Article

Seed Treatment with α -Tocopherol Regulates Growth and Key Physio-Biochemical Attributes in Carrot (*Daucus carota* L.) Plants under Water Limited Regimes

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Abstract: The influence of seed priming with varying levels (50 and 100 mg L⁻¹) of alpha-tocopherol (Toc) was investigated in carrot plants under water-deficit conditions. For this purpose, two cultivars of carrot, DC4 and DC90, were selected and subjected to well-watered (100% field capacity (FC)) and water-deficit stress (50% FC). After 21 days of water-deficit conditions, a significant suppression was observed in shoot and root fresh and dry weights, their lengths, chlorophyll *a*, *b* and total contents, and total soluble proteins (TSP). However, an up-regulatory effect of water stress was observed on the concentrations of glycinebetaine (GB), hydrogen peroxide (H₂O₂), malondialdehyde (MDA), ascorbic acid (AsA), total phenolics as well as the activities of catalase (CAT) and peroxidase (POD) enzymes. Exogenous application of alpha-tocopherol was effective in reducing the accumulation of H₂O₂ and MDA contents and improving all growth attributes, contents of chlorophyll, proline, GB, AsA, total phenolics, TSP, and the activities of CAT and POD enzymes. Of both carrot cultivars, cv. DC4 had better performance in terms of growth attributes, whereas the response of the two cultivars was similar in all other attributes varying water regimes. Overall, it is suggested that seed priming with 100 mg L⁻¹ Toc was effective in improving plant growth attributes, osmoprotectants and the oxidative defense system of carrot plants under water-deficit conditions.

Keywords: *Daucus carota*; α -tocopherol; drought stress; reactive oxygen species; antioxidants

1. Introduction

Water deficit stress commonly known as drought is the most devastating abiotic stress which adversely affects morphological, biochemical, physiological, anatomical and molecular characteristics of plants [1]. Due to drought stress, a significant decrease in growth and yield production of many crops was noticed that is believed to be associated with reduced transpiration rate, chlorophyll pigments, photosynthetic rate, water potential, and gaseous exchange characteristics [2,3]. Morphological adaptations such as trichome/bark thickness and leaf wax can effectively suppress water loss, thereby providing protection to plants against water shortage conditions [4]. Water shortage affects the concentration

and composition of plant primary as well as secondary metabolites [5]. Depending on cultivated practices and types of plant species, drought stress influences almost all the main classes of plant metabolites [6]. To adapt to arid environments, the plant expresses an extremely dynamic and complex process of homeostasis. Modulation of cell structure, hormone induction, scavenging of reactive oxygen species (ROS), kinase cascade signaling, osmolyte synthesis, regulation of gene expression, amino acid metabolism, nitrogen assimilation and ion channel activation are key mechanisms operating actively in response to drought stress [7,8]. Considerable variation in the functioning of a myriad of molecular, physiological and biochemical attributes was reported in the drought-stressed plants of different crops, e.g., mung bean [9], carrot [10], sunflower [11], and safflower [12]. Plants can also accumulate ROS under water-deficit regimes that may act as secondary messengers [13]. ROS of various forms like superoxide, hydroxyl radicals, hydrogen peroxide and singlet oxygen are generated in several parts of the cell such as microbodies, chloroplasts and mitochondria [14,15]. Another vital response of plants to drought stress is cell osmoregulation, which is mediated by the accumulation of solutes such as proline, sugars and glycinebetaine, and these biomolecules are believed to protect cellular structures and conserve cellular water content [5,16]. By accumulating osmoprotectant compounds such as proline, trehalose and polyamines, plants can resist a stress [17,18].

