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PERFORMANCE AND TRANSCRIPTOMIC ANALYSIS OF *SORGHUM BICOLOR* RESPONDING TO DROUGHT STRESS

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SUMMARY

Drought is a severe causal factor of reduced crop yields than other abiotic stresses. Therefore, four sorghum genotypes underwent evaluation for their drought tolerance under three irrigation levels (100%, 70%, and 40% from evapotranspiration) under three sowing dates at the Higher Institute for Agricultural Cooperation Farm, Regwa region, Alexandria Desert Road, Egypt, to study the mean performance of grain yield plant^{-1} and transcriptomic analysis. The performance results reported that Shandaweel-6 and Hybrid-306 revealed the highest drought-tolerant hybrids, while Dorado and Giza-113 cultivars showed the lowest. The transcriptomic profiling of sorghum under normal and drought stress used the RNA-Seq method. Two differentially-expressed genes (DEGs) in leaves respond to drought. In the DREB2 case, the greatest average fold change showed 10.7 and 9.3 for the tolerant hybrids, Hybrid-306 and Shandaweel-6, respectively. Both genotypes performed significantly higher than the average fold change calculated for the sensitive genotypes Dorado and Giza-113 cultivars. This study contributes to a better understanding of the molecular basis of drought tolerance of sorghum and promotes sorghum improvement.

Keywords: *Sorghum bicolor,* DREB2, CBF4, transcriptomic, cDNA, transcription factors, drought stress

Key findings: Sorghum hybrids Shandaweel-6 and Hybrid-306 received classification as drought-tolerant because of their best performance under drought stress and normal conditions. Both hybrids possess resistance genes for drought and other abiotic stresses. However, the cultivars Dorado and Giza-113 proved susceptible to drought stress due to their weak performances under drought-stress conditions.

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INTRODUCTION

Sorghum (*Sorghum bicolor* L. Moench) is an essential cereal crop grown globally for feed and food demands. Over the world, it ranks as the sixth most planted crop after wheat, maize, rice, soybean, and barley (FAOSTAT, 2021). Grown in arid and semi-arid tropical regions (Abdelhalim *et al*., 2021; Gano *et al*., 2021; Kebbede *et al*., 2021; Kumar *et al*., 2021a; Kumar *et al*., 2021b), cultivation of sorghum takes place in diverse geographic areas of America, Africa, Asia and Oceania (USDA, 2020; Yali, 2022). It has an inherent ability to

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adapt to a harsh climate, growing well in dry conditions and tolerating water stress, thus making it ideal for cultivation in the arid and semi-arid regions of the world. Well adapted to warm regions and, given its elasticity, sorghum can grow in temperate and tropical areas. Still, its production comes up against several constraints that lead to low yields, such as, irregular rainfall distribution exacerbated by climate change, low soil fertility and sandy soils, and various crop diseases and pests (Balcerek *et al.*, 2020; Das *et al.*, 2020; Kalaria *et al*., 2020; Naroui *et al.*, 2020; Gano *et al.*, 2021; Kumar *et al.*, 2021b).

It grew globally to over 40.07 million ha with a production of 57.89 million t and productivity of 1,444 kg ha^{-1} (FAOSTAT, 2021). The USA leads the world production with 9.47 million MT, followed by Nigeria with 6.9 and Ethiopia with 5.2 million MT (USDA, 2020). Sorghum serves as a staple crop in developing countries of Africa, Central America, and South Asia. It is the foremost food of about 500 million people in 30 countries (Abdelhalim *et al.*, 2019; Malick *et al.*, 2019; Kumar *et al.*, 2021b; Yali, 2022). In such terminal drought environments, with crops lacking water during grain filling, sorghum hybrids display high levels of stay-green, an ability to retain green leaf area during grain filling, generally producing higher grain yield than those with the intermediate or low phenotypic expression of the trait (Derese *et al.,* 2018; Nida *et al.,* 2019; Yitayeh *et al.,* 2019; Ananda *et al.*, 2020; Kumar *et al.*, 2021a).

