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DEPARTMENT OF FIELD CROPS SCIENCE



DREB Gene Expression of Introduced Wheat Genotypes in Response to Last Irrigation Cutoff

**A Thesis Submitted To The Council of The College of Agricultural
at the University of Anbar in Partial Fulfillment of the
Requirements for the Degree of Master in Agricultural Sciences
(Field Crops)**

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

{وَجَعَلْنَا مِنَ الْمَاءِ كُلَّ شَيْءٍ حَيٍّ أَفَلَا يُؤْمِنُونَ}

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We certify that this thesis under the title: (Estimation of DREB Gene Expression In Wheat Genotypes (Triticum aestivum L.) Introduced to Anbar Governorate Under Water Stress) was prepared under our supervision at the University of Anbar in partial Fulfillment of the requirements for the master degree of Science in field crops science.

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Dedication

To the teacher of creation and their master to the doomsday the prophet Mohammad (peace and blessings be upon him).

To the provenance of civilizations and agriculture ... my homeland Iraq.

To the one who encouraged me throughout my academic life and supported me in this work ... my dear father.

To the tenderness and the candle of our house, who reinforce me with her prayer... my darling mother.

To supporter and companions of my path... my brothers and sisters

I present to you all this work.

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Summary

A Field experiment was conducted in one of a farmers private fields in Hit city/Qnan region, Anbar site which was located west of Iraq (Latitude 33°39 N and Longitude 42°47 E) during the winter season of 2019-2020 to estimate DREB gene expression of wheat genotypes under drought conditions. The experiment was included 24 wheat genotypes and one drought treatment. The irrigated treatment was applied normally until the physiological maturity stage, while drought treatment was directly applied through cutting irrigation after flowering. The experiment was distributed as a split-plot arrangement in Randomized Complete Block Design with three replications. Irrigation treatments were occupied the main plots while genotypes were put in the subplots. The results were showed that the genotypes were differently responded differently to the treatments according to the measured traits. The most prominent genotype was Iraq which recorded a high expression of the DREB 1A gene (221.88 folds) followed by genotypes 39, 24, 6, 28, 25, and 20 at drought treatment. Genotype 11 was achieved the minimum number of days from planting to 50% flowering which was around 103.00 day. For plant height trait, genotype 6 was superior and gave the highest average of 94.08 cm. Genotypes Iraq was superior in flag leaf area with a high average 35.62 cm². While the genotypes Iraq and 6 achieved the highest means attained 11.82 and 11.08 cm respectively spike length. The highest average for tillers number was recorded by genotype 3 (495.33 tiller m⁻²). Genotype 29 was superior in dry weight trait which recorded 666.66 g m⁻². A high mean of spikes number was obtained by genotype 41 which gave 499.11spike m⁻². Genotype 6 was showed superiority in number of grain per spike (57.48 grain spike⁻¹). Genotype 39 was superior in fertility ratio attained (3.37 %). While the genotype 18 was superior in grain thousand weight trait with a higher

average attained 56.65 g. In grains yield trait, genotypes 3 and 29 were superior by giving higher average of the trait (7.39 and 7.29 ton ha⁻¹). Genotype 20 showed significant superiority in harvest index trait with a rate of 57.57%. It can be concluded from this study that the genotypes had the highest gene expression under drought conditions. So this indicates their ability to tolerate drought than the rest of the other genotypes.

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List of Abbreviations

Abbreviation	Name
ABA	Absciscic Acid
BYD	Biological Yield
bZIP	Basic Leucine Zipper Domain
CBF	C-repeat Binding Factor
cDNA	Complementary deoxyribonucleic acid
Chl	Chlorophyll
CKs	Cytokinins
CPE ratio	Cumulative pan evaporation ratio
CRT	C-repeat
DAP	Diammonium phosphate

DNA	Deoxyribonucleic acid
DPM	Days to Physiological Maturity
DREB	Dehydration Responsive element Binding
DW	Dry weight
EREBP	Ethylene Responsive Element Binding Protein
ERF	Ethylene-Responsive Factor
F	Flowering
FR	Fertility ratio
FLA	Flag leaf area
GB	Glycine betaine
GF	Grain Filling
GY	Grain Yield
HI	Harvest Index
HSFs	Heat Stress transcription Factors
HVA1	ABA-inducible protein PHV A1
IW	Irrigation water
LEA	Late Embryogenesis Abundant (LEA) proteins
MYB	Myeloblastosis
MYC	Myelocytomatosis
NAC	NAM, ATAF, and CUC
NGS	Number of Grain per Spike
OA	Osmotic adjustment

OsDREB1	Oryza sativa dehydration-responsive element-binding
Pas	Polyamines
PEG	Polyethylene glycol
PH	Plant height
QTL	Quantitative traits location
RNA	Ribonucleic acid
ROS	Reactive oxygen species
SL	Spike length
SN	Spike Number
SNP	Sodium Nitroprusside
SHWs	Synthetic Hexaploid Wheat
TaDREB	<i>Triticum aestivum</i> dehydration-responsive element-binding
TaERs	Ethylene Receptor Genes in wheat
TaMOR	<i>Triticum aestivum</i> transcription factor gene MORE ROOT
TFs	Transcription factors
TGW	Thousand Grain weight
TN	Tillers Number
USA	United States of America
VRN1	Vernalization genes
YM	Young microspore

Chapter One: Introduction

1. Introduction

Wheat (*Triticum aestivum* L.) is considered one of the most important crops global in terms of harvested area, and the second food crop after rice in importance. It is a source of daily protein by about 20% as well as a source of important calories. Recently, the level of wheat production does not have the perfect with an increasing world population potential of about a billion in 2050. Also, demands for wheat are expected to magnify up to 60%, in this case, the yield of wheat should increase from 1% to 1.6%. To achieve this, two strategies may be adopted, the first is producing more tolerant genotypes for biotic and abiotic stresses, while the second is enhancing the input use efficiency (GCARD, 2012; Narayanan, 2018). The global production of the wheat crop for the year 2019-2020 did estimate at 761.9 million tons according to FAOstat (2019), and the topmost countries in wheat production for the year 2017 were China, India, Russian, USA, France, Australia, Canada, Pakistan, Ukraine and Germany. In Iraq, there is an increase in cultivated area and production for the winter season of the year 2020. Wheat productivity was estimated at 6238 thousand tons by an increase of 43.6% over last year production, which estimated 4343 thousand ton (Agricultural Statistics Directorate, 2020). Climate changes, that Iraq exposes to a reduction of the rain and retention and increased of temperatures thus lands have deteriorated due to droughts such as desertification and the exhaustion in natural resources of the land (Saad, 2016); This makes Iraq face great challenges in terms of providing food, chief among them is the reduction of water from their sources, also its location within areas characterized by low rainfall. Which affects directly on grains and the most important is the wheat. Wheat grains contains 12-17% protein, 76-78 % starch, and oil 1.2-1.5% (Hadi et al., 2013; Rijib and Jbara, 2016). During exposure to abiotic stresses, The plants show a wide

range of morphological, physiological, and biochemical alternations. The molecular changes of genes organize at dehydration and cold conditions and confer tolerance thus keep the plant alive. Stress-responsive gene expression timing is regulate through a set of transcription factors and Cis-acting elements in stress-inducible promoters. This makes the stress-responsive transcription factors (TFs) genes an important target in genetic engineering programs to enhance tolerance to abiotic stress, where their overexpression reflects positively or negatively on the expression of genes that they control (Yamaguchi Shinozaki and Shinozaki, 2006; Akhtar et al., 2012). DREBs (dehydration responsive element binding) are important plant transcription factors (TFs) that belong to APETALA2 (AP2) family transcription factors. DREBs regulate the expression of many genes that induce stress tolerance at most an ABA-independent way. Also, they have a crucial function in improving tolerance for abiotic stress via the interaction with DRE/CRT cis-element that exist at the promoter zone of different responsive gene responsible for abiotic stress. Recently, DREB1/CBF genes are one of the genes used in genetic modification engineering programs to serving in producing more tolerant crop plants (Lata and Prasad, 2011; Akhtar et al., 2012). Drought is one of the most critical environmental stresses which affect all plant functions. With drought stress occurs, it leads to produce abscisic acid (ABA), which plays a vital function in tolerating drought besides induced most of the drought stress-inducible genes (Shinozaki and Yamaguchi-Shinozaki, 1997). CRT/DRE elements participate in ABA signal transduction; besides that its existence is required for the ABA responder element in the *cor78a / rd29A* promoter (Yamaguchi-Shinozaki and Shinozaki, 1994). Revealed later that the DREBs are ABA-independent, excepting CBF4, which is ABA-responsive, and includes CRT/DRE components in the ABA-

dependent pathway. The DRE participation in ABA-dependent organizing for stress response refers to more interaction between the ABA-dependent and ABA-independent signal transduction pathways (Agarwal et al., 2006). This study aims to estimate DREB gene expression of some introduced genotypes of wheat and cultivation under the influence of water stress in order to continue using the superior genotypes in the western parts of Iraq.

Chapter Two: Literature Review

2. Literature review

2.1. History of Wheat

According to archaeological and phytophagous directories the origin of wheat provenance is 'fertile crescent' in the middle east, specifically in the top region (Tigris-Euphrates) in about 7500 years BC (Zuhary and Hopf, 1993). The wheat crop is widespread due to its large adaptation and this acclimatization goes back to the diverse genes in types; consequently, the genetic diversity in wheat strains appear among themselves which is giving seeds, blooming under a different period of lighting and temperature, and survive under the extremely cold winter and summer heat. Cultivated wheat can be artificially hybridized with many closely related wild species and produce the crossbred to transfer the desired genes, as well as creating new types, for example Triticale, the output of artificial between wheat and rye. There are different cultivated wheat species such as Einkorn wheat (*Triticum monococcum*) is diploid and has seven pairs of chromosomes ($2n=2x=14$), the seeds are found in sites of Egyptian antiquarian. The other two cultivated types *Triticum turgidum* (which called Emmer wheat), and its derivative subspecies (*Triticum durum*), common wheat (*Triticum aestivum*). *Triticum turgidum* is tetraploid wheat with 14 pairs of chromosomes ($2n=2x=28$; AABB genomes). *Triticum urartu* ($2n=2x=14$; AA genome (closely connected to *Triticum monococcum*)). The third genome called *Triticum tauschii* (= *Aegilops squarrosa* , $2n=2x=14$; DD genome) derived from diploid goatgrass. Bread wheat is a hexaploid type ($2n=6x=42$; AABBDD genomes) with 21 pairs of chromosomes. Genetic and cellular evidence suggests that it did not exist as a natural species and that it is originated by self-hybridization while the old farmers were planting the tetraploid wheat approximately 9500 years ago. These crossbreeding produced inherited bread wheat recognized as

Triticum spelta, as a result of a natural mutation (Snape and Pa'nkova', 2013). Wang et al. (2013) pointed out that hexaploid wheat (*Triticum aestivum*, AABBDD genomes) has produced as a result of crossbreeding among tetraploid *Triticum turgidum* AABB genomes and diploid *Aegilops tauschii* DD genome (Figure 1 and Table 1).

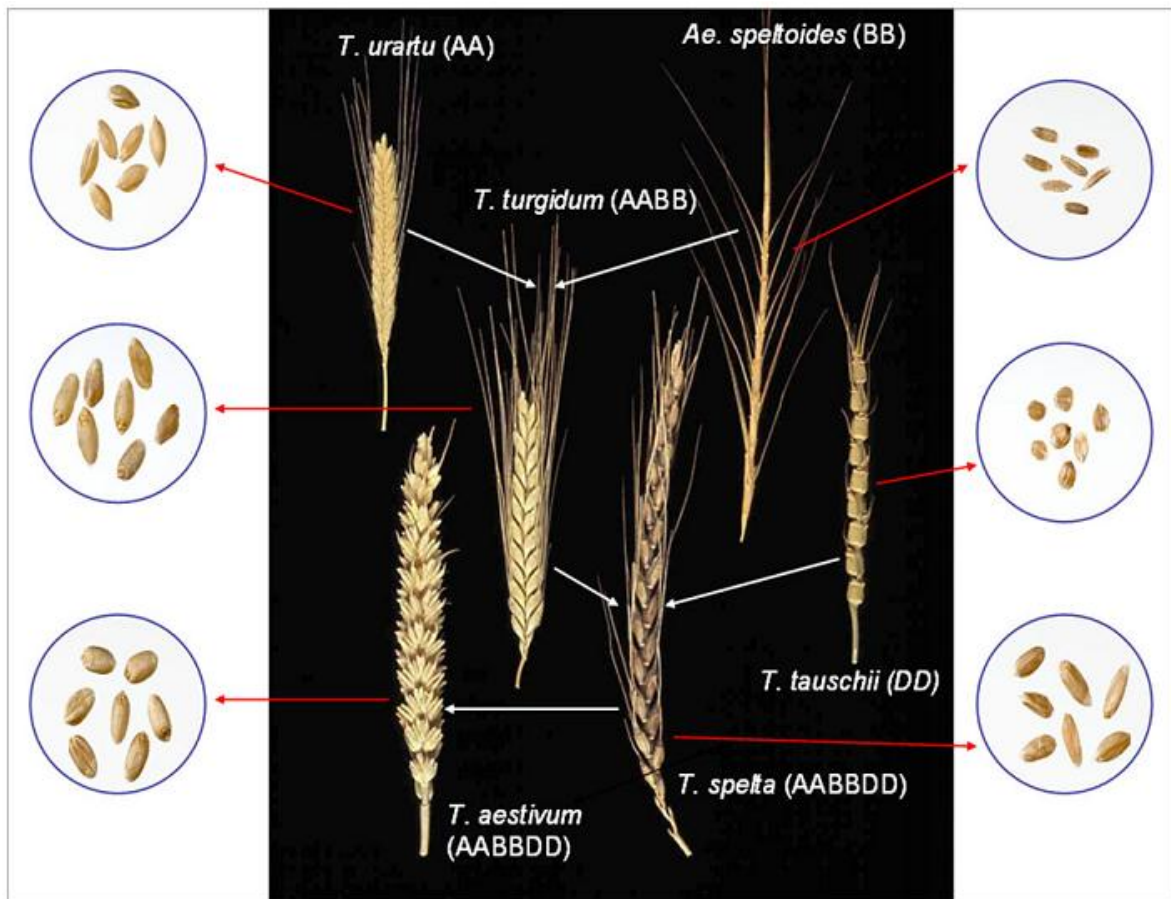


Figure 1. The evolution of cultivated wheat through the obvious difference in the appearance of spikes and grains among them (Shewry, 2009).

Table 1. Genomic constitution of wheat and their relatives adapted from Stallknecht et al (1996).

