Response of some wheat genotypes morphogically in grain yield and its components and molecularly in gene expression of drought tolerant genes grown in moisture depletion levels

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Abstract

A field experiment was carried out in the fields of the College of Agricultural Engineering Sciences - University of Baghdad for the seasons 2018-2019 and 2019-2020. A randomized complete block design (RCBD) was used in a split plot design with three replications for the two seasons. Eight genotypes of bread wheat and durum wheat add in the aim : molecular work were planted individually to test their ability to tolerance moisture stress, the genotypes occupied the secondary plots, and moisture depletion levels were 50, 70 and 90% of the ready water main plots. The results showed that reducing the moisture depletion to 50% of the ready water caused an increase in most of the yield components, as it was superior in giving the highest mean number of spikes (26.67 and 23.21 spike plant^{-1}), the number of grains per spike (77.67 and 73.00 grain spike^{-1}), and the highest weight of 1000 grains (44.46 and 43.42 gm) and the biological yield (82.61 and 78.33 gm plant^{-1}), and this confirms the improvement of the photosynthetic system ability, as it gave the highest mean grain yield (43.42 and 38.76 gm plant^{-1}). Plants grown under this moisture level were distinguished in increasing their efficiency in CO_2 assimilation, as it gave the highest mean harvest index (53.33 and 46.23%) for both years respectively. The two genotypes Um- Rabee and Al-Noor were superior in their ability to tolerance different levels of moisture depletion, and represented by giving the highest means in the number of spikes (23.00, 23.22 , 21.44 and 21.00 spike plant^{-1}) and the number of grains in a spike (63.00, 63.34, 69.90 and 67.90), and the superiority of Um- Rabee in giving the highest mean weight of 1000 grains (38.23 and 40.04 gm), and this shows the ability of this composition to give the highest yield of grains (40.46, 40.67, 34.61 and 33.94 gm plant^{-1}) for the two genotypes respectively. The high levels of moisture depletion led to the high levels of gene expression in the drought-tolerant NAC, GSK and dreb gene. From this, we conclude that the availability of the appropriate quantities of irrigation water worked to create a balance, activate metabolic processes, and increased the efficiency of photosynthesis and reflected positively on the yield of the plant, and that the decrease in the quantities of irrigation water caused the high levels of gene expression that increased the plant's ability to tolerance water stress.

Key words: gene expression, Drought stress, chromosomes and genotypes
The part of PhD dissertation of the first author

Introduction

The plant lives in balance with climatic conditions. Drought is a climatic condition that affects the physiological processes of plants, and more precisely, it is the most important climatic factor that adversely affects the yield and quality of the crop . That is why drought problems have been given great importance in modern research because it is one of the most important challenges facing agricultural production in the world due to the fluctuation in the distribution of rain and as a result of these conditions, the efficiency of water use reduces and productivity decreases. The increase in the world's population in recent years has caused a major crisis due to the lack of sufficient food to meet the needs of those poor communities that suffer from many and intertwined problems, in addition to the various strains that act as a catalyst for the deterioration and lack of agricultural production. Therefore, it differs of the scientific methods and techniques used to raise efficiency and production in the agricultural sector varied. Stress is a group of conditions that affect the plant and cause a clear change in the physiological processes that they cause damage and deterioration of agricultural lands and reduce food productivity at the global level by 12% (16), so it was necessary to know and study the damages resulting from these stresses and to know the mechanisms of plant tolerance in order to improve and produce varieties tolerant of stresses. The mechanism of plant tolerance to stress is a complex molecular genetic mechanism with an interaction structure that varies according to the type and severity of stress and is related to the type of plant and its growth stage as well as the type of soil and other factors. Also, those mechanisms included the physiological, chemical, and anatomical effects, as well as the phenotypic effect, as well as the molecular effect. All of these conditions interfere and are reflected in the plant's performance, production, growth rate, number of flowers and leaf area, consequently the deterioration of the outcome, which can be absent under severe stress and for long periods.

Drought tolerance is a quantitative characteristic that is controlled by a large group of genes that express themselves as tolerance in several biochemical, functional and physiological traits related to the combination of genes, and that Wheat has great variations that include 16 different species. This allows breeders and researchers a wide range to choose what suitable their environment, as it has a wide ability to adapt to the surrounding conditions and has the ability to withstand various stresses. The success of wheat mapping methods has encouraged the use of modern methods and gene expression (13).

Gene expression is used to determine genotypes that have a high ability to adapt to different stresses. Al-Sumaidai (7) confirmed that some plants delete change their mechanisms and work to

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increase the accumulation of some acids at the expense of other acids and some compounds and increase their gene expression, and that the products of gene action play a key role in stress-bearing mechanisms. This study aims to evaluate the performance of some varieties of bread wheat and durum wheat under different levels of moisture depletion, study gene expression, and determine the levels of moisture depletion that activate the gene expression of some drought-tolerant genes in fine and coarse wheat, and to identify the best drought-tolerant varieties.

