



# The Biostimulant Potential of the Seaweeds *Galaxaura oblongata* and *Turbenaria ornate* in Improving Drought Tolerance in Rice Plant (*Oryza sativa* L.)

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## Abstract

Drought stress is a major limiting factor for rice production globally. The current climatic changes have further increased the incidence and duration of droughts worldwide. On the other hand, seaweed extracts have been extensively studied as biostimulants that improve plant growth, nutrition, quality, yield, and stress tolerance of rice (*Oryza sativa*). Different types of seaweeds have been employed for biostimulant preparation, of which algae is most commonly used. Of particular interest were *Galaxaura oblongata* and *Turbenaria ornate* seaweeds, as biostimulants that inoculate with soil in rice plants growing under drought conditions to boost the resilience of rice to drought. The incorporation of drought-stressed-rice soil with two investigated seaweeds led to improved growth, almost regulated levels of photosynthetic pigments, compatible solutes, MDA, hydrogen peroxide, and phenolic compounds as well as flavonoids, along with notably upregulated expression of *PIP1;4*, *PIP2;7*, *NCDE5*, and *OsMyb-RI* genes of the rice cultivars Giza 177 and Giza 179, and this enhanced the water status of rice cultivars grown under drought conditions. Furthermore, this study suggested that amending soil with investigated seaweeds enhanced nutrition and osmolyte production, ameliorated MDA, and upregulated investigated drought-responsive genes as well as promoted the growth along with antioxidant reply thoroughly contribute to improving rice plant tolerance to drought stress.

**Keywords** Drought · *Oryza sativa* · Seaweed · Biostimulant · Gene expression

## Introduction

Rice (*Oryza sativa* L.) is a main staple of diets and a major source of food security for more than half of the world's population; the sustenance of many people depends upon the rice plant (Samal et al. 2021). The production and improvement of rice are affected by several environmental stresses, particularly water deficits, and these stresses have a negative impact on the rice plant's growth, development, and physiological as well as phytochemical aspects (Datta et al.

2017). Water deficits cause plants to continuously lose water via transpiration or evaporation. During periods of drought, plants close their stomata to prevent evaporation and this reduces the plants' ability to effect gas exchange. Irrigation is one of the main strategies used to offset water shortages; it enhances the efficiency of water use across the world (Bakry et al. 2012). Water resources in Egypt are very limited, so Egypt has established several water-preserving irrigation practices that can benefit rice plants.

Indeed, drought stress alters the biochemical, physiological, and molecular parameters of almost all crop plants, and these changes help plants to tolerate harsh environmental stresses. The extent of these alterations differs significantly depending upon the plant species and the stress duration and intensity, as well as the growth stage of plants (Abd Elhamid et al. 2016; Ahmed and Sadak 2016; Sadak 2016; Arora et al. 2002). Abiotic stresses such as salinity and drought are global issues threatening the survival of agronomic crops and maintainable production of food (Elewa et al. 2017; Dawood et al. 2017). A water deficit is a restrictive factor

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during the primary stage of plant growth and development, and it can have harmful effects on a plant's cell structure and metabolism (Anjum et al. 2003; Bhatt and Rao 2005; Kusaka et al. 2005; Ezzo et al. 2018). Rice may be more susceptible to drought stress than other crops because it is actually grown in water.

Drought damages a plant's photosynthetic apparatus by inhibiting the content of chlorophyll and the enzyme activities involved in photosynthesis (Monakhova and Chernyadev 2002; Bakry et al. 2019). Moreover, it alters the metabolism of antioxidants, which is considered one of the essential metabolic processes that may enhance plants' tolerance to drought stress (Da Costa and Huang 2007; Sadak et al. 2020). Notably, the imbalance between the production and elimination (or detoxification) of reactive oxygen species (ROS) induced by water stress causes cellular injuries (Foyer and Noctor 2002; Sadak and Bakry 2020). Meanwhile, some plants can tolerate stresses via developing cellular adaptive mechanisms, including the accumulation of protective compatible solutes such as soluble sugars and proline, as well as the upregulation of oxidative stress markers; these mechanisms help plants to tolerate environmental conditions (Sakamoto and Murata 2002; Abd El-Hameid and Sadak 2020). Consequently, enzymatic and nonenzymatic antioxidant defense mechanisms such as catalase (CAT), ascorbate peroxidase (APX), and peroxidase (POX), as well as ascorbate (AsA), the total phenolic compounds, and the flavonoids, are the most potent defense systems for scavenging ROS (Abd Elhamid et al. 2021).

Furthermore, plants undergo molecular changes to counteract the adverse effects of environmental stresses (El-Esawi et al. 2018, 2019). The water channel proteins called aquaporins form pores in the membranes of biological cells, mainly facilitating the transport of water between cells. Plant aquaporin proteins (AQPs) are intrinsic protein families that are widely distributed in plasma membranes (Quigley et al. 2002; Agre 2006). Plasma membrane intrinsic proteins (PIPs) are the main groups of AQPs, and they are subdivided into two subgroups *PIP1* and *PIP2* (Kaldenhoff and Fischer 2006). The overexpression of *PIP1* and *PIP2* genes has improved plant tolerance in response to abiotic stresses and mitigated the negative effects of drought stress (Aharon et al. 2003; Jang et al. 2007; Liu et al. 2013; Ayadi et al. 2019). Concurrently, the abscisic acid (ABA) biosynthetic pathway *NCED5* gene encoded 9-*cis*-epoxycarotenoid dioxygenase 5 (Lefebvre et al. 2006; Yang and Guo 2007). Moreover, the myeloblastosis transcription factor (TF) *Myb-RI* is one of the most important classes of TFs, as it has an essential role in regulating a plant's defense mechanism by mitigating the adverse effects triggered by biotic or abiotic stresses (Erpen et al. 2018).

The global population growth poses a serious threat to food security due to a certain amount of land available

for agriculture which decreased with time. Consequently, the development of environmentally friendly products like biofertilizers as well as its influences as a result of modern agriculture has been explored with respect to several crops, including rice. Therefore, the improvement of the yield and crop quality can be achieved by the use of fertilizers. Traditional fertilizers, coming from either organic or inorganic origins play a vital role in agriculture which qualifies the production of food at a broad scale that sustains the global population (Mahanty et al. 2017). The application of seaweeds as biofertilizers is seen as a potential substitute for chemical fertilizers in agriculture due to a vast potential for improving crop productivity and food safety. Seaweeds are macro-aquatic thallophytic algae of the kingdom *Plantae* (Arioli et al. 2015). Seaweeds are a renewable natural resource that adapts to environmental conditions, and they are rich in various types of natural biologically bioactive compounds (Nabti et al. 2017; Bakhroum et al. 2023). The derivation of biofertilizers from seaweeds such as *Ascophyllum* and *Sargassum* is considered an ecofriendly activity because these seaweeds are nontoxic, biodegradable, and nonpolluting, as well as harmless to humans and animals (Dhargalkar and Pereira 2005). Therefore, nowadays, seaweeds have the potential to be used, and are used, as bioresources and biofertilizers in sustainable applications instead of chemical fertilizers (Khan et al. 2009; Layek et al. 2018). In addition, the recent application of seaweeds as bio-fertilizers in agriculture and for the improvement of crop plants is considered a tool for preserving the environment. The application of seaweed products in the fields of horticulture and agriculture has led to enhanced seed germination, improved plant development, and increased abilities of plants to tolerate environmental conditions (Zhang and Ervin 2008). Hashem et al. (2019) reported that the potential of different algal taxa such as *Ulvalactuca*, *Cystoseira* spp., and *Gelidiumcrinale* for use as biofertilizers could improve the growth and productivity of canola plants under salt stress conditions. Also, the results obtained by Hussein et al. (2021) confirmed that the application of the seaweeds *Ulva fasciata*, *Cystoseira compressa*, and *Laurencia obtusa* as biostimulants had the potential to enhance the salinity tolerance of *Vigna sinensis* and *Zea mays* plants.

Irrigation management practices have been described as water-saving as compared with submerging in rice productivity. Moreover, the deficit irrigation adjusts the management use of nitrogen fertilizers is not clear. The influence of deficit irrigation on the efficient nutrient use of rice is important for improving the fertility of the soil and growing rice cultivation. Consequently, this study aimed to elucidate the mechanism by which the rice plant survives drought stress. This was estimated by assaying the plant's physiological, biochemical, and molecular responses to drought. Attempts were investigated to check the performance of both

rice cultivars, Giza 177 and Giza 179 exposed to drought conditions, in terms of their productivity by using seaweeds as biofertilizers in combination with soil also made to enhance the defense mechanisms in both rice cultivars. Finally, this study considered a sustainable approach for reducing water consumption through the management of irrigation practices in improvement the rice production (de Avila et al. 2015; Chen et al. 2021).

## Materials and Methods

### Plant Material

Two different cultivars of rice (*Oryza sativa* L) grains, Giza 177 and Giza 179 were attained from the Agriculture Research Center, Rice Research Institute in Giza, Egypt.

### Algal Collection

Two seaweed species were collected by handpicking from the Red Sea coast in Egypt, with the littoral zone of rocky shorelines in Hurghada city (27° 15′ 58.45″ N, 33° 48′ 57.09″). Algal identification was performed according to (Aleem 1993) which was established by morphological structures determined by microscopic examination. One of them belongs to the division Rhodophyta (red algae) and identified as *Galaxaura oblongata* (*Tricleocarpa fragilis*) (*J. Ellis & Solander*) *J.V. Lamouroux*, while the other one from the Pheophyta division (brown algae) and identified as *Turbenaria ornate* (*Turner*) *J. Agardh*. After collection, the algal samples were washed with tap water to eliminate salt and sand particles, then air-dried, and finally grounded into small powder using a mill and stored at room temperature for analysis (Table 1).

