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Genetic Assessment and Performance of Some Rice Genotypes under Water Deficiency Stress

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Abstract

The present study was conducted at the Experimental Farm of Sakha Research Station, located in Sakha, Kafr El-Sheikh, Egypt, during the 2021 and 2022 growing seasons, utilizing seven rice cultivars: Sakha104, Sakha103, Sakha108, Giza178, Giza179, E Hybrid1, and Sakha Super 300. A strip-plot design was employed, featuring three replications for each treatment. The main plots were designated for two irrigation intervals: continuous flooding (irrigation every 4 days) (CF) and drought stress conditions (irrigation every 12 days).The results of this experiment indicated that certain cultivars demonstrated commendable performance under drought conditions, with significant increases observed under continuous flooding compared to the twelve-day irrigation treatment. Under drought stress, E Hybrid1 exhibited the highest values for plant height (cm), number of tillers per plant, flag leaf area (cm²), chlorophyll content, relative water content (RWC), and proline content. Conversely, Giza178 displayed the highest values for traits such as panicle length (cm), panicle weight (g), and grain yield per plant (g). In terms of 1000-seed weight, the cultivar Sakha108 achieved superior weight, while Giza178 recorded the lowest. Additionally, Sakha103 exhibited the lowest values for nearly all measured traits. Overall, the proline levels detected in the rice cultivars studied were positively correlated with the plants' ability to withstand drought stress, as evidenced by enhanced growth. Furthermore, to elucidate the mechanisms by which plants respond and adapt to drought stress, six genes with differentially expressed cDNAs were identified: COX1, PKDP, bZIP1, AP2-EREBP, Hsp20, and COC1. Real-time quantitative PCR analysis confirmed that drought-stressed plants expressed all six genes.

Keywords: Rice, grain yield traits, drought tolerance genes, quantitative real-time PCR.

Introduction

"Rice is life" – This slogan from the International Year of Rice (2004) emphasizes the vital importance of rice (Reddy, Verulkar, and Saxena, 2018). Rice (*Oryza sativa* L.) is one of Egypt's most significant staple crops and the second-largest primary food crop globally, trailing only wheat. It serves not only as a fundamental food source but also as an important source of energy. Due to its semi-aquatic nature, rice flourishes in moist soil, making its production highly water-intensive (Ghazy et al., 2021).

Numerous challenges confront rice cultivation, particularly the scarcity of irrigation water exacerbated by the continuous growth of the population (Asibi et al., 2019; Bandumula, 2018). The rice varieties cultivated in Egypt necessitate substantial irrigation water (16,500 m³/ha). The Nile River can provide only 55.5 million m³/year of irrigation water, a figure that diminishes annually due to competition among the Nile basin countries and the demand for water for agriculture, industry, and human consumption. Consequently, the future of rice cultivation in Egypt hinges on the development of drought-resistant varieties, as the currently cultivated lowland varieties are characterized by their high-water requirements and susceptibility to water deficits (Youssef, Mansour, and Solliman, 2010).

Over the past decade, water shortages have emerged as one of the principal threats to the agricultural economy in Egypt. This issue has constrained rice cultivation to areas deemed sufficient for ensuring food security in the 21st century. During the 2021 agricultural season, the total cultivated area in Egypt amounted to 0.464 million hectares, a decrease from 0.347 million hectares in the 2018 season, representing a reduction of 25.2%. Conversely, the total production during the 2018 season was 3.3 million tons, while production increased to 4.953 million tons of paddy rice in the 2021 season, reflecting an increase of 32.6%. Moreover, the average national production reached 9.98 t/ha during the 2021 season, compared to 8.76 t/ha in the 2018 season, indicating a rise of 12.22%. This increase may be attributed to the initiatives undertaken during the national campaign (RRTC, 2022).

Rice is particularly vulnerable to abiotic stresses such as drought, salinity, heat, and cold, due to its semi-aquatic nature. Drought stress remains the most significant constraint on rice yields, serving as a major contributor to yield loss and instability in rice production across various ecosystems (Bernier et al., 2007).

Drought stress during the reproductive stage can significantly impact both yield and its components. When drought stress occurs shortly after panicle initiation, the number of spikelets formed may decrease, resulting in a reduction in the number of grains per panicle, which is further compounded by a decrease in grain weight, ultimately leading to a decline in grain yield (Pantuwan et al., 2002). The components of yield associated with final grain yield are adversely affected by stress conditions. For instance, panicle length, spikelet per panicle, and grain yield are significantly diminished under drought and heat stress (Mohamed et al., 2021).

To enhance productivity per unit area, several drought-tolerant cultivars have been introduced from overseas. Proline, a non-protein amino acid that accumulates in various tissues subjected to water stress, is readily metabolized upon recovery from drought conditions (Singh et al., 2000). The changes observed in total chlorophyll concentration are consistent with previous findings by Pandey and Shukla (2015), who indicated that the loss or reduced synthesis of photosynthetic pigments during drought stress is a common phenomenon, closely associated with reductions in plant biomass and yield. Gene expression analyses are essential for understanding the mechanisms of drought adaptation. To cope with water deficit conditions, plants regulate the expression of numerous genes (Pabuayon et al., 2016). Each gene has distinct roles in conferring drought tolerance and is predominantly expressed in response to drought stress, primarily involving ABA-dependent pathways. COX1 plays a critical role in regulating energy and carbohydrate metabolism, while Hsp20, bZIP1, and COC1 mediate ABA-dependent signaling pathways. Additionally, PKDP functions as a protein kinase, and AP2-EREBP is involved in stress-related signaling (Manoj, 2019). To enhance rice quality, it is imperative to develop new varieties capable of withstanding abiotic stresses, such as drought and salinity. The objectives of the present study are to investigate the effects of drought stress on grain yield traits and to examine the expression of drought tolerance genes in the rice cultivars under investigation.

Materials and Methods

The plant materials of this study included seven rice varieties, namely, Sakha104, Sakha103, Sakha108, Giza178, Giza179, E Hybrid1, and Sakha Super 300. The names, pedigree, type, and origin of the included lines are presented in, Table (1). The experimental design employed was a strip-plot arrangement with three replications. The main plots were assigned to two irrigation treatments: continuous flooding irrigation every four days (CF) and irrigation every twelve days (12D) throughout the growing seasons. Samples were randomly collected from the inner ridge of each plot at full growth and before harvest, representing the three replications, and data were recorded on various growth and yield components of rice. Rice yield traits were estimated from the entire plot. Data for all traits were recorded as mean values from ten individual plants per plot, selected at random from each cultivar in each experiment, except for the 50% heading date, which was recorded on a plot basis. The mean values of the ten plants were utilized in the statistical analysis.

