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Research Article

**USE OF NANOMATERIALS IN INDUCING TOLERANCE
AGAINST WATER STRESS IN FAVA BEAN (*VICIA FABA L.*)
PLANTS GROWN IN TABUK REGION****M. Nasir Khan* and Fahad M. AlZuaibr***Department of Biology, Faculty of Science, University of Tabuk, Tabuk-71491, Saudi Arabia***Article Received:** January 2019**Accepted:** February 2019**Published:** March 2019**Abstract:**

In the past decade nanomaterials (NMs) have emerged as important tools for manipulating growth and yield of crop plants. Of these NMs, nano-titanium dioxide (nano-TiO₂) has been shown to play various roles in growth and development of crop plants under abiotic stresses. Present work was carried out to evaluate the role nano-TiO₂ in the protection of plants against water stress (WS) and to select the best of nano-TiO₂. The results show that WS caused a significant reduction in growth attributes, chlorophyll (Chl) content, leaf relative water content (LRWC) and activity of nitrate reductase (NR) enzyme. Whereas, an increase was noticed in reactive oxygen species (ROS), carbonic anhydrase (CA) activity, electrolyte leakage, and lipid peroxidation in water stressed plants. In addition, plants under WS also showed an increase in the activities of antioxidant enzymes and accumulation of proline (Pro) and glycine betaine (GB) content. However, application of nano-TiO₂ (5, 10, 15, and 20 mg L⁻¹) to water-stressed plants further enhanced the activities of antioxidant enzymes, CA activity, and Pro and GB content. Nano-TiO₂-induced increase in ROS, CA, Pro and GB resulted in a decrease in ROS level, electrolyte leakage and lipid peroxidation leading to increase in LRWC, Chl content, NR activity and growth of the plants. Furthermore, an additional increase in the level of nano-TiO₂ to 25 mg L⁻¹ could not alleviate water stress and showed adverse effects.

Keywords: Antioxidant system, Nanomaterials, Titanium dioxide, *Vicia faba*, Water stress

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INTRODUCTION:

Water stress (WS) is one of the devastating environmental problems which adversely affects growth and production of crop plants across the globe. According to World Resources Institute (WRI), 14 of the 33 countries most likely to be water-stressed in 2040 are in the Middle East and Saudi Arabia is one of these countries. As far as Tabuk region is concerned, lower humidity and high temperature of the region jointly accelerate the rate of evaporation. Being sessile in nature plants are always exposed to the detrimental conditions of WS. Water stress inhibits photosynthetic capacity, and enzyme activities, and induces production of reactive oxygen species (ROS). Excessive generation of ROS creates oxidative stress and causes peroxidation of membrane lipids, and damages proteins and nucleic acids [1]. Oxidative stress also triggers cytotoxicity, genotoxicity [2,3] and suppresses growth and productivity of the crops [4]. All these detrimental effects ultimately contribute to reduced yield and low nutritive value of crop products. However, to counter oxidative stress, plants are fitted with a system of enzymatic antioxidants viz. superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR) which continuously scavenges harmful ROS. Whereas, plants counter osmotic stress by enhancing the accumulation of organic osmolytes such as proline (Pro) and glycine betaine (GB) which assist the plants in maintaining normal hydration level of cells.

Therefore, to cope with the detrimental situation, induction of water stress-tolerance capacity within the plant, through up-regulation of antioxidant enzymes, and accumulation of osmolytes would be of considerable importance for better growth and yield of crop plants

Application of nanomaterials (NMs), designed for sustainable crop production, reduces nutrient losses, suppresses disease and enhances the yields [5]. Nanomaterials influence key life events of the plants that include seed germination, seedling vigor, root initiation, growth and photosynthesis to flowering. Additionally, NMs have been implicated in the protection of plants against oxidative stress as they mimic the role of antioxidative enzymes such as superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX). Several studies reveal that NMs can improve growth and yield of crop plants by enhancing seed germination, water and fertilizer absorption, antioxidant system, enzyme activities, and photosynthesis and nitrogen metabolism [6,7]. NMs have been shown to play important role in plant

growth and development. Nano-TiO₂ enhances seed germination, seedling growth and photosynthesis and activities of antioxidant enzymes [8]. Lei et al. [9] observed that nano-TiO₂ alleviates accumulation of malondialdehyde content by induction of plant antioxidant systems. It has been reported that nano-TiO₂ treated seeds produced plants that had 73% more dry weight, three times higher photosynthetic rate, and 45% increase in chlorophyll-a formation [10]. A significant increase in photosynthetic pigments and crop yield was recorded in nano-TiO₂ treated maize plants.

Fava bean (*Vicia faba* L.; Family Fabaceae) is one of the important crops grown in Tabuk region. Fava bean is a leguminous crop and is consumed mostly as dried seed while, a little portions is consumed as fresh kernel. The crop is also becoming increasingly important in Saudi diets due to the high lysine content of the seed, which encourages the use of fava bean as a protein supplement [11,12]. Proteins in legume seeds represent from about 20% (dry weight) in pea and beans up to 38–40% in soybean and lupin [13,14]. Therefore legume seeds are among the richest food sources of proteins and amino acids for human and animal nutrition. In spite of being such an important crop, production and quality of fava bean does not meet the requirements of the increasing population. On the other hand, meager or no information is available on the effect nano-TiO₂ on yield and quality and on the tolerance of fava bean plants to WS.

Therefore, considering the role of nano-TiO₂ in plants, the present work was carried out to select the best dose of nano-TiO₂ that could improve the production and quality of fava bean by enhancing water stress-tolerance capacity of the plants.

