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Full Length Article

Germination Responses of Soybean Seeds to Fe, ZnO, Cu and Co Nanoparticle Treatments

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Abstract

Engineered nanometals have been used as stimulants or micronutrients to improve yield and quality of crops. In order to elucidate the effects of important metal nanoparticles (NPs) on the germination of soybean seeds, early responses of this procedure were evaluated. Soybean seeds were treated with Fe, ZnO, Cu and Co NPs at different concentrations. Germination percentage, germination rate, primary root and leaf growth at morphological and cytological levels were analyzed. Obtained results indicated that treatment of soybean seeds promoted germination rate, the growth of primary roots and leaves, depending concentrations of metal NPs used. The number of germinated seeds treated with 50 mg/L Fe, ZnO and Cu NPs achieved 62.19, 66.41 and 57.62%, respectively, significantly higher than control. ZnO NP seemed to be the strongest in root growth acceleration. The maximum primary root length of 9.26 mm was obtained at ZnO NP concentration of 50 mg/L. The growth of leaves from the treated seeds was significantly faster than the control after 39 hours (h) of germination, especially treated with ZnO and Co NPs. Fe and Cu NPs did not show the influence in growth of primary leaves during the first 60 h of germination. The positive effect of metal NPs also revealed through the root mitotic index, which was increased from 26 to 34% compared to the control. The obtained results will provide an evident for further investigation of the response of plant cells at molecular level to nanometal application. © 2018 Friends Science Publishers

Keywords: Metal nanoparticles; Seed germination; Soybean; Morphological characteristic; Mitotic index

Introduction

Currently, metal nanoparticles are widely used in agriculture as stimulants and micronutrients because of their advances in structure, surface to volume ratio, morphology, and reactivity compared to respective chemicals (Wang *et al.*, 2016). The application of metal-based NPs as seed treatment agents in improving different stages of plant development, including germination and seedling establishment has been investigated in several plant species (Salama, 2012; Singh *et al.*, 2013; Gopinath *et al.*, 2014).

The most metal NPs reported are silver (Ag), Fe, Zn, Cu and their oxide because they have unique electronic, optical, magnetic and chemical properties and are also essential elements in growth and development of plants (Ruttkay-Nedecky *et al*., 2017). ZnO NP was found to promote seed germination, seedling vigor and plant growth in peanuts (Prasad *et al.*, 2012). Similarly, Panwar *et al.* (2012) observed that shoot and root lengths of tomato seedling were promoted when seeds treated with ZnO NP at high concentration of 100 mg/L. Cu and Ag NPs at ultralow concentrations had stimulating effects on seed germination and sprout growth of wheat grain and tomato seeds (Maslobrod *et al.*, 2013). The germination percentages of soybean seeds treated with Cu, Co and Fe NPs at extra-low concentration were increased from 55% of the control to 65, 80 and 80%, respectively (Ngo *et al.*, 2014).

The mechanisms how metal NPs induced seed germination are still uncertain, although essential bulk metal elements are known to act as co-factors in enzyme systems and to participate in redox reactions (Hansch and Mendel, 2009). The germination and subsequent seedling establishment of several plant species were stimulated in case their seeds primed in essential metal-enriched solutions (Farooq *et al.*, 2012). Nanoscales of metal and metal oxide NPs, which differ from their native bulk metals in surface, optical, thermal, and electrical properties (Wang *et al.*, 2016), may have addition functions in plants depending on NP types and plant species. For instance, Ag NP seed priming was considered as a possible benefactor to enhance

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α-amylase activity and then soluble sugar content in germinating rice seeds, resulting in seedling growth acceleration (Mahakham *et al.*, 2017). Ag NP could also stimulate up-regulation of aquaporin genes, which help it to entry the cells efficiently.

The interaction of metal NPs and plants based on mitotic root meristem analysis has been studied in several species and showed both positive and negative depending on type and concentration of used NPs. Nagaonkar *et al.* (2015) found mitotic index at root tips of *Allium cepa* was increased at Cu NP concentrations lower than 20 µg/mL and decreased as Cu NP concentrations were elevated.

