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NANOSIZED TITANIUM DIOXIDE SEED PRIMING ENHANCES SALT TOLERANCE OF AN ORNAMENTAL AND MEDICINAL PLANT *PAEONIA SUFFRUTICOSA*

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Abstract

Paeonia suffruticosa is a popular ornamental and medicinal plant it is vulnerable to saline stress. To find an effective seed treatment for *P. suffruticosa* under salt stress, the present study explored the effects of nanosized titanium dioxide (nano-TiO_2) priming on the germination, growth and physiological response of *P. suffruticosa* under salt stress. The seeds were primed with different concentrations of nano-TiO_2 for either 48 h or 72 h in a growth chamber. The germination characteristics, activities of superoxide dismutase, peroxidase, and catalase enzymes, net photosynthetic rate (P_n), chlorophyll content, lateral root number, and seedling biomass were evaluated. The results indicated that treatment with 2, 10, and 100 mg·L^{-1} nano-TiO_2 significantly reversed the adverse effects of salinity stress on *P. suffruticosa* seed germination and increased the activities of superoxide dismutase, peroxidase, and catalase, the number of lateral roots and the content of chlorophyll in seedlings, thereby increasing seedling dry weights *P. suffruticosa* of when primed for 48 h. Nano-TiO_2 priming for 72 h increased the P_n of *P. suffruticosa* seedlings, while priming for 48 h decreased P_n or had no significant effect. The findings suggest that priming seeds with 2, 10, or 100 mg·L^{-1} nano-TiO_2 is an effective method for enhancing seed germination and early seedling growth in *P. suffruticosa* under salt stress.

Key words: Nanomaterials; Titanium dioxide; Paeonia; Seed priming; Salt stress.

Introduction

Paeonia suffruticosa Andrews (Paeoniaceae), belongs Moutan of the genus *paeonia*, is a popular ornamental and medicinal plant that is widespread in China. It is native to China and has also been introduced in other Asian counties (e.g., Japan), Europe, and America (Hao *et al*., 2013). In addition to ornamental use due to its attractive flowers, *P. suffruticosa* has a long history of use in traditional Chinese medicine (TCM) and has been recorded in the Pharmacopoeia of the People's Republic of China. *P. suffruticosa* shows analgesic, antianaphylactic, antioxidative, anti-inflammatory, and antitumor properties; it has also been widely used across Asia to treat infection, atherosclerosis, inflammation and cutaneous disease (Gao *et al*., 2015). Furthermore, *P. suffruticosa* seeds contain high concentrations of unbound unsaturated fatty acids, which are used for refining edible oils. *P. suffruticosa* seeds, in addition, have been used in Chinese folk medicine for whitening skin, curing oral ulcers, and easing waist and leg pain (Almosnid *et al*., 2016). In view of its or wamental and medicinal value *P. suffruticosa* is widely cultivated because.

With the expansion of *P. suffruticosa* cultivation, salt stress has become an increasingly critical question for its cultivation. Salinity is one of the major abiotic stresses in agriculture worldwide (Kasim *et al*., 2016). Unfortunately, our previous study found that *P. suffruticosa* is vulnerable to salt stress. Low seed germination rates and weak early seedling growth were observed when *P. suffruticosa* was subjected to salt stress, consequently resulting in low yield and quality. Although several studies explored the impacts of arbuscular mycorrhizal fungi on the physiological characteristics of *P. suffruticosa* plants under salt stress (Guo *et al*., 2013; Liang *et al*., 2013), but little is known about *P. suffruticosa* seed treatment under salt stress.

Seed priming is a low-cost, easy, and low-risk technique for overcoming the salinity problem on agricultural lands, and its positive effects under such conditions have been previously reported for many crops, such as radish (*Raphanus sativus*) and *Physalis angulata* L. (de Souza *et al*., 2016; Kasim *et al*., 2016; Ibrahim 2016; Yan 2016). For example, Yan (2016) found that hydropriming is an effective seed pretreatment method for increasing seed germination percentages and for promoting early seedling growth in *Brassica rapa* subsp. *pekinensis* grown under salt stress.

On the other hand, nanotechnology plays an important role in revolutionizing the agricultural field, economics, society and the environment (Singh *et al*., 2016). In recent years, the effects of nanomaterials on seed germination and plant growth have been widely studied, promoting their use in agricultural production (Zari *et al*., 2015).

Nanopriming is a new method, being used to improve seed germination percentage and seedling growth (Dehkourdi & Mosavi 2013). Nanosized titanium dioxide (nano-TiO_2) is one of the most fascinating materials used to stimulate the germination and growth of various seeds (Mir *et al*., 2016). Recently, studies have identified successful applications of nano-TiO_2 in promoting seed germination and seedling growth in several crops, such as spinach (*Spinacia oleracea*) (Zheng *et al*., 2005), wheat (*Triticum aestivum* L. var. Pishtaz) (Feizi *et al*., 2012), *Petroselinum crispum* (Dehkourdi & Mosavi 2013), *Solanum lycopersicum* and *Vigna radiata* (Singh *et al*., 2016), and *Hordeum vulgare* (Mir *et al*., 2016) under a suitable range of nano-TiO_2 concentrations.

Recently, in addition to their role in plant growth and development, nanomaterials have been used as a vital tool to improve growth and crop productivity under adverse environmental conditions such as salt stress (Khan *et al*., 2016). Previously, nano-TiO_2 exhibited the ability to alleviate the negative effects of cold stress by mitigating

membrane damage and electrolyte leakage in chickpea (*Cicer arietimun* L.) (Mohammadi *et al*., 2013). Furthermore, the application of nano-TiO_2 enhanced the activities of superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) (Mohammadi *et al*., 2014) and the expression of Rubisco and chlorophyll protein-binding genes (Hasanpour *et al*., 2015), maintaining the stability of the chlorophyll and carotenoid content and increasing the tolerance of chickpea plants to cold stress. In addition, nano-TiO_2 has been shown to positively affect the physiological characteristics and growth of plants exposed to other stresses, such as UV-B radiation (Zheng *et al*., 2008), drought stress (Jaberzadeh *et al*., 2013; Aghdam *et al*., 2016), and cadmium (Cd) toxicity (Singh & Lee, 2016).

However, despite numerous released reports on the effects of nano-TiO_2 on seed germination and seedling growth, few studies have focused on the effects of nano-TiO_2 priming treatments on the germination characteristics and seedling growth of crops under salt stress, especially aromatic and medicinal plants.

Due to the importance of *P. suffruticosa* as a popular ornamental and medicinal plant and its reduced germination percentage under salt stress, as well as the demonstrated successful application of nano-TiO_2 as a seed precondition strategy, we hypothesized that nano-TiO_2 priming could alleviate the negative effect of salt stress on germination and subsequently on early seedling growth in *P. suffruticosa*. Therefore, the present study was conducted to explore the optimum nano-TiO_2 concentration and priming duration for effective *P. suffruticosa* seed preconditioning to enable the seeds to survive adverse salinity conditions during cultivation.

Materials and Methods

Materials: *P. suffruticosa* seeds were collected from a cultivation base in Shangluo, Shaanxi Province, China, in July 2013. Species identification was performed by Professor Qiaosheng Guo of the Institute of Chinese Medicinal Materials at Nanjing Agricultural University in Nanjing, China.

