Arab Universities Journal of Agricultural Sciences
(2024) 32 (2) 231-248
Website: http://ajs.journals.ekb.eg

Assessment of Drought Tolerance in Barley Genotypes Through Phenotypical and Molecular Analysis

Mariam HM El Nabawy[1]*, Khadegah MA Najeeb[2], Khaled A Soliman[1], Alia A El-Seoudy[1]
1- Genetics Dept, Fac of Agric, Ain Shames Univ, P.O. Box 68, Hadayek Shubra 11241, Cairo, Egypt
2- Wheat Diseases Dept, Plant Pathology Research Institute, Agriculture Research Center, Giza, Egypt

*Corresponding author: mariamhassan@agr.asu.edu.eg

https://doi.org/10.21608/AJS.2024.291703.1578

Received 20 June 2024 ; Accepted 8 September 2024

Keywords:

Climate change,
Barley genotypes,
Drought stress,
Polyethylene glycol,
Simple sequence repeats,
SSR primers

Abstract: Climate change poses a significant challenge to agriculture while barley is an essential and crucial crop worldwide. This study evaluated the drought stress tolerance of 25 barley genotypes. A field experiment was carried out to investigate agronomical traits, such as plant height at 110 days (PH110) and spike length (SL), in response to different surface irrigation treatments. Subsequently, 15 barley genotypes were chosen for the second experiment which aimed to examine the impact of physiological stress generated by polyethylene glycol-6000. Several biological metrics, including seedling vigor index (SVI), and drought tolerance index (DTI), were quantified. Ultimately, six SSR primers were used to analyze the genetic diversity between different barley genotypes. The findings demonstrated that the G1, G2, and G6 genotypes were tolerant but G5, G9, and G14 were susceptible. The primers Bmag0603, EBmac0849, and Bmag770 were polymorphic. This study provides valuable initial insights into the drought resistance of various barley genotypes, highlighting the genetic diversity and potential for breeding drought-tolerant varieties. We suggest expanding the sample size and incorporating a broader range of environmental conditions in future studies to validate these findings. Additionally, the identified genetic markers could be further explored and utilized in breeding programs.

1 Introduction

Climate change is a pressing issue in the 21st century, attracting public, political and academic attention. It has serious environmental, social and economic risks in Africa, such as rising temperatures, soil erosion, pest and disease pressure, crop and livestock migration, desertification of the Sahara, flooding, deforestation, and soil erosion (Jungudo 2023). One method is to start solving climate change issues in the agricultural system and to focus on a critical crop in less-than-ideal growing conditions. Barley (*Hordeum vulgare* L.) is a major cereal crop that was one of the first to be domesticated and grown (Yang et al 2017). Barley is considered the first crucial crop cultivated by the Poaceae family. Barley is ranked fourth in cereals production (Rani et al 2024). Water is the most valuable resource on earth since it is the source

of life. Climate change and population growth have led to increased competition for water supplies worldwide. As freshwater availability for crop production decreases, agriculturists are seeking innovative solutions to preserve water and maximize crop yield per drop (Olamide et al 2022). Breeding for drought resistance is a primary goal in arid and semiarid regions of the world due to insufficient precipitation, a scarcity of irrigation water, and a high water demand for crop evapotranspiration in such environments. Egypt's paucity of water owing to the Grand Ethiopian Renaissance Dam makes strengthening drought tolerance even more vital (Mansour et al 2020). Creating controlled water deficit circumstances in a lab or glasshouse is the most popular technique for studying how plants react to drought stress (Hellal et al 2018). The more practical method for researching the effects of dryness on germination and stress tolerance is thought to be the generation of osmotic potential utilizing various osmotic chemicals. Because PEG (particularly PEG-6000) has a higher molecular weight than the other osmotic utilized chemicals, it cannot pass through the plant's cell wall. Due to the previous reasons, PEG is commonly used in the studies of germination and drought tolerance to control osmotic potential (Badr et al 2020). Employing germination indices, seeding features2 and drought tolerance indices can functionally facilitate the evaluation of drought responses (Ahmed et al 2022). Barley genotypes can be examined for their phylogenetic relationship using molecular markers that rely on SSR (Simple Sequence Repeats). The reasons for these markers used are because of their high polymorphism, codominance, many alleles, and rapid racking up (Aboulilal and Mansour 2017). For essential crop plants, new microsatellite markers have been developed. The marker system is expected to be helpful in breeding programs and accelerate the development of new markers (Maniruzzaman et al 2014). To construct genetic maps of all seven barley chromosomes using microsatellites, (Varshney et al 2007) took advantage of over 775 microsatellites. The purpose of this study was to evaluate the effects of water deficit stress on the physiological and morphological characteristics of the tested barley genotypes and their response to drought stress. The results are critical for understanding mechanisms of drought tolerance to select genotypes and evaluating tolerance variation. These breeding programs can make use of them.

2 Materials and Methods

2.1 Materials

2.1.1 Plant materials

A total of 25 barley genotypes, including 10 Egyptian commercial verities and 15 coded genotypes, are shown in **Table 1**. Seeds in this study were obtained from the National Gene Bank (NGB) in the Agricultural Research Center (ARC), Giza, Egypt. The experiments were conducted during the winter season from 2022 to 2023 in the experimental field of the Department of Genetics, Fac. Agric., Ain Shams Univ., Cairo, Egypt.

2.1.2 Chemicals and Reagents

The procedures and techniques section will list all chemicals and reagents utilized in this search.

2.2 Methods

2.2.1 Field experiment for drought tolerance assessment

The experimental design was a split-plot design with three replications in three irrigation treatments (Oraby et al 2018). The main plots were irrigation treatments, and the subplots were the plant materials.

Metrological Data

Monthly average minimum temperature, maximum temperature, and relative humidity (RH) during the growing barley season in the experimental site are shown in **Table 2**.

Irrigation water levels

Barley requires irrigation four times throughout the growing season. The field was irrigated twice as the severe drought treatment, three times as the moderate drought treatment, and four times as the control treatment. The first treatment (T1) was to be irrigated two times: once at sowing and then after fourteen days of sowing. The second treatment (T2) is three times, once at sowing, then after fourteen days of sowing, and after a month of the fourteen days. The third treatment (T3) was four times: once at sowing, then after fourteen days of sowing, then after a month, and finally after

Table 1. Accessions, row type, and pedigree of 25 barley genotypes

Accessions	Row Type	Pedigree
Giza 123	Six	Giza 117/FAO 86
Giza 124	Six	Giza 117/Bahteem 52// Giza 118/FAO 86
Giza 125	Six	Giza 117/Bahteem52// Giza118 /FAO86 (sister line to G.124).
Giza 126	Six	Baladi Bahteem/S D729-Por12762-BC.
Giza 127	Two	W12291/B0gs//Hamal-02
Giza 129	Six	Deir Alla 106/Cel//As46/Aths*2''
Giza 130	Six	Comp.cross"229//Bco.Mr./DZ02391/3/Deir Alla 106 CM67B/CEN-TENO//CAMB/3/ROW906.73/4/GLORIABAR/ COME-B/5/
Giza 134	Six	ICB91-0343-0AP-0AP-0AP-289AP-0AP
Giza 135	Six	ZARZA/BERMEJO/4/DS4931//GLORIABAR/COPAL/3/SEN/5/AYAROS PLAISANT/7/CLN-B/LIGEE640/3/S.P-B//GLORIAAR/ COME B/5/
Giza 2000	Six	Giza117/Bahteem52// Giza118/ FAO86 / 3/Baladi16/ Gem.
G1	Six	El Minya (Egy., GenBank, code No. 11331).
G2	Six	El Minya (Egy., GenBank, code No. 11333).
G3	Six	El Minya (Egy., GenBank, code No. 11334).
G4	Six	El Minya (Egy., GenBank, code No. 11335).
G5	Six	El Minya (Egy., GenBank, code No. 11337).
G6	Six	El Minya (Egy., GenBank, code No. 11338).
G7	Six	El Minya (Egy., GenBank, code No. 11339).
G8	Six	El Minya (Egy., GenBank, code No. 11340).
G9	Six	El Minya (Egy., GenBank, code No. 11342).
G10	Six	Alexandria (Egy., GenBank, code No. 11343).
G11	Six	Alexandria (Egy., GenBank, code No. 11344).
G12	Six	El Wadi El Gadid, El Dakhla, Shazly Yamani Muhammad.
G13	Six	El Wadi El Gadid, El Dakhla, Mut Agricultural School.
G14	Two	Kafr El-Sheikh - Muhammad Issa El Nataq.
G15	Six	Kafr El-Sheikh - Wanis Nazih - Al-Rawda.

Table 2. Average of maximum (MAX), minimum (MIN) temperatures, and relative humidity (RH) during the growing season in the experimental site

	Temperatures (ºC)		RH	
	MAX	**MIN**	**MAX**	**MIN**
November	28.49	10	69.44	44.31
December	27.33	7.62	80.25	35.94
January	23.96	5.8	82.75	45.25
February	30.75	3.41	79.19	50.31
March	32.65	6.97	74.75	31.12
April	36.58	8.64	66.12	25.25
May	38.39	11.69	54.31	27

two months of this previous irrigation. All irrigations were done using surface irrigation (Oraby et al 2018). Data was collected for the five agronomical traits: plant height at 50, 80, and 110 days, days to heading, days to maturity, number of spikes, and spike length.

