Estimation of DREB Gene Expression in Wheat Genotypes (Triticum aestivum L.) Introduced to Anbar Governorate Under Water Stress

M. L. Mansoor1, M. H. Al-Issawi1 and J. N. Mhmood2

1 College of Agriculture- University Of Anbar/IRAQ
2 Agriculture Research Directorate/Ministry of Science and Technology.

*Corresponding author's Email: mar18g5002@uoanbar.edu.iq

Abstract. Wheat crop is known as one of the domesticated crops and the second-largest crop in the world where cultivated in arid and semi-arid environments. A Field experiment was conducted in Hit city/Qnan region, Anbar governorate that located west of Iraq, during the winter season of 2019/2020 in order to estimate gene expression of 24 wheat genotypes under drought conditions. The experiment included two treatments, the first is wheat genotypes and drought treatment (droughted and Irrigated). A split-plot arrangement in Randomized Complete Block Design (R.C.B.D) with three replications was used in this experiment where drought treatments occupied the main plots while genotypes were put in the subplots. The results showed that the genotypes responded differently to the treatments according to the measured traits most prominent genotype was 43 which recorded a high expression of DREB 1A gene (221.88-fold) followed by genotypes 39, 24, 6, 28, 25, and 20 at drought treatment. Genotype 6 showed superiority in plant height of 94.78 and 93.39 where the mean did not affect by the interaction of both treatments on the trait, also recorded the highest average for NGS trait around 57.48 grain. spike\(^{-1}\), followed by genotypes 43 gave 56.57 grain. spike\(^{-1}\)which also was superior in flag leaf area with high average 35.62 cm\(^2\). For the dry weight trait, genotype 29 superior and recorded 666.66 g. m\(^{-2}\). While the genotypes (18, and Al-diyar) were superiors in TGW trait with a higher average attained 56.65, and 55.77 g, respectively. The genotypes did not differ significantly in GY although the genotype 3 and 29 recorded higher mean for GY with 7.39 and 7.29ton ha\(^{-1}\).

1. Introduction
Wheat (Triticum aestivum L.) is considered as one of the domesticated crops and the second-largest crop in the world in cultivation. It is a source of calories by about 20% after rice as well as a source of an important protein. The level of wheat production recently not satisfactory in the face of increasing world population potential of about a billion in 2050. Also, expect increased the request for wheat about 60%, in this case, should increase the yield of wheat from 1% to about 1.6%, which in return requires the tolerate for abiotic and biotic stresses and improves the input use efficiency [1,2]. Climate changes that Iraq undergo leads to rain retention and increased temperatures thus lands have deteriorated due to droughts such as desertification and the exhaustion of natural resources of the land [3]. Where currently, Iraq faces major challenges in terms of food, the most prominent of which is the scarcity of water and the lack of food products, which reflected on the main foods, including grains, and the most important grain is wheat that the Iraqi people depend on for their food, as wheat made bread contains protein by 12-17%, Starches 76-78% and fats 1.2-1.5% [4]. The multiplicity of wheat genotypes besides their genetic variation are different in the performance of growth and production in some environments besides the variation in soil and climate conditions for these environments [5]. The introduction of new genotypes and varieties is one of the traditional breeding methods. The hybrid and introduced genotypes in Iraq are subject to evaluation and selection for several generations, depending on the yield and the degree of stability without the occurrence of genetic variations, as well as studying production factors and then comparing them with cultivated varieties in order to approve it as a new variety [6]. Stress-responsive gene expression timing is regulated through a set of transcription factors and cis-acting elements in
stress-inducible promoters [7]. DREBs are important plant transcription factors (TFs) that regulate the expression for many of genes that induce stresses tolerance at most in an ABA-independent way also have a crucial function to improve tolerance for abiotic stress of plants via the interaction with DRE/CRT cis-element that existent at in the promoter zone of different responsive genets for abiotic stress [8]. Therefore, this research aims to estimate DREB gene expression of the introduced genotypes of wheat and its cultivation under the influence of the irrigation cutoff treatment.

2. Materials and methods

A Field experiment was conducted in Hit city/Qnan region, Anbar governorate that is located west of Iraq, (Latitude 33°39 N and Longitude 42°47 E) during the winter season of 2019/2020. The Experiment included 24 wheat genotypes (23 newly introduced to Iraq in addition to local cultivar) and two drought treatments. The genotypes introduced into Iraq were obtained from the Ministry of Science and Technology. The experiment was laid out as a split-plot arrangement in Randomized Complete Block Design (R.C.B.D) with three replications. Drought treatment occupied the main plots while genotypes were put in the subplots. Plants were subjected to water stress treatment after the flowering stage, where the irrigation was cut off from the specified main plots until the end of the experiment. Traits that were measured included plant height, flag leaf area, dry weight, number of grain per spike, 1000 grain weight, and grain yield.

