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ORIGINAL ARTICLE

Titanium dioxide nanoparticles (TiO_2-NPs) enhance drought tolerance and grain yield of sweet corn (*Zea mays* L.) under deficit irrigation regimes

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Abstract

Among environmental stresses, drought is the main limiting factor of crop yield. The use of nanomaterials has been considered a strategy to enhance the drought tolerance of plants. To evaluate the beneficial effect of titanium dioxide nanoparticles (TiO_2-NPs; 0 (T_0), 50 (T_{50}), and 100 (T_{100}) mg l^{-1}) and deficit irrigation (70 as control (I_{70}), 105 (I_{105}), and 140 (I_{140}) mm evaporation from Class A evaporation pan) on drought tolerance of sweet corn cv. chase, an open field experiment was performed in 2018–2019. The results showed that deficit irrigation decreased the maximum efficiency of PSII (F_v/F_m), but T_{50} improved F_v/F_m. 50 mg l^{-1} TiO_2-NPs enhanced leaf SOD, APX, and CAT antioxidant activities by 17%, 10%, and 24%, respectively, over the control. $I_{140}T_{50}$ increased leaf proline content 8% and 17% over $I_{140}T_0$ and $I_{140}T_{100}$, respectively. Grain number per ear with the highest correlation with grain yield ($r = 0.97$) was the most determining grain yield component. The greatest grain yield was obtained from I_0T_{50} and $I_{105}T_{50}$ (760 and 809 g m^{-2}, respectively). Therefore, $I_{105,}$ due to less water consumption, and T_{50} may be considered to produce sweet corn with a desired performance under water-limited conditions.

Keywords Antioxidant enzymes · Chlorophyll fluorescence · Irrigation interval · Maximum efficiency of PSII · Proline

Abbreviations

CAT	Catalase
ETC	Electron transport chain
F_v/F_m	The maximum efficiency of photosystem II
POX	Peroxidase
ROS	Reactive oxygen species
RWC	Relative water content
SOD	Superoxide dismutase
TiO_2-NPs	Titanium dioxide nanoparticles

Introduction

Sweet corn (*Zea mays* L.) is one of the varieties of the corn plant and a type of mutant isolated from dent corn by a mutation in the locus *SU*, which increases sugar accumulation twice as the regular corn (Levitt 1980). Sweet corn is the second most valuable crop among vegetables for the processing industries (canning and freezing) and the fourth for fresh consumption and is rich in protein, vitamins, sugar, and minerals, such as calcium, potassium, phosphorus, and magnesium (Kalloo and Bergh 2012). Nonetheless, sweet corn productivity is adversely affected by global water scarcity and increasing water use efficiency and drought tolerance of plants, here sweet corn, is critical.

Among environmental factors, drought is the second main limiting factor of plant productivity after pathogens (Biglouei et al. 2010) and the most critical abiotic stress that has limited the production of ~ 25% of arable lands worldwide. One of the most important adaptation strategies of plants to stressful conditions is alterations in plant physiology and biochemistry, leading to impact the plant productivity (Liu et al. 2011). When plants are exposed to drought stress, they adapt to such conditions by morphological and

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physiological changes (Wang and Huang 2004). Water shortage is a critical problem limiting maize growth through impacts on anatomical, morphological, physiological, and biochemical processes (Bänzinger 2000). Among the drought stress damages, the adverse effects on the photosynthetic apparatus can be significant, which is associated with a decrease in the chlorophyll content. These, ultimately lead to an accumulation of protective pigments, including carotenoids and anthocyanins (Farooq et al. 2009). Photosynthesis is a crucial factor in plant survival and improving its performance under stressful conditions would increase plant productivity.

The use of nano-photocatalysts has been introduced as a promising way to increase light absorption and photosynthetic efficiency (Kirschbaum 2011). Plant physiologists have considered titanium dioxide nanoparticles (TiO_2-NPs) as a photocatalyst with biological properties (Qi et al. 2013). TiO_2-NPs reduces the destructive effects of environmental stresses through the reinforcement of the electron transport chain (ETC), improving the photosynthetic efficiency of PSII and photophosphorylation of chloroplasts, stimulation of the activity of Rubisco, nitrate reductase, and antioxidant enzymes, as well as reducing the oxygen-free radicals (Lei et al. 2007; Mishra and Kannan 2014). Results of a study revealed that *Arabidopsis thaliana* plants treated with TiO_2-NPs showed severely disrupted stomatal guard cells and the microtubular network in epidermal cells (Wang et al. 2011). NPs enter the leaves through stomata and their deposition on the stomatal cavity cell wall or in neighboring cells (Birbaum et al. 2010) may reduce stomatal conductance, leading to reduced transpiration and photosynthetic rate.

Studies indicate that foliar application of titanium (Ti) has been able to stimulate plant growth (Jaberzadeh et al. 2013). It has been shown that the quantum effects and photocatalytic activities of TiO_2-NPs are greater than those of the non-nano types (Gao et al. 2006). This nanoparticle in the form of foliar spray and root nutrition increased nitrogen uptake due to the stimulation of nitrate reductase activity and photosynthetic rate and instigated the synthesis of amino acids and proteins (Saber et al. 2013). A most recent study showed that nanoparticles potentially mediate salt stress tolerance through efficient antioxidant defense systems and other key metabolic activities in plants (Zulfiqar and Ashraf 2021). Foliar application of TiO_2 at a concentration of 0.01% increased the peroxidase enzyme activity, soluble sugars and proline content, and ear weight and decreased malondialdehyde content of sweet corn (*Zea mays* var. *saccharata*) leaves under water deficit stress (Sharghi and Khalilvand Behrouzyar 2020).

To deal with drought stress, plants increase the synthesis and accumulation of soluble and osmotic active substances (*osmolytes*). These substances help maintain the plant water absorption under these conditions by reducing the cell osmotic potential. Proline is one of the most commonly known osmolytes, the innate plant response to osmotic and drought stresses in many plants (Cattivelli et al. 2008). In addition to osmotic regulation, this osmolyte also plays a protective role for macromolecules and prevents their deformation under stressful conditions (Esra et al. 2010). Soluble sugars, mainly sucrose, also play an essential role in the osmotic regulation of cells and protecting macromolecules and the cell membranes during drought stress (Rafinezhad et al. 2016). Soluble sugars help maintain the cell turgor by stabilizing the cell membranes, i.e., plants with higher soluble sugars have a better osmotic regulation ability (Slama et al. 2007).

In arid and semi-arid climates of the world, water scarcity is becoming a severely limiting factor to plant production. Significant maize (*Zea mays* L.) yield losses from drought are expected to increase with global climate change as temperatures rise and rainfall distribution changes (Campos et al. 2004). Hence, it is necessary to pay attention to drought stress effects on different stages of plant growth. It is also necessary to screen the drought-resistant plant species and recognize their strategies to deal with the water shortage damages (Dai et al. 2012). Such C_4 plants as corn can be considered a good suggestion for hot and dry regions, because they have a relatively high tolerance to water shortage besides their high fertility strength and nutritional value (Khazaei et al. 2016). Hence, we aimed to evaluate: (1) the drought tolerance of sweet corn cv. Chase under deficit irrigation regimes, (2) the efficiency of TiO_2-NPs to ameliorate the adverse effects of drought stress on sweet corn productivity, (3) how physiochemical traits related to the improvement of the crop yield would be affected by TiO_2-NPs, and (4) whether sweet corn can be cultivated with lower water consumption without significant yield loss.

