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Original article

Effects of titanium dioxide nanoparticles against salt and heat stress in safflower cultivars

Burcu Seckin Dinler[1*], Hatice Cetinkaya[1], Fatma Nur Koc[1], Volkan Gül[2], Fırat Sefaoğlu[3]

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ABSTRACT

This study aimed to determine the effects of titanium dioxide nanoparticle (TiO_2NP) pretreatment on seeds of different safflower cultivars (Balci, Dinçer) under salt and heat stresses. The apparent effects on stress markers (malondialdehyde (MDA), hydrogen peroxide (H_2O_2) and superoxide radical ($O_2^{\cdot-}$) content), as well as changes in germination and physiological parameters (radicle and plumula weight and length measurements), were investigated. TiO_2NP pretreatment caused an increase in radicle length and plumula fresh weight for the Balci cultivar under salinity. Furthermore, plumula dry weight was alleviated with TiO_2NP pretreatment for both cultivars. TiO_2NP pretreatment improved plumula dry and fresh weights for both cultivars under heat stress. In addition, MDA content decreased for both cultivars under heat stress but only for Balci under salt stress. The amount of $O_2^{\cdot-}$ radicals positively affected only the radicle for both cultivars under heat stress. This study is the first to document the alleviation of salt stress damage for the Balci safflower cultivar, and protection for both Balci and Dinçer cultivars under heat stress, using 200 ppm TiO_2NP pretreatment.

Keywords: *Carthamus tinctorious*, nanoparticle, reactive oxygen species

Introduction

Salinity and heat are important abiotic stress factors affecting plant growth, development, and yield potential (Zhang & Dai 2019). Salinity causes osmotic stress by reducing water potential, which disrupts plant water balance and turgor (Navada *et al.* 2020). The entry of high concentrations of salt ions into plant cells causes cell ion imbalance and ionic stress. Depending on both osmotic and ionic stress, reactive oxygen species (ROS) production and accumulation increase, causing oxidative damage. Increased ROS causes lipid peroxidation, membrane disruption, and denaturation of biomolecules such as DNA, proteins, and lipids (Arif *et al.* 2020).

Human activities, especially increased emissions of greenhouse gases, such as carbon dioxide, methane, and chlorofluorocarbons, into the atmosphere, cause increased heat, contributing to global warming. Heat must exceed a particular threshold value for heat stress to occur, with a

1 Sinop University, Faculty of Arts and Sciences, Department of Biology, Sinop, Turkey
2 Bayburt University Organic Farming Management, Faculty of Applied Sciences, Bayburt, Turkey
3 Kastamonu University, Faculty of Engineering and Architecture, Department of Genetics and Bioengineering, Kastamonu, Turkey

*Corresponding author: bseckin@sinop.edu.tr

rapid increase in ambient heat of 10 to 15°C being defined as heat stress (Lipiec *et al.* 2013). The duration and intensity of high heat determines the effects of heat stress on plant growth, potentially including permanent damage to plant growth and development (Lal *et al.* 2022). Heat stress causes direct and indirect effects that can result in plant death due to protein and lipid denaturation, mitochondrial death, and disruption of membrane stabilization (Kumar & Kaushik 2021).

Nanotechnology is an effective method to increase crop yield and ensure plant sustainability (Mohapatra *et al.* 2023). Nanoparticles can improve agricultural production by providing effective solutions to many agrarian problems via the active role they play in the interaction between atomic or molecular structures (Chen *et al.* 2016; Prasad *et al.* 2017; Shang *et al.* 2019). Nanoparticles range in size from 1 to 100 nm in diameter and can be synthesized by many methods to have different physico-chemical and biological properties than the same substance at a larger size (Singh *et al.* 2018; Cele 2020). Nanoparticles can protect cells against lipid, protein and DNA denaturation and cell membrane damage caused by oxidative stress due to the formation of ROS (Kumar *et al.* 2017). Nanoparticles, when applied to plants, have been reported to regulate the harmful effects of environmental stress and the adaptation mechanisms of plants, as well as seed germination, development, and other positive effects (Paparella *et al.* 2015; Guha *et al.* 2018; Zulfiqar & Ashraf 2021). Titanium dioxide nanoparticles (TiO_2NPs) can have various profound effects on the morphological, physiological, and biochemical properties of some plant species (Gohari *et al.* 2020). The application of TiO_2NPs has been reported to improve rubisco and antioxidant enzyme activities, photosynthetic rate, and chlorophyll formation, thereby increasing crop yield (Lateef *et al.* 2018). Particularly in the agricultural sector, nanoparticle application has been shown to provide salinity tolerance as an effective method to increase productivity under stressful environmental conditions (Ahmad & Akhtar 2019; Avestan *et al.* 2019; Abdoli *et al.* 2020; Alabdallah & Alzahrani 2020; Ye *et al.* 2020). In addition, TiO_2NPs have been reported to alleviate salt stress in broad bean plants under saline conditions by increasing plant growth; antioxidant enzyme activities; soluble sugars, amino acids, proline, phenolics and leaf chlorophyll contents; antioxidant capacity; and yield, while reducing H_2O_2 and MDA contents (Abdel Latef *et al.* 2018). Additionally, Pérez-Zavala *et al.* (2022) reported that TiO_2NPs activate a transcriptional response to osmotic stress in *Arabidopsis* plants. Similarly, Thakur *et al.* (2021) determined that TiO_2NPs cause an increase in biomass by increasing the adverse effects of heat stress. They reported that TiO_2NPs increase defense mechanisms, including against heat exposure and increased ROS production and some natural antioxidants (fenol and flavonoid) in plants (Zafar *et al.* 2016). In addition, it has been shown that TiO_2NPs affect membrane repair and improve growth and development of morphological structures under heat stress, and are effective at healing damage to chloroplasts due to heat stress (Faran *et al.* 2019; Younis *et al.* 2020; El-Saadony *et al.* 2021). In contrast, TiO_2NP treatment via foliar spraying did not ameliorate drought stress in *Helianthus annuus* plants (Ramadan *et al.* 2022). Lastly, Kumar *et al.* (2023) determined that TiO_2NP (100 µg/ml) application alters nutrient levels, growth and the antioxidant defense system of *Mentha arvensis* because of its toxicity.

