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Determining of Genetic Affinity and Divergence of Some Cultivars of *Zea mays* Grown Under Water Stress Conditions

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Abstract

The maize plant is the most important role in making sure people have enough food after rice and wheat, it is the third most important source of calories, because there wasn't enough rain, which caused maize output to drop. The present study aimed to identifying genetic differences between different varieties of maize plants under water stress condition. Three varieties of corn seeds (Local, Sudanese, and Spanish) were studies for their pattern of *ahb2* expression under three periods of water stress conditions. Our findings indicated that the *ahb2* gene in corn exhibits a pronounced response to water stress, but in a manner contingent upon genotype. In both the local and Spanish cultivars, *ahb2* expression exhibited a non-linear trajectory, characterized by an initial upregulation at day 7, suppression at ten days, and a pronounced induction after 14 days. This dynamic profile indicates that *ahb2* may be involved in early signaling, mid-phase metabolic adaptation, and late-stage stress resilience processes. This suggests that prolonged or delayed overexpression of *ahb2* may be detrimental to drought survival, presumably by delaying stomatal closure. The Sudanese type, on the other hand, showed a steady decrease in *ahb2* levels as the water stress progressed. This pattern is more in line with better water stress adaptability. This kind of inhibition might make ABA levels rise, help stomata close, and start stress-adaptive signaling pathways.

***Keywords*:** *ahb2, Maize, Water Stress, Gene Expression.*

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I. INTRODUCTION

The complex relationships among rising human populations, agricultural production, and climate change are very pertinent to the agricultural sector. In the previous twenty years, the requirement for higher agricultural yields to feed the growing number of people throughout the world has grown considerably (Liu *et al*., 2023a). Changes in temperature, as well as changes in the amount, distribution, and timing of precipitation, all make maize output less stable (McMillen *et al*., 2022).

Due to their immobility anchored by roots, plants encounter a range of environmental pressures that influence their growth and development throughout their life cycle (Wang *et al*., 2019). Due to the rise in global temperatures, the environment is suffering from severe water stress (Sheoran *et al*., 2022). The agricultural sector is particularly susceptible to the swift changes occurring in the climate. Among many unprecedented challenges, drought represents a significant challenge to global crop production, as it is considered the most dangerous abiotic stress due to its impact on crop productivity, as water stress causes economic losses in agriculture (Adebayo and Menkir, 2015). Maize stands as the predominant staple crop across sub-Saharan Africa, significantly contributing to food security, but it is vulnerable to severe drought, losing 15-20% of its production is due to drought every year (Lunduka *et al*., 2017; FAO, 2021). Maize works to reduce the impact of water shortages using three basic mechanisms, including escaping drought, tolerating or avoiding drought. Escape is a mechanism to prevent water shortages from coinciding with the main growth stages and is achieved through flowering and early maturity (Tang *et al*., 2025). Drought avoidance is the ability of the plant to avoid or reducing water deficiency while maintaining cell swelling by increasing water absorption using a larger or deeper root system, or reducing water use by closing stomata (Lunduka *et al*., 2017; Messina *et al*., 2021). Therefore, it is important to develop maize varieties that can withstand abiotic stresses such as water stress (Raza *et al*., 2019). Due to economic losses in this crop amounting to 10 million tons annually with rising temperatures, the use of new

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molecular methods mainly targeted the development of drought-resistant varieties (Shoeran *et al*., 2022). There are several sources of genetic variation that potentially contain alleles that increase drought resistance (Barbosa *et al*., 2021). Gene regulation in response to drought stress during the seedling stage was also studied, as many genes were identified during osmotic and drought stress (Zhu, 2016). It was divided into several paths, including the abscisic acid path ABA, an essential plant hormone that participates in stomatal closure and gene expression in response to drought. There are other pathways besides this hormone, such as osmotic stress signals (Fujii and Zhu, 2012). Even though *ahb2* is known to negatively regulate drought tolerance, little is known about how its expression changes naturally over time and across different maize genotypes under water deficit. This work was carried out to better understand the role of *ahb2* gene in maize responses to drought stress, given the growing importance of water scarcity for global food security.