2.1 Molecular analysis of DREB

Samples of flag leaf were taken after applying the irrigation cut and were directly put in zipper bags and directly were kept in a box containing ice, then samples of the leaves were placed in tubes containing trizol then transferred to the laboratory (ASCo Learning Center).

RNA was isolated from the sample according to the protocol of TRIzol™ Reagent. For each tube, 1mL from TRIzol™ Reagent was added per 50-100 mg of sample and gently mixed by the vortex. For each tube, 0.2 mL of chloroform was added to the lysate, then the tube cap was secured. Samples were incubated for 2–3 minutes then centrifuged for 10 minutes at 12000 rpm. The mixture was separated into a lower organic phase, interphase, and a colorless upper aqueous phase. The aqueous phase containing the RNA was transferred to a new tube, then added 0.5 mL of isopropanol to the aqueous phase and incubated for 10 minutes then putting in centrifuged for 10 minutes at 12000 rpm for precipitated the RNA, and discarded the supernatant. After that and for each tube, added 0.5 mL of 70% ethanol, vortex briefly then centrifuged for 5 minutes at 10000 rpm. Then ethanol will aspirate and air-dried the pellet. Then rehydrated the Pellet in 70μl of nuclease free water then incubated in a water bath or heat block set at 55–60ºC for 10–15 minutes. RNA yield was determined by using quantus fluorometer to detect the concentration of extracted RNA or cDNA, for 1µl of RNA, 199 µl of diluted quantifluor dye was mixed. After 5min incubation at room temperature in a dark place, RNA concentration values were detected. The primer of DREB was used to detect the expression. Primer (DREB 1A-Forward: 5`-CGAGGTCTTGGTGTTTCTCTCAG-3 and DREB1A Reverse: 5`-CAAACTCGGCATCTCAAACA-3`) were supplied by Macrogen Company in a lyophilized form. The primer was designed using gene-specific sequencing of DREB1A and according to the reference [9].

Absolute quantification by the standard curve (SC)

The standard curve method employs a dilution series of known template copy number in the qPCR assay. Linear regression of log concentration (copy μl⁻¹) versus Cᵀ gives the standard curve, and this is then used to calculate template concentration (copy μl⁻¹) of the sample. Eight of 0.2 ml tube prepared, 90 µl of Nuclease Free Water was added to each tube then added 10 µl from sample of 22*10⁹ copy μl⁻¹ to the first tube and made a serial dilution by transferred 10 µl from first tube to second tube and so on. The standard curve reaction started from the tube of 22*10⁹ copy μl⁻¹ to the tube of 22*10⁵ copy μl⁻¹.

Absolute quantification according to the method in [10]:

m = [n bp] [1.096e-21 g/bp]
m= {  g}*10⁹ng
Copy No. = concentration/m, Where:
 n = DNA size (bp), m = mass
3. Results and Discussion

3.1 DREB 1A gene expression
DREB 1A is considered one of the AP2/EREBP plant specific family of transcription factors and binds to the promoter of genes such as rd29A which in turn induces the expression of rd29A gene in response to either drought, salt, or cold. In current study, DREB 1A gene expression has been investigated and estimated according to the method mentioned in [10] in 24 wheat genotypes that were newly introduced to Anbar governorate. Results presented in Table 1 showed the superiority of genotype 43 with a high number of copies of DREB 1A (221.88 copies), followed by genotypes 39, 24, 6, 28, 25, and 20 which gave respectively 174.12, 29.19, 25.74, 22.09, 19.41, and 16.25 copies at drought treatment in comparison with irrigation treatment where they gave a convergent number of copies among them excepting the genotype Al-Mahmodia (MH) which recorded a high number of copies (16.05 copies) in irrigation treatment. It is clear that the genotypes under study were not the same in their response to water stress and might belong to their genetic background. Drought stress is considered a big problem for plant growth, evolution, then productivity. Therefore, there is improving the cultivars towards drought stress and environmental changes as a solution to improve crop productivity. Drought tolerance is managed through several genes included transcription factors (TFs) where lots of transcription factors such DREBs protein TFs function as main switches that stimulate the expression of stress-tolerance. The changeable gene expression is considered as a main molecular response for an inverse condition of the plant environment [12,13]. DREB/CBF associated with drought-responsive cis-acting elements, it belongs to the ERF family of TFs which consisting of two subclasses DREB1/CBF and DREB2 that are induced by cold and drought, sequentially. Where the DREBs could be used in genetic engineering programs and in partnership with various promoters through produce transgenic that tolerate drought, salinity, and coldness [14]. A study by Kurahashi et al., [15] using synthetic hexaploid wheat lines referred that the TaDREB1 TF genes accumulated in the tolerant accessions to drought more than the sensitive accessions. It was found that the two genes TaDREB1 and TaDREB2 were motivated under drought stress in the wheat plant [16], and this could promote the expression at the osmotic stresses. Research of Shinozaki & Yamaguchi [17] cleared the participation of CBF/DREB1 in the responses of the gene expression of the cold while CBF/DREB2 is an important TFs in the Effector gene expression of Dehydration and salt stress. So, the group of DREB1 is depending on their participation in osmotic and temperature stress responses, besides having a genetic function that may improve dehydration tolerance in wheat [18]. These results demonstrate the role of DREB genes as a central regulator for abiotic stress response and tolerance when plants are exposed to inappropriate conditions. Whereas, gene DREBs engineering works to regulate the expression of several osmotic stress-inducing genes, in addition to the original stress-responsive pathways that lead to physiological and chemical adaptation in plants and to make them adapt to these osmotic stresses. This makes gene DREB a target pathway for genetic engineering and crop improvement [8].
Table 1. Number of DREB 1A copies in wheat genotypes under two irrigation treatment (Irrigated and droughted)