2.2.2 Germination experiment and drought tolerance indices

The seeds from the previous experiment were used as experimental materials to screen through germination under laboratory conditions by adding polyethylene glycol-6000 (PEG-6000), according to Hellal et al (2018). The experiment was conducted in the growth chamber of the Tissue Culture lab with three replications in a factorial experimental design. The first factor was six barley varieties (Giza 123; Giza 125; Giza 126; Giza 127; Giza 129; Giza 130) and nine genotypes (G1, G2, G5, G6, G9, G10, G13, G14, G15), while the other factor was comprising of three concentrations (PEG-6000) at 0% PEG-6000 (control) which was (P1), 10% PEG-6000 (P2), and 20% PEG-6000 (P3). The seeds were placed on sterilized (Whatman paper) in Petri dishes. The seeds were also sterilized by submerging them in 1% sodium hypochlorite solution for five minutes, and then they were washed with distilled water before placing in the petri dish. Ten seeds of each genotype were placed in each sterilized glass petri dish 10 cm in diameter containing the Whatman filter papers. In each petri dish, 15 ml of distilled water and 20 ml of PEG-6000 solution at specific concentrations were added after 48 hours. The dishes were kept at 25±2º C for 16 h in the dark and 8 h in the light. The seeds were counted daily for 10 days until the germination was completed. Then, data were recorded on all desired parameters **Table 3** of the germination experiment according to the International Seed Testing Association guidelines (ISTA 2019).

2.3 DNA extraction and SSR markers

Plant leaves were collected for DNA extraction on the 10th day after germination. One gm of the fresh weight was taken. According to the manufacturer, DNA was isolated using the DNeasy™ Plant Mini Kit (Qiagen Inc., cat. no. 69104, Germany). The PCR amplifications were conducted in a 20 µl total volume using the OnePCR™ Ultra kit from GeneDireX, Inc., Taiwan. Each reaction comprised 10 µl of OnePCR™ Ultra, which contained Taq buffer, $MgCl_2$, dNTPs, and Taq polymerase from GeneDireX, Inc., Taiwan. Additionally, 8 µl of distilled water, 0.5 µl of each primer, and 1 µl of DNA template from Khoothiam et al (2023) were added to the tube. BOECO Thermal Cycler TC-TE "Boeco Germany 2018" was used to PCR the DNA sample. The SSR primers' sequence conditions are presented in detail in **Table 4**. The PCR products were subjected to electrophoresis at 100 V, in 1.7% (w/v) agarose gel containing 10 µl ethidium bromide for approximately 1 h, using 100 ml 1 X TAE buffer and a DNA ladder. The gel was visualized under UV, while DNA bands were assessed as absent (0) or present (1), and SSR amplifications were compared. Using an unweighted pair-group method with an arithmetical average (UPGMA), cluster analysis was performed to produce a dendrogram.

2.4 Statistical Analysis

All collected data for measured parameters over the two experiments were evaluated using analysis of variance (ANOVA), Excel version 365 and SPSS Statistics 21.0 (2012). After measuring the agronomical traits and calculating the genetic similarity, the heatmaps were done using ClustVis (Metsalu and Vilo 2015).

3 Results and Discussion

3.1 Evaluation of field phenotypic traits for drought tolerance

Fig 1 shows the plant height (in cm) of various barley genotypes after 50 days, measured under three different irrigation treatments (T1, T2, and T3). The genotypes are listed along the x-axis, while the y-axis represents plant height. The colors orange, yellow, and green correspond to treatments T1, T2, and T3, respectively. It is evident that T3 generally resulted in the tallest plants across most genotypes, while T1 produced the shortest. Genotypes like G3, G4, and G6 displayed the tallest heights under T3. The initial in PH50 ranged from 25 cm for T1 to a maximum of 86 cm for T2. The G13 and G14 cultivars had the tiniest growth, whereas the G3, G4, and G6 cultivars displayed the greatest height across all three treatments. Giza 2000 and G5 exhibited the lowest coefficient of variation (CV), whilst G13 and G14 exhibited the highest CV. **Fig 2** depicts the plant height (in cm) of various barley genotypes after 80 days, measured under three irrigation treatments. T3 led to the tallest plants in most genotypes, with Giza 124, Giza 126, and G4 standing out.Conversely, T1 resulted in the shortest plants, especially in genotypes G13 and G14, underscoring

Table 3. Names of traits, abbreviations, units, methodology, and references to determine the main measurements in the germination experiment

Trait	Abbreviation	Unit	Methodology/Description	References
Germination Indices				
Germination percentage	G%	%	(Number of germinated seeds/total number of seeds) × 100 $G\% = \frac{G}{T} \times 100$	**(Hellal et al 2018, Thabet et al 2018, Ahmed et al 2022)**
Germination pace	GP		$GP = \frac{G}{\sum(g \times d)}$	**(Hellal et al 2018, Thabet et al 2018)**
Seedling Vigor Index	SVI		$SVI = \frac{(\text{Root Length} + \text{Shoot Length}) \times G\%}{100}$	**(Hellal et al 2018, Ahmed et al 2022)**
Seedling Traits				
Root length	RL	cm	Length of fresh root in cm	**(Hellal et al 2018, Thabet et al 2018, Ahmed et al 2022)**
Shoot length	SHL	cm	Length of fresh shoot in cm	**(Ahmed et al 2022), (Thabet et al 2018), (Hellal et al 2018)**
Root shoot ratio	RSR	%	$RSR = \frac{\text{RL under drought or control}}{\text{SHL under drought or control}}$	**(Hellal et al 2018, Thabet et al 2018, Ahmed et al 2022)**
Root fresh weight	RFW	mg	Weight of fresh root in mg	**(Ahmed et al 2022), (Thabet et al 2018), (Hellal et al 2018)**
Shoot fresh weight	SHFW	mg	Weight of fresh shoot in mg	**(Hellal et al 2018, Thabet et al 2018, Ahmed et al 2022)**
Root dry weight	RDW	mg	Weight of dry root in mg after drying at 70°C for 24 h	**(Ahmed et al 2022), (Thabet et al 2018), (Hellal et al 2018)**
Shoot dry weight	SHDW	mg	Weight of dry shoot in mg after drying at 70°C for 24 h	**(Hellal et al 2018, Thabet et al 2018, Ahmed et al 2022)**
Tissue Water Content	TWC		$TWC = \frac{FW - DW}{FW}$	**(Hellal et al 2018)**

Cont. Table 3. Names of traits, abbreviations, units, methodology, and references to determine the main measurements in the germination experiment

Trait	Abbreviation	Unit	Methodology/Description	References
Drought Tolerance Indices				
Drought Tolerance Index of the Germination Percentage	GDTI	%	$\text{DTI (G\%)} = \frac{\text{G\% under drought}}{\text{G\% under control}} \times 100$	**(Thabet et al 2018, Ahmed et al 2022)**
Drought Tolerance Index of the Germination Pace	GPDTI	%	$\text{DTI (GP)} = \frac{\text{GP under drought}}{\text{GP under control}} \times 100$	**(Thabet et al 2018)**
Drought Tolerance Index of the Root Length	RLDTI	%	$\text{DTI (RL)} = \frac{\text{RL under drought}}{\text{RL under control}} \times 100$	**(Thabet et al 2018, Ahmed et al 2022)**
Drought Tolerance Index of the Shoot Length	SHLDTI	%	$\text{DTI (SHL)} = \frac{\text{SHL under drought}}{\text{SHL under control}} \times 100$	**(Thabet et al 2018, Ahmed et al 2022)**
Drought Tolerance Index of the Root Fresh Weight	RFWDTI	%	$\text{DTI (RFW)} = \frac{\text{RFW under drought}}{\text{RFW under control}} \times 100$	**(Ahmed et al 2022)**
Drought Tolerance Index of the Shoot Fresh Weight	SHFWDTI	%	$\text{DTI (SHFW)} = \frac{\text{SHFW under drought}}{\text{SHFW under control}} \times 100$	**(Ahmed et al 2022)**
Drought Tolerance Index of the Root Dry Weight	RDWDTI	%	$\text{DTI (RDW)} = \frac{\text{RDW under drought}}{\text{RDW under control}} \times 100$	**(Ahmed et al 2022)**
Drought Tolerance Index of the Shoot Dry Weight	SHDWDTI	%	$\text{DTI (SHDW)} = \frac{\text{SHDW under drought}}{\text{SHDW under control}} \times 100$	**(Ahmed et al 2022)**

Table 4. Names, sequences, chromosome number, annealing temperature, and references of the primers used in this study

Name	Sequence of primers (5'-3')	Chromosome No.	(ºC)T_a	References
Bmag0603F	ATACCATGATACATCACATCG	3H	55	(**Dizkirici et al 2008**)
Bmag0603R	GGGGGTATGTACGACTAACTA			
GBM1221F	ACCAGCAATCCAAGTTACGG	4H	55	(**Mariey et al 2022**)
GBM1221R	TGCCTTGGTCTTGGTGTGTA			
EBmac0849F	TTCCGTTGAGCTTTCATACAC	2H	57	(**Hellal et al 2018**)
EBmac0849R	ATTGAATCCCAACAGACACAA			
Bmag770F	AAGCTCTTTCTTGTATTCGTG	1H	55	(**Mariey et al 2013**)
Bmag770R	GTCCATACTCTTTAACATCCG			
GBM1459F	AACACATCCATACTTCCCCG	2H	57	(**Fu and Horbach 2012**)
GBM1459R	AGCTGAATAAATGCCCATGC			
GBM1405F	TACACGCACTGAAAAGACGG	3H	57	
GBM1405R	CTCGCTGCTGAGTTTGTCTG			