Species	Genome constitution	Common name
Diploid (2n = 14)		
<i>Triticum aegilopoides</i>	AA	Wild einkorn
<i>Triticum monococcum</i>	AA	Einkorn (cultivated)
<i>Triticum speltoides</i>	BB	Wild grass
<i>Triticum tauschii</i>	DD	Wild grass
Tetraploid (2n = 28)		
<i>Triticum turgidum</i>	AABB	Emmer (wild)
<i>Triticum dicoccum</i>	AABB	Emmer (cultivated)
<i>Triticum Durum</i>	AABB	Durum
Hexaploid (2n = 42)		
<i>Triticum spelta</i>	AABBDD	Spelt (cultivated)
<i>Triticum aestivum</i>	AABBDD	Common wheat (cultivated)
<i>xTriticosecale</i> (Wittmack)y	AABBRR	Triticale

2.2. Wheat classification and benefits

Wheat plant is growing in diverse environmental conditions where it is adapted to temperate regions. It is classified into two main categories: winter and spring wheat according to the growth habit. Winter wheat is planting in the autumn season, in regions where wheat can tolerate the low temperature of winter. While spring wheat is cultivated in the spring season to eschew freezes. Spring wheat in Mediterranean areas is cultivated in autumn while winter is moderate and rainy to achieve benefit from available water. In these dissimilar regions, wheat varieties show a different manifestation in allele at the major vernalization gene (VRN1), a MADS-box transcription factor symmetric to the meristem identity gene APETALA 1 (AP1) in Arabidopsis

(Yan et al. 2003; Trevaskis et al. 2003). Winter wheat requires exposure to low temperature (vernalization) to accelerate the transference from the vegetative to the reproductive phase, where the vernalization helping in prevent the accurate flower meristem from exposition to damage by low temperatures through preventing flowering (Distelfeld et al. 2009).

Commercially, wheat is classified through-checked of grain size, subsistence and protein into hard white, hard red winter, soft white, etc. (Battenfield et al., 2016). The main planted wheat species are Common wheat (*T. aestivum*, a hexaploid species) the most widely planted species globally which is usually used for bread, the second one is Durum wheat a tetraploid also wide planted, Einkorn (*T. monococcum*, a diploid species) was domesticated at the same time like Emmer wheat (*T. dicoccum*, a tetraploid species), Spelt (*T. spelta*, a hexaploid species) limited in planting (Cooper, 2015).

2.3. Screening of Bread Wheat Genotypes

There are many attempts to reduce drought effects by breeding adaptable varieties around the world. The genetic modification technique includes the amendment in the qualitative and the quantitative characteristic through a transfer for required genes (Ashraf, 2010). The selection of new genotypes and varieties is one of the traditional breeding methods. Introduced genotypes in Iraq 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 production factors, have studied and then comparison them with cultivated varieties to approve a new variety (Al-Sudani et al., 2009). The successful cultivation of new genotypes requires an environmental adaptation, genetic stability across different environments to determine the best genotypes (Daniel et al, 2014, and Reza et al., 2014). Assuring food

security is significant by enhancing wheat tolerance for drought stress through some strategies. To realize this target without increasing the land of cultivated areas, there must be emphasis intensified on main features that linked with plant output plus adaptive to the ecological challenges. For wheat breeders, genetic amelioration and improving wheat cultivars that tolerate drought is essential (Mwadzingeni et al., 2016a). The traits in which a plant excels can be used as a criterion to screen for stress-tolerant cultivars, where the results of Al-Temimi et.al. (2013) showed the superiority of tested drought-resistant cultivars in most yield components and vegetative growth traits compared to non-drought-tolerant cultivars.

2.4. Effect of Drought on Wheat Growth and Production

Drought is one of the environmental factors which causes loss in plant production, in addition to its recent widespread in several areas. There is an urgent need to intensify studies on the impedance of osmotic stresses in wheat (Vinocur and Altman, 2005). For preserving the growing and metabolic, plants should be adapting with various ecologies of stresses, through exciting the transcription factors by preliminary stresses and promoting the response of mechanisms which in return, re the protect and balance for cells (Erwin, 2007). Tuteja and Sarvajeet (2012) mentioned that the sensitiveness of plants for drought and rise temperatures leads to troubled metabolic processes with shorten plant life. Thus, minimize plant biomass accumulation and grain yield (Hasanuzzaman et al., 2013). Drought affects all phases of wheat growth but the greatest impact during floral periods and cereals filling and that leads to a big loss in yields. The causes for these losses back to decrease in net photosynthetic due to determinations of oxidative to metabolism and its effect to chloroplast and closes of stomata, evolution and poorly cereals (Farooq,

2014). The climate changes besides diminishing the freshwater, make drought classify as one of the stresses that impact the production of crops around the globe. So, plants uses various morphological and physiological strategies in responding to drought stress (Hu and Xiong, 2014). It is important to improving wheat genetically to tolerant stresses as a result of its direct impact on food security because it is a global staple food source (Kulkarni et al., 2017).

2.4.1 Drought Tolerance Mechanisms

Estimate the genotypes that tolerating drought through morphology, physiological and molecular markers could reduce drought stress. There is an attempt to amelioration for genotypes versus drought by researchers and through using for physiological and molecular markers; and by using Mendelian genetics and present-day biotechnology methods, it is possible to find an allelic site that resists drought and transfer it to high-yield genotypes (Iqbal, 2019). The plant responds to drought stress by adapting to it, this is happening through enhancing osmotic protection, rate of the antioxidant response, gene expression and regulation whether positive or negative, and also promoting root growth through absolute growth (Sharp et al., 2004; Yamaguchi and Sharp, 2010; Xu et al., 2013). It has been found that extrinsic application for a number of plant growth regulators such as Cytokinins (CKs), Abscisic Acid (ABA), Glycine Betaine (GB), Polyamines (Pas), and Salicylic Acid (SA) improve tolerance of wheat against drought. Through raising the osmotic adjustment (OA) which keeps on turgor pressure, improvement the antioxidant accumulation to remove reactive oxygen species (ROS) to strengthen the stabilization of biological membranes enzymes (Singh and Usha 2003; Yang et al., 2004; Ma et al., 2006; Travaglia et al., 2007). Wang et

al. (2010) indicated in a study, in transgenic wheat, when Glycine betaine excessively piling up in an organism may promote antioxidative defenses. Also, one strategy is to modify antioxidant defenses, where genotypes under dehydration showing an increase in enzymatic and non-enzymatic antioxidant activity (Huseynova, 2012; Maevskaya and Nikolaeva, 2013). There is a lot of genes in wheat that have an important role in tolerating drought besides production for various kinds of enzyme and protein such as the response to abscisic acid, abundance and lateness the embryogenesis, rubisco, helicase, proline, glutathione-s-transferase, and carbohydrates through drought (Nezhadahmadi et al., 2013).

2.4.2 Transcription Factors (TFs)

Transcription factors belong to the regulatory proteins, which play an important role to start gene expression (Chen et al., 2004; Xu et al., 2008). The transcription factors interfere with cis-acting elements that exist in the promoter area of many genes associated with stress. There are more than 1500 transcription factors found in *Arabidopsis* (Riechmann et al., 2000). Most of these TFs families are linked to drought tolerance, as AP2/ERF, bZIP, NAC, MYB, MYC, Cys2His2 zinc-finger and WRKY (Umezawa et al., 2006). So, there is a hope to enhance abiotic stress tolerance in transgenic plants. Many studies were done to clarify the role of DREB and showed that DREB TFs possibly can be used for improving the tolerance to dehydration stress (Agarwal et al., 2017). Signals of stress lead to regulate effector genes and transcription factors that together are called stress regulation genes. TFs contribute to respond to drought stress besides motivating the binding factor expression C-repeat binding factor expression (C-repeat). Increasing stress

tolerance can be through using the transcription factors and attached genes in the genetic diversion of field crops (Jan et al., 2017).

2.4.3 Dehydration Responsive Element Binding (DREB)

DREB genes play a role in tolerate of stresses. Ito et al. (2006) studied generated transgenic rice plants that overexpressing the OsDREB1 or DREB1 genes, these transgenic plants revealed a delay in growing at normal conditions besides enhancing abiotic stresses. Also, they determined the target stress-inducible genes of OsDREB1A in the transgenic rice, which encode proteins tolerate of stress in plants. Molecularly, plants responsive through rearranging of transcriptome and stimulation a lot of genes that respond to stress. Gene functions encoding enzymes that contribute to the production of protective metabolites, antioxidant enzymes, transport/channel proteins, lipids biosynthesis genes, etc. Therefore, plant growth and production can be continued for longer duration. DREB protein is one of the novel proteins that has an important role in the abiotic and biotic stress responses (Agarwal et al., 2006). The DREB mentioned as CBF (C-repeat Binding factor), belongs to ethylene response factor ERF (super family) and plays a vital role in tolerating environmental stresses (Sakuma et al., 2002). The DREB proteins comprise a big family of transcription factors that play an important role in abiotic stresses through the regulation of some genetic functions associated with drought, high-salinity and low temperature (Liu et al., 1998). At first, the DRE from the RD29A promoter is proved that it has an important role in motivating the expression of genes under dehydration, higher salinity, and low-temperature stresses, and not by ABA processing (Yamaguchi-Shinozaki and Shinozaki, 1994). Heretofore, the whole length sequence of DREB genes was cloned from wheat (Shen et al., 2003a), rice (Chen et al., 2003; Dubouzet et

al., 2003; Tian et al., 2005), maize (Qin et al., 2003), Arabidopsis (Liu et al., 1998) and the halophyte *Atriplex hortensis* (Shen et al., 2003b). The main sequences for DRE are 5-bp, which is CCGAC designed the C-repeat (CRT) that stimulate gene expression for abiotic stress (Jiang, 2011). DREB genes are characterized by three conserved regions, an EREBP/AP2 DNA binding domain, an N-terminal nuclear localization signal, and a conserved Ser/Thr rich region adjacent to the EREBP/AP2 domain (Wei et al., 2009). The features of the three regions above determined the DREB properties (Kanaya et al., 1999). DREB proteins classified into six groups namely A-1, A-2, A-3, A-4, A-5, and A-6. The biggest two group A-1 and A-2 (Sakuma et al., 2002). The A-1 subgroup contains DREB1/CBF genes, and the A-2 subgroup consists of DREB2 like genes. Those two groups play a major role in responding to abiotic stress (Sakuma et al., 2002). For the set DREB1/CBF (A-1) of DREB the expression and successive activities are organized in the transcription stage. Contrariwise, the DREB2-type (A-2) is activated and controlled at transcription, post transcription, and after translation stage (Agarwal et al., 2017). In Arabidopsis plant, transcription factors contain six DREB1/CBF genes summarized as DREB1/CBF1, DREB1A/CBF3, DREB1D/CBF4, DDF1/DREB1F, DREB1C/CBF2 and DDF2/DREB1E. Transcription factors, DREB1/CBF besides being present in the Arabidopsis, also found in other plants that have been adapted to the cold such as barley, *Brassica napus*, and in those which are not acclimatized i.e. rice and tomato, halophytes i.e. *Atriplex hortensis*, and some other plants such as ryegrass, and soybean (Choi et al., 2002; Jaglo et al., 2001; Dubouzet et al., 2003; Shen et al., 2003b; Xiong and Fei, 2006; Chen et al., 2007). DREB/CBF pathways present in plants confers them the ability to adapt to the cold environment (Puhakainen et al., 2004). Two major subgroups of the DREB subfamily

(DREB1 and DREB2) participate in two different pathways to convey the signal under the cold and drought stresses respectively (Stockinger et al., 1997). DRE/CRT is a cis-acting element and in Arabidopsis plants control the expression of the gene e.g. under abiotic stresses (Shinozaki et al., 2003). The expression of DREB1A/CBF3 under the control of stress-catalyzer RD29A promoter improves transgenic wheat to tolerate drought (Pellegrineschi et al., 2004). CBF/DREB1 involved in the responses of the gene expression of the cold environment while CBF/DREB2 is an important TFs in the gene expression of dehydration and salt stress (Shinozaki and Yamaguchi-Shinozaki, 2007). So, the group of DREB1 is depend on their participation in osmotic and temperature stress responses, besides it has a genetic function that may improve dehydration tolerance in wheat (Wei et al., 2009). Under the stress of intermittent dehydration, there were augmented yields in transgenic lines compared to the plants that non genetically modified, where the existence of DREB1A in the roots of plants under drought stress help them to grow and the possibility to use it for drought tolerance in breeding programs (Vadez et al., 2007; Jagana et al., 2012).

2.5 Effect of Genotypes on Agronomical Traits

Wheat productivity can be improved significantly by choosing the traits (agronomic and adaptive) via traditional plant breeding techniques at optimum and marginal rainfall conditions (Mwadzingeni et al. 2016 b). The life of any field crop is the interaction between environmental factors and genotype, that affecting the growth and yield traits of wheat. So, the appropriate environments for growth and productivity must be taken into account (Al-Fahdawi, 2013).

2.5.1 DREB Transcription Factors in Wheat Genotypes

Transgenic wheat and barley plants were produced by Morran et al. (2011) showing constitutive (double 35S) and drought-inducible (maize Rab17) expression of the TaDREB2 and TaDREB3 TFs isolated from wheat grain. Transgenic populations with constitutive over-expression were slow in growth, late efflorescence, and a decreased grain yield comparative to non-transgenic controls. Nevertheless, both the TaDREB2 and TaDREB3 transgenic plants showed a higher in survival rate under drought conditions compared to non-transgenic controls, besides, improve of frost tolerance at the over-expression. Shavrukov et al. (2016) revealed that transgenic was in all four F1BC3 groups, but the stress-inducible transgene expression was in only three of the four groups. The expression of the transgene did not induce under cold stress, hence, there was no enhancing for frost tolerance in the progenies of drought-tolerant F3BC3 lines.

2.5.2 Flowering Time

Flowering timing is a complicated trait effected by environment of factors such as photoperiod, vernalization, besides interior signals whether autonomous, circadian clock, plant age, gibberellin, and carbohydrates (Mouradov et al., 2002; Fornara et al., 2010; Andrés and Coupland, 2012). A study by Al-Amiry and Al-Ubaidi (2016) pointed to a significant difference between genotypes in days number from planting until 50% flowering, the genotype Al-RV20 was early and recorded fewer days attained 125.67 days followed by Araz which spend 127 days. The genotypes varied in a number of days to flowering, where the pure lines S175, S130, S123 and S52 earliest with 106.33, 106.33, 107.33 and 108.08 days, while cultivar IPA99, S-177 and 148 recorded longer duration attained 116.5, 116.92 and 116.33 days,

respectively (Baktash and Naes, 2016). Bread wheat genotypes recorded different duration of flowering, also the durum wheat recent genotypes were early in flowering in comparison with the old genotypes (Honsdorf et al., 2018).

2.5.3 Plant Height (cm)

Plant height trait in cereal crops influencing the structuring of the plant and grain yield. Short plants are one of the important agronomic traits which strongly attached with harvest index in dryland cereal crops particularly in environments with finite water (Blum, 2010). Plant height is an important trait because of the strong relationship with lodging on one hand, and the efficiency of light intercepting on the other hand. The taller plants, despite their receive to more amount of light it is not desirable under irrigation conditions because they are exposed to lodge in contrast to the short plants (Al-karkhi et al., 2017). Results of Al-Amiry and Al-Ubaidi (2016) study showed a significant variation among genotypes in plant height average, where the higher plants were genotype AL-RV 20 (122.53 cm) followed by genotype AL-8/172 (107.43 cm) with the non-significant difference between them, while less mean was scored by genotype AL-RV 84 (86.57 cm). Plant height different a significantly in genotypes Al-ezz, and Alrashidia (83.3 and 76.8 cm) respectively, while the cultivar Sham 8 gave the less height of 58.6 cm (Al-fahdawi and Muslih, 2018). The variation of wheat varieties in plant height return to genetic variation in stalks lengths especially upper stalk as Al-Asseel et al. (2018) cleared that were their results showed a significant difference in plant height average.

