Eurasian Journal of Molecular and Biochemical Sciences

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The effects CaO nanoparticles applications on *Onobrycis sativa* seedlings growth under mannitol stress

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Cite:Yazıcılar B, Koç Ü. The effects CaO nanoparticles applications on Onobrycis sativa seedlings growth under mannitol stress. Eurasian Mol Biochem Sci 2022;1(2): 6-10.

Received: 04 October 2022, Accepted: 28 October 2022 *DOI. 10.54672/ejmbs.2022.8*

Abstract

In our study, two *Onobrycis sativa* population (*Uzuntekne*, and *Barış)* were used as the material for the response to CaO-NPs nanoparticulate. More DNSA and proline were collected in these two Onobrycis sativa genotypes than in control seedlings while two genotypes were exposed to mannitol. Proline content highest at 150 mM mannitol and 1.5 ppm CaO while the minimum and maximum content was observed at 50–150 mM mannitol dosages. The collected of DNSA was greatly correlated with higher mannitol concentrations. Proline activities demonstrated an increasing trend against the increasing concentration of mannitol. In conclusion, the growth characteristics and physiological responses of Onobrycis sativa increased, depending on genotype, mannitol and CaO dosage in the media and their interactions.

Keywords: Nanoscience, CaO, proline, DNSA

Introduction

Nanoscience has influenced every field of science and technology, of which long-term agricultural sustainability is a significant part of these fields (1). Nanoparticles have unique agronomic traits, i.e., protect in response to plant disease and use water effectively, alleviate environmental hazards and impacts of environmental factors. It provides novel information and decreases the treatments of chemicals and increases nutrient utilization efficiency, which ensures environmentally friendly sustainable production (2,3). They can supply eliminate nutrient deficiencies in plants, and increase the resistance of plants to stress factors in this way. Several studies have been shown on the exogenous treatment of nanoparticles for plant regeneration and development, but its effect on seedling growth and regeneration *in vitro* is limited compared to exogenous treatments. CaO-NPs can use the sustainable crop

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production and agricultural industry with the support of many novel techniques in reversing oxidative stress symptoms caused by environmental stresses (4). Moreover, the impact of CaO-NPs on physiological, biochemical, and antioxidative activities in many plant species has also been not tested novelty. Tissue culture techniques are particularly beneficial in all areas of food science because these techniques can contribute to the agronomic improvement of plants by eliminating the difficulty in exogenous applications under uncontrolled conditions (5). The *Onobrycis sativa* is one of the most economic-growth forage legumes widely grown worldwide. It has been an important grain forage crop for livestock, environmental, nutraceutical attributes, and nutritional. *O. sativa* is cultivated for its honey production and is a valuable resource for pollinators (6,7). Farmers also benefit from its drought resistance in the field of drought and light-free draining soil, mainly due to its deep taproot. This study aimed to determine the seedlings regeneration, proline, and DNSA (3,5-Dinitrosalicylic acid) for *O. sativa* by testing different CaO concentrations *in vitro* medium.

Materials and Methods

Plant material

Plant material and CaO treatments: In our study, two *O. sativa* genotypes (*Uzuntekne*, and *Barış*) were used as the material for the response to CaO NPs nanoparticulate and mannitol. Seeds were surface sterilized with 22% NaOCl for 30 minutes and disinfected three times with sterile distilled water. Then, seeds were grown in plates including full MS medium (8) from two different CaO NPs concentrations containing 0.5, and 1.5 ppm CaO NPs nanoparticulate and 50 and 150mM mannitol.

DNSA: For DNSA determination, 10 mg of tissue per seedling was ground in a mortar, homogenized in 1 mL of 80% ethanol, and centrifuged at 5000 × g at 4 °C for 10 min. Supernatants were transferred into other tubes and the pellets were homogenized again in 0.5mL of 80% ethanol and centrifuged as above. The second supernatant was added to the first. Total DNSA were measured by a modified method by Watanabe et al. (9). One milliliter of the extract was reacted with 3 mL of freshly prepared anthrone reagent (50 mg anthrone + 50 mL of 95 % H_2SO_4) at 100 °C for 10 min. After cooling on ice, the total DNSA was determined at 620 nm with a spectrophotometer using glucose as a standard.

Proline estimation: The proline amount was determined with the producer of Bates et al. (10). Seedling samples (100 mg) were powdered in 5 mL of 3% aqueous sulfosalicylic acid and centrifuged at 4 °C for 15 min at 4800 × g. Extract (2 mL) was mixed with 2 mL of acid-ninhydrin and 2 mL of glacial acetic acid in test tubes. Samples were boiled for 1 h at 100 °C. The reaction was terminated in an ice bath and 4 mL of toluene was used for the reaction of the mixture extraction. The absorbance of the color reaction product was measured at 520 nm using toluene as a blank. The proline concentration was determined from a calibration curve.