Table 1. Names, pedigree, type and origin of included lines.

No.	Genotypes name	Pedigree	Type	Origin
1.	**Sakha104**	(GZ 4096-8-1 / GZ 4100-9-1)	Japonica	Egypt
2.	**Sakha103**	(Giza 177 / Suweon 349)	Japonica	Egypt
3.	**Sakha108**	(Sakha101/ HR5824-B-3-2-3// Sakha 101)	Japonica	Egypt
4.	**Giza178**	(Giza 175 / Milyang 49)	Indicia/ Japonica	Egypt
5.	**Giza179**	(GZ 1368-S-5-4 / GZ 6296-12-1-2)	Indicia/ Japonica	Egypt
6.	**E. Hybrid1**	(IR 69625A / Giza 178R)	Indicia/ Japonica	Egypt
7.	**Sakha super 300**	Unknown	Japonica	Egypt

Statistical analysis

The data were analyzed statistically using the variance analysis (ANOVA) technique, as outlined by Gomez and Gomez (1984). Treatment means were compared utilizing Duncan's Multiple Range Test (Duncan, 1955). All statistical analyses were conducted using the G.STAT statistical software tool, implementing the analysis of variance technique.

Determination of relative water content (RWC)

The second young leaf was cut to a length of 1 cm after its fresh weight (f.wt.) was determined. To aid in rehydration, the leaf segment was subsequently submerged in 10 milliliters of distilled water for the entire night. Turgid weight (t.wt.) is the weight measured after this time frame. After two days of drying at 80°C in an oven, the leaves were reweighed to determine their dry weight (d.wt.) using the method described by Basnyake et al. (1993).

Determination of proline

To determine the proline content, a similar assay by Bates et al., (1973) was used. A standard curve was used to calculate the proline concentration, which was then represented as µg/g of the plant's fresh weight.

Determination of total chlorophyll concentrations

The following formulas (adopted by Bhushan *et al*., 2007), were used to determine the pigment concentrations and measure the absorbance values at 663, 645, and 470 nm.

Chlorophyll a (mg/g) = [(12.7*A 663 - 2.69*A 645) v/w]

Chlorophyll b (mg/g) = [(22.9*A 645 - 4.68*A 663) v/w]

Carotenoid (mg/g) = {[(1000*A470) - (3.27*chlorophyll a + 1.04*chlorophyll b)] /227 v/w}f

Total RNA extraction and cDNA synthesis

Young rice leaf samples were collected from plants experiencing both normal and drought stress, and liquid nitrogen was used to grind the leaves into free cells. Total RNA was isolated from rice tissues using the RNeasy® Plant Mini kit (Qiagen, Cat. no. 51,304) and according to the manufacturer's instructions. The gDNA contamination was removed from the extracted RNA samples from rice leaves by following the manufacturer's instructions and using the gDNA Wipeout Buffer of the QuantiTect® Reverse Transcription Kit. The isolated RNA was processed using the QuantiTect® Reverse Transcription Kit (Qiagen, Cat. No. 205,311) to produce complementary DNA (cDNA). To prepare for future studies, the cDNA samples were kept at -20 °C.

Comparative expression study of the genes under investigation

To validate the differentially expressed genes, quantitative reverse transcription PCR (qRT-PCR) assays were performed using six gene-specific primers. qRT-PCR was conducted on the ABI7300 Real-Time PCR System, Software version 2.1. Actin cDNA (accession no. X16280) was used as the endogenous control (reference gene) for all reactions, utilizing primers reported by Omar et al. (2021). The specific primers used in this study are listed in, (Table2).

Table 2. Primer information for qRT-PCR.

Gene name	Accession ID.	Sequence (5′ to 3′)		Fragment length (bp)	References
		Forward primer	**Reverse primer**		
COX1	Os12g0561000	CTCCTAGTCGGCCTGATTTC	CATGAGCAGTAGCATCCTTGA	108	Omar et al., 2021
PKDP	Os08g39170	CGTTGATAGTCGCCGCTAAA	TTTAAGAGGCGGGAATGGTG	109	SAAKRE et al., 2017
bZIP1	CT833525	GAGCGTACTCTGTCCCATTTAG	GTTCCAGCGATGAGGTTGT	115	SAAKRE et al., 2017
AP2-EREBP	OsIBCD031146	AGGTAAAGCCCGAGCAATTC	GCATCGGTGAATGGTGGTATAA	101	Omar et al., 2021
Hsp20	Os01g04380.1	TGTGTGTCACCACGCTTTA	CCTCGCATAGACCCATTCATC	119	SAAKRE et al., 2017
COC1	AY885936	CACCTCATGACGATGCAAGA	GAGCTTGCTCACTCCTTCAA	101	SAAKRE et al., 2017
Actin	X16280	CAT GCT ATC CCT CGT CTC GACCT	CGC ACT TCA TGA TGG AGT TGTAT		Omar et al., 2021

Results and Discussion

1-Morphological growth traits.

1.1. Total Duration (day):

The data recorded in, Table (3) show that the differences between the seven rice cultivars ranged from the highest value of 148.85 days shown by the Sakha super 300 cultivar, while the lowest value of 120.52 days was achieved by the Giza179 cultivar with a range of 28 days and the difference is relatively large under CF irrigation. It is worth noting that the differences between the seven means are significantly different. That is to say none of the means of the seven cultivars showing the same mean. In the same context, Ghazy (2017) and Abdel-Hafez et al. (2016) clarified that conditions of heat stress and drought stress led to earlier headings regarding the interaction between cultivars and their environments.

All cultivars showed early maturation times all showed reduction of days to maturate as indicated in, Table (3).

Concerning the behavior of employed cultivars within this study under drought treatment, the highest value was achieved by Sakha super 300 under drought irrigation conditions with a value equal to 133.8 days. The least remaining cultivar was Sakha 103 under water stress conditions with a value equal to approximately 107.4 days. The difference between the highest and lowest values is approximately 26.4 days. It is considered a relatively huge difference; also, according to the data in Table (3), there are no two means whose difference is not significant. This shows the high level of significance of this trait and its strong influence by either drought treatment or the cultivar itself. Especially the comparison also has a great effect on this important trait. This may agree greatly with what Abo Yousef et al., 2023.