| Genotypes | Irrigated | Droughted |
|-----------|-----------|-----------|
| Genotypes | No. of folds | Genotypes | No. of folds |
| Al-diyar  | 10.32 | Al-diyar | 10.22 |
| MH        | 16.05 | MH       | 10.35 |
| 3         | 11.64 | 3         | 9.47 |
| 4         | 11.49 | 4         | 9.16 |
| 5         | 10.31 | 5         | 9.43 |
| 6         | 9.76  | 6         | 25.74 |
| 7         | 10.35 | 7         | 9.82 |
| 9         | 11.73 | 9         | 10.96 |
| 10        | 11.31 | 10        | 11.06 |
| 11        | 12.84 | 11        | 10.06 |
| 18        | 11.25 | 18        | 11.04 |
| 19        | 9.96  | 19        | 9.94 |
| 20        | 11.18 | 20        | 16.25 |
| 24        | 11.34 | 24        | 29.19 |
| 25        | 9.85  | 25        | 19.41 |
| 28        | 9.90  | 28        | 22.09 |
| 29        | 7.90  | 29        | 11.32 |
| 30        | 8.94  | 30        | 10.21 |
| 31        | 10.46 | 31        | 13.09 |
| 32        | 10.13 | 32        | 12.10 |
| 36        | 7.44  | 36        | 10.76 |
| 39        | 12.10 | 39        | 174.12 |
| 41        | 11.74 | 41        | 10.47 |
| 43        | 11.15 | 43        | 221.88 |

3.2 Plant height (cm) (PH)
An important criterion in wheat breeding and improvement programs is low plant height and early maturity in order to withstand drought and reduce water consumed [19]. The statistical analysis showed in (Table 2) a non-significant effect for genotypes, and irrigation treatments in the mean of plant height trait. The interaction between genotypes and irrigation intervals effects as shown in Table 2 was significant on plant height, where the highest combination for plant height was recorded by genotype 6 with a mean of 94.78 and 93.39 cm in drought and irrigation treatments respectively as noticed no difference in significantly for both treatments in the same genotype on height, followed by genotype 3 which attained 90.59 cm at irrigation treatment, whereas the lowest height average was 72.96 cm recorded by genotype 20 at drought stress treatment. The reason for the difference among genotypes in plant height is due to the variation among genotypes in response for environment factors, this agrees with results of Al-Temimi at
where showed a significant effect for the interaction between water deficit treatments and wheat cultivars on PH.

### Table 2. Plant height (cm) of wheat genotypes under drought treatments.

| Genotypes | Irrigated | Droughted | Mean | Genotypes | Irrigated | Droughted | Mean |
|-----------|-----------|-----------|------|-----------|-----------|-----------|------|
| Al-diyar  | 78.83     | 80.13     | 79.48| 9         | 84.91     | 85.98     | 85.44|
| 10        | 85.62     | 84.39     | 85.01| 28        | 75.11     | 85.11     | 80.11|
| 29        | 87.78     | 82.30     | 85.04| 18        | 79.45     | 85.97     | 82.71|
| 39        | 83.79     | 80.94     | 82.37| 6         | 93.39     | 94.78     | 94.08|
| 36        | 82.56     | 87.08     | 84.82| 3         | 90.59     | 80.92     | 85.75|
| 25        | 77.05     | 79.82     | 78.44| Al-mahmodia| 66.92     | 82.23     | 74.57|
| 20        | 78.04     | 72.96     | 75.50| 19        | 85.73     | 88.56     | 87.14|
| 24        | 82.01     | 74.76     | 78.38| 5         | 87.78     | 88.42     | 88.10|
| 31        | 82.12     | 79.24     | 80.68| 41        | 73.71     | 88.23     | 80.97|
| 7         | 84.07     | 89.88     | 86.98| 4         | 84.65     | 87.94     | 86.30|
| 30        | 81.75     | 78.36     | 80.06| 43        | 83.52     | 85.12     | 84.32|
| 11        | 84.70     | 85.36     | 85.03| 32        | 82.76     | 88.04     | 85.40|

