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Research Article

Effect of seed priming with selenium nanoparticles and plant growth promoting rhizobacteria on improving Quinoa seedling growth under salinity stress

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Abstract

Soil and water salinity are the most important problems that limit crop production, especially in the arid and semi-arid regions. This study was conducted to investigate the effect of growth-promoting bacteria and selenium nanoparticle pretreatment on increasing the tolerance of the *Chenopodium quinoa* plant to salinity. A factorial experiment was conducted in a completely randomized design with three replications. Experimental treatments consisted of selenium nanoparticles at a concentration of 1 mg L^{-1}, *Bacillus cereus* (BW) and *Pseudomonas fluorescens* (Ps) and three different levels of saline solution with concentrations of 0, 200 and 400 mM sodium chloride. The results showed that quinoa seeds soaking in selenium nanoparticle solution and PGPRs by increasing photosynthetic pigments (up to about 96% in Se+BW pretreated plants), compatible solutes (approximately 65% increase in prolin content in Se+Bw treatment), reducing oxidative stress (increased the activity of some antioxidant enzyme in about 100%), protecting the cell membrane (decreased the MDA up to 32%) and reducing sodium uptake (in about 23% in Se+Ps pretreated plant) improve plant growth and increase the quinoa resistance at salinity conditions. The results of this study showed that Se, although not essential element for plants, can improve growth and morphological parameters of the plant under salinity stress at low concentrations and it has more effects in the presence of PGPRs. So, it is suggested that these two biostimulators can be used together for protecting plants from salinity damaged and this strategy could be applied for sustainable agriculture. In addition, since most of the positive functions of these bacteria were not affected by salinity stress, so this type of bacteria can be used with selenium in saline soils.

Keywords: PGPR, Se, Oxidative stress, *Chenopodium quinoa*

Introduction

Plants face different biotic and abiotic stresses in adverse natural conditions. Among these, salinity is one of the most important constraints to crop productivity over large areas of land around the world (Mandhania *et al*., 2006; Zulfiqar and Ashraf, 2021). Salinity is a major environmental stress that severely limits factors affecting plant growth and production in the arid and semi-arid regions. Exposure of plants to salinity causes alterations in a wide range of physiological, biochemical, and molecular processes in plants. Also, excess salt causes an imbalance of ions, ion toxicity-induced metabolism, production of reactive oxygen species (ROS) as well as hyperosmotic stress which induced water deficiency (Parida, 2005; Orcutt, 2000; Seleiman *et al*., 2020). Plants have antioxidative defense system including enzymatic and non-enzymatic antioxidants aiding in ROS detoxification (Hasanuzzaman *et al*., 2020b).

Naturally, plants interact with different microbes that exist in soil (Dobbelaere, 2003). Kloepper (1978) suggest the term "PGPR" (Plant Growth-Promoting Rhizobacteria) for the main group of rhizobacteria that have good effects on plant growth when colonizing roots. Recent studies indicated these microorganisms are beneficial for plants and develop plant tolerance against the abiotic stresses through the direct and indirect mechanisms (Olanrewaju *et al*., 2017). PGPR increase plant growth due to numerous factors, among which the release of phytohormones, promoting mycorrhizal function, nitrogen fixation, regulation of ethylene production in roots, siderophore production, solubilizing nutrients such as phosphate, and decreasing heavy metal toxicity are the most important. Plants benefit due to the PGPR addition; including increase in germination rate, shoot and root growth, yield including grain, leaf area, chlorophyll content, minerals absorption, protein content, hydraulic activity, tolerance to biotic as well as abiotic stress (Figueiredo *et al*., 2010).

Selenium (Se) is a metalloid element that belongs to group 16 (Oxygen Family) of the Periodic table that was identified by Jons Jacob Berzelius in 1817. The ionic radius of Se and sulfur are close together, hence the chemical and physical properties are similar. Selenium is an essential microelement for humans and animals, but leads to toxicity when taken in excessive contents. In animals, Se acts as an antioxidant and helps in

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reproduction, immune responses, and thyroid hormone metabolism (Bodnar *et al*., 2012). Although the importance of Se in plants has not been entirely studied, many type of researches have reported its useful effects on plants. The results of Se treatment on the plant are dependent on the overall growth and development of the plant (Hasanuzzaman and Fujita, 2011). Se is effective in maintaining cellular structures and their function, also improves photosynthesis activity and plant growth and yields. Several studies have shown that Se has a positive impact on reducing damages caused by abiotic stresses. Many researches have proved the protective role of Se against cell damage caused by free radicals (Gupta and Gupta, 2017).