MATERIALS AND METHODS:

Plant culture and treatments

Seeds of fava bean (*Vicia faba* L.) were purchased from local market of Tabuk. Before sowing, healthy and uniform seeds were selected and surface sterilized with 1% sodium hypochlorite for 10 min, then vigorously rinsed with double distilled water (DDW). Surface sterilized seeds were sown in plastic pots containing soil/vermiculite mixture (1:1). After sowing, the well-watered (100% field capacity) pots were kept for two weeks under natural illuminated conditions with an average day/night temperature of 30/10±3°C. After two weeks plants were exposed to nano-TiO₂ and water stress (WS). The treatments were given as: (i) Double distilled water (DDW; control: T1); (ii) WS (T2); (iii) 5 mg L⁻¹ nano-TiO₂ +

WS (T3); (iv) 10 mg L⁻¹ nano-TiO₂ + WS (T4); (v) 15 mg L⁻¹ nano-TiO₂ + WS (T5); (vi) 20 mg L⁻¹ nano-TiO₂ + WS (T6); (vii) 25 mg L⁻¹ nano-TiO₂ + WS (T7). Plants were considered as water stressed by maintaining soil water content at 30% through withholding water supply. During the water stress period, soil relative water content was monitored daily, and WS was continued up to 9 days when the desired level of soil relative water content (30%) was achieved. Plants treated with DDW only were considered as control. After one week of treatment, the effect of water stress and nano-TiO₂ on fava bean plants was tested by assessing following parameters.

Estimation of growth characteristics

Response of plants to water stress and nano-TiO₂ was assessed in terms of plant height, loss of plant height, fresh weight per plant, loss of fresh weight.

Assay of carbonic anhydrase (CA) and nitrate reductase (NR) activity

The activity of CA (E.C. 4.2.1.1) was measured using the method as described by Dwivedi and Randhawa [15]. The enzyme activity was expressed as $\mu\text{M CO}_2 \text{ kg}^{-1} \text{ leaf FW S}^{-1}$.

Activity of NR (E.C. 1.6.6.1) was estimated by the method of Jaworski [16]. The OD of the content was recorded at 540 nm. Activity of NR was expressed as $\mu\text{M NO}_2^- \text{ g}^{-1} \text{ leaf FW h}^{-1}$.

Estimation of Leaf chlorophyll (Chl) content

Leaf Chl content was estimated using the method of Lichtenthaler and Buschmann [17]. The data were expressed as mg g⁻¹ leaf FW.

Determination of leaf relative water content (LRWC) and electrolyte leakage

Leaf relative water content (LRWC) was measured by adopting the method of Yamasaki and Dillenburg [18]. Electrolyte leakage was measured as described by Lutts *et al.* [19].

Determination of hydrogen peroxide (H₂O₂) and superoxide (O₂^{•-}) content, and lipid peroxidation

The hydrogen peroxide (H₂O₂) and superoxide (O₂^{•-}) content were determined according to Velikova *et al.* [20] and Elstner and Heupel [21], respectively. Lipid peroxidation was determined by estimating the content of thiobarbituric acid reactive substances (TBARS) as described by Cakmak and Horst [22]. TBARS content was expressed as nM g⁻¹ leaf FW.

Determination of proline (Pro) and glycine betaine (GB) content

Proline (Pro) content was determined spectrophotometrically adopting the ninhydrin method of Bates *et al.* [23]. Glycine betaine (GB) content was estimated by the method of Grieve and Grattan [24].

Assay of antioxidant enzymes

Activity of SOD, POX, CAT, APX, and GR was determined according to Beauchamp and Fridovich [25], Upadhyaya *et al.* [26], Cakmak and Marschner [27], Nakano and Asada [28] and Foyer and Halliwell [29], respectively.

Statistical analysis

Each treatment was replicated three times. The data were analyzed statistically with SPSS-17 statistical software (SPSS Inc., Chicago, IL, USA). Means were statistically compared and critical difference (CD) was calculated at 5% level.

RESULTS AND DISCUSSION:

Growth characteristics

The results of the study show that WS reduced plant height and fresh weight by 44.93% and 59.39%, respectively as compared to the control (Table 1). Plants under stress exhibited enhanced levels of electrolyte leakage, TBARS and ROS that might have resulted in reduced Chl content, and NR activities that caused suppressed growth of stressed plants. Poor dry matter accumulation in shoot and leaf growth are the major morphological changes under WS conditions [30]. Therefore, reduced shoot length possessed lesser number of leaves with reduced leaf area that could not capture sufficient solar energy resulting in poor carbon assimilation and thus reduced fresh weight [31]. These results are also in agreement with the findings of Aghdam *et al.* [32], Shivhare and Lata [33] and Gurusurthy *et al.* [34]. The stressed plants treated with nano-TiO₂ showed an improvement in the growth parameters from 5 to 20 mg L⁻¹ nTiO₂, whereas further increment in the concentration of nano-TiO₂ to 25 mg L⁻¹ exhibited adverse effects and caused reduction in growth parameters. Therefore, among five concentrations, 20 mg L⁻¹ nano-TiO₂ proved best in protecting plants against WS (Table 1). It has been worked out that nano-TiO₂ enhances dry matter accumulation, seed germination, seedling growth and photosynthesis [35]. Zheng *et al.* [10] observed that nano-TiO₂ assists in water absorption and improves seed germination. Moreover, Navarro *et al.* [36] suggested that NMs could sequester nutrients on their surfaces, and thus, serve as nutrient stock. NMs also regulate aquaporins, the water channels, which regulate water permeability and play important role in seed germination and plant growth [37]. It has also been reported that nano-TiO₂ accelerates photosynthetic

carbon assimilation by activating rubisco that could promote rubisco carboxylation, thereby increasing growth of the plants [38]. Plant exposure to NMs causes activation of genes responsible for water

channel protein and also for better cell growth by regulating cell cycle, these effects of NMs reflect in the form of improved growth of the plants [5,39].

