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The impact of cerium oxide nanoparticles on the salt stress responses of *Brassica napus* L.[☆]

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A B S T R A C T

Dwindling high quality water resources and growing population are forcing growers to irrigate crops with water of high salinity. It is well recognized that salinity negatively affects plant physiology and biochemistry, and represents one of the most serious threats to crop production and food security. Meanwhile, engineered nanoparticles (ENPs) are increasingly detected in irrigation water and agricultural soils due to the rapid advancement of nanotechnology. Previous research has demonstrated that ENPs such as cerium oxide nanoparticles (CeO_2-NPs) exert significant impact on plant growth and production. However, almost all previous studies were conducted in well controlled environment. Knowledge on how ENPs affect plant development in a stressed condition is almost empty. The goal of the present study was to understand the physiological and biochemical changes in *Brassica napus* L. (canola) cv. 'Dwarf Essex' under synergistic salt stress and CeO_2-NPs effects. Two salinity levels: 0 (control) and 100 mM NaCl, and three CeO_2-NPs concentrations: 0 (control), 200 and 1000 mg kg^{-1} dry sand and clay mixture, were employed. As expected, 100 mM of NaCl significantly hindered plant growth and negatively affected the physiological processes of canola. Plants treated with CeO_2-NPs had higher plant biomass, exhibited higher efficiency of the photosynthetic apparatus and less stress in both fresh water and saline water irrigation conditions Overall, our results demonstrated that CeO_2-NPs led to changes in canola growth and physiology which improved the plant salt stress response but did not completely alleviate the salt stress of canola.

1. Introduction

Global food demands are expected to grow by 70–110% by 2050 (Tilman et al., 2011) while arable lands are decreasing due to land degradation, urbanization and seawater intrusion (Munns et al., 2012). Global climate change further aggravates the problem, especially in arid and semi-arid regions, by forcing farmers to use salty water for irrigation (Qadir et al., 2007; Rengasamy, 2010; Sanoubar et al., 2016). It is estimated that more than 6% of the world's total land and approximately 20% of irrigated land are affected by salinity (Munns and Tester, 2008), which is a serious concern in agriculture. Meanwhile, nanotechnology is rapidly advancing and its growth is forecasted to reach $3 trillion in final goods by 2020 (Roco, 2011). The rapid development of nanotechnology has resulted in significant increases in the applications and release of engineered nanoparticles (ENPs) into the environment (Ma et al., 2010, 2015; Schwabe et al., 2015). Most ENPs eventually find their way into agricultural soils through water irrigation or biosolids land applications. Because of their known impacts on plant health and development, the introduction of ENPs into agricultural systems may result in stronger effects on plants which are already suffering from various environmental restraints than those grown in well controlled environment (*e.g.*, green house). However, the synergistic effects of ENPs with different environmental stresses (*e.g.*, salt stress) on plant health have not been explored.

Cerium oxide nanoparticles (CeO_2-NPs) are widely used in the production of catalysts, sunscreen creams, microelectronics and polishing agents due to their unique catalytic and optic properties (EPA, 2009). They are also used as a diesel fuel additive to increase fuel combustion efficiency and decrease diesel soot emissions (Cassee et al., 2011). This last application entails great environmental occurrence and impact of these nanoparticles. Previous

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studies have demonstrated that CeO_2-NPs displayed strong effects on plant health both positively and negatively, depending upon the plant species, exposure concentration, exposure duration and plant growth conditions (Ma et al., 2010, 2016, 2015; Wang et al., 2012; Zhao et al., 2014). For example, the root elongation of lettuce was significantly inhibited after exposure to 2000 mg L^{-1} CeO_2-NPs suspension for five days, while the root elongation of tomato, radish, rape, wheat, cucumber, and cabbage were largely unaffected at the same exposure condition (Ma et al., 2010). Similar species-dependent responses of plants to CeO_2-NPs were also reported by other researchers (López-Moreno et al., 2010b). Recently, Pagano et al. (2016) investigated the molecular mechanisms for plant responses to CeO_2-NPs and reported that chloroplast in tomato and zucchini play important roles in plant responses to CeO_2-NPs. Overall, the study indicated low CeO_2-NPs toxicity both at the physiological and molecular level. A separate study showed that the toxicity of CeO_2-NPs is concentration dependent, with high concentrations of CeO_2-NPs (2000 and 4000 mg L^{-1}) causing DNA damage to the soybean seedlings, but such effect was absent at lower concentrations (López-Moreno et al., 2010a). In fact, CeO_2-NPs were shown to enhance plant growth under certain exposure conditions. Wang et al. (2012) found that irrigation with 10 mg L^{-1} of CeO_2-NPs slightly improved tomato growth and yield. While the underlying mechanisms for the different impacts of CeO_2-NPs at different concentrations are not fully understood, this may be partially attributed to the duel valance states of Ce (Ce^{3+} and Ce^{4+}) on the nanoparticle surface, which makes CeO_2-NPs behave as an antioxidant at certain conditions (Wang et al., 2012) and an oxidative stress inducer at other conditions (Ma et al., 2015, 2016).