2.5.4 Flag Leaf Area (cm²)

Flag leaf is the main source of photosynthesis products which its functions are so correlated with the grain filling trait, which in turn affects yield due to its nearby location from the spike, and it is discriminated by staying green (Ali et al., 2010; Shahinnia et al., 2016). Results show a significant variation among local cultivars and Russian genotypes in flag leaf area (Aljana et al., 2017), where the local cultivar Rasheed showed a superiority of 55.6 cm² compare with Nacowy potas which recorded the less rate attained 22.9 cm². A study by Hashim et al. (2017) has concluded that flag leaf area plays an important role in spike yield with a ratio of 30.1-35.29 % and referred to the role of flag leaf in spikelets number by considered as a final estuary for photosynthetic products, where their study showed a significant effect of flag leaf removal treatments through decrease spikelets number. The varieties differed significantly in flag leaf area, and the highest average scored by Ebaa 99 about 31.88 cm² according to the study results of Al-Asseel et al. (2018), where they indicated to the reason of varieties different in flag leaf area to the genetic variation besides the difference in flowering duration where this period considering as flag leaf developing time.

2.5.5 Spike Length (cm)

The spike is considered a distinct part of a wheat plant. It can be used as an indicator in the classification of the different species. Additionally, have a wild range of spike length. Spike arises in the period of rapid and effective growth of the plant. Length of the spike is a quantitative trait that is related to the yield, as there is a positive correlation between the spike length and the yield, the number of spikelets and grains formed in it on the one hand, on the other hand, the spike length in wheat plants reaches its maximum at the

flowering stage (Mohammed, 2000). The spike in the wheat plant is considered the source and the sink at the same time, as its green parts which consisting of the rachis, awn, glume, and lemma are considered a source of photosynthesis products as they contribute to the process of photosynthesis (Al-Mousawi, 2001). The results showed that Endure genotype was registered significantly superior for spike length in Alkafagiey and Alsakran sites in the two seasons respectively (Al-Hadithi et al., 2017). Local cultivars and Russian genotypes showed significant variation in spike length, Rasheed gave the highest average of 16.87 cm and a lower average was the variety Latefeyia of 10.86 (Al-Jana et al., 2017). Al-Rashidia recorded a significant and higher rate in the length of the spike about 20.0 cm compared with cultivars (Al-Ezz, Sham 4, Sham8) about 10.9 cm (Al-Fahdawi and Muslih, 2018).

2.5.6 Tillers Number (m^{-2})

Tillering has an important role in the yield of wheat, which influenced by genotype and environmental conditions. Tillers are observed after days post the emergence (Fioreze et al., 2012). Results of statistical analysis of Al-Amiry and Al-Ubaidi (2016) showed a significant variation among genotypes for tillers number where the genotype Al-RV63 recorded a high average (518.7 tiller m^{-2}) with no significant difference in comparison with Araz and Al-ESW143 (501.3 and 499.7 tiller m^{-2}) while genotype Al-LSSN 108 gave less average (401.7 tiller m^{-2}). Nacowy potas cv. revealed a significant superiority in tiller number with 635 tiller m^{-2} , while less significant variation was detected in Coa variety (380 tillers m^{-2}) according to study results of Al-Jana et al. (2017). To assess the tillering proportion in wheat plant grain yield, Mahmood and Al-Hassan, (2017) concluded that the primary tillers exceeded the main stem in the number of spikelets, the number of grains, and the weight

of TGW. The results of Al-Fahdawi and Muslih (2018) mentioned that the genotype Sham 4 was significantly higher in tiller forming with an average of 21.9 tillers m^{-1} comparing with the rest of the genotypes, where the two cultivars AL-ezz, Al-Rashidia were lower in a number of tillers (11.5, and 11.3 m^{-1}) tillers respectively.

2.5.7 Dry Weight

Dry matter is the outcome of carbon absorption and distribute the photoassimilates on plant organ. Then release it through respiration, exudation, and organs death. It is influenced by several factors such as nutrients availability and flow through root system (McDonald et al., 1996). Dry weight for vegetative parts recorded a significant variation among genotypes, the higher weight was in genotypes 6, and 2 about 20.333, 18.166 g plant respectively (Al-Joburi et al., 2014). Results of Al-Tahir and Al Hamdaoui (2016) showed a significant difference among wheat varieties in dry weight, the variety Resheid was significantly superior in this trait with 6.061 g. Al-Jana et al. (2017) results showed a significant difference in dry weight of genotypes. Nacowy potas was superior with a higher average of 40.23 g, while the least significant variation was in cultivar Latefeyia (4.35 g). A significant variation in dry weight among varieties at first season only, IPA 99 cultivar was the highest in dry weight (1.8 and 1.4 $kg m^{-2}$) (Al-fahdawi, 2019).

2.5.8 Spikes Number Per m^2

Spikes number is determined early in crop life hence it is an important component of the grain yield due to its effect on the final yield. This trait is influenced by the environmental conditions and also the management systems of the crop during the tillers formation stage, as well as genetic factors. Bread

wheat varieties have a different ability to produce effective tillers and thus the number of spikes due to the difference of food production which later be transformed into tillers bearing spikes (Mohammed, 2000). A number of spikes per area showed a significant variation, Latefiya and IPA 99 cultivars were significantly superior in this trait than Resheid (428.7, 411.7 and 360.0 spike m^{-2}) respectively, where they referred to the genetic variation of these genotypes in producing tillers (Al-Tahir and Al Hamdaoui, 2016). In a study of Al-Jana et al. (2017) the fertile spike number trait showed a significant variation among genotypes, the higher average was recorded by Nacowy potas cultivar about 635 spike m^{-2} while the minimum average was recorded by cultivar Coa 380 spike m^{-2} . The analysis results showed differences in spike number m^{-2} trait with a higher rate in AL Barakah variety (305 spikes) compared to AL Fateh, and Bhooth22 while less average was recorded by variety Orok cultivar about 258.8 spikes. This variation in this trait returns to the ability of varieties to produce tillers (Al-Asseel et al., 2018).

2.5.9 Number of Grains per Spike

NGS trait is important and has a direct effect on the grain yield. The varieties significantly differed in NGS, where the cultivar Mexipack which gave the highest number of grains in the two seasons 54.73 and 65.65 grain spike $^{-1}$, while less number were obtained by Abu-Graib3 and Fatih in the two sessions (40.64 and 54.91 grain spike $^{-1}$, respectively), (Hashim et al., 2017). The genotypes showed a significant variation in grain number per spike at season 2. Bohooth10 genotype gave the highest number (62.07 grain spike $^{-1}$) whereas, genotype 38 had less mount of grains in spike (38.32 grain) (Mohammed and Kadhem, 2017). The difference of wheat varieties in the number of grains per spike trait is due to the difference in the number of

spikelets per spike by the nature of the genetic background, where the study results of Al-Asseel et al. (2018) showed a significant superiority between varieties in the average of grains number per spike. High significant differences were found in NGS according to results of Ziydan et al. (2018) study, where the variety Azar recorded a high average of 57.83 grain per spike⁻¹, and the less rate was obtained by the variety Adena 48.67 grain spike⁻¹.

2.5.10 Fertility Ratio

The increases of fertile tiller numbers are considered a very important trait in high-yielding wheat lines (Zhang et al., 2013). The formation of fertile tiller in wheat is an important factor that influencing fertile spike number and yield. Wheat breeders are interested in choosing varieties with high fertile spike numbers. Zhang et al. (2013) revealed a new gene (*ftin*) that controlling fertile tiller formation in chromosome 1AS, neighbouring to the *tin1* gene in Pubing3558 line (which was derived from a cross between common wheat and wild grass *Agropyron cristatum*) where this site can be targeted by wheat breeders for improving wheat yield. A study by Guo and Schnurbusch (2015) indicated that the three determining factors for floret fertility are maximum floret primordia, fertile floret, and final grain number per spikelet. Floral deterioration has a sensitive effect in defining these three traits that are linked with floret fertility. In their experiment, tillers were removed and found that it is retard the floral deterioration in some cases and was linked with increased the maximum floret primordia, fertile floret, and final NGS. A study by Ye et al. (2015) used the wild wheat relatives (*Agropyron cristatum* L.) which are distinguished with high fertile tiller number and grain number per spike compared to common wheat into the genetic improvement of wheat. The

results revealed that *A. cristatum* chromosome 6P has regulated fertile tiller number. Also the positive and negative regulators for this trait lie on arm (6PS and 6PL) of *A. cristatum* chromosome. Al-Amiry and Al-Ubaidi (2016) found a significant variation among genotypes in terms of fertility ratio, genotype Al-ESW 122 gave a higher ratio of 96.61 % and was non-significant different from genotype Al-SSN 108 which gave a high ratio (96.51 %), while Araz gave less ratio of 81.45 %. Growth process that plant passes through affecting in spike fertility and grain number, which are developed during the stem elongation period (time between terminal spikelet phase and anthesis) (Gonzalez-Navarro et al., 2016). Al-Fahdawi and Muslih (2018) indicated that the varied genotypes in tillers number per plant were significantly equal in a fertile ratio (fertile spikes) where they gave a higher average (87.8 %) in genotype (wheat17) to 94.9 % in Sham4.

2.5.11 1000 Grain Weight (g) (TGW)

Grain weight is one of the important components in the yield of wheat, as it is considered a measure of the amount of accumulation of nutrients in the grains. The variation in grain weight of a thousand grains is the result of the variation in the genetics of the varieties as well as environmental factors (Algaffar, 2014). The grain weight of the wheat plants is important because of it correlated with the quality of milling. Significant variation of genotypes in TGW trait. Al-Jana et al. (2017) indicated a significant variation for cultivars in TGW, where the cultivars Rasheed, IPA99, and Nwewya cultivars were superior with a high average 42.93, 41.73, and 24.27 g, while cultivar Nacowy potas recorded less average (30.93 g). Wyzińska and Grabiński (2018) indicated that grain weight dependent highly on the experimental factors. in their experiment found that implanting late led to a decrease in TGW. Al-

Fahdawi and Muslih, (2018) indicated a significant variation among cultivars in TGW. Al-ezz cultivar had superior on the rest and obtained 36.0 g, while the cultivar Sham95 was less with an average of 18.1 g. Al-Barakah and Ibaa 99 was superior in TGW over the other genotypes according to Al-Asseel et al. (2018) who explained the reason for this variation among genotypes in TGW to the difference of yield components trait and the volume of vegetative.

2.5.12 Grain Yield

One of the main aims is to improve the cultivars especially Triticum species that have higher yield even in the water deficient environment. Which is considered a major source of food for more than half of overall humans consumption (Fleury et al. 2010; Habash et al. 2009). The final yield is consists of its three components which are the spikes number, number of grain per spike, and grain weight. The grain yield is affected by agricultural processes in terms of their effect on the ability of the source to prepare photosynthetic products and the capacity of the sink to store them (Algaffar, 2014). Al-Amiry and Al-Ubaidi, (2016) statistical analysis showed a significant variation among genotype in GY. AL-LSSN genotype was superior and gave a high average (4.29 ton ha⁻¹), while less average recorded by AL-RV 84 genotype (2.77 ton ha⁻¹). Mohammed and Kadhem (2017) indicated that GY production was higher at genotype 26 about (6.117 and 5.074 ton h⁻¹) for the two seasons, while the cultivar IPA99 gave a lower yield 3.395, and 3.473 ton h⁻¹. A high significant difference was recorded among wheat varieties in grain yield according to the results of Al-Asseel et al. (2018). Iba 99 variety gave a higher average (3762 kg h⁻¹) while less average was obtained by Orok 2700 kg h⁻¹.

2.5.13 Biological Yield ton h⁻¹

It is a measure of the total amount of dry matter produced by the plant during its growing season, which represents the difference between the processes of photosynthesis and respiration, as the process of photosynthesis depends on the efficiency of the crop vegetative sum by intercepting light during its growing season, and this efficiency is affected by various genetic and environmental factors, Al-Amiry and Al-Ubaidi (2016). Biological yields recorded a significant variation among cultivars according to study results of Al-Temimi et al. (2013), where the cultivar Rabyaa scored higher mean about 16.96 ton ha⁻¹, whereas less mean was by Sham 6 attained 9.09 t ha⁻¹. Al-Tahir and Al Hamdaoui (2016) pointed to a significant variation among cultivars in BYD, where the cultivar Rasheed superior significantly than Latefeyia and IPA99 which both did not differ significantly among them, and attained 4.157, 3.69, and 3.32 g. The study by Baktash and Naes (2016) mentioned a significant difference for genotypes in BYD, and pointed to the reason which is attributed to the difference in plant height, and several spikes. The varieties of wheat differed significantly in the biological yield trait as the results of Al-Jana et al. (2017) showed, where the cultivar Rasheed was superior on the other cultivars with a mean 23.88 ton. ha⁻¹, while the cultivars IPA99 and Coa showed less mean 17.00 and 14.12 ton. ha⁻¹. The cultivars recorded significant differences in the biological yield traits, as the variety AL Fateh gave the highest average attained 11735 kg h⁻¹ and it differed significantly from the rest of the varieties, while the variety Orok was the lowest average of biological yield of 9890 kg h⁻¹ (Al-Asseel et al., 2018). There was a significant variation among genotypes in BYD trait for season two only Al-Azawi et al. (2018), and the variety Rashed recorded a higher rate in the two-season attained

1632.29 and 1677.65 g m⁻², this reason as a result of an increase in some components of growth and yield.

2.5.14 Harvest Index % (HI)

The harvest index is considered important evidence of evaluation and selection genotypes for grain yield (Sharma and Smith, 1987). Harvest index is defined as a measure of conversion efficiency of photosynthetic products in plants into an economic yield. Harvest index is using as a statistical indicator (parameter) linking the biological yield to the grain yield (Algaffar, 2014). The varieties recorded variation in harvest index and for both seasons according to the results of Al-Hassan et al. (2014), where they attributed the reason to the difference in the efficiency of converting and distributing dry matter into grains. The varieties were different significantly in HI, the variety N70 recorded a high rate of 39.00 with non-significant differences from varieties sham 6 and Al-Iraq, while less rate in HI showed by variety Al-Furat about 26.22 (Al-E et al., 2014). The varieties showed a significant difference in HI, where the varieties Al-Barakah, Ibaa 99, and Bhooth22 recorded higher average attained 33.3, 32.91, and 32.89 %, while the varieties Orok and Bhooth10 showed low ratio at 25.84 and 26.29 % (Al-Asseel et al., 2018).

2.6 Effect of Drought on Gene Expression and Agronomical Traits

Phenotyping stayed a key standard for checking breeding materials depending on morpho-physiological characteristics that adapt to drought included yield and its components (Monneveux et al., 2012; Passioura, 2012). In drought conditions, the plants respond to diverse mechanisms including physiological, biochemical, and gene expression modulation (Al Khateeb et al., 2017).