Statistical Analysis: Each experiment was replicated three times. Analysis of variance was carried out using a two-way ANOVA test using SPSS 13.0 and means were compared by the Duncan test at the $P<0.05$ confidence degree.

Results

Proline content: CaO treatments caused different effects on the proline content. There was a detectable difference among genotypes and concentrations. Proline amount displayed high variation between tested samples for CaO and mannitol applications, ranging from 0,130 to 1.195 mg/g FW. The proline amount in 150 mM Mannitol/1.5 ppm CaO of the *Barış* genotype was higher than that of the other

concentrations under CaO and mannitol treatments, which peaked at 1.195 mg/g FW. The highest content was found (1.153 mg/g FW) from seedlings treated with 150 mM Mannitol/0.5 ppm CaO in the *Uzuntekne* genotype (Figure 1). There was also a detectable difference in proline content between mannitol and CaO treatments.

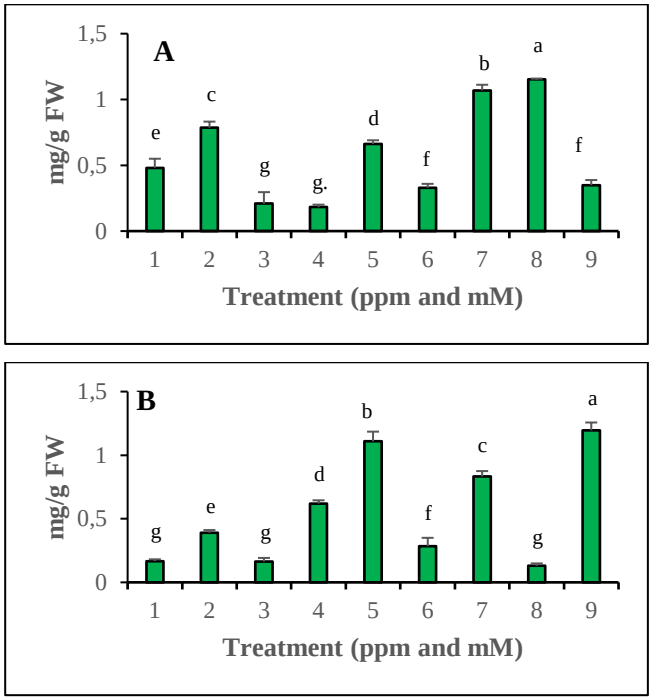

Figure 1. Changes in proline contents of *O. sativa* genotypes treated with the mannitol stress and CaO-NPs. A: *Uzuntekne*, B: *Barış*. Lower-case letters for the study indicate statistically significant differences between the groups at *P<0.05*. Bars mean SE. (1: Control, 2:0.5 ppm CaO, 3: 1.5 ppm CaO, 4:50 mM mannitol, 5: 150 mM mannitol, 6: 50 mM mannitol/0.5 ppm CaO, 7: 50 mM mannitol/1.5 ppm CaO, 8: 150 mM mannitol/0.5 ppm CaO, 9: 150 mM mannitol/1.5 ppm CaO).

DNSA: Figure 2 displays that DNSA were highly affected in the seedlings stage of two *O. sativa* genotypes in presence of 0.5 ppm, 1.5 ppm CaO-NPs and 50 mM mannitol, 150 mM mannitol treatments. DNSA revealed an extent range of variation between tested samples for CaO-NPs ve mannitol applications, ranging from 0.024 to 1.498 mg/g FW of the *Uzuntekne* genotype. The highest content was obtained from seedlings treated with control in the *Barış* genotype. The *Uzuntekne* genotype in 50 mM mannitol/1.5 ppm (1.498 mg/g FW) indicated the best result in standard CaO-NPs and mannitol for DNSA compared to the other concentrations. Although the maximum DNSA content was found in the treatments of control and 50 mM mannitol/1.5 ppm CaO in seedlings, the lowest DNSA content was found in seedlings for 0.5 ppm CaO-NPs (Figure 2).