The importance of safflower (*Carthamus tinctorious* L.) stems from the demand for its seed oil (Zemour *et al.* 2021). Safflower oil is rich in oleic acid (omega-9) and reaches up to 75% linoleic acid (C18-2), an essential fatty acid for humans, making it an important food source. Also important for human health is its high α-tocopherol content (Sujatha 2002). The safflower plant is very sensitive to salt and heat during germination and early seedling development. The safflower plant adapts easily to the climatic conditions of Turkey and so breeding studies are regularly carried out to obtain high-yield, high-quality, and stress-resistant varieties for agriculture. In addition to these long-term studies, there is a need to better understand the dimensions of the stress factors to which safflower is exposed, how they affect the plant, and what solutions can be taken. Therefore, this study aimed to elucidate the mechanism of action of TiO_2NPs for two different safflower seed cultivars — Balci and Dinçer — when under salt and heat stress.

Materials and Methods

Materials

Balci and Dinçer safflower cultivars were obtained from the Gecit Kuşağı Agricultural Research Institute of the Ministry of Agriculture and Forestry of the Republic of Turkey. Titanium dioxide (TiO_2) nanoparticles (20 nm in diameter, 99.9% purity) were purchased from Nanography Nanotechnology.

Experiment preparation

Seeds were submitted to surface sterilization in 1% sodium hydrochloride solution for 10 minutes, followed by five washes with distilled water. The seeds were then placed on filter paper and dried at room temperature. Next, the seeds were kept for 18 hours in a 200 ppm TiO_2NP solution that was prepared in advance using distilled water. The seeds in solution were then exposed to ultrasonic vibration twice, one hour at the beginning of treatment and 30 minutes at the end, followed by three washes with distilled water and drying on filter paper.

Seeds were sowed in petri plates lined with filter paper and provisioned with 3 ml of distilled water. Ten seeds were sowed per plate, which were placed in a dark environment

for germination. After three days, the plates were divided into groups for salt and heat stress treatments, with three replicates per cultivar. Preliminary experiments were performed with nanoparticle concentrations of 100, 200 and 500 ppm TiO_2NPs to determine the most effective salt concentration (50 and 100 mM NaCl) and heat treatment (36,40 and 45 °C), resulting in the selection of 50 mM NaCl and 45 °C, resepctively. Safflower seedlings are shown in Figs. 1-4.

Salt stress treatment

Treatments for the salt stress experiment included the following: TiO_2NP treatment (control) = pretreatment of 200 ppm TiO_2NPs; NaCl treatment = 50 mM NaCl concentration; and TiO_2NP + NaCl treatment (interaction) = 200 ppm TiO_2NPs + 50 mM NaCl. Salt and interaction treatments received 3 ml of 50 mM NaCl solution. Length and fresh and dry weight were determined for the radicle and plumula four days after salt stress treatment (Fig. 1).

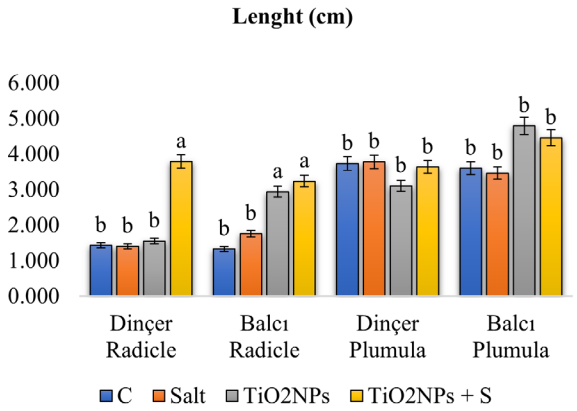

Figure 1. The effects of TiO2NP treatment on radicle and plumula length lenght of safflower cultivars (Balci, Dinçer) under salt stress. C:Control, Salt: 50 mM NaCl, TiO2NP, 200 ppm; TiO2NP+S: 200 ppm +50 mM NaCl.

Heat stress treatment

Treatments for the heat stress experiment included the following: TiO_2NP treatment (control) = pretreatment of 200 ppm TiO_2NPs; 45 °C treatment = 45 °C for six hours throught two days; and TiO_2NP + 45 °C treatment (interaction) = 200 ppm TiO_2NPs + 45 °C. Length and fresh and dry weight were determined for the radicle and plumula (Fig. 2).

Growth analysis

Growth was analyzed after salt stress and heat stress treatments according to Karagüzel *et al*. (2004), using length and fresh and dry weight of radical and plumula. Radicle and plumula lengths of six germinated seeds taken randomly from each replicate were measured (caliper) and averaged. Radicle and plumula fresh weights (mg/seed) of six germinated seeds taken randomly from each replicate were weighed (0.001 g precision balance). Radicle and plumula dry weights (mg/seed) of six germinated seeds taken randomly from each replicate were determined by rapid drying at 80°C for 24 hours, then weighing (0.001 g precision balance). Average radicle and plumula fresh and dry weights were calculated for the six seeds of each treatment. All measurements were done in triplicate.

