Aerosol and Air Quality Research, 14: 632–641, 2014

ISSN: 1680-8584 print / 2071-1409 online
doi: 10.4209/aaqr.2013.06.0212

Electrospray Facilitates the Germination of Plant Seeds

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ABSTRACT

We proposed a new approach to enhance the germination of plant seeds via the electrospray of nanoparticles (NPs). A single-capillary electrospray system with a particle deposition stage (where seeds are placed) was set up for this investigation. For demonstration, lettuce (*Lactuca sativa*) seeds were bombarded by TiO_2 NPs via the electrospray for 2–4 minutes in order to promote their germination. Based on our study, the enhancement in germination was significant in cases with aged seeds or seeds placed in an unfavorable growth condition (e.g., low pH medium). The electrospray of other NPs (i.e., Au) was also shown to be effective in enhancing the germination of aged lettuce seeds. TEM (Transmission Electron Microscopy) and SEM-EDX (Scanning Electron Microscopes and Energy-Dispersive X-ray Spectroscopy) analyses suggested that sprayed NPs could enter the seed coat via the frequent bombardment of NPs, thus breaking the coat-imposed seed dormancy. The enhancement of the germination of grass seeds was also observed in this study. The proposed seed treatment may have the potential to improve the germination of various recalcitrant crop seeds. Meanwhile, this study also indicates that nano-size aerosols may have a strong impact on plant physiologies.

Keywords: Crop seeds; Lettuce; Nano-size aerosols; Shelf life; TEM; TiO_2 nanoparticles; Seed dormancy.

INTRODUCTION

In recent years, Nanotechnology and Nanoparticle-related research have undergone rapid growth in various fields, including nanomedicines, drug delivery, biomedical imaging and sensing, and solar energy conversion (Guo and Dong, 2011; Yoo *et al.*, 2011; Wei *et al.*, 2007). Nanoparticles are defined as objects with at least one characteristic dimension less than 100 nm (Roco *et al.*, 1993). The unique surface area and solubility of nanoparticles distinguish them from their molecular and bulk counterparts, contributing to various biological effects (Nel *et al.*, 2006; Menard *et al.*, 2011). In work related to plants, several studies have focused on the translocation of NPs in plants (Lin and Xing, 2008; Khodakovskaya *et al.*, 2009; Ma *et al.*, 2010). For instance, nanomaterials can be used as a vector to deliver DNA or other chemicals into plant cells and tissues (Torney *et al.*, 2007; González-Melendi *et al.*, 2008; Liu *et al.*, 2009; Nair *et al.*, 2010; Martin-Ortigosa *et al.*, 2012). There are also extensive studies on nanotoxicity on plants in the hopes of advancing nanobiotechnology into agricultural applications while mitigating public concern over its potential risk (Rico *et al.*, 2011). Zheng *et al.* (2005) has showed that a low dosage of TiO_2 NPs had no harmful effects on spinach plants, but rather promoted photosynthesis and nitrogen metabolism that benefited the plant's growth (Zheng *et al.*, 2005). Multi-walled carbon nanotubes and zinc oxide NPs were found to be able to stimulate seed germination, thus enhancing the plant's growth in aqueous cultures (Khodakovskaya *et al.*, 2009; Prasad *et al.*, 2012). Moreover, a series of approaches (i.e., genetic, photo-thermal and photo-acoustic methods) were combined to characterize the interactions between multiwalled carbon nanotubes and tomato tissues, providing new insights into the gene transcription regulations of plants under the influence of nanomaterials (Khodakovskaya *et al.*, 2011). However, only colloidal suspensions of NPs were investigated in the previous literature. It is known that NPs in suspended solutions tend to agglomerate, resulting in the reduction of their nanoscale effect (Nel *et al.*, 2006). It is thus more desirable to have the NPs in their singlet form to study the effect of NPs. Electrospray has been demonstrated to accomplish the above task (Kim *et al.*, 2010).

In this study, we employed a single-capillary electrospray setup to simultaneously disperse and deliver individual NP onto the surface of plant seeds. TiO_2 NPs were applied in this investigation because of their low cost and low toxicity. *Lactuca sativa* (an edible lettuce) was selected as an example plant to demonstrate the feasibility of the proposed treatment

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for crop seeds. Tests on other NPs (Au and CuO) and grass seeds were also performed to support the logic in this study.

EXPERIMENT

Electrospray Setup

The details of the experimental setup have been described in the work of Wu *et al.* (2010). A brief description is provided herein for reference. The schematic diagram of the experimental setup is shown in Fig. 1. The single-capillary electrospray setup consisted of four components: a spray head (i.e., a capillary with 1/32" OD and 150 µm ID), a particle deposition stage, a high voltage power supply (Bertan Model 230), and an optical monitor system. The electrospray head was a single capillary, connected to a syringe driven by a programmable Harvard syringe pump (PHD 2000). Sprayed TiO_2 suspension was fed through the spray head at 2 µL/min. A non-uniform electric field was established between the spray head and deposition stage by applying a positive high voltage on the spray nozzle and electrically grounding the stage. The deposition stage provided a platform on which the lettuce seeds (typically 30 lettuce seeds for each run) were exposed to the NP electrospray. The distance between the capillary tip and the seed platform was kept at 2.0 cm. In this study, a typical working voltage was about 7 kV–10 kV for operating the electrospray in the so-called cone-jet mode (Chen *et al.*, 1995). The optical monitor system, which included a microscopic lens (InfiniGage, Infinity Photo-Optical Co. Japan), a CCD camera (XC-ST 70, Infinity Photo-Optical Co. Japan) and an LCD screen, magnified the liquid meniscus at the capillary exit in order to monitor the cone-jet operation.

