phenotypic and genotypic correlation as shown in *Table 4*. The relation between the chl a, and shoot length was negative in the normal condition at phenotypic level and had non-significant correlation in both normal and water deficit environments at genotypic and phenotypic levels. The trait OP correlated non-significantly in the water deficit condition at the phenotypic level as displayed in *Table 4*. The chl b correlated positively and highly significantly with all the indices in normal and water deficit environments at phenotypic and genotypic levels while SL traits had positive but non-significant association in normal environment at both phenotypic and genotypic levels as represented in *Table 4*.

### *Biplot analysis of 95 barley genotypes under normal and water deficit conditions*

The biplot analyses in *Figures 1A and 1B* effectively illustrate the distribution and variability of 95 barley genotypes based on seedling traits under both normal and water deficit conditions. The first two principal components (PC1 and PC2) capture a significant proportion of the total variation, with red vectors representing the direction and strength of association of each trait. The axes, F1 and F2, represent the first and second principal components, respectively, capturing 55.73% and 10.25% of the total variance in the data under normal conditions. The axes, F1 and F2, represent the first and second principal components, respectively, capturing 50.17% and 11.46% of the total variance in the data under water deficit stress.

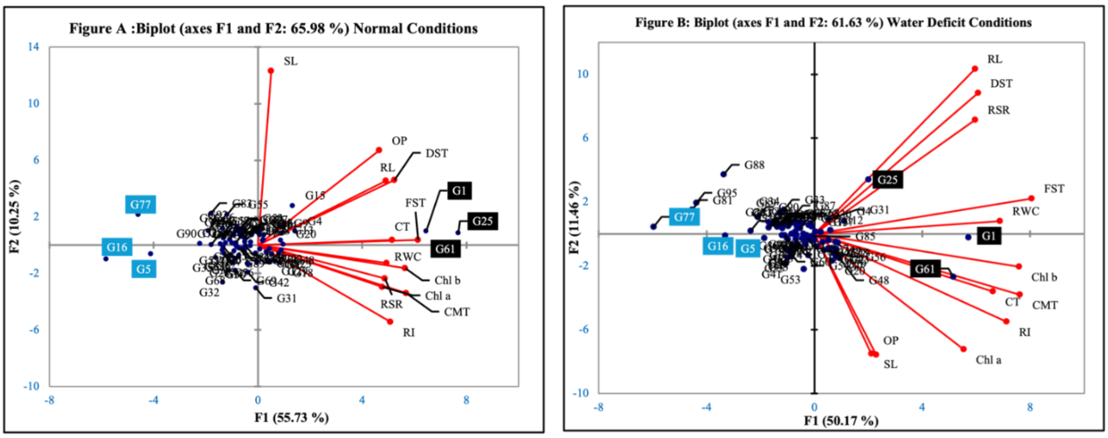

*__Figure 1.__ Biplot analysis showing the distribution of 95 barley genotypes based on seedling traits under (A) normal and (B) water deficit conditions. Abbreviation: RL=root length, SL=shoot length, RSR=root/shoot ratio, FST=fresh seedling weight, DST=dry seedling weight, RWC= relative water content, CMT=cell membrane thermo- stability, RI=Relative Injury, CT=carotenoids, OP=osmotic potential, chl a=chlorophyll a, chl b=chlorophyll b*

Under normal conditions (*Figure 1A*), genotypes G1, G25, and G61 are positioned on the right side of the plot, aligning with vectors of key traits such as root length (RL), shoot length (SL), relative water content (RWC), cell membrane thermo-stability (CMT), and chlorophyll a and b (chl a, chl b). These associations suggest these genotypes express favorable physiological and biochemical traits even under non-stressful environments. Conversely, G5, G16, and G77, located on the far left, show poor associations with most traits, indicating weak baseline seedling performance and supporting their identification as water deficit susceptible genotypes. Under water deficit conditions (*Figure 1B*), the same tolerant genotypes (G1, G25, and G61) again cluster in the positive quadrant, closely aligned with vectors representing favorable water deficit-responsive traits such as RL, SL, fresh and dry seedling weight (FST, DST), carotenoids (CT), and CMT. Their positioning confirms superior seedling vigor and stress-adaptive traits under water-deficit stress. In contrast, G5, G16, and G77 are located on the far left, away from most trait vectors, indicating poor expression of water deficit-associated traits and reaffirming their susceptibility. Additionally, trait interrelationships are consistent across both environments. Traits like RL, SL, RWC, chl a, chl b, CT, and CMT are positively correlated and contribute significantly to genotype differentiation, while Relative Injury (RI) and Osmotic Potential (OP) are negatively associated with water deficit tolerance, pointing in opposite directions to key adaptive traits. A central cluster of genotypes in both biplots (under normal and water deficit) indicates moderate performance, with limited distinctiveness in either stress adaptation or trait expression.

## Discussion

The ANOVA results revealed significant genotypic variability across all studied traits in both stress and non-stress environments, indicating the influence of genetic differences. Additionally, the significant genotype-environment interaction suggests that genotypic performance varied across conditions, highlighting the need for evaluation under diverse environments. Previously study evaluated 426 barley genotypes under deficit and full irrigation, identifying significant interactions between genotypes and water regimes (Boudiar et al., 2020; Manju et al., 2023). The artificial water stress stopped the cell division of the root length, and it decreased under low water stress and growth led to tuberization. The process of tube formation and the lignification of the root system allowed the situation to become useful again (Jalilian and Mohsennia, 2013). The findings from the Tukey HSD test revealed clear statistical groupings among the 95 barley genotypes based on their reduction percentages under drought stress, enabling the identification of tolerant and susceptible genotypes. Such stratification supports targeted selection in breeding programs and aligns with previous studies (Jalilian and Mohsennia, 2013; Ijaz et al., 2023) that emphasized the utility of multiple comparison tests in differentiating drought responses among cereals. These results reinforce the role of early seedling traits as reliable indicators for screening drought tolerance. In our study, reduction percentage for shoot length recorded to determine the drought tolerance. The similar findings were also reported by Manju et al. (2023), and Boudiar et al. (2020). Many scientists reported that water deficit condition had effect the plant dry mass and they found reducing level of dry weight at seedling stage (Boudiar et al., 2020; Fatemi et al., 2023). According to the present study, there was deficient in relative water content observed in the water deficit condition. Similarly, the more loss in relative water contents under less water condition made sensitive barley genotype in comparison to barley tolerant genotypes. The decrease in moisture of soil was a significant disastrous factor in arid and semi-arid regions that cause decrease in water potential of leaf, causes the decreased in turgor and, ultimately, lower crop production (Jalilian and Mohsennia, 2013). Breeders examined those barley varieties with Higher relative water content was more repellent to water deficit condition. So, they considered that osmoregulation conserve the turgor pressure in plants was one of important mechanisms to protect the plants from the loss of water and reserve from metabolic activities and continue to absorb (Manju et al., 2023; Alsamadany et al., 2024). These results represented that genotypes that were tolerant to water deficit had the higher thermos-stability in cell membrane that eventually rose up the potential of genotypes to survive after the water deficit and heat-shock applications. Thus, CMT assays might be a good selection criterion at seedling stage for water deficit and high temperature tolerance that save up the cost and time consumption for field experiments. Under the water deficit stress, the relative water content and cell membrane thermo-stability can also be considered important selection criteria for barley (Jalilian and Mohsennia, 2013; Manju et al., 2023). When the increase in water deficit occur, thylakoid membrane was responsible for reduction in photosynthetic pigments and cell dehydration in barley (Song et al., 2024).

Previously the study found significant genetic variations among eight barley genotypes in response to different irrigation treatments, indicating that water deficit conditions affect growth differently across varieties. Giza 138, Giza 131, and Line 4 showed high performance under stress (Jalilian and Mohsennia, 2013; Manju et al., 2023). The similar findings were also being examined by the researcher (Ashraf et al., 2022; Slawin et al., 2024). Photosynthesis contains low concentration of the water culture medium which was the basic part of the plant cell. The photosynthesis mechanisms will be more effective if chlorophyll contents were present in higher amount. Previously reported that genetic variation among barley genotypes significantly influences their growth under water deficit conditions, as evidenced by the superior performance of the G15 genotype in water deficit tolerance, alongside higher chlorophyll content and carotenoids in G3 and G15 compared to others (Fatemi et al., 2022; Saidi et al., 2024).

In the current study, the highest value heritability ($h^2$) obtained for the traits shoot length, dry seed weight, chlorophyll b and fresh seed weight. The moderate value of heritability for the traits were fresh seed weight, shoot length and chlorophyll b. In the current study, no one trait showed the low value of heritability. The genetic advance for the studied traits showed the RL had high value of GA%, SL in the non-stressed condition. The moderate value of genetic advance showed by the traits were like RWC and carotenoid under the normal condition. The trait that exhibited the lowest percentage of genetic advance was FST in the normal environment. Under the stressed condition, the highest genetic advance percentage exhibited by the trait was root length. The moderate value obtained by the trait chl b. While the trait OP had lowest value of genetic advance. Barley's growth is significantly influenced by water deficit conditions, and these responses vary among different genotypes (Munns et al., 2010; Song et al., 2024). Heritability estimates are influenced by both genetic and environmental variances. Under optimal (non-stress) conditions, environmental variation tends to be lower and more uniform, which allows genetic differences among genotypes to explain a larger proportion of the total phenotypic variance—resulting in higher heritability estimates. In contrast, under water deficit stress, environmental variability often increases due to inconsistent stress exposure across replicates or micro-environmental effects. This inflates the environmental variance component, thereby reducing the proportion of total variance attributable to genetic differences and leading to lower heritability. This pattern has been reported in other studies as well (Munns et al., 2010; Song et al., 2024), where stress environments increase non-genetic variability and thus reduce heritability estimates. Nonetheless, traits that maintain moderate heritability under stress (e.g., root length in our study) still provide valuable targets for selection in drought-prone environments.