Materials and methods

To evaluate the performance of some fine and coarse wheat varieties under moisture depletion levels and its effect on gene expression levels for some drought-tolerant chromosomes, a field experiment was carried out in the fields of the College of Agricultural Sciences - University of Baghdad - Jadiriyah for the seasons 2018-2019 and 2019-2020. The soil was plowed by two orthogonal plows and the leveling was done by adding 100 kg (P_2O_5) at once when preparing. Urea was added at an amount of 200 kg h-1 (46%N) in two equal batches (26). Soil samples were taken during preparation at a depth of 30 cm to conduct physical and chemical analyzes of the soil and estimate its water retention capacity by estimating the relationship between the structural tension of the soil sample and the moisture content at the tension (0.33, 100, 500, 1000 and 1500) kilopascals in the Agricultural Research Department / Ministry of Science and technology (Table 1). Through the difference between the volumetric moisture content at the field capacity of 33 kilopascals and the permanent wilting point of 1500 kilopascals, the ready water content of the soil was estimated. Three levels of moisture depletion were selected: 50, 70 and 90% of the prepared water. The planting date was 17-12-2018 and 24-11-2019 for the first and second seasons, respectively. The randomized complete block design (RCBD) with the arrangement of the split plots for both seasons, the depletion levels of 50, 70, and 90% of the ready water comment the main plots, and the genotypes occupied the secondary plots and with three replications for both seasons. The process of weed control in the experiment was carried out using the herbicide Pallas WG at a rate of use of 90 gm. h^{-1} for one spray after 28 days of planting and by manual weeding whenever needed for the two seasons.

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Table 1. Some soil physical, chemical properties and moisture content for the agricultural seasons 2018-2019 and 2019-2020.

Analysis type	measruing unit	Season 2018-2019	Season 2019-2020
PH	-	7.2	7.2
EC	dsm m^{-1}	2.2	2.0
N	mg kg-1	78.3	52.30
P	mg kg-1	12.25	8.05
K	mg kg-1	120.7	123.00
Sand	%	36.20	36.20
Clay	%	16.70	17.20
Silt	%	47.10	46.60
bulk density	Mega gm m^{-3}	1.2	1.2
Volumetric moisture content at field capacity	cm^{-3}	0.33	0.33
Volumetric moisture content at permanent wilting point	cm^{-3}	0.14	0.14
ready water	cm^{-3}	0.19	0.19

Irrigation was from a sub-waterwheel equipped with a pump and by means of plastic tubes ending in a meter to measure the quantities of water added to each experimental unit in liters. Equal amounts of water were added to all plates when planting and to the limits of the field capacity to ensure field emergence and after emergence, irrigation began under the specified depletion levels at a depth of (30 and 50) cm. It was 30 cm deep from cultivation to end stage and 50 cm deep from end stage to full maturity stage. The depth of water added for each experimental unit was calculated by estimating the soil moisture before irrigation and its completion of the field capacity. The equation mentioned by Allen et al. (5) was used to calculate the depth of water to be added to compensate for the depleted moisture.

$$d = (\theta_{fc} - \theta_w) \times D$$

d = depth of water added (cm)

θ_{fc} = volumetric humidity at field capacity (cm^3-cm^3)

θ_w = Volumetric humidity before irrigation (cm^3-cm)

D= Effective Radical Depth (cm)

The gravimetric method was used to measure the moisture content of the soil, as samples were taken from the soil by the ogare a day before irrigation

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and the soil was placed in a metal box with a known weight. The drying time is 12 minutes until reaching the stage of stability in weight after drying (28).

$$Pw=\left(\frac{Msw-Ms}{Ms}\right)\ 100$$

Since:

Pw = weight percentage of moisture.

Mw = mass of wet soil (g).

Ms = dry soil mass (g)

After recording the dry weight, the moisture content of the soil was estimated according to (15).

The experiment included the use of eight genotypes of wheat, obtained from the Agricultural Research Department/ Ministry of Science and Technology (Um- Rabee, 32, 49, and Al-Noor), the Agricultural Research Department/Ministry of Agriculture (Abu Ghraib, bohooth 10, Ibaa 99) and the College of Agricultural Engineering Sciences / University of Baghdad (KM5180) to test its drought tolerance. The seeds were planted singly in experimental units with dimensions of $(2\times1)M^{-2}$, the distance between the lines is 20 cm, and the distance between one plant and another is about 20 cm, leaving farrows between treatments.

The experiment was covered using transparent polyethylene (agricultural nylon with a thickness of 2 mm) to prevent the arrival of rain during the period of rain. It was fixed on iron pole made for this purpose at a height of 1.5 m above the surface of the soil, and it was covered from the top while leaving the sides open for the purpose of allowing the entry of air and allowing steam water from evaporation - transpiration out into the atmosphere. The coverage process was based on the information of meteorological sites that expected rain during the season.