In order to understand the reactions of plant cells to metal NPs at cellular and molecular levels in the future, it may be useful to investigate the timely responses of seeds to metal nanoparticle application. Therefore, in this study, the effect of some essential microelements in nano sizes including Fe, ZnO, Cu and Co on the germination of soybean seeds, an important crop in Vietnam, was investigated based on closely assessing morphological parameters such as germination percentage, germination rate, length of primary root and leaf. Root mitotic index (MI) was also analyzed to support the possible positive effect of studied NPs in germination of soybean. Obtained results could also be a scientific basis for the application of metal NPs to increase crop productivity and develop sustainable agriculture.

Materials and Methods

Materials

Seeds of soybean cultivar DT26 harvested in winter 2015 were obtained from Legumes Research and Development Center, Food Crops Research Institute, Vietnam Academy of Agriculture Sciences. Healthy seeds with no injury on the surface and equal size were selected. NPs include Fe, ZnO, Cu and Co with purity>95% and 40–60 nm in size were provided by the Institute of Environmental Technology - Vietnam Academy of Science and Technology.

Seed Preparation

To avoid possible effects of microbes on germination process, selected seeds were surface sterilized using chlorine gas as described by Clough and Bent (1998). Briefly, selected seeds were added to a Pyrex bottle and placed inside a desiccator along with a beaker containing 100 mL of 5.25% sodium hypochlorite under a fume hood. After added 3 mL of concentrated HCl (37%) into the beaker, the glass desiccator was closed tightly. The bottle of seeds was removed from the desiccator after 4 h and leaved remained gas released for a while and storage until use.

Seed Treatments

In order to assess the effects of metal NPs on germination, at first nanometal powders were suspended in distilled water by ultrasonic at 200W and 37kHz for 30 min. 30 g sterilized soybean seeds (150 seeds) were soaked for 30 min in 10 mL of metal NP suspension which contain Fe NP at 25, 50 and 250 mg/L; ZnO NP at 25, 50 and 500 mg/L; Cu NP at 5, 25 and 50 mg/L or Co NP at 0.05, 0.5 and 2.5 mg/L. Control were treated with 10 mL of distilled deionized water. These concentrations of each metal NP tested were based on the earlier results obtained by Ngo *et al.* (2014). Seeds were germinated on 0.5% agar solution. Agar solution was prepared by dissolving 5 g of plant agar (Duchefa Biochemie BV, catalog number: D1001) in 1000 mL of distilled water and heating until the agar was completely dissolved, then cool slightly to 50°C. 450 mL of warm liquid agar solution was poured into each of the 10 x 20 x 15 cm plastic boxes and left to cool down to room temperature. Seeds (50 each) were placed on agar surface of a box. For single NP treatment, 150 seeds were used. The boxes were placed in darkness at room temperature. The experiment was repeated three times.

Seeds were considered to be germinated when the radicles were visible. The data on germination percentage (GP) and germination rate (GR) were collected at 15, 21, 24 and 48 h after treatment and calculated as following equations:

$$GP\ (\%) = \frac{\text{Number of germinated seeds}}{\text{Total seeds}} \times 100$$

$$GR = \sum \frac{X_n}{Y_n\,(X_n - X_{n-1})}$$

In which X_n is the germination percentage in n- the hour and Y_n is the hours from beginning of seed treatment to the n hour).

Morphological characteristic and length of radicles were analyzed and measured at 15, 18, 21 and 24 h after treatment. At each time point, 30 seeds per experimental plot were randomly collected for studying. The length of primary leaves of germinating seeds was observed and measured using Leica M80 binocular stereomicroscope combined with Leica IC80 HD camera and Leica-EZ V3.2 program (Germany).

Cytological Analysis

Root development capacity was analyzed based on MI as described by Martins *et al.* (2008). Primary roots at 15, 18, 21 and 24 h of the most effective concentration of each metal NP were separated from germinated seeds and fixed in Carnoy solution (3 : 1 v/v of ethanol : glacial acetic acid). Cytological examination was conducted using the squash technique after root samples hydrated with 1 *M* HCl for 15 min at $60^{o}C$ and stained following Feulgen technique as described by Venora *et al.* (2000). A total of 1,000 root-tip cells on each slide were scored and 10 slides were analyzed per treatment. Mitotic cells at different phases were quantified and MI was calculated as the bellow equation.

$$MI = \frac{\text{Total number of mitotic cells}}{\text{Total number of observed cells}} \times 100$$

Statistical Analysis

Each treatment was performed in three replicates and experimental data was statistically analysed using Graph Pad Prism 5.0 software. The significant levels of difference for all measured characteristics were calculated, and the means were compared by Duncan's Multiple Range test with $p < 0.05$.