A researcher stated that genetic advance and heritability estimates were normally more helpful in predicting the gain under selection (Munns et al., 2010; Song et al., 2024). The heritability due to the additive gene effect is usually related to broad heritability and genetic advance values that help in appropriate selection. The value of high heritability along with the low genetic advance value indicated the epistatic gene effect which is not so appropriate for the trait selection. Low heritability is accompanied by high genetic advancement reveals that the character is governed by the additive gene effect. The low heritability is being exhibited due to high environmental effects and selection may be affected in such cases. Low heritability accompanied with low genetic advance indicated the character is highly influenced by the environmental effects and selection would be in-effective (Manju et al., 2023).

In the current study, the shoot length only showed highly significant and positive association with root length while non-significant and positive with all the traits at both levels. The current research represents similar results with the scientist Song et al. (2024), and Munns et al. (2010). They concluded that shoot length had non-significant association with root length, dry weight, shoot length, relative injury, fresh weight, cell membrane thermo-stability and chlorophyll a and b. The breeders observed in an experiment that under normal and water deficit conditions the association were positive and highly significant between fresh shoot weight and dry shoot (Munns et al., 2010; Abdelrady et al., 2024). The current findings were similar with the results of Kumar et al. (2022). On the basis of association with the other attributes, the earlier experiments supported the similar results revealing that cell membrane thermo-stability of seedling of the barley plants was the more important trait followed by root length, fresh weight and dry weight (Munns et al., 2010; Abdelrady et al., 2024). The results of Abdelrady et al. (2024), and Munns et al. (2010) also support the current findings with similar results, whereby their correlation coefficients depicted that root length showed significant and highly positive correlation with the fresh weight and dry weight under water deficit conditions. The previously study demonstrated that barley varieties exhibit genotypic differences in response to water deficit, with low-stomatal-density genotypes like Hindmarsh showing less biomass reduction and greater drought tolerance compared to high-stomatal-density genotypes, indicating significant genetic variability in drought tolerance (Kumar et al., 2022).

Earlier researches also revealed the similar results, indicating that the cell membrane thermo-stability of the barley at the seedling stage was the most important trait, followed by root length, fresh weight, and dry weight, on the basis of their relationships with other traits (Alsamadany et al., 2024). The correlation of RWC with OP was non-significant. Plant breeders studied and revealed physiological traits as indicators of water deficit tolerance, and they analyzed that those genotypes that contained high concentrations of RWC and CMT had more potential against water deficit conditions than the genotypes having low percentage, and vice versa (Manju et al., 2023). The higher the relative water content which causes resistant mechanism in water deficit conditions. It can be the result of low elasticity of cell wall tissue or high osmotic regulation (Jalilian and Mohsennia, 2013; Song et al., 2024). In the water deficit conditions there was reduction the in the levels of chlorophyll and carotenoids level and was accounted as a typical sign of oxidative stress and may be the result of pigment photo-oxidation chlorophyll degradation (Alsamadany et al., 2024). These findings collectively underscore the importance of genetic diversity in barley for developing drought-tolerant varieties, highlighting specific genotypes and traits that contribute to improved growth and yield under water-limited conditions.

The biplot analysis under both normal and water deficit conditions provides a comprehensive multivariate view of the relationships among seedling traits and genotypic responses in barley, offering critical insight into stress tolerance mechanisms (Kendal et al., 2019; Wang et al., 2024). The consistent clustering of genotypes like G1, G25, and G61 under both conditions reflects their genetic stability and adaptive potential. This pattern supports previous findings by Elakhdar et al. (2023), who reported that genotypes with high root and shoot growth, chlorophyll retention, and stable membrane structures tend to exhibit superior water deficit resilience. Traits like root length (RL) and shoot length (SL) are critical for water uptake and biomass accumulation under limited moisture, and their strong loading in PC1 reinforces their importance in early-stage drought screening.

The positive associations among traits such as RWC, carotenoids, and CMT highlight their interconnected roles in mitigating drought stress. Relative water content (RWC) is a direct indicator of cellular hydration status, while carotenoids (CT) and chlorophyll content protect photosynthetic machinery under oxidative stress, as supported by Kendal et al. (2019), and Baloch et al. (2024). The alignment of these traits with drought-tolerant genotypes affirms their utility in phenotypic selection strategies. Moreover, the clustering of a majority of genotypes near the biplot origin, under both normal and water deficit conditions, implies limited variation or intermediate expression of key traits. This may reflect either insufficient genetic divergence or moderate adaptive capacity, underscoring the need for more refined selection criteria or targeted breeding for stress-specific traits. Overall, the PCA-biplot approach has proven effective in distinguishing genotypes with contrasting water deficit responses and identifying the most relevant physiological traits for water deficit tolerance screening in barley. These findings can guide future breeding efforts aimed at improving early vigor and resilience in water-limited environments.

## Conclusions

In this study, 95 genotypes were observed in response to water deficit stress. We found the root length, shoot length, root-shoot length, fresh seed weight, dry seed weight, relative injury, cell membrane thermo-stability, relative water injury, osmotic potentials, carotenoids, chlorophyll a and chlorophyll b were deferentially influenced by water deficit condition and showed positive and negative correlations among the physiological traits. The observed attributes showed that highly significant difference present between all the traits. The root length had low value of reduction percentage and had best performing ability. The genotypes G1, G25 and G61 had maximum ability to perform best in water deficit stress and become water deficit tolerant genotypes. The genotypes G5, G16 and G77 had low ability to perform under the stress condition and considered as worst performing genotypes. The biplot analyses further confirmed the classification consistency across environments. Genotypes G1, G25, and G61 exhibited strong positive associations with key seedling traits, reinforcing their potential as resilient lines for breeding programs. In contrast, G5, G16, and G77 were associated with unfavorable trait profiles, validating their susceptibility to drought. The several attributes like root length, fresh seed weight, dry seed weight, relative water content, relative injury, cell membrane thermos-stability, carotenoids and chlorophyll b associate positively with each other. So, by selecting water deficit-tolerant barley lines the breeders should minimized the severity of water deficit stress.

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## APPENDIX

***Table S1.*** *List of 95 barley genotypes used in the experiment*

| Code | Genotype Name | Code | Genotype Name | Code | Genotype Name | Code | Genotype Name |
|---|---|---|---|---|---|---|---|
| G1 | Peral-21 | G25 | Sultan-17 | G49 | IU-21C1 | G73 | TJ-70 |
| G2 | Clipper*Sultan | G26 | Frontier-87 | G50 | IU-21C2 | G74 | BW-22C5 |
| G3 | Peral-21 | G27 | PB-2999 | G51 | IU-21C3 | G75 | BW-22C6 |
| G4 | IC-062189 | G28 | IB-22 | G52 | IU-21C4 | G76 | BW-22C7 |
| G5 | EC-663751 | G29 | BW-57 | G53 | Haider-93 | G77 | Clipper |
| G6 | Haider-93 | G30 | IB22-1 | G54 | JAU-83 | G78 | Frontier-87 |
| G7 | 9031 | G31 | IU-22-1 | G55 | IU-21C7 | G79 | FAC1B |
| G8 | 8421 | G32 | IB22-2 | G56 | IU-21C8 | G80 | FAC11B |
| G9 | Sheikh-634 | G33 | IU-22-2 | G57 | IU-21C9 | G81 | 864C |
| G10 | IU-21C5 | G34 | IB22-3 | G58 | Sanober-96 | G82 | IB-21C |
| G11 | IU-21C6 | G35 | IU-22-3 | G59 | Rakhshan-10 | G83 | FAC21B |
| G12 | IB21*Pearl21 | G36 | IB22-4 | G60 | DL-36 | G84 | FAC03C |
| G13 | IU-21 | G37 | IU-22-4 | G61 | Jau-21 | G85 | 8312 |
| G14 | Jau-E-Paghambri | G38 | JAU-87 | G62 | 9587-21 | G86 | 942 |
| G15 | FAC19B | G39 | Sheikh-638 | G63 | 1733-21 | G87 | 7421 |
| G16 | FAC9B | G40 | Aanaj-2013 | G64 | L862 | G88 | Jau-17 |
| G17 | Talbina-21 | G41 | FA-23 | G65 | L5291 | G89 | Bajwar-2000 |
| G18 | BW-22C3 | G42 | FAC1 | G66 | L7329 | G90 | 7302 |
| G19 | BW-22C4 | G43 | FA-96 | G67 | L257 | G91 | 8021 |
| G20 | Sadabhar | G44 | FAE-578 | G68 | L3763*C1 | G92 | 84302 |
| G21 | 7403 | G45 | Super1 | G69 | L453*BC | G93 | 7920 |
| G22 | 8402 | G46 | JAU-22C | G70 | BW-22C1 | G94 | 9314 |
| G23 | Barley Dwarf | G47 | Kisan-22C | G71 | BW-22C2 | G95 | BW24C |
| G24 | Clipper | G48 | IBM-21 | G72 | Neelum | | |