Lipid peroxidation level (MDA)

The amount of MDA, the end product of lipid peroxidation, was determined according to Madhava Rao and Stresty (2000), using thiobarbituric acid (TBA) reaction for radicle and plumula samples of six germinated seeds taken randomly from each replicate after salt stress or heat stress treatment. Leaf samples (0.5 g) of each treatment were homogenized by adding 2.5 ml of trichloroacetic acid (TCA). Homogenates were then centrifuged at 10,000 x g for 5 min at 4 °C. The reaction mixture containing TBA and TCA was then pipetted into test tubes containing the

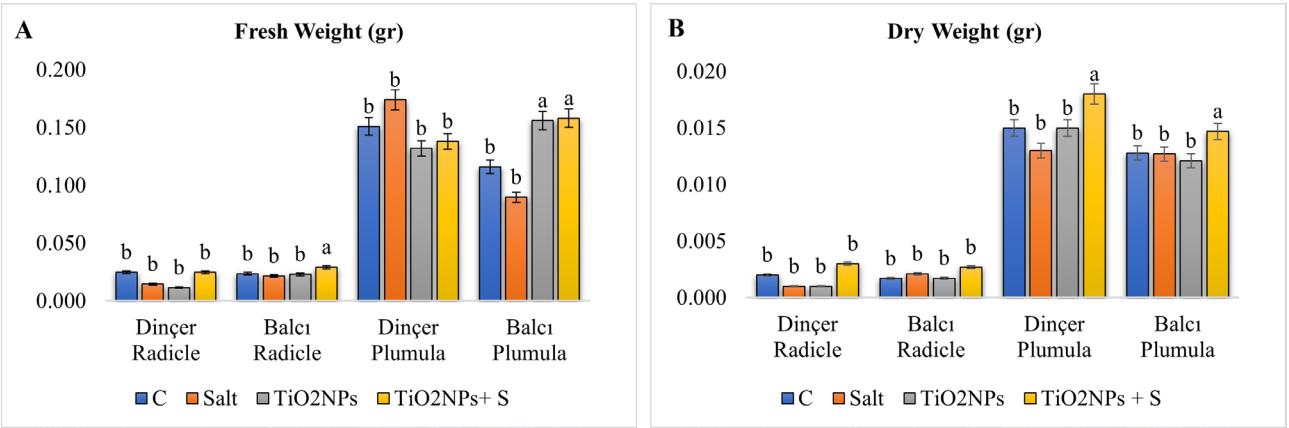

Figure 2. The effects of TiO2NP treatment on dry and fresh weight of safflower cultivars (Balci, Dinçer) under salt stress. C:Control, Salt: 50 mM NaCl, TiO2NP, 200 ppm; TiO2NP+S: 200 ppm +50 mM NaCl.

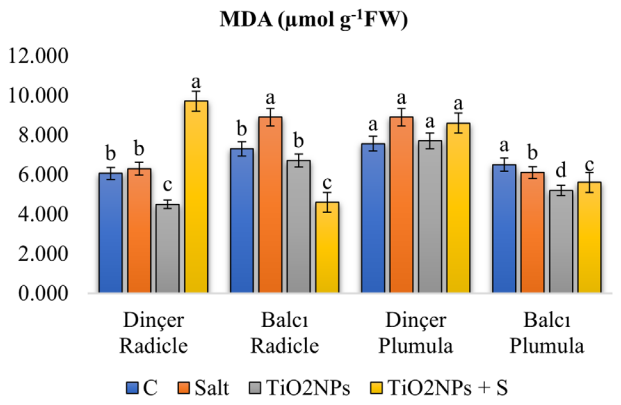

Figure 3. The effects of TiO2NP treatment on MDA content of safflower cultivars (Balci, Dinçer) under salt stress. C:Control, Salt: 50 mM NaCl, TiO2NP, 200 ppm; TiO2NP+S: 200 ppm +50 mM NaCl.

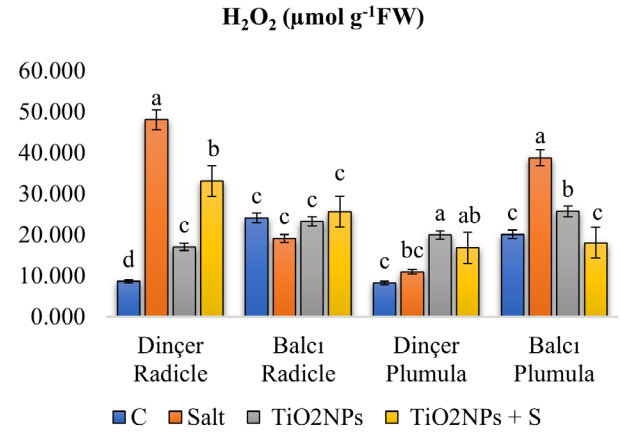

Figure 4. The effects of TiO2NP treatment on H_2O_2 content of safflower cultivars (Balci, Dinçer) under salt stress. C:Control, Salt: 50 mM NaCl, TiO2NP, 200 ppm; TiO2NP+S: 200 ppm +50 mM NaCl.

obtained supernatants. All test tubes were heated at 95 °C for 30 min. The mixture was then centrifuged at 1,000 x g for 15 min and the absorbance of the formed supernatant read 532 and 600 nm using a Thermo Scientific Genesys (10S UV-VIS) spectrophotometer. MDA concentration was calculated using the extinction coefficient 155 $mM^{-1}cm^{-1}$.