Results

Seed Germination

Soybean seed treatment with Fe, ZnO, Cu and Co NPs prior sowing promoted GP and GR, regardless different concentrations of metal NPs used (Table 1). Significant differences between treatment with NPs and control were clearly presented at the 15 h after treatment. The number of seeds treated with 50 mg/L Fe, ZnO and Cu NPs achieved 62.19, 57.62 and 66.41% germination, respectively, while it was only 39.19% for the control (Table 1). However, at 24 and then 48 h the number of seeds germinated in the control was equal with that in the treated seeds, average of 94% except for Fe NP of 50 mg/L and 250 mg/L, where the germination percentage was up to 97 and 99%. The GR increased when seeds were treated with Fe, ZnO, Cu and Co NPs. At 50 mg/L Cu NP, the seed GR was the highest (0.602).

Root Emerging and Growth

The primary root is an important structure which is vital to the survival of plants. To assess the growth response of primary root under nanometal treatments, the length of primary roots and the root mitotic index were investigated. Root length was recorded from 15 h after metal NP treatment. It is the time emerged radicals can be noticeable in the control seeds.

The obtained data in Table 2 indicates that nanometal treatments produced significant effects at early stage of germination of soybean seeds and the development of primary root. Under nanometal treatments, except at low concentrations of Fe (25 mg/L) and Cu (5 mg/L) NPs, the radicals emerged earlier and grew faster than the control seeds. At 15 h after treatment, the length of primary roots reached 8.26–9.26 mm, while it was only 7.53 mm for the control (Table 2 and Fig. 1). Among used nanometals, ZnO NP seemed to be the strongest in root growth acceleration. At the lowest concentration of ZnO NP (25 mg/L), the root length was significantly longer than the control (8.82 and 7.53 mm, respectively). The growth characteristics were better when ZnO NP concentrations increased. The maximum primary root length of 9.26 mm was obtained at ZnO NP concentration of 500 mg/L. The extremely low Co NP concentration of 0.05 mg/L also produced the same effect as ZnO NP. However, such effect was reduced when its concentration was up to 5 and 2.5 mg/L (Table 2). Fe and Cu NPs only promoted primary root development at concentrations equal or higher than 50 and 25 mg/L, respectively.

At 18 h after treatment, the length of primary roots treated with ZnO NP remained the highest compared to those treated with other nanometals. Cu NP at concentrations 25 and 50 mg/L promoted primary roots to develop more rapidly than the earlier period. The average length of primary roots in 25 and 50 mg/L Cu NP treatments was equal with that in 25 mg/L ZnO NP treatment. In opposite, the root growth under Co NP slowed down and the primary root length was comparable with that of the control (Table 2).

From 21 to 24 h, the growth of primary roots under effect of Fe, ZnO and Cu NPs still was highest compared to the control. The root length reached up to 15.51 mm with ZnO NP at 50 mg/L and 15.79 cm with Cu NP at 50 mg/L, while it was only 12.56 mm for the control seeds (Table 2).

These above results obviously reveal that the nanometals, especially Fe, ZnO and Cu NPs have a particular role in promoting soybean seed germination and primary root growth at least during 24 h after sowing. The comparable development of root length from different treatments could also be seen clearly in Fig. 1.

Root Cell Division

In vivo cytogenetical assay in root meristems has been carried out to study the effect of nanometal on MI, which can reflect the frequency of cell division and usually considered to be an important root growth parameter. In order to confirm the contribution of metal NPs on the growth enhancement of soybean primary roots, the mitotic cell division and root MI of germinated seeds at optimal treatments, was under examination.

The observation indicated that mitotic division and chromosomal behavior at root tips of both treated and control seeds were normal. The root MI increased considerably with all nanometal treatment seeds (Table 3). It enhanced from 4.75% in the control to 6.82, 7.21, 6.80 and 6.52% for Fe NP of 50 mg/L, ZnO NP of 500 mg/L, Cu NP of 50 mg/L and Co NP of 0.05 mg/L, respectively.

