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DROUGHT STRESS EFFECTS ON RESISTANT GENE EXPRESSION, GROWTH, AND YIELD TRAITS OF WHEAT (*TRITICUM AESTIVUM* L.)

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SUMMARY

The study comprised two experiments that were carried out for two consecutive years (2019–2020 and 2020–2021) at the Agricultural Research Station, Babil Governorate, Iraq. In the first experiment, seven wheat (*Triticum aestivum* L.) cultivars, viz., Iraq, Ezz, Abba-99, Furat, Sham-6, N-70, and Tamoz, were studied for most drought- tolerant genes in 2019–2020. During this year, three drought-tolerant wheat cultivars, i.e., Iraq, Tamoz, and Abba-99, were selected having the most drought-tolerant genes. In the second experiment, the three selected drought-tolerant wheat cultivars under three different drought stress conditions (D-1, D-2, and D-3) were studied during 2020–2021 in a randomized complete block design (RCBD) with three replications using a split-plot arrangement. The study aimed to determine the impact of drought effects on the expression of drought-resistant genes, growth, and yield traits in wheat. The results showed that wheat cultivars differed in their possession of drought-resistant genes (ABC4, GPAT, GBSS1, and umc1283), and the bands appeared in cultivars, Iraq, Tamoz, and Abba-99, while the rest of the four cultivars had lost one or two genes. Cultivar Iraq was distinguished as the most drought-tolerant genotypes, by having an increased relative expression of genes, ABC4 and GPAT, compared with other cultivars. The D-3 - drought stress condition caused a significant reduction in the biological and grain yield, and harvest index, with a decrease of 38.85%, 12.60%, and 29.83%, respectively. Cultivar Iraq was the least affected for plant height, flag leaf area, tillers meter^2, biological and grain yield, and harvest index when increasing drought severity, and these traits decreased by 16.96%, 24.08%, 44.17%, 28.08%, 15.10%, and 15.29%, respectively. Results authenticated wheat cultivars differed in the expression of drought-resistant genes, and drought resistance is largely controlled by genes.

Keywords: Wheat (*Triticum aestivum* L.), drought stress, drought-resistant genes, genes expression, growth traits, grain yield, and related traits

Key findings: As a result of severe drought stress conditions in wheat cultivars, the biological and grain yield, and harvest index decreased by 12.60%, 38.85%, and 29.83%, respectively. Wheat cultivar Iraq was the most tolerant genotype to the drought stress conditions with the growth and yield-related traits being the least affected. The study also demonstrated that genes ABC4 and GPAT play an important part in regulating the response of different wheat cultivars to drought stress conditions.

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INTRODUCTION

Drought is an abiotic stress factor imposed by the environment that affects optimal performance (Leslie and Vahdati, 2013; Swelam *et al*., 2022), which leads to a decline and deviation from the potential performance of the crop plants (Larcher, 2003). The drought effect is very complex as it subjects many physiological, biochemical, and molecular changes in higher plants, and it constitutes a great challenge for crop scientists (Shao *et al*., 2008; Sial *et al*., 2022***)***. A proposal to increase irrigation to reduce the drought problems is not a viable option due to the current scarcity of water.

Wheat (*Triticum aestivum* L.) crop is one of the sensitive crops to drought stress, especially in the production stages causing a significant decrease in the growth and yield-related traits and eventually in the grain yield. Therefore, understanding the knowledge of the genetic basis of drought tolerance in wheat is essential for plant breeders to enable them to develop drought-tolerant wheat genotypes. Zafarnaderi *et al*. (2013) studied the traits associated with the performance of wheat genotypes under drought stress, and reported that drought negatively affects the number of tillers, spikes, and grains per spike, and ultimately the grain yield***.*** In the diversifying global climate change, the drought stress may become more dangerous and may cause 15%–50% yield losses in rice and other cereal crops (Darzi-Ramandi *et al*., 2016; Kumar *et al*., 2017).

Plant response to drought stress conditions occurs through several changes at the physiological levels by changing the gene expression responsible for drought tolerance in wheat (Shi *et al*., 2010). When plants are subjected to abiotic stresses, molecular understanding causes genes to be stimulated to produce specific proteins as a defense mechanism. These proteins participate in the biosynthesis of osmotic protective compounds and regulation of detoxification and transport enzymes, as well as, activation of regulatory proteins. These molecules were found necessary in the regulation of signal transmission and gene response for their expression (Krasensky and Jonak, 2012).

