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Evaluation of seven different wheat cultivars for their resistance to drought in terms of growth indicators and yield

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ABSTRACT

The study used a randomized complete block design with three replicates to investigate drought tolerance genes and wheat growth and yield. The experiment included seven wheat cultivars: Buhooth 158, Rasheed, Buhooth 22, Tahade, Fatih, N70, and Cimmyt 72 in main plots and three drought treatments: Irrigation after draining 50% of available water (D1), irrigation after draining 65% of available water (D2), and irrigation after draining 75% of available water (D3) in subplots. Drought treatments resulted in an increase in the expression of genes that promote drought resistance. It is also noted that the Buhooth 158 cultivar was superior by giving the highest relative expression of LOC100382183 and LOC103630223 gene, with averages of 2.41 and 2.25 times higher than the rest of the cultivars, and this leads us to think that the Buhooth 158 cultivar has a higher drought tolerance than the rest of the cultivars. It was found that the drought treatment (D3) had a significant impact on plant height and flag leaf area, as well as on the number of tillers, spikes, and grains in a spike. The decreases in these indicators were 12.76% for plant height, 25.89% for flag leaf area; 44.00% for tillers number; 49.31% for spike number; 40.00% for grain number; and 32.21% for the weight of 1000 grains, 43.55% for grain yield, 4.91% for biological yield, and 40.44% for harvest index. Cultivars Buhooth158, Rasheed, and Buhooth 22 surpassed the rest, with the highest average yield components, grain yield, biological yield, and harvest index all indicating their superior performance.

1. INTRODUCTION

Although the global average production of cereal crops such as wheat, barley, and rice per unit area for human nutrition has nearly doubled since the beginning of the 20th century thanks to research and those interested in breeding and improving these crops, wheat (*Tritium aestivum* L.) is still the world's first crop in terms of total cultivable area and global production. One of the reasons for the decline in wheat productivity is drought, which is the most important determinant of crop production in dry and semi-arid areas, representing about 70% of potential losses [1], as it negatively affects agricultural production through its effect on plant growth, as it works to reduce the number of cells and tissue expansion. Consequently, the number of leaves and leaf area will drop, and the crop's growth time will shorter, all of which will lead to a reduction in the crop's components and, as a result, a reduction in the yield [2]. Negative water stress results from the drying out of the cells' protoplasm. The loss of water leads to the shrinkage of the protoplasm, resulting in an increase in the concentration of solutions, which causes great damage [3]. Adaptation mechanisms of plant include metabolic and physiological changes, gene expression and transcription regulation, as well as epigenetic plasticity [4], these mechanisms allow plants to better withstand the effects of drought. In response to environmental conditions of water deficiency, a number of genes undergo expression and translation [5]. Drought stress response molecular pathways have been studied in a number of researches. These researches have led to the discovery of drought-responsive genes that are both conserved and unique to each species. This gene family contains proteins that help cells retain water, such as membrane stabilizers and late embryogenic abundant proteins (LEA) [6]. In addition, a number of heat shock proteins (HSPs) were discovered [7]. These HSPs are extremely important for maintaining the structure of proteins. In reaction to abiotic stress, the HSPs are principally in charge of unraveling some folded proteins and preventing protein denaturation [8]. Transcription factors that regulate and provide an adaptive response under drought stress include myeloblastosis, dehydration-responsive element binding, C-repeat binding factor, abscisic acid responsive elements binding factor, ABRE binding, NAM, ATAF1/2, and CUC2 protein-containing proteins (NAC), WRKY, and SNF1-related kinase 2. However, despite these successes, the gene network of drought stress response has not yet been entirely unraveled [9]. A further factor supporting the notion that the drought stress response is complex is the existence of numerous cultivars of drought-inducible genes [10]. As a result, uncovering mechanisms for

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drought tolerance will aid in the creation of new crop varieties that are better suited to areas that are more prone to experience drought as a result of climate change. As a result, crop productivity and food security will improve over the long term. As a result, according to Marcek *et al*. [11], enhancing plants' drought resistance is sufficient to address the issue of drought. The identification of these genes in various types of crops using new biotechnology tools, including DNA-based markers, is one of the most effective indicators and an important tool that can be trusted to measure genetic diversity in crops that were in biological evolution, as opposed to phenotypic and biochemical characteristics, which may be affected by environmental factors and long-term growth processes, because DNA markers give a quintessential answer. Therefore, this study aimed to determine the drought tolerance levels of the studied wheat cultivars and to determine the gene expression values of the studied genes. This study aims to investigate drought tolerance genes and wheat growth and yield.

