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Application of anatase nanoparticles (TiO_2) on strawberry seed germination (*Fragaria ananassa* L.)

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Abstract: Priming enhances germination, establishment and yields in a range of crops in many diverse environments. This experiment evaluated the effects of soaking strawberry seeds in different concentrations (0, 3.5, 5.5, 7.5 and 9.5 percentage) of nano anatase on germination parameters (germination percentage, germination rate index, radicle and plumule length, fresh weight of seedlings and vigor index) using a factorial design with eight replications. Results showed that an increase in the concentration of nano anatase led to significant differences in the percentage of germination, germination rate index, root and shoot length, fresh weight and vigor index of seedlings. The best nano anatase concentration was found to be 7.5%.

1. Introduction

Strawberry (*Fragaria x ananassa* Duch.), a small fruit crop and a hybrid of two highly variable octoploid species, has adapted to extremely different environmental conditions (Rieger, 2005). Prerequisites for successful strawberry growing are suitable climate, cultivars, soil and nutrition (Almaliotis *et al*., 2002). Seed priming enhances seed performance by rapid and uniform germination in normal and vigorous seedlings, leading to faster and better germination in different crops (Cantliffe, 2003). Priming is responsible for the repair of age-related cellular and sub cellular damage of low vigour seeds that may accumulate during seed development. Priming of seed promotes germination by repairing the damaged proteins, RNA and DNA (Koehler *et al*., 1997).

Several treatments have been used with the aim of improving strawberry achene (seed) germination: humidity (Guttridge and Bright, 1978); exposure to red light (Iyer *et al*., 1975); and osmotic pre-treatment (Hanke, 1993).

With the rapid growth of nanotechnology, there are increasing concerns about the potential adverse impact of engineered nanoparticles (ENPs) in the environment. However, our understanding of how ENPs may affect organisms within natural ecosystems lags far behind our rapidly increasing ability to engineer novel nanomaterials (Bernhardt *et al*., 2010). On the other hand, Lin and Xing (2007) studied the positive effects of suspensions of nanoparticles on seed germination and root growth of six different crop species [radish (*Raphanus sativus*), rape (*Brassica napus*), rye grass (*Lolium perenne*), lettuce (*Lactuca sativa*), corn (*Zea mays*) and cucumber (*Cucumis sativus*)]. Also, the effects of nano-TiO_2 (rutile) and non-nano-TiO_2 on the germination and growth of naturally aged spinach seeds were studied and an increase of these factors was observed at 0.25-4‰ nano-TiO_2 treatment (Zheng *et al*., 2005). Feizi *et al*. (2011) reported that nano-TiO_2 at suitable concentration could promote the seed germination of wheat in comparison to bulk TiO_2 while at high concentrations it had an inhibitory or no effect on the crop.

Limited studies have been done on the effects of nanoparticles on crops and thus, we decided to investigate the phytotoxicity or positive effects of different concentrations of nano-TiO_2 on seed germination and seedling growth of strawberry.

2. Materials and Methods

In order to evaluate the effect of nano priming on the quality of seedling production during germination in strawberry (*Fragaria ananassa* L.), this experiment was conducted in 2013 at Shahid Chamran University Ahvaz in Iran using a factorial design with eight replications. Seeds of strawberry (*Fragaria ananassa* cv. Queen Eliza) were from the Gene Bank of Iran, at Seed and Plant Improvement Institute, Tehran (Karaj). The factors studied included different concentrations of TiO_2 (Control, 3.5, 5.5, 7.5 and 9.55%) and time (24 and 48 h). In order to prepare nano anatase solutions a stock solution with the highest

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concentration (9.5%) was fixed and then with dilution of this stock solution, various concentrations of nano anatase were obtained. Strawberry seeds were sterilized using sodium hypochlorite solution (1%) for 10 min, then washed several times with distilled water, and soaked in nano-TiO_2 solutions at different concentrations. Seeds were placed in disinfected Petri dishes, each dish contained 100 seeds. All of the Petri dishes were irrigated using distilled water. Seeds were allowed to germinate at 25±3°C for 14 days. Every day the number of seeds with visible radicle were counted and recorded as sprouted seeds; the length of seedling shoot and root were also measured.

Germination rate [Eq. (1)] and germination percentage [Eq. (2)] were calculated using the following formula (Hosseini *et al*., 2013):

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

Where GR is the germination rate, Σn is the number of seeds germinated on the day, and Σdn 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, Σn is the number of seeds germinated until the last day of experiments, and N is the total number of seeds.

