



Effects of Cobalt Application on Yield Attributes, Nutritive Value, and Protein Pattern of Rice (*Oryza sativa* L.) Grains under Drought Conditions



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THE PRESENT study is aimed at investigating the drought stress responses of two Egyptian rice varieties (Sakha 104 and Giza 178) after grain soaking in cobalt nutrient solution at the optimum concentration before sowing in soil and gaining physiological insights into drought tolerance in rice plants. The drought stress was applied according to different irrigation intervals (control; irrigation every 4 days, moderate; irrigation every 6 days; and severe; irrigation every 8 days). The results showed variable reductions in the tiller number/plant, tiller bearing panicles/plant, panicle length/plant, panicle weight/plant, 100-grains weight, and harvest index in both varieties under drought stress. Also, drought stress significantly reduced the nutritional value of the grains such as sucrose, soluble sugars, polysaccharides, total carbohydrates, total protein, vitamins (B9 and B12), as well as element content (Ca^{2+} , Na^+ , Fe^{2+}). Meanwhile, there was a notable increase in the grain contents of glucose, K^+ , K/Na ratio, and Co^{2+} of both rice varieties under drought conditions. Cobalt application significantly alleviated the detrimental effects of drought stress by improving the above-mentioned parameters comparably in both rice varieties, particularly in Giza 178. The results of sodium dodecyl-sulfate polyacrylamide gel electrophoresis of drought stress either alone or in combination with cobalt nutrition showed *de novo* synthesis of protein pattern bands in the grains with the molecular weights of 33, 91, and 110kDa in the Sakha 104 variety and 42, 53, 57, 60, 71, 91, and 97 kDa in the Giza 178 variety.

Keywords: Cobalt, Drought, *Oryza sativa* L., Protein, Vitamin B.

Introduction

Rice (*Oryza sativa* L.) is one of the world's most significant grains, covering about 10 % of fertile land and serving as the staple food for more than half of the world's population. It also plays a vital role in economic and social stability (Goodman, 2020). According to a previous study, the total area under rice cultivation in 2017 was around 167 million hectares (Zhang et al., 2021). However, in these years, rice production faces numerous challenges, including an unstable climate, heavy metal pollution, and drought (Chandio et al., 2020). As a result, sustaining rice productivity stability in a deteriorating environment is critical for national food security.

Drought stress is regarded as the most damaging

environmental danger to crop productivity among all abiotic stresses, particularly in arid and semiarid regions of the world (Quiroga et al., 2020). Understanding the mechanisms of plant responses to drought conditions is crucial to enhancing agricultural output in broad parts of the world where rainfall is limited or erratic. Aside from the drought's inherent complications (Passioura, 2007), rice is extremely sensitive to water stress throughout the reproductive stage, resulting in a considerable decrease in grain productivity (Kamoshita et al., 2008; Palanog et al., 2014). The quantity of crop loss is determined by the growth stage and period, as well as the intensity of drought stress (Gana, 2011; Kumar et al., 2014). In one study, severe drought stress administered during the vegetative stage and mild drought stress applied during the blooming stage resulted in yield losses

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of 20 and 28%, respectively (Babu et al., 2003). Also, drought reduces rice yield components such as panicle length, primary and secondary branches per panicle, seed setting rate, and grain weight per panicle (Muthurajan et al., 2011). Moreover, water deficiency causes various morphological changes, including reduced leaf and tiller expansion, increased leaf rolling and drying, stomatal closure, decreased photosynthesis, and limited dry matter buildup (Kumar, 2015).

Cereals and other plants interconvert monosaccharides, disaccharides, and more complex carbohydrates such as fructan to cope with osmotic stresses, including those caused by salt, freezing, and drought, as well as other stresses such as hypoxia and early senescence (Halford et al., 2011). On the other hand, vitamin B is the precursor to vital metabolic cofactors which are susceptible to oxidation under stress conditions. Also, cobalamin, or cyanocobalamin, is the active form of vitamin B12, and it is required for the modulation of DNA synthesis during cell division (Smith et al., 2007). Moreover, folic acid (vitamin B9) helps plants make RNA, a nucleic acid that transports information from DNA to plant cell structures known as ribosomes and aids in protein synthesis (Poudineh et al., 2015). As a result, it stands to reason that stressed plants suffer from B vitamin shortages and that certain stress symptoms are metabolic side consequences of these deficiencies (Hanson et al., 2016). Drought stress reduces nutrient uptake by the roots, which is caused by a decrease in soil moisture, which causes a decrease in the rate of nutrient diffusion from the soil matrix to the absorbing root surface and translocation to the leaves (Hu et al., 2007). Protein content and composition are major determinants of cereal grain quality, and they are susceptible to drought, heat stress, and atmospheric CO₂ concentrations (Halford et al., 2015).

It is a big problem to enhance rice output in the face of increased drought caused by climate change; nevertheless, it may be accomplished by the adoption of drought-tolerant rice varieties capable of producing high yields under drought-stress conditions or treatment with adaptive chemicals (Luo, 2010). Since the publication of Bertrand's rule (Bertrand, 1912) and the essentiality concept (Arnon & Stout, 1939), significant progress has been made in plant mineral nutrition. Cobalt (Co) has been considered a beneficial element for prokaryotes, humans, and other mammals, but its importance for plants, particularly non-leguminous plants, is

unclear (Hu et al., 2021). It is a key component of cobalamin (vitamin B12 and its derivatives), as well as a cofactor for a variety of enzymes and a component of various proteins in prokaryotes and animals (Odaka & Kobayashi, 2013). Co at low concentrations promotes the growth of non-leguminous crops (Hu et al., 2021). The abilities of Co to combat abiotic stresses like salinity, drought, and cadmium have been documented (Gad et al., 2018; Akeel & Jahan, 2020). Foliar application of Co increased tomato growth and fruit yield under salt stress (Gad et al., 2017), controlled ions homeostasis in cucumber (Gad et al., 2018), and modulated phytohormones in maize (Gad & El-Metwally, 2015).

There are several reasons for the increased growth of non-leguminous plants under the effect of Co at the appropriate levels, which can be summarized as follows: (1) increased resistance to abiotic stresses, (2) antioxidative enzyme activation, (3) replacement of active metals, and (4) hormesis (Hu et al., 2021). Co-application has been reported to alleviate drought, salt, and heavy metal stresses, resulting in unaffected plant growth (Hu et al., 2021). Abiotic stresses, in general, cause plant imbalances in the production and accumulation of reactive oxygen species (ROS) (Sachdev et al., 2021). ROS can activate the antioxidant system of the plant, thus minimizing the damage (Tewari et al., 2002; Choudhury et al., 2017). The action of the antioxidant system is the first line of defense against adverse effects. As a result, increased activities of ascorbate peroxidase, catalase, peroxidase, and superoxide dismutase are not surprising (Hasanuzzaman et al., 2020). When Co is used at the proper concentrations, can activate antioxidative enzymes, thus reducing ROS-caused damage (Hu et al., 2021). A study by Tourky et al. (2023) revealed the enhanced effect of CoSO₄ at concentrations 10 and 44.5 µm on the antioxidant defense system in two rice varieties under different intensities of drought stress during the reproductive stage. Throughout this stage, Co increased the strength of the antioxidant defense system and inhibited ROS production by increasing the activities of catalase, peroxidase, and polyphenol oxidase enzymes, increasing the production of phenols and flavonoids compounds, enhancing the protein and proline levels, and hindering the injure of membranes and lipid peroxidation, thus enhanced rice growth (Tourky et al., 2023).