Studied traits

Number of spikes: by calculating the number of spikes for each plant in the first experiment. Number of grains in a spike (grains $spike^{-1}$), It was calculated as an mean of ten spikes from each experimental unit and randomly for the same spikes that were used to calculate the spike length. Weight of 1000 grain (gm), mean weight of 1000 grains was taken randomly from the grain yield of each experimental unit and weighed by a sensitive electronic scale. Grain yield: measure the weight of the grains for each plant, after excluding the guard lines.

Use of the least significant difference below the probability level of 5%. To method of analysis of variance, and the finding statistical differences were analyzed statistically by the between the arithmetic means of the data treatments (24), using the computer, within the program Genstat-Version 7.

mMeasurements

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When the plant reached the flowering stage, the leaves of the cultivated genotypes were cut after making sure that they were free of pathogens, then washed with distilled water and kept in sterile plastic bags in the freezer (-20 C) until the start of extraction.

The RNA extraction process was carried out according to the following steps: The RNA was extracted using AccuZolTM total RNAExtraction regent bioneer TRI sure from the Korean company Pioneer, according to the method proven by the company.

1- Weigh 100 mg of fresh leaves from plants for each treatment. Leaves were cut into small pieces and then transferred to 1.5 ml Eppendorf tubes containing 1 ml of Accu ZolTM extraction solution. Then the leaves were mashed well with the extraction solution, then the samples were left at room temperature for 10 minutes.

2-The samples were centrifuged at a speed of 13000 cycle min-1 for 10 minutes, then the filtrate was transferred to new Eppendorf tubes and 200 µl of chloroform was added to it and then incubated for 5 min on ice, then the samples were centrifuged at a speed of 12000 cycle min-1 For 15 minutes, the top layer was withdrawn and transferred to a new Eppendorf tube.

3- 500 µl of isopropanol alcohol was added and left for 10 minutes at -20°C. Samples were centrifuged at 12000 cycle min-1 for 10 minutes, then the isopropanol was discarded by pouring.

4- 1 ml of ethanol alcohol (80% concentration) was added to the precipitate, then the samples were centrifuged at 12000 cycles for 5 min, then the alcohol was discarded by pouring.

5- The precipitate was dissolved by adding 50 microliters of sterile water treated with Diethylpyrocarbonate-DEPC, then the samples were incubated in a water bath at 55°C for 10 minutes.

6- The samples were stored at -20°C.

Characterization of RNA: the process of characterizing RNA included measuring its concentration (Nanog-microliter-1) and its purity using a nano-drop spectrophotometer. The absorbance of the sample is calculated at the wavelength of 260 nm, and the purity ratio ranges between greater or equal to 2 for RNA (Sambrook et al., 1989).

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DNA removal from RNA extracted using the kit RQ1 RNase –Free DNase Promega Kit.

DNA was removed from the ribonucleic acid (RNA) according to the following steps:

1- Transfer 8 µl of the RNA extract into 0.2 ml Eppendorf tubes.

2- Add 1µl of 10X RQ1 RNase-Free DNase 10x Reaction buffer and 1µl of RQ1 RNase-Free DNase enzyme, then the samples were placed at 37°C for 30 minutes (PCR is used).

3- End the reaction by adding 1µl of RQ1 DNase stop solution and placing the samples at a temperature of 65°C for 10 minutes (using a PCR).

Reverse transcriptase, using the kit AccuPower® RocketScriptTM RT PreMix: for the purpose of manufacturing the cDNA complementary DNA based on the mRNA template, a special cloning kit from Pioneer Corporation was used. 4µl of RNA was mixed with 1µl of oligo (dt) primer (concentration of 50 bcm) for each treatment in 0.2 ml Eppendorf tubes, 15 µl sterile distilled water was added and then placed in a PCR machine at 37 °C for 10 minutes. Then 42°C for an hour, then 95°C for 5 minutes.

RTPCR reaction using the kit: AccuPower®GreenStarTMqPCR PreMix, use 3 dry pairs plus the housekeeping reference gene (TaActin) as shown in the table2. The primer 3f DREB and 3r DREB were designed based on the transcription factor associated with drought tolerance in wheat, namely *Triticum aestivum* AP2-containing protein (dreb1) with registration number AF303376.1 and *Triticum aestivum* GSK- like kinase with registration number AF525086.1 in the databases of the National Center for Biotechnology Information (NCBI) website. Use Primer3-based Primer BLAST software at NCBI's site for primer design. The primers are manufactured by the Korean company Pioneer.

The primers were dissolved by adding sterile distilled water and the added amount was based on the manufacturer's instructions to get the final recommended concentration. Then, safe solutions were prepared for each starter with a concentration of 100 ng μl^{-1}.

