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Effects of nanoparticle treatments on propagation of *Prunus mahaleb* L. by seed

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Abstract

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We examined the effects of nanoparticles (NPs) of TiO_2 and ZnO at 0.5, 1, 2 or 3% concentrations for 10, 20, and 30 min in stratified seeds of *Prunus mahaleb* Linnaeus. Then, seedlings produced were irrigated to field capacity with NP solutions at control, 1, 5, and 10% concentration for 7 months in the greenhouse conditions. Treating seeds at 1% concentrations of TiO_2-NPs for 20 min resulted in the highest germination percentage (65%) and at concentrations of 3% for 30 min it showed the lowest germination percentage (13%). The highest total seedling height was obtained after exposure of seeds to 0.5% TiO_2-NPs for 10 min. Irrigation of seedlings with TiO_2-NPs at the concentration of 1% seems to be a suitable method how to increase their total height, survival, and total dry weight. A decrease in the relative water content and an increase in proline were observed in response to the application of high levels of NPs.

Keywords: germination percentage; growth characteristics; proline; relative water content; seed dormancy; survival

The Mahaleb cherry (*Prunus mahaleb* Linnaeus) belongs to the family Rosaceae with a wide distribution throughout Mediterranean region, central Europe, northwest Africa, and south-western Asia including countries such as Pakistan, Turkey, Iraq, and Iran (Kollmann, Pflugshaupt 2005). The fruit of *P. mahaleb* is a small thin-fleshed (stone fruit), spherical cherry-like drupe that is 8–10 mm in diameter; and seeds with a hard, bony endocarp that changes in colour as it ripens from green to red, dark purple and finally black, fully maturing in mid to late summer.

Propagation of this species in the wild and nurseries is by seed. For reasons: (*i*) wasteful seed harvesting by forest dwellers, (*ii*) low germination percentage of seeds due to having both an internal mechanism (embryo dormancy) and an external mechanism (endocarp dormancy and the testa), (iii) multiplicative effects of climate change, grazing livestock, and expansion of agriculture, (*iv*) the decreasing quality and quantity of mother plants of this species in natural habitats (Sekhavati et al. 2011), the emergence rate in natural regeneration of *P. mahaleb* is less than 10%. This could lead to the loss of this valuable

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Prunus Linnaeus genetic resource, therefore, to the restoration of its natural habitats; investigation on propagation of this species is needed.

The seed-coat structure, abscisic acid content in the testa or phenolic content and the chemical compounds of seed kernels, capacity of gibberellin formation by the embryo affect germination percentage and dormancy (Hartmann et al. 1997). The seed kernels of *P. mahaleb* contain coumarin, hydrogen cyanide, tannins, herniarin, dihydrocoumarin, fatty acids, sucrose, high protein content and fixed oil (27–40%), and a small amount of amygdaline (Al-Said, Hifnawy 1986). Fruits often collected by growers in full maturity when their colour is dark red, which leads to a low permeability of seed coat to water and gases, delay in the germination percentage and then to an increase in the duration of the stratification period. However, the stony endocarp in *Prunus* fruits is water permeable (Dirr, Heuser 1987) but some time is needed until the endocarp becomes more permeable to water and oxygen. Under natural conditions, the release of seeds from dormancy generally occurs during alternating temperatures by gradual abrasion of the seed coat, the action of fire, wind and water, synergistic and competing effects of environmental and endogenous signals and the action of microorganisms (Schmidt 2000), seeds passing through the gut of animals, and leaching of phenolic compounds and alkaloids by rainwater (Venier et al. 2012).

Several studies have been performed to overcome and accelerate the breaking of dormancy in *Prunus* seeds. Sekhavati et al. (2011) reported that *P. mahaleb* seeds did not germinate even after a three-month period of chilling and their seed germination percentage is regulated by internal and external hormonal stimuli. According to the results of Pipinis et al. (2012), removal of the endocarp, application of gibberellic acid (1,000 or 2,000 $mg \cdot l^{-1}$ for 24 h) and then cold stratification for 1 month showed the highest germination percentage of *P. mahaleb* seeds. In seven species of *Prunus* (*P. armeniaca* Linnaeus, *P. avium* Linnaeus, *P. domestica* Linnaeus, *P. mahaleb*, *P. padus* Linnaeus, *P. pensylvanica* Linnaeus f., *P. serotina* Ehrhart) Grisez et al. (2008) concluded that warm stratification followed by a period of cold stratification is more effective on seed germination percentage than using cold stratification alone.