Table 1 Effect water stress and nano-TiO₂ on the growth parameters of *Vicia faba*

Treatments	Parameters			
	Plant height (cm)	Loss of plant height	Fresh weight per plant (g)	Loss of fresh weight
DDW (control)	6.9	00.00	3.62	00.00
WS	3.8	44.93	1.47	59.39
5 mg L ⁻¹ nano-TiO ₂ + WS	3.9	43.48	1.43	60.49
10 mg L ⁻¹ nano-TiO ₂ + WS	4.2	39.13	1.68	53.59
15 mg L ⁻¹ nano-TiO ₂ + WS	5.1	26.09	2.13	41.16
20 mg L ⁻¹ nano-TiO ₂ + WS	5.8	15.94	2.87	20.72
25 mg L ⁻¹ nano-TiO ₂ + WS	4.7	31.88	1.59	56.08
CD at 5%	0.049	1.26	0.043	1.64

WS: water stress; values are mean of three replicates

Activities of CA and NR, and leaf Chl content and LRWC

It is evident from Table 2 that WS enhanced the activity of CA enzyme by 15.8% as compared with the control. However, a decrease in the activity of NR enzyme, Chl content and LRWC was recorded. Water-stressed plants show 68.6%, 51.4% and 44.2% reduction in NR, leaf Chl content, and LRWC, respectively in comparison with their respective controls. The CA, a chloroplast-localized enzyme, catalyzes the reversible hydration of CO₂, and maintains its constant supply to rubisco, a key enzyme responsible for the fixation of CO₂. In addition to its role in CO₂ fixation, CA plays vital role in the protection of plants under stresses [40]. Under drought stress CA activity was upregulated that controlled ROS levels and helped the plants to counter cytotoxic concentration of ROS [41]. NR is the key enzyme that catalyzes the rate-limiting step of N assimilation. Activity of NR is associated with the rate of photosynthesis and the availability of carbon skeletons through both transcriptional and posttranslational controls [42]. A decrease in NR activity under drought stress has also been reported by Foyer *et al.* [43]. Chlorophyll is one of the important tools to evaluate the severity of the plants to drought-induced water stress. Excessive accumulation of ROS causes pigment photo-oxidation, instability of protein complexes and increase in the activity of Chl-degrading enzyme

chlorophyllase [44] leading to Chl degradation [45-47].

However, water-stressed plants supplemented with nano-TiO₂ showed a parallel increase with increasing concentration of nano-TiO₂ from 5 to 20 mg L⁻¹ nTiO₂. Water-stressed plants treated with 20 mg L⁻¹ nano-TiO₂ showed an increase of 42.1%, 88.7%, 32.4% and 78.5% in CA, NR, Chl content and LRWC, respectively as compared with water-stressed plants (Table 2). The results also show that nano-TiO₂ at the rate of 25 mg L⁻¹ did not show any further increase in these parameters, but a decrease was noticed in CA, and NR activities, and Chl content and LRWC (Table 2). Application of nano-TiO₂ suppressed the generation of H₂O₂ and O₂⁻ content and improved LRWC which protected Chl molecules from photo-oxidative damage and resulted in improvement of Chl content (Table 2). In addition, nano-TiO₂-induced reduction in ROS was associated with the reduction in electrolyte leakage and TBARS and increase in LRWC (Tables 2 and 3) that provided a favourable environment for the biosynthesis of Chl and normal activities of CA and NR enzymes. Enhanced level of Chl content by nano-TiO₂ has also been reported in drought stressed plants of *Linum usitatissimum* [32]. Our results also corroborate the findings of Hruby *et al.* [48], Khan [49], Khan and Alzuaibr [50], and Ahmad *et al.* [51] who observed that nano-TiO₂ improves activities of NR and CA enzymes and level of Chl content.

Table 2 Effect water stress and nano-TiO₂ on CA and NR activity, leaf Chl content and leaf relative water content of *Vicia faba*

Treatments	Parameters			
	CA activity ($\mu\text{M CO}_2 \text{ kg}^{-1} \text{ leaf FW s}^{-1}$)	NR activity ($\mu\text{M NO}_2 \text{ g}^{-1} \text{ leaf FW h}^{-1}$)	Leaf Chl content ($\text{mg g}^{-1} \text{ FW}$)	LRWC (%)
DDW (control)	236.58	366.71	2.86	98.42
WS	274.02	115.29	1.39	44.07
5 mg L ⁻¹ nano-TiO ₂ + WS	288.39	143.54	1.42	51.61
10 mg L ⁻¹ nano-TiO ₂ + WS	291.61	151.82	1.49	63.71
15 mg L ⁻¹ nano-TiO ₂ + WS	311.70	211.74	1.61	69.82
20 mg L ⁻¹ nano-TiO ₂ + WS	389.47	217.52	1.84	78.51
25 mg L ⁻¹ nano-TiO ₂ + WS	321.29	166.38	1.46	71.43
CD at 5%	3.61	2.74	0.059	3.28

WS: water stress; values are mean of three replicates

Electrolyte leakage, thiobarbituric acid reactive substances (TBARS), and hydrogen peroxide (H₂O₂) and superoxide (O₂^{•-}) content

Electrolyte leakage represents the degree of damage to cell membrane integrity and stability in response to the stress. The results exhibited that water-stressed plants showed an increase of 83.8% in electrolyte leakage as compared with the control plants (Table 3). Water-stressed plants supplemented with nano-TiO₂ showed a reduction in electrolyte leakage with increasing concentration from 5 to 20 mg L⁻¹ of nano-TiO₂. However, the highest level of nano-TiO₂ (25 mg L⁻¹) did not show a further decrease in electrolyte leakage (Table 3).