Since today, PGPR has been used worldwide as a bioinoculant to promote plant production under unfavorable conditions, especially salinity (El-Ghany, 2015), the objective of this paper is to study the effects of two strains of isolated plant growth-promoting rhizobacteria (PGPR) alone and in combination with Se on accumulation of osmoprotectants, phenolic and mineral content, as well as enzymatic antioxidant activity in Quinoa leaves under salinity conditions. On the other hand, the aim of this study was to investigate whether useful metals such as selenium have a better performance on physiological and biochemical parameters in the presence of bacteria. The results of this research can provide a perspective of synergistic effects of beneficial elements and PGPR bacteria for increasing salt resistance in crops.

Materials and methods

Quinoa culture conditions and inoculums preparation: The bacterial (*Pseudomonoas fluorescens* (PS) and *Bacillus cereus* (BW)) inoculum was produced by transferring one loop full of each strain to 100 ml of Nutrient Broth liquid medium in a 250 ml Erlenmeyer flask incubated at 28°C on a rotary shaker for 24 h. Certified seeds of quinoa (*Chenopodium quinoa*) were achieved from the Research and Technology Institute of Plant Production (RTIPP), Shahid Bahonar University of Kerman. Seeds of quinoa were surface sterilized with 70% ethanol for five min, followed by washing with sterile distilled water. The sterilized seeds were soaked in distilled water for 5 hours in case of un-inoculated control. The rest of the sterilized seeds were soaked in Nutrient Broth liquid medium of PS ،BW and Se nanoparticles for 5 hours in case of inoculation (this time selected based on preliminary experiment). Seeds of quinoa (10 seeds per pot) were sown in plastic pots. The soil contained 100% sand; it had pH (1:2) (soil: water, v: v) 7.2 and electrical conductivity (1:2) (soil: water, v: v) 0.43 ds m^{-1} (Hanlon, 2015). For the preparation of sterile soil, field soil was autoclaved for 20 min at 120° C.

After two weeks of growth, uniformly developed seedlings were selected for salinity treatments. Irrigation was in three levels of 0 (control), 200, and 400 mM of sodium chloride. The pots were irrigated with 100 ml of each solution. The salinity treatment was performed ten times every other day. One day after the last salinity treatment, the leaves (the second row of leaves after the cotyledon leaves) were collected, and frozen in liquid nitrogen.

Photosynthetic pigments content: Chlorophylls and carotenoids were extracted by 80% acetone and assessed according to Lichtenthaler (1987).

Chl a (µg/ml) = 12.25 A663.2 – 2.79 A646.8
Chl b (µg/ml) = 21.21 A646.8 – 5.1 A663.2
Chl t (Chl a + Chl b) = 7.15 A663.2 + 18.71 A646.8
Car (µg/ml) = (1000A470 - 1.8 Chl a - 85.02 Chl b) /198

Lipid peroxidation content: Malondialdehyde and other aldehydes content was estimated by using Heath and Packer (1968) and Meir *et al*. (1992) method.

Enzyme's extraction and assays: 300 mg FW of leaf were homogenized in an ice-cold mortar using 3 mL of 50 mM potassium phosphate buffer. After centrifugation at 17000×g for 20 min, the supernatant was used for the determination of enzymes' activities and protein content. Protein content was determined according to the method of Bradford (1976) using Bovine serum albumin as standard.

GPX (EC 1.11.1.7): activity was measured in a reaction mixture containing 50 mM potassium phosphate (pH 7.0), 0.3% (v/v) H_2O_2, 1% (v/v) guaiacol and the enzyme extract by a method described by Plewa *et al*. (1991). One unit (U) of GPX activity was defined as the amount of enzyme that produced 1 µmol of tetraguaiacol per minute. The enzyme activity was expressed in U per mg protein.

CAT (EC 1.11.1.6): activity was determined according to the method of Dhindsa *et al*. (1981). The assay mixture consisted of a 50 mM potassium phosphate buffer (pH 7.0), 15 mM H_2O_2 and 100 µL of the enzyme extract. The decline in absorbance at 240 nm was recorded (Ɛ=40 $mM^{-1}cm^{-1}$). The enzyme activity was expressed in U per milligram protein (1 µM of H_2O_2 reduction min^{-1} mg^{-1} protein).

APX (EC 1.11.1.11) activity was measured according to Nakano and Asada (1981). The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, 0.1 mM H_2O_2 and 150 µL of enzyme extract. The absorbance was read as a decrease at 290 nm against the blank (Ɛ=2.8 $mM^{-1}cm^{-1}$). The enzyme activity was expressed in U per milligram protein.