Nano-TiO_2 was purchased from Shanghai Xilong Chemical Co., Ltd, in Shanghai, China. The particle size was 5-10 nm, and the purity was >99.0%. Nano-TiO_2 solutions were prepared at concentrations of 2, 10, 100, 500, and 1000 $mg \cdot L^{-1}$ in distilled water. An ultrasonication treatment was applied for 15 min to obtain properly dispersed and stable TiO_2 suspensions of each concentration.

Salt stress treatments: *P. suffruticosa* seeds were washed with running tap water, sterilized in 0.5% potassium permanganate for 2 h and then rinsed three times with distilled water. After soaking in distilled water for 24 h, the seeds were mixed with wet sand in a 1:3 ratio (V/V) and incubated in a growth chamber (relative humidity 75%) at 20°C under darkness for 90 d. When the radicle was at least 4 cm in length, the seeds were soaked in 300 $mg \cdot L^{-1}$ GA_3 for 24 h. Then, the seeds were transferred to 10-mm Petri dishes filled with sterilized sand, and the seeds were sown at a depth of approximately 1 cm. Ten microliters of NaCl at serial concentrations (0.1%, 0.3%, 0.5%, 0.7%, and 0.9% NaCl solutions, using distilled water as the control) was added to Petri dishes. Seed germination was tested in a growth chamber at 15°C and 75% humidity with a photoperiod of 12-h light, with a light intensity of 300 $\mu E \cdot m^{-2} \cdot s^{-1}$ and protection from drying. Each Petri dish contained 50 seeds with triplicate treatments. The sand was changed every 10 d to maintain the corresponding NaCl concentration. The number of germinated seeds was noted daily. The germination potential and germination percentage were measured at 45 and 60 days after sowing (DAS).

Nano-TiO_2 seed priming treatments: A randomized complete design with three replicates was employed from February to September of 2014. The factors included different concentrations of nano-TiO_2 and different priming durations (48 h or 72 h). The nanosized TiO_2 treatments included five concentrations (2, 10, 100, 500, and 1000 $mg \cdot L^{-1}$), control 1 (non-primed dried seeds), and control 2 (seeds primed with distilled water for 24 h). The selection of control 2 was based on our previous *P. suffruticosa* seed germination experience because we found that priming with distilled water for 24 h was beneficial for *P. suffruticosa* seed germination.

P. suffruticosa seeds were soaked in different concentrations of nano-TiO_2 for either 48 h or 72 h in a growth chamber at 20°C under darkness. After priming, the seeds were washed with distilled water and kept at room temperature until they were redried to the initial seed moisture content. The primed *P. suffruticosa* seeds were sterilized in 0.5% potassium permanganate for 2 h and then rinsed three times with distilled water. The seeds were mixed with wet sand at a 1:3 ratio (V/V), stratified in a growth chamber at 20°C and maintained under darkness for 90 d. Nine hundred seeds (30 groups of 30 seeds) were randomly selected and placed in moistened sand in Petri dishes for each treatment.

When the seed radicles were at least 4 cm in length, the seeds were randomly selected and soaked in 300 $mg \cdot L^{-1}$ GA_3 for 24 h and then rinsed three times with distilled water. Then, the seeds were transferred and sown in 10-mm Petri dishes filled with sterilized sand at a depth of approximately 1 cm. Ten milliliters of 0.3% NaCl solution was added to each Petri dish. The seeds were tested for germination in a growth chamber at 15°C and 75% humidity with a photoperiod of 12-h light, using a light intensity of 300 $\mu E \cdot m^{-2} \cdot s^{-1}$ and protection from drying. Each Petri dish contained 50 seeds with triplicate treatments. The sand was changed every 10 d to maintain a stable 0.3% NaCl concentration.

The number of germinated seeds was recorded every day. The germination percentage was measured at 60 DAS. The *P. suffruticosa* seedlings were harvested, and growth parameters were measured at 120 DAS when the seedlings were approximately 20 cm in height with three leaves. The superoxide dismutase, peroxidase and catalase activities, chlorophyll content and *Pn* were measured using fresh leaves. The number of lateral roots and seedling dry weight were also recorded.

Growth and physiological parameter measurements: The following equations were used to calculate germination parameters:

$$\text{Germination percentage (\%)} = (Gf/n) \times 100,$$

where Gf is the total number of germinated seeds at the end of the experiment (60 DAS), and n is the total number of seeds used in the test.

$$\text{Germination potential (\%)} = (Gi/n) \times 100,$$

where Gi is the total number of germinated seeds at 45 DAS, and n is the total number of seeds used in the test.

Thirty seedlings were randomly selected to count the number of lateral roots; then, the seedlings were oven-dried at 70°C and weighed.

Fresh leaves (0.5 g) were homogenized with a mortar and pestle in 10 mL of 50 mM sodium phosphate buffer containing 1% polyvinylpyrrolidone (w/v). The homogenate was centrifuged at 15,000 rpm at 4°C for 10 min; then, the supernatant was used for subsequent antioxidative enzyme assays.

SOD activity was determined according to the method of Giannopolitis & Ries (1977), POD activity was determined according to Shannon *et al*., (1966), and CAT activity was determined according to Pukacka & Ratajczak (2005).

Thirty *P. suffruticosa* seedlings were randomly selected, and the leaves were used to measure the *Pn* and chlorophyll content index (SPAD) between 11:00 a.m. and 1:00 p.m.

SPAD-502 (Konica-Minolta, Osaka, Japan) was used to measure the SPAD value, and *Pn* was measured with a LI-6400XT (LI-COR, Lincoln, Nebraska, USA).

Statistical analysis

SPSS 20.0 (IBM Corp., Armonk, NY) was used to conduct the statistical analyses. All experiments were prepared in triplicate, and the data were presented as the mean value ± the standard deviation. The data were compared using Duncan's test; $p \leq 0.05$ was considered significant.

Results

Effects of different concentrations of NaCl on the germination of *P. suffruticosa* seeds: The germination percentage and germination potential of *P. suffruticosa* seeds exposed to different concentrations of NaCl are shown in (Fig. 1.). The germination percentage and germination potential of *P. suffruticosa* seeds decreased with increasing NaCl concentrations in a similar pattern. No significant difference was found in the germination percentage of the 0.1% NaCl treatment and the control (distilled water). When the NaCl concentration was 0.3%, the germination potential and germination percentage were 47.62% and 54.76%, respectively. With a further increase in NaCl concentration, the germination percentage and germination potential were decreased markedly. The *P. suffruticosa* seeds failed to germinate when the NaCl concentration was 0.9%. Thereafter, 0.3% NaCl was used as the salt stress condition for further seed germination, seedling growth, and physiological analysis.

