GENOME-WIDE CHARACTERIZATION OF DROUGHT-RESPONSIVE GENES ACCELERATES FABA BEAN BREEDING PROGRAM

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Drought is an influential environmental stress that limits faba bean growth and development, leading to a substantial reduction in their productivity in both arid and semi-arid region of the world. However, the responses of faba bean to drought have not been illustrated very well at molecular level. Six cultivars of faba beans and their fifteen hybrids in F_2 were used to investigate the transcriptome, proteome, and metabolome changes. In the present study, two-weeks-old seedlings were deficit by seven days drought stress, whereas control seedlings were watered regularly. The results indicated that water deficiency negatively influences substantial physiological and metabolic parameters in faba bean crop. Under drought stress, values of biomass and chlorophyll in the genotypes Nubaria 1, Giza 2, Giza 429, Giza 716, Nubaria 1 × Giza 716, Sakha 2 × Giza 2, Sakha 2 × Giza 716, Giza 2 × Giza 429 and Sakha 2 × Sakha 1 were higher than those of the rest of genotypes. Notably, a marked increase was recorded in the biomolecule indicators of plant oxidative stresses; proline and soluble sugars with same genotypes after water deficiency. Transcriptome analysis via real-time reverse transcriptase-polymerase chain reaction revealed that expression levels of *VfD*HN4, *APETALA2*, *VfHSP18*, and *VfAQP2* genes were altered in response to drought conditions. The results demonstrated that all genes were differentially expressed in the studied faba bean genotypes due to water shortage intensity according to cultivar's tolerance to drought. This study could be a distinctive strategy to

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speed up breeding programs via exploring the molecular, biochemical and physiological mechanism of drought tolerance in faba bean or other economically important crops.

Keywords: faba bean, drought stress, proteomic, metabolomic, genomic and transcriptomic

The growing population explosion is in dire need of increasing global food production. As water is the source of life, it is the main factor in agricultural expansion to increase food production, particularly in dry and semi-arid lands (Ammar et al., 2017). One of the important food sources is faba bean (*Vicia faba* L.), especially in Egypt and the Middle East, due to its high nutritional value because it contains high protein and carbohydrates. That is why faba bean ranks fourth among the legume crops after dry beans, dry peas and chickpeas (Toker et al., 2007). Despite the importance of faba bean, the expansion of this crop in Egypt is unstable due to the water deficit, especially in the desert lands which constitute 96% of the total area of Egypt (Rasmy et al., 2010).

Continuous exposure to drought stress leads to a decline in the yield of faba bean crop due to the increased rate of cellular damage, decreased photosynthesis and the increased accumulation of reactive oxygen species (Belal et al., 2009 and Ewas et al., 2017a). Drought stress restricts plant growth by negatively affecting diverse biochemical and physiological processes, including antioxidant phenomena, osmolyte accumulation, photosynthesis, ion homeostasis and nitrogen metabolism (Ashraf, 2004; Ammar et al., 2017 and Ewas et al., 2017b). For proper growth and water uptake, plants must maintain their relative water content below the soil water content. This mechanism demands the increase of osmotica through improving the synthesis of compatible solutes (Tester and Davenport, 2003 and Dawood et al., 2014).

At the molecular level, many genes play an important role in improving the plant's ability to face drought stress conditions, by increasing the synthesis of various compounds associated with enduring water shortages, controlling of stomatal closure, reducing transpiration rate, eliminating damaging compounds arising from exposure to drought and regulating many important biological processes that protect the plant from environmental stresses (Pastori and Foyer, 2002; Azevedo et al., 2011 and Nagahatenna et al., 2015). Several genes that express under water deficit conditions are implicated in the regulation of all these processes and pathways. In the last decade, many drought-tolerant genes have been reported in main food crops and still there are different genes taking part in drought stress whose functions are unrecognized. The available knowledge about genomic and transcriptomic data provides an essential and quick breakthrough towards exploring the

functions for many of these unknown genes under various abiotic stresses (Azevedo et al., 2011; Mohamed and Akladious, 2014 and Singh et al., 2018).

Among these genes, dehydrins (*Dhn*), *APETALA2*/ethylene-responsive factors (*AP2/ERFs*), heat shock protein (*HSP*), and aquaporin (*AQPs*) genes are induced in response to drought conditions. Dehydrins (*DHNs*) proteins play a substantial role in plant adaptation and response to abiotic stresses. They accumulate typically in maturing seeds or are induced in the vegetative tissues following dehydration, salinity, and cold stresses (Close, 1996 and 1997; Svensson et al., 2002; Mouillon et al., 2008; Brini et al., 2010 and Hanin et al., 2011).

Other transcription factors (TFs) like *AP2/ERFs* are an integral component of these signaling cascades, because they regulate expression of a wide variety of down-stream target genes related to stress response and development via different mechanisms (Phukan et al., 2017). The induction of *AP2/ERFs* in response to drought stress have been reported in different plant species including, *Pennisetum glaucum* (Agarwal et al., 2007), *Arabidopsis thaliana* (Buttner and Singh, 1997; Bethke et al., 2009; An et al., 2010 and Bolt et al., 2017), *Eucalyptus grandis* (Cao et al., 2015), *Solanum tuberosum* (Charfeddine et al., 2015) and *Medicago sativa* (Chen et al., 2012).

