RESEARCH ARTICLE

SABRAO Journal of Breeding and Genetics
55 (6) 2181-2194, 2023
http://doi.org/10.54910/sabrao2023.55.6.29
http://sabraojournal.org/
pISSN 1029-7073; eISSN 2224-8978

# DROUGHT STRESS EFFECTS ON APETALA2 (*AP2*) GENE EXPRESSION, PHYSIOLOGICAL, AND YIELD TRAITS OF MAIZE (*ZEA MAYS* L.)

**A.M. ABDUL MOHSIN and A.N. FARHOOD***

Department of Field Crops, College of Agriculture, University of Kerbala, Kerbala, Iraq
*Corresponding author's emails: ali.nazem@uokerbala.edu.iq, ali.nazem1987@gmail.com
Email address of co-author: alaaaltaee2008@gmail.com

## SUMMARY

Maize (*Zea mays* L.) field experiments commenced during crop season 2022 at the Experimental Farm, Al-Mahnawiya, Extension Training Center, Babylon, Iraq. The study examined the impacts of water stress tolerance on four maize cultivars' *AP2* gene expression, physiology, and yield. The experiment used a randomized complete block design (RCBD) with a split-plot arrangement and three replications. Four irrigation treatments applied on the main plots comprised 1) full irrigation (control), 2) withholding irrigation during elongation (Gs-V7), 3) non-irrigation during grain-filling (Gs-R2), and 4) suppressing irrigation during both elongation and grain-filling (Gs-V7+R2). The subplots included four maize cultivars: Furat, Dijlah, ZP, and Konsens. The results showed that withholding water during maize plants' elongation stage (GS-V7) significantly reduced leaf water and chlorophyll content. However, withholding irrigation increased peroxidase and catalase enzyme concentrations significantly. Suppressing irrigation at the elongation stage (GS-V7) and the elongation and grain-filling stages (GS-V7+R2) resulted in a considerable drop in cycle threshold (CT) values, with average CT values of 31.68 and 31.69 cycles, respectively. The *AP2* gene expression and CT values indicated a negative correlation. The study found that the *AP2* gene increases maize's water stress tolerance, and the gene expression in maize cultivar "Furat" confirms this. Maize cultivar Furat achieved recommendations for cultivation in desert regions because of its superior ability to survive and adapt.

**Keywords:** Maize (*Zea mays* L.), *AP2* gene expression, water stress tolerance**,** irrigation regimes, physiological traits, chlorophyll content, peroxidase and catalase enzymes, grain yield

**Key findings:** Based on the presented study, the *AP2* gene presence and expression enhances maize (*Zea mays* L.) genotype water stress tolerance. In the maize cultivar Furat, the said *AP2* gene expression was favorable, which increases its water stress tolerance and, thus, attained recommendations as an ideal maize cultivar for desert areas.

Communicating Editor: Prof. P.I. Prasanthi Perera

Manuscript received: July 11, 2023; Accepted: October 3, 2023.

**Citation:** Abdul Mohsin AM, Farhood AN (2023). Drought stress effects on APETALA2 (*AP2*) gene expression, physiological, and yield traits of maize (*Zea mays* L.). *SABRAO J. Breed. Genet.* 55(6): 2181-2194. http://doi.org/10.54910/sabrao2023.55.6.29.

## INTRODUCTION

Numerous studies arose to examine water scarcity issues and their diverse detrimental impacts on the environment and plant life. This problem holds importance as it signifies a crucial abiotic challenge in crops' vitality, survival, and production. Crop plants' exposure to specific stress factors has various adverse effects on the structure and physiological processes of the tissues (El-Moukhtari *et al.,* 2020). These effects also include an alteration and irregularities in the chemical composition of the cell wall and cell membranes, degradation of proteins and protoplasm, and disruption of crucial physiological processes, such as carbon metabolism (Liu *et al*., 2018).

Water stress can affect and diminish plant enzyme activities, morphological shape, and related parameters. Furthermore, among other consequences, the production of reactive oxygen species (ROS) can lead to cell membrane damage, as well as the decomposition of proteins and nucleic acids (Basu *et al.,* 2016; Fathi and Tari, 2016; Vaughan *et al.,* 2018). The plant drought tolerance phenomenon can be a multifaceted trait encompassing various morphological, anatomical, physiological, biochemical, and genetic attributes regulated by multiple genes with distinct impacts (Xiang *et al*., 2017).

Some plant species exhibit numerous adaptive mechanisms to effectively respond to water stress, involving alterations in morphological characteristics and physiological and biochemical processes. According to past studies by Barnabás *et al.* (2008) and Flexas *et al.* (2006), water stress has negatively impacted the carbon assimilation rate. This effect can occur indirectly, through the closure of stomata, or directly, by reducing the capacity and efficiency of carbon assimilation in leaves. The impact of proline acid on plant tissues enhances the plant's resilience to diverse stressors, such as water scarcity (Tahaei *et al*., 2022).

Cell elongation, stomatal opening, and carbon metabolism efficiency are crucial for sustainable plant growth and development during water stress conditions (Al-Shaheen *et al.*, 2016). In leaf tissues, the proline presence is a reliable indicator of a lack of water in plants. Proline accumulation seemed non-harmful to crop plants and is crucial in storing excess nitrogen. Research results indicated that the widespread proline accumulation in the cells of plant leaves helps. The osmotic potential of the plant tissue changes, increasing the plant's ability to absorb more water from the soil because proline serves as a storehouse of metabolic materials within the cell that regulates osmosis, working to balance catabolic building (Amzeri *et al*., 2020; Jumaah and AL-Hassa, 2020; Javaid *et al*., 2023). This stored nitrogen becomes advantageous to the plant during stress and energy deprivation. It is worth noting that the oxidation of each unit of proline releases approximately 30 ATP, which provides the necessary energy for the plant (Zhang and Becker, 2015).

One of the primary consequences of water stress is the degradation of plant pigments, leading to impairment of the carbon metabolism pathway. Notably, chlorophyll, a crucial pigment involved in photosynthesis, is particularly susceptible to such damage caused by drought stress conditions. According to Alam *et al.* (2014), the yellow corn plant leaves exhibited a reduction in the chlorophyll content, i.e., 12.75% and 49.44%, during the cessation of irrigation in both stages of plant growth. Drought-tolerant plants' categorization can depend on their relative water content, a crucial indicator for assessing their tolerance to water and other stress types.

Consequently, relative water content measurement has become a significant indicator in crop research studies. In their study, Abd-Ulameer and Ahmed (2018) demonstrated that water stress can significantly reduce the relative water content of maize plant leaves. This phenomenon refers to a decrease in soil water availability, subsequently impairing the plant's capacity to uptake water, causing a decline in water content within the plant tissues, and leading to water loss. The rise in temperature and decrease in relative humidity resulting from transpiration through the leaves have led to an imbalance between water absorption and transpiration rates. This imbalance has adversely impacted the water condition of the plant.