Alpha-tocopherol (Toc) is an effective oxygen-quenching micro-molecule, which accumulates to reasonable levels in plants. It is generally a lipophilic compound which is synthesized in the chloroplast, and most of the enzymes responsible for its biosynthesis are located on the inner membrane of the chloroplast envelope [9]. It is a major antioxidant that contributes in intracellular signaling, electron transport, photosystem-II, membrane stability and photo-protection. Tocopherols (alpha, beta and gamma) perform a role in a variety of diverse physiological phenomena including prevention of lipid peroxidation, delaying senescence and stimulation of signal cascade interactions that transfer biotic and abiotic signals [19]. Seeds and leaves contain varying concentrations of alpha-tocopherol. Under stress conditions, the concentrations of ROS in the biological membranes increase, which ultimately decreases α -tocopherol contents [20]. Tocopherols are essential antioxidants that control ROS production. One molecule of tocopherol can effectively control 120 oxygen molecules [21]. Naturally, stress conditions regulate the synthesis of tocopherol [20]. It is the best active form of vitamin E, which has considerable importance in humans' diet. It is mostly present in higher concentrations in vegetable oils (canola, almond and safflower), seeds, nuts or grains that are sources of high fats [22].

Exogenous application of antioxidants and vitamins on plants can minimize the negative effect of salinity and water stress on plant growth, yield quality and quantity [6]. Vitamins are considered as natural bioregulators, and various chemical activities may be affected even by their very small concentrations, but it depends on plant species or a stress, level of vitamin E, as well as different climatic conditions [23]. Alpha-tocopherol concentration changes due to associated genes expression and it is well known in that its high concentration in plants results in stress tolerance or resistance [21]. For example, seed treatment with alpha-tocopherol of wheat reduced oxidative stress and enhanced tolerance to temperature stress in the plants [24]. Moreover, adverse effects of ROS were reported to be nullified by the external supplementation of alpha-tocopherol [25], which could be ascribed to preservation of membrane stability and mitigation of lipid peroxidation [19].

Carrot (*Daucus carota* L.), a member of family Apiaceae, is an herbaceous biennial species. On the basis of pigments present in roots, cultivated carrots are generally classified into western and eastern carrots [10]. The storage root of carrot is a big source of dietary fibers, carotenoids, vitamins, antioxidants, carbohydrates, bioactive micronutrients and minerals (Fe, Ca, Mg and P) [26]. Due to a rich source of nutrients, it is good for human health [27]. Biotic and abiotic stresses greatly affect quality features of carrot [10]. It is a nitrophilous horticultural plant and has the capability to store a high amount of nitrate [27]. Keeping in mind the importance of Toc and carrot, the main objectives of the current

study were to assess the influence of water deficit stress and pre-sowing treatment with α -tocopherol on plant growth and physio-biochemical attributes of carrot plants.

2. Materials and Methods

2.1. Experimental Design and Growth Conditions

A greenhouse completely randomized design experiment was conducted to assess the role of pre-sowing seed treatment with alpha-tocopherol in improving growth and various physio-biochemical features of carrot plants subjected to water deficit conditions. During the experimentation, average temperature, 16.5–19.4 °C, relative humidity, 75% and day length, 7.0 h were recorded. Seeds of two carrot cultivars (DC-4 and DC-90) were obtained from the Vegetable Section, Ayyub Agricultural Research Institute, Faisalabad, Pakistan. About 250 seeds of each carrot cultivar were sterilized, wrapped in a miracloth package followed by 5 min soaking in 0.5% sodium hypochlorite solution. Then, the seeds were rinsed briefly with deionized water and air-dried. For pre-sowing seed treatment, the seeds were soaked in three (0, 50 and 100 mg L⁻¹) different concentrations of alpha-tocopherol along with 0.1% Tween-20 as a surfactant for 14 h. Sandy-loam soil (8 kg per pot) was obtained from the Ever-Green Nursery, Faisalabad, Pakistan. In each pot, 10 seeds were sown and allowed to germinate. After 10 days of seed germination, thinning was done and 5 plants were maintained in each plastic pot (diameter, 28 cm). After one month of seed germination, the plants were subjected to drought stress at the rate of 50% field capacity (FC) in addition to control (100% FC). The drought stress was maintained by measuring the saturation percentage of the soil extract. Then, the pots were regularly monitored and we kept the weight of each pot equal to that calculated for 50% and 100% FC through addition of water. After 21 days of drought stress treatment, sampling was done to evaluate changes in the following morphological and physio-biochemical parameters in the carrot plants.