Heat-shock proteins (*HSPs*) work as molecular chaperones for a variety of client proteins in abiotic stress response and play central roles in protecting plants against stress (Xiang et al., 2018). *HSP* proteins are large gene family that are up-regulated by heat stress and positively regulates drought stress tolerance perhaps by modulating osmotic adjustment and ROS homeostasis in many crops such as rice (Boston et al., 1996; Apel and Hirt, 2004; Breiman, 2014; Fang et al., 2015 and Xiang et al., 2018), tomato (Hahn et al., 2011) and soybean (Xu et al., 2013).

Aquaporin proteins (*AQPs*) are present in several isoforms in both tonoplast membranes and plasmalemma to control water flow in and out of plant cells. Thus, it is not unusual that molecular and physiological studies have reported *AQPs* as playing pivotal roles in regulating hydraulic conductance in leaves and roots. Consequently, *AQPs* activation influences a group of physiological processes including phloem Absorbing, xylem water egression, gas exchange and stomatal closure (Shekoofa and Sinclair, 2018).

The present study reveals the possibility of accelerating faba bean breeding programs for drought tolerance by studying the transcript level of many drought-tolerance-related-genes such as *Dhn*, *APETALA2*/*ERFs*, *HSP* and *AQP* proteins. The induction of these genes improves various physiological processes in faba bean, including the increased of proline, chlorophyll and soluble sugars synthesis, as well as the production of several proteins that increase drought tolerance. This study could be distinctive and useful in crop breeding programs to improve many other important crops.

MATERIALS AND METHODS

1. Plant Materials and Growth Condition

Seeds of twenty-one genotypes of faba bean including six cultivars (Nubaria 1, Sakha 1, Sakha 2, Giza 2, Giza 429 and Giza 716) and its fifteen hybrids in F_2 were used in the experiments of the present study (Supplemental Table S1). All genotypes were grown in a greenhouse under a 12 h light/12 h dark regime of 180 mmol m^{-2} s^{-1} light intensity (Qian et al., 2015).

2. Physiological Measurements

For physiological studies, two-weeks-old seedlings were deficit by seven days of drought stress, whereas control seedlings were watered regularly. After treatment, seedlings were weighed (fresh weight) and dried in an oven at 70ºC for 48 h then weighed (dry weight). Soluble sugars, chlorophyll (A and B) and proline contents were measured with three replicates, according to the previous methods described by Bates et al. (1973), Wellburn (1994) and Orozoco and Ryan (1999). Meanwhile, the total carotenoids content was determined in 0.1 g fresh tissue of leaves using Spekol 11 spectrophotometer following the method of Metzner et al. (1965).

3. Biochemical Analysis

Total protein was extracted from bulk-crushed leaves as described by Chen et al. (1995). Then powder (0.05 g) was soaked in 0.4 ml water for at least 24 h, and then the mixture was placed in an Eppendorf centrifuge for 30 min at 8000 g. The supernatant was preserved at 4°C for sample preparation. Each sample was suspended in a medium containing 2% SDS (w/v), 5% 2-mercaptoethanol (w/v), 0.001% pyronin (w/v), 10% glycerol (v/v), and 1 M Tris-HCl, pH 6.8. The samples were left for 3 h at room temperature and shaken every 15 min. Then, the mixture was placed in a boiling water bath for 3-5 min and cooled at 4°C. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was performed on 4% slab gels in a discontinuous buffer system as described by Laemmli (1970). A constant electric current of 30 mA was used to run two gels for 10 h.

4. Expression Analyses

Total RNA was extracted using Trizol reagent (Invitrogen), while the first-strand cDNA was synthesized using 200 U of M-MLV reverse transcriptase (Invitrogen) and 3 µg of RNA according to the producer's protocol. Real-time Polymerase Chain Reaction (PCR) was carried out on an optical 96-well plate using an AB StepOnePlus PCR system (Applied Biosystems) by using SYBR Premix Reagent F-415 (Thermo Scientific). Also, actin gene was used as an internal control which was amplified with 24 cycles. Gene expression level was calculated using a relative quantification

method described by Schmittgen and Livak (2008). All primers used in this study are shown in supplemental table (S2).

RESULTS AND DISCUSSIONS

1. Physiological Changes in Faba Bean Genotypes as a Response to Drought Stress

Drought is one of the most severe stresses that negatively affect various physiological processes in the plant including biomass, chlorophyll, soluble sugars and proline synthesis. In the present study, seedlings of twenty-one genotypes of faba bean, including six cultivars and their fifteen hybrids at two-weeks old were exposed to drought stress for seven days. The results show that the negative impact of drought stress depends on the ability of faba bean genotypes to endure this stress. This idea was confirmed by the results of biomass analysis, which showed a significant decrease in both fresh and dry weights for all genotypes except Nubaria 1, Giza 2, Giza 429, Giza 716, Nubaria 1 × Giza 716, Sakha 2 × Giza 2, Sakha 2 × Giza 716, Giza 2 × Giza 429 and Sakha 2 × Sakha 1 under drought conditions, while no significant differences were recorded between all genotypes under normal conditions (Fig. 1).