Therefore, the objective of this study was to give

a physiological overview of the negative impacts of drought stress besides the ameliorative effect of Co nutrition on yield attributes and the nutritive value of rice grains by assessing the content of carbohydrate fractions, minerals, vitamins, total protein, and study the changes in protein pattern in the grains of two Egyptian rice varieties contrasting in drought tolerance.

Materials and Methods

Plant material and environmental conditions

Plants of twenty-eight-day-old Egyptian rice varieties (Sakha104; drought-sensitive; and Giza 178; drought-tolerant) were used. The grains of these varieties were obtained from the Rice Research and Training Center in Sakha, Kafr El-Sheik, Egypt. The main characteristics of these varieties are shown in Table 1. On 14th May 2019, the grains of each variety (200–300 grains) were soaked in two groups of plastic boxes (22 x 17 x 9cm); one containing 2 liters of CoSO₄ at the optimum concentration (44.5μM for the Sakha 104 variety, and 10μM for the Giza 178 variety), and the other was 2 liters of distilled water (control). The used concentration of CoSO₄ for each variety was chosen according to previous preliminary germination experiments (Saleh, 2021). Both groups were incubated in the dark at 27 ± 2°C for 72h under sterilized conditions at the Lab of Plant Physiology, Faculty of Science, Mansoura University, Egypt. Subsequently, the grains were sowed in the soil until they reached a 28-day-old. After that, a pot experiment was carried out in the Botanical Garden of the Faculty of Agriculture, Mansoura University, Egypt, during the summer season of 2019 (from 17th May to 21st October). Mansoura is located between latitude 13°01' North and longitude 05°15' East. The average temperature and relative humidity during

the experiment were about 30 ± 5°C and 69–76%, respectively. The characteristics of the used soil have been illustrated in Table 2, which was taken from the upper 0-30 cm arable layer.

Drought stress application

On the 14th of June, and after twenty-eight days of grain sowing, an identical lot of healthy seedlings from each variety was taken and transplanted in plastic pots: 10 seedlings/pot (25cm diameter and 20cm height), containing 7kg silty texture soil. Thus, 72 pots in a completely randomized design, were divided into two groups (36 pots for each variety). The first group was for the Sakha 104 variety, which was divided into two subgroups; the first (18 pots) were seedlings from grains soaked previously in distilled water for 72h before sowing in soil, and the second (18 pots) were the seedlings from grains soaked previously in CoSO₄ (44.5μM) for 72h before sowing in soil. Afterward, each subgroup was divided into three sets (each set with hexa-replicates) that represented three levels of drought stress application based on different irrigation intervals, as follows: set 1; watering every 4 days (assigned as a control, C4) as recommended by The Ministry of Agriculture in Egypt, set 2; watering every 6 days (assigned as moderate drought stress, C6), and, set 3; watering every 8 days (assigned as severe drought stress, C8). Combination with CoSO₄ treatments was labelled as T4, T6, and T8. The second group was for the Giza 178 variety, which was divided similarly to the Sakha 104 variety in terms of drought stress, using an optimum concentration of 10μM CoSO₄. Thinning was performed a week after transplantation to leave 5 uniform young plants/pot for experimentation. To avoid any shock at this stage, drought stress was applied two weeks after transplantation.

TABLE 1. Details of two varieties

Genotype	Pedigree	Origin	Salience and feature
Giza 178	Giza175/Milyang 49	Egypt	Indica/Japonica type, medium maturing, semi-dwarf, resistant to blast, medium grain, tolerant to drought, and high yield
Sakha 104	GZ4096-8-1/GZ4100-9-1	Egypt	Japonica type—sensitive to drought—long stature, moderate duration—sensitive to blast.

TABLE 2. Characteristics of the used soil

Soil characters	pH	EC (dS/m)	O.C (%)	T.N (%)	C/N ratio	T.S.S (%)	W.H.C (%)
Silty texture soil	8.17	0.52	0.71	0.26	2.73	0.52	48.5

W.H.C., water holding capacity; EC, electrical conductivity; O.C., organic carbon; T.N., total nitrogen; TSS., total soluble solids.

Recommended doses of fertilizers (N–P₂O₅–K₂O) besides insect and weed control were applied at the appropriate times throughout the experiment. Thus, a hex-replicated in a completely randomized design of 6 treatments /variety was represented. The plants were harvested on 6th October for Giza 178 variety and on 21st October for Sakha 104 variety to record yield attributes (tillers number/plant, tillers barring panicles/plant, panicle length/plant, panicle weight/plant, weight of 100-grains weight, and harvest index (HI)). The grain nutritive value as carbohydrate fractions, minerals (K⁺, Na⁺, K/Na ratio, Ca²⁺, Fe²⁺, and Co²⁺), vitamin B9, vitamin B12, total protein as well as the changes in protein banding pattern in the grains was investigated.

Nutritious value of grains

Estimation of carbohydrate fractions

Carbohydrate fractions were isolated from grain samples of both varieties using the method developed by Yemm & Willis (1954). The O-toluidine approach developed by Feteris (1965) and modified by Riazi et al. (1985) was used to measure glucose. Sucrose content was evaluated using a Van Handel modification (1968). The concentration of soluble sugars (SS) was assessed by modifying the processes of Yemm & Willis (1954). The total carbohydrates in grain extracts were determined using the method developed by Hedge et al. (1962). The polysaccharides content was estimated by subtracting SS from total carbohydrates.

Determination of mineral content

Grain samples were digested in HNO₃ and made up to volume with deionized water as described by Motsara & Roy (2008). The concentrations of K⁺, Na⁺, Ca²⁺, Fe²⁺ and Co²⁺ in the samples were measured by Atomic Absorption Spectrophotometry (GBC Scientific Equipment Model SensAA, Dandenony, Victoria, Australia) according to Jackson et al. (1973).

Determination of vitamins B9 and B12

Vitamins B9 and B12 in grains were determined according to a simple spectrophotometric method described by Ruengsitagoon & Hattanat (2012) and Kumar et al. (2010). Absorption was measured at 460 nm and 275 nm, respectively.