All spray suspensions were freshly prepared by dispersing the NPs (via 1min of sonication, 15 W, Misonix XL-2000 Ultrasonic Liquid Processors, NY, US) in 1 mM of a sodium acetate buffer (pH = 7) to ensure that the conductivity of sprayed suspensions was in the range of 150–200 µS/cm.

Chemicals and Materials

TiO_2 NPs (30–50 nm, rutile) and CuO NPs (30–50 nm) were obtained from Nanostructured & Amorphous Materials, Inc. (Houston, TX, US), while Au NPs (50 nm) were obtained from BBInternational Inc. (Cardiff, UK). The lettuce seeds (*Lactuca sativa*, Black Seeded Simpson, #2846) were purchased from Ferry-Morse Seed Co. (Fulton, KY, US), while the yarrow (*Achillea millefolium*) and common ragweed (*Ambrosia artemisiifolia*) seeds were purchased from Herbiseed Company (Twyford, UK).

To investigate the effect of the electrospray treatment on seed germination in an acidic or basic environment (e.g., to mimic the growth conditions in acidic & alkaline soil), we prepared the following buffer solutions for the seed culture: 2 mM of citric acid buffer for a pH of 3; 2 mM of MES (2-(N-morpholino) ethanesulfonic acid) buffer for a pH of 5; 2 mM of HEPES (4-2-hydroxyethyl-1-piperazineethanesulfonic acid) buffer for a pH of 7; 2 mM of Tricine (N-tris (hydroxymethyl) methylglycine) buffer for a pH of 9; and 2 mM of N-cyclohexyl-3- aminopropanesulfonic acid buffer for a pH of 11(Reddy and Singh, 1992).

Determination of Size Distribution of Electrosprayed NPs

The size distribution of TiO_2 NPs after electrospray was characterized by a scanning mobility particle sizer (SMPS, TSI, USA) via the electrospray setup described by Chen *et al.* (1995). The size distribution of the TiO_2 NPs in suspension was determined by dynamic light scattering (DLS) (Zetasizer, Malvern Instruments, Worcestershire, UK).

Protocol for SEM and TEM Images of Treated Seeds

To observe the NPs' bombardment on the seeds, the sprayed lettuce seeds were air dried and subjected to scanning electron microscopy (SEM, Nova 2300 FEI, OR, US). When necessary, samples were coated with gold by a low vacuum sputter coater (SPI supplies, PA, US) to increase the surface conductivity before imaging. To validate the entrance of nanoparticles inside of the seed coat, a TEM (Transmission Electron Microscope, JEOL 1200 EX, MA, US) was used to image the cross sections of treated seeds.

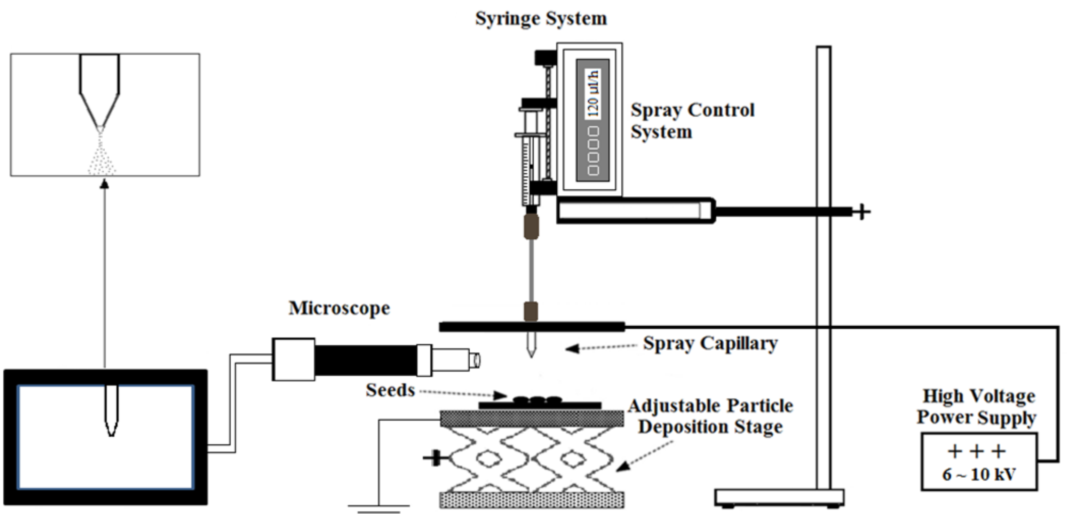


Fig. 1. A schematic diagram of the single-capillary electrospray setup with the particle deposition stage (used in this study).