In nature, seeds are often exposed to drought and salinity stresses. Hence, choosing an appropriate technique to increase seed tolerance to adverse environmental conditions would enhance the probability of success of seed germination percentage. Among different methods, nanoparticles (NPs) of different metal oxides by absorbing water, oxygen and nutrients and having the antimicrobial properties can affect seed germination percentage, improve growth, dry weight, photosynthesis, chlorophyll biosynthesis, and plant metabolism (Rezaei et al. 2015). Thus, soaking seeds in NP solution can be used as an option to increase seed germination percentage. The application of 100% TiO_2-NP treatment increased seed germination percentage, germination index, germination energy, vigour index, seedling height and fresh weight of *Pinus tabulaeformis* Carrière (Yinfeng, Xiaohua 2009). The effect of silver NPs on *Boswellia ovalifoliolata* N.P. Balakrishnan & A.N. Henry caused an increase and acceleration of the seed germination percentage (Savithramma et al. 2012). Despite positive effects of NPs, some researchers reported that many metal oxide NP treatments have phytotoxic effects on plant growth (Dimkpa et al. 2012). However, Zarafshar et al. (2015) did not observe any toxic effects on some of the physiological and biochemical parameters after addition of SiO_2-NPs in irrigation of *Pyrus biosseriana* Buhse seedlings.

The purpose of this study was to assess effects of NPs of metal oxides like TiO_2 and ZnO at different concentrations and time periods on germination percentage and growth of stratified seeds of *P. mahaleb*. Also, seedlings of *P. mahaleb* were irrigated with NP solutions at different concentrations for determining their effect on growth, survival, and biochemical properties. In our study, Ti and Zn concentrations in the aboveground biomass of *P. mahaleb* seedlings in treatments with NPs of TiO_2 and ZnO were determined.

MATERIAL AND METHODS

Seed collection. Fruits of *P. mahaleb* were collected on 25 June 2012 from upright branches of stock plants 10 years old in the Sholabad of Lorestan province, Iran, located at 49°12'E, 33°12'N, 1,630 m a.s.l. Mean annual rainfall, temperature and humidity are 683 mm, 13°C, and 49.1%, respectively. The drought period for the area is approximately 120 days, occurring from June through to the end of September, which has a rather cold, semi-humid mountain climate. The experiment was carried out in the greenhouse of the Agricultural and Natural Resources Research Centre of Markazi province, Arak, Iran, located at 49°43'E, 34°7'N, 1,690 m a.s.l.

Determination of seed characteristics. The pulp was removed by wet maceration. The pH level of the juicy pulp of fruits was determined with a pH meter (Lab 420A, Metrohm AG, USA) based on four replicates of 50 pulps each. The soluble solids content expressed as Brix degree was determined with a refractometer (DR101-60, Krüss GmbH, Germany) based on the International Seed Testing Association method. Evaluations of physical characteristics of the seeds immediately after harvest such as seed number per kg, weight per 1,000 seeds, moisture content, purity, viability, and mean seed diameter of the fresh seeds were done. Eight samples of 100 pure seeds each were used to estimate 1,000 seed weight, seed moisture content by the air-oven method (103°C, 17 h) using three replicates of 10 g each, purity by weighing four replicates of 100 g seeds each, viability by the 2,3,5-triphenyltetrazolium chloride solution test with three replicates of 50 seeds each, and mean seed diameter of 20 randomly selected seeds.

Stratification. Seeds with endocarp were immersed in running water for 24 h, afterwards seeds were surface sterilized in a 2% NaOCl solution for 15 min, and then rinsed with distilled water three times. Then the seeds were subjected to warm stratification in a moist medium in a growth chamber for 1 month at the constant temperature of 23°C and 50% relative humidity and then followed cold stratification in the refrigerator at 4°C for 2 months (Baskin, Baskin 1998). After stratification, the seeds were treated with NPs.

The effect of TiO_2-NP and ZnO-NP treatments on improving germination. Seeds were treated with the TiO_2 and ZnO NPs at control, 0.5, 1, 2 or 3% concentration for 10, 20, and 30 min at the ambient laboratory temperature. They were purchased from Neutrino Corporation, New York. The NPs were dissolved in distilled water to obtain solutions. Polyethylene bags (4 cm diameter, 5.5 cm height) were filled with mixed soil of moist sand, perlite, and cocopeat (2:1:1) and then treated seeds were placed on 1 cm-deep layer. The experiment was carried out in a temperature controlled growth chamber with air circulation at 23 ± 0.5°C and 16/8 h (light-dark) at 3,000 lux light intensity. Seeds were checked daily for moisture content. Criterion of germination was the emergence of cotyledons above the soil mixture. The number of germinating seeds was counted at 3-day intervals for 60 days, from the time germination commenced. Germination percentage was calculated when no further germination took place for 7 days. For this experiment, approximately 3,600 seeds were collected on the end date of harvest. All treatments were repeated 3 times with 50 seeds for each treatment in a factorial arrangement based on a completely randomized design. Germination testing was terminated after 180 days. The germination percentage was calculated by Eq. 1 (Panwar, Bhardwaj 2005):

$$\frac{\text{number of germinated seeds}}{\text{total number of sown seeds}}\times 100 \quad (1)$$

At the end of the growing season, 3 seedlings were randomly selected from each repetition in treatment and assessed for total height and total dry weight. Total height of seedlings was recorded using a ruler to the nearest cm and dry weight to the nearest g at 70°C for 24 h.