Mean of irrigation treatments: Irrigated= 82.37, Droughted= 84.02
L.S.D (Genotypes x Irrigation) = 10.58

### 3.3 Flag leaf area (cm²) (FLA)

The trait of the FLA considered as important for the wheat plant, where the large area of flag leaf for the genotypes indicates its efficiency in preserving the length of the leaf cells elongation under the environmental conditions which is planted in. Also, the area of the leaf during the filling period of the grains is a determining factor for its extent fullness, as the leaves dried out gradually and the leaf of the flag retains its greenness to contribute to the process of photosynthesis and thus the synthesis of dry matter that is finally transported to the sink [21]. According to statistical analysis results in (Table 3) showed a significant variation for genotypes in the flag leaf area. Genotype 43 was superior with a high mean of 35.62 cm², followed by genotype 32 was 27.02 cm² and both were significantly different in comparison with genotype 20 which showed a lower mean of flag leaf area 13.41 cm². The difference of genotypes in the formation of different rates of flag leaf area is due to being a genetic trait linked to its genetic makeup. This finding is in agreement with [22] results that showed a significant difference for genotypes in flag leaf area. The flag leaf area of genotypes according to (Table 3) results showed significant differences at the interaction of genotypes and irrigation intervals, the highest average gave by genotype 43 about 36.96, 34.27 cm² at irrigation and drought treatments respectively where the genotype 43 was not affected by interaction in flag leaf area followed by genotype 19 recorded 30.92 cm² at drought treatment and was different in significant from genotype 43, then genotype 11 which attained 29.00 cm² at drought, while lower mean was recorded in the genotype 20 about 11.65 cm² recorded at drought treatment effect. This agreement with results of [20].

### Table 3. Flag of leaf area (cm²) of wheat genotypes under drought treatments.

| Genotypes | Irrigated | Droughted | Mean | Genotypes | Irrigated | Droughted | Mean |
|-----------|-----------|-----------|------|-----------|-----------|-----------|------|
| Al-diyar  | 18.61     | 23.78     | 21.19| 9         | 24.53     | 25.43     | 24.98|
| 10        | 25.41     | 25.79     | 25.60| 28        | 21.00     | 24.73     | 22.86|
| 29        | 27.28     | 24.97     | 26.12| 18        | 17.85     | 25.67     | 21.76|
| 39        | 16.45     | 19.45     | 17.95| 6         | 23.95     | 22.96     | 23.46|
| 36        | 16.75     | 27.57     | 22.16| 3         | 24.19     | 19.42     | 21.80|
25 12.98 15.27 14.13 Al-mahmodia 18.00 25.47 21.74
20 15.17 11.65 13.41 19 20.02 30.92 25.47
24 18.29 11.66 14.98 5 22.50 24.61 23.55
31 22.25 17.67 19.96 41 15.79 24.40 20.09
7 20.72 21.95 21.33 4 20.82 18.74 19.78
30 17.91 20.20 19.05 43 36.96 34.27 35.62
11 23.16 29.00 26.08 32 25.59 28.44 27.02

Mean of irrigation treatments: Irrigated= 21.09, Droughted= 23.08
L.S.D (Genotypes)= 7.262, (Genotypes x Irrigation)= 8.988

3.4 Dry weight (g m^-2) (DW)
As shown in Table 4, the results of the statistical analysis pointed to a significant effect for genotype on the dry plant weight average, where genotype 29 was superior over the rest of the genotypes with a higher mean for a dry weight of 666.66 g m^-2 and did not significantly differ from genotypes 36, 6 and 3 were they gave 655.11, 649.77, and 647.11 g m^-2 respectively, while the lowest mean for the trait was recorded by genotype 20 (408.89 g m^-2). It could be attributed to the different genotypes in the dry weight is the result of the different response to the surrounding conditions and growth factors, and thus the variation in the accumulation of dry matter, this finding is consistent with [23] results which showed a significant difference between the cultivars in the dry weight of the plant. Results of the same (Table 4) showed a non-significant effect for irrigation intervals and the interaction on dry weight.

