FOLIA OECOLOGICA – vol. 51, no. 2 (2024), doi: 10.2478/foecol-2024-0023

Triacontanol 'TRIA' application to mitigate the adverse effects of drought and salinity stress under *in vitro* culture of date palm plants

Ahmed Madi Waheed Al-Mayahi*

Date Palm Research Centre, University of Basrah, Basrah, Iraq

Abstract

Al-Mayahi, A.M.W., 2024. Triacontanol 'TRIA' application to mitigate the adverse effects of drought and salinity stress under *in vitro* culture of date palm plants. *Folia Oecologica*, 51 (2): 250–262.

This study was conducted to attempt adaptation to combined drought and salt stresses (DS) (PEG- 6000 + NaCl) in date palm cv. Barhee implanted *in vitro*, keeping in mind the detrimental influence of DS. *In vitro* experimentation was executed on *P. dactylifera* L. to examine the efficacy of the application of triacontanol (TRIA), on growth attributes, and some biochemical constituents under DS. The optimal treatment was 10 µg l^{-1} TRIA. Such treatment under DS improved the callus growth and increased its weight to 215.0 mg. This treatment also showed the highest response rate and the number of shoots per jar (72.23% and 10.30 shoots, respectively) under DS stress. TRIA enhanced DS tolerance by increasing the contents of osmoregulatory substances such as proline, total soluble carbohydrates, and total soluble proteins, were obtained by adding 20 and 10 mg l^{-1} TRIA. This treatment was also more effective under DS in increasing Ca^{2+}, Mg^{2+}, and K^{+}, as well as Fe^{2+}, and chlorophyll pigment. These results also indicate that using 10 µg l^{-1} TRIA as a supplement under DS can increase SOD, APX, and PAL activity, to 31.68, 3.377 unit g–1 min^{-1}, and 33.78%, respectively. Data analysis also indicated that the application of 10 µg l^{-1} TRIA countered the DS-induced harmful effects by reducing the content of malondialdehyd (MDA) and H_2O_2 in stressed tissues to 1.06, and 1.278 µMg of fresh weight (FW). Our work could reveal detailed changes in the quantity and number of protein bands by SDS-PAGE. New protein bands appeared in both stressed with TRIA-treated plants. The result of the present study will be useful for rapid clonal propagation of date palm which can be used to enhance the tolerance of plants to drought and salt stress.

Keywords

abiotic stress, callus induction, growth regulator, multiple shoots, nutrients, protein patterns

Introduction

Date palm (*Phoenix dactylifera* L.), which belongs to the Arecaceae family, is a multipurpose tree with nutritional, medicinal, and ornamental importance, and today its worldwide production, utilization, and industrialization are continuously increasing. Due to the increasing demand for date palm plantlets, production is carried out in large areas (Jasim et al., 2009). Various climate changes greatly affect agricultural systems around the world (Goharrizi et al., 2021). Drought and salinity are known to be the two most important abiotic stresses affecting plant growth, development, and productivity (Ma et al., 2020). The focus on plant stress-related research has gained substantial momentum over the past decades. Screening plants for different stress conditions is essential in breeding and selecting elite varieties. Most experiments screening plants for stressors are performed at field conditions, but their management is very difficult and is subject to various risks due to dynamic external environments. The application of plant tissue culture-based techniques in the laboratory has allowed the development of biotechnological tools to

*Corresponding author:
e-mail: hng_1988@yahoo.com

address obstacles to improving plants for sustainable agriculture (Ibrahim et al., 2013; Al-Mayahi et al., 2010; Al-Asadi et al., 2019; Al-Mayahi, 2021; 2022a). The use of tissue culture increases the possibility of intensive screening and selection of more drought and salt-tolerant genotypes. Several factors regulate *in vitro* plant growth and development among them, the culture medium is the most important (Al-Mayahi, 2019; 2022b, c; Awad et al., 2020; Al-Mayahi and Ali, 2021). Plant hormones play a vital role in the growth and development of plants and also represent an important line of defense during the plant is exposed to stress (Rasool, 2022). Triacontanol (TRIA) has been of interest to researchers working to promote plant growth under abiotic stress conditions, due to its stimulatory effects on water and nutrient uptake, photosynthesis, enzyme activities, membrane stability, and gene regulation (Perveen et al., 2012 a, b). TRIA ($C_{30}H_{61}OH$) is a saturated primary alcohol and has a newly discovered as a plant growth regulator that is a component of the epicuticle waxes that contribute to promoting plant growth under abiotic stresses (Karam and Keramat, 2017). In addition to the vital role that TRIA plays in plants, it also can be used in media as an effective growth regulator in micropropagation (Malabadi et al., 2005; Parimalan et al., 2009). As an important growth regulator, TRIA has attracted much attention regarding the classification of its physiological effects on several plants. Plant growth regulators can be used to ameliorate stress-induced growth decrease (Islam and Mohannad, 2020). It is believed that hormonal changes are sometimes necessary to regulate plant growth under environmental stresses (Keskin et al., 2010). The palm tree is negatively affected by the combination of salinity and drought during all stages of growth in cultivated areas. The adverse effects of salinity combined with water scarcity on osmotic stress greatly increased the sensitivity of reproductive development. It is necessary to establish breeding programs to develop stress-tolerant date palm genotypes. TRIA is known to have growth-promoting activities in several plants under normal and stress conditions (Verma et al., 2009). The development of *in vitro* selection technology, combined with molecular approaches will provide a new opportunity to promote stress tolerance in plants. Hence, this study aimed to evaluate the protective effect of TRIA in drought and/or salt stressed date palm explants under *in vitro* conditions.

Materials and methods

The experiments were carried out in the tissue culture laboratory of Date Palm Research Centre at Basrah University, Basrah, Iraq.

Young offshoots (2–3 years old) were collected from palm trees grown in Abu Al-Khaseeb district, Basrah City, southern Iraq. Outer leaves and fibrous tissues at their bases were removed gradually until the shoot tip zone was exposed. Sterilization of explants was performed using 70% ethanol for 1 min and 2.5% sodium hypochlorite for 20 min. Explants were then rinsed three times with sterile distilled water. To induce callus induction, explants were cultured on the MS basal medium (Murashige and Skoog, 1962). It was combined with Gamborg's vitamins and supplemented with 3 mg l^{-1} 6-(dimethylallyl amino) purine (2iP), 30 mg l^{-1}, naphthalene acetic acid (NAA), 30 g l^{-1} sucrose, 2.0 g mg l^{-1} activated charcoal, and solidified with agar–agar at 6.0 g l^{-1} were used. The pH of the medium was adjusted to 5.7 with 0.1N NaOH or HCl, before the addition of agar. Cultures were kept under complete darkness at 27 ± 2 °C. The cultures were transferred to fresh media, with the same composition every 6 weeks until callus induction.

The induced callus was separated, weighed, and cultured (100 mg per jar) on MS medium added with 100 mg l^{-1} glutamine, 5 mg l^{-1} thiamine HCl, 1 mg l^{-1} biotin, 30 g l^{-1} sucrose, and solidified with agar at 7.0 g l^{-1} and 0.5 g l^{-1} activated charcoal, with the addition of growth regulators Naphthalene Acetic Acid (NAA) at 6 mg l^{-1} and 6-(dimethylallyl amino purine) (2iP) at 2 mg l^{-1}, and triacontanol (TRIA) to tolerance drought (PEG-6000) and salt (NaCl) stresses. The pH of the medium was adjusted to 5.7–5.8 before the addition of agar. Media was dispensed into culture containers and autoclaved at 121 °C and 1.04 kg cm^{-2} for 20 min. The cultures were kept in a culture room at 27 ± 2 °C with 16 h light and eight-hour dark provided by cool white fluorescent bulbs. To study the effects of TRIA on callus growth, supplementation of this substance at different concentrations in the growth medium was assessed under combined drought and salt stresses (DS). To study the effects of TRIA on callus growth, supplements of this substance at different concentrations (after dissolving it in chloroform) in the culture medium were evaluated under combined drought and salt stress (DS) conditions. Treatments consisted of 12 media, TRIA purchased from (Sigma-Aldrich Company Ltd.), with two doses of polyethylene glycol (PEG-6000, Sigma-Aldrich Company Ltd.) and sodium chloride (NaCl), as shown in Table 1.