Effect of NPs at different concentrations on growth, survival, and biochemical response of *P. mahaleb* seedlings. Seedlings produced from untreated seeds were transferred on 5 April 2013 to polyethylene bags (20 cm diameter, 40 cm height) containing soil/cattle manure (2:1) for the study of the effect of NPs at control, 1, 5, and 10% concentrations on growth and biochemical properties. After determining the soil texture, bulk density, soil moisture in pot and soil moisture curve plotting, seedlings were watered to field capacity with NP solutions every 2 days regularly according to Saxton et al. (1986) method for the whole 7 months duration of growing.

All treatments were repeated 3 times with 10 seedlings in each replicate in a complete randomized design. After 7 months in the greenhouse conditions, the seedlings were harvested and assessed for total height, survival, total dry weight, leaf relative water content – RWC (Dhopte 2002) and proline by the ninhydrin and sulphosalicylic acid method (Bates et al. 1973).

For determination of Ti concentration in dry aboveground tissues of *P. mahaleb* seedlings, plant tissues were acid-digested at 150°C for 4 h in pressurized Teflon lined vessels with 70% nitric acid and then diluted to a final concentration of 2% nitric acid and read using graphite furnace atomic absorption spectroscopy (Burke et al. 2015). For determination of Zn concentration in dry aboveground tissues of *P. mahaleb* seedlings irrigated with NP treatments, plant tissues were digested in a microwave oven following the EPA 3051 method proposed by the US Environmental Protection Agency using 10 ml of HNO_3. The digested samples were then solubilized in 10 ml of double-distilled water and filtered with cellulose filter paper (pore diameter of 0.45 μm) and read using inductively coupled plasma optical emission spectrometry.

Data analysis. Distribution was tested for normality by the Kolmogorov-Smirnov test and germi-

Table 1. Characteristics of the *Prunus mahaleb* Linnaeus seeds

pH level of the juicy pulp	Soluble solids content – Brix (%)	Seed No. per kilogram	Weight per 1,000 seeds (g)	Moisture (%)	Purity (%)	Viability (%)	Mean seed diameter (mm)
4.04	7.2	12,682	73	10.1	99	85	5.3

nation percentage data were transformed to arcsine ($\sqrt{x}$) values before statistical analysis. The assumption of homogeneity of variance among treatments was tested using Levene's test. Statistical significance between mean values was performed using one-way ANOVA. Duncan's multiple range test was used for the comparison of the means at a 5% probability level ($P \leq 0.05$) using SPSS (Version 19, 2010).

RESULTS

Characteristics of seeds

Evaluations for the characteristics of *P. mahaleb* seeds are shown in Table 1.

The effects of TiO_2-NP and ZnO-NP treatments on germination percentage, total height, and total dry weight

In treatments with TiO_2-NPs, an increase in germination percentage and total dry weight was observed at a concentration of 1% for 20 min. The highest total height was at a concentration of 0.5% for 10 min (32.63 cm) (Table 2). The control (only stratification used) shows a low germination percentage that did not differ from the treatments of seeds at a concentration of 2% TiO_2-NPs for 10 min and 3% TiO_2-NPs for 30 min, although seed viability of 85% was obtained.

In treatments with ZnO-NPs, germination percentage and total height were greater at a concentration of 2% for 10 min and were lower at a concentration of 0.5% ZnO-NPs for 30 min and in the control and at a concentration of 3% ZnO-NPs for 10 min than in the other treatments (Table 2).

Effect of irrigation with TiO_2-NP and ZnO-NP treatments on growth, survival, and biochemical response of *P. mahaleb* seedlings

Under NP treatments, the highest total height, survival, and total dry weight were obtained in TiO_2-NPs at concentrations of 1%. ZnO-NPs at a concentration of 10% showed the lowest all traits measured except for proline (Table 3). A significant

Table 2. Effects of TiO_2 nanoparticles (NPs) and ZnO-NP treatments at concentrations of 0.5, 1, 2, and 3% and for different time periods on the germination percentage, total height, and total dry weight in seeds of *Prunus mahaleb* Linnaeus

