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Effects of drought stress on gene expression and morphological traits of the barley cultivar Giza 134

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ABSTRACT

Drought tolerance is a main trait for growing and stabilizing barley productivity in dry areas globally. The current study was conducted to evaluate the morphological and yield-related traits of the barley cultivar "Giza134" in response to drought stress. To determine the impact of drought and stress, the experiment was conducted in the growth chamber and in rainfed conditions. In the growth chamber, seedlings were irrigated, watered (normally), and subjected to 30% PEG-600 (polyethylene glycol 600) as a drought stress condition. Furthermore, barley plants were evaluated during two consecutive seasons, 2021 and 2022, at Nubaria (normal condition), in addition to two different rainfed locations on the northwest coast of Egypt, West Barrani and East Matrouh. Most morphological and yield component traits declined significantly, including plant height (cm), spike length (cm), number of grains per spike, biological yield (BY; ton/fed-1), and grain yield (GY; Ardab Fed-1). Grain yield losses were over 85% in West Barrani and East Matrouh, respectively, compared with Nubaria. To understand the mechanisms of drought tolerance at the molecular level, the gene expression of drought-responsive genes, including *HvAPX1* encodes peroxidase, *HvFNR* encodes ferredoxin-NADP+ reductase, *HvDHN1* encodes dehydrin, *HvSAM* encodes S-adenosyl-L-methionine methyltransferases, *HvEDE* encodes ER degradation enhancer, and *HVABH* encodes alpha/beta-hydrolases, were measured in leaf tissues of "Giza 134." The relative expression levels of *HvAPX1*, *HvFNR,* and *HvDHN1* were significantly (p 0.01) upregulated, with over 8-fold for *HvDHN1*. while *HvSAM, HvEDE* and *HVABH* genes are downregulated in response to drought stress. These findings might provide new insights into the mechanisms of drought tolerance in barley and facilitate future breeding programs for resilient barley crops in a changing global climate.

Keywords: Barley, drought stress, peroxidase, dehydrin, Real time-qPCR

INTRODUCTION

Drought is a major environmental stress issue that impacts the growth and development of plants. The impacts of drought are projected to rise with climate change and growing water scarcity (Elakhdar *et al.,* 2022). Water is an increasingly scarce resource given the current and future human population and societal needs, emphasizing sustainable water use (Rosegrant and Cline 2003). Thus, understanding drought stress and water use about plant growth is important for sustainable agriculture. Drought mainly influences water uptake and increases crop dehydration by damaging the equilibrium of cellular osmosis. This regularly improves different physiological and metabolic disorders, for example, by destroying the activity of photosynthetic enzymes and producing oxidative stress, all of which result in yield reduction (Farooq *et al.,* 2009). Significant evidence has verified that genetic variability of sensitivity to drought conditions occurs within or between crop species (Askarnejad *et al.,* 2021; Bhargava *et al.,* 2013). Therefore, it is recommended to use contrasting genotypes or species to determine the physiological, morphological, and biochemical features that are consistently linked with drought tolerance (Bista *et al.,* 2018; Blum 2017; Chaves *et al.,* 2003).

Plant responses to different stress elements can be reflected on a range of levels of their regulation, starting with the molecular background, over cells and organs, and terminating the whole plant. At the molecular level, responses of plant comprise a large number of genes that regulates plant physiology such as osmotic adjustments, plant hormones, and defense systems of antioxidant, in addition to morphology and anatomy such as modifications in leaf rolling, plasma membranes and modifications in stomatal density (Umezawa et al. 2006; Thameur *et al.,* 2012; Tezara et al. 1999). Plants have also used special defense systems to adapt to abiotic stress such as drought stress

for subsistence. During abiotic stresses to accumulate the reactive oxygen species (ROS) is one of the major plant defensive machines. Some subcellular organelles for example mitochondria, chloroplasts, and peroxisomes are the regular supports of the ROS generation (Asada 2006). In response to the stress, the ROS accumulated, including hydroxyl radical, superoxide, singlet oxygen, and hydrogen peroxide (H_2O_2), work as signal molecules (Gill and Tuteja 2010). Unnecessary ROS production causes protein degradation, enzyme inhibition, cellular damage, damage to DNA and RNA, and ultimately cell death (Asada 2006). Therefore, the ROS detoxification mechanism in plant cells is essential against the lethal impact of ROS in plants. The ROS detoxification mechanisms include enzymatic and non-enzymatic antioxidant events (Vranova *et al.,* 2002). The enzymatic antioxidants superoxide dismutase (SOD), comprise catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPX), and peroxiredoxin (PrxR). These enzymes essentially exist in sub-cellular organelles. The over-expression of the genes encoding antioxidant-enzyme might prompt tolerance to abiotic stresses.

Barley is among the most essential cereal crops produced in several developing countries, where it is regularly subject to exciting drought stress that significantly impacts production (Ceccarelli *et al.,* 2007). Elucidating the mechanisms of drought tolerance in barley might accelerate a better understanding of the genetic bases of its regulation, and allow the successful use of genetic and genomic methods to develop its tolerance. Experiments have been used to monitor the expression of genes that respond to abiotic stresses. Some of these experiments were conducted with a short period of dehydration stress (Harb *et al.,* 2015; Harb *et al.,* 2020). These studies identified several differentially expressed genes corresponding to drought-related stress. Furthermore, most previous studies of drought stress were conducted on seedlings associated with grain yield (Ahmed *et al.,* 2013). Hence, gene expression analysis for drought tolerance through the seedling stage could afford extra insight into the molecular regulation of drought tolerance in barley. The mechanism of drought tolerance in barley is still unknown well (Elakhdar *et al.,* 2022).

This study aims to; (i) evaluate the morphological and yield related-treats responses of the barley cultivar "Giza 134" to drought stress at the seedling stage using 30% PEG-600 as well as at the adult stage under rainfed conditions. (ii) to investigate the expression of some drought-responsive genes at the seedling stage.