Seeds were first fixed overnight at 4°C in a phosphate buffer solution containing 2% paraformaldehyde and 2.5% glutaraldehyde (pH 7.2), followed by post-fixing with 1% osmium tetroxide for 2 hours after a phosphate buffer wash. Subsequently, these samples were stained with 1% aqueous uranyl acetate overnight at 4°C. After dehydration with sequential ethanol concentrations ranging from 50 to 100%, sections of each sample were cut with a Leica Ultracut UCT ultramicrotome (Leica Microsystems Inc., Bannockburn, IL, US) and placed on grids for TEM imaging. To further confirm the existence of nanoparticles in the seed sections, the sections were characterized by an energy dispersive X-ray spectroscopy, coupled with SEM (SEM-EDX) for elemental analysis of titanium.

Seed Germination

Thirty lettuce seeds were placed on the electrically-grounded deposition stage to undergo NP electrospray treatment for time periods of approximately 5 minutes. After the spray, all seeds were transferred into petri dishes (15 mm × 100 mm) containing 5 mL of medium solution. Fifteen lettuce seeds were incubated in each petri dish to ensure adequate space to germinate and grow. All dishes were sealed and incubated at 25°C in the dark (Reddy and Singh, 1992). After the end of the germination period (usually three days), the seedling's length were measured (note: the majority of lettuce seeds were germinated in the first three days of incubation). The lettuce seeds with seedling lengths longer than 1 cm were considered to be well-germinated seeds. A total of 60 seeds were used for each treatment condition for analysis. Similar protocols were performed for the other seeds with slight variances in seed number per petri dish (i.e., Yarrow seed: about 120 per petri dish/2 weeks; Common ragweed seed: 50 per petri dish/2 weeks.).

The impact of NP electrospray was evaluated based on the seed germination percentage, calculated by the following equation:

$$\text{Germination percentage} = \frac{\text{Number of seeds well germinated}}{\text{Total number of seeds}} \tag{1}$$

Note that we used the germination percentage as the sole parameter to evaluate the effect of the NP electrospray treatment. The Statistics Toolbox of MATLAB was employed to conduct the data analyses based on the Z test for germination percentage, where statistically significant was defined as $P < 0.05$.

RESULTS AND DISCUSSION

The Electrospray of TiO_2 NPs onto Plant Seeds

Fig. 2 shows the size distribution measurement of TiO_2 NPs in aqueous solutions. It is evidenced that TiO_2 NPs in

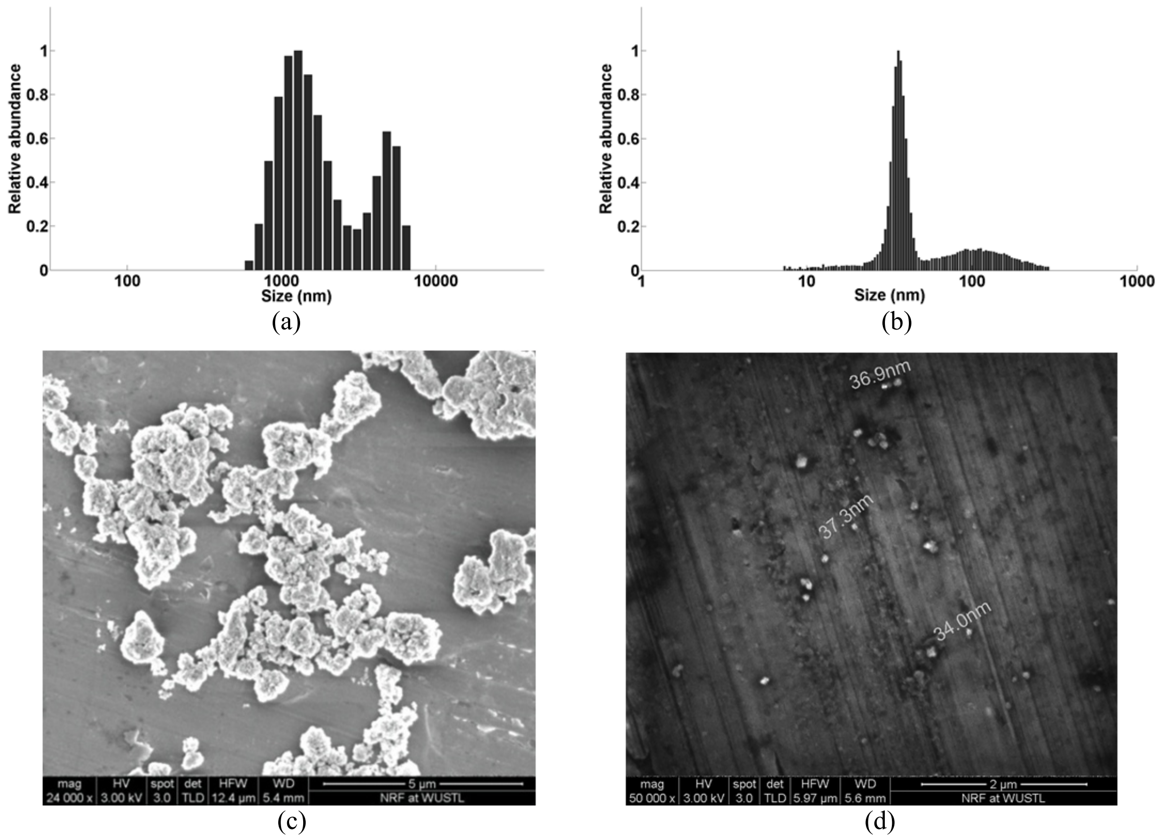


Fig. 2. The measurement of TiO_2 NP size distributions: (a) for a freshly prepared NP suspension (at 1 g/L TiO_2 NPs) measured by dynamic light scattering; (b) for gas-borne TiO_2 NPs after electrospray; (c) SEM image of freshly prepared NP solution (1 g/L) and (d) SEM image of TiO_2 NPs after electrospray.