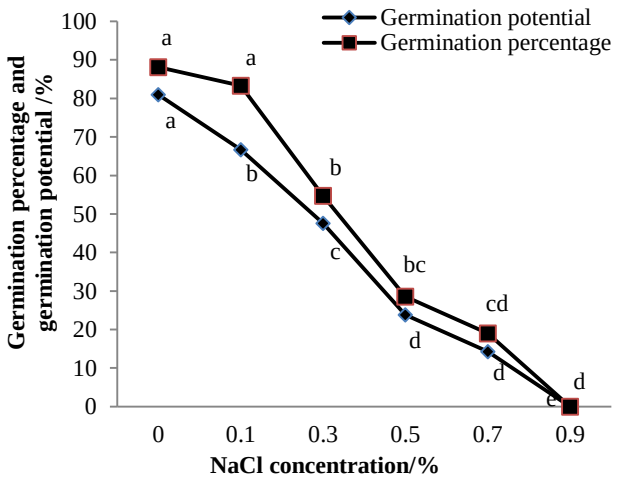

Fig. 1. Effects of different concentrations of NaCl on germination of *P. suffruticosa* seeds.

Effects of nano-TiO_2 priming on the germination percentage of *P. suffruticosa* seeds under salt stress: Under 0.3% NaCl salinity conditions, the effect of nano-TiO_2 priming on the germination percentage of *P. suffruticosa* seeds varied with nano-TiO_2 concentration and priming duration (Fig. 2). When priming for 48 h, compared with control 1 (non-primed) and control 2 (hydroprimed for 24 h), the low concentrations of the nano-TiO_2 treatment (2, 10, and 100 $mg \cdot L^{-1}$) increased germination percentage, whereas the high concentrations of the nano-TiO_2 treatment (500 and 1000 $mg \cdot L^{-1}$) decreased germination percentage. The highest germination percentage (57.14%) under salt stress was observed in the 100 $mg \cdot L^{-1}$ nano-TiO_2 treatment, followed by the 2 $mg \cdot L^{-1}$ nano-TiO_2 treatment. These values were significantly higher than the corresponding value in control 1 (31.82%). Furthermore, the 2 and 100 $mg \cdot L^{-1}$ nano-TiO_2 treatments increased the seed germination percentage by 25.00% and 26.98%, respectively, compared with control 2, although no significant difference was observed. The seed germination percentages in the 500 and 1000 $mg \cdot L^{-1}$ treatments were 44.91% and 65.46% of that in the non-primed control and 31.76% and 46.29% of that in the hydroprimed control, respectively.

When priming for 72 h, all of the concentrations of nano-TiO_2 led to a lower germination percentage of *P. suffruticosa* seeds than did control 1 (non-primed) and control 2 (hydroprimed). The seed germination percentage in the 2, 100, and 500 $mg \cdot L^{-1}$ treatment groups was 47.14%, 41.89% and 44.91% of that in the non-primed group and 33.33%, 29.62%, and 31.76% of that in the hydroprimed group, respectively.

Under treatment with 2, 10, and 100 $mg \cdot L^{-1}$ nano-TiO_2, a lower germination percentage was found when the priming duration was prolonged from 48 h to 72 h. Under treatment with 500 and 1000 $mg \cdot L^{-1}$ nano-TiO_2, the germination percentage remained stable when the priming duration changed from 48 h to 72 h.

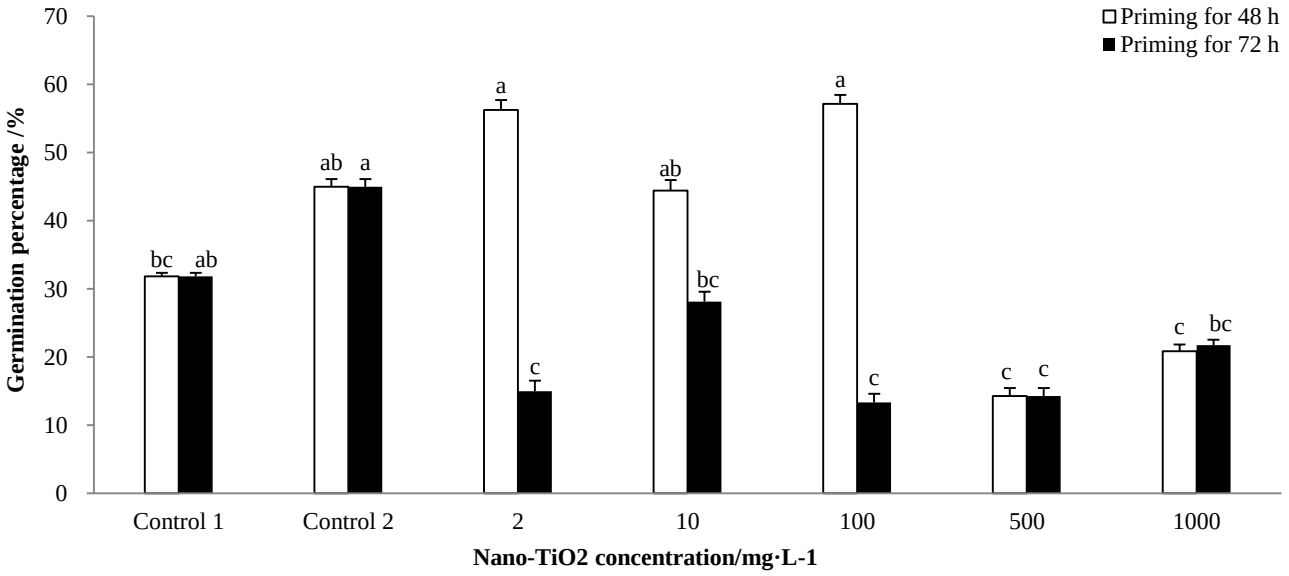

Fig. 2. Effects of nano-TiO_2 priming on the germination of *P. suffruticosa* seeds under salt stress. Control 1 was the non-priming treatment and control 2 involved hydro-priming for 24 h.

Table 1. Effects of nano-TiO_2 priming on SOD, POD, and CAT activities in the *P. suffruticosa* seedlings under salt stress ($\bar{x}$ ± SD，n=3). Control 1 was the non-priming treatment and control 2 was the hydro-priming for 24 h treatment.

Priming time /h	Nano-TiO_2 concentration/mg·L^{-1}	SOD/U·g^{-1}	CAT/mg·g^{-1}·min^{-1}	POD/U·g^{-1}·min^{-1}
48	Control 1	171.67 ± 1.01 b	24.00 ± 1.66 b	5.33 ± 0.51 d
	Control 2	220.99 ± 2.08 ab	34.67 ± 3.23 a	33.20 ± 0.57 a
	2	309.55 ± 6.19 a	33.33 ± 1.78 a	24.13 ± 0.19 b
	10	348.79 ± 2.04 a	39.33 ± 0.94 a	23.20 ± 1.51 b
	100	316.26 ± 3.08 a	36.67 ± 2.71 a	23.33 ± 2.71 b
	500	318.57 ± 3.21 a	37.33 ± 2.33 a	16.80 ± 1.51 c
	1000	225.67 ± 2.01 ab	26.57 ± 2.34 b	14.20 ± 1.67 c
72	Control 1	171.67 ± 2.01 de	24.00 ± 1.66 d	5.33 ± 0.51 d
	Control 2	220.99 ± 1.08 cd	34.67 ± 2.11 ab	33.20 ± 0.57 a
	2	301.78 ± 4.07 b	32.00 ± 1.23 abcd	13.87 ± 0 c
	10	369.57 ± 2.04 a	40.67 ± 0.94 a	20.93 ± 1.96 b
	100	147.53 ± 1.06 e	25.33 ± 2.11 cd	4.53 ± 0 d
	500	284.58 ± 1.04 b	33.33 ± 2.66 abc	21.73 ± 2.07 b
	1000	258.34 ± 2.01 bc	26.00 ± 1.71 bcd	16.13 ± 0.94 c