Proline content: Proline was determined following Bates *et al*. (1975).

Total carbohydrate content: Leaves extract was taken in 25 ml test tubes and 6 ml anthrone reagent (150 mg of anthrone in 72 % H_2SO_4) was added, and then, heated in boiling water bath for 10 min. The test tubes were ice-cooled for 10 min and incubated for 20 min at 25º C. Absorbance was read at 625 nm (Fales, 1951).

Ion's content: leaves oven-dried were powdered for estimation of ions. One gram of dried sample was digested by using 10 ml of nitric acid. Ion's

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concentrations were determined using the Flame Photometric Technique (PerkinElmer Optima 7000 DV, USA).

Statistical analysis: All experiments, including plant treatment, growth and the assessment of biochemical parameters, were performed by a factorial arrangement, based on a completely randomized design (CRD). All experiments comprised three replications (pots) per treatment. Data were subjected to analysis of variance. Analysis of variance was performed using the ANOVA procedure. Statistical analyses were performed according to the SPSS 23 software (IBM, Armonk, NY, USA). Significant differences between means were determined by Duncan's multiple range tests. *P* values less than 0.05 ($P < 0.05$) were considered statistically significant. The results were shown as mean ± SEM (standard error of the mean).

Results

In the present study, we observed that 200 mM NaCl had no significant effect on chlorophyll a, b and total chlorophylls however 400 mM NaCl decreased the chlorophyll a, b and total chlorophyll when compared with the control plants (Table 1). Se pretreatment increased the total chlorophyll in plants which were under 200 and 400 mM NaCl (35% and 49% respectively). When selenium+bacteria was used as a pretreatment, its effect on increasing the amount of total chlorophyll in 200 and 400 mM salinity was more than selenium pretreatment alone. Pretreatment of Se + Bw and Se + Ps increased the total chlorophyll content (at the rate of 74 % and 54% respectively) in plants that were under 200 mM NaCl in comparison with the non-pretreatment plants. In 400 mM salinity treated plants, Se+BW and Se+Ps pretreatment also increased the total chlorophyll content (96% and 64% respectively) when compared with the non-pretreated plants. Se pretreatment increased the carotenoid content of plants in 200 and 400 mM NaCl at the rate of 27% and 24% respectively however, when selenium was used with bacteria especially BW, it was more effective than selenium alone. Se+BW pretreatment increased the carotenoid content of plants under 200 and 400 mM NaCl approximately 90% and 62% respectively.

In the present research, our results showed that the MDA content increased significantly in plants that were under salinity stress (400 mM) and PGPRs and Se nanoparticles pretreatment of seeds caused a decrease in MDA and other aldehydes contents in both control and saline conditions. Data showed that Se+BW was the most effective treatment (decreased the MDA approximately 32% in 400 mM NaCl treatment when compared with the non-pretreated plants) (Table 2).

In this study, the proline content in quinoa plants was significantly increased under 400 mM salinity (27%) and seed priming by Se+BW, Se+PS and Se nanoparticles alone increased proline content (65%, 42% and 9% respectively) in quinoa plants that were under 400 mM salinity conditions when compared with the non-pretreated plants. Pretreatment of plants with Se nanoparticles, Se+BW and Se+PS enhanced the sugar content in the control and salt stressed quinoa (Table 2). The highest amount of sugar was observed in plants which were pretreated with Se+Ps and then treated with 400 m salinity.

In the present study, salt stress significantly increased the amount of protein. BW and PS pretreatments of seeds lead to an increase of protein content in the control group and quinoa plants that were treated with 200 mM salinity. Se nanoparticles pretreatment of seeds caused an increase in protein contents in plant under control and salinity stress (200 and 400 mM) (Table 3).

Date showed that salinity stress increased the APX and GPX enzymes activity at 400 mM NaCl but had no significant effect on CAT activity (Table 3) however, Se pretreatment increased the CAT activity in control and salt stressed plants. GPX activity increased significantly (100%) under 400 mM NaCl treatment. Se nanoparticle, Se+BW and Se+PS pretreatment increased the GPX activity (50%, 50% and 100% respectively) when compared with non-pretreated plant under salt stress. APX activity increased about 100% in 400 mM NaCl treatment. Se, Se+BW and Se+PS pretreatment increased the APX activity by about 100% (Table 3) in plants what were under 400 mM NaCl when compared with non-pretreated plants.