aqueous solution formed agglomerates up to several µm in size. The agglomeration of NPs reduces the particle size effect, particularly on cellular function (Wu *et al.*, 2010). By adjusting the electrical conductivity and the feeding flow rate of TiO_2 NP suspensions, the single-capillary electrospray operated in the cone-jet mode ensured the production of monodisperse droplets in sub-micrometer and nanometer size ranges (Chen *et al.*, 1995; Jaworek, 2007). When the concentration of TiO_2 NPs in the solutions was diluted, the solvent in the droplets evaporated during electrospray so that singlet TiO_2 NPs could be dispersed onto seed surfaces.

Fig. 2 shows the size distributions measured by the SMPS when freshly prepared TiO_2 NP suspensions of 1 g/L were electrosprayed. The measured particle size distribution exhibited a narrow peak at 36.0 nm, suggesting that most particles were singly dispersed after the electrospray process. Once electrosprayed, gas-borne NPs were accelerated by the presence of a DC electric field. The terminal velocity of sprayed NPs was estimated to be in the range of 100 to 500 m/s prior to bombarding the seed coat (Pui and Chen, 2000). The entrance of NPs inside of the seed coat under the proposed treatment was verified by the SEM images (shown in Fig. 3). To our knowledge, this is the first report demonstrating the application of using aerosolized NPs to treat plant seed coats.

The seed coat, consisting of layers of the testa and endosperm envelope (Welbaum *et al.*, 1998), provides protection against the entry of parasites and mechanical injury. However, the coat may impose the seed dormancy (Zeng *et al.*, 2005). To improve seed germination, we employed NPs with high electrical charges to facilitate the breaking of the seed coat by accelerating NPs in a DC electric field. As opposed to the delivery of particles into plant cells via high-pressure gas (Gordon-Kamm *et al.*, 1990; Torney *et al.*, 2007), electrospray accelerates particles primarily through the space charge effect (due to the presence of highly charged particles in high concentration) (Chen *et al.*, 2000). The NPs' entry into seeds via electrospray demonstrates the potential of this proposed method for delivering various materials (DNAs or plant hormones) into embryos (Gu *et al.*, 2011). By tuning the electric field strength and controlling the charges on the NPs, a broad range of particle velocities can be achieved for the bombardment of targeted organelles. The optimal speeds needed for the successful delivery of nanomaterials (varying in size, density, and shape) into various types of seeds would be an interesting topic to explore in the near future.

Seed Germination Enhancement

Two factors may affect the germination of seeds. First, germination rates of seeds that have been stored for long periods of time are typically reduced (i.e., seeds become more recalcitrant). Second, soil pH influences seed germination, as plants have to adapt to acidic or alkaline

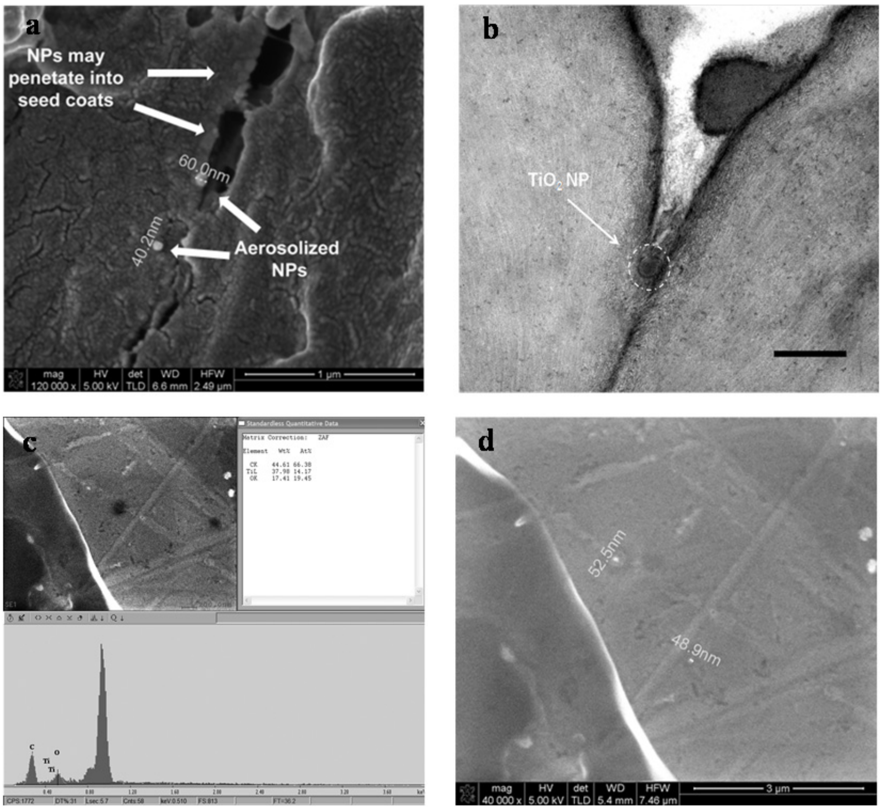


Fig. 3. SEM (a), TEM (b) and SEM-EDX (c, d) images of lettuce seeds treated by electrospray of TiO_2 NP suspension at a concentration of 1 g/L for 5 min. The SEM image (a) shows that TiO_2 NPs were individually adsorbed onto the seed surface. The TEM image (b) and SEM-EDX images (c, d) show the cross sections of treated lettuce seeds. The images evidence that the TiO_2 NPs can enter the coat of lettuce seeds. The scale bar in (b) indicates a length of 200 nm.

