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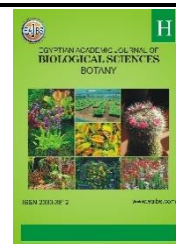
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Triacontanol Foliar Spray Alleviated Drought Stress Effects by Maintaining Photosynthesis and Cellular Redox Balance in Sunflower Seedlings

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ABSTRACT

Drought is one of the most dangerous threats to cultivated lands and agriculture in Egypt. Exploring strategies to enhance crop tolerance to drought is therefore imperative. This study aimed to examine whether triacontanol (TRIA) can mitigate the drought harmful effects on sunflower seedlings. Eleven-day-old sunflower (cv. Sakha 53) seedlings were exposed to two watering regimes: (1) well-watered and (2) drought-stressed for 5 days. Two concentrations of TRIA (10 and 25 μM) were applied as a foliar spray to drought-stressed seedlings. The effect of TRIA on growth attributes, physiology, and biochemistry of 16-day-old sunflower seedlings in the absence and presence of drought stress was evaluated. TRIA (25 μM) was relatively efficient in alleviating drought injurious effects on the growth of sunflower seedlings. TRIA also counteracted the drought-induced photosynthetic impairment by increasing the level of the photosynthetic pigments, the photosynthetic performance index (PI_{abs}), the photochemical efficiency of photosystem II (F_v/F_m), and the expression of the Rubisco small subunit (*RBCS*) gene. Also, TRIA treatment increased total soluble sugars (TSS), proline, antioxidant defense system (measured by DPPH scavenging capacity), ascorbic acid (ASA), total phenolics, enzyme activities of peroxidase (PX) and polyphenol oxidase (PPO), while decreased contents of abscisic acid (ABA) and malondialdehyde (MDA) as well as the activities of superoxide dismutase (SOD) and ascorbate oxidase (AO; particularly 10 μM) of drought-stressed seedlings. It is evident that TRIA is a potential candidate to enhance sunflower seedling tolerance to drought stress by maintaining photosynthetic activities and enhancing antioxidant defense systems.

INTRODUCTION

Water scarcity and demand for both fresh water and agriculture have overgrown in the last decades, as more than two-thirds of the fresh water in the world are consumed in agriculture (FAO, 2017). About 96% of Egypt's are deserts, leaving only 4% of cultivated lands that are threatened by urbanism and stresses especially drought (Gad, 2020). The frequency and severity of drought events, especially in the Mediterranean basin, have been predicted to increase shortly (Tramblay *et al.*, 2020). Drought has great impacts on the growth, physiology, and biochemistry of several plant species (Jafari *et al.*, 2019), and its effects are expected to exaggerate as global temperature increases. It has been reported that drought stress can directly cause a wide range of injury symptoms in plants, such as limited

water uptake, impeding photosynthesis, disrupted plasma membranes and cell compartments, and ultimately decreased growth and productivity (Talbi *et al.*, 2020).

For decades, extensive attempts to improve plant tolerance to drought stress have been conducted through the regulation of numerous genes and metabolic pathways involved in enhancing photosynthetic efficiency, osmolyte accumulation, and plant antioxidant defense system as well as modulating ABA level (Van Oosten *et al.*, 2016). Therefore, considerable efforts in crop management practices are being encouraged to overcome water deficit stress and enhance tolerance, such as the application of various types of exogenous chemicals that can mitigate stress deleterious effects. Triacontanol (TRIA) is one of these chemicals that have shown promising results in improving plant tolerance to various abiotic stresses such as chilling, drought, heavy metals, and salt stress in different crop species (Islam and Mohammad, 2020). TRIA is a saturated primary alcohol that exists in epicuticular waxes of different plants and has been considered as a potential plant growth regulator promoting plant growth and development (Islam and Mohammad, 2020). Exogenous application of TRIA to stressed plants was reported to enhance biomass, photosynthetic pigments, quantum efficiency, osmolyte accumulation, antioxidant defense systems, and to regulate the gene expression in a variety of plants (Younis and Ismail, 2019; Islam *et al.*, 2020). Nevertheless, the exact mechanisms by which TRIA delivers these responses are not completely revealed.

Sunflower (*Helianthus annuus* L.) is an economically important oilseed crop. It is generally planted for seed and oil production purposes which ranks the fourth largest source of edible oil after soybean, rapeseed and peanut (Wildermuth *et al.*, 2016). It is also used as a high-quality forage by livestock producers (Konca *et al.*, 2016). Though sunflower generally has been considered as a sensitive plant to abiotic stresses (Tyagi *et al.*, 2018), cultivar Sakha 53 was found to show a mild tolerance to drought and salinity (Ebeed *et al.*, 2019). One strategy to overcome drought stress's harmful impacts on a sunflower is by exploring the influence of TRIA, as an exogenous alleviator of drought, on its critical initial stages of development. Therefore, this study aims to investigate the role of TRIA foliar spray in alleviating the deleterious effects of drought on the growth, physiological and biochemical characteristics, and gene expression of sunflower seedlings.