The response of primary root at cellular level can also be visible in Fig. 2, where number of mitotic cells in NP treatments increased, especially for ZnO NP treatment (also in Table 3). This proves that nanometal seed treatment at optimum concentrations has a positive effect on the root proliferation at least during 24 h after germination.

Table 1: Effect of metal NPs on the germination percentage and germination rate of soybean seeds

NP treatments (mg/L)	GP (%)			GR
	15 h	24 h	48 h	
Control	39.19^{a*}	80.26^{a}	93.42^{a}	0.296^{a}
Fe 25*	49.98^{b}	86.58^{a}	96.64^{a}	0.365^{b}
Fe 50	62.19^{c}	83.89^{a}	97.99^{b}	0.373^{b}
Fe 250	46.95^{b}	90.79^{b}	99.34^{b}	0.395^{b}
ZnO 25	39.47^{a}	75.16^{a}	91.58^{a}	0.271^{a}
ZnO 50	57.62^{c}	82.27^{a}	94.74^{a}	0.364^{b}
ZnO 500	49.33^{b}	76.78^{a}	92.17^{a}	0.308^{b}
Cu 5	48.54^{b}	88.67^{b}	96.67^{a}	0.410^{b}
Cu 25	52.95^{b}	83.33^{a}	95.33^{a}	0.346^{b}
Cu 50	66.41^{c}	91.45^{b}	96.71^{a}	0.602^{c}
Co 0.05	50.00^{b}	80.41^{a}	95.36^{a}	0.310^{b}
Co 0.5	40.00^{a}	79.80^{a}	92.17^{a}	0.306^{a}
Co 2.5	40.79^{a}	80.54^{a}	94.12^{a}	0.295^{a}

Data in one cell indicate the mean of GP and GR of soybean in three replicates; a, b, c in the same column show a statistically significant difference with $p<0.05$; n = 3. * Representative for a metal NP and its concentration

Table 2: Effect of metal NP treatments to the length of soybean primary roots

NP treatments	Primary root length after treatment (mm)			
(mg/L)	15 h	18 h	21 h	24 h
Control	7.53^{a}	8.98^{a}	10.65^{a}	12.56^{a}
Fe 25*	7.87^{a}	9.33^{a}	11.46^{a}	13.32^{a}
Fe 50	8.36^{b}	11.41^{b}	12.70^{c}	15.04^{b}
Fe 250	8.27^{b}	9.94^{b}	12.20^{bc}	14.97^{b}
ZnO 25	8.82^{b}	10.38^{bc}	12.50^{b}	15.11^{b}
ZnO 50	9.08^{bc}	10.96^{c}	12.84^{bc}	15.18^{b}
ZnO500	9.26^{c}	10.81^{c}	13.06^{c}	15.51^{c}
Cu 5	7.55^{a}	8.94^{a}	10.66^{a}	12.43^{a}
Cu 25	8.30^{b}	10.24^{bc}	12.47^{b}	15.02^{b}
Cu 50	8.26^{b}	10.37^{bc}	13.07^{c}	15.79^{c}
Co 0.05	9.10^{c}	9.34^{b}	11.12^{a}	13.54^{a}
Co 0.5	8.46^{b}	8.90^{a}	10.14^{a}	12.55^{a}
Co 2.5	8.52^{b}	9.12^{a}	11.12^{a}	13.21^{a}

Data in one cell indicate the mean of primary root length for 30 seed samples; a, b, c, d in the same column show a statistically significant difference with $p<0.05$; n = 3. * Representative for a metal NP and its concentration

Table 3: Effect of different metal NP treatments on cell division in soybean primary roots

NP treatments	Average number of mitotic cells				Total	MI
(mg/L)	Prophase	Metaphase	Anaphase	Telophase		(%)
Control	13.63^{a}	24.73^{a}	9.10^{a}	0.03^{a}	47.50^{a}	4.75^{a}
Fe 50	13.60^{a}	33.33^{b}	20.73^{bc}	0.53^{a}	68.20^{b}	6.82^{b}
ZnO 500	16.03^{b}	33.63^{b}	21.93^{b}	0.53^{a}	72.13^{b}	7.21^{b}
Cu 50	15.07^{b}	31.43^{b}	21.03^{bc}	0.50^{a}	68.03^{b}	6.80^{b}
Co 0.05	11.97^{a}	35.13^{b}	17.90^{bc}	0.20^{a}	65.20^{b}	6.52^{b}

