INFLUENCE OF METAL NANOCOLLOIDS ON SELECTED ABIOTIC STRESS FACTORS IN PUMPKIN

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ABSTRACT

Nanotechnology could be a solution for providing sustainable agriculture, clean water and a better environment. Various nanomaterials can sustain the agricultural sectors. Plants are essential fundamental components of all ecosystems, and the interaction between NPs and plants is an indispensable aspect of the risk assessment. Originally, this research focuses on NP phytotoxicity, which is an important precondition to promote the application of nanotechnology and to avoid the potential ecological risks. SOD removes $O_2^{\cdot-}$, prevents generation of highly toxic $OH^{\cdot}$, and catalyzes the dismutation of superoxide radical into molecular oxygen and hydrogen peroxide. SOD is ubiquitous and can be found in virtually all oxygen-consuming organisms, aero-tolerant anaerobes, and some obligate anaerobes.

The objective of the study was to determine the effect of nanoparticles (NPs) on the oxidative stress parameters, of pumpkin seedlings. The study comprised analyses of the effect of two NPs (NKAg, NKCu) on the total soluble leaf protein content, lipid peroxidation (LPO), superoxide dismutases (SODs) activity. The effect of the concentration of NPs (50, 100 and 150 ml L⁻¹), was studied.

INTRODUCTION

Designed "nano" materials are characterized by specific physical and chemical properties. In addition, their size causes that they have greater penetration area, reactivity and solubility than their mass counterparts (Nowack and Bucheli, 2007). These materials, especially silver and copper, are increasingly being used in numerous industrial production processes as an additive to opacifiers, catalysts, semiconductors, cosmetics, microelectronics and in agriculture as nanofertilizers capable of delivering one to several nutrients to plants thus influencing their growth and yields (Curtis et al. 2006; Buzea et al., 2007; Anjum et al., 2013; Ma et al., 2015). In the context of sustainable agriculture, the use of innovative agricultural nanotechnology (including fertilizer development) is considered as an intentional, as one of the most promising ways to significantly increase plant production to meet the needs of ever-increasing populations in the world. It should be noted, however, that safe and sustainable use of nanotechnology in agriculture requires standards for the use of NPs in crop production and in soil and water environments (OECD 2014). Due to the appropriate size, nanoparticles can easily penetrate the organisms affecting their changes at the DNA level (Auffan et al. 2012), protein oxidation, electrolyte loss, and damage of cell membrane contributing to stress (ROS) (Dimkpa et al., 2012; Rui et al., 2015; Zhang et al., 2015), and finally to a cell death (Meriga et al., 2004; Sharma et al., 2012). Environmental conditions can cause stress in plants that alters the system of phytochemical synthesis. Reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2) and superoxide radical anion ($O_2^{\cdot-}$), are primarily produced as mitochondrial aerobic respiratory byproducts and can damage many cellular macromolecules, including lipids, proteins, and nucleic acids (Finkel and Holbrook, 2000). Plants have evolved and successfully developed a defense system that would prevent the accumulation of ROS and repair damages caused by the formation of ROS. This system includes fat soluble antioxidants (e.g. tocopherols and carotenoids), water-soluble

antioxidants (e.g. ascorbate and glutathione) and enzymes, such as catalase (CAT) or superoxide dismutase (SOD) (Imahori, 2014).

The purpose of this study was to determine the effect of two nanocolloids: silver and copper, on selected factors of abiotic stress in pumpkin leaves.

MATERIALS AND METHODS

Plant material

In the experiment, non-encapsulated seeds of Polish pumpkin cultivar 'Miranda' were used. Nano-materials for the study were two commercially available products in 1L bottles represented by silver and copper nanocolloids at a concentration of $\geq 0.2\%$ for silver (2KAg) and $\geq 0.1\%$ for copper (1KCu) produced by ITP-SYSTEM Ltd. in Dąbrowa Górnicza, Poland. In order to carry out the experiment to determine the impact of abiotic stress on the plant, pumpkin seeds were placed in a 70% ethanol solution for surface sterilization (30 s). They were then transferred to a 1.5% solution of sodium hypochlorite (NaClO) for 15 min. After this time, the seeds were washed five times in distilled water and then laid on round plastic trays 30 cm in diameter lined with a paper towel. Each tray contained about 500 seeds. They were moistened by pouring about 100 ml of distilled water into each tray, then covered with aluminum foil and inserted into the phytotron FD 711 DD INOX to germinate under the following conditions: relative humidity 60% at 20/18 °C day/night, in the dark. Seeds were ventilated daily. The germination process lasted 7 days.