Protein profile

Protein concentration in enzyme extracts was determined using Bradford's method (1976). Soluble proteins from grains were extracted according to Reuveni et al. (1992) with some modifications. Removing and quickly rinsing the cells with 0.1 M NaCl once. The lytic solution contained 5% (v/v), 2 mM EDTA, 2% SDS, and 5% sucrose. The solution was rehabilitated to provide a lysis solution for cell pellets. Then the tube was put on ice for 3 minutes and repeated three times quickly in the water bath at 100°C for 3 minutes. To verify that breakage occurred, test the microscopic suspension. If not, freeze and restore the sample quickly with a high-speed cooling centrifuge. Take the supernatant out into a different tube. Separation on a 12.5% SDS-PAGE was done according to the methods adopted by Hanna et al. (2000). Protein gels were stained with Coomassie brilliant blue R-250 solution.

Statistical analysis

The data obtained is a mean value of six independent treatments with six replicates per variety, which were subjected to one-way ANOVA. The error bars shown in the graph are standard error (\pm SE) of mean values. The significance of differences between treatments was evaluated using Post Hoc Duncan's test using COSTAT Version 6.3 to compare the means (developed by Cohort software, Berkeley, California, USA) at the significance level of $P \leq 0.05$, using the IBM SPSS Statistics 23.0.

Results and Discussion

Changes in yield attributes

As shown in Table 3 and Photo 1, tiller numbers/plant, tillers barring panicles/plant, panicles length/plant, panicles weight/plant, 100-grain weight, and HI, in both varieties recorded a variable significant or non-significant decrease with the progress in the level of drought stress, as compared with controls. The negative effect was more pronounced in the Sakha 104 variety than in the Giza 178 variety. These findings are consistent with those of Ihsan et al. (2016), who noticed a reduction in grain yield in barley under drought stress by reducing the number of tillers, spikes, grains/plant, individual grain weight, and plants matured earlier than non-stressed plants with a higher rate of grain filling. The depletion in HI

could be attributed to a shortage of nutrients and an increase in empty grain output because of drought stress conditions (Hossain et al., 2020). Whereas panicle parameter reductions might be owing to a considerable fall in photosynthesis rate, which resulted in lower production of assimilates for panicle development and rice grain filling, resulting in a severe loss in yield (Akram et al., 2013). Conversely, Co application led to a noticeable enhancement in the previous parameters as compared with untreated plants, and the values of increase were more pronounced in the Giza 178 variety than in

Sakha 104 (Table 3 and photo 1). It was shown previously that Co treatments have improved growth performance under salinity stress in maize (Gad & El-Metwally, 2015), tomato (Gad et al., 2017), and onion (Gad et al., 2020) by improving physiological responses (e.g., ions and reactive oxygen species homeostasis) and modulating the level of phytohormones (e.g., auxins and cytokinins). This promoting effect of Co was more tangible in the Giza 178 (drought tolerant) variety than in the Sakha 104 (drought-sensitive) as a strategy of drought tolerance.

TABLE 3. Effects of drought stress either alone or in combination with CoSO_4 on yield attributes of Sakha 104 and Giza 178 plants at the harvest stage. Data are the means of ten replicates \pm standard error. C4; Control, C6; plants irrigated every 6 days, C8; plants irrigated every 8 days, T4; Control+ CoSO_4 , T6; plants irrigated every 6 days + CoSO_4 , and T8; plants irrigated every 8 days + CoSO_4

Variety	Parameters	Treatments					
		C4	C6	C8	T4	T6	T8
Sakha 104	Tillers number/plant	12.25 ^{ab} ± 0.160	11.00 ^b ± 0.136	7.75 ^d ± 0.160	12.75 ^a ± 0.160	11.50 ^{ab} ± 0.096	9.50 ^c ± 0.096
	Tillers birring panicles	11.25 ^{ab} ± 0.083	9.50 ^c ± 0.096	6.50 ^d ± 0.167	12.25 ^a ± 0.210	10.00 ^{bc} ± 0.192	8.75 ^c ± 0.083
	Panicles number/plant	13.00 ^a ± 0.192	10.75 ^{ab} ± 0.210	10.50 ^b ± 0.096	12.50 ^{ab} ± 0.397	12.00 ^{ab} ± 0.236	11.25 ^{ab} ± 0.160
	Panicles length/ plant	13.58 ^{ab} ± 0.011	12.88 ^{abc} ± 0.015	11.91 ^c ± 0.017	14.08 ^a ± 0.019	13.80 ^a ± 0.018	12.42 ^{bc} ± 0.007
	Panicles weight/plant	0.64 ^a ± 0.003	0.51 ^{ab} ± 0.002	0.33 ^b ± 0.003	0.69 ^a ± 0.003	0.63 ^a ± 0.004	0.41 ^b ± 0.001
	100 grains weight	2.38 ^a ± 0.010	2.16 ^b ± 0.019	2.15 ^b ± 0.016	2.29 ^{ab} ± 0.011	2.19 ^b ± 0.012	2.21 ^b ± 0.024
	HI	0.328 ^{bc} ± 0.002	0.311 ^c ± 0.005	0.240 ^d ± 0.007	0.41 ^a ± 0.005	0.364 ^b ± 0.005	0.341 ^{bc} ± 0.005
Giza 178	Tillers number/plant	13.00 ^b ± 0.178	11.13 ^{cd} ± 0.327	10.25 ^d ± 0.214	14.38 ^a ± 0.153	13.25 ^{ab} ± 0.194	11.63 ^c ± 0.086
	Tillers birring panicles	12.75 ^b ± 0.194	10.75 ^c ± 0.278	8.38 ^d ± 0.198	14.38 ^a ± 0.153	13.00 ^b ± 0.236	10.88 ^c ± 0.107
	Panicles number/plant	9.88 ^c ± 0.288	12.25 ^{ab} ± 0.248	11.13 ^{bc} ± 0.327	12.88 ^a ± 0.226	12.50 ^{ab} ± 0.089	13.63 ^a ± 0.217
	Panicles length/plant	16.23 ^b ± 0.382	14.60 ^c ± 0.264	12.69 ^d ± 0.088	18.45 ^a ± 0.145	14.80 ^c ± 0.204	16.39 ^b ± 0.086
	Panicles weight/plant	1.09 ^a ± 0.085	0.65 ^c ± 0.035	0.64 ^c ± 0.039	1.14 ^a ± 0.082	1.10 ^a ± 0.067	0.850 ^b ± 0.039
	100 grains weight	1.82 ^b ± 0.005	1.77 ^{cd} ± 0.005	1.71 ^c ± 0.004	1.90 ^a ± 0.013	1.79 ^{bc} ± 0.006	1.73 ^{de} ± 0.005
	HI	0.416 ^b ± 0.005	0.368 ^c ± 0.004	0.336 ^d ± 0.002	0.470 ^a ± 0.004	0.425 ^b ± 0.004	0.376 ^c ± 0.002

Mean values with the same superscripts are not significantly different at the $P \leq 0.05$ probability level, using Post Hoc Duncan test.