environments. Thereby, we tested the effectiveness of NP treatment for seed germination under unfavorable conditions. Fig. 4 summarizes the experimental results of seed germination of lettuce seeds when treated with TiO_2 NP electrospray and incubated in buffer solutions. Fig. 4(a) shows the germination percentage of aged lettuce seeds (stored for 10 months) treated by NP electrospray as a function of NP concentration and spray time. After planting aged lettuce seeds in an unfavorable pH condition (pH 5) for germination, treated lettuce seeds showed a clear enhancement in germination. Aged lettuce seeds (stored for over 10 months) in a MES buffer (pH = 5, a typical pH in acidic soils) had a natural germination percentage of ~40%. The germination of the same lettuce seeds reached its peak value (65%) when seeds were pretreated by electrospraying TiO_2 NP suspension (at the concentration of 1 g/L) for about 4 min. Prolonging NP electrospraying displayed little additional enhancement of the seeds' germination.

Fig. 4(b) shows the germination of lettuce seeds that were incubated in buffer solutions of various pH values. Three sets of fresh lettuce seeds were used in each test incubation condition: untreated seeds (black bars), pre-treated seeds by electrospraying the solvent only (grey bars), and pre-treated seeds by electrospraying with TiO_2 NPs (white bars). When lettuce seeds were fresh (i.e., recently purchase from the seed company), the control seeds (seeds without pretreatment) usually had high germination percentages (~80%) even without NP electrospray treatment under a wide range of pH growth conditions (i.e., pH = 5–9, normal soil range). When the lettuce seeds were placed in an extremely harsh pH condition (i.e., pH = 3), the control seeds showed minimal germination (~0%) while the NP-treated seeds recovered to a germination percentage of 20%. This result indicates that NP-treated plant seeds may be useful in vegetating polluted lands (e.g., phytoremediation of acid contaminated soil).

Effect of Various NPs on the Seed Germination Enhancement

To verify the feasibility of this proposed treatment, CuO and Au NPs were also applied in this study to pre-treat different sets of aged lettuce seeds. Fig. 5(a) shows the seed germination results when aged lettuce seeds (stored for 16 months) were electrosprayed by CuO NP suspensions. Fig. 4(b) gives the germination percentage when the same aged lettuce seeds were pre-treated by electrospraying Au NP suspensions at various concentrations. The germination percentages for the controls were also included in the figure as the reference. The results indicate that the electrospray of either CuO NP (1 g/L) or Au NP suspensions had significant enhancement effects on aged lettuce seeds. Similar to TiO_2 NP electrospray, the best seed germination percentage for

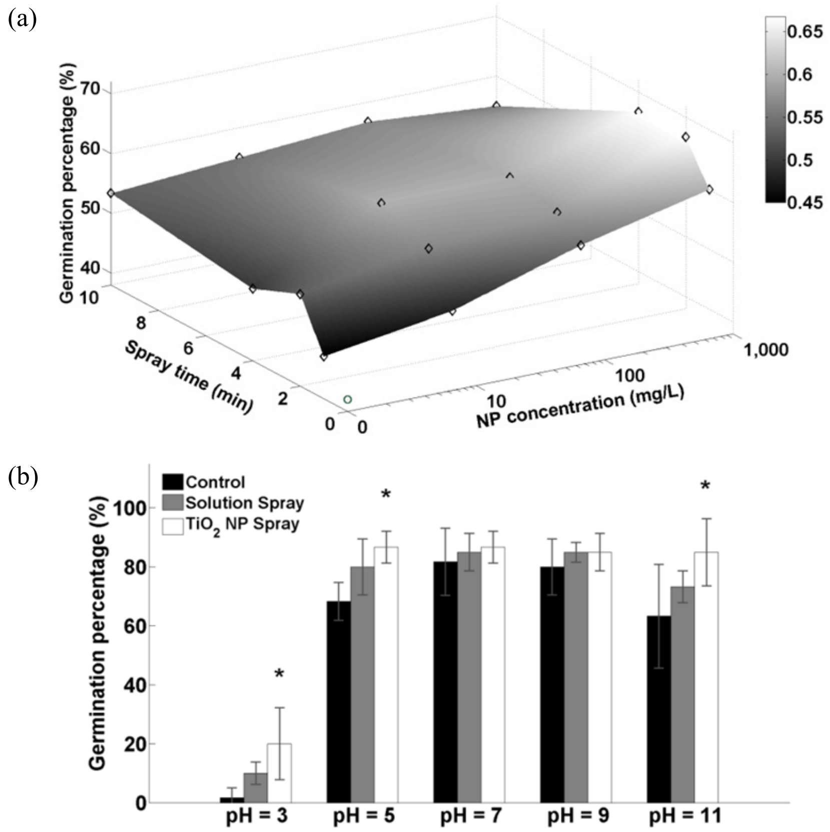


Fig. 4. Germination percentages of lettuce seeds after treatment by TiO_2 NP electrospray as a function of spray time and NP mass concentration in spray solutions: (a) for the cases with aged seeds (stored for 10 months) and incubated in buffer solution of pH 5; (b) for the cases with fresh seeds and incubated in buffer solutions of pH = 3–11. Error bars in the figure are adapted from four replicates in each treatment.