MATERIALS AND METHODS

Plant Growth Conditions and Stress Imposition:

Sunflower (*Helianthus annuus* L.) seeds (cv. Sakha 53) were obtained from the Agricultural Research Center, Giza, Egypt, and kept in the dark at 4 °C. The seeds were surface sterilized by immersion in 1% (w/v) sodium hypochlorite solution for 10 min and then rinsed thoroughly with distilled water. The seeds were then sown in loamy soil (sand: clay, 1: 2) in plastic pots (diameter 15 cm, height 30 cm) containing 1.5 Kg dry soil and 75±5% water-holding capacity (WHC) per pot and kept under natural environmental conditions: 14-h photoperiod, light/dark temperature 27/15 °C, and 60-70% relative humidity. The seedlings were randomly grown under two watering regimes: (1) well-watered (75±5% WHC) and (2) short-term drought-stressed regime treatment at the two-leaf stage (11-day-old seedlings) for 5 days (40±5% WHC). Soil moisture was measured daily to achieve the target water content. Two concentrations of TRIA (Triplntanol.com, USA) at 10 and 25 µM were selected based on a trial experiment that exhibited the best results on sunflower germination and growth. TRIA was applied twice as foliar spray treatment at the two-leaf stages (11-day-old and 13-day-old seedlings). The treatments included: 1, well-water (control); 2, drought stress (Dr); 3, drought stress combined with 10 µM TRIA (Dr + TRIA 10); 4, drought stress combined with 25 µM TRIA (Dr + TRIA 25). Three independent

biological replicates were conducted per treatment (10 plants per treatment) under the same experimental conditions. When seedlings were 16 days old, the photosynthetic performance as (PI_{abs}) and (F_v/F_m) were measured of the dark-adapted youngest fully expanded leaf using a pulse amplitude modulation portable fluorometer (Handy PEA, Hansatech, Norfolk, UK) as described by Maxwell and Johnson (2000). Next, 16-day-old seedlings were harvested and three plants per treatment were subjected for measuring growth criteria (shoot and root lengths, fresh and dry weights, leaf area and number, root/shoot ratio). The remaining seedlings were immediately uprooted, separated, frozen in liquid nitrogen, and stored at -80°C for other analyses.

Photosynthetic Pigments Determination:

Chlorophyll a (Chl a), chlorophyll b (Chl b), and carotenoids (Car) were extracted in 80% (v/v) acetone and measured by UV spectrophotometer UNICAM Helios α (Unicam, Cambridge, UK) according to Metzner *et al.* (1965). The pigment concentration was calculated and expressed in $\mu\text{g g}^{-1}$ DW, then total pigment contents and Chl a/b ratio were calculated.

RNA Extraction and Quantitative Real-Time PCR (qRT-PCR):

Rubisco small subunit *RBCS* gene sequence was previously searched in National Center for Biotechnology Information (NCBI) to design gene-specific primers for qRT-PCR. Primer sequences for the reactions were *HARBCS1*; Y00431 FP: 5'-CATTGACACGTGGCTCTCC -3'; RP: 5'-AGGATGTTGTGGCTCTTGA -3' and for AAF82805 (action sequence from sunflower) FP: 5'-AGGGCGGTCTTTCCAAGTAT -3'; RP: 5'-ACATACATGGCGGGAACATT -3'. Total RNA was extracted from 30 mg of fresh leaves of each treatment using Gene JETTM RNA purification Kit (Thermo Fisher Scientific, MA, USA). One μg of total RNA was reverse transcribed into complementary DNA using Revert Aid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, MA, USA). PCR amplification specificity was verified using melting curve analysis and data were analyzed using the $2^{-\Delta\Delta C_t}$ method (Livak and Schmittgen, 2001) after normalized to the expression of each of Actin gene.

Determination of Soluble Sugars and Proline Contents:

Total soluble sugars and free proline were determined by UV spectrophotometer UNICAM Helios α (Unicam, Cambridge, UK), according to McCready *et al.* (1950) and Carillo and Gibon (2011), respectively.

ABA Determination:

ABA was extracted using freshly collected samples according to the method described by Almeida Trapp *et al.* (2014). The quantity of ABA was determined using high-performance liquid chromatography (HPLC; instrument E-Chrom Tech, LC 1620, USA). Samples were assayed against ABA as internal standards.

Lipid Peroxidation Analysis:

Lipid peroxidation was evaluated by measuring the production of malondialdehyde (MDA) using the thiobarbituric acid (TBA)-based colorimetric method based on Heath and Packer (1968).

DPPH Radical Scavenging Assay:

The activity of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging was determined using the method of Hatano *et al.* (1988). The radical-scavenging activity was calculated as a percentage according to the equation:

$$\text{DPPH radical-scavenging (\%)} = [(\text{absorbance of the control} - \text{the absorbance of the sample}) / \text{absorbance of the control}] \times 100.$$

Enzymatic and Non-Enzymatic Antioxidants:

The leaves were randomly selected from each replication, and 0.5 g was ground in liquid nitrogen and extracted with the following optimized extraction media: 100 mM

potassium phosphate buffer (pH 7.8) [0.1 mM EDTA, 1% (w/v) PVP and 0.1% (v/v) Triton X100] for antioxidant enzyme activities. Superoxide dismutase (SOD, EC 1.15.1.1) activity was measured according to Kong and Xu (1999). Polyphenol peroxidase (PX, EC 1.11.1.7) activity was determined based on the method of Shannon *et al.* (1966). Polyphenol oxidase (PPO, EC 1.10.3.1) activity was measured using the method of Trejo-Gonzalez and Soto-Valdez (1991). Catalase (CAT, EC 1.11.1.6) activity was assayed following the method of Aebi (1984). Ascorbate oxidase (AO, EC 1.10.3.3) and peroxidase (APX, EC 1.11.1.11) activities were measured by the methods of Diallinas *et al.* (1997) and Chen and Asada (1989), respectively. Non-enzymatic antioxidants: total phenolics and ascorbic acid were determined according to Makkar *et al.* (1993) and Mukherjee and Choudhuri (1983), respectively.

