Biol Trace Elem Res (2013) 155:283–286
DOI 10.1007/s12011-013-9788-3

Effect of Anatase Nanoparticles (TiO_2) on Parsley Seed Germination (*Petroselinum crispum*) In Vitro

Elahe Hashemi Dehkourdi · Mousa Mosavi

Received: 18 July 2013 / Accepted: 7 August 2013 / Published online: 22 August 2013

Abstract Nano priming is a new method for the increase of seedling vigor and improvement of germination percentage and seedling growth. The experiments to evaluate the effect of different concentrations of nano-anatase on germination parameters of parsley as a completely randomized design with five replications were performed in a tissue culture laboratory of the Department of Horticulture, Shahid Chamran University of Ahvaz. In addition, nano-anatase at four concentrations (10, 20, 30, and 40 mg/ml) was added to the Murashige and Skoog medium. At the end of the experiment, the percentage of germination, germination rate index, root and shoot length, fresh weight of seedlings, vigor index, and chlorophyll content were evaluated. The results showed that an increase in the concentration of nano-anatase caused a significant increase in the percentage of germination, germination rate index, root and shoot length, fresh weight, vigor index, and chlorophyll content of seedlings. The best concentration of nano-anatase was 30 mg/ml.

Keywords Anatase · Germination · Nano-TiO_2 · Parsley

Introduction

Parsley with scientific name *Petroselinum crispum* is a biennial vegetable of the family Apiaceae (Umbelliferae). Parsley is one of the most important vegetable that seed germination has taken a long time, and it is difficult to be germinated, especially under unfavorable environmental conditions [1]. The germination percentage of parsley seed is lower than 55–75 % in these kinds of conditions [2]. Thus, slow emergence and low emergence rates lead to smaller seedlings [3]. However, uniformity and rapidity of seed germination and emergence are essential to increase yield, quality, and profits in crops [4].

In recent decades, entry of different technologies in the field of agricultural has led to enormous changes. Nanoscience and technology is the ability to take control of a matter at the nanometer scale (molecular), exploiting the phenomena properties of the materials, devices, and systems for the new method [5]. The main applications of this technology in agriculture and related industries include the following: production of nanoparticles, creating a new package commensurate with changes in food production, new biosensors for pathogen detection, nano-food, and cell transfer; production of enzymes with special nano, nanocrystalline and nanocatalyst; the use of carbon nanotubes in food production, medicine, and new plants; production of pesticides and fertilizers with the scale of nano and long-lasting effect; etc. [6]. The diversity and complexity of the living organisms by atomic and molecular structures and its motion are determined at the nanoscale. Ultrafine particles at the nanoscale can be found anywhere of either single atoms or macromolecules such as hemoglobin or DNA. In general, few studies have been conducted on the effect and mechanism of nanoparticles on plant growth [7].

The effects of nano-TiO_2 (rutile) and non-nano-TiO_2 on the germination and growth of naturally aged spinach seeds were studied. An increase of these factors was observed at 0.25–4‰ nano-TiO_2 treatment [8].

Feizi et al. [9] reported that nano-TiO_2 in a suitable concentration could promote the seed germination of wheat in comparison to bulk TiO_2 but, in high concentrations, had inhibitory or any effect on wheat.

E. H. Dehkourdi (✉)
Department of Horticulture Science, Pomology, Faculty of Agriculture, Shahid Chamran University of Ahvaz, Ahvaz, Iran
e-mail: hashemielahe7866@yahoo.com

M. Mosavi
Department of Horticulture Science, Faculty of Agriculture, Shahid Chamran University of Ahvaz, Ahvaz, Iran
e-mail: Mousa_mousawi@yahoo.com

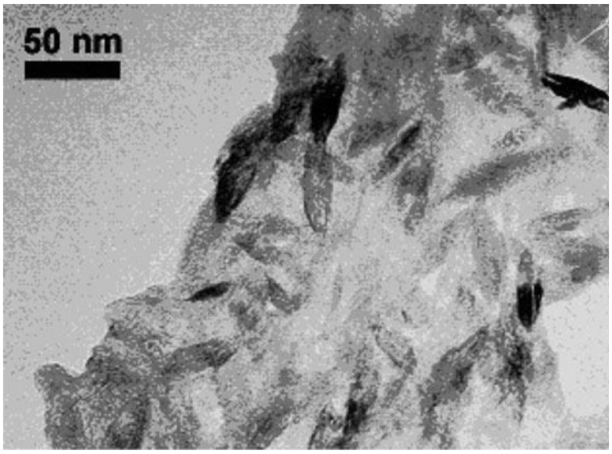

Fig. 1 TEM image of nano-TiO_2

A few studies have been done on the effects of nanoparticles on crops, particularly on parsley. In this work, we decided to find out the phytotoxicity or positive effects of different concentrations nano-TiO_2 on seed germination and seedling growth of parsley.