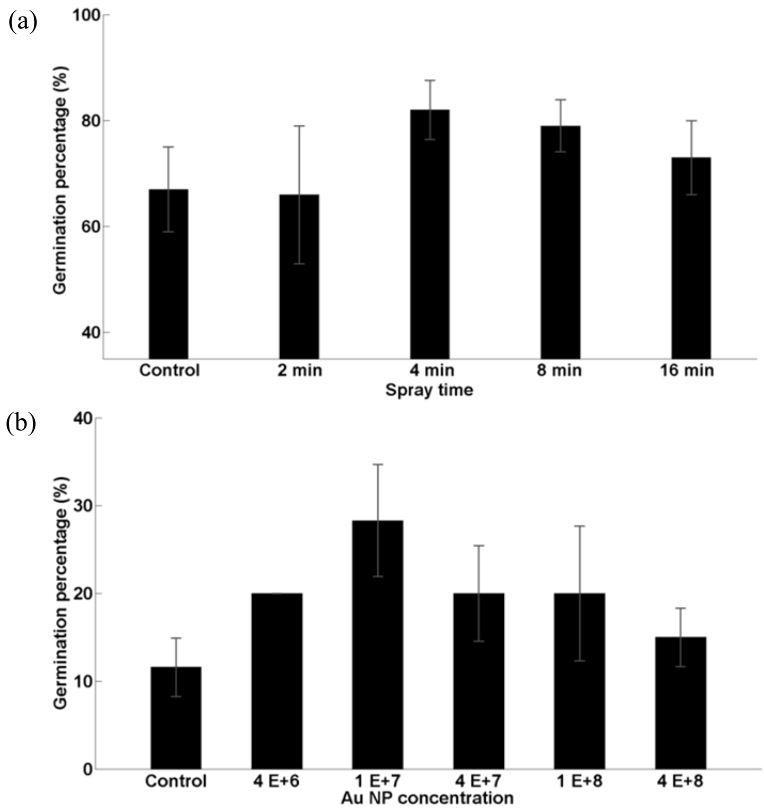


Fig. 5. Germination percentages of lettuce seeds after treatment by NP electrospray: (a) for the cases with fresh seeds, using CuO NPs and incubated in the buffer solutions of pH = 7 for three days, and (b) for the cases with aged seeds (stored for 16 months), using Au NPs and incubated in the buffer solutions of pH = 7 for three days. Error bars in the figure are adapted from four replicates in each treatment.

CuO NP electrospray was obtained in the case of a short spray time of 4 min. For the 4-min spray period, the best germination percentage occurred at the concentration of 10^7 NPs/cm^3 for Au NP suspensions. Based on the above observation, it is possible to use nano-materials of different compositions for enhancing seed germination.

We further performed the comparative test to characterize the effectiveness of various NPs on the germination of aged lettuce seeds in a prolonged incubation period (shown in Fig. 6). Fig. 6(a) shows the germination percentage under the electrospray treatment by suspensions of TiO_2, CuO and Au NPs. The results indicate that treatment by electrospraying either TiO_2 or Au NP suspensions gave the most improved germination percentage. These pretreated seeds also germinated faster, while the control samples (seeds without pretreatment) showed a longer germination window (over seven days). Although the electrospray of the buffer solution (1 mM NaAc) accelerated the seed germination, we did not observe an improvement in the overall germination percentages in a seven-day incubation period. For agricultural and horticultural crops, delayed and sporadic germination is undesirable because it reduces the harvest efficiency. NP-electrospray treated seeds may have the advantage of better crop productivity because of their early and homogeneous germination behavior.

No significant difference on the shoot length of germinated lettuce seeds among all treatment conditions (only the case with CuO NPs showed somewhat negative impact on the shoot length) was observed in this study (shown in Fig. 6(b)). This observation suggests that the proposed treatment has no adverse effects on the early state of shoot development and could potentially improve overall seed germination.

Mechanism for Lettuce Seed Germination Enhancement by TiO_2 NP Electrospray

Plant seeds are incapable of 100% germination even under favorable conditions involving temperature and hydration. In this study, we have demonstrated that the electrospray of NP suspensions enhanced the germination of lettuce seeds while immersing seeds in buffer solutions only (without spray) showed no significant impact on the seed germination percentage. Although the electrospray of buffer solutions without NPs showed minor positive enhancement of seed germination, it was not as effective as the electrospray of NP suspensions. A possible explanation for the observed enhancement of seed germination due to NP electrospray treatment is the breaking of the coat-imposed seed dormancy. Electrosprayed NPs bombarding the seed coat may weaken the structure of the seed coat (Fig. 3), which is considered an influential factor in controlling seed dormancy (Gleiser *et al.*, 2004).