To each small Eppendorf tube (from the reaction kit) 12 µl of water and 100 ng (1 µl of the starter buffer solution) of the forward (F) and reverse (R) initiator were added with 5 µl of the

 ISSN 2072-3875

cDNA sample. The samples were centrifuged for two minutes, then The samples were placed in the RT PCR machine and the following program was

Executed: 95°C for 5 minutes, then 35 revolutions (95°C for 30 seconds, then 55° C for 30 seconds).

Note that the interaction of each drought gene with the interaction of a houskeeping reference gene is performed for the purpose of comparison using the method ΔCt-method (Livak and Schmittgen, 2001).

Table 2 Sequences of nitrogenous bases

primers name	sequence
TaActinF	CGTGTTGGATTCTGGTGATG
TaActinR	AGCCACATATGCGAGCTTCT
TaNAC6F	TACGGCGAGAAGGAGTGGTA
TaNAC6R	ACCCAGTCATCCAACCTGAG
3f DREB	GTCATGCGGAGCAATAGGGA
3R DREB	TGAACTCAACGCACAGGACA
GSK F5	CAGACGGCTGCTTGAAGTTT
GSK R5	GGGCCTCGATCCATGAACAA

Results and discussion

Number of spikes (spike plant^{-1})

It is one of the important traits associated with the yield, the increase of which is positively reflected on the grain yield. Table (3) shows that the increased levels of moisture depletion caused a significant decrease in the number of spikes. The level of depletion of 50% of the prepared water gave the highest mean number of spikes, which amounted to (26.67 and 23.21 spike plant^{-1}) for the two seasons in , respectively, with an increase of (54.61 and 39.99%) over the depletion of 90% of the ready water, which gave the lowest mean number of spikes of which reached (17.25 and 16.58 spike plant^{-1}) for the two seasons, respectively, these results are in agreement with the findings of Hashem (14), Krait (19), Al-Timimi and others (8), who indicated the existence of a decrease in the number of spikes with increased levels of moisture stress because low humidity limits leaf

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expansion, so the leaf area decreases, and thus the amount of light reaching the leaf decreases. The efficiency of photosynthesis and biological reactions decreases, and there is a reduction in the number of tillers by the death of some of them due to the lack of food availability during the stages of spike formation or the development of initiators of tillers. As for the effect of the genotypes, it was significant during the two years of the study, as the two genotypes, Umm Rabee' and Al Noor, were superior in the first season in giving the highest mean number of spikes, which amounted to (23.00 and 23.22 spike plant^{-1}) for the two genotypes, respectively. The values of spikes for the rest of the genotypes during the first season ranged between (19.49 and 21.67 spike plant^{-1}). In the second season, the genotypes Um Rabee, 32, and bohooth 10 outperformed in giving the highest mean number of spikes, which amounted to (21.44, 21.67 and 21.00 spike plant^{-1}) , respectively, for the genotypes, respectively. The mean number of spikes for other genotypes ranged between (18.22- 20.67 spike plant^{-1}) and these results are in agreement with Al-Falahi (3) and Al-Anbari (2), who confirmed the different susceptibility of the genotypes to the production of tillers and representative materials that support the growth of tillers and their transformation into fertile bearing tillers the reason for this difference is the variation in the susceptibility of genotypes in their response to environmental conditions and water stress, as they caused a difference in the number of fertile tillers and thus the difference in the number of spikes. The interaction of the genotypes with the levels of moisture depletion was significant, as genotype Um Rabee with the level of depletion of 50% of the ready water gave the highest mean number of spikes (29.00 spike plant^{-1}) superior to the genotype of Abu Ghraib with the level of depletion of 90% of the ready water, which gave the lowest mean number of spikes was (15.00 spike plant^{-1}) for the first season because water stress caused the short period of vegetative growth and the lack of spike initiators in the formed tillers, and the lack of appropriate conditions led to a reduction in the number of tillers transformed from the vegetative state to the fruit-bearing tillers. As for the second season, there is no significant interaction of genotypes with different levels of moisture depletion.

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Table 2 Effect of moisture depletion levels into number of spikes for some genotypes of wheat.