Materials and methods

Field practices and sowing

The experiment was carried out at the research field of the Vali-e-Asr University of Rafsanjan during the 2018–2019 growing season (55° 55′ N, 30° 22′ W, 1526 m asl). Rafsanjan, with a mean annual precipitation of 75.5 mm, is classified as an arid climate based on the Domarten climatic classification. Three levels of irrigation treatments (70 as control, 105, and 140 mm evaporation from the class A evaporation pan; I_{70}, I_{105}, and I_{140}, respectively (Khalili et al. 2013), and three levels of foliar application of titanium dioxide nanoparticles (TiO_2-NPs; TiO_2, anatase, 99 + %, 10–25 nm) (zero; as the control), 50, and 100 mg l^{-1}; T_0, T_{50}, and T_{100}, respectively) were considered as the experimental factors (Bayazidi Aqdam 2014). The class A evaporation pan

Table 1 Irrigation water properties and water consumption for each treatment during the growing season

Irrigation levels (evaporation form class A pan (mm))	Irrigation (number)	Total water consumption (m^3 ha^{-1})	EC (dS m^{-1})	TDS (mg l^{-1})	pH
70[a]	12	9128	1.9	1197	7.8
105	10	7250			
140	8	5412			

[a]70: control, 105: moderate water deficit, 140: severe water deficit, TDS: total dissolved salts, EC: electrical conductivity

was located in an Agricultural Research Center in the vicinity of the experimental site. Weather data was collected daily and the time of irrigation water was calculated accordingly.

Uniform sweet corn seeds (cv. Chase) were hand-sown in early July in 4–5 cm soil depth and four rows with 40 cm apart and three meters long giving 12.5 plants.m^{-2} in plots of 2×3 m. The field was surface irrigated right after sowing, and the plants were then irrigated weekly for 4 weeks. One month after planting (at the 4–6 leaf stage), irrigation treatments were applied based on water evaporation from the class A evaporation pan (mm day^{-1}) (Table 1). Simultaneously, foliar spraying of TiO_2-NPs at the rate of ~ 416 l ha^{-1} was applied by a handheld sprayer twice with an interval of 2 weeks (at the 4–6 leaf stage and 2 weeks after that). The control plants were sprayed with water. Weeding was done twice per month by hand. Nitrogen as urea (46% N) was applied twice (at the V_5 and V_{12}; 5-leaf and 12-leaf stages, respectively) at the rate of 66 N kg ha^{-1} each time during the experiment. There was no precipitation during the experimental procedure (Fig. 1). The physicochemical characteristics of the experimental soil are represented in Table 2.

Sampling and measurements

Shortly before the beginning of pollination, the efficiency of photosystem II (F_v/F_m) was measured. Simultaneously, three samples from each replication (each treatment including the

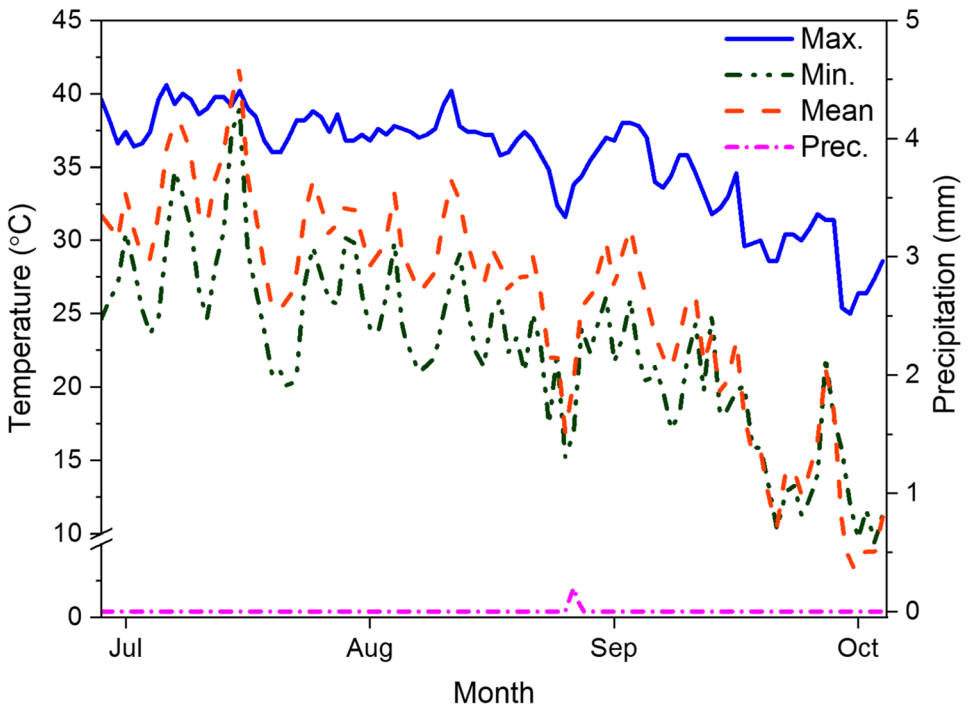

Fig. 1 Climatic characteristics during the experiment

Table 2 Physicochemical characteristics of the experimental field soil

Soil												
pH	Mn	Zn	Cu	Fe	P	K	N	OM[a]	ST	BD	FC	PWP
	mg kg^{-1}						%			(g cm^{-3})	%	
8.12	7.88	0.47	0.73	1.24	9	391	0.13	0.87	Loam sand	1.5	25	9

[a]*OM* organic matter; *ST* soil texture; *BD* bulk density; *FC* field capacity; *PWP* permanent wilting point

control) were collected (55 days after emergence (DAE) equal to 10 days after the last foliar spraying) from the youngest fully expanded leaves. The samples were then immediately frozen in liquid nitrogen and kept at −75 ℃ for further physiochemical analysis.

Leaf physiology

Leaf chlorophyll fluorescence

A handheld PEA Chlorophyll Fluorimeter (Hansatech, UK) was used to measure the leaf chlorophyll fluorescence. The youngest fully expanded leaves were considered for measurements. The clips were installed on three leaves of three plants of each treatment at the middle hours of the day (11:00–13:00 h). After 15 min of darkness, the fluorescent device was placed on the clips, and the parameters F_m and F_v, the dark-adapted maximum and variable fluorescence of photosystem II, respectively, were recorded (Ahmadi-Lahijani et al. 2018).