In an aqueous suspension of NPs, it has been previously reported that NPs can slowly penetrate into seeds of various types and affect their metabolism *in vivo* (Navarro

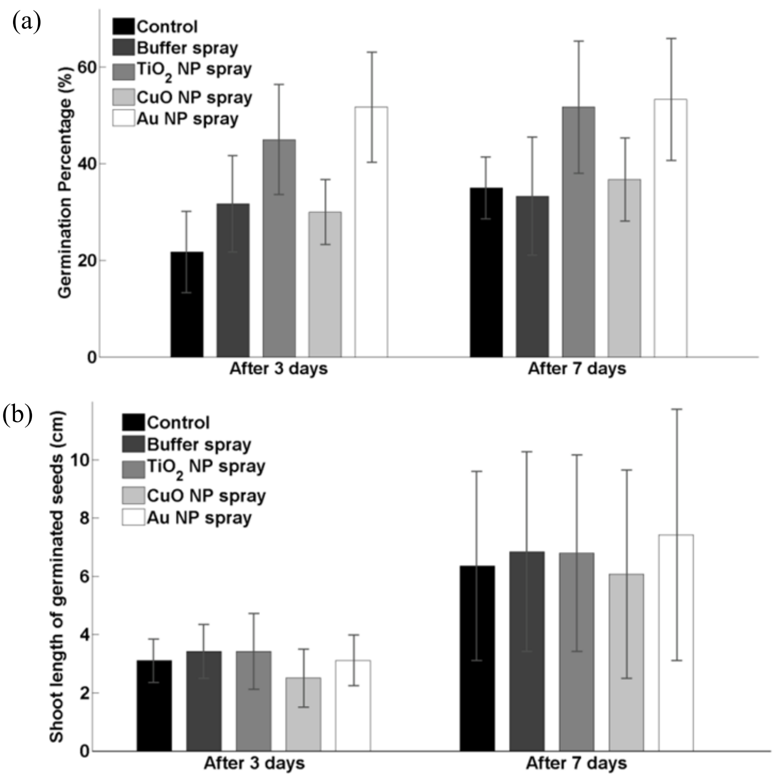


Fig. 6. Comparison of lettuce seed germination after electrospray treatment using NPs of various materials (i.e., TiO_2, CuO and Au). Also included in the figure are the data for the control (without the treatment) and the case treated by electrospraying buffer solutions only for the reference. (a) germination percentage of seeds at both Day 3 and Day 7; (b) shoot length of seeds at Day 3 and Day 7; Error bars in (a) and (b) are adapted from four replicates in each treatment with aged seeds (Stored for 14 months and incubated in the buffer of pH = 5).

et al., 2008; Ma *et al.*, 2010). For example, multiwalled carbon nanotubes (MWCNTs) were able to penetrate through the coats of tomato seeds after several days of co-incubation (Khodakovskaya *et al.*, 2009). In such bulk solutions, NPs are often observed in the agglomerate form and their interactions with seeds are weak. In this study, the electrospray process effectively disperses most of NPs in singlet form, accelerates the velocity of NPs via the presence of electric field and space charge effect, and bombards the seeds by NP collision at high frequency. The proposed process thus increases the chance for NPs entering into seeds by piecing their coats or entering through natural pores. Once nano-sized holes are created in the seed coat, oxygen transfer and water uptake might occur and drive the metabolic process for plant growth (Khodakovskaya *et al.*, 2009).

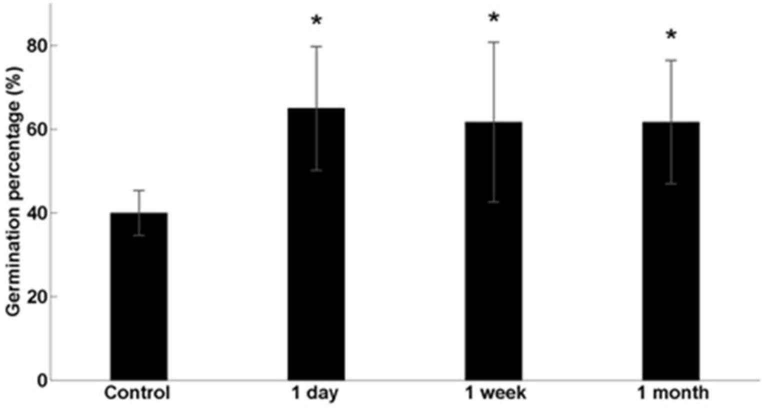
Further, the effect of metal ions on seed germination can be excluded in our study as TiO_2 NPs are considered to be insoluble (< 5 ppb, confirmed by our ICP-MS measurement). Because the incubation process for seed germination took place in the dark, the production of oxidative H_2O_2 through the reaction of light with TiO_2 NPs was minimized in this study. This may explain why the toxicity of TiO_2 NPs, reported by Menard *et al.* (2011) and Hund-Rinke and Simon (2006), was not observed in our investigation (Hund-Rinke and Simon, 2006; Menard *et al.*, 2011). For the case of CuO NP spray, the decrease in seed germination effectiveness is presumably because of the known phytotoxicity of CuO NPs (e.g., release of Cu^{2+} ions to interfere with seed functions) (Karlsson *et al.*, 2008; Wang *et al.*, 2010; Baek and An, 2011, Wu *et al.*, 2012).