Drought stress condition always significantly impacts the levels of plant hormones promoting growth, namely, auxins, gibberellins, and cytokinins (Aldesuquy, 2014). Additionally, water stress caused an increase in ABA and ethylene levels. According to Bano *et al.* (1993), it has been evident that plants subjected to water deficiency exhibit reduced levels of cytokinins in their plant tissues, which promptly recover to their baseline levels upon alleviating this stress. Based on the above discussion, the opportune study aimed to investigate the impact of water stress on various physiological indicators and its subsequent influence on grain yield in four different maize cultivars.

## MATERIALS AND METHODS

The maize (*Zea mays* L.) field experiment during the spring of 2022 commenced at the Experimental Farm, Al-Mahnawiya, Extension Training Center, Babylon, Iraq. The study's primary objective was to investigate the effects of water stress tolerance on APETALA2 (*AP2*) gene expression, physiology, and yield of four maize cultivars. The experiment setup in a randomized complete block design (RCBD) had a split-plot arrangement with two factors and three replications. The main plots consisted of four irrigation regimes and withholding strategies, i.e., a comparison treatment involving irrigation after depleting 50% of the available water (Gs0), withholding of one irrigation during the elongation stage (Gs-V7), kernel-filling phase (Gs-R2), and the irrigation restriction once during the elongation and kernel-filling stages (Gs-V7+R2). The subplots comprised four maize cultivars: Furat, Dijlah, ZP, and Konsens. The crop's growth stages assessment used the Abendroth scale (Abendroth *et al.,* 2011).

### Maize tolerance genes identification

### *DNA extraction*

In the presented study investigating the *AP2* gene's potential role in conferring water stress tolerance to maize plants, young leaves of four distinct maize cultivars sustained DNA extraction. The DNA extraction process involved the utilization of a kit manufactured by Add-Bio, Korea. The extraction procedure followed the instructions provided by the said company.

### *PCR amplification*

The *AP2* gene diagnosis used a polymerase chain reaction (PCR) test, employing specific primers (F: TCAATGTGCCCTTGTCCTCG) and (R: TTGGTTGGTGTGGTAGAGGC). The experiment utilized the Maxime™ PCR PreMix (i-Taq) kit provided by the iNtRoN company. The reaction mixture had a total volume of 25 microliters (µl) and consisted of the following components: Taq PCR PreMix (10 µl), Forward primer (1 µl), Reverse primer (1 µl), and DNA (5 µl). A nuclease-free water addition to the remaining capacity brought the total volume to 25 µl to ensure the absence of nucleic acid; the amplification mixture creation was in a separate sterile tube that served as the negative control. Using a micropipette helped stir the mixture's components, with the reaction mixture's ultimate volume determined by centrifugation. Finally, the mixture's transfer continued to a thermo polymerase chain reaction (PCR) device. The *AP2* gene's amplification used the following procedure: Initial denaturation at 95 °C for 3 min in one cycle. Afterward, 35 cycles proceeded, which included denaturation at 95 °C for 30 s, annealing at 60 °C for 30 s, and extension at 72 °C for 2 min. After completing the amplification, the tubes' storage in the refrigerator ensued until the need for electrophoresis.

### **AP2 *gene expression***

The process involves collecting leaf samples for each experimental unit from the maize crop. RNA isolation from the samples utilized a kit from the Korean Add-Bio company. The method proposed by Livak and Schmittgen (2001) proceeded to assess the relative gene expression. The *Actin* gene became a reference gene, with its manifestation determined using the following equations:

$$\Delta ct = ct_{target\ gene} - ct_{reference\ gene}$$

$$\Delta\Delta ct = \Delta ct_{Test} - \Delta ct_{Control}$$

$$\textbf{gene expression} = 2^{-\Delta\Delta ct}$$

Where: ct $_{\text{target gene}}$ is the target gene's cycle threshold,
ct $_{\text{reference gene}}$ is the Actin's cycle threshold,
Ct $_{\text{test}}$ is the cycle threshold for target gene samples, and
the control sample's cycle threshold for the target gene is $CT_{Control}$.

### ***RT-qPCR quantification***

Assessing the study parameters used the RT-qPCR assay, performed at specific growth stages and on different plant parts. Specialized primers (CACATGGTTCTGTGCCTGAG) and (TCCTCCTCATCTGGCTCATC) attained usage in this reaction. Utilization of the GoTaq® Probe RT-qPCR Master Mix kit provided by Promega aided the assay. The appropriate volume of all RT-qPCR components was: GoTaq® RT-qPCR Master Mix (10 µl), Forward primer of target gene (1 µl), Reverse primer of target gene (1 µl), Forward primer of gene reference (1 µl), Reverse primer of gene reference (1 µl), Nuclease-free water (6 µl), and RNA Sample Volume (5 µl).

The earlier mentioned components, blended in a rotary mixer, operated at a speed of 3000 rpm for 10 seconds. Finally, the mixture's transfer continued to a quantitative real-time PCR device. Applying the following program helped to amplify the *AP2* gene: cDNA synthesis at 50 °C for 20 min in one cycle, initial denaturation at 95 °C for 10 min in one cycle, continuing to 40 cycles, including denaturation at 95 °C for 45 s, annealing at 60 °C for 45 s, and later, the extension at 72 °C for 1 min.

### **Traits measurement**

Estimating the total chlorophyll content of leaves (mg/100 g fresh weight) progressed using the method described by Zhang and Kirkham (1996). In the maize leaves, determining the proline content (µmol $g^{-1}$) used the method described by Bates *et al*. (1973***)***. For the percentage of relative water content in the leaves (%), selecting several tender leaves transpired, specifically the third leaf from the top of the plant. The subsequent cutting of four tablets with a diameter of 2 cm attained their extraction from the middle portion of the plant. These tablets carefully proceeded transfer inside nylon bags to mitigate potential moisture loss. The specimens' prompt weighing after being severed continued for submerging in distilled water. The leaves underwent a 24-hour exposure to lighting and room temperature. Afterward, drying the leaves with filter paper, continued measuring their weight to determine the initial heaviness. Subsequently, the leaves' placement in an oven set at a temperature of 60 °C continued for another 24 hours. After this drying process, weighing the leaves again helped determine the dry weight. According to the study conducted by Barnes and Woolley (1969), the estimation of the relative water content used the following equation:

$$R.W.C = \frac{FW - DW}{TW - DW} \times 100$$

Where: FW = fresh weight (g), DW = dry weight (g), and TW = full weight (g)

As for the leaf content of plant hormones (micromole), the plant hormones' (auxins [IAA], gibberellins [GA], cytokinins [CKs]) estimation ensued according to the method of Unyayar *et al*. (1996). On the effectiveness of antioxidant enzymes, the

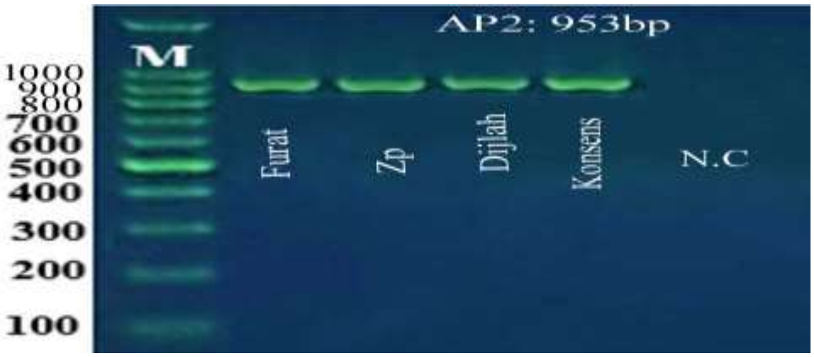

**Figure 1**. Electrophoresis of the *AP2* gene primer PCR products without DNA. Besides the DNA ladder are sizes shown on the left.

antioxidant enzymes peroxidase (POD) and Catalase (CAT) effectiveness acquired estimations (Beers and Sizer, 1952). Grain yield (tons ha$^{-1}$), calculated from the average weight of one plant's harvest, came as the average of five plants harvested from each experimental unit multiplied by the plant density.