The wheat plant's response to drought stress can be influenced by several factors, including crop genotype, growth stage and its physiology, duration and severity of the stress, gene expression patterns, respiration and photosynthesis activities, and environmental factors (Lou *et al*., 2018; Urban *et al*., 2018; Tembo, 2021). Researchers have focused their attention on a wide variety of drought-responsive genes as a result of the effects that drought has on the expression of genes in crop plants (Kathiresan *et al*., 2006). Gene expression may shed light on the vital role that the gene plays in the production of high levels of resistance in crop cultivars. Drought effects on plants can also be managed through protein levels, production of antioxidants, osmotic adjustment, hormone composition, root depth and extension, stomata closing and opening, inhibition of photosynthesis, reduction in chlorophyll content, reduced transpiration, and growth inhibition (Farooq *et al*., 2009). While it works to adjust the plant's response to stress, the ABC4 gene's expression levels in plants increase when subjected to higher degrees of stress, and this is all because of the gene's function (Das *et al*., 2020).

Through its influence on chloroplasts, the GPAT gene also makes a significant contribution to a large number of biological processes, all of which contribute to the organism's success in surviving adverse environmental conditions (Xue *et al*., 2019***)***. The study by Tang *et al*. (2015) also demonstrated that the GBSS1 gene is responsible for the production of several enzymes that work to protect the plant from the effects of drought. Additionally, the study demonstrated that the umc1283 gene collaborates with the aforementioned genes to increase the plant's resistance to drought by controlling the synthesis of ABA within the plant (Campbell, 2000).

In addition, drought can cause pollen to become sterile, which can lead to a reduction in grain yield, and it can also lead to the accumulation of abscisic acid in cereals (Ji *et al*., 2011). Recent molecular technology can serve as an appropriate and useful identification tool for establishing the clonal variation, stress tolerance, and genetic stability in crop plants (Rai *et al*., 2011). Molecular techniques, such as, suppression subtractive hybridization, cDNA microarrays, cDNA-amplified fragment length polymorphism, and the differential display reverse transcription polymerase chain reaction (DD-RT-PCR), are some of the tools that are currently available for analyzing transcriptomes. By using the technique DD-RT-PCR, it is possible to successfully isolate many genes from plants that exhibit differential levels of expression. The said approach is not complicated and is also very sensitive and effective.

In the present era, quantitative real-time PCR, also known as RT-qPCR, has

established itself as a standard method for determining the levels of gene expression. The said technology is primarily attributable to the fact that it is extremely sensitive, accurate, and simple to operate (Saakre *et al*., 2017). In the recent investigations, the PCR was used to locate several genes that confer resistance to drought in seven distinct wheat cultivars. Following this, the three promising wheat cultivars that contained the greatest number of stress-tolerant genes were selected and used with RT-qPCR technology to investigate the relative expression of these genes in each selected cultivar. Hence, the objectives of the study were: a) to figure out how these genes play in the overall process of regulating the plant's ability to withstand drought, and a) how drought stress and its negative impact affects the growth and yield characteristics of the wheat crop.

MATERIALS AND METHODS

Breeding material and procedure Experiment No. 1

During the winter season of 2019–2020, the first experiment comprising screening of wheat (*Triticum aestivum* L.) genotypes for most drought-tolerant genes and their expression, was carried out at the Agricultural Research Station, Babil Governorate, Iraq. Seven wheat (*T. aestivum* L.) cultivars, i.e., Iraq, Ezz, Abba-99, Furat, Sham-6, N-70, and Tamoz were grown in pots. After the appearance of the third leaf, the DNA was extracted with the ZR Plant DNA Extractor Kit, and isolated from leaves for each cultivar. The company's instructions for the R2144 kit were then followed.