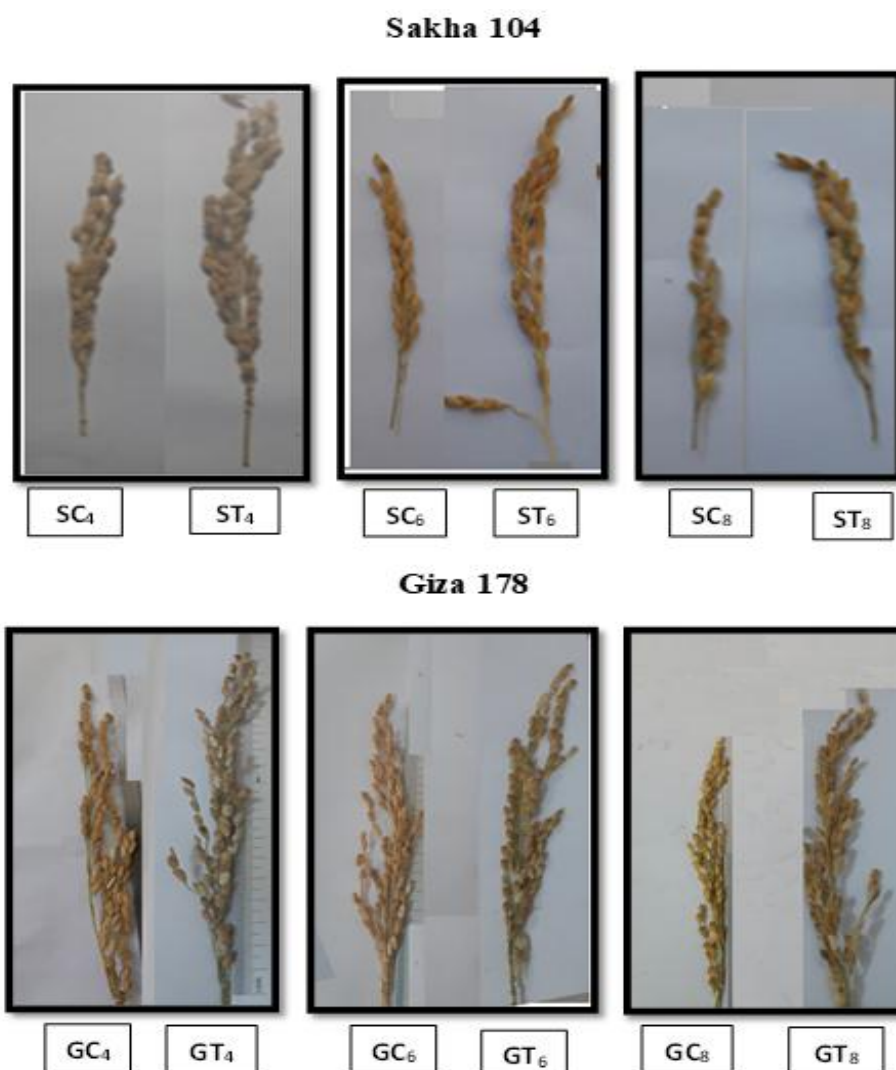


Photo 1. Panicle morphology of Sakha 104 (S) and Giza 178 (G) either under drought stress or in combination with CoSO_4 at the harvest stage: C4; Control, C6; plants irrigated every 6 days, C8; plants irrigated every 8 days, T4; Control+ CoSO_4 , T6; plants irrigated every 6 days + CoSO_4 , and T8: plants irrigated every 8 days + CoSO_4

Nutritious value of grains

Changes in carbohydrate fractions

The data tabulated in Fig. 1 showed a progressive significant increase in grain glucose content with the elevation in drought stress level. Contrariwise, the content of sucrose, SS, polysaccharides, and total carbohydrates showed a progressive significant decrease with altitude in the level of drought stress in both varieties, as compared with control values. For more clarity, treatment C6 increased grain glucose content by 8.4 and 4.2%, and decreased grain total carbohydrates content by 15.7 and 9.6%, whereas treatment C8 increased grain glucose content by 11.6 and 7.4%, and decreased grain

total carbohydrates by 42.1 and 23.9%, in Sakha 104 and Giza 178, respectively, compared with the C4 treatment. These results agree with those reported by Ali et al. (2010) in maize and Emam et al. (2014) in rice due to sink limitations. Parvaiz & Satyawati (2008) suggested that SS has a role as an osmoprotectant, regulating osmotic adjustment, providing membrane protection, and scavenging toxic reactive oxygen species under various types of stress. The tendency of starch degradation under drought stress in this study confirmed previous studies, which suggested that starch is degraded under drought conditions to provide energy and carbon when photosynthesis activity is restricted (Siaut et al., 2011). In the

present investigation, there were differences in the amount of carbohydrate fractions between the two rice varieties. This may be attributed to the metabolism of SS and starch in rice being affected by both drought stress conditions and the characteristics of varieties, or in other words, stimulated starch/sugar interconversion (Dien et al., 2019).

Reciprocally, the grains of the plants that were previously treated with Co, showed a significant and comparable improvement in the content of the previous sugars. Except for SS content, which recorded a reduction with the progress in drought stress level in both varieties as compared with untreated plants (Fig. 1). For more visibility, treatment T4 increased glucose content by 8.4 and 6.5% and total carbohydrates content by 9.7 and 11.2%, and T6 increased glucose content by 11.7

and 10.6% and total carbohydrates content by 8.1 and 9.7%, whereas treatment T8 increased glucose content by 12.6 and 11.9% and total carbohydrates content by 6.3 and 7.1%, in Sakha 104 and Giza 178, respectively, as compared with C4, C6, and C8 treatments. Many authors demonstrated that applying Co to broccoli plants (Gad & Abd El-Moez, 2011) and tomatoes (Gad & Hassan, 2013) over two seasons causes changes in sugar content under drought stress (Gad et al., 2015). The increase in sugar content in Co pretreated stressed rice grains could be attributed to an increase in photosynthetic pigments, resulting in an increase in carbohydrate synthesis and accumulation in the grains (Khalid & Ahmed, 2016). This effect was dependent on the drought tolerance trait, which was more noticeable in the Giza 178 variety than in the Sakha 104.