### Statistical analysis

Data analysis for all the parameters was according to the analysis of variance (ANOVA). The Least Significant Difference (LSD) test employed helped in the comparison and separation of means. The statistical analysis used the Gen Stat software program to compare the arithmetic means.

## RESULTS AND DISCUSSION

### Diagnosis of the *AP2* gene

The electrophoresis results of the amplified PCR products for the *AP2* gene expression in four maize cultivars (Furat, Zp, Dijlah, and Konsens) appear in Figure 1. A distinct band with a molecular weight of 953 bp, corresponding to the *AP2* gene associated with drought tolerance in maize plants, was evident. The *AP2* gene is typical for regulating plant growth, development, and defense mechanisms against biological stresses in maize genotypes (Liu *et al.,* 2021). Additionally, this gene plays a vital role in metabolism through protein phosphorylation, a common post-translation modification enabling the gene's products to assume new functions in enzyme binding, catalysis, and regulation. Also, the examined physical properties of enzymes and their modifying properties included enzymatic activity and interactions with other enzymes. Moreover, it regulates stress responses in maize genotypes by facilitating the transmission of stress signals from the cellular membrane to the nucleus (Lam *et al*., 2016). This process is a biochemical signal within cells functioning as a pivotal post-translational modulator (Xu and Zhang, 2015; Amzeri *et al*., 2020; Javaid *et al*., 2023).

### *AP2* gene expression in maize leaves

The RT-qPCR technology employed amplified the *AP2* gene presence and its expression. The analysis of cycle threshold (CT) values and the relative expression levels of the *AP2* gene in maize leaves during the vegetative stage indicate that withholding irrigation during the elongation stage (GS-V7) and the elongation and grain-filling stages (GS-V7+R2) resulted in specific effects (Table 1). A notable reduction was apparent in the CT values, with average values of 31.68 and 31.69 cycles, respectively, while the full irrigation treatment (GS0) yielded cycles of 32.36. However, the difference was not significant when comparing the irrigation suppression treatments during the grain-filling stage (GS-R2), which resulted in a CT value of 32.21 cycles. The reason is measuring the said

**Table 1.** Effect of irrigation withholding treatments on the cycle threshold (CT) and relative *AP2* gene expression in maize cultivar leaves at the vegetative stage.

| Withhold irrigation | CT *Actin* gene | CT of *AP2* gene | Δ CT of *AP2* gene | ΔΔ CT of *AP2* gene | Gene Expression |
|---|---|---|---|---|---|
| GS0 | 18.56 | 32.36 | 13.80 | 0.00 | 1.00 |
| GS-V7 | 18.53 | 31.68 | 13.15 | -0.65 | 1.62 |
| GS-R2 | 18.44 | 32.21 | 13.77 | -0.03 | 1.02 |
| GS-V7+R2 | 18.57 | 31.69 | 13.12 | -0.68 | 1.66 |
| LSD$_{0.05}$ | ------ | 0.241 | -------- | ------ | 0.217 |

| Maize cultivars | CT *Actin* gene | CT of *AP2* gene | Δ CT of *AP2* gene | ΔΔ CT of *AP2* gene | Gene Expression |
|---|---|---|---|---|---|
| Furat | 18.60 | 31.83 | 13.23 | -0.38 | 1.37 |
| Zp | 18.65 | 32.20 | 13.54 | -0.23 | 1.20 |
| Dijlah | 18.39 | 31.59 | 13.20 | -0.53 | 1.56 |
| Konsens | 18.46 | 32.32 | 13.86 | -0.22 | 1.18 |
| LSD$_{0.05}$ | ------ | 0 .393 | ------- | ----- | 0 .223 |

| Interactions | | CT *Actin* gene | CT of *AP2* gene | Δ CT of *AP2* gene | ΔΔ CT of *AP2* gene | Gene Expression |
|---|---|---|---|---|---|---|
| GS0 | Furat | 18.69 | 32.31 | 13.61 | 0.00 | 1.00 |
| | Zp | 18.71 | 32.48 | 13.77 | 0.00 | 1.00 |
| | Dijlah | 18.53 | 32.26 | 13.73 | 0.00 | 1.00 |
| | Konsens | 18.31 | 32.39 | 14.08 | 0.00 | 1.00 |
| GS-V7 | Furat | 18.51 | 31.41 | 12.90 | -0.71 | 1.64 |
| | Zp | 18.58 | 32.05 | 13.47 | -0.30 | 1.23 |
| | Dijlah | 18.60 | 31.14 | 12.54 | -1.19 | 2.29 |
| | Konsens | 18.44 | 32.12 | 13.68 | -0.40 | 1.32 |
| GS-R2 | Furat | 18.65 | 32.19 | 13.54 | -0.07 | 1.05 |
| | Zp | 18.44 | 32.20 | 13.76 | -0.01 | 1.01 |
| | Dijlah | 18.09 | 31.80 | 13.71 | -0.02 | 1.01 |
| | Konsens | 18.57 | 32.63 | 14.05 | -0.03 | 1.02 |
| GS-V7+R2 | Furat | 18.55 | 31.41 | 12.86 | -0.75 | 1.78 |
| | Zp | 18.89 | 32.05 | 13.17 | -0.60 | 1.56 |
| | Dijlah | 18.33 | 31.15 | 12.82 | -0.91 | 1.95 |
| | Konsens | 18.49 | 32.14 | 13.64 | -0.91 | 1.38 |
| LSD$_{0.05}$ | | ------ | 0.706 | ----- | ----- | 0.422 |

parameter ensued before implementing the irrigation withholding treatment.

Furthermore, a notable correlation was distinct between the CT values and the relative expression of the *AP2* gene. This association is evident from the findings presented in Table 2, which indicate that withholding irrigation during the elongation stage (GS-V7) and elongation and grain-filling stages (GS-V7+R2) resulted in a substantial rise in the observed values. At the vegetative stage, the relative expression of the *AP2* gene emerged to be 1.62 and 1.66 times greater, respectively, compared with the control treatment (GS0).