Leaf relative water content (RWC)

The youngest fully developed leaves (ear leaves) of each treatment were separated and wrapped in a foil and immediately transferred to the laboratory and weighted (fresh weight; FW). The leaves were then placed in vials containing distilled water for 24 h in the dark and immediately weighed after drying out of the leaf outer surfaces by a paper towel (turgid weight; TW). The leaves were then placed in an oven at 70 °C to constant weight and weighed (dry weight; DW). Finally, the leaf RWC was calculated using the following Eq. (1) (Ritchie et al. 1990):

$$\mathrm{RWC} = \left[\frac{\mathrm{FW} - \mathrm{DW}}{\mathrm{TW} - \mathrm{DW}}\right] \times 100 \quad (1)$$

Leaf biochemistry

Leaf photosynthetic pigments

Fresh leaves (0.25 g) were completely homogenized in 5 ml 80% acetone using a mortar and pestle, then the solution was brought to a volume of 25 ml, and the absorption was read at 646, 663, and 470 nm. The following equations were used to calculate chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), total chlorophyll (Chl *t*), and carotenoid content (Lichtenthaler and Wellburn 1983).

$$\mathrm{Chl}a = (12/25 \quad A663 - 2/79 \quad A646) \quad (2)$$

$$\mathrm{Chl}b = (21/21 \quad A646 - 5/1 \quad A663) \quad (3)$$

$$\mathrm{Chl}t = \mathrm{Chl}a + \mathrm{Chl}b \quad (4)$$

$$\mathrm{Carotenoids} = (1000A470 - 1/8\mathrm{Chl}\ a - 85/02\mathrm{Chl}\ b)/198 \quad (5)$$

Leaf sucrose and soluble sugars content

Fresh leaves (0.5 g) were ground with 5 ml 95% ethanol in a mortar and pestle, and the mixture was poured into a falcon tube. The solution was centrifuged at 3500 rpm for 10 min. Then, 0.2 ml from the extract was separate, and 0.1 ml 30% KOH was added to all samples and kept at 100 °C for 10 min. After cooling at room temperature, 3 ml of anthrone was added to each sample and placed at 40 ℃ for 15 min. When the samples were cooled, the absorption was read at 620 nm using a blank anthrone solution. The standard sucrose curve was plotted at concentrations of 0, 20, 40, 60, 80, 100 µg ml^{-1} (van Handel 1968). Three ml of fresh anthrone were added to 0.1 ml of the extract (described above) (150 mg of anthrone and 100 ml 72% sulfuric acid) were used to measure soluble sugars. The mixture was heated in a water bath for 10 min, then the absorption rate at 625 nm was read, and the soluble sugars content was calculated based on the standard curve of different glucose concentrations (Irigoyen et al. 1992).

Leaf proline content

Fresh leaves (0.5 g) were ground with 5 ml 95% ethanol in a mortar and pestle and transferred to the falcon tube. The extraction was repeated twice, and each time with 5 ml 70% ethanol, the resulting mixture was centrifuged for 10 min at 3500 rpm, and the supernatant was used to extract proline. Proline standards were also prepared with L-Proline at concentrations of 0, 2, 4, 16, 32 mg l^{-1}, and the standard curve was plotted. The sample concentration was calculated in ppm by replacing the data in the standard curve slope (Bates et al. 1973).

Preparing extract to assess leaf protein and antioxidant content

Fresh leaf samples (500 mg) stored in −75 °C were grounded and extracted in 5 ml 50 mM potassium phosphate buffer with a pH of 7.5 containing Polyvinylpyrrolidone (PVP) and EDTA 1 mM. All extraction procedures were done on ice. The extracts were centrifuged for 20 min at 4000 rpm at 4 °C. The solution was used to measure the leaf enzymatic and protein content of leaves.

Leaf total protein content

The extract (20 µl) was diluted in 80 µl of the extraction buffer, and 5 ml of the fresh dye Coomassie blue (Bradford reagent) was added to it and stirred for 2 min. After 5 min, the absorption rate was read at 595 nm using a spectrophotometer (Spekol-1500). The sample's protein concentration was calculated according to the spectrophotometer absorption and using the standard curve obtained from bovine albumin serum (BSA) (Bradford 1976).

Superoxide dismutase (SOD) (EC 1.15.1.1)

The extract (50 µl) was mixed with 1 ml of the SOD measurement solution (including 50 ml potassium phosphate buffer with a pH of 7.8, 75 µM NBT, 13 ml L-methionine, 0.1 ml EDTA, and 2 µM riboflavin) and placed in the light chamber for 15 min to react with this mixture. Then, the absorption was read at 560 nm (Beauchamp and Fridovich 1971).

Catalase (CAT) (EC 1.11.1.6)

The extract (50 µl) was mixed with 1 ml of the catalase measurement solution (including 50 mM KH_2PO_4 buffer with a pH of 7 and 15 mM H_2O_2). The absorption was recorded at 240 nm for 1 min. One catalase enzyme unit is equivalent to the breakdown of 1 $mM.min^{-1}$ H_2O_2 (Dhindsa et al. 1981).

Peroxidase (POX) (EC 1.11.1.7)

The extract (50 µl) was mixed with 1 ml of the ascorbic peroxidase solution (including 50 mM KH_2PO_4 buffer with a pH of 7, 0.1 mM EDTA, 0.5 mM ascorbic acid (ASA), and 0.15 mM H_2O_2). The absorption was read at 290 nm for 1 min with a spectrophotometer (Nakano and Asada 1981).

Yield and yield components

Upon reaching physiological maturation (120 DAE), sampling was performed to measure the grain yield and its components from an area of 1 m^2, considering the marginal effects. To measure ear fresh and dry weight, sampling was made in the dough stage of the grains, in which three ears per replication ($n=9$) were randomly harvested and immediately weighed, and then dried in an oven at 70 ℃ to constant weight. The number of grains per ear (GN) was counted after drying in the oven. The grain weight (GW) was divided by the number of grains (GN) to measure the mean grain weight (MGW) according to Eq. 6: (ISTA 2019)

$$MGW = \left(\frac{GW}{GN}\right) \times 1000 \quad (6)$$

Four plants from the middle rows of each replicate were harvested, and the grain yield was calculated per square meter. Harvest Index (HI) was obtained by dividing the economical yield (EY) by the biological yield (BY) according to Eq. 7:

$$HI = \left(\frac{EY}{BY}\right) \times 100 \quad (7)$$

Statistical analysis

The experiment was carried out as a split-plot arrangement in a randomized complete block design with three replications. Deficit irrigation and foliar application of TiO_2-NPs, each in three levels, were considered as the main and subplots, respectively (3×3). Data were analyzed by ANOVA using SAS v.9.4 software. Mean comparison, where indicated, was determined by Duncan's test at $p \leq 0.05$. Pierson's correlation coefficient between parameters was computed when applicable.

Results

Leaf chlorophyll fluorescence

Leaf chlorophyll fluorescence was significantly affected by the interaction of irrigation treatments and foliar application of TiO_2-NPs ($p \leq 0.01$) (Table 3). The highest maximum efficiency of PSII (F_v/F_m) was recorded at $I_{70}T_{50}$, in which it was 10 and 16% greater than $I_{70}T_0$ and $I_{70}T_{100}$ F_v/F_m, respectively (Fig. 2a). Deficit irrigation significantly decreased F_v/F_m, but the foliar application of TiO_2-NPs up to 50 mg l^{-1} improved F_v/F_m at all irrigation levels. However, by applying 100 mg l^{-1} TiO_2-NPs, F_v/F_m showed a significant decreasing trend that reached less than the control at all irrigation treatments (Fig. 2a).