Statistical Analysis:

Analysis of variance (ANOVA) of the data was performed using SPSS v. 20.0 (SPSS, Chicago, USA) software. Statistical significance of the means was compared by Duncan's test at $P \leq 0.05$.

RESULTS

Effect of TRIA on the Growth Of Drought-Stressed Sunflower Seedlings:

Drought stress significantly reduced shoot and root lengths, area of total leaves of sunflower seedlings (Fig. 1). TRIA alleviated the drought-induced inhibition of the shoot growth, but not that of the root: the increase in shoot height and leaf area was 13.08% and 30.27%, respectively, by 25 μM TRIA relative to only stressed plants.

Effect of TRIA on Photosynthetic Parameters of Drought-Stressed Sunflower Seedlings:

Drought-stressed seedlings exhibited lower Chl a, Chl b, Car, total pigment contents, and Chl a/b ratio than control seedlings (Table 1). TRIA (10 μM) greatly improved the photosynthetic pigment contents, more so with 25 μM TRIA (Table 1). Drought stress inhibited both F_v/F_m and PI_{abs} , which were significantly restored to control level or even higher with 10 or 25 μM TRIA (Table 1). The average expression levels of *HARBCS1* in plants under drought stress were 1.7-fold higher than those in the control plants (Fig. 2). However, the 10 or 25 μM TRIA application remarkably stimulated the expression levels of *HARBCS1* gene in drought-treated plants by about 2.5 and 2.6-fold, respectively (Fig. 2).

Effect of TRIA on Total Soluble Sugars and Proline of Drought-Stressed Sunflower Seedlings:

Total soluble sugar (TSS) and proline contents were not significantly changed in the drought-stressed shoots compared with the control ones (Figs. 3a & b). Stressed plants treated with TRIA, however, accumulated three folds TSS and one-fold proline higher than the control plants, respectively (Figs. 3a & b).

Effect of TRIA on ABA content of drought-stressed sunflower seedlings

The ABA content increased in drought-stressed seedlings relative to the control ones that showed no ABA detected (Fig. 4). TRIA application decreased the ABA content in drought-stressed seedlings, more so with 10 μM TRIA (Fig. 4).

Effect of TRIA on Lipid Peroxidation of Drought-Stressed Sunflower Seedlings:

A significant increase of MDA content was induced by drought stress, but its value significantly decreased with the application of either 10 or 25 μM TRIA; this took place in a concentration-dependent manner (Fig. 5a).

Effect of TRIA on DPPH of Drought-Stressed Sunflower Seedlings:

The highest percentage of DPPH radical scavenging activity was recorded in the control untreated seedlings (87%). Drought treatment significantly reduced DPPH radical

scavenging activity percentage to reach the lowest value (78%). Foliar application of TRIA (10 or 25 μM) to drought-stressed seedlings significantly increased DPPH radical scavenging activity but was still lower than the control value (Fig.5b).