Material and Method

In order to study the effect of different concentrations of nano-TiO_2 (anatase) on parsley germination, a completely randomized design with five replications was employed (Fig. 1). Parsley (*P. crispum*) seeds were taken from the Agricultural Research Center of Khuzestan Province, Iran. Standard agar Murashige and Skoog (MS) medium was prepared and poured into the glass bottles. Each bottle contained 50 ml of MS media. Nano-anatase in different concentrations (10, 20, 30, and 40 mg/ml) was added into the medium. The medium was sterilized in autoclave (15 lb pressure, 121 °C temp). Parsley seeds were soaked in running tap water for 15 min and then washed with distilled water. Then, water was decanted, and 70 % ethanol was added and soaked for 5 min exactly. Ethanol was decanted, and 4 % sodium hypochlorite (NaOCl) was added separately and soaked for 10 min exactly. In a sterile laminar airflow chamber, sodium hypochlorite was decanted, and the seeds were thoroughly washed with sterile distilled water for four to five times to remove the traces of NaOCl. Sterilized seeds (10 seeds in each bottle) were carefully inoculated onto the MS medium treated with different concentrations of nano-anatase and also media without nano (control) with the help of sterilized forceps under a laminar airflow chamber, and then, these bottles are transferred into the growth chamber. The experiment was conducted in a tissue culture laboratory at the College of agriculture, Shahid Chamran University of Ahvaz, Iran.

Seeds were checked every day, and the number of seeds with visible radicle was counted as sprouted seeds. The length of shoot and radicle and the fresh weight of seedling were measured.

Germination rate (Eq. (1)) and germination percentage (Eq. (2)) were calculated by the following formula [10]:

$$GR = \frac{\sum n}{\sum dn} \quad (1)$$

where GR is the germination rate, $\sum n$ is the number of seeds germinated on the day, and $\sum dn$ is the number of days from the start of experiment.

$$GP = \frac{\sum n}{N} \times 100 \quad (2)$$

where GP is the germination percent, $\sum n$ is the number of seeds germinated until the last day of experiments, and N is the total number of seeds.

Table 2 Effect of nano-anatase on seed germination of parsley

Concentration (mg/ml)	Germination (%)	Germination rate	Root length (cm)	Shoot length (cm)	Fresh weight (g)
0	44.97 c	0.6 b	6.78 e	4 d	0.012 d
10	70.1 b	0.65 b	8.5 d	4.5 c	0.021 c
20	74.8 b	1 a	9.1 c	4.7 b	0.029 b
30	92.46 a	1.2 a	12.24 a	6 a	0.034 a
40	52.64 c	0.67 b	10 b	4.6 c	0.030 b

Means with different letters at each column have a statistical difference at 1 % level

Table 1 Analysis of variance of indices of parsley seed germination affected by nano-anatase

Sources of variation	*df*	Percentage of germination	Germination rate	Root length	Shoot length	Fresh weight of seedling	Chlorophyll	VI
Treatment	4	1,762.51[a]	0.2126[a]	32.060[a]	0.3414[a]	0.00039[a]	0.9[a]	0.7[a]
Error	20	75.7564	0.02140	1.4250	1.33140	0.0000174	0.002	0.001

[a] Significant at 1 % level

Table 3 Effect of nano-anatase on the chlorophyll content of seed parsley

	0 mg/ml	10 mg/ml	20 mg/ml	30 mg/ml	40 mg/ml
Chlorophyll *a*	3.87 e	4.3 d	4.8 c	5.8 a	5.3 b
Chlorophyll *b*	2.48 e	2.6 d	3 c	3.5 a	2.9 b
Total chlorophyll	6.35 e	6.9 d	7.8 c	9.3 a	8.2 b

Means with different letters at each row have a statistical difference at 1 % level

Seed vigor indices (Eq. (3)) were calculated according to the following formula [10]:

$$VI = (RL + SL) \times GP \quad (3)$$

where VI is the seed vigor index, RL is the radicle length, SL is the shoot length, and GP is the germination percentage.

Chlorophyll contents of the plants were measured [11].

Statistical Analysis

The data were analyzed using the SAS 9.1 software. The significant levels of difference for all measured traits were calculated, and the means were compared by the multiple-range Duncan test at 1 % level.