Hydrogen peroxide (H_2O_2) content

The amount of hydrogen peroxide (H_2O_2) was determined according to Velikova *et al.* (2000), by homogenizing samples (0.5 g) of each group by adding 5 ml of TCA. Homogenates were then centrifuged at 12,000 rpm for 15 min. The supernatants were then mixed with 0.5 ml of potassium phosphate buffer (10 mM, pH 7) and 1 ml of KI buffer, and their absorbance read at 390 nm using a Thermo Scientific Genesys (10S UV-VIS) spectrophotometer.

Superoxide ($O_2^{\bullet-}$) radical content

Superoxide anion radical content was determined according to Ke and Sun (2004) by homogenizing samples (0.5 g) of each group by adding 5 ml of TCA. Next, 1 ml of 1 mM hydroxylammonium chloride solution was added to 0.5 ml of supernatant and incubated at 25°C for 1 hour. Color change was observed for 20 minutes at 25°C after the addition of 1 ml of 17 mM 4-aminobenzenesulfonic acid solution and 1 ml of 7 mM naphthylamine. Specific absorbance was read at 530 nm using a Thermo Scientific Genesys (10S UV-VIS) spectrophotometer. Sodium nitrite was used as a standard solution to calculate the amount of superoxide radicals.

Statistical Analysis

All data were analyzed using the SPSS package (SPSS, Version 20.0, SPSS Inc, Chicago, IL, USA). Statistical significance was evaluated by the F-test ($P < 0.05$) and, when significant, the protected least significant difference (Protected DUNCAN) was used to separate means.

Results

Effects of TiO_2NP pretreatment on radicle and plumula lengths under salt stress

Radicle length for the Balci cultivar increased significantly (1.8 fold) under the TiO_2NP + NaCl treatment compared to the NaCl treatment (Fig. 1). Radicle length for the Dinçer cultivar increased significantly (2.7 fold) under the TiO_2NP treatment compared to the NaCl treatment (Fig. 1). Plumula length for both Balci and Dinçer cultivars did not differ significantly between the TiO_2NP + NaCl treatment and the NaCl treatment (Fig. 1).

Effect of TiO_2NP pretreatment on fresh and dry weights of radicle and plumula under salt stress

Radicle fresh weight for the Balci cultivar increased significantly (1.3 fold) under the TiO_2NP treatment compared to the TiO_2NP + NaCl treatment and the NaCl treatment (Fig. 2A). On the other hand, radicle fresh weight for the Dinçer cultivar did not differ significantly between the NaCl treatment and the TiO_2NP + NaCl treatment (Fig. 2A). Plumula fresh weight for the Dinçer cultivar did not differ significantly between the TiO_2NP + NaCl treatment and the NaCl treatment. Plumula weight of the Balci cultivar increased significantly (1.8 fold) under the TiO_2NP + NaCl treatment compared to the NaCl treatment (Fig. 2A).

Radicle dry weight for both Balci and Dinçer cultivars did not differ significantly between the TiO_2NP + NaCl treatment and the NaCl treatment (Fig. 2B). Plumula

dry weight for the Dinçer cultivar increased significantly (1.3 fold) under the TiO_2NP + NaCl treatment compare to the NaCl treatment. Likewise, plumula dry weight for Balci cultivar increased significantly (1.1 times) under the TiO_2NP + NaCl treatment (0.14) compared to the NaCl treatment (Fig. 2B).

Effect of TiO_2NP pretreatment on malondialdehyde content under salt stress

Radicle MDA for the Balci cultivar decreased significantly (1.9 fold) under the TiO_2NP + NaCl treatment compared to NaCl treatment. Plumula MDA for the Balci cultivar decreased significantly (by 7.2%) under the TiO_2NP + NaCl treatment compared to the NaCl treatment. Radicle MDA for the Dinçer cultivar increased significantly (1.5 fold) under the TiO_2NP treatment compared to the NaCl treatment Plumula MDA for the Dinçer cultivar did not differ significantly between the TiO_2NP + NaCl treatment and the NaCl treatment (Fig. 3).

Effect of TiO_2NP pretreatment on hydrogen peroxide content under salt stress

Radicle hydrogen peroxide for the Dinçer cultivar decreased significantly (1.45 fold) under the TiO_2NP + NaCl treatment compared to the NaCl treatment Radicle hydrogen peroxide for the Balci cultivar did not differ significantly between the TiO_2NP + NaCl treatment and the NaCl treatment. Plumula hydrogen peroxide for the Dinçer cultivar increased significantly (1.6 fold) under the TiO_2NP + NaCl treatment compared to the NaCl treatment. Plumula hydrogen peroxide for the Balci cultivar decreased (2.14 fold) under the TiO_2NP + NaCl treatment compared to the NaCl treatment (Fig.4).

Effect of TiO_2NP pretreatment on superoxide radical content under salt stress

Superoxide radical content ($O_2^{\bullet -}$) for the Dinçer cultivar did not differ significantly between the TiO_2NP + NaCl treatment and the NaCl treatment. Similarly, radicle $O_2^{\bullet -}$ content for the Balci cultivar did not differ significantly between the TiO_2NP + NaCl treatment and the NaCl treatment. Plumula $O_2^{\bullet -}$ content for the Dinçer cultivar decreased significantly (3.34 fold) under the TiO_2NP + NaCl treatment compared to the NaCl treatment. Plumula $O_2^{\bullet -}$ content for the Balci cultivar increased significantly (1.93 fold) under the TiO_2NP + NaCl treatment compared to the NaCl treatment (Fig.5).