Seed vigor indices [Eq. (3)] were calculated according to the following formula (Hosseini *et al*., 2013):

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

Where VI is the Seed Vigor Index, RLis radicle length, PL is the plume length, and GP the germination percentage.

Statistical analysis

The data were analyzed using 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.

3. Results and Discussion

Analysis of variance showed that treatment of nano anatase led to significant differences in germination percentage, germination rate, root and shoot length, allometric index and vigor index of the seedlings. Time treatment was not significant for root, shoot, seedling length and seedling allometric coefficient. Interaction of anatase nanoparticles and time treatment was not significant for root and shoot length and seedling allometric coefficient (Table 1-3).

The interaction of anatase nanoparticles and time showed the lowest percentage of germination in the treat-

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

Sources of variation	df	Percentage of germination	Germination rate	Shoot length	Root length	Seedling length	Allometric	VI
Nano-TiO_2	4	2260.6 *	0.184 *	11.077 *	15.366 *	0.525 *	3.33 *	103028.5 *
Time	1	136.9 *	1.089 *	0.196 NS	0. 016 NS	0.0022 NS	0.049 NS	5712.1 *
Nano*time	4	76.9 *	0.078 *	0.179 NS	0.0053 NS	0.001 NS	0.087 NS	3868.1 *
Error	30	13.7	0.180	0.148	0.145	0.004	0.069	578.23

*= significant at 1% level.
NS= non-significant.

Table 2 - Effect of nano anatase on seed germination of strawberry after 24 hours

Concentration	Germination (%)	Germination rate	Shoot length (cm)	Root length (cm)	Fresh weight (g)
0 %	10.97 e	0.60 d	1.78 e	2.0 d	0.012 d
3.5 %	40.10 d	0.60 c	8.50 d	4.5 c	0.021 c
5.5 %	68.80 ab	0.67 b	9.10 c	4.4 c	0.029 b
7.5 %	92.46 a	1.20 a	12.24 a	6.0 a	0.034 a
9.5 %	74.64 b	1.00 ab	10.00 b	4.8 b	0.030 b

Means with different letters at each column are statistically different at 1% level.

Table 3 - Effect of nano anatase on seed germination of strawberry after 48 hours

Concentration	Germination (%)	Germination rate	Shoot length (cm)	Root length (cm)	Fresh weight (g)
0 %	0 e	0 e	0 e	0 d	0 d
3.5 %	23.2 d	0.17 d	3.5 d	2.5 c	0.011 c
5.5 %	40.6 b	0.26 b	5.3 c	2.9 c	0.017 b
7.5 %	87.6 a	0.56 a	10.3 a	5 a	0.030 a
9.5 %	46.3 bc	0.35ab	7.1 b	3.3 b	0.025 b

Means with different letters at each column are statistically different at 1% level.

ment without nanoparticles and the highest at a concentration of 7.5% of nanoparticles for 48 h (Fig. 1).

Zheng *et al*. (2007) reported that the significant effect of nano-sized TiO_2 on germination is probably due to the small particle size which allows penetration into the seed during the treatment period, exerting its enhancing functions during growth.

Results showed that the lowest germination rate resulted from treatment without nanoparticles while the highest rates were obtained at a concentration of 7.5% of nanoparticles in 24 and 48 h. The key to increase seed geermination is the penetration of nanomaterial into the seed (Hashemi and Mousavi, 2013) (Fig. 2).

Khodakovskaya *et al*. (2009) mentioned that the carbon nanotubes can effectively penetrate through the seed coat, thus influencing seed germination. Exposure of tomato seeds to carbon nanotubes (CNTs) resulted in enhanced seed germination and growth rate.

Among the different nanoparticle TiO_2 concentrations, 7.5% for both times tested (24 and 48 h) showed the maximum seedling vigor index while the control showed the lowest (Fig. 3).

Effects positive of nano anatase are reported that nano-TiO_2 (anatase) improved plant growth by enhanced nitrogen metabolism (Yang, 2006) that promotes the absorption of nitrate in spinach and, accelerating conversion of inorganic nitrogen into organic nitrogen, thereby increasing the fresh weights and dry weights. Studies also showed the effects of nitrogen photoreduction on the improved growth of treated spinach plant (Yang, 2007). Effects of nano-TiO_2 (anatase) on the content of light harvesting complex II (LHC II) on thylakoid membranes of spinach was studied and it showed an increase in LHC II content (Mingyu, 2007 a). These promote energy transfer and oxygen evolution in photosystem II (PS II) of spinach (Hong, 2005).