Effect of nano-TiO₂ on water stress-induced peroxidation of membrane lipids was assessed by determining thiobarbituric acid reactive substances (TBARS). Results show that WS enhanced the level of TBARS by 77.3% as compared with the control (Table 3). Stressed plants show a parallel increase in electrolyte leakage with increased level of TBARS because electrolyte leakage is the result of peroxidation of membrane lipids. Water-stressed plants show a decrease in TBARS with increasing levels of nano-TiO₂ from 5 to 20 mg L⁻¹ nano-TiO₂. Lowest level of TBARS was recorded in water-stressed plants treated with 20 mg L⁻¹ nano-TiO₂

which showed 36.0% reduction as compared with water-stressed plants. However, a further increase in the level of nano-TiO₂ (25 mg L⁻¹) showed deleterious effect and caused increase in TBARS (Table 3).

Water stress also causes oxidative stress which was evaluated by analyzing ROS (H₂O₂ and O₂^{•-}) level. Perusal of the data shows that WS significantly elevated ROS level (Table 3). Exposure of plants to WS enhanced H₂O₂ and O₂^{•-} content by 87.5% and 94.6%, respectively compared with the control. Increasing levels of nano-TiO₂ from 5 to 20 mg L⁻¹ reduced the generation of ROS and 20 mg L⁻¹ proved most effective which reduced H₂O₂ and O₂^{•-} content by 35.4% and 45.2%, respectively as compared to water-stressed plants not treated with nano-TiO₂. On the contrary, 25 mg L⁻¹ nano-TiO₂ caused an increase in H₂O₂ and O₂^{•-} content (Table 3). Water stress induced overproduction of ROS (H₂O₂ and O₂^{•-}) that caused peroxidation of membrane lipids and leakage of electrolytes (Table 3) [5, 50, 52]. On the other hand, stressed plants supplemented with nano-TiO₂ showed an increase in the activities of antioxidant enzymes (Fig. 2 A-E) that inhibited ROS production and caused reduction in the level of ROS [53] leading to reduced TBARS and electrolyte leakage [5,50].

Table 3 Effect water stress and nano-TiO₂ on electrolyte leakage, TBARS, and H₂O₂ and O₂^{•-} content of *Vicia faba*

Treatments	Parameters			
	Electrolyte leakage (%)	TBARS (nM g ⁻¹ DW)	H ₂ O ₂ content (μM g ⁻¹ leaf DW)	O ₂ ^{•-} content (μM g ⁻¹ leaf DW)
DDW (control)	9.58	12.58	76.31	3.92
WS	17.61	22.31	143.10	7.63
5 mg L ⁻¹ nano-TiO ₂ + WS	16.56	22.07	136.47	7.49
10 mg L ⁻¹ nano-TiO ₂ + WS	16.37	19.84	134.52	6.28
15 mg L ⁻¹ nano-TiO ₂ + WS	9.04	16.39	113.80	5.36
20 mg L ⁻¹ nano-TiO ₂ + WS	6.47	14.27	92.49	4.18
25 mg L ⁻¹ nano-TiO ₂ + WS	10.82	21.71	118.05	6.86
CD at 5%	1.82	1.19	3.53	1.04

WS: water stress; values are mean of three replicates

Proline (Pro) and glycine betaine (GB) content

The results show that water-stressed plants accumulated 16.6% and 23.8% more Pro and GB content, respectively than the control plants (Fig. 1). Plants counter osmotic stress by accumulating osmolytes such as Pro and GB which play crucial role in osmotic adjustment of plants under stress. Our results are in agreement with the findings of Khan *et al.* [54,55], and Shivhare and Lata [33] who observed increased accumulation of these osmolytes under drought stress. In addition, a further increase in these osmolytes was noted in water-stressed plants treated with nano-TiO₂. Of the five given levels, 20 mg L⁻¹ nano-TiO₂ gave highest values and caused an increase of 67.8% and 50.6% in Pro and GB content,

respectively as compared with the stressed plants. However, a further increase in the concentration of nano-TiO₂ (25 mg L⁻¹) suppressed the level of osmolytes (Fig. 1 A and B). It has been observed that nano-TiO₂ induces the synthesis of hydrogen sulfide in plants under drought stress [50]. In addition, hydrogen sulfide has been shown to induce the synthesis of Pro and GB content that stabilize biological membranes and protects the plants against adverse effects of abiotic stresses [56,57]. Moreover, increased accumulation of Pro and GB content elevates osmotic pressure which enhances water uptake capacity of treated plants [50] as shown by improved LRWC (Table 2).