2.2. Morphological Attributes

Two carrot plants from each pot were uprooted carefully after watering the soil. Roots were washed carefully with distilled water and measured the root and shoot lengths using a scale (cm). Root and shoot fresh weights were recorded by using an electrical weighing machine and placed in an oven at 65 °C for three days before recording their dry weights.

2.3. Chlorophyll Pigments

A fresh leaf (0.5 g) was extracted in 10 mL of acetone (80%) and placed all the samples overnight at 4 °C. Then, the extracts were centrifuged at 10,000 × g for 5 min. The supernatant was poured into a quartz cuvette and absorbance was recorded at 480, 645 and 663 nm on a spectrophotometer. Chlorophyll *a* and *b* concentrations were calculated following Arnon [28].

2.4. Leaf Free Proline Contents

Fresh leaf (0.5 g) was homogenized in 10 mL of 3% sulfosalicylic acid (*w/v*) solution. After filtration, 2 mL of the filtrate was mixed with 2 mL acid ninhydrin solution and an equal volume of glacial acetic acid was added to it. The mixture was heated at 100 °C for one h in a water bath and subsequently cooled in an ice bath. After that, 4 mL of toluene was added and shaken well the mixture. Two layers were formed in the mixture and the absorbance of the upper layer was noted at 520 nm using a spectrophotometer. Then, proline contents were calculated following Bates et al. [29].

2.5. Glycinebetaine (GB)

Following Grieve and Grattan [30], a fresh leaf (0.5 g) was ground in 10 mL distilled water and centrifuged at 10,000 × g for 10 min. Then, 1.0 mL of the filtrate was mixed with 1.0 mL of 2N H₂SO₄ solution. Then, 0.5 mL of the mixture was taken in a test tube and potassium tri-iodide (0.2 mL) was added to it. After it, the mixture was shaken and cooled for 90 min on ice. Ice-cooled distilled water (2.8 mL) and 1, 2 dichloroethane (6 mL) were

added to the reaction mixture. By passing an air stream for 2 min through the mixture, two layers were formed. Optical density of the upper organic layer was read at 365 nm using a spectrophotometer.

2.6. Hydrogen Peroxide (H_2O_2) Contents

According to the method of Velikova et al. [31], 0.5 g fresh leaf was homogenized in 5 mL of 1.0% trichloroacetic acid (TCA) in an ice-cooled pestle and mortar. To separate the supernatant, centrifugation was done at $12,000 \times g$ for 15 min. After that, 0.5 mL of the supernatant was taken with the same volume of potassium-phosphate buffer (pH 7) along with 1 mL of 1 M potassium iodide and mixed well. The optical density (OD) was recorded at 390 nm to calculate H_2O_2 content.

2.7. Malondialdehyde (MDA)

Fresh leaf (0.25 g) was ground in 3 mL of 5% (*w/v*) TCA using a pre-chilled pestle and mortar following Cakmak and Horst [32]. The extract was centrifuged for 15 min and 0.5 mL of the supernatant was mixed with 2 mL 0.5% (*w/v*) thiobarbituric acid prepared in 20% trichloro-acetic acid. The reaction mixture was heated in a water bath for 50 min at 95 °C and cooled. Absorbance of the mixture was recorded at 532 and 600 nm using a spectrophotometer.

2.8. Total Phenolics

Following Julkenen-Titto [33], fresh leaf (0.1 g) was extracted in 5 mL of 80% acetone (*v/v*). Then, the extract was centrifuged for 10 min at $10,000 \times g$. Then, 100 μ L of supernatant, 2 mL of distilled water and 1 mL Folin-Ciocalteu's phenol reagent were mixed and shaken well. To that, 5 mL of sodium carbonate (20%) was added and made the final volume as 10 mL using distilled water. The absorbance was noted at 750 nm using a spectrophotometer.

2.9. Ascorbic Acid (AsA)

The Mukherjee and Choudhuri [34] method was used to determine the ascorbic acid (AsA) contents in fresh leaves of carrot plants. Fresh leaf (0.25 g of each sample) was extracted in 10 mL solution of 6% TCA. The filtrate (4 mL) was taken in test tubes and we added 2 mL of 2% dinitrophenyl hydrazine prepared in H_2SO_4 (9 N) solution to each test tube. Then, 1 drop of 10% thiourea (prepared in 70% (*v/v*) ethanol) was added and kept it in a water bath for 15 min at 100 °C. Then, the filtrate was cooled at room temperature and 5 mL of 80% (*v/v*) H_2SO_4 was added to it. Then, the absorbance of the mixture was recorded at 530 nm using a spectrophotometer.