Effects of TiO_2NP pretreatment on growth parameters under heat stress

Radicle and plumula lengths for both Dinçer and Balci cultivars did not differ significantly between the TiO_2NP + 45 °C treatment and the 45 °C treatment. (Fig. 6). Radicle fresh weight for both Dinçer and Balci cultivars

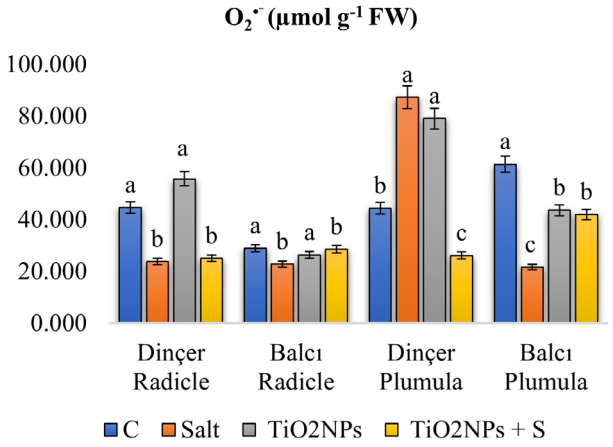

Figure 5. The effects of TiO2NP treatment on $O_2^{\bullet -}$ content of safflower cultivars (Balci, Dinçer) under salt stress. C:Control, Salt: 50 mM NaCl, TiO2NP, 200 ppm; TiO2NP+S: 200 ppm +50 mM NaCl.

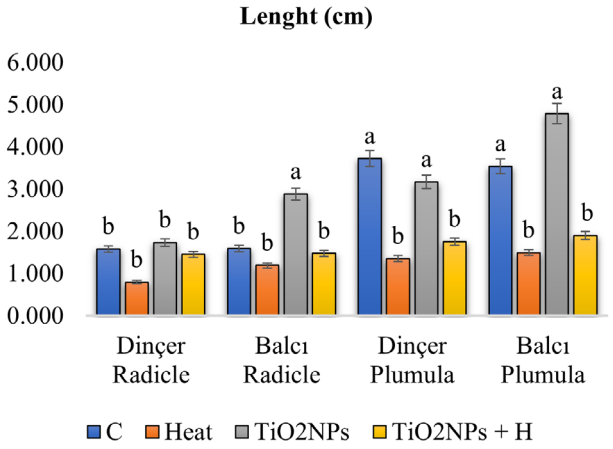

Figure 6. The effects of TiO2NP treatment on radicle and plumula length lenght of safflower cultivars (Balci, Dinçer) under heat stress. C:Control, Heat: 45 °C, TiO2NP, 200 ppm; TiO2NP+H: 200 ppm +45 °C.

did not differ significantly among treatments (Fig. 7A, B). Plumula fresh weight for both Dinçer and Balci cultivars increased with the TiO_2NP treatment compared to the 45 °C treatment. Radicle and plumula dry weights for both cultivars did not differ significantly between the 45 °C treatment and the TiO_2NP treatment (Fig. 7B). Plumula dry weight for both cultivars did not differ among treatment groups (Fig. 7B).

Effect of TiO_2NP pretreatment on malondialdehyde content under heat stress

Radicle MDA content for the Dinçer cultivar decreased (by 16.33%) under the TiO_2NP treatment compared to the 45 °C treatment (Fig. 8). In addition, radicle MDA content

for the Balci cultivar decreased (40.94%) under the TiO_2NP + 45 °C treatment compared to the 45 °C treatment (10.6). Plumula MDA content for the Dinçer cultivar decreased (22%) under the TiO_2NP + 45 °C treatment compared to the 45 °C treatment (Fig. 8). Plumula MDA content of the Balci cultivar decreased (4.16%) under the TiO_2NP + 45 °C treatment compared to the 45 °C treatment.

Effect of TiO_2NP pretreatment on hydrogen peroxide content under heat stress

Radicle hydrogen peroxide content for the Dinçer cultivar decreased (by 43.40%) under the TiO_2NP + 45 °C treatment compared to the 45 °C treatment. Radicle hydrogen peroxide content for the Balci cultivar increased (3.83 fold) under the TiO_2NP + 45 °C treatment compared to the 45 °C treatment (Fig. 9). Plumula hydrogen peroxide content for the Dinçer cultivar did not differ significantly between the TiO_2NP + 45 °C treatment and the 45 °C treatment. Plumula hydrogen peroxide content for the Balci cultivar decreased (73.61%) under the TiO_2NP + 45 °C treatment compared to the 45 °C treatment (Fig. 9).

Effect of TiO_2NP pretreatment on superoxide radical content under heat stress

Radicle superoxide radical content for the Dinçer cultivar decreasd (by 35.84%) under the TiO_2NP + 45 °C treatment compared to 45 °C treatment. Radicle superoxide radical content for the Balci cultivar decreased (51.32%) under the TiO_2NP + 45 °C treatment compared to the 45 °C treatment (Fig. 10). Plumula superoxide radical contents for both Balci and Dinçer cultivars did not differ significantly between the TiO_2NP + 45 °C treatment and the 45 °C treatment (Fig. 10).