Grain sorghum ranks fourth in Egypt's major cereal crops (Hafez *et al*., 2021; Abd El-Mageed *et al*., 2022) in terms of area and production, next to wheat, rice, and maize. It has a cultivated area of about 150,811 ha, producing about 802,128 t grains (Economic Affairs Sector, 2020). Most grain sorghum cultivated areas in Egypt center in Assiut and Sohag governorates (Upper Egypt), where the atmospheric temperature during the growing season is high since grain sorghum tolerates high temperatures more than maize (Al-Naggar *et al*., 2018; Ibrahim *et al*., 2019). Moreover, the El-Fayoum province has a sizeable cultivated area due to soil problems, such as salt, drought, and low fertility, in addition to heat stress, which prevent the cultivation of other crops (Reddy *et al*., 2014; Rini *et al*., 2017; Ibrahim *et al*., 2019; Rifka *et al*., 2020). One of the most drought-tolerant grain crops, sorghum is an excellent crop model for studying the mechanism of drought tolerance. The ability to survive and tolerate water stress conditions make it the most promising crop for improving water use efficiency among other cereal crops.

It is decisive to develop and adapt new technologies to expose variability among sorghum genotypes for stress resistance and to identify the best genotypes that can increase the water use efficiency under environments of low water supply. Improving the expanding breeding programs for increased water use efficiency in grain sorghum is critical to efficiently utilizing Egypt`s water resources (Al-Naggar *et al*., 2018; Ibrahim *et al*., 2019; Badran, 2020; El-Kady *et al*., 2020). Drought stress affects crop plants' growth and productivity, including sorghum (Attia *et al*., 2021; Mwamahonje *et al.,* 2021a and b). The most severe environmental stress dramatically limiting crop yields impacts crop growth and productivity, causing losses estimated at US$2.9 billion annually (Badran, 2020; Naroui *et al.*, 2020; Ocheing *et al.,* 2020; FAOSTAT, 2021). Sorghum production faces a problem in semi-arid tropics where insufficient rainfall has an irregular distribution (Eggen *et al.,* 2019; Naroui *et al.*, 2020; Ocheing *et al.,* 2020; Yali, 2022; Zhou *et al.,* 2022). Alleviating this problem requires developing new cultivars or hybrids resistant to drought and adapted to dry conditions, such as, the newly reclaimed soils at Toshky and Darb El-Arbain in Upper Egypt (El-Kady, 2015).

The response of plants to water stress depends on several factors, such as development stage, intensity and duration of stress, and cultivar genetics (Naroui *et al.*, 2020). The plant response shows complexity because it reflects the integration of stress effects and response at all underlying levels over space and time. Water stress inhibits the photosynthesis of plants, causes changes in chlorophyll content and components, and damages the photosynthetic mechanism (Abraha *et al*., 2015). Also, it inhibits the photochemical activities and decreases the enzyme actions in the Calvin cycle in photosynthesis (Amelework *et al*., 2015; Hadebe *et al.*, 2017; Seyoum *et al.*, 2019; Naroui *et al.*, 2020; Zhou *et al.*, 2022).

Sorghum presents an excellent model for studying the molecular basis for abiotic stress tolerance (Seyoum *et al.*, 2019). Its genome showed 730 Mbp with a small diploid genome and a low level of gene duplication. DNA transposons constitute 7.5% of the sorghum genome (Rakshit and Wang 2016). The genome of sorghum has a small, uniquely sequenced diploid (Mace *et al*., 2013). The ERF, NAC, WRKY, HD-ZIP, bHLH, and MYB transcription factors had different expression patterns under drought stress in sorghum (Zhang *et al.*, 2019). Transcription factors play significant roles in regulating gene expression in response to drought. Major plant transcription factor families, such as, DREB1/CBF, DREB2, AREB/ABF, NAC, AP2/ERF, and MYB, have been documented as key regulators in plant responses to various abiotic stresses (Tawfik and El-Mouhamady, 2019). This study aimed to detect the most drought-tolerant sorghum genotype using grain yield traits and DREB2 and CBF4 genes as molecular markers with real-time PCR.