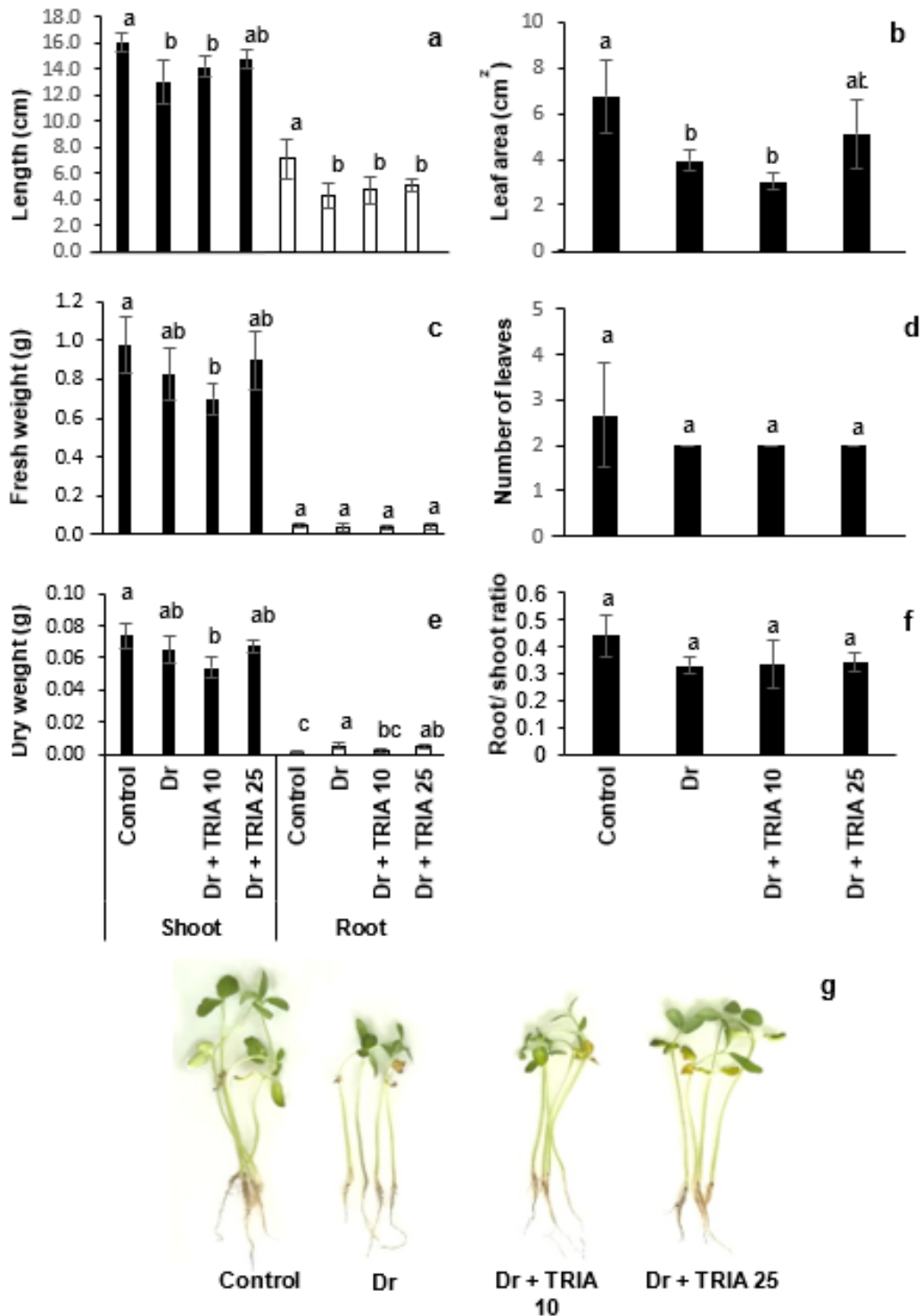


Fig. 1 Effect of drought (Dr) alone or combined with different concentrations of triacontanol (10 and 25 μM) on: (a) shoot and root lengths, (b) total leaves area, (c) shoot and root fresh weights, (d) number of leaves per plant, (e) shoot and roots dry weights, (f) Root/shoot length ratio, and (g) morphological changes, as compared with the control untreated sunflower plants. Each value is the mean \pm SD (n=3), and bars with different letters are significantly different at $P \leq 0.05$.

Table 1 Effect of drought alone (Dr) or combined with 10 or 25 μM TRIA (Dr + TRIA 10, Dr + TRIA 25) on chlorophyll a (Chl a), chlorophyll b (Chl b), carotenoids (Car), and Chl a/b ratio ($\mu\text{g g}^{-1}$ DW), the quantum efficiency of the photochemical reactions in PSII (F_v/F_m) and photosynthetic performance index (PI_{abs}) of leaves of 16-day-old sunflower seedlings.

Treatment	Pigments content (mg g^{-1} DW)					F_v/F_m	PI_{abs}
	Chl A	Chl b	Car	Total pigments	Chl a/b		
Control	3.30	1.69	1.66	6.65	1.95	0.85 ± 0.005 a	5.29 ± 0.66 ab
Dr	1.63	1.18	1.36	4.17	1.38	0.832 ± 0.012 b	4.22 ± 1.10 b
Dr + TRIA 10	3.05	1.55	1.54	6.13	1.96	0.85 ± 0.002 a	6.13 ± 0.92 a
Dr + TRIA 25	4.00	1.98	1.69	7.67	2.02	0.85 ± 0.004 a	5.57 ± 0.24 a

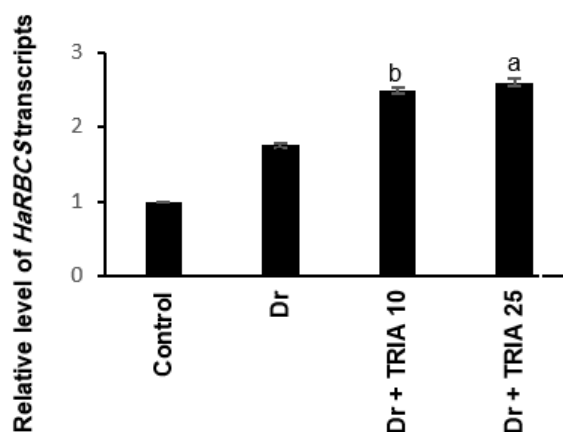


Fig. 2: Effect of drought alone (Dr) or combined with 10 or 25 μM TRIA (Dr + TRIA 10, Dr + TRIA 25) on *HaRBCS* gene expression, as compared with the control untreated plants. Data are means \pm SD ($n=3$), bars with different letters are significantly different at $P \leq 0.05$.