II. Materials And Methods

A. Sample collection

Corn seeds (local, Sudanese, and Spanish varieties) were obtained from commercial agricultural markets in Mosul, Iraq. Although not certified, seeds were selected based on uniform morphology and viability to reduce variability. The three varieties of corn seeds were planted in pots that contain mixed soil and left to grow for 14 days with good watered, then all the three cultivars were exposed to water stress (without watering) for duration of 7, 10 and 14 days consequently as a treatment, while, the control sample was good watered. The plants were then harvested and plant leaves separated in order to conduct molecular studies on them.

B. Gene expression level of (ahb2) using quantitative PCR technology

The process involved multiple steps as follows:

RNA extraction and quantification. Plant tissue was crashed using liquid nitrogen, then mixed with 1 ml of the Trizol, then nucleic acid mRNA was extracted by the kit supplied by the Transgene (China) and following the recommended steps. RNA concentration and purity was determined using a nanodrop device.

Complementary DNA synthesis. 1000ng of RNA extracted from all samples were converted to cDNA using the kit supplied by Transgene company (China) according to the installed steps.

Quantitative PCR reaction. To detect the level of *ahb2* expression, quantitative PCR was conducted using SYBR green and specific primers for *ahb2* and *cul*, as a housekeeping gene, as show in table (1) and following the PCR conditions shown in table (2).

Table 1. Sequence of primers used in the qPCR experiment.

Primer	Sequence	Reference
Hb2-F	CGAGGAGCAGACGAAGAAC	This study
Hb2-R	CTAGGAGCTAGGAAGCATCAAC	
CUL-F	GAAGAGCCGCAAAGTTATGG	This study
CUL-R	ATGGTAGAAGTGGACGCACC	

Table 2. qPCR conditions used for gene expression

No.	Stage	Temperature	Time	Number of Cycles
1.	Initial denaturation	95	5 min.	1
2.	Denaturation	95	1 min.	35
3.	Annealing	58	1 min.	
4.	Extension	72	1 min.	
5.	Final extension	72	5 min.	1
6.	Stop reaction	4	5 min	1

Calculation of gene fold.

The fold change in gene expression of *ahb2* was determined based on the target CT gene value relative to the standard housekeeping gene for both the control and plant samples, utilizing the following equations (Haimes *et al*., 2013):

$\Delta CT\ (test) = CT\ (target, test) - CT\ (ref, test)$

$\Delta CT\ (control) = CT\ (target, control) - CT\ (ref, control)$

$\Delta\Delta CT = \Delta CT(test) - \Delta CT(control)$

Finally the gene expression fold was calculated following the equation below:

$$\text{Gene fold} = 2^{-\Delta\Delta CT}$$

Graphs and statistical analysis were conducted using Graphpad prism 8.0 software.

III. Results

From results greenhouse experiment show grow maize plants under water stress (Figure 1). Our results from molecular experiment that the *ahb2* gene in maize showed a strong response to water stress, however, exhibited a non-linear expression pattern. Results for the local variety (Figure 2) show that after day 7 of water deficit, expression of *ahb2* increased threefold compared to the control. However, at day 10, expression dropped sharply below control levels, while a significant increase in *ahb2* expression at 14 days was noticed. Similar results were seen with the Spanish variety indicating similar pattern of gene expression in both local and Spanish variety (Figure 3).

Unlike the previous dataset obtained from the local and Spanish variety, values of the Sudanese variety showed a progressive downregulation of *ahb2* with increasing water availability in soil (Figure 4). In the first week of exposure to water stress, expression was reduced by almost half. Further after 10 days, reduction strengthens the effect lowering *ahb2*.

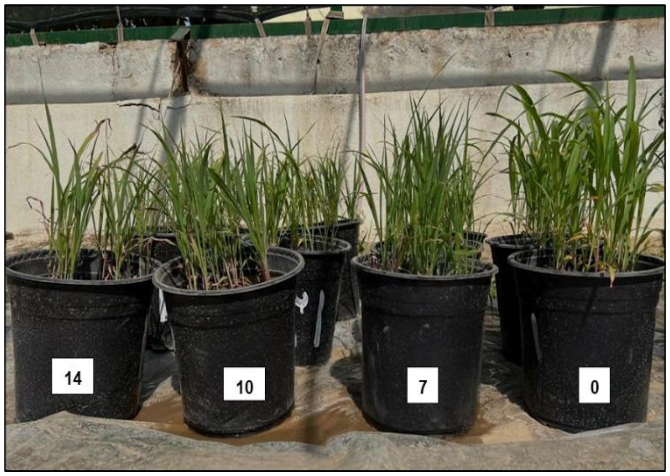

Figure 1. The greenhouse experiment.