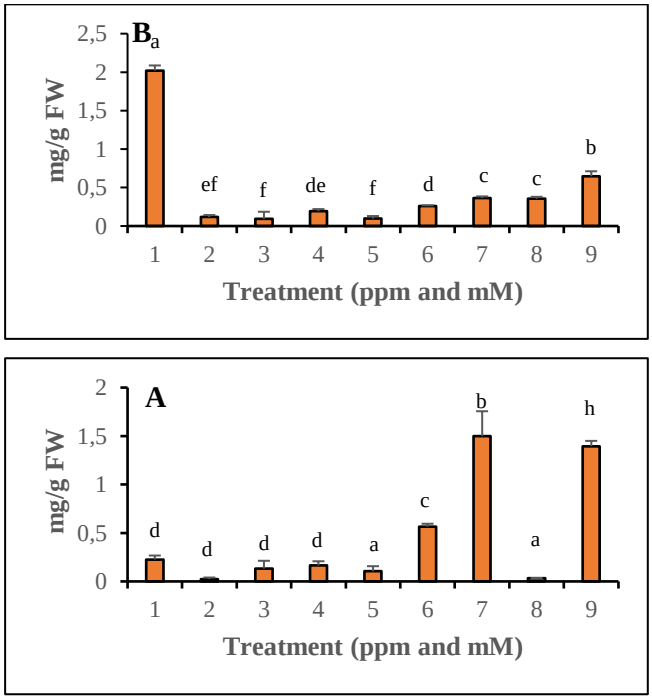

Figure 2. Changes in soluble sugar content of O. sativa genotypes treated with the mannitol stress and CaO-NPs. A: *Uzuntekne*, B: *Barış*. Lower-case letters for the study indicate statistically significant differences between the groups at P<0.05. Bars mean SE. (1: Control, 2:0.5 ppm CaO, 3: 1.5 ppm CaO, 4:50 mM mannitol, 5: 150 mM mannitol, 6: 50 mM mannitol/0.5 ppm CaO, 7: 50 mM mannitol/1.5 ppm CaO, 8: 150 mM mannitol/0.5 ppm CaO, 9: 150 mM mannitol/1.5 ppm CaO).

Discussion

Ca are a major essential nutrient for growth and development in plants. It induces enzymes, plant vegetative biomass, and photosynthesis ratio, and increases biochemical reaction. CaO-NPs (Ca^{+2}) is important elements and several biochemical and molecular changes during the plant cycle (11,12). In this study, applications of CaO highly affected the seedlings regeneration, development, proline, and DNSA. CaO at

two doses was tested in vitro on seedlings tissues in the MS media in the combination with 4 mg L-1 2,4-D (2,4-dichlorophenoxyacetic acid) and 0.125 mg kinetin including 0.5, 1.5 ppm CaO NPs nanoparticulate. The observed improvement in plant growth traits in vitro culture due to the supplementation of CaO-NPs is matched with previous studies (4,13). In seedlings samples, proline was greatly increased under drought stress applications and the impacts of CaO-NPs on proline content are well linked to the mannitol-resistance ability. High-concentration CaO and mannitol-treated *O. sativa* seedlings showed a significant decrease in stress (Figure 1). Proline content has been exhibited to have defensive impacts in response to mannitol stress (14). These results also indicated a raised collection of proline amount in mannitol-tolerant seedlings as compared to control seedling samples of two samples. Similarly, Nayyar et al. (15) found that varied mannitol treatments increased proline levels in wheat and maize. Soheilikhah et al. (16) obtained similar results for *Carthamus tinctorius L.* varieties callus cultures under salt and mannitol stress. In terms of DNSA, CaO-NPs at the highest concentration increased the content of these molecules (Figure 2). Both proline and DNSA content maintain a certain point of metabolic equilibrium in the plant cells, and when the plant is subject to external factors, this equilibrium will be unstable. DNSA at low concentrations of CaO, increased significantly in an exposure time-dependent manner. On the other hand; High-concentration CaO treatment can significantly increase the content of DNSA, which could be due to the injury of metabolic equilibrium, thus blocking enzyme activity. Similar results were observed in DNSA amounts in the present study. Results are matched with those published by Signh and Kumar (17) in the study on mannitol for *Eucalyptus tereticornis*. Their results demonstrate that as the concentration of nanoparticles, DNSA amount decreased. In conclusion, the growth characteristics and physiological responses of *O. sativa* increased, depending on genotype, mannitol, and CaO dosage in the media and their interactions. Proline and DNSA in *Barış* seedlings grown under osmotic stress were exhibited to be greater than those in *Uzuntekne* seedlings. These parameters may be employed as criteria for assessing drought resistance.

Declaration of Interest: The author declares that there is no conflict of interest regarding the publication of this paper.

Authors' Contributions: BY and ÜGK contributed to the study conception, design and laboratory work. Writing the article (BY and ÜGK). All authors read and approved the final manuscript. BY; Büşra Yazıcılar, ÜGK; Ümmü Gülsüm Koç.

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