MATERIALS AND METHODS

The latest investigation took place at the Higher Institute for Agricultural Cooperation Farm, Regwa region, Alexandria Desert Road, Egypt (30°11′12.0′′N, 30°34′32.7′′E), during the summer seasons of 2019 and 2020, and the Molecular Genetics Laboratories, Genetics Department, Faculty of Agriculture, Ain Shams University, Shoubra El-Khema, Cairo, Egypt, during June 2022.

The Grain Sorghum Research Department, Agricultural Research Center (ARC), Egypt, provided four genotypes of the sorghum grain (*Sorghum bicolor* L. Moench)—Dorado and Giza-113 cultivars, hybrid Shandaweel-6, and Hybrid-—serving as the breeding materials**.** The name, origin, and pedigree of these genotypes appear in Table 1. According to the FAO Penman-Monteith method for estimating evapotranspiration, sorghum genotypes evaluation for drought tolerance consists of three irrigation levels: 100%, 70%, and 40% from evapotranspiration, as described by Allen *et al.* (1998). Sowing of grain sorghum genotypes took place on 13 July in the first season (2019), while in the second season, sowing grain sorghum genotypes consisted of two dates. The early-date sowing occurred on 13 July 2020, while the late date happened on 15 August 2020.

The experimental design employed a randomized complete block design (RCBD) with three replications. Each replication has three rows. The row length measured 11 m, and the distance between each experiment of irrigation treatment measured 3 m in width. The distance between plant hills was 20 cm with thinning, leaving two plants hill^{-1}. Table 2 shows the amounts of irrigation water m^3 ha^{-1} for each date**.** The agriculture practices followed used the recommended sorghum production, except for irrigation treatments throughout the growing seasons.

Table 1. Names, pedigree, and origins of four sorghum genotypes used in the study.

No	Genotype	Pedigree	Origin
P1	Dorado	American introduced variety	U.S.A
P2	Giza-113	Giza 15 × Giza 114	Egypt
P3	Shandaweel-6	ICSA-631 × Dorado	Egypt
P4	Hybrid-306	ICSA-1 × ICSR-93002	Egypt

Table 2. Amounts of irrigation water m^3 ha^{-1} according to FAO Penman-Monteith method**.**

Irrigation levels	Sowing dates	Growing season	m^3 ha^{-1}
40%	13th July	2019	1855.12
70%	13th July	2019	3246.45
100%	13th July	2019	4637.78
40%	13th July	2020	1899.12
70%	13th July	2020	3323.45
100%	13th July	2020	4747.78
40%	15th August	2020	1345.78
70%	15th August	2020	2355.12
100%	15th August	2020	3364.45

Data collection

At harvest, random samples of 20 guarded plants from each plot took place, recording the data for grain yield plant^{-1} (g). Calculating the drought tolerance index (DTI) used the formula according to Fernandez (1992).

Molecular genetics experiment

Selective PEG experiment

According to data analysis of the preliminary experiment, four sorghum genotypes (*Sorghum bicolor* L. Moench) were selected: two represented the drought-tolerant genotypes (Hybrid Shandaweel-6 and Hybrid-306). Conversely, the two drought-susceptible sorghum genotypes include Dorado and Giza-113 cultivars. The selected genotypes underwent germination in the RCBD, evaluating them under normal and drought conditions (15% w/v PEG^{6000}). Germination occurred in a sawdust-filled plastic pot slit in an aluminum tray filled with water slightly exceeding the pots' side slits, maintaining the level throughout the experiment. Germination occurred in dark conditions for the first three days without treatments. After 15 days, fresh leaves of each genotype were ground immediately in liquid nitrogen for a complete homogenization and stored at -20°C for molecular analysis.

