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DROUGHT-STRESS EFFECTS ON RESISTANT GENE EXPRESSION, GROWTH, AND YIELD TRAITS OF MAIZE (*ZEA MAYS* L.)

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SUMMARY

A maize (*Zea mays* L.) field experiment conducted during the crop season 2022 at the Experimental Farm, Al-Mahnawiya, Extension Training Center, Babylon, Iraq, sought to evaluate the water stress tolerance of four maize cultivars under different irrigation regimes. The experiment used a randomized complete block design (RCBD) with a split-plot arrangement and three replications. Four irrigation treatments comprised the main plots: full irrigation (control), no irrigation during elongation (Gs-V7), no irrigation during grain-filling (Gs-R2), and no irrigation during elongation and grain-filling (Gs-V7+R2). The subplots included four maize cultivars: Furat, Dijlah, ZP, and Konsens. Leaf area decreased by 1873.76 cm^2 $plant^{-1}$ during the elongation stage (Gs-V7) due to non-irrigation. Non-irrigation during elongation (Gs-V7) and both elongation and grain-filling (Gs-V7+R2) reduced rows per ear, grains per row, and 500-grain weight at 11.65 and 11.02 rows ear^{-1}, 26.77 and 23.23 grains row^{-1}, and 54.90 and 63.94 g, respectively. Withholding irrigation during the elongation stage (Gs-V7), the filling (Gs-R2), and the elongation and filling phases all had decreased grain output. The lack of irrigation during the elongation stage (Gs-V7) boosted the *ZmMYBE1* gene expression in vegetative phases. However, irrigation suppression did not impact the *ZmMYBE1* gene expression in reproductive stages. The cultivar Furat had the most rows (17.58) and grains per row (37.58), and the cultivar Konsens had the maximum mean of 500-grain weight (84.36 g).

Keywords: Maize (*Zea mays* L.), irrigation regimes, cultivars, gene expression, water-stress tolerance, grain yield, yield-contributing traits

Key findings: The study demonstrated the stimulation of the *ZmMYBE1* gene expression in response to water scarcity. The study also revealed significant differences among the maize cultivars based on their ability to withstand stress, as evidenced by grain yield variations and their components.

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INTRODUCTION

Drought always poses a significant challenge to crop production in arid and semi-arid regions across the globe. Drought is a phenomenon that falls within the water security framework, which is a crucial component of food security. The reason is that water resources play a vital role in determining optimum crop production and its sustainability. In Iraq, the present and prospective water situation necessitates effective management of water resources to maximize its utilization (Abdulhamed *et al*., 2021).

In arid and semi-arid regions, enhancing water use efficiency can result in implementing deficit irrigation, known as partial irrigation. This approach addresses the primary objective of irrigation processes in said areas to combat drought resistance (Zou *et al.,* 2021). An increasing global population needs to enhance the productivity and output of crucial crops in optimal irrigated conditions and water-scarce and drought situations.

Maize (*Zea mays* L.) holds significant importance as a grain crop in Iraq and worldwide, ranking as the third most essential crop after wheat and rice (Liu *et al.*, 2021). Maize is crucial in ensuring food security; however, its productivity has sustained adverse impacts from water scarcity conditions. Hence, an increasing demand for the development of maize cultivars capable of thriving and yielding satisfactory harvests in water-deficient environments has persisted. Chukwudi *et al*. (2021) mentioned that the maize cultivars possessing water-tolerant traits hold promise in mitigating drought-related challenges to a specific degree.

Moreover, the drought-tolerance attributes have been a crucial factor in this context. The genetic control of maize genotypes and the utilization of advanced tools, such as, DNA-based markers, offer reliable means to assess genetic diversity in maize. This preferred approach relies solely on phenotypic and biochemical characteristics, which can also incur influences from environmental factors and protracted growth processes. In genetics, DNA markers are mainly valuable as they can provide a rapid means of visualizing the genetic sequence in plants to identify the genetic variation among individuals (Mengesha *et al*., 2017).

The polymerase chain reaction (PCR) technique has been instrumental in detecting the specific genes that confer tolerance to water drought. Additionally, researchers have utilized advanced technology, such as, quantitative real-time PCR (qPCR), to investigate the expression levels of numerous genes. This progressive approach facilitates exploring novel genetic resources that exhibit drought tolerance (Luo *et al*., 2018***)***. The regulation of maize's drought tolerance control is from specific genetic elements known as drought-tolerant genes. These genes exhibit variability across different genotypes, contributing to variations in drought tolerance levels among the diverse genotypes.