For differentiation and multiplication, the callus on growth media was divided and subcultured on differentiation and multiplication media supplemented as mentioned above, except for the plant growth regulators 1 mg l^{-1} (NAA), 0.5 mg l^{-1} (BA), and 0.5 mg l–1 kinetin (K) (Al-Mayahi, 2022b). The same treatments mentioned in Table 1 were also used to study their effect on bud multiplication and some changes

Table 1. Treatments that are applied in this work

No	Treatments	No	Treatments
T1	0% PEG-6000, 0% of NaCl, no TRIA (control)	T5	0% PEG-6000, 0% of NaCl, with 20 µg l^{-1} TRIA
T2	10% PEG-6000, 1% of NaCl, no TRIA	T6	10% PEG-6000, 1% of NaCl, with 5 µg l^{-1} TRIA
T3	0% PEG-6000, 0% of NaCl, with 5 µg l^{-1} TRIA	T7	10% PEG-6000, 0% of NaCl, with 10 µg l^{-1} TRIA
T4	0% PEG-6000, 0% of NaCl, with 10 µg l^{-1} TRIA	T8	10% PEG-6000, 1% of NaCl, with 20 µg l^{-1} TRIA

in phytochemical properties. The cultures were maintained at room temperature 27 ± 2 °C, under a photoperiod of 16-h day/8-h night cycles. The light intensity was 2,000 lux provided by cool white fluorescent lamps. There were 18 replicates of each treatment. The percentage of bud regeneration and bud number per jar were recorded at 12 weeks from the inoculation of callus on the media.

Physiological and biochemical attributes

Total soluble carbohydrates
The anthrone technique adopted by Watanabe et al. (2000) quantified the total carbohydrates as glucose.

Total soluble protein
Total soluble proteins were quantified using the procedure of Bradford (1976).

Proline content
Proline content was measured by the method of Bates et al., (1973).

Malondialdehyde (MDA) content
MDA was quantified as a marker of membrane lipid peroxidation, according to Heath and Packer (1969).

Hydrogen peroxide (H_2O_2) content
Hydrogen peroxide (H_2O_2) content was measured calorimetrically at 390 nm according to Sergiv et al. (1997).

SOD, APX, and PAL assay
Extractions of superoxide dismutase (SOD; EC1.15.1.1.), ascorbate peroxidase (APX; EC 1.11.1.11.), and determinations of their activities were carried out according to the methods of Kawakami et al., (2000) and Nakano and Asada (1981), respectively.

Phenylalanine ammonia-lyase (PAL) activity was assayed following the method of (Syklowska-Baranek et al., 2012).

Mineral content in *in vitro*-grown shoots
The content of total Na^+ (sodium), Ca^{2+} (Calcium), Mg^{2+} (Magnesium), K^+ (Potassium), and Fe^{2+} (iron) in shoots were analyzed according to the method described by Cresser and Parsons (1979). Chemical analyses were performed using the following methods: Na, K, Ca, and Mg, which were determined by atomic absorption spectrometry, according to the method described by Black (1968). Fe was determined by ICP method (PerkinElmer Optima 7000 DV, US) (Gupta et al., 2007).

Chlorophyll content
The concentration of chlorophylls in the leaves was estimated by the method described by Porra (2002).

Extraction of protein and gel electrophoresis
Proteins were extracted by homogenizing the 0.333 gm freeze-dried shoot sample in pre-chilled mortar and pestle using 1 ml of extraction buffer consisting of 0.2 M, tris hydroxymethyl aminomethane (Tris); 0.001 M ethylene diamine tetra acetic acid (Na2 EDTA); 12%, glycerol; 0.01 M, dithiothreitol (DTT); and 0.05 mM phenyl methyl sulfonyl fluoride (PMSF). Protein samples (~500 µg) were electrophoresed in a discontinuous SDS polyacrylamide gel following Laemmli (1970) using a 12% resolving gel (0.375 M, Tris–HCl; pH 8.8) and 4% stacking gel (0.125 M, Tris–HCl; pH 6.8) in Tris–glycine buffer (0.025 M, Tris; pH 8.3; 0.192 M, glycine; 0.1%, SDS) for 16 h, constantly at 20 mA. Staining of the gel was done using 0.2% (w/v) Coomassie Brilliant Blue R-250 in 12.5% (w/v) trichloroacetic acid (TCA). The position of the protein band in the gel was expressed to compare with standard protein markers with known molecular weight.

Experimental design and statistical analysis

The experiments were carried out using a completely randomized design (CRD). Data were analyzed using variance (ANOVA) analysis using Statistical Package for Social Sciences (SPSS) software version 20. Treatment means were compared using the least significant difference (LSD) at the $P < 0.05$ level.

Results

Growth parameters

Based on the results of this study, it was observed that adding TRIA to the growth medium resulted in improved growth characteristics in all DS, non-stressed cultures compared to the control. Maximum reduction in growth characteristics was recorded in cultures implanted under DS at a concentration of 10% PEG-6000 and 1% NaCl in the absence of TRIA, the callus tissues exhibited a slight growth and a brown color. Furthermore, the development of shoots was also low (Fig. 1b). The highest fresh weight of date palm callus, the highest response rate, and the number of shoots per jar (294 mg, 88.87%, and 13.5, respectively), were associated with callus tissues implanted under no DS stress, combined with the application of 10 µg l^{-1} TRIA, compared to other treatments (Table 2, Figs 1 and 2).

Total carbohydrates, total proteins, and proline

The results obtained, in Figs 3, a and b, revealed the carbohydrates and free proline in date palm cultures. Both of these two traits increased under DS. In the control and stress treatments, application TRIA resulted in higher contents of total soluble carbohydrates and free proline. In contrast, the highest content of these compounds was associated with the plants implanted under DS with a concentration of 10% PEG-6000 and 1% NaCl combined with 10 and 20 µg l^{-1} TRIA. According to the results (Fig. 3c), the total soluble protein value was significantly decreased ($p < 0.05$) under DS induced by PEG (10%) and 1% NaCl as compared with the other treatments, while

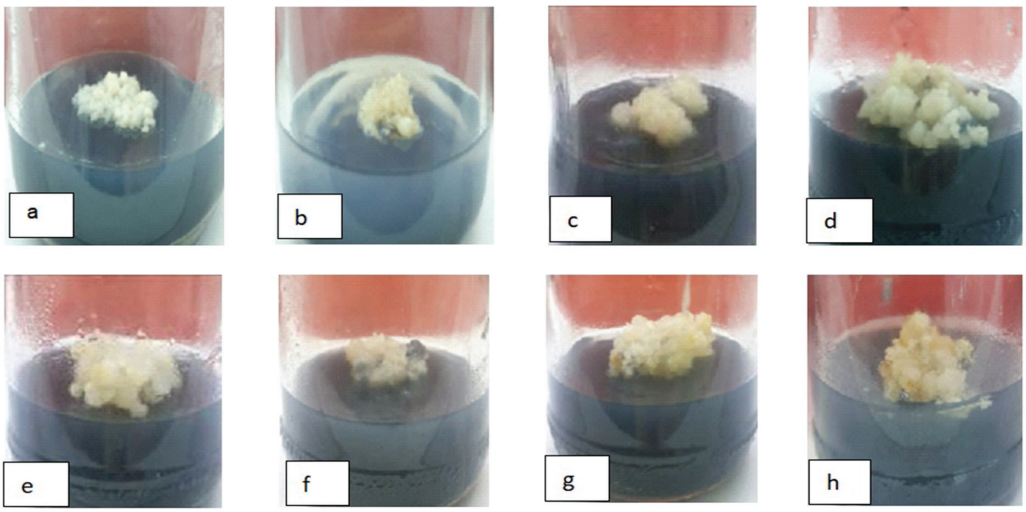


Fig. 1. Callus proliferation on MS culture × medium with: (a) control, (b) 10% PEG6000 + 1.0% NaCl, (c) 0.0% PEG6000 + 0.0% NaCl + 5 µg l^{-1} TRIA, (d) 0.0% PEG6000 + 0.0% NaCl + 10 µg l^{-1} TRIA, (e) 0.0% PEG6000 + 0.0% NaCl + 20 µg l^{-1} TRIA, (f) 10% PEG6000 + 1.0% NaCl + 5 µg l^{-1} TRIA, (g) 10% PEG6000 + 1.0% NaCl + 10 µg l^{-1} TRIA, (h) 10% PEG6000 + 1.0% NaCl + 20 µg l^{-1} TRIA.

Table 2. Effect of triacontanol (TRIA) on in *in vitro* growth traits of date palm cv. Barhee under combined drought and salt stresses (DS). ± Standard error (n = 18). Values followed by the same letter are not significantly different at $P < 0.05$.