RNA isolation

Total RNA extraction from the stored leaves used the In vitrogen TRIzol^{TM} Reagent following the manufacturer's manual. RNA integrity validation resulted from electrophoresis on a 2% agarose gel, and RNA concentration and purity determination used a NanoDrop spectrophotometer device. The 260/280 ratio was around 2, confirming a pure RNA. DNA contamination removal in samples used a TURBO DNA-free kit, according to the manufacturer's protocol.

cDNA synthesis and quantitative PCR

The concentration of mRNA dilution to a final concentration of 100 ng µl^{-1} used RNase-free distilled water. The evaluation of the relative expression of *DREB2, CBF4*, and *Ubiquitin* genes in every sorghum genotype employed the Rotor-Gene Q (Qiagen, UK). Sequences used *DREB2* Forward: 5′-GCGTACAACACCTTGATTTCC-3′ and *DREB2* Reverse: 3′-AAACTCAACTCCATCTAAGC-5′; *CBF4* Forward: 5′-TCGTACTACGCGAGCTTGGCGC -3′ and *CBF4* Reverse: 3′-TGTGCCCTTCCGGGAGTAGAAACC-5′; *Ubiquitin* Forward: 5′- GCACCTTGGCGGACTACAACATTC-3′ and *Ubiquitin* Reverse: 3′-GACACCGAAGACGAGACTTGTGAACC-5′.Performing a one-step RT-PCR utilized the Quanti Nova PCR Kits (Qiagen, UK), according to the manufacturer's protocol. The qPCR program setting is as follows: reverse transcription step at 48°C for 45 min; followed by an amplification step for 5 min at 95°C; 55 cycles each consists of three steps as follows: step (1) 10 s at 95°C, step (2) 20 s at 54°C, and finally 72°C at 10 s. Eventually, melting curve analysis took place to validate the presence of only one single amplicon of interest. The ubiquitin gene served as a housekeeping gene, and the fold expression of the target genes was normalized accordingly. Determining the relative fold changes by the 2-ΔΔCt formula followed the Livak method (Livak and Schmittgen, 2001).

RESULTS AND DISCUSSION

Performance of genotypes under different environments

The mean performance of grain yield plant^{-1} of four sorghum genotypes under three irrigation levels and three sowing dates appeared in Table 3. The combined data of July 2019, July 2020, and August 2020 (Average of the three irrigation levels: 40%, 70%, and 100% optimum water irrigation level) showed that Hybrid-306 gave the highest grain yield (75.3, 78.56, and 76.38 g plant^{-1}, respectively). Moreover, the combined data of the hybrid Shandaweel-6 showed that the grain yields were 72.69, 78.11, and 75.38 g plant^{-1}, respectively. On the other hand, a notable opposite trend resulted for the two cultivars: Dorado and Giza-113. The data for Dorado cultivar revealed 39.78, 42.88, and 40.23 g plant$^{-1,}$ respectively, under the same conditions mentioned above. Also, Giza-113 cultivar grain yields combined data showed 30.08, 32.08, and 30.36 g plant^{-1}, respectively.

In conclusion, these results indicate that two genotypes: Hybrid Shandaweel-6 and Hybrid-306 could be considered the most drought tolerant, which can be used in breeding programs to improve sorghum productivity under drought stress. In contrast, Dorado and Giza-113 proved susceptible to drought-stress conditions.

Table 3. Mean performance of grain yield /plant (g) of four sorghum genotypes (G) under three irrigation levels (I) and three sowing dates (D) in the 2019 and 2020 growing seasons.

Grain yield Plant^{-1} (g)													
	Season 2019				Season 2020								Average
	July 2019				July 2020				August 2020				
G	40%	70%	100%	Combined	40%	70%	100%	Combined	40%	70%	100%	Combined	
Dorado	34.67	39.71	44.96	39.78	35.66	43.61	49.36	42.88	32.77	40.8	47.12	40.23	40.96
Giza-113	25.52	30.81	33.92	30.08	24.58	32.49	39.17	32.08	23.19	30.54	37.36	30.36	30.84
Shandaweel-6	69.94	72.36	75.76	72.69	73.64	78.39	82.3	78.11	68.99	76.59	81.46	75.68	75.49
Hybrid-306	71.98	75.19	78.72	75.3	70.57	80.05	85.05	78.56	69.36	76.36	83.42	76.38	76.75
Mean	48.94	53.87	57.83	53.55	49.86	57.26	62.49	56.54	47.48	55.21	60.86	54.52	54.87
LSD$_{0.05}$													
D													0.19
I				0.43				0.29				0.27	0.19
G	1.16	1.03	1.1	0.6	0.65	0.83	0.87	0.4	0.96	0.71	0.51	0.38	0.27
ID													0.33
GD													0.47
GI				1.04				0.7				0.66	0.47
GID													0.81

Table 4. Drought tolerance index (DTI) of four-grain sorghum genotypes under three irrigation levels and three dates over the two growing seasons.

