

# Seed priming with selenium nanoparticle and plant growth promoting rhizobacteria improve seedling development of foxtail millet (*Setaria italica*) under salinity stress

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## Research Article

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# Abstract

Much research has been carried out in agriculture and physiology to minimize the adverse effects of salinity on crops. This study was conducted to investigate the effect of selenium nanoparticles and plant growth-promoting bacteria (PGPRs) pretreatment on increasing the resistance of foxtail millet to salinity stress. Experimental treatments consisted of selenium nanoparticles at a concentration of  $1 \text{ mg L}^{-1}$ , *Bacillus cereus* and *Pseudomonas fluorescens* and three different levels of saline solution with concentrations of 0, 100 and 200mM sodium chloride. In this study, foxtail millet seeds were priming with solutions of selenium nanoparticles, a variety of bacteria and a mixture of both, and then the pretreated seeds were planted in pots and after two weeks were affected by salinity stress. After 10 days of salinity stress, plants were harvested for analysis. The results of measuring the growth, biochemical and physiological parameters of this study showed that soaking millet seeds in a solution of selenium nanoparticles can increase the plant's ability to cope with salinity stress by increasing photosynthetic pigments, compatible solutes, reducing oxidative stress, protecting the cell membrane and reducing sodium uptake, and it has more effects in the presence of PGPRs bacteria (such as *Bacillus cereus* and *Pseudomonas fluorescens*). Therefore, it is suggested that these two biostimulators be used together for greater effects. 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.

## 1. Introduction

Salinity, either in the soil or in irrigation water, is one the important abiotic stresses affecting crop production in arid and semi-arid areas (Zulfiqar and Ashraf 2021). Salinity causes damage to plant by both hyperosmotic and hyper-ionic stresses (Seleiman et al. 2020). Harmful effects of salts on plants are the result of combining factors observed in growth reduction (Bitterli et al. 2010). Salinity stress also causes accumulation of toxic ions such as sodium and chloride s, which leads to nutritional imbalance. Generation of reactive oxygen species such as singlet oxygen, superoxide anions ( $\text{O}_2^{\cdot -}$ ) and hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) under salinity stress, results in oxidative damages (Alam et al., 2020). However, ROS molecules trigger signaling molecules activating antioxidant system enabling the plant to cope with stress condition, but high production of reactive oxygen species induces oxidative injuries in cells, such as peroxidation of lipid, oxidation of protein and DNA impairment (Nadarajah 2020). Plants have antioxidative defense system including enzymatic and non-enzymatic antioxidants aiding in ROS detoxification (Hasanuzzaman et al. 2020).

Selenium has intermediate physical and chemical properties between metal and non-metal and is considered as a beneficial element for normal healthy life of plants, animals, human and certain microorganisms (Masuda 2012; Zhao et al. 2020). In soil, Se mainly exists in four different oxidation states: selenid ( $\text{SeO}^{-2}$ ), selenium ( $\text{Se}^0$ ), selenite ( $\text{seO}_3^{-2}$ ) and selenate ( $\text{SeO}_4^{-2}$ ). Selenium nanoparticles have less toxicity than other forms of selenium due to their high reactivity, wide specific surface area, and good adsorption capability (Deng et al. 2018; Li et al.

2020; Zhao et al. 2020). (Poldma et al. 2011). Current reports suggest that low concentration of Se exerts profound beneficial effects to plants (in early stage) through improvement of photosynthesis, physiological and biochemical processes and antioxidative responses that ultimately promote proper growth and development of plants (Mroczek-Zdyrska et al. 2017; Huang et al. 2018). Selenium can regulate the reactive oxidative species (ROS) levels in

stressed plants through three pathways: 1) by stimulating the spontaneous dismutation of  $O_2^{\cdot -}$  into  $H_2O_2$ ; 2) by a direct reaction between selenium containing compounds and ROS; 3) reduce 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 (Cartes et al. 2010). It has been reported that selenium NPs also alleviates various abiotic stresses caused by metals, drought and temperature (Ikram et al. 2020; Zahedi et al. 2019).

Plant growth promoting bacteria (PGPRs) represent a wide variety of soil bacteria that are involved in the biological activity of soil ecosystem and in turn can be effective in absorbing nutrients and increasing crop growth along with soil fertility (Do Amaral 2016). Using plant growth promoting rhizobacteria (PGPR) is an eco-friendly method with the capacity to alleviate abiotic stresses such as salinity. PGPRs can affect the breakdown of organic matter, plant tolerance to stress, plant yield, plant metabolism, etc. (Verjan et al. 2016). Recent studies showed these microorganisms are beneficial for plants and increase plant tolerance against the abiotic stresses through direct and indirect processes (Olanrewaju et al. 2017). Some direct mechanisms including solubilizing of nutrients like phosphate and potassium, providing  $N_2$  (biological nitrogen fixation), the release of phytohormones (like auxin, cytokinin, abscisic acid, and gibberellic acid), and synthesized enzyme 1-amino cyclopropane-1-carboxylate (ACC) deaminase (that decrease the stressed ethylene level) and siderophores production (Figueiredo et al. 2010).

Foxtail millet (*Setaria italic*, Poaceae) is a highly nutritious plant with abundant micro and macronutrients and several bioactive compounds. This plant also requires slim irrigation, and flourish even under low nutrient conditions and requires a very small amount of fertilizer (Kole et al. 2015). It has been reported that foxtail millet can adapt to a wide array of abiotic stresses such as drought, salinity, heat and excessive light, so, this plant is a candidate for cultivation in arid and semi-arid areas for food and feed where key cereals often show lower yield (Amadou et al. 2013; Kaur et al. 2018).

The response of plants to salinity stress varies according to growth and development stage, but germination and early stages of seedling growth, are the most sensitive stages affected by salinity stress. So, today, it is tried as much as possible to use natural materials and easy methods to strengthen the plant's immune system against stress specially in early growth stage. Seed priming is a pre-sowing hydration technique which triggers germination and leading to uniform seedling emergence. Positive outcomes of seed priming as a promising strategy in biotic and abiotic stress management without imposing any genetic/ transgenic alterations were reported by several authors ().

Foxtail millet is a plant species sensitive to salinity stress in the early stages of growth, so this research aimed to evaluate the effect of the seed priming with selenium nanoparticles (as beneficial mineral) and growth-promoting bacteria to reduce the negative effects of salinity stress on the seedlings of foxtail millet. Meanwhile, the objective 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.

## 2. Materials And Methods

The bacterial *Pseudomonas 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 foxtail millet (*Poaceae Setaria*) were obtained from the Research and Technology Institute of Plant Production (RTIPP), Shahid Bahonar University of Kerman. Seeds of foxtail millet were sterilized with 70% ethanol for five min, followed by washing with distilled water. Then seeds are divided to 6 groups as follow, 1: seeds were soaked in distilled water as control, 2: seeds were soaked in PS bacterial cell suspension, 3: seeds were soaked in BW bacterial cell suspension, 4: seeds were soaked in selenium nanoparticles solution, 5: seeds were soaked in PS. bacterial cell suspension + selenium nanoparticles, 6: seeds were soaked in BW bacterial cell suspension + selenium nanoparticles solution for three hours. In each group, seeds of foxtail millet (10 seeds per pot) were sown in plastic pots (with 10 cm diameter) and were kept in a greenhouse. The soil contained 100% sand which was washed three times with distilled water and was autoclaved for 20 min at 120°C. After two weeks of seedlings growth, uniformly developed seedlings were selected in each group. In each group, pots were divided into three subgroups for salinity stress treatments including subgroup1: which were irrigated with distilled water, subgroup 2: which were irrigated with 100 mM NaCl solution and subgroup 3: which were irrigated with 200 mM NaCl. The salinity treatment was performed ten times a day. The pots were irrigated with 100 ml of each solution every 10 days. In addition, 30% leaching was considered in order to avoid the accumulation of salt in the soil pots. One day after the last salinity treatment, the leaves (the second row of leaves after the cotyledon leaves) were collected and immediately frozen in liquid nitrogen.