Table 4. Dry matter (g m^-2) of wheat genotypes under drought treatments.

| Genotypes  | Irrigated | Droughted | Mean | Genotypes | Irrigated | Droughted | Mean |
|------------|-----------|-----------|------|-----------|-----------|-----------|------|
| Al-diyar   | 476.44    | 490.66    | 483.55 | 9         | 574.22    | 663.11    | 618.66|
| 10         | 657.77    | 625.78    | 641.78 | 28        | 481.78    | 606.22    | 544.00|
| 29         | 682.66    | 650.66    | 666.66 | 18        | 538.66    | 588.44    | 563.55|
| 39         | 563.55    | 542.22    | 552.89 | 6         | 634.66    | 664.88    | 649.77|
| 36         | 631.11    | 679.11    | 655.11 | 3         | 707.55    | 586.66    | 647.11|
| 25         | 433.77    | 501.33    | 467.55 | Al-mahmodia | 330.66    | 572.44    | 451.55|
| 20         | 433.78    | 384.00    | 408.89 | 19        | 624.00    | 625.77    | 624.89|
| 24         | 511.99    | 453.33    | 482.66 | 5         | 600.89    | 636.44    | 618.66|
| 31         | 592.00    | 524.44    | 558.22 | 41        | 417.77    | 695.11    | 556.44|
| 7          | 492.44    | 572.22    | 533.33 | 4         | 512.00    | 581.33    | 546.66|
| 30         | 519.11    | 524.44    | 521.78 | 43        | 560.00    | 615.11    | 587.55|
| 11         | 540.44    | 608.00    | 574.22 | 32        | 563.55    | 668.44    | 616.00|

Mean of irrigation treatments: Irrigated= 545.03, Droughted= 585.92
L.S.D (Genotypes)= 178.48

3.5 Number of grain per spike^-1 (NGS)
Through the results of statistical analysis in (Table 5) appeared that genotypes were significantly different in NGS, where the highest mean was given by genotype 6 (57.48 grain spike^-1) and did not significantly differ from genotype
who gave 56.57 grain spike$^{-1}$ respectively, whereas less mean in grain number recorded by genotype 24 gave 40.62 grain spike$^{-1}$, and did not significantly differ from genotype 41 which had recorded 40.88 grain spike$^{-1}$ respectively. The reason for the superiority of the two genotypes aforementioned above is the superiority in spike length trait (11.08 and 11.82 cm), this is due to the increase in the number of florets in the spike, and accordingly its positive reflection of increasing the number of grain per spike, this result is consistent with the findings of Al-Amiry, & Al-Ubaidi [24] indicated a significant difference between genotypes in NGS. It was found a significant interaction between genotypes and irrigation intervals in NGS as shown in Table 5 were genotype 6 showed a higher mean of 58.70 and 56.27 grain spike$^{-1}$ at both irrigation treatments with significant differences between the two means, followed by genotype 43 which was superior significantly also with 58.40 grain spike$^{-1}$ in drought treatment and the two genotypes did not differ significantly at drought treatment for this trait, while less mean registered by genotype Al-mahmodia with mean of 37.03 grain spike$^{-1}$ followed by genotype 41 (39.80 grain spike$^{-1}$) at irrigation conditions, respectively, also both genotypes were not significantly different. The reason for this interaction is due to different in response of genotypes towards irrigation intervals. This is consistent with the results of [25].

| Genotypes | Irrigated | Droughted | Mean | Genotypes | Irrigated | Droughted | Mean |
|-----------|-----------|-----------|------|-----------|-----------|-----------|------|
| Al-diyar  | 47.43     | 52.23     | 49.83| 9         | 50.43     | 52.90     | 51.67|
| 10        | 54.60     | 52.20     | 53.40| 28        | 42.17     | 44.93     | 43.55|
| 29        | 53.93     | 54.53     | 54.23| 18        | 44.23     | 44.10     | 44.17|
| 39        | 48.30     | 50.77     | 49.53| 6         | 56.27     | 58.70     | 57.48|
| 36        | 41.17     | 44.17     | 42.67| 3         | 46.67     | 47.53     | 47.10|
| 25        | 47.30     | 44.87     | 46.08| Al-mahmodia | 37.03     | 46.97     | 42.00|
| 20        | 46.93     | 46.13     | 46.53| 19        | 48.50     | 47.83     | 48.17|
| 24        | 40.47     | 40.77     | 40.62| 5         | 46.57     | 45.60     | 46.08|
| 31        | 49.27     | 52.00     | 50.63| 41        | 39.80     | 41.97     | 40.88|
| 7         | 45.87     | 50.50     | 48.18| 4         | 45.60     | 47.43     | 46.52|
| 30        | 45.77     | 50.37     | 48.07| 43        | 54.73     | 58.40     | 56.57|
| 11        | 49.43     | 51.07     | 50.25| 32        | 42.53     | 43.60     | 43.07|