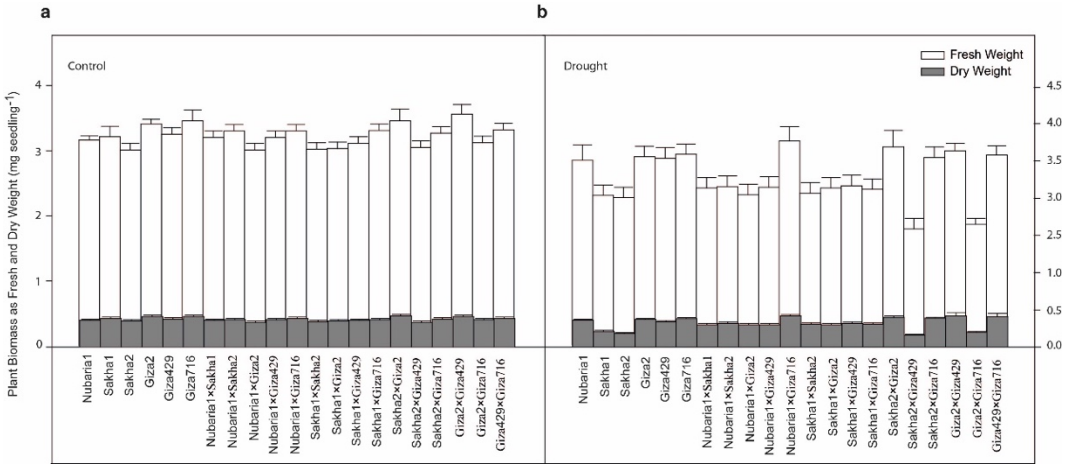

Fig. (1). Drought stress affects the biomass of faba bean genotypes. (**a**) Plant biomass under normal conditions; (**b**) Plant biomass under drought stress conditions. Fresh and dry weights were measured to obtain plant biomass (mg seedling^{-1}). Values are the mean ± SD (n = 8).

The association of growth performance with genotype in response to drought stress has been reported in several plant species such as maize, rice, potato and tomato (Yan et al., 2000; Sarma et al., 2016; Shahzad et al., 2016 and Ewas et al., 2017a and b).

In relation to growth, the photosynthetic active pigments, chlorophylls a and b of plant leaves decreased significantly with the continued effect of drought stress for all genotypes except Nubaria 1, Giza 2, Giza 429, Giza 716, Nubaria 1 × Giza 716, Sakha 2 × Giza 2, Sakha 2 × Giza 716, Giza 2 × Giza 429 and Sakha 2 × Sakha 1 (Fig. 2a, b), while no significant differences were registered between all genotypes under normal conditions (Fig. 2c, d). In the same manner, a marked increase in the total carotenoids content was detected in the leaves of the same genotypes characterized by higher chlorophyll content than the rest of the studied genotypes under drought stress conditions. However, there was no significant difference in the total carotenoids content among all faba bean genotypes under normal conditions (Fig. 2e, f). Working with algae, it has been found that exposure to dehydration stress also reduced the photosynthetic pigments content (Karsten and Holzinger, 2011 and Wu, 2016). The synthesis level of chlorophyll a and b rely on the ability of the genotype to cope with drought stress, this idea was confirmed previously in different plant species including, *Triticum aestivum* (Rahnama et al., 2010), *Oryza sativa* (Moonmoon et al., 2017), *Solanum lycopersicum* (Zhou et al., 2017) and *Arabidopsis thaliana* (Yao et al., 2018).

Soluble sugars changed dramatically between faba bean genotypes in the present study. The soluble sugars content decreased slightly in all genotypes, except the same genotypes that had higher biomass rates and pigments under drought stress conditions, including Nubaria 1, Giza 2, Giza 429, Giza 716, Nubaria 1 × Giza 716, Sakha 2 × Giza 2, Sakha 2 × Giza 716, Giza 2 × Giza 429 and Sakha 2 × Sakha 1, also had high soluble sugars content (Fig. 3). These findings are consistent with the results of distinctive drought-prone genotypes, including faba beans (Belal et al., 2009), soybean (Mohamed and Latif, 2017), tomato, maize (Nayer and Heidari, 2008), rice (Rebolledo et al., 2012), wheat (Marcek et al., 2019) and barley (Wehner et al., 2015).

On the other hand, the results of proline accumulation analysis showed a slight increase of proline content in leaves of all faba bean genotypes after exposure to drought stress, except for Nubaria 1, Giza 2, Giza 429, Giza 716, Nubaria 1 × Giza 716, Sakha 2 × Giza 2, Sakha 2 × Giza 716, Giza 2 × Giza 429 and Sakha 2 × Sakha 1, which exhibited a marked increase in proline accumulation level under drought stress condition (Fig. 4b). However, no significant differences were recorded in the proline content between all investigated faba bean genotypes under normal conditions (Fig. 4a). These results are in close agreement with those obtained by some researchers (Xiong et al., 2012; Bandurska et al., 2017 and Fu et al., 2018).

Positive correlation between increased ABA levels and proline accumulation in plants and environmental stresses that lead to limit plant growth was reported by Do¨rffling et al. (1990) and Yashiba et al. (1997). Under drought stress, a common effect of prevent irrigation and water loss from plants is the accumulation of ABA (Loukehaich et al., 2012). The significance of proline accumulation due to drastic environmental factors is

still controversial (Verma and Hong, 1996 and Hare and Cress, 1997), and other functions have been suggested for this response such as nitrogen storage, free radical scavenging or pH regulation (Stewart and Hanson 1980). Thus, proline accumulation in response to stress conditions is still challenge not only in the discussions reported previously, but also in the current study.