Data in one cell represents the mean of mitotic cell number in prophase, metaphase, anaphase and telophase, which observed in 10 root tip samples; a, b and c in each column show statistically significant differences with $p<0.05$; n = 3. MI value (%) were calculated as the total number of mitotic cells per 1000 observed cells. Fe NP: 50 mg/L; ZnO NP: 500 mg/L; Cu NP: 50 mg/L; Co NP: 0.05 mg/L

Primary Leaf (Unifoliate Leaf) Development

Preliminary experiments revealed that the growth of primary leaves started later than of primary roots. Therefore, the examination procedure for development of primary leaves was kept until 63 h after nanometal treatment.

There was an insignificant difference between the treated and non-treated seeds during the first 24 h. From 39 h onwards the length of primary leaves from seeds treated with ZnO and Co NPs significantly longer than those of control (Table 4). Their leaf lengths attained around 3.0 mm after 51 h and 4.0 mm at 63 h, and were 2.0 and 3.0 mm, respectively, for the control. Unlike the positive effect on primary roots, Fe and Cu NPs did not influence the length of primary leaves during the first 60 h (Fig. 3).

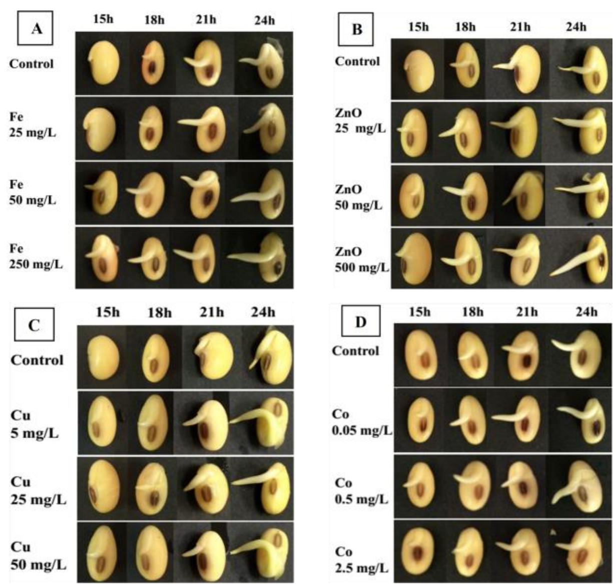

Fig. 1: Effect of different metal NPs on the development of soybean primary roots. Seeds treated with Fe NP (A); ZnO NP (B); Cu NP (C); and Co NP (D)

Discussion

Germination, an early stage of development, is the complex process of seeds developing into new plants. Rapid and uniform seed germination and seedling emergence are important determinants of successful establishment. The process is known influenced by many factors from moisture, temperature, and available nutrients including microelements. The obtained results in this study indicated that micronutrients at nanoscales such as Fe, Cu, Zn and Co positively affect the seed germination of soybean. These results suggest that metal NPs promote seed germination earlier of 4 h compared with the control. The similar benefit on seed germination of other plant species has also been reported by several investigators. When using ZnO NP, Prasad *et al.* (2012) found a higher germination of peanut seeds compared to both chemical zinc sulfate and the control. Takahashi *et al.* (2009) has considered mineral elements Fe, Zn, manganese (Mn), and Cu as crucial need in rice seed germination.