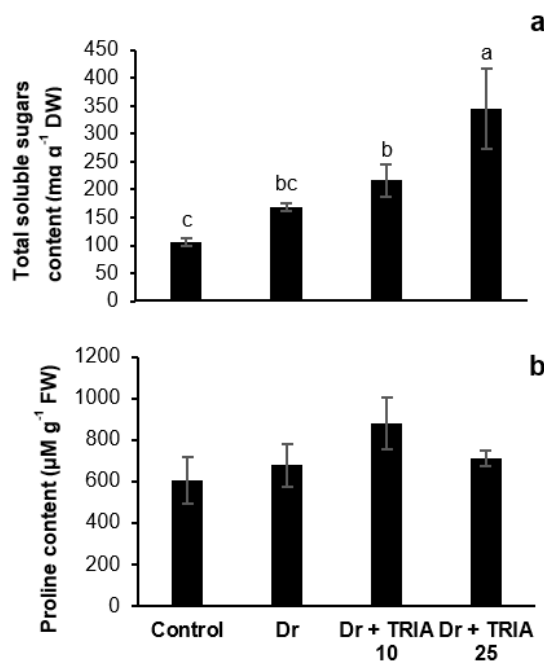


Fig. 3 Effect of drought alone (Dr) or combined with 10 or 25 μM TRIA (Dr + TRIA 10, Dr + TRIA 25) on: (a) total soluble sugar content and (b) proline content, as compared with the control untreated shoots. Data with statistical analysis are means \pm SD ($n=3$), bars with different letters are significantly different at $P \leq 0.05$.

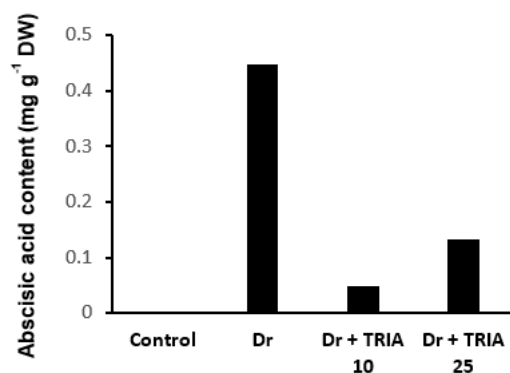


Fig. 4 Effect of drought alone (Dr) or combined with 10 or 25 μM TRIA (Dr + TRIA 10, Dr + TRIA 25) on abscisic acid content, as compared with the control untreated shoots

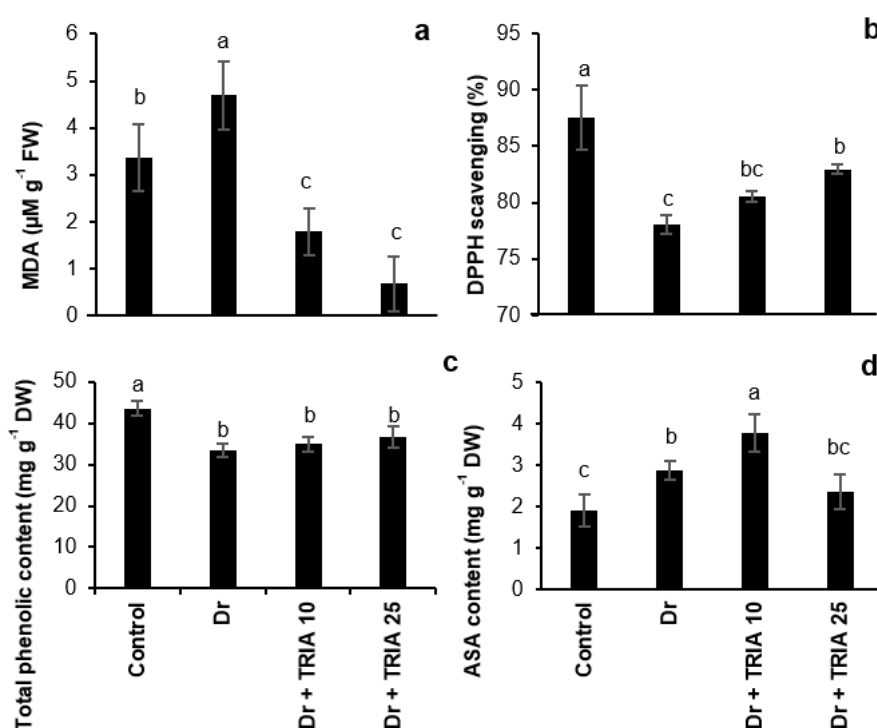


Fig. 5 Effect of drought alone (Dr) or combined with 10 or 25 μM TRIA (Dr + TRIA 10, Dr + TRIA 25) on: (a) lipid peroxidation (MDA), (b) DPPH scavenging percentage, (c) total phenolic content, and (d) ascorbic acid content (ASA) in sunflower shoots, as compared with the control untreated shoots. Data are means \pm SD ($n=3$), bars with different letters are significantly different at $P \leq 0.05$.