Treatment	Concentration (%)	Time (min)	TiO_2-NPs			ZnO-NPs		
			germination (%)	total height (cm)	total dry weight (g)	germination (%)	total height (cm)	total dry weight (g)
T1	control		15 ± 1.73^{ij}	18.24 ± 0.03^{j}	0.08 ± 0.00^{j}	15 ± 1.73^{e}	18.24 ± 0.03^{e}	0.08 ± 0.00
T2	0.5	10	27.67 ± 1.2^{f}	32.63 ± 0.02^{a}	0.25 ± 0.00^{d}	36 ± 0.58^{c}	18.24 ± 0.41^{e}	0.1 ± 0.02
T3	1		41.67 ± 0.88^{c}	16.34 ± 0.02^{k}	0.25 ± 0.00^{d}	56.67 ± 0.88^{a}	19.02 ± 0.06^{d}	0.11 ± 0.00
T4	2		17.33 ± 0.33^{hi}	21.21 ± 0.18^{i}	0.14 ± 0.00^{i}	61 ± 1.15^{a}	27.13 ± 0.1^{a}	0.18 ± 0.03
T5	3		26 ± 0.58^{f}	25.22 ± 0.09^{e}	0.18 ± 0.00^{h}	31.67 ± 2.03^{c}	11.33 ± 0.07^{j}	0.09 ± 0.00
T6	0.5	20	55.67 ± 0.88^{b}	22.83 ± 0.03^{h}	0.23 ± 0.00^{e}	31.33 ± 0.67^{c}	13.25 ± 0.05^{i}	0.1 ± 0.00
T7	1		65 ± 0.58^{a}	24.25 ± 0.04^{g}	0.36 ± 0.00^{a}	61.67 ± 3.18^{a}	20.15 ± 0.17^{c}	0.12 ± 0.00
T8	2		30.33 ± 0.33^{e}	26.09 ± 0.03^{c}	0.21 ± 0.00^{f}	41.33 ± 1.2^{b}	16.72 ± 0.1^{f}	0.11 ± 0.00
T9	3		19.67 ± 0.88^{gh}	27.59 ± 0.11^{b}	0.26 ± 0.00^{c}	24 ± 0.58^{d}	16.78 ± 0.09^{f}	0.09 ± 0.00
T10	0.5	30	34.67 ± 0.88^{d}	25.74 ± 0.02^{d}	0.29 ± 0.00^{b}	13.33 ± 0.88^{e}	16.01 ± 0.04^{g}	0.08 ± 0.00
T11	1		35.67 ± 0.88^{d}	18.14 ± 0.09^{j}	0.23 ± 0.00^{e}	41.67 ± 2.6^{b}	25.26 ± 0.05^{b}	0.12 ± 0.00
T12	2		21.67 ± 0.88^{g}	18.21 ± 0.05^{j}	0.2 ± 0.00^{g}	45.67 ± 1.86^{b}	18.76 ± 0.15^{d}	0.43 ± 0.28
T13	3		13 ± 0.58^{j}	24.75 ± 0.04^{f}	0.21 ± 0.00^{f}	26.33 ± 1.86^{d}	15.01 ± 0.09^{h}	0.11 ± 0.00

Values are given as means ± standard errors, means with different letter(s) in the same column are significantly different from each other ($P < 0.05$)

Table 3. Effects of irrigation with TiO_2 nanoparticles (NPs) and ZnO-NP treatments at different concentrations on the total height, survival, total dry weight, relative water content, and proline of *P. mahaleb* seedlings after 7 months in the greenhouse conditions

Treatment		Concentration (%)	Total height (cm)	Survival (%)	Total dry weight (g)	Relative water content (%)	Proline (µmol·g^{-1} FW)
T1	control		22.08 ± 0.56^{cd}	71.67 ± 1.67^{b}	7.46 ± 0.25^{c}	92.07 ± 0.67^{a}	19.77 ± 0.77^{e}
T2	TiO_2-NPs	1	39.03 ± 1.39^{a}	93.33 ± 1.67^{a}	9.9 ± 0.4^{a}	90.57 ± 0.14^{a}	20.41 ± 0.66^{de}
T3	TiO_2-NPs	5	37.11 ± 1.63^{a}	88.33 ± 1.67^{a}	9.26 ± 0.15^{b}	87.15 ± 1.23^{b}	24.19 ± 1.05^{d}
T4	TiO_2-NPs	10	24.47 ± 2.86^{bc}	68.33 ± 4.41^{bc}	7.11 ± 0.15^{cd}	80.81 ± 0.67^{c}	34.6 ± 2.45^{c}
T5	ZnO-NPs	1	26.85 ± 0.62^{b}	60 ± 5.77^{c}	7.67 ± 0.2^{c}	82.45 ± 1.85^{c}	38.29 ± 0.74^{c}
T6	ZnO-NPs	5	22.78 ± 0.82^{cd}	43.33 ± 1.67^{d}	7.07 ± 0.14^{cd}	80.39 ± 0.89^{c}	46.01 ± 1.15^{b}
T7	ZnO-NPs	10	15.22 ± 2.69^{e}	28.33 ± 1.67^{e}	5.41 ± 0.15^{d}	74.48 ± 1.21^{d}	65.41 ± 3.31^{a}

Values are given as means ± standard errors, means with different letter(s) in the same column are significantly different from each other ($P < 0.05$)

FW – fresh weight

decrease in the RWC of *P. mahaleb* seedlings was observed in different treatments with NPs compared to the control.

Concentrations of Ti and Zn in seedlings grown with TiO_2 and ZnO NPs are shown in Figs 1a, b, respectively. The highest concentration of Ti and Zn in aboveground parts of *P. mahaleb* seedlings irrigated with TiO_2-NP and ZnO-NP treatments was obtained in TiO_2 and ZnO-NPs at a concentration of 10%.