Treatments	Callus weight (mg)	Frequency (%)	Shoot number
T1 0% PEG-6000, 0% of NaCl, no TRIA (control)	146.0 d ± 7.60	44.45 e ± 3.89	4.88 f ± 0.40
T2 10% PEG-6000, 1% of NaCl, no TRIA	111.0 e ± 7.23	33.34 f ± 4.05	3.60 g ± 0.50
T3 0% PEG-6000, 0% of NaCl, with 5 µg l^{-1} TRIA	179.0 c ± 9.44	55.56 cd ± 3.05	7.20 e ± 0.19
T4 0% PEG-6000, 0% of NaCl, with 10 µg l^{-1} TRIA	294.0 a ± 12.33	88.87 a ± 6.40	13.50 a ± 0.42
T5 0% PEG-6000, 0% of NaCl, with 20 µg l^{-1} TRIA	226.0 b ± 9.23	77.78 b ± 6.40	11.85 b ± 0.80
T6 10% PEG-6000, 1% of NaCl, with 5 µg l^{-1} TRIA	151.0 d ± 7.49	50.00 de ± 4.60	5.56 f ± 0.80
T7 10% PEG-6000, 1% of NaCl, with 10 µg l^{-1} TRIA	215.0 b ± 13.21	72.23 b ± 6.40	10.30 c ± 0.90
T8 10% PEG-6000, 1% of NaCl, with 20 µg l^{-1} TRIA	182.0 c ± 9.44	61.12 c ± 3.89	8.90 d ± 1.30

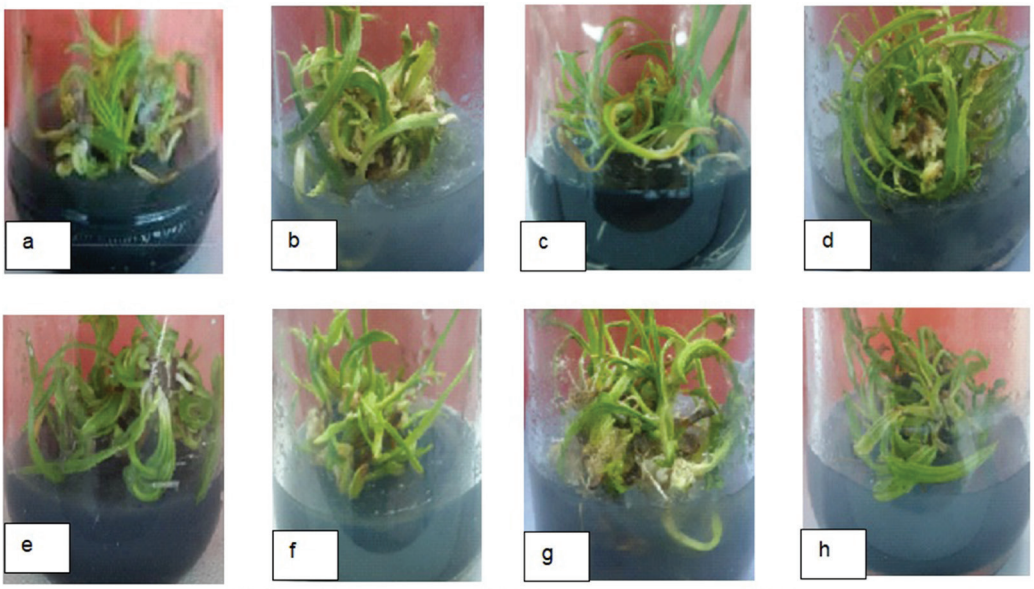


Fig 2. Effect of triacontanol (TRIA) on shoots multiplication in the date palm cv. Barhee under combined drought and salt stresses (DS): (a) control, (b) 10% PEG6000 + 1.0% NaCl, (c) 0.0% PEG6000 + 0.0 % NaCl + 5 µg l^{-1} TRIA, (d) 0.0% PEG6000 + 0.0% NaCl + 10 µg l^{-1} TRIA, (e) 0.0% PEG6000 + 0.0% NaCl + 20 µg l^{-1} TRIA, (f) 10% PEG6000 + 1.0% NaCl + 5 µg l^{-1} TRIA, (g) 10% PEG6000 + 1.0% NaCl + 10 µg l^{-1} TRIA, (h) 10% PEG6000 + 1.0% NaCl + 20 µg l^{-1} TRIA.

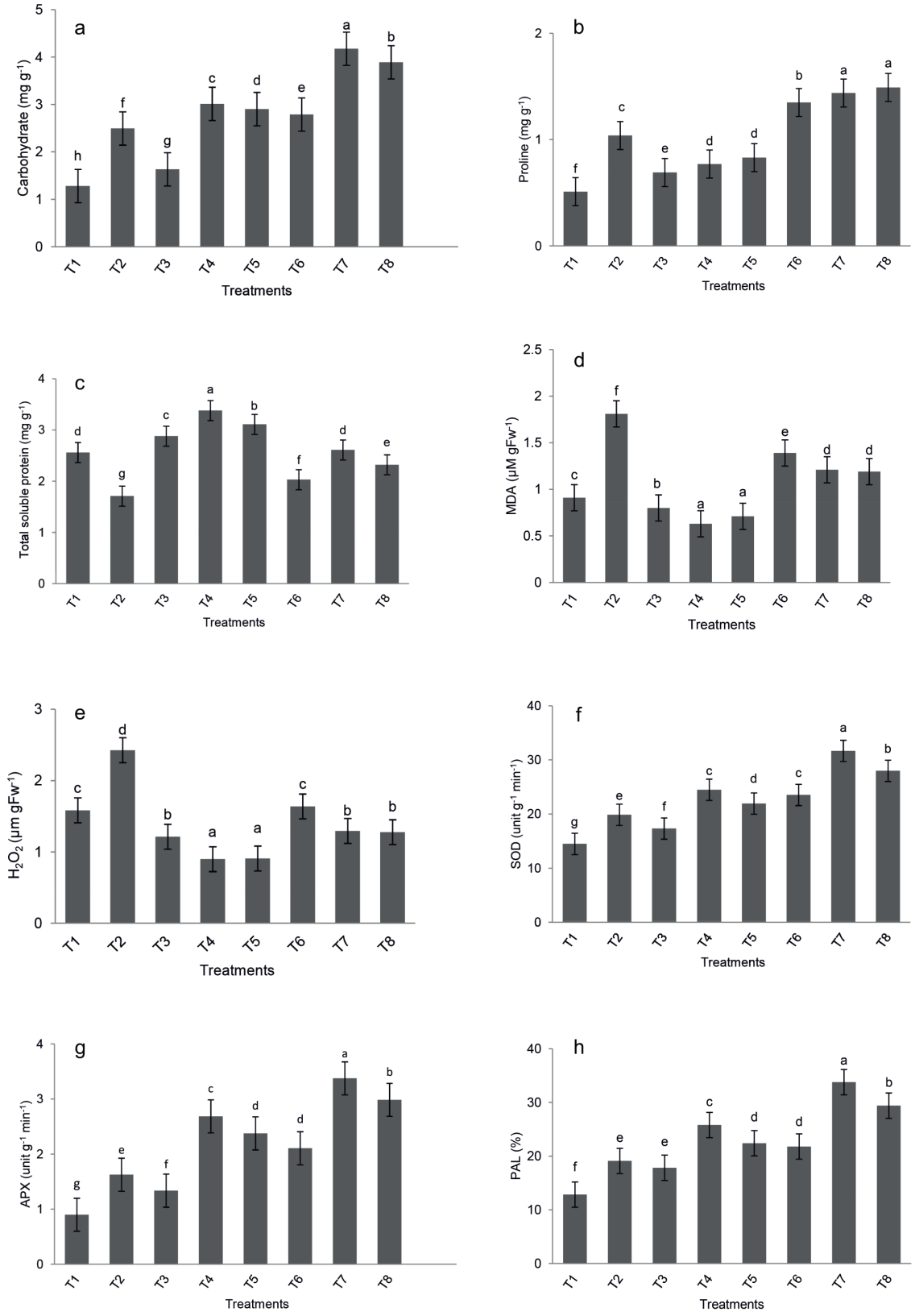


Fig. 3. Effect of triacontanol (TRIA) on (a) Total soluble carbohydrates, (b) Proline, (c) Total soluble protein, (d) Malondialdehyde (MDA), (e) Hydrogen Peroxide (H_2O_2), (f) Superoxide oxidase (SOD), (g) Ascorbate peroxidase (APX), (h) Phenylalanine ammonia-lyase (PAL) in the date palm cv. Barhee, treated with PEG6000 + NaCl, TRIA: (T1) control, (T2) 10% PEG6000 + 1.0% NaCl, (T3) 0.0% PEG6000 + 0.0 % NaCl + 5 µg l^{-1} TRIA, (T4) 0.0% PEG6000 + 0.0% NaCl + 10 µg l–1 TRIA, (T5) 0.0% PEG6000 + 0.0% NaCl + 20 µg l–1 TRIA, (T6) 10% PEG6000 + 1.0% NaCl + 5 µg l^{-1} TRIA, (T7) 10% PEG6000 + 1.0% NaCl + 10 µg l^{-1} TRIA, (T8) 10% PEG6000 + 1.0% NaCl + 20 µg l^{-1} TRIA.

the application of TRIA caused an increase in total soluble protein value under stress and non-stress conditions. The highest value of total soluble protein is associated with the cultures implanted under non-DS stress in combination with the addition of 10 µg l^{-1} TRIA.

Malondialdehyde (MDA) and hydrogenperoxide (H_2O_2) content

Malondialdehyde (MDA) and hydrogenperoxide (H_2O_2) as an indication of the rate of harm caused by ROS to cultures were measured; the data presented in Fig. 3, d and e, showed that MDA and H_2O_2 content was increased in treated cultures with DS, hence, this was evident by increasing their contents under stress conditions as compared with the control treatment (Fig. 3, d and e). The use of TRIA somewhat improved the negative effect of DS, by reducing MDA and H_2O_2 levels. The lowest levels of MDA and H_2O_2 were associated with cultures implanted under non-DS stress in combination with the application of 10 µg l^{-1} µg l^{-1} TRIA.