In the analysis of the CT and relative expression of the *AP2* gene in maize leaves during the reproductive stage, the findings indicated that withholding irrigation during the grain-filling stage (GS-R2) and the elongation and grain-filling stages (GS-V7+R2) caused a significant reduction in CT values (Table 2). Specifically, the average CT values for these treatments were 34.60 and 34.77 cycles, respectively. The full irrigation treatment (GS0), which yielded CT values of 35.04 cycles, revealed a nonsignificant difference compared with the treatment involving irrigation inhibition during the elongation stage (CT = 34.97 cycles). Furthermore, it was also noteworthy that an inverse correlation exists between the CT values and the relative expression of the gene. This observation, as supported by the findings in Table 2, indicates that irrigation withholding during the seed-filling stage (GS-R2) and at the elongation and seed-filling phases (GS-V7+R2) led to a significant rise in the relative expression. At the proliferative stage, the expression levels of

**Table 2.** Effect of irrigation withholding treatments on the cycle threshold (CT) and relative *AP2* gene expression in maize cultivar leaves at the reproductive stage.

| Withhold irrigation | CT *Actin* gene | CT of *AP2* gene | Δ CT of *AP2* gene | ΔΔ CT of *AP2* gene | Gene Expression |
|---|---|---|---|---|---|
| GS0 | 18.56 | 35.04 | 16.48 | 0.00 | 1.00 |
| GS-V7 | 18.53 | 34.97 | 16.44 | -0.03 | 1.03 |
| GS-R2 | 18.44 | 34.60 | 16.16 | -0.31 | 1.29 |
| GS-V7+R2 | 18.57 | 34.77 | 16.20 | -0.27 | 1.25 |
| $LSD_{0.05}$ | ------ | 0.176 | -------- | ------ | 0.163 |

| Maize cultivars | CT *Actin* gene | CT of *AP2* gene | Δ CT of *AP2* gene | ΔΔ CT of *AP2* gene | Gene Expression |
|---|---|---|---|---|---|
| Furat | 18.60 | 34.83 | 16.23 | -0.16 | 1.15 |
| Zp | 18.65 | 35.05 | 16.39 | 0.00 | 1.01 |
| Dijlah | 18.39 | 34.48 | 16.09 | -0.32 | 1.28 |
| Konsens | 18.46 | 35.03 | 16.57 | -0.14 | 1.12 |
| $LSD_{0.05}$ | ------ | 0.433 | ------- | ----- | غ.م |

| Interactions | | CT *Actin* gene | CT of *AP2* gene | Δ CT of *AP2* gene | ΔΔ CT of *AP2* gene | Gene Expression |
|---|---|---|---|---|---|---|
| GS0 | Furat | 18.69 | 35.08 | 16.39 | 0.00 | 1.00 |
| | Zp | 18.71 | 35.10 | 16.39 | 0.00 | 1.00 |
| | Dijlah | 18.53 | 34.94 | 16.41 | 0.00 | 1.00 |
| | Konsens | 18.31 | 35.02 | 16.71 | 0.00 | 1.00 |
| GS-V7 | Furat | 18.51 | 34.89 | 16.38 | -0.01 | 1.01 |
| | Zp | 18.58 | 34.97 | 16.39 | 0.00 | 1.00 |
| | Dijlah | 18.60 | 34.97 | 16.37 | -0.04 | 1.03 |
| | Konsens | 18.44 | 35.07 | 16.63 | -0.08 | 1.06 |
| GS-R2 | Furat | 18.65 | 34.60 | 15.95 | -0.44 | 1.35 |
| | Zp | 18.44 | 35.06 | 16.62 | 0.23 | 0.87 |
| | Dijlah | 18.09 | 33.85 | 15.76 | -0.65 | 1.57 |
| | Konsens | 18.57 | 34.88 | 16.31 | -0.40 | 1.38 |
| GS-V7+R2 | Furat | 18.55 | 34.74 | 16.19 | -0.20 | 1.22 |
| | Zp | 18.89 | 35.05 | 16.17 | -0.23 | 1.18 |
| | Dijlah | 18.33 | 34.15 | 15.82 | -0.60 | 1.54 |
| | Konsens | 18.49 | 35.14 | 16.64 | -0.07 | 1.06 |
| $LSD_{0.05}$ | | ------ | 0.762 | ----- | ----- | 0.369 |

the *AP2* gene occurred to be 1.29 and 1.25 times higher, respectively, than the control treatment (GS0). The observed up-regulation of the *AP2* gene may refer to the plant's utilization of cellular mechanisms that modulate gene expression by modifying specific chemical reactions. The demonstrated methylation process induces protein and enzyme production alterations, thereby regulating gene expression in response to drought conditions (Bartels *et al*., 2018)**.**

The results further revealed notable variations in the CT values of different corn cultivars during the vegetative stage (Table 1). In particular, the maize cultivars Konsens and Furat exhibited the lowest CT values of 31.83 and 31.59 cycles, respectively, and the two other cultivars, Zp and Tigris, demonstrated higher CT values of 32.19 and 32.32 cycles, respectively. The two cultivars, Konsens and Furat, exhibited heightened relative expression of the *AP2* gene, as supported by the findings of the relative expression analysis. By assessing the CT values and relative gene expression during the reproductive stage, no significant variations occurred among the different maize cultivars (Table 2).

### Total chlorophyll

The results indicated that the act of withholding irrigation during the elongation stage (GS-V7) and at the elongation and grain-filling stages (GS-V7+R2) caused a remarkable reduction in the leaves’ chlorophyll content (Table 3). The two treatments yielded average values of 29.52 and 29.65 mg 100 $g^{-1}$ fresh weight, respectively, compared with the full

**Table 3.** Effect of withholding irrigation on the chlorophyll content (mg 100 g$^{-1}$ fresh weight) of maize cultivars.

| Withhold irrigation | Maize cultivars | | | | Average |
|---|---|---|---|---|---|
| | Furat | ZP | Dijlah | Konsens | |
| GS0 | 39.18 | 35.27 | 37.63 | 36.00 | 37.02 |
| GS-V7 | 33.00 | 29.22 | 30.10 | 25.76 | 29.52 |
| GS-R2 | 38.84 | 34.92 | 37.26 | 35.63 | 36.66 |
| GS-V7+R2 | 33.12 | 29.34 | 30.21 | 25.91 | 29.65 |
| LSD$_{0.05}$ | 1.963 | | | | 1.392 |
| Average | 36.03 | 32.19 | 33.80 | 30.83 | |
| LSD$_{0.05}$ | 0.914 | | | | |