Relative water content (RWC)

Leaf RWC was affected by the irrigation treatments, foliar application of TiO_2-NPs, and their interaction ($p \leq 0.01$) (Table 3). Plants grown at I_{70} had the greatest RWC when 50 mg l^{-1} TiO_2-NPs was foliar sprayed; however, spraying 100 mg l^{-1} TiO_2-NPs under I_{140} conditions significantly reduced the leaf RWC, even to a value lower than the control plants (Fig. 2b). Decreasing the available soil moisture due

Table 3 ANOVA results of physiochemical traits of sweet corn leave foliar spayed with various concentrations of TiO_2-NPs (mg l^{-1}) under different irrigation regimes

S.O.V	df	Chl *a*[a]	Chl *b*	Chl t	Chl *a/b*	Carotenoids	F_v/F_m	RWC
Replication	2	ns[b]	ns	ns	ns	ns	ns	ns
Irrigation (I)	2	ns	*	ns	ns	*	**	**
Main plot error	4	ns	ns	ns	ns	ns	ns	ns
Nano-TiO_2 (Ti)	2	**	*	**	**	**	**	**
Ti × I	4	ns	ns	ns	ns	**	**	**
Subplot error	12	*	*	*	*	ns	ns	ns
C.V. (%)	–	12.5	9.5	10.7	12.5	9.3	6.2	12.2

[a]*Chl a* chlorophyll *a*, *Chl b* chlorophyll *b*, *Chl t* total chlorophyll, *Chl a/b* chlorophyll *a/b* ratio, $F_v/F_{m;}$ maximum efficiency of PSII, *RWC* relative water content

[b]ns, *, and ** represent non-significant, and significant at 0.05 and 0.01 probability levels

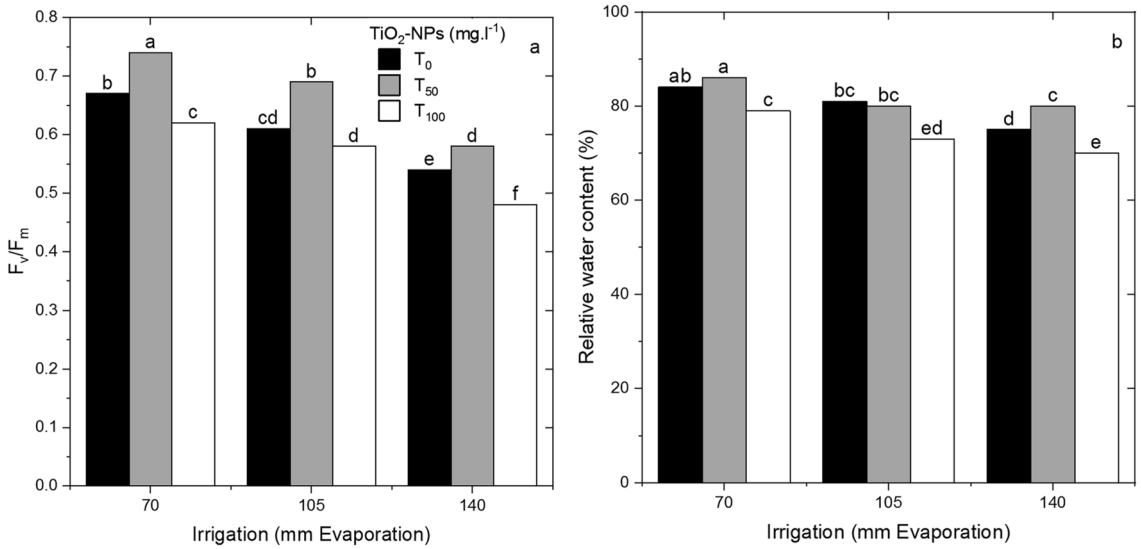

Fig. 2 Maximum efficiency of PSII (F_v/F_m) (**A**) and RWC (**B**) of sweet corn leaves foliar spayed with various concentrations of TiO_2-NPs (mg l^{-1}) under different irrigation regimes (mm water evaporation from class A evaporation pan). The control plants (70) were sprayed with water. Data presented are the means of three replicates. Means with the same letter are not significantly different based on the Duncan test $p \leq 0.05$

to deficit irrigation decreased the leaf RWC, but spraying 50 mg l^{-1} TiO_2-NPs significantly reduced the adverse effects of deficit irrigation s at all irrigation levels. For instance, the application of 50 mg l^{-1} TiO_2-NPs significantly increased leaf RWC by 6 and 12% compared with T_0 and T_{100}, respectively, under I_{140} conditions (Fig. 2b).

Leaf pigments content

Although leaf Chl *a*, Chl t, and Chl *a/b* ratio were not affected by deficit irrigation, the effect was significant on Chl *b* content ($p \leq 0.05$) (Table 3). Nevertheless, Chl *a*, Chl t, and Chl *a/b* were affected by TiO_2-NPs ($p \leq 0.01$). According to Table 4, the greatest leaf Chl *b* content was observed in T_{50}; however, the application of 100 mg l^{-1} TiO2-NPs significantly reduced Chl *b* content by 16 and 10% compared

Table 4 Pigment content of sweet corn leaves foliar spayed with various concentrations of TiO_2-NPs (mg l^{-1}) under different irrigation regimes

Treatment	Level	Chl *a*[a]	Chl *b*	Chl t	Chl *a/b*
		mg gFW^{-1}			
Irrigation (mm evaporation)[b]	70	22.5	1.64 a	24.1	13.7
	105	21.3	1.50 ab	22.8	14.2
	140	19.2	1.46 b	20.6	13.1
Nano-TiO_2 (mg l^{-1})	0	22.1 b[c]	1.55 ab	23.7 b	14.6 ab
	50	27.2 a	1.64 a	29.1 a	16.8 a
	100	18.1 c	1.41 b	19.5 c	12.5 b

[a]*Chl a* chlorophyll *a*; *Chl b* chlorophyll *b*, *Chlt* total chlorophyll, *Chl a/b* chlorophyll *a/b* ratio

[b]mm water evaporation from class A evaporation pan

[c]Data presented are the means of three replicates. Means with different letters are significantly different based on Duncan's test $p \leq 0.05$

with T_{50} and T_0, respectively. The greatest leaf Chl *a*, Chl t, and Chl *a*/*b* were observed when 50 mg l^{-1} TiO_2-NPs was foliar applied. The irrigation treatments ($p \leq 0.05$), foliar application of TiO_2-NPs, and the interaction of irrigation treatments and foliar application of TiO_2-NPs ($p \leq 0.01$) significantly affected the carotenoid content of sweet corn leaves (Table 3). In general, leaf carotenoid content was the greatest at all irrigation treatments when 50 mg l^{-1} TiO_2-NPs was foliar applied. For instance, the greatest leaf carotenoid content was observed at $I_{70}T_{50,}$ which was 32% greater than the control (Fig. 3a).