Based on this principle, numerous genes associated with drought tolerance in the maize crop have been identified, including the *ZmMYBE1* gene. This gene plays a crucial role in regulating plant growth and development and in the defense response against abiotic stresses, thereby contributing to the drought tolerance of maize plants (Sun *et al*., 2022). Therefore, the prevailing research aimed to utilize the *ZmMYBE1* gene as a marker for identifying drought-tolerant maize cultivars and assessing their gene expression levels. The study also aimed to evaluate the maize cultivars' growth and yield characteristics under reduced irrigation conditions.

MATERIALS AND METHODS

The presented research employed a standardized approach to investigate the material for study and methods utilized in this work***.*** A maize field experiment setup transpired during the crop season 2022 at the Experimental Farm, Al-Mahnawiya, Extension Training Center, Babylon, Iraq. The study aimed to investigate the water-stress tolerance of four maize cultivars and assess the expression of the *ZmMYBEI* gene.

The experimental land's partitioning was on an arrangement of split plots, utilizing a randomized complete block design (RCBD) with three replications. The main parcels consisted of irrigation withholding strategies, specifically, a comparison treatment involving irrigation after depleting 50% of the available water (Gs0) and withholding one irrigation during the elongation stage (Gs-V7). Implementing non-irrigation during the grain-filling period (Gs-R2) and once during the elongation and grain-filling stages (Gs-V7+R2) incurred evaluation. The assessment of maize crop growth stages used the Abendroth scale (Abendroth *et al.,* 2011). The subplots comprised four distinct maize cultivars, i.e., Furat, Dijlah, ZP, and Konsens.

Maize tolerance genes identification

DNA Extraction

The existing study investigated the *ZmMYBE1* gene's potential role in conferring water stress tolerance in maize plants. The DNA extraction process involved using a kit manufactured by Add Bio-Company, Korea, wherein the leaves of four distinct maize cultivars underwent DNA extraction. The extraction procedure ensued according to the instructions provided by the kit's manufacturer.

PCR – Amplification process

The *ZmMYBE1* gene's diagnosis involved a polymerase chain reaction (PCR) test employing specific primers (F: AGACGAAGATGGCCTCCAAC) and (R: AGTGATTCCTGGTGGTGGTG). The experiment utilized the Maxime™ PCR PreMix (i-Taq) kit provided by the iNtRoN irrigation company. The reaction mixture had a total volume of 25 microliters and consisted of the components specified in Table 1. The remaining quantity incurred nuclease-free water to reach a final amount of 25 microliters.

The preparation of the amplification mixture began in a sterile tube, with a separate cylinder designated as the negative control to ensure the absence of nucleic acid. Combining the concoction components used a micropipette and, subsequently, subjected to centrifugation to ensure the final volume of the reaction mixture. Finally, the mixture's transfer continued to a thermo polymerase chain reaction (PCR) device. The program outlined in Table 2 was a particular design for gene amplification. After the completed amplification process, the tubes gained refrigeration until the conduct of electrophoresis (Figure 1).

Table 1. PCR mixture concentrations.

Components	Volume (µl)
Taq PCR PreMix	10
Forward primer	1
Reverse primer	1
DNA	5
Distilled water	8
Final volume	25

Table 2. The *ZmMYBE1* gene PCR program.

Stages	Temperature (°C)	Time	Number of cycles
Initial Denaturation	95	3 min	1
Denaturation - 2	95	45 s	
Annealing	59	45 s	35
Extension - 1	72	2 min	
Extension - 2	72	7 min	1

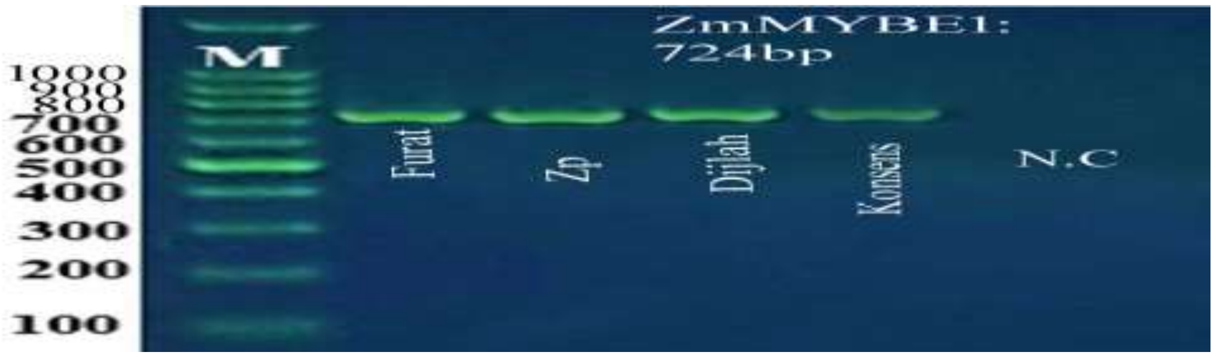

Figure 1. Electrophoresis of the *ZmMYBE1* gene primer PCR products without DNA. Besides the DNA ladder, sizes shown on the left.