### Morphological parameters

Shoots of plants were separated and weighed. The samples were then dried in a oven at 50°C for 24 h and then the dry weights were measured.

### Photosynthetic Pigments Content

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

Chl a ( $\mu\text{g/ml}$ ) =  $12.25 A_{663.2} - 2.79 A_{646.8}$

$\text{Chl b } (\mu\text{g/ml}) = 21.21 \text{ A}_{646.8} - 5.1 \text{ A}_{663.2}$

$\text{Chl t } (\text{Chl a} + \text{Chl b}) = 7.15 \text{ A}_{663.2} + 18.71 \text{ A}_{646.8}$

$\text{Car } (\mu\text{g/ml}) = (1000\text{A}_{470} - 1.8 \text{ Chl a} - 85.02 \text{ Chl b}) / 198$

### **Lipid peroxidation content**

Malondialdehyde (MDA) and other aldehydes content was estimated by using Heath and Packer (1968) and Meir et al. (1992) method. For MDA calculation, the value of non-specific absorption at 600 nm was subtracted from the 532 nm reading. The MDA content was calculated using the Lambert-Beer law, with extinction coefficient of  $155 \text{ mM}^{-1}\text{cm}^{-1}$  and expressed as  $\mu\text{mol MDA per g fresh weight}$ . For other aldehyde calculation, non-specific absorption at 600 nm was subtracted from the 455 nm reading and the extinction coefficient of  $0.458 \times 10^5 \text{ mM}^{-1}\text{cm}^{-1}$  was used and expressed as  $\mu\text{mol per g fresh weight}$ .

### **Antioxidant enzyme extraction and assays**

Three hundred milligrams weight of fresh leaves were homogenized in an ice-cold mortar using 3 mL of 50 mM potassium phosphate buffer. After centrifugation at  $17000\times g$  for 20 min in  $4^\circ\text{C}$ , 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.

Guaiacol peroxidase (GPX) (EC 1.11.1.7) activity was measured by a method described by Plewa et al. (1991) with reaction mixture containing 50 mM potassium phosphate (pH 7.0), 0.3% (v/v)  $\text{H}_2\text{O}_2$ , 1% (v/v) guaiacol and the enzyme extract. One unit (U) of GPX activity was defined as the amount of enzyme that produced 1  $\mu\text{mol}$  of tetraguaiacol per minute. The enzyme activity was expressed in U per milligram protein.

Catalase (CAT) (EC 1.11.1.6) activity was assayed according to the method of Dhindsa et al. (1981). The reaction mixture consisted of a 50 mM potassium phosphate buffer (pH 7.0),  $\text{H}_2\text{O}_2$  (15 mM) and 100  $\mu\text{L}$  of the enzyme extract. The decrease in absorbance at 240 nm was recorded ( $=40 \text{ mM}^{-1}\text{cm}^{-1}$ ). The enzyme activity was expressed in U per milligram protein (1  $\mu\text{M}$  of  $\text{H}_2\text{O}_2$  reduction  $\text{min}^{-1} \text{ mg}^{-1}$  protein).

Ascorbate peroxidase (APX) (EC 1.11.1.11) activity was measured by Nakano and Asada method (1981). The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, 0.1 mM  $\text{H}_2\text{O}_2$  and 150  $\mu\text{L}$  of enzyme extract. Decrease at 290 nm against the blank ( $=2.8 \text{ mM}^{-1}\text{cm}^{-1}$ ) was recorded. The enzyme activity was expressed in U per milligram protein.

### **Proline content**

Free proline amount was measured according to Bates et al. (1973). 500 mg of leaf tissues was homogenized in 10 ml of 3% sulfosalicylic acid, and the homogenate was centrifuged at 2000g for 5 min. 2 ml of was treated with 2 ml of acid ninhydrin and 2 ml of glacial acetic acid and incubated at 100°C for 1 h in a bath. The reaction mixture was extracted with adding 4 ml toluene. The absorbance of chromophore containing toluene was recorded at 520 nm.

### **Total soluble sugar content**

Total soluble sugar was assayed using anthrone reagent and glucose as standard. 6 ml of anthrone reagent (150 mg of anthrone in 72% H<sub>2</sub>SO<sub>4</sub>) was added to the leaf tissue extract, and then heated in a boiling water bath for 10 min. The test tubes were ice cooled for 10 min and incubated for 20 min at 25° C. Absorbance was read at 625 nm (Fales 1951).

### **Ion content**

dried leaves tissue was powdered for measurement of ions. One g of dried sample was digested by using 10 ml of nitric acid. The concentrations of the Na<sup>+</sup> and K<sup>+</sup> were determined by Flame Technique (PerkinElmer Optima 7000 DV, USA).

**Statistical analysis:** All experiments were performed by a factorial arrangement, based on complete randomized design (CRD). All experiments comprised four 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 LSD tests. *P* values less than 0.05 (*P* < 0.05) were considered statistically significant. The results were shown as the mean ± SEM (standard error of the mean).

## **3. Results**

### **Fresh and dry weight**

As shown in Fig. 1, salinity stress in both level (100 and 200 mM) decreased the fresh and dry weight of foxtail millet shoot. Seed pretreatment of plants with Se, BW and PS increased the fresh and dry weight of plants in both control and salinity stress groups in comparison with non-pretreated plants. The combination of Se with bacteria increased their effects on growth parameters of the foxtail plant.

### **Photosynthetic pigments**

It was observed that chlorophyll *a* and *b* and total chlorophyll were reduced in plants under salinity stress compared to control plants while carotenoids content increased in plants which were under stress conditions (Table 1). Pretreatments of plants with PGPRs, Se and PGPRs + Se improved the photosynthetic pigments content under control and salinity stresses compared to non-pretreated plants. In the present study, in both conditions (control and salinity stress) the BW bacteria pretreatment was

more effective than PS bacteria on increment of chlorophylls and carotenoids content. However, when bacteria were used with selenium, their effect on reducing stress damage was much greater than that of any pretreatment alone.