The leaf sucrose and soluble sugars content

Leaf sucrose and soluble sugars content were affected by irrigation treatments and foliar application of TiO_2-NPs ($p \leq 0.01$) (Table 5). Deficit irrigation significantly increased leaf sucrose and soluble sugars content (Table 6). The greatest leaf sucrose content was observed in I_{140}, which was 14 and 39% over I_{105} and I_{70}, respectively. Yet, foliar application of TiO_2-NPs showed a different trend, i.e., foliar application of 50 mg l^{-1} TiO_2-NPs significantly decreased leaf sucrose and soluble sugars content, while 100 mg l^{-1} TiO_2-NPs increased leaf sucrose and soluble sugars content (Table 6). For instance, 50 mg l^{-1} TiO_2-NPs decreased soluble sugars content 47% compared with T_0, whereas increasing TiO_2-NPs concentration to 100 mg l^{-1} increased soluble sugars content 31% over T_0 (Table 6).

Leaf proline content

Leaf proline content was influenced by the irrigation treatments, foliar application of TiO_2-NPs, and their interaction ($p \leq 0.01$) (Table 5). Deficit irrigation significantly increased

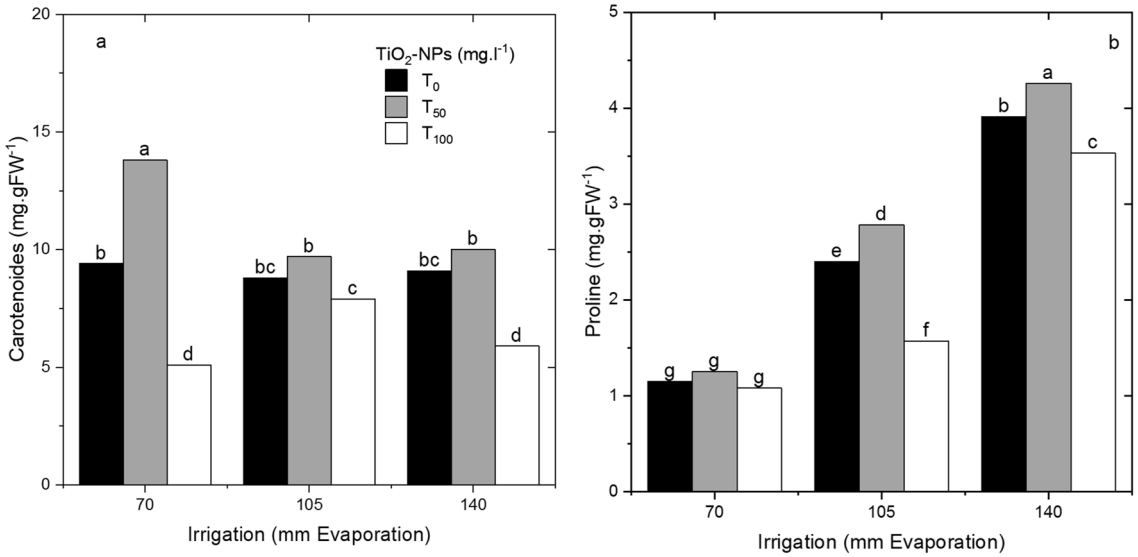

Fig. 3 Carotenoids (**A**) and proline (**B**) content of sweet corn leaves foliar spayed with various concentrations of TiO_2-NPs (mg l^{-1}) under different irrigation regimes (mm water evaporation from class A evaporation pan). The control plants (70) were sprayed with water. Data presented are the means of three replicates. Means with the same letter are not significantly different () based on the Duncan test $p \leq 0.05$

Table 5 ANOVA results of biochemical traits and antioxidant content of sweet corn leaves foliar spayed with various concentrations of TiO_2-NPs (mg l^{-1}) under different irrigation regimes

S.O.V	df	Sucrose	Soluble sugar	Proline	Protein	SOD[a]	CAT	APX
Replication	2	ns[b]	ns	ns	ns	ns	ns	ns
Irrigation (I)	2	**	**	**	**	**	**	**
Main plot error	4	ns	ns	ns	ns	ns	ns	ns
Nano-TiO_2 (Ti)	2	**	**	**	**	**	**	**
Ti × I	4	ns	ns	**	ns	ns	ns	ns
Subplot error	12	ns	*	ns	ns	ns	ns	*
C.V. (%)	–	8.9	18.6	9.3	7.5	12.4	11.6	9.2

[a]*SOD* superoxide dismutase; *APX* ascorbic peroxidase; *CAT* catalase

[b]ns, *, and ** represent non-significant, and significant at 0.05 and 0.01 probability levels

Table 6 Sucrose, soluble sugars, and protein content of leaves, and biological yield (BY) and harvest index (HI) of sweet corn plants foliar spayed with various concentrations of TiO_2-NPs (mg l^{-1}) under different irrigation regimes

Treatment	Level	Sucrose	Soluble sugar	Protein	BY	HI
	mg gFW^{-1}				g m^{-2}	%
Irrigation (mm evaporation)[a]	70	2.46 c	2.06 b	2.11 a	147 a	39.1
	105	3.46 b	2.87 b	1.96 b	139 a	38.5
	140	4.02 a	4.61 a	1.71 c	116 b	35.9
Nano-TiO_2 (mg l^{-1})	0	3.31 b	3.05 b	1.91 b	138 b	35.8 b
	50	2.74 c	2.07 c	2.14 a	150 a	41.3 a
	100	3.89 a	4.41 a	1.74 c	115 c	33.8 b

Data presented are the means of three replicates. Means with different letters are significantly different based on Duncan's test $p \leq 0.05$

[a]mm water evaporation from class A evaporation pan

leaf proline content nearly four times over the control. The greatest proline content of leaves was observed in I_{140} when 50 mg l^{-1} TiO_2-NPs was foliar sprayed. The $I_{140}T_{50}$ treatment increased leaf proline content 8 and 17% over $I_{140}T_0$ and $I_{140}T_{100}$, respectively (Fig. 3b).

Leaf soluble protein content

Leaf soluble protein content was influenced by the irrigation treatments and TiO_2-NPs ($p \leq 0.01$) (Table 5). Deficit irrigation significantly decreased leaf protein content. Plants grown at I_{105} and I_{140} had 7 and 24% lower soluble protein content, respectively, compared with the I_{70} treatment (Table 6). Foliar application of 50 mg l^{-1} TiO_2-NPs significantly increased the soluble protein content of leaves; nevertheless, increasing the concentration of TiO_2-NPs to 100 mg l^{-1} sharply decreased leaf protein content, i.e., application of 50 mg l^{-1} TiO_2-NPs significantly increased the soluble protein content of leaves by 11%, while leaf soluble protein content was decreased 23% at T_{100} compared with the control (Table 6).