Effect of TRIA on Non-Enzymatic and Enzymatic Antioxidants of Drought-Stressed Sunflower Seedlings:

Drought stress significantly decreased the total phenolic content of sunflower seedlings (Fig. 5c), while it elevated ASA level, which was further increased by 10 μM TRIA (Fig. 5d). A significant decrease in the activities of PX (73%) and PPO (46%) in response to drought stress was observed as compared with the well-watered control. This drought-induced inhibitory effect on both enzymes was reversed by 25 μM TRIA: PX (169.67%) and PPO (86.61%) (Fig. 6b & d). No significant change was observed in APX and CAT in

response to drought stress in relation to control seedlings as well as 10 μM TRIA compared with the stressed ones, yet 25 μM TRIA stimulated the activity of APX (229.23% of the control value), but this treatment had no impact on CAT (Fig. 6a & c). The activity of AO increased in drought-stressed seedlings as compared with the controls and in those treated with 25 μM TRIA compared with the stressed ones, while the enzyme activity was declined by 10 μM TRIA (Fig. 6e). How about 25 μM TRIA?? On the other hand, drought stress significantly increased the activity of SOD relative to the control seedlings, however, the TRIA application markedly reduced the enzyme activity in a concentration-dependent manner (Fig. 6f).

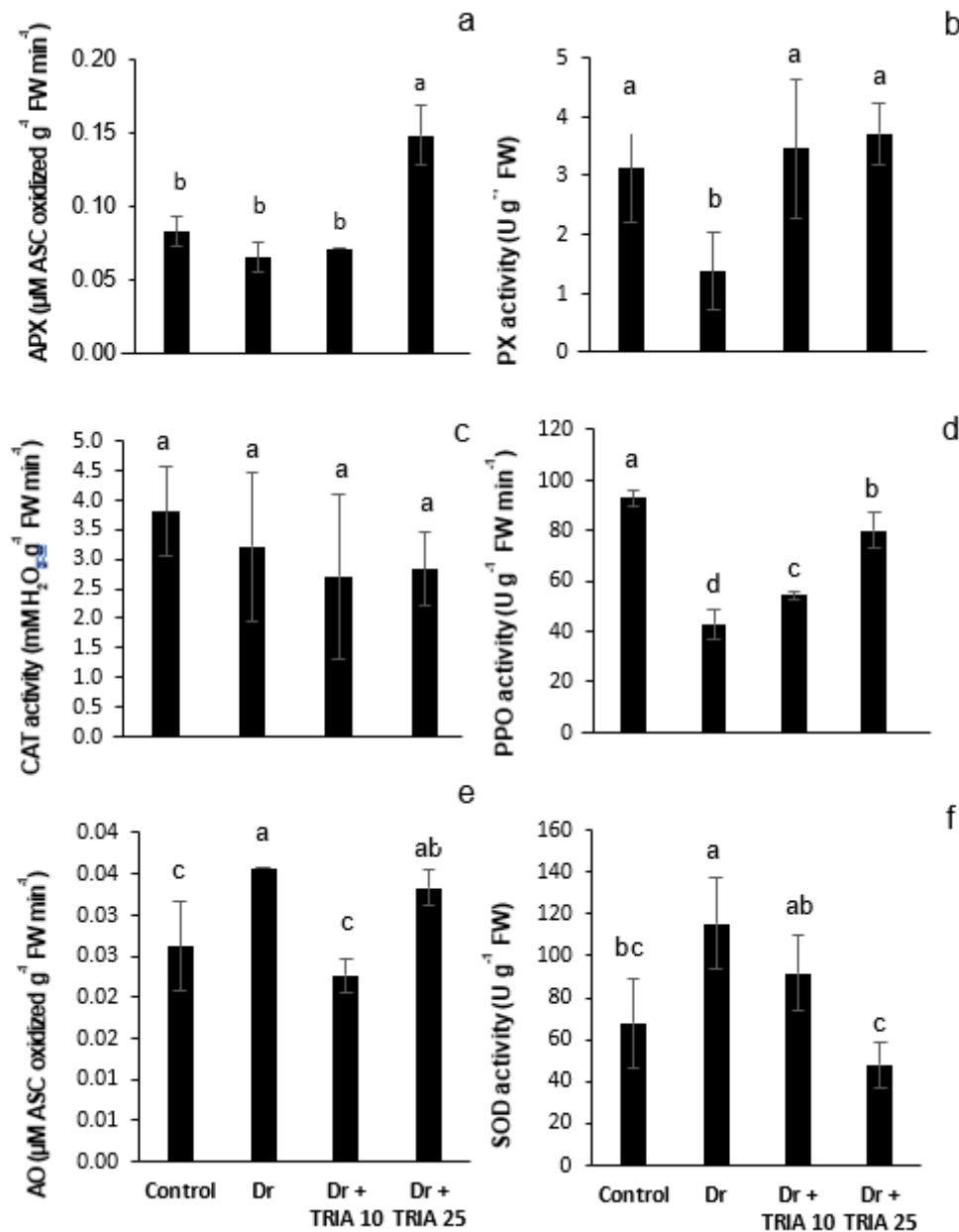


Fig. 6 Effect of drought alone (Dr) or combined with 10 or 25 μM TRIA (Dr + TRIA 10, Dr + TRIA 25) on: (a) ascorbate peroxidase (APX), (b) peroxidase (PX), (c) catalase (CAT), (d) polyphenol oxidase (PPO) (e) ascorbate oxidase (AO), and (f) superoxide dismutase (SOD) activities measured in sunflower shoots, as compared with the control untreated plants. Data are means \pm SD ($n=3$), bars with different letters are significantly different at $P \leq 0.05$.