Seven rice cultivars were evaluated for the total duration of plant residence in the ground and its effect on exposure to drought treatment as shown in, Table (3). The values of the least significant difference between the means indicate a high level of significant differences in the mentioned trait. The values of the over all means under normal irrigation and drought conditions were 133.04 and 121.34 days, respectively. This means indicates that the difference is significant and large between the two means, and thus drought treatment has a significant effect on the total duration of rice plant residence in the ground i.e. earlier in maturation.

1.2. Plant Height (Cm):

Data shown in, Table (3), there is behavior of the cultivars under irrigation treatment. This comparison had a significant effect on plant height within a range of approximately 41.1 cm. The highest value of plant height was recorded for the Sakha super 300 cultivar under normal conditions with a value equal to 113.8 cm. However the shortest cultivars were Giza 179, Sakha 103 and Sakha 108 with a value equal to (94.43, 100.51, 101.85cm, respectively under normal condition.

Plant height values were maximum with cultivars, E. Hybrid1 with a value equal to 99.75 cm. It is important to mention that the two varieties Sakha super 300 and Giza 178 under drought conditions showed the same height value of 99.25 cm. As for the Sakha 103 variety, it was the shortest of all with a value equal to 72.72 cm.

In general, and according to results obtained and written in Table (3), the plant height trait in all rice cultivars was negatively affected by exposure to drought. First, the average height for all cultivars planted in naturally irrigated plots was 103.2 cm, and this value was significantly higher than the average of the plots planted under drought conditions by only 91.3 cm. These results are consistent with those of Mohamed et al. (2021). It is generally believed that rice cultivars have tolerance based on the amount of height reduction after stress.

1.3. Number of tillers/ plant:

Significant differences were found as a result of the comparison between cultivars and treatment. The highest value was 35.6 for E Hybrid1 under normal conditions, while the lowest value was 17.9 for Sakha super 300. The same pattern was noted under the drought conditions. There were many values in between that showed varying levels of significance according to Table (3).

Considering the data in Table (3), we can say that the difference between the drought treatment and the natural irrigation treatment was significant with respect to the number of shoots per plant, with averages of 18.98 and 25.43, respectively. These results are consistent with those of Mohamed et al. (2021).

1.4. Flag Leaf Area (Cm^2):

A plant's flag leaf area of rice is important for transpiration, simulation and for photosynthesis. It is important to note that there is comparison between cultivars and irrigation treatments ranging from the highest mean value of 44.35cm^2 for E Hybrid1 cultivar however, Sakha 103 cultivated in an irrigated plot had the lowest results, measuring 36.58 cm^2. Notably, the E Hybrid1, Giza 178, and Sakha 104 cultivars exhibited the largest flag leaf areas, demonstrating significant advantages over other cultivars, as illustrated in Table 3. Meanwhile, cultivars like Sakha 108 and Sakha 103 showed lower flag leaf areas, with means ranging from 34.283 to 26.75 cm^2 under drought conditions.

Table No. (3) contains the averages of the seven rice cultivars under natural irrigation conditions and also under drought conditions for the flag leaf surface area cm^2. As a result of drought, the flag leaf surface area decreased from 41.7 to 37.3 and this decrease was significant. These findings were comparable to those of Abo Yousef et al. (2023).

Table 3. Genotypes performance for selected morphological traits over normal and water stress in combined analysis.

Genotypes	Total duration (day)		Plant height (cm)		Number of tillers/ plant		Flag Leaf Area (cm2)	
	CF	D	CF	D	CF	D	CF	D
Sakha 104	134.17[d]	120.25[j]	104.51[b]	94.35[i]	21.46[h]	17.09[k]	42.9[b]	40.55[e]
Sakha 103	124.85[h]	107.35[m]	100.51[f]	72.71[l]	16.69[l]	10.56[n]	36.58[g]	26.75[i]
Sakha 108	135.51[b]	119.55[k]	101.85[e]	83.25[k]	27.88[d]	20.18[i]	42.28[c]	34.28[h]
Giza 178	134.51[c]	127.65[g]	103.15[d]	99.25[h]	30.11[b]	22.41[g]	42.10[c]	40.25[e]
Giza 179	120.52[i]	114.45[l]	94.43[i]	90.55[j]	28.34[c]	23.94[f]	41.15[d]	39.45[f]
E. Hybrid1	132.85[f]	126.25[g]	103.85[c]	99.75[g]	35.60[a]	25.68[e]	44.35[a]	40.65[e]
Sakha super 300	148.85[a]	133.85[e]	113.8[a]	99.25[h]	17.91[j]	12.94[m]	42.86[b]	39.25[f]
Treat. Mean	133.04[**]	121.34[*]	103.20[**]	91.30[*]	25.430[**]	18.980[*]	41.748[**]	37.312[*]
T. LSD5%	0.00146		0.214328		0.283824		0.558304	

CF: Continuous flooding. **D**: drought. **Treat.Mean**: Treatments mean. **T.LSD**: Treatments LSD.

Figure (1) shows the percentage of height reduction for each cultivar used during this experiment. This is considered a distinct indicator of the degree of drought tolerance of each cultivar. The most drought-tolerant cultivar, which lost more than a quarter of its height, was Sakha 103 with a percentage of 27.65, followed by Sakha 108, which lost 18.26%. These two cultivars are the most sensitive to drought according to the percentage of plant height reduction. Sakha super 300 comes next with a percentage of 12.7%, then Sakha 104 with a percentage of 9.72%. Accordingly, they are considered to be moderately drought-tolerant. The most drought-tolerant cultivars are Giza 178, E Hybrid1, Giza 179 with averages of 3.78, 3.94, 4.1 respectively.