DISCUSSION

The effect of TiO_2-NPs on germination percentage, total height, and total dry weight of seeds

It was found that the use of TiO_2-NPs enhanced the germination percentage of seeds compared to the control except the concentration of 3% for 30 min. Application of 0.5% concentration of TiO_2-NPs for 10 min and 1% for 30 min proved best by giving the

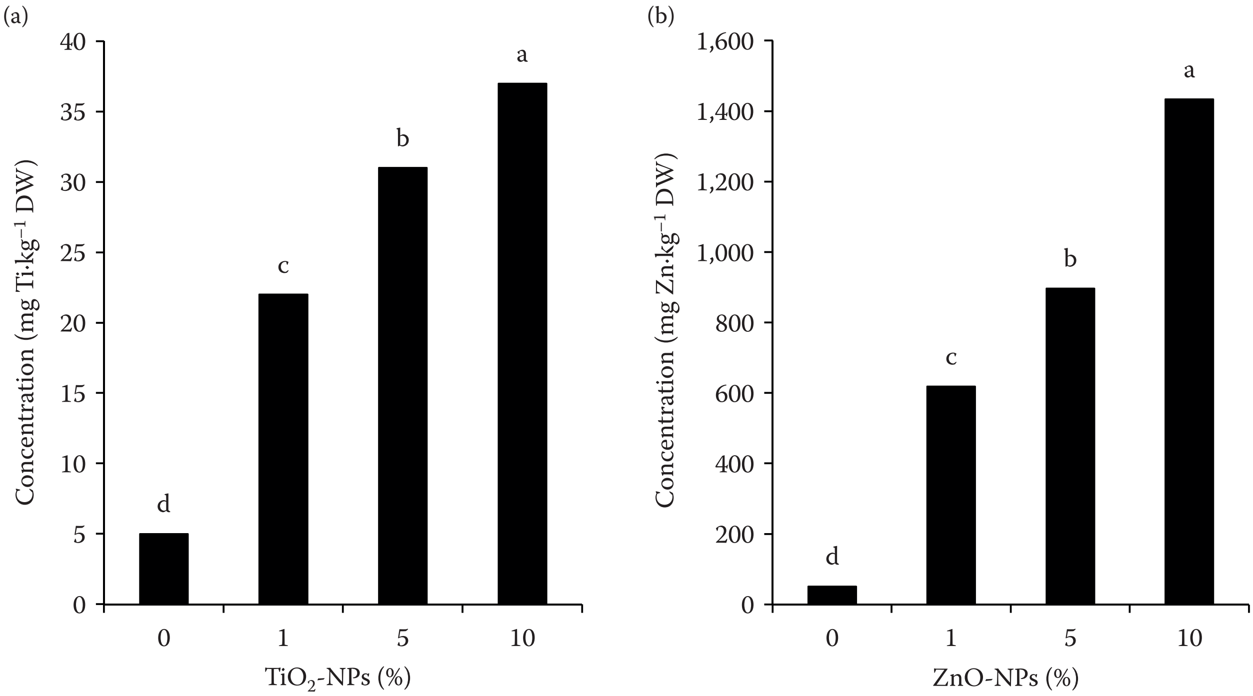

Fig. 1. Ti (a), Zn (b) concentration in aboveground parts of *Prunus mahaleb* Linnaeus seedlings irrigated with TiO_2 nanoparticles (NPs) and ZnO-NP treatments at different concentrations for 7 months in the greenhouse conditions. Data are means of three replicates ± standard error. Different small letters indicate significant difference in each of Ti and Zn contents between TiO_2-NP and ZnO-NP treatments ($P < 0.05$)

DW – dry weight

highest values for total height. The low germination percentage of control seeds (15%) under stratification shows that the seeds are in a dormant state.

These results confirm that seed soaking with TiO_2-NPs could increase the germination percentage of *P. mahaleb* at an appropriate concentration probably due to the cell wall loosening that apparently forces the endocarp to split and thus removes a physical barrier to germination. In higher concentrations, TiO_2-NPs accumulated in plant organs lead to the inhibition of plant growth probably due to the low solubility. The results of this study are in contrast with the results of Seeger et al. (2009) on seedlings of *Salix* sp. and Zarafshar et al. (2015) on *P. biosseriana* seedlings in which they did not induce any significant change in growth and biochemical parameters by applying NPs.

TiO_2-NPs enhance the seed germination percentage especially at low concentrations due to having antimicrobial and antifungal properties and encourage capsule penetration to the seed coat for intake of water, oxygen and fertilizer needed for activation of the embryo and then quick germination (Khot et al. 2012). They can affect the seed germination percentage and seedling growth depending on the chemical compound and the size and shape of the particles, concentration, their mechanism of uptake and/or plant species (Ruffini, Cremonini 2009).