MATERIALS AND METHODS

Plant culture and stress treatments:

Two experiments were performed to investigate the response of barley cv. "Giza 134" to drought stress at seedling and grain yield stages. (I) In the growth chamber experiment, the drought stress was conducted using PEG 600. Seeds were surface-sterilized by using bleach and Tween 20 (Sigma), washed several times with distilled water, and grown on (23 23 19 cm) pots containing seedling-raising soil (JA Kumiai King Soil; Agr. Japan Co., Ltd.) with four plants per pot. Seeds were sown for 10 days in normal conditions, then treatment was induced for 15 days using 30% (- 1.03 MPa) of PEG 600 (Sigma Aldrich) in a growth chamber (SANYO) at 27 °C for 16 hours and 25 °C for 10 hours in the dark. After 15 days of treatment, plant leaves were collected and stored at -80 °C for gene expression analyses. The experiment was laid out in a completely randomized design (CRD) in three replicates with 12 seeds each.

(II) To assess the response to drought stress at the adult stage, the "Giza 134" genotype was evaluated under normal and rainfed conditions within the nursery experiment at the experimental farms of the Agricultural Research Center in Egypt. The experiment was conducted during two consecutive seasons, 2021 and 2022 in a randomized complete block design with three replications. The rainfed locations on the northwest coast include West Barrani (31°.36N 25°.55E) and East Matrouh (Matrouh government; 29.66°N 27.51°E). While the normal condition was the experimental farm of Nubaria Agricultural Research Station, Nubaria (30.40°N 30.04°E). The recommended irrigation amount was applied at the normal condition, Nubaria, with an average of 1130 m^3. The location characterizations are listed in Figure 1.

Statistical analysis:

The student's t-test was performed on the variables of days to heading (days), days to maturity (days), plant high (PH; cm), spike length (SL; cm), No. spike/ m^2, biological yield (BY; ton/ Fed^{-1}), grain yield (GY; Ardab Fed^{-1}). Differences with $P \leq 0.01$ values were considered significant. Correlation coefficients and *P* values were calculated using "Hmisc" packages in R, and the "corrplot" package was used for drawing scatter plots.

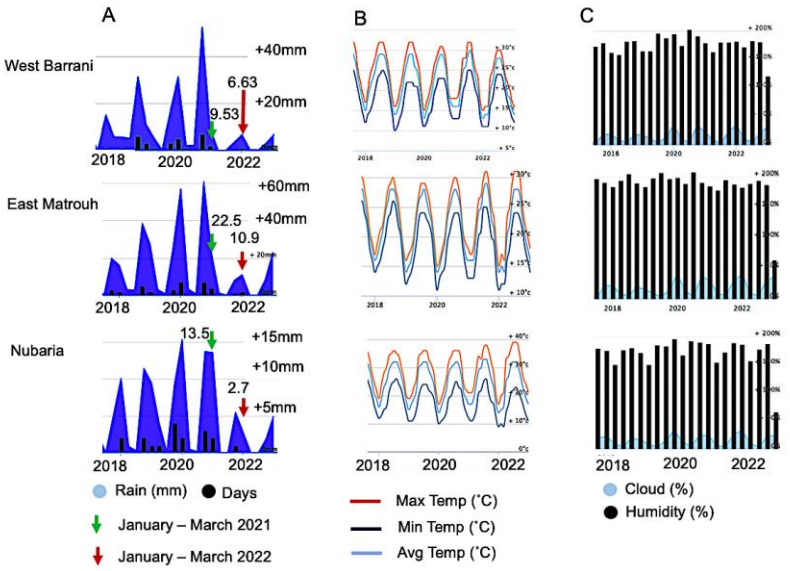

Fig. 1. The locations studied have characterizations. A. Averages of rainfall amount (mm) and rain days average B. Annual averages of cloud and humidity (January to March show the high level of precipitation). Data source: World Weather Online.

RNA isolation and real-time quantitative PCR:

The total RNA was extracted from the frozen leaves under the well-watered and 30% PEG-600 drought treatment using an RNeasy® Plant Mini Kit (QIAGEN, Hilden, Germany). The quality of the isolated RNA was checked by Nanodrop2000 Spectrophotometer (USA). Real-time quantitative PCR (RT-qPCR) was performed using a Primer Script™ RT reagent kit (TaKaRa, Japan) according to (Elakhdar et *al.,* 2019). A 1.5µg of the total RNA was used to synthesize the first complementary DNA (cDNA). The expression of drought-related genes was measured based on three technical replicates, subsequent the manufacturer protocol from the KAPA SYBR® FAST qPCR kit (Kapa Biosystems). RT-qPCR was conducted using as the following: an initial incubation at 95 °C for 3 min, followed by 40 cycles of denaturation at 95 °C for 3 sec, and extension at 60 °C for 20 sec. The barley housekeeping gene *β-tubulin* was used as an internal control to normalize the gene expression of drought-related genes *HvAPX1*, *HvFNR*, *HvSAM, HvEDE, HvDHN1,* and *HVABH* according to the comparative Ct method ($2^{-\Delta\Delta Ct}$) (Schmittgen and Livak 2008), using CFX96 real-time detection system (Bio-Rad, Hercules, CA, USA). The sequences of the oligonucleotide primers used in this study are listed in Table 2.

Table 1. Oligo primers utilized for quantitative real-time RT-qPCR.

Gene ID		Sequence of primers (5'- 3')	Size	Putative Function*
HORVU2Hr1G018510	*HvAPX1*	F: AAGCAAGGTTGGGCGTTTGG	172	Peroxidase
		R: GCCACATCCGCCAATTCATGT		
HORVU7Hr1G002210	*HvFNR*	F: TTGTGCATATGTGCCGACGC	184	Ferredoxin-NADP⁺ reductase
		R: TGAGTGAAACGCTGTTGGGAA		
HORVU5Hr1G120390	*HvSAM*	F: ACGTTCTTCTGCGCCCTTGA	177	S-adenosyl-L-methionine-dependent methyltransferases
		R: ACCCAGTGGTTTCAGCACCT		
HORVU3Hr1G075850	*HvEDE*	F: GGGCACGCAGGTCAAAACTC	81	ER degradation enhancer
		R: CTGCATCCTGCTTTTGTGTCCA		
HORVU3Hr1G089300	*HvDHN1*	F: GATGACACCTTGGGTCGGGT	116	Dehydrin
		R: GCAGTGCCAAACAGGTTGTCC		
HORVU3Hr1G087020	*HVABH*	F: CGGGCGTCGAGTAGAGACAA	147	Alpha/beta-Hydrolases superfamily protein
		R: GCGAACCGTTTTCTTTGTGGAA		
Tubulin		F: AGT GTCCTGTCCACCCACTC		Housekeeping
		R: AGCATGAAGTGGATCCTT GG		

*: Source; Corresponding annotations were retrieved from the BARLEYMAP platform version of the MorexV3 genome.