***ZmMYBE1* gene expression**

The process involves collecting leaf samples from the maize crop from each experimental unit. The RNA incurred isolation from the trials utilizing a kit provided by the Add Bio Company, Korea. The method proposed by Livak and Schmittgen (2001) was helpful to assess the relative gene expression. The Actin gene served as a reference gene, with its expression 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:
$ct_{target\ gene}$ was the target gene's cycle threshold,
$ct_{reference\ gene}$ was Actin's cycle threshold,
Ct_{test} was the cycle threshold for target gene sample, and

The control sample's cycle threshold for the target gene was $CT_{Control}$

RTqPCR quantifies gene expression

The study parameters assessment used the RTqPCR assay, performed at specific growth stages and on different plant parts. The use of specialized primers (GCTTCAGGTGCTCTGCCTAC) and (TTCCATCCTGCTAGCGAAGT) was a requirement in this reaction. The GoTaq® Probe RT-qPCR Master Mix kit provided by Promega aided the assay process. The appropriate volume of all RTqPCR components determined followed the details based on Table 3. Combining the mentioned components in a rotary mixer included operating at a speed of 3000 rpm for 10 seconds. Subsequently, the mixture transferred to the instantaneous thermal polymerization device executed the procedures outlined in Table 4.

Data recorded

The measurement for plant height (cm) calculated the average of five plants randomly selected from each experimental unit. Acquiring the leaf area per plant (cm^2 $plant^{-1}$) averaged the values obtained from five plants. The leaf area's calculation used the following formula (Jasab and AL-Jubouri, 2013).

$$\text{leaf area} = \text{leaf length squared under the ear leaf} \times 0.75$$

Determining the number of rows in the ear continued through manual calculation, deriving the mean value afterward. The number of grains in each row's computation also proceeded manually, with the average values drawn. For 500-grain weight (g), each sample collected for five plants harvested from each experimental unit had their weight

Table 3. RT-qPCR components.

Components	Volume
GoTaq® RT-qPCR Master Mix	10
Forward primer of target gene	1
Reverse primer of target gene	1
Forward primer of gene reference	1
Reverse primer of gene reference	1
Nuclease-free water	6
RNA Sample Volume	5

Table 4. The ZmMYBE1 RT-qPCR program.

Stages	Temperature (°C)	Time	Number of cycles
cDNA synthesis	50	20 min	Hold
Denaturation Initial	95	10 min	Hold
Denaturation	95	45 s	40
Annealing	60	45 s	40
Extension	72	1	40
Extension	72	5	Hold

measured using a precise electric scale***.*** Grain yield (tons ha^{-1}) reached estimation from the average weight of the yield of one plant taken as the average of five plants harvested from each experimental unit multiplied by the plant density per hectare.

Statistical analysis

The data on all the maize quantitative traits under investigation sustained analysis of variance (ANOVA). The least significant difference (LSD) test was also helpful for means comparison and separation. The statistical analysis employed the Gene Stat software program to compare the arithmetic means.

RESULTS AND DISCUSSION

ZmMYBE1 **gene identification**

Following the establishment of PCR reaction conditions to amplify the *ZmMYBE1* gene in four distinct maize cultivars (Furat, Zp, Dijlah, and Konsens), the resulting reaction products continued to transfer onto an agarose gel. The maize exhibited resilience to drought conditions due to its crucial involvement in regulating the transcription process during treatment. Furthermore, a negative correlation was distinct between the CT values and the plant metabolism and development and its influence on modulating the response to light.

Maize leaf ***ZmMYBE1*** **gene expression**

The applied RT-qPCR technology amplified the *ZmMYBE1* gene, investigating its relative expression in the leaves at the maize cultivars' vegetative and reproductive stages. The analysis transpired under the influence of withholding irrigation at two distinct stages of plant growth and their intersection. The expression of the *ZmMYBE1* gene was evident in the maize leaves during the vegetative stage, whereas nonsignificant variations were apparent in the relative expression estimation during the reproductive stage (Table 6). Applying the GS-V7+R2 treatment resulted in a notable reduction in cycle threshold (CT) values (with average values of 29.69 and 29.68 cycles). By comparison, the complete irrigation treatment (GS0) yielded CT values of 30.37 cycles, which did not exhibit a significant difference compared with the variant involving the withholding of irrigation during the grain-filling stage (GS-R2), producing an average value of 30.20 cycles, These figures are also available in Table 6.

It is important to note that the related parameter measurement ensued before implementing the irrigation withholding relative expression of the gene (Table 5). It was also notable that the irrigation withholding during the elongation stage (GS-V7) and the elongation and grain-filling stages (GS-V7+R2) significantly increased the relative expression of the gene *ZmMYBE1*. Specifically, the relative expression of the *ZmMYBE1* gene at the vegetative stage was 1.63 and 1.69-fold higher, respectively, compared with the control treatment (GS0).