The effect of ZnO-NPs on germination percentage, total height, and total dry weight

In the present study, lower concentrations with short-time immersion in ZnO-NPs showed significant enhancement in seed germination percentage, however a higher concentration (3% for 30 min) of ZnO-NPs led to a reduction in total height compared to the control. This can probably be due to toxicity or oxidative stress exerted by the higher concentration of ZnO-NPs causing moisture stress, low uptake of water and oxygen, and hence suppressing germination and growth due to the degradation of cell walls (Raskar, Laware 2014). Zn is an essential element for plant growth but at high concentrations it can also affect the root uptake of minerals by interference with several metabolic processes.

Generally, NPs increase some enzymes such as nitrate reductase, superoxide dismutase, ascorbate peroxidase, guaiacol peroxidase, and catalase activities, and reduce H_2O_2 and superoxide radicals and promote the antioxidant system that can improve seed germination percentage in some plant species (Lei et al. 2008). Enzyme activity causing structural changes in the pericarp and in the seed cell walls is therefore effective in breaking dormancy. NPs due to an increase in some scavenging reactive oxygen species enzymes can probably reduce oxidative damage and inhibit lipid peroxidation and thereby reduce environmental stresses and accelerate the breakdown of organic substances and formation of essential amino acids (Harrison 1996).

Another possible reason for increasing the germination percentage by NPs can be that they may generate new pores on the seed coat during penetration which may help to influx the nutrients and chemicals inside the seed. NPs may carry the nutrients, mineral elements and metabolites to cells which may lead to rapid germination and growth rate. NPs can affect xylem humidity and water translocation by attaching to the plant tissue and forming a cellulose layer which results in water use efficiency improvement (Sahebi et al. 2015).

The increase some chemical compounds such as abscisic acid and phenols and high seed physical dormancy that may result in reducing viability (Hossain et al. 2005). Seed coats in the genus *Prunus* contain large amounts of phenolic compounds that probably cause a reduction in oxygen availability to the embryo (Nesme 1985).

Effect of NPs of TiO_2 and ZnO on growth, survival and total dry weight of *P. mahaleb* seedlings

In the present study, the highest total height, survival, and total dry weight at concentrations of 1% TiO_2-NP treatment may be related to the increase in water absorbance and providing better conditions for growth. Similar results were achieved in TiO_2-NPs (2.5–40 $g\cdot kg^{-1}$ soil) that improve the growth of spinach by enhancing photosynthesis in leaves and nitrogen-fixation capability in roots (Yang et al. 2007).

In the results of Bao-shan et al. (2004), the treatment with 500 $\mu l\cdot l^{-1}$ SiO_2-NPs produced the highest mean height, root collar diameter, main root length, and the highest number of lateral roots in seedlings of *Larix olgensis* A. Henry. The resistance of *P. mahaleb* seedlings to different concentration of NPs could be determined by the survival percentage. The imbalance between the free radical generation, enzyme activity, endogenous hormone levels, cell moisture availability and the degree of oxidative stress when using high concentrations of NPs (Jayarambabu et al. 2014) will negatively affect the survival of seedlings.

Seedling weight loss at high concentrations of NPs in the present study can be due to a decrease in the water content of the plant and its effect on physiological processes such as transpiration, respiration and photosynthesis. In the present results, a reduction in RWC with the use of NPs especially at a concentration of 10% of ZnO-NPs was obtained. This is consistent with the results of Ma and Yamaji (2008) on *Oryza sativa* Linnaeus and Zarafshar et al. (2015) on *P. biosseriana*, who with the application of high levels of SiO_2-NPs reported a reduction in RWC due to a decrease in the xylem water potential.

Effect of NPs of TiO_2 and ZnO on RWC, proline and concentrations of Ti and Zn in *P. mahaleb* seedlings

The accumulation of non-enzymatic molecules such as proline in plant tissues shows the cell protection in response to abiotic stress conditions (Jin et al. 2012). High proline content and low RWC in ZnO-NPs at a concentration of 10% may be due to oxidative damage and then to the cell death in the leaves of *P. mahaleb*. This state indicates that the plant is under stress conditions that increase the parameter of proline that can act as a metabolic substrate and a good defensive mechanism for survival under stressful conditions (Ghars et al. 2008). In the control with the highest RWC, the lowest proline was achieved and proline increased with increasing levels of TiO_2 and ZnO NPs, which reflects the emphasis on contents listed above.

Prolines are strongly hydrophilic and have an important role in the improvement of plant tolerance to environmental stresses and protection of cellular structures during cell dehydration by reducing the water potential and keeping the activities of some biological macromolecules (Rathinasabapathi 2000). Its accumulation in plants under stress is associated with reducing damage to the cell membrane and proteins. Proline synthesis implicated in alleviating the cytoplasmic osmotic potential and retention of $NADP/NADPH^+$ ratio (Bhati-Kushwaha et al. 2013). They act as organic osmolytes, scavenging free radicals and a macromolecule stabilizer contributes to stabilizing sub-cellular structures (Hu et al. 2006).