2.10. Total Soluble Proteins

Fresh leaf (0.5 g) was homogenized in 5 mL phosphate buffer (pH 7) in a pre-chilled pestle and mortar according to Bradford [35]. After centrifugation, 100 μ L of the supernatant was mixed with 5 mL of the Bradford reagent. The absorbance of the mixture was noted at 595 nm using a spectrophotometer.

2.11. Enzymatic Antioxidants

A fresh leaf (0.5 g) was homogenized in 5 mL of 50 mM phosphate buffer (pH 7.8) using a pre-chilled pestle and mortar. Then, the extract was centrifuged for 15 min at $15,000 \times g$ at 4 °C. The supernatant was used for the determination of the activities of catalase (CAT) and peroxidase (POD) enzymes following Chance and Maehly [36]. For the determination of the activity of CAT enzyme, leaf extract (100 μ L), 50 mM potassium phosphate buffer (1.9 mL) along with 1 mL of 5.9 mM H_2O_2 were mixed in a cuvette. Changes in absorbance were noted after every 20 s for 3 min at 240 nm. For the determination of activity of peroxidase (POD), 100 μ L of 40 mM H_2O_2 , 100 μ L of 20 mM guaiacol, 50 μ L of the enzyme extract and 750 μ L of potassium phosphate buffer were mixed and a change in absorbance

of the reaction mixture for three minutes was observed at 470 nm. The activities of CAT and POD enzymes were calculated on the basis of total soluble proteins.

2.12. Statistical Analysis

A three-way (cultivars, drought and tocopherol) analysis of variance of data of all parameters was employed using the statistical software Statistix 8.1 to assess the level of significance. The least significance difference (LSD) at the 5% probability level was employed to assess the differences among the treatment means. The heat-map analysis between different variables was constructed using RStudio.

3. Results

3.1. Plant Growth and Biomass

The effectiveness of varying levels (50 and 100 mg L⁻¹) of alpha-tocopherol (Toc) as a seed pre-treatment in stress tolerance (water-deficit) of carrot plants was investigated in the present study. Two cultivars of carrot, DC4 and DC90, were subjected to 100% FC (control) and 50% FC (water stress). Data indicated that all morphological attributes including shoot fresh and dry weights ($p \leq 0.001$), root fresh and dry weights ($p \leq 0.05$), as well as shoot and root lengths ($p \leq 0.01$ and 0.05), decreased significantly under water deficit conditions. Seed priming with Toc considerably ($p \leq 0.001$; 0.01) improved all the growth attributes of carrot plants subjected to both water regimes. Of both levels of Toc, 100 mg L⁻¹ was found to be more effective in enhancing the growth attributes than the other Toc level under water shortage conditions (Figure 1). Of both carrot cultivars, cv. DC4 had better performance in terms of shoot and root fresh weights, as well as root length under both water regimes. The response of both carrot cultivars to water stress and exogenously applied Toc was similar for other growth attributes.

3.2. Photosynthetic Pigment

Drought stress significantly ($p \leq 0.001$) suppressed chlorophyll *a*, *b* and total content of both carrot cultivars (Figure 2). Moreover, exogenously applied alpha-tocopherol as a pre-sowing seed treatment significantly ($p \leq 0.01$) improved the chlorophyll content of both cultivars under water limited conditions. It was noticed that tocopherol applied as 100 mg/L was more effective than the other level in regulating the concentrations of the photosynthetic pigments. However, no significant change was observed in chlorophyll *a/b* ratio of both carrot cultivars subjected to water stress as well as an exogenous supply of Toc. The trend of decrease in chlorophyll pigments was consistent in both carrot cultivars under varying water stress and Toc treatments (Figure 2).