### Chemicals

The chemicals used in the present work with high purity, obtained from Sigma-Aldrich Chemical Co., Germany, and the organic solvents used with AR grade.

## Experimental Design and Growth Condition

Rice grains were immersed for 5 min in sodium hypochlorite solution (1%) to ensure surface sterilization was performed, then washed carefully with tap water. The pot experiment was directed in the Botany Department, Faculty of Science, Ain Shams University. This experiment was performed during the autumn season of 2022 from September to October. Two native rice grains (*Oryza sativa* L.) cultivars were applied in the current experiment defined; Giza 177 and Giza 179. The pots were separated into 2 groups; the first group was used for cv. Giza 177 and the later one for cv. Giza 179. The rice grains were planted in pots (30 × 18 cm) occupied with standardized clay/sandy (2:1) soil (8 kg). The physical and chemical constituents of the experimental soil are presented in Table 2. The clay/sandy soil was divided into three sub-groups for each cultivar. The two series were amended by 10 g w/w from the two investigated seaweeds *T. ornate* and *G. oblongata* as well as the third one remains without algal treatment which serves as a control. A randomized complete block design was performed with 3 replicates per each treatment for all analyses, except; 10 different replicates from each treatment were chosen for measuring the growth parameters. The range of relative humidity recorded was 24 and 60%. The maximum and minimum day temperatures were 38 °C and 18 °C, respectively. All pots were provided with sufficient irrigation. Moreover, after 2 weeks, the drought stress condition was exercised in half of each treatment through withholding water capacity as irrigation was displayed every 12 days (Alharbi et al. 2021). The plants after thinning were left-hand to develop with the different treatments for one month, and then seedlings were collected for measuring growth criteria, and physiological, biochemical, and molecular analyses.

## Methods

### Growth Parameters

The growth parameters were measured after 30 days from sowing. Ten plants were randomly sampled in the morning from each treatment sensibly washed with tap water to

**Table 1** The main phytochemical constituents of the two investigated seaweeds

Parameters/treatments		Total soluble sugar (mg/g DW)	Total phenols (mg/g DW)	Flavonoids (mg/g DW)	Total antioxidant capacity (µg/g DW)
Algal name	<i>G. oblongata</i>	1.135	12.12	0.830	0.982
	<i>T. ornate</i>	2.828	16.76	0.515	1.028

**Table 2** Physical and chemical constituents of the experimental soil

Parameters	Physical analysis			pH	E.C. (dSm <sup>-1</sup> )	Chemical analysis													
	Clay	Silt	Sand			Texture	Soluble anions (meq L <sup>-1</sup> )			Soluble cations (meq L <sup>-1</sup> )									
<b>Value</b>	53.4%	36.7%	9.9%	8.2	7.2	SO <sub>4</sub> <sup>-</sup>	13.7	HCO <sub>3</sub> <sup>-</sup>	8.3	Cl <sub>-</sub>	37.7	Ca <sup>++</sup>	7.4	Mg <sup>++</sup>	10.3	K <sup>+</sup>	0.56	Na <sup>+</sup>	42.6

remove soil debris, and left on the filter sheets to get rid of water. Plant growth parameters in terms of root and shoot lengths as well as fresh weights of roots and shoots.

### Extraction and Estimation of Photosynthetic Pigments

The chlorophylls a and b and carotenoids were extracted and estimated according to the method of Metzner et al. (1965). One gram of leaves was extracted with 85% (v/v) acetone, then the extract was filtered and up to the total volume (100 mL) by acetone. The absorbance was recorded at three wavelengths of 663, 644, and 452.5 nm. The photosynthetic pigment content was assayed according to the following equations:

$$\text{Chlorophyll } a = 10.3 E_{663} - 0.918E_{644}$$

$$\text{Chlorophyll } b = 19.7 E_{644} - 3.870E_{663}$$

$$\text{Carotenoids} = 4.2 E_{452.5} - (0.0364\text{chlorophyll } a + 0.426\text{chlorophyll } b)$$

### Extraction and Estimation of Carbohydrates

The method described for carbohydrate extraction was adopted by (Homme et al. 1992). A known weight of fresh plant tissue was extracted with boiled 80% (v/v) ethanol. The homogenate was filtered through filter paper (Whatman No. 1), then was oven-dried at 60°C, followed by dissolving the residue in a known volume of distilled water to get ready for soluble sugar determination. Furthermore, the method used for soluble sugar determination was that of (Blakeney and Mutton 1980). Two mL of dissolved residue was mixed with 10 mL of anthrone reagent, then placed in a boiling water bath for 20 min, then cooled and finally, the absorbance was measured at 620 nm.

### Extraction and Estimation of Total Soluble Proteins

Total soluble proteins were extracted by 0.15 N NaCl. The homogenate was centrifuged at 4,000 rpm. A known volume of supernatant was taken to determine the soluble protein content by using the Folin–Ciocalteu reagent according to the method adopted by (Daughaday et al. 1952).

### Extraction and Estimation of Proline

Proline was determined according to the method described by (Bates et al. 1973). Fresh plant tissue (0.5 g) was extracted with 3% sulfosalicylic acid, the homogenate was filtered by Whatman No.1 filter paper. Two milliliters of filtrate were added to 2 mL of acid ninhydrin reagent and 2 mL of glacial acetic acid. The absorbance at 520 nm was recorded against toluene as blank.

### Extraction and Estimation of Hydrogen Peroxide

The hydrogen peroxide was assayed by Titanium reagent according to the Yu et al. (2003) method. The absorbance was measured by spectrophotometric at 415 nm.

### Extraction and Estimation of Lipid Peroxidation Product (MDA)

The level of lipid peroxidation product was determined by determining the amount of malondialdehyde (MDA) conferring to the method of Minotti and Aust (1987). Plant tissue (100 mg) was ground with 2 mL of distilled water, then centrifuged at 4000 rpm for 15 min. The supernatant was added to the same volume of 0.5% thiobarbituric (v/v) dissolved in 20% (w/v) trichloroacetic acid. The mixture was heated at 95 °C for 30 min, after cooling in an ice bath, the absorbance was measured at two wavelengths; 532 and 600 nm.

### Extraction and Estimation of Ascorbic Acid (AsA)

Nonenzymatic antioxidants like ascorbic acid were performed by the method of Mukherjee and Choudhuri (1983). Half a gram of fresh plant sample was homogenized with 10 mL of 6% (w/v) trichloroacetic acid. The As A content was determined by a 2% dinitrophenylhydrazine reagent. The absorbance was recorded at 530 nm.

### Extraction and Estimation of Total Phenolic Compounds

Total phenols were measured according to the method applied by (Makkar et al. 1993). The extraction method was performed with 80% methanol, then evaporation was carried out and the evaporated residue was up to the known total volume by distilled water. The estimation method was applied by adding 0.5 mL of extract to 0.5 mL Folin–Ciocalteu reagent. The optical density was recorded at 725 nm.

### Extraction and Estimation of Flavonoids

According to Harborne (1998), flavonoids were measured based on the reaction of the aluminum chloride colorimetric method. After extraction of flavonoids from the plant sample as carried out in the total phenols method, a known extract volume (1 mL) was added to 0.1 mL (1 M potassium acetate), 1.5 mL (methanol), 0.1 mL (10% aluminum chloride) and 2.8 mL (distilled water). The mixture was left at room temperature for half an hour. Finally, the absorbance was measured at 415 nm.

### Extraction and Estimation of Total Antioxidant Capacity

The ferric reducing antioxidant power (FRAP) method was followed for the estimation of total antioxidant capacity as reported by Oyaizu (1986).

### DPPH Radical Scavenging Assay

The antioxidant capacity of plant extract was determined through the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) method followed by (Yamaguchi et al. 1998) and calculated conferring to the below equation:

$$\% \text{DPPH radical scavenging} = [(A_0 - A_1)/A_0] \times 100.$$

So,  $A_0$  is the absorbance of the DPPH blank solution, and  $A_1$  is the sample absorbance.

### Extraction and Estimation of Antioxidant Enzymes

The method described for enzyme extraction was that according to (Mukherjee and Choudhuri 1983). Catalase (CAT) activity was assayed following the method of Chen et al. (2000). Peroxidase (POX) activity was determined according to the method of Kar and Mishra (1976). Ascorbate peroxidase (APX) activity was assayed as described by (Koricheva et al. 1997).

### RT-PCR Analysis

Total RNA was extracted from leaf tissues using the GeneJet RNA purification kit (Cat. No. K0731, Thermo Scientific, US) conferring to the manufacturer's instructions. The concentration of total RNA and their purity were determined by using NanoDrop (Thermo Scientific, USA); the purity of extracted RNAs (A260/A280) was 1.8–2. The High-Capacity cDNA Reverse-Transcription Kit (Applied Biosystems, US) cat.no. 4368814 was used to synthesize cDNA from isolated RNA. The real-time SYBR Green PCR method was utilized for the PCR analysis, with the Maxima SYBR Green qPCR master mix (Thermo Scientific, US) cat. no. K0251 and primers are listed in Table 3. qPCR was performed on an optical 96-well plate using a real-time polymerase chain reaction (Agilent Stratagene MX3000P, USA) under cycling conditions (10 min at 95 °C, followed by 45 cycles of 15 s at 95 °C, 60 s at 60 °C, and 30 s at 72 °C). The melting curves were examined to confirm the amplicon specificities for SYBR Green-based PCR amplification. A thresholding approach was used to assess relative gene expression. Every sample was tested twice to express relative mRNA levels, the relative quantitation was calculated as  $2^{-\Delta\Delta Ct}$  according to the technique applied by Livak and Schmittgen (2001). The resulting cDNA was subjected to PCR for 35 cycles with respective primers designated from the sequence of the *OsMyb-R1*, *OsPIP1;3*, *OsPIP2;7*, *OsNCED5* genes (Table 3) using primer premier 5.0 software and were procured from Invitrogen Corporation (Van Allen Way, Carlsbad,

**Table 3** Sequences of the 5' and 3' primers applied in PCR-technique

Gene	Primer sequence	
	Forward 5'-3'	Reverse 5'-3'
<i>OsMyb-R1</i>	CTACAAGGAATTACCGGCAATCG	GCAGCTATACACAGGCCCATCAAC
<i>OsPIP1;3</i>	AAGGACTACCGGGAGCCG	GGTAGAACGACCACGACGTCA
<i>OsPIP2;7</i>	ACGAGCGAGCTGGGTAAGTG	ATGAGCGTCGCCATGAAGCT
<i>OsNCED5</i>	CATCTTCAACGAGTCGGA	GTAGGCGTACCTCGTCTTC
<i>OsActin</i>	AGCTATCGTCCACAGGAA	ACCGGAGCTAATCAGAGT

Canada). Dream Taq™Green PCR Master Mix (Invitrogen Corporation) was used in the PCR. Amplification yields were run in 1.5% agarose gel 1×TAE (Tris–Acetate-EDTA) buffer at approximately 120 V, which was separated by electrophoresis, and then visualized via staining the gel in ethidium bromide. For comparative determinations, the control samples and treated PCR yields were run on the same gel. Gel images were analyzed by using Gel Analyzer Pro software (version 3.1).

