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The Impact of Selenium Nanoparticles and Endophytic Fungus of *Piriformospora indica* in Drought Stress Tolerance in Two Barley Genotypes

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Introduction

Among the environmental stresses, drought is a major abiotic stress limiting agricultural crop production and is the most important stresses worldwide. Complete understanding of physio-biochemical responses of plants to drought is vital for improving plant tolerance mechanisms to drought stress. Barley is one of the most important crops in arid and semiarid regions. Nevertheless, drought is an important abiotic stress for this cereal, which is often grown in environments where drought is common. Furthermore, barley is also a model experimental system because of its short life cycle and morphological, physiological, and genetic characteristics. In order to investigate the effects of selenium nanoparticles and the mycorrhizal fungus *Piriformospora indica* on drought tolerance in two selected barley genotypes (Yousef as the tolerant genotype and Morocco as the sensitive genotype) during the vegetative stage, a greenhouse experiment was conducted in a factorial design within completely randomized blocks with three replicates per sample. The emphasis was on evaluating several growth-related traits and antioxidant indices. Experimental treatments were designed with three irrigation levels (70 %, 30 %, and 10 % of field capacity), three levels of selenium nanoparticles (0, 3, and 6 mg/L), and two levels of fungal treatment (inoculated and non-inoculated). Growth-related traits including dry weight, leaf area, leaf relative water content, relative growth rate in the greenhouse, and antioxidant indices including catalase, peroxidase, superoxide dismutase, ascorbate peroxidase, and glutathione S-transferase activities, as well as hydrogen peroxide and malondialdehyde contents were measured and analyzed after harvesting fully matured leaves. Drought stress led to a reduction in growth-related traits in both genotypes, especially in the sensitive genotype, and a significant increase in hydrogen peroxide and malondialdehyde contents. The application of

selenium nanoparticles, particularly at 6 mg/L, and the inoculation of *Piriformospora indica* resulted in improved growth and antioxidant traits in the studied genotypes under drought stress.

Material and Methods

In order to investigate the effects of drought stress, selenium nanoparticles and the effect of *Piriformospora indica* fungus on selected genotypes of barley, research was carried out in a research greenhouse. The isolate of *Piriformospora indica* was grown in a special liquid culture medium and chlamydospores were collected after 20 days. The experimental treatments included two genotypes of barley named Yousef (resistant) and Morocco (sensitive) as the first factor, three levels of water stress 10 %, 30 % and 70 % of the field capacity of the soil as the second factor, Selenium nanoparticle (3 and 6 mg/L, 3 times at 10-day intervals) as the third factor and finally, *Piriformospora indica* mushroom (no inoculation and inoculation) as the fourth factor. Finally, chlamydospores and spores were mixed with sterile sand and used as seed inoculation medium. Drought stress was applied at the three-leaf stage by stopping irrigation and measuring the water holding capacity of the soil by weighing the pots. After measuring the growth indices, fully opened and healthy leaves were harvested and then quickly transferred in liquid nitrogen to a freezer at -80°C for biochemical analysis. Statistical analysis of the data was done in 3 replications using SPSS software and checking the significance of the differences at $P \geq 0.01$ and $P \geq 0.05$ levels.

Result and Discussion

According to analysis of variances, the treatments had significant effects on biochemical properties of two genotypes. Biochemical values in the Yousef genotype were more than the Morocco genotype except H2O2 and malondialdehyde contents, so, Yousef genotype showed the increase in term of glutathione S-transferases activity (15 %), total protein content (28 %), ascorbate peroxidase activity (11 %), peroxidase activity (50 %), catalase activity (14 %), shoot/root ratio (7 %), root weight (14 %), relative growth rate (12 %), relative water content (3 %) and leaf area (22 %) by compare to Morocco. The Morocco showed the highest mean of H_2O_2 and malondialdehyde with 62.3 and 5.78 (Nmol/gr FW) values, respectively, in compare to Yousef genotype. The result revaluated that inoculation of *Piriformospora indica* and application of selenium nanoparticles can be effective on the increase of glutathione S-transferases activity (1.73 fold), total protein content (1.56 fold), superoxide dismutase activity (2 fold), ascorbate peroxidase activity (1.54 fold), peroxidase activity (2.33 fold), root weight (1.15 fold), relative growth rate (5 fold), relative water content (4 fold) and leaf area (8 fold) by compare to control. One of the detrimental side effects of drought is oxidative stress, leading to the production of reactive oxygen species, including superoxide anions and hydroxyl radicals. This results in cellular membrane peroxidation and various cellular damages. Drought stress increases the content of reactive oxygen species, leading to an elevation in malondialdehyde level as a byproduct of lipid peroxidation in membrane lipids.

The degradation of cell membranes under abiotic stresses and the production of malondialdehyde, resulting from the breakdown of cellular membrane lipids, serve as a suitable criterion for examining plant response to stress. This condition was significantly more pronounced in drought-sensitive barley genotypes, especially under severe drought stress. The results of present research indicated that each of selenium nanoparticles and *Piriformospora indica*, through their specific mechanisms, inhibit the production of reactive oxygen species. Superoxide dismutase catalyzes the dismutation of superoxide radicals into molecular oxygen or hydrogen peroxide, while other studied antioxidant enzymes such as catalase, peroxidase, and ascorbate peroxidase, by decomposing hydrogen peroxide produced by superoxide dismutase, contribute to the breakdown of reactive oxygen species. Furthermore, the results demonstrated that selenium nanoparticles and *Piriformospora indica* lead to a significant increase in the activity of these antioxidant enzymes, accompanied by a notable reduction in the levels of free oxygen species. Consequently, it appears that these treatments effectively decrease malondialdehyde levels, as a product of membrane lipid peroxidation.

Conclusions

At this article, we evaluated the physiological responses of two barley cultivar to selenium nanoparticle application and *Piriformospora indica* inoculation under drought stress. The results revaluated that selenium nanoparticles and *Piriformospora indica* by increasing the activity of antioxidant enzymes and reducing reactive oxygen species, reduced the destructive effects of drought stress and increases the growth of two barley cultivars.

Keywords: Antioxidant, Drought stress, Barley, Growth indices.

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Declaration of conflict of interest

The authors claim that there is no conflict of interest.