2. MATERIALS AND METHODS

The 2019–2020 winter season was used to conduct a field experiment. Iraqi wheat cultivars of various drought-tolerance genes were exposed to quantitative reverse transcription-polymerase chain reaction (PCR) (RT-qPCR) analysis to determine the impact on yield and yield components in Babil Governorate-Iraq. A randomized complete block design with three replicates, set up using split plots, was used. There were seven different wheat cultivars used in the experiment, all of which were found in the main plots, including Buhooth 158, Rasheed, Buhooth 22, Tahade, Fatih, N70, and Cimmyt 7, and three treatments of drought treatments: Irrigation after draining 50% of the available water (D1), irrigation after draining 65% of the available water (D2), and irrigation after draining 75% of the available water (D3), within subplots.

2.1. DNA Extraction

At the vegetative stage, 10 leaves were collected from each of the following cultivars: Buhooth 158, Rasheed, Buhooth 22, Tahade, Fatih, N70, and Cimmyt 7. After that, the DNA was extracted with the ZR Plant DNA Extractor Kit from the samples. The company's instructions for the R2144 kit were then followed.

2.2. PCR Technology

For seven wheat cultivars, the Korean company iNtRoN offered the MaximeTM PCR PreMix (i-Taq) kit (Kit No. 25025) for the PCR test. Drought tolerance genes were detected using the primers 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 micro pipette and then placed in a centrifuge to maintain the final volume of the reaction mixture. Then, it was placed in a PCR device, and the reaction was carried out according to the program shown in Table 2 for the purpose of amplifying drought tolerance genes.

After the completion of the PCR reaction, electrophoresis was performed, and pictures of the electrophoresis were taken.

2.3. RNA Extraction

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

2.4. RT-qPCR to Measuring 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 [Table 3].

Afterward, it was inserted into the real-time PCR and run through the steps outlined in Table 4.

Using Marcek *et al*. [11] approach, the relative gene expression was estimated following the conclusion of the interaction:

$$\Delta_{ct} = ct_{target} - ct_{GAPDH}$$

$$\Delta\Delta_{ct.control} = \Delta_{ct.test} - \Delta_{ct.control}$$

$$\text{Relative Gene Expression} = 2^{-\Delta\Delta ct}$$

A target gene's cycle threshold 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 are referred to as (ct. control).

2.5. Studied Traits

The following are the results of measurements made in the field:

- Plant height (cm): Average of 10 plants from each experimental unit, from soil surface to spike.
- Area of flag leaf (cm^2): The following equation gives the average of ten readings from each experimental unit.

  Area of flag leaf =Leaf length × leaf width at center × 0.75
- Number of tillers (tiller m^{-2}): Each experimental unit's harvested tillers per square meter at crop maturity.
- Number of spikes (spike m^{-2}): The number of spikes per square meter of each experimental unit.

Table 1: Primer list for PCR.

Gene symbol	Forward primer	Product length (bp)	Gene description
LOC100194201	F 5'AATGAGAGCACCTAGAGGGGG'3 R 5'TCGGGAAGTGATTAACGGCG'3	950	Ran BP2/NZF zinc finger-like superfamily protein
LOC103630223	F 5'3GCAACGGCTTTAGTGACGTG R 5' AAAGACTTGGCTGTGTGCAG'3	905	Granule-bound starch synthase 1 chloroplastic/amyloplastic
LOC100382183	F' 5CTTGTGACCCGATTTGCAGC'3 R 5'CCGCAGAGAAGGTTTGGACA'3	831	Glycerol-3-phosphate acyltransferase 1
LOC103634810	F 5'TCGGCCATGGAAGACAGACT'3 R 5'TAAAATGTGTCGGCGTTTCGAG'3	741	Cytochrome P450 family 77 subfamily A polypeptide 5 pseudogene

PCR: Polymerase chain reaction.

- Number of grains in the spike (grain. spike^{-1}): It was the average of 20 randomly selected spikes per harvested square meter.
- 1000 grain weight (g): One thousand grains were randomly selected from each experimental unit's previously harvested square meter and weighed with a sensitive scale.
- Grain yield (ton ha^{-1}): Each experimental unit's square meter was harvested, dried, and translated to tons ha^{-1}.
- Biological yield (ton ha^{-1}): Each experimental unit's square meter was harvested, dried, weighed (stems, leaves, and spike), and converted to tons ha^{-1}.
- Harvest index: The equation was:

$$\text{Harvest index} = \frac{\text{Grain yield}}{\text{Biological yield}} * 100$$

3. RESULTS AND DISCUSSION

After PCR reaction conditions were created, the PCR products were electrophoresed for wheat cultivars (Buhooth158 [V1], Rasheed [V2], Buhooth22 [V3], Tahade [V4], Fatih [V5], N70 [V6], and Cimmyt 7 [V7]) on agarose gel with a 1 KB ladder. The results of Figure 1 showed the presence of bands with molecular weights (950 bp, 905 bp, 831 bp, and 741 bp) in the seven cultivars representing the genes responsible for drought tolerance investigated in this study.