RESULTS

Phenotypic Responses to drought stress:

Figure 1 shows how the severity of drought stress was measured by looking at the barley "Giza 134" in the nursery experiment at two different sites over two seasons. The levels of precipitation varied between the locations, the West Barrani location recorded the lowest rain level while East Matrouh had the highest level (Figure 1). The response to drought stress was assessed on several growth and yield-related parameters including days to heading (days), days to maturity (days), plant height (PH; cm), spike length (SL; cm), Number of grains per spike (No. Grain/spike), biological yield (BY; ton/ Fed^{-1}), grain yield (GY; Ardab Fed^{-1}) (Figure 2).

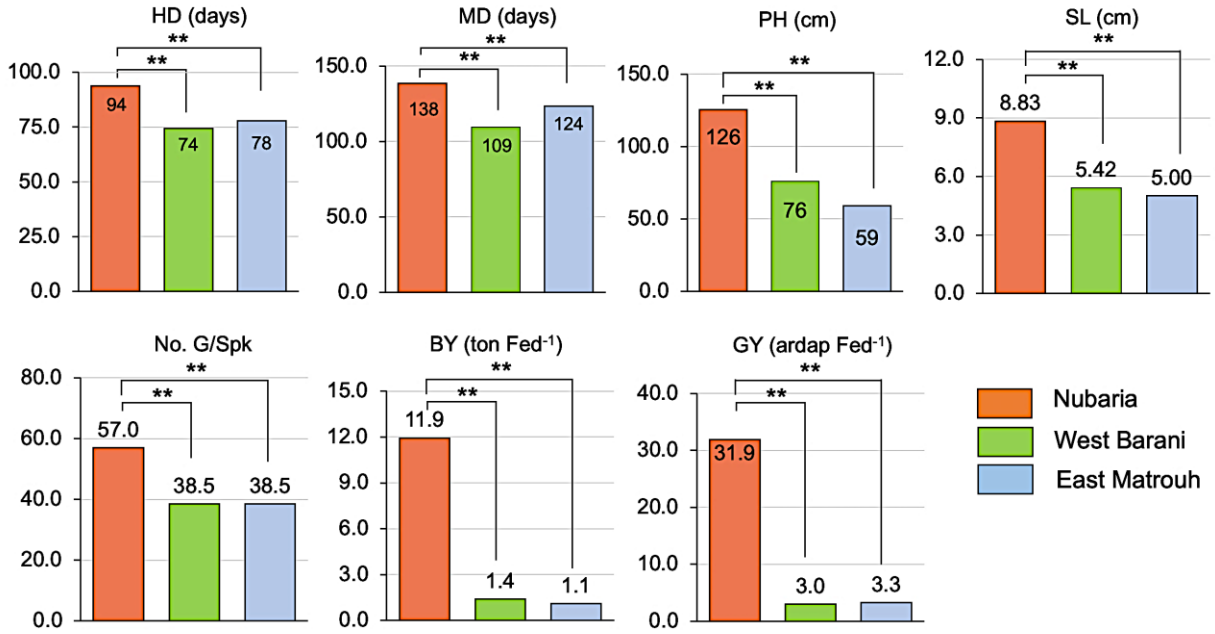

Fig. 2. Morphological and yield related-traits response of the barley cultivar "Giza 134" under drought stress including, days to heading (days), days to maturity (days), plant height (PH; cm), spike length (SL; cm), No. grains/spike (No. G/Spk), biological yield (BY; ton/ Fed^{-1}), grain yield (GY; Ardab Fed^{-1}). Values represent the mean (2021 and 2022 seasons) ± SE of three replicated experiments. The recommended irrigation amount was applied at the normal condition, Nubaria, with an average of 1130 m^3. The precipitation rate is indicated in Figure 1. Asterisks indicate statistically significant differences among different conditions by Student's t-test (**p< 0.01).

The result revealed that the average of the days to heading was 74 days and 78 days in West Barrani and East Matrouh, respectively compared with 93 days in Nubaria (irrigated). While the days to maturity were 109 days and 124 days in West Barrani and East Matrouh, respectively compared with 138 days in Nubaria. This means the "Giza 134" plants exhibited early maturity in response to drought stress. The plants escaped the impact of drought stress by adjusting their morpho-physiology traits to complete their life cycle, which is an important mechanism for drought tolerance. The other parameters studied declined under the impact of drought compared to irrigated. The yield related-traits include plant height (PH; cm), spike length (SL; cm), and No. grains/ spike, biological yield (BY; ton/ Fed^{-1}), and grain yield (GY; Ardab Fed^{-1}) were decreased significantly by p< 0.01 under the two rainfed locations compared with the irrigated location (Figure 2). The grain yield losses were 77.07 % and 89.70 % in West Barrani and East Matrouh, respectively compared with Nubaria.

Figure 3 displays the correlation between traits studied of the stressed plants. A positive correlation was observed between the heading date and maturity date (r>0.8), and between plant height and heading date and maturity date. In contrast, a negative significant correlation was observed between BY and GY with the HD and MD, in addition to BY and GY with PH. These results indicate that the earlier plant in flowering is the higher yield plant. The barley plant escapes the drought stress by tuning to early maturity. Thus, these criteria are important for the selection of drought tolerance.