### Statistical Analysis

The data were expressed as mean values ± standard error (SE). One-way analysis of variance (ANOVA) test was used to perform the statistical analysis using SPSS version 25. Furthermore, apply the least significant difference (LSD) test applied by Snedecor and Cochran (1980) at  $P$ -value < 0.05. Finally, Duncan's multiple range test was carried out to comparisons between intergroups. Further comparisons between intergroup heatmap and PCA (principal comparison analysis) were performed.

## Results and Discussion

Drought stress has become a detrimental factor to the growth of rice, as shown in the morphological, physiological, and biochemical, as well as molecular, alterations in rice plants suffering drought (Quampah et al. 2011).

### Changes in Growth Parameters

In this study, drought stress altered the length of the roots of the two investigated rice cultivars, Giza 177 and Giza 179, compared with the corresponding control values (Table 4). This might be attributed to the deficits in irrigation intervals experienced by the rice plants. The roots of both investigated rice cultivars were significantly lengthened due to the fact that desiccation caused the root systems to become thick and long so they could reach water (Bouman and Tuong 2001; Lipiec et al. 2013). Moreover, these conditions may also have facilitated improved drought tolerance in the plants via the deep absorption of water from the soil and the reduction of toxic elements and organic

**Table 4** Influence of amended soil with either *Turbenaria ornate* or *Galaxaura oblongata* on shoot, root lengths, and root/shoot ratio as well as fresh weights of shoots and roots of the two rice cultivars Giza 177 and Giza 179 grown under drought condition

Cultivar	Parameters/treatments	Shoot length (cm)	Root length (cm)	Shoot/root ratio	Shoot Fwt (g)	Root Fwt (g)
<b>Giza 177</b>	<b>Control</b>	29.0±0.11 <sup>b</sup>	8.9±0.12 <sup>c</sup>	3.2±0.01 <sup>a</sup>	0.145±0.001 <sup>f</sup>	0.050±0.002 <sup>e</sup>
	<i>T. ornate</i>	29.8±0.08 <sup>a</sup>	9.2±0.14 <sup>ab</sup>	3.4±0.03 <sup>a</sup>	0.277±0.001 <sup>a</sup>	0.082±0.001 <sup>a</sup>
	<i>G. oblongata</i>	29.5±0.11 <sup>a</sup>	9.1±0.24 <sup>a</sup>	3.2±0.05 <sup>a</sup>	0.186±0.001 <sup>c</sup>	0.068±0.001 <sup>bc</sup>
	<b>Drought</b>	27.0±0.18 <sup>e</sup>	9.4±0.26 <sup>a</sup>	3.1±0.08 <sup>b</sup>	0.085±0.001 <sup>i</sup>	0.027±0.001 <sup>g</sup>
	<b>Drought + <i>T. ornate</i></b>	27.9±0.03 <sup>d</sup>	8.6±0.08 <sup>c</sup>	3.2±0.06 <sup>a</sup>	0.183±0.002 <sup>c</sup>	0.084±0.001 <sup>a</sup>
	<b>Drought + <i>G. oblongata</i></b>	28.4±0.11 <sup>c</sup>	8.9±0.10 <sup>bc</sup>	3.1±0.13 <sup>a</sup>	0.169±0.001 <sup>e</sup>	0.061±0.001 <sup>d</sup>
<b>Giza 179</b>	<b>Well-watered</b>	15.8±0.06 <sup>h</sup>	7.9±0.17 <sup>d</sup>	2.0±0.03 <sup>c</sup>	0.172±0.001 <sup>d</sup>	0.063±0.002 <sup>d</sup>
	<i>T. ornate</i>	16.6±0.12 <sup>g</sup>	9.5±0.12 <sup>a</sup>	1.7±0.06 <sup>ef</sup>	0.195±0.001 <sup>b</sup>	0.079±0.001 <sup>b</sup>
	<i>G. oblongata</i>	15.9±0.05 <sup>h</sup>	8.9±0.10 <sup>bc</sup>	1.8±0.04 <sup>def</sup>	0.182±0.001 <sup>c</sup>	0.066±0.008 <sup>cd</sup>
	<b>Drought</b>	14.3±0.10 <sup>i</sup>	8.6±0.06 <sup>c</sup>	1.6±0.02 <sup>f</sup>	0.087±0.008 <sup>i</sup>	0.034±0.002 <sup>f</sup>
	<b>Drought + <i>T. ornate</i></b>	17.2±0.14 <sup>f</sup>	9.2±0.14 <sup>ab</sup>	1.9±0.02 <sup>cd</sup>	0.113±0.002 <sup>g</sup>	0.076±0.001 <sup>b</sup>
	<b>Drought + <i>G. oblongata</i></b>	17.7±0.14 <sup>f</sup>	9.1±0.11 <sup>a</sup>	1.9±0.03 <sup>de</sup>	0.101±0.001 <sup>h</sup>	0.064±0.002 <sup>d</sup>
<b>LSD at 0.05%</b>		0.162	0.213	0.083	0.002	0.002

Results are shown as the mean of ten replicates ± SE. The values with the same letters in the same column are non-significant

acid intermediates, thereby increasing the bioavailability and utilization of some nutrients such as nitrogen and phosphorus, as well as potassium, compared with plants flooded control treatment (Gowda et al. 2011; Uga et al. 2013). On the other hand, the continuous flooding of rice seedlings followed by water deficit irrigation every 12 days significantly reduced the lengths of the shoots and the fresh weights of the shoots and roots of both cultivars (Table 4). Our results are in agreement with those of Ghouri et al. (2021), who postulated that the root length would increase with an increased irrigation period and the shoot length would be reduced in rice plants. Moreover, the amending of the soil with the seaweed *Galaxaura oblongata* or *Turbenaria ornate* resulted in significant increases in the previously mentioned growth parameters of both stressed and unstressed rice plants (Table 4). This increase might be a result of the application of seaweed as bio nutrient fertilizers increasing the tolerance to abiotic stress in plants, which the plants can benefit from the water and nutrients in the soil more effectively to the endogenous nutrients of seaweed; as well as the high quantities of organic matter present in seaweeds (Kumareswari and Rani 2015; Layek et al. 2018). The application of seaweed stimulated root proliferation in tomato plants, and this increased the ability of the plants to absorb sufficient nutrients from the soil, leading to plant growth and improvement (Hernández-Herrera et al. 2013). The efficient biostimulation of the investigated seaweeds might be attributed to their total soluble sugars, total antioxidant capacity, flavonoid content, and content of phenolic compounds (Table 1). Moreover, the positive effect of seaweeds as biofertilizers was noticeable in the growth of apple trees, and this could be due to the seaweeds' adequate supply of essential nutrients and their enhancement

of the soil textures, as well as to an improved water-holding capacity (Mazzola and Brown 2010). Nedzarek and Rakusa-Suszczewski (2004) mentioned that the biodegradability of seaweed in soil released great amounts of organic material and various minerals, especially phosphate,  $\text{NO}_2$ ,  $\text{NO}_3$ , and  $\text{NH}_4$ , which enhanced the fertility of the soil.