2.6.1 Effect of Drought on DREB Gene Expression

Plants stimulate the expression of different transcription factors to cope with environmental stresses. Upon exposure to abiotic stresses, transcription factors which are up or down-regulated the expression of a chain of genes through the linkage of the enhancer or promoter area of the gene with DNA-binding domains (Yang et al., 2010; Okay et al., 2014; Gahlaut et al., 2016). Ravikumar et al. (2014) developed a transgenic rice plant (Samba Mahsuri) by using the AtDREB1A gene, the gene was expressed and inherited in transgenic rice lines T1 and T2 which were highly tolerated the shortage of water. According to a study by Hassan et al. (2015), DREB appears to be stimulated the transcription process of Tadh and wcor genes in drought conditions, also, found that the genes were organized by drought stress and after re-watering. Yousfi et al. (2015) results pointed to significant interaction between genotypes and environment conditions during growing season, nitrogen content and the expression of most genes, where expression of TaDREB1A increased under stress compared to control conditions. Islam et al. (2015) investigated the contribution of a few molecular and biochemical specified, where gene expression DREB1A stimulated strongly due to PEG treatment in roots of wheat cultivars BG-25 and Bijoy. The higher promotion noticed in BG-25 cultivar roots which indicates to its tolerance to drought. Liu et al. (2015 b) explored the transcriptional response of wheat to the individual and interaction stresses. They found that 1,328 wheat transcription factors responded to stress treatments. Also, analysis of the regulatory network revealed that Heat shock factors (HSFs) and DREBs are involved in regulation abiotic response. Zotova et al. (2018) showed that a significant increase in TaDREB5 expression levels in the high yield varieties comparison with controls whatever the kind of stress. Yang et al. (2020) showed that the

expression of DREB/ CBF genes, TaDREB3 and TaCBF5L were modulated in transgenic wheat and barley, through uses of stress-responsive promoters HDZI-3 and HDZI-4. The expression of the DREB/CBF genes under those promoters improved dehydration and frost tolerance, which means HDZI promoter incorporated with the DREB/CBF factors can be used for enhancing of tolerance to abiotic stress in transgenic cereal plants.

2.6.2 Flowering Time

Wheat is flowering after exposure to a low temperature during winter in the process of vernalization. Vernalization takes place when the plant is exposed to low temperature for adequate several days which helps motivate flowering. Plants that need vernalization could prevent flowering during summer or fall through encode repressors till exposure to low winter temperatures. Then in spring, plants are flowering after removing the repressor effect (Andrés and Coupland, 2012). The three genetic that controlling wheat flowering time are Vernalization (Vrn), photoperiod (Ppd), and earliness per se (Eps) (Herndl et al. 2008). The wheat crop has a special genetic possibility to synchronize the flowering time according to suitable environment status thus it is planting in global. Irrigation treatment significantly affected flowering time. Bread wheat and at full irrigation were flowered after emergence between 80 and 87 days, while at reducing irrigation it had been noticed earlier flowering which was between 75 and 81 days after flowering. Whereas durum wheat took time for flowering 75 to 83 days at full irrigation and took 72 to 77 days at reduced irrigation (Honsdorf et al., 2018). Anthesis was affected significantly at different irrigation duration with higher days attained (54.80 days), while the treatment drought stress was less in flowering days (53.00 days) (Islam et al., 2018).

2.6.3 Plant Height (cm)

Until now, more than 20% of genes had specificities that decrease wheat height (Mcintosh et al. 2013). Irrigation treatment every two weeks recorded a high average for plant height 100.74 and 98.39 cm, while increased irrigation periods led to reducing plant height 90.37 and 89.61 cm for the two season (Hashim and Al-Haydary, 2012). Statistical analysis revealed for plant height of winter wheat cultivars in various treatments that Cappelle Desprez / CAP/ was a tall, whereas Ba'нку'ti 1201/BKT/ genotype registered a greater plant height (Varga et al., 2015). Gizaw et al. (2016) indicated that plant height was varied in the different environments (drought, medium, and precipitation), while it was higher in the watering environment. Thirty-four of synthetic hexaploid wheat (SHWs) has been estimated in addition to bread wheat Jinmai47 under treatment of well-watered and water-stressed. There was a significant variation under well-watered state where all the SHWs showed a higher average for plant height in both years 2013-2014 and 2014-2015, respectively (Song et al., 2017). Irrigation intervals showed a significant effect in plant height, the period every 10 days was superior on the other and gave a higher average in this trait which were about 94.17 cm and with no significant difference from I5 day interval which recorded about 92,14 cm, while I 20 was less height (73.62 cm) (Hussein et al., 2017).

2.6.4 Flag Leaf Area (cm²)

Flag leaf size and its angle are positively related to the yield of grain crops (Ding and Xiong, 2011; Sidro et al., 2012). Flag leaf, during the reproductive phase, contributes to equipping assimilation for plant growth, evolution, spike development, adapting to drought allusion, and photosynthesis (Tian et al., 2015). Xu and Zhao (1995) mentioned that flag leaf and in a suitable state

participates in photosynthesis vigor for about 45-58% in some genotypes of wheat, and about 41-43% in post-flowering which uses in grain filling (Sharma et al., 2003). Quarrie et al. (1999) pointed that wheat genotypes, with small and straight flag leaf, can decrease water forfeiture by winding for their leaf under the influence of drought, unlike genotypes with a loose leaf. In morphological marks, flag leaf influences the plant architecture and possibly yields. The statistical analysis of Yang et al. (2016) results showed an affecting of water regime and environment factors on flag leaf morphology phenotypic, where it has appeared a significant decrease in mean besides kept their small size and straight state at drought-stressed compared to irrigated. Statistical analysis that genotypes were different significantly in flag leaf area under water treatments. Synthetic hexaploid wheat (SHW) was superior to cultivar Jinmai47 for flag leaf area in both years (Song et al. 2017). A high role for FLA in grain filling through assimilates synthesis as Ul-Allah et al. (2018) mentioned in their study, where irrigation treatment affected significantly in flag leaf area which recorded at water stress decreasing by 20%.

2.6.5 Spike Length (cm)

Terminal drought virtually obstructed the length of the spike whereas osmopriming led to improve the length of the spike in both situations, well-watered and stressed, through the two years of study (Farooq et al., 2015). The results were significant for spike length (cm) at various moisture regimes, spike recorded the highest length at irrigation water treatment (I1) with 12.5 and 12.7 cm and I4 (1.0 IW: CPE ratio) through both years 2014-2015 and 2015-2016 (Deo et al., 2017). Synthetic hexaploid wheat (SHWs) recorded a significant length of spikes compare with the cultivated Jinmai47 in 2013-

2014, while in the years 2014-2015 only twelve (35.29%) of the SHWs showed a high average for spike length from the cultivated Jinmai47(Song et al., 2017). The water stress treatment was 25% of available water recorded high average for spike length attained 13.580 and 14.602 cm, while increasing water stress at treatment 75% led to a significant decrease in spike length 9.135 and 9.362 cm in the two seasons (Mohammed and Kadhem, 2017).

2.6.6 Tillers Number (m²)

Tillering is an important trait for plant architecture which eventually affecter grain yields, where the tiller number per plant determines spikes number and influence on grain yield (Naruoka et al. 2011). Water stress level revealed a significant effect in tillers number trait, which recorded a high average at booting stage (3.82 and 3.06 tiller plant⁻¹) at treatment (75 and 50 %) of the field capacity (Al-Da'mi, 2015). The effective tillers m⁻² registered a significant high in number at irrigation water (I1) treatment, while the lowest number at treatment (I2) in both years 2014-15 and 2015-16 (Deo et al., 2017). Hashim and Al-Haydary (2012) mentioned that the tillers number had increased with the decrease in the irrigation duration of the crop. A high average for the trait was recorded at irrigation every two weeks, while plant height was decreased with increasing irrigation duration.

2.6.7 Dry Weight

Hashim and Al-Haydary (2012) study results showed superiority for 2-week irrigation significantly and gave a high average in dry weight at the two-season, while less weight was at the treatment of 5-weeks irrigation. The decrease in soil water content with a high ratio affects the lack of dry matter formed, as it causes the speed of the vital processes of the plant and then the decrease of the main components such as plant height, tillers number, flag leaf

area, and the result is a less dry matter. Hassan et al. (2015) studied the roles of dehydrin genes in wheat tolerance to drought stress and showed a significant reduction in dry weight at the end of the experiment by Gmiza cultivar in comparison with control, while drought effected was trivial on dry weight in both cultivars (Sids and Gmiza). Dry weight, according to results of Al- Da'mi (2015) study, appeared to be significantly affected by water stress, where attained at elongation stage (5.63, and 4.18 g plant⁻¹) at water stress of field capacity (75 and 50%) with reduction of 23.40 and 43.12 % respectively in comparison to control. The reason for this decrease in dry weight was due to the reduction of photosynthesis along with the decrease in leaf area, which affected the formation of carbohydrates and proteins, and thus the lack of vegetative growth.

2.6.8 Spikes Number Per m⁻²

The water stress affected significantly in spike number per meter according to the study Al-E et al. (2014) where full irrigation (S0) gave a higher average for SN per m⁻² (477.7 spikes) with a significant difference compared to water treatments (S1 and S2). The reason for SN reduction in the increase of water stress was due to loss and diminishing some tillers number. There was a significant effect for the water level in spike number, where S1 gave a higher average in spikes number m⁻² (407.8, 448.7) in the two-season whereas less rate was at S2 197.7, 310.3 spikes m⁻² (Mohammed and Kadhem, 2017). Irrigation durations were significantly different in their effect on spike number. Hussein et al. (2017) indicated a significant effect for irrigation duration in spikes number. The irrigation treatment every 10 days was superior and recorded a high SN 5.43 spike plant⁻¹, while the irrigation duration (20 days) was less number 2.25 spike plant⁻¹. The irrigation treatment

each (15 days) recorded a high spike number m^{-2} (310.46 and 322.4 spike m^{-2}) in both seasons, while irrigated every 30 days recorded fewer spikes number (198 and 196.1 spike m^{-2}) (Mohammed et al., 2020).

2.6.9 Number of Grains Per Spike⁻¹

The trait of grain number per spike is one of the most important components of the yield in cereal crops, especially under stress conditions. It is also the most important and determining factor of grain yield, as it is one of the quantitative characteristics that are highly correlated with yield (Hasanpour et al., 2012). Grain number per spike is considered an important trait in wheat yield, which extremely impacted by floret fertility (Guo and Schnurbusch, 2015). Stress that occurs before or at anthesis mainly affects the grain number as Liu et al. (2015 a) cleared. The statistical analysis of Gizaw et al. (2016) study showed a significant variation, where the subgroup of soft winter wheat displayed a high grain number per spike in comparison with hard winter in all of the ecological cases. A number of grains per spike were high average in the two seasons at 25% depletion from available water 65.09, and 61.00 grain spike⁻¹, and at 75% depletion least number 35.35, and 32.34 grain spike⁻¹ (Mohammed and Kadhem, 2017). irrigation duration showed a significant variation in grain number, higher grain number was achieved at the irrigation every 15 days, whereas the second duration of irrigating every 30 days showed less average grain number per spike (Mohammed et al., 2020).

2.6.10 Fertility Ratio

More tillers were obtained at well-watered treatment, thus a high number of fertile spikes and grains per plant was produced according to Samarah (2005) study. Mildly-stressed plants were differential significantly from watered plants treatment in tillers number. Ji et al. (2010) pointed that, water decrease

led to failing the reproductive stage, also drought stress at early stages of reproductive development (meiosis in pollen mother cells) leads to pollen sterility. Ravikumar et al. (2014) indicated that at both stressed and unstressed conditions, some of the homozygous lines were drought tolerating, and resulted in significant grain yield besides spikelet fertility comparative to non-transgenic control plants. Loss of the grain in wheat is a result of drought influence on fertility, therefore some plants avoided phenological drought by flowering and productive stages for seed before water supplies are exhausted (Ma'arup, 2016). Fertile tillers number m^{-2} were significantly affected by irrigation treatments, where it was shown that production of 7% tillers at water-stressed irrigation lower than normal irrigation (Ul-Allah et al., 2018).

2.6.11 1000 Grain Weight (g)

Both full irrigation and 75 % of available water treatments recorded a highly significant effect on grain weight, while treatment 90 % of available water gave less average in grain weight 29.87 g (Al-E et al., 2014). The reason was a result of leaves and stem drought which happened along with water shortage, high temperature, low relative humidity, and increased wind speed. This led to a reduction in accumulated dry matter in grain because of the decrease in photosynthesis duration of the flag leaf. Barutcular et al. (2016) revealed in their study that grain weight significantly decreased by -45.3% at water deficiency where higher cereal weight was noticed under the normal situations. Irrigation duration revealed a significant variation on grain weight, the irrigation every 15 days gave a high average for grain weight by 39.39 and 38.44 g, for both seasons whereas the water treatment every 30 days achieved less average 24.19 and 27.40 g (Mohammed et al., 2020).

2.6.12 Grain Yield

Farshadfar et al. (2014); and Jatoi et al. (2014) defined yield as a complicated trait that is controlled via many yield components, as well as polygenes. Al-E et al. (2014) indicated a significant effect of water stress in grain yield. The irrigated treatment recorded a high average of GY (7.094 ton h⁻¹) and differed significantly than stress treatments achieved less average attained 3.171 ton h⁻¹. The reason for this decrease in grain yield at drought treatments back to the deficiency in one of the yield components. There was a significant difference for the irrigation treatments in grain yield. The watered treatment (15 days) recorded a high average, while the treatment (30 days) was low in yield (Mohammed et al., 2020). Deng et al. (2019) included cultivar Zhongmai 175, where the statistical analysis of major agronomic traits exhibited a significant increase in the number of unfertilized spikelets at the water shortage treatment, which led eventually to a reduction by 7.33% in starch contents and about 19.22% in grain yield.

2.6.13 Biological Yield Ton h⁻¹

In a study conducted by Hashim and Al-Haydary (2012) for some bread wheat, where the biological yield trait was higher mean at irrigation every two weeks, while less average in BYD was achieved when irrigated for every five weeks, and they referred to the increase in this trait when reducing the irrigation period to the increase in the dry matter components represented by plant height, the number of tillers, the flag leaf area, and the weight of dry matter upon flowering. Al-Temimi et al. (2013) noticed a significant reduction in biological yields at water shortage stress, where the decrease also significantly increased with increasing water shortage stress attained 26.44 and 40.97% of control by applying 25 and 15% of FC water deficiency,

respectively. Abdelraouf et al. (2013) study results reveal a significant effect for reducing irrigation treatments in BYD trait, where reducing the irrigation requirements (IR) from 100% to 50% leads to a decrease in BYD from 6.57 to 4.75 ton/faddan. There was a significant variation in biological yield as indicated by Islam et al. (2018), the highest average attained 10.05 ton ha⁻¹ at three irrigations (T3) given at 25, 40, and 55 days, whereas the lowest biological yield was 7.60 ton ha⁻¹ at no irrigation.

2.6.14 Harvest Index (HI)

Harvest index is considered a measure of the conversion efficiency of photosynthetic products in green plant parts into economic yield (grains). This trait also uses as a statistical parameter between the biological yield and the grain yield (Algaffar, 2014). Statistical analysis showed that irrigation treatments significantly affected HI. A high ratio for the trait was obtained at irrigation treatment 38%. The cut of irrigation after the flowering stage recorded less ratio in HI 32% (Moghaddam et al., 2012). Irrigation treatments showed a significant effect on the harvest index. The highest ratio of the trait was obtained at reducing the irrigation duration by 33.81 and 33.28 % while increasing of irrigation duration led to reducing the harvest index by 31.01 and 29.42 % in the two-season (Hashim and Al-Haydary, 2012). The reason for the reduction along with the increase in irrigation treatment duration to the decrease in the efficiency of the leaves in supplying photosynthesis products to the grain due to the lack of water, thus reducing the fullness period which leads to a decrease in yield and then less harvest index. Al-E et al. (2014) showed in their study differences in water stress levels on HI trait with a higher average at full irrigation (41.45 %) and significantly different from the other irrigation treatment (26.92 %).