APX, SOD, and PAL activities

Figures 3f, g, and h show that the highest activity of SOD, APX, and PAL occurred in the shoots cultured under DS 10% PEG6000 and 1% NaCl in combination with 10 µg l^{-1} TRIA, compared to other treatments. The study results also indicate that the least activity of SOD, APX, and PAL were achieved in no application of TRIA with no DS stress condition (control treatment).

Nutrient content in *in vitro* shoots

Combined drought and salt stresses (DS) reduced the Mg^{2+}, K^+, and Fe^{2+} levels, and increased the Na^+ and Ca^{2+}, while TRIA application resulted in the accumulation of these nutrients in the shoots and reduced the Na^+ in the shoots (Table 3). The highest Ca^{2+}, Mg^{2+}, K^+, and Fe^{2+} values are associated with cultures implanted under no DS stress in combination with the use of 10 µg l^{-1} TRIA. In contrast, the lowest content of Ca^{2+}, Mg^{2+}, K^+, and Fe^{2+} are associated with cultures implanted under DS without TRIA (0 µg l^{-1}). The lowest Na^+ content occurred in the shoots implanted under no DS stress, as well as the use of 20 µg l^{-1} TRIA compared to the other treatments.

Chlorophyll content

Table 4 shows that the Chl (a, b), and total chlorophyll values were significantly reduced ($p < 0.05$) under DS compared to other treatments, while the application of TRIA caused an increase in chlorophyll content under stress and non-stress conditions. The highest content of Chl a, Chl b, and total chlorophyll are associated with cultures that were implanted under non-DS stress in combination with the addition of 10 µg l^{-1} TRIA.

SDS-PAGE protein patterns

Alterations in protein patterns in tissues of date palm cv. Barhee were analyzed in laboratory culture conditions to detect any possible changes in gene expression in cultures implanted under DS conditions (PEG-6000 + NaCl) in the absence or addition of TRIA compared with non-stressed (control treatment), by applying SDS-PAGE.

Some protein types appear as a result of PEG-6000+NaCl (DS), while others decrease in concentration or disappear compared with the control treatment. Stress treatments induced new bands with molecular

Table 3. Content of Ca, Na, Mg, K^+, and Fe of *in vitro* cultures of date palm Barhee exposed to drought and salt stresses implanted in the presence of triacontanol (TRIA). ± Standard error (n = 9). Values followed by the same letter are not significantly different at $P < 0.05$.

Treatments	Na (mg g^{-1} DW*)	K (mg g^{-1} DW)	Mg (mg g^{-1} DW)	Ca (mg g^{-1} DW)	Fe (µ.g^{-1} DW)
T1 0% PEG-6000, 0% of NaCl, no TRIA (control)	2.234 c ± 0.18	3.783 d ± 0.20	11.834 e ± 1.01	10.032 e ± 1.01	1.137 e ± 0.21
T2 10% PEG-6000, 1% of NaCl, no TRIA	3.024 d ± 0.42	2.076 f ± 0.40	10.938 g ± 1.4l	11.400 d ± 1.4l	0.741 f ± 0.04l
T3 0% PEG-6000, 0% of NaCl, with 5 µg l^{-1} TRIA	1.754 b ± 0.19	3.955 c ± 0.50	12.519 cd ± 1.50	11.594 d ± 1.30	1.290 d ± 0.30
T4 0% PEG-6000, 0% of NaCl, with 10 µg l^{-1} TRIA	1.109 a ± 0.13	4.808 a ± 0.80	13.034 a ± 1.20	13.288 abc ± 1.08	1.794 a ± 0.08
T5 0% PEG-6000, 0% of NaCl, with 20 µg l^{-1} TRIA	1.089 a ± 0.12	4.676 b ± 0.75	12.754 b ± 1.00	12.693 bc ± 1.20	1.648 b ± 0.20
T6 10% PEG-6000, 1% of NaCl, with 5 µg l^{-1} TRIA	2.433 c ± 0.18	2.824 e ± 0.18	11.288 f ± 1.06	12.519 c ± 1.06	1.084 e ± 0.06
T7 10% PEG-6000, 1% of NaCl, with 10 µg l^{-1} TRIA	1.907 b ± 0.20	3.955 c ± 0.40	12.619 bc ± 1.49	13.619 a ± 1.02	1.477 c ± 0.023
T8 10% PEG-6000, 1% of NaCl, with 20 µg l^{-1} TRIA	1.824 b ± 0.19	3.888 cd ± 0.8	12.385 d ± 1.21	13.570 ab ± 1.04	1.313 d ± 0.019

*mg – milligrams; g – gram; DW – Dry Weight; µ.g – microgram.

weights of 19, 29, 38.0, 40.0, and 48.6 kD in cultures under DS (Fig. 4, lane 2). Analysis of protein patterns also shows that in non-stressed cultures and implanted in culture media equipped with TRIA, protein bands were not altered, compared to the control treatment, while in stressed cultures use of TRIA contributed to the induction of new protein bands and the disappearance of other protein bands. Stressed cultures (PEG-6000 + NaCl) and those treated with 10 and 20 µg l^{-1} TRIA showed the greatest improvement in protein synthesis, compared to other treatments. In cultures treated with 10 and 20 µg l^{-1} TRIA bands were recorded as 14.3, 19.0, 29.0, 38.0, 48.6, and 68.0 KD; and 14.3, 19.0, 29.0, 38.0, 54.0 and 72.0.0 at 10 % PEG-6000, 1% of NaCl, respectively (Fig. 4, lane 7 and 8).

SDS-PAGE protein patterns

Alterations in protein patterns in tissues of date palm cv. Barhee were analyzed in laboratory culture conditions to detect any possible changes in gene expression in cultures implanted under DS conditions (PEG-6000 + NaCl) in the absence or addition of TRIA compared with non-stressed (control treatment), by applying SDS-PAGE.

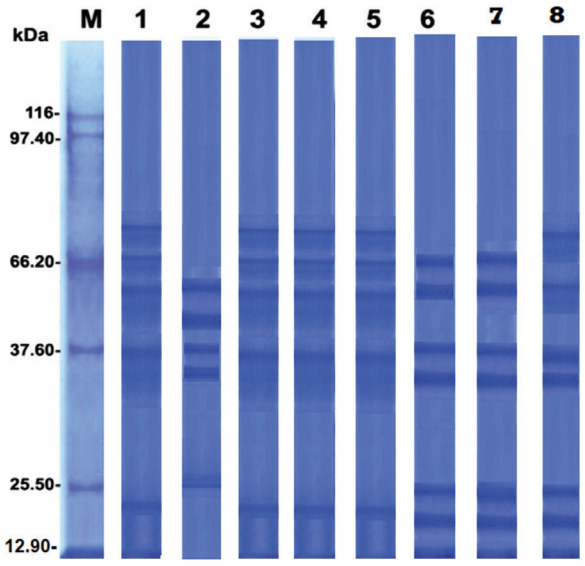
Some protein types appear as a result of PEG-6000+NaCl (DS), while others decrease in concentration or disappear compared with the control treatment. Stress treatments induced new bands with molecular weights of 19, 29, 38.0, 40.0, and 48.6 kD in cultures under DS (Fig. 4, lane 2). Analysis of protein patterns also shows that in non-stressed cultures and implanted in culture media equipped with TRIA, protein bands were not altered, compared to the control treatment, while in stressed cultures use of TRIA contributed to the induction of new protein bands and the disappearance of other protein bands. Stressed cultures (PEG-6000 + NaCl) and those treated with 10 and 20 µg l^{-1} TRIA showed the greatest improvement in protein synthesis, compared to other treatments. In cultures treated with 10 and 20 µg l^{-1} TRIA bands were recorded as 14.3, 19.0, 29.0, 38.0, 48.6, and 68.0 KD; and 14.3, 19.0, 29.0, 38.0, 54.0 and 72.0.0 at 10 % PEG-6000, 1% of NaCl, respectively (Fig. 4, lane 7 and 8).