Table 1

Effect of Se and PGPRs pre-treatment on photosynthetic pigments of foxtail millet plants under control and salinity stress. Each value represents mean  $\pm$  SEM. Means were compared using Duncan's multiple range tests. Different letters indicate significant differences among treatments ( $p \leq 0.05$ ).

| Treatments            | Chl a<br>(mg/g FW)              | Chl b<br>(mg/g FW)              | Total Chl<br>(mg/g FW)           | Carotenoids<br>( $\mu$ g/g FW)   |
|-----------------------|---------------------------------|---------------------------------|----------------------------------|----------------------------------|
| Control               | 0.929 <sup>d</sup> $\pm$ 0.0236 | 0.403 <sup>ef</sup> $\pm$ 0.014 | 1.306 <sup>gh</sup> $\pm$ 0.019  | 1.332 <sup>m</sup> $\pm$ 0.046   |
| 100 mM NaCl           | 0.678 <sup>f</sup> $\pm$ 0.017  | 0.286 <sup>ig</sup> $\pm$ 0.012 | 0.955 <sup>k</sup> $\pm$ 0.019   | 1.458 <sup>kl</sup> $\pm$ 0.024  |
| 200 mM NaCl           | 0.4599 <sup>g</sup> $\pm$ 0.020 | 0.13 <sup>m</sup> $\pm$ 0.009   | 0.845 <sup>l</sup> $\pm$ 0.016   | 1.404 <sup>m</sup> $\pm$ 0.025   |
| Selenium (Se)         | 1.026 <sup>c</sup> $\pm$ 0.024  | 0.548 <sup>c</sup> $\pm$ 0.012  | 1.446 <sup>de</sup> $\pm$ 0.017  | 2.045 <sup>c</sup> $\pm$ 0.036   |
| 100 mM NaCl + Se      | 0.813 <sup>e</sup> $\pm$ 0.026  | 0.417 <sup>e</sup> $\pm$ 0.013  | 1.322 <sup>fgh</sup> $\pm$ 0.030 | 1.712 <sup>gh</sup> $\pm$ 0.026  |
| 200 mM NaCl + Se      | 0.701 <sup>f</sup> $\pm$ 0.021  | 0.333 <sup>gh</sup> $\pm$ 0.016 | 1.224 <sup>ij</sup> $\pm$ 0.019  | 1.587 <sup>ij</sup> $\pm$ 0.032  |
| BW + Se               | 1.546 <sup>a</sup> $\pm$ 0.021  | 0.631 <sup>a</sup> $\pm$ 0.014  | 1.847 <sup>a</sup> $\pm$ 0.020   | 2.710 <sup>a</sup> $\pm$ 0.028   |
| 100 mM NaCl + BW + Se | 0.946 <sup>cd</sup> $\pm$ 0.021 | 0.533 <sup>bc</sup> $\pm$ 0.008 | 1.652 <sup>b</sup> $\pm$ 0.018   | 1.909 <sup>de</sup> $\pm$ 0.026  |
| 200 mM NaCl + BW + Se | 0.904 <sup>d</sup> $\pm$ 0.024  | 0.406 <sup>ef</sup> $\pm$ 0.011 | 1.560 <sup>c</sup> $\pm$ 0.017   | 1.822 <sup>ef</sup> $\pm$ 0.034  |
| PS + Se               | 1.225 <sup>b</sup> $\pm$ 0.015  | 0.570 <sup>b</sup> $\pm$ 0.007  | 1.707 <sup>b</sup> $\pm$ 0.015   | 2.203 <sup>b</sup> $\pm$ 0.029   |
| 100 mM NaCl + PS + Se | 0.702 <sup>f</sup> $\pm$ 0.021  | 0.461 <sup>d</sup> $\pm$ 0.011  | 1.487 <sup>cd</sup> $\pm$ 0.013  | 1.754 <sup>fg</sup> $\pm$ 0.027  |
| 200 mM NaCl + PS + Se | 0.511 <sup>g</sup> $\pm$ 0.020  | 0.236 <sup>kl</sup> $\pm$ 0.007 | 1.375 <sup>efg</sup> $\pm$ 0.019 | 1.640 <sup>b</sup> $\pm$ 0.026   |
| B. cereus (BW)        | 0.950 <sup>cd</sup> $\pm$ 0.019 | 0.506 <sup>c</sup> $\pm$ 0.013  | 1.396 <sup>ef</sup> $\pm$ 0.017  | 1.974 <sup>d</sup> $\pm$ 0.024   |
| 100 mM NaCl + BW      | 0.791 <sup>e</sup> $\pm$ 0.034  | 0.369 <sup>fg</sup> $\pm$ 0.015 | 1.262 <sup>hi</sup> $\pm$ 0.029  | 1.649 <sup>hi</sup> $\pm$ 0.032  |
| 200 mM NaCl + BW      | 0.663 <sup>f</sup> $\pm$ 0.026  | 0.273 <sup>c</sup> $\pm$ 0.015  | 1.158 <sup>j</sup> $\pm$ 0.032   | 1.490 <sup>kl</sup> $\pm$ 0.034  |
| P. fluorescen (PS)    | 0.940 <sup>cd</sup> $\pm$ 0.022 | 0.463 <sup>d</sup> $\pm$ 0.012  | 1.25 <sup>hi</sup> $\pm$ 0.023   | 1.940 <sup>cd</sup> $\pm$ 0.020  |
| 100 mM NaCl + PS      | 0.690 <sup>f</sup> $\pm$ 0.019  | 0.321 <sup>hi</sup> $\pm$ 0.009 | 1.166 <sup>j</sup> $\pm$ 0.018   | 1.563 <sup>ijk</sup> $\pm$ 0.016 |
| 200 mM NaCl + PS      | 0.477 <sup>g</sup> $\pm$ 0.027  | 0.195 <sup>i</sup> $\pm$ 0.008  | 1.003 <sup>k</sup> $\pm$ 0.022   | 1.440 <sup>l</sup> $\pm$ 0.0238  |

## **Lipid peroxidation**

Data showed that the MDA and other aldehyde content as index of membranes lipid peroxidation, increased significantly in plants that were under salinity stress (100 and 200 mM) (Fig. 2). PGPRs and selenium nanoparticles pretreatment of seeds, caused a decrease in MDA and other aldehydes contents in salinity stressed plants (especially under 200 mM NaCl) while they had no significant effects on control plants.

## **Compatible solutes**

In this study, the proline content in foxtail millet plants was significantly increased under 100 mM and 200 mM salinity stress (Fig. 3). Seed priming by BW, PS and selenium nanoparticles alone or in combination increased proline content in foxtail millet in control and stress conditions compared to non-pretreated plants. In this research, in control plants, pretreatment of seeds with Se increased the soluble sugars up to 2.5 times (Fig. 3). PS and BW pretreatment also showed an increase in the soluble sugar 1/5 and 2 times respectively in these conditions. The effects of BW + Se and PS + Se nanoparticles were much greater than that of BW or PS alone. Results showed that salinity stress (100 and 200 mM) enhanced sugar content significantly (Fig. 3) and pretreatment of seeds with PS, BW, Se nanoparticle, BW + Se and PS + Se increased the soluble sugar when compared with non-pretreatment plants. The best results were related to BW + Se and PS + Se treatments which increased the sugar content 3 times in 100 mM salinity stress in comparison with non-pretreated plants.

## **Antioxidant enzymes activity**

The results showed that salinity stress (moderate) at 100 mM NaCl concentration had no significant effects on CAT and GPX activity while it decreased the activity of APX enzyme in comparison with control plants (Fig. 4). In plants which were under 200 mM NaCl stress (severe stress), the activity of CAT decreased while the GPX and APX activity increased in these plants. In salinity stressed plants, pretreatment of seeds with Se nanoparticles, and the combination of them with bacteria (BW + Se or PS + Se) increased the activity of CAT and GPX, and the BW bacteria was more effective than PS. Pretreatment of seeds with these two bacteria alone, had no significant effect (in case of GPX activity) or a slight effect (in case of CAT activity) however when used with selenium their effects of them increased dramatically. In plant under moderate stress (100 mM NaCl), pretreatment of seeds with Se and BW + Se increased the APX activity while the seed priming with BW, PS and Se + PS decreased the activity of this enzyme but in severe stress condition (200 mM NaCl), seed priming with Se, BW bacteria and BW + Se increased the APX activity while PS or PS + Se decreased the APX activity.