Plant growth

After germination of seedlings to a similar size with well-developed roots and having about 1.5 cm of length, seedlings were transferred to a modified, aerated Hoagland medium with optimum pH 6. Plants were grown in phytotron for the next 21 days under the following conditions: relative humidity 60%, temperature 20/18 °C day/night, photoperiod 18/6h day/night. After 21 days, the seedlings were sprayed with a prepared variant of nanocolloids in amount of about 4 ml (NKAg and NKCu at 3 different concentrations: 50 ml L⁻¹, 100 ml L⁻¹ and 150 ml L⁻¹). After spraying, the samples were grouped in variants to avoid possible contact with plants treated with other concentrations. Analytical material was collected on day 22 (after first spraying with nanocolloid) and on day 29 of the plant life. For the biochemical analysis, the first leaves were separated from each of 30 plants, then their central parts were separated by weighing 0.5 g samples and then frozen at -60 °C.

Preparation of plant homogenates

Prepared 0.5g samples were removed from the freezer and placed in ice. Then the samples were ground in a water-bath with 4.5 ml of cooled (4 °C) 50 mM sodium phosphate buffer (pH 7.5) containing 0.1 mM EDTA and 2% PVP. The homogenate was filtered through a nylon fabric thereby producing a "crude homogenate" used directly to determine lipid peroxidation degree (LPO). The remaining part was centrifuged at 11,000 RPM for 20 min at 4 °C. The resulting homogenate (supernatant) was used for other biochemical analyses (Balakhnina et al., 2015).

Soluble protein content according to Bradford

The reagent was prepared by dissolving 100 mg of G-250 Coomassie Brilliant Blue G-150 in 50 ml of 95% EtOH. Then 100 ml 85% (w/v) H₃PO₄ was added and when the dye was completely dissolved, diluted to 1 liter with distilled water and filtered through a laboratory filter (65 g cm⁻²). Immediately before use, the reagent was diluted 5 times with distilled water.

The volume of 5 ml of reagent was added to 0.25 ml sample and incubated for 10 min in the dark. Absorbance was measured at 595 nm. The calibration curve was made using bovine albumin from serum (Bradford, 1976).

Lipid peroxidation (LPO)

The level of oxidative stress (LPO) was determined by the TBARS method. The reaction mixture consisted of 3 ml 1% H_3PO_4 , 1 ml 0.6% thiobarbituric acid, 0.1 ml 0.28% FeSO_4 and 0.3 ml "crude" homogenate. Prepared samples were placed in a water bath at 100 °C for 45 min. After this time, they were immediately cooled on ice and extracted using 4 ml of n-butanol. The upper fraction was collected and after centrifugation (3,000rpm/5min), spectrophotometric measurements were performed at 532 nm and 600 nm wavelengths. The amount of malonic dialdehyde was expressed in nMg of g fresh weight⁻¹. For calculations, a molar extinction coefficient equaled to $1.56 \times 10^{-5} \text{ mol cm}^{-1}$ was used (Uchiyama and Mihara, 1978).

Superoxide dismutase (SOD) activity

Prepared reaction mixture consisted of 2.1 ml phosphate buffer, 0.1 ml 13 mM methionine, 0.1ml 75 μM tetrazole blue chloride (NBT), 0.1 ml homogenate, 0.2 ml gelatin (5mg ml⁻¹) and 0.2 ml 2 μM riboflavin. The first measurement was done in the dark, after which the reaction mixture was exposed for 10 min using a fluorescent lamp (30 W and 30 cm distance) as the only light source. One unit of superoxide dismutase activity was defined as the amount of enzyme responsible for 50% inhibition of NBT photoreduction (Giannopolitis and Ries, 1977).

Statistical analysis

Achieved results were analyzed statistically by considering all variables, for which the mean values $\pm$ SD were calculated. Because the results did not show normal distribution (Shapiro-Wilk test, $p < 0.05$), non-parametric Kruskal-Wallis test ($p < 0.05$) was used for statistical analysis. The results are reported as mean values (n=9).

RESULTS AND DISCUSSION

Reactive oxygen species are able to oxidize almost any compound in a plant cell leading to numerous internal injuries. The lipid peroxidation LPO, by biological membrane disorders, attacks particularly unsaturated fatty acids, destabilizing their stability and contributing to disintegration in subsequent reactions to produce aldehydes and alcohols. The suppression of this phenomenon occurs in the presence of enzymatic and non-enzymatic antioxidants, i.e. low molecular weight antioxidants, including carotenoids and enzymes, among which SOD can be distinguished (Herrero et al., 2008; Gill and Tuteja, 2010).