Effects of nano-TiO_2 priming on antioxidative systems of *P. suffruticosa* seedlings under salt stress: After *P. suffruticosa* seeds were primed with different concentrations of nano-TiO_2 for 48 h, the plants grown from primed seeds exhibited increased SOD, CAT, and POD activities compared with the plants grown from non-primed seeds (control 1) (Table 1). The activities of SOD and CAT initially increased and then decreased with increasing nano-TiO_2 concentrations. The highest SOD and CAT activities were found in the 10 mg·L^{-1} nano-TiO_2 treatment, with 103.51% and 63.88% increases compared with control 1, respectively. With increasing nano-TiO_2 concentrations, the POD activity decreased, and the 2 mg·L^{-1} nano-TiO_2 treatment resulted in a 3.52-fold increase in POD activity over that of control 1. However, treatment with different concentrations of nano-TiO_2 exhibited no advantage over hydropriming (control 2) in SOD, CAT, and POD activities. No significant difference in SOD activity was found between each concentration of nano-TiO_2 and hydropriming (control 2). The POD activity in control 2 was higher (p<0.05) than that in each of the Nano-TiO_2 treatments. In terms of CAT activity, the 2, 10, 100 and 500 mg·L^{-1} nano-TiO_2 treatments showed no significant difference from hydropriming (control 2), and further increases in nano-TiO_2 concentration (1000

$mg \cdot L^{-1}$) resulted in a significant decline in CAT activity compared with control 2.

After priming for 72 h, the variation in the SOD, CAT, and POD activities indicated an M-curve with increasing nano-TiO_2 concentration. Compared with control 1, the 10 $mg \cdot L^{-1}$ nano-TiO_2 treatment increased the SOD, CAT, and POD activities by 115.28%, 292.68%, and 69.46%, respectively. However, the SOD and POD activities in the 100 $mg \cdot L^{-1}$ nano-TiO_2 treatment were lower than those in control 1. When compared with control 2, the treatments with different concentrations of nano-TiO_2 exhibited decreased POD activity ($p<0.05$). Except for the 100 $mg \cdot L^{-1}$ nano-TiO_2 treatment, the other concentrations of nano-TiO_2 increased SOD activity compared with control 2. In the case of CAT activity, except for the 10 $mg \cdot L^{-1}$ nano-TiO_2 treatment, the other concentrations of nano-TiO_2 resulted in decreased CAT activity compared with control 2.

Effects of nano-TiO_2 priming on the P_n of *P. suffruticosa* seedlings under salt stress: The effects of nano-TiO_2 seed priming on the P_n of *P. suffruticosa* seedlings under 0.3% NaCl salinity conditions are shown in Fig. 3. When nano-TiO_2 was used to prime *P. suffruticosa* seeds for 48 h, compared with the nonprimed control, none of the nano-TiO_2 concentrations significantly impacted P_n. The P_n value of the 100 $mg \cdot L^{-1}$ nano-TiO_2 treatment (8.76 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$) was higher ($p<0.05$) than that of the hydroprimed control, whereas the other concentrations of nano-TiO_2 did not differ significantly compared with the hydroprimed control.

When nano-TiO_2 was used to prime *P. suffruticosa* seeds for 72 h, all concentrations of nano-TiO_2 increased the P_n of *P. suffruticosa* seedlings compared to no priming and hydropriming. The highest P_n was found in the 100 $mg \cdot L^{-1}$ nano-TiO_2 treatment (12.43 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$), representing 47.27% and 113.57% increases compared with no priming and hydropriming, respectively.

The P_n of *P. suffruticosa* seedlings increased when the priming duration was prolonged from 48 h to 72 h at all nano-TiO_2 treatment concentrations. The increasing magnitude was concentration dependent, with the highest increment of 98.63% observed in the 1000 $mg \cdot L^{-1}$ nano-TiO_2 treatment.

Priming effects of nano-TiO_2 on the relative chlorophyll content of *P. suffruticosa* seedlings under salt stress: When *P. suffruticosa* seeds were primed with different concentrations of nano-TiO_2 for 48 h, the seedlings grown from primed seeds exhibited increased SPAD compared with those grown from the non-primed seeds (control 1) (Fig. 4); in particular, the 2, 10, 100, and 500 $mg \cdot L^{-1}$ nano-TiO_2 treatments significantly increased the SPAD value. The SPAD values in the 2 and 500 $mg \cdot L^{-1}$ nano-TiO_2 treatments were 1.36- and 1.08-fold higher than that of control 1 (non-primed). Compared with control 2 (hydroprimed for 24 h), the 2 and 500 $mg \cdot L^{-1}$ nano-TiO_2 treatments exhibited an increased SPAD value ($p<0.05$), whereas the 100 and 1000 $mg \cdot L^{-1}$ nano-TiO_2 treatments significantly decreased SPAD values.

When priming for 72 h, compared with no priming (control 1), all the nano-TiO_2 treatment concentrations significantly increased the SPAD value in *P. suffruticosa* leaves. The highest SPAD value was found in the 500 $mg \cdot L^{-1}$ nano-TiO_2 treatment, which increased by 84.72% compared with control 1. However, none of the nano-TiO_2 concentrations significantly impacted the SPAD value compared with hydropriming (control 2).

When the priming time was prolonged from 48 h to 72 h, a 38.64% decrease was found in the 2 $mg \cdot L^{-1}$ nano-TiO_2 treatment, and a low variation range of 3.69% to 14.97% was observed in the other nano-TiO_2 treatment concentrations.