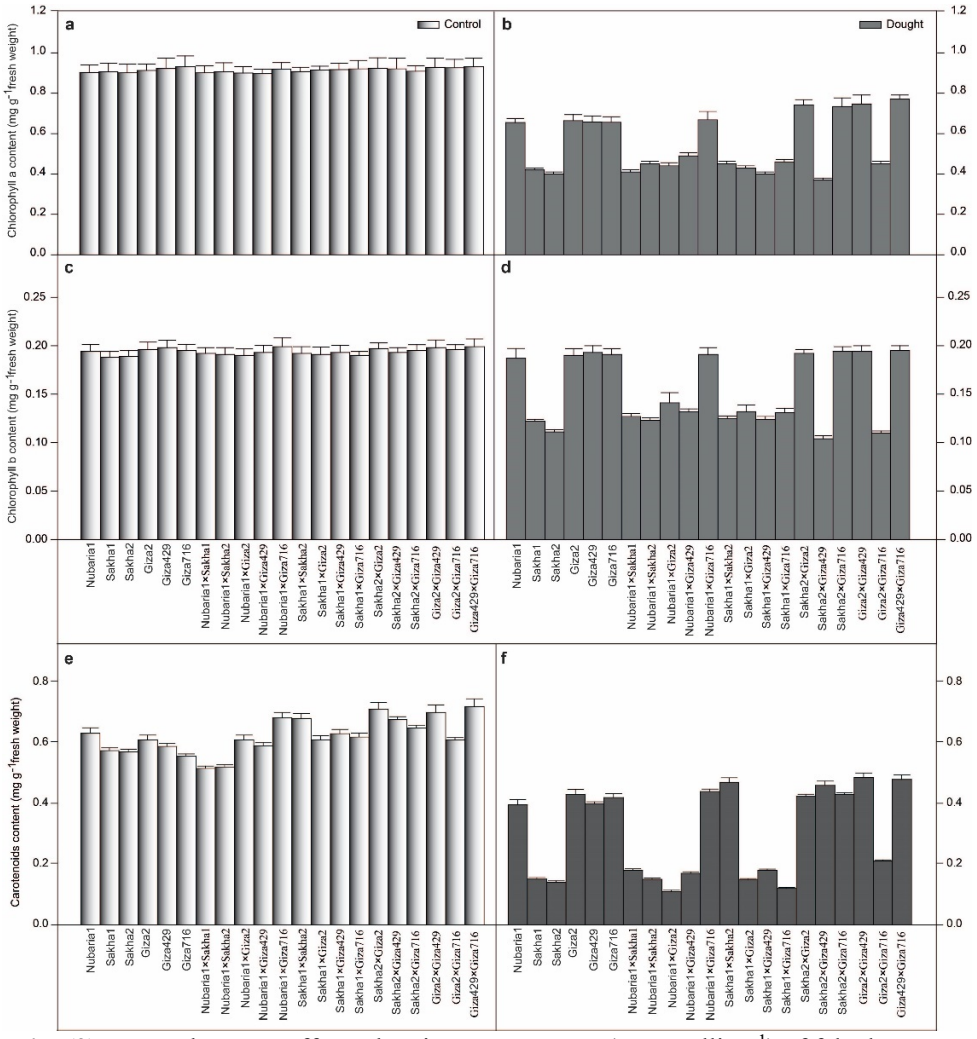

Fig. (2). Drought stress affects the pigments content (mg seedling^{-1}) of faba bean genotypes. (**a**) Chlorophyll a content under normal conditions; (**b**) Chlorophyll a content under drought stress conditions; (**c**) Chlorophyll b content under normal conditions; (**d**) Chlorophyll b content under drought stress conditions; **(e)** Carotenoids content under normal conditions; **(f)** Carotenoids content under drought stress conditions. Values are the mean ± SD (n = 8).

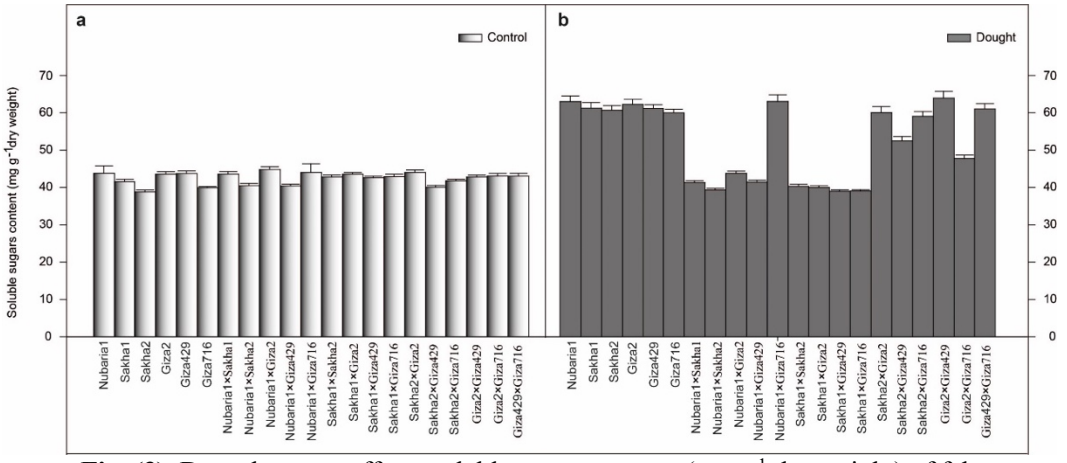

Fig. (3). Drought stress affects soluble sugars content (mg g^{-1} dry weight) of faba bean genotypes. (**a**) Soluble sugars content under normal conditions; (**b**) Soluble sugars content under drought stress conditions. Values are the mean ± SD (n = 8).