The observed upregulation of gene expression may refer to the influence of stress-induced modifications in DNA methylation, leading to alterations in gene expression levels (Dodig *et al.,* 2019; Kumar *et al*., 2015b; Javaid *et al*., 2023). Additionally, it is conceivable that specific cells possess stress-sensing mechanisms, wherein sensitive molecules on their surfaces detect stress indicators and subsequently transmit signals to modulate cellular metabolic processes by modifying the gene expression (Rodríguez *et al.,* 2005). Consequently, heightened stress levels may augment the signaling pathways required for the *ZmMYBE1* gene to function correctly.

The results also indicated significant variations among the maize cultivars based on CT values (Table 6). The cultivars Furat and Dijlah exhibited the lowest CT values of 29.59 and 29.84 cycles, respectively, and the cultivars Zp and Dijlah yielded higher CT values of 30.20 and 30.31 rotations, respectively. The relative expression of the *ZmMYBE1* gene in the mentioned cultivars, Konsens and Dijlah, caused an increase as proof of the results (Table 6). The relative expression values at the vegetative stage appeared to be 1.58 and 1.38 times higher for maize cultivars Konsens and Dijlah, respectively.

Table 5. Effect of irrigation-withholding treatments on cycle threshold (CT) and relative *ZmMYBE1* gene expression in maize cultivar leaves at vegetative stage.

Withhold Irrigation		CT Actin gene	CT of *ZmMYBE1* gene	Δ CT of *ZmMYBE1* gene	ΔΔ CT of *ZmMYBE1* gene	Gene Expression
GS0		18.56	30.37	11.81	0.00	1.00
GS-V7		18.53	29.69	11.15	-0.66	1.63
GS-R2		18.44	30.20	11.76	-0.05	1.03
GS-V7+R2		18.57	29.68	11.11	-0.70	1.69
$LSD_{0.05}$		------	0.254	--------	------	0.233
Maize Cultivars		**CT Actin gene**	**CT of *ZmMYBE1* gene**	**Δ CT of *ZmMYBE1* gene**	**ΔΔ CT of *ZmMYBE1* gene**	**Gene Expression**
Furat		18.60	29.84	11.24	-0.40	1.38
Zp		18.65	30.20	11.54	-0.24	1.21
Dijlah		18.39	29.59	11.21	-0.55	1.58
Konsens		18.46	30.31	11.85	-0.21	1.18
$LSD_{0.05}$		------	0.401	-------	-----	0.231
Interactions		**CT Actin gene**	**CT of *ZmMYBE1* gene**	**Δ CT of *ZmMYBE1* gene**	**ΔΔ CT of *ZmMYBE1* gene**	**Gene Expression**
GS0	Furat	18.69	30.33	11.64	0.00	1.00
	Zp	18.71	30.49	11.78	0.00	1.00
	Dijlah	18.53	30.28	11.75	0.00	1.00
	Konsens	18.31	30.38	12.06	0.00	1.00
GS-V7	Furat	18.51	29.43	10.92	-0.72	1.65
	Zp	18.58	30.04	11.47	-0.31	1.25
	Dijlah	18.60	29.15	10.55	-0.20	2.31
	Konsens	18.44	30.12	11.68	-0.38	1.30
GS-R2	Furat	18.65	30.18	11.53	-0.11	1.08
	Zp	18.44	30.20	11.77	-0.01	1.01
	Dijlah	18.09	29.79	11.70	-0.05	1.04
	Konsens	18.57	30.63	12.06	-0.01	1.01
GS-V7+R2	Furat	18.55	29.41	10.86	-0.78	1.81
	Zp	18.89	30.04	11.16	-1.62	1.58
	Dijlah	18.33	29.16	10.83	-0.93	1.96
	Konsens	18.49	30.10	11.61	-0.46	1.40
$LSD_{0.05}$		------	0.721	-----	-----	0.442

Table 6. Effect of irrigation-withholding treatments on cycle threshold (CT) and relative *ZmMYBE1* gene expression in maize cultivar leaves at reproductive stage.