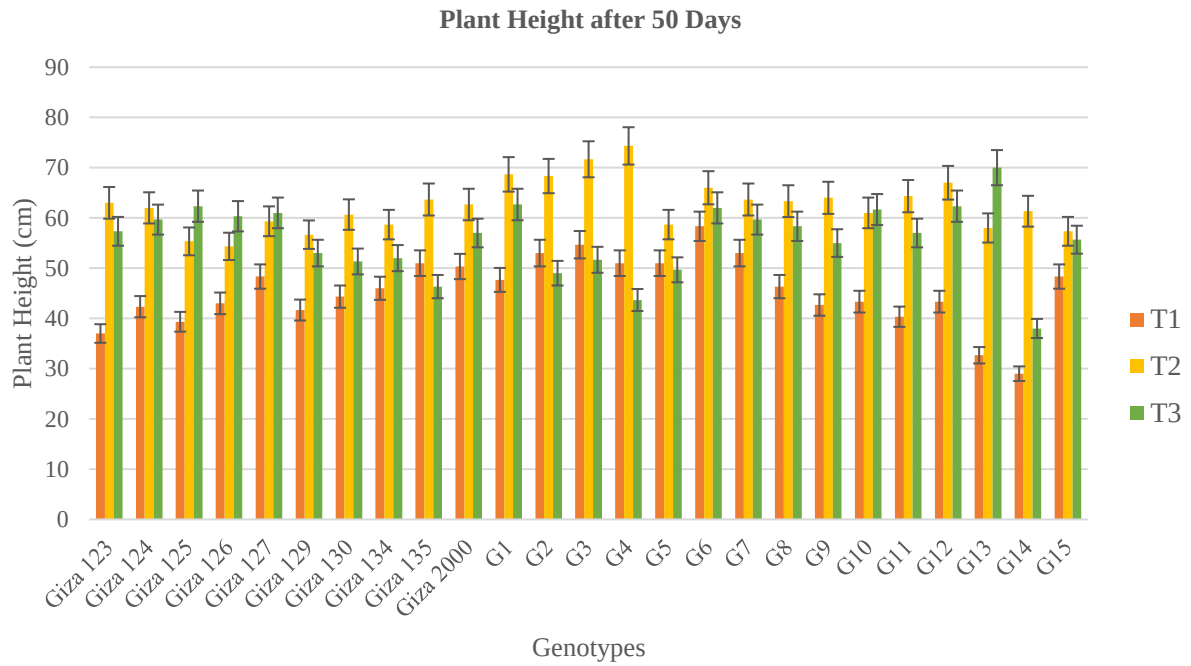

Fig 1. Impact of different irrigation levels on the plant height after 50 days

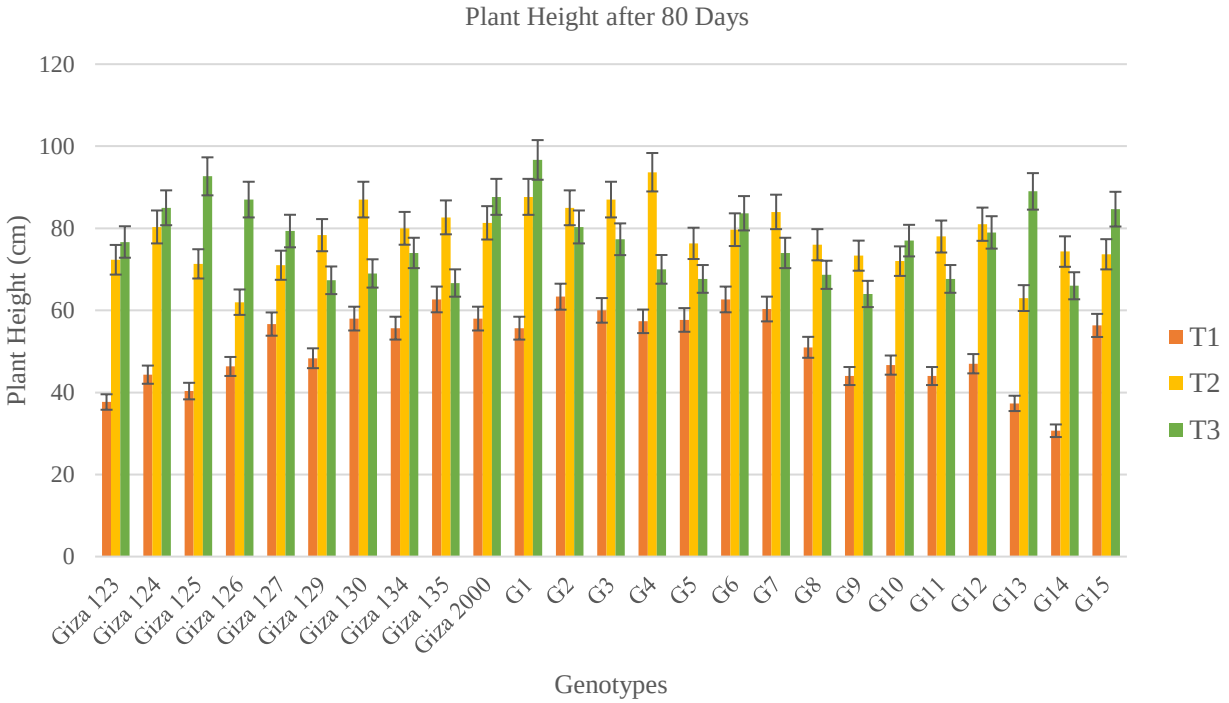

Fig 2. Impact of different irrigation levels on the plant height after 80 days

the importance of irrigation. The PH80 varied between 110 cm for T2 and T3 and 25 cm for T1. Among the several genotypes, G13 and G14 had the shortest plants, while Giza 126, Giza 2000, and G4 had the tallest plants throughout all three treatments. G13 and G14 had the largest coefficient of variation (CV), while G6 and G15 had the lowest CV. **Fig 3** shows that PH110 ranged from 20 cm for T1 to 113 cm for T3, while the G12 and G14 produced the shortest ones, Giza 126, Giza 2000, and G4 produced the tallest plants throughout the three treatments. Giza 129 and G2 showed the lowest CV, while G13 and G14 had the highest ones. In **Fig 4**, barley genotypes considerably impacted the days to heading, ranging from 69 to 96 days for T3. Under the three treatments, Giza 127, Giza 129, and G13 showed the earliest heading. In contrast, genotypes G10 and G14 showed the later heading, Giza 123, and Giza 2000 displayed the lowest CV values, whereas G4 and G13 displayed the most significant CV values. In **Fig 5**, the days to maturity they were also varied considerably between irrigation treatments, ranging from 98 days for T2 to 132 days for T3. Under the three treatments, Giza 123, G4, G6, G9, and G12 had the earliest maturity dates. However, Giza 135, G13, and G14 showed later dates; G6 and G10 displayed the lowest CV values, while G4 and G14 displayed the most significant CV values. In response to irrigation treatments, there were substantial differences in the number of spikes (**Fig 6**), which ranged between 0 for T1 and 36 spikes for T3. However, for T1, most of them showed the fewest spikes, which were zero, while T2 and T3 showed the middle number of spikes for Giza 124, Giza 125, Giza 135, and G14. In contrast, G1, G2, G6, and G15 displayed the highest number of spikes; the genotypes G4 and G6 displayed the lowest CV, whereas the genotypes Giza 126 and G14 displayed the highest CV. In **Fig 7**, significant variations in the spike length were seen in response to irrigation treatments. It ranged from 0 for T1 to 9.33 cm for T3. At T1, most of the barley genotypes gave no spikes, but Giza 125 and Giza 126 showed the shortest spikes at T2 and T3. While G1, G2, G14, and G15 showed the tallest spikes at T3, Giza 135 and G8 showed the lowest CV; the genotypes Giza 124 and Giza 126 showed the highest CV. The field experiment demonstrated a strong relationship between agronomical traits and barley genotypes under varying irrigation treatments. Significant variations were observed in plant height, days to heading, days to maturity, number of spikes, and spike length. Genotypes like Giza 126, Giza 2000, and G4 consistently produced the tallest plants and displayed more stable traits across different treatments. Conversely, genotypes G13 and G14 frequently exhibited the shortest plants and the highest coefficient of variation (CV), indicating greater variability. These findings underscore the importance of genotype selection in optimizing barley performance under specific environmental conditions, particularly in response to irrigation practices. Based on selection indices, cluster analysis was used to categorize and compare genotypes to prior results (Oraby et al 2018, Mansour et al 2017). The heatmap in **Fig 8** presents the clustering of barley genotypes based on various agronomic traits across three different treatments (T1, T2, T3). The traits include plant height at 50, 80, and 110 days (PH50, PH80, PH110), days to heading (DTH), days to maturity (DTM), number of spikes (NS), and spike length (SL). The color gradient represents the standardized values, with red indicating higher values, blue indicating lower values, and yellow representing intermediate values. The color scale on the right side of the heatmap ranges from blue to red, with a gradient passing through light yellow. Blue denotes negative values, with the darkest blue representing the lowest value in the data set (about -1.5). Light yellow denotes readings near zero, indicating a neutral or baseline value. Red colors denote positive values, with the darkest red indicating the data set's greatest value (approx. 1.5). This color scale represents the relative strength or magnitude of data for each genotype and trait combination. Red spots show greater values, indicating higher levels or stronger expression of the feature, whereas blue areas indicate lower or weaker expression. Light yellow indicates moderate or average values. Clustering exposes patterns and correlations between genotypes and phenotypes, with related genotypes and traits grouped. The genotypes are clustered on the x-axis (traits) and y-axis (genotypes) to highlight patterns in their responses across treatments. For instance, genotypes G4, Giza 135, and Giza 130 show higher values in many traits under treatment T3, indicated by the red hues, particularly in plant height and days to heading. Conversely, genotypes like G13 and G14 display lower values across most traits, particularly in spike length and plant height under T1, reflected by the blue hues. This visualization effectively illustrates the variation in barley genotype responses to different irrigation treatments and the relationships between multiple agronomic traits. Genotypes in this study (**Fig 8**) were separated into four groups of eight, three, five, and nine genotypes, each as groups A, B, C, and D, respectively. The first group consisted of Giza 123, Giza 124, Giza 126, Giza 127, Giza 2000, G1 and