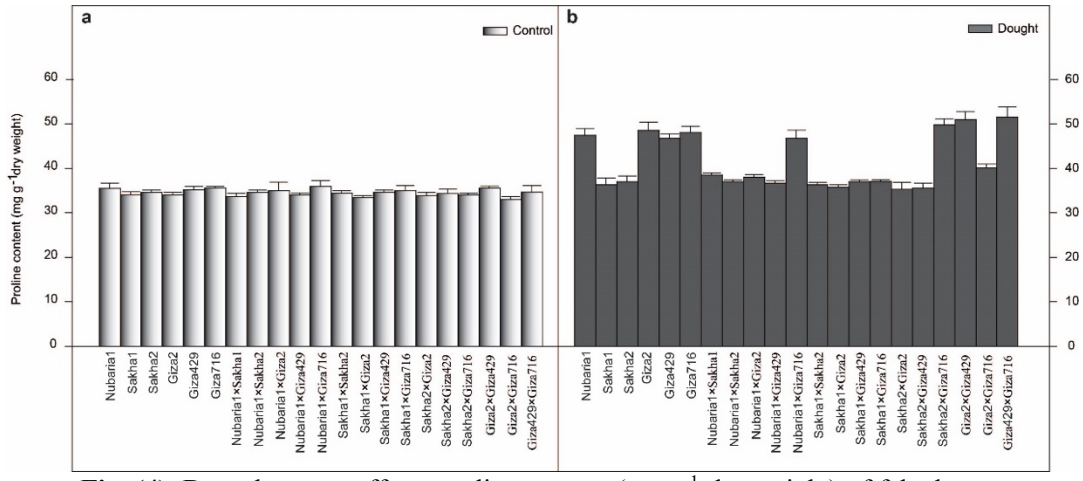

Fig. (4). Drought stress affects proline content (mg g^{-1} dry weight) of faba bean genotypes. (**a**) Proline content under normal conditions; (**b**) Proline content under drought stress conditions. Values are the mean ± SD (n = 8).

2. Biochemical Changes in Faba Bean Genotypes as a Response to Drought Stress

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) is widely used to fractionate the proteins according to their molecular weights (Sharobeen et al., 1991). In order to study the variation of the studied genotypes in response to drought stress, protein extracts were analyzed by

SDS-PAGE. The analysis showed induction of ~15, 20, 35, 45, 80 and 120 kDa proteins under stress condition, which could be used as a positive markers of drought tolerance (Fig. 5). These results also reveal that the drought tolerant and susceptible genotypes of faba bean differed from each other in their protein patterns and each of them characterized by the presence of some specific protein bands. Robinson et al. (1990) suggested that the disappearance of polypeptides during stress were compensated by the increased synthesis of others. Moreover, under drought stress, despite the reduction in protein levels (Singla and Grover, 1994), the cells preferentially synthesized a few specific proteins that are termed stress proteins (Pureek et al., 1995).

3. Differential Expression of the Genes Involved in Responses to Water-Deficit Stress in Faba Bean Genotypes

At the molecular level, plants regulate the expression of stress-tolerance-related genes to cope with different stresses. Previous studies identified *Dhns*, *APETALA2/ERFs*, *HSPs* and *AQPs* gene-families as induced proteins under drought stress conditions (Hanin et al., 2011; Cao et al., 2015; Xiang et al., 2018 and Shekoofa and Sinclair, 2018). To further explore the molecular response to drought stress in faba bean genotypes, the expression level of *VfDHN4* was measured in the leaves of investigated faba bean genotypes under normal and drought stress conditions. No significant differences were recorded in the expression level of all genotypes (Fig. 6a), while a marked induction of *VfDHN4* was recorded after preventing irrigation for seven consecutive days, especially in leaves of Nubaria 1, Giza 2, Giza 429, Giza 716, Nubaria 1 × Giza 716, Sakha 2 × Giza 2, Sakha 2 × Giza 716, Giza 2 × Giza 429 and Sakha 2 × Sakha 1 up to 11.07, 12.71, 13.47, 12.52, 13.52, 13.31, 13.03, 14.00, 14,32, respectively (Fig. 6b). A previous study indicated that *DHN4* is regulated by an ABA-dependent signal pathway and that the high sensitivity of *VfDHN4* to ABA might be an important mechanism enhancing the drought tolerance of faba bean (Lv et al., 2017).

Similar to the results of *VfDHN4* gene expression, there were no significant differences in the gene expression of both *VfAPETALA2* and *VfHSP18* among all faba bean genotypes under normal conditions (Fig. 6c, e). Under drought stress conditions, the transcript levels of *VfAPETALA2* and *VfHSP18* were up-regulated in leaves of Nubaria 1, Giza 2, Giza 429, Giza 716, Nubaria 1 × Giza 716, Sakha 2 × Giza 2, Sakha 2 × Giza 716, Giza 2 × Giza 429 and Sakha 2 × Sakha 1 up to 2.77, 3.01, 2.95, 2.84, 3.21, 3.51, 2.84, 3.22 and 3.02, and 2.10, 2.22, 2.31, 2.12, 2.41, 2.50, 2.43, 2.36 and 2.51, respectively (Fig. 6d, f). Heat shock proteins HSPs and *APETALA2*/ERFs function as molecular chaperones. These proteins are encoded by multi-gene families, whose members play crucial roles in plant growth, development and

stress response (Okamuro et al., 1997; Nagaya et al., 2009; Mustari et al., 2016; Chen et al., 2018 and Lin et al., 2018).