In the present study, it was observed that salinity stress (400 mM NaCl) reduced potassium (15%) but increased sodium (33%) contents in leaves of quinoa plants. Se nanoparticles pretreatment of seeds had no effects on Na^+ content but Se+BW and Se+PS pretreatment decreased the Na^+ content of leaves approximately 11% and 23% respectively. Pretreatment of seed with Se, Se+BW an Se+PS increased the K^+ content of leaves about 23%, 20% and 39% respectively (Table 4).

Discussion

Studies have shown that Se plays crucial roles in mitigating the negative effects of abiotic stresses, and when used at low concentrations, it can enhance plant growth and production (Lanza and reis, 2021; Hasanuzzaman *et al*., 2020a). It has been shown that Se has a beneficial effect on plant growth by increasing the function of antioxidant enzymes (Pereira *et al*., 2018) and enhancing plant resistance to a range of abiotic stresses, such as cold, drought, desiccation, as well as metal stress (Sotoodehnia-Korani *et al*., 2020; Hasanuzzaman and Fujita, 2011; Pukacka *et al*., 2011; Pandey and Gupta, 2015). In this research, our result showed that Se pretreatment alleviated the salinity stress though physiological parameters. Data showed that pretreatment of seed with Se enhanced the photosynthesis pigment content that can improve the growth of plants under salinity stress. It has been reported that Selenium supplementation safeguards the activity and cellular structure of photosynthetic

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Table 1. Effect of Se and PGPRs pre-treatment on photosynthetic pigments of quinoa plants under control and salinity stress

Treatment	Chl a	Chl b	Total Chl	Carotenoids
	(mg/g FW)			(µg/g FW)
Control	$0.665^{gh} \pm 0.006$	$0.367^{fg} \pm 0.005$	$1.017^{e-h} \pm 0.008$	$2.188^{i} \pm 0.020$
200 mMNaCl	$0.635^{h} \pm 0.004$	$0.348^{g} \pm 0.004$	$0.949^{gh} \pm 0.019$	$2.204^{i} \pm 0.030$
400 mMNaCl	$0.574^{i} \pm 0.006$	$0.260^{i} \pm 0.003$	$0.816^{i} \pm 0.008$	$2.936^{e} \pm 0.030$
Selenium (Se)	$0.778^{e} \pm 0.004$	$0.438^{c} \pm 0.004$	$1.306^{cde} \pm 0.017$	$2.753^{h} \pm 0.026$
200 mM NaCl+ Se	$0.756^{ef} \pm 0.013$	$0.415^{cd} \pm 0.006$	$1.279^{c-f} \pm 0.014$	$2.843^{gh} \pm 0.026$
400 mM NaCl+ Se	$0.678^{g} \pm 0.009$	$0.360^{fg} \pm 0.007$	$1.218^{d-g} \pm 0.012$	$3.670^{d} \pm 0.026$
BW+ Se	$0.931^{a} \pm 0.015$	$0.543^{a} \pm 0.004$	$1.678^{a} \pm 0.012$	$4.148^{c} \pm 0.021$
200 mM NaCl+ BW+ Se	$0.888^{b} \pm 0.017$	$0.536^{a} \pm 0.002$	$1.641^{ab} \pm 0.014$	$4.289^{b} \pm 0.016$
400 mM NaCl+ BW+ Se	$0.798^{cd} \pm 0.012$	$0.489^{b} \pm 0.004$	$1.596^{abc} \pm 0.012$	$4.768^{a} \pm 0.027$
PS + Se	$0.836^{c} \pm 0.012$	$0.382^{ef} \pm 0.007$	$1.489^{a-d} \pm 0.019$	$3.074^{f} \pm 0.052$
200 mM NaCl+ PS+ Se	$0.811^{cd} \pm 0.009$	$0.358^{fg} \pm 0.012$	$1.457^{a-d} \pm 0.012$	$3.131^{f} \pm 0.026$
400 mM NaCl+ PS+ Se	$0.718^{f} \pm 0.012$	$0.271^{i} \pm 0.007$	$1.337^{b-e} \pm 0.007$	$3.649^{d} \pm 0.020$
B. cereus (BW)	$0.832^{c} \pm 0.017$	$0.417^{cd} \pm 0.008$	$1.363^{bcd} \pm 0.017$	$3.448^{e} \pm 0.024$
200 mM NaCl+ BW	$0.808^{cd} \pm 0.009$	$0.397^{de} \pm 0.006$	$1.336^{b-e} \pm 0.005$	$3.459^{e} \pm 0.029$
400 mM NaCl+ BW	$0.734^{f} \pm 0.011$	$0.319^{h} \pm 0.007$	$1.239^{d-g} \pm 0.015$	$4.170^{bc} \pm 0.020$
P. fluorescen (PS)	$0.743^{ef} \pm 0.013$	$0.376^{efg} \pm 0.008$	$1.215^{h} \pm 0.007$	$2.255^{i} \pm 0.021$
200 mM NaCl+ PS	$0.719^{f} \pm 0.004$	$0.364^{fg} \pm 0.016$	$1.189^{d-g} \pm 0.012$	$2.297^{i} \pm 0.036$
400 mM NaCl+ PS	$0.641^{gh} \pm 0.009$	$0.272^{i} \pm 0.011$	$0.978^{fgh} \pm 0.033$	$3.400^{e} \pm 0.011$