Chapter Three: Material and Methods

3. Materials and Methods:

The experiment was conducted in private field in Hit city/Qnan region, Anbar governorate that located west of Iraq, (Latitude 33°39 N and Longitude 42°47 E) during the winter season of 2019/ 2020. The Experiment including 24 wheat genotypes (21 newly entered into Iraq by agricultural directorate of ministry of science and technology in 2017-2018 from international maize and wheat improvement center (CIMITY) and there are local varieties Iraq, al-diyar, and Al-Mahmodia) and one drought treatment. The first treatment (Irrigated) was applied normally to the experimental units until physiological maturity, and the other one (droughted) is cutoff the irrigation after flowering until the physiological maturity. The experiment was placed a split-plot arrangement in Randomized Complete Block Design (R.C.B.D) with three replications. Irrigation treatments occupied the main plots while genotypes were put in the subplots.

Table 2 showed the physical and chemical characteristics of the soil of the experiment for samples that were randomly collected from different places in the field. The field was plowed, smoothed, leveled, and divided into three replication, each replicate consisted of two main plots and was divided into 24 experimental units (subplots). The area of each experimental unit was 3 x 3 m² where each unit consisted of 12 lines and the distance between every two lines was 25 cm. The depth of holes was 5 cm, and the amount of seeds was 10 g line⁻¹.

The seed quantity per line was calculated according to the following equation:

$$Q \text{ (kg)} = \frac{D \times L \times R}{10000} \quad (\text{Singh and Stoskopf, 1971})$$

Where :

Q= seeding quantity for one line

D= distance between lines

L= line length

R= seeding rate for one hectare

Table 2. Physical and chemical characteristics of the soil of experiment field before planting (0-30 cm Depth) *

Soil separates		Ratio %	
Clay		40	
Sand		22	
Silt		38	
Soil Texture		Clay-Loam	
EC	0.3	Ml/m	
PH	8.4	-	
OM	1.27	%	
P	7.9	ppm	
N	15	ppm	
K**	225.0	ppm	

Soil sample analysis was performed in:

*Iraqi Ministry of Agriculture, Plant Protection Directorate. Organic Farming Dept.

** State Board for Agricultural Research in Abu Ghraib-Soil section laboratories.

The experiment field was fertilized by Diammonium phosphate (DAP) fertilizer with a level of 5 g per line at planting, while urea (N 46%) was used as the complementary source of nitrogen fertilizer at a rate of 30 g for each experimental unit. Nitrogen was added in the elongation stage of the plant. Planting was done manually on the 4th of December 2019. Field processes were performed as needed. The irrigation was cut for the drought treatment after flowering stage to activate DREB expression.

3.1 Molecular Analysis of DREB

Gene Expression One Step

Plants were subjected to water stress treatment after the flowering stage, where the irrigation was cutoff from the specified main plots until the end of the experiment.

Samples of flag leaf area were taken after 5 days of applying the irrigation treatments and were directly put in zipper bags, then samples of the leaves were placed in tubes containing trizol then transferred to the laboratory (ASCo Learning Center).

3.1.1 Materials: Kits, Primers, and Instruments

- **Table 3. Kits**

Kits	Company/ Origin
Chloroform	LiChrosolv, Germany
GoTaq® 1-Step RT-qPCR System, MgCL ₂ , Nuclease Free Water, Quantifluor RNA System.	Promega, USA
Isopropanol, 70% Ethanol	ROMIL pure chemistry, UK
Primers	Macrogen, Korea
TRIzol Reagent	Thermo Scientific, USA

- **Table 4. Primers***

Primer Name	Seq.	Annealing Temp. (°C)	RNA size (bp)
DREB1A-F	5`-CGAGTCTTCGGTTTCCTCAG-3`	56	499
DREB1A-R	5`-CAAACCTCGGCATCTCAAACA-3`		

* In this study, the primer was designed using gene-specific sequencing of DREB1A and according to the reference Pellegrineschi et al. (2004).

• **Table 5. Instruments**

Instruments	Company/ Origin
1.5ml, 0.5ml and 0.2ml Tube	JET BIOFIL, Singapore
Centrifuge	Fisher Scientific, USA
Mic qPCR Cycler	Bio Molecular System, Australia
Mic Tube	Bio Molecular System, Australia
Micro spin Centrifuge	My Fugene, China
Micropipette	Human, Germany
Quantus Fluorometer	Promega, USA
Refrigerator	TEKA, Spain
Vortex	Quality Lab System, England
Water bath	China

3.1.2 Methods and Workflow

i) RNA Purification

RNA was isolated from the sample according to the protocol of TRIzol™ Reagent as the following steps:

A-Sample Lysis

Tissues: For each tube, 1mL from TRIzol™ Reagent was added per 100 mg of sample and gently mixed by a vortex.

B-For Three Phase's Separation

- For each tube, 0.2 mL of chloroform was added to the lysis, then the tube cap was secured.
- All mixes were incubated for 2-3 minutes at room temperature in dark place then centrifuged for 10 minutes at 12,000 rpm, the mixture was separated into a lower organic phase, interphase, and a colourless upper aqueous phase.
- The aqueous phase containing the RNA was transferred to a new tube.

C-For RNA Precipitation

- 0.5 mL of isopropanol was added to the aqueous phase and incubated for 10 minutes at room temperature in dark place then centrifuged for 10 minutes at 12,000 rpm.
- Total RNA was precipitated and formed a white gel-like pellet at the bottom of the tube.
- Supernatant was then discarded.

D-For RNA Washing

- For each tube, 0.5mL of 70% ethanol was added and vortex briefly then centrifuged for 5 minutes at 10000 rpm.
- Ethanol then aspirated and air-dried the pellet.

E-For RNA Solubility

- Pellet was rehydrated in 70 μ l of nuclease-free water then incubated in a water bath set at 60°C for 10-15 minutes.

ii) Determine RNA yield

Fluorescence Method. Quantus Fluorometer was used for detect the concentration of extracted RNA or cDNA in order to detect the quality of samples for downstream applications. For 1 μ l of RNA, 199 μ l of diluted Quantifluor dye was mixed. After 5min incubation at room temperature in dark place, RNA concentration values were detected.

iii) Table 6. Primer preparation

Primer Name	Vol. of nuclease free water (μ l)	Concentration (pmol/ μ l)
DREB1A-F	300	100
DREB1A-R	300	100

These primers were supplied by Macrogen Company in a lyophilized form. Lyophilized primers were dissolved in a nuclease-free water to give a final

concentration of 100 pmol/ μl as a stock solution. A working solution of these primers was prepared by adding 10 μl of primer stock solution (stored at freezer -20 C) to 90 μl of nuclease-free water to obtain a working primer solution of 10 pmol/ μl .

iv) Absolute Quantification by the Standard Curve (SC)

The standard curve method uses for a dilution series of known template copy numbers in the qPCR assay. Linear regression of log concentration (copy μl^{-1}) versus C_T gives the standard curve, and this is then used for 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 22×10^9 copy μl^{-1} to the first tube and made a serial dilution by transferred 10 μl from the first tube to the second tube and so on. The standard curve reaction started from the tube of 22×10^5 copy μl^{-1} to the tube of 22×10^2 copy μl^{-1} .

v) Reaction Setup and Thermal Cycling Protocol

- One Step RT-PCR.

Table 7. PCR Component Calculation

No. of Reaction	48	rxn	Annealing temperature of primers			
Reaction Volume/run	10	μl	No. of primers			
Safety Margin	5	%	No. of PCR Cycles			
Master mix components	Stock	Unit	Final	Unit	Volume	
					1 Sample	48.05 Samples
qPCR Master Mix	2	X	1	X	5	240.25
RT mix	50	x	1	x	0.25	12.0125
MgCl ₂					0.25	12.0125
Forward primer	10	μM	1	μM	0.5	24.025
Reverse primer	10	μM	1	μM	0.5	24.025
Nuclease Free Water					2.5	120.125
RNA		ng/μl		ng/μl	1	
Total volume					10	
Aliquot per single rxn	9μl of Master mix per tube and add 1μl of Template					

Table 8. Real Time PCR Program

Steps	°C	m: s	Cycle
RT. Enzyme Activation	37	15:00	1
Initial Denaturation	95	05:00	
Denaturation	95	00:20	40
Annealing	56	00:20 acquiring on Green	
Extension	72	00:20	

3.1.3 Summary of Data Production

Table 9. RNA Concentration (ng/μl)

Treatment	Sample	Conc.	Treatment	Sample	Conc.
Irrigated	Al-Diyar	76.4	Droughted	Al-Diyar	166
	Al-Mahmodia	152		Al-Mahmodia	173
	3	143		3	150
	4	169		4	154
	5	100		5	188
	6	149		6	184
	7	167		7	93.7
	9	174		9	141
	10	103		10	134
	11	178		11	196
	18	176		18	145
	19	32.1		19	180
	20	149		20	112
	24	139		24	135
	25	159		25	145
	28	71.4		28	139
	29	81.2		29	147
	30	182		30	74.9
	31	194		31	154
	32	183		32	127
36	75.9	36	125		
39	162	39	152		
41	167	41	81.8		
43	151	43	68.7		

3.1.4 Absolute quantification. According to the method in (Applied Biosystems, 2003)

$$m = [n \text{ bp}] [1.096 \times 10^{-21} \text{ g/bp}]$$

$$m = \{ \text{g} \} * 10^9 \text{ ng}$$

Copy No. = concentration/m, where:

n = DNA size (bp)

m = mass

$$m = 499 * 1.096 * 10^{-12}$$

$$m = 546.904 * 10^{-12}$$

$$\text{Copy No.} = 12 / 546.904 * 10^{-12}$$

$$= 22 * 10^9$$

3.1.5 Standard Curve

Equation:

$$y = -2.58 x + 24.83$$

$$y = Cq \text{ or } Ct$$

x = Concentration

$$x = (y - 24.83) / -2.58$$

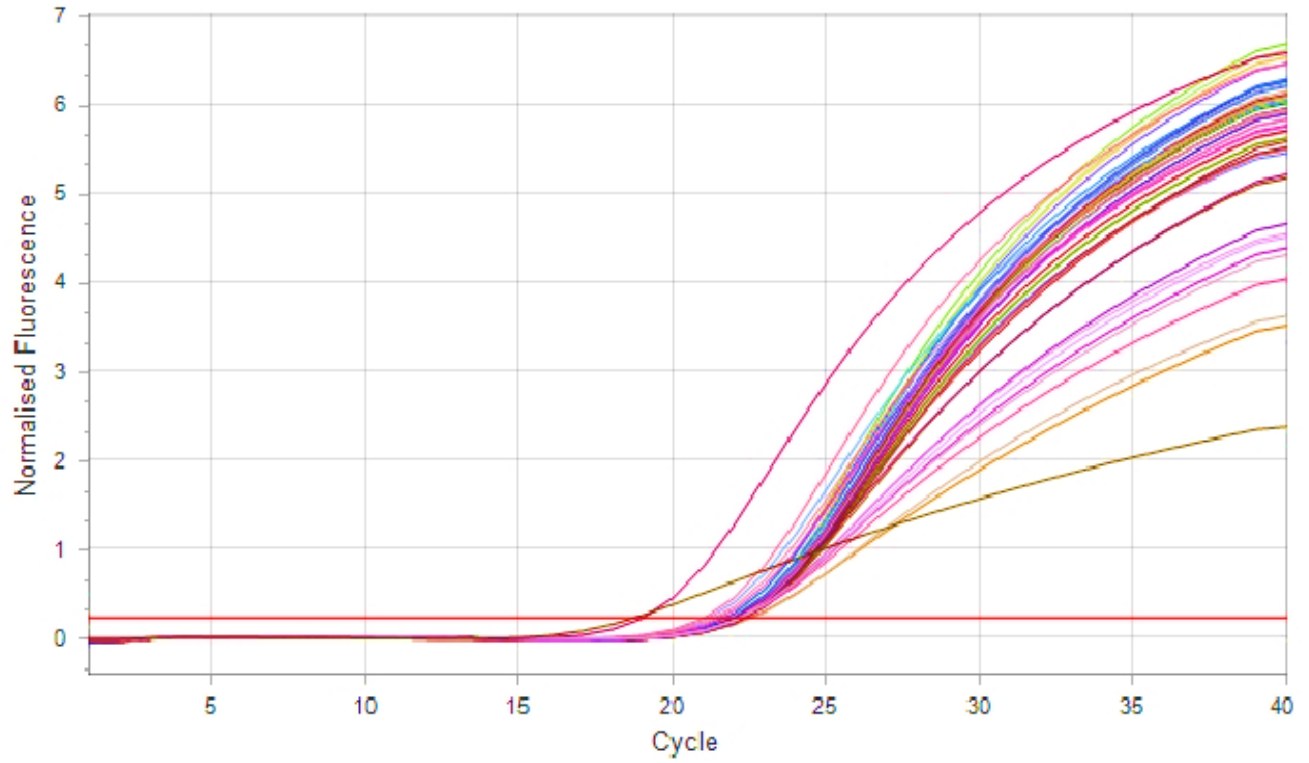


Figure 2. Ct values for samples from 24 wheat genotypes.

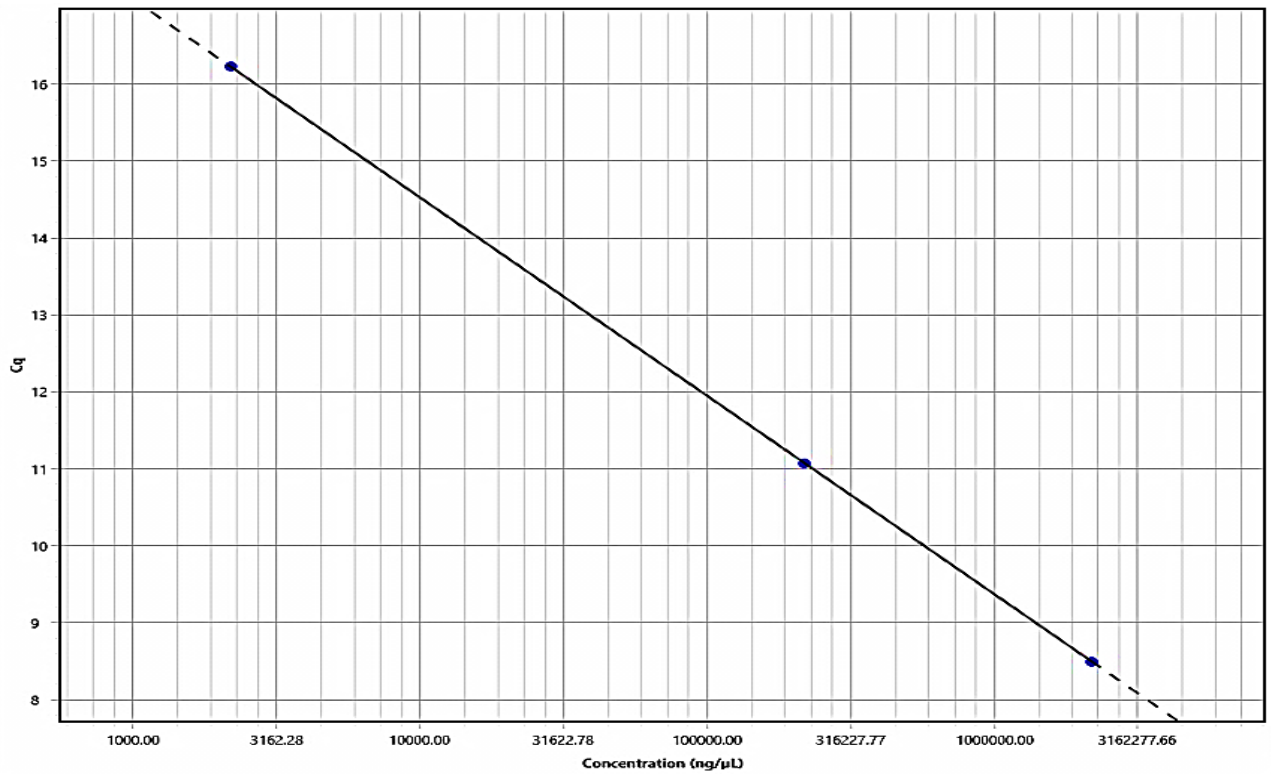


Figure 3. Standard Curve of Absolute quantification

3.2 Phenotypic Traits

3.2.1 Growth Traits:

3.2.1.1 Days from planting until 50% flowering: Calculated from planting date to a flowering of 50% in each experimental unit.