## **Sodium and potassium content**

The results showed that salinity stress increased the amount of sodium ions in leaves while it decreased the potassium ions when compared with control plants (Fig. 6). Seed priming with Se, BW and PS bacteria decreased the Na<sup>+</sup> and increased the K<sup>+</sup> amounts in leaves of plants which were under salinity

stress. When Se nanoparticles were applied in combination with BW or PS bacteria, was more effective than Se or bacteria alone.

## 4. Discussion

Salinity is one of the most important limiting factors affects the physiological, biochemical, and molecular mechanisms of plants. Plants adopt various processes to combat salinity stress by involving complex physiological traits, metabolic pathways, and molecular or gene expression (Amirjani 2010). Seed germination and early seedling growth are the most prominent and sensitive stages of plant growth affected by abiotic stresses. The use of PGPR is a good tool to increase soil fertility and plant health. PGPR improve the growth and development of plants directly and indirectly through several mechanisms such as production of secondary metabolites, plant growth substances, changes root morphology resulting in greater root surface area for the uptake of nutrients, siderophores production, antagonism to soil-borne root pathogens, phosphate solubilization, and nitrogen fixation (Figueiredo et al. 2010).

Selenium is an essential element for animals and humans, and its beneficial role for plants has also been proven. This element, if used at low concentrations with protective role in conferring tolerance to certain abiotic and biotic stresses although higher concentrations cause metal toxicity in plants. (Zhao and McGrath 2009; Hasanuzzaman et al. 2010; Wu et al. 2014). Nanotechnology has solved some of the challenges we face in agriculture today and has provided a great opportunity to protect and increase the productivity of agricultural products. Recently, it has been reported that the function of selenium nanoparticles in plant growth and metabolism, is much more effective than its usual forms such as selenate and selenite. However, it should be noted that the use of these nanoparticles at high concentrations causes toxicity (Hu et al. 2018, Babajani et al. 2019, Zahedi et al. 2019).

Improvement of water absorption and production of phytohormones can be due to the increment of growth in plants treated with growth-promoting bacteria (El-Ghany et al. 2015). Microbial secretions have been reported to improve growth by encouraging the synthesis of plant hormones such as auxin, gibberellin, and cytokinin (Verjan et al. 2016). It has also been observed that in salinity stress conditions, plant growth-promoting bacteria help to increase the amount of water available to plant, and there is a higher hydration in inoculated plants, which results in better water use efficiency and improved photosynthesis and biomass (Shukla et al. 2012). Selenium plays beneficial roles in plants through maintaining the structure and fluidity of chloroplast and plasma membranes, activation of membrane enzymes, transfer of metabolites in chloroplasts, improving photosynthetic function, delaying aging, increasing plant yield and reducing oxidative stress (Yao et al. 2009). Literature review reveals that selenium at low concentrations protect the plants from a variety of abiotic stresses such as cold (Chu et al. 2010), drought (Hasanuzzaman and Fujita 2011), desiccation (Pukacka et al. 2011), and heavy metal stress (Kumar et al. 2012).

In this study, seed pretreatment with selenium and PGPR improved the growth of foxtail millet seedlings under control and salinity stress. This growth improving their synergistic effects. In line with our results, in

previous research, selenium NPs improved root length, shoot length and biomass production in wheat plants subjected to drought stress (Ikram et al. 2020).

Salinity causes premature aging of plants, breaking down chloroplasts and reducing the amount of chlorophyll (Muttlu et al. 2009). Chlorophyll is one of the components of chloroplasts that are important for photosynthesis and the amount of it is directly related to the amount of photosynthesis that takes place. Low concentrations of photosynthetic pigments can directly limit photosynthesis and primary production (Yildiz and Terz 2013). Our results indicated that salinity decreased photosynthetic pigments confirming some previous reports (Ashraf et al. 2007; Shukla et al. 2012).

In the present study, pre-inoculation of fox tail seeds under stress, significantly increased chlorophyll content compared to plants grown under salinity stress without seed priming. Similar results have also been reported by El-Ghany et al. (2015). PGPRs have been shown to improve the chlorophyll synthesis pathway with the availability of iron (Sharma 2017). Additionally, it has been communicated that *Pseudomonas fluorescens* strains ameliorated chlorophyll fluorescence and chlorophyll pigments in sweet corn under water-deficit stress (Zarei et al. 2020). Also, ALKahtani et al. (2020) observed that treatment of sweet pepper plants with PGPRs, increased the chlorophyll fluorescence and chlorophyll pigment. Furthermore, it has been reported that low concentrations of selenium, increases antioxidant activity, protects chloroplast enzymes, and improves the efficiency of photosystem II (Feng et al. 2013; Xu et al. 2021). Photosynthetic apparatus protection and increase of chlorophyll content, may be another reason for the incremental of growth in pretreated seedlings. Induction of carotenoid synthesis under stress can be related to their protective role in photosynthetic organization. In addition to quenching the unique oxygen, carotenoids are able to quench the excited triplet chlorophyll, thus indirectly reducing the production of reactive oxygen species (Abdul Jaleel 2009). Considering the antioxidant role of carotenoids, it seems that in foxtail millet, selenium and bacteria pretreatment protect this plant against stress conditions by increasing the amounts of carotenoids as a non-enzymatic antioxidant.

One of the reactions that accelerate in the presence of reactive oxygen species, is the peroxidation of membrane lipids, which produces aldehydes such as malondialdehyde (MDA) (Yun et al. 1996). The increase of lipid peroxidation has often been suggested as an indicator of increased oxidative damage to membranes (Abdul Jaleel 2009). Numerous studies have revealed the protective role of selenium against oxidative stress in plants, which has reduced lipid peroxidation (Hassanuzamman et al. 2010). The regulation of ROS levels by Se may be a key mechanism for counteracting environmental stress in plants. The effect of PGPRs bacteria on reducing MDA content also emphasizes the protective role of these bacteria for membrane integrity and fluidity against stress-induced damage (Vejan et al. 2016). In this study, priming of seeds with PGPRs bacteria and selenium nanoparticles, reduced lipid peroxidation in plants that were under salinity stress, confirming the preservation of membrane integrity and reduction of oxidative stress in these plants.

Oxidative stress occurs in the cell when the amount of active oxygen species produced exceeds the plant's antioxidant capacity. Plants use a variety of defense mechanisms to control and neutralize free

radicals. They have an antioxidant defense system with enzymatic and non-enzymatic mechanisms that can eliminate reactive oxygen species and mitigate the damage caused by oxidative stress (Abdul Jaleel 2009; de Brito Mateus et al., 2021). In this research, seed priming with selenium nanoparticles and bacteria, increased the activity of antioxidant enzymes which is consistent with the results in wheat plants (Yadav 2011). In tomato plants under salinity stress, selenium also increased the CAT activity (Mozafariyan et al. 2016). It seems that APX acts as a signaling substance for the fine modulation of ROS, whereas CAT (mainly localized in peroxisomes) might be responsible for the scavenging of excess ROS (Mittler 2002). It has been reported that, in stressed plants, Se significantly reactivates these two enzymes, particularly CAT (Yao et al., 2009; Djanaguiraman et al. 2010; Hasanuzzaman et al. 2011; Yao et al. 2011). Numerous studies have shown the protective role of selenium against oxidative stress in plants. For example, Jozwiak and Politycka (2019) reported that Se increased the activities of SOD, POD and CAT in cucumber roots under drought stress.