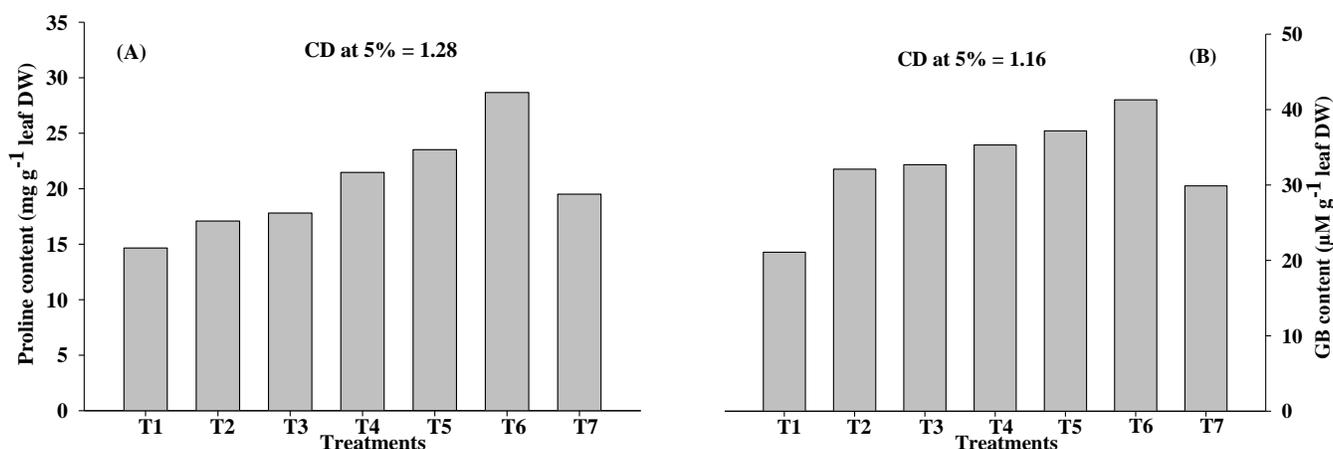


Figure 1 Effect of water stress and nTiO₂ on proline content (A) and glycine betaine content (B) of *Vicia faba* plants under water stress. [(i) Double distilled water (DDW; control: T1); (ii) WS (T2); (iii) 5 mg L⁻¹ nano-TiO₂ + WS (T3); (iv) 10 mg L⁻¹ nano-TiO₂ + WS (T4); (v) 15 mg L⁻¹ nano-TiO₂ + WS (T5); (vi) 20 mg L⁻¹ nano-TiO₂ + WS (T6); (vii) 25 mg L⁻¹ nano-TiO₂ + WS (T7)]. WS: water stress; values are mean of three replicates

Activities of antioxidant enzymes

It is evident from the results that exposure of plants to water stress elevated the activities of antioxidant enzymes (SOD, POX, CAT, APX and GR) (Fig. 2 A-E). To counter oxidative stress plants are equipped with a system of antioxidant enzymes which is activated in response to a stress stimulus [33,34] and maintains optimum level of ROS. Moreover, application of nano-TiO₂ to stressed plants further enhanced the activities of these antioxidant enzymes. For instance, WS enhanced the activities of SOD, POX, CAT, APX and GR by 45.2%, 27.5%, 41.6%,

13.6% and 19.4%; whereas 20 mg L⁻¹ nTiO₂ enhanced these activities by 95.8%, 70.5%, 80.7%, 69.2% and 64.8%, respectively as compared with their respective controls (Fig. 2 A-E). It is well documented that NMs stimulate the activities of antioxidant enzymes and protect plants against various abiotic stresses [5]. Mohammadi *et al.* [57] and Khan and Alzuaibr [50] observed that nanoTiO₂ enhances the activities of antioxidant enzymes which regulate excessive generation of ROS and results in reduced TBARS and electrolyte leakage (Table 3).

