The effects of plant growth promoting rhizobacteria on antioxidative activity in chickpea (*Cicer arietinum* L.) under salt stress

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**ABSTRACT**

In chickpea soil salinity is one of the most important factors affecting yield, nodulation and physiological events. Salinity affects the growth of salt sensitive varieties. The inoculation of plant growth promoting rhizobacteria (PGPR) allows to reduce the harmful effects of salinity. To prevent adverse effects of chickpea salinity, the effects of four bacteria (*Rhizobium ciceri, A-08, EB-80 and Isolate-30*) in root rhizosphere under controlled environmental growth conditions were studied. This study has shown that PGPRs play an important role in growth regulators for the positive development of plants under salt stress. It has been observed that these isolates, common in roots, are tolerant to salinity antioxidant activity and an increase in proline, MDA, APX, SOD and CAT concentrations were found under saline conditions when unvaccinated plants were compared with grafted plants. The results also suggested that inoculated PGPR strains can reduce salinity stress by increasing salt tolerance.

**Key words:** Antioxidant enzyme activity, Chickpea, \( \text{H}_2\text{O}_2 \), PGPR, Salt stress.

**INTRODUCTION**

The cultivation of chickpea, its production and its efficiency have been known to decline considerably. In addition to the many reasons for this significant reduction in chickpea yield and quality, the most important of these is the undeniable fact that the chickpea vegetable is caused by biotic, drought and salinity factors encountered during augmentation and improvement. Soil salinity has been reported to reduce yields in legume plants. The chickpea is also very sensitive to salinity stress. Salinity stress also reduce photosynthetic capacity because of osmotic stress and part affinity of the stomat. Plants can also suffer from disorders of the metabolism and the intake of general nutrients (Han and Lee, 2005). Plants have various antioxidative enzymes (glutathione reductase, ascorbate peroxidase, superoxide dismutase, catalase and peroxidase) and molecules (ascorbate, glutathione, alpha-tocopherols and carotenoids) to remove the oxidative stress. These antioxidative systems can detoxify AOTs (Hussein *et al.*, 2008; Jamei *et al.*, 2009).

Antioxidant systems have been declared to have an important role in the defense mechanism in many studies made with various plants under salt stress (Koca *et al.*, 2007; Hussein *et al.*, 2008; Melchiorre *et al.*, 2009; Heidaria, 2010). In plants, the modulation and concentration of antioxidative enzyme activity is often accepted as an oxidative stress indicator. Plant cells produce both antioxidant enzymes and non-enzymatic antioxidants to maintain towards oxidative stress (Mittler, 2002). Aspartate peroxidase (APX), part of the cleansing cycle, reacts with ascorbic acid \( \text{H}_2\text{O}_2 \) and catalyses the regeneration of glutathione reductase ascorbic acid (Smirhoff, 1993).

Plant growth and yield can be affected by Plant growth promoting rhizobacteria (PGPR) with direct or indirect mechanisms. (Noel *et al.*, 1996). It is well known that plant growth promoting rhizobacteria have affirmative effect on plant growth and yields and improve endurance in plants antagonistic to abiotic and biotic stress circumstances (Reddy *et al.*, 2000; Kloepper *et al.*, 2004). The plant growth promoting rhizobacteria (PGPR) can produce plant hormones such as auxin, gibberellin and ethylene (Cakmakci *et al.*, 2006; Aslantas *et al.*, 2007), free or symbiotic N fixation (Sahin *et al.*, 2004; Cakmakci *et al.*, 2006). It has been reported that it can dissolve mineral phosphate and iron, and mineralize organic phosphate and other nutrients (Cakmakci *et al.*, 2007 a,b), reduce the significant effects of salt stress, antagonistic effects against pathogens by mechanisms such as synthesis of vitamins, siderophore, antibiotics, enzymes and fungicides or competition (Kotan and Sahin 2002; Akgül and Mirik 2008). PGPR strains can manufacture exploysaccharies (EPSs) to connect Na-having cations, and it may be envisaged that increasing the population density of EPS-producing bacteria in the root region would reduce the ingredient of Na present for plant uptake, thus help alleviating salt stress in plants growing in saline perimeter (Ashraf *et al.*, 2004).

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The objective of this study was to understand and examine the effect of PGPR alone and in combination with NaCl doses having both antioxidant enzyme and MDA and H$_{2}$O$_{2}$ activities in salinity stress on chickpea seedling grown in the greenhouse.

MATERIALS AND METHODS

Plant material: Chickpea cultivar Işık-05 was obtained from transitional zone of agricultural research institute, Eskisehir, Turkey.

Bacterial strains: The standard culture Rhizobium cicer was provided from the Soil and Fertilizer Research Institute, Ankara, Turkey. A-08 isolate was obtained from Prof. Dr. Metin TURAN, the Department of Genetics and Bioengineering, Faculty of Engineering, Yeditepe University, Istanbul, Turkey. EB-80 and Izolate-30 was obtained from Asst. Prof. Dr. Ahmet AKKOPRÜ, the Department of Plant Protection, Faculty of Agronomy, Yuzuncu Yil University, Van, Turkey. Bacteria were named as Rhizobium Cicer (B1), A-08 (B2), EB-80 (B3), Izolate-30 (B4).

Grown condition: Chickpea seeds were surface sterilized and treated with the bacterial cultures (108 cell Ml$^{-1}$). After the seeds germinate, the plants where watered with half-strength Hoagland’s solution and after thirty days passed, salt-treatment was initiated and the NaCl concentration (60 Mm (S1) ve 120 Mm (S2)) was upgraded gradually for 10 days. Plants without salt were considered as controls.