Each value represented mean ± SEM. Means were compared using Duncan's multiple range tests. Different letters indicated significant differences among treatments ($P \leq 0.05$).

Table 2. Effect of Se and PGPRs pre-treatment on lipid peroxidation, sugars and proline content of quinoa plants under control and salinity stress

Treatment	MDA Content (µmol/gFW)	Other Aldehydes Content (µmol/gFW)	Soluble Sugars Content (mg/g FW)	Proline Content (µmol/g FW)
Control	$0.080^{cd} \pm 0.000$	$1.455^{abc} \pm 0.020$	$40.127^{h} \pm 0.145$	$2.708^{j} \pm 0.144$
200 mMNaCl	$0.083^{cd} \pm 0.001$	$1.450^{abc} \pm 0.022$	$40.527^{h} \pm 0.171$	$2.877^{ij} \pm 0.128$
400 mMNaCl	$0.089^{a} \pm 0.001$	$1.526^{a} \pm 0.020$	$41.827^{g} \pm 0.247$	$3.451^{hg} \pm 0.128$
Selenium (Se)	$0.080^{cd} \pm 0.009$	$1.455^{abc} \pm 0.020$	$42.745^{ef} \pm 0.259$	$2.873^{ji} \pm 0.065$
200 mM NaCl+ Se	$0.082^{cd} \pm 0.001$	$1.436^{abc} \pm 0.035$	$43.881^{d} \pm 0.277$	$3.220^{hi} \pm 0.047$
400 mM NaCl+ Se	$0.087^{ab} \pm 0.001$	$1.538^{a} \pm 0.038$	$45.544^{c} \pm 0.202$	$3.784^{f} \pm 0.137$
BW+ Se	$0.057^{k} \pm 0.001$	$1.188^{g} \pm 0.034$	$42.668^{ef} \pm 0.079$	$4.710^{cd} \pm 0.082$
200 mM NaCl+ BW+ Se	$0.060^{jk} \pm 0.001$	$1.238^{fg} \pm 0.023$	$43.123^{e} \pm 0.091$	$5.271^{b} \pm 0.072$
400 mM NaCl+ BW+ Se	$0.068^{def} \pm 0.000$	$1.313^{def} \pm 0.017$	$44.016^{d} \pm 0.139$	$5.753^{a} \pm 0.117$
PS + Se	$0.0658^{ij} \pm 0.0016$	$1.247^{fg} \pm 0.019$	$45.680^{c} \pm 0.387$	$3.952^{f} \pm 0.170$
200 mM NaCl+ PS+ Se	$0.066^{jhi} \pm 0.002$	$1.297^{ef} \pm 0.026$	$46.732^{b} \pm 0.221$	$4.381^{de} \pm 0.083$
400 mM NaCl+ PS+ Se	$0.075^{c-f} \pm 0.007$	$1.413^{bcd} \pm 0.037$	$47.710^{a} \pm 0.083$	$4.931^{bc} \pm 0.091$
B. cereus (BW)	$0.068^{ghi} \pm 0.009$	$1.350^{cde} \pm 0.023$	$40.127^{h} \pm 0.145$	$3.699^{gf} \pm 0.145$
200 mM NaCl+ BW	$0.074^{def} \pm 0.001$	$1.359^{cde} \pm 0.025$	$40.678^{h} \pm 0.307$	$4.060^{ef} \pm 0.087$
400 mM NaCl+ BW	$0.079^{cde} \pm 0.002$	$1.421^{bc} \pm 0.022$	$42.124^{fg} \pm 0.109$	$4.877^{bc} \pm 0.113$
P. fluorescen (PS)	$0.072^{fgh} \pm 0.002$	$1.407^{bcd} \pm 0.017$	$42.668^{ef} \pm 0.079$	$2.610^{j} \pm 0.049$
200 mM NaCl+ PS	$0.073^{efg} \pm 0.002$	$1.418^{bc} \pm 0.036$	$43.123^{e} \pm 0.091$	$3.224^{hi} \pm 0.104$
400 mM NaCl+ PS	$0.079^{cde} \pm 0.006$	$1.476^{ab} \pm 0.021$	$44.483^{d} \pm 0.307$	$3.899^{f} \pm 0.134$