**Table 4.** Effect of withholding irrigation on the proline concentration (µmol g$^{-1}$) of maize cultivars.

| Withhold irrigation | Maize cultivars | | | | Average |
|---|---|---|---|---|---|
| | Furat | ZP | Dijlah | Konsens | |
| GS0 | 44.51 | 56.43 | 73.20 | 67.99 | 60.53 |
| GS-V7 | 73.00 | 67.54 | 87.25 | 79.35 | 76.79 |
| GS-R2 | 44.32 | 56.18 | 72.93 | 67.75 | 60.29 |
| GS-V7+R2 | 72.27 | 67.17 | 86.51 | 78.68 | 76.16 |
| LSD$_{0.05}$ | 9.210 | | | | 6.234 |
| Average | 58.53 | 61.83 | 79.97 | 73.44 | |
| LSD$_{0.05}$ | 4.409 | | | | |

irrigation treatment (GS0), which gave an average of 37.02 mg 100 g$^{-1}$ fresh weight and was not significantly different from the treatment with inhibited irrigation during the seed-filling stage (36.66 mg per 100 g$^{-1}$ fresh weight). The reduction in chlorophyll levels due to water scarcity can be due to the diminished capacity of plants to uptake water and essential elements, particularly nitrogen. Nitrogen is a Porphyrins content, a crucial component in chlorophyll molecule synthesis (Pessarakli, 2016). Water stress can also reduce cytokinin levels vital in chlorophyll synthesis, particularly in crana (Guo *et al*., 2017). The latest results were in analogy with the findings of Rafiee (2012), who demonstrated a reduction in chlorophyll levels in maize plant leaves under water stress conditions.

The further findings showed significant variations in chlorophyll content among the different maize cultivars (Table 3). Cultivar Furat exhibited the highest average chlorophyll content of 36.03 mg 100 g$^{-1}$ fresh weight, and the cultivars ZP, Dijlah, and Konsens had average chlorophyll contents of 32.19, 33.80, and 30.83 mg 100 g$^{-1}$ fresh weight, respectively. The higher chlorophyll content observed in the maize cultivar Furat can be because of its larger leaf area, which consequently leads to an increased chlorophyll accumulation.

The outcomes indicated a significant interaction between the irrigation withholding treatments and the maize cultivars based on the total chlorophyll content in the leaves (Table 3). It was also apparent that the various maize cultivars exhibited different levels of susceptibility to water stress conditions. Specifically, the cultivar Konsens with GS-V7 treatment displayed the lowest total chlorophyll content in the leaves (28.44 mg 100 g$^{-1}$ fresh weight). In contrast, the cultivar Furat had the minimum impact on stress conditions, with a total chlorophyll content of 33.00 mg 100 g$^{-1}$ fresh weight.

## Proline concentration

The withholding irrigation during the elongation stage (GS-V7) and at the elongation and grain-filling stages (GS-V7 + R2) resulted in a notable elevation in proline concentration (Table 4). Explicitly, the two stress conditions yielded average proline concentrations of 76.79 and 76.16 µmol g$^{-1}$, respectively, which were also superior. The observed increase in the average value of 60.53 µmol g$^{-1}$ in the full irrigation treatment is attributable to the plant's physiological response to safeguard its cells against water deficiency-induced damage. This response involves the plant's ability to mitigate the impairment caused by reactive oxygen species (ROS) compounds and their positive impact on the osmotic regulation of plant cells, thereby promoting an increase in the observed value. Proline is crucial in mitigating the effects of water scarcity on plant growth. It also facilitates water and nutrients withdrawal from the environment, sustains cell elongation, and promotes stomata opening. These processes collectively enhance the efficiency of carbon metabolism, thereby supporting plant growth even in water-limited conditions. Proline acts as a cohesive water shell, exhibiting strong resistance to alterations caused by water stress conditions. The findings of Mahdi and Muhammad (2009) and Al-Shaheen *et al*. (2016) were consistent with the presented results as they reported an elevation in maize leaves' proline content in response to water-deficient conditions**.**

The results also revealed significant variations among the maize cultivars in proline leaf content (Table 4). In particular, the cultivar Dijlah exhibited the highest average proline leaf content of 79.97 µmol $g^{-1}$. However, the cultivars, Furat, ZP, and Konsens had average proline leaf contents of 58.53, 61.83, and 73.44 µmol $g^{-1}$, respectively. The differences in proline leaf content could refer to various factors and the genetic disparity among the maize genotypes, which might be the underlying cause of this phenomenon (Rafiee, 2012).

The findings further presented a significant interaction between the irrigation withholding treatments and the maize cultivars on proline concentration in the maize genotype leaves (Table 4). It was evident that the various maize cultivars exhibited varying degrees of susceptibility to water stress conditions. Cultivar Furat displayed the lowest proline concentration in the leaves (64.01%) under the GS treatment. Among the variables examined, it was plain that cultivar Konsens acquired the lowest impact level, with a recorded value of 16.71%.

## Relative water content (%)

The findings presented in Table 5 indicate that withholding irrigation during the elongation stage (GS-V7) and the elongation and grain-filling stages (GS-V7+R2) notably reduced the relative water content. Specifically, these treatments' average relative water content was 70.03% and 69.88%, respectively, in contrast to the full irrigation treatment (GS0) with a relative water content of 84.37%. It is worth noting that the complete irrigation treatment did not exhibit a significant difference in relative water content compared with the treatment of suppressed irrigation during the grain-filling stages, resulting in a relative water content of 83.57%. The reduction in the relative water content within the leaves may be due to the limited availability of water in the soil, resulting in a diminished capacity of the plant to uptake water. Consequently, this leads to a decline in the water content present in the plant's tissues (Karrou *et al.*, 2012). The absence of sufficient foliage also impacts the leaves' capacity to retain moisture. This finding agrees with the results of Aslam *et al.* (2014), who reported a reduction in relative water content with inhibited irrigation during the vegetative phases.

The outcomes presented in Table 5 specify significant variations in the relative water content among different maize cultivars. Specifically, the Furat cultivar exhibited the highest percentage of relative water content, reaching 88.11%. On the other hand, the ZP, Dijlah, and Konsens cultivars displayed shares of 83.64%, 69.33%, and 66.78%, respectively**.** The observed disparity in relative water content among cultivars is ascribable to variations in leaf area, which consequently affects their capacity to retain water.

The results detailed in Table 5 imply a statistically significant interaction between the withholding of irrigation and the choice of maize cultivars based on the leaves' relative water content. It was visible that different maize cultivars exhibited varying levels of susceptibility to water stress. Specifically, the Furat cultivar demonstrated the lowest decline in relative water content, with a decrease of

**Table 5**. Effect of withholding irrigation on the relative water content (%) of maize cultivars.

| Withhold irrigation | Maize cultivars | | | | Average |
|---|---|---|---|---|---|
| | Furat | ZP | Dijlah | Konsens | |
| GS0 | 93.35 | 89.92 | 73.88 | 80.34 | 84.37 |
| GS-V7 | 83.66 | 77.53 | 65.10 | 53.85 | 70.03 |
| GS-R2 | 92.01 | 89.18 | 73.83 | 79.27 | 83.57 |
| GS-V7+R2 | 83.41 | 77.93 | 64.51 | 53.65 | 69.88 |
| $LSD_{0.05}$ | 9.318 | | | | 6.407 |
| Average | 88.11 | 83.64 | 69.33 | 66.78 | |
| $LSD_{0.05}$ | 4.422 | | | | |

10.38% under the GS-V7 treatment. On the other hand, the Konsens cultivar was the most adversely affected, experiencing a decline of 32.97%.