DISCUSSION

Detrimental consequences of drought on plant growth are ubiquitous, yet its effect on the shoot is more conspicuous than on the root (Manzoor *et al.*, 2016), which agrees with the results observed in this study. Reduced shoot length and leaf area under drought stress can be interpreted as one early stress-avoiding response adopted by plants to reduce the amount of water loss via minimizing cell expansion and closing stomata (Oliver *et al.*, 2010). TRIA (25 μM) treatment restored both leaf area and shoot length to comparable levels of the control. TRIA-induced growth is most likely attributed to TRIA-mediated activation of L (+)-adenosine, leading to cell enlargement and proliferation (Khan *et al.*, 2014). Comparable alleviating effects of TRIA on the growth of stressed plants have been previously reported (Younis and Ismail, 2019). TRIA effect on growth was manifested on shoot rather than on root, which can be explained by the fact that TRIA was applied as a foliar spray and that TRIA is a native constituent of epicuticular waxes of many plants (Naeem *et al.*, 2012).

Significant diminish in PI_{abs} , F_v/F_m , photosynthetic pigments (Chl a, Chl b, and Car) contents, and Chl a/b ratio in response to drought stress agreed with the finding of Hassan *et al.* (2020) on wheat plants. Reduced photosynthetic pigments under water limiting conditions is possibly due to diminished photosystems I and II and pigment decomposition rather than hindered biosynthesis (Zargar *et al.*, 2017). TRIA treatment maintained the photosynthetic pigments concurrent with enhanced PI_{abs} and F_v/F_m values under stress conditions, which was comparable to the control value or even higher. These mitigating effects induced by TRIA could be explained by the impact of TRIA on increasing the number of chloroplasts, chlorophyll biosynthesis, as well as preventing their damage under stress conditions (Ramos-Zambrano *et al.*, 2020). A similar ameliorative effect of TRIA on photosynthetic efficiency and pigments content under various abiotic stresses was previously reported (Ramos-Zambrano *et al.*, 2020; Nabi *et al.*, 2020). It is noteworthy that carotenoids not only act as antenna capturing light but also have a substantial role in photoprotection of the photosynthetic apparatus under stress, as carotenoids quench triplet chlorophyll and prevent $^1\text{O}_2$ formation (Uarrota *et al.*, 2018). Also, carotenoid oxidation products, caused by stress-induced reactive oxygen species (ROS) formation, may play an important role in sensing stress signals, which leads to the alteration of responsive gene expression (Havaux, 2014).

The photosynthetic process is directly influenced by the activity of Rubisco, which is often associated with *RBCS* expression level. Interestingly, drought-induced overexpression of *RBCS* in sunflower seedlings probably acts as a reparation mechanism in order to compensate for reduced leaf area and lower pigment contents, which presumably explain how efficient photosynthesis was maintained under stress. Contrary, Xu *et al.* (2013) showed reduced transcription of *RBCS* under water limiting conditions in watermelon (Mo *et al.*, 2016). Plant age and stress severity most likely interpret such contradictory results (Wang *et al.*, 2015). The *RBCS* expression was further induced by TRIA application in sunflower seedlings, which was similarly reported by Chen *et al.* (2002; 2003) on rice. To our knowledge, this is the first attempt to relate TRIA application with *RBCS* expression in stressed sunflower. It is important to mention that a positive correlation ($R^2 = 0.77$, data not shown) was observed between *RBCS* expression and TSS content in this work, implying its crucial role in replenished metabolism.

One drought tolerance trait is the accumulation of compatible organic osmolytes such as proline and sugars (Khalid *et al.*, 2016). Drought-stressed sunflower seedlings accumulated sugars and proline that were comparable to the control, which was further enhanced by TRIA treatment. Similar TRIA-induced accumulation of osmoprotectants in

stressed peppermint plants was shown by Khanam and Mohammad (2018). Osmoprotectants have been reported to contribute to osmotic adjustment, which ensures continuous water absorption under stress conditions (Mansour and Salama, 2020). In addition, both osmolytes exhibit antioxidant, protective and signaling functions in response to various stresses (Poonam *et al.*, 2016; Mansour and Salama, 2020).

The leap of ABA content provoked by drought stress illustrated in this work has been demonstrated to be one of the early commonly known responses to drought (Imadi *et al.*, 2016). ABA triggers a complex signaling network to regulate stomatal movement (da Silva Folli-Pereira *et al.*, 2016) and plays an important role in drought tolerance in plants (Imadi *et al.*, 2016). Upon TRIA application, ABA level was greatly reduced, yet did not reach the control level; this ABA reduction was also revealed by El-Shafey *et al.* (2018) who showed TRIA-induced ABA decline in maize under drought. The decline in ABA content by TRIA treatment was found to be a result of the downregulation of ABA encoding genes and their related stress proteins (Chen *et al.*, 2002). Furthermore, TRIA treatment increased gibberellic acid at expense of ABA in *Artemisia annua* L. seedlings (Shukla *et al.*, 1992), but further research is needed to explore the precise feature of ABA and TRIA crosstalk.