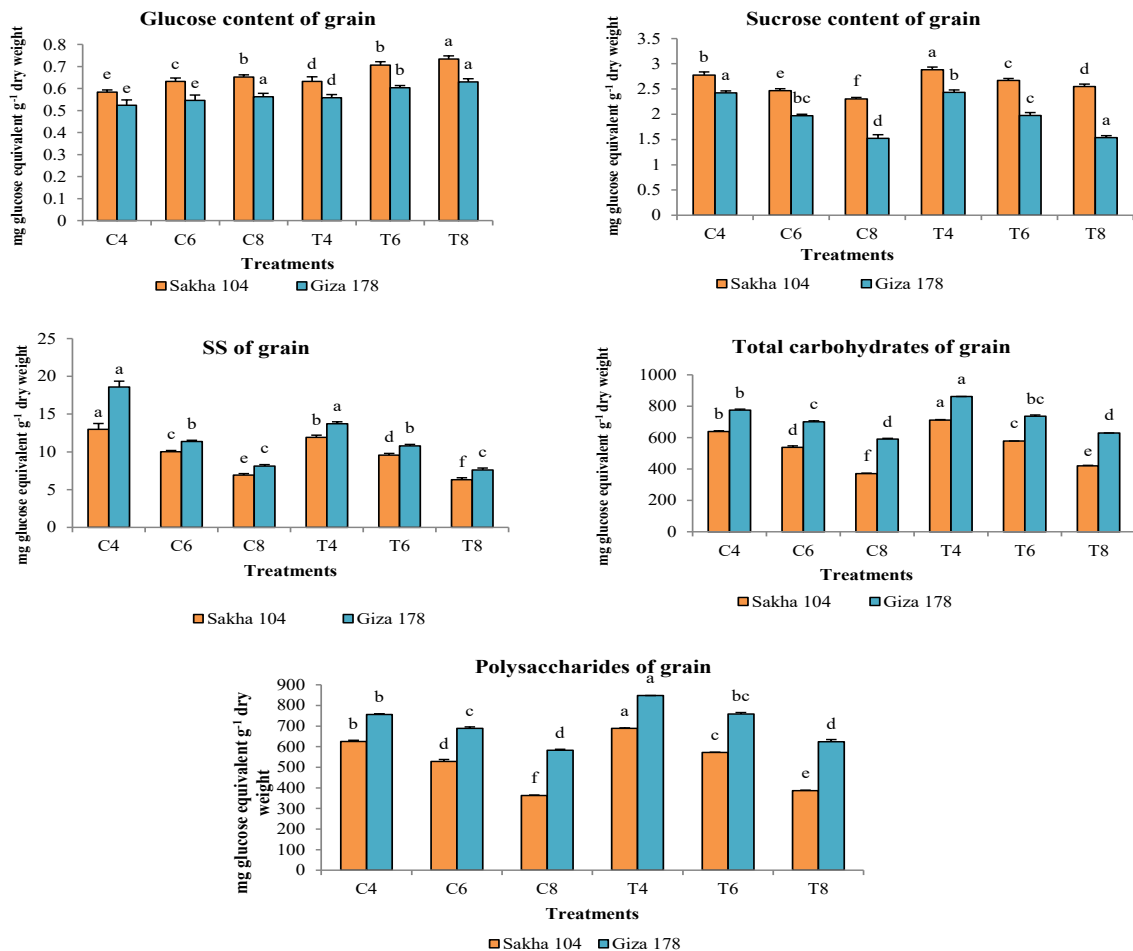


Fig. 1. Effects of drought stress either alone or in combination with CoSO_4 on carbohydrate fractions in grains of Sakha 104 and Giza 178 rice plants at the harvest stage. Vertical bars represent the standard error (\pm S.E.). C4; Control, C6; plants irrigated every 6 days, C8; plants irrigated every 8 days, T4; Control+ CoSO_4 , T6; plants irrigated every 6 days + CoSO_4 , and T8; plants irrigated every 8 days + CoSO_4

Changes in minerals content

The progress in drought stress level significantly decreased the grains content of Ca^{2+} , Na^+ , Fe^{2+} , while showing a significant increase in K^+ , Co^{2+} and K/Na ratio of both rice varieties comparably, as compared with C4 treatment (Table 4). This is because most of these nutrients are generally immobile in plant tissues and soil, which may have hindered the ability of plants to acquire them from the soil at deeper rooting depths or influenced their ability to be remobilized for transit within the phloem (Pang et al., 2011; Maillard et al., 2015). According to several studies, the significance of Na^+ as an osmotic regulator in drought-stressed plants is still debatable. Martínez

et al. (2005) consider that Na^+ has just a minor osmotic regulatory effect in *Atriplex halimus* L. Our findings revealed a significant increase in the K/Na ratio with increasing drought stress levels in the grains of both varieties, indicating a high level of K^+ in the cell which implies that K^+ homeostasis is an important factor in drought tolerance. This is consistent with the findings of Munns & Tester (2008) under salinity stress. As a result, we may conclude that this is a type of adaptation for retaining the water content of stressed rice grains in both varieties, which was more apparent in the Giza 178 variety as a drought-tolerant variety than in Sakha 104.

TABLE 4. Effects of drought stress either alone or in combination with CoSO_4 on elements content of grains of Sakha 104 and Giza 178 plants at the harvest stage. Data are the means of three replicates \pm standard error. C4; Control, C6; plants irrigated every 6 days, C8; plants irrigated every 8 days, T4; Control+ CoSO_4 , T6; plants irrigated every 6 days + CoSO_4 , and T8; plants irrigated every 8 days + CoSO_4

Variety	Parameters	Treatments					
		C4	C6	C8	T4	T6	T8
Sakha 104	K^+	1.050 ^f	1.222 ^c	1.325 ^d	1.540 ^e	1.655 ^b	1.952 ^a
	$\mu\text{g/g}$ dry weight	± 0.012	± 0.022	± 0.054	± 0.023	± 0.032	± 0.041
	Na^+	1.225 ^a	1.112 ^c	1.002 ^e	1.114 ^b	1.054 ^d	0.997 ^f
	$\mu\text{g/g}$ dry weight	± 0.023	± 0.111	± 0.087	± 0.012	± 0.095	± 0.078
	K/Na ratio	0.857 ^f	1.099 ^e	1.322 ^d	1.382 ^c	1.570 ^b	1.958 ^a
		± 0.096	± 0.074	± 0.055	± 0.47	± 0.023	± 0.25
	Ca^{+2}	1.206 ^c	1.084 ^e	0.893 ^f	1.738 ^a	1.393 ^b	1.121 ^d
$\mu\text{g/g}$ dry weight	± 0.102	± 0.099	± 0.101	± 0.130	± 0.101	± 0.111	
	Fe^{+2}	3.901 ^a	3.689 ^b	3.140 ^d	3.253 ^b	3.024 ^e	2.918 ^f
	ppm	± 0.124	± 0.142	± 0.111	± 0.102	± 0.124	± 0.101
	Co^{+2}	0.049 ^e	0.072 ^d	0.111 ^b	0.095 ^c	0.111 ^b	0.140 ^a
	ppm	± 0.125	± 0.154	± 0.213	± 0.152	± 0.135	± 0.231
Giza 178	K^+	1.324 ^f	1.652 ^c	1.745 ^d	2.015 ^c	2.423 ^b	2.652 ^a
	$\mu\text{g/g}$ dry weight	± 0.045	± 0.045	± 0.102	± 0.074	± 0.012	± 0.65
	Na^+	1.265 ^a	1.122 ^c	1.044 ^e	1.222 ^b	1.101 ^d	1.003 ^f
	$\mu\text{g/g}$ dry weight	± 0.014	± 0.012	± 0.025	± 0.011	± 0.010	± 0.065
	K/Na ratio	1.047 ^f	1.472 ^c	1.671 ^c	1.649 ^d	2.201 ^b	2.644 ^a
		± 0.036	± 0.036	± 0.032	± 0.064	± 0.051	± 0.033
	Ca^{+2}	1.168 ^d	0.919 ^e	0.617 ^f	1.877 ^a	1.469 ^b	1.204 ^c
$\mu\text{g/g}$ dry weight	± 0.045	± 0.023	± 0.044	± 0.31	± 0.022	± 0.046	
	Fe^{+2}	3.678 ^a	3.577 ^b	3.489 ^c	3.353 ^d	3.187 ^e	3.119 ^f
	ppm	± 0.052	± 0.042	± 0.081	± 0.023	± 0.027	± 0.076
	Co^{+2}	0.041 ^f	0.074 ^e	0.104 ^b	0.084 ^d	0.100 ^c	0.153 ^a
	ppm	± 0.025	± 0.011	± 0.014	± 0.015	± 0.025	± 0.023