### Changes in Photosynthetic Pigments

A water deficit is a great loss of water that induces a disturbance in the structure and metabolism of plant cells. It also ultimately diminishes the water content and leaf turgor and causes the stomata to close; all these activities arrest photosynthesis (Jaleel et al. 2007, 2008). A significant reduction in chlorophylls a and b and the carotenoid content was recorded in the two investigated rice cultivars, Giza 177 and Giza 179, that were cultivated under drought conditions (Table 5). This reduction might be attributed to the inhibition of chlorophyll synthesis or the destruction of chloroplasts in the rice plants and the consequent reduction in the photosynthetic apparatus and inhibition of growth (Monakhova and Chernyadev 2002; Faseela et al. 2019). The results of the current study agreed with those of several other studies (Keyvan 2010; Arjenaki et al. 2012; Embiale et al. 2016; Alharbi et al. 2022) on rice plant species. Consistent with our results, it was recorded that soil amended with *G. oblongata* or *T. ornate* ameliorated the effects of water deficit stress. This may have been due to the protection of photosystems against photo-oxidation and the minimization of ROS production triggered by oxidative stress as compared with the controls (see Table 5). Moreover, seaweed application is considered a source of cytokinins, which have protective properties on chloroplasts (Wally et al. 2013), thus,

**Table 5** Influence of amended soil with either *Turbenaria ornate* or *Galaxaura oblongata* on the contents of photosynthetic pigments of the two rice cultivars Giza 177 and Giza 179 grown under drought condition

Cultivar	Parameters/treatments	Chlorophyll a ( $\mu\text{g/g}$ Fwt)	Chlorophyll b	Carotenoids	chl-a/b
Giza 177	Control	89.8 $\pm$ 0.26 <sup>f</sup>	40.5 $\pm$ 0.36 <sup>e</sup>	69.2 $\pm$ 1.04 <sup>ef</sup>	2.21 $\pm$ 0.02 <sup>e</sup>
	<i>T. ornate</i>	116.8 $\pm$ 0.28 <sup>d</sup>	58.3 $\pm$ 0.26 <sup>b</sup>	90.1 $\pm$ 3.11 <sup>b</sup>	2.00 $\pm$ 0.01 <sup>f</sup>
	<i>G. oblongata</i>	109.7 $\pm$ 0.75 <sup>e</sup>	45.1 $\pm$ 0.43 <sup>d</sup>	84.2 $\pm$ 2.04 <sup>c</sup>	2.43 $\pm$ 0.03 <sup>c</sup>
	Drought	68.3 $\pm$ 0.44 <sup>h</sup>	37.7 $\pm$ 0.61 <sup>f</sup>	27.2 $\pm$ 1.52 <sup>g</sup>	1.81 $\pm$ 0.03 <sup>g</sup>
	Drought + <i>T. ornate</i>	91.3 $\pm$ 0.43 <sup>f</sup>	44.7 $\pm$ 0.96 <sup>d</sup>	78.3 $\pm$ 0.15 <sup>cd</sup>	2.04 $\pm$ 0.05 <sup>ef</sup>
	Drought + <i>G. oblongata</i>	89.4 $\pm$ 0.10 <sup>f</sup>	41.3 $\pm$ 0.44 <sup>e</sup>	65.8 $\pm$ 3.52 <sup>ef</sup>	2.16 $\pm$ 0.02 <sup>e</sup>
Giza 179	Well-watered	123.8 $\pm$ 0.36 <sup>c</sup>	43.2 $\pm$ 0.68 <sup>de</sup>	75.4 $\pm$ 2.86 <sup>de</sup>	2.86 $\pm$ 0.04 <sup>a</sup>
	<i>T. ornate</i>	172.7 $\pm$ 0.87 <sup>a</sup>	65.5 $\pm$ 0.80 <sup>a</sup>	119.3 $\pm$ 3.11 <sup>a</sup>	2.63 $\pm$ 0.03 <sup>b</sup>
	<i>G. oblongata</i>	147.1 $\pm$ 0.68 <sup>b</sup>	62.4 $\pm$ 1.63 <sup>a</sup>	118.8 $\pm$ 0.84 <sup>a</sup>	2.35 $\pm$ 0.06 <sup>de</sup>
	Drought	83.0 $\pm$ 1.65 <sup>g</sup>	33.1 $\pm$ 0.34 <sup>g</sup>	66.2 $\pm$ 3.18 <sup>f</sup>	2.50 $\pm$ 0.07 <sup>b</sup>
	Drought + <i>T. ornate</i>	117.3 $\pm$ 1.08 <sup>d</sup>	49.1 $\pm$ 0.58 <sup>c</sup>	99.4 $\pm$ 2.80 <sup>b</sup>	2.38 $\pm$ 0.04 <sup>cd</sup>
	Drought + <i>G. oblongata</i>	107.5 $\pm$ 0.31 <sup>e</sup>	44.1 $\pm$ 1.53 <sup>d</sup>	81.7 $\pm$ 0.33 <sup>c</sup>	2.43 $\pm$ 0.08 <sup>c</sup>
LSD at 0.05%		1.04	1.18	3.33	0.069

Results are shown as a mean of three replicates  $\pm$  SE. The values with the same letters in the same column are non-significant

they reflect on the chlorophyll contents. Our findings on the stimulatory effect of the investigated seaweeds on the content of photosynthetic pigments were similar to the findings of Kumar (2014) and Ali et al. (2022) on tomato and okra, respectively, plants.

### Changes in Soluble Sugars, Soluble Proteins, Proline, and Lipid Peroxidation Products

Sugars are considered the first organic compounds to be synthesized in plants as metabolic photosynthetic products. A water deficit impacts the metabolism of carbohydrates, and this causes the formation of a group of compatible solutes that act as osmolytes to maintain leaf cell turgor, which can be affected by stress conditions (Rolland et al. 2006; Mahmood et al. 2020). Our study clearly showed that drought stress significantly increased the total soluble sugars in seedlings of the rice cultivars Giza 177 and Giza 179. The percentage was noticeably higher in Giza 177, at 152.3%, than in Giza 179, at 88.9%, compared with their corresponding controls grown under well-watered conditions. The mechanism of accumulation of soluble sugars in plants is recognized as an osmotic regulation that is closely related to the severity of the plant drought stress (Shehab et al. 2010; Usman et al. 2013; Maisura et al. 2014). The percentages of our findings might be attributed to the role of soluble sugars in scavenging ROS, which enhanced water uptake from drying soil and was associated with improved plant tolerance under drought conditions. Our results were in agreement with those of Li et al. (2020) who found that increased drought intensity was responsible for an increased sugar content in maize plants. In addition, the application

of either *G. oblongata* or *T. ornate* to soil significantly increased the total soluble sugars (Table 6) in both the stressed rice cultivars. Such a percentage of total soluble sugars of seaweed-treated rice plants might be attributed to the effect of the applied algae on the mitigation of the injurious impacts of drought stress on chloroplasts. The results in this study were in agreement with those of El Boukhari et al. (2023), who reported that the application of *Fucusspiralis*, *Ulvalactuca*, and *Ascophyllum nodosum* improved the levels of soluble sugars in faba bean plants as compared with the control as a response to drought stress.

The synthesis of protein, as well as its degradation, is one process affected by drought stress in plants. The soluble protein content is an important sign of the plant's physiological status (Doganlar and Atmaca 2011). In the current study, the increase in the total soluble protein content was noticed in both Giza 177 and Giza 179 planted under drought stress (see Table 6). This increase was more pronounced in Giza 177. The decrease in the total soluble protein during drought stress could be due to the appearance of new stress proteins (Jiang and Huang 2002). Furthermore, the increment in the total soluble protein content might be the cells compensating for both that have been deactivated due to ROS binding (Seregin and Ivanov 2001; Lamhamdi et al. 2010). Moreover, it could be that drought causes disturbances in the nitrogen metabolism of many plants. The results in Table 6 indicated that the cultivation of the investigated rice cultivars in soil amended with *G. oblongata* or *T. ornate* significantly induced the further accumulation of total soluble proteins in both cultivars. The increases in total soluble proteins in stressed plants grown in algae-treated soil were consistent with the findings of Xu and Leskovar (2015) and Kapur et al.

**Table 6** Influence of amended soil with either *Turbenaria ornate* or *Galaxaura oblongata* on soluble sugar, soluble protein, proline, and MDA contents of the two rice cultivars Giza 177 and Giza 179 grown under drought condition

Cultivar	Parameters/treatments	Soluble sugar (mg/g Fwt)	Soluble protein	Proline	MDA (nmol/g Fwt)
Giza 177	Control	8.64 ± 0.008 <sup>d</sup>	10.56 ± 0.10 <sup>i</sup>	1.12 ± 0.008 <sup>d</sup>	0.037 ± 0.003 <sup>c</sup>
	<i>T. ornate</i>	5.37 ± 0.09 <sup>f</sup>	11.43 ± 0.04 <sup>h</sup>	0.81 ± 0.02 <sup>def</sup>	0.014 ± 0.003 <sup>i</sup>
	<i>G. oblongata</i>	4.40 ± 0.10 <sup>g</sup>	14.06 ± 0.22 <sup>ef</sup>	1.09 ± 0.02 <sup>de</sup>	0.016 ± 0.001 <sup>i</sup>
	Drought	10.65 ± 0.26 <sup>c</sup>	13.25 ± 0.44 <sup>f</sup>	2.62 ± 0.01 <sup>c</sup>	0.080 ± 0.003 <sup>a</sup>
	Drought + <i>T. ornate</i>	12.58 ± 0.29 <sup>a</sup>	15.31 ± 0.37 <sup>bc</sup>	3.56 ± 0.10 <sup>b</sup>	0.033 ± 0.008 <sup>e</sup>
	Drought + <i>G. oblongata</i>	11.27 ± 0.30 <sup>b</sup>	14.93 ± 0.17 <sup>de</sup>	3.76 ± 0.10 <sup>b</sup>	0.028 ± 0.003 <sup>f</sup>
Giza 179	Well-watered	7.55 ± 0.03 <sup>e</sup>	12.75 ± 0.10 <sup>g</sup>	0.89 ± 0.008 <sup>def</sup>	0.031 ± 0.003 <sup>e</sup>
	<i>T. ornate</i>	3.34 ± 0.06 <sup>h</sup>	13.75 ± 0.22 <sup>fg</sup>	0.71 ± 0.01 <sup>ef</sup>	0.028 ± 0.003 <sup>f</sup>
	<i>G. oblongata</i>	3.70 ± 0.06 <sup>h</sup>	15.62 ± 0.31 <sup>cd</sup>	0.65 ± 0.005 <sup>f</sup>	0.022 ± 0.003 <sup>h</sup>
	Drought	11.48 ± 0.16 <sup>b</sup>	14.93 ± 0.07 <sup>d</sup>	2.95 ± 0.08 <sup>c</sup>	0.048 ± 0.003 <sup>b</sup>
	Drought + <i>T. ornate</i>	13.64 ± 0.14 <sup>a</sup>	16.56 ± 0.22 <sup>b</sup>	4.10 ± 0.26 <sup>a</sup>	0.025 ± 0.003 <sup>g</sup>
	Drought + <i>G. oblongata</i>	13.50 ± 0.28 <sup>a</sup>	17.68 ± 0.24 <sup>a</sup>	4.32 ± 0.16 <sup>a</sup>	0.036 ± 0.001 <sup>d</sup>
LSD at 0.05%		0.263	0.344	0.144	0.0009

Results are shown as a mean of three replicates ± SE. The values with the same letters in the same column are non-significant



(2018) for spinach and strawberry plants, respectively; they reported that seaweeds enhanced the edible quality of plants by stimulating the accumulation of total soluble proteins. The algal extract may have a potential role in diminishing the injurious effects of drought stress on plant productivity. This is due to the rich seaweed, which has vital components that participate in mitigating the adversarial effects of drought stress (El-Sayed et al. 2015; Fouda et al. 2022).