Mean of irrigation treatments: Irrigated= 46.88, Droughted= 48.73
L.S.D(Genotypes)= 4.58, (Genotypes x Irrigation) = 6.37

3.6 1000 Grain weight (g) (TGW)
The trait of TGW indicates the fullness of the grains, as it depends on the strength of the estuary (grains). From the results and data of statistical analysis shown in Table 6, a significant difference appeared among genotypes in the mean of TGW, where the genotypes 18, and Al-diyar were superior over the other genotypes with a higher mean (56.65, and 55.77 g respectively), while less mean found in genotype 36 (47.13 g). The variation of genotypes in the TGW is due to the difference in the genetic background, and the difference in the ability to synthesis and stimulation photosynthetic products from the source, as well as the variation of the sink (grains) in the receiving and storage of photosynthetic products, also the influence of duration and filling speed of the grain, this finding is in agreement with [24, 22] where the genotypes were varying in GW. The results in same Table 6 showed a significant variation for irrigation intervals on average TGW. Where irrigation treatment was superior by giving the highest mean of 51.01g compared with drought treatment which showed a significant decrease in TGW and gave less mean of 49.66 g. This result is compatible with [26] where they pointed to the influence of drought on grain weight. The interaction between genotypes and irrigation intervals showed a significant effect of drought on TGW. The best combination was for genotype 18 with a higher mean of 56.73, and 56.57 g in both treatments, followed by genotype Al-diyar
which also was superior in this trait with a mean of 56.30 g in irrigated plants respectively whereas less mean of TGW was recorded in genotype 10 (46.10 g) in droughted plant. The difference in the response of genotypes to water stress may be due to the difference in the duration of grain filling under the conditions of irrigation treatments, this is consistent with what found by [20] results that indicated a significant effect for the interaction of the various irrigation treatments and wheat cultivars on TGW.

Table 6. 1000 Grain weight (g) of wheat genotypes under drought treatments.

| Genotypes | Irrigated | Droughted | Mean | Genotypes | Irrigated | Droughted | Mean |
|------------|-----------|-----------|------|-----------|-----------|-----------|------|
| Al-diyar   | 56.30     | 55.23     | 55.77| 9         | 48.47     | 47.33     | 47.90|
| 10         | 48.80     | 46.10     | 47.45| 28        | 47.57     | 48.00     | 47.78|
| 29         | 51.13     | 50.83     | 50.98| 18        | 56.57     | 56.73     | 56.65|
| 39         | 46.67     | 48.30     | 47.48| 6         | 47.33     | 47.47     | 47.40|
| 36         | 47.93     | 46.33     | 47.13| 3         | 49.93     | 47.77     | 48.85|
| 25         | 52.90     | 51.97     | 52.43| Al-mahmodia | 51.97     | 51.57     | 51.77|
| 20         | 50.33     | 49.00     | 49.67| 19        | 54.70     | 53.77     | 54.23|
| 24         | 50.73     | 49.50     | 50.12| 5         | 48.73     | 46.30     | 47.52|
| 31         | 50.37     | 50.87     | 50.62| 41        | 55.23     | 50.77     | 53.00|
| 7          | 51.57     | 48.30     | 49.93| 4         | 49.20     | 47.50     | 48.35|
| 30         | 54.13     | 47.90     | 51.02| 43        | 50.37     | 48.30     | 49.42|
| 11         | 52.77     | 51.63     | 52.20| 32        | 50.37     | 50.47     | 50.42|

Mean of irrigation treatments: Irrigated= 51.01, Droughted= 49.66
L.S.D (Genotypes)= 1.98,(Irrigation)= 0.59,(Genotypes x Irrigation)= 2.87

3.7 Grain yield (ton. h⁻¹) (GY)
The GY is the final result of several factors, including traits linked to the yield itself, as well as genetic factors that control the trait in addition to environmental factors. It has been noticed from the results of the statistical analysis in Table 7 that there was not a significant variation in the genotypes regarding this trait. Although there were numerical differences between the yield of the genotypes, but they did not reach the significance, which indicates the natural growth of these genotypes within the limits of this environment and giving an economic outcome, this refers to the suitability of the conditions of the environmental region for the growth and production of these genotypes. This is consistent with the findings of ALFahdawi & Almehemdi [27] were showed a non-significant variation among genotypes of bread wheat in GY. The results in the same Table (7) showed the treatments of drought had not affected grain yield. The higher mean of GY was 6.53ton ha⁻¹ recorded in droughted plants in comparison with irrigated plants which gave the lowest mean of 6.35 tons ha⁻¹. A study of Varga et al., [28] referred that in recent cultivars, drought stress during maturity did not decrease the yield compared to cutting water off during the heading stage. The results of Table 7 showed no significant effect of the combined interaction of genotypes and water treatment, where the mean of grain yield ranged between 7.55, 5, and 3.86 ton ha⁻¹ in genotypes 3, 20 and Al-mahmodia respectively in droughted and irrigated plants.