genotypes	Number of spikes (spike plant^{-1}) first season moisture depletion			mean
	%50	%70	%90	
Um Rabee	29.00	%70	17.67	23.00
32	26.33	22.33	17.67	21.67
49	27.00	21.00	17.67	21.22
Al Noor	28.33	19.00	19.00	23.22
Bohooth 10	25.67	22.33	17.00	20.56
Abu Ghraib	23.67	19.00	15.00	19.45
Ibaa 99	25.67	19.67	16.33	21.00
KM5180	27.67	21.00	17.67	21.67
mean	26.67	19.67	17.25	23.00
L.S.D. 0.05	genotypes	moisture depletion	genotypes * moisture depletion	
	1.39	1.59	2.55	
genotypes	Number of spikes (spike plant^{-1}) Second season moisture depletion			mean
	%50	%70	%90	
Um Rabee	25.33	20.67	18.33	21.44
32	24.00	23.67	17.67	21.78
49	23.33	19.00	14.33	18.89
Al Noor	26.00	19.00	18.00	21.00
Bohooth 10	22.00	21.00	19.00	20.67
Abu Ghraib	21.00	18.67	15.00	18.22
Ibaa 99	23.00	19.00	15.00	19.00
KM5180	21.00	19.33	15.33	18.55
mean	23.21	20.04	16.58	21.44
L.S.D. 0.05	genotypes	moisture depletion	genotypes * moisture depletion	
	1.71	2.74	n.s	

The number of grains in the spike (grain spike^{-1})

The number of grains in the spike is one of the most important traits of the yield and it depends on the efficiency of the pollination process. The results of Table (3) showed a significant decrease in the number of grains by spike with an increase in moisture depletion levels. The level of depletion of 50% of the ready water exceeded in giving the highest mean number of grains in the spike that amounted to (77.67 and 73.00 grains spike^{-1}) for the two seasons respectively, with an increase of (101.06 and 66.01%) for the level of 90% depletion of the ready water, which gave the lowest mean number of grains per spike of (38.62 and 44.00 grains of spike^{-1}) for the two seasons respectively. This agrees with Wajid (25), Al-Obaidi (6) and Hashem (14), as the occurrence of moisture stress in the different stages of wheat growth, especially the flowering stage, leads to a decrease in the number of fertile florets and thus the number of

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grains in the spike, and that the decrease in the number of grains is associated with the readiness of irrigation water. The number of grains per spike differed with the different genotypes. The two genotypes Um Rabee and Al Noor gave the highest mean number of grains per spike, which amounted to (63.00, 69.90, 63.34 and 67.90 grains spike^{-1}) for the two seasons, respectively, superior to the rest of the genotypes, While the genotype was 49, which gave the lowest mean number of grains per spike of (50.67 and 53.60 grains spike^{-1}) for the two seasons , respectively. The number of fertile florets formed at the flowering stage and is reflected in the number of grains in the spike.

As for the interaction of the genotypes with the levels of depletion, it was significant, the genotypes Al Noor and Abaa 99 at the level of depletion of 50% of the ready water gave the highest mean number of grains in the spike, which reached (83.00 and 84.00 grains spike^{-1}) for the two genotypes respectively during the first season. The same level of moisture depletion during the second season in giving the highest mean number of grains in the spike of (80.70 and 84.70 grains spike^{-1}) for the two genotypes, respectively, superior on the two genotypes of 49 in the level of depletion of 90% of the ready water, which gave the lowest mean of the number of grains in the spike of (33.67 grains spike^{-1}) in the first season, and the genotype was 99 under the same level of moisture depletion, which gave the lowest mean number of grains in a spike in the second season, which amounted to (37.00 grains spike^{-1}), because water stress caused a decrease in the rate of pollination of florets, thus a decrease in the number of grains formed in spike and this agrees with the results of Farhoud and Al-Maini (12).

Weight of 1000 grain (gm)

The weight of the grains is a good indicator of the efficiency of the photosynthesis process and the transfer of metabolites to the sink, which is highly sensitive to growth factors. The results of table (4) showed a significant decrease in the weight of 1000 grains with an increase in the levels of moisture depletion, as the level of depletion of 50% of the prepared water exceeded in giving the highest mean weight of 1000 grains that amounted to (44.46 and 43.42 g) for the two seasons, respectively, with an increase of (110.01 and 73 75%) at the 90% depletion level of the ready water, which gave the lowest mean weight of 1000 grains (21.17 and 25.00 gm) for the two seasons, respectively. These results are in agreement with the results of Aown et al. (9), Mahasneh (21) and Hashem (14), who proved the role of water in increasing the activity of photosynthesis and various vital activities within the plant tissues, and it has a positive role in the process of transporting processed nutrients and thus increasing the weight of grains.

The genotypes differed significantly in the mean weight of 1000 grains, the genotype Um- Rabee gave the highest mean weight of 1000 grains (38.23 and 40.04 gm) for the two seasons in , respectively, superior to Abu Ghraib, which gave the lowest mean weight of

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1000 grains (30.01 and 28.30 gm) for two seasons, respectively. These results are in agreement with what Abulnadr (1) found when studying a group of coarse and fine genotypes that confirmed the difference in the response of genotypes in the efficiency of photosynthesis to the same growth factors. There was no significant interaction between genotypes and moisture depletion levels in the first season. As for the second season, there was a significant decrease in the weight of 1000 grains with increased levels of moisture depletion. The genotype of 32 with the level of depletion of 50% of the ready water was superior in giving the highest mean weight of 1000 grains amounted to (49.67 gm) compared to the light genotype with the level of depletion of 90% of the ready water, which gave the lowest mean weight of 1000 grains (20.80 gm), for the role of water in prolonging the filling period of the grain by delaying the aging of the flag leaf, which increases the amount of manufactured materials transferred from the leaves to the grains in the spikes, which are the final sink of photosynthesis products.