Brassica napus L. (canola) is the third most important source of vegetable oil in the world, after soybean and palm oil. During the past 20 years, it has surpassed peanut, cottonseed, and most recently, sunflower, in worldwide production (Ash, 2012). This is mostly attributed to the pioneering plant breeding work initiated in the 1950s and 1960s in Canada which greatly reduced the levels of two anti-nutritional compounds, erucic acid in the oil and glucosinolates in the meal, creating a new, high-value oil and protein crop known as canola in Canada and the United States (Sovero, 1993). In addition, canola has shown good salt and heat resistance (Maas and Grattan, 1999). However, the levels of these stresses in arid and semi-arid environments frequently exceed the thresholds for optimal canola production.

Given the chances of ENPs accumulation in saline agricultural soils, some studies examining the synergistic effects of these two conditions on plants have emerged. For instance, Soliman et al. (2015) reported that *Moringa* plants sprayed with Hoagland's solutions containing ZnO-NPs and Fe_3O_4-NPs enhanced growth parameters under both normal and saline conditions compared with controls in each condition. The researchers also found that spraying plants with nanoparticles-containing Hoagland solutions resulted in a significant decrease of Na^+ and Cl^- and a significant increase of N, P, K, Mg, Mn, Fe, Zn, total chlorophyll, carotenoids, proline and carbohydrates in plant tissues. Considering the extent of CeO_2-NPs effects on plants and its unique redox chemistry on the surface, it is intriguing to investigate how these nanoparticles will affect plant responses to external abiotic stresses (*e.g.*, salt stress).

The main objectives of this study were (1) to investigate whether CeO_2-NPs at different concentrations (0, 200 and 1000 mg kg^{-1} dry sand and clay mixture) could affect the physiological and biochemical processes in canola and (2) to determine if the synergistic presence of CeO_2-NPs and NaCl (100 mM) could alleviate the plant oxidative stress induced by NaCl.

2. Materials and methods

2.1. CeO_2-NPs

The dispersion of CeO_2-NPs coated with Polyvinylpyrrolidone (PVP) was purchased from the US Research Nanomaterials, Inc. (Houston, TX). The Transmission Electron Microscopy (TEM) image of CeO_2-NPs used in this study is shown in Fig. 1. The CeO_2-NPs are roughly spherical, but polygonal nanoparticles with clear edges are also found. According to the TEM image, most CeO_2-NPs fell in the size range of 20–110 nm, with an average size of 55.6 nm. The size and size distribution of CeO_2-NPs shown in Fig. 1 was obtained by measuring 270 individual NPs with an image processing software ImageJ (ver. 1.49, National Institutes of Health, Bethesda, MD). The zeta potential of 200 mg L^{-1} CeO_2-NPs dispersed in water at $pH = 7$ was -51.8 mV, as measured by a dynamic light scattering instrument (Zetasizer Nano ZS90, Malvern Instruments Ltd., Worcestershire, UK). CeO_2-NPs concentrations (200 and 1000 CeO_2 mg kg^{-1} dry mixture) were chosen because the majority of the previous studies on the toxicity of CeO_2-NPs to terrestrial plants fell in the range of 1–1000 mg/$kg^{-1,}$ (Holden et al., 2014). Even though the chosen concentrations are substantially higher than most predicted concentrations of CeO_2-NPs in the environment, it should be cautioned that those predictive studies were often based on the assumption that the released CeO_2-NPs would be uniformly distributed in the studied media (*e.g.* water, or soil) (Gottschalk et al., 2013), while in reality, this assumption may not be true. For example, a study indicated that about 95% of CeO_2-NPs entering into a wastewater treatment plant was retained in the activated sludge (Limbach et al., 2008) and when these activated sludge were applied to agricultural soils as biosolids, the concentrations of CeO_2-NPs in agricultural soils could be significantly higher than the predicted concentrations of CeO_2-NPs in soil.

2.2. Plant species and growth conditions

Brassica napus (canola) cv. 'Dwarf Essex' seeds were purchased from Johnny's Selected Seeds (Winslow, ME). This cultivar was selected because of its germination characteristics and salt stress tolerance. The seeds were sterilized in 2.7% Clorox bleach for 10 min, and washed three times with DI water. They were then placed in 100 mm diameter × 15 mm depth polystyrene petri dishes with a thin layer of DI water above the supporting filter paper. Following germinations, young seedlings were individually transplanted into 9.5 cm diameter × 12 cm height plastic pot with closed bottom and 500 g of the mixture of sand (Quikrete, Atlanta, GA) and leca (Clay pebbles, Hydrofarm, Petaluma, CA) (1:1 v/v) on the third day after seed imbibition. Before plant transfer, known amount of CeO_2-NPs solution and 25% Hoagland solution (Phyto Technology Lab, Shawnee Mission, KS) were introduced to each pot to achieve the concentration of 200 or 1000 mg kg^{-1} dry mixture and full saturation (100% water holding capacity) in each pot. Ten plants for each treatment (0, 200 and 1000 mg/kg) were grown at room temperature under fluorescent bulbs providing 250 μmol m^2 s^{-1} photosynthetic photon flux density (I) (16 h light – 8 h dark photoperiod). Control plants were irrigated with 20 mL of DI water on the same day of CeO_2 feeding. After 10 days of plant transfer, five plants from each treatment were randomly selected to be treated with 100 mM NaCl once a week. All plants were fertirrigated with full strength Hoagland (Hoagland and Arnon, 1950) for 40 days. The NaCl concentration was chosen because 60–120 mM (Yousuf et al., 2016; Zaghdoud et al., 2016) was deemed as the average salinity in saline soil and brackish water in most previous studies. Starting from day 12 (one week after NaCl treatment), the chlorophyll fluorescence, net photosynthesis rate