Genotypes	Drought tolerance index		
	40%	70%	Combined
Dorado	72.89	87.75	80.32
Giza-113	66.36	84.96	75.66
Shandaweel-6	88.75	94.91	91.83
Hybrid-306	85.73	93.69	89.71
Mean	78.4325	90.3275	84.38

Drought tolerance index

The combined data of the drought tolerance index (Table 4) showed that hybrid Shandaweel-6 and Hybrid-306 ensued as the best genotypes, with 91.83% and 89.71%, respectively, rather than the worst cultivars Dorado and Giza-113, with 80.32% and 75.66%, respectively. These results agree with the findings of the behavior of the genotypes under different irrigation levels. Drought is a major constraint in sorghum production and is considered the most important cause of yield reduction in crop plants, including sorghum (Naroui *et al.*, 2020; Badran, 2022). Drought severely reduces grain yield in many cereal-growing regions, which results in fluctuations in the world food supply (Malick *et al.*, 2019). Water stress decreased grain yield plant^{-1} (Hafez *et al*., 2021).

The reductions in the average grain yield plant^{-1} due to drought conditions for the females, R-lines, and crosses under severe water stress 70% were 7.72, 7.53, and 10.84 g, respectively (El-Kady, 2015). Drought severely reduces grain yield in many cereal-growing regions, which results in fluctuations in the world food supply. Grain yield reduction is between 10% and 17% when drought stress occurs shortly before the booting stage through anthesis until the soft dough stage (Malick *et al.*, 2019). Drought stress decreased grain yield by 30.77% and 9.80% for cultivars and hybrids, respectively (Gangadasari *et al.,* 2020). Under post-anthesis water stress, shifting even small amounts of water from pre- to post-anthesis can substantially increase grain yield (Kapoor *et al.,* 2020). Drought stress at any stage of development might significantly affect yield reduction, decrease the number of irrigation, and reduce grain yield (El-Samnoudi *et al.,* 2019).

Grain yield decreased in all the genotypes as the crop got subjected to progressive drought stress under a receding soil moisture situation (Kidanemaryam *et al.,* 2020). Grain yield was reduced from 51.67 for the control irrigation to 40 and 33.57 ard ha^{-1} (This is unclear) for numbers 3 and 4 irrigation treatments, respectively (Shaikh *et al.,* 2021). Water stress decreased grain yield plant^{-1} (Sakhi *et al.,* 2014; Belay and Meresa, 2017; Kidanemaryam *et al.,* 2020). Drought stress decreased grain yield at 40% ET; from 24.46% and 22.87% for hybrids in 2010 and 2011; from 24.77% and 28.35% for lines in 2010 and

2011, respectively (Mahmoud *et al.,* 2013); from 23.77% and 25.17% for B-Lines and R-Lines, respectively in 2009; from 27.34%, 26.74%, and 23.11% for hybrids, B-Lines, and R-Lines, respectively in 2011; from 27.39%, 26.33%, and 22.71% for hybrids, B-Lines, and R-Lines, respectively in 2012 (El-Kady, 2015).