Although the collection of wheat cultivars possessed these genes responsible for drought tolerance, these cultivars differed in their tolerance to drought tolerance, so by studying the gene expression of these genes in the seven wheat cultivars, we were able to understand the reason for the varietal differences in their tolerance to drought tolerance. Figure 2 shows that these four genes increase their gene expression with increasing levels of drought, and this confirms the findings of Livak and Schmittgen [12] that these genes respond to drought significantly and work to raise drought tolerance through several mechanisms.

It also appears from Figure 2, that the cultivars Buhooth 158, Rasheed, and Buhooth 22 were characterized by higher gene expression compared to the rest of the cultivars, especially in the genes (LOC100194201 gene, LOC103630223 gene, and LOC100382183 gene). It is also noted that the Buhooth 158 cultivar was superior by giving the highest relative expression of the LOC100382183 gene and the LOC103630223 gene, with averages 2.41 and 2.25 times higher than the rest of the cultivars, and this leads us to think that the Buhooth 158 cultivar has a higher drought tolerance than the rest of the cultivars.

According to Table 5, drought at treatment D3 resulted in significant decreases in wheat plant height, flag leaf area, and tiller number, with respective percentages of 12.76%, 25.89%, and 44.00%. Treatment D3 was the only one to exhibit a significant difference in these variables. The decrease in the rate of total photosynthesis and the decrease in water potential, which resulted in a reduction in stem cell elongation, division, and expansion due to the decrease in the water potential of plant cells, are responsible for the reduction in vegetative growth characteristics in wheat with increasing drought intensity in the treatment of D3 [13,14]. The reason for the wheat plant's reduced height after water stress may be due to the breakdown of auxin because it does not have the opportunity to work on the elongation of the internodes and the lack of development of the ridges [15]. It also has to do with the fact that plants cannot get enough nutrients, especially nitrogen, because there is not enough water in the soil. Another Table 5 result shows Buhooth 158, Rasheed, AB99, and Buhooth 2299 cultivars to be the most productive wheat cultivars for crop height, flag leaf area, and tillers.

Table 6 shows that the number of spikes, the number of grains in each spike, and the weight of 1000 grains for the wheat crop showed significant difference between the drought treatments. Treatment D3's drought reduced these traits by 49.31%, 40.00%, and 32.21%, respectively. Treatment D3's drought was the most severe. This is the reason for this. As the severity of drought increased, the number of

Table 2: PCR reaction conditions program.

Step	Temperature	Time	Cycle
Denaturation Initial	95	5 min	
Denaturation	95	40 s	
Annealing	65	40 s	40 cycles
Extension	72	2 min	
Extension	72	5 min	

PCR: Polymerase chain reaction.

Table 3: Primer for qPCR.

Gene symbol	Forward primer	Product length (bp)
LOC100194201	F: 5' GCTTCAGGTGCTCTGCCTAC'3 R: 5' TTCCATCCTGCTAGCGAAGT '3	122
LOC103630223	F: 5' CACATGGTTCTGTGCCTGAG'3 R: 5' TCCTCCTCATCTGGCTCATC '3	132
LOC100382183	F: 5' CAGGAGGAAGGTGGCGGT '3 R: 5' TGCGTGCACACGTAGAGG '3	128
LOC103634810	F: 5' GTCCATCCAGATTGCTCGTT '3 R: 5' CTGTGAACTGGTTGCTCGAA '3	141

Table 4: Program for real-time PCR reactions.

Step	Temperature (C°)	Time	Cycle number
cDNA synthesis	45.0°C	30 min	Hold
Denaturation Initial	95.0°C	2 min	Hold
Denaturation	95.0°C	20 s	40
Annealing	65.0°C	20 s	
Extension	72°C	1 min	

PCR: Polymerase chain reaction.