Withhold Irrigation		CT Actin gene	CT of *ZmMYBE1* gene	∆ CT of *ZmMYBE1* gene	∆∆ CT of *ZmMYBE1* gene	Gene Expression
GS0		18.56	34.44	15.88	0.00	1.00
GS-V7		18.53	34.35	15.82	-0.06	1.04
GS-R2		18.44	34.29	15.85	-0.04	1.03
GS-V7+R2		18.57	34.38	15.82	-0.07	1.05
$LSD_{0.05}$		------	N.S	--------	------	N.S
Maize Cultivars		CT Actin gene	CT of Z *ZmMYBE1* gene	∆ CT of *ZmMYBE1* gene	∆∆ CT of *ZmMYBE1* gene	Gene Expression
Furat		18.60	34.29	15.69	-0.05	1.04
Zp		18.65	34.48	15.83	-0.04	1.03
Dijlah		18.39	34.24	15.86	-0.07	1.05
Konsens		18.46	34.45	15.99	0.00	1.00
$LSD_{0.05}$		------	N.S	-------	-----	N.S
Interactions		CT Actin gene	CT of *ZmMYBE1*gene	∆ CT of *ZmMYBE1*gene	∆∆ CT of *ZmMYBE1* gene	Gene Expression
GS0	Furat	18.69	34.44	15.74	0.00	1.00
	Zp	18.71	34.58	15.87	0.00	1.00
	Dijlah	18.53	34.45	15.93	0.00	1.00
	Konsens	18.31	34.31	15.99	0.00	1.00
GS-V7	Furat	18.51	34.22	15.70	-0.04	1.03
	Zp	18.58	34.35	15.77	-0.10	1.07
	Dijlah	18.60	34.41	15.82	-0.11	1.08
	Konsens	18.44	34.44	16.00	-0.01	0.99
GS-R2	Furat	18.65	34.32	15.67	-0.08	1.06
	Zp	18.44	34.27	15.83	-0.03	1.03
	Dijlah	18.09	33.92	15.83	-0.09	1.07
	Konsens	18.57	34.63	16.06	0.06	0.96
GS-V7+R2	Furat	18.55	34.19	15.64	-0.10	1.07
	Zp	18.89	34.72	15.83	-0.03	1.02
	Dijlah	18.33	34.19	15.86	-0.07	1.05
	Konsens	18.49	34.42	15.93	-0.06	1.04
$LSD_{0.05}$		------	0.661	-----	-----	0.085

The noted upregulation of the *ZmMYBE1* gene suggests a potential association with drought tolerance in these maize cultivars. The variation in the expression of the *ZmMYBE1* gene among different cultivars can be due to genetic variations and the potential occurrence of mutations that modify gene expression by altering the signaling mechanism. Additionally, the disparity was also apparent in the cultivars' capacity to transmit the signal that stimulates enhanced gene expression (Vranová *et al.,* 2002), or their divergence in transcription factors responsible for upregulation and down-regulation of gene expression may also contribute to this difference (Denekamp and Smeekens, 2003).

Plant height

The results presented on the implementation of two irrigation withholding treatments, particularly at the elongation stage (GS-V7) and at the elongation and grain-filling stages (GS-V7+R2), showed a significant reduction in plant height (Table 7). The average plant height recorded for the GS-V7 and GS-V7+R2 treatments were 171.26 cm and 170.69 cm, respectively. These values of plant height were notably lower compared with the complete irrigation regime. The average plant height found in treatment GS0 was 205.82 cm, which was not significantly different from the treatment GS-R2 (207.20 cm). The observed reduction in plant height resulting from

Table 7. Effect of withholding irrigation on plant height in maize cultivars.

Irrigation withholding	Maize cultivars				Means (cm)
	Furat	ZP	Dijlah	Konsens	
GS0	184.51	230.73	185.82	222.22	205.82
GS-V7	140.39	205.28	161.98	177.39	171.26
GS-R2	188.93	232.50	185.26	222.10	207.20
GS-V7+R2	139.47	203.77	159.00	180.51	170.69
$LSD_{0.05}$	14.80				13.31
Means (cm)	163.32	218.07	173.02	200.56	
$LSD_{0.05}$	5.09				

Table 8. Effect of withholding irrigation on leaf area (cm^2 $plant^{-1}$) in maize cultivars.

Irrigation withholding	Maize cultivars				Means (cm^2 $plant^{-1}$)
	Furat	ZP	Dijlah	Konsens	
GS0	3776.51	3543.82	3190.73	3300.22	3452.82
GS-V7	2362.39	1949.98	1792.28	1463.39	1892.01
GS-R2	3725.93	3488.26	3137.50	3245.10	3399.20
GS-V7+R2	2373.47	1937.00	1805.77	1456.51	1893.19
$LSD_{0.05}$	231.98				176.16
Means (cm^2 $plant^{-1}$)	3057.58	2729.77	2481.57	2366.31	
$LSD_{0.05}$	102.64				

withholding irrigation can be attributable to the insufficient water within the chloroplast, decreasing relative water content. The water deficit conditions disrupt the enzymatic system, leading to the inhibition of photosynthesis and the cessation of cell growth and development (Gupta, 2011). The presented results also align with the conclusions drawn by Azarpanah *et al*. (2013), who reported a significant reduction in plant height during the vegetative growth phase due to the impact of water stress on cellular division and elongation**.**