Shelf Life of Treated Lettuce Seeds

The shelf life test was performed on lettuce seeds that had undergone the NP electrospray treatment, in order to evaluate the practical potential of the proposed method (Schwember and Bradford, 2010). Aged lettuce seeds treated by NP electrospray were sealed and stored in the dark and at room temperature for various time periods (i.e., 1 day, 1 week, and 1 month) prior to incubation. Fig. 7 shows the germination percentage of aged and TiO_2 NP spray treated lettuce seeds after the defined storage periods. Significant enhancement of the germination of NP spray treated seeds was observed compared to the control. The germination percentage of aged seeds slightly dropped after one-month storage, indicating that the seed coats of NP-treated seeds remained a sufficient protection for the seed embryo during the storage (i.e., long shelf life).

NP Electrospray Treatment of Grass Seeds

Several types of grass seeds (e.g., recalcitrant yarrow seeds and ragweed seeds) which have naturally low germination percentages under favorable growth conditions (typically1–2% each year) were also used in this study to demonstrate the broader applications of the proposed seed treatment. In



Note: * indicates a significant improvement in seed germination as compared with the control (based on Z-test, SE is based on four replicates).

Fig. 7. Shelf life of NP-treated lettuce seeds: germination of aged lettuce seeds treated by TiO_2 NP electrospray after being placed in the dark for one day, one week and one month prior to the incubation.

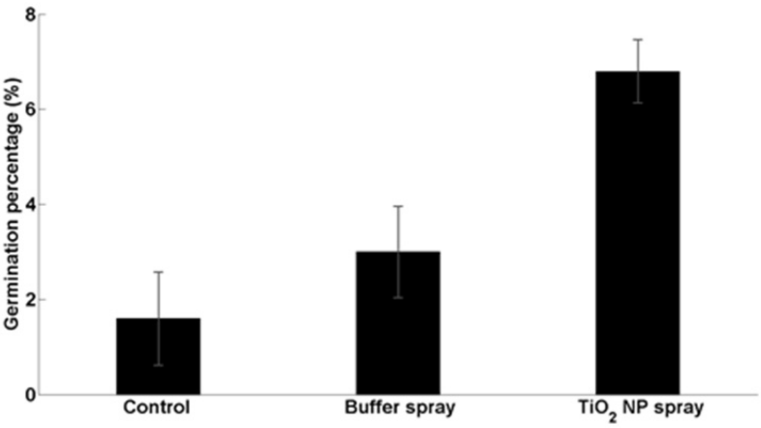


Fig. 8. Germination of yarrow seeds after being treated by TiO_2 NP electrospray and incubated for 15 days. Also included in the figure is the germination of untreated seeds and seeds treated by spraying buffer solutions only (for reference).

this part of experiment, recalcitrant yarrow and ragweed seeds were subjected to 5 min electrospray of suspension with 1 g/L TiO_2 NPs. After culturing in de-ionized H_2O at pH = 7, the germination percentage of recalcitrant yarrow seeds was enhanced from 1.6% (untreated) to 6.8% (pre-treated) (shown in Fig. 8). The germination percentage of ragweed seeds was improved from 1% (untreated) to 3% (treated). The germination percentage of grass seeds studied herein has the potential to be further improved by optimizing the electrospray operating conditions and NP concentration in spray suspensions. Because of the diversity in the structures and coats of seeds from different species (Finch-Savage and Leubner-Metzger, 2006), different electrospray conditions may be required to optimize results for various species.

CONCLUSION

Electrospray of NP suspension was proposed to treat plant seeds for the enhancement of seed germination. A single-capillary electrospray system having a particle deposition stage was set up for this investigation. By applying a positive high voltage on the spray capillary and the electrically grounded stage, the electrospray was operated in the cone-jet mode for the production of monodisperse droplets. The solvent in droplets evaporated immediatey following droplet production, resulting in highly charged NPs in singlet form. Charged NPs were then accelerated in the presence of DC electric field and space charge effect, and bombarded lettuce seeds on the deposition stage. Our study demonstrates that NP electrospray effectively dispersed NPs for breaking the seed coat to enhance seed germination. Our study further shows that the proposed treatment enables seeds to germinate under harsh environments (i.e., low soil pH). The enhancement of seed germination was also observed when electrospraying a NP suspension of Au. Such treatment was further investigated and proven to be effective for certain grass seeds. These results suggest that aerosol exposure of NPs can have a strong impact on plant physiologies.

Our proposed seed treatment method can be further optimized by varying the NP concentration/size, electric field, and spray time. Such a method can be cost effective for scale up in industrial applications because very small amounts of NP suspensions were needed for the treatment (estimate: 1 g of NPs can spray 3.6 million lettuce seeds based on this study). We believe that the proposed NP electrospray has the potential to be applied to various plant seeds (Pui and Chen, 2000; Nadjafi *et al.*, 2006). Meanwhile, environmental friendly NPs (Rieter *et al.*, 2008; Yan *et al.*, 2010) can be employed in the future to alleviate concerns surrounding the cytotoxicity of metal oxide NPs (Walser *et al.*, 2012). In addition, the use of engineered NPs carrying DNA, plant hormones or other chemicals in the proposed process for seed treatment may open up new opportunities for a broad application of nanotechnology in the agricultural industry.

ACKNOWLEDGEMENTS

We appreciate valuable suggestions from Prof. G. Welbaum at Virginia Tech. We would also like to thank Prof. D. Ho from the Department of Biology at Washington University in St. Louis and Prof. I. Kong at Yeungnam University for close readings and helpful discussions of this manuscript.

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Received for review, June 25, 2013
Accepted, December 27, 2013