Table 3 Effect of moisture depletion levels on the number of spike grains for some genotypes of wheat.

genotypes	Number of grains (grains spike-1) first season			mean
	moisture depletion			
	%50	%70	%90	
Um Rabee	79.67	63.00	46.33	63.00
32	77.33	60.67	35.67	57.89
49	67.67	50.66	33.67	50.67
Al Noor	83.00	64.68	42.34	63.34
bohooth 10	76.33	49.67	36.32	54.11
Abu Ghraib	73.67	50.00	38.00	53.89
Ibaa 99	84.00	56.66	40.32	60.33
KM5180	79.67	54.64	36.35	56.89
mean	77.67	56.25	38.63	63.00
L.S.D. 0.05	genotypes	moisture depletion	genotypes * moisture depletion	
	3.32	7.55	8.16	
genotypes	Number of grains (grains spike^{-1}) Second season			mean
	moisture depletion			
	%50	%70	%90	
Um Rabee	80.70	75.70	53.30	69.90
32	62.00	59.70	45.70	55.80
49	60.00	57.70	43.00	53.57
Al Noor	84.70	71.30	47.70	67.90
bohooth 10	76.00	56.30	37.70	56.67
Abu Ghraib	71.70	58.70	42.00	57.47
Ibaa 99	70.00	54.70	37.00	53.90
KM5180	78.70	59.00	45.30	61.00
mean	72.98	61.64	43.96	
L.S.D. 0.05	genotypes	moisture depletion	genotypes * moisture depletion	
	3.51	3.36	6.19	

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Table 4 Effect of moisture depletion levels into the weight of 1000 grains of some genotypes of wheat.

genotypes	weight of 1000 grains (gm) first season moisture depletion			mean
	%50	%70	%90	
Um Rabee	51.00	38.00	25.68	38.23
32	47.00	36.66	22.65	35.44
49	45.33	35.00	22.68	34.34
Al Noor	48.67	36.68	23.00	36.12
bohooth 10	42.66	34.00	19.00	31.89
Abu Ghraib	40.00	33.33	16.69	30.01
Ibaa 99	39.66	33.34	21.00	31.33
KM5180	41.32	32.31	18.65	30.76
mean	44.46	34.92	21.17	
L.S.D. 0.05	genotypes	moisture depletion	genotypes * moisture depletion	
	1.96	2.24	N.S	
genotypes	weight of 1000 grains (gm) second season moisture depletion			mean
	%50	%70	%90	
Um Rabee	46.63	39.50	34.00	40.04
32	49.67	39.97	26.30	38.65
49	38.67	31.43	25.80	31.97
Al Noor	44.33	35.17	20.80	33.43
bohooth 10	45.00	32.53	21.37	32.97
Abu Ghraib	38.70	24.47	21.73	28.30
Ibaa 99	40.33	30.50	25.30	32.04
KM5180	44.00	38.00	24.63	35.54
mean	43.42	33.95	24.99	
L.S.D. 0.05	genotypes	moisture depletion	genotypes * moisture depletion	
	1.71	1.83	3.08	

Grain yield (gm plant^{-1})

Grain yield depends on the efficiency of the photosynthesis process and the efficiency of transferring the metabolites to the grain, which is highly affected by genetic genotype and environmental factors. Table (5) reveals a significant decrease in the mean yield of the plant with the increase in the levels of moisture depletion. The level of depletion of 50% of the ready water exceeded in giving the highest mean grain yield amounted to (43.42 and 38.76 gm plant^{-1}) for the two seasons respectively, with an increase of (31.02 and 61.23%) from the level of depletion of 90% of the ready water, which gave the lowest mean grain yield amounted to (33.12 and 24.05 gm plant^{-1}) for the two seasons respectively, as the reduction of the yield with reducing the quantities of irrigation water due to the lack of one or more of the components of the yield, So the decrease in the number of spikes, the number of grains and the

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weight of 1000 grains (2, 3 and 4) caused a decrease in the grain yield. These results agree with the findings of Zeidain (27) and Al-Fatlawi (4).