***Table S2.*** *Normal and drought mean values along with reduction percentages for seedling traits (RL, SL, RSR, FST, DST, RWC) in 95 barley genotypes. Letters (a, b, c, etc.) represent groupings based on Tukey’s test, indicating statistically significant differences among genotypes*

| GET | RL-N | RL-WD | R% | SL-N | SL-WD | R% | RSR-N | RSR-WD | R% | FST-N | FST-WD | R% | DST-N | DST-WD | R% | RWC-N | RWC-WD | R% |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| G1 | 20.61 | 33.23 | -61.21a | 20.61 | 18.9 | 8.34a | 0.41 | 0.79 | -92.68a | 1.06 | 0.91 | 14.73a | 0.49 | 0.44 | 10.51a | 82.33 | 74.34 | 9.71a |
| G2 | 15.67 | 11.65 | 25.68bc | 23.17 | 19.19 | 17.18bc | 0.91 | 0.6 | 34.01de | 0.96 | 0.61 | 36.33b | 0.18 | 0.11 | 40.19c | 68.93 | 59.38 | 13.86cd |
| G3 | 9 | 6.66 | 26c | 23.39 | 16.67 | 28.74d | 0.39 | 0.4 | -1.24bc | 0.86 | 0.51 | 40.54d | 0.18 | 0.11 | 36.45bc | 64.67 | 55.12 | 14.77de |
| G4 | 17.67 | 13.99 | 20.83b | 24 | 20.68 | 13.83ab | 0.74 | 0.68 | 7.63bc | 0.98 | 0.63 | 35.9b | 0.32 | 0.15 | 53.4de | 72.67 | 63.12 | 13.14bc |
| G5 | 18.5 | 8.67 | 53.16e | 26.84 | 13.88 | 48.28e | 0.69 | 0.44 | 36.09e | 0.97 | 0.56 | 42.27e | 0.32 | 0.13 | 59.38e | 61 | 51.45 | 15.66e |
| G6 | 20.5 | 12 | 41.46d | 22.11 | 18.13 | 18c | 0.93 | 0.66 | 28.65de | 1.07 | 0.75 | 29.91b | 0.46 | 0.27 | 40.73c | 82.33 | 71.22 | 13.5c |
| G7 | 14 | 9.99 | 28.62c | 23.44 | 19.46 | 16.98bc | 0.6 | 0.51 | 14.41c | 0.93 | 0.58 | 37.84cd | 0.34 | 0.15 | 56.8e | 71.33 | 61.78 | 13.39bc |
| G8 | 12 | 8.66 | 27.83c | 25.84 | 20.52 | 20.59c | 0.46 | 0.42 | 8.84bc | 0.94 | 0.59 | 37.23c | 0.26 | 0.13 | 48.37cd | 66.33 | 56.78 | 14.4de |
| G9 | 13.11 | 10 | 23.72bc | 26.17 | 22.51 | 13.96b | 0.5 | 0.44 | 11.15c | 0.94 | 0.59 | 37.23c | 0.28 | 0.13 | 53.53de | 70 | 60.45 | 13.64c |
| G10 | 15 | 11.32 | 24.53bc | 25 | 21.02 | 15.92bc | 0.6 | 0.54 | 10.2c | 0.94 | 0.59 | 37.23c | 0.26 | 0.13 | 48.37d | 71 | 61.45 | 13.45bc |
| G11 | 23 | 15.33 | 33.33cd | 25.5 | 21.52 | 15.61b | 0.9 | 0.71 | 21.01d | 1.11 | 0.81 | 26.81ab | 0.42 | 0.21 | 49d | 79 | 69.45 | 12.09b |
| G12 | 14.33 | 10.65 | 25.67bc | 21.5 | 17.52 | 18.51c | 0.67 | 0.61 | 8.79bc | 0.96 | 0.61 | 36.59bc | 0.27 | 0.15 | 45.4c | 67.93 | 58.38 | 14.06d |
| G13 | 13.67 | 10.32 | 24.49bc | 24.34 | 18.46 | 24.15cd | 0.56 | 0.56 | 1.02bc | 0.94 | 0.59 | 37.1bc | 0.26 | 0.14 | 47.74cd | 74.26 | 64.71 | 12.86b |
| G14 | 14.33 | 10.32 | 28c | 27 | 23.02 | 14.74b | 0.53 | 0.45 | 15.55cd | 0.93 | 0.58 | 37.5c | 0.25 | 0.15 | 38.26bc | 72.59 | 63.04 | 13.16bc |
| G15 | 14 | 10.99 | 21.52bc | 20.84 | 16.86 | 19.1c | 0.67 | 0.65 | 3.01bc | 0.96 | 0.61 | 36.46b | 0.28 | 0.14 | 47.88cd | 64.93 | 55.38 | 14.71de |
| G16 | 22.67 | 11.48 | 49.35e | 25.67 | 13.77 | 46.35e | 0.89 | 0.57 | 35.6e | 1.06 | 0.59 | 44.16e | 0.44 | 0.17 | 61.51e | 81.67 | 69.22 | 15.24e |
| G17 | 13 | 10.66 | 18b | 20.5 | 16.52 | 19.41c | 0.63 | 0.65 | -1.7b | 0.91 | 0.56 | 38.32d | 0.23 | 0.15 | 34.31b | 68.62 | 59.07 | 13.92cd |
| G18 | 13 | 9.65 | 25.74bc | 20 | 16.02 | 19.9c | 0.65 | 0.6 | 7.18bc | 0.93 | 0.58 | 37.5c | 0.25 | 0.14 | 42.95c | 70.62 | 61.07 | 13.52c |
| G19 | 9.67 | 8.67 | 10.34b | 23 | 19.02 | 17.3bc | 0.42 | 0.46 | -8.53b | 0.94 | 0.59 | 37.23c | 0.26 | 0.15 | 41.18c | 68.59 | 59.04 | 13.92cd |
| G20 | 12.33 | 8.31 | 32.59cd | 23 | 19.02 | 17.3bc | 0.54 | 0.44 | 18.51cd | 0.97 | 0.62 | 36.21b | 0.28 | 0.14 | 51.48d | 69.93 | 60.38 | 13.66c |
| G21 | 20 | 12 | 40d | 21 | 17.02 | 18.95c | 0.95 | 0.71 | 26d | 1.05 | 0.76 | 27.39ab | 0.43 | 0.22 | 48.24cd | 81 | 70.56 | 12.89b |
| G22 | 13.67 | 9.65 | 29.41c | 24.17 | 20.19 | 16.47bc | 0.57 | 0.48 | 15.55cd | 0.95 | 0.6 | 36.84bc | 0.27 | 0.15 | 44.03cd | 72.31 | 62.76 | 13.21bc |
| G23 | 11.67 | 8.65 | 25.83c | 22.33 | 18.35 | 17.82c | 0.52 | 0.47 | 9.74bc | 0.95 | 0.6 | 36.71bc | 0.27 | 0.15 | 45.96c | 74.26 | 64.71 | 12.86b |
| G24 | 12 | 8.33 | 30.61c | 23.84 | 19.86 | 16.7bc | 0.5 | 0.42 | 16.66cd | 0.96 | 0.61 | 36.33b | 0.28 | 0.14 | 48.5d | 67.93 | 58.38 | 14.06d |
| G25 | 13.67 | 21.23 | -55.34a | 22.5 | 20.23 | 10.09a | 0.45 | 0.89 | -97.78a | 0.91 | 0.67 | 26.25a | 0.22 | 0.2 | 9.77a | 71.59 | 64.34 | 10.13a |
| G26 | 21.94 | 14 | 36.2d | 23.67 | 19.69 | 16.82bc | 0.93 | 0.71 | 23.2d | 1.05 | 0.74 | 29.75ab | 0.41 | 0.21 | 49.38d | 79.67 | 70.12 | 11.99b |