Polymerase chain reaction (PCR)

For seven wheat cultivars, the Korean company iNtRoN offered the MaximeTM PCR PreMix (i-Taq) kit (KIt. No 25025) and was used for the polymerase chain reaction (PCR) test. Drought-tolerant genes were detected using the primers (designed by the researchers using NCBI) listed in Table 1. The reaction mixture was prepared in a sterile tube (one for each genotype with a tube free of DNA, a negative control) and its components were mixed using a micropipette, where the reaction mixture contained: 10 µl of Taq PCR PreMix, 1 µl of forward and 1 µl of reverse primer of the target gene, 5 µl of DNA, and 8 µl of distilled water, then placed in a centrifuge to maintain the final volume (25 µl) of the reaction mixture. Then it was placed in a PCR device, and the reaction was carried out to amplify (Table 2). To determine the diameters of the fragment for PCR products and the DNA Ladder marker, electrophoresis was performed after 1 g of agarose was combined and dissolved in 100 ml of TBE (1X), and the mixture was heated until it reached boiling point. After bringing the temperature down to between 40 °C and 50 °C, 2 µl of a red safe dye were added. In the meantime, 3 µl of PCR products were combined with 5 µl of loading buffer.

Table 1. PCR primers used in the first year of study.

Gene name	Forward primer (5'---3')	Reverse primer (5'---3')	Product length
ABC4	TCGGCCATGGAAGACAGACT	TAAAATGTGTCGGCGTTTCGAG	741
GPAT	CTTGTGACCCGATTTGCAGC	CCGCAGAGAAGGTTTGGACA	831
GBSS1	GCAACGGCTTTAGTGACGTG	AAAGACTTGGCTGTGTGCAG	905
Umc1283	AATGAGAGCACCTAGAGGGGG	TCGGGAAGTGATTAACGGCG	950

Table 2. mPCR reaction conditions program.

Temperature (°C)	Time	Cycle
95	5 min	
95	40 s	40 cycle
65	40 s	40 cycle
72	2 min	40 cycle
72	5 min	

After preparing the gel casting tray and placing the comb so that it would create wells within the agarose gel layer, the dissolved agarose was poured into the prepared tray, and it was allowed to harden at room temperature. After the agarose had completely hardened, the comb had to be carefully removed without causing any deformation or breaking any of the wells. The tray was then placed back where it belonged in the electrophoresis device, and TBE was poured into the electrophoresis chamber until the agarose layer was submerged at a height of approximately ***1*** mm***.*** Finally, the PCR products were injected into each well of the agarose gel, and a 5 µl (1 Kbp) ladder marker was placed on the wells on the left side of the additional samples so that the size of PCR products could be more accurately determined. Then the power supply ran on 120 mA for an hour and a half. After electrophoresis, the agarose layer was lifted and placed on a UV transilluminator.

Experiment No. 2

The study was carried out during the 2020–2021 cropping season under field conditions at the Babil Governorate, Iraq, to study the drought effects on the expression of drought-resistant genes, growth, and yield-related traits in wheat (*T. aestivum* L.). In the first year of study, three out of seven drought stress tolerant wheat genotypes were selected. Three drought-tolerant wheat cultivars, i.e., Iraq, Tamoz, and Abba-99, were studied under three drought stress conditions in a randomized complete block design (RCBD) with three replications on a split-plot arrangement. The three drought stress conditions were: D-1 (irrigation after draining 50% of the available water), D-2 (irrigation after draining 60% of the available water), and D-3 (irrigation after draining 70% of the available water). The wheat cultivars were placed in sub-plots while drought stress conditions were in the main plots.

RNA extraction

Each experimental unit had 10 leaf samples obtained at the vegetative stage. Plant RNA was isolated using the ZR Plant kit. Afterward, the instructions in the kit's manual (Kat. No. R2024) were followed to extract RNA.

RT-qPCR - to measure drought tolerance genes expression

Ribonucleic acid was detected by RT-qPCR using the GoTaq® qPCR Master Mix (Cat. No A6120) kit from Promega and a qPCR primer designed specifically for this test was used (Table 3). Afterward, it was inserted into the real-time PCR and run through the different steps as outlined in Table 4. Using Livak and Schmittgen (2001) approach, the relative gene expression was estimated by following the conclusion of the interaction.

$$Relative\ Gene\ Expression = 2^{-(CT_{Trget\ gene} - CT_{GAPDH})Test - (CT_{Target\ gene} - CT_{GAPDH})Control}$$

A target gene's cycle threshold (CT) is indicated by the variable CT target, CT GAPDH is the reference gene's cycle threshold (GAPDH***)***. It is the difference between the cycle threshold of the target gene and that of the reference gene for the samples tested***.*** Differences in cycle thresholds between the target gene and reference gene for control samples were referred to as control.

Table 3. Primers used for qPCR.