The protein content (Fig. 1) for day 22 of the controls' growth and after NKA_g and NKCu application was 7.37 mg g⁻¹ FW and 7.61 mg g⁻¹ FW. Nanocolloid spraying of individual metals did not cause statistically significant differences in protein levels, except for NKCu application at 100 ml L⁻¹ (+7.3%) concentration on the 29nd day of plant growth.

The level of LPO (Fig. 2) in the control plants was 242.1 nmol g⁻¹ FW on the 22nd day of plant growth for NKA_g and 198.8 nmol g⁻¹ FW for NKCu.

Fig. 1. Content of soluble protein in pumpkin leaves. Mean values $\pm$ SD ($n=9$). a – significant difference in relation to corresponded control (Kruskal-Wallis test, $p<0.05$).

After silver application, statistically significant decrease in peroxidation was observed for each of the concentrations as compared to the control, and it amounted to -8.1%, -19.3% and -23.1%. When NKC was used on plants at day 22 of growth, for the first two concentrations of 50 ml L⁻¹ and 100 ml L⁻¹ a slight increase by +1.5% and +1.4% was observed, respectively. At the highest concentration of the applied formulation, LPO was reduced as compared to the control sample and was 192.8 ml L⁻¹ (-3%). On the 29th day of plant growth, the amount of LPO for individual nanocolloids in the control was 225.9 nmol g⁻¹ FW and 218.4 nmol g⁻¹ FW. After silver application, statistically significant decrease in peroxidation was observed for the concentration 100 ml L⁻¹ (-2%) and increase for the concentration 150 ml L⁻¹ (+5%). Following NKC administration, statistically significant increase in activity was observed using all concentrations, which were higher by +14.3%; +23.4% and +6.7%.

Superoxide dismutase (SOD) is an antioxidant enzyme that catalyzes the conversion of superoxide anion radical to H₂O₂ and molecular oxygen (Fridovich, 1972; Krötz et al., 2002). The SOD expression is a response to various stressors, including metallic impurities (Doyotte et al., 1997) permeating the plants during their spraying. The activity of SOD (Fig.3) in control samples was 24.84 U mg⁻¹ protein (NKA) and 19.18 U mg⁻¹ protein (NKC) for 22 days of plant growth. A slight decrease in activity at a concentration of 50 ml L⁻¹ was observed after spraying with silver nanocolloid, which was lower by -1.3% as compared to the control. For higher (100 and 150 ml L⁻¹) applications, a statistically significant increase in SOD activity was observed, which was higher than the control by +8.3% in both cases. Following NKC administration, statistically significant increase in activity was observed using 50 and 150 mL L⁻¹ concentrations, which were higher by +9.7% and +6.7%. For the 29th day of plant growth, the SOD values obtained in control samples for nanocolloids amounted to 27.85 U mg⁻¹ protein and 21.10 U mg⁻¹ protein.

Fig. 2. Lipid peroxidation in pumpkin leaves. Mean values $\pm$ SD (n=9). a – significant difference in relation to corresponded control (Kruskal-Wallis test, $p<0.05$).

Fig. 3. SOD activity in pumpkin leaves. Mean values $\pm$ SD (n=9). a – significant difference in relation to corresponded control (Kruskal-Wallis test, $p<0.05$).

CONCLUSIONS

The use of nanotechnology in sustainable agriculture, especially in, and the interaction between NPs and plants is an indispensable aspect of the risk assessment. Current study reveal that:

1. The silver nanocolloid application on the pumpkin leaves at day 22 and day 29 resulted in a slight decrease in protein synthesis in the plant, except for the concentration of 50 ml of L⁻¹ on day 22 of their growth. Despite the lower protein content, the plant growth has not been disturbed.
2. After NKCu application on day 22 of growth, a decrease in protein synthesis was observed. On 29 day of growth, the amount of protein increased and was slightly higher than in the control.

3. The effect of NKAg on lipid peroxidation (LPO) on day 22 of growth was negative and its reduction was greater along with increasing the applied concentration. The use of NKCu at day 22 of growth did not cause oxidative stress, but for day 29, there was a significant increase and its highest value was observed at a concentration of 100 ml L⁻¹ (+23.4%).
4. After application of NKAg and NKCu in most concentrations, it contributed to an increase in SOD activity in photosynthetic tissues. This indicates a higher ability of plants to neutralize the superoxide anion radical that cause oxidative stress.

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