Reports of the significant effects the water regimes, genotypes, and their interactions for grain yield per plant occurred. In stress IR1 (withholding irrigation at the three-leaf stage) in the first season of 2014 and full watering in the second season of 2015 produced the highest grain yield plant (45.2 and 50.9 g, respectively). Watered plants and stressed plants three-leaf stage had more grain yield than stressed plants at the eight-leaf stage due to pollination susceptibility to water stress and seed weight (Jabereldar *et al.,* 2017). Under water stress (65% ET), grain yield decreased by 22.79% and 28.15% in 2018 and 2019, respectively (Gano *et al*., 2021). Drought stress at 70% ET decreased grain yield from 12.69%, 13.74%, and 11.68% for hybrids, B-Lines, and R-Lines, respectively, in 2011, and from 13.12%, 14.7%, and 12.6% for hybrids, B-Lines, and R-Lines, respectively, in 2012 (El-Kady, 2015). Increased levels of water stress caused adverse effects on grain yield, with an apparent variance among tested genotypes under water stress levels (Badran, 2020; Abebe, 2021). The sorghum yield exhibited significant differences for different sowing dates (Besheit *et al.*, 2019).

Increases in grain sorghum yield require growing of the genotypes Shandwel-6 and Hybrid-306. High values of vegetative growth, yield and their components, photosynthesis portioning, and migration toward the economic yield characterized these hybrids, compared with the Dorado cultivar (Ahmed *et al.,* 2013). The results of the drought tolerance index of grain yield plant^{-1} cleared that the different genotypes differed significantly in their response to water stress. Some genotypes scored a drought tolerance index over 76% at 40% ET, which could be considered drought tolerant, while some severely got affected by drought (Mahmoud *et al.,* 2013). The DTI for grain yield showed that some genotypes scored a DTI over 88% at 40% ET, while Dorado (DTI) value displayed 65.82% (EL-Kady, 2015).

Gene expression responses to drought

Gene expression quantification is a widely applied molecular genetics technique to measure gene expression level based on a housekeeping gene to contrast its molecular-chemical status, whether up-regulated or down-regulated. Additionally, using contrasting genotypes, qPCR can distinguish relative gene expression between both genotypes and the same genotype under different conditions (control *versus* treatment). The latest analysis applied the latter case: two drought-sensitive varieties along with two drought-tolerant hybrids that showed contrasting tolerance levels in the field under drought conditions. Two genes were measured and identified as transcription factors, known as DREB2 and CBF4. Both genes were previously reported as molecular markers for abiotic stress tolerance.

The collected fresh leaves underwent DNA and RNA extraction and isolation. A total of four samples included 01 and 02, representing the drought-tolerant hybrids (Hybrids-306 and Shandaweel-6), and 03 and 04 representing the drought-sensitive genotypes (Dorado and Giza-113). All genotype extractions succeeded (DNA, Figure 1 left, and RNA, Figure 1 right). The DNA and RNA showed sufficient quality on the agarose gel, where a single dense band occurred in all DNA samples and several ribosomal bands displayed in all RNA samples.

Figure 1. Nucleic acid extraction and isolation from four sorghum genotypes (01: Hybrid-306, 02: Shandaweel-6 hybrid, 03: Dorado cultivar, 04: Giza-113 cultivar). DNA extraction agarose gel electrophoresis (left), RNA isolation agarose gel electrophoresis (right).

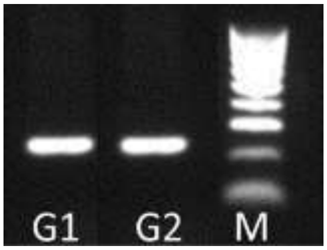

Figure 2. PCR amplification of DREB2 (G1) and CBF4 (G2) success on the extracted DNA samples at 55°C annealing temperature.

PCR amplification

Performing this step ensured the effectiveness of the applied primers for amplification and optimized the annealing temperature of each gene to avoid mismatches or multiple-band amplification. Sorghum Hybrid-306 was used as a sample to perform the PCR optimization step. The PCR revealed fruitful for the two primer pairs (*i.e.,* two genes), and both pairs effectively bind to the DNA, resulting in a single amplified band (Figure 2).

Real-time PCR (qPCR)

RNA conversion into cDNA used reverse transcription to quantitatively analyze the gene expression (qPCR) of the DREB2 and CBF4 genes for all tested genotypes, along with the ubiquitin as a house-keeping gene as an indicator to know the extent of the change in the gene expression of the drought tolerance capacity. The quantitative gene expression analysis showed effective for all triplicates of each genotype. After the real-time PCR run, the obtained CT values were used to estimate ΔCT and the fold change of gene expression of DREB2 and CBF4 using subtractive equations.