The genotypes were significantly affected, as the Um- Rabee and Al-Noor genotypes were superior in giving the highest mean grain yield of (40.67,40.46 , 34.61 and 33.94 gm plant^{-1}) for the two seasons, respectively, superior to Abu Ghraib, which gave the lowest mean for grain yield it reached (36.55 and 27.75 gm plant^{-1}) for the two seasons, respectively. The superiority of some genotypes indicates that they are highly efficient in the process of photosynthesis when suitable conditions are available for growth, in addition to their superiority in the mean number of spikes, the number of grains and the weight of 1000 grains, which positively reflected on the grain yield. There was no significant interaction between genotypes and moisture depletion levels in the first season. As for the second season, the grain yield decreased with the increase in the levels of moisture depletion, as the genotype of Al Noor with the level of depletion of 50% of the ready water was superior in giving the highest mean of the yield of grains reached (43.87 g plant^{-1}) than the genotype of Abu Ghraib with the level of depletion of 90% of the ready water, which gave the lowest mean grain yield amounted to (21.12 gm plant^{-1}) due to the effect of water abundance on growth stages, controlling physiological functions, prolonging the period of filling the grain and improving most of the growth traits associated with the components of the yield and increasing its components that caused an increase in grain yield.

gene expression

The concept of adaptation is the ability of a plant to give a good yield under stress conditions. An adapted plant is a plant that bears or resists a certain stress and with which it can produce at an acceptable level compared to another plant that is not adapted to that stress. The stress tolerance mechanism is a complex genetic mechanism of interaction according to the type Stress and its severity. Therefore, the detection of a gene associated with stress tolerance must be accompanied by estimating the percentage of its gene expression and determining the level of depletion that stimulated this expression.

The results of tables (6, 7 and 8) showed significant differences and an increase in the levels of gene expression for genotypes with an increase in moisture depletion levels.

The results of table (6) for estimating the levels of gene expression for the NAC chromosome with the levels of moisture depletion showed that the genotype of bohooth 10 was superior in giving the highest levels of gene expression with higher levels of moisture depletion 90% as the value of the Ct cycle threshold for it decreased and reached (-4.79) and thus the levels of its gene expression increased it reached (27,66519) at the level of 90% depletion of the prepared water.

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As for the genotype of KM5180, whose gene expression levels increased with higher levels of moisture depletion, and its best expression was in the level of 70% depletion of ready water, where the value of the CT cycle threshold decreased and reached (5.9), and thus its gene expression levels increased and reached (0.016746).

Table 5 Effect of moisture depletion levels on grain yield of some genotypes wheat.

genotypes	grain yield (gm plant^{-1}) first season			mean
	moisture depletion			
	%50	%70	%90	
Um Rabee	47.67	39.66	34.67	40.67
32	43.00	39.65	34.33	38.99
49	40.33	37.64	32.34	36.77
Al Noor	45.68	40.69	35.00	40.46
bohooth 10	43.33	39.33	32.00	38.22
Abu Ghraib	39.00	39.00	31.66	36.55
Ibaa 99	44.65	42.00	33.00	39.88
KM5180	43.66	38.32	32.10	38.03
mean	43.42	39.54	33.14	
L.S.D. 0.05	genotypes	moisture depletion	genotypes * moisture depletion	
	2.47	1.94	N.S	
genotypes	grain yield (gm plant^{-1}) second season			mean
	moisture depletion			
	%50	%70	%90	
Um Rabee	41.15	36.19	26.5	34.61
32	37.78	34.48	24.66	32.31
49	38.33	32.11	24.76	31.73
Al Noor	43.87	35.77	22.19	33.94
bohooth 10	41.1	29.02	23.7	31.27
Abu Ghraib	31.55	30.59	21.12	27.75
Ibaa 99	37.88	31.74	25.13	31.58
KM5180	38.44	32.54	24.24	31.74
mean	%50	%70	%90	
L.S.D. 0.05	genotypes	moisture depletion	genotypes * moisture depletion	
	1.15	1.37	2.37	

Table (7) of the levels of gene expression for the dreb gene indicates the superiority of the genotypes bohooth 10, 49, Abaa 99 and KM5180 in the levels of gene expression with the level of depletion of 90% of the ready water, as the cycle threshold values decreased and reached (1.51, 8.99, 9.50 and 9.60). Therefore, its gene expression levels increased and reached (2.848, 0.001967, 0.001381 and 0.001289) for the genotypes , respectively.

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The two genotypes 32 and Al-Noor were superior in gene expression levels with the level of depletion of 70% of the ready water, and the CT values decreased and reached (5.81 and 7.67), so their gene expression levels increased and reached (0.0178 and 0.00491) for the two genotypes, respectively.

Despite the high levels of gene expression for genotypes 49, 99, and KM5180 with a depletion level of 90%, the best gene expression occurred for these genotypes with a depletion level of 70%, where the CT cycle threshold indicators decreased and reached (8.86, 9.3 and 9.22), and thus their gene expression levels increased and reached (0.002152, 0.001586 and 0.001677) , respectively.

Table (8) shows the superiority of the gene expression of the GSK chromosome to the genotypes Um-Rabee, 49, bohooth 10, Abu Ghraib and KM5180 in their high levels of gene expression with the level of 90% depletion of ready water, where the CT cycle threshold indicators decreased and reached (8.94, 9.5, -1 and 9.92). and 10.54), and therefore their gene expression levels increased and were (0.002036, 0.001381, 2, 0.001032, and 0.000672), respectively.