Table 7. Grain yield (ton ha⁻¹) of wheat genotypes under drought treatments.

| Genotypes | Irrigated | Droughted | Mean | Genotypes | Irrigated | Droughted | Mean |
|-----------|-----------|-----------|------|-----------|-----------|-----------|------|
| Al-diyar  | 6.08      | 6.16      | 6.12 | 9         | 6.66      | 7.50      | 7.08 |
| 10        | 6.57      | 6.23      | 6.40 | 28        | 5.94      | 6.49      | 6.21 |
| 29        | 7.21      | 7.37      | 7.29 | 18        | 6.44      | 6.30      | 6.37 |
### 4. Conclusion

Based on the finding of the current study, it can be concluded that the genotypes used in this study varied in the growth and yield characteristics, as well as in their response to cutting off irrigation at flowering stage. Genotype 43 distinction with some of the traits of growth and yield FLA, and NGS as well as genotype 6 in PH, DW, NGS while the rest of the genotypes differed slightly in the characteristics of growth and yield, also Genotypes 43 and 39 were superior with the highest number of DREB 1A gene copies followed by genotypes 24, 28, 6, 25, and 20 so this indicates their ability to withstand drought from the rest of the other genotypes and the suitability to the environment they are grown in. Based on what has been stated above, genotypes under study can be investigated more regarding other tolerating genes as well as the level of antioxidant so the tolerant genotypes among them can identified in order to be able to recommend them for planting in arid and semi-arid areas.

### References

[1] GCARD. 2012. Breakout session P1.1 national food security-speaker brief-the wheat initiative-an international research initiative for wheat improvement. [https://www.gfar.net/documents/gcard2-breakout-session-p11-national-food-security-speaker-brief-wheat-initiative](https://www.gfar.net/documents/gcard2-breakout-session-p11-national-food-security-speaker-brief-wheat-initiative)

[2] Narayanan, S. 2018. Effects of high temperature stress and traits associated with tolerance in wheat. *Review Article. J. Sci.*, 2 (3), 177–186. DOI: 10.15406/oajs.2018.02.00067

[3] Saad, K. S. 2016. Am pact of climatical changes on production of strategical grains and security food of Iraq. *AL-Adab Journal*, (119 Supplement): 353-386.

[4] Rijib, M. Z. & Jbara, O. K., 2016. Measuring the technical efficiency and the rate of change in the TFP for farms rain-fed wheat in the region in light of differing size area. *The Iraqi J. of Agri. Sci.*, 47(6), 1475-1485.

[5] Al fahdawi, H. M. M. 2012. Effect of seeding rates on growth and yield of wheat genotypes (*Triticum aestivum* L.) planted in two location. Center of Desert Studies-University of Anbar.

[6] AL-Sudani, M. A., Taha, T. M. & Noor, W. K. 2009. Study for Proteins and Enzymes System in Some Genotype inters to Iraq and compare with wheat *Triticum aestivum* L. *Al Kufa University Journal for Biology*, 1(1), 115-122.

[7] Yamaguch -Shinozaki, K.& Shinozaki, K. 2006. Transcriptional Regulatory Networks in Cellular Responses and Tolerance to Dehydration and Cold Stresses. *Annu. Rev. Plant Biol.*, 57, 781–803. DOI: 10.1146.

[8] Lata, C., and Prasad, M. 2011. Role of DREBs in regulation of abiotic stress responses in plants. *Rev. J. Exp. Bot.*, 62 (14), 4731–4748. Doi:10.1093/jxberr210.

[9] Pellegrineschi, A., Reynolds, M., Pacheco, M., Brito, R. M., Almeraya, R., Yamaguch-Shinozaki, K., & Hoisington, D. 2004. Stress-induced expression in wheat of the Arabidopsis thaliana DREB1A gene delays
water stress symptoms under greenhouse conditions. *Genome, 47*(3), 493-500. doi: 10.1139/G03-140.