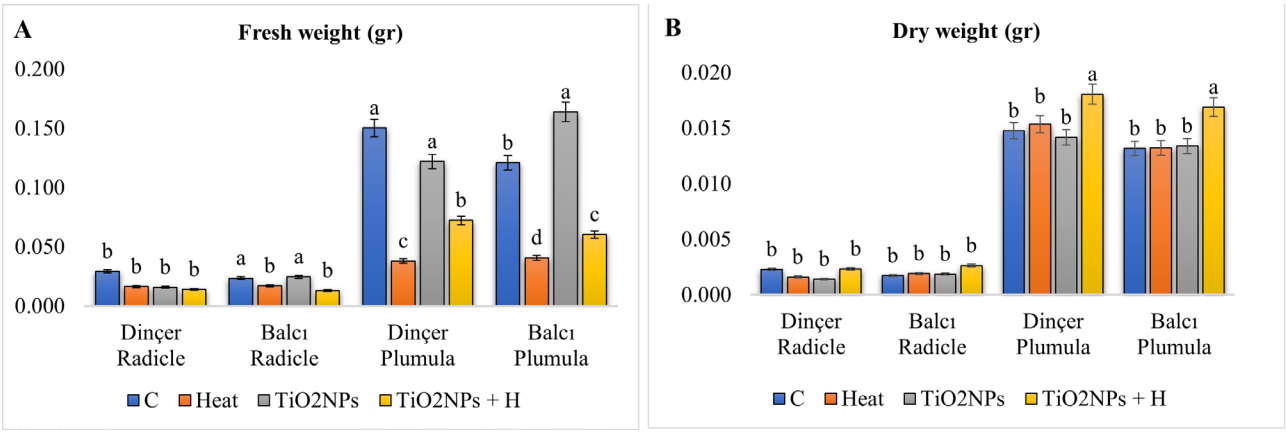

Figure 7. The effects of TiO2NP treatment on dry and fresh weight of safflower cultivars (Balci, Dinçer) under heat stress. C:Control, Heat: 45 °C, TiO2NP, 200 ppm; TiO2NP+H: 200 ppm +45 °C.

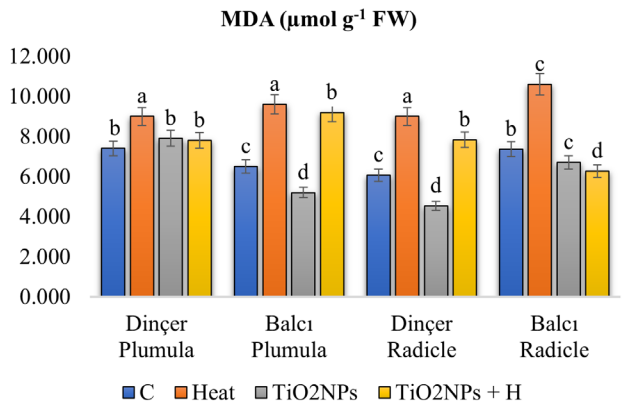

Figure 8. The effects of TiO2NP treatment on MDA content of safflower cultivars (Balci, Dinçer) under heat stress. C:Control, Heat: 45 °C, TiO2NP, 200 ppm; TiO2NP+H: 200 ppm +45 °C.

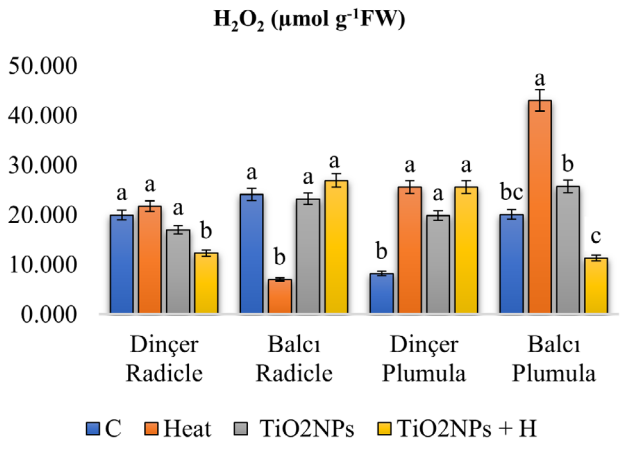

Figure 9. The effects of TiO2NP treatment on H_2O_2 content of safflower cultivars (Balci, Dinçer) under heat stress. C:Control, Heat: 45 °C, TiO2NP, 200 ppm; TiO2NP+H: 200 ppm +45 °C.

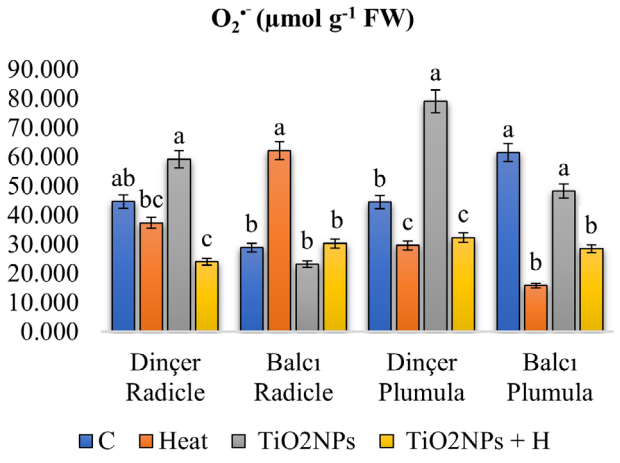

Figure 10. The effects of TiO2NP treatment on $O_2^{\bullet-}$ content of safflower cultivars (Balci, Dinçer) under heat stress. C:Control, Heat: 45 °C, TiO2NP, 200 ppm; TiO2NP+H: 200 ppm +45 °C.