Each value represents mean ± SEM. Means were compared using Duncan's multiple range tests. Different letters indicate significant differences among treatments ($P \leq 0.05$).

machinery in plants under salt stress (Xu *et al*., 2021). In addition, it has been reported that, Se seed priming enhances chlorophyll synthesis by protecting chloroplast enzymes in stressed plants (Gupta and Gupta, 2017). In this study results showed that Se effect was more when it was used with PGPR bacteria. A previous study showed that Pseudomonas fluorescens strains ameliorated chlorophyll fluorescence and chlorophyll pigments in sweet corn under water-deficit conditions (Zarei *et al*., 2020). Also, AL Kahtani *et al*. (2020) observed that sweet pepper plants treated with PGPRs showed a significant increase in chlorophyll fluorescence and chlorophyll pigment. So, it seems that the synergistic effects of selenium and bacteria in this study, have preserved the pigments and photosynthetic structure of the plant under salinity stress.

In the present study, data showed that, salinity ramped up the content of MDA and other aldehyde which is the indicator of lipid peroxidative and damage of bio membranes and primming of seeds with Se, Se+BW and Se+Ps decreased the peroxidation of membrane lipids under salt stress. Previous studies have shown that PGPR reduce the content of malondialdehyde by protecting the membrane against

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Table 3. Effect of Se and PGPRs pre-treatment on protein content and antioxidant enzymes activity of quinoa plants under control and salinity stress

Treatment	Proteins Content (mgFW)	CAT Activity	GPX Activity	APX Activity (U/mg Protein)
Control	$5.74^{k}\pm0.21$	$0.03^{i}\pm0.04$	$0.01^{j}\pm0.07$	$0.01^{j}\pm0.04$
200 mMNaCl	$6.63^{j}\pm0.14$	$0.03^{ki}\pm0.07$	$0.01^{hij}\pm0.09$	$0.01^{j}\pm0.06$
400 mMNaCl	$8.74^{e-h}\pm0.10$	$0.04^{hi}\pm0.07$	$0.02^{g}\pm0.03$	$0.02^{hi}\pm0.09$
Selenium (Se)	$8.98^{efg}\pm0.14$	$0.05^{d}\pm0.04$	$0.02^{g}\pm0.08$	$0.03^{d}\pm0.07$
200 mM NaCl+ Se	$10.0^{d}\pm0.16$	$0.05^{c}\pm0.01$	$0.02^{g}\pm0.03$	$0.03^{cd}\pm0.01$
400 mM NaCl+ Se	$13.54^{dc}\pm0.11$	$0.06^{a}\pm0.02$	$0.03^{cd}\pm0.04$	$0.04^{a}\pm0.03$
BW+ Se	$11.22^{c}\pm0.27$	$0.03^{ki}\pm0.03$	$0.01^{hi}\pm0.09$	$0.03^{ef}\pm0.05$
200 mM NaCl+ BW+ Se	$12.81^{b}\pm0.17$	$0.03^{k}\pm0.01$	$0.02^{e}\pm0.03$	$0.03^{e}\pm0.04$
400 mM NaCl+ BW+ Se	$15.55^{a}\pm0.18$	$0.04^{gh}\pm0.09$	$0.03^{b}\pm0.05$	$0.04^{b}\pm0.05$
PS + Se	$8.94^{efg}\pm0.10$	$0.04^{fg}\pm0.03$	$0.03^{d}\pm0.06$	$0.03^{d}\pm0.01$
200 mM NaCl+ PS+ Se	$9.61^{de}\pm0.17$	$0.04^{ef}\pm0.03$	$0.03^{c}\pm0.02$	$0.04^{bc}\pm0.08$
400 mM NaCl+ PS+ Se	$9.92^{d}\pm0.04$	$0.05^{b}\pm0.02$	$0.04^{a}\pm0.04$	$0.04^{b}\pm0.01$
B. cereus (BW)	$8.46^{g}\pm0.15$	$0.03^{ki}\pm0.07$	$0.01^{ij}\pm0.06$	$0.02^{gh}\pm0.01$
200 mM NaCl+ BW	$8.60^{fgh}\pm0.04$	$0.03^{k}\pm0.04$	$0.01^{h}\pm0.08$	$0.02^{fg}\pm0.08$
400 mM NaCl+ BW	$9.44^{def}\pm0.04$	$0.04^{gh}\pm0.01$	$0.02^{g}\pm0.03$	$0.03^{d}\pm0.03$
P. fluorescen (PS)	$7.624^{i}\pm0.075$	$0.04^{ij}\pm0.08$	$0.01^{h}\pm0.06$	$0.023^{i}\pm0.004$
200 mM NaCl+ PS	$7.86^{hi}\pm0.09$	$0.03^{j}\pm0.03$	$0.01^{h}\pm0.06$	$0.02^{i}\pm0.02$
400 mM NaCl+ PS	$8.67^{fgh}\pm0.08$	$0.04^{de}\pm0.06$	$0.02^{f}\pm0.02$	$0.03^{ef}\pm0.001$