3.3. Proline Content

No significant change was observed in proline content of both carrot cultivars under the water limited regimes (Figure 2). The seed treatment with alpha-tocopherol enhanced ($p \leq 0.01$) the accumulation of proline content under both water regimes. Of both Toc levels, maximum accumulation in proline content was observed at 100 mg L⁻¹ under varying water regimes. The response of both carrot cultivars was almost similar under water stress and Toc treatments.

3.4. Glycinebetaine Content

A prominent ($p \leq 0.01$) increase in glycinebetaine (GB) content was observed in water-stressed carrot plants. The pre-sowing seed treatment with Toc triggered ($p \leq 0.01$) the accumulation of GB content under varying water supply conditions. Of both Toc levels, 100 mg L⁻¹ was more effective in causing the accumulation of the GB content under varying water regimes. No significant difference in GB accumulation was noticed in both carrot cultivars (Figure 2).

Figure 1. Effect of different levels of alpha-tocopherol on shoot fresh weight, shoot dry weight, root fresh weight, root dry weight, shoot length and root length of 51-day-old carrot cultivar grown under control (normal watering) and drought (50% field capacity) stressed environments. Bars sharing different letter(s) for each parameter are significantly different from each other according to Duncan's multiple range test ($p < 0.05$). All the data represented are the average of three replications ($n = 3$). Error bars represent standard deviation (SD) of three replicates. ns, non-significant, *, ** and ***; significant at 0.05, 0.01 and 0.001 levels.

Figure 2. Effect of different levels of alpha-tocopherol on chlorophyll (a, b, a/b ratio, total) content, proline, glycinebetaine (GB), hydrogen peroxide and malondialdehyde content of 51-day-old carrot cultivar grown under control (normal watering) and drought (50% field capacity) stressed environments. Bars sharing different letter(s) for each parameter are significantly different from each other according to Duncan's multiple range test ($p < 0.05$). All the data represented are the average of three replications ($n = 3$). Error bars represent standard deviation (SD) of three replicates. ns, non-significant, *, ** and ***, significant at 0.05, 0.01 and 0.001 levels.

3.5. Oxidative Stress Indicators

Under water stress conditions, hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) were found to accumulate considerably ($p \leq 0.001$ and 0.05 , respectively) as compared to that in the control plants of both carrot cultivars. Exogenous application of Toc was effective ($p \leq 0.05$) in minimizing the H_2O_2 and MDA accumulation in drought-stressed carrot plants (Figure 2). The response of both carrot cultivars was consistent in both these attributes.

3.6. Non-Enzymatic Antioxidant

Ascorbic acid (AsA) and total phenolic concentrations increased significantly ($p \leq 0.001$) in both carrot cultivars under water scarce conditions (Figure 3). Both levels of alpha-tocopherol significantly ($p \leq 0.01$, 0.001 , respectively) improved the AsA and total phenolic content in both carrot cultivars under water stress as well as non-stress conditions. Of both Toc levels, 100 mg L^{-1} was the most effective in enhancing the AsA and total phenolic content in the water-stressed plants of both carrot cultivars. However, no difference was observed in the two carrot cultivars in AsA and total phenolics under varying water regimes.

3.7. Protein Content

A significant decrease ($p \leq 0.001$) was observed in total soluble proteins (TSP) of both carrot cultivars under drought stress (Figure 3). However, application of alpha-tocopherol improved the TSP in both carrot cultivars under varying water regimes. Overall, 100 mg L^{-1} of alpha-tocopherol was most effective in regulating the levels of TSP in both carrot cultivars. A non-significant difference was observed in the two carrot cultivars in terms of TSP.

3.8. Enzymatic Antioxidant

The activities of catalase (CAT) and peroxidase (POD) enzymes enhanced significantly ($p \leq 0.001$) in both carrot cultivars under water deficit conditions (Figure 3). Exogenous application of alpha-tocopherol, particularly 100 mg L^{-1} , tended to upregulate the activities of both enzymes under water deficit conditions. No significant change in both carrot cultivars was observed in terms of the activities of CAT and POD enzymes.