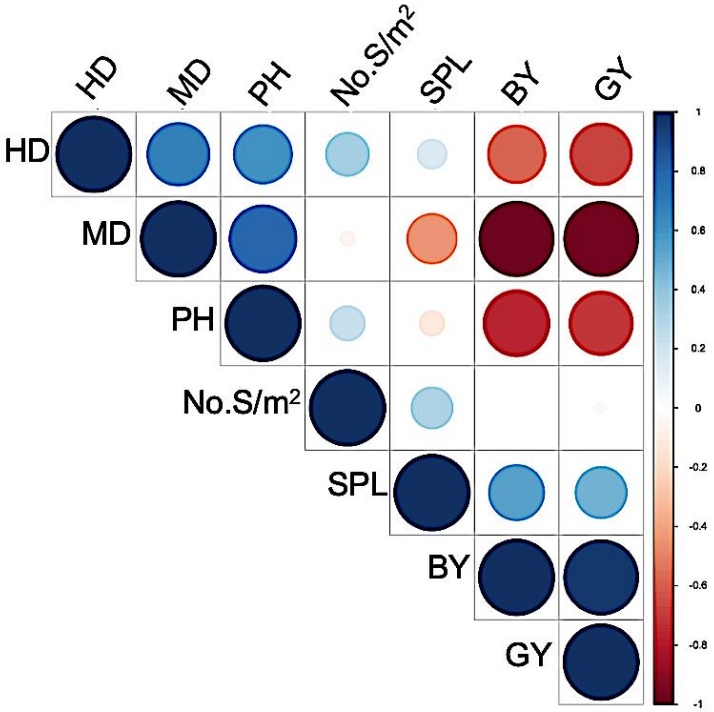

Fig. 3. The correlation analysis between the traits studied under the drought condition (combined data). The positive correlations are revealed in blue while negative correlations are in red. The color intensity and the size of the circle are proportional to the correlation coefficients. The legend color shows the correlation coefficients and the corresponding colors on the right side. Plant height (PH; cm), spike length (SL; cm), Number of grains per spike (No. G/Spk), biological yield (BY; kg), and grain yield (GY; kg).

Expression of some drought-responsive genes:

The whole transcriptome analysis using RNA-Seq of the barley "Giza 134", shows hundreds of deferentially expressed genes (DEGs) were up-or-downregulated (Data under publishing), revealing a high level of plasticity has occurred in response to drought stress. The current study shows the relative expression of some drought-responsive genes in irrigated and drought-stressed conditions. The expression patterns of genes *HvAPX1*, *HvFNR*, *HvSMM, HvEDE, HvDHN1,* and *HVABH* genes were performed in Giza 134 cultivar after 15 days of the severe drought stress using 30 % PEG (Figures 4 and 5). The *HvAPX1* gene encodes peroxidase, *HvFNR* encodes Ferredoxin-$NADP^+$ reductase*, HvSAM* encodes S-adenosyl-L-methionine methyltransferases*, HvEDE* encodes, ER degradation enhancer*, HvDHN1* encodes dehydrin and *HVABH* encode alpha/beta-hydrolases. These genes are involved in plant adaptation to drought stress through the generation of reactive oxygen species (ROS), photosynthesis, protein folding into the endoplasmic reticulum, and polyamine oxidation.

Gene expression analysis based on RT-qPCR reveals high significant different ($P < 0.01$) between the RNA samples from control and stressed plants. The expression level of the genes studied *HvAPX1, HvFNR,* and *HvDHN1* were up regulated in the drought-treated plants of the "Giza 134" genotype (Figure 4). It's worth mentioning, the *HvDHN1*gene increased over8-fold (Figure 4). The results suggested that changes in the expression of genes studied were probably associated directly with the "Giza 134" response to drought stress. In contrast, three genes were exhibited in the low expression levels of *HvSAM*, *HvEDE,* and *HVABH* under drought stress (Figure 5). Their expression, however, was inhibited significantly via drought stress.

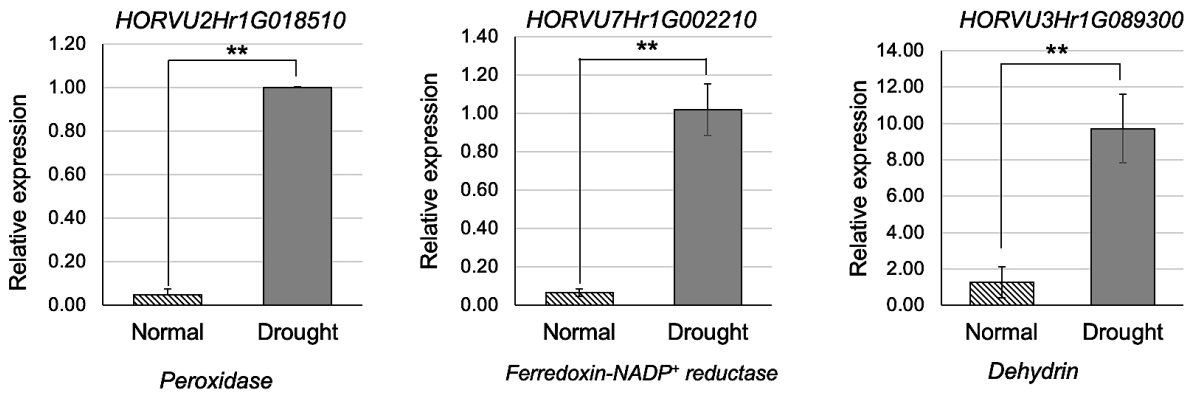

Fig. 4. Expression levels of mRNA for upregulated genes in "Giza 134" under the well-watered and drought stress. Real-time qPCR was carried out with cDNA obtained from well-watered control plants and plants exposed to drought stress for 15 days using 30% PEG 600. Gene expression was normalized to each tissue by*β-tubulin*. Each bar is the mean ± SD of analyses completed in triplicate. Significant differences are indicated as (**$p < 0.01$).

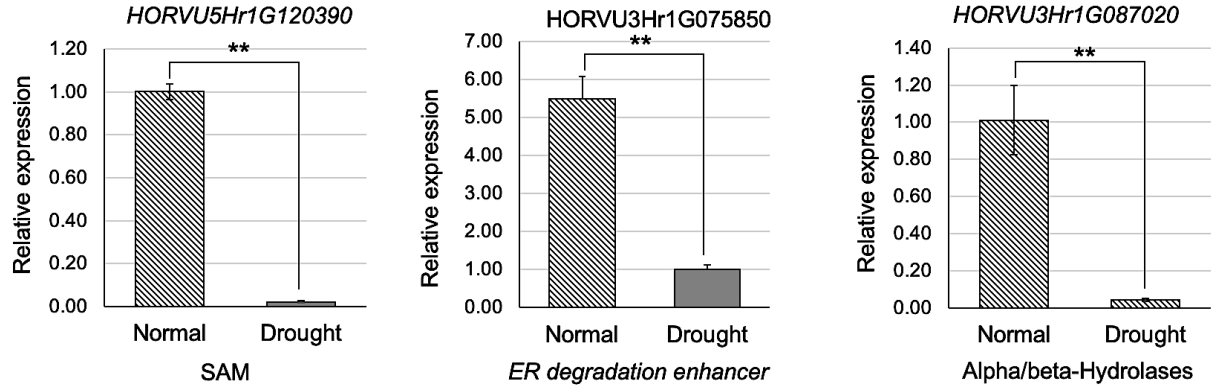

Fig. 5. Expression levels of mRNA for downregulated genes in "Giza 134" under the well-watered and drought stress. Real-time qPCR was carried out with cDNA obtained from well-watered control plants and plants exposed to drought stress for 15 days using 30% PEG 600. Gene expression was normalized to each tissue by*β-tubulin*. Each bar is the mean ± SD of analyses completed in triplicate. Significant differences are indicated as (**$p < 0.01$).