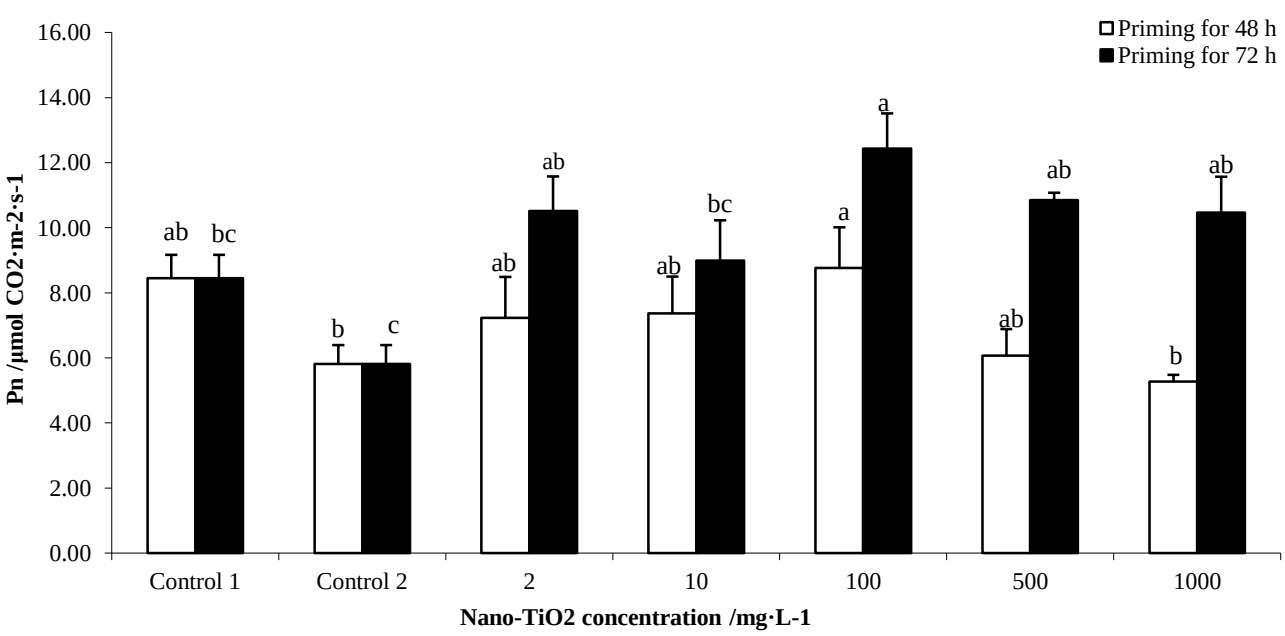

Fig. 3. Effects of nano-TiO_2 priming on the net photosynthetic rate of *P. suffruticosa* seedlings under salt stress. Control 1 was the non-priming treatment and control 2 was the hydro-priming for 24 h treatment.

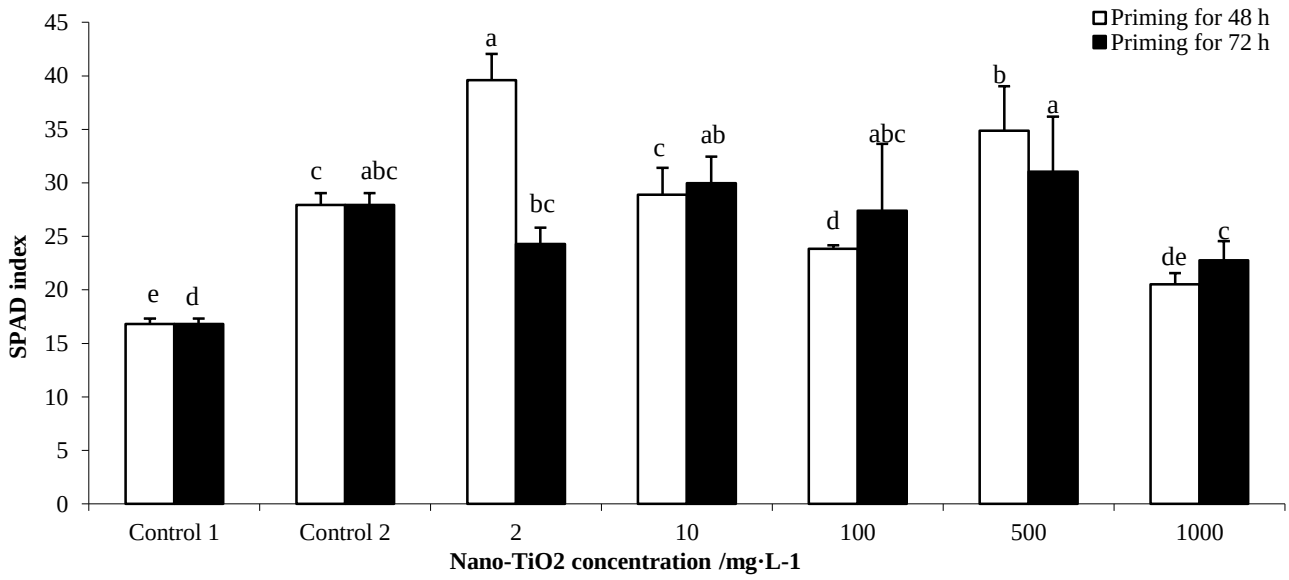

Fig. 4. Effects of nano-TiO_2 priming on the relative chlorophyll content of *P. suffruticosa* seedlings under salt stress. Control 1 was the non-priming treatment and control 2 was the hydro-priming for 24 h treatment.

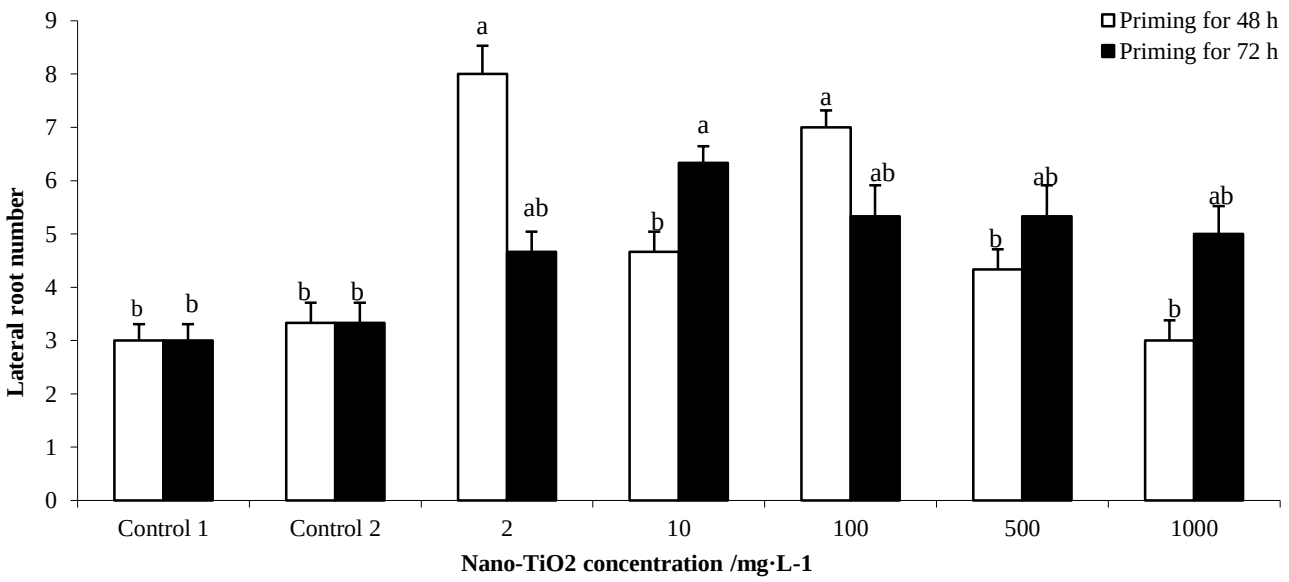

Fig. 5. Effects of nano-TiO_2 priming on the lateral root number of *P. suffruticosa* seedlings under salt stress. Control 1 was the non-priming treatment and control 2 was the hydro-priming for 24 h treatment.