Gene name	Forward (5'---3')	Reverse (5'---3')
ABC4	GTCCATCCAGATTGCTCGTT	CTGTGAACTGGTTGCTCGAA
GPAT	CAGGAGGAAGGTGGCGGT	TGCGTGCACACGTAGAGG
GBSS1	CACATGGTTCTGTGCCTGAG	TCCTCCTCATCTGGCTCATC

Table 4. Program for real-time PCR reactions**.**

Steps	Temperature (°C)	Time	Cycle number
Reverse transcription	45.0	30 min	Hold
Enzyme activation	95.0	2 min	Hold
Denaturation	95.0	20 s	
Annealing	65.0	20 s	40
Extension	72.0	1 min	

Data recorded

Plant height (cm) was measured from an average of 10 plants in each experimental unit, from the soil surface to the spike tip excluding awns. Flag leaf area (cm^2) was measured from an average of 10 plants in each experimental unit by following the formula of Francis *et al*. (1969). Moreover, the tillers m^{-2} were counted and measured for each experimental unit. Furthermore, the number of harvested spikes m^{-2} was recorded. The grains in $spike^{-1}$ were averaged among 20 randomly selected spikes. The 1000-grains weight (g) was taken from 20 randomly selected wheat seed samples in each experimental unit and then averaged. Also, the biological and grain yield (ton ha^{-1}) were measured for each experimental unit's square meter of wheat harvested, dried, and converted to tons ha^{-1}. The harvest index (%) was calculated by using the following formula:

$$Flag\ leaf\ area = Leaf\ length \times Maximum\ width\ at\ the\ center \times 0.75$$

$$Harvest\ Index\,(\%) = \frac{Grain\ yield}{Biological\ yield} \times 100$$

RESULTS

Experiment No. 1

Five out of seven wheat (*T. aestivum* L.), i.e., Iraq (V1), Ezz (V2), Abba-99 (V3), Furat (V4), and Tamoz (V7), showed the appearance of bands with a molecular weight of 741 bp (Figure 1). Results indicate that these cultivars have the gene ABC4, whereas the absence of bands in cultivars Sham-6 (V5) and N-70 (V6) denotes that these cultivars are missing the gene ABC4 and may instead have some other allele present. For the GPAT gene, the wheat cultivars Iraq (V1), Ezz (V2), Abba-99 (V3), Sham-6 (V5), and Tamoz (V7) were well responsive and showed bands with a molecular weight of 831 bp (Figure 2). This specifies that these cultivars owned the gene GPAT. However, the cultivars Furat (V4) and N-70 (V6) showed no response to the said gene.

Five out of seven wheat cultivars, viz., Iraq (V1), Abba-99 (V3), Sham-6 (V5), N-70 (V6), and Tamoz (V7), confirmed the presence of gene GBSS1 and showed the appearance of bands with a molecular weight of 905 bp (Figure 3). However, no bands were developed in wheat cultivars Furat (V4) and Ezz (V2), which means that these genotypes lack the said gene and might be replaced by some other allele. All the seven wheat cultivars presented the bands with a molecular weight of 950 bp for the gene umc1283, which authenticated that the said gene is not involved in the wheat's ability to withstand drought stress conditions (Figure 4).

After compiling the results of the electrical migration of all the genes, a preliminary assessment can be made about the wheat cultivars that are resistant to drought (Table 5). This is because the wheat cultivars Iraq, Tamoz, and Abba-99 have the genes GBSS1, GPAT, and ABC4, while the rest of the genotypes did not show any sign of these genes. Therefore, in the recent study, the three wheat cultivars, viz., Iraq, Abba-99, and Tamoz, were found to be the most drought-tolerant genotypes.

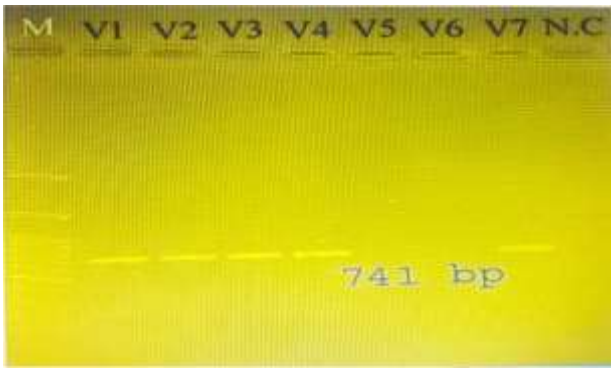

Figure 1. Electrophoresis of PCR reaction products with a specific primer for detecting the ABC4 gene in seven wheat cultivars in the first year of study, viz., Iraq (V1), Ezz (V2), Abba-99 (V3), Furat (V4), Sham-6 (V5), N-70 (V6), Tamoz (V7), with negative control (N.C), and DNA ladder (M).