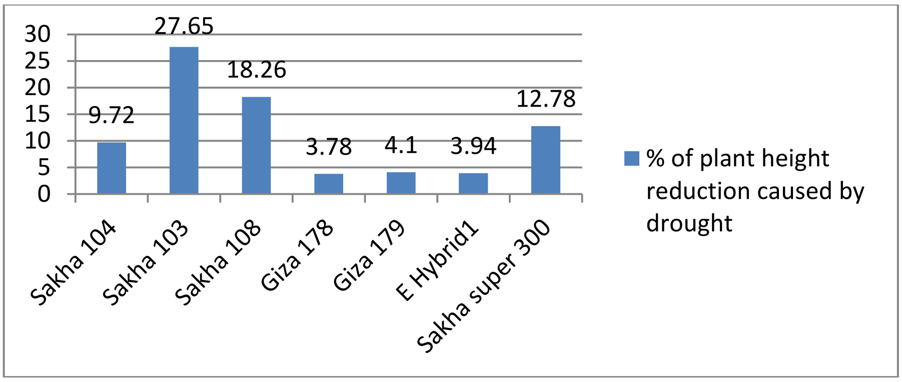

Fig. (1): Percentage of plant height reduction caused by drought.

Figure (2) shows the percentage of decrease in the number of tillers per plant because of exposure to drought. The most affected cultivar is Sakha 103 with a percentage of 36.74, followed by the E Hybrid1, Sakha super 300, Sakha 108 cultivars with almost equal percentages of 27.82, 27.74, 27.61 respectively. After them comes the Giza 178 variety with a percentage of decrease of 25.57. Then comes the Sakha 104 variety with a percentage of decrease of 20.35. Finally, the least affected cultivar by drought with regard to the number of tillers trait is the Giza 179 cultivar with a percentage of decrease of only 15.52.

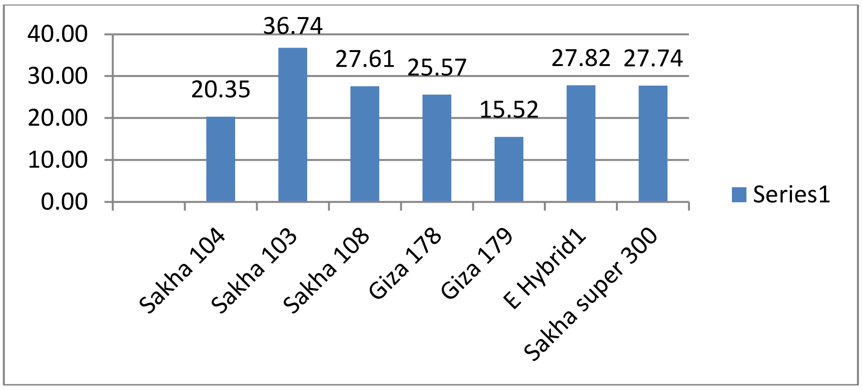

Fig. (2): Percentage of tillers number per plant reduction caused by drought.

As shown in Figure (3), the highest percentage of decrease in flag leaf surface area due to drought exposure was shown by Sakha 103 cultivar with 26.88%. This was followed by Sakha 108 cultivar with 18.9% decrease. Then Sakha super 300, E Hybrid1 cultivars with percentages of 8.42, 8.34 respectively. Finally, the three cultivars with the least decrease were Sakha 104, Giza 178, Giza 179 with averages of 5.48, 4.4, 4.13 cm2 respectively.

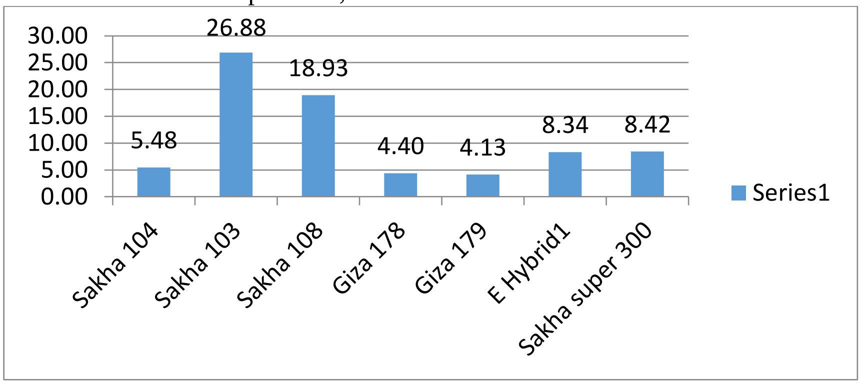

Fig. (3): Percentage of Flag leaf area trait (cm2) reduction caused by drought.

2- . Yield and its components traits

2.1. Panicle length (cm):

One of the most significant characteristics influencing rice yield is the length of the inflorescence. Table (4) contains the averages of the inflorescence length trait for rice plants grown under normal irrigation or exposed to drought.

When comparing the cultivars under normal irrigation treatments, the largest value planted under normal irrigation was 27.15 cm was achieved by the E. Hybrid1 cultivar, while the lowest value of 22.51 cm was obtained from the Giza 179 cultivar. There are no significant differences between cultivars G178 and Sakha 108, or between Sakha108, Sakha103 and Sakha 104.

Regarding the comparison between cultivars under drought treatment, the highest mean value was achieved by Giza 178 under drought irrigation conditions with a value equal to 33.55 cm, while the least value was 15.14 cm was obtained from cultivar Sakha 103 planted under water stress conditions as shown in, Table (4). There are no significant differences between cultivars Giza179 and E. Hybrid1, or between E. Hybrid1 and Sakha super 300.

A significant difference is starting from the highest average value of the cultivar E. Hybrid1 planted with normal irrigation with a value of 27.15 cm as compared with for the lowest value, that appeared from the cultivar Sakha 103 with the drought treatment with a value equal to 15.14 cm. This may agree greatly with what reported by Abo Yousef et al., 2023 and El-Gammaal., et al. 2022.

2.2. Panicle weight (g):

Data in Table (4) presented the effect of both water treatment (Flood and drought irrigation) on spike weight. Because of its direct effect on the yield of an individual plant that greatly affects the total yield production.

Comparing the seven cultivars for cultivation at normal irrigation, it showed that the highest two values were achieved by the cultivars E.hybrid1 and Sakha 108 with values of 5.65 and 5.59 grams that were not significantly different. While the lowest cultivar in spike weight was the cultivar Sakha 104, which achieved 3.69 grams under CF irrigation as shown in, Table (4).

In addition, the comparison between the genetic composition of the cultivars at drought treatment showed reduction in panicle weight (g). Significant among cultivars was highly significance. The highest value of which was 4.83 grams was achieved by the Giza 178 cultivar, while the lowest value of 1.58 g was obtained from the Sakha 103 cultivar planted under drought. There are none of cultivars whose differences between their values are significant.