## Phytohormones concentrations

The results revealed that suppressing irrigation had a notable impact on the levels of auxins (IAA), gibberellins (GA), and cytokinins (CKs) (Tables 6, 7, and 8). Specifically, the irrigation withholding during the elongation stage (GS-V7) and at the elongation and grain-filling stages (GS-V7+R2) gave significantly lower mean values for IAA, GA, and CKs at 52.97 and 52.60, 33.45 and 33.24, and 43.01 and 42.51 μmol $g^{-1}$, respectively. When compared, the complete irrigation treatment (GS0) yielded higher averages of 71.39, 47.97, and 61.08 μmol $g^{-1}$ of IAA, GA, and CKs, respectively. However, these values did not show a substantial difference when compared with the treatment of suppressed irrigation during the grain-filling stage. Wang *et al.* (2008) also reported that water deficiency conditions have caused to reduce the concentration of phytohormones (specifically IAA, GA, and CKs) in maize leaves. This decrease can refer to particular enzymes' inhibition, lessening their promotion of the phytohormones' synthesis.

The findings disclosed significant variations in the phytohormone levels among the different maize cultivars. In particular, the maize cultivars Furat and Zp exhibited the highest average concentrations of IAA, GA, and CKs in their leaves, with values of 74.04 and 71.32, 47.44 and 45.77, and 59.54 and 44.96 μmol $g^{-1}$, respectively. The average values for the two cultivars, Dijlah and Konsens, emerged as the lowest (44.96 and 42.85 μmol $g^{-1}$, respectively). The variation in phytohormone levels among maize cultivars can be due to genetic disparities in the expression of genes for promoting phytohormone synthesis across different maize cultivars. According to Ma *et al.* (2022), it was definite that phytohormones varied according to different maize cultivars.

The results further enunciated that a significant interaction was available between the treatments of withholding irrigation and the maize cultivars according to the plant hormones' content in the leaves (Tables 6, 7, and 8). Specifically, cultivars Furat and Zp exhibited superior performance by giving the highest hormone concentrations (IAA, GA, and CKs) with full irrigation conditions (GS0). However, these hormone concentrations significantly lowered when subjecting the maize genotypes to the GS-V7 and GS-V7+R2 stress conditions.

## Peroxidase and catalase enzymes activity

The study found withholding of irrigation resulted in a significant increase in maize genotype leaves' peroxidase and catalase enzyme levels (Tables 9 and 10). The highest enzyme mean values came from suppressing irrigation during the elongation stage (GS-V7) and at the elongation and grain-filling stages (GS-V7+R2). With partial irrigation, the uptake of peroxidase and catalase enzyme was higher (76.54 and 73.77 $g^{-1}$) and (53.83 and 52.25 $g^{-1}$, respectively), versus full irrigation (GS0) (62.40 and 55.09 $g^{-1}$, respectively). For both enzymes, nonsignificant differences appeared between the full irrigation and the treatment of withholding irrigation at the grain-filling stage in maize genotypes. The observed elevation in

**Table 6.** Effect of withholding irrigation on the IAA (μmol $g^{-1}$) of maize cultivars.

| Withhold irrigation | Maize cultivars | | | | Average |
|---|---|---|---|---|---|
| | Furat | ZP | Dijlah | Konsens | |
| GS0 | 80.84 | 79.60 | 60.22 | 64.90 | 71.39 |
| GS-V7 | 67.78 | 63.79 | 48.12 | 32.16 | 52.97 |
| GS-R2 | 80.06 | 87.42 | 60.12 | 64.06 | 70.67 |
| GS-V7+R2 | 67.50 | 63.48 | 47.50 | 31.94 | 52.60 |
| $LSD_{0.05}$ | 10.603 | | | | 7.109 |
| Average | 74.04 | 71.32 | 53.99 | 48.27 | |
| $LSD_{0.05}$ | 5.101 | | | | |

**Table 7**. Effect of withholding irrigation on the gibberellin concentration (μmol $g^{-1}$) of maize cultivars.

| Withhold irrigation | Maize cultivars | | | | Average |
|---|---|---|---|---|---|
| | Furat | ZP | Dijlah | Konsens | |
| GS0 | 53.57 | 52.85 | 41.19 | 44.26 | 47.97 |
| GS-V7 | 42.28 | 39.61 | 30.46 | 21.46 | 33.45 |
| GS-R2 | 51.79 | 51.16 | 40.14 | 43.10 | 46.55 |
| GS-V7+R2 | 42.11 | 39.43 | 30.09 | 21.33 | 33.24 |
| $LSD_{0.05}$ | 6.112 | | | | 4.147 |
| Average | 47.44 | 45.77 | 35.47 | 32.54 | |
| $LSD_{0.05}$ | 2.922 | | | | |

**Table 8**. Effect of withholding irrigation on the cytokinin concentration (μmol $g^{-1}$) of maize cultivars.

| Withhold irrigation | Maize cultivars | | | | Average |
|---|---|---|---|---|---|
| | Furat | ZP | Dijlah | Konsens | |
| GS0 | 68.00 | 52.78 | 67.16 | 56.38 | 61.08 |
| GS-V7 | 52.05 | 38.15 | 49.25 | 32.57 | 43.01 |
| GS-R2 | 66.24 | 51.53 | 65.83 | 55.02 | 59.66 |
| GS-V7+R2 | 51.86 | 37.39 | 49.04 | 31.76 | 42.51 |
| $LSD_{0.05}$ | N.S | | | | 4.485 |
| Average | 59.54 | 44.96 | 57.82 | 43.93 | |
| $LSD_{0.05}$ | 3.533 | | | | |

**Table 9.** Effect of withholding irrigation on the peroxidase enzyme activity (absorption unit $g^{-1}$) of maize cultivars.

| Withhold irrigation | Maize cultivars | | | | Average |
|---|---|---|---|---|---|
| | Furat | ZP | Dijlah | Konsens | |
| GS0 | 66.19 | 62.25 | 64.39 | 56.78 | 62.40 |
| GS-V7 | 99.31 | 76.07 | 73.33 | 57.47 | 76.54 |
| GS-R2 | 62.60 | 58.56 | 60.01 | 43.79 | 56.24 |
| GS-V7+R2 | 97.05 | 72.77 | 71.27 | 53.99 | 73.77 |
| $LSD_{0.05}$ | 12.059 | | | | 8.149 |
| Average | 81.29 | 67.41 | 67.25 | 53.00 | |
| $LSD_{0.05}$ | 5.777 | | | | |

**Table 10.** Effect of withholding irrigation on the catalase enzyme activity (absorption unit $g^{-1}$) of maize cultivars.

| Withhold irrigation | Maize cultivars | | | | Average |
|---|---|---|---|---|---|
| | Furat | ZP | Dijlah | Konsens | |
| GS0 | 52.13 | 39.37 | 49.21 | 32.99 | 43.43 |
| GS-V7 | 59.89 | 46.13 | 59.42 | 49.87 | 53.83 |
| GS-R2 | 50.09 | 36.54 | 47.11 | 30.95 | 41.17 |
| GS-V7+R2 | 58.24 | 44.95 | 57.21 | 48.58 | 52.25 |
| $LSD_{0.05}$ | N.S | | | | 4.810 |
| Average | 55.09 | 41.75 | 53.24 | 40.60 | |
| $LSD_{0.05}$ | 3.417 | | | | |

peroxidase and catalase enzyme activity could be attributable to an enhanced abundance and efficacy of amino acids and soluble proteins within the cell's cytoplasm, which may facilitate the augmentation and efficiency of these enzymes. Reactive oxygen species (ROS), including superoxide ($O_2$), hydrogen peroxide ($H_2O_2$), and hydroxyl radical (OH), attained activation in response to cellular exposure to water deficit conditions. These ROS are vital in eliminating hydrogen peroxide in maize populations (Kamphorst *et al.,* 2018**)**.