3.2.1.2 Plant height (cm): It was measured from the base of the plant up-to-the last node of the stem, by taking an average of ten randomly plants from one of the middle lines and for each experimental unit.

3.2.1.3 Flag leaf area (cm²): It was calculated as an average of ten flag leaf randomly taken per experimental unit according to the equation:

$$\text{Flag leaf area (cm}^2\text{)} = L \times W \times 0.95$$

Where:

L: The length of flag leaf

W: maximum width of flag leaf

3.2.1.4 Spike length (cm): It was measured from the base of the spike to the end of the terminal spikelet excluding awn as an average for ten spikes was taken from the guarded middle lines and for each experimental unit.

3.2.1.5 Tillers number per square meter: This trait was calculated through the number of total tillers for one of the middle lines of a harvested area by 1 meter randomly taken from each experimental unit then converted to the square meter.

3.2.1.6 Dry weight (g m²): Stems and leaves of the harvested area are dried under sunlight and for each experimental unit. When the weight was stabilized, the dry weight was calculated.

3.2.2: Yield and its Components:

At full maturity, an area of a (1 m) was harvested from one of the middle lines for each experimental unit to study the following traits:

3.2.2.1 Number of spikes m^{-2} : It was calculated for the group of plants for the same harvested area, then converted to the square meter.

3.2.2.2 Number of grain per spike: It was calculated from the average of grains in 10 spikes randomly taken from the harvested spikes for each experimental unit.

3.2.2.3 Fertility ratio %: It is estimated according to the following equation:

$$\text{Fertility ratio} = \frac{\text{Grains}}{\text{Spikelets}} \quad (\text{Scott and Langer, 1977})$$

3.2.2.4 1000 Grain weight (g): A sample of 1000 grain was randomly taken from grain yields samples of each experimental unit and then weights were measured by sensitive electronic balance.

3.2.2.5 Grain yield (ton h^{-1}): After the manual threshing of the harvested plants for one of the midline lines of 1.5 m length from each experimental unit, and after isolating the straw from the grains and cleaning them well, the grains were weighed in addition to the grains used in estimating the weight of 1000 grains for the same treatment, and then converting the weight from g m^{-2} to a ton h^{-1} .

3.2.2.6 Harvest index % (HI): It was calculated according to Singh and Stoskopf, (1971) as the following equation:

$$\text{Harvest index (HI)} = \frac{\text{Grain Yield (ton h}^{-1}\text{)}}{\text{Biological Yield(ton h}^{-1}\text{)}} \times 100 \quad (\text{for each experimental unit})$$

3.3: The Statistical Analysis:

Collected data from studied traits were ordered in tables and then were subjected to analysis of variance ANOVA using MS: excel 2016. The experiment was applying according a split-plot arrangement in Randomized Complete Block Design (R.C.B.D.) with three replications. Irrigation treatments were occupied the main plots while genotypes were put in the subplots. The significant differences between means were distinguished by using the least significant difference (L.S.D) test at a probability level of 0.05 for the studied traits. The simple correlation (r) and its significance between all studied traits were also calculated (Al-Rawey and Khalaf Allah, 2000).

Chapter Four: Results and Discussion

4. Results and Discussion:

4.1 Expression of DREB gene

In the current study, DREB 1A gene expression investigated and estimated in 24 wheat genotypes that were newly entered to the Anbar governorate. Results presented in (Table 10) showed the superiority of genotype Iraq with a high number of copies of DREB 1A (221.88 copies); followed by genotypes 39 which gave 174.12 copies at drought treatment, comparison with irrigated treatment that gave a convergent number of copies between them excepting the genotype Al-Mahmodia which recorded a high number of copies (16.05 copies). It is clear that the genotypes under study were not the same in their response to water stress and might belong to their genetic printing. A study by Kurahashi et al. (2009), used synthetic hexaploid wheat lines, referred that the TaDREB1 genes accumulated in the tolerant accessions more than the sensitive accessions. It was found that the two genes TaDREB1 and TaDREB2 were motivated under drought stress in the wheat plant (Egawa et al., 2006), and this could promote the expression at the osmotic stresses. Shinozaki and Yamaguchi (2007) cleared that the accumulation 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 accumulation in osmotic and temperature stress responses, besides having a genetic function that may improve dehydration tolerance in wheat (Wei et al., 2009). 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. This makes the DREB gene a target pathway for genetic engineering and crop improvement (Lata and Prasad, 2011).

Table 10. Gene expression of DREB in wheat genotypes for one replicate under (irrigated and droughted) treatment.

Irrigated	Ct	Log Conc.	Copy number. 10 ⁹	Droughted	Ct	Log Conc.	Copy number. 10 ⁹
Al-Diyar	22.22	1.01	10.32	Al-Diyar	22.23	1.01	10.22
Al-Mahmodia	21.72	1.21	16.05	Al-Mahmodia	22.21	1.01	10.35
3	22.08	1.07	11.64	3	22.31	0.98	9.47
4	22.10	1.06	11.49	4	22.35	0.96	9.16
5	22.22	1.01	10.31	5	22.32	0.97	9.43
6	22.28	0.99	9.76	6	21.20	1.41	25.74
7	22.22	1.01	10.35	7	22.27	0.99	9.82
9	22.07	1.07	11.73	9	22.15	1.04	10.96
10	22.11	1.05	11.31	10	22.14	1.04	11.06
11	21.97	1.11	12.84	11	22.25	1.00	10.06
18	22.12	1.05	11.25	18	22.14	1.04	11.04
19	22.26	1.00	9.96	19	22.26	1.00	9.94
20	22.13	1.05	11.18	20	21.71	1.21	16.25
24	22.11	1.05	11.34	24	21.05	1.47	29.19
25	22.27	0.99	9.85	25	21.51	1.29	19.41
28	22.26	1.00	9.90	28	21.37	1.34	22.09
29	22.52	0.90	7.90	29	22.11	1.05	11.32
30	22.38	0.95	8.94	30	22.23	1.01	10.21
31	22.20	1.02	10.46	31	21.95	1.12	13.09
32	22.24	1.01	10.13	32	22.04	1.08	12.10
36	22.58	0.87	7.44	36	22.17	1.03	10.76
39	22.04	1.08	12.10	39	19.06	2.24	174.12
41	22.07	1.07	11.74	41	22.20	1.02	10.47
Iraq	22.13	1.05	11.15	Iraq	18.78	2.35	221.88

4.2 Number of days from planting until 50% flowering

The statistical analysis of variance in Appendix 1 and Table 11 indicated a significant differences between genotypes in the mean of 50 % flowering. Genotype 11 was earlier and flowered after 103.00 day from planting, followed by the two genotypes 20 and 24 that took 103.67 day, whereas genotype Al-Mahmodia took the longest time to reach this stage which was 109.67 day, followed by the two genotypes 29, 41 where they did not differ significantly between each other with an average of 109.00 and 109.33 day respectively. The variation of wheat genotypes in this trait maybe due to the difference in their genetic background. These results were consistent with Baktash and Naes (2016), and Al-Amiry and Al-Ubaidi (2016), Al-Asseel et al. (2018), and Abood et al. (2019) as the significant differences between the genotypes, for this trait, is a result of their genetic variation, which is reflected in their different response to the environmental conditions, thus the difference in the period of their arrival to the stage of flowering. Results in Table 11 showed a non-significant effect of irrigation treatments, and interaction between the irrigation treatments and the genotypes on the average of 50 % flowering.

Table 11. The effect of genotype and irrigation treatments on the number of days from planting until 50% flowering.

Genotypes	Irrigated	Droughted	Mean	Genotypes	Irrigated	Droughted	Mean
Al-Diyar	108.33	109.00	108.67	9	105.67	105.00	105.33
10	107.67	109.00	108.33	28	105.67	109.67	107.67
29	108.33	109.67	109.00	18	105.33	105.67	105.50
39	107.67	107.67	107.67	6	106.33	105.67	106.00
36	107.00	107.67	107.33	3	108.33	103.33	105.83
25	104.33	103.67	104.00	Al-Mahmodia	109.67	109.67	109.67
20	104.33	103.00	103.67	19	106.33	106.33	106.33
24	104.00	103.33	103.67	5	106.33	105.67	106.00
31	106.33	106.33	106.33	41	107.00	111.67	109.33
7	106.33	106.00	106.17	4	107.67	109.00	108.33
30	107.67	105.67	106.67	Iraq	107.67	109.00	108.33
11	103.00	103.00	103.00	32	107.00	108.33	107.67
Mean of irrigation treatments: Irrigated= 106.58, Droughted= 106.79							
L.S.D (A)= n.s, (B)= 2.90, (A × B) = n.s							

*n.s= non-significant

4.3 Plant Height (cm)

Analysis of variance in Appendix 1 and Table 12 indicated a significant effect of genotypes in plant height, genotype 6 was superior in this trait and gave the highest (94.08 cm), followed by genotype 5 with 88.10 cm, and both genotypes were significantly different against each other, while less average in plant height recorded by genotype Al-Mahmodia was 74.57 cm. The differences between genotypes for plant height rates are due to common ancestor nature. This is agreement with the results finding by Al-Amiry and Al-Ubaidi (2016), and Al-Fahdawi and Muslih (2018) showed in their study result a significant difference between genotypes in plant height. A non-significant effect was recorded of irrigation treatments and the interaction between genotypes and irrigation in the mean of plant height trait as shown in

Table 12 due to cutoff irrigation after flowering stage so as plant reached in height. An important criterion in wheat breeding and improvement programs is low plant height and early maturity in order to withstand drought and reduce water consumption (Johen et al., 2004). As shown in Appendix (2), plant height was correlated significantly with all the studied traits except the following traits: Chl., 50% F, and was negatively correlated with TGW, HI, and fertility ratio which did not reach the significance. This consistent with what has been found by Hassan and Al-Dawdi (2014). Alemu et al. (2020) referred to that some phenotypic traits including plant height was positively affected the grain yield.

Table 12. The effect of genotypes and irrigation treatments on plant height (cm).

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	Iraq	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 (A)= n.s, (B)= 7.68, (A × B)= n.s							

*n.s = non-significant

4.4 Flag Leaf Area (cm²)

Flag leaf during the reproductive phase contribute to equipping assimilation for plant growth normal, evolution, spike development, adapting to drought, and photosynthesis. In morphological markers, flag leaf influences the plant architecture yields, where it is positively correlated with grain filling. The flag leaf participates in photosynthesis vigor which is used in grain filling. According to statistical analysis results in appendix 1 and table 13 showed a significant variation of genotypes in flag leaf area, where the genotype Iraq was superior with a high average of 35.62 cm², followed by genotype 32 which gave 27.02 cm² and both were significantly different in comparison with genotype 20 which showed a lower average of flag leaf area (13.41 cm²). The differences in genotypes in the formation of different rates of flag leaf area maybe due to being a genetic trait linked to its genetic makeup. This finding is in agreement with Al-Amiry and Al-Ubaidi (2016), Baktash and Naes (2016), Al-Jana et al. (2017), Hashim and et al. (2017), Al-Fahdawi and Muslih (2018), Al-Fahdawi (2019), and Mohammed et al. (2020), who their study results showed a significant difference of genotypes in flag leaf area. Irrigation treatment results in table 14 did not affect the flag leaf area, that is return to emergence, growth and expansion of flag leaf occurring in pre-spiking stage which linked with total leaf area of plant and photosynthesis rates (Al- maeini and Mohsin, 2016). The flag leaf area of genotypes according to table 13 was not significantly affected by irrigation treatments and the interaction between genotypes and irrigation treatments.

Table 13. The effect of genotype and irrigation treatments on flag leaf area (cm²).

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	Iraq	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 (A) = n.s, (B)= 6.48, (A × B) = n.s							

*n.s = non-significant

4.5 Spike Length (cm):

The results of statistical analysis in appendix 1 and table 14 showed a significant variation between genotypes in spike length. The genotype Iraq recorded high average of spike length (11.82 cm) followed by genotypes 6 and 9 which recorded 11.08, 11.06 cm, respectively, however they did not significantly differ between each other. Low value of spike length was recorded in genotype 20 which gave 8.81 cm. The reason for these differences may return to the genetic nature of the variety, where the superiority of the genotype 6 in spike length return to the common ancestor. This result was consistent with the results of Al-Tahir and Al Hamdaoui (2016), Al-Jana et al. (2017), Al-Fahdawi and Almehemdi (2017), Al-Fahdawi and Muslih (2018), Wahid and Al-Hilfy, (2018), who clearly indicated the effect of hereditary variation on spike length. Appendix 2 showing a significant positive correlation between spike length and FLA (0.6), also spike length correlated

highly significant with plant height (0.45). This was consistent with the results of Al-Amiry and Al-Ubaidi (2016); and Rakašćan et al. (2019), while Al-Mailiky et al. (2019) referred to a highly significant genetic and phenotypic correlation of plant height with spike length. From table 14 there was a non-significant effect of irrigation treatment on spike length and that was consistent with Qadir et al. (2016). Results of table 14 also showed a non-significant interaction between genotypes and irrigation treatment on the spikes length.

Table 14. The effect of genotypes and irrigation treatments on spike length (cm).