The data arranged in, Table (4) show the following. The average spike weight for all varieties grown under natural irrigation was 4.87 grams. The average for the crops that suffered from drought was 3.49. This amount of decrease shows a significant effect of drought on the trait. These results are in agreement with the results obtained by Mohamed et al., 2021, Abo Yousef et al., 2023 and El-Gammaal., et al. 2022

2.3. Grain yield/ plant (g):

The grain yield per plant is one of the most important crop traits in rice. Table (4) shows the averages of this trait as affected by both water flood and drought.

Among the cultivars, the highest two values were achieved by Giza 179 and Sakha108 that were not significantly different and with values of 50.05 and 48.91 grams, respectively. While the least value was 39.8 (g) was obtained with Sakha 103 grown under CF conditions. Furthermore, Giza 178 obtained the highest value under drought conditions with a value equal to 50.05 (g). In addition, the extreme value at the bottom was 17.13 for Sakha 103 cultivated under drought treatment.

This trait showed a significant values ranging from 50.05 (g) for Giza 179 cultivated under normal cultivation to 17.13 for Sakha 103 cultivated under drought conditions, the difference is considered exceptionally large at 32.917 (g). These results are in agreement with the results obtained by Abo Yousef et al., 2023 and El-Gammaal., et al. 2022

2.4. 1000 Grain Weight (g):

The weight of a thousand grains was also estimated as shown in, Table (4). Comparing the values of the cultivars planted at Cf conditions, we find that they fluctuate in a range that is considered large for this trait. The largest value was found to 29.6 grams for the cultivar Sakha 108, while the lowest cultivar in this trait was Giza 178 with a value equal to 23.01 grams.

Under the drought stress conditions the same trend was observed. There were many values in between that showed varying levels of significance as shown in, Table (4). Moreover, significant difference between any other two cultivars was found.

The highest value was achieved by Sakha 108 (27.6 grams) when grown with drought water. While the lowest value was 21.01 shown by Giza 178 when grown under drought conditions. The range for these two cultivars is 8.59 grams which is a relatively large value for the weight of 1000 grains. Between the two extreme values there are also many values that may be important to note, Table (4). We can also distinguish that there is a significant difference between the average of the cultivars grown with normal irrigation 27.65 and those grown with drought 24.87. Same results explained by El-Gammaal., et al. 2022.

Table 4 Genotypes performance for yield and related traits over normal and water deficit in combined analysis.

Genotypes	Panicle length (cm)		Panicle weight (g)		Grain yield/plant (g)		1000 grain weight (g)	
	CF	D	CF	D	CF	D	CF	D
Sakha 104	24.02c	17.18j	3.69i	2.59k	41.05e	33.71hi	28.23d	26.23g
Sakha 103	24.14c	15.14k	3.74h	1.58l	39.8ef	17.13j	28.71c	23.04k
Sakha 108	24.35bc	18.35i	5.59a	4.39e	48.91ab	28.91i	29.6a	27.6e
Giza 178	24.55b	23.55d	5.09c	4.83d	46.63cd	39.29fg	23.01k	21.01l
Giza 179	22.51f	21.51g	4.95d	4.13f	50.05a	37.38gh	28.67c	25.67h
E. Hybrid1	27.15a	21.31gh	5.65a	3.97g	48.48bc	34.48h	26.45f	25.45i
Sakha super 300	23.02e	21.02h	5.35b	2.95j	46.08d	30.78i	28.89b	25.05j
Treat. mean	**24.25****	**19.72***	**4.87****	**3.49***	**45.86****	**31.67***	**27.65****	**24.87***
T. LSD5%	**0.520344**		**0.229804**		**1.7155**		**0.085264**	

CF: Continuous flooding. **D**: drought. **Treat.Mean**: Treatments mean. **T.LSD**: Treatments LSD.

Figure (4) shows the percentage of reduction in inflorescence length resulting from exposure to drought. The four highest percentages of reduction were above one fifth, with values equal to 37.28, 28.45, 24.64, 21.48 respectively for the cultivars Sakha 103, Sakha 104, Sakha 108, E Hybrid1. The percentage of reduction was low at 8.69% for the cultivar Sakha super 300. The percentage was very low for the cultivars Giza 178, Giza 179 with values of 4.07, 4.44%.

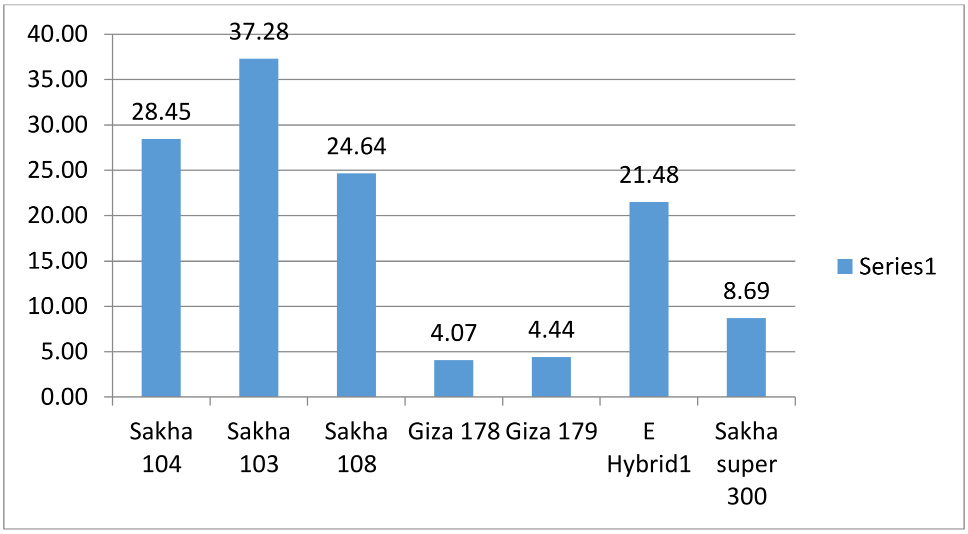

Fig. (4): Percentage of % Panicle length reduction caused by drought.