Mean values with the same superscripts are not significantly different at the $P \leq 0.05$ probability level, using Post Hoc Duncan test.

On the other hand, in plants previously treated with Co, grains showed additional significant enhancement in the content of Ca^{2+} , K^+ , Co^{2+} , and K/Na ratio, while Na^+ and Fe^{2+} showed a significant reduction, as compared with untreated plants (Table 4). In support of our findings, Rietra et al. (2017) mentioned that nutritional uptake is catalyzed by proteins embedded in root membranes that accelerate the transfer of nutrients across the membrane. Similar cations and anions remain competitive for adhesion to specific carrier proteins, whereas anions and cations are taken up by different transport proteins, such as P1B- Zn^{2+} -ATPases (Zn^{2+} , Co^{2+} , Cu^{2+}), K channel (K^+), P3A-type H-ATPases (Na^+ , K^+ , Ca^{2+}), P2B-(Ca^{2+})-ATPases (Ca^{2+}). The transport proteins are unable to distinguish between comparable ions effectively, implying that the selectivity of some transport proteins in the plasma membrane of root cells is dependent in part on physicochemical similarities between ions (Rietra et al., 2017). As a result, the action of Co^{2+} may be referred to as the antagonistic effect of Co^{2+} , Fe^{2+} , and Na^+ ions on their transport proteins in the plasma membrane of root tissues in both varieties. In contrast, Co^{2+} manipulation had a synergistic effect on K^+ , K/Na ratio, Ca^{2+} , and Co^{2+} absorption in stressed rice plants of both varieties through increasing ion uptake through transport proteins. The improved effects of Co^{2+} on mineral uptake were significantly more noticeable in the Giza 178 variety as a type of adaptation under drought stress than in the Sakha 104 variety.

Changes in vitamin B9 and B12 content

The content of vitamins B9 and B12 in the grains of both rice varieties decreased significantly as the severity of drought stress increased, particularly in Sakha 104 (Fig. 2). For more lucidity, treatment C6 decreased vitamin B9 content by 25.2 and 24.3% and vitamin B12 content by 22.7 and 9.6%, while treatment C8 decreased vitamin B9 content by 52.6 and 33.9% and vitamin B12 content by 32.1 and 28.2%, in Sakha 104 and Giza 178, respectively, as compared with the C4 treatment. The findings are consistent with those of Li et al. (2021), who discovered that under drought stress, the content of vitamins B9 and B12 decreased significantly below control levels. The grains of the plants previously treated with Co revealed significant enhancement in vitamins B9 and B12 content of both varieties, particularly in the Giza 178 variety

(2-fold content in both vitamins), as compared to untreated plants (Fig. 2). T4 treatment increased (vitamin B9 content by 2.6 and 4.6% and vitamin B12 content by 21.6 and 42.1%), and T6 treatment increased (vitamin B9 content by 1.7 and 2.0% and vitamin B12 content by 20.5 and 39.4%), T8 treatment increased (vitamin B9 content by 0.9 and 1.9%, and vitamin B12 content by 2.2 and 9.5%), in Sakha 104 and Giza 178 respectively, when compared to C4, C6, and C8 treatments. Gad (2006) pointed out that Co addition is directly proportional to vitamin B12 content in *Phaseolus vulgaris*. The levels of vitamins B9 and B12 in grains of both varieties appeared to respond strongly to drought stress, the intensity of the stress, the physiological state of plants, and the sensitivity of species to stress, which was more noticeable in the Giza 178 variety than Sakha 104. Furthermore, the enhancement effect of Co application on the grain content of both vitamins may be linked to the fact that Co supports the oxidative defense system of the rice plant under drought stress by increasing the grain content of both vitamins as one of the drought-tolerant strategies, which was more perceptible in the drought-tolerant variety Giza 178 than in the sensitive variety Sakha 178.

Changes in total protein content

The deleterious effect of drought stress on grains protein content in both varieties was significant with the elevation in the level of drought stress, particularly in the Sakha 104 variety, as compared with the control (Fig. 2). For more visibility, treatment C6 decreased protein content by 8.3 and 5.5%, while treatment C8 decreased protein content by 11.8 and 9.5%, in Sakha 104 and Giza 178, respectively, as compared with the C4 treatment. This reduction might be attributable to the fact that under drought stress, certain proteins are upregulated while others are downregulated, modifying protein quantity and performance (Feki & Brini, 2016). On the other hand, Fan et al. (2004) revealed that, although total protein content increases in drought conditions, protein production reduces due to lessened grain output. Per contra, the interactive effect of Co with control or stressed plants caused a significant increase in grain protein content compared with grains of untreated plants of both varieties, particularly in Giza 178 (Fig. 2). For more lucidity, treatment T4 increased protein content by 16.0 and 38.4% and treatment T6 increased protein content by

11.5 and 29.9%, while treatment T8 increased protein content by 8.4 and 10.7%, in Sakha 104 and Giza 178, respectively, as compared with C4, C6, and C8 treatments. These findings correspond with those obtained by Gad (2012) for *Arachis hypogaea*. Furthermore, Gad & Kandil (2010) reported that Co had a substantial positive influence on the total protein content in tomatoes. Meanwhile, Mounika et al. (2010) discovered that Co amplified the amino acid and protein content of *Zea mize* L. The differences in drought tolerance strategies in both varieties assured the obtained results.