Each value represents mean ± SEM. Means were compared using Duncan's multiple range tests. Different letters indicate significant differences among treatments (P ≤ 0.05).

Table 4. Effect of Se and PGPRs pre-treatment on leaf Na^+ and K^+ content of quinoa plants under control and salinity stress

Treatment	Na^+ Content (mg/g FW)	K^+ Content (mg/g FW)
Control	$13.218^{cd}\pm 0.211$	$41.837^{fgh}\pm 1.341$
200 mMNl	$14.177^{c}\pm 0.034$	$40.734^{gh}\pm 0.293$
400 mM NaCl	$17.654^{a}\pm 0.249$	$35.842^{i}\pm 0.411$
Selenium (Se)	$12.765^{cde}\pm 0.261$	$51.622^{c}\pm 0.726$
200 mM NaCl+ Se	$13.345^{cd}\pm 0.061$	$51.907^{bc}\pm 0.670$
400 mM NaCl+ Se	$16.547^{ab}\pm 0.175$	$44.341^{efg}\pm 0.578$
BW+ Se	$11.099^{efg}\pm 0.129$	$46.287^{de}\pm 1.157$
200 mM NaCl+ BW+ Se	$11.909^{def}\pm 0.346$	$45.796^{ef}\pm 0.255$
400 mM NaCl+ BW+ Se	$15.830^{b}\pm 0.301$	$43.072^{efg}\pm 0.614$
PS + Se	$10.825^{fg}\pm 0.351$	$56.842^{a}\pm 0.304$
200 mM NaCl+ PS+ Se	$10.900^{fg}\pm 0.129$	$55.612^{ab}\pm 0.614$
400 mM NaCl+ PS+ Se	$13.615^{cd}\pm 0.361$	$49.865^{cd}\pm 0.294$
B. cereus (BW)	$8.268^{hi}\pm 0.582$	$41.440^{gh}\pm 0.123$
200 mM NaCl+ BW	$9.754^{hg}\pm 0.237$	$38.739^{hi}\pm 0.301$
400 mM NaCl+ BW	$12.085^{def}\pm 0.450$	$35.092^{i}\pm 1.844$
P. fluorescen (PS)	$7.819^{i}\pm 0.318$	$50.957^{c}\pm 0.335$
200 mM NaCl+ PS	$8.779^{hi}\pm 0.353$	$50.059^{bc}\pm 0.416$
400 mM NaCl+ PS	$11.139^{efg}\pm0.481$	$42.294^{ab}\pm 0.625$

Each value represents mean ± SEM. Means were compared using Duncan's multiple range tests. Different letters indicate significant differences among treatments (P ≤ 0.05).

stress-induced damages (Shukla, 2012). Selenium also has antioxidant properties and can neutralize the damage caused by oxidative stress (Hasanuzzaman and Fujita, 2001). Application of Se and PGPR can mitigate the peroxidation of lipid in pretreated plants.

In the present investigation, the responses of the quinoa plant to salinity were reflected by an increase of CAT, ASPX and GPX activities. These activated antioxidant systems are useful for plant performance and have an essential role in alleviating oxidative stress damage in plants, by removing excess ROS and inhibiting lipid peroxidation. Se and PGPRs pretreatment of seeds increased antioxidant enzymes activities of cultivated quinoa under salt stress which trend has also been observed in other researches resulting in enhanced salt tolerance (Eraslan, 2008; Baltruschat, 2008; Bharti, 2016 Mozafariyan *et al*., 2016).

Selenium is an important element that plays a role in increasing the antioxidant activity of plants, animals and humans. Furthermore, Se acts as the catalytic center of several selenoproteins, such as glutathione peroxidase (GSHPx), thioredoxin reductase, and iodothyronine-deiodinases hence, it is important in the scavenging of

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free radicals (Mehdi *et al*., 2013). It has been reported that, selenium can regulate the reactive oxidative species (ROS) levels in stressed plants through three pathways: (1) by stimulating the spontaneous dismutation of O^{2-} into H_2O_2; (2) by a direct reaction between selenium containing compounds and ROS; (3) by reducing the excess ROS generation in plants exposed to different environmental stresses by the regulation of antioxidative enzymes. Regulation of ROS levels by selenium may be a key mechanism for preventing stress in plants.