Figure (5) summarizes the estimated spike weight reduction due to drought. The most affected cultivar is Sakha 103 with a reduction of more than half (57.8%). Number two in terms of severity is Sakha super 300 with a reduction of 44.8%. Number three is Sakha 104 and E Hybrid1 with almost equal percentages (29.9 and 29.7%). Sakha 108 decreased by only 21.4%. Giza 179 decreased by a smaller percentage (16.6%). The cultivar that was excellent and achieved the lowest percentage of spike weight reduction among its peers was Giza 178 with a reduction of only 5.1%.

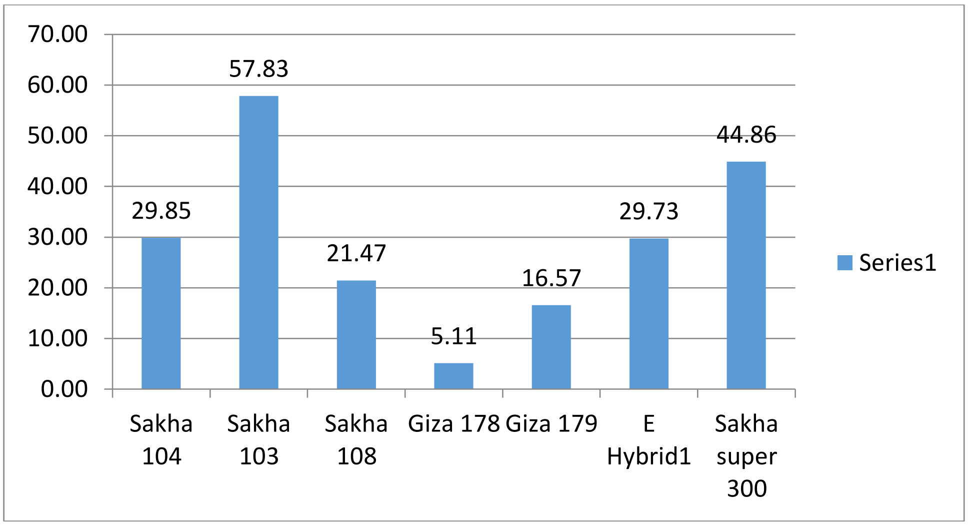

Fig. (5): Percentage of % Panicle weight reduction caused by drought.

The amount of decrease in grain yield per plant is shown in Figure (6). The highest percentage of decrease was for the Sakha 103 cultivar with a percentage of 56.95, followed by the Sakha 108 cultivar with a percentage of 40.89. Then the Sakha super 300 cultivar had a percentage of decrease of 33.2. Then the E Hybrid1 cultivar had a percentage of decrease of 28.88. The Giza 179 cultivar decreased by 25.31. As for the Sakha 104 cultivar, it decreased by 17.86. The least decreased cultivar was the Giza 178 with a percentage of only 15.73%.

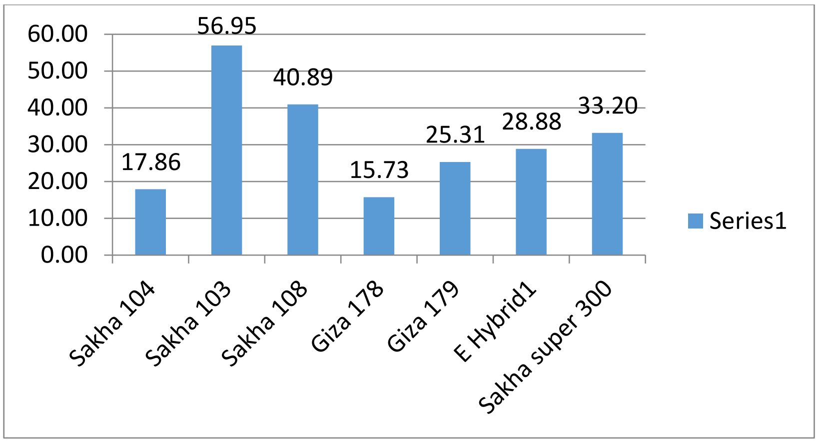

Fig. (6): Percentage of % Grain yield per plant reduction caused by drought.

Figure (7) summarizes the percentage of reduction in the weight of a thousand grains affected by drought. The most affected cultivar is Sakha 103 with a reduction of 19.74%. It is followed by Sakha super 300 and Giza 179 with percentages of 13.27 and 10.46% respectively. Then the three cultivars Giza 178, Sakha 104 and Sakha 108 with very close percentages of 8.69, 7.08 and 6.76%. Finally, the cultivar E Hybrid1 with a distinctive reduction percentage, which is the lowest of only 3.78 grams.

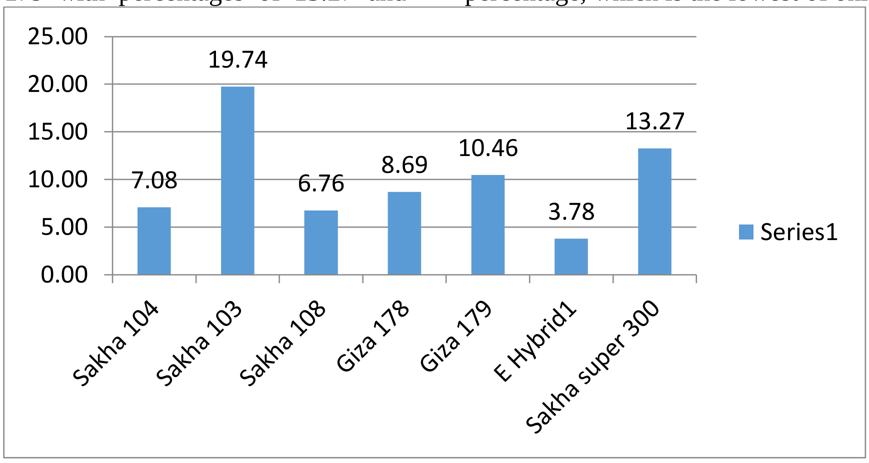

Fig. (7): Percentage of %1000 grain weight reduction caused by drought.

According to previous studies, drought stress had greater negative impacts on early growth stages than on filling stages because the yield components were mostly in place (except for 1000 grain weight).(Zhang et al., 2024; Cheng-a et al., 2007; , Fageria 2007; Moonmoon et al., 2017). According to Ghazy (2017) and AbdAllah et al. (2010), a common drought avoidance strategy is the reduction of leaf area. Growth inhibition resulting in enormous and irreversible cell expansion created by meristematic divisions may be the cause of the decrease in rice growth caused by a decrease in the amount of available soil moisture. Additionally, water stress results in tissue loss, which lowers turgor pressure within the cell and prevents cell division and enlargement, which lowers plant development, stem elongation, and leaf expansion.