Proline is an amino acid with many functions; it performs as a signaling compound, an osmoprotectant, and a stress-tolerance marker (Ahmad et al. 2009; Liu et al. 2011). It adjusts the cells' osmotic pressure; limits the protein denaturation, membrane constancy, and stabilization of enzymes; protects cells against stress and ROS-triggered damage; and maintains the balance of nutrients via water transport (Kartashov et al. 2008; Ivanov et al. 2013). In the current study, the imposition of drought stress stimulated the buildup of proline in the two investigated rice cultivars. The proline level in the Giza 177 stressed seedlings was higher than that in the Giza 179 stressed seedlings. Soil amendment with *G. oblongata* or *T. ornate* stimulated an enormous augmentation of proline. The overexpression of proline was determined by its metabolism and transport through cells, as well as diverse cellular compartments (Szabados and Savoure 2010). Moreover, the massive upregulation of proline biosynthesis under drought conditions was due to the genes involved in this process (Armengaud et al. 2004). The tolerance mechanism was also associated with osmotic adjustment and turgor maintenance. This mechanism led to the upregulation of some osmolytes such as proline, which caused a decline in the cellular osmotic potential and affected the increase of leaf turgor (Armengaud et al. 2004; Trovato et al. 2008), scavenging of ROS, stabilization of protein, and maintenance of membrane integrity (Ashraf and Foolad 2007; Liu et al. 2011). The overproduction of proline in stressed plants was correlated with the plant's stress tolerance (Anjum et al. 2011). The high levels of proline in stressed rice plants grown in soil amended with *G. oblongata* or *T. ornate* caused the plants to be more tolerant than the plants grown in untreated soil (see Table 6). Previous studies reported an enhancement in the accumulation of proline content in seaweed-treated plants grown under drought conditions, thus might be due to the lipophilic components (LPC) existing in seaweeds, as these could enhance the proline content (Sangha et al. 2014). Previous studies reported that the application of *A. nodosum* algae caused an increase in proline levels in drought-stressed bean plants (Renuka and Rathinavel 2006; Carvalho et al. 2018).

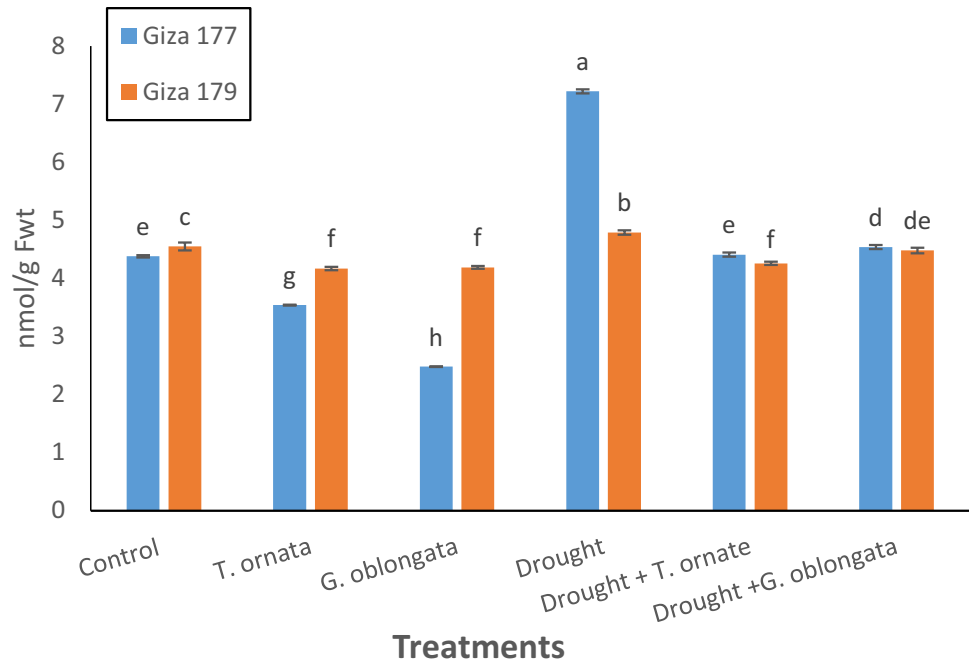
Notably, drought stimulated the liberation of ROS as hydrogen peroxide, as well as the superoxide radical, so it was able to first attack the phospholipids of the plasma membrane and deactivate the enzymes of the SH group. In response to drought conditions, the generation of ROS

exceeded the capacity of the antioxidant defense system and caused a loss of membrane stability; this was allied with an intensification in permeability and an injury of integrity (Blokchina et al. 2003). Therefore, the capability of plasma membranes to regulate the rates of entry and egress of ions in and out of a cell was considered a sign of membrane injury. The results of this investigation, as shown in Table 6, showed that drought stress induced a significant intensification in the lipid peroxidation product malondialdehyde (MDA) in Giza 177 and Giza 179. The increase in MDA levels was parallel with the increase in hydrogen peroxide ( $H_2O_2$ ) levels in the two rice cultivars, and this resulted in increased membrane fluidity and, consequently, enhanced membrane permeability (Reddy et al. 2004; Zhou et al. 2007). Cultivar Giza 177 exhibited greater increases in MDA and  $H_2O_2$  content than cultivar Giza 179; this could be evidence of a greater degree of membrane damage and, thereby, a high susceptibility of Giza 177 to drought compared with Giza 179 (see Table 6 and Fig. 1). The reduction in membrane damage in both rice cultivars when grown in soil amended with *G. oblongata* or *T. ornate*, as evident by the lower values of MDA and  $H_2O_2$ , could be due to the high activity of antioxidant enzymes in stressed rice seedlings. An increase in the activities of antioxidant enzymes imposed by drought stress might buffer the increased generation of ROS and enhance the protective strategy for eliminating the oxidative damage generated by drought stress (Gunes et al. 2007). The fact that the  $H_2O_2$  level was reduced in the drought-stressed seedlings of both rice cultivars suggested that the higher activities of scavengers such as CAT, POX, and APX were sufficient to eliminate  $H_2O_2$  (Bray et al. 2000). The data in this study were in agreement with those of Jacomassi et al. (2022), who reported that the MDA concentration was reduced in sugarcane plants grown in soil amended with seaweed extract.

### Changes in the Activities of Some Antioxidant Enzymes

Plants have enzymatic and nonenzymatic antioxidants which can serve as an effective defense system. It can attenuate the augmented production of ROS and build up a protective mechanism to eliminate the damage inflicted by oxidative stress (Diaz et al. 2001). The enzymatic antioxidants assayed in this work were peroxidase (POX), catalase (CAT), and ascorbate peroxidase (APX), and the nonenzymatic compounds assayed included ascorbic acid, total phenols, and flavonoids. In plants, there is a balance between antioxidant enzymes and ROS that extremely alterations, hindering plant growth in stressful conditions and reducing plant homeostasis; decline in ROS accumulation in plants treated with seaweeds (Dutot et al. 2012). The activities of CAT, POX, and APX in the present study were markedly increased in the stressed rice cultivars grown in soil amended with

**Fig. 1** Influence of amended soil with either *Turbenaria ornata* or *Galaxaura oblongata* on hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) content of the two rice cultivars Giza 177 and Giza 179 grown under drought conditions. Results are shown as a mean of three replicates; the bars on the column show  $\pm$ SE



*G. oblongata* or *T. ornata* (Table 7). The application of *G. oblongata* or *T. ornata* caused increases in the activities of CAT and POX in stressed rice seedlings, and this was associated with a significant reduction in the H<sub>2</sub>O<sub>2</sub> level. Furthermore, antioxidant enzyme activities improved with the application of seaweeds, which mitigate ROS damage and return cellular homeostasis in plants (Elansary et al. 2017). The increase in the enzyme activities of CAT, POX, and APX under conditions of abiotic stress reduces the risk of membrane damage and returns plants to homeostasis (Gill

et al. 2002). The present results showed increased activities of APX, CAT, and POX in rice plants grown in soil amended with our investigated seaweeds (Table 7). The results were in accordance with the results of Sujata et al. (2023), who showed that seaweeds were responsible for increased antioxidant enzymes in *Brassica juncea* (L.). This increase might be attributed to the potential for seaweed extracts to upregulate the activities of the antioxidant enzymes APX, CAT, and POX; this would be related to their endogenous bioactive components (O'Sullivan et al. 2011). The results

**Table 7** Influence of amended soil with either *Turbenaria ornata* or *Galaxaura oblongata* on catalase (CAT), peroxidase (POX), and ascorbate peroxidase (APX) activities of the two rice cultivars Giza 177 and Giza 179 grown under drought condition