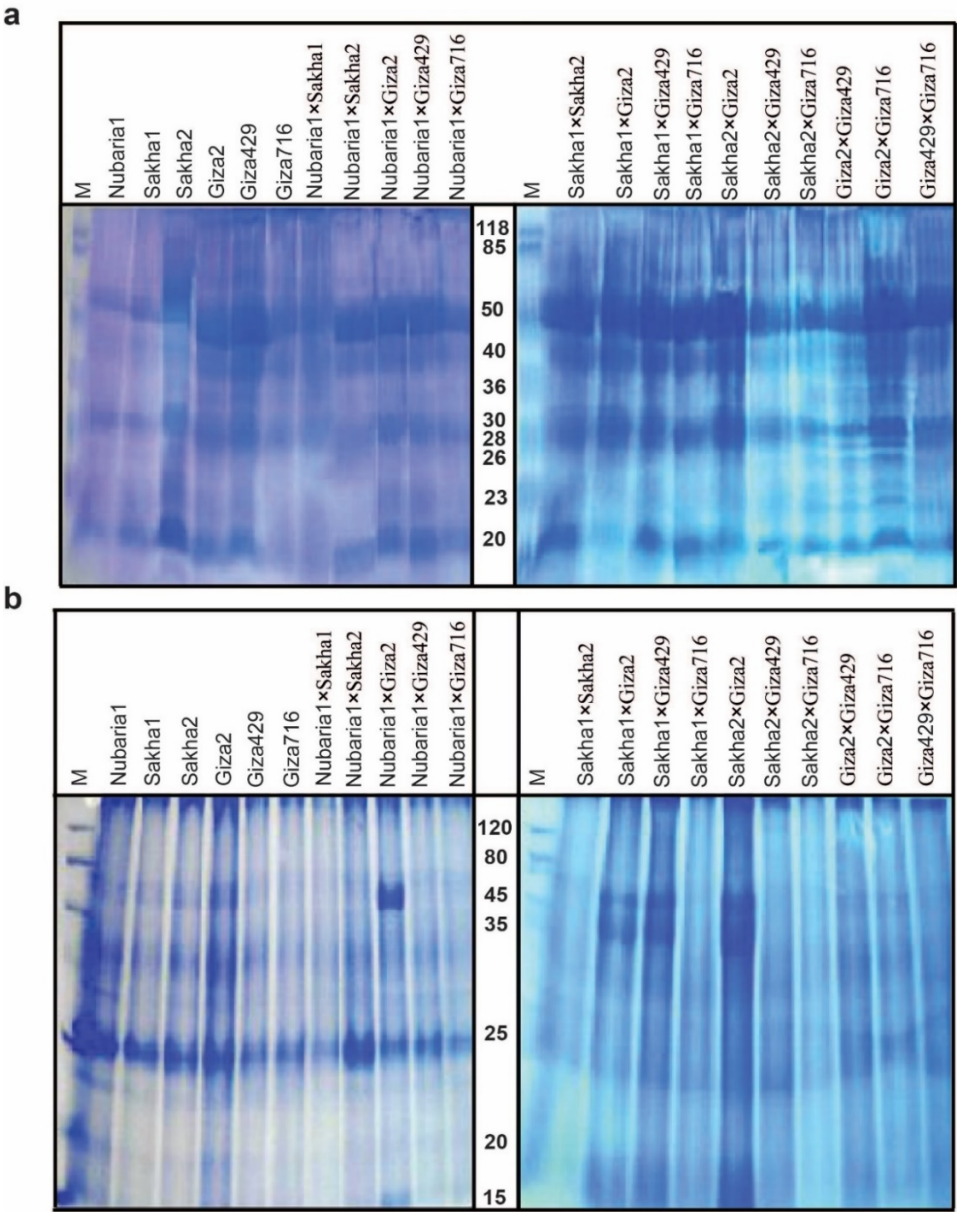

Fig. (5). Drought stress affects faba bean proteome. (**a**) Electrophoretic profiles of total proteins of parental and F_2 genotypes of faba bean under normal condition; (**b**) Electrophoretic profiles of total proteins of parental and F_2 genotypes of faba bean under stress condition.