In the present study, as the external concentration of NPs of TiO_2 and ZnO increased, Ti and Zn levels in tissues increased, thus presenting the translocation of Ti and Zn into aboveground plant tissues in seedlings grown with the NPs.

CONCLUSIONS

The current study showed that the seeds of *P. mahaleb* were in a dormant state at the time of harvesting. Seed stratification followed by the application NPs of TiO_2 and ZnO at different concentrations and time immersion could significantly affect seed germination percentage, total height and seedling dry weight. Within the concentration range the investigated irrigation of seedlings with NPs of TiO_2 and ZnO (1, 5, and 10%), the 1% concentration of TiO_2-NPs seems to be a suitable concentration for total height, survival, and total dry weight. With the application of high levels of NPs, a decrease in the RWC and an increase in the proline were observed. In this experiment, we studied uptake mechanisms of NPs in the seedling texture of *P. mahaleb*. Concentration of Ti and Zn in aboveground tissues increased as the external concentrations of NPs increased. Further studies are needed to obtain how NPs affect the chemical activities of seed germination percentage and seedling growth to gain a better understanding of the dormancy release mechanism and produce vigorous seedlings for application in nurseries. Also, it is necessary to compare the price of the nanoparticle products and the total cost of their application with possible gains from the eventual higher seedling production in further investigations by other researchers.

References

Al-Said M.S., Hifnawy M.S. (1986): Dihydrocoumarin and certain other coumarins from *Prunus mahaleb* seeds. Journal of Natural Products (Lloydia), 49: 721.

Bao-shan L., Shao-qi D., Chun-hui L., Li-jun F., Shu-chun Q., Min Y. (2004): Effect of TMS (nanostructured silicon dioxide) on growth of Changbai larch seedling. Journal of Forestry Research, 15: 138–140.

Baskin C.C., Baskin J.M. (1998): Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination. San Diego, Academic Press: 666.

Bates L.S., Waldren R.P., Teare I.D. (1973): Rapid determination of free proline for water-stress studies. Plant and Soil, 39: 205–207.

Bhati-Kushwaha H., Kaur A., Malik C.P. (2013): The synthesis and role of biogenic nanoparticles in overcoming chilling stress. Indian Journal of Plant Sciences, 2: 2319–3824.

Burke D.J., Pietrasiak N., Situ S.F., Abenojar E.C., Porche M., Kraj P., Lakliang Y., Samia A.C.S. (2015): Iron oxide and titanium dioxide nanoparticle effects on plant performance and root associated microbes. International Journal of Molecular Sciences, 16: 23630–23650.

Dhopte A.M. (2002): Principles and Techniques for Plant Scientists. Jodhpur, Agrobios: 373.

Dimkpa C.O., McLean J.E., Latta D.E., Manangón E., Britt D.W., Johnson W.P., Boyanov M.I., Anderson A.J. (2012): CuO and ZnO nanoparticles: Phytotoxicity, metal speciation and induction of oxidative stress in sand-grown wheat. Journal of Nanoparticle Research, 14: 1125.

Dirr M.A., Heuser C.W.J. (1987): The Reference Manual of Woody Plant Propagation: From Seed to Tissue Culture. Athens, Varsity Press, Inc.: 239.

Ghars M.A., Parre E., Debez A., Bordenave M., Richard L., Leport L., Bouchereau A., Savoure A., Abdelly C. (2008): Comparative salt tolerance analysis between *Arabidopsis thaliana* and *Thellungiella halophila*, with special emphasis on K^+/Na^+ selectivity and proline accumulation. Journal of Plant Physiology, 165: 588–599.

Grisez T.J., Barbour J.R., Karrfalt R.P. (2008): *Prunus* L. cherry, peach and plum. In: Bonner F.T., Karrfalt R.P. (eds): The Woody Plant Seed Manual. Washington, D.C., USDA Forest Service: 875–890.

Harrison C.C. (1996): Evidence for intramineral macromolecules containing protein from plant silicas. Phytochemistry, 41: 37–42.

Hartmann H.T., Kester D.E., Davies F.T., Geneve R.L. (1997): Plant Propagation: Principles and Practices. 6th Ed. Englewood Cliffs, Prentice-Hall, Inc.: 770.

Hossain M.A., Arefin M.K, Khan B.M., Rahman M.A. (2005): Effects of seed treatments on germination and seedling growth attributes of Horitaki (*Terminalia chebula* Retz.) in the nursery. Research Journal of Agriculture and Biological Sciences, 1: 135–141.

Hu J., Xie X.J., Wang Z.F., Song W.J. (2006): Sand priming improves alfalfa germination under high-salt concentration stress. Seed Science and Technology, 34: 199–204.

Jayarambabu N., Siva Kumari B., Venkateswara Rao K., Prabhu Y.T. (2014): Germination and growth characteristics of mungbean seeds (*Vigna radiata* L.) affected by synthesized zinc oxide nanoparticles. International Journal of Current Engineering and Technology, 4: 3411–3416.