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| GET | RL-N | RL-WD | R% | SL-N | SL-WD | R% | RSR-N | RSR-WD | R% | FST-N | FST-WD | R% | DST-N | DST-WD | R% | RWC-N | RWC-WD | R% |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| G27 | 6 | 7.33 | -22.22ab | 23.34 | 19.36 | 17.06bc | 0.26 | 0.38 | -47.59a | 0.94 | 0.59 | 37.23c | 0.26 | 0.17 | 32.68b | 71.93 | 62.38 | 13.28bc |
| G28 | 9.67 | 6.99 | 27.66c | 18.84 | 14.86 | 21.13c | 0.51 | 0.47 | 8.15bc | 0.9 | 0.55 | 38.75d | 0.22 | 0.17 | 23.66a | 66.67 | 57.12 | 14.33d |
| G29 | 12.33 | 8.31 | 32.59cd | 19.33 | 15.35 | 20.59c | 0.64 | 0.54 | 15.12c | 0.92 | 0.57 | 38.04cd | 0.24 | 0.14 | 40.43c | 73 | 63.45 | 13.08bc |
| G30 | 13.33 | 10.32 | 22.6bc | 19.33 | 15.35 | 20.59c | 0.69 | 0.67 | 2.53bc | 0.94 | 0.59 | 37.37c | 0.25 | 0.13 | 47.02cd | 70 | 60.45 | 13.64c |
| G31 | 5 | 7.66 | -53.2ab | 14.34 | 11.35 | 20.83c | 0.94 | 0.92 | 1.66bc | 0.94 | 0.59 | 37.1bc | 0.26 | 0.16 | 38.06bc | 68.59 | 59.04 | 13.92cd |
| G32 | 5 | 6.33 | -26.67ab | 13.17 | 11.51 | 12.56ab | 0.38 | 0.56 | -48.09a | 0.96 | 0.61 | 36.59bc | 0.27 | 0.13 | 53.37de | 69.93 | 60.38 | 13.66c |
| G33 | 8 | 7.33 | 8.33b | 17.67 | 14.35 | 18.79c | 0.45 | 0.51 | -12.91b | 0.94 | 0.59 | 37.37c | 0.25 | 0.15 | 42.38c | 70.93 | 61.38 | 13.46bc |
| G34 | 11 | 6.98 | 36.55d | 16.84 | 12.86 | 23.64cd | 0.65 | 0.54 | 16.87cd | 0.94 | 0.59 | 37.23c | 0.26 | 0.15 | 41.83c | 70.33 | 60.78 | 13.58c |
| G35 | 9.67 | 7.66 | 20.76b | 20.5 | 16.52 | 19.41c | 0.47 | 0.46 | 1.76bc | 0.92 | 0.57 | 38.18cd | 0.23 | 0.15 | 36.69bc | 67.33 | 57.78 | 14.18d |
| G36 | 8 | 5.33 | 33.42cd | 25.17 | 18.85 | 25.11d | 0.32 | 0.3 | 6.01bc | 0.94 | 0.59 | 37.37c | 0.25 | 0.14 | 44.37cd | 67.26 | 57.71 | 14.2d |
| G37 | 9.33 | 5.99 | 35.79cd | 22.17 | 17.46 | 21.24c | 0.42 | 0.34 | 18.66cd | 0.92 | 0.57 | 38.04cd | 0.24 | 0.13 | 45.39c | 72.31 | 62.76 | 13.21bc |
| G38 | 9 | 6.32 | 29.78c | 21.67 | 16.9 | 21.99cd | 0.42 | 0.38 | 7.89bc | 0.91 | 0.56 | 38.6d | 0.22 | 0.16 | 29.32a | 66.93 | 57.38 | 14.27d |
| G39 | 21.67 | 12.67 | 41.54d | 23 | 17.79 | 22.65cd | 0.94 | 0.72 | 23.65d | 1.05 | 0.74 | 29.75ab | 0.41 | 0.2 | 50.62d | 80 | 70.45 | 11.94b |
| G40 | 10 | 5.99 | 40.13d | 23 | 19.02 | 17.3bc | 0.43 | 0.31 | 27.64d | 0.92 | 0.57 | 38.04cd | 0.24 | 0.13 | 45.39c | 72.62 | 63.07 | 13.15bc |
| G41 | 6.67 | 3.99 | 40.2d | 23 | 19.02 | 17.3bc | 0.29 | 0.21 | 28.1de | 0.96 | 0.61 | 36.46b | 0.28 | 0.14 | 50.3d | 69.95 | 60.4 | 13.65c |
| G42 | 9.67 | 5.99 | 38.07d | 18.67 | 14.69 | 21.32c | 0.52 | 0.41 | 21.17d | 0.92 | 0.57 | 37.91cd | 0.24 | 0.16 | 34.27b | 68.59 | 59.04 | 13.92cd |
| G43 | 8 | 6.33 | 20.92bc | 22.17 | 18.19 | 17.95c | 0.36 | 0.35 | 3.68bc | 0.94 | 0.59 | 37.1bc | 0.26 | 0.11 | 58.71e | 69.93 | 60.38 | 13.66c |
| G44 | 20 | 12.99 | 35.03cd | 21.84 | 17.86 | 18.23c | 0.92 | 0.73 | 20.58cd | 1.06 | 0.71 | 32.81b | 0.38 | 0.2 | 45.78c | 80.33 | 70.78 | 11.89a |
| G45 | 9 | 5.99 | 33.41cd | 20.5 | 16.52 | 19.41c | 0.44 | 0.36 | 17.31cd | 0.96 | 0.61 | 36.46b | 0.28 | 0.15 | 47.27cd | 73.98 | 64.43 | 12.91b |
| G46 | 11.67 | 7.65 | 34.46cd | 23 | 19.02 | 17.3bc | 0.51 | 0.4 | 20.76d | 0.95 | 0.6 | 36.97bc | 0.26 | 0.14 | 46.5cd | 74.26 | 64.71 | 12.86b |
| G47 | 14 | 9.98 | 28.71c | 21.5 | 18.35 | 14.67b | 0.65 | 0.54 | 16.48cd | 0.93 | 0.58 | 37.5c | 0.25 | 0.14 | 45.64c | 67.93 | 58.38 | 14.06d |
| G48 | 9.33 | 5.99 | 35.86cd | 21.5 | 17.52 | 18.51c | 0.43 | 0.34 | 21.33d | 0.95 | 0.6 | 36.71bc | 0.27 | 0.17 | 36.65bc | 73.26 | 63.71 | 13.04bc |
| G49 | 8 | 5.66 | 29.25c | 23.17 | 18.13 | 21.77cd | 0.35 | 0.32 | 8.77bc | 0.96 | 0.61 | 36.46b | 0.28 | 0.13 | 52.73de | 67.93 | 58.38 | 14.06d |
| G50 | 8.67 | 5.33 | 38.54d | 22.5 | 17.62 | 21.67cd | 0.39 | 0.31 | 20.7cd | 0.93 | 0.58 | 37.5c | 0.25 | 0.14 | 44.97cd | 66.93 | 57.38 | 14.27d |
| G51 | 19.67 | 15.65 | 20.44b | 21.33 | 17.35 | 18.66c | 0.92 | 0.9 | 2.33bc | 1.03 | 0.71 | 31.17b | 0.37 | 0.2 | 47.09cd | 79.33 | 69.78 | 12.04b |
| G52 | 9 | 4.98 | 44.67d | 20.17 | 16.19 | 19.73c | 0.45 | 0.31 | 30.89de | 0.92 | 0.57 | 38.04cd | 0.24 | 0.14 | 39.01bc | 70 | 60.45 | 13.64c |
| G53 | 10 | 7.32 | 26.8c | 29.17 | 25.19 | 13.64ab | 0.34 | 0.29 | 14.95c | 0.91 | 0.56 | 38.46d | 0.23 | 0.14 | 39.26bc | 70.28 | 60.73 | 13.59c |
| G54 | 11.67 | 7.65 | 34.46cd | 23.84 | 19.86 | 16.7bc | 0.49 | 0.39 | 21.29d | 0.93 | 0.58 | 37.77cd | 0.24 | 0.15 | 36.55bc | 66.67 | 57.12 | 14.33de |