Changes in the protein patterns of grains

Stress protein synthesis is a common response to prevalent stressful conditions such as drought conditions. Most stress proteins are water-soluble and hence contribute to stress tolerance by hydrating cellular structures (Farooq et al., 2009). For grains of Sakha 104, drought, either alone or in combination with Co led to the appearance of one new protein band at C6, C8, T4, T6, and T8 treatments, which is of low molecular weight 33kDa, besides the appearance of two new protein bands at C8 and T4 treatments with high molecular weights of 91 and 110 compared to parent grains, as illustrated in (Photo 2 and Table 5). On the other hand, in grains of Giza 178 as shown in (Photo 2 and Table 6) at C4, C6, C8, T4, T6, and T8 treatments, there is a *de novo* synthesis of one protein band with a molecular weight of 42 kDa, besides the appearance of different new protein bands with high molecular weights in Co treatments (T4, T6, and T8) ranges from 42 to 97kDa compared with parent grains. In support of the present results, according to Qaseem et al. (2019), protein stress accumulation is required to maintain osmotic equilibrium and membrane stability in a stressful environment. Jalal et al. (2012) investigated the effect of drought stress on the protein electrophoresis patterning band of *Plectranthus tenuiflorus* plants and discovered that there was an induction of new bands and degradation of others. This means that, in both varieties, drought stress perhaps induced the synthesis of some heat shock proteins (Hsps) with low (33kDa in Sakha 104), moderate (42kDa in Giza 178), and high (91 and 110kDa in Sakha 104) molecular weights to cope with this stress. Meanwhile, Co treatments promoted the high level of expression of Hsps (91, 110kDa in Sakha 104 and 53, 57, 60, 71, 91, and 97kDa in Giza 178). Hsps have been reported to act as molecular

chaperones in adenosine triphosphate-dependent protein unfolding or assembly/disassembly processes and to avoid protein denaturation during stress (Gorantla et al., 2007). There have also been multiple reports that the dehydrin protein family, which is categorized as a member of the Late embryogenesis abundant (LEA) proteins, accumulates in a wide range of drought-stressed plant species ranging in size from 9 to 200kDa (Arora et al., 1998). Also, variations in gene expression can generally be correlated with changes in the accumulation of different types of proteins in cereal grains in response to nutrition (Halford et al., 2015). According to Khalid et al. (2014), Co increased protein and nutrient accumulation in tomato and radish plants. As a result, the positive role of Co in combination with drought stress may be attributed to the fact that Co magnified the adaptation of rice plants of both varieties under drought stress conditions via the accumulation of necessary proteins to help them defend themselves from the negative effects of drought stress. The differences in protein accumulation levels were related to the degree of stress tolerance in each rice variety used, which was more obvious in Giza 178 (drought-tolerant) than in Sakha 104 (drought-sensitive).

Conclusion

Our results illustrated that soaking rice grains in CoSO_4 solution at the optimum concentration before sowing in the soil is considered extremely useful in mitigating the harmful consequences of drought stress on the yield attributes and the nutritive value of grains of both rice varieties, particularly in Giza 178. The enhancing effect of Co was apparent by enhancing tillers number/plant, tillers bearing panicles/plant, panicle length/plant, panicle weight/plant, 100 grains weight, HI, and the nutritive value of grains represented in sugars, minerals, vitamins B9 and B12, and protein contents. Also, the changes in protein profiles of the grains of both rice varieties appeared to be dependent on (1) the level of drought stress, (2) the concentration of CoSO_4 utilized, and (3) the drought-tolerant trait in each variety. Overall, this study could provide a clear understanding for researchers to find out the actual physiological impact of Co in ameliorating the adverse effects of drought stress on rice yield, which they can further investigate at a cellular and molecular level.

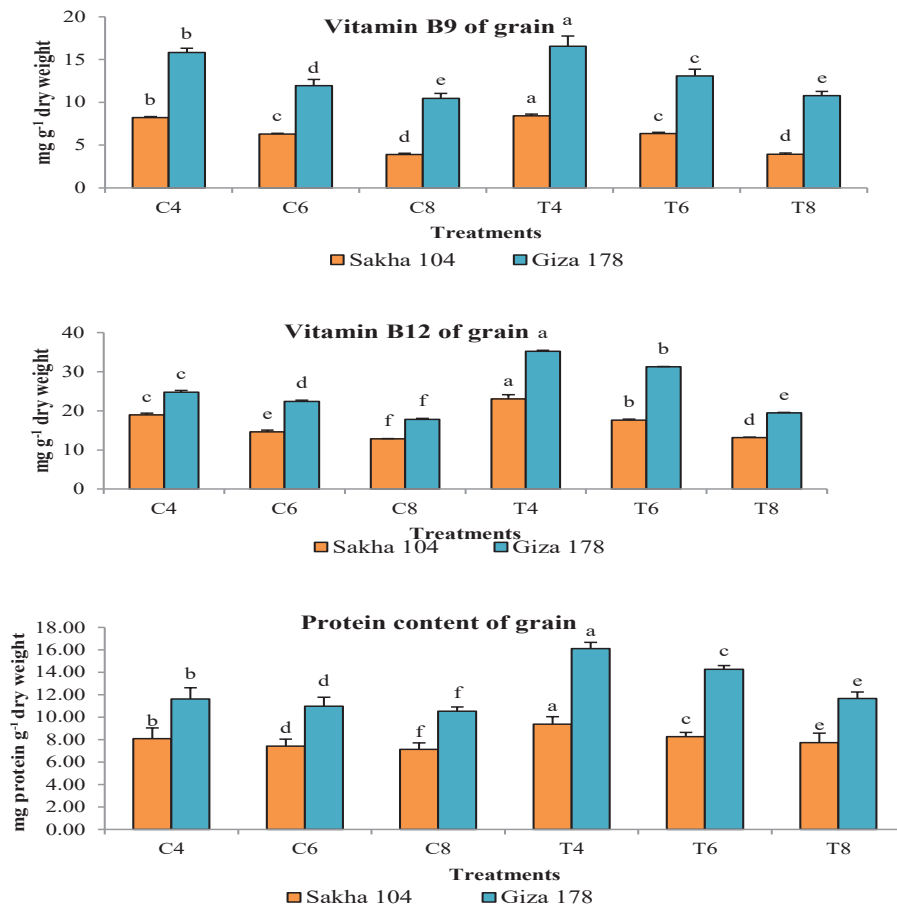
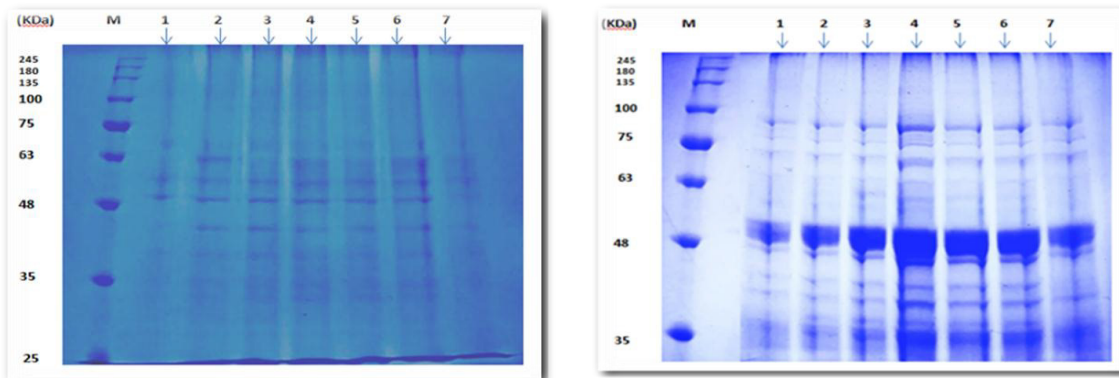