Leaf antioxidant enzyme content

Leaf antioxidant enzyme content was affected by the irrigation treatments and foliar application of TiO_2-NPs ($p \leq 0.01$) (Table 5). The leaf antioxidant enzyme content was significantly increased by deficit irrigation (Fig. 4a). The greatest SOD, APX, and CAT content were recorded at I_{140}, 43, 22, and 65% greater over the I_{70}, respectively. Foliar application of TiO_2-NPs at the rate of 50 mg l^{-1} enhanced SOD, APX, and CAT content of leaves by 17, 10, and 24%, respectively, compared with the control. However, foliar application of 100 mg l^{-1} TiO_2-NPs sharply decreased the enzyme activity even to levels lower than the control (Fig. 4b).

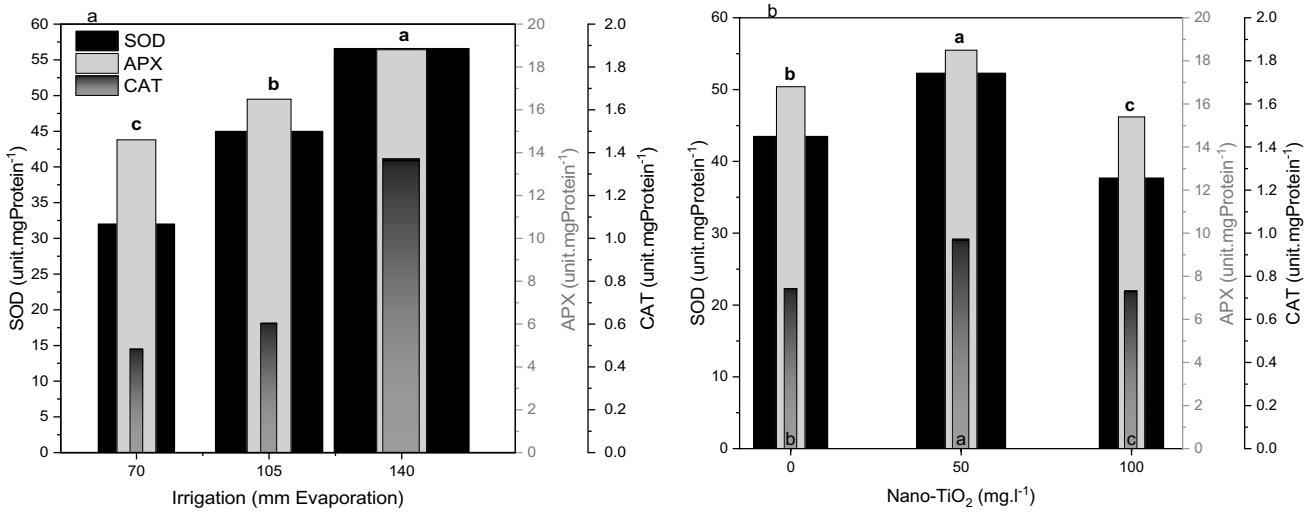

Fig. 4 Antioxidant content of sweet corn leaves at different irrigation regimes (mm water evaporation from class A evaporation pan) (**a**) and foliar sprayed with various concentrations of TiO_2-NPs (mg l^{-1}) (**b**). The control plants (70) were sprayed with water. Data presented are the means of three replicates. Means with the same letter are not significantly different based on the Duncan test $p \leq 0.05$

Table 7 ANOVA results of yield and yield component of sweet corn plants foliar spayed with various concentrations of TiO_2-NPs (mg l^{-1}) under different irrigation regimes

S.O.V	df	GN[a]	MGW	GY	BY	HI
Replication	2	ns[b]	ns	ns	ns	ns
Irrigation (I)	2	**	**	**	**	ns
Main plot error	4	ns	ns	ns	ns	ns
Nano-TiO_2 (Ti)	2	**	**	**	**	**
Ti×I	4	*	**	*	ns	ns
Subplot error	12	ns	ns	*	ns	ns
C.V. (%)	–	9.1	8.6	9.5	9.2	7.0

[a]*GN* grain number per ear; *MGW* mean grain weight; *GY* dry grain yield; *BY* biological yield; *HI* harvest index

[b]ns, *, and ** represent non-significant and significant at 0.05 and 0.01 probability levels

Harvest index and biological yield

Foliar application of TiO_2-NPs significantly affected HI ($p \leq 0.01$) (Table 7). The application of 50 mg l^{-1} TiO_2-NPs increased HI 13% over the control. However, 100 mg l^{-1} TiO_2-NPs negatively affected HI to a value lower than the control plants (Table 6). The effects of irrigation treatments and foliar application of TiO_2-NPs were significant on BY ($p \leq 0.01$) (Table 7). According to Table 6, deficit irrigation reduced BY, i.e., BY was significantly decreased by 27% compared with I_{70}. The application of 50 mg l^{-1} TiO_2-NPs increased BY 10% compared with the control; nevertheless, 100 mg l^{-1} TiO_2-NPs sharply decreased BY compared with both T_0 and T_{50} (Table 6).

Grain number, mean grain weight, and grain yield

Irrigation treatments, foliar application of TiO_2-NPs, and their interaction affected GN, MGW, and GY (Table 7). The greatest GN was obtained when 50 mg l^{-1} TiO_2-NPs was applied at I_{70} while respecting the GY, foliar spraying 50 mg l^{-1} TiO_2-NPs showed the greatest GY at I_{105} (Fig. 5a, b). The lowest GN and GY were obtained from the I_{140} treatment that was significantly lower than those of I_{70} and I_{105} ($p \leq 0.05$). Although the foliar application of 50 mg l^{-1} TiO_2-NPs partially compensated for reducing GN and GY at I_{140}; finally, a smaller number of grains was obtained compared with I_{70} and I_{105} (Fig. 5a, b). According to Fig. 4c, the most significant MGW was obtained at the I_{105} treatment and foliar application of 50 mg l^{-1} TiO_2-NPs ($p \leq 0.01$), which were 13 and 19% greater than T_0 and T_{50}, respectively, at the respective irrigation treatment.

Discussion

TiO_2-NPs application as a stress reliever has been widely accepted and is under consideration (Gohari et al. 2020; Ullah et al. 2020; Waani et al. 2021). TiO_2 toxicity concern has mostly been about its direct usage as a food additive or inhalation by humans (Wu and Ren 2020). While, when it is applied to plants, it undergoes many detoxification metabolisms. For instance, Ma et al. (2015) studied the metal-based nanotoxicity and detoxification pathways in higher plants. They found that the metal nanoparticles can be detoxified in plants through non-enzymatic antioxidants (anthocyanin) and heat shock proteins (HSPs). A recent study examined the toxicity of metal nanoparticles and found that TiO_2 was the less toxic, while ZnO was the most toxic one (Wu and Ren 2020). TiO_2-NPs have also been considered as heavy metal detoxifiers. Exogenously applied TiO_2-NPs prevails over root application in reducing Cd accumulation and mitigating Cd-induced phytotoxicity in maize (*Zea mays* L.) (Lian et al. 2020). In another study, TiO_2-NPs were applied to reduce bioaccumulation of arsenic in rice seedlings (*Oryza sativa* L.) (Wu et al. 2021).