Effects of nano-TiO_2 priming on the number of lateral roots in *P. suffruticosa* under salt stress: Under 0.3% NaCl salinity conditions, priming with different concentrations of nano-TiO_2 (2, 10, 100 and 500 mg·L^{-1}) for 48 h resulted in more lateral roots in seedlings; in particular, significantly more lateral roots were observed in the 2 and 100 mg·L^{-1} nano-TiO_2 treatments than in control 1 and control 2 (p<0.05) (Fig. 5). The number of lateral roots in the 2 mg·L^{-1} nano-TiO_2 treatment increased by 1.67-fold and 1.40-fold compared with control 1 and control 2, respectively.

When priming for 72 h, treatment with all concentrations of nano-TiO_2 increased the number of lateral roots, and the highest value (6.33) was found in the 10 mg·L^{-1} nano-TiO_2 treatment, with increases of 111.00% and 90.09% compared with control 1 and control 2, respectively. Except for the 10 mg·L^{-1} nano-TiO_2 treatment, no significant difference was observed among the other nano-TiO_2 treatment groups, the no priming treatment group, and the hydropriming treatment group.

In the 2 and 100 mg·L^{-1} nano-TiO_2 treatments, fewer lateral roots were found in *P. suffruticosa* seedlings when the seeds were primed for 72 h than for 48 h. The opposite tendency was observed in the 10, 500, and 1000 mg·L^{-1} nano-TiO_2 treatments.

Effects of nano-TiO_2 priming on the dry weight of *P. suffruticosa* seedlings under salt stress: When *P. suffruticosa* seeds were primed with 2, 10, and 100 mg·L^{-1} nano-TiO_2 for 48 h, the seedlings grown from primed seeds exhibited higher dry weights than those grown from non-primed seeds (control 1) and hydroprimed seeds (control 2) (Fig. 6). In particular, the 2 mg·L^{-1} nano-TiO_2 treatment increased the dry weight

by 102.12% and 89.74% compared to no priming (control 1) and hydropriming (control 2), respectively. In addition, the 10 and 100 mg·L^{-1} nano-TiO_2 treatments increased the dry weight by 94.13% and 82.23% compared to no priming (control 1) and by 87.28% and 75.80% compared to hydropriming (control 2), respectively. The seedling dry weights in the 500 and 1000 mg·L^{-1} nano-TiO_2 treatments did not differ significantly from those in control 1 or control 2.

After priming for 72 h, the highest dry weight was observed in the 10 mg·L^{-1} nano-TiO_2 treatment, which was significantly higher than that in control 1. Compared with control 1 and control 2, other concentrations of nano-TiO_2 did not significantly influence the dry weight of *P. suffruticosa* seedlings.

When comparing the effect of nano-TiO_2 priming duration, 48 h of seed priming led to higher *P. suffruticosa* seedling dry weight than did 72 h of seed priming at the concentrations of 2, 10, and 100 mg·L^{-1} nano-TiO_2, whereas 48 h of seed priming resulted in a lower dry weight than did 72 h of seed priming at the concentrations of 500 and 1000 mg·L^{-1} nano-TiO_2.

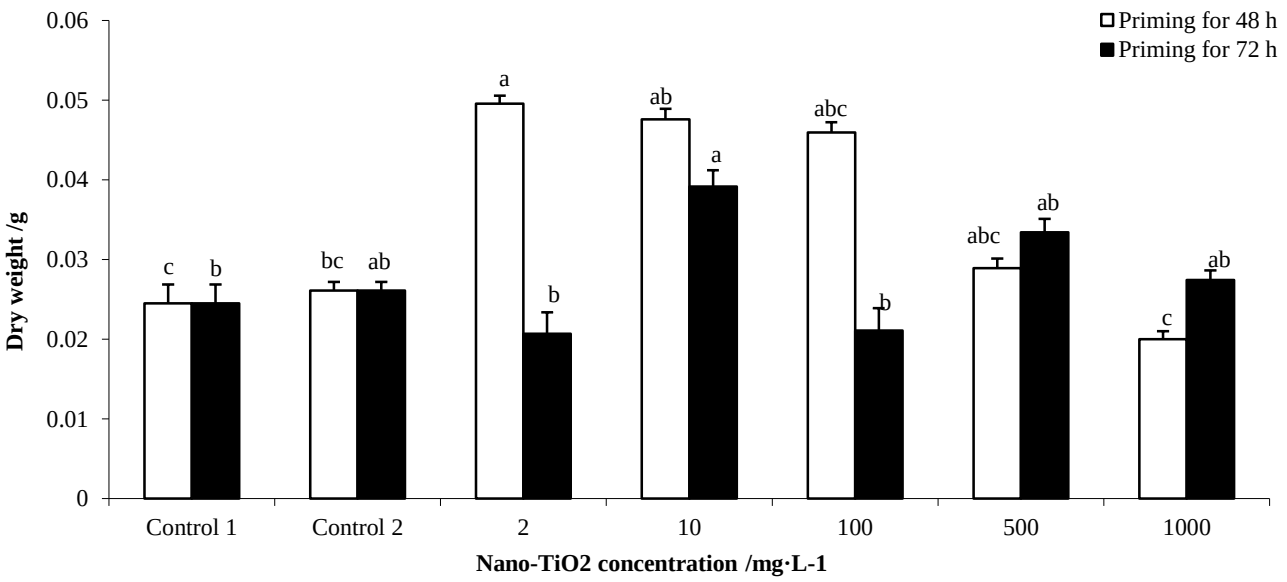

Fig. 6. Effects of nano-TiO_2 priming on the dry weight of *P. suffruticosa* seedlings under salt stress. Control 1 was the non-priming treatment and control 2 was the hydro-priming for 24 h treatment.

Discussion

In most plant species, salinity adversely affects seed germination and seedling development, which are considered the developmental stages that are most sensitive and vulnerable to abiotic stresses (de Souza *et al*., 2016; Ibrahim, 2016). Several previous studies have shown that priming is a useful technique to prevent the detrimental effect of salinity on seed germination, especially for low vigor seeds (de Souza *et al*., 2016).

Several researchers found a possible mechanism of the positive role of nano-TiO_2 in seed germination. The positive effect of nano-TiO_2 on seed germination can possibly be explained by the small particle size, which allows nano-TiO_2 to penetrate into the seed during the treatment period and to promote growth after germination. First, nano-TiO_2 with increased seed penetration power facilitates the entry of water and oxygen into the cell and increases the absorption of nutrients in the seed, ultimately stimulating seed germination (Dehkourdi & Mosavi, 2013; Singh *et al*., 2016). Second, the entry of nano-TiO_2 into cells induces oxidation–reduction reactions via superoxide ion radicals during germination in the dark, resulting in the quenching of free radicals in germinating seeds. During this process, additional oxygen could in turn be produced for respiration, further promoting germination (Zheng *et al*., 2005). In the present work, priming seeds with proper concentrations of nano-TiO_2 mitigated the adverse effects of salinity stress on *P. suffruticosa* seed germination and subsequent early seedling growth. When priming for 48 h, low concentrations of nano-TiO_2 (2, 10, and 100 mg·L^{-1}) accelerated seed germination, whereas high concentrations of nano-TiO_2 (500 and 1000 mg·L^{-1}) suppressed seed germination.