Discussion

Environmental extremes are known to cause various abiotic

Table 4. Effect of triacontanol (TRIA) on the chlorophylls (a, b) and total chlorophyll content in date palm cv. Barhee under differenct combined dorught and salt stresses (DS). ± Standard error (n = 9). Values followed by the same letter are not significantly different at $P < 0.05$

Treatments	Chl a	Chl b	Chl t
T1 0% PEG-6000, 0% of NaCl, no TRIA (control)	0.748 f ± 0.04	0.248 e ± 0.030	1.032 f ± 0.05
T2 10% PEG-6000, 1% of NaCl, no TRIA	0.585 g ± 0.075	0.195 f ± 0.020	0.780 g ± 0.03
T3 0% PEG-6000, 0% of NaCl, with 5 µg l^{-1} TRIA	0.917 e ± 0.065	0.271 cd ± 0.03	1.188 e ± 0.07
T4 0% PEG-6000, 0% of NaCl, with 10 µg l^{-1} TRIA	1.395 a ± 0.065	0.330 a ± 0.020	1.725 a ± 0.080
T5 0% PEG-6000, 0% of NaCl, with 20 µg l^{-1} TRIA	1.267 b ± 0.035	0.308 ab ± 0.03	1.575 b ± 0.023
T6 10% PEG-6000, 1% of NaCl, with 5 µg l^{-1} TRIA–	0.741 f ± 0.030	0.250 de ± 0.01	0.991 f ± 0.040
T7 10% PEG-6000, 1% of NaCl, with 10 µg l^{-1} TRIA	1.205 c ± 0.070	0.290 bc ± 0.20	1.495 c ± 0.05
T8 10% PEG-6000, 1% of NaCl, with 20 µg l^{-1} TRIA	1.068 d ± 0.085	0.280 c ± 0.070	1.348 d ± 0.100



M	Con.1	2	3	4	5	6	7
116	76.0	48.6	76.0	76.0	76.0	66.0	68.0
97.40	66.0	40.0	66.0	66.0	66.0	42.37	48.0
66.20	42.37	38.0	42.37	42.37	42.37	38.0	38.0
37.60	21.0	29.0	21.0	21.0	21.0	29.0	29.0
25.5		19.0				19.0	19.0
12.9						14.3	14.3

Fig. 4. SDS-PAGE of protein extracted from regenerated shoots of *Phoenix dactylifera* L. cv. Barhee, treated with PEG6000 + NaCl, TRIA as shown in Table 1.

stresses (particularly drought and salinity) on plant fitness and performance (Kaushal and Wani, 2016). *In vitro* selection carried out under controlled environment conditions in confined spaces is highly effective. Micropropagation of date palms requires improving protocols and adaptation to specific cultivar requirements. Our study showed that triacontanol (TRIA) treatment has beneficial effects on date palm cultures propagated *in vitro*. Moreover, TRIA contributes to alleviating the negative effects caused by DS. TRIA is known to have growth-promoting activities in plants (Malabadi et al., 2005). TRIA also regulates the growth and development of plants under normal and stressful conditions (Verma et al., 2009; Parimalan et al., 2009). Among the different treatments tested, 10 µg l^{-1} TRIA was found most effective in increasing the callus fresh weight (294 and 215 mg jar^{-1}), percentage of bud production (88.87% and 72.23%), and the number of shoots (13.50, and 10.30), which were higher as compared with under non-stress and stress treatments, respectively (Figs 1 and 2). This proves that TRIA improves callus growth and shoot regeneration. TRIA is one of the plant growth regulators that plays an important role in plant growth and development (Obulreddy et al., 2002; Verma et al., 2022). TRIA plays a vital role in alleviating stress-induced changes in plants by modulating the activation of stress resistance mechanisms. (Khan et al., 2020; Verma et al., 2022). TRIA has been shown to play an essential role in the defensive response to stresses in many plant species (Shahbaz et al., 2013; Perveen et al., 2016; Weremczuk-Jezyna et al., 2022). The positive effects of TRIA might be attributed to its role in enhancing mineral nutrient uptake, cell division, and the permeability of the membrane (Chen et al., 2003; Perveen et al., 2012a, b). Previous studies on TRIA application on different plants showed higher accumulation of nutrients by excitation of the signaling molecule L (+) adenosine leading to improved plant growth and development (Waqas et al., 2016). Also, TRIA can interact with cytokines and gibberellic acid to regulate growth and metabolic processes in cultures (Segura and Joaquin, 2021). TRIA effectively mitigates the harmful effects of stress and regulates plant growth under stressed and normal conditions (Naeem et al., 2011; Khan et al., 2020). During the experiment under study, TRIA suggested enhancement in growth may be due to the upregulation of genes that are related to the photosynthetic (Chen et al., 2003), and improvement in the activities of antioxidant enzymes (Perveen et al., 2012a, 2014). In addition, TRIA also enhances nutrient absorption and uptake under stress conditions (Krishnan and Kumari, 2008). Mineral contents may stimulate plant growth and development. The DS affects the physiological and biochemical processes of plants, especially the synthesis and accumulation of secondary metabolites. During DS, the accumulation of many solutes, such as total soluble carbohydrates, proteins, and free amino acids, including proline, which is one of the most widely compatible solutes that accumulate in plants during adverse environmental restrictions and plays an essential role in plant stress tolerance (Ghaderi et al., 2018).

To ensure relative stability of osmotic potential under DS, our results revealed increased values of osmoregulation compounds such as free proline, soluble total carbohydrates, and total soluble proteins, with the application of TRIA. Proline is an important osmotic regulatory substance, which can contribute to reducing the osmotic potential and maintaining turgor pressure. Proline is the biochemical parameter most predictive of stress among the traits studied. Besides being an excellent osmolyte, proline plays many vital roles during stress. In addition, proline contributes to many functions, such as scavenging reactive oxygen species (ROS) and protecting the plasma membrane, etc. (Molinari et al., 2007; Tuteja, 2007; Gupta and Huang, 2014). Our results were consistent with previous studies on the positive effect of TRIA on enhancing proline accumulation under stress conditions in the studied plants (Perveen et al., 2016; Khan et al., 2020). Carbohydrate accumulation in response to various abiotic stressors has been reported to have potential roles in stress adaptation. Total protein values decreased significantly with increasing DS. Furthermore, a lower protein content than the control may indicate a higher catabolic than anabolic process. Plants activate some internal mechanisms to prevent stress. Some of them are the synthesis of protective molecules, including the synthesis of some stress-induced proteins (Büyük et al., 2012). Under stress, TRIA has been observed to enhance mineral nutrient uptake, increase the activities of antioxidant enzymes, and promote the synthesis of various organic substances (Naeem et al., 2009; Gupta and Huang, 2014; Aziz and Shahbaz, 2015; Karam and Keramat, 2017; Zaid et al., 2019). TRIA, like other plant hormones, may activate enzymes or alter the membrane, leading to increased metabolism and accumulation of various important intermediate substances.

Our results suggested an increase in MDA and H_2O_2 values in cultures exposed to DS. These increases in MDA and H_2O_2 in leaves reflect oxidative damage to membrane lipids and other biomaterials. MDA and H_2O_2 as indicators representing the severity of stress (Al-Mayahi et al., 2020). In contrast, MDA and H_2O_2 content were significantly reduced by TRIA addition to date palm cultures media that were suffering from osmotic stress. Our results also showed that antioxidant enzymes responded to DS stress. To cope with oxidative stress, it was found that treatment with TRIA enhanced the activities of SOD, and APX in date palm plantations. Hence, TRIA reduces H_2O_2 and MDA content via antioxidant enzymatic activity in *P. dactylifera*, confirming that TRIA alleviates DS via activation of antioxidant enzymatic activity. Membrane stability is an important property required for the functional survival of plants under abiotic stress because damage to it will lead to cell death (Shah et al., 2020; Koleva et al., 2022). Among all types of ROS, H_2O_2 is a key player in stress signal transduction pathways related to tolerance to various stresses (Guler and Pehlivan, 2016). Furthermore, H_2O_2 directly regulates the expression of many genes involved in plant defense and related pathways such as antioxidant enzymes, defense proteins, and transcription factors (Huang and Guo, 2005). Thus, TRIA reduced the harmful effects on the treated date palm tissues. The high nega-

tive correlation values between H_2O_2 and MDA with SOD, APX, and PAL in the current study under both normal and DS conditions, confirm the importance of antioxidant enzymatic protection in ROS detoxification. Perveen et al. (2016) showed that plant cells produce different reactive oxygen species (ROS) in response to exposure to stress. Many studies have shown that TRIA enhances enzymatic antioxidant activity in plants exposed to drought or salt stresses (Perveen et al.. 2016; Shahbaz et al., 2013; Islam et al., 2020). Therefore, we hypothesized that TRIA utilizes enzymatic antioxidant mechanisms in addition to PAL that mitigate oxidative stress and plant growth under DS stress. SOD is the first enzyme in detoxification processes, catalyzes the dismutation of O_2 to H_2O_2 and O_2. SOD and APX can eliminate H_2O_2 via different mechanisms (Gill and Tuteja, 2010). Similarly, Grzegorczyk et al. (2006) reported that TRIA treatment increased the antioxidant activities of *S. officinal*. Stimulation of the antioxidant defense system is one of the responses of plants to combat drought and salinity stress. Phenylalanine ammonia-lyase (PAL) is an inducible enzyme that responds to various stresses. Regarding the activity of PAL, a key enzyme involved in the biosynthesis of anti-oxidative compounds (Gholizadeh, 2011; Al-Mayahi et al., 2020), our results indicate the importance of PAL in date palm plant defense response to DS. A positive correlation can be observed in PAL activity and accumulation of TSPCs (total soluble carbohydrates), indicating increased content of TSPCs due to increased PAL activity. In the current study, the DS increased the Na^+ (sodium), and Ca^{2+} (calcium) content, but decreased Mg^{2+} (magnesium), K^+ (potassium), and Fe^{2+} (iron) content in tissues cultured. TRIA application reduced the Na^+ value in stressed plants and increased nutrients content of Ca^{2+}, Mg^{2+}, K^+, and Fe^{2+}. TRIA enhances nutrient absorption and uptake under stress conditions, which to some extent reflects the plants' ability to resist stress (Krishnan and Kumari, 2008; Kilic, et al., 2010). The increase in nutrient levels can be attributed to the prominent effect of TRIA in enhancing their absorption (Chen et al., 2003; Perveen et al., 2012b). Increased calcium content can induce some types of proteins (calmodulin) that modulate transcription factors (MYB, GTL, and $CAMTA_3$) and thus lead to the phosphorylation of growth, development- and defense-related genes (Tang et al., 2020). Magnesium and potassium are necessary to activate the different enzymes and metabolic processes in plants (Keramat et al., 2017). Iron plays a vital role in the synthesis of chlorophyll, an essential molecule in photosynthesis during plant growth (Bello-Bello et al., 2017).