3- Biochemical characterization

Drought stress exerted a negative influence on the relative water content (RWC) of the treated cultivars, as illustrated in Figure (8A). The E. Hybrid cultivar exhibited the least susceptibility to drought stress, whereas the Sk103 cultivar demonstrated the greatest sensitivity in comparison to the control group. Drought conditions were associated with a notable decrease in the relative water content across the majority of the rice cultivars studied when compared to their non-stressed counterparts (control). The E. Hybrid1 cultivar recorded the highest mean value of relative water content at 92.880%, while the Sakha 103 cultivar exhibited the lowest mean value at 80.620% under drought stress conditions. These findings are validated by the research conducted by Ibrahim et al. (2019).

Additionally, proline buildup in the treated cultivars responded to drought stress in a highly significant way, as shown in Figure (8B). Upon exposure to drought conditions, the proline concentration in the plant leaves increased dramatically (Figure 8B). A significant enhancement in proline accumulation was noted in the E. Hybrid1 cultivar compared to the other varieties. This observation closely resembles what Abo-Youssef et al. (2023) found.

On the other hand, all cultivars exhibited a decrease in total chlorophyll concentration under drought stress compared to normal conditions. Notably, E.Hybrid1 and Giza198 demonstrated a significant increase in total chlorophyll concentration relative to the other cultivars under drought conditions (Fig. 8C).

The length and severity of drought stress have an impact on the amount of chlorophyll in crops during certain times. (Zhang et al., 2024). Among the seven rice cultivars studied, E.Hybrid1, Giza198, and Giza179 displayed the highest chlorophyll concentrations under drought conditions. These findings are consistent with those reported by Ibrahim et al. (2019).

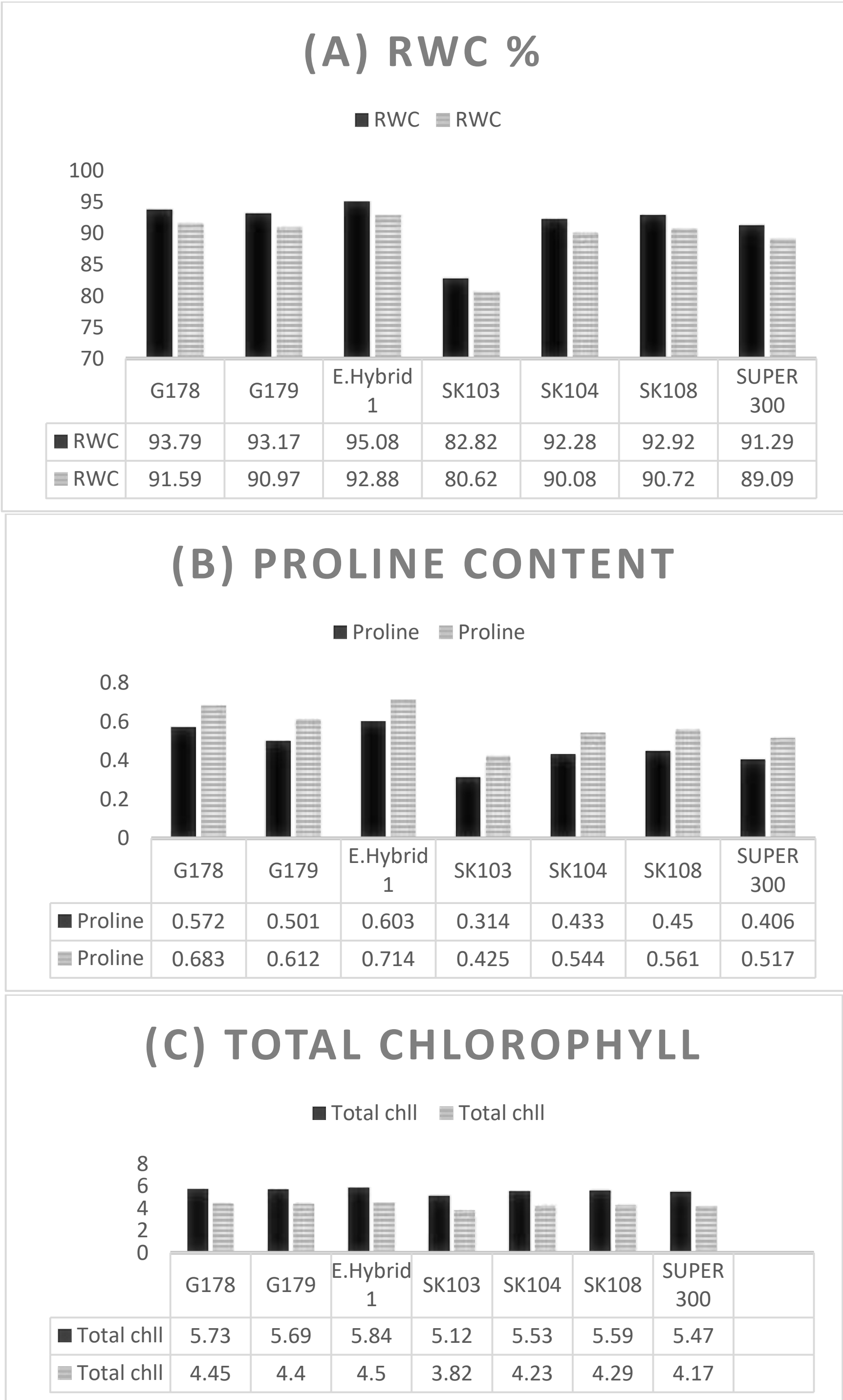

Fig. (8): Changes in RWC, proline and total chlorophyll in response to drought treatment for seven rice cultivars differing in drought tolerance.

4- The effects of drought stress on gene expression:

Six genes have been recognized with differentially expressed cDNAs, COX1 (which regulates energy and carbohydrate metabolism), PKDP (a protein kinase), AP2-EREBP (ethylene-responsive element binding protein), bZIP1, Hsp20, and COC1 (associated with the abscisic acid (ABA) dependent signaling pathway). This research aims to explicate how plants react and adjust to drought stress. The expression of these genes under drought conditions was analyzed using quantitative real-time PCR (qPCR), with details of the primers utilized provided in, Table (2). The qPCR analysis indicated that drought-stressed plants exhibited expression of all six genes. Under stress conditions, each gene displayed differential expression compared to those under normal conditions. Notably, as compared to the control, all six genes showed increased expression in response to drought stress, specifically in E. Hybrid.1, Giza178, and Giza 179, whereas Sakha103 showed the lowest expression levels. The relative expression of the studied genes is depicted in Figure (9).