Figure 2. Electrophoresis of PCR reaction products with a specific primer for detecting the GPAT gene in seven wheat cultivars in the first year of study, viz., Iraq (V1), Ezz (V2), Abba-99 (V3), Furat (V4), Sham-6 (V5), N-70 (V6), Tamoz (V7), with negative control (N.C), and DNA ladder (M).

Figure 3. Electrophoresis of PCR reaction products with a specific primer for detecting the GBSS1 gene in seven wheat cultivars in the first year of study, viz., Iraq (V1), Ezz (V2), Abba-99 (V3), Furat (V4), Sham-6 (V5), N-70 (V6), Tamoz (V7), with negative control (N.C), and DNA ladder (M).

Figure 4. Electrophoresis of PCR reaction products with a specific primer for detecting the umc1283 gene in seven wheat cultivars in the first year of study, viz., Iraq (V1), Ezz (V2), Abba-99 (V3), Furat (V4), Sham-6 (V5), N-70 (V6), Tamoz (V7), with negative control (N.C), and DNA ladder (M).

Table 5. Positive and negative signs possessed by genes (ABC4, GPAT, umc1283, and GBSS1) in seven wheat cultivars in the first year of study.

No.	Cultivars	Genes			
		ABC4	GPAT	GBSS1	umc1283
V1	Iraq	+	+	+	+
V2	Ezz	+	+	-	+
V3	Abba-99	+	+	+	+
V4	Furat	+	-	-	+
V5	Sham-6	-	+	+	+
V6	N-70	-	-	+	+
V7	Tamoz	+	+	+	+

Experiment No. 2

Cycle threshold (CT) for various genes and their expression

The cycle threshold (CT) values for genes. ABC4, GPAT, and GBSS1 significantly differed among the wheat cultivars under various drought stress conditions (D1, D2, and D3) (Figure 5). In all the genes, the CT values decreased as the drought intensity was increased which authenticated that both traits were inversely proportional. The gene ABC4 had the lowest CT values among study cultivars at varying drought stress levels. Cultivar Iraq has the lowest CT values for genes, ABC4 and GPAT compared with the two other wheat cultivars, Abba-99 and Tamoz, under all the drought stress conditions. Therefore, cultivar Iraq was found relatively most expressive and responsive for these two drought-tolerant genes. For CT values in gene GBSS1, all the three selected cultivars were not significantly different under diverged drought stress conditions.

Wheat cultivars under different drought conditions have a unique significant effect on the relative expression of genes. ABC4, GPAT, and GBSS1 (Figure 6). With the increased drought, the relative expression of genes was also enhanced in the wheat genotypes. Wheat cultivar Iraq showed the highest relative expression of genes ABC4 and GPAT in D3 - drought stress, indicating that the said genotypes were found as the most drought-tolerant and the higher expression of these two genes improves its stress tolerance.

Significant differences were observed among the three selected wheat cultivar's performance for growth traits under different water stress levels (Table 6). After exposure of these selected wheat cultivars to three different drought stress conditions, a significant reduction has occurred in the growth characteristics of the plants. Specifically, the D3 - drought stress condition resulted in a significant reduction in plant height, flag leaf area, and the number of tillers, with the respective decrease of 20.25%, 25.82%, and 44.66%, respectively. Cultivar Iraq being tolerant to drought stress, was the least affected genotype for plant height, flag leaf area, and the number of tillers. With increasing drought severity, the said traits decreased in cultivar Iraq by 16.96%, 24.08%, and 44.17%, respectively, while cultivar Tamoz was the most affected by drought stress, with a decrease of 19.96%, 25.95%, and 44.88%, respectively.

Results revealed that there were significant differences in the performance of various cultivars for yield-related traits under varied drought stress conditions (Table 7). The recent findings further revealed that water stress conditions were responsible for a sizable reduction in the yield components of all the three tested wheat cultivars. Particularly, the D3 - drought stress condition resulted in a significant reduction in the spikes m^{-2}, grains per spike, and 1000-grain weight, with reductions of 46.99%, 39.81%, and 31.43%, respectively. Cultivar Iraq was the least affected for spikes m^{-2}, grains in the spike, and 1000-grain weight. With increased drought severity, these traits decreased in the Iraq variety by 37.82%, 31.00%, and 26.83%, respectively. However, the cultivar Tamoz was the most affected genotype by drought stress, and the decreases were 52.17%, 46.43%, and 34.46%, respectively.