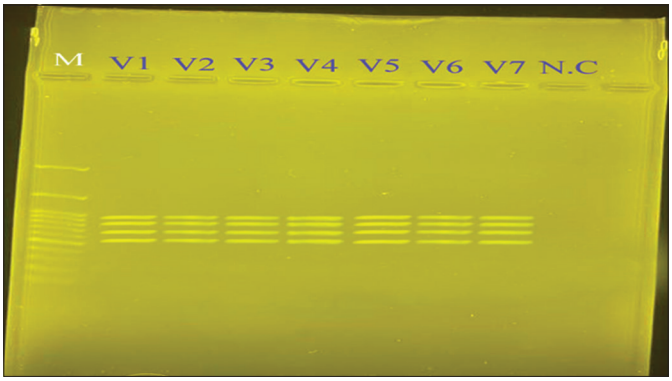

Figure 1: Electrophoresis of the PCR reaction products for four primers and for seven cultivars of wheat (Buhooth 158 [V1], Rasheed [V2], Buhooth 22 [V3], Tahade [V4], Fatih [V5], N70 [V6], and Cimmyt 7 [V7]) with a negative control (N.C) without adding DNA to the rest of the components required for the polymerase chain reaction. Also, the presence of a DNA ladder (m).

Table 5: Effect of drought stress treatment on a number of vegetative growth treats for seven wheat cultivars.

Drought stress	Cultivars	Plant height (cm)	Flag leaf area (cm^2)	No. of tillers (tiller m^{-2})
D1	Buhooth 158	94.25	46.76	522.43
	Rasheed	123.54	52.36	587.62
	Buhooth 22	104.56	51.57	593.37
	Tahade	111.74	51.78	614.65
	Fatih	84.25	45.78	507.72
	N70	114.65	51.44	583.33
	Cimmyt 7	96.28	42.76	430.00
D2	Buhooth 158	93.89	45.82	496.31
	Rasheed	122.55	51.31	558.24
	Buhooth 22	102.73	50.53	563.70
	Tahade	109.86	50.74	583.92
	Fatih	83.91	44.86	482.33
	N70	110.60	50.41	554.16
	Cimmyt 7	94.70	41.90	408.50
D3	Buhooth 158	76.10	32.02	292.56
	Rasheed	105.14	39.27	329.07
	Buhooth 22	92.29	38.67	332.29
	Tahade	98.76	38.83	344.20
	Fatih	75.21	34.33	284.32
	N70	99.43	38.58	326.66
	Cimmyt 7	89.25	32.07	240.80
LSD 0.05		8.12	3.64	24.28

Table 6: Effect of drought stress treatment on yield components for seven wheat cultivars.

Drought stress	Cultivars	No. of spike (spike m^{-2})	Number of grains in spike (grain $spike^{-1}$)	1000 grain weight (g)
D1	Buhooth 158	417.94	50.96	44.65
	Rasheed	470.10	56.07	44.97
	Buhooth 22	474.70	56.52	44.23
	Tahade	491.72	58.19	43.93
	Fatih	406.18	49.81	44.76
	N70	466.66	55.73	44.03
	Cimmyt 7	344.00	43.71	44.69
D2	Buhooth 158	397.05	48.91	43.31
	Rasheed	446.59	53.77	43.62
	Buhooth 22	450.96	54.19	42.90
	Tahade	467.13	55.78	42.61
	Fatih	385.87	47.81	43.42
	N70	443.33	53.45	42.71
	Cimmyt 7	326.80	42.03	43.35
D3	Buhooth 158	256.78	35.16	32.67
	Rasheed	230.35	32.57	30.13
	Buhooth 22	232.60	32.79	29.63
	Tahade	240.94	33.61	29.43
	Fatih	199.03	29.50	29.99
	N70	228.67	32.41	29.50
	Cimmyt 7	168.56	26.52	29.94
LSD 0.05		27.68	2.87	8.56

Table 7: Effect of drought stress treatment on grain yield, biological yield, and harvest index for seven wheat cultivars.

Drought stress	Cultivars	Grain yield (ton h^{-1})	Biological yield (ton h^{-1})	Harvest index
D1	Buhooth 158	5.64	15.66	36.03
	Rasheed	5.96	17.41	34.23
	Buhooth 22	5.87	16.27	36.07
	Tahade	4.85	16.70	29.03
	Fatih	4.96	15.06	32.95
	N70	5.81	16.88	34.42
	Cimmyt 7	4.65	15.78	29.47
D2	Buhooth 158	5.47	15.63	34.99
	Rasheed	5.78	17.35	33.32
	Buhooth 22	5.69	16.16	35.23
	Tahade	4.70	16.59	28.35
	Fatih	4.81	15.03	32.00
	N70	5.64	16.64	33.88
	Cimmyt 7	4.51	15.68	28.76
D3	Buhooth 158	3.97	14.57	27.26
	Rasheed	3.22	16.31	19.73
	Buhooth 22	3.17	15.54	20.40
	Tahade	2.62	15.93	16.45
	Fatih	2.68	14.51	18.46
	N70	3.14	15.97	19.65
	Cimmyt 7	2.51	15.36	16.35
LSD 0.05		0.12	0.73	0.85