3.9. Relationship

A heat-map correlation analysis was constructed to quantify the relation between different parameters studied in this experiment. The graphical representation of DC4 variety is presented in Figure 4. DC4 variety showed more resistance in the drought-stressed environment, so we constructed only one heat-map analysis using DC4 variety. The results from the constructed heat-map analysis show that plants grown in control conditions under the application of 100 mg L^{-1} of alpha-tocopherol were highly significant with their growth parameters, while the rest of the parameters in controlled and drought-stressed environments showed that different growth and physiological parameters were decreasing or non-significant under increasing levels of alpha-tocopherol. Hence, this relationship is showing a close connection between growth of DC4 under drought and controlled environments, with the application of alpha-tocopherol.

Figure 3. Effect of different levels of alpha-tocopherol on ascorbic acid, total phenolics, total soluble proteins and activities of catalase and peroxidase enzymes of 51-day-old carrot cultivar grown under control (normal watering) and drought (50% field capacity) stressed environments. Bars sharing different letter(s) for each parameter are significantly different from each other according to Duncan's multiple range test ($p < 0.05$). All the data represented are the average of three replications ($n = 3$). Error bars represent standard deviation (SD) of three replicates. ns, non-significant, *, ** and ***; significant at 0.05, 0.01 and 0.001 levels.

Figure 4. Heat-map analysis between various growth and physiological attributes in carrot grown in control and drought-stressed environment. Different treatments in the base of the figure are as follows—DS 0 (Drought-stressed environment with 0 mg L^{−1} of alpha-tocopherol), DS 50 (Drought-stressed environment with 50 mg L^{−1} of alpha-tocopherol), DS 100 (Drought-stressed environment with 100 mg L^{−1} of alpha-tocopherol), Ck 0 (Sufficient watered supply with 0 mg L^{−1} of alpha-tocopherol), Ck 50 (Sufficient watered supply with 50 mg L^{−1} of alpha-tocopherol) and Ck 100 (Sufficient watered supply with 100 mg L^{−1} of alpha-tocopherol). Different abbreviations used in the figure are as follows—RDW (root dry weight), Pro (proline content), SDW (shoot dry weight), TC (total chlorophyll content), Chl a (chlorophyll a content), Chl b (chlorophyll b contents), SL (shoot length), RL (root length), RFW (root fresh weight), SFW (shoot fresh weight), TSP (total soluble protein), AsA (ascorbic acid content), Phe (phenolic content), H₂O₂ (hydrogen peroxide initiation), MDA (malondialdehyde content), CAT (catalase activity), GB (glycinebetaine) and POD (peroxide activity).

4. Discussion

Exogenously applied plant growth regulators are reported to be very effective in improving stress tolerance in plants [18,37]. Of different growth regulators, the application of lipophilic antioxidative compounds including tocopherols (Toc) is very effective in

increasing plant growth and yield on stressed environments particularly by ameliorating the stress-induced adversaries [20]. Exogenously applied alpha-tocopherol was reported to be effective in improving the plant growth in different plants, e.g., *Vicia faba* [38], wheat [39] and *Vigna radiata* [40] under drought stress conditions. Water stress is one of the largest emerging abiotic stresses adversely affecting places all over the world due to anthropogenic as well as rapid climatic changes [41]. Deficiency of water perturbs many plant processes from seed germination to seed formation [42]. Generally, reduction in plant growth and yield production was associated with water stress-induced osmotic stress, nutritional and hormonal imbalance, over-production of ROS as well as disturbance in proteins synthesis [1,8]. In the present study, both carrot cultivars (DC4 and DC90) showed a significant decrease in shoot and root lengths as well as overall plant growth. However, seed treatment with Toc (50 and 100 mg L⁻¹) improved the shoot and root fresh and dry weights as well as lengths (shoot and root) of carrot plants. This Toc-induced improvement in plant growth has been already reported to be associated with different metabolic processes such as better water status, uptake of essential nutrients, up-regulation of the oxidative defense system, stomatal functioning, improved rate of photosynthesis, synthesis of chlorophyll and accumulation of osmoprotectants under stress conditions [3,6,41].