G6 in section A of the cluster diagram as expected to be tolerant. The second group consisted of Giza 125, G13, and G14 in the B section of the diagram, which was expected to be moderately tolerant. The third group consisted of G8, G9, G10, G11, and G12 in the C section of the cluster diagram, which is expected to be moderately sensitive. The fourth group consisted of Giza 129, Giza 130, Giza 134, Giza 135, G2, G3, G4, G5, and G7 in the D section of the cluster diagram as expected to be sensitive. The result of Giza 134 contradicts (Mansour et al 2017) as expected to be tolerant. The results indicate that cluster analysis can differentiate genotypes based on agronomical traits in drought and non-stress situations and distinguish all genotypes. Researchers (Jalil and Salehi 2012, Eivazi et al 2013) evaluated barley genotypes for drought tolerance using cluster analysis based on drought resistance indices.

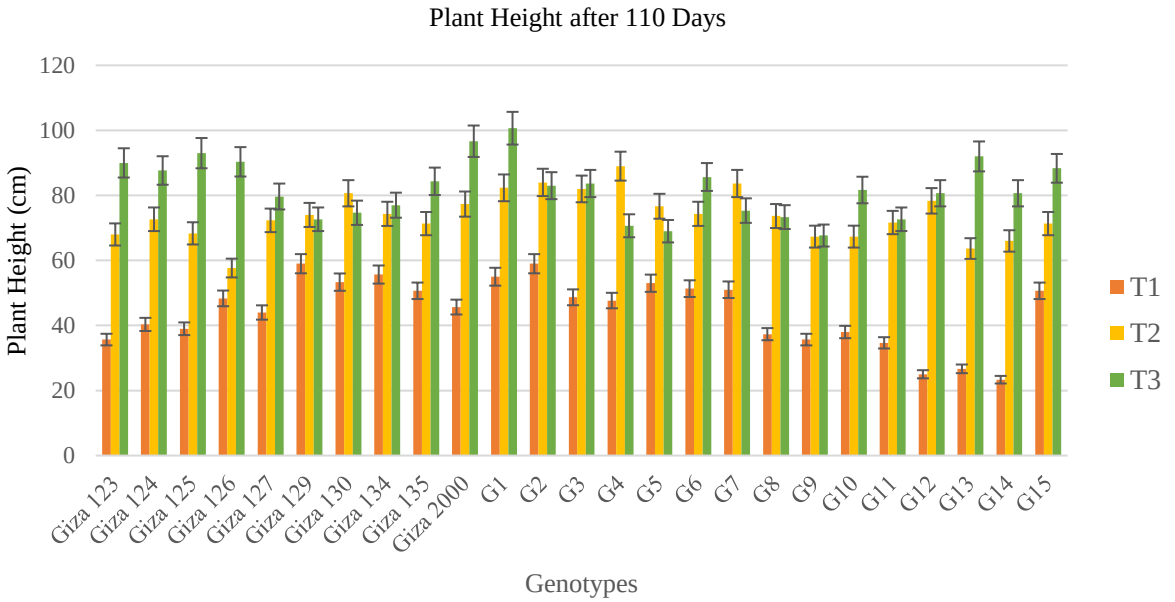

Fig 3. Impact of different irrigation levels on the plant height after 110 days

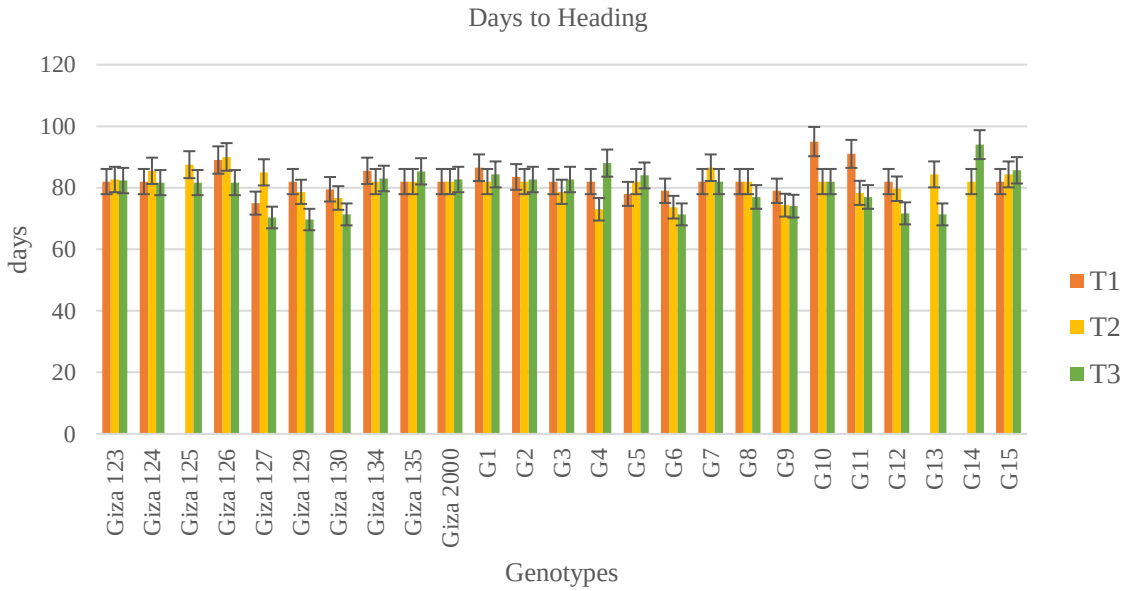

Fig 4. Impact of different irrigation levels on the days to heading

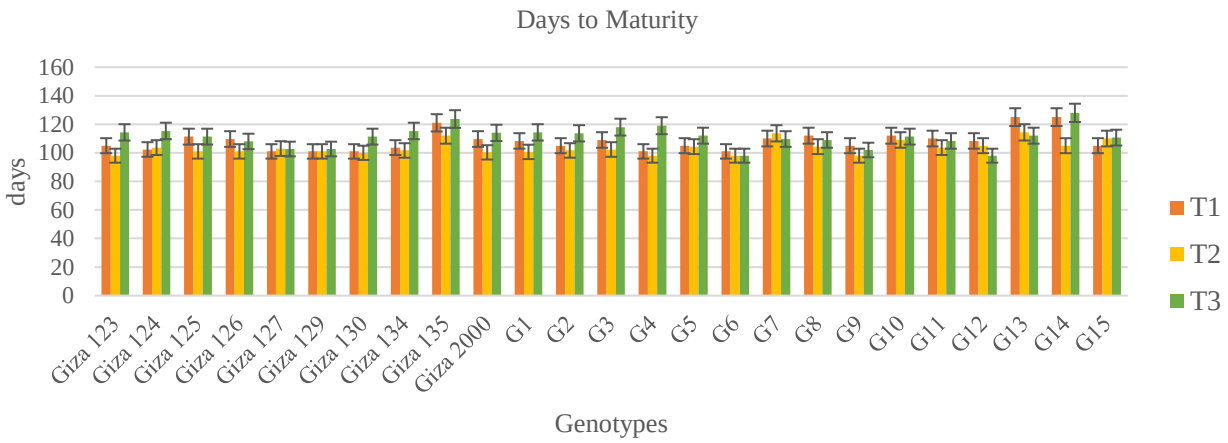

Fig 5. Impact of different irrigation levels on the days to maturity

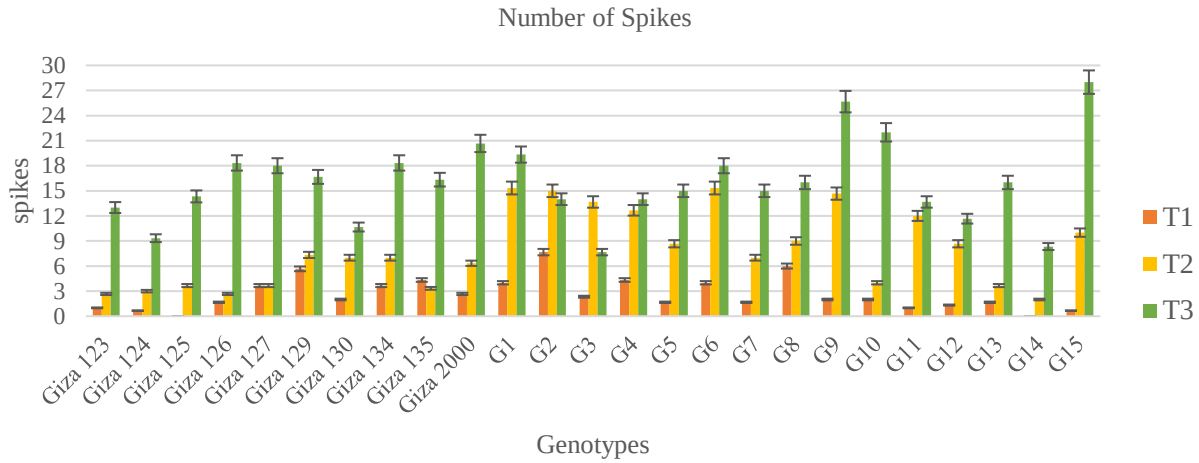

Fig 6. Impact of different irrigation levels on the number of spikes

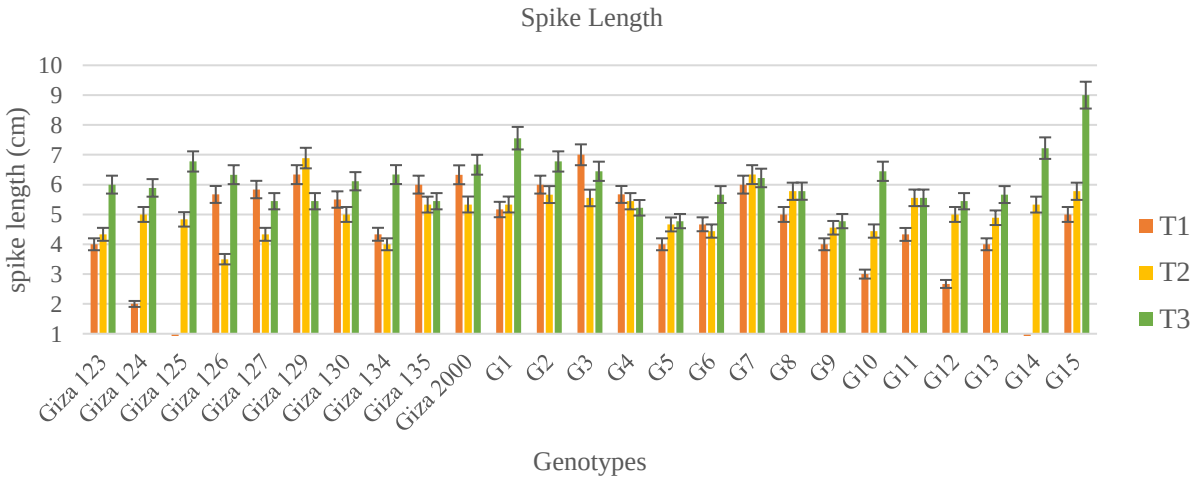

Fig 7. Impact of different irrigation levels on the spike length

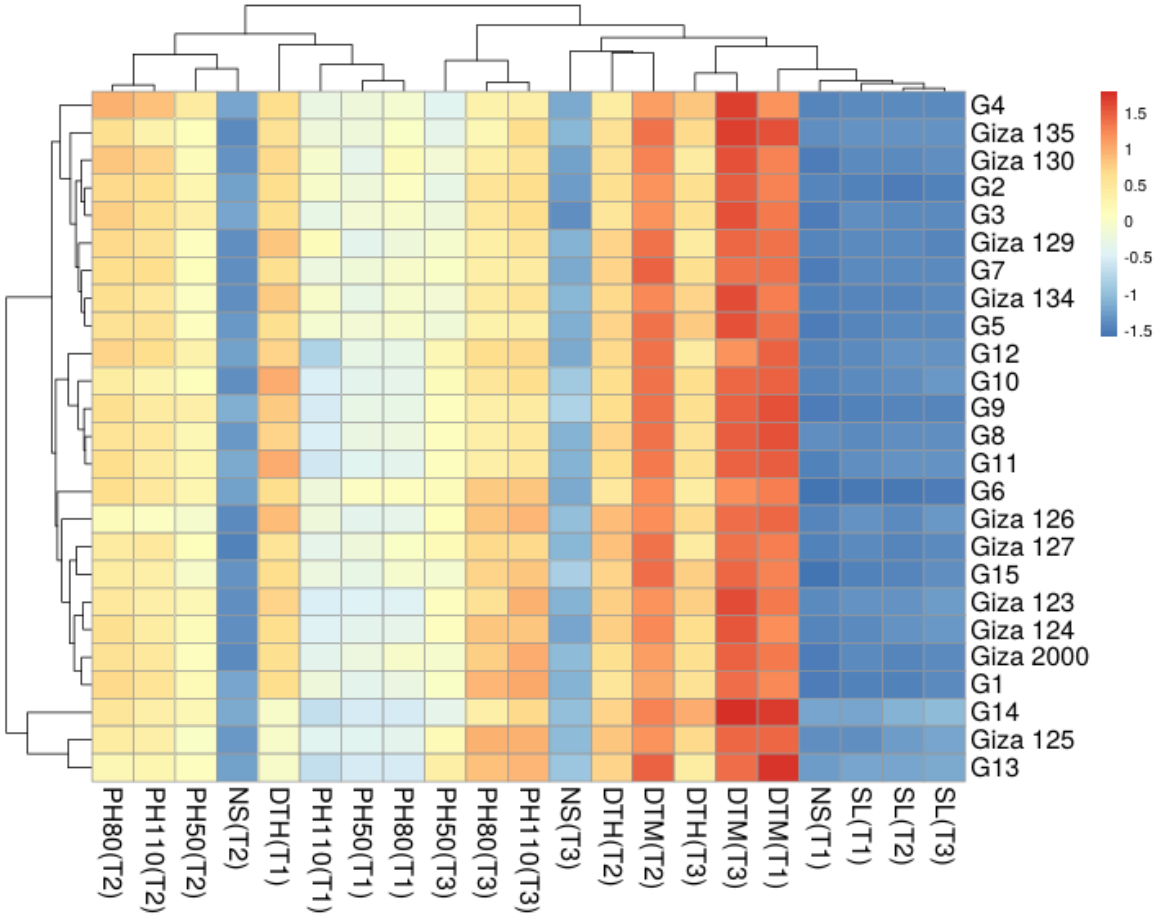

Fig 8. Heatmap illustrating the relation between the agronomical traits and all barley genotypes

3.2 Parameters used to evaluate drought tolerance during germination

