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Research article

The study of Nano silica effects on the total protein content and the activities of Catalase, Peroxidase and Superoxid Dismutase of *Vicia faba* L.

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Abstract: Silica is the second common element of soil content which has positive effects on the resistance of plants against biotic and abiotic stresses. This element can increase the yield, decrease the evaporation and perspiration and moreover, causes increasing of production of antioxidant enzymes, and less sensitivity to some fungal diseases. In the present study, the effects of silica on the total protein content and activity of some antioxidant enzymes in *Vicia faba* L. were studied. The seeds of plant were treated by 0 (as control), 1.5 and 3 mM of Nano silica. There were three repeats for all treatments. The result showed that 1.5 mM treatment did not significantly increase the total protein content in comparison to control samples. The activity of Peroxidase in the 1.5 and 3 mM treatments of Nano silica was significantly increased. In 3 mM treatments of Nano silica also increased the activity of Superoxide Dismutase and Peroxidase significantly. Based on the results, it can be concluded that Nano silica particles can increase the activity of some antioxidant enzymes in broad bean, which in turn, brings about less damages caused by reactive oxygen species, and protects the plant's physiological processes against stresses.
Keywords: Antioxidant enzymes - Total protein - Nano silica - *Vicia faba* L.

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INTRODUCTION

Vicia faba L. is one of the Fabaceae. This plant is annual grass, with 80–110 cm height. The flowers of broad bean are white with black or purple spots. The seeds are sheathed and the fruits, seeds and flowers have medical usages. *Vicia faba* L. is hetero fertilized with 2n=12. Because of possessing of high percentage of proteins (30–34%), this plant is alimentary- worth. Environmental stress causes reduction of balance between reactive oxygen species and antioxidant defence of plants (Bai & Sui, 2006). Superoxide Dismutase as one of the metalloproteins, can catalyse $2O_2^{0-} \rightarrow o_2 + O_2^{0-}$ (Kakkar & Sawhney 2002). SOD indeed, produces H_2O2. (Elkahoui *et al*. 2005). Catalase (CAT) is one of the H_2O_2 scavenger which can catalyse the reaction of $2H_2O_2 \rightarrow 2H_2O+O_2$ as a metalloprotein (Garnczarska 2005). After an smooth increasing of catalase activity which is associated with shortage of water in root and leaves, this activity in leaves would be stable at constant level, and is reduced in heavy shortage of water in root, that may bring about inactivation of catalase (Feierabend *et al*. 1992). After oxygen, silica is the second structural element in the earth which is non-mobile in the plants. Although silica is not necessary for plants, most of the higher plants need it to have optimum growth (Richmond & Sussman 2003, Ma *et al*. 2004, Currie & Perry 2007). The most effect of silica on plants is related to the resistance against biotic and abiotic stress (Ma & Yamaji 2006, Liang *et al*. 2007). As the cell wall of plants prevents the entrance of elements into cells, the Nano particles which have less diameter than the pores of cell wall, therefore can easily cross the pores. Nano particles in the leave's surface enter the plants through the stomata and or base of hairs, and then transported to the different organs. Silica plays important role in the

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tolerance against salt stress (Zhu *et al*. 2003), manganese toxicity (Shi *et al*. 2005), boron toxicity (Gunes *et al*. 2007) and cadmium toxicity (Vaculik *et al*. 2009, Shi *et al*. 2010) via changing the activity of antioxidant enzymes. In the present study, silica was used as Nano particles with 14 nm diameter (1.5 and 3 mM concentrations) to assay the effects of Nano particles of silica on some antioxidant enzymes such as catalase, peroxidase and superoxide dismutase changes, and the yield of broad bean plant.

MATERIALS AND METHODS

In order to assess the effects of silica nanoparticles, on antioxidant activity of broad bean (*Vicia faba*), the samples were grown in greenhouse. Before cultivation, the impact seeds were sterilized in 5% hypochlorite sodium solution. The seed then were washed up by deionised water. In each pot 2 seeds were cultivated. Solution containing 0 (as control), 1.5 and 3 mM of nanoparticle of silica, were used for treating. The temperature of greenhouse was adjusted to 22±2 °C (at night) and 25±2 °C (at day). The relative humidity was 44 %. The samples were treated for 65 days and the fresh leaves of them kept in liquid nitrogen for enzyme assay.

Total protein

The Bradford (1976) method was used for total protein assay. 1 mL of Bradford solution was mixed with 100 μL of enzyme extract, and then the absorption was recorded in 595 nM wave length. The protein concentration was expressed as mg ml^{-1}

Catalase activity

The activity of catalase was measured by Aebi (1984) method. CAT activity was determined as the rate of disappearance of H_2O_2 at 240 nm, for 1 minute. Reaction mixture (3 ml) included 50 mM potassium phosphate buffer (pH 7), and the activity was expressed as μmol min^{-1} mg^{-1} protein.

Peroxidase activity

Koroi (1998) method was used to assay the activity of peroxidase. The mixture of 2 mL acetate buffer (pH 4.8), 0.2 mL hydrogen peroxide 3% was used. The change in absorbance was determined at 590 nm (FW OD min^{-1} g^{-1}).

Superoxide dismutase activity

The activity of superoxide dismutase was assayed by Giannopolitis & Ries (1977). Reaction mixture containing 50 mM potassium phosphate buffer (pH 7.8), 1.3 μM riboflavin, 0.1 mM EDTA. 13 mM methionine, 63 μM NBT, 0.05 M sodium carbonate (pH 10.2) and enzyme extract was used. The photo-reduction of NBT was measured at 560 nm.