Cultivar	Parameters/treatments	CAT (mM of H <sub>2</sub> O <sub>2</sub> g <sup>-1</sup> FW min <sup>-1</sup> )	POX (amount of Quinon g <sup>-1</sup> FW min <sup>-1</sup> )	APX (mM of ascorbate oxidized g <sup>-1</sup> FW min <sup>-1</sup> )
<b>Giza 177</b>	<b>Control</b>	0.19 ± 0.003 <sup>hi</sup>	2.12 ± 0.01 <sup>g</sup>	8.78 ± 0.04 <sup>e</sup>
	<b>T. ornata</b>	0.29 ± 0.023 <sup>f</sup>	2.53 ± 0.02 <sup>f</sup>	7.92 ± 0.01 <sup>g</sup>
	<b>G. oblongata</b>	0.33 ± 0.003 <sup>de</sup>	2.80 ± 0.02 <sup>e</sup>	8.41 ± 0.006 <sup>f</sup>
	<b>Drought</b>	0.24 ± 0.008 <sup>gh</sup>	2.74 ± 0.008 <sup>e</sup>	9.02 ± 0.005 <sup>d</sup>
	<b>Drought + T. ornata</b>	0.60 ± 0.005 <sup>a</sup>	3.12 ± 0.04 <sup>d</sup>	10.48 ± 0.07 <sup>a</sup>
	<b>Drought + G. oblongata</b>	0.55 ± 0.003 <sup>b</sup>	3.41 ± 0.07 <sup>c</sup>	9.83 ± 0.05 <sup>b</sup>
<b>Giza 179</b>	<b>Well-watered</b>	0.16 ± 0.003 <sup>i</sup>	0.61 ± 0.02 <sup>i</sup>	5.65 ± 0.005 <sup>k</sup>
	<b>T. ornata</b>	0.33 ± 0.030 <sup>c</sup>	1.45 ± 0.003 <sup>h</sup>	6.65 ± 0.21 <sup>i</sup>
	<b>G. oblongata</b>	0.30 ± 0.008 <sup>e</sup>	1.55 ± 0.02 <sup>h</sup>	6.19 ± 0.006 <sup>j</sup>
	<b>Drought</b>	0.26 ± 0.015 <sup>fg</sup>	2.32 ± 0.07 <sup>f</sup>	7.71 ± 0.02 <sup>h</sup>
	<b>Drought + T. ornata</b>	0.36 ± 0.005 <sup>cd</sup>	4.95 ± 0.09 <sup>b</sup>	9.35 ± 0.04 <sup>c</sup>
	<b>Drought + G. oblongata</b>	0.38 ± 0.003 <sup>c</sup>	6.08 ± 0.01 <sup>a</sup>	10.46 ± 0.08 <sup>a</sup>
<b>LSD at 0.05%</b>		0.018	0.062	0.104

Results are shown as a mean of three replicates  $\pm$  SE. The values with the same letters in the same column are non-significant

in this study agreed with the findings of Mansori et al. (2015), who reported that when the seaweeds *Ulva rigida* and *Fucus spiralis* were applied to bean plants exposed to drought conditions, the result was the activation of the antioxidant enzymatic system, including CAT and APX, and an increase in total phenolic compounds, which led to improved protection of the plants against peroxidation triggered by drought stress.

### Changes in Levels of Nonenzymatic Antioxidant Compounds such as Ascorbic Acid (AsA), Total Phenolic Compounds, and Flavonoids

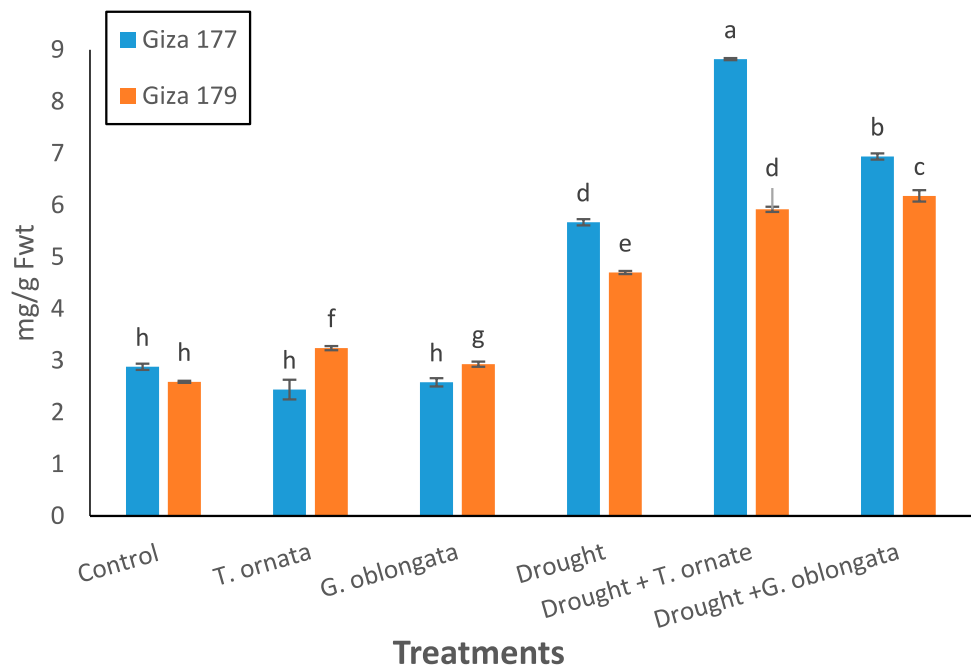
Abiotic stresses such as drought, salinity, and temperature extremes can enhance the assembly of secondary metabolites such as phenols (Çirak et al. 2007). In this study, the content of total phenols and flavonoids showed a significant increase in both rice cultivars under investigation exposed to drought stress compared with fully irrigated plants (Figs. 2 and 3). Polyphenols are considered the most important secondary metabolite class of antioxidants, and they efficiently inhibit lipid peroxidation products by scavenging ROS; therefore, the polyphenols increase the adaptation to abiotic oxidative stress (Pandey and Rizvi 2009; Krol et al. 2014; Stagnari et al. 2016). Other studies showed an accumulation of phenolic compounds in response to drought in Spanish (Xu and Leskovar 2015), tomatoes (Patanè et al. 2021), and wheat (Ali et al. 2022). In addition, algal treatments of soil with either *G. oblongata* or *T. ornata* induced further accumulations of total phenols and flavonoids in both stressed rice cultivars (Fig. 2). This accumulation might be the cause of

the strong effect of algal extracts in increasing the polyphenol content, especially the content of flavonoids, in stressed plants. Also, due to the accumulation of some enzymes such as phenyl ammonia lyase (PAL) as well as chalcone synthase (Naikoo et al. 2019). Mansori et al. (2015) mentioned that the application of the seaweeds *U. rigida* and *F. spiralis* induced the accumulation of total phenols in bean plants.

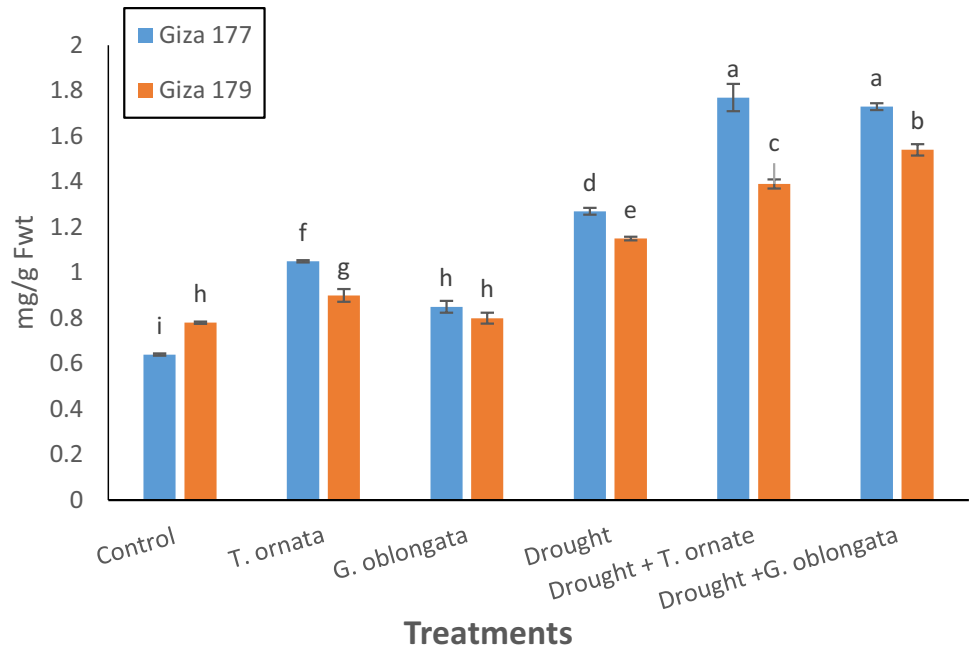
The nonenzymatic antioxidant ascorbic acid (AsA) was measured in both the investigated rice cultivars exposed to drought stress (Fig. 4). A more pronounced increase was observed in Giza 179, which was 73.6% over the control value. Our results supported the findings of Pourghasemian et al. (2020) for sesame plants exposed to water deficit conditions. These findings may be attributed to the highly significant antioxidant capacity of AsA to detoxify ROS, minimize MDA levels, and mitigate other types of physiological deterioration in plants; it is considered one of the defense mechanisms plants employ for drought tolerance (Miller et al. 2010; Xu et al. 2010). Moreover, soil amended with either *G. oblongata* or *T. ornata* led to increased AsA levels in the two investigated rice cultivars (see Fig. 4).