Seed priming could develop different defense mechanisms, such as antioxidant defense systems and osmotic adjustment, in seeds to protect against salinity stress (Ibrahim, 2016). Hence, seed priming with nano-TiO_2 possibly has an integrated advantage in mitigating salinity-induced oxidative damage. In the present study, *P. suffruticosa* seedlings grown from seeds primed with nano-TiO_2 showed increased SOD, POD, and CAT activities. Similarly, previous research has shown that nano-TiO_2 can protect plants from various abiotic stresses by stimulating the activities of antioxidant enzymes and the accumulation of osmolytes, free amino acids and nutrients (Zheng *et al*., 2008; Mohammadi *et al*., 2013, 2014; Khan *et al*., 2016). Under oxidantive stress caused by UVB radiation, nano-TiO_2 could directly clear large amounts of $O_2^{\bullet-}$ and indirectly remove reactive oxygen species (ROS) by activating SOD, CAT, GPX, and APX in spinach chloroplasts (Zheng *et al*., 2008). Furthermore, in chickpea plants treated with TiO_2 nanoparticles, decreased H_2O_2 and MDA content and electrolyte leakage index and more effective antioxidant enzymes were observed in

comparison with the control plants, indicating that the intracellular presence of nano-TiO_2 probably promotes protective metabolic processes, such as the induction of defense machinery when ROS accumulation occurs under cold stress conditions (Mohammadi *et al*., 2014).

Photosynthesis is a cellular process highly susceptible to abiotic stresses. However, nano-TiO_2 has been found to protect photosynthetic systems and to improve photosynthesis by suppressing oxidative stress (Zheng *et al*., 2008; Mohammadi *et al*., 2014; Aghdam *et al*., 2016; Khan *et al*., 2016; Singh *et al*., 2016). Previously, research on improving spinach photosynthesis suggested that nano-TiO_2 plays an important role in increasing light absorbance, accelerating the transport and conversion of light energy, protecting chloroplasts from oxidative stress, prolonging the duration of photosynthesis, activating Rubisco to promote Rubisco carboxylation, and increasing the rate of the photosynthetic carbon reaction (-Zheng *et al*., 2008; Hasanpour *et al*., 2015). In the current study, under salinity stress, different concentrations of nano-TiO_2 (ranging from 2-1000 $mg \cdot L^{-1}$) used in seed priming for 72 h increased the net photosynthetic rate and chlorophyll content of *P. suffruticosa* seedlings grown from primed seeds. These results were in agreement with the results of Aghdam *et al*., (2016), who observed that nano-TiO_2 particles at appropriate concentrations decreased H_2O_2 accumulation in *Linum usitatissimum* under drought stress, which subsequently prevented chlorophyll degradation and/or stimulated its biosynthesis.

In the present study, the increase in the biomass of *P. suffruticosa* treated with low concentrations of nano-TiO_2 is possibly the comprehensive result of morphological and physiological effects caused by nano-TiO_2 priming, e.g., the positive effects on photosynthetic ability and lateral roots. The present results indicated that the 2 and 100 $mg \cdot L^{-1}$ nano-TiO_2 treatments were beneficial for increasing the number of lateral roots and further increasing the active absorption area and salt tolerance of *P. suffruticosa*. In addition, improved photosynthesis and increased chlorophyll content are two important factors for biomass accumulation in *P. suffruticosa*. These results may be best explained by the ability of nano-TiO_2 to increase plants' capacity to absorb water and nitrogenated fertilizers, promote vigor in root systems, increase nitrate reductase activity, accelerate the breakdown of organic substances, and facilitate the formation of essential amino acids (Zheng *et al*., 2005). Similarly, previous studies reported that the application of nano-TiO_2 reversed the negative effects of abiotic stress on the growth characteristics of wheat (Jaberzadeh *et al*., 2013) under water deficit stress and those of soybean plants (*Glycine max*) under Cd stress (Singh & Lee, 2016). Furthermore, Hasanpour *et al*., (2015) found that chickpea plants treated with TiO_2 nanoparticles under cold stress gained more carbon and had a higher metabolic potential for photosynthesis, which, in turn, ensured the ability of plants to acclimate.

Despite abundant literature on the positive effects of TiO_2 nanoparticles on plant seed germination and seedling growth, there are negative impacts of TiO_2 nanoparticles. For example, Castiglione *et al*., (2011) observed that after short-term exposure, nano-TiO_2 decreased the seed germination percentage for the first 24 h in both *Vicia narbonensis* and *Zea mays*. Root elongation was affected only after treatment with a higher nano-TiO_2 concentration. Additionally, Song *et al*., (2013) found that nano-TiO_2 did not have an effect on seed germination or on most of the plant species tested, including *Brassica campestris* ssp. *napus* var. *nippo-oleifera* Makina, *Lactuca sativa* L., and *Phaseolus vulgaris* var. humilis. No physiological differences in enzyme activities or chlorophyll content were found, even though the plants absorbed the nano-TiO_2.

However, the inconsistent effect of nano-TiO_2 on plants is not surprising because the impacts of nanoparticles on seed germination and plant development depend on the concentration, type, and size of nanoparticles, the mechanism of uptake, the plant species, and the specific conditions of experiments (Castiglione *et al*., 2011; Mir *et al*., 2016; Singh *et al*., 2016). It has been demonstrated that nano-TiO_2 exhibited positive effects at low concentrations, whereas it can be toxic to plants at high concentrations. For example, Zheng *et al*., (2005) found that spinach plant growth greatly improved under 250-4,000 $mg \cdot L^{-1}$ nano-TiO_2 treatment, but no improvement was observed at higher concentrations. Similarly, Feizi *et al*., (2012) showed that low concentrations of nanosized TiO_2 (2 and 10 $mg \cdot L^{-1}$) could accelerate seed germination in wheat, but a negative or neutral effect on wheat seeds was observed at high concentrations (100 and 500 $mg \cdot L^{-1}$).

Conclusions

In summary, priming seeds with low concentrations of nano-TiO_2 could reverse the adverse effects of salinity stress on *P. suffruticosa* seed germination and subsequent early seedling growth. When primed for 48 h, low concentrations of nano-TiO_2 (2, 10, or 100 $mg \cdot L^{-1}$) accelerated seed germination and increased the activities of SOD, POD, and CAT, the number of lateral roots and the content of chlorophyll in seedlings, thereby increasing *P. suffruticosa* seedling dry weight. However, high concentrations of nano-TiO_2 (500 and 1000 $mg \cdot L^{-1}$) suppressed seed germination but had no significant impact on early seedling biomass. Hence, there is a large potential for nano-TiO_2 seed priming in *P. suffruticosa* production under salt stress. However, the optimization of *P. suffruticosa* seed nano-TiO_2 priming technology and the mechanism involved require further extensive research into the environmental and health risks of nano-TiO_2 to meaningfully help reduce adverse effects of nano-TiO_2 on agricultural systems.

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References

Aghdam, M.T.B., H. Mohammadi and M. Ghorbanpour. 2016. 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.* 39: 139-146.

Almosnid, N.M., Y. Gao, C. He, H.S. Park and E. Altman. 2016. *In vitro* antitumor effects of two novel oligostilbenes, *cis*- and *trans*-suffruticosol D, isolated from *Paeonia suffruticosa* seeds. *Int. J. Oncol*., 48: 646-656.