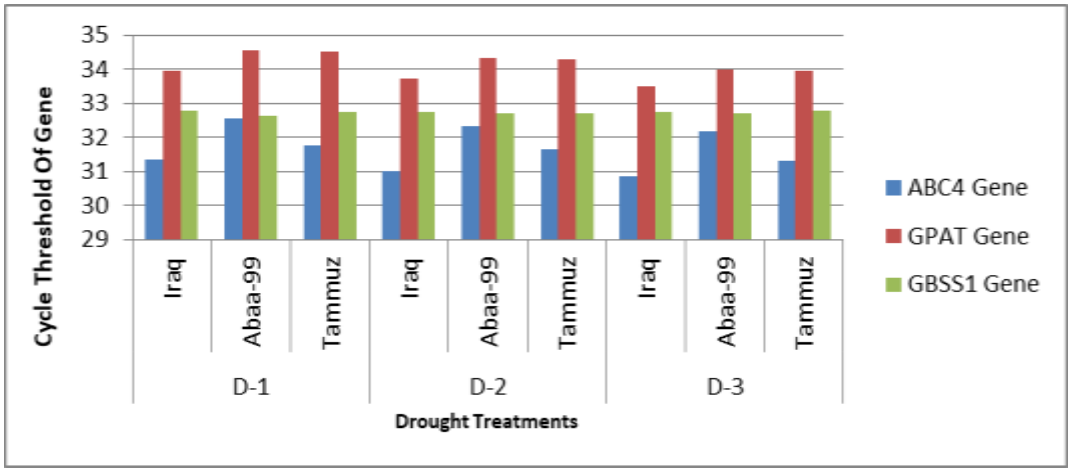

Figure 5. Cycle threshold (CT) for genes ABC4, GPAT, and GBSS1 in three selected wheat cultivars under drought stress conditions in the second year of study.

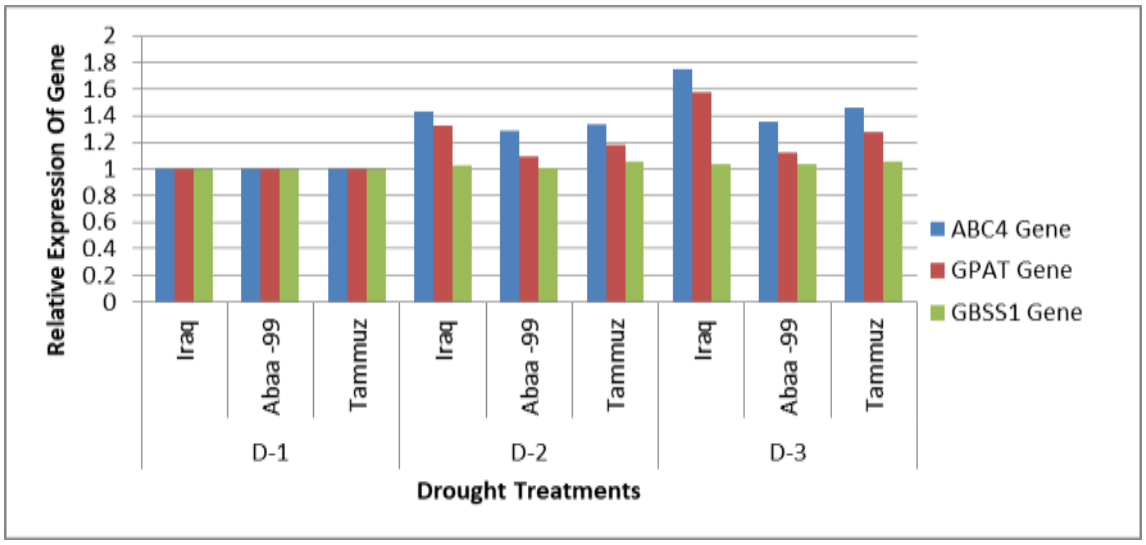

Figure 6. Relative expression of genes ABC4, GPAT, and GBSS1 in three selected wheat cultivars under drought stress conditions in the second year of study.