Table 4: Effect of metal NP treatments on the length of soybean primary leaves

NP treatments	**Primary leaf length** (mm)				
(mg/L)	15 h	27 h	39 h	51 h	63 h
Control	1.03^{a}	1.13^{a}	1.71^{a}	2.08^{a}	3.21^{a}
Fe 25*	1.09^{a}	1.17^{a}	1.58^{a}	2.08^{a}	3.52^{a}
Fe 50	1.07^{a}	1.12^{a}	1.71^{a}	2.08^{a}	3.19^{a}
Fe 250	1.10^{a}	1.19^{a}	1.66^{a}	2.11^{a}	3.58^{a}
ZnO 25	1.43^{a}	1.50^{a}	2.62^{b}	3.29^{b}	4.67^{b}
ZnO 50	1.39^{a}	1.49^{a}	2.73^{b}	3.13^{b}	4.44^{b}
ZnO500	1.33^{a}	1.44^{a}	2.63^{b}	3.12^{b}	4.75^{b}
Cu 5	1.08^{a}	1.19^{a}	1.69^{a}	1.96^{a}	3.10^{a}
Cu 25	1.12^{a}	1.29^{a}	1.81^{a}	2.50^{a}	3.58^{a}
Cu 50	1.13^{a}	1.18^{a}	1.74^{a}	2.24^{a}	3.43^{a}
Co 0.05	1.36^{a}	1.40^{a}	2.56^{b}	3.24^{b}	4.25^{a}
Co 0.5	1.35^{a}	1.45^{a}	2.48^{b}	3.02^{b}	4.11^{b}
Co 2.5	1.35^{a}	1.48^{a}	2.48^{b}	3.07^{b}	4.05^{b}

Data in one cell indicate the mean of primary leaf length for 30 seed samples; a, b, c, d in the same column show a statistically significant difference with $p<0.05$; n = 3. *Representative for a metal nanoparticle and its concentration

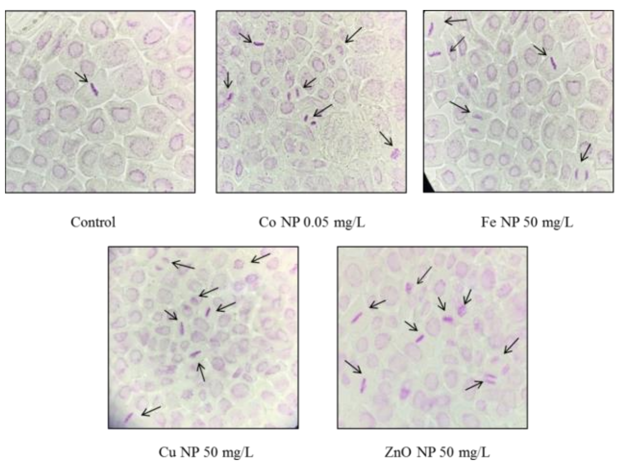

Fig. 2: Cell division of soybean root tip under Co NP, Fe NP, Cu NP and ZnO NP treatment. Arrows indicate observed cells at the mitosis phases

Among the studied NPs, ZnO NP is most effective in germinating activities of soybean seeds. It doesn't only promote the growth of primary roots but also supports the development of first trifoliate leaves earlier than the control. Zn ion is known to be critical for plant life and involved in many biochemical processes such the carbohydrate, protein, lipids metabolisms and physiological processes such as photosynthesis (Rehman *et al.*, 2012; Maret, 2013). It is the only metal represented in the largest number of proteins for attain structural property. A significantly improvement of germination and seedling growth was found in seeds enriched Zn content prior to sowing of rice (Prom-u-thai *et al.*, 2012) and maize (Imran *et al.*, 2017), similar to findings in bean (*Phaseolus vulgaris*) (Kaya *et al.*, 2007), barley (Ajouri *et al.*, 2004). However, at high concentrations of Zn can be toxic and inhibit germination (Prom-u-thai *et al.*, 2012).