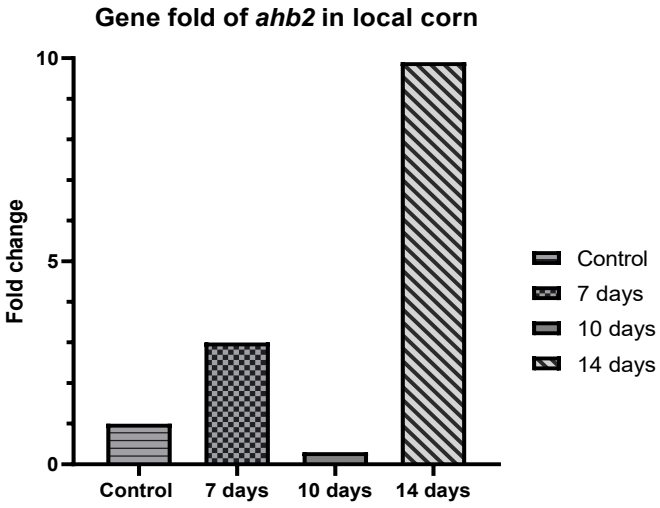

Figure 2. Gene fold of *ahb2* in local corn variety.

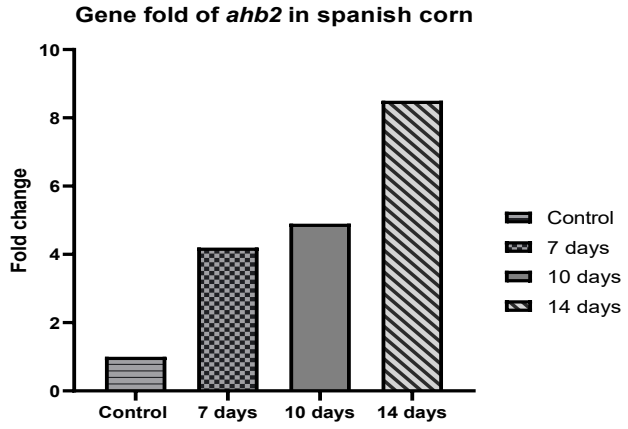

Figure 3. Gene fold of *ahb2* in Spanish corn variety.