The CT of genotype 32 decreased in the depletion level of 70% and reached (4.63) and therefore its gene expression levels increased and reached (0.040386). As for the two genotypes Abu Ghraib and KM5180, despite the high levels of their gene expression in the level of depletion of 90% of the ready water, the best genetic expression for them it was in the level of depletion of 70% of the prepared water, where the CT cycle threshold decreased and reached (8.28 and 9.55) and its gene expression levels increased and reached (0.003217 and 0.001334) for the two formulations, respectively. These results are consistent with what Al-Sumaidai (7), which showed that the value of gene expression depends on the degree of environmental stress.

Table 6 cycle threshold and relative gene expression levels for genotypes of wheat with different moisture depletion levels of the NAC chromosome.

genotypes	Cycle threshold (Ct) for the NAC gene			Relative gene expression		
	moisture depletion			moisture depletion		
	50%	70%	90%	50%	70%	90%
Um Rabee	7.64	6.27	12.45	0.00501338	0.01295812	0.00017872
32	3.98	3.05	5.08	0.06337247	0.12074204	0.0295643
49	6.57	6.55	5.33	0.01052526	0.01067219	0.02486052
Al Noor	3.9	4	11.83	0.06698584	0.0625	0.00027467
bohooth 10	-2	-2.2	-4.79	4	4.59479342	27.6651914
Abu Ghraib	3	5.48	5.82	0.125	0.02240555	0.01770131
Ibaa 99	6.81	5.56	6.02	0.00891222	0.02119694	0.01540989
KM5180	10.23	5.9	6.84	0.00083265	0.01674646	0.00872881

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Table 7 cycle threshold and relative gene expression levels for genotypes of wheat with different moisture depletion levels of the dreb gene.

genotypes	Cycle threshold (Ct) for the dreb gene			Relative gene expression		
	moisture depletion			moisture depletion		
	50%	70%	90%	50%	70%	90%
Um Rabee	7.64	10.02	16.37	0.00501338	0.00096312	1.1807E-05
32	7.63	5.81	6.4	0.00504825	0.01782443	0.01184154
49	9.94	8.86	8.99	0.00101803	0.00215216	0.00196671
Al Noor	10.43	7.67	13.38	0.00072487	0.00491021	9.3803E-05
bohooth 10	1.59	1.25	-1.51	0.33217145	0.42044821	2.84810039
Abu Ghraib	8.88	8.19	10.66	0.00212253	0.00342424	0.00061805
Ibaa 99	10.08	9.3	9.5	0.00092388	0.00158643	0.00138107
KM5180	14.48	9.22	9.6	4.3761E-05	0.00167689	0.00128858

Table 8 cycle threshold and relative gene expression levels for genotypes of wheat with different moisture depletion levels of the GSK chromosome.

genotypes	Cycle Threshold (Ct) for the GSK gene			Relative gene expression		
	moisture depletion			moisture depletion		
	50%	70%	90%	50%	70%	90%
Um Rabee	12.29	10.62	8.94	0.00019968	0.00063542	0.00203607
32	7.67	4.63	8.18	0.00491021	0.04038603	0.00344806
49	13.35	10.48	9.5	9.5774E-05	0.00070017	0.00138107
Al Noor	7.43	10.93	14.78	0.00579892	0.00051256	3.5545E-05
bohooth 10	-0.5	0.14	-1	1.41421356	0.90751916	2
Abu Ghraib	11.07	8.28	9.92	0.00046516	0.00321715	0.00103224
Ibaa 99	10.67	10	10.87	0.00061378	0.00097656	0.00053432
KM5180	12.98	9.55	10.54	0.00012377	0.00133402	0.00067165

Conclusion

The level of depletion of 50% of the ready water exceeded in increasing the means of the number of spikes, the number of grains per spike, the weight of 1000 grains and the grain yield for the two seasons. The diagnosis of the genotypes of Um Rabee, Al Noor and KM5180 is one of the best drought-tolerant genotypes. The levels of gene expression increased for genotypes of bohooth 10, Ibaa 99, and Um Rabee and 49 in chromosomes DREB, NAC and GSK with high levels of moisture depletion (90% of ready water). The best gene expression for genotypes KM5180, 32, Noor and Abu Ghraib in chromosomes GSK, DREB and NAC under the depletion level of 70% of ready water.

Therefore, we recommend planting the genotypes Um- Rabee and Al Noor in areas of water scarcity or seasons of lack of rain. As for the genotype KM5180, it is planted in seasons of lack and abundance of rain. Interest in studying the estimation of gene expression levels to understand the

genetic effects of study factors on plants, which are an important cause of phenotypic variations.

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