Discussion

Salt stress treatment

Salt stress causes negative effects on plant growth and development in parallel with increasing amounts of NaCl and other soluble salts in the soil. Increased salt concentration in the soil solution and decreased water potential reduce the osmotic potential of plant cells and cause a series of plant reactions (Acosta-Motos *et al.* 2017). Therefore, depending on the intensity and duration of salt stress, it can affect many biological events in plants, such as growth, development, germination, cell division and photosynthesis (Farooq *et al.* 2015), and limit plant productivity and product quality in agricultural areas (Koca *et al.* 2007).

Nanoparticles, when applied to plants, may cause an elongation of the radicle and plumula after germination. In addition, the concentration and amount of salt entering the plant cell are important factors affecting plant development. Certain concentrations of TiO_2NPs are known to have positive effects on radicle and plumula length against the effects of salt that may come from outside to the plant cell structure, as well as on many other factors such as cell pressure, external pressure, and enzymes (Clément *et al.* 2013; Doğaroğlu & Köleli 2016; Zulfiqar & Ashraf 2021). The present study observed increases in radicle length for both Balci and Dinçer cultivars when receiving the TiO_2NP treatment under salinity (Fig. 1). Likewise, Liu *et al.* (2021) reported that the application of TiO_2NPs to peony (*P. suffruticosa*) plants under salt stress increased the number and length of lateral roots. It was also reported that TiO_2 nanoparticles induced growth by increasing photosynthesis and nitrogen metabolism in fennel (*Foeniculum vulgare* Mill) and broad bean plants (Khater 2016). The present results corroborate the findings of these studies, with a TiO_2NP concentration of 200 ppm having a positive effect on radicle length against a NaCl concentration of 50 mM applied to seeds of both safflower cultivars (Fig. 1).

In the present study, radicle and plumula fresh weight for the Balci cultivar increased with TiO_2NP treatment compared to the salt treatment alone, while these values did not change for the Dinçer cultivar (Fig. 2A). Similar to the present study, TiO_2NP pretreatment of *Dracocephalum moldavica* L. plants under salt stress caused an increase in root and stem fresh weights (Gohari *et al.* 2020). An interpretation of these results is that TiO_2NP pretreatment of safflower seeds under salt stress increases plant nutrient content by positively affecting the uptake of mineral elements from the soil. Nanoparticles have physicochemical properties and the potential to improve plant metabolism (Giraldo *et al.* 2014). Due to the compatible structure of nanoparticles, they can increase plant water uptake and cause an increase in fresh weight. It is thought that this increase in fresh weight could be related to increased water uptake.

The present study determined that TiO_2NPs regulate the activity of enzymes involved in nitrogen metabolism, such as nitrate reductase, glutamate dehydrogenase, glutamine synthase, and glutamic-pyruvic transaminase. This helps the plant absorb nitrate and provides the conversion of inorganic nitrogen in protein and chlorophyll structure to organic nitrogen, which increases plant fresh and dry weights (Mishra *et al.* 2014). In parallel, salt treatment with nanoparticle treatment increased safflower fresh weight. Similarly, TiO_2NP treatment increased the fresh weight of wheat plants under salt stress (Mustafa *et al*. 2021b). Another study found that TiO_2 nanoparticle treatment stimulates plant growth and reduces the negative effects of selenium (Marchiol *et al.* 2016). Although radicle dry weight was not changed with TiO_2NP treatment compared to salinity in the present study, plumula dry weight increased for both cultivars (Fig. 2B). From these results, the increase in dry weight could be explained by increased organic material accumulation with TiO_2NP treatment of safflower plants.

TiO_2NP treatment decreased radicle and plumula MDA content for the Balci cultivar, while it was not effective for the Dinçer cultivar (Fig. 3). According to the literature, nanoparticle pretreatment applied to seeds has a positive effect for different plants under salt stress (Avestan *et al.* 2019; Abdoli *et al.* 2020; Alabdallah & Alzahrani 2020). A study similar to that presented here reported that TiO_2NP treatment alleviated the effects of salinity on the cell membrane and stimulated the defense system to reduce MDA (Sheikhalipour *et al*. 2021). In a study conducted with fava bean plants under salt stress found that TiO_2NP treatment regulated growth parameters and decreased MDA content, and that this was achieved by increasing antioxidant enzyme activities (Abdel Latef *et al.* 2018). In addition, Shah *et al.* (2021) reported that TiO_2NPs applied to maize seeds with seed-induced proline accumulation (Kishor *et al.* 2005) decreased electrical conductivity and reduced MDA content by adjusting osmotic potential.

Similar to MDA, the hydrogen peroxide results of this study showed an alleviation effect of TiO_2NPs (reduced hydrogen peroxide content) in the plumula of the Balci cultivar under salinity, while this did not occur for the radicle. In addition, while these values were increased in the plumula of the Dinçer cultivar, a reduction was observed in radicle (Fig. 4). In parallel with the present results, the H_2O_2 content of plants under salinity stress and treated with TiO_2NPs was determined to be higher than for plants treated with salt alone (Karami & Sepehri 2018). The present study found a negative regulation of H_2O_2 for the plumula of the Dinçer cultivar due to different production sites, so it can be said that when H_2O_2 is increased it acts as a signal molecule. Decreased H_2O_2 and MDA content with TiO_2NP treatment of sugar grass plants (*Stevia rebaudiana* Bertoni) under salt stress was reported to be due to antioxidant enzyme activity (Sheikhalipour *et al.* 2021). Lashkary *et al.* (2021) reported that decreased H_2O_2 and MDA content caused an increase in the antioxidant system and osmolyte and biomass accumulation in the marigold *Calendula officinalis* L. under salt stress. In addition, it is thought that decreased radicle H_2O_2 content may be achieved by increased enzyme activities of the related defense system.