Castiglione, M.R., L. Giorgetti, C. Geri and R. Cremonini. 2011. The effects of nano-TiO_2 on seed germination, development and mitosis of root tip cells of *Vicia narbonensis* L. and *Zea mays* L. *J. Nanopart. Res*., 13: 2443-2449.

de Souza, M.O., C.R. Pelacani, L.A.J. Willems, R.D. de Castro, H.W.M. Hilhorst and W. Ligterink. 2016. Effect of osmopriming on germination and initial growth of *Physalis angulata* L. under salt stress and on expression of associated genes. *Ann. Braz. Acad. Sci*., 88: 503-516.

Dehkourdi, E.H. and M. Mosavi. 2013. Effect of anatase nanoparticles (TiO_2) on parsley seed germination (*Petroselinum crispum*) *In vitro*. *Biol. Trace Elem. Res*., 155: 283-286.

Feizi, H., P. Rezvani Moghaddam, N. Shahtahmassebi and A. Fotovat. 2012. Impact of bulk and nanosized titanium dioxide (TiO_2) on wheat seed germination and seedling growth. *Biol. Trace Elem. Res*., 146: 101-106.

Gao, Y., C.N. He, R. Ran, D.B. Zhang, D.W. Li, P.G. Xiao and E. Altman. 2015. The resveratrol oligomers, *cis*- and *trans*-gnetin H, from *Paeonia suffruticosa* seeds inhibit the growth of several human cancer cell lines. *J. Ethnopharmacol*., 169: 24-33.

Giannopolitis, C.N. and S.K. Ries. 1977. Superoxide dismutases: I. Occurrence in higher plants. *Plant Physiol.*, 59: 309-314.

Guo, S.X., L.J. Xu and M. Li. 2013. Effects of AM fungi on salt tolerance of tree peony seedlings. *Chin. Agric. Sci. Bull.*, 29: 123-129 (in Chinese with English abstract).

Hao, Q., N. Aoki, J. Katayama, T. Kako, K.S. Cheon, Y. Akazawa and N. Kobayashi. 2013. Crossability of American tree peony 'High Noon' as seed parent with Japanese cultivars to breed superior cultivars. *Euphytica*., 191: 35-44.

Hasanpour, H., R. Maali-Amiri and H. Zeinali. 2015. Effect of TiO_2 nanoparticles on metabolic limitations to photosynthesis under cold in chickpea. *Russ. J. Plant Physiol*., 62: 779-787.

Ibrahim, E.A. 2016. Seed priming to alleviate salinity stress in germinating seeds. *J. Plant Physiol*., 192: 38-46.

Jaberzadeh, A., P. Moaveni, H.R. Tohidi Moghadam and H. Zahedi. 2013. Influence of bulk and nanoparticles titanium foliar application on some agronomic traits, seed gluten and starch contents of wheat subjected to water deficit stress. *Not. Bot. Horti. Agrobo.*, 41: 201-207.

Kasim, W.A.E.A., K.M. Saad-Allah and M. Hamouda. 2016. Seed priming with extracts of two seaweeds alleviates the physiological and molecular impacts of salinity stress on radish (*Raphanus sativus*). *Int. J. Agric. Biol.*, 18: 653-660.

Khan, M.N., M. Mobin, Z.K. Abbas, K.A. AlMutairi and Z.H. Siddiqui. 2017. Role of nanomaterials in plants under challenging environments. *Plant Physiol. Biochem.*, 110: 194-209.

Liang, Q.Q., W.H. Wang, S.X. Guo, S.M. Li and R.J. Liu. 2013. Effects of arbuscular mycorrhizae on photosynthesis of *Paeonia suffruticosa* under salt stress. *J. Qingdao Agric. Univ.*, 30: 79-83 (in Chinese with English abstract).

Mir, H., A.G. Ahangar and N. Mir. 2016. Superior performance of dye-sensitized versus conventional TiO_2 nanoparticles for promoting germination and early growth of barley: From photovoltaic to biotechnological application. *J. Nano. Res-SW*., 35: 77-91.

Missaoui, T., M. Smiri, H.Chmingui and A. Hafiane. 2017. Effects of nanosized titanium dioxide on the photosynthetic metabolism of fenugreek (*Trigonella foenum-graecum* L.). *C.R. Biol*., 340: 499-511.

Mohammadi, R., R. Maali-Amiri and A. Abbasi. 2013. Effect of TiO_2 nanoparticles on chickpea response to cold stress. *Biol. Trace Elem. Res*., 152: 403-410.

Mohammadi, R., R. Maali-Amiri and N. Mantri. 2014. Effect of TiO_2 nanoparticles on oxidative damage and antioxidant defense systems in chickpea seedlings during cold stress. *Russ. J. Plant Physiol.*, 61: 768-775.

Pukacka, S. and E. Ratajczak. 2005. Production and scavenging of reactive oxygen species in *Fagus sylvatica* seeds during storage at varied temperature and humidity. *J. Plant Physiol*., 162: 873-885.

Shannon, L.M., E. Kay and J.Y. Law. 1966. Peroxidase isoenzyme from horse radish roots: isolation and physical properties. *J. Biol. Chem.*, 241: 2166-2172.

Singh, J. and B.K. Lee. 2016. Influence of nano-TiO_2 particles on the bioaccumulation of Cd in soybean plants (*Glycine max*): A possible mechanism for the removal of Cd from the contaminated soil. *J. Environ. Manage*., 170: 88-96.

Singh, P., R. Singh, A. Borthakur, P. Srivastava, N. Srivastava, D. Tiwary and P.K. Mishra. 2016. Effect of nanoscale TiO_2-activated carbon composite on *Solanum lycopersicum* (L.) and *Vigna radiata* (L.) seeds germination. *Energ. Ecol. Environ.*, 1: 131-140.

Song, U., M. Shin, G. Lee, J.Roh. Y. Kim and E.J. Lee. 2013. Functional analysis of TiO_2 nanoparticle toxicity in three plant species. *Biol. Trace Elem. Res.*, 155: 93-103.

Yan, M. 2016. Hydro-priming increases seed germination and early seedling growth in two cultivars of Napa cabbage (*Brassica rapa* subsp. pekinensis) grown under salt stress. *J. Horti. Sci. Biotech.*, 91: 421-426.

Zari, H., P. Babak and R. Asad. 2015. The effect of priming with nano-sliver on agronomic traits of safflower cultivars. *J. Essent. Oil Bear Pl.*, 18: 1148-1156.

Zheng, L., F.S. Hong, S.P. Lu and C. Liu. 2005. Effect of nano-TiO_2 on strength of naturally aged seeds and growth of spinach. *Biol. Trace Elem. Res.*, 104: 83-91.

Zheng, L., M.Y. Su, X. Wu, C. Liu, C.X. Qu, L. Chen, H. Huang, X.Q. Liu and F.S. Hong. 2008. Antioxidant stress is promoted by nano-anatase in spinach chloroplasts under UV-B radiation. *Biol. Trace Elem. Res.*, 121: 69-79.

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