In this study, the total antioxidant capacity of rice seedlings and the evaluation of drought tolerance in rice plants were determined by fluorescence recovery by photobleaching (FRAP) and the DPPH assay for screening plants for stress tolerance (Rekika et al. 2005; Ozgen et al. 2006a, b). FRAP found that the antioxidant capacity markedly increased in both rice cultivars under drought stress compared with the fully irrigated plants (Fig. 5). Moreover, the application of *G. oblongata* or *T. ornata* extract to soil increased the total antioxidant capacity levels in both the

**Fig. 2** Influence of amended soil with either *Turbenaria ornata* or *Galaxaura oblongata* on total phenol content of the two rice cultivars Giza 177 and Giza 179 grown under drought conditions. Results are shown as a mean of three replicates; the bars on the column show  $\pm$  SE



**Fig. 3** Influence of amended soil with either *Turbenaria ornate* or *Galaxaura oblongata* on flavonoid content of the two rice cultivars Giza 177 and Giza 179 grown under drought conditions. Results are shown as a mean of three replicates; the bars on the column show  $\pm$  SE

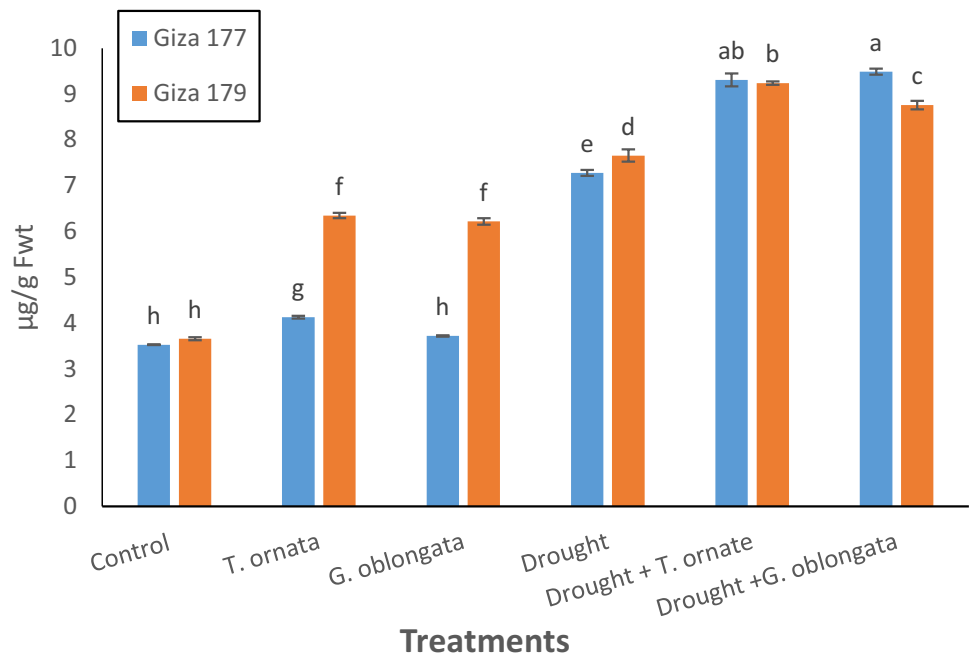


investigated stressed rice cultivars. This finding may be because the seaweeds preserved the highest antioxidant activity in rice plants by forming a protective barrier on the surface of the plants and inhibiting the reduction in antioxidant activity. On the other hand, the evaluation of the total antioxidant capacity by the DPPH scavenging method showed an opposite trend to that of the FRAP method in both rice cultivars grown under drought stress compared with their corresponding controls (see Figs. 5 and 6).

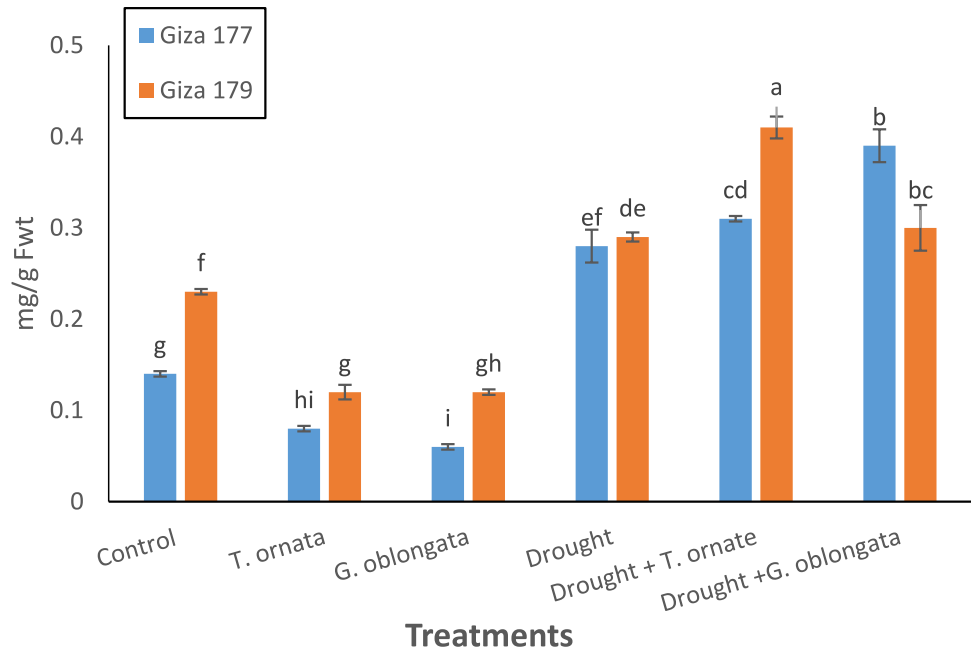
#### Gene Expression by RT-PCR

Plants have numerous types of aquaporin proteins, and they participate in the tolerance of and maintain the water balance of stressed plants (Hu et al. 2012; Ayadi et al. 2019). The plasma membrane intrinsic proteins (PIPs) belong to the AQP family and have a potential role in transporting water through the plasma membranes in many types of plants (Martre et al. 2002; Tyerman et al. 2002).

**Fig. 4** Influence of amended soil with either *Turbenaria ornate* or *Galaxaura oblongata* on ascorbic acid (AsA) contents of the two rice cultivars Giza 177 and Giza 179 grown under drought conditions. Results are shown as a mean of three replicates; the bars on the column show  $\pm$  SE



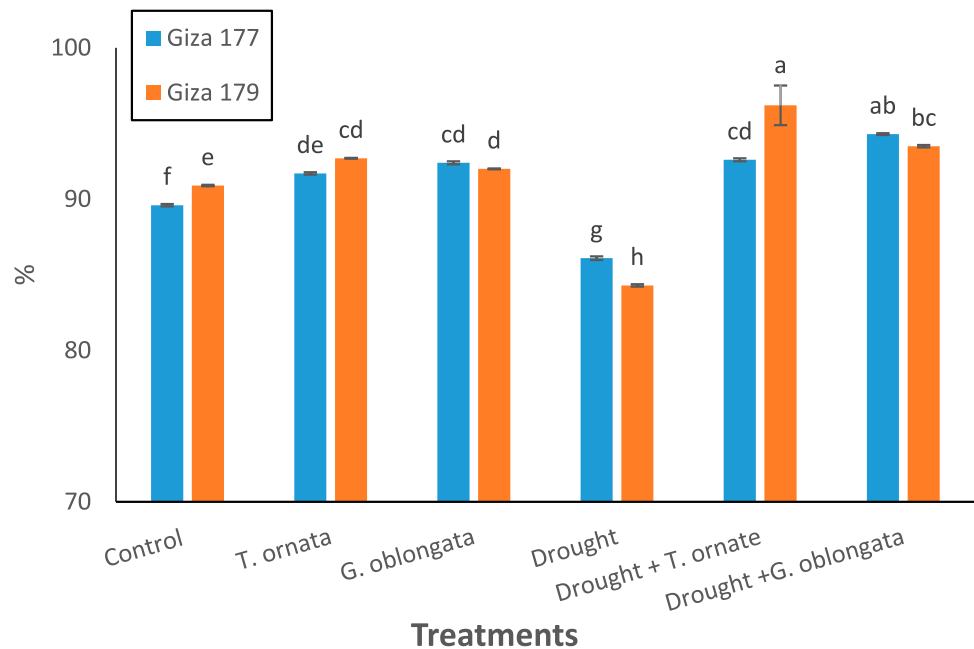
**Fig. 5** Influence of amended soil with either *Turbenaria ornate* or *Galaxaura oblongata* on total antioxidant capacity (TAC) content of the two rice cultivars Giza 177 and Giza 179 grown under drought conditions. Results are shown as a mean of three replicates; the bars on the column show  $\pm$  SE