Result and Discussion

The analysis of variance showed that the treatment of nano-anatase caused a significant difference in the percentage of germination, germination rate, root and shoot length, fresh weight, and chlorophyll content of the seedlings (Table 1).

In terms of parsley germination indices, percentage of germination and germination rate were affected by studied treatments. In addition, shoot length, root length, vigor index,

Fig. 2 Seedling of parsley in 0 and 30 mg/ml nano-anatase

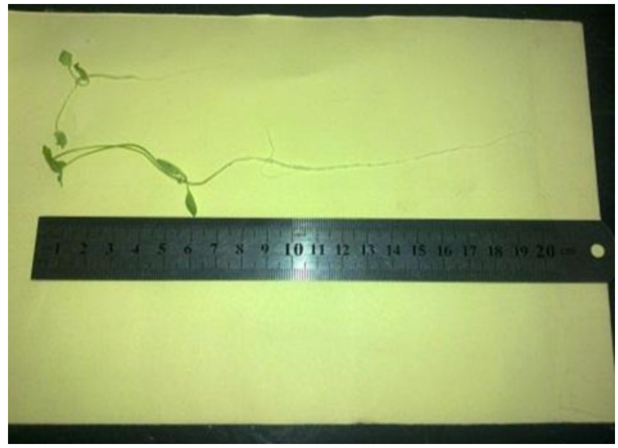

Fig. 3 Seedling length of parsley in 0 and 30 mg/ml nano-anatase

fresh weight, and chlorophyll content were affected significantly by nano-TiO_2 concentrations (Table 1).

The highest germination percentage (92.46 %) was achieved by 40 mg/ml treatment, and the lowest germination percentage (44.97 %) was achieved by control treatment.

The reason could be that the nano-anatase can penetrate through seed and may activate the embryo [12].

The minimum of germination rate was found in the control, with 10 and 40 mg/ml of nano-anatase (0.6), and the highest germination rate (1.2) was obtained at concentrations of 20 and 30 mg/ml.

The effect of studied treatments on root length and shoot length was significant.

The minimum of root length (6 mm) and shoot length (4 mm) was obtained in the control, and maximum, in the concentration of 30 mg/ml, respectively. The increased seedling growth rate may be due to the enhancement of the water and nutrient uptake by the treated seeds.

The lowest fresh weight (0.12 g) was seen at the control, and the highest fresh weight (0.34 g) was seen in a concentration of 30 mg/ml. In between concentrations of 20 and 40 mg/ml, no significant difference was found.

A suitable increase of the chlorophyll content of plants is beneficial to photosynthesis. Table 2 shows that at 30 mg/ml anatase, the formation of chlorophyll *a* and chlorophyll *b* increases compared to the control. This effect is probably the result of facilitation of the absorbance of minerals that promote the formation of chlorophyll and activation of key enzymes for carbon fixation (Table 3) [7].

Applications of nanomaterial can promote earlier plant germination and improve plant production.

Zheng et al. [7] showed that the growth of spinach plants was greatly improved at 250–4,000 ppm nano-TiO_2 concentrations, but there was no improvement at higher concentrations.

Nano-TiO_2 treatment led to a highly enhanced mRNA expressions and protein level in spinach [13].

Feizi et al. [9] reported that nano-TiO_2 in an appropriate concentration could promote the seed germination and seedling growth of wheat in comparison to bulk TiO_2.

Ruffini and Cermonini [14] suggested that nanoparticles can be explained in their actions, depending on both the chemical compound and on the size and/or shape of the particles.

Owolade et al. [15] reported that the seed yield of cowpea (*Vigna unguiculata* Walp) was increased when treated (as foliar application) with nano-TiO_2.

The results indicated that the nano-sized TiO_2 in an appropriate concentration could promote the seed germination and seedling growth of parsley (Figs. 2 and 3).

Zheng et al. [7] reported that the significant effect of nano-sized TiO_2 on spinach germination is probably attributed to the small particle size, which allows its penetration into the seed during the treatment period, exerting its enhancing functions during growth. Above 30 mg/ml, the chlorophyll contents of parsley decreased significantly. It is possible that the anatase enhances the activity of chlorophyllase.

Unfortunately, very little research has been done on the effect of nano-anatase in plants. Increased shoot and root length may be due to the early emergence induced by nano-anatase treatment as compared to control seeds. Rapid embryo growth resulted when the obstacle to germination was removed.