Tognon et al. (2016) reported that Se increased post-harvest performance and vase life of snapdragon cut flower by declining of lipid peroxidation and alleviating oxidative damage. Recent studies showed that Se improved the ability of many plants to scavenge ROS through enzymatic system (Chi et al. 2017; Pereira et al. 2018; de Brito Mateus et al. 2021; Liu et al. 2021). Wu et al. (2017) reported that Se increased the activities of SOD and CAT in the roots and leaves of Chinese cabbage in cadmium stress condition. It has also been observed that Se increased peroxidase (POD), CAT and SOD activities in cucumber roots exposed to drought stress (Jozwiak and Politycka 2019). In agreement with our results, Neysanian et al. 2020 reported that treatment of tomato plants with Se nanoparticle, improved enzymatic antioxidants in tomato and, they showed that Se nanoparticle is more effective than selenate in activating the antioxidant system (Neysanina et al. 2020). High activity of antioxidant enzymes such as CAT, GPX and APX in the Se and PGPR pretreated plants can be explained by a higher efficiency of Se and PGPR in enhancing the antioxidant system, when compared with non-pretreated plants.

Proline and soluble sugars are very important biochemical indicators of salinity tolerance in plants (Ashraf and Harris 2004). Proline as an unstructured amino acid, has been reported to accumulate in plants during stress, and protect proteins and membranes from oxidative damage by binding to membrane phospholipids and stabilizing the three-dimensional structures of proteins and increasing the activity of some antioxidant enzymes (Verburggen and Hermans 2008). According to Szabados and Savaure (2010), proline maintains the integrity of proteins because this Phyto protectant acts as a molecular chaperone. Proline also acts as an osmolyte in maintaining water balance, reducing injuries of reactive oxygen species, and play as a scavenger of hydroxyl radicals and thereby protecting cells from stress-induced damage (Szabados and Sav 2010).

Proline also plays an important role as carbon and nitrogen storage in plant growth and repair (Weisany et al. 2012). In this study, the amount of proline in foxtail millet plants increased under salinity stress. The increase of proline in plant tissues, probably is the result of decreased proline degradation, increased biosynthesis, less use in protein synthesis and increased protein hydrolysis. Pretreatment of foxtail millet seeds with selenium nanoparticles solution and bacteria increased the amount of proline under control

and salinity stress, as well. Increasing the proline content of millet plants pretreated with growth-promoting bacteria, can be due to the activation of proline biosynthetic pathway, which maintains cell water and protects membranes against salinity stress. Similar to our observations, it has been reported that growth-promoting bacteria, increased the amount of proline in safflower under salinity stress (Lack et al. 2013). Sardar et al. (2022) also observed that SeNPs treatment increased proline content both in control and Cd-stressed plants. It appears that the SeNPs enhanced the proline synthesis through improving the activity of nitrate reductase required for the synthesis of proline (Subramanyam et al. 2019).

Soluble sugars as vital metabolic substrates, have a critical role in controlling numerous developmental phases of plants (Afzal et al. 2021). Soluble sugars improve the plant growth via enhancing photosynthetic activity and delivering energy. Soluble sugars perhaps decrease cellular osmotic potential and sustain their metabolic activities (Zhang et al. 2018). Soluble sugars protect cell membranes against water loss by binding to the lipid bilayer of membranes (Ruelland et al. 2009). Sugars also prevent the breakdown of proteins, by formation of hydrogen bonds with polar polypeptide residues (Ashraf and Foolad 2007).

In addition, sugar as an osmolyte, plays a critical role in osmotic adjustment and stabilization of proteins and membranes under stress as well as acting as a carbon source for plant growth (Joshi et al. 2007), (Ashraf and Foolad 2007). In this study, pretreatment of seed with PGPR and Se, enhanced proline and sugars accumulation with or without salt stress compared to non-pretreated plants. In paddy and maize plants, high proline and sugars contents were also detected under salt stress in the presence of PGPRs (Sandhya et al. 2010; Jha and Subramanian 2013). Shahid et al. (2018) has been reported that selenium is able to increase the formation of soluble sugars by increasing the stability of the cytoplasmic membrane and reducing malondialdehyde, ultimately stimulates general growth. Tinggi (2003) also suggested that, reduction of starch in leaves under the influence of selenium, triggers 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 as a result of PGPRs and Se pretreatment under salinity stress, is of great importance and increases the resistance to stress in the plant.

Salt stress decreases water potential and causes ion imbalance or disturbances in ion homeostasis and toxicity (Parida and Das 2005; Orozco-Mosqueda et al. 2020)). Increasing the ratio of sodium to potassium content are the most significant effects of salinity, mentioned in many reports (Juan et al. 2005; Kafi et al. 2003; Abdel 2010; Farsaraei et al. 2020). In the present experiment, data showed that PGPRs and Se seed priming, increased the  $K^+$  to  $Na^+$  content in leaves of plants under salt stress. Similarly, PGPR are reported to reduce sodium absorption in various plants (Bano and Fatima 2009; Kohler et al. 2009; Neshat et al 2022). Growth-promoting bacteria have been reported to produce exoskeletons that bind to cations such as sodium, to reduce its uptake by the plant (Goswami 2016) which is consistent with the results of the present study. Low concentrations of selenium have also been reported to induce sodium transfer to vacuole, possibly due to increased plasma membrane  $H^+$ -ATPase

activity (Kong 2005). The previous study also showed that application of *Bacillus subtilis* treatment, increased the SOS1 expression under 300 mM NaCl (Woo et al. 2020).

## Conclusion

In general, the results of this study revealed that selenium although not essential for plants can improve growth and morphological parameters of foxtail millet plants under salinity stress in the form of nanoparticle and at low concentrations. The findings of biochemical, physiological and morphological analyses indicated that, this beneficial element can increase the plant's ability to cope with salinity stress by increasing photosynthetic pigments, compatible solutes, reducing oxidative stress and reducing sodium uptake, it is more effective in the presence of PGPRs bacteria such as *Bacillus* and *Pseudomonas*. Therefore, it is suggested that these two biostimulators be used together for greater effects. 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 and hopefully better results can be obtained by upcoming studies on candidate plants at the field level.

## Declarations

**Author's Contributions:** F.A, F.N and Gh. M conceived and planned the experiments. M. H helped prepare and cultivate the bacteria. F. N wrote the paper with input from all authors. F.A collected the data. Gh. M designed the analysis, and F.N assisted with F. A measurement. All authors discussed the results and commented on the manuscript.

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### Data availability

All data generated or analyzed during this study are included in this published article.

**Ethical Approval:** This article is original and not published elsewhere. All authors discussed the results and read and approved the final manuscript. The authors confirm that there are no ethical issues in the publication of the manuscript.