Drought stress causes induction of ROS generation, which triggers oxidative damage of cellular macromolecules such as proteins, nucleic acids, and membranes (Mansour *et al.*, 2019). Our results showed significant increase in lipid peroxidation along with lower antioxidant capacity (lower DPPH value) in drought-stressed sunflower plants, suggestive of drought-induced oxidative stress that triggers impaired metabolism and growth inhibition (Uddin *et al.*, 2016a; Uddin *et al.*, 2016b). Similar findings reported by Farooq *et al.* (2017) and Aziz *et al.* (2018) that drought stress enhanced MDA associated with decreased DPPH scavenging activity in wheat and quinoa. Surpassing drought-triggered oxidative stress in plants largely depends on the antioxidant defense system including enzymatic and non-enzymatic antioxidants (Noctor *et al.*, 2014). As components of the non-enzymatic antioxidant system, phenols and ASA (besides its enzymatic role) were measured in this study. ASA as a non-enzymatic antioxidant was elevated by drought stress in sunflower seedlings, which was further increased by 10 μ M TRIA. Elevation in ASA under drought stress was similarly observed in stressed rice (Zhou *et al.*, 2020). It is well-known that ascorbate functions as a central antioxidant compound that regulates cellular redox homeostasis and influences signaling intensity in response to abiotic stresses (Foyer and Noctor, 2011). Surprisingly, total phenols were reduced by drought and did not recover by TRIA treatment, which might be due to the lower accumulation of secondary metabolites (including phenols) in this early stage of the young seedling. Drought stress doubled SOD activity in sunflower seedlings, this leap in SOD activity coincided with the highest content of ABA in drought-stressed sunflowers. It has been suggested that ABA is implicated in the singling of increased SOD activity under drought stress (Cruz De Carvalho, 2008; Bousba *et al.*, 2020). Additionally, SOD has been demonstrated to be the first defense agent against ROS as it detoxifies the highly toxic superoxide ($O_2^{\bullet-}$) molecules and converts them into less toxic H_2O_2 (Jacob and Dietz, 2010). Drought-induced increase in PX and PPO activities under drought stress corresponds with Iqbal *et al.* (2018) finding who recorded reduced PPO in drought-sensitive quinoa under drought stress. Therefore, elevated ASA or SOD had no mitigative impact on MDA & DPPH under drought stress, which indicates that the cellular compartments were highly affected by drought-induced oxidation.

TRIA application to drought-stressed seedlings significantly enhanced both membrane integrity and DPPH antioxidant capacity, which is consistent with the previous report of Nabi *et al.* (2020). Also, drought-induced deterioration of PX and PPO was significantly restored by both TRIA treatments in a concentration-dependent manner. These results agree with the findings of Nabi *et al.* (2020) who showed that TRIA improved the

activities of PX under stressful conditions. Interestingly, a noticeable strong positive correlation between DPPH scavenging percentage and PPO ($R^2 = 0.94$, data not shown) recorded in sunflower seedling indicates the vitality of its role as an antioxidant. Moreover, stressed seedlings treated with 25 μM TRIA demonstrated the highest activity of APX at the expense of ASA content. These results demonstrate that TRIA stimulates the enzymatic antioxidant role of ASA, via the ascorbate-glutathione cycle (Islam *et al.*, 2020) besides the nonenzymatic one (Nabi *et al.*, 2020). As TRIA treatment decreased lipid peroxidation, enhanced APX, PX and PPO activities, and ASA accumulation, it is therefore obvious that TRIA enabled sunflower to develop an efficient antioxidant defense system protecting cellular compartments from drought-induced oxidative stress.

Conclusions:

It can be concluded that sunflower, cultivar Sakha 53, exhibited a relative sensitivity to drought. TRIA (especially 25 μM) significantly alleviated drought stress effects by maintaining photosynthesis by upholding both photosynthetic pigments and efficiency, *RBCS* expression, and boosting the redox cellular balance in sunflower seedlings. Other drought-responsive genes, electron microscopy of the chloroplast, and ion fluxes through the membranes of stressed plants treated with TRIA should be explored in future research in order to have a full understanding of TRIA action mechanism against drought, which can help in developing drought-tolerant crops.

List of abbreviations:

ABA - Abscisic acid
 AO - Ascorbate oxidase
 APX - Ascorbate peroxidase
 ASA - Ascorbic acid
 Car - Carotenoids
 CAT - Catalase
 Chl - Chlorophyll
 DPPH - 1, 1-diphenyl-2-picrylhydrazyl
 F_v/F_m - The maximum quantum efficiency of PSII
 MDA - Malondialdehyde
 PGRs - Plant growth regulators
 PI_{abs} - Photosynthetic performance index
 PPO - Polyphenol oxidase
 PX - Peroxidase
RBCS - Rubisco small subunit
 ROS - Reactive oxygen species
 SOD - Superoxide dismutase
 TBA - Thiobarbituric acid
 TRIA - Triacontanol
 TSS - Total soluble sugar

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