The results showed that $O_2^{\bullet-}$ radical content only had a healing effect for the Dinçer cultivar when under salinity stress and TiO_2NP pretreatment (Fig. 5). Singh *et al.* (2021), found that ZnNP treatment of flax (*Linum usitatissimum*) plants under salt stress reduced $O_2^{\bullet-}$ radical content. Evaluation of these results leads to the interpretation that increased H_2O_2 content improves oxidative damage to the cell membrane by increasing superoxide dismutase (SOD) enzyme activity, which ensures water balance in plants under salt stress, provides ionic balance, and decreases electrical conductivity.

Heat stress treatment

TiO_2NP pretreatment increased root length, shoot length, fresh weight, and dry weight of wheat under well-irrigated and drought conditions (Mustafa *et al.* 2021a). The current study found no change in radicle and plumula lengths for the Balci and Dinçer cultivars under heat and TiO_2NP treatment, compared to heat alone (Fig. 6). In contrast, a study of wheat determined that Zn/TiO_2NPs improve root length and plant water condition by increasing water uptake under heat stress (32 °C) (Thakur *et al.* 2021). In addition, the application of SiO_2NPs to barley plants under drought stress reduces $OH^{\bullet-}$ radical content and membrane damage, and increases stem length and relative water content (Yildiz 2018). TiO_2NP treatment of plants has been shown to accelerate growth under normal conditions and drought stress, and has been used in different concentrations for different plants under adverse environmental conditions (Selahvarzi *et al.* 2020; Sattari & Khayati 2020). Thakur *et al.* (2021) reported that pretreatment with different concentrations of ZnNPs caused increases in root and stem lengths of wheat plants under heat stress. TiO_2NP treatment of plants from seed was found to ameliorate damage caused by heat stress by increasing plant photosynthetic capacity (closure of stomata, transpiration rate, regulation of electron transfer), thus regulating plant growth (Qi *et al.* 2013).

The photosynthesis mechanism is fundamental in plants and one of the structures most affected by abiotic stress factors. TiO_2NP treatment has been reported to increase the rate of photosynthesis by decreasing oxidative damage under stress conditions, with positive effects on plant growth and improved crop yield by increased production (Mohammadi *et al.* 2014; Singh & Lee 2016). In the present study, TiO_2NP pretreatment (200 ppm) of seeds of Balci and Dinçer safflower cultivars under heat stress (45 °C) did not show any significant differences from the other treatments for radicle fresh weight (Fig. 7A, B). In contrast, external treatment with TiO_2NPs and ZnNPs caused an increase in fresh and dry weights of plants of two wheat cultivars under water deficit (El-Bassiouny *et al.* 2022). The present study found an increase (1.9 times) in plumula fresh weight for the TiO_2NP treatment of the Dinçer cultivar under heat stress compared those not receiving the treatment and under heat stress. Plumula fresh weight increased (1.5 fold) with the heat + TiO_2NPs treatment (Fig. 7A). Similar to the present study, foliar TiO_2NP treatment of tomato plants under heat stress was reported to increase photosynthesis rate and leaf water condition (Qi *et al.* 2013).

Considering these results, heat treatment positively affected radicle and plumula MDA content by causing a decrease for both safflower cultivars. Thus, it can be said that the reason for this protection from MDA is that TiO_2NP treatment provides cell membrane repair, ionic balance, and preservation of cell membrane integrity (Faran *et al* 2019; Thakur *et al.* 2021). The present study found an increase in hydrogen peroxide content in the radicle of the Balci cultivar, but a decrease in its plumula (Fig. 9). In addition, these values were decreased in radicle of the Dinçer cultivar, with no change in its plumula. In parallel with this, hydrogen peroxide has been reported to act as a signal molecule due to differences in the location of its production (Nazir *et al.* 2020), and decreased H_2O_2 content is mediated by catalase (CAT) and glutathione peroxidase (GPX) enzyme activities and the involvement of phenol and flavonoids in ROS scavenging (Zafar *et al.* 2016).

Based on these results, TiO_2NPs applied to broad bean (*Vicia faba* L.) plants under water deficit stress caused a decrease in the amount of $O_2^{\bullet-}$ radicals by stimulating the defense system, providing water balance through osmolyte accumulation, and causing an increase in internal nitric oxide (Khan *et al.* 2020). In addition, Shoarian *et al.* (2020) reported that TiO_2NP treatment under drought stress was effective at scavenging ROS by stimulating SOD enzyme activity. The present study found a decrease in $O_2^{\bullet-}$ radical content for the radicle of both cultivars under heat stress (Fig. 10), which can be explained by the decrease in H_2O_2 content and the increase in SOD enzymatic activity in the scavenging of $O_2^{\bullet-}$ radicals.

Conclusion

This study is the first to investigate the effect mechanisms of TiO_2NP application for two safflower cultivars (Balci and Dinçer) under salt and heat stress. TiO_2NP pretreatment positively affected both cultivars under heat stress, but only the Balci cultivar under salt stress. This improvement seems to be achieved by the stimulation of the antioxidant defense system or by preserving cell membrane integrity. Considering the results, how TiO_2NP pretreatment under salt and heat will affect safflower plants at the seedling stage remains a matter of curiosity. In addition, changes in the related antioxidant defense system need to investigate using biochemical and molecular approaches.

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