Priming also causes the adherence of seed coat to reduce due to imbibition, which may permit to emerge out radicle without resistance as the priming minimizes seed coat adherence during emergence of parsley seeds. According to the results, we can say that the nano-anatase with the increased penetration power of the seed facilitates the entry of water and oxygen into the cell and that with increasing absorption of nutrients in the seed, the processes related to germination resonant occur ultimately to stimulate germination [7]. Germination indices in spinach increase more by nano-titanium compared to those by bulk titanium. During the growth period of spinach, dry weight, chlorophyll content, and photosynthetic rates finally increased. The germination index in *Plantago psyllium* by nanoparticles TiO_2 was increased, and that may be due to an increased rate of cell division in the root tip. The increased length of the root is due to the rapid emergence [16].

Thus, increased nitrate reductase enzyme concentrations and reduced antioxidant stress by reducing H_2O_2, superoxide radicals, and malonyldialdehyde content and by increasing some enzymes such as superoxide dismutase, ascorbate peroxidase, guaiacol peroxidase, and catalase activities [17] result in an improve seed germination in some plant species.

References

1. Ely P, Heydecker W (1981) Fast germination of parsley seeds. Scientia Horticulturae 15:127–136
2. Rabin J, Berkowitz GA, Akers SW (1988) Field performance of osmotically primed parsley seed. Horticulture Science 23:554–555
3. Pill W, Kilian A (2000) Germination emergence of parsley in response to osmotic or matric seed priming and treatment with gibberellin. Horticulture Science 35:907–909
4. Podlaski S, Chrobak Z, Wyszkowska Z (2003) The effect of parsley seed hydration treatment and pelleting on seed vigour. Plant Soil Environ 49:114–118
5. Khayyam Nekoei S, Sharifnasab M, Ahmadisome H, Barkhivar K, Momeni M (2009) A review of Nanotech in Agricultural-Jehad ministry, 2nd edn. Publication of Agricultural Education 23:45–49
6. Ranjbar M, Shams G (2009) Applications of nanotechnology. Journal of Green Environment 34:85–87
7. Zheng L, Mingyu S, Xiao W, Chao L, Chunxiang Q, Liang C, Huang H, Xiaoqing L, Hong F (2007) Effect of nano anatase on spectral characteristics and distribution of LHCLL on the thylakoid membranes of spinach. Biol Trace Elem Res 120:273–280
8. Zheng L, Hong F, Lu S, Liu C (2005) Effect of nano-TiO_2 on strength of naturally aged seeds and growth of spinach. Biology Trace Elemental Research 105:839–843
9. Feizi H, Razavi P, Shahtahmasebi N, Fotovat A (2011) Impact of bulk and nanosized titanium dioxide (TiO_2) on wheat seed germination and seedling growth. Biol Trace Elem Res 146:101–106
10. Hosseini HR, Chehrazi M, Nabati Ahmadi D, Mahmoodi Sorestani M (2013) Study the effects of colchicine treatment on generation of autopolyploidy in *Catharanthus roseus* cvs. Rosea and Alba. M.Sc. Thesis. Shahid Chamran University of Ahvaz. Iran.
11. Hashemi Dehkourdi E, Mosavi M, Moallemi N, Ghafarian Moghareb MH (2013) Effect of nanoparticles of anatase (TiO_2) on some of characteristics quantity and quality of fruit of strawberry in hydroponic condition. M.Sc. Thesis. Shahid Chamran University of Ahvaz, Iran.
12. 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
13. Gao F, Hong F, Liu C, Zheng L, Su M, Wu X, Yang F, Wu C, Yang P (2006) Mechanism of nano-anatase TiO_2 on promoting photosynthetic carbon reaction of spinach. Biol Trace Elem Res 111:239–253
14. Ruffini Castiglione M, Cremonini R (2009) Nanoparticles and higher plants. Caryologia 62:161–165
15. Owolade OF, Ogunleti DO, Adenekan MO (2008) Titanium dioxide affected diseases, development and yield of edible cowpea. Electronic J Environ Agric Food Chem 7(5):2942–2947
16. Farahani HA, Sani B, Maroufi K (2012) The germination variations in fleawort (*Plantago psyllium* L.) by nano-particle. International conference on biotechnology, nanotechnology and its application 12: 43–46.
17. Lei Z, Mingyu S, Xiao W, Chao L, Chunxiang Q, Liang C, Hao H, Xiao-qing L, Fashui H (2008) Antioxidant stress is promoted by nano-anatase in spinach chloroplasts under UV-B radiation. Biol Trace Elem Res 121:69–79