Statistic analyze

SPSS ver16. Was used for comparing of the means using duncan test at P<0/05, level of significance. The diagrams were plotted using Excel software.

RESULTS

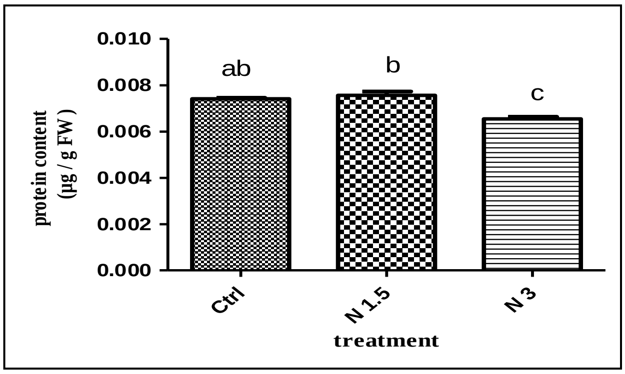

Figure 1. The effect of nano silica particles on total protein content of broad bean leaves. (Means ± SE and P < 0.05. The letters show significance of differences)

Total protein

The result showed that the protein content in 1.5 mM treatment of nano silica has no significant different to control sample. But this content in 3 mM treatment of nano silica was reduced in range of 9% compared to control. This rage was about 7% in comparison with 1.5 mM of nano silica treatment (Fig. 1).

Peroxidase activity

The result showed that in the leaves of broad bean, 1.5 and 3 mM treatments of nano silica, significantly increased the activity of peroxidase in range of 25 and 27 % compared to control samples respectively (Fig. 2A).

Catalase activity

The assessment of catalase activity indicated that in 1.5 mM treatment of nano silica the activity of this enzyme in leaves was significantly decreased in a range of 29% compared to control samples. However, the increasing of catalase activity was not significant in 3 mM treatment (Fig. 2B).

Superoxide dismutase activity

The result showed that the activity of superoxide dismutase in leaves of broad bean plant, has highest level in 3 mM treatment in comparison to control (71 % higher). There was no significant difference between control sample and 1.5 mM silica treatment. However this difference was significant between 1.5 and 3 nm silica treatments (Fig. 2C).

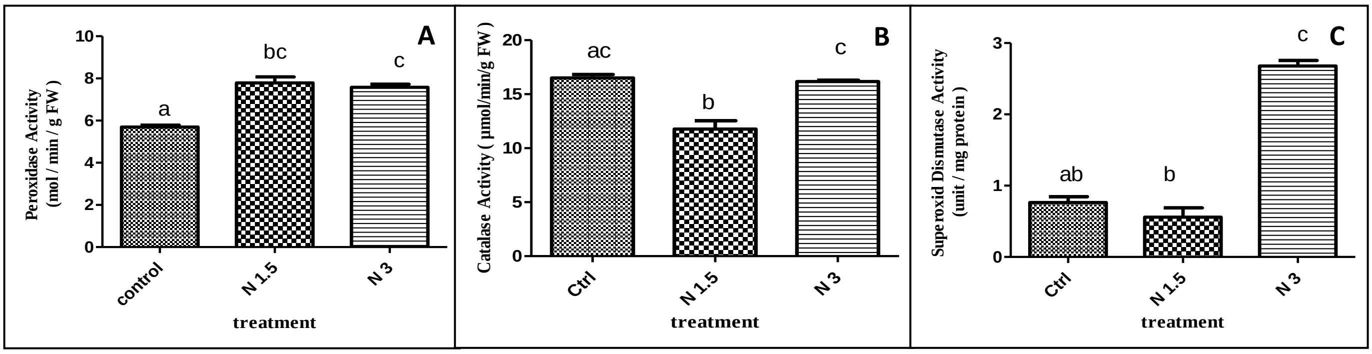

Figure 2. The effect of nano silica particles on total protein content of broad bean leaves: **A**, Peroxidase; **B**, Catalase; **C**, Superoxide dismutase. (Means ± SE and $P < 0.05$. The letters show significance of differences)

DISCUSSION

The total protein content if 1.5 mM treatment of nano silica showed no significant increasing compared to control sample. When plant's cell is under stress signalling pathway in corporation with calcium send signals to nucleus of cell. Due to this signalling, genes expression undergoes changes and because of increasing or decreasing of some genes, plant can resist against stress. The result of this change in the genetics, changes in the amount and type of special proteins (Amini *et al*. 2007).

Watanabe *et al*. (2001) showed that treatment of selenium can cause increasing of amino acid content, especially Asp in rice. The assessment of changes pattern of total protein content shows that under silica stress some new proteins can be generated, or the amount of some others can be increased or decreased. Treatment of rice plant with silica brought about activity of catalase and Glycine betaine (Biglari *et al*. 2012).

Silica and nanoparticles of that, can act as a stressgen factor in leaves and as a result, the activity of antioxidant enzymes would be increased. These enzymes protect plants against toxicity and damages of reactive oxygen (Van Breusegem *et al*. 1999). Catalase and ascorbate peroxidase can scavenge H_2O_2 in plant and therefore, the increasing of superoxide dismutase is also predictable. The activity of ascorbate peroxidase was increased in nano silica treatment. Miao *et al*. (2010) indicated that silica can compensate the effect of potassium shortage in soy bean. Kiani *et al*. (2012) reported that treatment of rice with nano silica increased the activity of catalase and ascorbate peroxidase.

CONCLUSION

The result of present study conclude that silica prevent oxidant damages via increasing of antioxidant enzymes activity and decreasing of free radicals. Due to the lack of information about the main mechanism of silica effects is yet unknown, more studies are needed to assay the uptake and transportation of nanoparticles in plants.

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