The chlorophyll values in date palm cultures exposed to DS were significantly decreased as shown in Table 4. The accumulation of toxic ions resulting from salinity stress and physiological water deficiency in leaves delayed chlorophyll biosynthesis as well as accelerated chlorophyll degradation (Mahboob et al., 2017). The decreased chlorophyll value could be due to the increased activity of chlorophyllase, a chlorophyll-degrading enzyme (Reddy and Vora, 1986). The decrease in chlorophyll content may be associated with increased H_2O_2 levels. TRIA is known to increase chlorophyll content, as in our results, 10 µg l^{-1} TRIA proved to be the most effective in retaining the highest Chl (a, b) and total chl contents (Table 4). TRIA acts as a signaling molecule that induces plants to resist various abiotic stresses (Waqas et al., 2016). The enhanced chlorophyll content due to exposure to TRIA is presumably related to the strength of the stability membrane, which remains intact in response to TRIA under DS conditions. Decreased chlorophyll due to stress is related to increased production of ROS in the cell. These free radicals cause oxidation, disintegration, and reduction of chlorophyll content in plants under stressful conditions. Photosynthesis is the main cause of oxidative stress when plants are exposed to unfavorable conditions. The beneficial effects of TRIA treatments on the stimulation of chlorophyll pigments may be a result of its role in increasing the uptake of water and mineral nutrients (Krishnan and Kumari, 2008; Perveen et al., 2012c), which increases stomatal conductance and thus affects the rate of photosynthesis by stabilizing carbon dioxide (Moutuchilian et al., 2003).

SDS-PAGE analysis showed some novel polypeptide bands in all TRIA-treated plants, representing a defense strategy facing drought and salt stress (DS). Exposure to DS also stimulated the synthesis of several low molecular weight proteins, which contributed to some extent to protecting the implanted tissues from injury caused by DS.

New bands of proteins in stressed cultures treated with TRIA may be due to stimulation of the production of these proteins. Changes in protein induction under DS stress conditions may be due to changes in mRNA translation efficiency or regulation of RNA transcription, which depends on the tissue's need and response to the type of stress it is exposed to ensure control of these stresses. The expression of DS-proteins is associated with the process of adaptation of tissue to DS as well as with the genetic composition of selected DS-resistance genotypes. These induced proteins may have a specific function in protecting date palm tissue cultures from more harmful stresses and are considered a means of defense against stresses. Several stress-induced polypeptides have been shown in several reports and are postulated to play an important role in stress resistance (Jiang and Huang, 2002). These results confirmed the study reported by Hussein et al. (2015), who showed that one of the effective mechanisms contributing to protecting the cell from stresses is the induction of new proteins. In our current study, the promoted expression of a 72-kDa protein at TRIA indicated that TRIA may have stimulated protein synthesis under DS stress conditions. TRIA can promote plant growth and increase tolerance under DS through mechanisms such as the induction of specific stress proteins. Such changed and improved protein expression may be responsible for the growth and development of cultures under high-stress levels. These novel peptides may have vital roles in DS binding and may be low molecular weight proteins produced in cultures in response to abiotic stress (Duressa et al., 2010). On the other hand, the disappearance of some polypeptides in DS-stressed cultures in the absence of TRIA may

be associated with enhanced RNAse hydrolysis activity (Kong-Ngern et al., 2005).

Conclusions

To the best of our knowledge, this study is the first to show the beneficial effects of TRIA on the growth and development of *in vitro*-cultivated date palm plants, with or without drought and salt stress (DS) (PEG-6000 + NaCl) stress. In conclusion, TRIA application was shown to alter the physiological and biochemical dynamics of *P. dactylifera* under DS in date palm cultures under laboratory conditions. Treatment with TRIA reduced the harmful effects of DS stress by reducing membrane injury. Improved DS tolerance may relate to high chlorophyll content besides antioxidant enzyme activity and PAL, the accumulation of solutes, and the appearance of new protein bands. Altogether, our results indicate that the application of 10 μg l^{-1} TRIA was more effective in reducing the negative effect of DS.

Acknowledgements

The author thanks and appreciates the efforts of all the staff at the Date Palm propagation Lab.

References

Al-Asadi, A.Z., Abdulwahid, A.H., Al-Mayahi, A.M.W., 2019. The effect of thidiazuron on call us and in vitro shoots development of date palm (Phoenix dactylifera L.) cv. Barhee. *Basrah Journal of Agricultural Sciences*, 32: 258–265.

Al-Mayahi, A.M.W., 2019. Effect of aluminium on the growth of the in vitro culture tissues of the date palm (Phoenix dactylifera L.) cv. Um-Adelhin. *Folia Oecologica*, 46 (2): 164–169. https://doi.org/10.2478/foecol-2019-0019

Al-Mayahi, A.M.W., 2021. In vitro plant regeneration system for date palm (Phoenix dactylifera L.): effect of chelated iron sources. *Journal of Genetic Engineering and Biotechnology Research*, 19: 83. https://doi.org/10.1186/-021-00177-4

Al-Mayahi, A.M.W., 2022a. The effect of polyamines and silver thiosulphate on micropropagation of date palm followed by genetic stability assessment. *World Journal of Microbiology and Biotechnology*, 38: 124. https://doi.org/10.1007/s11274-022-03305-5

Al-Mayahi, A.M.W., 2022b. The effect of phenyl acetic acid (PPA) on micropropagation of date palm followed by genetic stability assessment. *Journal of Plant Growth Regulator*, 41:3127–3137. https://doi.org/10.21203/rs.3.rs-545347/v1

Al-Mayahi, A.M.W., 2022c. In vitro propagation and assessment of genetic stability in date palm as affected by chitosan and thidiazuron combinations. *Journal of Genetic Engineering and Biotechnology*, 20: 165. https://doi.org/10.1186/s43141-022-00447-9

Al-Mayahi, A.M.W., Ahmed, A.N., Al-Khalifa, A.A., 2010. Isolation and diagnose of fungi associated with the cultivation of tissues of five cultivars of date palm Phoenix dactylifera L.) and the effect of fungicide Benlate in control. *Basrah Journal for Date Palm Research*, 9 (2): 79–97.

Al-Mayahi, A.M.W., Ali, A.H., 2021. Effects of different types of gelling agents on in vitro organogenesis and some physicochemical properties of date palm buds, Showathy cv. *Folia Oecologica*, 48 (1): 110–117. https://doi.org/10.2478/foecol-2021-0012

Al-Mayahi, A.M.W., Jafar, O.N., Mohsen, K.A., 2020. Ef fect of glutathione (GSH) on Date palm (Phoenix dactylifera L.) micropropagation. *Folia Oecologica*, 47 (1): 64–69. https://doi.org/10.2478/foecol-2020-0008

Awad, K.M., Al-Mayahi, A.M.W., Mahdi, M.A., Al-Asadi, A.S.M., Abass, M.H., 2020. In vitro assessment of ZnO nanoparticles on Phoenix dactylifera L. Micropropag. *Scientific Journal of King Faisal University (Basic and Applied Sciences)*, 21 (1): 149–161. https://doi.org/10.37575/b/agr/2000

Aziz, R., Shabbaz, M., 2015. Triacontanol-induced regulation in the key osmoprotectants and oxidative defense system of sunflower plants at various growth stages under salt stress. *International Journal of Agriculture and Biology*, 17: 881–890. DOI:10.17957/IJAB/15.0022

Bates, L.S., Waldren, R.P., Teare, I.D., 1973. Rapid determination of free proline for water-stress studies. *Plant and Soil*, 39: 205–207. https://doi.org/10.1007/BF00018060

Bello, J.J., Chavez-Santoscoy, R.A., Lecona-Guzman., C.A., Bogdanchikova, N., Salinas-Ruz, J., Gomez-Merino, F.C., Pestryakov, A., 2017. Hormetic response by silver nanoparticles on in vitro multiplication of sug arcane (Saccharum spp. Cv. Mex 69-290) using a temporary immersion system. *Dose-Response: An International Journal*, 15 (4): 1–9. https://doi.org/10.1177/1559325817744945

Black, C.A., 1968. Methods of Sei analysis part 1. Physical properties. Am. Soc. Agron. Inc. Publisher, Madison Wisconsin, USA pollen and flowers of five male cultivars of Iraqi date palm (Phoenix dactylifera L.). *Date Palm Journal*, 2 (2):111–207.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 38 (1-2): 248–252. https://doi.org/10.1016/0003-2697(76)90527-3

Büyük I., Soydam-Aydin, S., Aras, S., 2012. Bitkilerin stres kos¸ullarına verdig˘i moleku¨ler cevaplar [Molecular responses of plants to stress conditions]. *Türk Hijyen ve Deneysel Biyoloji Dergis*, 69: 97–110.