DISCUSSION

An adequate strategy of drought-stress investigation and accurate statistical analysis are important for assessing the drought tolerance of barley genotypes. The soil water content and availability affect the amount of water that plants can uptake from the soil for their growth and development. True determination of whether, to what degree, and when a plant is susceptible to water stress is a vital step in assessing drought tolerance (Elakhdar et al., 2022). The barley cultivar "Giza 134" was previously released as a candidate cultivar for newly reclaimed areas in Egypt (El-Sayed *et al.,* 2011). In the current study, a procedure was developed to simulate field drought environments in a growth chamber by controlling the soil water content available during the seedling stage. Giza 134 was also evaluated under rainfed conditions to assess the effect of water shortages on yield-related traits at the reproductive stage. With this method, several morphological and yield-related traits were measured under control and drought conditions to estimate the response of the Giza 134 barley genotype to drought stress. The yield losses were high due to drought stress, in good agreement with previous reports (Ahmed *et al.,* 2013). Differential morphological and physiological responses to water deficit were established on the seedling of "Giza 134" using 30% PEG 600 (Data under publishing). The results revealed that several traits including the crop growth rate, Leaf relative water content leaf area duration, flag leaf area, chlorophyll a, b and total chlorophyll (Moradi and Ismail 2007), and

net photosynthesis rate (µmol m^2s^1) (Lawson and Blatt 2014) were decreased significantly when p< 0.01 under water deficit, compared to the welled-water condition. While the proline content was 29.8% higher than the welled-water condition (Bandurska and Stroiński 2003). Rum barley genotype displayed a similar sensitivity to drought stress when it was exposed to drought (Harb *et al.,* 2010).

In this study, the expression of some drought-responsive genes was measured in the Giza 134. We observed that the relative expression ratio in *HvAPX1*, *HvFNR*, and *HvDHN1*, increased under stress conditions while *HvSMM*, *HvEDE*, and *HVABH* were down-regulated. Alterations in enzyme activities perhaps associate directly with the expression of their corresponding genes.

The peroxidase (*HvAPX1*) gene, an enzymatic antioxidant was known for its important role in plants subjected to abiotic stresses (Gill and Tuteja 2010). Under water deficit conditions this enzyme protects plants against ROS-produced oxidative agents in barley (Mittler 2002; Ashraf and Foolad 2007). Peroxidase (APX) is one of the key enzymatic antioxidants for cellular scavengers of H_2O_2 (Willekens *et al.,* 1997). This enzyme essentially exists in all subcellular organelles that can eliminate a single type of ROS (Mittler 2002). APX functions in concert with glutathione reductase in the ascorbate-glutathione cycle to electively scavenge H2O2, thus sustaining low levels in cells (Noctor *al.,* 2014). In this pathway, APX uses two molecules of ascorbate to convert H2O2 to water; for the regeneration of ascorbate by dehydroascorbate, using glutathione as substrate; and for the subsequent recovery of glutathione catalyzed by glutathione reductase. This cycle is suggested to be the most effective result of alleviating oxidative stress (Mittler, 2002) (Figure 6). Consequently, the over-expression of genes encoding the antioxidant enzyme might induce abiotic stress tolerance.

Upon drought stress, the ferredoxin-$NADP^{+-}$reductase (*HvFNR*) gene increased in Giza 134. Ferredoxins are small, soluble (2Fe–2S) proteins that show an important role in electron distribution in the plastids (Morigasaki *et al.,* 1993). Photo-reduced Fxdin chloroplasts can cooperate with Fxd–$NADP^+$ reductase (FNR) to produce the NADPH necessitated for carbon assimilation and other protective and biosynthetic pathways (Arakaki *et al.,* 1997) (Figure 6). In Arabidopsis expression level of two FNR genes was up-regulated under drought stress, while low levels of FNR protein were observed in the water-stressed leaves (Lehtimaki *et al.,* 2010). In chloroplasts, FNR isoenzymes are existing as soluble pools and are membrane-bound. A similar release of FNR from the thylakoid membrane happens when the plants are subjected to high light (Benz *et al.,* 2009).

As noted in this study, the *HvDHN1* gene shows higher expressions in Giza 134 cultivar under severe drought stress as compared to normal conditions (Harb *et al.,* 2010). This result is consistent with the findings of Harb *et al.,* (2010), that two dehydrins genes, *HvDHN1* and *HvDHN9*, exhibit higher transcription levels in barley under severe drought stress (Harb *et al.,* 2010). In *Arabidopsis thaliana*, early responses to dehydration genes are quickly induced in response to different abiotic and biotic stresses, such as drought, temperature, and high salt. *DHN* genes could act as water attractants in cells with low water potential, resulting in an osmotic potential regulation (Nylander et al. 2001). Upregulation of the *Dhn6* gene was detected during water deficit induced by dehydration (Qian *et al.,* 2008), which was the same as the results obtained in the cultivated barley (Choi *et al.,* 1999). Likewise, the Dhn6 gene was significantly upregulated in tolerant plants in comparison with drought-sensitive wild barleys at earlier stages (Suprunova *et al.,* 2004). As a validation of these findings, we noted high levels of *HvDHN1* over an 8-fold gene in drought-stressed plants after 15 days of dehydration.