| GET | RL-N | RL-WD | R% | SL-N | SL-WD | R% | RSR-N | RSR-WD | R% | FST-N | FST-WD | R% | DST-N | DST-WD | R% | RWC-N | RWC-WD | R% |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| G55 | 9 | 6.33 | 29.63c | 28.84 | 24.86 | 13.8ab | 0.31 | 0.26 | 17.63cd | 0.91 | 0.56 | 38.32d | 0.23 | 0.16 | 29.93b | 70.28 | 60.73 | 13.59c |
| G56 | 10.67 | 7.32 | 31.38c | 21.5 | 16.35 | 23.97cd | 0.5 | 0.44 | 10.99c | 0.92 | 0.57 | 38.04cd | 0.24 | 0.16 | 31.91b | 68.59 | 59.04 | 13.92cd |
| G57 | 8.67 | 6.33 | 27c | 26 | 14.33 | 44.88de | 0.33 | 0.48 | -43.2a | 0.92 | 0.57 | 38.18d | 0.23 | 0.15 | 35.25bc | 69.93 | 60.38 | 13.66c |
| G58 | 11.67 | 7.65 | 34.46cd | 24 | 20.02 | 16.58bc | 0.49 | 0.38 | 21.45d | 0.92 | 0.57 | 38.04cd | 0.24 | 0.15 | 34.75bc | 70.93 | 61.38 | 13.46bc |
| G59 | 12.33 | 8.34 | 32.38c | 23.67 | 19.69 | 16.82bc | 0.52 | 0.42 | 18.58cd | 0.92 | 0.57 | 38.04cd | 0.24 | 0.15 | 36.17bc | 75.93 | 66.38 | 12.58b |
| G60 | 8.67 | 4.99 | 42.46d | 26.33 | 22.35 | 15.12b | 0.33 | 0.22 | 32.19de | 0.95 | 0.6 | 36.84bc | 0.27 | 0.16 | 40.88c | 74.26 | 64.71 | 12.86b |
| G61 | 3.67 | 6 | -63.64a | 17.89 | 19.69 | -10.04a | 0.34 | 0.68 | -98.35a | 1.01 | 0.76 | 25a | 0.12 | 0.11 | 4.23a | 79.33 | 71.23 | 10.21a |
| G62 | 10.67 | 7.32 | 31.38c | 20.67 | 16.69 | 19.26c | 0.51 | 0.44 | 14.43c | 0.93 | 0.58 | 37.63c | 0.25 | 0.13 | 47.62cd | 74.59 | 65.04 | 12.8b |
| G63 | 11.67 | 8.32 | 28.69c | 24.5 | 20.52 | 16.24bc | 0.48 | 0.41 | 14.82c | 0.93 | 0.58 | 37.5c | 0.25 | 0.16 | 35.57bc | 70.28 | 60.73 | 13.59c |
| G64 | 15 | 10.98 | 26.8c | 24.34 | 20.36 | 16.36bc | 0.62 | 0.54 | 12.48c | 0.92 | 0.57 | 38.18d | 0.23 | 0.13 | 42.45c | 73.93 | 64.38 | 12.92bc |
| G65 | 8.67 | 6.66 | 23.15bc | 25.5 | 17.95 | 29.61d | 0.34 | 0.37 | -9.15b | 0.94 | 0.59 | 37.1bc | 0.26 | 0.16 | 37.42bc | 70.33 | 60.78 | 13.58c |
| G66 | 10 | 7.66 | 23.4bc | 26.34 | 22.36 | 15.11b | 0.38 | 0.34 | 9.8c | 0.92 | 0.57 | 38.18d | 0.23 | 0.14 | 39.57bc | 72.33 | 62.78 | 13.2bc |
| G67 | 13 | 8.98 | 30.92c | 23.83 | 19.85 | 16.7bc | 0.55 | 0.45 | 17.1cd | 0.95 | 0.6 | 36.71bc | 0.27 | 0.13 | 51.55d | 68.59 | 59.04 | 13.92cd |
| G68 | 8.33 | 6.99 | 16.08b | 18 | 14.02 | 22.11cd | 0.46 | 0.5 | -7.51b | 0.92 | 0.57 | 38.04cd | 0.24 | 0.16 | 33.33b | 69.93 | 60.38 | 13.66cd |
| G69 | 10 | 5.98 | 40.2d | 15.17 | 11.19 | 26.24d | 0.66 | 0.53 | 19cd | 0.91 | 0.56 | 38.46d | 0.23 | 0.15 | 31.85b | 70.93 | 61.38 | 13.46c |
| G70 | 13 | 8.98 | 30.92c | 20.67 | 16.69 | 19.26c | 0.63 | 0.54 | 14.52c | 0.94 | 0.59 | 37.1bc | 0.26 | 0.15 | 43.23c | 72.31 | 62.76 | 13.21bc |
| G71 | 11.33 | 7.31 | 35.47cd | 21.45 | 14.79 | 31.02d | 0.54 | 0.49 | 9.91c | 0.93 | 0.58 | 37.63c | 0.25 | 0.12 | 50.34d | 74.26 | 64.71 | 12.86b |
| G72 | 9.33 | 5.99 | 35.86d | 20 | 16.02 | 19.9c | 0.47 | 0.37 | 19.96cd | 0.92 | 0.57 | 37.91cd | 0.24 | 0.18 | 23.08a | 67.93 | 58.38 | 14.06d |
| G73 | 8 | 6.67 | 16.67b | 20.34 | 16.36 | 19.57c | 0.39 | 0.41 | -3.8b | 0.93 | 0.58 | 37.77cd | 0.24 | 0.17 | 28.28a | 67 | 57.45 | 14.25d |
| G74 | 10.67 | 6.65 | 37.69d | 26 | 19.01 | 26.9d | 0.41 | 0.35 | 14.72c | 0.93 | 0.58 | 37.63c | 0.25 | 0.16 | 33.33b | 68.33 | 58.78 | 13.98d |
| G75 | 9.67 | 5.65 | 41.59d | 24.17 | 20.19 | 16.47bc | 0.4 | 0.28 | 29.67de | 0.94 | 0.59 | 37.37c | 0.25 | 0.16 | 37.75bc | 73.67 | 64.12 | 12.96bc |
| G76 | 10.33 | 6.99 | 32.39cd | 24 | 20.02 | 16.58bc | 0.43 | 0.35 | 18.84cd | 0.91 | 0.56 | 38.6d | 0.22 | 0.18 | 18.8a | 73.28 | 63.73 | 13.03bc |
| G77 | 4.33 | 2.34 | 46e | 24.34 | 12 | 50.69e | 0.16 | 0.09 | 44.18e | 0.86 | 0.49 | 43.63e | 0.34 | 0.13 | 62.19e | 64.67 | 53.11 | 17.87e |
| G78 | 12 | 8.99 | 25.11bc | 21.67 | 18.13 | 16.35bc | 0.56 | 0.5 | 10.8c | 0.93 | 0.58 | 37.84cd | 0.24 | 0.18 | 25a | 69.93 | 60.38 | 13.66cd |
| G79 | 9 | 7.67 | 14.81b | 24 | 16.56 | 30.99d | 0.38 | 0.46 | -23.45b | 0.96 | 0.61 | 36.33b | 0.28 | 0.14 | 50.3d | 70.93 | 61.38 | 13.46c |
| G80 | 12 | 7.98 | 33.5cd | 25.84 | 17.95 | 30.51d | 0.46 | 0.45 | 3.42bc | 0.95 | 0.6 | 36.84bc | 0.27 | 0.17 | 35.22bc | 73.33 | 63.78 | 13.02bc |
| G81 | 5.33 | 6 | -12.5ab | 19.22 | 15.57 | 19c | 0.28 | 0.39 | -38.63b | 0.84 | 0.55 | 34.26b | 0.15 | 0.11 | 27.47a | 63.67 | 54.12 | 15de |
| G82 | 9 | 8 | 11.11b | 23 | 17.68 | 23.13cd | 0.39 | 0.45 | -15.94b | 0.91 | 0.56 | 38.46d | 0.23 | 0.14 | 39.26bc | 74.62 | 65.07 | 12.8b |

| **GET** | **RL-N** | **RL-WD** | **R%** | **SL-N** | **SL-WD** | **R%** | **RSR-N** | **RSR-WD** | **R%** | **FST-N** | **FST-WD** | **R%** | **DST-N** | **DST-WD** | **R%** | **RWC-N** | **RWC-WD** | **R%** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| G83 | 12 | 7.98 | 33.5cd | 25.5 | 21.52 | 15.61b | 0.47 | 0.37 | 21.21d | 0.94 | 0.59 | 37.23c | 0.26 | 0.14 | 45.1c | 68.59 | 59.04 | 13.92d |
| G84 | 10.33 | 8.33 | 19.42b | 21.5 | 15.34 | 28.65d | 0.48 | 0.54 | -12.53b | 0.96 | 0.61 | 36.59bc | 0.27 | 0.13 | 50.92d | 69.93 | 60.38 | 13.66cd |
| G85 | 10 | 5.98 | 40.2d | 26.84 | 17.33 | 35.41de | 0.41 | 0.5 | -21.36b | 0.95 | 0.6 | 36.97bc | 0.26 | 0.16 | 39.49bc | 70.93 | 61.38 | 13.46c |
| G86 | 10.33 | 9 | 12.9b | 27 | 20.01 | 25.9d | 0.38 | 0.45 | -18.86b | 0.94 | 0.59 | 37.37c | 0.25 | 0.14 | 46.36c | 73.98 | 64.43 | 12.91b |
| G87 | 11 | 8.32 | 24.36bc | 20.84 | 16.86 | 19.1c | 0.53 | 0.49 | 6.38bc | 0.94 | 0.59 | 37.23c | 0.26 | 0.14 | 44.44cd | 74.26 | 64.71 | 12.86b |
| G88 | 21.67 | 17.65 | 18.55b | 18.67 | 16 | 14.28b | 0.2 | 0.37 | -90.65a | 0.8 | 0.5 | 37.76c | 0.35 | 0.21 | 40.67c | 63.98 | 54.43 | 14.93de |
| G89 | 8 | 6.33 | 20.92bc | 20.5 | 12 | 41.46de | 0.39 | 0.52 | -33.96b | 0.91 | 0.56 | 38.6d | 0.22 | 0.13 | 39.85bc | 72.28 | 62.73 | 13.21bc |
| G90 | 11 | 8.66 | 21.27bc | 20.67 | 16.69 | 19.26c | 0.53 | 0.52 | 2.3bc | 0.91 | 0.56 | 38.32d | 0.23 | 0.14 | 40.15bc | 67.67 | 58.12 | 14.11d |
| G91 | 11 | 8.32 | 24.36bc | 23.84 | 18.95 | 20.49c | 0.46 | 0.44 | 3.94bc | 0.94 | 0.59 | 37.23c | 0.26 | 0.15 | 43.14c | 68.59 | 59.04 | 13.92d |
| G92 | 12.33 | 8.31 | 32.59cd | 28.84 | 17.33 | 39.89de | 0.43 | 0.48 | -11.56b | 0.91 | 0.56 | 38.46d | 0.23 | 0.13 | 42.96c | 68.26 | 58.71 | 13.99d |
| G93 | 10.67 | 6.65 | 37.69d | 21.5 | 15.68 | 27.07d | 0.5 | 0.42 | 14.78c | 0.91 | 0.56 | 38.32d | 0.23 | 0.16 | 31.39b | 69.93 | 60.38 | 13.66cd |
| G94 | 10 | 7.33 | 26.73c | 26 | 17.67 | 32.05de | 0.38 | 0.42 | -9.33b | 0.92 | 0.57 | 38.04cd | 0.24 | 0.17 | 29.08a | 71 | 61.45 | 13.45bc |
| G95 | 9.33 | 6.43 | 31.11c | 20.22 | 11 | 45.61de | 0.48 | 0.49 | -1.52b | 0.75 | 0.46 | 38.94d | 0.15 | 0.11 | 30.43b | 74 | 64.45 | 12.91bc |

Abbreviation: GET=genotypes, RL=root length, SL=shoot length, RSR=root/shoot ratio, FST=fresh seedling weight, DST=dry seedling weight, RWC= relative water content, CMT=cell membrane thermo- stability, RI=Relative Injury, CT=carotenoids, OP=osmotic potential, chl a=chlorophyll a, chl b=chlorophyll b, Normal, WD=Water Deficit, R%=reduction percentage