Table 6. Effect of drought stress conditions on the growth traits of wheat cultivars in the second year of study.

Drought stress conditions	Cultivars	Plant height (cm)	Flag leaf area (cm^2)	Tillers (m^{-2})
D-1	Iraq	95.25	48.76	529.43
	Tamoz	127.54	51.36	588.62
	Abba-99	106.56	53.57	597.37
D-2	Iraq	93.89	44.82	498.61
	Tamoz	121.55	43.31	555.24
	Abba-99	104.73	48.53	561.7
D-3	Iraq	79.1	37.02	295.56
	Tamoz	97.14	37.27	324.07
	Abba-99	85.29	39.67	329.29
$LSD_{0.05}$		8.12	3.64	24.28

Table 7. Effect of drought stress conditions on yield components of wheat cultivars in the second year of study.

Drought stress conditions	Cultivars	Spike (m^{-2})	Grains $spike^{-1}$	1000-grain weight (g)
D-1	Iraq	412.94	50.96	44.65
	Tamoz	471.1	57.07	45.97
	Abba-99	474.7	56.52	44.23
D-2	Iraq	397.05	48.91	43.31
	Tamoz	442.59	51.77	43.62
	Abba-99	450.96	54.19	42.9
D-3	Iraq	256.78	35.16	32.67
	Tamoz	225.35	30.57	30.13
	Abba-99	232.6	32.79	29.63
$LSD_{0.05}$		27.68	2.87	8.56

Table 8. Effect of drought stress conditions on biological and grain yield, and harvest index of wheat cultivars in the second year of study.

Drought stress conditions	Cultivars	Biological yield (ton ha^{-1})	Grain yield (ton ha^{-1})	Harvest index (%)
D-1	Iraq	15.56	5.52	35.48
	Tamoz	17.41	5.82	33.43
	Abba-99	16.27	5.64	34.67
D-2	Iraq	14.42	4.47	31.00
	Tamoz	16.35	4.88	29.85
	Abba-99	15.16	4.69	30.94
D-3	Iraq	13.21	3.97	30.05
	Tamoz	15.31	3.22	21.03
	Abba-99	14.54	3.17	21.80
$LSD_{0.05}$		0.73	0.12	0.85

Significant differences were observed in wheat cultivars for biological yield, grain yield, and harvest index under different drought stress conditions (Table 8). The selected three wheat cultivars were recorded with a significant reduction in biological yield, grain yield, and harvest index when subjected to varied water stress conditions. Specifically, the D3 - drought stress condition was the leading in this reduction and resulted in a decrease of 12.60% in biological yield, 38.85% in grain yield, and 29.83% in harvest index. Cultivar Iraq was the least affected genotype in biological yield, grain yield, and harvest index with increased drought intensity, as these traits decreased by 28.08%, 15.10%, and 15.29%, respectively. However, cultivar Tamoz was the most affected genotype by drought stress, as the percent decrease reached 44.67%.

DISCUSSION

Findings of the study provided an indication of the wheat cultivars that can withstand periods of prolonged exposure to dry conditions. This is because the cultivars Iraq, Abba-99, and Tamoz contain the genes, GBSS1, GPAT, and ABC4, whereas other cultivars did not show any evidence of having even one or two of these genes in them. Hence, this is what makes the cultivars Iraq, Tamoz, and Abba-99 resistant to the effects of drought stress conditions. Given that the gene ABC4 is responsible for such an important process, its presence in the DNA of particular cultivars is indicative of the fact that these plants have the genetic capacity to flourish in arid environments. The ABC transporters, also known as adenosine triphosphate (ATP)-binding cassette transporters, are prevalent in both eukaryotic and prokaryotic organisms (Higgins and Linton, 2004). The binding frame of ATP inspires the names of these transporters. The researchers found in a previous study that ABC transporters, which is one of the protein superfamilies with the widest range of functional applications, are involved in a variety of plant physiological processes (Davidson *et al*., 2008). The transport of plant hormones, the intake of

nutrients by organisms, the regulation of stomatal opening and closing, environmental stress responses, and the interaction between plants and microorganisms are all included in these processes.