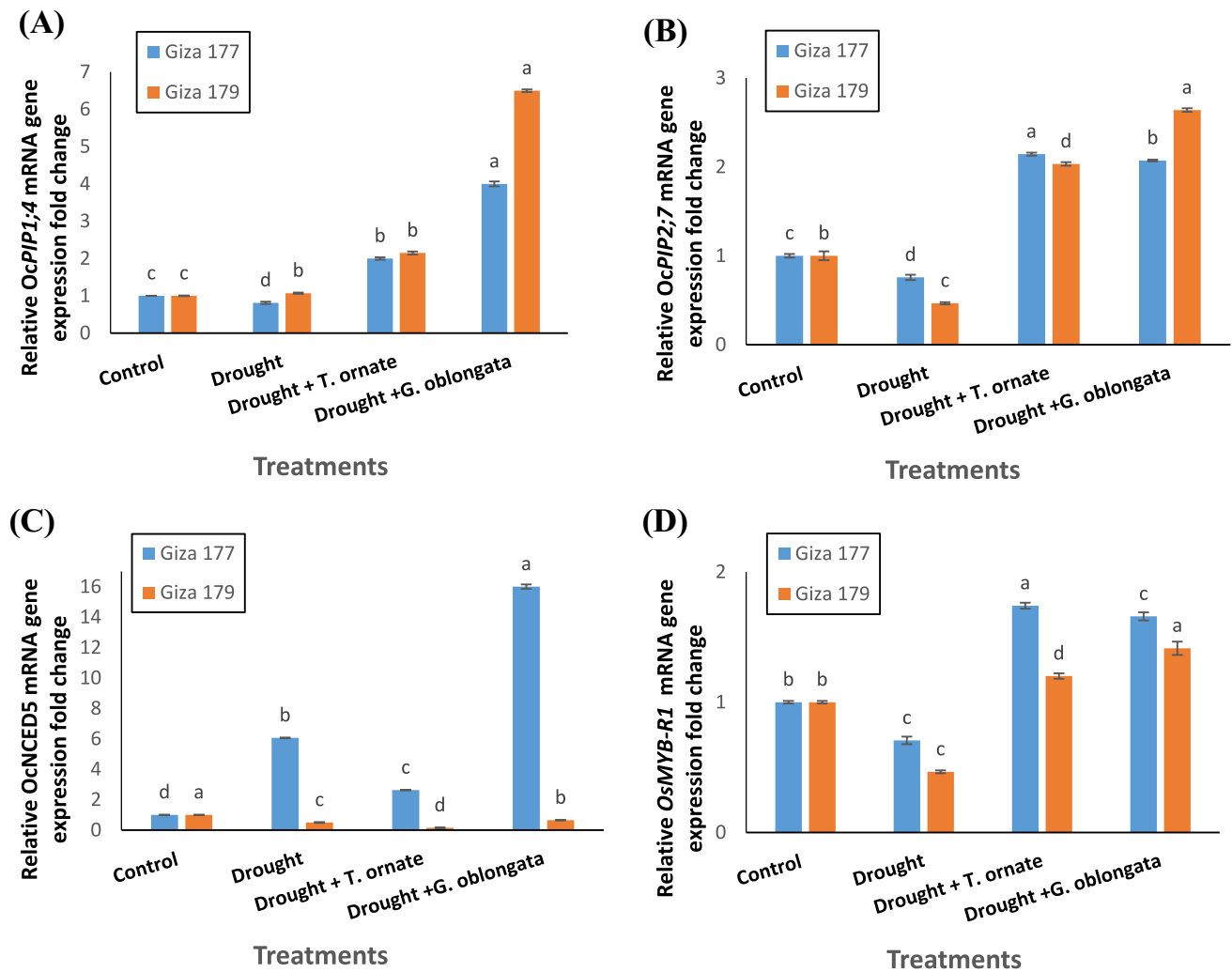


In the present study, the results obtained from real-time polymerase chain reaction (RT-PCR) analysis revealed that drought stress induced a downregulation of *PIP1;4* and *PIP2;7* expression. Our results were in accordance with those of Porcel et al. (2006), who studied *PIPs* in *Glycine max* and *Lactuca sativa* plants. This downregulation was accompanied by overexpression of the *PIP1;4* and *PIP2;7* genes in stressed rice plants grown in soil amended with *G. oblongata* or *T. ornate* (Fig. 7a and b) compared with their corresponding controls. This overexpression might have had a main role in retarding the growth of seedlings

in response to drought conditions (Jang et al. 2007). The most pronounced downregulated gene expression value was observed in the *PIP2;7* genes of Giza 179. In addition, the *NCDE5* gene related to abscisic acid biosynthesis showed downregulation under drought stress in the Giza 179 cultivar; however, a reverse pattern was significantly observed in the rice-sensitive cultivar Giza 177 (see Fig. 7c). The soil amended with either *G. oblongata* or *T. ornate* enhanced the overexpression of the *NCDE5* gene in the two stressed rice cultivars compared with the controls (see Fig. 7c). This might be due to stimulation by the *PIP1;4*

**Fig. 6** Influence of amended soil with either *Turbenaria ornate* or *Galaxaura oblongata* on DPPH percentage of the two rice cultivars Giza 177 and Giza 179 grown under drought conditions. Results are shown as a mean of three replicates; the bars on the column show  $\pm$  SE





**Fig. 7** Influence of amended soil with either *Turbenaria ornate* or *Galaxaura oblongata* on the relative mRNA gene expression fold change, which **A** represented PIP1;4, **B** PIP2;7, **C** NCDE 5; and **D**

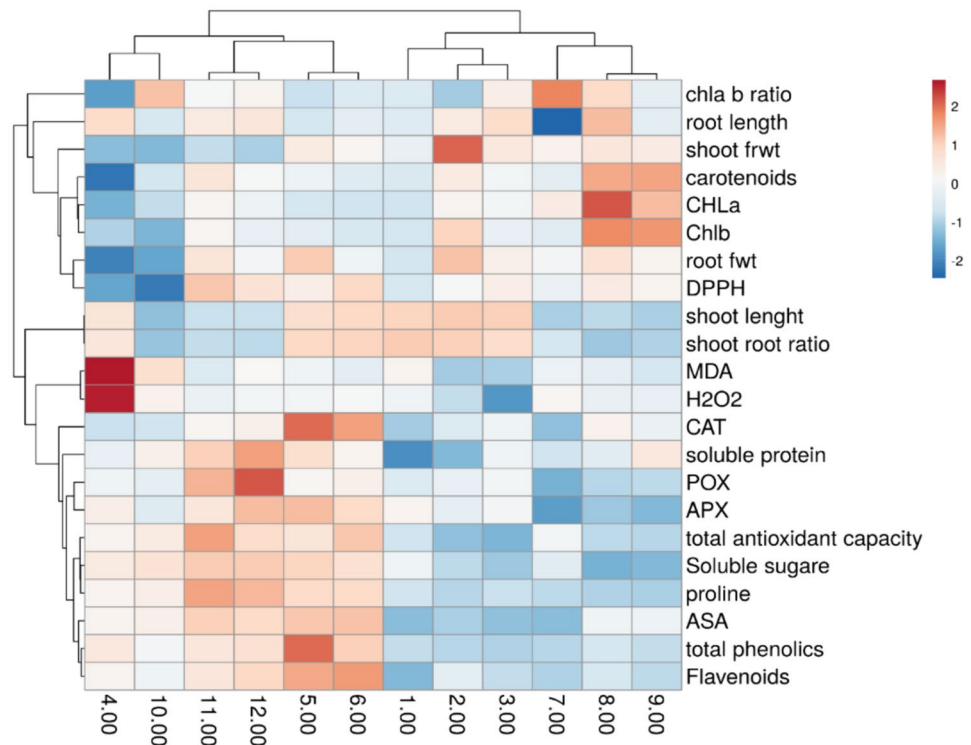
OsMyb-R1 of the two rice cultivars Giza 177 and Giza 179 grown under drought condition. Results are shown as a mean of three replicates; the bars on the column show  $\pm$ SE

and *PIP2;7* genes of the synthesis of ABA in stressed plants, which played an efficient role in the tolerance of plants exposed to drought (Li et al. 2015). In the present study, the application of investigated seaweeds induced a partial stomatal closure, associated with changes in the expression levels of genes involved in ABA-responsive and antioxidant systems in response to drought stress to eliminate ROS and improve the drought tolerance of rice plants. Our results are in alliance with the findings of Ali et al. (2022) on ork plants. The maximum overexpression of the *NCDE5* gene was noticeable in Giza 177; it reached an approximately 16-fold change. Transcription factors (TFs) are considered to be common regulators of many vital processes of plants, beginning during development and continuing with defense mechanisms. MYB is one TF that can play a crucial role in the maintenance of a plant's tolerance to environmental stresses (Erpen et al. 2018). As the data in Fig. 7d show,

the downregulation of *Myb-R1* gene expression in both Giza 177 and Giza 179 was observed under drought conditions. The amendment of soil by either *G. oblongata* or *T. ornate* improved the overexpression of the *Myb-R1* gene in the two stressed rice cultivars compared with the controls (see Fig. 7d). Chauhan et al. (2020) like us, found a significant difference in the overexpression of the *OsMyb-R1* gene in rice plants grown in treated soil. Our results suggested that the overexpression of the *OsMyb-R1* gene, when triggered by drought stress, could be a main factor in the control of the physiological, biochemical, and molecular responses to stresses (Zhang et al. 2014).

Finally, to summarize the comparisons between groups, the interrelationships among the investigated variables in terms of Pearson's correlation test were plotted as a heatmap (Fig. 8). The blue boxes in Fig. 8 indicate a positive correlation between variables, and the red boxes indicate

**Fig. 8** Heatmap showing the correlation between different treatments. The numbers represented in this figure showed the different treatments as follows: 1 and 7, control; 2 and 8, *Turbenaria ornate*; 3 and 9, *Galaxaura oblongata*; 4 and 10, drought; 5 and 11, drought + *Turbenaria ornate*; and 6 and 12, drought + *Galaxaura oblongata* of cv. Giza 177 and cv. Giza 179, respectively



a negative correlation. The principle component analysis (PCA) summarized the similarities and arranged the different samples in four groups: group 1 consisted of samples 11, 12, 5, and 6; group 2 of samples 8, 9, 3, and 2; group 3 of samples 10 and 4; and group 4 of samples 1 and 7 (Fig. 9).

## Conclusions

In this finding, we examined and studied the mechanism of drought as well as the relative effect of seaweeds *G. oblongata* and *T. ornate* incorporated soil as biostimulant on two rice cultivars' growth and crop productivity by determining some physiological and biochemical analyses as well as molecular aspects. Rice plants grown in algal-enriched soil under drought conditions presented adaption by accumulating some compatible solutes such as proline and soluble sugar to regulate cellular turgor and produce antioxidants like phenolic compounds and AsA accompanied with diminished the levels of MDA and H<sub>2</sub>O<sub>2</sub> to counter oxidative damage. They also upregulation *PIP1;4*, *PIP2;7*, *NCDE5*, and *Myb-R1* drought-responsive genes, improving rice tolerance and stress resilience. We found that this mechanism of tolerance worked better in the drought-resistant cultivar Giza 179 than in the drought-sensitive cultivar Giza 177. Further research is needed for comprehensive insights.

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**Data Availability** The manuscript has associated data in a data repository. The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

## Declarations

**Conflicts of Interest** The authors declare no competing interests.

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