***Table S3.*** *Normal and drought mean values along with reduction percentages for seedling traits (CMT, RI, OP, CT, Chl a, Chlb) in 95 barley genotypes. Letters (a, b, c, etc.) represent groupings based on Tukey's test, indicating statistically significant differences among genotypes*

| GET | CMT-N | CMT-WD | R% | RI-N | RI-WD | R% | OP-N | OP-WD | R% | CT-N | CT-WD | R% | chl a- N | hl a-WD | R% | chl b-N | chl b-WD | R% |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| G1 | 74 | 63.47 | 14.23a | 72.03 | 62.05 | 13.85b | 2.16 | 2.75 | -27.28d | 0.49 | 0.41 | 15.41a | 1.43 | 1.41 | 0.98d | 0.64 | 0.57 | 10.71a |
| G2 | 62.33 | 51.8 | 16.89c | 64.24 | 54.26 | 15.53c | 2 | 2.83 | -41.57bc | 0.39 | 0.32 | 19.23cd | 1.54 | 1.53 | 0.91b | 0.51 | 0.44 | 13.42d |
| G3 | 59 | 48.47 | 17.85de | 69.24 | 59.26 | 14.41b | 1.65 | 2.67 | -61.92a | 0.34 | 0.27 | 22.06e | 1.38 | 1.37 | 1.01de | 0.49 | 0.42 | 14.16de |
| G4 | 65.67 | 55.14 | 16.04bc | 67.58 | 57.6 | 14.77bc | 1.91 | 2.67 | -40.21c | 0.44 | 0.36 | 17.18b | 1.4 | 1.39 | 1de | 0.52 | 0.45 | 13.17d |
| G5 | 67.33 | 56.8 | 15.64b | 56.91 | 46.93 | 17.54e | 2.09 | 2.28 | -8.68e | 0.44 | 0.37 | 16.92b | 1.64 | 1.59 | 2.81e | 0.52 | 0.45 | 13.34d |
| G6 | 72.67 | 62.14 | 14.49a | 74.58 | 64.6 | 13.38a | 2.12 | 2.63 | -24.02de | 0.47 | 0.4 | 15.96a | 1.63 | 1.62 | 0.86a | 0.61 | 0.54 | 11.3b |
| G7 | 62 | 51.47 | 16.98cd | 63.91 | 53.93 | 15.62cd | 1.92 | 2.84 | -47.57b | 0.38 | 0.31 | 19.74cd | 1.43 | 1.42 | 0.98d | 0.57 | 0.5 | 12.02b |
| G8 | 66.33 | 55.8 | 15.87bc | 68.24 | 58.26 | 14.62b | 1.82 | 2.45 | -35.05cd | 0.42 | 0.35 | 17.86bc | 1.5 | 1.48 | 0.94bc | 0.57 | 0.5 | 12.16b |
| G9 | 68 | 57.47 | 15.49a | 69.91 | 59.93 | 14.28b | 1.92 | 2.71 | -41.34bc | 0.44 | 0.37 | 17.05b | 1.43 | 1.42 | 0.98d | 0.55 | 0.48 | 12.6bc |
| G10 | 64.33 | 53.8 | 16.37bc | 66.24 | 56.26 | 15.07c | 1.89 | 2.74 | -45.36bc | 0.38 | 0.31 | 19.74d | 1.48 | 1.46 | 0.95bc | 0.54 | 0.47 | 12.68bc |
| G11 | 73 | 62.47 | 14.42a | 74.91 | 64.93 | 13.32a | 2.09 | 2.6 | -24.2de | 0.48 | 0.41 | 15.63a | 1.62 | 1.61 | 0.86a | 0.61 | 0.54 | 11.23b |
| G12 | 67.33 | 56.8 | 15.64b | 69.24 | 59.26 | 14.41b | 1.75 | 2.54 | -44.77bc | 0.38 | 0.31 | 19.74d | 1.53 | 1.52 | 0.91b | 0.54 | 0.47 | 12.76bc |
| G13 | 66.67 | 56.14 | 15.8b | 68.58 | 58.6 | 14.55b | 1.94 | 2.77 | -42.87bc | 0.41 | 0.34 | 18.15bc | 1.5 | 1.48 | 0.94bc | 0.53 | 0.46 | 13.08c |
| G14 | 67 | 56.47 | 15.72b | 68.91 | 58.93 | 14.48b | 1.83 | 2.71 | -48.27b | 0.42 | 0.35 | 17.72bc | 1.45 | 1.43 | 0.97cd | 0.52 | 0.45 | 13.21d |
| G15 | 66 | 55.47 | 15.95bc | 67.91 | 57.93 | 14.7bc | 1.82 | 2.82 | -54.95a | 0.39 | 0.32 | 19.07c | 1.46 | 1.44 | 0.96c | 0.53 | 0.46 | 13.12cd |
| G16 | 73 | 62.47 | 14.42a | 74.91 | 64.93 | 13.32a | 2.11 | 2.73 | -28.94d | 0.46 | 0.39 | 16.19a | 1.63 | 1.62 | 0.86a | 0.64 | 0.57 | 10.82a |
| G17 | 66.67 | 56.14 | 15.8bc | 68.58 | 58.6 | 14.55b | 1.79 | 2.69 | -50.09b | 0.39 | 0.32 | 19.07c | 1.52 | 1.51 | 0.92bc | 0.52 | 0.45 | 13.17d |
| G18 | 67.67 | 57.14 | 15.56b | 69.58 | 59.6 | 14.34b | 1.9 | 2.67 | -40.6c | 0.41 | 0.34 | 18.15bc | 1.58 | 1.56 | 0.89b | 0.52 | 0.45 | 13.25d |
| G19 | 65.33 | 54.8 | 16.12bc | 67.24 | 57.26 | 14.84bc | 1.84 | 2.67 | -45.19bc | 0.44 | 0.37 | 17.05b | 1.59 | 1.57 | 0.88b | 0.55 | 0.48 | 12.53bc |
| G20 | 65 | 54.47 | 16.2bc | 66.91 | 56.93 | 14.92bc | 1.9 | 2.89 | -52.11a | 0.45 | 0.37 | 16.79b | 1.56 | 1.54 | 0.9b | 0.53 | 0.46 | 13.08c |
| G21 | 71.67 | 61.14 | 14.69a | 73.58 | 63.6 | 13.56b | 2.09 | 2.63 | -25.92d | 0.47 | 0.4 | 15.96a | 1.63 | 1.62 | 0.86b | 0.62 | 0.55 | 11.11b |
| G22 | 67.33 | 56.8 | 15.64b | 69.24 | 59.26 | 14.41b | 1.8 | 2.74 | -52.5a | 0.39 | 0.32 | 19.07c | 1.52 | 1.51 | 0.92bc | 0.52 | 0.45 | 13.25d |
| G23 | 65.67 | 55.14 | 16.04bc | 67.58 | 57.6 | 14.77bc | 1.87 | 2.79 | -48.93b | 0.38 | 0.3 | 19.91d | 1.56 | 1.54 | 0.9b | 0.57 | 0.5 | 12.02b |
| G24 | 62 | 51.47 | 16.98cd | 63.91 | 53.93 | 15.62cd | 1.92 | 2.73 | -41.93bc | 0.42 | 0.35 | 17.72bc | 1.47 | 1.45 | 0.95bc | 0.57 | 0.5 | 12.16b |
| G25 | 62.33 | 51.8 | 16.89c | 64.24 | 54.26 | 15.53c | 1.89 | 2.63 | -39.08c | 0.39 | 0.32 | 19.07c | 1.41 | 1.39 | 1de | 0.55 | 0.48 | 12.6bc |
| G26 | 73 | 62.47 | 14.42a | 74.91 | 64.93 | 13.32a | 1.88 | 2.71 | -43.88bc | 0.46 | 0.39 | 16.19a | 1.62 | 1.61 | 0.86b | 0.62 | 0.55 | 11.05b |