Results presented in **Table 5**, showed that after full days, Giza 125 and G5, G9, and G13 had the highest number of germinated seeds in the control (P1), whereas Giza 127, Giza 129, and Giza 130 had the lowest numbers. Additionally, G1, G2, and G9 showed the highest performance in germination compared to Giza 123, G14, and G15, which showed the lowest germination in the second treatment (P2). Meanwhile, Giza 125 and Giza 127 were the only germinated seeds in 20% PEG (P3). The fastest germination pace was in Giza 127, Giza 130, G1, and G6 (all in P2), while the remaining evaluated ones showed a slower germination pace. The highest values were reported for root length for Giza 127 in (P2) and G9, followed by G14 in (P1). Giza 125 was found to be the best, followed by Giza 127 in (P3). In terms of root shoot ratio (RSR), as influenced by PEG, G2 had the highest value (6.00), followed by Giza 130 (4.20). This conclusion indicates that some genotypes can benefit from PEG% since they resist osmotic stress. Furthermore, data showed a highly significant association between root and shoot lengths during the various germination research periods. One of the most critical steps in barley's life cycle is germination. When there is water deficiency, seed germination is obstructed by a lack of available water. The collected data showed that three categories could be distinguished. the first group (Giza 125, G5) with germination percentages greater than 61%, the second group(G9, G13 and G15) with germination percentages between 51% and 61% and the third group (Giza 126, G1, G2 and G10) with germination percentages between 40% and 50%, while the remaining studies ones were less than 40%. This result explains the genotypes' vigor and their ability to store carbs. Therefore, it is evident that PEG% initially had a negative impact on the germinated seeds, but this negative impact was reduced by the time the germination test was complete. Under induced drought stress (20% PEG), all genotypes showed a significant decrease in performance for all traits compared to the control.