It has also been found that nano anatase TiO_2 promoted antioxidant stress by decreasing the accumulation of superoxide radicals, hydrogen peroxide, malonyldialdehyde content and enhance the activities of superoxide dismutase, catalase, ascorbate peroxidase, guaiacol peroxidase and thereby increase the evolution oxygen rate in spinach chloroplasts under UVB radiation (Mingyu, 2007 b).

The results indicated that nano sized TiO_2 in an appropriate concentration could promote the seed germination and seedling growth of strawberry.

The results show that with increasing concentration of nano anatase to 7.5% increased germination parameters. The seedlings grown with nano anatase increased length when compare to the control seedlings. Effect of nano anatase on root, shoot and seedling strawberry may be due to early emergence induced by nano anatase treatment as compared to control seeds. Rapid embryo growth resulted when the obstacle to germination was removed.

Although the concentration of 9.5% caused lower germination parameters toward concentration of 7.5%, showed more desirable effects of the concentration of 5.5% nano anatase and control.

In all treatments, the better result was obtained in time of 48 hour.

In order to understand the possible benefits of applying nanomaterials in agriculture, it is important to analyze penetration and transport of nanoparticles in the plants.

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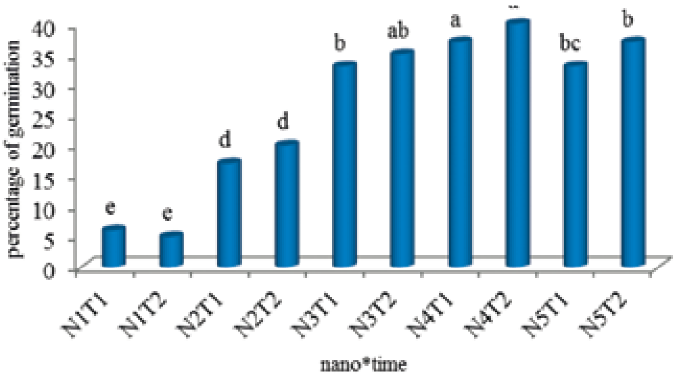

Fig. 1 - Effect of interaction nano anatase and time on germination percentage of strawberry seeds.

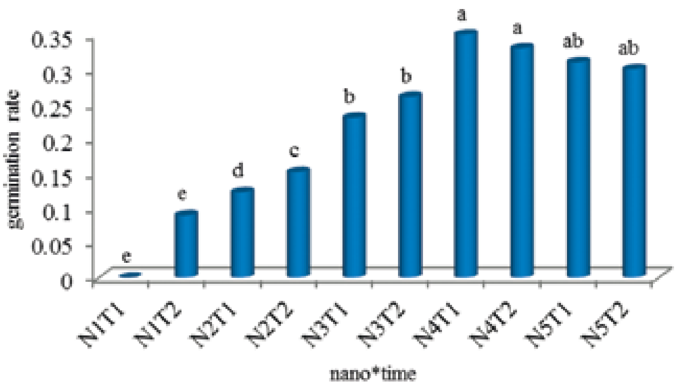

Fig. 2 - Effect of interaction nano anatase and time on germination rate of strawberry seeds.

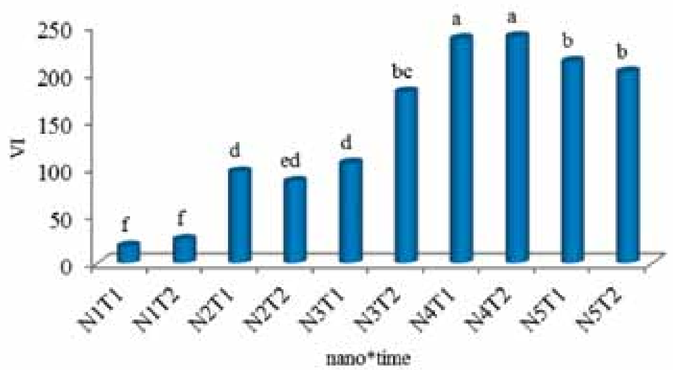

Fig. 3 - Effect of interaction nano anatase and time on vigor index of strawberry seeds.

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