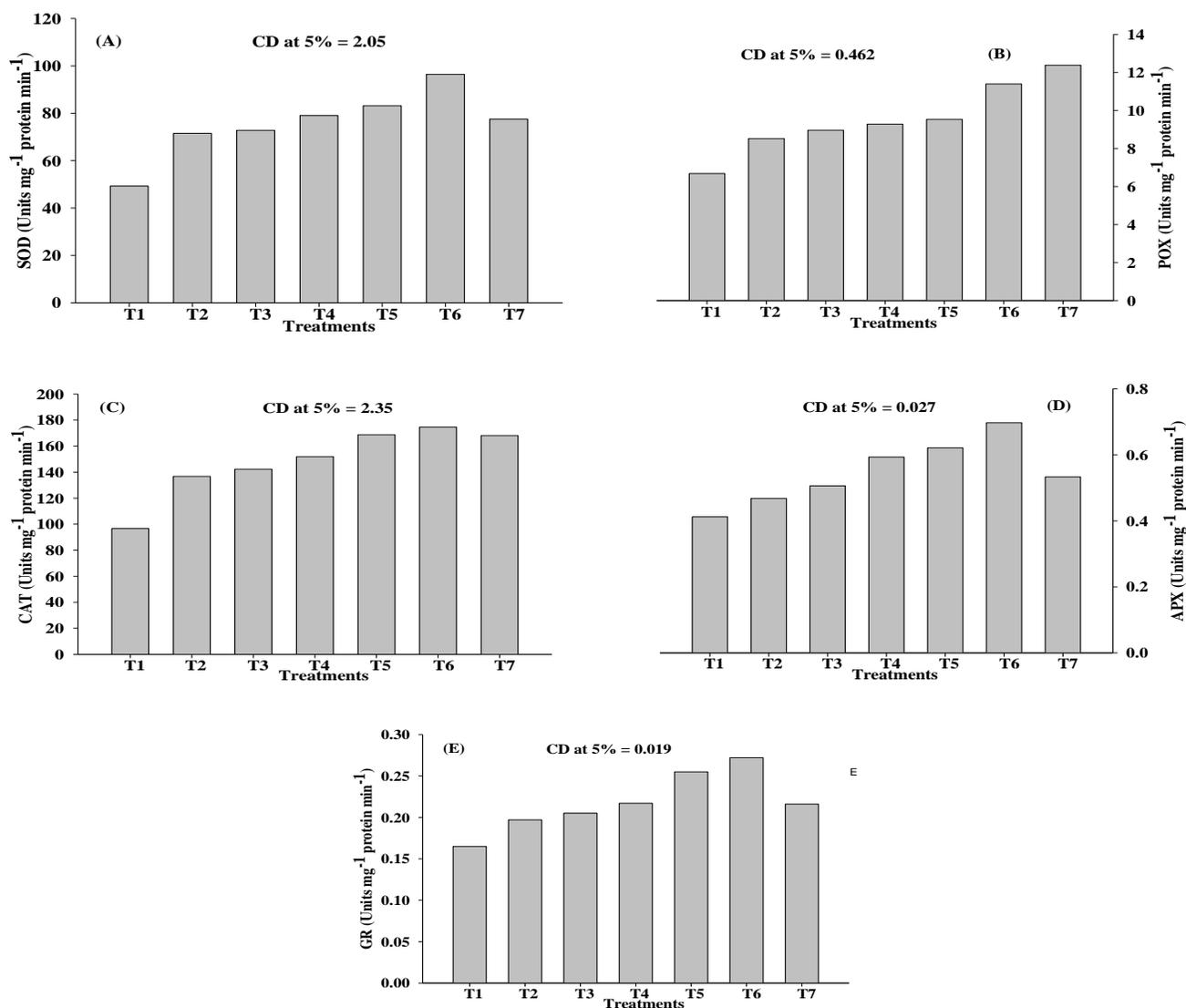


Figure 2 Effect of water stress and nTiO₂ on the activities of SOD (A), POX (B), CAT (C), APX (D) and GR (E) of *Vicia faba* plants under water stress. [(i) Double distilled water (DDW; control: T1); (ii) WS (T2); (iii) 5 mg L⁻¹ nano-TiO₂ + WS (T3); (iv) 10 mg L⁻¹ nano-TiO₂ + WS (T4); (v) 15 mg L⁻¹ nano-TiO₂ + WS (T5); (vi) 20 mg L⁻¹ nano-TiO₂ + WS (T6); (vii) 25 mg L⁻¹ nano-TiO₂ + WS (T7)]. WS: water stress; values are mean of three replicates

CONCLUSION:

On the basis of assessment of results it is observed that plants under stress showed excessive generation of ROS. These ROS caused damage to the cell membrane as reflected by elevated level of TBARS. Membrane damage caused by WS resulted in the leakage of electrolytes, loss of Chl content, reduced activity of NR and suppressed water uptake capacity leading to reduced plant height and fresh weight of the plants. Along with the increase in ROS levels, stressed plants also show an increase in the activities of antioxidant enzymes and CA, and accumulation of Pro and GB content. However, application of different concentrations of nano-TiO₂ (except 25 mg L⁻¹) further elevated the activities of antioxidant enzymes and CA which reduced the generation of ROS. Reduction in ROS was witnessed by a decrease in TBARS and electrolyte leakage. Water-stressed plants treated with nano-TiO₂ also showed elevated levels of Pro and GB content which maintained osmotic pressure and caused more water uptake as shown by increased LRWC and improved growth parameters. Therefore, it can be concluded that an increase in the concentration of from 5-20 mg L⁻¹ gave promising results and nano-TiO₂ at the rate of 20 mg L⁻¹ proved best. However, further increase to 25 mg L⁻¹ nano-TiO₂ was phytotoxic that adversely affected the stressed plants.

COMPETING INTERESTS:

The authors declare no competing interests.

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REFERENCES:

1. Foyer CH, Noctor G Oxygen processing in photosynthesis: Regulation and signalling. *New Phytol*, 2000; 146:359-388.
2. Shen X, Zhou Y, Duan L, Li Z, Eneji AE, Li J Silicon effects on photosynthesis and antioxidant parameters of soybean seedlings under drought and ultraviolet-B radiation. *J. Plant Physiol*, 2010; 167:1248-1252.
3. Yadav T, Mungray AA, Mungray AK. 2014. Fabricated nanoparticles: current status and potential phytotoxic threats. In: Whitacre DM, eds. *Reviews of Environmental Contamination and Toxicology*. Springer International Publishing, Switzerland.
4. Begum P, Fugetsu B. Phytotoxicity of multi-walled carbon nanotubes on red spinach (*Amaranthus tricolor* L.) and the role of ascorbic acid as an antioxidant. *J Hazard Mater*, 2012; 243:212-222.
5. Khan MN, Mobin M, Zahid KA, AlMutairi KA, Zahid HS. Role of nanomaterials in plants under challenging environments. *Plant Physiol Biochem*, 2017; 110:194-209.
6. Hong FS, Yang P, Gao FQ, Liu C, Zheng L, Yang F, Zhou J. Effect of nano-TiO₂ on spectral characterization of photosystem II particles from spinach. *Chem Res Chin Univ*, 2005; 21: 196-200.
7. Yang F, Hong F, You W, Liu C, Gao F, Wu C, Yang P. Influences of nanoanatase TiO₂ on the nitrogen metabolism of growing spinach. *Biol. Trace Elem. Res*, 2006; 110:179-190.
8. Laware SL, Raskar S. Effect of titanium dioxide nanoparticles on hydrolytic and antioxidant enzymes during seed germination in onion. *Int J Curr Microbiol Appl Sci*, 2014; 3:749-760.
9. Lei Z, Mingyu S, Xiao W, Chao L, Chunxiang Q, Liang C, Hao H, Xiaoqing L, Fashui H. Antioxidant stress is promoted by nano-anatase in spinach chloroplasts under UV-B radiation. *Biol Trace Elem Res*, 2008; 121: 69-79.
10. Zheng L, Hong F, Lu S, Liu C. Effect of nano-TiO₂ on strength of naturally aged seeds and growth of spinach. *Biol Trace Elem Res*, 2005; 105:83-91.
11. El-Fiel WEA, El Tinay AH, El-Sheikh, EAE. Effect of nutritional status of faba bean (*Vicia faba* L.) on protein solubility profiles. *Food Chem*, 2002; 76:219-223.
12. Alghamdi SS. Effect of various water regimes on productivity of some faba bean (*Vicia faba* L.) varieties in central region of Saudi Arabia. *Res Bult Agric Res Center King Saud Univ*, 2003; pp: 5-22.
13. Guéguen J, Cerletti P. 1994. Proteins of some legume seeds, soybean, pea, fababean and lupin. In: Hudson BJB, ed, *New and developing source of food proteins*. New York: Chapman and Hall, p. 145.
14. Derbyshire E, Wright DJ, Boulter D. Legumin and vicilin, storage protein of legume seeds. *Phytochem*, 1976; 15:3-24.
15. Dwivedi RS, Randhawa NS. Evaluation of a rapid test for the hidden hunger of zinc in plants. *Plant Soil*, 1974; 40:445-451.
16. Jaworski EG. Nitrate reductase assay in intact plant tissues. *Biochem Biophys Res Commun*, 1971; 43:1274-1279.
17. Lichtenthaler, HK, Buschmann C. 2011. Chlorophylls and carotenoids: Measurement and characterization by UV-vis spectroscopy. In: Wrolstad RE, Acree TE, An H, Decker EA, Penner MH, Reid DS, Schwartz SJ, Shoemaker