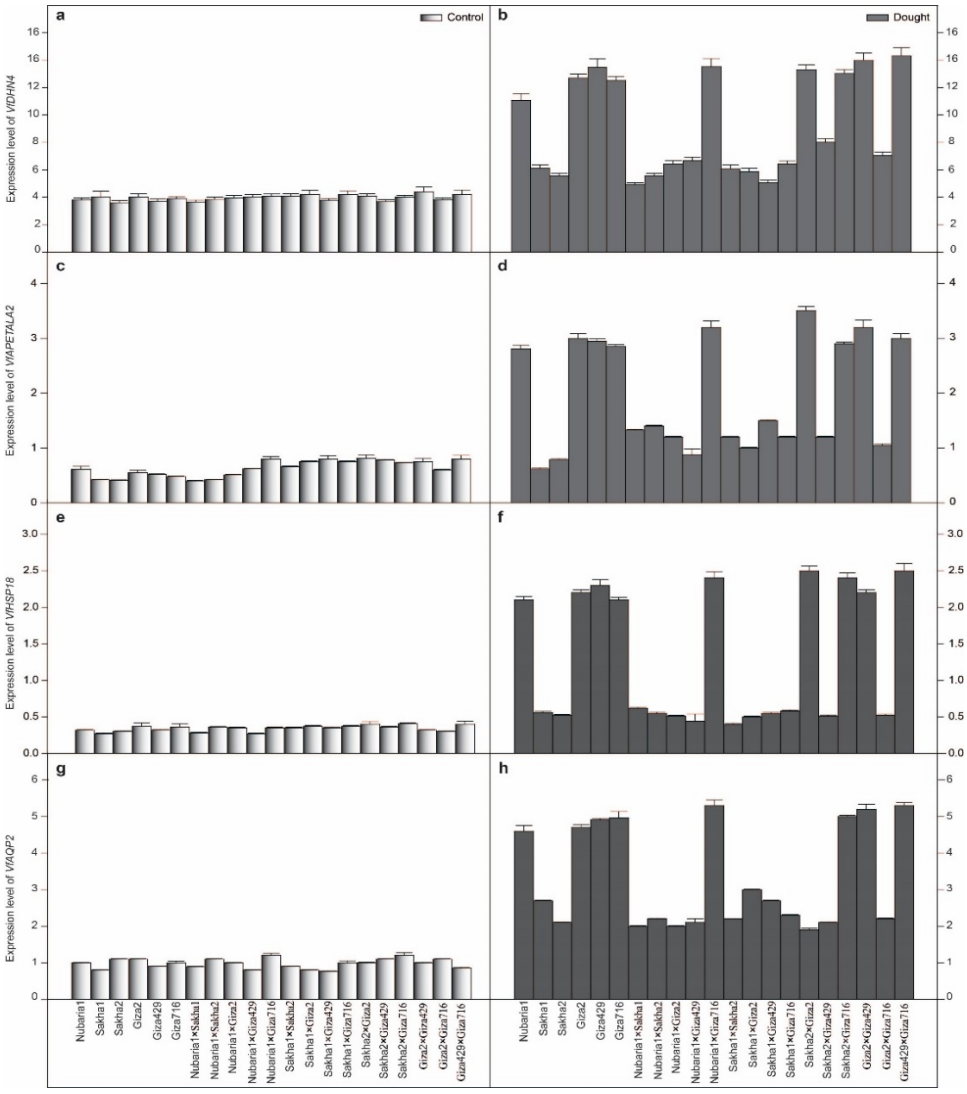

Fig. (6). Real-time quantitative PCR analysis for the transcripts of key genes involved in drought-stress tolerance. (**a** and **b**) Expression level of *VfDHN4* under normal and drought stress conditions, respectively; (**c** and **d**) Expression level of *VfAPETALA2* under normal and drought stress conditions, respectively; (**e** and **f**) Expression level of *VfHSP18* under normal and drought stress conditions, respectively; (**g** and **h**) Expression level of *VfAQP2* under normal and drought stress conditions, respectively. Actin was used as an internal control. Data are means of three biological replicates and error bars are ± SE from three independent experiments, each performed with 6–8 leaves from five separate plants.

In agreement with the results of transcript analysis of *VfDHN4, VfAPETALA2* and *VfHSP18* under normal conditions, no significant differences were obtained in the expression level of *VfAQP2* among all studied faba bean genotypes (Fig. 6g). Conversely, a marked induction of *VfAQP2* was registered after preventing irrigation for seven days, especially in leaves of Nubaria 1, Giza 2, Giza 429, Giza 716, Nubaria 1 × Giza 716, Sakha 2 × Giza 716, Giza 2 × Giza 429 and Sakha 2 × Sakha 1 up to 4.62, 4.71, 4.90, 4.96, 5.3, 5.00, 5.21 and 5.32, respectively (Fig. 6h). Aquaporins are membrane channels that facilitate the transport of water and small neutral molecules across biological membranes of most living organisms. In plants, aquaporins occur as multiple isoforms reflecting a high diversity of cellular localizations, transport selectivity, and regulation properties (Maurel et al., 2015). Induction of the aquaporin proteins (AQPs) under drought stress (Satake et al., 2010) has been reported previously in different plant species including, strawberry (Alleva et al., 2010), *Beta vulgaris* (Alleva et al., 2006), maize (Aroca et al., 2005) and *Phaseolus vulgaris* (Aroca et al., 2006).

CONCLUSION

In conclusion, drought stress impacts cell metabolism of economic crops, specifically their photosynthetic machinery. In the present study, several drought-responsive genes and genotypic differentially expressed genes between genotypes were analyzed by the real-time PCR approach using twenty-one faba bean genotypes with contrasting drought tolerance. Different regulation patterns and functional enrichment of these genes between the tolerant and sensitive genotypes were obtained. The results of gene expression were consistent with those of physiological and biochemical analyses, including the values of biomass, soluble sugars, chlorophyll, carotenoids, proline and peptides. The results of the present study help to elucidate the drought-responsive molecular mechanisms and provide candidate genes for further study to improve drought tolerance in faba bean breeding program.