Fig. 2. Effects of drought stress either alone or in combination with CoSO_4 on vitamin B9 and B12 and protein contents in grains of Sakha 104 and Giza 178 rice plants at the harvest stage [Vertical bars represent the standard error (\pm S.E.). C4; Control, C6; plants irrigated every 6 days, C8; plants irrigated every 8 days, T4; Control+ CoSO_4 , T6; plants irrigated every 6 days + CoSO_4 , and T8: plants irrigated every 8 days + CoSO_4]



Sakha 104

Giza 178

Photo 2. Electrograph of soluble protein pattern by one dimensional SDS-PAGE showing the change of protein bands (marked by arrowheads) of rice grains (Sakha 104 and Giza 178) in response to drought stress either alone or in combination with CoSO_4 . Each lane contains equal amounts of protein extracted from the grain. Protein bands in the gel were visualized by Coomassie Blue Stain [Lane M = protein markers, Lane 1 = grains of C4 treatment, Lane 2 = grains of C6 treatment, Lane 3 = grains of C8 treatment, Lane 4 = grains of T4 treatment, Lane 5 = grains of T6 treatment, Lane 6 = grains of T8 treatment and Lane 7 = parent grains]

TABLE 5. Effects of drought stress either alone or in combination with CoSO_4 on protein pattern of rice grains (Sakha 104) at the harvest stage. P; parent grains, C4; Control, C6; plants irrigated every 6 days, C8; plants irrigated every 8 days, T4; Control+ CoSO_4 , T6; plants irrigated every 6 days + CoSO_4 , and T8; plants irrigated every 8 days + CoSO_4

M.W (kDa)	Treatments							
	P	C4	C6	C8	T4	T6	T8	
110	-	-	-	+	+	-	-	
91	-	-	-	+	+	-	-	
63	+	+	+	+	+	+	+	
59	+	+	+	+	+	+	+	
50	+	+	+	+	+	+	+	
46	+	-	+	+	+	+	+	
40	+	+	+	+	+	+	+	
35	+	+	+	+	+	+	+	
33	-	-	+	+	+	+	+	
27	+	+	+	+	+	+	+	
25	+	+	+	+	+	+	+	
Number of total bands	8	7	9	11	11	9	9	
Number of new bands	-	-	1	3	3	1	1	

TABLE 6. Effects of drought stress either alone or in combination with CoSO_4 on protein pattern of rice grains (Giza 178) at the harvest stage. P; parent grains, C4; Control, C6; plants irrigated every 6 days, C8; plants irrigated every 8 days, T4; Control+ CoSO_4 , T6; plants irrigated every 6 days + CoSO_4 , and T8; plants irrigated every 8 days + CoSO_4

M.W (kDa)	Treatment							
	P	C4	C6	C8	T4	T6	T8	
97	-	-	-	-	+	-	-	
95	+	+	+	+	+	+	+	
91	-	-	-	-	+	-	-	
89	+	+	+	+	+	+	+	
82	+	+	+	+	+	+	+	
75	-	-	-	-	-	-	-	
73	+	+	+	+	+	+	+	
71	-	-	-	-	+	-	-	
60	-	-	-	-	+	+	+	
57	-	-	-	-	+	+	+	
53	-	-	-	-	+	-	+	
50	+	+	+	+	+	+	+	
48	+	+	+	+	+	+	+	
42	-	+	+	+	+	+	+	
40	+	+	+	+	+	+	+	
38	+	+	+	+	+	+	+	
35	+	+	+	+	+	+	+	
30	+	+	+	+	+	+	+	
Number of total bands	10	11	11	11	17	13	14	
Number of new bands	-	1	1	1	7	3	4	

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آثار استخدام الكوبالت على خواص الغلة والقيمة الغذائية ونمط البروتين في حبوب الأرز (*Oryza sativa* L.) تحت ظروف الجفاف

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تهدف الدراسة الحالية إلى التحقق من استجابات إجهاد الجفاف لنوعين من الأرز المصري (سحا 104 وجيزة 178) بعد نقع الحبوب في محلول الكوبالت المغذي بالتركيز الأمثل قبل الزراعة في التربة واكتساب رؤى فسيولوجية لتحمل الجفاف في نباتات الأرز. تم تطبيق إجهاد الجفاف على فترات ري مختلفة (التحكم؛ ري كل 4 أيام ومتوسط؛ ري كل 6 أيام و شديد؛ ري كل 8 أيام). أظهرت النتائج أن إجهاد الجفاف أدى إلى انخفاضات متفاوتة في عدد التفريعات / نبات، عدد التفريعات المحمله بالسنابل / نبات، طول ووزن السنابل / نبات، وزن 100 حبة، ومؤشر الحصاد. وقد انخفضت بشكل ملحوظ أيضا القيمة الغذائية للحبوب متمثلة في محتوى السكر، السكريات الذائبة، عديدات التسكر، الكربوهيدرات الكلية، البروتينات، والفيتامينات (B9 و B12)، وكذلك محتوى العناصر (Ca^{2+} ، Na^{+} ، Fe^{2+}) مع إجهاد الجفاف. وفي الوقت نفسه، أدى الجفاف إلى زيادة ملحوظة في محتوى الحبوب من الجلوكوز و العناصر (K^{+} ، Co^{2+} ، ونسبة K/Na) في كلا صنفى الأرز. وقد خفف تطبيق الكوبالت بشكل معنوي التأثير الضار للجفاف من خلال تحسين المعايير المذكورة أعلاه بشكل قابل للمقارنة في كلا صنفى الأرز، خاصة في صنف جيزة 178. أظهرت نتائج الفصل الكهربى للبروتينات لمعاملات إجهاد الجفاف إما بمفرده أو بالاشتراك مع التغذية بالكوبالت، تخليق جديد لنطاقات نمط البروتين في الحبوب بأوزان جزيئية (33، 91 و 110) كيلو دالتون في صنف سحا 104 و (42، 53، 57، 60، 71، 91 و 97) كيلو دالتون في صنف جيزة 178.