- CF, Sporns P, eds. Current Protocols in Food Analytical Chemistry (CPFA); John Wiley and Sons: New York, NY, USA, pp. F4.3.1–F4.3.8.
18. Yamasaki S, Dillenburg LC. Measurements of leaf relative water content in *Araucaria angustifolia*. Rev Bras Fisiol Veg, 1999; 11:69–75.
 19. Lutts S, Kinet JM, Bouharmont J. Changes in plant response to NaCl during development of rice (*Oryza sativa* L.) varieties differing in salinity resistance. J Exp Bot, 1995; 46:1843–1852.
 20. Velikova V, Yordanov I, Edreva A. Oxidative stress and some antioxidant systems in acid rain-treated bean plants: Protective role of exogenous polyamines. Plant Sci, 2000; 151:59–66.
 21. Elstner EF, Heupel A. Inhibition of nitrite formation from hydroxyl ammonium chloride, A simple assay for superoxide dismutase. Ann Biochem, 1976; 70:616–620.
 22. Cakmak I, Horst JH. Effects of aluminum on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (*Glycine max*). Physiol Plant, 1991; 83:463–468.
 23. Bates LS, Walden RP, Teare ID. Rapid determination of free proline for water stress studies. Plant Soil 1972; 39:205–207.
 24. Beauchamp C, Fridovich I. Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. Ann Biochem, 1971; 44:276–287.
 25. Upadhyaya A, Sankhla D, Davis TD, Sankhla N, Smith BN. Effect of paclobutrazol on the activities of some enzymes of activated oxygen metabolism and lipid peroxidation in senescing soybean leaves. J Plant Physiol, 1985; 121:453–461.
 26. Cakmak I, Marschner H. Magnesium deficiency and high light intensity enhance activities of superoxide dismutase, ascorbate peroxidase, and glutathione reductase in bean leaves. Plant Physiol. 1992; 98:1222–1227.
 27. Nakano G, Asada K. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant Cell Physiol, 1981; 22:867–880.
 28. Foyer CH, Halliwell B. The presence of glutathione and glutathione reductase in chloroplasts: a proposed role in ascorbic acid metabolism. Planta, 1976; 133:1–25.
 29. Álvarez S, Rodríguez P, Broetto F, Sánchez-Blanco MJ. Long term responses and adaptive strategies of *Pistacia lentiscus* under moderate and severe deficit irrigation and salinity: Osmotic and elastic adjustment, growth, ion uptake and photosynthetic activity. Agric Water Manag, 2018; 202:253–262.
 30. Toscano S, Ferrante A, Romano A. Response of mediterranean ornamental plants to drought stress. Horticulturae, 2019; 5: 6. doi:10.3390/horticulturae5010006
 31. Aghdam MTB, Mohammadi H, Ghorbanpour M. Effects of nanoparticulate anatase titanium dioxide on physiological and biochemical performance of *Linum usitatissimum* (Linaceae) under well-watered and drought stress conditions. Braz. J. Bot, 2015. <http://dx.doi.org/10.1007/s40415-015-0227-x>.
 32. Shivhare R, Lata C. Assessment of pearl millet genotypes for drought stress tolerance at early and late seedling stages. Acta Physiol Plant, 2019; 41:39. <https://doi.org/10.1007/s11738-019-2831-z>
 33. Gurumurthy S, Sarkar B, Vanaja M, Lakshmi J, Yadav SK, Maheswari M. Morpho-physiological and biochemical changes in black gram (*Vigna mungo* L. Hepper) genotypes under drought stress at flowering stage. Acta Physiol Plant, 2019; 41:42. <https://doi.org/10.1007/s11738-019-2833-x>
 34. Frazier TP, Burklew CE, Zhang B. Titanium dioxide nanoparticles affect the growth and microRNA expression of tobacco (*Nicotiana tabacum*). Funct Integr Genom, 2014; 14:75-83.
 35. Navarro E, Baun A, Behra R, Hartmann NB, Filser J, Miao A, Quigg A, Santschi PH, Sigg L. Environmental behavior and ecotoxicity of engineered nanoparticles to algae, plants, and fungi. Ecotoxicol, 2008; 17:372-386.
 36. Heinen RB, Ye Q, Chaumont F. Role of aquaporins in leaf physiology. J Exp Bot, 2009; 11: 2971-2985.
 37. Gao F, Hong F, Liu C, Zheng L, Su M, Wu X, Yang F, Wu C, Yang P. Mechanism of nano-anatase TiO₂ on promoting photosynthetic carbon reaction of spinach: Inducing complex of Rubisco-Rubisco activase. Biol Trace Element Res, 2006; 111:239-253.
 38. Khodakovskaya, MV, de Silva K, Biris AS, Dervishi E, Villagarcia H. Carbon nanotubes induce growth enhancement of tobacco cells. ACS Nano, 2012; 6:2128-2135.
 39. Das A, Eldakak M, Paudel B, Kim D-W, Hemmati H, Basu C, Rohila JS. Leaf proteome analysis reveals prospective drought and heat stress response mechanisms in soybean. BioMed Res Int, 2016; 6021047.
 40. Sun WH, Wu YY, Wen XY, Xiong SJ, He HG, Wang Y, Lu GQ. Different mechanisms of photosynthetic response to drought stress in