Jin C.W., Sun Y.L., Cho D.H. (2012): Changes in photosynthetic rate, water potential, and proline content in kenaf seedlings under salt stress. Canadian Journal of Plant Science, 92: 311–319.

Khot L.R., Sankaran S., Mari Maja J., Ehsani R., Schuster E.W. (2012): Applications of nanomaterials in agricultural production and crop protection: A review. Crop Protection, 35: 64–70.

Kollmann J., Pflugshaupt K. (2005): Population structure of a freshly-fruited species at its range edge – the case of *Prunus mahaleb* L. in northern Switzerland. Botanica Helvetica, 115: 49–61.

Lei Z., Mingyu S., Xiao W., Chao L., Chunxiang Q., Liang C., Hao H., Xiaoqing L., Fashui H. (2008): Antioxidant stress is promoted by nano-anatase in spinach chloroplasts under UV-B radiation. Biological Trace Element Research, 121: 69–79.

Ma J.F., Yamaji N. (2008): Functions and transport of silicon in plants. Cellular and Molecular Life Sciences, 65: 3049–3057.

Nesme X. (1985): Respective effects of endocarp, testa and endosperm, and embryo on the germination of raspberry (*Rubus idaeus* L.) seeds. Canadian Journal of Plant Science, 65: 125–130.

Panwar P., Bhardwaj S.D. (2005): Handbook of Practical Forestry. Jodhpur, Agrobios: 191.

Pipinis E., Milios E., Mavrokordopoulou O., Gkanatsiou C., Aslanidou M., Smiris P. (2012): Effect of pretreatments on seed germination of *Prunus mahaleb* L. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 40: 183–189.

Raskar S.V., Laware S.L. (2014): Effect of zinc oxide nanoparticles on cytology and seed germination in onion. International Journal of Current Microbiology and Applied Sciences, 3: 467–473.

Rathinasabapathi B. (2000): Metabolic engineering for stress tolerance: Installing osmoprotectant synthesis pathways. Annals of Botany, 86: 709–716.

Rezaei F., Moaveni P., Mozafari H. (2015): Effect of different concentrations and time of nano TiO_2 spraying on quantitative and qualitative yield of soybean (*Glycine max* L.) at Shahr-e-Qods, Iran. Biological Forum – An International Journal, 7: 957–964.

Ruffini C.M., Cremonini R. (2009): Nanoparticles and higher plants. Caryologia, 62: 161–165.

Sahebi M., Hanafi M.M., Siti Nor Akmar A., Rafii M.Y., Azizi P., Tengoua F.F., Nurul Mayzaitul Azwa J., Shabanimofrad M. (2015): Importance of silicon and mechanisms of biosilica formation in plants. BioMed Research International, 2015: 396010.

Savithramma N., Ankanna S., Bhumi G. (2012): Effect of nanoparticles on seed germination and seedling growth of *Boswellia ovalifoliolata* – an endemic and endangered medicinal tree taxon. Nano Vision, 2: 61–68.

Saxton K.E., Rawls W.J., Romberger J.S., Papendick R.I. (1986): Estimating generalized soil-water characteristics from texture. Soil Science Society of America Journal, 50: 1031–1036.

Schmidt L. (2000): Guide to Handling of Tropical and Subtropical Forest Seed. Humlabaek, Danida Forest Seed Centre: 511.

Seeger E.M., Baun A., Kästner M., Trapp S. (2009): Insignificant acute toxicity of TiO_2 nanoparticles to willow trees. Journal of Soils and Sediments, 9: 46–53.

Sekhavati N., Hoseini M., Akbarinia M., Rezaei A. (2011): Effects of gibberellic acid and cold stratification on seed dormancy and seed germination on seeds with and without coat of *Cerasus mahaleb* (L.) Mill. Iranian Journal of Rangelands and Forests Plant Breeding and Genetic Research, 19: 193–204.

Venier P., Carrizo García C., Cabido M., Funes G. (2012): Survival and germination of three hard-seeded *Acacia* species after simulated cattle ingestion: The importance of the seed coat structure. South African Journal of Botany, 79: 19–24.
Yang F., Liu C., Gao F., Su M., Wu X., Zheng L., Hong F., Yang P. (2007): The importance of spinach growth by nano-anatase TiO_2 treatment is related to nitrogen photoreduction. Biological Trace Element Research, 119: 77–88.
Yinfeng, X., Xiaohua Y. (2009): Effects of nano-meter TiO_2 on germination and growth physiology of *Pinus tabulaeformis*. Acta Botanica Boreali-Occidentalia Sinica, 29: 2013–2018.
Zarafshar M., Akbarinia M., Askari H., Hosseini S.M., Rahaie M., Struve D. (2015): Toxicity assessment of SiO_2 nanoparticles to pear seedlings. International Journal of Nanoscience and Nanotechnology, 11: 13–22.

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