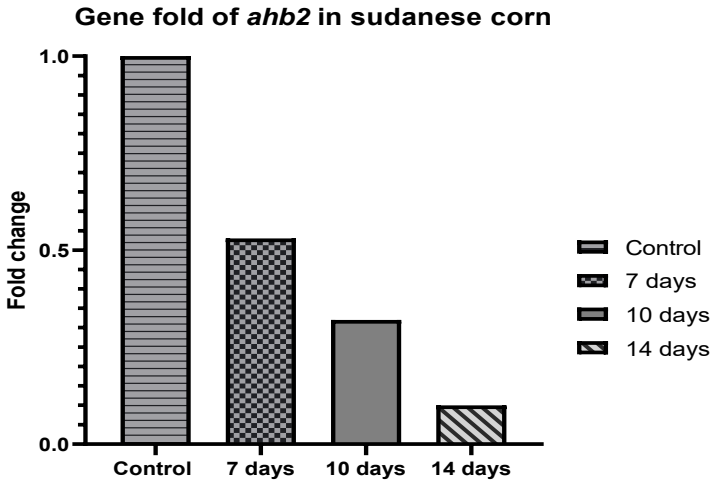

Figure 4. Gene fold of *ahb2* in Sudanese corn variety

IV. Discussion

According to our results, the *ahb2* gene in maize showed a strong response to water stress, however, exhibited a non-linear expression pattern. Results for the local variety (Figure 1) at day 7 of water deficit, expression of *ahb2* increased threefold compared to the control, suggesting that *ahb2* may be involved in early stress signaling or adaptation. Such early upregulation is often linked to the activation of protective mechanisms such as antioxidant defense, osmolyte biosynthesis, or signaling cascades that mitigate stress-induced damage (Hu, 2022). However, at day 10, expression dropped sharply below control levels. This may indicate down regulation due to metabolic suppression, resource reallocation, or feedback inhibition. In prolonged moderate stress, plants often shift from active defense to conserving energy for survival, leading to transient repression of certain stress-related genes (Shinozaki and Yamaguchi-Shinozaki, 2007). After day 14 indicates the activation of robust stress-induced pathways or a transition into a severe-stress survival phase. This increase may be associated with late-stage antioxidant defense, senescence signaling, or the activation of stress memory (Vishwakarma *et al*., 2017). This may potentially indicate permanent metabolic alterations when the plant allocates resources to defensive mechanisms. It could also show alterations in metabolism that cannot be undone since the plant is using its resources to protect itself. Our results for the local variety differ from those reported by Liu *et al*. (2020), who found that three homozygous maize lines with CRISPR-mediated *ahb2* knockout exhibited greater drought tolerance than wild-type plants. After the drought, the three mutants were far more likely to survive than wild-type plants.

This supports the idea that *abh2* has a deleterious effect on stress tolerance. They also showed that detached leaves from the abh2-CRISPR lines exhibited reduced water loss after a 1 to 4 hours dehydration treatment, suggesting that the abh2 deletion may facilitate more rapid stomatal closure in response to dehydration stress. Similar results were seen with the Spanish variety indicating similar pattern of gene expression in both local and Spanish variety (Figure 2).

Unlike the previous dataset obtained from the local and Spanish variety, values of the Sudanese variety showed a progressive downregulation of *ahb2* with increasing drought duration (Figure 3). In the first week of exposure to drought, expression was reduced by almost half. Early repression of *ahb2* may already begin enhancing ABA accumulation and stomatal closure, helping the plant conserve water. Further after 10 days, reduction strengthens the effect lowering *Ahb2* means stronger activation of drought-adaptive signaling. This is consistent with what was found earlier in *ahb2* knockout maize lines, which exhibited higher drought tolerance (Liu *et al*., 2020; Liu *et al*., 2023b). This strong suppression fits nicely with what we know about *Ahb2* detrimental involvement in how plants respond to drought stress. There are several reasons that may cause the change in gene expression and particularly for *ahb2*, Genotype, leaf age, tissue type (guard-cell-rich vs. bulk leaf), drought severity/soil water potential, time of day/circadian rhythm, ABA status, and ROS/NO backdrop can all change *Ahb2* dynamics and cause transient peaks to flip (Sheoran *et al*., 2022).

V. Conclusions

Our findings showed that *ahb2* gene in maize exhibited a clear response to water stress, with expression patterns varying between genotypes. In some varieties, *ahb2* followed a non-linear trajectory, with early up-regulation, mid-phase suppression, and strong late induction, suggesting roles in stress signaling and late survival. In contrast, other varieties displayed progressive down-regulation, which is more consistent with enhanced drought tolerance reported in *ahb2* knockout lines. These differences highlight that *ahb2* expression is context- and genotype-dependent, influenced by factors such as stress severity and physiological state. Our results suggests that *ahb2* appears to function as a negative regulator of drought tolerance, and its suppression may represent an adaptive strategy in maize. These findings show that different maize genotypes control *ahb2* in different ways, which suggests that they have different ways of responding to drought. The non-linear expression seen in certain types may signify stress intensity thresholds or circadian/physiological control, whereas progressive suppression aligns more closely with adaptive drought tolerance mechanisms. Overall, our research indicates that *ahb2* is a significant regulator, and its expression pattern during drought may influence the equilibrium between sensitivity and tolerance in maize. To our knowledge, the genotype exhibiting progressive down-regulation of *ahb2* is likely more drought tolerant and may better maintain yield under water stress conditions, although future work may focus on direct yield measurements to confirm this relationship.

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