In general, foliar application of 50 mg l^{-1} TiO_2-NPs stimulated the concentration of all leaf pigments. It has been reported that the application of TiO_2-NPs on spinach plants increased photosynthesis, growth rate, the absorption of solar light energy by increasing the leaf chlorophyll a content, and had positive effects on the thylakoid membrane (Lei et al. 2007). A study reported a 23% increase in grain yield, harvest index, electron transport rate, and photosystem II (PSII) activity by spraying nano-TiO_2 in barley (Morteza et al. 2013). Photocatalysts break down organic molecules into carbon dioxide (CO_2) in the presence of sunlight and water, returning the water molecule back to the environment. Therefore, it can increase the concentration of CO_2 to stabilize photosynthesis under stress conditions. As the results showed, the content of photosynthetic pigments was increased by applying TiO_2-NPs, which is probably due to the photocatalytic ability of this substance.

The general trend of leaf carotenoid content indicated an increase by the irrigation delay, which can be expected due to a decrease in photosynthetic efficiency and possibly an increase in free radicals. Carotenoids are non-enzymatic antioxidants that prevent chlorophyll photooxidation under

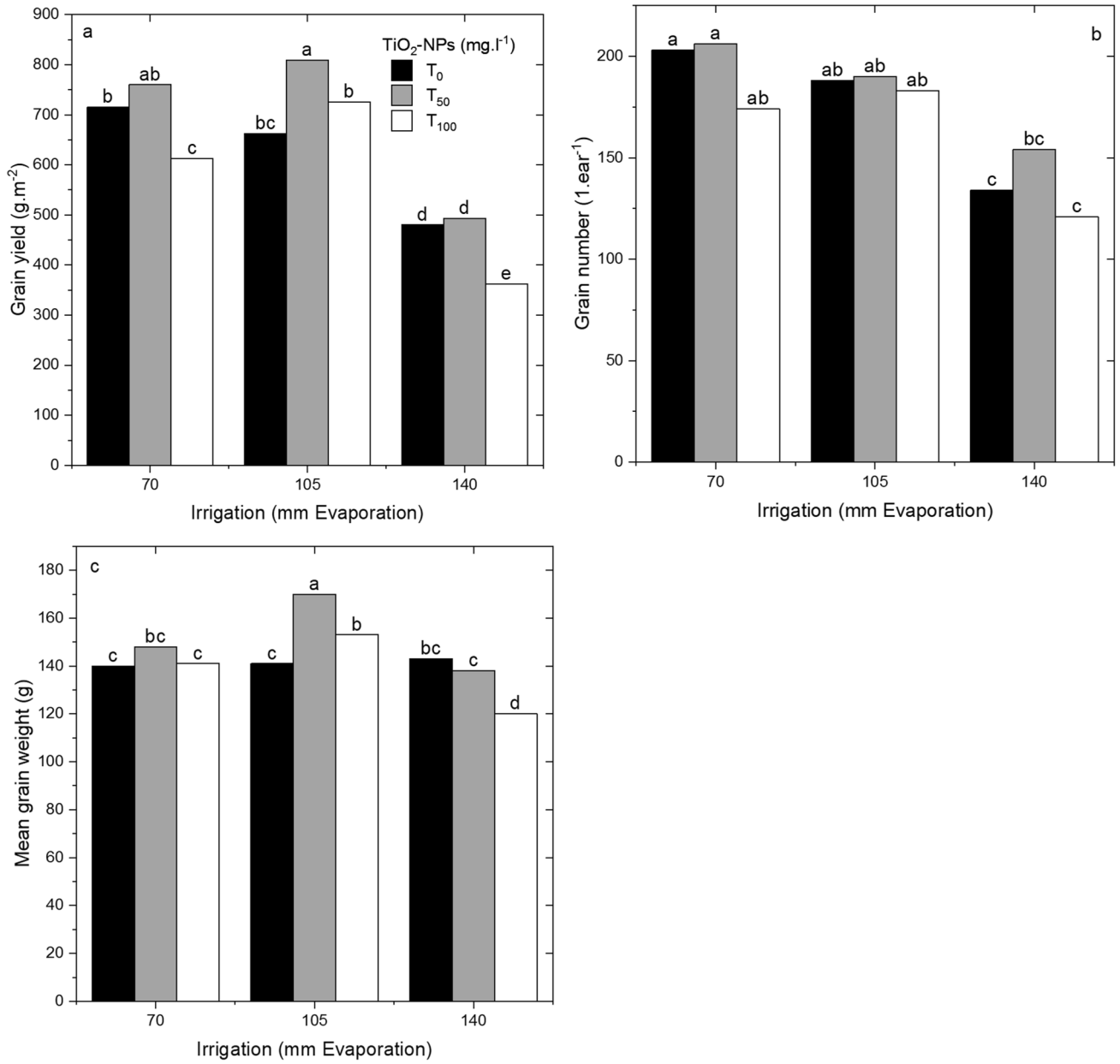

Fig. 5 Grain yield (**a**), grain number per ear (**b**), and mean grain weight (**c**) of sweet corn leaves foliar spayed with various concentrations of TiO_2-NPs (mg l^{-1}) under different irrigation regimes (mm water evaporation from class A evaporation pan). The control plants (70) were sprayed with water. Data presented are the means of three replicates. Means with the same letter are not significantly different based on the Duncan test $p \leq 0.05$

stress conditions (Farooq et al. 2009). Correlation results showed that the leaf carotenoid content was positively correlated with the Chl *b* content (Fig. 6). This correlation indicates the two auxiliary pigments joint function to protect the photosynthetic apparatus under water deficit conditions.

The quantum yield of PSII, F_v/F_m, is directly related to chlorophyll activity in the reaction centers of photosystems, and with any stress, the maximum performance will face a reduction (Fracheboud and Leipner 2003). In this experiment, deficit irrigation significantly reduced F_v/F_m. PSII is the most sensitive target of heat stress. One of the consequences of water shortage is stomatal closure that reduces the heat exchange of leaves, i.e., a reduction in transpiration rate leading to an increase in leaf temperature. Meanwhile, when PSII suffers from severe thermal damage, photosynthetic electron transport and ATP synthesis are greatly affected (Wang et al. 2018). Although the foliar application of 50 mg l^{-1} TiO_2-NPs improved F_v/F_m, a sharp

Fig. 6 Pearson's correlation coefficient of maximum efficiency of PSII (F_v/F_m), relative water content (RWC), soluble sugar content (SS), protein content (Prot), proline content (Prol), chlorophyll *b* content (Chl *b*), total chlorophyll content (Chl *t*), carotenoids content (Car), superoxide dismutase (SOD), ascorbic peroxidase (APX), catalase (CAT), grain number per ear (GN), mean grain weight (GW), dry grain yield (GY), biological yield (BY), and harvest index (HI) of sweet corn leaves under different irrigation regimes (mm water evaporation from class A evaporation pan) and different TiO_2-NPs (mg l^{-1})

decreasing trend was observed at the level of 100 mg l^{-1}. This probably was due to a reduction in leaf chlorophyll content and disruption of chloroplast photochemical processes. The correlation results also showed that the F_v/F_m was positively related to the leaf Chl *t* and carotenoid content (Fig. 6).