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| GET | CMT-N | CMT-WD | R% | RI-N | RI-WD | R% | OP-N | OP-WD | R% | CT-N | CT-WD | R% | chl a- N | hl a-WD | R% | chl b-N | chl b-WD | R% |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| G27 | 63.33 | 52.8 | 16.63cd | 65.24 | 55.26 | 15.3c | 1.9 | 2.59 | -35.81c | 0.37 | 0.3 | 20.09de | 1.51 | 1.49 | 0.93bc | 0.57 | 0.5 | 12.09b |
| G28 | 64.67 | 54.14 | 16.28bc | 66.58 | 56.6 | 14.99c | 1.89 | 2.64 | -39.93c | 0.39 | 0.32 | 19.07c | 1.52 | 1.51 | 0.92bc | 0.54 | 0.47 | 12.76bc |
| G29 | 66.67 | 56.14 | 15.8bc | 68.58 | 58.6 | 14.55b | 1.88 | 2.73 | -45.04bc | 0.47 | 0.39 | 16.07a | 1.46 | 1.44 | 0.96c | 0.52 | 0.45 | 13.17d |
| G30 | 64 | 53.47 | 16.45c | 65.91 | 55.93 | 15.14c | 1.77 | 2.65 | -50.19b | 0.36 | 0.29 | 20.64de | 1.52 | 1.5 | 0.92bc | 0.55 | 0.48 | 12.53bc |
| G31 | 65 | 54.47 | 16.2bc | 66.91 | 56.93 | 14.92bc | 1.86 | 2.81 | -50.81a | 0.38 | 0.31 | 19.74d | 1.56 | 1.54 | 0.9b | 0.53 | 0.46 | 12.92c |
| G32 | 64.33 | 53.8 | 16.37c | 66.24 | 56.26 | 15.07c | 1.82 | 2.63 | -44.6bc | 0.38 | 0.31 | 19.74d | 1.45 | 1.44 | 0.96c | 0.53 | 0.46 | 13c |
| G33 | 60.67 | 50.14 | 17.36d | 62.58 | 52.6 | 15.95d | 1.89 | 2.31 | -22.18e | 0.42 | 0.35 | 17.86bc | 1.45 | 1.44 | 0.96c | 0.54 | 0.47 | 12.88c |
| G34 | 60.33 | 49.8 | 17.45d | 62.24 | 52.26 | 16.03d | 1.82 | 2.5 | -36.84cd | 0.43 | 0.36 | 17.44b | 1.42 | 1.4 | 0.99d | 0.54 | 0.47 | 12.68bc |
| G35 | 64.67 | 54.14 | 16.28bc | 66.58 | 56.6 | 14.99c | 1.79 | 2.6 | -45.52bc | 0.38 | 0.31 | 19.74d | 1.42 | 1.4 | 0.99d | 0.53 | 0.46 | 13.08c |
| G36 | 64 | 53.47 | 16.45c | 65.91 | 55.93 | 15.14c | 1.95 | 2.62 | -34.36cd | 0.42 | 0.35 | 17.86bc | 1.42 | 1.41 | 0.99d | 0.55 | 0.48 | 12.6bc |
| G37 | 66.33 | 55.8 | 15.87bc | 68.24 | 58.26 | 14.62b | 1.89 | 2.57 | -35.48c | 0.38 | 0.31 | 19.74d | 1.42 | 1.41 | 0.98d | 0.56 | 0.49 | 12.38b |
| G38 | 61.33 | 50.8 | 17.17cd | 63.24 | 53.26 | 15.78cd | 1.94 | 2.62 | -34.97cd | 0.41 | 0.34 | 18.15bc | 1.46 | 1.44 | 0.96c | 0.57 | 0.5 | 12.09b |
| G39 | 71 | 60.47 | 14.83a | 72.91 | 62.93 | 13.69b | 2.13 | 2.63 | -23.32e | 0.47 | 0.4 | 15.96a | 1.62 | 1.61 | 0.86b | 0.63 | 0.56 | 10.94a |
| G40 | 66 | 55.47 | 15.95bc | 67.91 | 57.93 | 14.7bc | 1.88 | 2.6 | -38.28cd | 0.39 | 0.32 | 19.07c | 1.47 | 1.46 | 0.95c | 0.52 | 0.45 | 13.25d |
| G41 | 64.33 | 53.8 | 16.37c | 66.24 | 56.26 | 15.07c | 1.9 | 2.77 | -45.88b | 0.4 | 0.33 | 18.6c | 1.5 | 1.49 | 0.93bc | 0.51 | 0.44 | 13.64de |
| G42 | 68 | 57.47 | 15.49b | 69.91 | 59.93 | 14.28b | 1.81 | 2.39 | -31.62cd | 0.39 | 0.32 | 19.07c | 1.51 | 1.5 | 0.93bc | 0.53 | 0.46 | 13.12cd |
| G43 | 64 | 53.47 | 16.45c | 65.91 | 55.93 | 15.14c | 1.89 | 2.64 | -39.93c | 0.41 | 0.34 | 18.15c | 1.55 | 1.53 | 0.91bc | 0.52 | 0.45 | 13.29d |
| G44 | 72 | 61.47 | 14.63a | 73.91 | 63.93 | 13.5a | 2.12 | 2.67 | -26.1d | 0.46 | 0.39 | 16.3b | 1.64 | 1.62 | 0.86b | 0.63 | 0.56 | 10.94b |
| G45 | 61.33 | 50.8 | 17.17cd | 63.24 | 53.26 | 15.78cd | 1.91 | 2.55 | -33.25cd | 0.42 | 0.35 | 17.86bc | 1.48 | 1.46 | 0.95c | 0.54 | 0.47 | 12.76bc |
| G46 | 63.67 | 53.14 | 16.54c | 65.7 | 55.72 | 15.19cd | 1.82 | 2.47 | -35.81c | 0.38 | 0.31 | 19.57cd | 1.46 | 1.44 | 0.96c | 0.53 | 0.46 | 13c |
| G47 | 66 | 55.47 | 15.95bc | 68.03 | 58.05 | 14.67bc | 1.9 | 2.63 | -38.84c | 0.39 | 0.32 | 19.07cd | 1.56 | 1.54 | 0.9b | 0.55 | 0.48 | 12.45bc |
| G48 | 64 | 53.47 | 16.45c | 66.03 | 56.05 | 15.11c | 1.91 | 2.82 | -47.13b | 0.47 | 0.4 | 15.85a | 1.53 | 1.51 | 0.92bc | 0.57 | 0.5 | 12.09b |
| G49 | 61 | 50.47 | 17.26cd | 63.03 | 53.05 | 15.83cd | 1.97 | 2.61 | -32.82cd | 0.44 | 0.37 | 16.92b | 1.57 | 1.55 | 0.89b | 0.54 | 0.47 | 12.76bc |
| G50 | 66.33 | 55.8 | 15.87bc | 68.37 | 58.39 | 14.6b | 1.77 | 2.7 | -52.69a | 0.39 | 0.32 | 19.07cd | 1.45 | 1.43 | 0.97cd | 0.55 | 0.48 | 12.53bc |
| G51 | 71.33 | 60.8 | 14.76a | 73.37 | 63.39 | 13.6b | 2.09 | 2.34 | -11.96e | 0.46 | 0.39 | 16.19a | 1.62 | 1.61 | 0.86b | 0.61 | 0.54 | 11.23b |
| G52 | 62 | 51.47 | 16.98cd | 64.03 | 54.05 | 15.59cd | 1.92 | 2.56 | -33.1cd | 0.37 | 0.3 | 20.09de | 1.49 | 1.47 | 0.94bc | 0.53 | 0.46 | 13.12cd |
| G53 | 67.33 | 56.8 | 15.64b | 69.37 | 59.39 | 14.39b | 1.91 | 2.71 | -41.87bc | 0.39 | 0.32 | 19.07cd | 1.5 | 1.48 | 0.94bc | 0.51 | 0.44 | 13.51d |
| G54 | 64 | 53.47 | 16.45c | 66.03 | 56.05 | 15.11c | 1.87 | 2.73 | -45.72bc | 0.44 | 0.37 | 16.92b | 1.53 | 1.52 | 0.91bc | 0.53 | 0.46 | 13c |