On the other hand, the mRNA expression of the S-adenosyl-L-methionine-dependent methyltransferases (*HvSAM*) was downregulated in Giza 134 leaves under drought stress. The SAMs catalyze the biosynthesis of SAM from methionine and ATP (Tabor and Tabor, 1984). The SAM serves as a methyl donor for pectin methylesterases and O-methyltransferase, participating in the lignin and pectin metabolism (Lamblin *et al.,* 2001). SAM is decarboxylated via S-adenosyl- methionine decarboxylase to work as a precursor for polyamine biosynthesis (Evans and Malmberg 1989). In higher plants, ethylene is synthesized from SAM and consumed in different physiological processes including stress responses (Yang and Hoffman, 1984). These findings suggest that SAM is essential for the growth and development of plants and it can be a potential candidate for assisting the stress-response mechanisms of barley. In alfalfa, overexpression of the SAM gene enhanced the tolerance to cold stress via stimulating polyamine oxidation and rising tolerance to hydrogen peroxide-induced antioxidant protection (Guo *et al.,* 2014). High level of SAMs was observed in cold-stressed rice (Cui *et al.,* 2005) and salt-stressed barley (Witzel *et al.,* 2009). In contrast, a declined level of SAMs was detected in flooding and drought-exposed soybean (Nanjo *et al.,* 2010; Wang *et al.,* 2016). These results indicate that SAMs are involved in the regulation of stress-response mechanisms to flooding and drought in soybean.

The total *HvEDE* mRNA down regulated more than 4-fold upon the drought stress in Giza 134. Unassembled, misfolded, or mutated proteins are degraded in cells via a proteolytic mechanism. The endoplasmic reticulum (ER), the main compartment for protein folding and quality control, likewise has a particular proteolytic system. Evidence showed that the ubiquitin-proteasome mechanism of the ER degradation apparatus also extends into the cytoplasm (Sommer and Wolf, 1997). Meanwhile, abiotic stress cause misfolded protein accumulation, while protein breakdown and recycling are important feature of the plant response to environmental stress (Hieng *et al.,* 2004). In the current study, down regulated *HvEDE* and the yield components significantly declined in the barley plants due to drought stress. This might be because the drought stress impacts the protein aggregation in the starchy endosperm, for example, and leads to yield losses.

The expression of the *HVABH* gene was significantly decreased in the drought-stressed Giza 134 barley plants. The α/β-hydrolase superfamily is folded enzymes among the major protein families, comprising hydrolases, carboxylesterase, acetylcholinesterase, lipase, diene-lactone hydrolase, cutinase, proline aminopeptidase, thioesterase, serine carboxypeptidase, proline oligopeptidase and epoxide hydrolase beside enzymes that require activation of HCN, H_2O_2 or O_2 instead of H_2O for the reaction mechanism (haloperoxidase, hydroxy nitrile lyase, haloalkane dehalogenase) (Bugg, 2004). The functions of α/β-hydrolase enzymes widely include metabolism, biosynthesis, gene regulation, and signal transduction (Lord *et al.,* 2013). Some α/β-hydrolase fold enzymes such as OsPOP5, esterase, and phospholipase D have been indicated to participate in plant salt tolerance (Liu *et al.,* 2014; Hong *et al.,* 2010). Thus, the drought stress in this study could lead to a lack of α/β-hydrolase transcription, while the novel α/β-hydrolase IbMas overexpression exhibited an enhanced salt tolerance (Liu *et al.,* 2014).

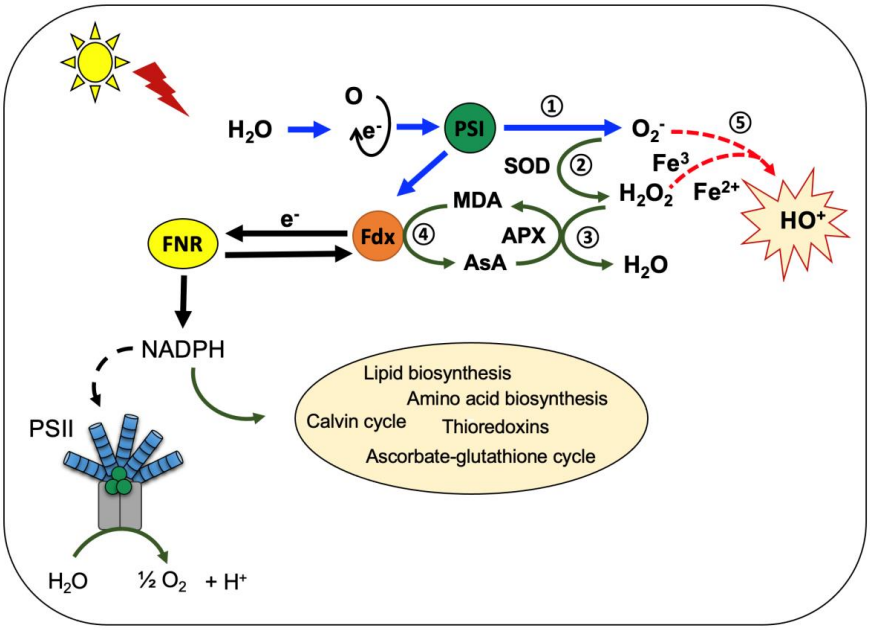

Fig. 6. Schematic showing the pathways of *APX* and *FNR* genes. The Mehler-peroxidase reaction or the water-water cycle includes the leakage of electrons from the photosynthetic electron transport chain to oxygen with the generation of superoxide (1), which is additionally disputed by SOD-developing H_2O_2(2). H_2O_2 is then scavenged via a thylakoidal APX through the generation of water (3). This cycle acquires it's decreasing power by ascorbate regeneration via ferredoxin (4). Side reactions occur in the chloroplasts are the metal-catalyzed Haber-Weiss/Fenton reactions that result in constructing the highly reactive hydroxyl radical (5). PSI, photosystem I, MDA, monodehydroascorbate, Fdx, ferredoxin, APX, thylakoidal ascorbate peroxidase, AsA, ascorbate, SOD, superoxide dismutase. On the stromal side, the structures of the soluble proteins and ferredoxin-$NADP^+$ reductase (FNR) transfer the e^- to $NADP^+$ to form NADPH.

CONCLUSION

In summary, phenotypic and RT-qPCR results show that Giza 134 has a high level of phenotypic plasticity, which is one of the main ways that phenotypes change in response to changes in the environment. For example, after 15 days of drought stress, *HvAPX1, HvFNR, and HvDHN1* genes were up-regulated under drought stress. These results indicate that these genes can be used as useful markers to study the differential response of other barley cultivars at an early stage as an indicator to improve new drought-tolerant cultivars.