Table 5. Means of the effects of Polyethylene Glycol (PEG) on germination percentage (G%), germination pace (GP), seedling vigor index (SVI), root shoot ratio (RSR), root length (RL), and shoot length (SHL)

Genotypes	G%			GP			SVI			RSR			RL			SHL		
	P1	P2	P3	P1	P2	P3	P1	P2	P3	P1	P2	P3	P1	P2	P3	P1	P2	P3
Giza 123	30%	0%	0%	0.417	0.000	0.000	2.36	0.00	0.00	0.50	0.00	0.00	2.61	0.00	0.00	5.25	0.61	0.00
Giza 125	63%	37%	7%	0.355	0.530	0.167	4.33	1.07	0.02	0.48	2.89	0.00	2.22	2.17	0.33	4.61	0.75	0.00
Giza 126	43%	17%	0%	0.504	0.431	0.000	3.73	0.50	0.00	0.67	2.00	0.00	3.44	2.00	0.00	5.17	1.00	0.00
Giza 127	27%	23%	10%	0.457	0.917	0.063	2.44	1.44	0.17	0.56	1.64	0.67	3.28	3.83	0.67	5.89	2.33	1.00
Giza 129	20%	13%	0%	0.286	0.556	0.000	0.87	0.44	0.00	0.34	4.00	0.00	1.11	2.67	0.00	3.22	0.67	0.00
Giza 130	23%	7%	0%	0.389	0.667	0.000	1.58	0.19	0.00	0.55	4.20	0.00	2.39	2.33	0.00	4.36	0.56	0.00
G1	40%	47%	0%	0.317	0.650	0.000	2.36	0.88	0.00	0.54	0.00	0.00	2.06	1.89	0.00	3.83	0.00	0.00
G2	40%	50%	0%	0.579	0.594	0.000	2.51	1.17	0.00	0.66	6.00	0.00	2.50	2.00	0.00	3.78	0.33	0.00
G5	70%	10%	0%	0.419	0.500	0.000	3.42	0.13	0.00	1.00	0.00	0.00	2.44	1.33	0.00	2.44	0.00	0.00
G6	37%	23%	0%	0.387	0.810	0.000	1.75	0.73	0.00	1.05	3.67	0.00	2.44	2.44	0.00	2.33	0.67	0.00
G9	60%	43%	0%	0.384	0.507	0.000	4.70	0.00	0.00	1.14	0.00	0.00	4.17	0.00	0.00	3.67	0.00	0.00
G10	40%	23%	0%	0.392	0.369	0.000	3.44	0.47	0.00	0.87	0.00	0.00	4.00	2.00	0.00	4.61	0.00	0.00
G13	60%	10%	0%	0.309	0.417	0.000	3.93	0.30	0.00	0.90	0.00	0.00	3.11	3.00	0.00	3.44	0.00	0.00
G14	33%	0%	0%	0.643	0.000	0.000	2.93	0.00	0.00	0.93	0.00	0.00	4.22	0.00	0.00	4.56	0.00	0.00
G15	57%	0%	0%	0.292	0.000	0.000	4.22	0.00	0.00	0.72	0.00	0.00	3.11	0.00	0.00	4.33	0.00	0.00

None of the genotypes exhibited a G% of 100% or a GP of 1. Significantly, certain genotypes exhibited superior performance under induced drought compared to controlled conditions for certain attributes. For drought tolerance indices (DTI), as shown in **Table 6**, the means of the genotypes for G% were 117% and 125% in G1 and G2, respectively. The means of the genotypes for SVI were 0.59, 0.51, and 0.46 in Giza 127, Giza 129, and G2, respectively. In terms of decrease, the drought tolerance indices for root dry weight (RDWDTI) ranged from 0% in most genotypes in (P3) and 83% in G1 in (P2). Significant variance was found regarding how genotypes react to artificially induced drought stress.

3.3 Significance of genotypes, treatments, and their interactions

Regarding the surface irrigation experiment, (**Table 7**) shows the total variance analysis for the main irrigation treatments, genotypes, and their interactions. For each examined variable, there were highly significant changes between genotypes and irrigation treatments. This reveals the presence of genetic diversity in the genotypes and regimens being studied. Furthermore, the mean squares of regimens and genotypes revealed that, except for spike length and plant height at 110 days, irrigation treatments had a greater impact on the analyzed attributes than genotypes. Except for plant height after 110 days, spike length, and spike count, the interaction between irrigation treatments and the genotypes was very significant for all metrics studied. This key interaction reveals how genotypes performed significantly depending on the sort of irrigation used. **Table 8** displays the results of ANOVA for PEG treatments, genotypes, and their interactions in the second experiment. There were extremely substantial differences between genotypes and PEG treatments for each studied variable. In addition, the mean squares of PEG treatments and genotypes revealed that, except for shoot length, fresh weight of root, fresh weight of shoot, and dried weight of shoot, treatments had a greater impact on the studied characteristics.

Table 6. Drought tolerance indices (DTIs) of PEG on germination percentage (G%), germination pace (GP), root length (RL), and shoot length (SHL)

Barley Genotypes	GDTI (P2)	GDTI (P3)	GPDTI (P2)	GPDTI (P3)	RLDTI (P2)	RLDTI (P3)	SHLDTI (P2)	SHLDTI (P3)
Giza 123	0%	0%	0%	0%	0%	0%	12%	0%
Giza 125	58%	11%	149%	47%	98%	15%	16%	0%
Giza 126	38%	0%	85%	0%	58%	0%	19%	0%
Giza 127	88%	38%	201%	14%	117%	20%	40%	17%
Giza 129	67%	0%	194%	0%	240%	0%	21%	0%
Giza 130	29%	0%	171%	0%	98%	0%	13%	0%
G1	117%	0%	205%	0%	92%	0%	0%	0%
G2	125%	0%	103%	0%	80%	0%	9%	0%
G5	14%	0%	119%	0%	55%	0%	0%	0%
G6	64%	0%	209%	0%	100%	0%	29%	0%
G9	72%	0%	132%	0%	0%	0%	0%	0%
G10	58%	0%	94%	0%	50%	0%	0%	0%
G13	17%	0%	135%	0%	96%	0%	0%	0%
G14	0%	0%	0%	0%	0%	0%	0%	0%
G15	0%	0%	0%	0%	0%	0%	0%	0%

Table 7. Mean squares of examined parameters for 25 barley genotypes grown under various surface irrigation conditions

SOV	Df	PH50	PH80	PH110	DTH	DTM	NS	SL
Treatments (T)	2	5527.70**	17383.24**	29516.30**	82.72**	1135.39**	3400.97**	332.42**
Genotypes (G)	24	126.18**	277.70**	272.96*	102.14**	223.18**	66.06**	5.55^{NS}
T × G	48	118.02**	196.14*	220.01^{NS}	41.91**	70.38**	39.40^{NS}	3.05^{NS}
CV	---	12.12	16.76	17.87	4.19	2.92	62.31	46.41

NS: Not Significant, *p < 0.05, **p < 0.01

PH50 (Plant Height after 50 days), PH80 (Plant Height after 80 days), PH110 (Plant Height after 110 days), DTH (Days to Heading), DTM (Days to Maturity), Number of Spikes (NS), SL (Spike Length), and CV (Coefficient of Variation)

Table 8. Mean squares of examined parameters for 15 barley genotypes in the germination experiment

SOV	df	G%	GP	RFW	SHFW	RDW	SHDW	RL	SHL	SVI
PEG Treatments (P)	2	1.97**	2.69**	932.55**	2357.87**	61.08**	176.05**	89.54**	222.49**	12.62**
Genotypes (G)	14	0.07**	0.09**	42.32^{NS}	16.63^{NS}	49.41**	4.26*	1.67**	1.73^{NS}	0.14**
P × G	28	0.05**	0.10**	22.08^{NS}	16.32^{NS}	39.24**	2.56^{NS}	2.51**	1.32^{NS}	0.17**
CV	---	64.93	55.59	122.74	70.11	128.89	103.87	48.54	79.15	61.30

NS: Not Significant, *p < 0.05, **p < 0.01

G% (Germination Percentage), GP (Germination Pace), RFW (Root Fresh Weight), SHFW (Shoot Fresh Weight), RDW (Root Dry Weight), SHDW (Shoot Dry Weight), RL (Root Length), SHL (Shoot Length), SVI (Seedling Vigor Index), and CV (Coefficient of Variation)

3.4 Polymorphism as detected by SSR analysis

Three of the 6 primers employed (GBM1221, GBM1459 and GBM1405) had monomorphic band profiles, and the rest were polymorphic.The genetic diversity among the screened barley genotypes using the three discriminating primers. Bmag0603 primer produced two alleles (120 and 147 bp), as mentioned by (Dizkirici et al 2008). As for agronomical traits, each genotype is expected to carry a 120 bp allele and is considered sensitive, while the other one is considered a tolerant genotype. Primer GBM1221 is a monomorphic primer with one allele in all barley (Mariey et al 2022). EBmac0849 primer is a polymorphic primer showing a significant variation between genotypes. Bmag770 primer produced three polymorphic bands with sizes ranging from 158 to 220 bp, one of which was exclusively seen in genotypes that were high in chlorophyll content and had a size of about 158 bp, which is in agreement with Mariey et al (2013). This band is considered a good indicator of chlorophyll content in barley. So, the primer created the most polymorphic bands across all genotypes and could differentiate between the genotypes under study. This primer had a polymorphism percentage (PP) of 100%. GBM1459 and GBM1405 primers showed a monomorphic band in 185 and 283bp, respectively, which shares the same results as Fu and Horbach (2012). Based on the phylogenic tree, the dendrogram created with SSR markers identified two major genetic groups. Since Giza 126 was tolerant to drought, as proved by Hellal et al (2018); meanwhile, G1, G2, and G6 have an 83% similarity with Giza 126. Therefore, those genotypes are expected to be tolerant to drought. **Fig 9** is a hierarchical clustering heatmap where rows represent different genotypes and columns represent different amplicons. The color scale on the right side of the heatmap goes from dark blue to pale yellow. Dark blue reflects the data's greatest negative values, with the scale bottoming at (-1.5). Light blue and light green represent values near zero, indicating moderate or neutral levels. Light yellow denotes the most significant values, with the scale capped at approximatel (0.5). The heatmap's color gradient depicts the data's strength for each genotype-marker combination. Dark blue patches represent lower levels or weaker expression of the markers, whereas light yellow highlights higher levels or stronger expression. Clustering in both rows (genotypes) and columns (markers) groups similar data points together to identify patterns and correlations between genotypes and markers. The first cluster in **Fig 10** includes all tolerant genotypes and was discovered to be intricately linked, which is in good agreement with the field evaluation of the data. However, sensitive genotypes like (G5, G10, and G14) were found in the second cluster. A similarity value of 100% between Giza 127, G5, and G9 indicated that these three genotypes were closely related to one another, which is evident from their response to drought stress, as shown in **Table 9**. Molecular markers are extremely useful tools for determining genetic differences within and between species/populations.The polymerase chain reaction (PCR) spawned the development of numerous molecular methods, including RAPD, SSR, STS, RAMP, and ISSR. These molecular markers were employed for genotype identification, genetic mapping, and determining gene expression differences (Pan et al 2008). The study assessed the drought tolerance of several barley genotypes by analyzing their field agronomic features, drought tolerance during germination, and conducting genetic analysis. The findings demonstrated substantial variation among the genotypes under different irrigation treatments (T1, T2, T3), with T3 consistently yielding the tallest plants. Genotypes such as Giza 126, Giza 2000, and G4 regularly exhibited superior plant height and more consistent characteristics, suggesting enhanced drought tolerance. On the other hand, G13 and G14 were often shorter and had significant coefficients of variation, indicating their susceptibility to drought. Under drought-induced stress (20% PEG), specific genotypes such as Giza 125 and G5 exhibited greater germination percentages, whereas other genotypes demonstrated decreased performance. The genotypes were classified into four categories using cluster analysis, based on their drought tolerance. This study confirmed the distinction between genotypes that are tolerant to drought and those that are susceptible to it. The findings were further reinforced by the molecular investigation utilizing SSR markers, which identified crucial genetic markers linked to drought resistance. The primer Bmag770, which exhibits polymorphism, was particularly successful in differentiating between tolerant genotypes and sensitive genotypes. Lastly, to assess drought effectively, focus on evaluating key phenotypic traits like plant height, days to heading and maturity, number of spikes, and spike length across different genotypes under varying irrigation conditions. Analyzing the coefficient of variation (CV) helps in understanding trait stability. For molecular assessment, use SSR markers such as Bmag0603 and Bmag770, which have shown polymorphism and can differentiate between drought-tolerant and sensitive genotypes. Those markers are very recommended