Deficit irrigation increased the leaf sucrose and soluble sugar content. This increase could be due to the starch hydrolysis and the accumulation of soluble sugars to maintain cell turgor. Soluble sugars play a role in osmotic adjustment and regulating the stability of cell membranes (Slama et al. 2007). It has been reported that an increase in soluble sugars in response to deficit irrigation might be due to their lower transport from leaves, lower consumption due to reduced plant growth rate, and hydrolysis of storage starch under these conditions (Mohammadkhani and Heidari 2008). The application of 50 mg l^{-1} TiO_2-NPs diminished the increasing effect of deficit irrigation on the leaf sucrose and soluble sugar content, while the application of 100 mg l^{-1} TiO_2-NPs increased their concentration. Since high concentrations of TiO_2 can lead to damage to cellular organic compounds, such as starch in chloroplasts (Gao et al. 2018), the accumulation of sucrose and soluble sugars can be a protection against those damages. The negative correlation between leaf sucrose content and Chl

t showed that a decrease in Chl content was accompanied by an increase in leaf soluble sugars (Fig. 6).

The protein hydrolysis and production of soluble amino acids are among the adaptive reactions of plants to dehydration. In addition, a decrease in the concentration of the Rubisco enzyme due to damage to chloroplasts can be among the other reasons for a decrease in protein content (Kafi et al. 2009). Rubisco is the key enzyme in the Calvin cycle and photosynthetic process. Rubisco is the most abundant leaf protein that forms more than 50% of the leaf soluble proteins. The positive effect of 50 mg l^{-1} TiO_2-NPs could be due to increased concentration of leaf pigments and consequently the Rubisco enzyme. The general trend of leaf proline content indicated that delay in irrigation increased the leaf proline content. Proline has been considered to be involved in the non-enzymatic antioxidant defense in plants (Signorelli et al. 2015). Proline biosynthesis and accumulation help to absorb more water by reducing the cell osmotic potential. On the other hand, proline reduces the risks of protein breakdown due to the accumulation of free radicals and prevents the accumulation of ammonia in the cell under drought stress (Kafi et al. 2009). Foliar application of 50 mg l^{-1} TiO_2-NPs increased the leaf proline content. Interestingly, there was no significant difference between the TiO_2-NPs treatments at the control, but at I_{105} and I_{140} treatments, indicating the vital role of proline in the plant cell protection under lack of moisture.

Due to the adverse effects of high levels of TiO_2-NPs (100 mg l^{-1}) on cell structure, i.e., damage to the cell membranes (Gao et al. 2018), damage to chloroplasts and decomposition of chlorophylls may reduce the absorption of light energy and the production of free radicals. Therefore, a reduction in the activity and content of antioxidant enzymes is expected. The positive correlation of leaf enzymatic antioxidants with proline and carotenoid content indicated the coexistence and collaboration of non-enzymatic and enzymatic antioxidants in response to soil moisture shortage (Fig. 6). An increase in the antioxidant enzyme content of spinach (*Spinacia oleracea*) leaves has also been reported under the foliar application of nano-anatase TiO_2 at a concentration of 0.25 mg l^{-1} (Yang et al. 2006).

Grain yield showed positive correlations with leaf photosynthetic pigments, chlorophyll fluorescence, and grain number per ear (Fig. 6). GN showed the highest correlation with GY, which seems to be the most important determining component of grain yield. Mean grain weight is directly related to the photosynthetic rate; any factor that alleviates environmental stress effects on plants or increases the photosynthesis can directly improve MGW. The correlation coefficients showed positive correlations between MGW and soluble sugar content and chlorophyll fluorescence (Fig. 6). The greater MGW at I_{105} compared with I_{70} might be due to the formation of fewer seeds at I_{105} treatment, causing the allocation of more photoassimilates to the fewer grains and leading to a greater MGW.

Foliar application of 50 mg l^{-1} TiO_2-NPs diminished the subtractive effects of water stress and increased GY and BY. Gohari et al. (2020) also reported that TiO_2-NPs increased wheat plant growth and yield components. However, in our experiment, GY increased more than BY, which led to an improvement in HI. Dawari and Luthra (1991) shown that HI is a critical component of crop yield, and selection based on which can be effective to improve yield. Greater grain yield was associated with greater plant biomass (Ahmadi-Lahijani and Emam 2016). This highlights the importance of BY in increasing plant grain yield. Greater biomass leads to a greater photosynthetic unit, which provides greater photoassimilates to fill the grains. Like GN, there was no significant difference in GY between I_{70} and I_{105} treatments, which could indicate that the lack of soil moisture at I_{105} was not severe enough, and the plants were able to produce GY at the level of I_{70} despite a reduction in vegetative growth. As the growth rate of sweet corn plants slows down under water stress, the tassels appear with a delay, accompanied by an increase in the time interval between pollination and the tassel emergence. As a result, corn tassels appear when pollination has occurred, and the number of pollen grains was severely reduced (Bänzinger 2000). Correlation coefficients showed that the grain number per ear and the total leaf chlorophyll and soluble sugar content, and chlorophyll fluorescence were positively correlated (Fig. 6), which indicated that the current photosynthesis either through the formation of larger ears (more reproductive components) or by preventing the abortion and grain shedding after pollination (shedding due to lack of photoassimilates) affects the grain number per ear.

In summary, deficit irrigation decreased the water absorption of sweet corn plants. The effects of water stress were ameliorated by foliar application of 50 mg l^{-1} TiO_2-NPs. However, TiO_2-NPs at the concentration of 100 mg l^{-1} showed adverse effects on physiological traits and reduced the crop yield. The grain number per ear had the highest correlation ($r = 0.97$) with GY, and it can be considered the most important determining component of sweet corn yield under such conditions as of this study. The greatest GY was obtained from $I_{105}T_{50,}$ followed by $I_{70}T_{50}$ (809 and 760 g.m^{-2}, respectively). Since I_{105} treatment reduced the water consumption and induced mild water stress on the plants, so this irrigation treatment with foliar application of 50 mg l^{-1} TiO_2-NPs can be considered to produce sweet corn as the second cultivation (summer) to obtain a desired performance. Furthermore, foliar application of 50 mg l^{-1} TiO_2-NPs can be recommended even under the control irrigation treatment to enhance the crop yield. Finally, regarding the positive effects of TiO_2-NPs on the crop performance, evaluating the foliar application of TiO_2-NPs at different

levels (less than 50 mg l^{-1}) with more spraying interval and repetition (at different growth stages), both on sweet corn and other crops, can still be interesting to be studied in the future.

Author contribution statement MK: data collecting, software, and writing—original draft. AA: resources, supervision, and validation. AR: validation and resources. ShM-H: validation and investigation. MJA-L: software, formal analysis, writing—original draft, and writing—review and editing.

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Code availability SAS v. 9.1; Origin 2018; Minitab v. 17; Microsoft excel 2019.

Declarations

Conflict of interest There is no conflict of interest.

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