Chlorophyll content declined significantly in both carrot cultivars under water stress regimes. However, the application of alpha-tocopherol significantly increased the chlorophyll content in both carrot cultivars under water stress. The decrease in pigments in response to drought stress is a common phenomenon reported in various crops like carrot [10], mung bean [40], and canola [3]. However, supply of Toc has been reported to mitigate the stress-induced adversaries in plants. For example, while working with mung bean, Sadiq et al. [40] applied Toc at the rate of 300 mg L⁻¹ as a foliar spray and found a significant improvement in chlorophyll *a* and *b* content under water-deficit conditions. In fact, alpha-tocopherol is believed to play an effective role in the xanthophyll cycle taking place in chloroplasts particularly to preserve membrane lipids and protect PSII from photo-inactivation, thereby keeping the chlorophyll contents intact [22].

A significant increase in GB and proline content was observed due to pre-sowing seed treatment with Toc under limited water supply. Of both Toc levels, 100 mg L⁻¹ was more effective in causing the accumulation of proline and GB content under varying water regimes. Glycinebetaine and proline are known to act as compatible organic solutes for osmotic adjustment and as ROS scavengers for improving plant water status [18,40]. For instance, the accumulation of GB and proline is a common response of plants exposed to stress conditions as a defense mechanism to alleviate the stress adversaries [43,44]. In this study, water stress remained ineffective to alter the levels of proline, whereas it caused enhanced accumulation of GB; however, exogenous application of Toc increased the accumulation of both GB and proline in both carrot cultivars. Similar results regarding Toc-induced proline accumulation were reported by Orabi and Abdelhamid [45] in the leaves of faba bean plants under salt stress, and by Sadiq et al. [40] in pods of mung bean under drought stress.

High accumulation of ROS beyond the tolerance level in different plant species is one of the selection criteria for selecting sensitive cultivars of different crops under water stress conditions [46]. In the current study, accumulation of H₂O₂ and MDA (both indicators of oxidative stress) increased considerably in both carrot cultivars under water-limited regimes. However, exogenously applied Toc effectively minimized the accumulation of H₂O₂ and MDA content in water-stressed carrot plants (Figure 2). The response of both carrot cultivars was similar to drought stress with respect to ROS species. In response to high ROS production under water deficiency, triggering of antioxidants (enzymatic and non-enzymatic) is considered as one of the promising strategies of water stress-tolerant plants [4]. In the present study, we found that non-enzymatic antioxidants (ascorbic acid and total phenolic contents) increased significantly under drought stress conditions in both carrot cultivars. Both AsA and total phenolics are well-known to detoxify the ROS produced under stress conditions [47]. Moreover, in the present study the activities of enzy-

matic antioxidant (POD and CAT) increased significantly in both carrot cultivars (DC4 and DC90) under water scarce conditions. Moreover, exogenously applied alpha-tocopherol improved the activities of these enzymes under water-deficit conditions. Analogous to the results of the current study, Sadiq et al. [40] observed that exogenously applied Toc (200 and 300 mg L⁻¹) considerably enhanced the activities of SOD, POD and CAT antioxidant enzymes in mung bean pods under water deficit conditions. It is well evident that improvement in antioxidative defense system suppresses the oxidative stress specifically by increasing the activities of peroxidase and catalase enzymes [48,49]. It is important to note that the exogenously applied plant growth regulators altered enzymatic and non-enzymatic machinery that may have prevented the cell membrane from the damages induced by over-production of ROS [50,51].

5. Conclusions

In general, water stress considerably reduced the plant growth, chlorophyll pigments and total soluble proteins, while it increased the accumulation of GB, H₂O₂, MDA, AsA, total phenolics and the activities of enzymatic antioxidant (CAT and POD) enzymes. Seed soaking treatment with Toc was effective in reducing the accumulation of H₂O₂ and MDA content, while it improved plant growth, chlorophyll, proline, GB, AsA, total phenolics, TSP content and the activities of CAT and POD enzymes. Cultivar DC4 was better in plant growth, while the response of the two cultivars was similar in all other attributes to water stress. Overall, seed treatment with 100 mg L⁻¹ Toc was very effective in enhancing plant growth, accumulation of osmoprotectants and upregulation of the oxidative defense system of carrot plants under water-deficit conditions.

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