By extracting the CT values for all the triplicated samples, the CT values were observed between 19 and 21 for the Ubiquitin gene (HKG; as an indicator) and ranged from 20 to 23 for both the DREB2 and CBF4 transcription factors. The CT values of the transcription factors were subtracted from the CT values of the HKG independently to define the changes in CT values (ΔCT). The ΔCT values ranged from -4 to zero, where each ΔCT was negatively powered to number 2 (*i.e.,* anticipated duplication of DNA strand by PCR) to estimate the relative rate of change in the gene expression (fold change) for each replicate. The fold change average of the triplicates underwent calculation to represent each genotype per quantified genes. In the DREB2 case, the highest fold change average was 10.7 for the tolerant Hybrid-306 and 9.3 for the tolerant hybrid Shandaweel-6. Both genotypes were significantly higher than the average fold change calculated for the sensitive genotype Dorado and Giza-113. In the case of CBF4, the average fold change was between 2.0 and 2.7, with no significant difference between the four genotypes (Table 5 and Figure 3).

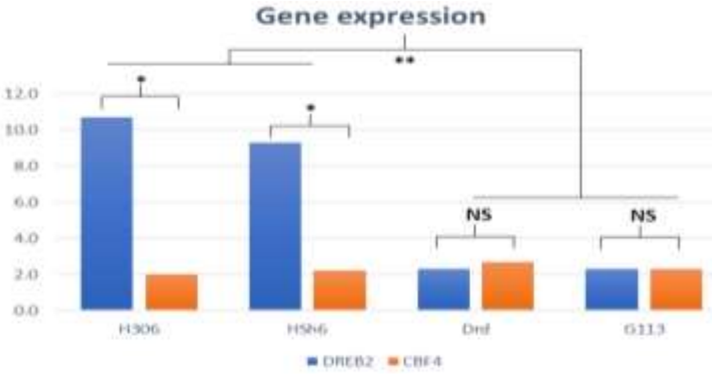

Figure 3. A histogram represents the relative expression (avgFC) of DREB2 and CBF4 transcription factors in sorghum genotypes under drought stress conditions (*<0.05; **<0.01).

Table 5. The CT values, delta-CT, and ratio of relative expression of DREB2 and CBF4 for each tested genotype.

Gene	Genotype	Rep.	HKG	Gene CT	ΔCT	Fold Change	AvgFC
DREB2	Hybrid-306	R1	19	22	-3	8	10.7
		R2	19	23	-4	16	
		R3	20	23	-3	8	
	Shandaweel-6	R1	21	23	-2	4	9.3
		R2	20	23	-3	8	
		R3	19	23	-4	16	
	Dorado	R1	21	21	0	1	2.3
		R2	20	22	-2	4	
		R3	21	22	-1	2	
	Giza-113	R1	21	22	-1	2	2.3
		R2	21	23	-2	4	
		R3	21	21	0	1	
CBF4	Hybrid-306	R1	19	20	-1	2	2.0
		R2	19	20	-1	2	
		R3	20	21	-1	2	
	Shandaweel-6	R1	21	20	1	0.5	2.2
		R2	20	22	-2	4	
		R3	19	20	-1	2	
	Dorado	R1	21	22	-1	2	2.7
		R2	20	22	-2	4	
		R3	21	22	-1	2	
	Giza-113	R1	21	23	-2	4	2.3
		R2	21	22	-1	2	
		R3	21	21	0	1	