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## Figures

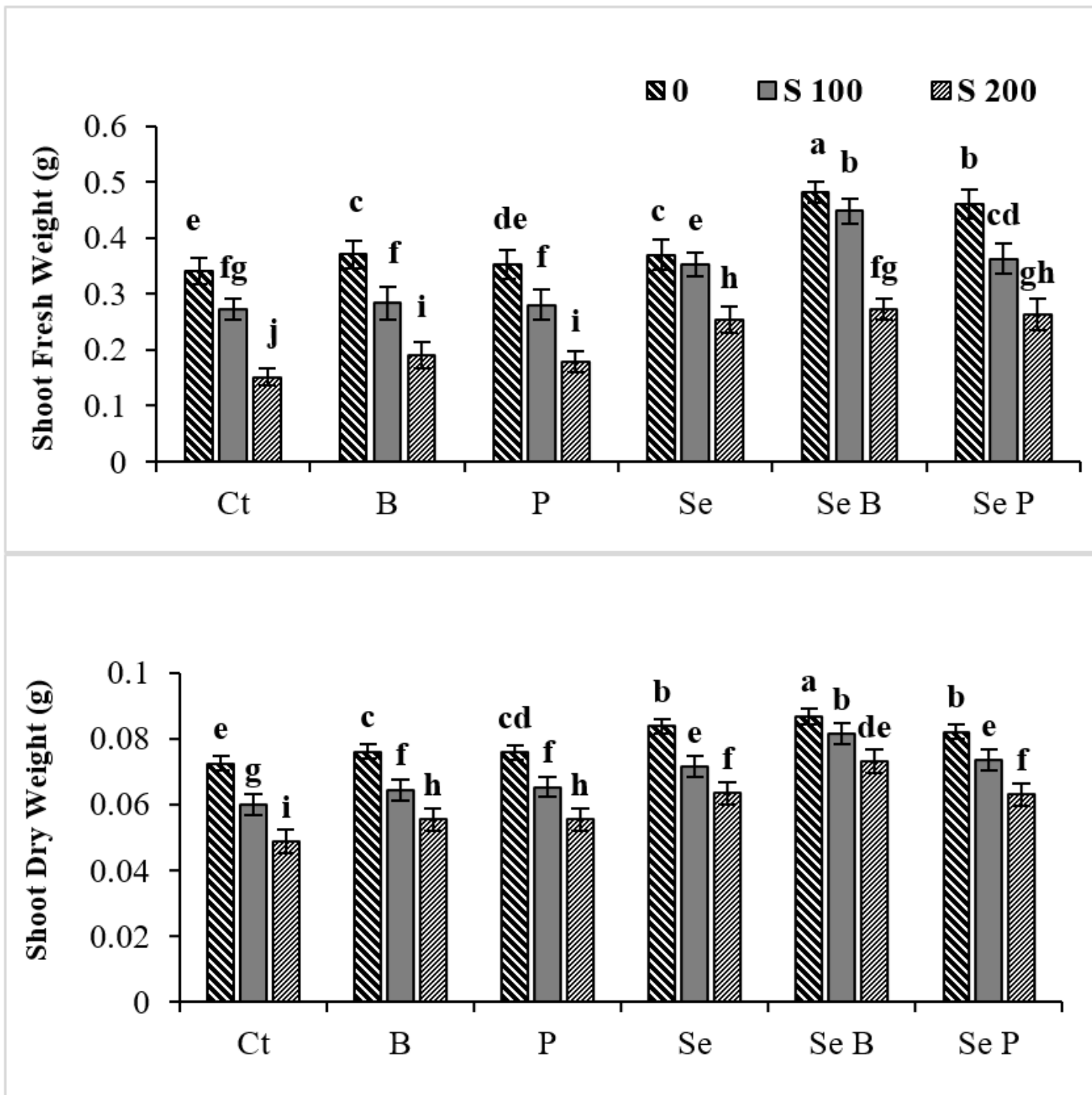


**Figure 1**

Effect of Se and PGPRs pre-treatment on fresh and dry weight of foxtail millet shoot under control and salinity stress. Each value represents the mean  $\pm$  SEM. Means were compared using Duncan's multiple range test. Different letters indicate significant differences among treatments ( $p \leq 0.05$ ). (Ct=Control, B=*Bacillus cereus*, P= *Pseudomonas fluorescens*, Se=Selenium, SeB=Selenium+ *Bacillus cereus*, SeP=Selenium+ *Pseudomonas fluorescens*, S100=Salinity 100mM NaCl, S200= Salinity 200mMNaCl)