The results further presented significant variations among the maize cultivars for plant height. Distinctively, the maize cultivars ZP and Konsens exhibited the highest average plant height, measuring 218.07 cm and 200.56 cm, respectively. The cultivars Furat and Dijlah had average plant heights of 163.32 cm and 173.02 cm, respectively (Table 7). The observed inconsistency among the genotypes for plant height, such as, Zp and Konsens, which exhibited taller plants than other genotypes, indicates a contribution of genetic factors. The variation in maize cultivars for plant height can point to genetic differences in the length of a specific segment and the number of these segments, regulated by specific genes (AL-Jobouri *et al.*, 2018**)**.The interaction effects between the irrigation-withholding treatments and the maize cultivars on plant height were also noteworthy. It was visible that the maize cultivars exhibited varying degrees of susceptibility to water stress, and the cultivar Furat displayed the lowest rate (23.91%) under the GS-V7 treatment. Conversely, the cultivar Zp demonstrated a different response. The group with the minimum degree of impact experienced a decrease of 11.03%.

Leaf area

The findings indicated that withholding irrigation during the elongation stage (GS-V7) and in the elongation and grain-filling stages (GS-V7+R2) significantly reduced leaf area (Table 8). Specifically, the average leaf areas for these treatments were 1892.01 cm^2 $plant^{-1}$ and 1893.19 cm^2 $plant^{-1}$, respectively. In contrast, the full irrigation treatment (GS0) yielded a leaf area average of 3452.82 cm^2 $plant^{-1}$, and also did not differ significantly from the treatment involving non-irrigation during the grain-filling stage (GS-R2) (3399.20 cm^2 $plant^{-1}$). The decrease in leaf area due to withholding irrigation can be due to the contraction of leaf tissue cells, hampering their capacity for elongation and expansion.

Additionally, a decline in growth-promoting hormones like auxins and gibberellins contributed to this reduction. The decrease in leaf turgor pressure adversely impacted the growth and enlargement of leaf cells, consequently reducing leaf area. The relevant finding also agrees with the observations made by Al-Awda and Khaiti (2008).

The results further presented significant variations among the maize cultivars for leaf area (Table 8). The cultivar Furat exhibited the highest average leaf area (3057.58 cm^2 $plant^{-1}$), whereas the three cultivars ZP, Dijlah, and Konsens had mean leaf areas of 2729.77, 2481.57, and 2366.31 cm^2 $plant^{-1}$, respectively. The variation among the maize cultivars for leaf area can be because of the genotypes' varied genetic makeup and differences in the duration of the two growth periods.

The findings also revealed a significant interaction between the irrigation-withholding treatments and the maize cultivars in the leaf area (Table 8). It was evident that the maize cultivars exhibited varying degrees of susceptibility to water stress. Specifically, the cultivar Furat demonstrated the minimum reduction (37.45%) under the GS-V7 treatment, whereas the cultivar Konsens incurred the most adverse effects, with a decrease of 56.66%. These results agreed and also got support from past findings in maize genotypes under abiotic stress conditions (Casaretto *et al.*, 2016).

Rows per ear

The outcomes indicated that the absence of irrigation during the elongation stage (GS-V7) and elongation and grain-filling stages (GS-V7+R2) significantly reduced the number of rows per ear in maize genotypes (Table 9). These treatments yielded average values of 11.65 and 11.02 rows per ear, respectively. In contrast, the treatment with complete irrigation (GS0) produced an average of 16.40 rows per cob, which did not differ significantly from the treatment of withholding irrigation during the grain-filling stage (GS-R2) (15.98 rows per cob). The observed reduction in rows per ear due to withholding of irrigation during the GS-V7 and GS-V7+R2 stages may refer to two factors. Firstly, there was a decrease in the duration from emergence to 50% of male flowering, which coincides with the period of ovary formation and growth. Secondly, the limited leafy area (as indicated in Table 8) could contribute to inadequate resource allocation, resulting in incomplete formation of the maximum number of rows per ear (Salem *et al*., 2012).

The results also revealed significant variations among the maize cultivars on the number of rows per ear (Table 9). The cultivar Furat exhibited the highest mean number of rows per ear, averaging 17.5 rows per ear. In contrast, the cultivars ZP, Dijlah, and Konsens had average numbers of rows per ear of 14.44, 12.84, and 10.19, respectively. This phenomenon can be due to the duration of the growth periods of the cultivar Furat, the leaf area (as indicated in Table 8), and the leaf area index. These factors also influenced the efficiency of photosynthesis, causing the production of an ample amount of photosynthetic products, enabling the formation of a higher number of rows in the ear (Abd-ul-Ameer and Ahmed, 2018).