Similarly, the presence of the gene GPAT in wheat cultivars is an indication that these cultivars have the genetic potential to tolerate drought; this is the essential function that the GPAT gene plays. When producing PG, the first acyl esterifying enzyme that is created is called glycerol-3-phosphate acyltransferase. This enzyme is responsible for the production of PG. This allows the production of 1-acylglycerol-3-phosphate by moving the acyl group from an acyl-coenzyme A (CoA) donor (or an acyl–acyl carrier protein [ACP] in plastids) to the sn-1 position of a glycerol-3-phosphate (G3P) molecule (or lysophosphatidic acid, LPA). It has been possible to clone the GPAT gene in a wide variety of plant species, including *Lycopersicum esculentum, Spinacia oleracea, Helianthus annuus, Oryza sativa*, and *Arabidopsis* (Liu *et al*., 2013; Payá-Milans *et al*., 2016). Recent research has shown that a plant's fertility, resistance to stress, oil content, and seed development are all intimately connected to a gene known as GPAT in rice and other cereals (Ariizumi *et al*., 2002).

Likewise, the presence of the GBSS1 gene in wheat cultivars is evidence that these genotypes have the genetic capacity to withstand dry conditions. It has been hypothesized that the gene GBSS1 is required for blooming time and seed filling in addition to growth rate, which plays a role in these processes. This gene is necessary for the production of amylase, which is required for the growth rate in crop plants (Bernier *et al*., 1993; Schulze *et al*., 1994; Ma *et al*., 2022).

These findings also demonstrated that drought is responsible for a reduction in the growth characteristics and grain yield in wheat, which may be attributable to the factors, i.e., a reduction in the rate of total photosynthesis and a corresponding reduction in water potential, contribute to a shorter plant height. This decrease is caused by a decline in the water potential of plant cells due to a lack or unavailability of soil water, causing a decrease in the elongation, division, and expansion of stem cells in winter wheat (Jiang *et al*., 2020). The low amount of water that is ready for the plant usually has a significant impact on the tillering process. A lack of water that is ready in the soil slows the rate of main stem emergence and also greatly reduces the development of tillers from the coleoptile node. Singh and Khanna (2010) found that the emergence of tillers in bread wheat plants exposed to drought takes longer than in plants that are fully irrigated. A sizable relief from water stress was the root cause of this drop in the total number of spikes, grains, and grain weight. With a decrease in the products of photosynthesis, which caused competition between the stem that began with rapid elongation, and the production of the tillers, as well as, between the tillers themselves on these products, caused a decrease in the chance of their survival, and thus, do not have a great opportunity to carry the spikes as the severity of the drought increased in wheat (Akram, 2011) and barley (Thabet *et al*., 2020).

When a plant is subjected to conditions that cause it to be under water stress, the stomata closes, which leads to a decrease in the spread of carbon dioxide, followed by a decrease in the process of photosynthesis. The low grain weight that results from the grain filling stage is a reflection of the fact that the dry matter that is produced from the flag leaf decreases at this stage in wheat (Mohammed *et al*., 2021). The results also showed that drought was to blame for the decrease in biological yield, as well as, grain yield, which is the harvest index in wheat. The reason is water stress caused a decrease in the accumulation of dry matter in plants as a result of the lack of vegetative growth related to plant height, as well as, lesser number of tillers and leaf area that work to reduce the interception of solar radiation, and the lack of conversion of solar energy into chemical energy from the closing of stomata.

A decrease in grain yield is brought on by a reduction in the various components that make up the grain yield. These components include the grain weight, the number of grains in the spike, and the number of spikes that are affected by drought first. There is a strong positive correlation between the ability of the crop to increase the area of the flag leaf and the total amount of grain that is produced**.** Since these two genes work to regulate the plant's response to drought resistance by controlling several biological processes necessary to protect the plant and reduce its vulnerability to harsh conditions, the ability of the wheat cultivar Iraq to resist harsh drought conditions is attributed to its relative expression of the genes, ABC4 and GPAT, found in that genotype. The results also revealed the varied performance of the wheat cultivars under drought stress levels, which

might be due to the relative expression of the genes, ABC4 and GPAT, found in them.

CONCLUSIONS

Results of the study authenticated that drought stress harms the wheat crop and it causes a significant reduction in grain yield. Some cultivars showed a higher drought tolerance than the rest of the genotypes, and there is a trait that distinguishes one type of wheat from another. Wheat cultivar Iraq was the most tolerant genotype to the drought stress conditions as least affected for its growth and yield-related traits. The study also demonstrated that genes, ABC4 and GPAT, play an important part in regulating the response of different wheat cultivars to drought.

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