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Table (S1). Names, origin, pedigree and features of the twenty-one faba bean genotypes.

Genotype	Origin	Pedigree	Features
Nubaria$_1$	FCRI	Individual plant selection from Spanish variety Reina Blanca	Large-seeded, foliar disease resistant, colorless-hilum seed and drought tolerant
Sakha$_1$	FCRI	Reina Blanca×461/845/83	Foliar disease resistant and drought tolerant
Sakha$_2$	FCRI	-716/724/88×620/283/85	Early and foliar disease resistant
Giza$_2$	FCRI	Individual plant selection from Giza$_{402}$ variety	Orbanche resistant
Giza$_{429}$	FCRI	461/842/83×503/453/83	Early, foliar disease resistant and drought tolerant
Giza$_{716}$	FCRI	Individual plant selection from local genetic resources	Early
Nubaria$_1$×Sakha$_1$	EDGB	Half diallele cross	F_2
Nubaria$_1$×Sakha$_2$	EDGB	Half diallele cross	F_2
Nubaria$_1$×Giza$_2$	EDGB	Half diallele cross	F_2
Nubaria$_1$×Giza$_{429}$	EDGB	Half diallele cross	F_2
Nubaria$_1$×Giza$_{716}$	EDGB	Half diallele cross	F_2
Sakha$_1$×Sakha$_2$	EDGB	Half diallele cross	F_2
Sakha$_1$×Giza$_2$	EDGB	Half diallele cross	F_2
Sakha$_1$×Giza$_{429}$	EDGB	Half diallele cross	F_2
Sakha$_1$×Giza$_{716}$	EDGB	Half diallele cross	F_2
Sakha$_2$×Giza$_2$	EDGB	Half diallele cross	F_2
Sakha$_2$×Giza$_{429}$	EDGB	Half diallele cross	F_2
Sakha$_2$×Giza$_{716}$	EDGB	Half diallele cross	F_2
Giza$_2$×Giza$_{429}$	EDGB	Half diallele cross	F_2
Giza$_2$×Giza$_{716}$	EDGB	Half diallele cross	F_2
Giza$_{429}$×Giza$_{716}$	EDGB	Half diallele cross	F_2

FCRI = Field Crops Research Institute, Agricultural Research Center "ARC", Giza, Egypt.
EDGB = Egyptian Desert Gene Bank, Desert Research Center "DRC" North Sinai, Egypt.

Table (S2). Primer pairs used in this study for gene expression analysis.

Experiment	Primer Name	Primer Sequence
Expression analysis	*VfDHN4* -FW	CATGAGGGACGAGCACCAGACT
	VfDHN4-RV	GATCTTCTCCTTGATGCCCTTCT
	VfAPETALA2-FW	AAGAGGACCATCTCTCAG
	VfAPETALA2-RV	AACACTCGCTAGCTTCTC
	VfHSP18-FW	CAAGTTGCCCATCATTACGG
	VfHSP18-RV	TATGCCGGGCACTTTCCCAC
	VfAQP2-FW	GCCTCAGGCCCCAATCTAAT
	VfAQP2-RV	AGGAGGAGTGTGGAGGGTTT
	Actin-FW	AAATGACGCAGATTATGTTTGA
	Actin-FW	GCTCGTAGTGAGGGAGTACC

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توصيف الجينوم واسع النطاق للجينات المستجيبة للجفاف يسرع برنامج تربية الفول البلدى

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يمثل الجفاف أحد الضغوط البيئية الحادة التي تؤثر سلبًا على نمو وتطور الفول البلدي، مما يؤدي إلى انخفاض الإنتاجية في كلًا من المناطق الجافة وشبة الجافة على مستوى العالم. إلا أنه لم يتم توضيح إستجابة الفول البلدي للجفاف بشكل تفصيلي على المستوى الجزيئي. في الدراسة الحالية تم تعريض بادرات ستة أصناف من الفول البلدي إضافة إلى الخمسة عشر هجين الناتجة منها بنظام التهجين نصف الدائري في الجيل الثاني لظروف الجفاف لمدة أسبوع، لدراسة التغيرات في الفول البلدي على مستوى التعبير الجيني والبروتيني والأيضي. وعكست النتائج التأثير السلبي للجفاف على الدلائل القسيولوجية والأيضية في الفول البلدي. بينما أظهرت بعض التراكيب الوراثية تحملها للجفاف حيث زادت معدلات الكلوروفيل والكاروتين والكتلة الحيوية في بعضها عن باقي التراكيب الوراثية الأخرى. وفي نفس السياق زادت معدلات البرولين والسكريات الذائبة في نفس التراكيب الوراثية المتحملة للجفاف عن باقي التراكيب الوراثية الأخرى. كما أظهر التحليل البيوكيميائي إختلاف البروتين في التراكيب الوراثية محل الدراسة تحت ظروف الإجهاد المائي. في حين أكدت نتائج التعبير الجيني زيادة حث الجينات المرتبطة بتحمل الجفاف في نفس التراكيب الوراثية المتميزة السابقة. وأخيرًا فإن هذه الدراسة تمثل إستراتيجية متميزة لتسريع برامج تربية المحاصيل الإستراتيجية المختلفة إعتمادًا على الآلية الجزيئية والبيوكيميائية والفسيولوجية الخاصة بها تحت ظروف الإجهاد المائي.