REFERENCES

Ahmed, I. M., Cao, F., Zhang, M., Chen, X., Zhang, G., & Wu, F. (2013). Difference in yield and physiological features in response to drought and salinity combined stress during anthesis in Tibetan wild and cultivated barleys. **PLoS One,** 8(10), e77869.

Arakaki, A. K., Ceccarelli, E. A., & Carrillo, N. (1997). Plant-type ferredoxin-NADP+ reductases: A basal structural framework and a multiplicity of functions. *FASEB Journal,* 11(2), 133-140.

Asada, K. (2006). Production and scavenging of reactive oxygen species in chloroplasts and their functions. *Plant Physiology,* 141(2), 391-396.

Ashraf, M., & Foolad, M. R. (2007). Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environmental and Experimental Botany,* 59(2), 206-216.

Askarnejad, M. R., Soleymani, A., & Javanmard, H. R. (2021). Barley (Hordeum vulgare L.) physiology including nutrient uptake affected by plant growth regulators under field drought conditions. *Journal of Plant Nutrition*, 44(15), 1-17.

Bandurska, H., & Stroiński, A. (2003). ABA and proline accumulation in leaves and roots of wild (*Hordeum spontaneum*) and cultivated (*Hordeum vulgare* 'Maresi') barley genotypes under water deficit conditions. *Acta Physiologiae Plantarum,* 25(1), 55-61.

Benz, J. P., Stengel, A., Lintala, M., Lee, Y. H., Weber, A., Philippar, K., Gugel, I. L., Kaieda, S., Ikegami, T., Mulo, P., Soll, J., & Bolter, B. (2009). Arabidopsis Tic62 and ferredoxin-NADP(H) oxidoreductase form light-regulated complexes that are integrated into the chloroplast redox poise. *Plant Cell,* 21(12), 3965-3983.

Bhargava, S., Sawant, K., & Tuberosa, R. (2013). Drought stress adaptation: Metabolic adjustment and regulation of gene expression. *Plant Breeding,* 132(1), 21-32.

Bista, D. R., Heckathorn, S. A., Jayawardena, D. M., Mishra, S., & Boldt, J. K. (2018). Effects of drought on nutrient uptake and the levels of nutrient-uptake proteins in roots of drought-sensitive and -tolerant grasses. *Plants* (Basel), 7(2), 44.

Blum, A. (2017). Osmotic adjustment is a prime drought stress adaptive engine in support of plant production. *Plant, Cell & Environment,* 40(1), 4-10.

Bugg, T. D. H. (2004). Diverse catalytic activities in the αβ-hydrolase family of enzymes: Activation of H2O, HCN, H2O2, and O2. *Bioorganic Chemistry,* 32(5), 367-375.

Ceccarelli, S., Grando, S., & Baum, M. (2007). Participatory plant breeding in water-limited environments. *Experimental Agriculture,* 43(4), 411-435.

Chaves, M. M., Maroco, J. P., & Pereira, J. S. (2003). Understanding plant responses to drought - from genes to the whole plant. *Functional Plant Biology,* 30(3), 239-264.

Choi, D. W., Zhu, B., & Close, T. J. (1999). The barley (*Hordeum vulgare* L.) dehydrin multigene family: sequences, allele types, chromosome assignments, and expression characteristics of 11 Dhn genes of cv Dicktoo. *Theoretical and Applied Genetics,* 98(8), 1234-1247.

Cui, S., Huang, F., Wang, J., Ma, X., Cheng, Y., & Liu, J. (2005). A proteomic analysis of cold stress responses in rice seedlings. *Proteomics,* 5(12), 3162-3172.

El-Sayed, A. A., El-Moselhy, M. A., Abd El-Hameed, M. M., Amer, K. A., Megahed, M. A., Saad, M. F., Ashmawy, H. A., Eid, A. A., Abo El-Enein, R. A., El-Gamal, A. S., Ahmed, I. A., Noaman, M. M., Shendy, M. Z., Abaas, S. I., & Badawy, A. A. (2011). Giza 133 and Giza 134, two new barley varieties for new reclaimed areas in Egypt.

Elakhdar, A., Solanki, S., Kubo, T., Abed, A., Elakhdar, I., Khedr, R., Hamwieh, A., Capo-chichi, L. J. A., Abdelsattar, M., Franckowiak, J. D., & Qualset, C. O. (2022). Barley with improved drought tolerance: Challenges and perspectives. *Environmental and Experimental Botany, 201*, 104738.

Elakhdar, A., Ushijima, T., Fukuda, M., Yamashiro, N., Kawagoe, Y., & Kumamaru, T. (2019). Eukaryotic peptide chain release factor 1 participates in translation termination of specific cysteine-poor prolamines in rice endosperm. *Plant Science*, 281, 223-231.

Evans, P. T., & Malmberg, R. L. (1989). Do polyamines have roles in plant development? Annual Review of Plant *Physiology and Plant Molecular Biology,* 40(1), 235-269.

Farooq, M., Wahid, A., Kobayashi, N., Fujita, D., & Basra, S. M. A. (2009). Plant drought stress: Effects, mechanisms and management. *Agronomy for Sustainable Development,* 29(1), 185-212.

Gill, S. S., & Tuteja, N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiology and Biochemistry,* 48(12), 909-930.

Guo, Z., Tan, J., Zhuo, C., Wang, C., Xiang, B., & Wang, Z. (2014). Abscisic acid, H2O2 and nitric oxide interactions mediated cold-induced S-adenosylmethionine synthetase in Medicago sativa subsp. falcata that confers cold tolerance through up-regulating polyamine oxidation. *Plant Biotechnology Journal,* 12(5), 601-612.

Harb, A., Awad, D., & Samarah, N. (2015). Gene expression and activity of antioxidant enzymes in barley (Hordeum vulgare L.) under controlled severe drought. *Journal of Plant Interactions,* 10(1), 109-116.

Harb, A., Krishnan, A., Ambavaram, M. M., & Pereira, A. (2010). Molecular and physiological analysis of drought stress in Arabidopsis reveals early responses leading to acclimation in plant growth. Plant Physiology, 154(3), 1254-1271.

Harb, A., Simpson, C., Guo, W., Govindan, G., Kakani, V. G., & Sunkar, R. (2020). The Effect of Drought on Transcriptome and Hormonal Profiles in Barley Genotypes with Contrasting Drought Tolerance. *Frontiers in Plant Science,* 11, 618491.