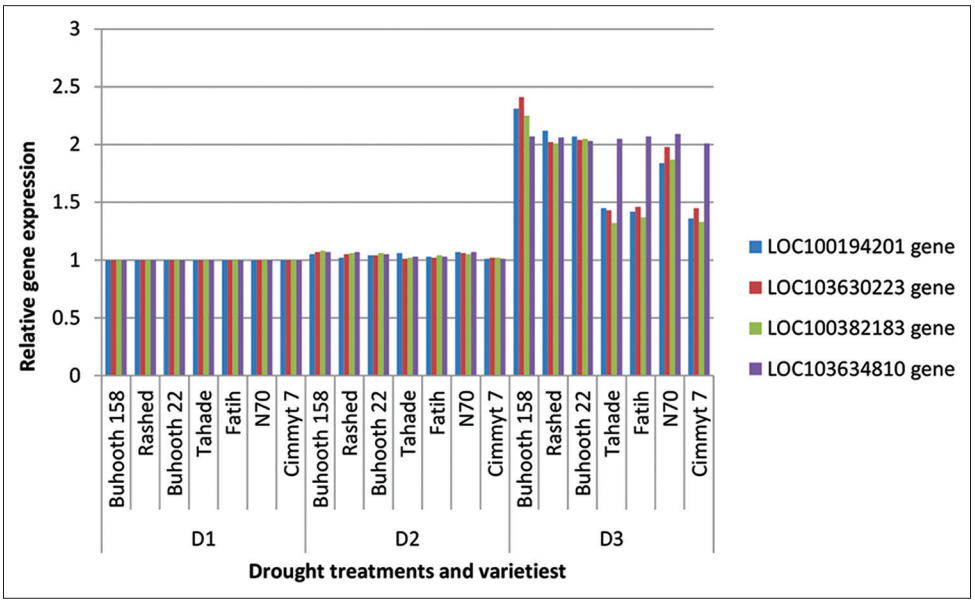

Figure 2: Relative gene expression response of drought stress tolerance genes in wheat cultivars.

tillers in the D3 treatment decreased [Table 5], and this was reflected in the number of spikes. The lack of photosynthesis and interception of sunlight caused by the drought also reduced the amount of dry matter needed to produce grains, resulting in a drop in grain weight. This, in turn, reduced the amount of dry matter needed to produce grains.

It was also noted that the Buhooth 158 cultivar under the influence of drought (D3) was superior in the number of spikes (256.78 spike m^{-2}) and the number of grains in the spike (35.16 grain $spike^{-1}$) and the weight of 1000 grains (32.76 g) by giving it the highest averages for this treats, outperforming the rest of the cultivars, and this was confirmed by relative gene expression results [Figure 2] that the Iraqi cultivar was superior by giving it the highest relative expression of LOC100382183 gene and LOC103630223 gene, which enabled it to drought tolerance.

Table 7 shows that the grain yield, biological yield, and harvest index for the wheat crop were all different between the different study treatments, with 43.55%, 4.91%, and 40.44%, respectively, of these traits getting worse in treatment D3, the drought made a big difference. This decrease is attributed to the drought. It worked to reduce the yield components (no. of spikes, no. of grains, and weight of the grain) and thus work to decrease grain yield, in addition to a drop in biological yield due to a decrease in plant growth parameters (plant height, flag leaf area, and the number of tillers) [16], in addition to the fact that drought caused a decrease in the accumulation of dry matter for plants [17] as a result of the lack of vegetative growth and then reducing the interception of solar rays and the decrease in the conversion of solar energy into chemical energy as a result of closing stomata, increasing respiration and the occurrence of disturbances in biochemical processes [18].

Table 7 further revealed that the cultivars Buhooth 158, Rasheed, and Buhooth 22 produced the highest grain yields, biological yields, and harvest indexes for wheat crops when compared to the other cultivars studied. It was shown that the Buhooth 158 cultivar had the highest genetic expression of the genes responsible for drought resistance, which made it the least affected by drought (D3) for grain production, biological yield, and harvest index.

4. CONCLUSION

These results enable us to conclude that the LOC100382183 gene and the LOC103630223 gene are the most responsive to drought stress and that the Buhooth158 cultivar has been able to resist drought conditions due to the increased expression of these two genes.

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6. AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

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The authors report no financial or any other conflicts of interest in this work.

9. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

10. DATA AVAILABILITY

This article contains all of the generated data as well as the analyses of that data.

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