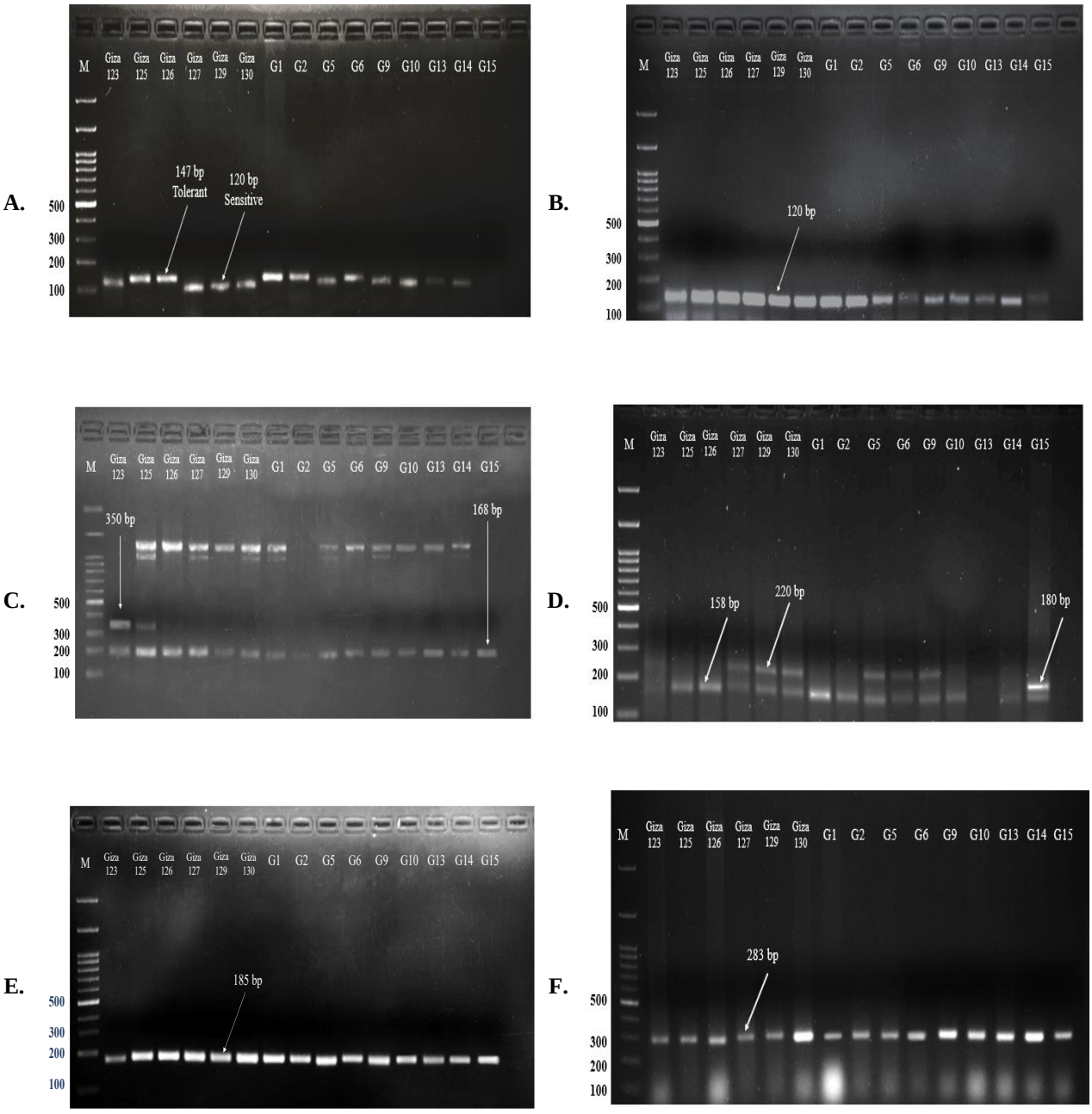

Fig 9. Banding pattern for barley genotypes using: A. Bmag0603, B. GBM1221, C. EBmac0849, D. Bmag770, E. GBM1459, F. GBM1405 primers

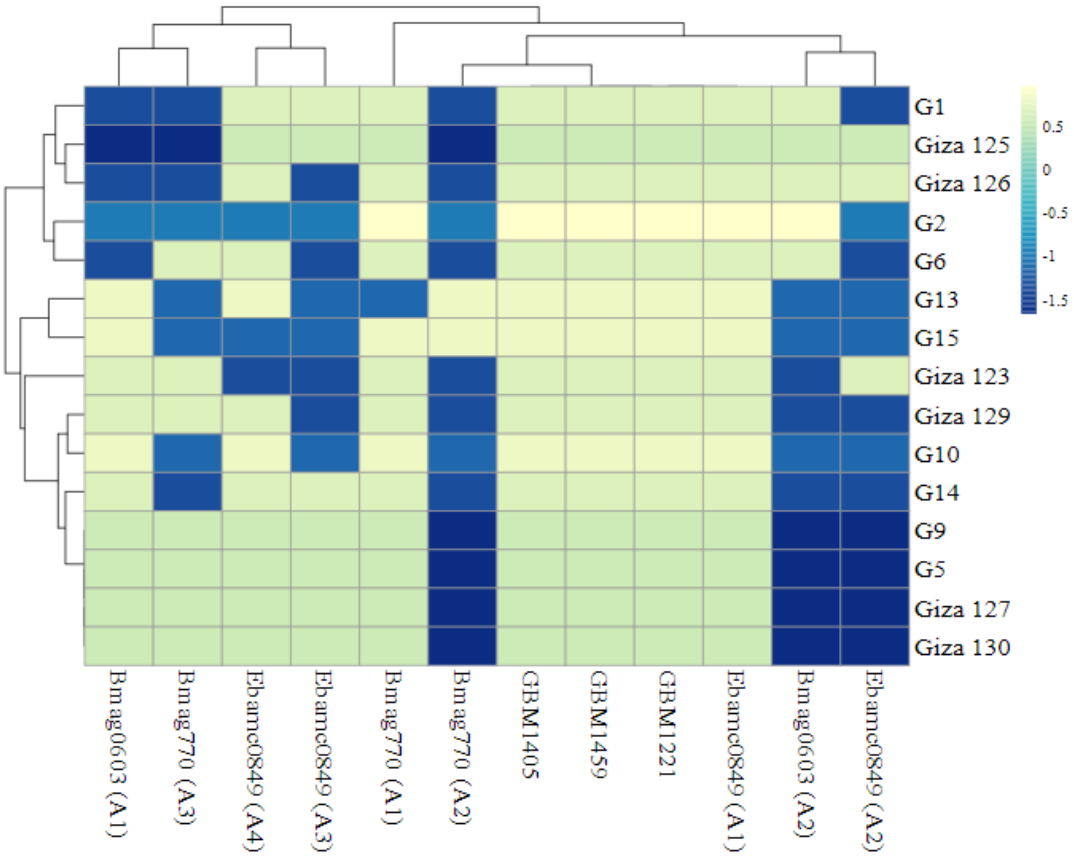

Fig 10. Heatmap illustrating the genetic diversity of 15 barley genotypes, based on the six SSR primers

Table 9. Genetic similarity for 15 barley based on simple matching similarity coefficient using UPGMA