Genotypes	Irrigated	Droughted	Mean	Genotypes	Irrigated	Droughted	Mean
Al-Diyar	10.24	9.93	10.09	9	11.06	11.06	11.06
10	10.71	10.39	10.55	28	9.70	10.11	9.90
29	10.43	10.38	10.40	18	10.33	10.87	10.60
39	10.24	10.79	10.52	6	10.86	11.29	11.08
36	9.78	10.39	10.09	3	9.60	9.03	9.32
25	9.13	9.25	9.19	Al-Mahmodia	8.80	9.92	9.36
20	8.73	8.89	8.81	19	8.54	9.87	9.21
24	10.86	10.15	10.51	5	10.57	10.40	10.48
31	10.26	10.09	10.18	41	9.08	10.18	9.63
7	10.35	10.05	10.20	4	9.84	9.49	9.67
30	10.29	10.20	10.24	Iraq	11.88	11.76	11.82
11	10.01	10.05	10.03	32	10.45	10.55	10.50
Mean of irrigation treatments: Irrigated= 10.07, Droughted = 10.21							
L.S.D (A) = n.s, (B) = 0.64, (A × B) = n.s							

*n.s = non-significant

4.6 Tillers Number Per Square Meter

In some small grain crops like wheat, tillering is a distinguishing characteristic vegetative growth stage, besides being a main component for the yield, therefore it is an important target to improve and increase grain yield (Mahmood and Al- Hassan, 2017). The tiller number per plant determines spikes number and influence grain yield. Statistical analysis in appendix 1 and table 15 showed a significant variation between wheat genotypes in tillers

number, and a high average was recorded by genotypes 3 and 36 (495.33 and 494.67 tiller m⁻²) respectively with the non-significant difference between them, while less average was recorded by genotype Al-Diyar (306.67 tiller m⁻²). The difference between genotypes in tillers rates maybe due to their genetic background. This is in agreement with the findings of Al-Fahdawi and Muslih (2018), and Wahid and Al-Hilfy, (2018), whose results showed a significant difference between genotypes in tillers number. According to the results of table 15, the drought treatment, and the interaction showed a non-significant effect in the mean of tillers number.

Table 15. The effect of genotypes and irrigation treatments on tillers number m⁻²

Genotypes	Irrigated	Droughted	Mean	Genotypes	Irrigated	Droughted	Mean
Al-Diyar	310.67	302.67	306.67	9	433.33	378.67	406.00
10	348.00	390.67	369.33	28	388.00	517.33	452.67
29	330.67	380.00	355.33	18	374.67	306.67	340.67
39	490.67	388.00	439.33	6	405.33	425.33	415.33
36	472.00	517.33	494.67	3	500.00	490.67	495.33
25	392.00	446.67	419.33	Al-Mahmodia	258.67	390.67	324.67
20	401.33	298.67	350.00	19	304.00	381.33	342.67
24	381.33	386.67	384.00	5	430.67	378.67	404.67
31	337.33	357.33	347.33	41	432.00	514.67	473.33
7	440.00	389.33	414.67	4	457.33	464.00	460.67
30	390.67	334.67	362.67	Iraq	362.67	349.33	356.00
11	460.00	433.33	446.67	32	418.67	465.33	442.00
Mean of irrigation treatments: Irrigated= 396.67, Droughted= 403.67							
L.S.D (A) = n.s, (B) = 80.66, (A × B) = n.s							

*n.s = non-significant

4.7 Dry Weight (g m^{-2})

According to the results of statistical analysis in appendix 1 and table 16, it had pointed to a significant effect of genotype on the plant dry weight average, where the genotype 29 was superior over other genotypes with higher mean in dry weight (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 average for the trait was recorded by genotype 20 with 408.89 g m^{-2} . The reason for the difference between genotypes in the dry weight is due to the difference in response to the surrounding conditions and growth factors, and thus the variation in the accumulation of dry matter. This finding is consistent with the finding of Al-Tahir and Al Hamdaoui (2016), Al-Jana et al. (2017), Al-Joburi et al. (2017) and Al-Fahdawi (2019), whose results showed a significant difference between the cultivars in the dry weight of the plant. Results of the same table (16) showed a non-significant effect of drought treatment and the interaction between genotypes and irrigation treatment on dry weight. The appendix 2 showed that dry weight was associated significantly with the studied traits, with the exception of TGW (-0.34), HI (-0.63) and FR (-0.14), Chl, 50% flowering, and DW were non-significant. The harvest index increases inversely with the decrease in straw weight due to the increased transfer of dry matter from plant parts to the grain, what proves this is the correlation coefficient which showed the existence of an inversed correlation between TGW and all the studied traits excepting the harvest index, this result is consistent with previous findings stated by Hassan and Al-Dawdi (2014).

Table 16. The effect of genotypes and irrigation treatments on dry weight (g m^{-2})

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	574.22	533.33	4	512.00	581.33	546.66
30	519.11	524.44	521.78	Iraq	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 (A) = n.s, (B)= 132.48, (A × B) = n.s							

*n.s = non-significant

4.8 Spikes Number Per Square Meter

The spikes number is an important component of the grain yield to be determined at an early stage in the crop's life. This characteristic is influenced by the environmental conditions and also the management practices of the crop during the stages of tiller formation, as well as genetic factors. According to the statistical analysis in appendix 1 and table 17, the results showed a significant variation between genotypes in spikes number per square meter. The higher mean was obtained by genotypes 41, 3, and 36 which gave 499.11, 488.89, and 488.89 spike m^{-2} respectively, and the lowest mean was in genotype Al-Diyar with 330.66 spike m^{-2} . The difference of genotypes in the number of spikes maybe due to the genetic difference between them through the ability to produce active tillers, and this is consistent with the results of Al-Temimi et al. (2013), and Al-E et al. (2014) where they pointed to a significant

variation between cultivars in SN per m². Al-Hassan et al. (2014) pointed to a non-significant difference between genotypes in spikes number for the first season, while a significant effect was recorded in the second season, where they attributed to this difference to the extent of varieties ability to give tillers. The results in table 17 showed a non-significant variation for irrigation treatments, and the interaction on the number of spikes m⁻². As appeared in appendix 2 SN correlated significantly with tillers number per m⁻² (TN), DW and GY, also significantly with PH, GF, DPM, and BYD. This result agrees with Al-Hassan et al. (2014) where they found a significant correlation for SN with biological yield and GY; while Al-Salim et al. (2018) pointed to a significant positive correlation for SN with GY, whereas a negative significant correlation with TGW was detected.

Table17. The effect of genotypes and irrigation treatments on spikes number per m²

Genotypes	Irrigated	Droughted	Mean	Genotypes	Irrigated	Droughted	Mean
Al-Diyar	353.77	307.55	330.66	9	415.11	467.55	441.33
10	376.00	388.44	382.22	28	445.33	429.33	437.33
29	382.22	401.77	392.00	18	392.00	368.89	380.44
39	423.11	404.44	413.78	6	398.22	399.11	398.67
36	494.22	483.55	488.89	3	462.22	515.55	488.89
25	352.00	387.55	369.78	Al-Mahmodia	269.33	408.00	338.66
20	364.44	342.22	353.33	19	402.66	426.66	414.66
24	393.77	408.89	401.33	5	390.22	435.55	412.89
31	387.55	355.55	371.55	41	480.89	517.33	499.11
7	399.11	376.00	387.55	4	429.33	435.55	432.44
30	371.55	357.33	364.44	Iraq	354.66	370.66	362.66
11	393.78	453.33	423.55	32	412.44	470.22	441.33
Mean of irrigation treatments: Irrigated= 397.66, Droughted= 412.96							
L.S.D (A) = n.s, (B)= 68.96, (A × B) = n.s							

*n.s = non-significant

4.9 Number of Grains per Spike⁻¹

Basically, it is a trait that largely affected by the plant genetic background besides environmental factors. The increase in grains number depends on the decrease of apical dominance in the plant and spike's florets. It also depends on the completion of the pollination process (Al-Fahdawi, 2010). Through the results of statistical analysis in appendix 1 and table 18, genotypes significantly differed in grains number per spike, where the highest average was given by genotype 6, which gave 57.48 grain spike⁻¹ and did not significantly differ with genotype Iraq, which gave 56.57 grain spike⁻¹, whereas less mean in grain number was recorded by genotype 24, which gave 40.62 grain spike⁻¹, and did not significantly differ with genotypes 41 and Al-Mahmodia, where they gave 40.88, and 42.00 grain spike⁻¹ respectively. The reason for the superiority of the aforementioned two genotypes in this trait is the superiority in spike length trait (Table 14) this was due to the increase in the florets number in the spike, and accordingly, it was positively reflected in increasing grains number per spike, what confirms the above results are the existence of the significant and positive correlation between the number of grain per spike and spike length (0.52). The result is consistent with the findings of Al-Amiry and Al-Ubaidi (2016), Al-Tahir and Al Hamdaoui (2016), Hashim et al. (2017), Al-Jana et al. (2017), Al-Fahdawi and Muslih (2018), Wahid and Al-Hilfy (2018) and Mohammed et al. (2020) were they indicated significant difference between genotypes in this trait. Appendix 2 showed a significant correlation for the number of grain per spike (NGS) with some studied traits included FR (0.53), BYD (0.42), GY (0.39), days to physiological maturity (DPM) (0.38) with high correlation, then GF (0.31), PH (0.5), FLA (0.5), DW (0.4), while the NGS did not show any significant correlation with 50% F and NGS, and it was negative with Chl, TN, TGW, SN, and HI. This result agrees with what Hassan and Al-

Dawdi (2014) found; whereas study results of Al-Hassan et al. (2014) pointed to a highly significant positive correlation for NGS with some traits as TN, DW, FLA, and TGW in seasons one and two. There was no effect for irrigation treatments, and the interaction on number of grains per spike according to statistical analysis in table 18.

Table 18. The effect of genotypes and irrigation treatments on the number of grain per spike

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	Iraq	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 (A) = n.s, (B)= 4.45, (A × B) = n.s							

*n.s = non-significant

4.10 Fertility Ratio

The statistical analysis in appendix 1 and table 19 indicated a significant difference between the averages of fertility ratio, where genotype 39 showed a high ratio (3.37%), with non-significant difference with genotype 20, which recorded 3.35 %, while the lowest ratio was recorded by genotypes 32 and 36 (2.61 and 2.62% respectively). The reason for this variation between genotypes

maybe due to the variation in their genetic background. These results are consistent with other researchers who have found the same results (Al-Amiry and Al-Ubaidi, 2016), Hashim et al. (2017), Al-Fahdawi and Muslih (2018), and (Wahid and Al-Hilfy, 2018). Table 20 showed that irrigation treatments affected the fertility ratio but did not reach the significance, a high rate for the trait was recorded in drought treatment (3.02 %). Also, in the same table, the results showed a non-significant effect of the interaction between the genotypes and the treatment of drought on fertility ratio.

Table 19. The effect of genotypes and irrigation treatments on fertility ratio%

Genotypes	Irrigated	Droughted	Mean	Genotypes	Irrigated	Droughted	Mean
Al-Diyar	3.07	3.38	3.23	9	3.11	3.16	3.13
10	3.13	3.13	3.13	28	2.77	2.76	2.76
29	3.00	3.17	3.09	18	2.64	3.58	3.11
39	3.28	3.47	3.37	6	2.98	3.15	3.06
36	2.63	2.61	2.62	3	2.81	3.02	2.92
25	2.98	2.87	2.92	Al-Mahmodia	2.85	3.13	2.99
20	3.35	3.35	3.35	19	3.20	3.28	3.24
24	2.67	2.79	2.73	5	2.63	2.68	2.66
31	2.83	3.00	2.91	41	2.76	2.62	2.69
7	2.81	2.79	2.80	4	2.82	2.90	2.86
30	2.64	2.86	2.75	Iraq	3.02	3.06	3.04
11	2.97	3.12	3.04	32	2.61	2.62	2.61
Mean of irrigation treatments: Irrigated= 2.90, Droughted= 3.02							
L.S.D (A) = 0.03 ,(B) = 0.36, (A × B) = n.s							

*n.s = non-significant

4.11 1000 Grain Weight (g)

This trait indicates the fullness of the grains, as it depends on the strength of the sink (grains), which is the recipient of the products of assimilation, and on the efficiency source in the distribution of the metabolites. Data presented in

appendix 1 and table 20 showed significant differences between genotypes in the averages of grain weight, where the genotypes 18, and Al-Diyar gave the highest average of grain weight (56.65, and 55.77 g respectively), while lower average was found in genotype 36 (47.13 g). The variation of genotypes in the grain weight is due to recording a low average in tillers and spikes number which reduced the competition between plants for light and the accumulation of photosynthetic products and growth elements, thus, reflected in the increase in grain weight. The results are in agreement with Al-Jana et al. (2017), Al-Fahdawi and Almehemdi (2017), Al-Fahdawi and Muslih (2018), Wahid and Al-Hilfy (2018), and Mohammed et al. (2020) where they indicated that the genotypes were different in grain weight. The results in table 20 also showed significant variations in drought treatment in average of grain weight, where irrigated treatment was superior by giving the highest mean (51.01g) compared to droughted plants which showed a significant decrease in this trait and gave less mean (49.66 g), where the decreasing of water availability can be a reason for the lack of stored materials in its parts, thus slowing transport and storing in the grain. Under normal conditions, carbohydrates accumulation in the stems continues after flowering for a period of one to two weeks, but it stops during the exposure to water stress. Therefore the inability to sustain growth after that would reduce the weight of the grain. This result is compatible with Al-maeini and Mohsin (2016), Qadir et al.(2016) and Mohammed and Kadhem (2017), Hussein et al. (2017); Mohammed et al. (2020) where they pointed to the influence of water stress on grain weight. With regard to the interaction between genotypes and drought treatment, table (20) showed a non-significant effect on grain weight.

Table 20. The effect of genotypes and irrigation treatments on 1000 grain weight (g)

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	Iraq	50.53	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 (A)= 1.22, (B)=1.98, (A × B)= n.s							

*n.s = non-significant

4.12 Grain Yield (ton ha⁻¹)

The grain yield 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. The statistical analysis (appendix 1 and table 21) showed a significant variation between genotypes on this trait. Genotypes 3, and 29 were superiors on the others by giving a high average for GY (7.39 and 7.29 ton ha⁻¹) respectively with a non-significant difference between them, while less average recorded by genotype 24 was 5.23 ton ha⁻¹. The reason for the superiority of genotype 3 in grain yield return to the significant superior in spikes number and tillers number traits per m². Also both genotypes 3 and 29 were superior in dray weight, what confirms these results is the positive correlation between GY and DW, SN, and TN in appendix 2. Al-Nori and Brwari (2019) pointed in their study that yield showed a significant superiority

by genotype Cham 6 in the two locations, which was superior in the number of spikes per m², where the variation in yield of genotypes are due to differences in yield components, such as spike number per square meter, grains number per spike, and grain weight. Table 21 showed that the two irrigation treatments (irrigated and droughted), and the interaction had non-significant effects on grain yield. In a study by Varga et al. (2015), they referred that in new cultivars, drought stress during maturity did not decrease the yield in comparison with preventing water at heading. The grain yield showed a positive and highest significant correlation with BYD (0.95), DW (0.85), also significant with the traits plant height (0.73), DPM (0.56), SN (0.62), FLA (0.47), TN (0.41), and NGS (0.39), GF (0.38), while was negative and non-significance with the TGW and HI (Appendix 2). The findings of this study are consistent with what found by Hassan and Al-Dawdi (2014). While Al-Salim et al. (2018) mentioned that there was a significant correlation between BYD and GY. Also Al-Mailiky et al. (2019) indicated that the yield was correlated genetically, morphologically, and highly significant with PH, number of branches, and the 1000 grain weight, and significantly with the SL. Besides that, the positive and significant correlation for GY with FLA (0.47) and NGS (0.39) refers to the importance of the two traits in developing and improving the superior genotypes, (Iftikhar et al., 2012).