The results further indicated significant variations among the maize cultivars for peroxidase and catalase enzyme activity (Tables 9 and 10). Specifically, the cultivar Furat displayed the highest average activity for peroxidase and catalase enzymes, with values of 81.29 and 55.09 g-1 absorption units, respectively. The maize genotype surpassed the other three maize cultivars, ZP, Dijlah, and Konsens. These results could be because of the genetic variations among the different maize cultivars. The capacity of maize plants to withstand stressful conditions is quantifiable by maintaining a substantial quantity of efficient antioxidant enzymes (Azooz *et al.,* 2009).

A significant interaction was apparent between the treatments of withholding irrigation and the maize cultivars related to peroxidase and catalase enzyme activity (Tables 9 and 10). Distinctly, the cultivar Furat demonstrated a superior performance by exhibiting the highest activity of peroxidase and catalase enzymes with inhibited irrigation in the GS-V7 and GS-R2 treatments. However, the enzyme activity receded with optimal water conditions (GS0).

### Grain yield (t ha$^{-1}$)

The withholding of irrigation during the elongation (GS-V7), the grain-filling (GS-R2), and the elongation and grain-filling stages (GS-V7+R2) resulted in a considerable reduction in maize grain yield (Table 11). The average harvests for these treatments were 5.36, 4.54, and 4.13 t ha$^{-1}$, respectively. However, the complete irrigation treatment (GS0) had an average yield of 7.42 t ha$^{-1}$ compared with the three treatments. The potential reduction in grain yield with stress conditions is reasonably due to the impact of water stress on chlorophyll and hormone levels. Consequently, it leads to a decrease in light interception by the leaves and a subsequent decline in carbon metabolism rate. These adverse effects occurred to impair the plant growth and development and reduced dry matter accumulation, ultimately reducing grain yield (Farhood *et al.,* 2022).

The findings further indicated notable variations in the average grain yield of the different maize cultivars (Table 11). Specifically, the cultivar Furat exhibited the highest average grain yield (6.18 t ha$^{-1}$), whereas the cultivars ZP, Dijlah, and Konsens showed the lowest average harvests (5.54, 5.38, and 4.35 t ha$^{-1}$, respectively). The observed superiority of the maize genotypes can point to variations in their genetic makeup on chlorophyll content, relative water content, and enzyme activity (Li-Ping *et al*., 2006).

The outcomes denoted a significant interaction between the irrigation withholding treatments and the maize cultivars on grain yield (Table 11). It was evident that the

**Table 11**. Effect of withholding irrigation on the grain yield (t ha$^{-1}$) of maize cultivars.

| Withhold irrigation | Maize cultivars | | | | Average |
|---|---|---|---|---|---|
| | Furat | ZP | Dijlah | Konsens | |
| GS0 | 8.54 | 7.13 | 7.96 | 6.06 | 7.42 |
| GS-V7 | 6.89 | 5.79 | 4.84 | 3.92 | 5.36 |
| GS-R2 | 4.78 | 4.65 | 4.75 | 3.99 | 4.54 |
| GS-V7+R2 | 4.51 | 4.60 | 3.97 | 3.45 | 4.13 |
| $LSD_{0.05}$ | 0.962 | | | | 0.813 |
| Average | 6.18 | 5.54 | 5.38 | 4.35 | |
| $LSD_{0.05}$ | 0.375 | | | | |

different maize cultivars exhibited varying levels of susceptibility to water stress conditions. In particular, the cultivar Dijlah demonstrated the lowest rate of grain yield reduction at 50.13% under the GS-V7+R2 water stress treatment, while the cultivar Konsens sustained the minimum impact (43.07%).

## CONCLUSIONS

The cultivars displayed varying levels of drought tolerance when subjected to irrigation withholding treatments. Notably, cultivar Furat demonstrated heightened drought tolerance, as evidenced by an up-regulation of the *AP2* gene expression and increases in plant hormone levels and enzyme activity. Furthermore, it was noteworthy that suppressing irrigation during the grain-filling stage (Gs-R2) resulted in a substantial decrease in maize yield compared with inhibited irrigation during the elongation stage (Gs-V7). Hence, it attains recommendations to mitigate drought occurrences during the grain-filling phase while also exploring the cultivation of novel varieties that exhibit tolerance or resistance to water stress conditions.

## REFERENCES

Abd-Ulameer OQ, Ahmed SS (2018). Anti-transpirant role in improving the morphological growth traits of maize plants subjected to water stress. *Res. on Crops* 19(4): 593-603.

Abendroth LJ, Elmore RW, Boyer MJ, Marlay SK (2011). Corn Growth and Development. Ames, Iowa State University, USA.

Alam MR, Nakasathien S, Sarobol ED, Vichukit V (2014). Responses of physiological traits of maize to water deficit induced at different phonological stages. *Kasetsart J. Nat. Sci*. 48: 183-196.

Aldesuquy HS (2014). Glycine betaine and salicylic acid induced modification in water relation and productivity of drought wheat plants*. J. Stress. Physiol. Biochem.* 10(2): 55-73.

Al-Shaheen MR, Soh A, Ismael MH, Shareef RS (2016). Alleviation of water deficit conditions on the corn (*Zea mays* L.) by using gibberellic acid and proline. *World. J. Pharm. Pharm. Sci.* 5(4): 483-490.

Amzeri A, Daryono BS, Syafii M (2020). Genotype by environment and stability analyses of dryland maize hybrids. *SABRAO J. Breed. Genet*. 52(4): 355-368

Aslam M, Zamir MSI, Afzal I, Amin M (2014). Role of potassium in physiological functions of spring maize (*Zea mays* L.) grown under drought stress. *J. Anim. Plant Sci.* 24(5): 1452-1465 .

Azooz M, Ismail AM, Elhamd MA (2009). Growth, lipid peroxidation and antioxidant enzyme activities as a selection criterion for the salt tolerance of maize cultivars grown under salinity stress. *Int. J. Agric. Biol*. 11(1): 21-26.