[10] Applied Biosystems. 2003. Creating Standard Curves with Genomic DNA or Plasmid DNA Templates for Use in Quantitative PCR. Revision, 1-8. https://assets.thermofisher.com/TFS-Assets/LSG/Application-Note/cms_042486.pdf

[11] Al-Rawey, K. M. and Khalaf Allah, A. A. M. 2000. Design and analysis the Agricultural tests. Dar Al-kutub for printing and copyrighting, the second print, University of Mosel.

[12] Joshi, R., Wani, S. H., Singh, B., Bohra, A., Dar, Z. A., Lone, A. A., Pareek A. & Singla-Pareek, S. L. 2016. Transcription factors and plants response to drought stress: current understanding and future directions. *Frontiers in Plant Science, 7*, 1029. Doi.org/10.3389/fpls.2016.01029.

[13] Moon, S. J., Min, M. K., Kim, J., Kim, D. Y., Yoon, I. S., Kwon, T. R., Byun, M. O. and Kim, B. G. 2019. Ectopic Expression of OsDREB1G, a Member of the OsDREB1 Subfamily, confers cold stress tolerance in rice. *Frontiers in plant sci., 10*, 297. Doi: 10.3389/fpls.2019.00297.

[14] Agarwal, P. K., Agarwal, P., Reddy, M. K. & Sopory, S. K. 2006. Role of DREB transcription factors in abiotic and biotic stress tolerance in plants. *Plant cell reports, 25*(12), 1263-1274. DOI 10.1007/s00299-006- 0204-8.

[15] Kurahashi, Y., Terashima, A., and Takumi, S. 2009. Variation in dehydration tolerance, ABA sensitivity and related gene expression patterns in D-genome progenitor and synthetic hexaploid wheat lines. *Int J. Mol Sci., 10*, 2733-2751. Doi:10.3390/ijms10062733.

[16] Egawa, C., Kobayashi, F., Ishibashi, M., Nakamura, T., Nakamura, C., & Takumi, S. 2006. Differential regulation of transcript accumulation and alternative splicing of a DREB2 homolog under abiotic stress conditions in common wheat. *Genes & genetic systems, 81*(2), 77-91.

[17] Shinozaki, K., and Yamaguch Shinozaki, K. 2007. Gene networks involved in drought stress response and tolerance. *Journal of experimental botany, 58* (2), 221- 227. DOI: 10.1093/jxb/erl164

[18] Wei, B., Jing, R., Wang, C., Chen, J., Mao, X., Chang, X., & Jia., J. 2009. Dreb1genes in wheat (*Triticum aestivum* L.): development of functional markers and gene mapping based on SNPs. *Molecular Breeding*, 23(1), 13-22. DOI 10.1007/s11032-008-9209-z

[19] Koemel, J. E., Guenzi Jr., A. C., Carver, B. F., Payton, M. E., Morgan, G. H., & Smith, E. L. (2004). Hybrid and pureline hard winter wheat yield and stability. *Crop science, 44*(1), 107-113.

[20] Al-Temimi, H. N., Al-Shahwany, A. W. and Alsadawi, I. S. 2013. Screening of bread wheat cultivars (*Triticum aestivum* L.) to water deficit stress under field conditions. *Iraqi Journal of Sci., 54* (3), 577-584.

[21] Aziz, J. M., Jaber, S. H. and Saleh, Y. H. 2013. Development of a bread wheat cultivar of high productivity and resistant to leaf rust in central a northern irrigated areas. The Iraqi J. of Agri. Sci., 44 (4): 464-471.

[22] Mohammed, A. K., Neama, A. S., Swaid, A. H., Abd Majeed, M. M., Jaber, T. N. 2020. Effect of Irrigation Period on the Growth and Yield of Cultivars of Bread Wheat. J. of Education and Sci. Studies, 3: 199-212.

[23] AL- Joburi, J. M. A., Al-karkhi, H. A. H., Tahir, N. A. 2017. Evaluated several genotypes postings wheat bread (*Triticum aestivum* L.) studying some physiological traits under the influence of salt water. Tikrit Journal for Agri. Sci. *Proceedings of the Sixth Scientific Conference of Agricultural Sciences*, 17, 560-578.

[24] AL- maeini, A. H., Mohsin, R. H. 2016. Effect of seeds quantities and water stress in growth and yield characteristics of bread and durum wheat genotypes grown under desert environment. *Al-Furat J. of Agri. Sci.*, 8(2), 180-189.

[25] Varga, B., Vida, G., Varga-László, E., Bencze, S., & Veisz, O. 2015. Effect of simulating drought in various phenophases on the water use efficiency of winter wheat. *Journal of Agronomy and Crop Science, 201*(1), 1-9. Doi:10.1111/jac.12087.