Table 9. Effect of withholding irrigation on rows per ear in maize cultivars.

Irrigation withholding	Maize cultivars				Means (#)
	Furat	ZP	Dijlah	Konsens	
GS0	18.95	19.08	14.00	13.56	16.40
GS-V7	16.68	10.34	12.32	7.26	11.65
GS-R2	19.10	17.91	14.02	12.89	15.98
GS-V7+R2	15.57	10.45	11.00	7.06	11.02
$LSD_{0.05}$	4.11				3.77
Means (#)	17.58	14.44	12.84	10.19	
$LSD_{0.05}$	1.34				

Table 10. Effect of withholding irrigation on the grains per row in maize cultivars.

Irrigation withholding	Maize cultivars				Means (grain row^{-1})
	Furat	ZP	Dijlah	Konsens	
GS0	51.95	47.08	41.56	39.00	44.90
GS-V7	25.57	26.45	28.06	27.00	26.77
GS-R2	44.68	34.34	31.26	31.32	35.40
GS-V7+R2	28.10	26.91	21.89	16.02	23.23
$LSD_{0.05}$	7.44				5.56
Means (grain row^{-1})	37.58	33.69	30.69	28.34	
$LSD_{0.05}$	3.34				

The findings showed a significant interaction between the irrigation withholding treatments and the maize cultivars concerning the rows per ear (Table 9). It was also notable that the maize cultivars exhibited varying susceptibility to water stress conditions. In particular, the cultivar Furat demonstrated the minimum reduction (11.98%) under the GS-V7 treatment, whereas the cultivar Konsens experienced the maximum impact, with a decrease of 46.46%.

Grains per row

For grains per row, the implementation of irrigation-withholding treatments during the elongation stage (GS-V7) and the elongation and grain-filling stages (GS-V7+R2) significantly reduced maize genotypes (Table 10). The average number of grains per row for these treatments resulted in 26.77 and 23.23, respectively. Compared with the optimal irrigation treatment (GS0), which yielded an average of 44.90 grains per row, the irrigation-withholding treatment during the grain-filling stage (GS-R2) gave an average of 35.40 grains per row. The decrease in grains per row in the irrigation treatments (GS-V7 and GS-V7+R2) can be due to the positioning of seed originators within the row during the vegetative growth stages. It coincided with the application of water stress, resulting in a deficiency of photosynthetic products.

Consequently, the sites responsible for grain development decreased, leading to a decline in the overall grain count. The observed reduction in grain count within the row during the grain-filling stage (GS-R2) can point to the limited water availability. Water scarcity hampers the efficient transport of dry matter from the source to the downstream (grain) (Kumar *et al.,* 2015a, b), resulting in poor grain development. Accordingly, it impedes fertilization as the pollen grains become rigid and lifeless, failing to achieve successful fertilization and seed formation. Therefore, this phenomenon results in the hindered growth of the plant when it comes into contact with the stigmas (Mcphere and Boyer, 1977).

The results revealed considerable variations among the maize cultivars in the grains per row (Table 10). Specifically, the cultivar Furat exhibited the highest average number of grains per row (37.58). In contrast, the maize cultivars ZP, Dijlah, and Konsens had lower average grains per row, i.e., 33.69, 30.69, and 28.34, respectively. The superiority of the cultivar Furat may refer to its greater leaf area, as indicated in Table 8. This larger leaf area caused higher yields by reducing flower termination, eventually increasing the number of grains per row**.**

The results provided a significant interaction between irrigation-withholding treatments and maize cultivars based on grains per row (Table 10). It was also noticeable that the maize cultivars exhibited varying susceptibility to water stress. The cultivar Furat had the most impact, with 50.78% under GS-V7 treatment, whereas cultivar the Konsens had the slightest effect, with 30.77%. These results were in analogy with the findings of AL-Fatlawi *et al.* (2022), who identified the genotypes with drought tolerance.

500-grain weight

The outcomes enunciated that the act of withholding irrigation during the grain-filling stage (GS-R2) and the elongation and grain-filling stages (GS-V7+R2) significantly reduced the average 500-grain weight (Table 11).

Table 11. Effect of withholding irrigation on the 500-grain weight in maize cultivars.

Irrigation withholding	Maize cultivars				Means (g)
	Furat	ZP	Dijlah	Konsens	
GS0	73.02	79.32	72.34	88.26	78.23
GS-V7	75.29	82.00	80.41	93.23	82.73
GS-R2	50.10	33.69	61.25	74.56	54.90
GS-V7+R2	47.23	68.00	59.12	81.39	63.94
$LSD_{0.05}$	18.23				14.29
Means (g)	61.41	65.75	68.28	84.36	
$LSD_{0.05}$	7.83				

Table 12. Effect of withholding irrigation on the average grain yield in maize cultivars.