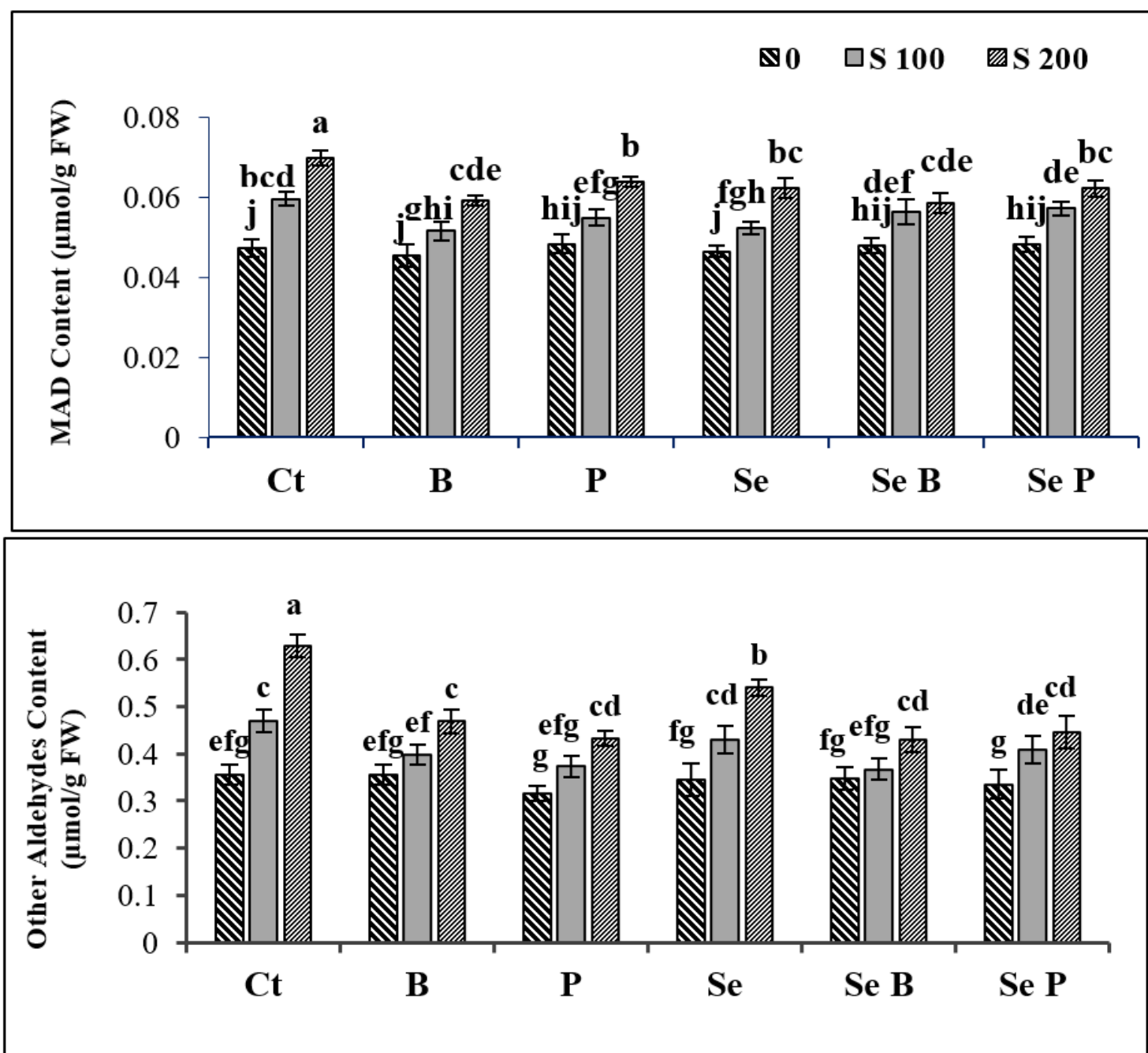


**Figure 2**

Effect of Se and PGPRs pre-treatment on MDA and other aldehydes in foxtail millet under control and salinity stress. Each value represents the mean  $\pm$  SEM. Means were compared using Duncan's multiple range tests. Different letters indicate significant differences among treatments ( $p \leq 0.05$ ). (Ct=Control, B=*Bacillus cereus*, P= *Pseudomonas fluorescens*, Se=Selenium, Se B=Selenium+ *Bacillus cereus*, Se P=Selenium+ *Pseudomonas fluorescens*, S100=Salinity 100mM NaCl, S200= Salinity 200mMNaCl)

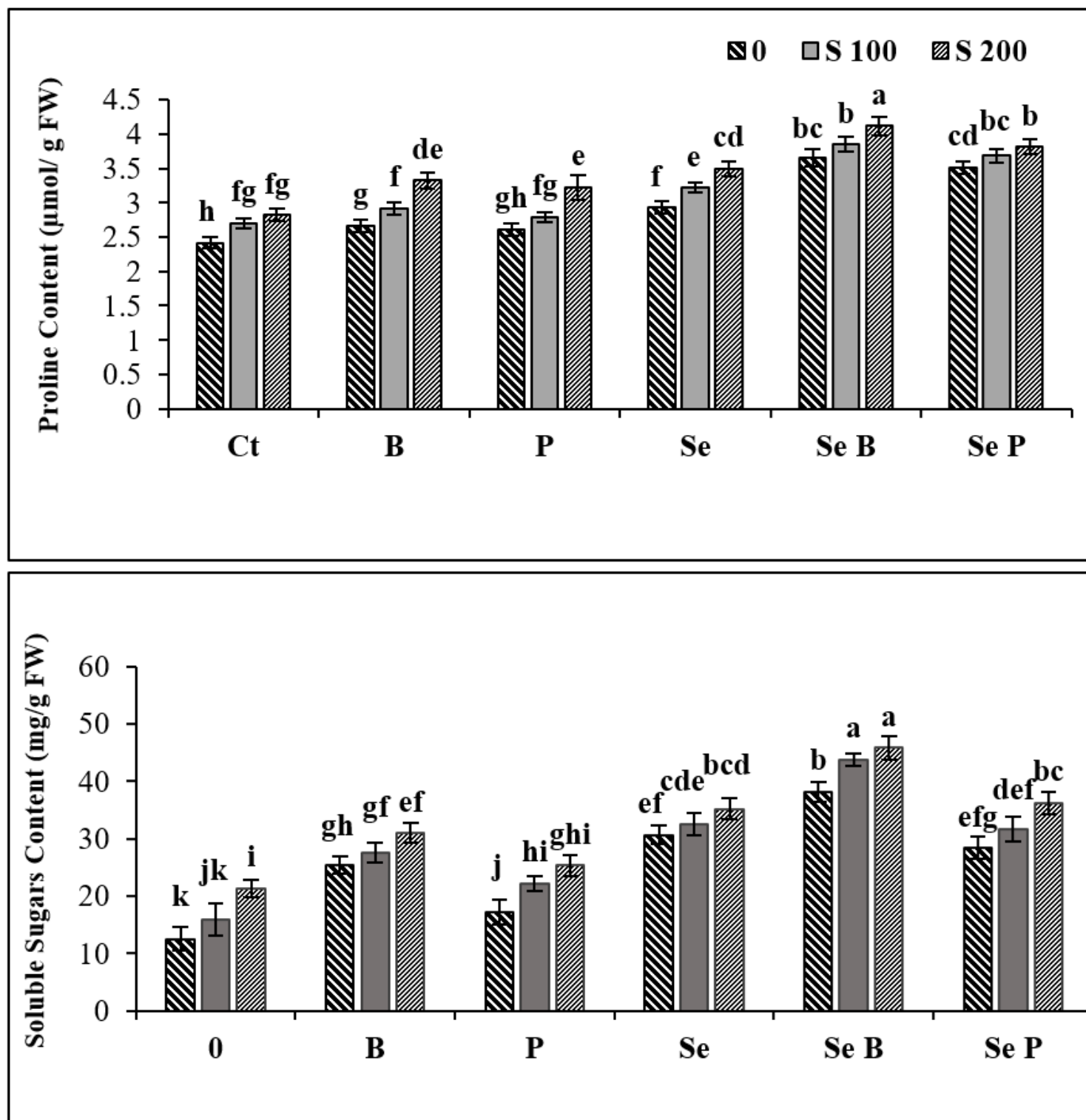


**Figure 3**

Effect of Se and PGPRs pre-treatment on proline and total soluble sugars of foxtail millet plants under control and salinity stress. Each value represents the mean  $\pm$  SEM. Means were compared using Duncan's multiple range tests. Different letters indicate significant differences among treatments ( $p \leq 0.05$ ). (Ct=Control, B=*Bacillus cereus*, P= *Pseudomonas fluorescens*, Se=Selenium, Se B=Selenium+ *Bacillus*

cereus, Se P=Selenium+ *Pseudomonas fluorescens*, S100=Salinity 100mM NaCl, S200= Salinity 200mMNaCl)