Bano A, Dorffling K, Bettin D, Hahn H (1993). Abscisic acid and cytokinins as possible root-to-shoot signals in xylem sap of rice plants grown in drying soil. *Aust. J. Plant Physiol*. 20: 109-115.

Barnabás B, Jäger K, Fehér A (2008). The effect of drought and heat stress on reproductive processes in cereals. *Plant, Cell Environ*. 31: 11-38.

Barnes DL, Woolley DG (1969). Effect of moisture stress at different stages of growth. I. Comparison of a single-eared and a two-eared corn hybrid. *Agron. J*. 61: 788-790.

Bartels A, Han Q, Nair P, Stacey L, Gaynier H, Mosley M, Xiao W (2018). Dynamic DNA methylation in plant growth and development. *Int. J. Mol. Sci*. 19(7): 2144.

Basu S, Ramegowda V, Kumar A, Pereira A (2016). Plant adaptation to drought stress. Crop, Soil, and Environmental Sciences, University of Arkansas, *Fayetteville, Arkansas*, 72701, USA.

Bates LS, Waldes RP, Teare TD (1973). Rapid determination of free proline for water stress studies. *Plant Soil* 39: 205-207.

Beers RFJ, Sizer IW (1952). Catalase assay. *J. Biol. Chem*. 159: 133-140.

El Moukhtari A, Cabassa-Hourton C, Farissi M, Savouré A (2020). How does proline treatment promote salt stress tolerance during crop plant development? *Frontiers in plant science*, 11, 11-27.

Farhood A, Merhij M, Al-Fatlawi Z (2022). Drought stress effects on resistant gene expression, growth, and yield traits of wheat (*Triticum aestivum* L.). *SABRAO J. Breed. Genet.* 54(3): 512-523.

Fathi A, Tari DB (2016). Effect of drought stress and its mechanism in plants. *Int. J. Life Sci*. 10(1): 1-6.

Flexas J, Ribas Carbó M, Bota J, Galmés J, Henkle M, MartínezCañellas S, Medrano H (2006).

Decreased Rubisco activity during water stress is not induced by decreased relative water content but related to conditions of low stomatal conductance and chloroplast $CO_2$ concentration. *New Phytol*. 172(1): 73-82.

Guo L, Xiu P, Chai F, Xue H, Wang D, Sun J (2017). Enhanced chlorophyll concentrations induced by Kuroshio intrusion fronts in the Northern South China Sea. *Geophys. Res. Lett*. 44(22): 11-565.

Javaid A, Razzaq H, Khan FA, Awan FS (2023). Evaluation of maize accessions for drought tolerance through principal component analysis. *SABRAO J. Breed. Genet*. 55(2): 476-484. http://doi.org/10.54910/sabrao2023.55.2.19.

Jumaah NA, AL-Hassa AFF (2020). Effect of addition of humic acid and proline acid in anatomical characteristics of *Zea mays* L. *Plants under Water Stress*, 20(2), 7382-7388.

Kamphorst SH, De-Lima VJ, do Amaral JAT, Schmitt KFM, Leite JT, Carvalho CM, Campostrini E (2018). Popcorn breeding for water-stress tolerance or for agronomic water-use efficiency. *J. Genet. Mol. Res*. 17(4): 1-18.

Karrou M, Oweis T, El Enein RA, Sherif M (2012). Yield and water productivity of maize and wheat under deficit and raised bed irrigation practices in Egypt. *African J. Agri. Res. 7*(11), 1755-1760.

Lam DV, Stes E, Van Bel M, Nelissen H, Maddelein D, Inze D (2016). Up-to-date workflow for plant (phospho) proteomics identifies differential drought-responsive phosphorylation events in maize leaves. *J. Proteome Res*. 15(12): 4304-4317.

Li-Ping BAI, Fang-Gong SUI, Ti-Da GE, Zhao-Hui SUN, Yin-Yan LU, Guang-Sheng ZHO (2006). Effect of soil drought stress on leaf water status, membrane permeability and enzymatic antioxidant system of maize. *Pedosphere* 16(3): 326-332.

Liu J, Guo YY, Bai YW, Camberato JJ, Xue JQ, Zhang RH (2018). Effects of drought stress on the photosynthesis in maize. *Russian journal of plant physiology*, 65, 849-856.

Liu W, Li S, Zhang C, Jin F, Li W, Li X (2021). Identification of candidate genes for drought tolerance at maize seedlings using genome-wide association. *Iranian J. Biotechnol*. 19(3): 2637.

Livak KJ, Schmittgen TD (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta C(T)) method. *Methods* 25: 402-408**.**

Ma C, Hua J, Li H, Zhang J, Luo S (2022). Inoculation with carbofuran-degrading rhizobacteria promotes maize growth through production of IAA and regulation of the release of plant-specialized metabolites. *Chemosphere* 307: 136027.

Mahdi AS, Muhammad HA (2009). The effect of moisture stress, seed hardening process and potassium fertilizer on the quantitative and qualitative traits of corn yield*. Tikrit J*. 9(3): 79-90.

Pessarakli M (2016). Handbook of Photosynthesis. *3rd Ed*. Taylor and Francis Group, Boca Raton. 846.

Rafiee M (2012). Effect of every other furrow irrigation and planting density on physiological traits in corn (*Zea mays* L.). *World Appl. Sci*. *J*. 17(2):189-193.

Tahaei SAR, Nasri M, Soleimani A, Ghooshchi F, Oveysi M (2022). Effects of growth regulators and proline amino acid on yield and yield components of single cross 704 maize under drought stress conditions in Isfahan province. *Environmental Stresses in Crop Sciences*, 15(3), 657-668.

Unyayar S, Topcuoglu SF, Unyayar A (1996). A modified method for extraction and identification of indole-3-acetic acid (IAA), gibberellic acid (GA3), abscisic acid (ABA), and zeatin produced by Phanerochaete chrysosporium ME446. *Bull. J. Plant Physiol*. 22(3-4): 105-110.

Vaughan MM, Block A, Christensen SA, Allen LH, Schmelz EA (2018). The effects of climate change associated abiotic stresses on maize phytochemical defenses. *Phytochem. Rev*. 17(1): 37-49.

Wang C, Yang A, Yin H, Zhang J (2008). Influence of water stress on endogenous hormone contents and cell damage of maize seedlings. *J. Integr. Plant Biol*. 50(4): 427-434.

Xiang Y, Sun X, Gao S, Qin F, Dai M (2017). Deletion of an endoplasmic reticulum stress response element in a *ZmPP2C-A* gene facilitates drought tolerance of maize seedlings. *Molecular Plant*, 10(3), 456-469.

Xu J, Zhang S (2015). Mitogen-activated protein kinase cascades in signaling plant growth and development. *Trends in Plant Sci*. 20(1): 56-64.

Zhang J, Kirkham MB (1996). Antioxidant responses to drought in sunflower and sorghum seedling. *New Phytol*. 132: 361-373.

Zhang L, Becker DF (2015). Connecting proline metabolism and signaling pathways in plant senescence. *Front. Plant Sci.* 6(552):1-8.