Transcription factors play essential roles in regulating gene expression in response to drought. Major plant transcription factor families, such as, DREB1/CBF, DREB2, AREB/ABF, NAC, AP2/ERF, and MYB, have been documented as essential regulators in plant responses to various abiotic stresses (Tawfik and El-Mouhamady, 2019). Sixty-six DEGs of transcription factors responding to mild drought, severe drought, and re-watering treatments in roots were identified, although only four DEGs of transcription factors were identified in leaves. These DEGs were mainly classified into HSF (six genes up-regulated), ERF (six up-regulated and three down-regulated), NAC, WRKY, HD-ZIP, bHLH, and MYB transcription factor families (Zhang *et al.*, 2019). The ERF superfamily plays an essential role in plant systems' biotic and abiotic stress responses. In addition to ERF, chloroplast glutathione reductase (cpGRs), G-protein complexes, drought response element-binding (DREB) proteins, and SbEST are reported to play significant roles in abiotic stress response not only in sorghum but in other plant species as well. Three ERF genes, one NAC gene, five WRKY genes, four HD-ZIP, seven bHLH, and three MYB genes were down-regulated under drought stress and up-regulated under re-watering treatment. The ERF, NAC, WRKY, HD-ZIP, bHLH, and MYB transcription factors had different expression patterns under drought stress in sorghum roots. Additionally, two growth-regulating factor genes (SB04g034800 and Sb01g009330) were down-regulated under drought stress and up-regulated under re-watering treatment, indicating that drought stress negatively impacts the growth and development of sorghum roots (Zhang *et al.*, 2019).

Gene expression profiling through microarrays has successfully identified genes regulating crop drought resistance. Most drought-responsive genes identified from transcriptomics are classified into ABA-dependent, ABA-independent, and DREB2A/ubiquitination-related mechanisms. Genes associated with the production of osmolytes, amino acids (proline), and amines (glycine betaine and polyamines) are differentially expressed in response to drought stress. Proteomics, the systematic analysis of (differentially) expressed proteins, serves as a tool for identifying proteins involved in cellular processes. Proteomics provides information on the amount of the gene products, their isoforms, and which post-transcriptional modifications regulate protein activation. Proteomics in different plant tissues has identified several drought-responsive proteins.

Plant metabolism gets highly altered in response to drought, and downstream transcript-level changes lead to the alteration in the quality and quantity of various metabolites. Metabolic profiling can give an instantaneous snapshot of the physiology and biochemical changes in the cell. In addition to gene transcripts, proteins, and metabolites, a study reported small RNAs (miRNAs, siRNAs) involved in adaptive responses to abiotic stresses (Abebe, 2021). Genetic engineering has been successfully applied to identify and transfer different genes responsible for the biosynthesis of various metabolites, such as, proline, tetrahalide, and polyamines, from different organisms to crop plants through a targeted approach. Exploration of wide genetic variation of relevant characters, consideration of more genes at a time to transfer through breeding or genetic engineering method, application of antisense RNA technique, assessment of polypeptides induced under drought, and multidisciplinary approach needs inclusion in future research programs for drought resistance. The applications of genetic engineering of food crops have already led to improved drought tolerance and increased yield under drought (Kidanemaryam, 2019).

The genetic mechanisms for the expression of drought tolerance in crop plants are poorly understood. Since drought tolerance is a complex trait controlled by many genes and depends on the timing and severity of moisture stress, it is one of the most challenging traits to study and characterize (Naeem *et al*., 2015; Asrat, 2022). The high genetic variability among sorghum genotypes and its relatively small genome size makes it a good model for identifying drought-related genomic regions and genes to unravel the high complexity of drought tolerance-related traits (Fracasso *et al.,* 2016). Consequently, improved genotypes with better performance under drought-prone areas are the ultimate solution for farmers facing drought for principal yield reduction (Ulemale *et al.,* 2013). In addition, evaluating newly developed drought-tolerant varieties for essential characteristics, such as, physiology, morphology, and phenological approaches, is useful for understanding crops' responses (Naeem *et al*., 2015). Moreover, improving genotypes tolerant to such environmental conditions provides the ideal way for the quality production of crops (Admas and Tesfaye, 2017).

CONCLUSIONS

Sorghum hybrid Shandaweel-6 and Hybrid-306 can be classified as drought-tolerant hybrids because of their high performance under drought stress and normal conditions, which prove useful in breeding programs to improve sorghum productivity under drought-stress conditions. Both hybrids possess resistance genes for drought and other abiotic stresses.

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