	Giza 123	Giza 125	Giza 126	Giza 127	Giza 129	Giza 130	G1	G2	G5	G6	G9	G10	G13	G14	G15
Giza 123	1.00														
Giza 125	0.58	1.00													
Giza 126	0.58	0.92	1.00												
Giza 127	0.75	0.67	0.58	1.00											
Giza 129	0.83	0.58	0.67	0.92	1.00										
Giza 130	0.75	0.67	0.42	1.00	0.92	1.00									
G1	0.50	0.92	0.83	0.75	0.67	0.75	1.00								
G2	0.67	0.75	0.83	0.58	0.67	0.58	0.83	1.00							
G5	0.75	0.67	0.58	1.00	0.92	1.00	0.75	0.58	1.00						
G6	0.67	0.75	0.83	0.75	0.83	0.75	0.83	0.83	0.75	1.00					
G9	0.75	0.67	0.58	1.00	0.92	1.00	0.75	0.58	1.00	0.75	1.00				
G10	0.75	0.67	0.75	0.83	0.92	0.83	0.75	0.75	0.83	0.75	0.83	1.00			
G13	0.58	0.50	0.58	0.67	0.75	0.75	0.58	0.58	0.67	0.58	0.67	0.83	1.00		
G14	0.67	0.75	0.67	0.92	0.83	0.92	0.83	0.67	0.92	0.67	0.92	0.92	0.75	1.00	
G15	0.75	0.50	0.58	0.67	0.75	0.67	0.58	0.75	0.67	0.58	0.67	0.83	0.75	0.83	1.00

Genetic similarity is colored according to the following scale (0.00, no similarity, and 1.00, perfect similarity)

0.00	0.08	0.17	0.25	0.33	0.42	0.50	0.58	0.67	0.75	0.83	0.92	1.00

to use as a marker-assisted selection. Combining field traits with SSR markers provides a comprehensive understanding of drought tolerance.

4 Conclusion

Barley is among the most significant crops. The purpose of this study was to determine how drought-tolerant different barley genotypes were. Some of the genotypes were found to be tolerant (would be preferred in breeding programs), whereas others were sensitive and would be avoided. The effects of water stress on various barley genotypes were investigated at the adult and germination stages. According to water stress, the genotypic responses vary among growth stages. Also, the current study demonstrates how drought stress might have an impact on seed germination and seedling growth metrics. Our findings demonstrated that the sensitive genotypes like G5 and 14 and the examined drought-tolerant barley genotypes like G1 and G2 both exhibited distinct bands created by the Bmag0603 and Bmag770 primers.

Acknowledgement

We sincerely thank the funding agencies, the Partnership for Research and Innovation in the Mediterranean area (PRIMA), and the nationalorganization in Egypt, the Science and Technology Development Fund (STDF). Their generous support enabled the conduct of this study and considerably contributed to its effective conclusion.

References

Aboulilal AA, Mansour M (2017) Efficiency of triple-SCoT primer in characterization of genetic diversity and genotype-specific markers against SSR fingerprint in some Egyptian barley genotypes. *American Journal of Molecular Biology* 7, 123-137. https://doi.org/10.4236/ajmb.2017.73010

Ahmed M, Kheir AMS, Mehmood MZ, et al (2022) Changes in germination and seedling traits of sesame under simulated drought. *Phyton*: *Phyton-International Journal of Experimental Botany* 91, 713-726.
https://doi.org/10.32604/phyton.2022.018552

Badr A, El-Shazly HH, Tarawneh RA, et al (2020) Screening for drought tolerance in maize (*Zea mays* L.) germplasm using germination and seedling traits under simulated drought conditions. *Plants* 9, 565.
https://doi.org/10.3390/plants9050565

Dizkirici A, Guren H E, Önde S, et al (2008). Microsatellite (SSR) variation in barley germplasm and its potential use for marker assisted selection in scald Resistance breeding. *International Journal of Integrative Biology* 4, 9-15.
https://hdl.handle.net/11511/79750

Eivazi AR, Mohammadi SA, Rezaei MA, et al (2013) Effective selection criteria for assessing drought tolerance indices in barley (*Hordeum vulgare* L.) accessions. *International Journal of Agronomy and Plant Production* 4, 813-821.
https://api.semanticscholar.org/CorpusID:140573729

Fu Y, Horbach C (2012) Genetic diversity in a core subset of wild barley germplasm. *Diversity* 4, 239-257. https://doi.org/10.3390/d4020239

Hellal F, El-Shabrawi HM, Abd El-Hady M, et al (2018) Influence of PEG-induced drought stress on molecular and biochemical constituents and seedling growth of Egyptian barley cultivars. *Journal of Genetic Engineering and Biotechnology* 16, 203-212.
https://doi.org/10.1016/j.jgeb.2017.10.009

SPSS Statistics 21.0 (2012) IBM SPSS Statistics for Windows, Version 21.0, Document number: 213045**.**
http://surl.li/ghvrca

ISTA (2019) International rules for seed testing. International Seed Testing Association. https://www.seedtest.org/en/home.html

Jalil A, Salehi M (2012) Evaluation of drought stress indices in barley (*Hordeum vulgare* L.). *Annals of Biological Research* 3, 5515-5520.
http://surl.li/vtlcrl

Jungudo M (2023) The Impact of Climate Change in Egypt. In: Kelechi JA (Ed). Resource Conflict and Environmental Relations in Africa. Palgrave Macmillan, Singapore, pp 173-188.
https://doi.org/10.1007/978-981-19-7343-7_11

Khoothiam K, Prapasawat W, Yosboonruang A, et al (2023) Prevalence, antimicrobial resistance, and enterotoxin gene profiles of *Staphylococcus aureus* isolated from mobile phones of the food vendors in Phayao province, Thailand. *Annals of Clinical Microbiology and Antimicrobials* 22, 68.
https://doi.org/10.1186/s12941-023-00621-y

Maniruzzaman M, Talukder Z, Rohman S, et al (2014) Polymorphism study in barley (*Hordeum vulgare*) genotypes using microsatellite (SSR) markers. *Bangladesh Journal of Agricultural Research* 39, 33–45.
https://doi.org/10.3329/bjar.v39i1.20078

Mansour H, El Sayed MS, Lightfoot D (2020) Molecular studies for drought tolerance in some Egyptian wheat genotypes under different irrigation systems. *Open Agriculture* 5, 280-290. https://doi.org/10.1515/opag-2020-0030

Mansour E, Abdul-Hamid MI, Yasin MT, et al (2017) Identifying drought-tolerant genotypes of barley and their responses to various irrigation levels in a Mediterranean environment. *Agricultural Water Management* 194, 58-67. https://doi.org/10.1016/j.agwat.2017.08.021

Mariey SA, Khaffagy AE, Aiad MA, et al (2022) The influence of the salinity and weed control treatments on some barley cultivars and its associated weeds. *Journal of Global Agriculture and Ecology* 13, 26-50. https://doi.org/10.56557/jogae/2022/v13i27464

Mariey SA, Mohamed M, Khatab I, et al (2013) Genetic diversity analysis of some barley genotypes for salt tolerance using SSR markers. *Journal of Agricultural Science* 5, 12-28. https://doi.org/10.5539/jas.v5n7p12

Metsalu T, Vilo J (2015) ClustVis: a web tool for visualizing clustering of multivariate data using principal component analysis and heatmap. *Nucleic Acids Research* 43, W566-W570. https://doi.org/10.1093/nar/gkv468

Olamide FO, Olalekan BA, Tobi SU, et al (2022) Fundamentals of Irrigation Methods and Their Impact on Crop Production. In: Sultan M, Ahmad F, (Eds), Irrigation and Drainage - Recent Advances. IntechOpen, Rijeka, pp 1-20.

Oraby MA, El-Khawaga AA, Mansour E, et al (2018) Assessing drought tolerance of sixteen barley genotypes under different irrigation treatments. *Zagazig Journal of Agricultural Research* 45, 1193-1208. https://doi.org/10.21608/zjar.2018.48565

Pan Z, Deng G, Zhai X, et al (2008) Molecular analysis of cultivated naked barley (*Hordeum vulgare* L.) from Qinghai-Tibet plateau in China using SSR markers. *Frontiers of Agriculture in China* 2, 372-379. https://doi.org/10.1007/s11703-008-0084-5

Rani V, Yadav MK, Singh R, et al (2024) Genetic Diversity Assessment in Cereal Crops. In: Al-Khayri JM, Jain SM, Penna S, (Eds), Sustainable Utilization and Conservation of Plant Genetic Diversity. ustainable Development and Biodiversity. vol 35 Springer, Gateway East, Singapore, pp 363-398. https://doi.org/10.1007/978-981-99-5245-8_11

Thabet SG, Moursi YS, Karam MA, et al (2018) Genetic basis of drought tolerance during seed germination in barley. *PloS One* 13, e0206682. https://doi.org/10.1371/journal.pone.0206682

Varshney RK, Marcel TC, Ramsay L, et al (2007) A high-density barley microsatellite consensus map with 775 SSR loci. *Theoretical and Applied Genetics* 114, 1091-1103. https://doi.org/10.1007/s00122-007-0503-7

Yang Y, Wang Q, Chen Q, et al (2017) Genome-wide survey indicates diverse physiological roles of the barley (*Hordeum vulgare* L.) calcium-dependent protein kinase genes. *Scientific Reports* 7, 5306. https://doi.org/10.1038/s41598-017-05646-w