- tomato and violet orychofragmus. *Photosynthetica*, 2016; 5:226–233.
41. Huber SC, McMichael RW, Bachmann M, Huber JL, Shannon JC, Kang KK, Paul M. 1996. Regulation of leaf sucrose-phosphate synthase and nitrate reductase by reversible protein phosphorylation. In: Shewry PR, Halford NG, Hooley R. eds. *Protein Phosphorylation in Plants*. Proceedings of the Phytochemical Society of Europe. Oxford Science Publications, Clarendon Press, Oxford, UK, pp 19–34.
 42. Foyer CH, Valadier M-H, Migge A, Becker TW. Drought-induced effects on nitrate reductase activity and mRNA and on the coordination of nitrogen and carbon metabolism in maize leaves. *Plant Physiol*, 1998; 117:283–292.
 43. Neill SJ, Desikan R, Hancock JT. Nitric oxide signalling in plants *New Phytol*, 2003; 159: 11–35.
 44. Dbira S, Al Hassan M, Gramazio P, Ferchichi A, Vicente O, Prohens J, Boscaiu M. Variable levels of tolerance to water stress (Drought) and associated biochemical markers in Tunisian barley landraces. *Molecules*, 2018; 23:613. doi:10.3390/molecules23030613
 45. Kamarudin ZS, Yusop MR, Mohamed MTM, Ismail MR, Harun AR. Growth performance and antioxidant enzyme activities of advanced mutant rice genotypes under drought stress condition. *Agronomy*, 2018; 8:279. doi:10.3390/agronomy8120279.
 46. Fathi A, Tari DB. Effect of drought stress and its mechanism in plants. *Int J Life Sci*, 2016; 10:1–6.
 47. Hruby M, Cigler P, Kuzel S. Contribution to understanding the mechanism of titanium action in plant. *J Plant Nutr*, 2002; 25:577–598.
 48. Khan MN. Nano-titanium dioxide (Nano-TiO₂) mitigates NaCl stress by enhancing antioxidative enzymes and accumulation of compatible solutes in tomato (*Lycopersicon esculentum* Mill.). *J Plant Sci*, 2016; 11:1-11.
 49. Khan MN, Alzuaibr FM. Nano-titanium dioxide-induced synthesis of hydrogen sulfide and cysteine augment drought tolerance in *Eruca sativa*. *Asian J Plant Sci*, 2018; 17:213-221.
 50. Ahmad B, Shabbir A, Jaleel H, Khan MMA, Sadiq Y. Efficacy of titanium dioxide nanoparticles in modulating photosynthesis, peltate glandular trichomes and essential oil production and quality in *Mentha piperita* L. *Curr Plant Biol*, 2018; 13:6–15.
 51. Jafarnia S, Akbarinia M, Hosseinpour B, Modarres Sanavi SAM, Salami SA. Effect of drought stress on some growth, morphological, physiological, and biochemical parameters of two different populations of *Quercus brantii*. *I Forest*, 2018; 11:212-220. doi: 10.3832/ifer2496-010.
 52. Mohammadi H, Esmailpour M, Gheranpaye A. Effects of TiO₂ nanoparticles and water-deficit stress on morpho-physiological characteristics of dragonhead (*Dracocephalum moldavica* L.) plants. *Acta Agric. Slovenica*, 2016; 107: 385 – 396.
 53. Khan MN, Mobin M, Abbas ZK, Siddiqui MH. Nitric oxide-induced synthesis of hydrogen sulfide alleviates osmotic stress in wheat seedlings through sustaining antioxidant enzymes, osmolyte accumulation and cysteine homeostasis. *Nitric Oxide*, 2017; 68:91-102. <https://doi.org/10.1016/j.niox.2017.01.001>
 54. Khan MN, AlZuaibr FM, Al-Huqail AA, Siddiqui MH, Ali HM, Al-Muwayhi MA, Al-Haque HN. Hydrogen sulfide-mediated activation of O-Acetylserine (Thiol) lyase and L/D-cysteine desulfhydrase enhance dehydration tolerance in *Eruca sativa* Mill. *Int J Mol Sci*, 2018; 19:3981; <https://doi.org/10.3390/ijms19123981>.
 55. Khan MN, Siddiqui MH, Mohammad F, Naeem M. Interactive role of nitric oxide and calcium chloride in enhancing tolerance to salt stress. *Nitric Oxide*, 2012; 27:210-218.
 56. Sakamoto A, Murata N. Genetic engineering of glycinebetaine synthesis in plants: Current status and implications for enhancement of stress tolerance. *J Exp Bot*, 2000; 51:81-88.
 57. Mohammadi R, Maali-Amiri R, Mantri NL. Effect of TiO₂ nanoparticles on oxidative damage and antioxidant defense systems in chickpea seedlings during cold stress. *Russian J Plant Physiol*, 2014; 61:768-775.