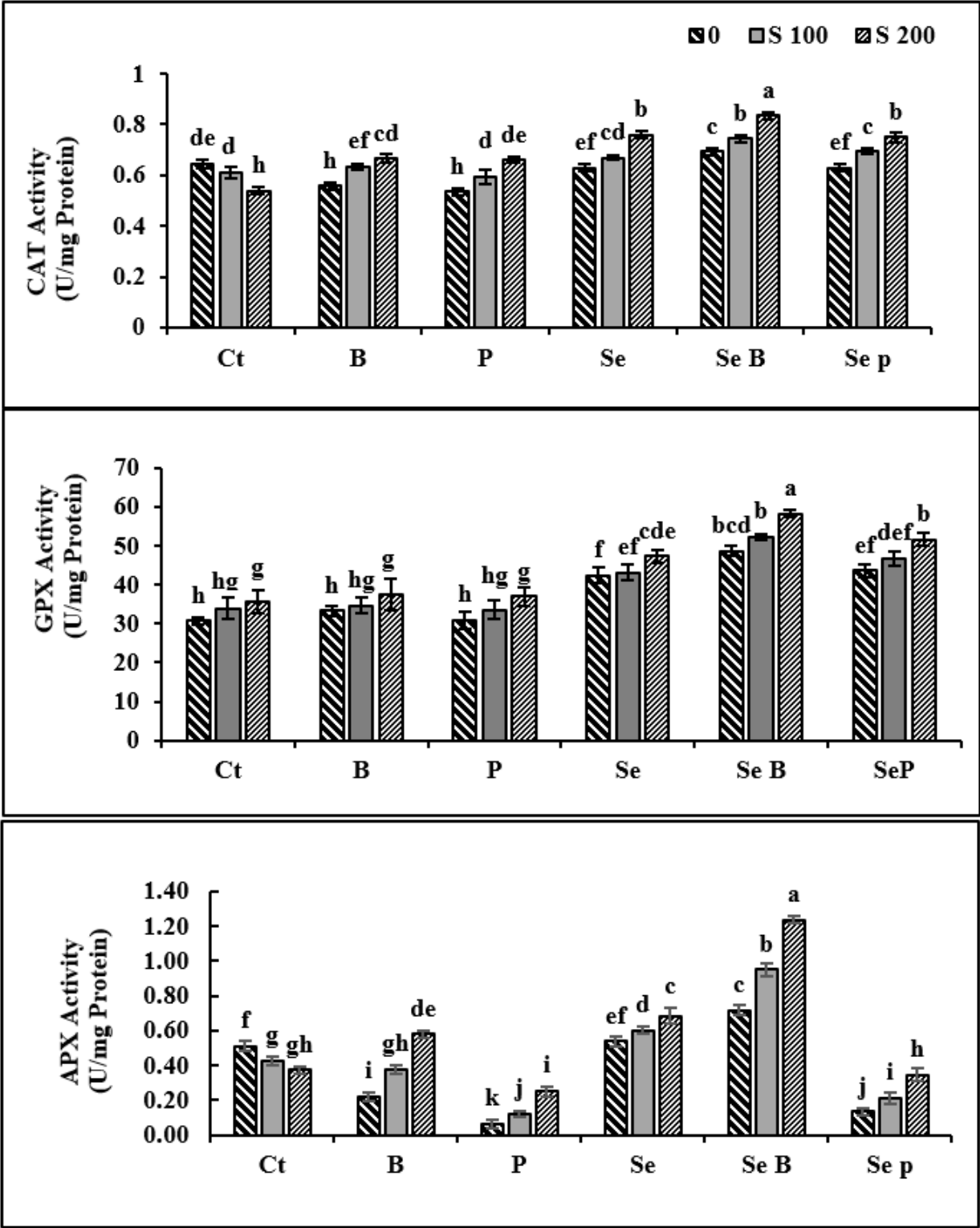


Figure 4

Effect of Se and PGPRs pre-treatment on protein content and antioxidant enzymes activity in foxtail millet plants under control and salinity stress. Each value represents the mean  $\pm$  SEM. Means were compared using Duncan's multiple range tests. Different letters indicate significant differences among treatments ( $p \leq 0.05$ ). (Ct=Control, B=*Bacillus cereus*, P= *Pseudomonas fluorescens*, Se=Selenium, Se B=Selenium+ *Bacillus cereus*, Se P=Selenium+ *Pseudomonas fluorescens*, S100=Salinity 100mM NaCl, S200= Salinity 200mMNaCl)

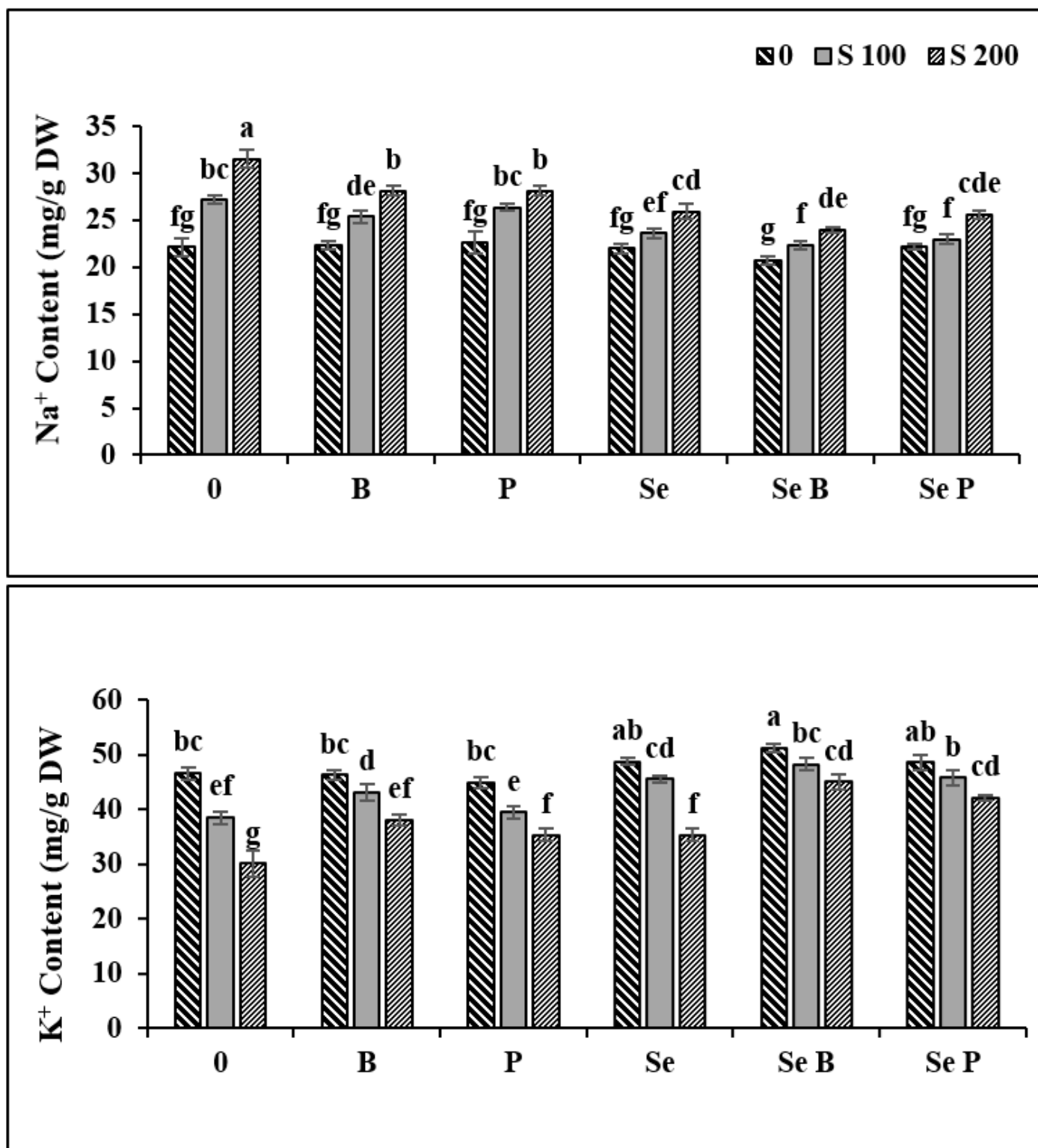


**Figure 5**

Effect of Se and PGPRs pre-treatment on Na<sup>+</sup> and K<sup>+</sup> content of leaves in foxtail millet plants under control and salinity stress. Each value represents the mean  $\pm$  SEM. Means were compared using Duncan's multiple range test. Different letters indicate significant differences among treatments ( $p \leq 0.05$ ). (Ct=Control, B=*Bacillus cereus*, P= *Pseudomonas fluorescens*, Se=Selenium, Se B=Selenium+ *Bacillus*

*cereus*, Se P=Selenium+ *Pseudomonas fluorescens*, S100=Salinity 100mM NaCl, S200= Salinity 200mMNaCl)