In the current research, PGPRs and Se nanoparticles pretreatments of seeds lead to an increase in the antioxidant enzymes activity and protein content. Some of these proteins can be antioxidant enzymes and may have a direct correlation with stress tolerance. In maize and rice plants it has been observed that many proteins are directly or indirectly related to plant growth promotion which was differentially found expressed by the interaction of *Pseudomonas fluorescens* (Kandasamy, 2009). It has been reported that selenium NPs also alleviates oxidative stress induced by various abiotic stresses such as metals, drought and temperature (Ikram *et al*., 2020; Zahedi *et al*., 2019).

Proline and soluble sugars are very important biochemical indicators of salinity tolerance in plants (Ashraf and Harris, 2004). Proline plays an important function like detoxification of ROS, stabilization of proteins and protein complexes (Ashraf and Foolad, 2007). Soluble sugars protect cell membranes from water loss by binding to the lipid bilayer of membranes (Ruelland *et al*., 2009). Sugars also prevent the breakdown of proteins, through the formation of hydrogen bonds with polar polypeptide residues (Ashraf and Foolad, 2007). In addition, sugar as an osmolytes, which plays an important role in osmotic adjustment and stabilization of proteins and membranes under stress as well as acting as a carbon source for plant growth (Ashraf and Foolad, 2007). In this study, seed inoculation with PGPR induced proline and sugars accumulation with or without salt stress compared to the un-inoculated plants. High proline and sugars contents were also observed under salt stress in the presence of PGPRs in paddy, safflower and maize plants (Sandhya *et al*., 2010; Jha and Subramanian, 2013; Lack *et al*., 2013). It has been reported that, bacteria through the production of plant hormones, increased the activity of invertase enzyme involved in the synthesis of monosaccharides, putting the plant in a more suitable nutritional condition and improving photosynthesis, increasing the accumulation of carbohydrates in inoculated plants (El-Ghany, 2015). Moreover, in this study, pretreatment of seeds with Se nanoparticle solution increased the amount of proline and sugars under control and salinity stress. Shahid *et al*. (2018) has been reported that Se is can increase the formation of soluble sugars by increasing the stability of the cytoplasmic membrane and reducing malondialdehyde, which stimulates general growth. Tinggi (2003) also suggested that, reduction of starch in leaves under the influence of Se, causes the path of carbohydrates to dissolved sugars, which is more effective than starch in maintaining water balance and supporting current growth. The accumulation of proline and soluble sugars in the leaves of quinoa plant as a result of PGPRs and Se pretreatment under salinity stress, is of great importance because it increases the resistance to stress in the plant.

Salt stress reduces water potential and causes ion imbalance or disturbances in ion homeostasis and toxicity (Parida and Das, 2005). Reducing many ions content and increasing sodium content are the most significant effects of salinity, which are mentioned in many reports (Juan *et al*., 2005; Kafi *et al*., 2003). Potassium is an osmotically active solute that contributes to water absorption at the cell and whole plant level. Therefore, it plays a key role in plant water stress tolerance (Caravaca *et al*., 2004). In the present study, we observed that pretreatment of Seeds with Se nanoparticle and decreased the sodium content and increased potassium absorption under salt stress. Similarly, several studies reported that PGPR pretreatment reduced sodium absorption and increased potassium content in plants (Bano and Fatima, 2009; Kohler *et al*., 2009; Nadeem *et al*., 2006). It has also been reported that PGPRs produce exopolysaccharides which bind with cations such as Na^+ and reduced their absorption through plant (El-Ghany *et al*., 2015). In addition, low concentrations of Se have been reported to induce the sodium transfer to the vacuole possibly due to increased plasma membrane H^+ ATPase activity (Kong *et al*., 2005). In this study, the effect of selenium nanoparticle along with bacteria in reducing the amount of sodium and increasing the potassium content was much greater than either alone, and it seemed that this effect could be one of the reasons for increasing the resistance to salinity stress in pretreated quinoa plant.

In general, the results of this study showed that Se, although not essential for plants can improve growth and morphological parameters of the plant under salinity stress at low concentrations and it had more effects in the presence of PGPRs. So can be an effective tool for protecting plants from salinity damaged and this strategy could be applied for sustainable agriculture.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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