Hieng, B., Ugrinovic, K., Sustar-Vozlic, J., & Kidric, M. (2004). Different classes of proteases are involved in the response to drought of Phaseolus vulgaris L. cultivars differing in sensitivity. *Journal of Plant Physiology,* 161(5), 519-530.

Hong, Y., Zhang, W., & Wang, X. (2010). Phospholipase D and phosphatidic acid signaling in plant response to drought and salinity. *Plant, Cell and Environment*, 33(4), 627-635.

Lamblin, F., Saladin, G., Dehorter, B., Cronier, D., Grenier, E., Lacoux, J., ... & David, A. (2001). Overexpression of a heterologous sam gene encoding S-adenosylmethionine synthetase in flax (*Linum usitatissimum*) cells: Consequences on methylation of lignin precursors and pectins. *Physiologia Plantarum,* 112(2), 223-232.

Lawson, T., & Blatt, M. R. (2014). Stomatal size, speed, and responsiveness impact on photosynthesis and water use efficiency. *Plant Physiology,* 164(4), 1556-1570.

Lehtimäki, N., Lintala, M., Allahverdiyeva, Y., Aro, E. M., & Mulo, P. (2010). Drought stress-induced upregulation of components involved in ferredoxin-dependent cyclic electron transfer. *Journal of Plant Physiology,* 167(12), 1018-1022.

Liu, D., Wang, L., Zhai, H., Song, X., He, S., & Liu, Q. (2014). A novel alpha/beta-hydrolase gene IbMas enhances salt tolerance in transgenic sweetpotato. *PLoS One,* 9(12), e115128.

Lord, C. C., Thomas, G., & Brown, J. M. (2013). Mammalian alpha beta hydrolase domain (ABHD) proteins: Lipid metabolizing enzymes at the interface of cell signaling and energy metabolism. *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids,* 1831(4), 792-802.

Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. Trends in Plant Science, 7(9), 405-410.

Moradi, F., & Ismail, A. M. (2007). Responses of photosynthesis, chlorophyll fluorescence and ROS-scavenging systems to salt stress during seedling and reproductive stages in rice. *Annals of Botany,* 99(6), 1161-1173.

Munns, R., & Tester, M. (2008). Mechanisms of salinity tolerance. Annual Review of Plant Biology, 59, 651-681.

Nakabayashi, K., Okamoto, M., Koshiba, T., Kamiya, Y., & Nambara, E. (2005). Genome-wide profiling of stored mRNA in Arabidopsis thaliana seed germination: Epigenetic and genetic regulation of transcription in seed. *The Plant Journal,* 41(5), 697-709.

Niu, Y., Jin, G. L., Li, R., Hao, R., Li, J., & Wang, X. (2013). Responses of photosynthesis, antioxidant system and gene expression in *Trifolium repens* L. under drought stress. *Acta Physiologiae Plantarum,* 35(3), 889-901.

Prasch, C. M., & Sonnewald, U. (2013). Signaling events in plants: stress factors in combination change the picture. *Environment and Experimental Botany*, 94, 4-14.

Sakuma, Y., Liu, Q., Dubouzet, J. G., Abe, H., Shinozaki, K., & Yamaguchi-Shinozaki, K. (2002). DNA-binding specificity of the ERF/AP2 domain of Arabidopsis DREBs, transcription factors involved in dehydration-and cold-inducible gene expression. *Biochemical and Biophysical Research Communications,* 290(3), 998-1009.

Seki, M., Umezawa, T., Urano, K., & Shinozaki, K. (2007). Regulatory metabolic networks in drought stress responses. *Current Opinion in Plant Biology,* 10(3), 296-302.

Shinozaki, K., Yamaguchi-Shinozaki, K., & Seki, M. (2003). Regulatory network of gene expression in the drought and cold stress responses. *Current Opinion in Plant Biology,* 6(5), 410-417.

Shrivastava, P., & Kumar, R. (2015). Soil salinity: A serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. *Saudi Journal of Biological Sciences,* 22(2), 123-131.

Wang, X., & Komatsu, S. (2018). Proteomic and metabolomic analyses of drought stress responses in soybean. *Journal of Proteomics*, 172, 82-92.

Zhang, J., Li, X., Wang, Y., Zhang, Y., & Zhang, J. (2015). Overexpression of ThVHAc1 and its potential upstream regulator, ThWRKY7, improved plant tolerance of cadmium stress. *Scientific Reports,* 5, 15649.

Zhu, J. K. (2016). Abiotic stress signaling and responses in plants. *Cell,* 167(2), 313-324.

Tezara, W., Mitchell, V. J., Driscoll, S. D., & Lawlor, D. W. (1999). Water stress inhibits plant photosynthesis by decreasing coupling factor and ATP. *Nature,* 401(6756), 914-917.

Thameur, A., Lachiheb, B., & Ferchichi, A. (2012). Drought effect on growth, gas exchange and yield, in two strains of local barley Ardhaoui, under water deficit conditions in southern Tunisia. *Journal of Environmental Management,* 113, 495-500.

Umezawa, T., Fujita, M., Fujita, Y., Yamaguchi-Shinozaki, K., & Shinozaki, K. (2006). Engineering drought tolerance in plants: discovering and tailoring genes to unlock the future. *Current Opinion in Biotechnology*, 17(2), 113-122.

Vranova, E., Inze, D., & Van Breusegem, F. (2002). Signal transduction during oxidative stress. *Journal of Experimental Botany*, 53(372), 1227-1236.

Wang, X., Oh, M. W., & Komatsu, S. (2016). Characterization of S-adenosylmethionine synthetases in soybean under flooding and drought stresses. *Biologia Plantarum,* 60(2), 269-278.

Willekens, H., Chamnongpol, S., Davey, M., Schraudner, M., Langebartels, C., Van Montagu, M., ... & Van Camp, W. (1997). Catalase is a sink for H2O2 and is indispensable for stress defense in C3 plants. *Embo Journal,* 16(16), 4806-4816.

Witzel, K., Weidner, A., Surabhi, G. K., Borner, A., & Mock, H. P. (2009). Salt stress-induced alterations in the root proteome of barley genotypes with contrasting response towards salinity. *Journal of Experimental Botany,* 60(12), 3545-3557.

Yang, S. F., & Hoffman, N. E. (1984). Ethylene Biosynthesis and its Regulation in Higher Plants. *Annual Review of Plant Physiology,* 35(1), 155-189.