Irrigation withholding	Maize cultivars				Means (tons ha^{-1})
	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
$LSD_{0.05}$	0.96				0.81
Means (tons ha^{-1})	6.18	5.54	5.38	4.35	
$LSD_{0.05}$	0.38				

These two treatments yielded means of weight of 500 grains, i.e., 54.90 and 63.94 g, respectively, compared with the complete irrigation treatment (GS0) (78.23 g). Conversely, withholding irrigation during the elongation stage (GS-R2) led to a significant increase in grain weight, with an average of 82.73 g. The decrease in the average weight of the grain can point to a reduction in irrigation, which resulted in limited access to water and nutrients during the grain's formation and maturation phases. This limited access led to shrinkage and smaller grain size. The lack of transfer of carbon metabolism products from the source to the downstream also contributed to the decrease in grain weight. The closure of stomata during this period also lessened the diffusion of CO_2, resulting in a decline in photosynthesis. Consequently, the production of dry matter from the leaves decreased during the grain-filling stage, leading to a lower grain weight. Alternatively, the decrease in weight may come from reduced rates and duration of starch accumulation and redistribution in the endosperm (Gao *et al.,* 2017).

Significant differences appeared among the maize cultivars on the average weight of 500 grains (Table 11). Specifically, the cultivar Konsens exhibited the highest average weight of 500 grains (84.36 g). In contrast, the three cultivars Furat, ZP, and Dijlah had average masses of 61.41, 65.75, and 68.28 g, respectively. The observed augmentation in grain weight within the maize cultivar Konsens may be due to a reduction in the number of grains, commonly known as the compensation phenomenon. Anjum *et al*. (2011) reported a negative correlation between the grains per ear and the overall weight of the grain when subjected to drought-stress conditions***.***

The interaction effects between the irrigation-withholding treatments and the maize cultivars were significant about the 500-grain weight (Table 11). It was also evident that the maize cultivars exhibited varying susceptibility levels to water stress conditions. In particular, the cultivar Furat demonstrated the lowest rate of dry weight reduction (35.32%) with the GS-V7+R2 treatment, whereas cultivar Konsens demonstrated the minimum impact, with a rate of 7.78%.

Grain yield

The results revealed that the implementation of different irrigation strategies during the growth stages of maize, specifically withholding irrigation at elongation (GS-V7), at grain-filling (GS-R2), and both at elongation and grain-filling stages (GS-V7+R2), significantly reduced maize grain yield (Table 12) The average grain yield in these treatments were 5.36, 4.54, and

4.13 tons ha^{-1}, respectively, compared with the control irrigation treatment (GS0) (7.42 tons ha^{-1}). The observed reduction in grain yield under the GS-V7 treatment can likely have effects from the influence of water stress on leaf growth and expansion, as available in Table 8. This water stress leads to a decrease in light interception by the leaves and, subsequently, lowers the rate of carbon metabolism.

These unfavorable effects showed manifestations in the reduced rate of plant growth and dry matter accumulation, ultimately affecting the number of grain rows and grain size (Tables 9 and 10) and consequently adversely affecting the grain yield. The grain yield has links to its constituent elements, with the latter connected to the subcomponents of the yield. The decrease in harvest emerged when maize cultivars incurred withholding-irrigation treatments, resulting in a notable reduction in the 500-grain weight. The decline in grain yield may be due to the decrease in the number of grain rows per ear, grains per row, and grain size (Farhood *et al*., 2022).

The findings indicated significant variations among the different maize cultivars on average grain yield (Table 12). Specifically, the cultivar Furat exhibited the highest average grain yield (6.18 tons ha^{-1}), whereas the cultivars ZP, Dijlah, and Konsens had mean outputs of 5.54, 5.38, and 4.35 tons ha^{-1}, respectively. The observed superiority can refer to the cultivar Furat's higher number of rows and grains per row (Tables 10 and 11), consequently leading to an increased grain yield***.***

The results also revealed considerable interaction between the irrigation-withholding treatments and the maize cultivars about grain yield. It was evident that the maize cultivars exhibited varying susceptibility degrees to water stress conditions. Specifically, the cultivar Dijlah demonstrated the lowest grain yield reduction (50.13%) with the GS-V7+R2 treatment, while the cultivar Konsens exhibited the minimum impact, with a reduction rate of 43.07% (Trachsel *et al*., 2016).

CONCLUSIONS

Based on the study, a conclusion can relate to the *ZmMYBE1* gene as significant in enhancing the tolerance of different maize cultivars to water stress conditions. This inference has validity from the observed upregulation of the *ZmMYBE1* gene expression in the maize cultivar Furat. In regions experiencing water scarcity, the maize cultivar Furat is highly recommendable for cultivation due to its notable resistance to drought-stress conditions.

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