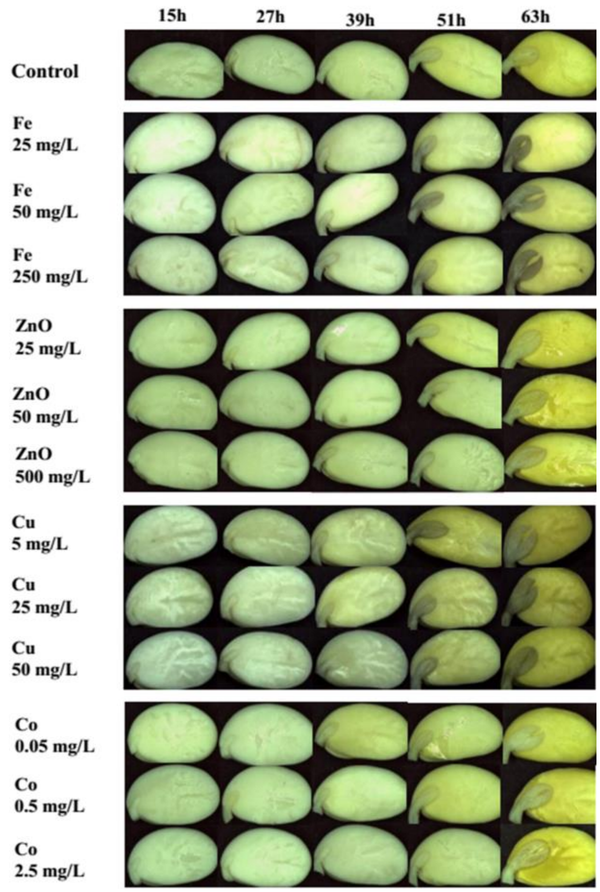

Fig. 3: The development of primary leaves under metal NP treatments during the first 63 h of germination. Pictures were acquired with Leica M80 binocular stereomicroscope combined with Leica IC80 HD camera and Leica-EZ V3.2 software program. 15 h, 27 h, 39 h, 51 h, 63 h: hours after NP treatment

According to Hossain *et al.* (2016), ZnO NP induced oxidative stress in soybean seedlings in a high concentration of 500 ppm and the use of extremely low concentrations of ZnO NP, lower than 500 ppm, can guarantee for the enhancement of the Zn content in the seed without toxicity to plant cells.

Apart of boosting primary roots and leaves, Fe is the only NP to accelerate the germination percentage in the first two days of soybean (Table 1). In the past, Farhat *et al.* (2015) reported the germination percentage of wheat was increased under Fe NP treatment. Fe NP at low concentrations of 40 or 80 μmol/L was observed to stimulate both seed germination and growth of peanut plants by Li *et al.* (2015). The contribution of Fe NP could be placed on its well-known role as an essential micronutrient in metabolic processes such as DNA synthesis, respiration, and photosynthesis and other physiological and biochemical pathways, which was indicated in the comprehensively review by Rout and Sahoo (2015).

In first few days of germination, Cu NP only had a significant effect on primary root growth of soybean. The radicles showed the greatest growth in response to Cu NP compared to other studied metal NPs (Table 2). No other research has mentioned a similar effect of Cu NP on primary root growth in other species. Observation of lettuce seeds germination in the presence of Cu NP, Shah and Belozerova (2009) only noticed an increase in the shoot-to-root ratio compared to the control plants. An observable increase in germination percentage of wheat was attained by Farhat *et al.* (2015). However, germination rate, root and shoot length of rice were decreased if an excess of Cu NP uptake occurred (Da Costa and Sharma, 2016). Like other mineral nutrients, Cu involved in many physiological processes and acted as a structural component in regulatory proteins, photosynthetic electron transport, cell wall metabolism, and hormone signaling (Solymosi and Bertrand, 2012).

In our experiments, Co NP had less effective in soybean at the first 3 days of germination. In a previous report, Ngo *et al*. (2014) reported that the germination rates of soybean seeds treated with Cu, Co and Fe NPs were increased significantly. The difference could be shown in later stage of germination, where the evaluation was carried out in 7 days after treatment. Co ion may act in the later stages of germination.

In the present studies, metal NPs at the concentrations supporting the primary root growth, can accompany with increasing mitotic activity in root tips. In assessment of the safety of ZnO NP to onion seed germination, Raskar and Laware (2014) have also indicated that at low concentration of 10 μg/mL enhanced cell division, mitotic index with minimum cell divisional abnormalities and also increased seed germination, and seedling growth. However, MIs were reduced significantly at higher concentrations. Therefore, in order to confirm the possible benefit of studied NPs on soybean germination, cell dividing and chromosome behavior under different concentrations of each NP should be further investigated.

Conclusion

Our study showed that Fe, ZnO, Cu and Co NPs have positive effects on the early stages of soybean germination, depending on types and concentrations of nanometal particles. Fe and ZnO NPs promote primary roots and the development of first true leaves. Fe NP is the only nanoparticle to accelerate the germination rate in the first two day of soybean germination. Cu NP only has a significant effect on primary root growth. Co NP has less effective compared to the other studied metal NPs at the first 3 days of soybean germination. At optimum concentrations, all studied NPs enhance cell division at root tips. Based on these results, further comprehensive experimentation needs to be conducted to explore underlying cellular and molecular mechanisms responsible for enhanced seed germination of soybean.

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