Chen, X., Yuan, H., Chen, R., Zhul, H.E.G., 2003. Biochemical and photochemical changes in response to triacontanol in rice (Oryza saliva L.). *Plant Growth Regulation*, 40 (3): 249–256. https://doi.org/10.1023/A:1025039027270

Cresser, M.S., Parsons, J.W., 1979. Sulphuric perchloric acid digestion of plant material for the determination

of nitrogen, phosphours, potassium, calcium and magnesium. *Analytica Chimica Acta*, 109: 43–436. https://doi.org/10.1016/S0003-2670(01)84273-2

Duressa, D., Soliman, K., Chen, D., 2010. Identification of aluminum responsive genes in Altolerant soybean line PI 416937. *International Journal of Genomics*, 2010: article ID 164862, 13 p. https://doi.org/10.1155/2010/164862

Ghaderi, N., Hatami, M.R., Mozafri, A., Siosehmardeh, A., 2018. Change in antioxidant enzymesactivity and some morpho-physiological characteristics of strawberry under long-term salt stress. *Physiology and Molecular Biology of Plants*, 24 (5):833–843. https://doi.org/10.1007/s12298-018-0535-2

Gholizadeh, A., 2011. Effects of drought on the activity of phenylalanine ammonia lyase in the leaves and roots of maize inbreeds. *Australian Journal of Basic and Applied Sciences*, 5 (9): 952–956.

Gill, S.S., Tuteja, N., 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiology and Biochemistry,* 48: 909–930.

Goharrizi, K.J., Hamblin, M. R, Soraya, Karami, S., Nazaro, M., 2021. Histological, biochemical, and metabolic responses of abiotic plant stress: salinity and drought. *Turkish Journal of Botany*, 45: 623–642.

Grzegorczyk, I., Bilichowski, I., Mikiciuk-Olasik, E., Wysokinska, H., 2006. The effect oftriacontanol on shoot multiplication and production of antioxidant compounds in shoot cultures of Salvia officinalis L. *Acta Societatis Botanicorum Poloniae*, 75 (1): 11–15. https://doi.org/10.5586/asbp.2006.002

Guler, N.S., Pehlivan, N., 2016. Exogenous low-dose hydrogen peroxide enhances drought tolerance of soybean (Glycine Max L.) through inducing antioxidant system. *Acta Biologica Hungarica*, 67 (2): 169–183.

Gupta, B., Huang, B., 2014. Mechanism of salinity tolerance in plants: physiological, biochemical, and molecular characterization. *International Journal of Genomics*, 2014: article ID 701596, 18 p. https://doi.org/10.1155/2014/701596

Gupta, R., Wall, T., Baxter, L., 2007. *Impact of mineral impurities in solid fuel combustion.* New York: Springer.

Heath, R.L., Packer, L., 1969. Photoperoxidation in isolated chloroplasts. I Kinetics and stoichiometry of fatty acid peroxidation. *Archives of Biochemistry and Biophysics,* 125: 189–198. https://doi.org/10.1016/0003-9861(68)90654-1

Huang, M., Guo, Z., 2005. Responses of antioxidative system to chilling stress in two rice cultivars differing in sensitivity. *Biologia Plantarum*, 49: 81–84. https://doi.org/10.1007/s00000-005-1084-3

Hussein, Y., Amin, G., Azab, A., Gahin, H., 2015. Induction of drought stress resistance in sesame (sesamum indicum L.) plant by salicylic acid and kinetin. *Journal of Plant Sciences*, 10 (4): 128–141. DOI: 10.3923/jps.2015.128.141

.Islam, S., Mohammed, F., 2020. Triacontanol as a dynamic growth regulator for plants under diverse environmental conditions. *Physiology and Molecular Biology of Plants*, 26(5): 871–883. https://doi.org/10.1007/s12298-020-00815-0

Islam, S., Zaid, A. Mohammed, F., 2020. Role of triacontanol in counteracting the ill effects of salinity in plants: a review. *Journal of Plant Growth Regulation*, 40: 1–10. https://doi.org/10.1007/s00344-020-10064-w

Jasim, A.M, Al-Mayahi, A.M.W., Attaha, A.H.M., 2009. Propagation of four rare cultivars of date palm (Phoenix dactylifera L.) by tissue culture techniques. *Basrah Journal for Date Palm Research*, 8 (1): 72–99.

Jiang, Y., Huang, B., 2002. Drought and heat stress injury to two cool-season turf grasses in relation toantioxidant metabolism and lipid peroxidation. *Crop Science*, 4: 436–442. https://doi.org/10.2135/cropsci2001.412436x

Karam, E.A., Keramat, B., 2017. Foliar spray of triacontanol improves growth by alleviating oxidative damage in coriander under salinity. *Indian Journal of Plant Physiology*, 22: 120–124. https://doi.org/10.1007/s40502-017-0286-z

Kaushal, M., Wani, S.P., 2016. Rhizobacterial-plant interactions: strategies ensuring plant growth promotion under drought and salinity stress. *Agriculture, Ecosystems & Environment,* 231: 68–78. http://dx.doi.org/10.1016/j.agee.2016.06.031

Kawakami, S., Mizuno, M., Tsuchida, 2000. Comparison of antioxidant enzyme activities between Solanum tuberosum L. cultivar danshaku and kitaakari during low-temperature storage. *Journal of Agricultural and Food Chemistry*, 48: 2117–2121. https://doi.org/10.1021/jf9910279

Keramat, B., Sorbo, S., Maresca, V., Asrar, Z., Mozafari, H., Basile, A., 2017. Interaction of triacontanol and arsenic on the ascorbate-glutathione cycle and their effects on the ultrastructure in Coriandrum sativum L. *Environmental and Experimental Botany*, 141: 161–169. https://doi.org/10.1016/j.envexpbot.2017.07.012Get rights and content

Keskin, B. C., Sarikaya, A.T., Yuksel, B., Memon, A.R., 2010. Abscisic acid regulated gene expression in bread wheat (Triticum aestivum L.). *Australian Journal of Crop Science*, 4: 617–625.

Khan, A., Hayat, F., Nawaz, H., Javed, S., Zafar, S., 2020. Application of triacontanol to increase salinity tolerance in sunflower (Helianthus annuus L.). *Journal Plantarum*, 2 (1)-05: 24–32.

Kilic, N.K., Duygu, E. Donmez, G., 2010. Triacontanol hormone stimulates population, growth and Brilliant Blue R dye removal by common duckweed from culture media. *Journal of Hazardous Materials*, 182: 525–530. https://doi.org/10.1016/j.jhazmat.2010.06.063

Koleva, L. Umar, A., Yasin, N.A., Shah, A.A., Siddiqui, M.H., Alamri, S., Riaz, L., Raza, A., Javed, T., Shabbir, Z., 2022. Iron oxide and silicon nanoparticles modulate mineral nutrient homeostasis and metabolism in cadmium-stressed Phaseolus vulgaris. *Frontiers in Plant Science*, 13: 806781. https://doi.org/10.3389/fpls.2022.806781

Kong-Ngern, K., Daduang, S., Wongkham, C.H., Bunnag, S., Kosittrakuna, M., Theerakulpisata, P., 2005. Protein profiles in response to salt stress in sheaths of

rice seedlings. *Science Asia*, 31: 403–408. DOI: 10.2306/scienceasia1513-1874.2005.31.403

Krishnan, R.R., Kumari, B.D., 2008. Effect of N-triacontanol on the growth of salt stressed soybean plants. *Journal of Biosciences*, 19: 53–62.

Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head bacteriophage T4. *Nature*, 227: 680–685. https://doi.org/10.1038/227680a0

Ma, Y., Dias, M.C., Freitas, H., 2020. Drought and salinity stress responses and microbe-induced tolerance in plants. *Frontiers in Plant Science*, 11: 591911. https://doi.org/10.3389/fpls.2020.591911

Mahboob, W., Khan, M.A., Shirazi, M.U., 2017. Characterization of salt tolerant wheat (Triticum aestivum) genotypes on the basis of physiological attributes. *International Journal of Agriculture and Biology,* 19: 726–734. DOI: 10.17957/IJAB/15.0347

Malabadi, R.B., Mulgund, G.S., Nataraja, K., 2005. Effect of triacontanol on the micropropagation of costus speciosus (Koen.) Sm. using rhizome thin. *In Vitro Cellular & Developmental Biology - Plant*. 41: 129–132. https://doi.org/10.1079/IVP2004586

Molinari, H.B.C., Marur, C.J., Daros, E., Campos, M.K.F., Carvalho, J.F.R., Bespalhok Filho, J.C., Vieira, L.G.E., 2007. Evaluation of the stress-inducible production of proline in transgenic sugarcane (Saccharum spp.) osmotic adjustment, chlorophyll fluorescence and oxidative stress. *Physiologia Plantarum*, 130: 218–229. https://doi.org/10.1111/j.1399-3054.2007.00909.x

Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bioassay of tobacco tissue cultures. *Physiologia Plantarum*, 18: 100–127. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x

Muthuchelian, K., Velelayutham, M., Nedunchezhian, N., 2003. Ameliorating effect of triacontanol on acidic mist-treated Erythrina variegata seedlings changes in growth and photosynthetic activities. *Plant Science*, 165 (6): 1253–1259.

Naeem, M., Khan, M.M., Moinuddin, M.M.A., 2009. Triacontanol: a potent plant growth regulator in agriculture. *Journal of Plant Interactions,* 7: 129–142. https://doi.org/10.1080/17429145.2011.619281

Naeem, M., Khan, M.M., Moinuddin, M.M.A., Idrees, M., Aftab, T., 2011. Triacontanol-mediated regulation of growth and other physiological attributes, active constituents and yield of Mentha arvensis L. *Plant Growth Regulation*, 65: 195–206. https://doi.org/10.1007/s10725-011-9588-8

Nakano, Y., Asada, K., 1981. Hydrogen peroxide is scavenged by ascorbate-specificperoxidase in Spinach chloroplasts. *Plant and Cell Physiology*, 22: 867–880.

Obulreddy, B.O., Giridhar, P., Ravishankar, G.A., 2002. The effect of triacontanol on micropropagation of Capsicum frutescens and Decalepishamiltonii W & A. *Plant Cell, Tissue and Organ Culture*, 71 (3): 258. https://doi.org/10.1023/A:1020342127386

Parimalan, R., Giridhar, P, Gururaj, H.B., Ravishankar, GA., 2009. Micropropagation of Bixa orellana using phytohormones and triacontanol. *Biologia Plantarum*, 53 (2): 347–350. https://doi.org/10.1007/s10535-009-0064-5

Perveen, S., Iqbal, M., Attia Nawaz, A., Perveen, A., Mahmood, S., 2016. Induction of drought tolerance in Zea mays L. by foliar application of triacontanol. *Pakistan Journal of Botany,* 48 (3): 907–915.

Perveen, S., Shahbaz, M., Ashraf, M., 2012a. Is pre-sowing seed treatment with triacontanol effective in improving some physiological and biochemical attributes of wheat (Triticum aestivum L.) under salt stress? *Journal of Applied Botany and Food Quality*, 85: 41–48.

Perveen, S., Shahbaz, M., Ashraf, M., 2012b. Regulation in gas exchange and quantum yield of photosystem II (PSII) in salt-stressed and non-stressed wheat plants raised from seed treated with triacontanol. *Pakistan Journal of Botany,* 42 (5): 3081.

Perveen, S., Shahbaz, M., Ashraf, M., 2012c. Changes in mineral composition, uptake and use efficiency of salt stressed wheat (Triticum aestivum l.) plants raised from seed treated with triacontanol. *Pakistan Journal of Botany*, 44: 27–35.

Perveen, S., Shahbaz, M., Ashraf, M., 2014. Triacontanol-induced changes in growth, yield, leaf water relations, antioxidative defense system and some key osmoprotectants in bread wheat (Triticum aestivum L.) under saline stress. *Turkish Journal of Botany*. 38: 896–913. https://doi.org/10.3906/bot-1401-19

Porra, R.J., 2002. The chequered history of the development and use of stimulation quantions for the accurate determination of chlorophylls A and B. *Photosynthesis Research*, 73: 149–156.

Rasool, N., 2022. Plant hormones: role in alleviating biotic stress. In *Plant hormones: recent advances, new perspectives and applications*. London: IntechOpen. 25 p. DOI: 10.5772/intechopen.102689

Reddy, M.P. Vora, A.B., 1986. Salinity induced changes in pigment composition and chlorophyllase activity in wheat. *Indian Journal of Plant Physiology*, 29: 331–334.

Segura, H.C., Joaquin, A.H., 2021. Interaction of triacontanol with other plant growth regulators on morphology and yield of field pea (Pisum sativum L.). *Agronomía Colombiana*, 39 (2): 187–195. https://doi.org/10.15446/agron.colomb.v39n2.94096

Sergiev, I., Alexieva, V., Karanov, E.V., 1997. Effect of spermine, atrazine and combination between them on some endogenous protective systems and stress markers in plants. *Comptes Rendus de l'Academie Bulgare des Sciences*, 51: 121–124.

Shah, A. A., Khan, W. U., Yasin, N. A., Akram, W., Ahmed, A., Abbas, M., 2020. Butanolide alleviated cadmium stress by improving plant growth, photosynthetic parameters and antioxidant defense system of Brassica oleracea. *Chemosphere*, 261:127728. https://doi.org/10.1016/j.chemosphere.2020.127728

Shahbaz, M., Noreen, N., Perveen, S., 2013. Triacontanol modulates photosynthesis osmoprotectants in canola (Brassica napus L.) under saline stress. *Journal of Plant Interactions*, 8 (4): 350–359. https://doi.org/10.1080/17429145.2013.764469

Syklowska-Baranek, K., Pietrosiuk, A., Naliwajski, M.R., Kawiak, A., Jeziorek, M., Wyderska, S., Łojkowska, E., Chinou, I., 2012. Effect of l-phenylalanine on PAL activity and production of naphthoquinone pigments in suspension cultures of Arnebia euchroma (Royle) Johnst. *In Vitro Cellular & Developmental Biology - Plant*, 48 (5): 555–564. https://doi.org/10.1007/s11627-012-9443-2

Tang, R.J., Wang, C., Li, K., Luan, S., 2020. The CBL–CIPK calcium signaling network: unified paradigm from 20 years of discoveries. *Trends in Plant Science*, 25: 604–617. https://doi.org/10.1016/j.tplants.2020.01.009

Tutea, N., 2007. Mechanisms of high salinity tolerance in plants. *Methods in Enzymology*, 428: 419–438. https://doi.org/10.1016/S0076-6879(07)28024-3

Verma, A., Malik, C.P., Gupta, V.K., Sinsinwar, Y.K., 2009. Response of groundnut varieties to plant growth regulator (BAP) to induce direct organogenesis. *World Journal of Agricultural Sciences*, 5 (3): 313–317.

Verma, T., Bhardwaj, S., Singh, J., Kapoor, D., Prasad, R., 2022. Triacontanol as a versatile plant growth regulator in overcoming negative effects of salt stress. *Journal of Agriculture and Food Research*, 10 (6): 88–91. https://doi.org/10.1016/j.jafr.2022.100351

Waqas, M., Shahzad, R., Khan, A.L., Asaf, S., Kim, Y.H., Kang, S.M., Bilal, S., Hamayun, M., Lee, I.J., 2016. Salvaging effect of triacontanol on plant growth, thermotolerance, macro-nutrient content, amino acid concentration and modulation of defense hormonal levels under heat stress. *Plant Physiology and Biochemistry*. 99: 118–125. https://doi.org/10.1016/j.plaphy.2015.12.012

Weremczuk-Jezyna, I., Hnatuszko-Konka, K., Lebelt, L., Piotrowska, D.G., Grzegorczyk-Karolak, I., 2022. The effect of the stress-signalling mediator Triacontanol on biochemical and physiological modifications in Dracocephalum forrestii culture. *International Journal of Molecular Sciences*, 23: 15147.

Watanabe, S., Kojima, K., Ide, Y., Sasakii, S., 2000. Effect of saline and osmotic stress on proline and sugar accumulation in Populus euphratica in vitro. *Plant Cell, Tissue and Organ Culture*, 63: 199–206. https://doi.org/10.1023/A:1010619503680

Zaid, A., Mohammad, F., Fariduddin, Q., 2019. Plant growth regulators improve growth, photosynthesis, mineral nutrient and antioxidant system under cadmium stress in menthol mint (Mentha arvensis L.). *Physiology and Molecular Biology of Plants*, 26: 25–39. https://doi.org/10.1007/s12298-019-00715-y

Received November 10, 2023
Accepted June 12, 2024