Table 21. The effect of genotypes and irrigation treatments on grain yield (ton ha⁻¹)

Genotypes	Droughted	Irrigated	Mean	Genotypes	Droughted	Irrigated	Mean
Al-Diyar	6.16	6.08	6.12	9	7.50	6.66	7.08
10	6.23	6.57	6.40	28	6.49	5.94	6.21
29	7.37	7.21	7.29	18	6.30	6.44	6.37
39	6.36	6.36	6.36	6	7.14	7.26	7.20
36	7.20	6.83	7.02	3	7.55	7.22	7.39
25	5.77	5.70	5.74	Al-Mahmodia	6.84	3.86	5.35
20	5.00	6.07	5.54	19	7.18	7.04	7.11
24	5.07	5.39	5.23	5	6.57	6.21	6.39
31	6.36	6.95	6.65	41	7.23	6.68	6.96
7	6.20	6.21	6.21	4	6.36	6.46	6.41
30	5.59	6.28	5.93	Iraq	6.13	6.31	6.22
11	7.43	6.39	6.91	32	6.82	6.14	6.48
Mean of irrigation treatments: Droughted= 6.53, Irrigated= 6.35							
L.S.D (A) = n.s, (B)= 1.20, (A × B) = n.s							

*n.s = non-significant

4.13 Harvest Index % (HI)

Harvest index is considered as a scale for the efficiency converting photosynthetic products into economical yield, where the high value of harvest index in cereals is preferred as evidence of variety efficiency in covering the greatest produced dry matter into grains. The results of statistical analysis in appendix 1 and table 22 indicates a significant difference between the investigated genotypes in this trait, where genotype 20 was superior over the rest of the genotypes under study, by giving 57.57% followed by genotypes 41 and Al-Diyar, which gave 56.32 and 56.00 % respectively. While a lower ratio of 50.09 % was by genotype 10. The variation of genotypes 20 in the harvest index values is due to recording a low average of plant height (table 12), and dry weight (table 16) which reflected in giving the highest average of harvest index through the ability to distribute the net photosynthesis to sink (grains) or

to other parts of the plant. This is consistent with the results of Al-Tahir and Al Hamdaoui (2016), Al-Amiry and Al-Ubaidi (2016), Al-Fahdawi and Almehemdi (2017) and Al-Fahdawi (2019), who found significant differences between genotypes in HI. The genotypes were almost similar in the HI trait except the genotypes 10, 5 and 43, which gave lower averages as a result of increasing biological yield. The same table showed a significant effect for irrigation treatments on HI, and a high average was recorded at irrigated treatment 54.00 %, while less average obtained at droughted treatment 52.87 %. The reason for the reduction of HI at drought treatment returns to the decrease in the efficiency of the leaves in supplying photosynthesis products to the grain due to the lack of water, thus reducing the fullness period which leads to a decrease in yield and then less harvest index. This is consistent with what Hashim and Al-Haydary (2012) found. According to the statistical analysis (Table 22) which demonstrated significant variations of interaction effect between genotype and irrigation treatments on HI trait. Genotype 41 gave higher value (61.67%) followed by genotype 25 with 56.98% in irrigated treatment and both showed a significant difference against each other, while lowest ratio was 50.01 % recorded by genotype10 in drought treatment. This is in agreement with the results of Al-E et al. (2014) who indicated a significant interaction between water stress level and varieties on HI, where a higher rate was recorded at full irrigation treatment and lowest average for trait was at dehydration. The reason of varieties differ in their response under water stress conditions to the variation of biological yield components values and then the efficiency in transfer these materials to the grains. The data of appendix (2) indicated that the HI trait had no significant correlation with all the studied traits except the 1000 grain weight, where the correlation was significant and positive (0.4). While HI showed a negative and non-significant correlation with

DW (-0.63) and BYD (-0.43), this indicated that HI increases with a decrease in the dry weight and the biological yield due to the transfer of the dry matter from the plant parts to the sink (grains). This result is consistent with what found by Hassan and Al-Dawdi (2014) results, where the correlation of harvest index was negative and highly significant with DW of straw and BYD, whereas was positive with TGW but did not reach the limit of significance.

Table 22. The effect of genotypes and irrigation treatments on HI %

Genotypes	Irrigated	Droughted	Mean	Genotypes	Irrigated	Droughted	Mean
Al-Diyar	56.17	55.83	56.00	9	53.71	53.70	53.70
10	50.17	50.01	50.09	28	55.21	51.84	53.52
29	51.45	52.98	52.22	18	54.37	51.56	52.97
39	53.16	53.66	53.41	6	53.43	51.87	52.65
36	52.29	51.46	51.88	3	50.56	56.45	53.50
25	56.98	53.52	55.25	Al-Mahmodia	51.97	54.68	53.32
20	58.44	56.70	57.57	19	52.97	53.52	53.24
24	51.58	52.82	52.20	5	50.85	51.02	50.94
31	54.03	55.24	54.64	41	61.67	50.98	56.32
7	56.43	51.90	54.17	4	56.10	52.54	54.32
30	54.75	51.74	53.25	Iraq	53.44	48.54	50.99
11	54.18	55.08	54.63	32	52.07	51.22	51.65
Mean of irrigation treatments: Irrigated= 54.00, Droughted= 52.87							
L.S.D (A)= 1.09, (B)= 3.2, (A × B)= 4.53							

Chapter Five: Conclusions and Recommendations

5. Conclusions and Recommendations

5.1. Conclusions

According to the results of this study, it can conclude that:

1. Studied genotypes were different in their performance and responses to the drought treatment.
2. Local genotype (Iraq) was superior with a high DREB 1A copies number, followed by introduced genotypes 39, 24, 6, 28, 25, and 20 at droughted treatment in comparison with irrigation treatment which indicates the tolerance of these genotypes to drought conditions.
3. Results indicates that introduced genotypes positively responded to the applied drought treatment by showing superiority in most studied traits.
4. In this study, traits of plant height, flag leaf area, grain number, and spikes number are demonstrated a positive and significant correlation with grain yield. So, these traits can be used in selection breeding programs to improve bread wheat production.

5.2. Recommendations:

1. The researchers carry on the same genotypes in order to select suitable genotypes for the local environment conditions.
2. DREB Expression could be a useful tool to select for drought tolerance, so the above-mentioned genotypes with high gene expression are recommended to be used in other environments and under different treatments in order to select suitable and stable genotypes.
3. Using genotypes with high DREB gene expression in breeding programs to produce hybrids with high drought tolerance.
4. Evaluate introduced genotypes regarding to traits related to water stress such as chlorophyll, antioxidants, ABA content, etc.

Chapter Six: References

6. References

(http://www.fao.org/worldfood_situation/csdb/en/).

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Appendix (1): Variance analysis of the genotypes wheat studied represented by the mean of the squares (M.S).

SOV	DF	No Of Days from planting until 50% Flowering	Plant Height (cm)	Spike length (cm)	Flag leaf Area	Tillers No per M ²	Dry Weight	Spikes number per M ²	No of grains per spike	Fertility Ratio	1000 grain weight	Grain yield	Harvest Index
Replicates	2	16.40	19.08	1.10	163.09	30041.99	31349.48	20.94	31349.48	0.26	1.74	6.91	9.66
Irrigation Interval (a)	1	1.56	98.32	0.71	142.95	60188.04	8423.42	124.14	8423.42	0.54*	65.21*	1.30	46.02*
E(a)	2	0.27	8.95	0.23	146.84	8502.82	687.40	24.63	687.40	0.003	2.92	0.48	2.31
Genotypes (b)	23	2.15*	114.05*	2.84*	132.37*	29968.77*	12517.99*	131.11*	12517.99*	0.31*	43.29*	2.20*	18.93*
AB	23	5.20	56.76	0.42	36.45	12266.57	2479.59	10.70	2479.59	0.07	4.34	0.89	15.45*
E(b)	92	6.39	44.88	0.31	31.97	13347.54	3616.99	15.07	3616.99	0.10	2.98	1.10	7.79

* significant at 5% level

Appendix (2). The correlation coefficient for the traits of studied wheat genotypes.

	Chl SPAD	50% F	PH (cm)	SL (cm)	FLA (cm ²)	TN (m ⁻²)	DW (g m ⁻²)	TGW (g)	SN (m ⁻²)	GF/ day	DPM / day	HI%	FR %	BYD ton ha ⁻¹	GY ton ha ⁻¹	NGS
Chl (SPAD)	1															
50% F	0.22	1														
PH (cm)	-0.59	0	1													
SL(cm)	-0.39	0.14	0.45**	1												
FLA(cm²)	-0.3	0.38**	0.52**	0.6**	1											
TN /m⁻²	-0.2	-0.07	0.34*	-0.1	-0.06	1										
DW(g m⁻²)	-0.32	0.21	0.82**	0.45**	0.64**	0.35*	1									
TGW(g)	0.06	-0.03	-0.34	-0.27	-0.08	-0.5	-0.34	1								
SN (m⁻²)	-0.14	0.03	0.39**	-0.07	0.09	0.87**	0.55**	-0.34	1							
GF/ days	-0.55	-0.59	0.46**	0.26	0.18	0.4**	0.36*	-0.29	0.29*	1						
DPM/days	-0.36	0.45**	0.51**	0.44**	0.61**	0.36*	0.62**	-0.35	0.35*	0.46**	1					
HI %	0.18	-0.23	-0.46	-0.62	-0.59	-0.02	-0.63	0.4**	-0.09	-0.14	-0.41	1				
FR %	0.2	-0.08	-0.08	-0.03	0	-0.49	-0.14	0.21	-0.45	-0.14	-0.25	0.26	1			
BYD ton ha⁻¹	-0.32	0.18	0.8**	0.35*	0.6**	0.38**	0.97**	-0.25	0.6**	0.39**	0.63**	-0.43	-0.06	1		
GY ton ha⁻¹	-0.28	0.13	0.73**	0.17	0.47**	0.41**	0.85**	-0.13	0.62**	0.38**	0.56**	-0.15	0.03	0.95**	1	
NGS	-0.21	0.04	0.5**	0.52**	0.5**	-0.28	0.4**	-0.21	-0.32	0.31*	0.38**	-0.21	0.53**	0.42**	0.39**	1

Appendix (3). Rainfall amount, humidity, maximum and minimum temperatures, and their average for a growing season 2019-2020.

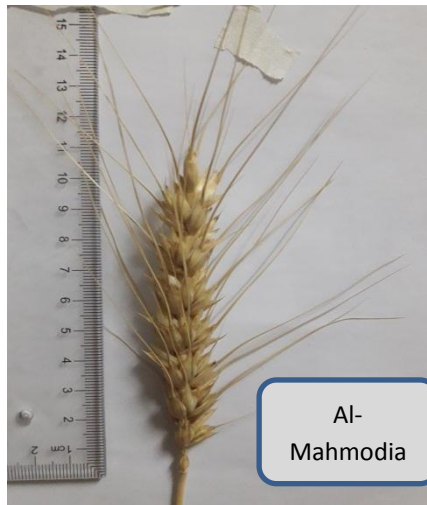
Month	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.
Amount of rain (mm)	0.60	0.81	0.87	0.85	0.07	0.05	0.00
Average temperature °C	10.62	8.24	9.87	15.89	21.33	28.10	32.37
Maximum temperature °C	16.63	13.97	16.20	22.26	27.62	35.36	39.41
Minimum temperature °C	6.37	3.97	4.46	10.39	15.04	20.48	25.05
Average relative humidity %	65.46	68.10	54.39	52.70	42.15	26.51	21.75
Average wind speed at a height of 2m (m/s)	2.54	2.46	2.75	2.86	2.88	3.61	3.78

*Climatic data were taken from the NASA website

Appendix (4). Pictures showing the spike length of genotypes







الخلاصة

أجريت تجربة حقلية في حقل خاص لأحد المزارعين في مدينة هيت / منطقة قنان في محافظة الأنبار غربي العراق الواقعة على دائرة العرض (33° 39) شمالاً وخط الطول (42° 47) شرقاً خلال فصل الشتاء (2020/2019). لتقدير التعبير الجيني في تراكيب وراثية من الحنطة المدخلة الى محافظة الأنبار تحت تأثير ظروف الاجهاد المائي. وشملت التجربة 24 تراكيب وراثية للقمح ومعاملتي ري (مروية وجافة). المعاملة الأولى (المروية) طبقت بشكل طبيعي حتى النضج الفسيولوجي، واما الأخرى معاملة (الجفاف) تم قطع الري بعد الإزهار. أعدت التجربة بترتيب القطع المنشقة بتصميم القطاعات العشوائية الكاملة (R.C.B.D) بثلاثة مكررات. احتلت معاملات الري الالواح الرئيسية بينما تم وضع التراكيب الوراثية في الالواح الثانوية. أظهرت النتائج أن التراكيب الوراثية استجابت بشكل مختلف للمعاملات وفقاً للصفات المقاسة. سجل التركيب الوراثي العراق تعبيراً عالياً عن جين DREB 1A بلغ (221.88 ضعفاً) تليه التراكيب الوراثية 39 و 24 و 6 و 28 و 25 و 20 في معاملة الجفاف. صفة عدد الايام من الزراعة الى 50 % تزهير فإن التركيب الوراثي 11 تفوق بأقل عدد ايام للتزهير بلغ 103.00 يوم. لصفة ارتفاع النبات فقد تفوق التركيب الوراثي 6 بإعطاء اعلى معدل بلغ 94.08 سم. تفوق التركيب الوراثي العراق في مساحة ورقة العلم بأعلى متوسط بلغ (35.62 سم²). لصفة طول السنبله حقق التركيبيان الوراثيان العراق و 6 اعلى متوسط بلغ 11.82، 11.08 سم على التوالي. تفوق التركيب الوراثي 3 في صفة عدد الاشطاء وسجل معدل بلغ 495.33 شطاً م². أما صفة الوزن الجاف فكان التركيب الوراثي 29 متفوقا وسجل 666.66 جم. م². في حين سجل التركيب الوراثي 41 معدل مرتفع لعدد السنابل بلغ 499.11 سنبله م². أظهر التركيب الوراثي 6 تفوقاً في عدد الحبوب في السنبله (57.48 حبة سنبله⁻¹). في حين سجل تفوق في صفة نسبة الخصب بواسطة التركيب الوراثي 39 بمعدل بلغ 3.37%. تفوق التركيب الوراثي 18 في صفة وزن الألف حبة بأعلى متوسط بلغ 56.65 غم. سجل اختلاف معنويًا في صفة حاصل الحبوب، فقد تفوق التركيبيين الوراثيين 3 و 29 بإعطاء اعلى متوسط للصفة بلغ 7.39 و 7.29 طن هكتار⁻¹. التركيب الوراثي 20 تفوق معنويًا في صفة دليل الحصاد بمعدل بلغ 57.57%. نستنتج من هذه الدراسة ان التراكيب الوراثية التي تفوقت في إعطاءها اعلى تعبير جيني تحت ظروف معاملة الجفاف يشير الى قدرتها في تحمل اجهاد الجفاف اكثر من بقية التراكيب الوراثية، مما يدل على ملائمتها لظروف البيئة التي زرعت فيها.



جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة الانبار
كلية الزراعة
قسم المحاصيل الحقلية

التعبير الجيني لجين DREB في تراكيب مدخلة وراثية من الحنطة كاستجابة لقطع الري في المراحل الأخيرة من النمو

رسالة مقدمة الى مجلس كلية الزراعة/ جامعة الانبار

وهي جزء من متطلبات نيل درجة الماجستير

في العلوم الزراعية (المحاصيل الحقلية)

من قبل

مريم لؤي منصور

بإشراف

د. جلال ناجي محمود

أ.م.د. محمد حمدان عيدان العيساوي

2021 م

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