Drought stress led to a slight increase in the expression of PKDP in Sakha 104, Sakha 108, and Sakha Super 300, with relative expression levels increasing by 1.98, 1.87, and 1.54-fold, respectively, compared to the control (Figure 9B). Furthermore, bZIP1 exhibited the highest expression levels, with increases of 4.12 and 4.3-fold compared to the control in E. Hybrid.1 and Giza 179, respectively (Figure 9C). Additionally, the expression levels of COX1, Hsp20, and COC1 under water stress reached increases of 3.04, 2.97, and 3.8-fold in E. Hybrid 1, respectively (Figures 9A, 9E, and 9F). The AP2-EREBP gene expression was also affected by the water-stressed condition (Figure 9D).

Gene expression is typically evaluated using quantitative reverse transcription PCR (qRT-PCR) across various environmental and developmental conditions. This technique employs an internal control gene, which demonstrates consistent expression across the analyzed samples, to ascertain the expression level of a target gene (Isaiah et al., 2016). Additionally, it is imperative to assess transgene expression variables via qRT-PCR (Kohli et al., 2006; Trijatmiko et al., 2016). A reference gene should remain unaffected by the experimental treatment, such as drought stress, which is the focus of the present study. Numerous housekeeping genes have been investigated in rice under various conditions and across different samples, predominantly at the seedling stage (Kim et al., 2003; Jain et al., 2006; Narsai et al., 2010; Moraes et al., 2015). Our findings indicate that the activation of the examined genes may contribute to enhancing the drought tolerance of rice plants.

Conclusion

Physiological, biochemical, and morphological responses to drought stress were investigated to assess the degree of tolerance developed in the studied organisms. Additionally, the expression of six drought tolerance genes was analyzed to identify the most promising drought-tolerant strains. Because of the complex interplay between stress factors and plant physiological, molecular and biochemical reactions, this study shows that different rice cultivars react differently to abiotic stress.

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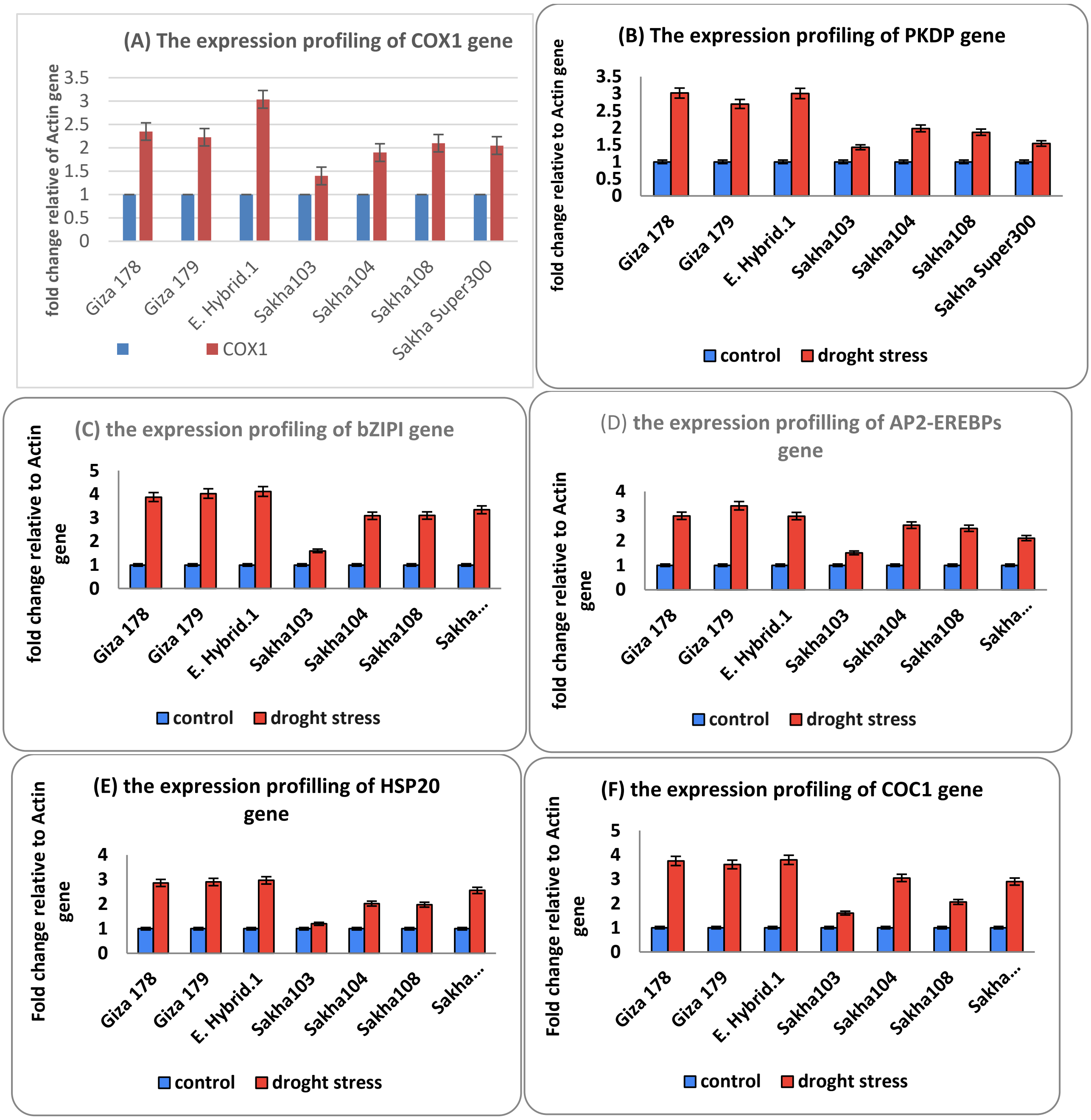

Fig. (9): Changes in the expression level of some stress-responsive genes in seven rice cultivars.

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