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| GET | CMT-N | CMT-WD | R% | RI-N | RI-WD | R% | OP-N | OP-WD | R% | CT-N | CT-WD | R% | chl a- N | hl a-WD | R% | chl b-N | chl b-WD | R% |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| G55 | 60.33 | 49.8 | 17.45d | 62.37 | 52.39 | 16d | 1.96 | 2.73 | -39.39c | 0.42 | 0.35 | 17.86bc | 1.46 | 1.44 | 0.96c | 0.56 | 0.49 | 12.38bc |
| G56 | 68 | 57.47 | 15.49b | 70.03 | 60.05 | 14.25b | 1.82 | 2.81 | -54.4a | 0.38 | 0.31 | 19.74d | 1.5 | 1.48 | 0.94bc | 0.57 | 0.5 | 12.09b |
| G57 | 61.67 | 51.14 | 17.08cd | 63.7 | 53.72 | 15.67cd | 1.85 | 2.68 | -44.59bc | 0.38 | 0.31 | 19.74d | 1.42 | 1.4 | 0.99d | 0.54 | 0.47 | 12.76bc |
| G58 | 63 | 52.47 | 16.71c | 65.03 | 55.05 | 15.35c | 1.78 | 2.76 | -54.49a | 0.42 | 0.35 | 17.86bc | 1.46 | 1.45 | 0.96c | 0.52 | 0.45 | 13.17d |
| G59 | 61 | 50.47 | 17.26d | 63.03 | 53.05 | 15.83cd | 1.85 | 2.72 | -46.49b | 0.44 | 0.37 | 17.05b | 1.44 | 1.43 | 0.97cd | 0.52 | 0.45 | 13.38d |
| G60 | 63.33 | 52.8 | 16.63cd | 65.37 | 55.39 | 15.27cd | 1.82 | 2.61 | -43.13bc | 0.38 | 0.31 | 19.74d | 1.46 | 1.44 | 0.96c | 0.53 | 0.46 | 13.12cd |
| G61 | 70 | 59.47 | 15.04a | 75.91 | 65.93 | 13.15a | 1.63 | 2.69 | -64.49a | 0.47 | 0.4 | 15.85a | 1.64 | 1.63 | 0.85a | 0.64 | 0.57 | 10.82a |
| G62 | 63.33 | 52.8 | 16.63cd | 65.37 | 55.39 | 15.27cd | 1.81 | 2.63 | -44.76bc | 0.38 | 0.31 | 19.74d | 1.46 | 1.45 | 0.96c | 0.54 | 0.47 | 12.76bc |
| G63 | 64.67 | 54.14 | 16.28bc | 66.7 | 56.72 | 14.96bc | 1.82 | 2.54 | -39.56c | 0.41 | 0.34 | 18.15c | 1.45 | 1.43 | 0.97cd | 0.54 | 0.47 | 12.76bc |
| G64 | 60.33 | 49.8 | 17.45d | 62.37 | 52.39 | 16d | 1.83 | 2.49 | -35.55c | 0.42 | 0.35 | 17.72bc | 1.44 | 1.42 | 0.97cd | 0.53 | 0.46 | 13.08c |
| G65 | 63.33 | 52.8 | 16.63cd | 65.37 | 55.39 | 15.27cd | 1.78 | 2.31 | -29.72d | 0.39 | 0.32 | 19.07cd | 1.44 | 1.43 | 0.97cd | 0.54 | 0.47 | 12.84c |
| G66 | 59.67 | 49.14 | 17.65d | 61.7 | 51.72 | 16.17d | 1.92 | 2.49 | -29.77d | 0.4 | 0.33 | 18.6c | 1.54 | 1.53 | 0.91bc | 0.57 | 0.5 | 12.16b |
| G67 | 59.67 | 49.14 | 17.65d | 61.7 | 51.72 | 16.17d | 1.91 | 2.43 | -26.85d | 0.39 | 0.32 | 19.07c | 1.5 | 1.48 | 0.94bc | 0.54 | 0.47 | 12.76c |
| G68 | 64.67 | 54.14 | 16.28bc | 66.7 | 56.72 | 14.96bc | 1.76 | 2.46 | -40.08c | 0.41 | 0.34 | 18.15c | 1.47 | 1.46 | 0.95c | 0.53 | 0.46 | 13.08c |
| G69 | 60.33 | 49.8 | 17.45d | 62.37 | 52.39 | 16d | 1.86 | 2.57 | -38.6cd | 0.43 | 0.35 | 17.58bc | 1.56 | 1.55 | 0.9b | 0.51 | 0.44 | 13.55de |
| G70 | 63.33 | 52.8 | 16.63c | 65.37 | 55.39 | 15.27cd | 1.89 | 2.56 | -35.19c | 0.42 | 0.34 | 18bc | 1.47 | 1.46 | 0.95c | 0.53 | 0.46 | 13.12cd |
| G71 | 59.67 | 49.14 | 17.65de | 61.7 | 51.72 | 16.17de | 2.09 | 2.65 | -26.35d | 0.38 | 0.31 | 19.57cd | 1.43 | 1.41 | 0.98d | 0.55 | 0.48 | 12.6bc |
| G72 | 60.67 | 50.14 | 17.36d | 62.7 | 52.72 | 15.92cd | 1.97 | 2.52 | -27.99d | 0.39 | 0.32 | 19.07c | 1.45 | 1.43 | 0.97d | 0.54 | 0.47 | 12.68bc |
| G73 | 62 | 51.47 | 16.98cd | 64.03 | 54.05 | 15.59cd | 1.96 | 2.44 | -24.72de | 0.39 | 0.31 | 19.4cd | 1.48 | 1.46 | 0.95c | 0.54 | 0.47 | 12.76c |
| G74 | 62.33 | 51.8 | 16.89c | 64.37 | 54.39 | 15.5c | 1.83 | 2.45 | -33.82cd | 0.39 | 0.32 | 19.23cd | 1.55 | 1.54 | 0.9b | 0.54 | 0.47 | 12.68bc |
| G75 | 64.33 | 53.8 | 16.37c | 66.37 | 56.39 | 15.04c | 1.76 | 2.37 | -35.23c | 0.39 | 0.32 | 19.07c | 1.48 | 1.46 | 0.95c | 0.54 | 0.47 | 12.88c |
| G76 | 68 | 57.47 | 15.49b | 70.03 | 60.05 | 14.25b | 1.86 | 2.58 | -38.62c | 0.39 | 0.31 | 19.4cd | 1.42 | 1.41 | 0.99d | 0.53 | 0.46 | 13.12cd |
| G77 | 55 | 44.47 | 19.15e | 61.03 | 51.05 | 16.35de | 1.94 | 2.6 | -34.25cd | 0.32 | 0.25 | 23.44e | 1.38 | 1.37 | 1.01de | 0.47 | 0.4 | 14.76e |
| G78 | 64.33 | 53.8 | 16.37c | 66.37 | 56.39 | 15.04c | 1.81 | 2.63 | -45.76b | 0.39 | 0.32 | 19.07c | 1.42 | 1.4 | 0.99d | 0.53 | 0.46 | 12.92c |
| G79 | 63.67 | 53.14 | 16.54c | 65.7 | 55.72 | 15.19cd | 1.86 | 2.53 | -36.2c | 0.42 | 0.34 | 18bc | 1.43 | 1.42 | 0.98d | 0.54 | 0.47 | 12.76c |
| G80 | 63.33 | 52.8 | 16.63c | 65.37 | 55.39 | 15.27c | 1.85 | 2.52 | -36.46c | 0.43 | 0.36 | 17.44bc | 1.45 | 1.43 | 0.97d | 0.53 | 0.46 | 13.08cd |
| G81 | 58 | 47.47 | 18.16e | 60.01 | 50.03 | 16.63e | 1.67 | 2.33 | -39.5c | 0.34 | 0.27 | 22.06e | 1.39 | 1.38 | 1.01de | 0.47 | 0.4 | 14.76e |
| G82 | 64.67 | 54.14 | 16.28bc | 66.68 | 56.7 | 14.97c | 1.9 | 2.38 | -25.33de | 0.39 | 0.32 | 19.07c | 1.46 | 1.44 | 0.96cd | 0.53 | 0.46 | 13.12cd |

| GET | CMT-N | CMT-WD | R% | RI-N | RI-WD | R% | OP-N | OP-WD | R% | CT-N | CT-WD | R% | chl a- N | hl a-WD | R% | chl b-N | chl b-WD | R% |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| G83 | 56 | 45.47 | 18.8e | 58.01 | 48.03 | 17.2e | 1.95 | 2.57 | -31.31d | 0.41 | 0.34 | 18.15c | 1.48 | 1.46 | 0.95c | 0.5 | 0.43 | 13.69de |
| G84 | 61 | 50.47 | 17.26d | 63.01 | 53.03 | 15.84cd | 1.81 | 2.56 | -41.16bc | 0.43 | 0.36 | 17.44bc | 1.46 | 1.44 | 0.96cd | 0.53 | 0.46 | 13.08cd |
| G85 | 64.67 | 54.14 | 16.28bc | 66.68 | 56.7 | 14.97c | 1.83 | 2.61 | -42.09bc | 0.43 | 0.35 | 17.58bc | 1.46 | 1.44 | 0.96cd | 0.54 | 0.47 | 12.76c |
| G86 | 62.33 | 51.8 | 16.89c | 64.34 | 54.36 | 15.51c | 1.82 | 2.52 | -38.44cd | 0.38 | 0.31 | 19.57cd | 1.42 | 1.41 | 0.98d | 0.52 | 0.45 | 13.17d |
| G87 | 63.33 | 52.8 | 16.63c | 65.34 | 55.36 | 15.27c | 1.85 | 2.35 | -26.99d | 0.39 | 0.32 | 19.07c | 1.42 | 1.4 | 0.99de | 0.54 | 0.47 | 12.68bc |
| G88 | 58.33 | 47.8 | 18.05de | 60.34 | 50.36 | 16.54de | 1.66 | 2.39 | -43.39bc | 0.32 | 0.25 | 23.44e | 1.39 | 1.38 | 1.01e | 0.47 | 0.4 | 14.66e |
| G89 | 61 | 50.47 | 17.26d | 63.01 | 53.03 | 15.84cd | 1.85 | 2.27 | -22.65e | 0.42 | 0.35 | 17.72bc | 1.5 | 1.48 | 0.94bc | 0.54 | 0.47 | 12.76c |
| G90 | 58.33 | 47.8 | 18.05e | 60.34 | 50.36 | 16.54e | 1.83 | 2.56 | -39.36c | 0.39 | 0.32 | 19.07c | 1.46 | 1.44 | 0.96cd | 0.52 | 0.45 | 13.17d |
| G91 | 61.67 | 51.14 | 17.08cd | 63.68 | 53.7 | 15.67cd | 1.82 | 2.74 | -50.55a | 0.42 | 0.35 | 17.72bc | 1.42 | 1.41 | 0.98d | 0.51 | 0.44 | 13.55de |
| G92 | 64 | 53.47 | 16.45c | 66.01 | 56.03 | 15.12c | 1.87 | 2.61 | -39.45c | 0.37 | 0.3 | 20.09de | 1.51 | 1.5 | 0.93bc | 0.53 | 0.46 | 13.12cd |
| G93 | 59.67 | 49.14 | 17.65de | 61.68 | 51.7 | 16.18de | 1.83 | 2.68 | -46b | 0.39 | 0.32 | 19.07c | 1.47 | 1.45 | 0.95c | 0.53 | 0.46 | 13.12cd |
| G94 | 63.67 | 53.14 | 16.54c | 65.68 | 55.7 | 15.2cd | 1.88 | 2.68 | -42.38bc | 0.39 | 0.31 | 19.4cd | 1.46 | 1.45 | 0.96cd | 0.52 | 0.45 | 13.38d |
| G95 | 57 | 46.47 | 18.47e | 59.01 | 49.03 | 16.91e | 1.63 | 2.57 | -57.35a | 0.33 | 0.25 | 22.96e | 1.37 | 1.36 | 1.02e | 0.48 | 0.41 | 14.3de |

Abbreviation: GET= genotypes, RL=root length, SL=shoot length, RSR=root/shoot ratio, FST=fresh seedling weight, DST=dry seedling weight, RWC= relative water content, CMT=cell membrane thermo- stability, RI=Relative Injury, CT=carotenoids, OP=osmotic potential, chl a=chlorophyll a, chl b=chlorophyll b, Normal, WD= Water Deficit

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