Enzyme extractions and assays: Prepared supernatant was used as a raw enzyme removal for SOD and CAT enzyme analyses. Catalase (CAT) activity (EC 1.11.1.6) was determined as a reducer in absorbance at 240 nm for 1-min, after the separation of H$_{2}$O$_{2}$. The reaction mixture (2.5 ml) contained 50 mM phosphate buffer (pH 7.0), 1.5 mM H$_{2}$O$_{2}$ and 0.2 ml raw enzyme extract (Jebara et al., 2005). Superoxide dismutase (SOD) activity was adjusted by the nitroblue tetrazolium (NBT) method (Rahnama and Ebrahimzadeh, 2005). Ascorbate peroxidase (APX) activity was determined after the decrease of ascorbate by measuring the change in absorbance at 290 nm for 1 min in 3 ml of a reaction mixture containing 50 mM KH$_{2}$PO$_{4}$ (pH 7.0), 1 mM EDTA-Na2, 0.5 mM ascrobic acid, 1.5 mM H$_{2}$O$_{2}$ and 0.2 ml of enzyme extract (Sairam et al., 2005). The levels of lipid peroxidation were measured in terms of malondialdehyde (MDA) content, a product of lipid peroxidation. The rate for non-specific absorption at 600 nm was extract. The MDA equivalent was calculated (Sairam and Saxena, 2000). The absorbancy of supernatant was read at 390 nm. The content of H$_{2}$O$_{2}$ was given on a standard curve (Velikova et al., 2000).

Statistical analysis: The results obtained from the study were subjected to analysis of variance using the SPSS 18.0 statistical program. Duncan’s multiple range test was employed to distinguish differences between treatments.

RESULTS AND DISCUSSION

The effects of salt stress applied to bacterial inoculated chickpea on superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), melondialdehyde (MDA) and hydrogen peroxide (H$_{2}$O$_{2}$) levels are given in Fig 1 (a, b, c, d, e). SOD, CAT, APX activities and MDA and H$_{2}$O$_{2}$ contents were measured to determine the oxidative stress response caused by salt in the leaves of chickpea cultivated by 0-60-120 mM NaCl application. MDA content in bacterial treated seed was detected under saline or non-saline condition. Bacterial applications caused significant positive changes in MDA content. Under salt stress condition, the highest MDA content was measured in Control-S2 and B2-S2, lowest in B4-S0. When salt stressed groups were examined, the amount of MDA in other bacterial groups was reduced by 17.62% to 9.47% compared to the control-120 mM salt-stressed plants, except for B2-S2 application. The MDA content in inoculated plants was also significantly lower than the control plants under salt stress (Fig 1b).

Salt stress significantly increased H$_{2}$O$_{2}$ ingredient in the leaves of chickpea after all treatments. Bio-priming significantly decreased H$_{2}$O$_{2}$ content in salt-stressed leaves. Hydrogen peroxide content in plants varied between 8.58-4.18 μmol g$^{-1}$ f.w. The content of H$_{2}$O$_{2}$ in Control-S2 application increased by 46.17% when compared to plants in B3-S2. In the other bacteria + S2 treated groups, except for B2 application, H$_{2}$O$_{2}$ accumulation decreased by 30.79% and 25.26% when compared to control-S2 plants. The activity of APX varied between 4.26-1.77 mmol g$^{-1}$ f.w / min$^{-1}$ in chickpea grown under salt stress of the PGPR bio-priming application. The APX activity of B2-S2 plants increased by 63.85% when compared to Control-S2. Also, B3-S1 treatment increased APX activity by 88.48% when compared to control-S1. CAT activity in chickpea grown under salt stress varied between 2.86-0.97 mmol g$^{-1}$ f.w / min$^{-1}$. The CAT activity of B4-S2 plants increased by 34.27% compared to control-S2. Bio-priming treatment increased catalase activity when compared to the control group in chickpea plants under salt stress. The highest catalase activity was 2.86 mmol g$^{-1}$ f.w / min$^{-1}$ in B4-S2 application. SOD activity in chickpea grown under salt stress varied between 0.60-0.26 Unite mg$^{-1}$ f.w. The SOD activity of B3-S2, B2-S2 and B1-S2 plants increased about 30.44% when compared to control-S2. Bio-priming treatment increased catalase activity when compared to control group in chickpea plants under salt stress. Bacteria application were induced to impose significantly positive changes in APX, CAT, SOD enzyme activity (Fig 1 c,d,e).

In agricultural crop productivity a major abiotic stress is soil salinity which have a considerable effect on plant growth and development (Liang et al., 2018). The first stress that arises when the plant is subjected to saline soil is...
the osmotic stress. It has been shown in this study that PGPR can have a positive regulatory role in salt stress. Molecular oxygen tends to form reactive oxygen species (ROS) at high levels. ROS are originated under salinity stress because of electrons leak (Farooq et al., 2017). Reactive oxygen species resulting from stress conditions are removed from by enzymatic and nonenzymatic antioxidants (Turhan and Emekci, 2008). Like SOD, CAT, APX all stress related enzymes of activity was by stages increased with the increment level of the NaCl concentration. This antioxidant enzymes are considered to be important in removing $\text{H}_2\text{O}_2$ from salt stressed roots role (Kim et al., 2005).

These results showed that bio-priming with PGPR can enhance the activity of SOD, APX and CAT in chickpea, thereby reducing the toxic effects of ROS. Similar results where reported in canola by inoculation HSNJ4 salt stress conditions. In their work they stated that inoculation with HSNJ4 could increase the SOD, POD and CAT activity in canola (Li et al., 2017). Superoxide dismutase (SOD) is the first defence mechanism of the plant antioxidant enzyme system and it can remove excess superoxide anions in the cells. The specific activity of SOD in potato plants vaccinated with PGPR, which grows under abiotic stress conditions, has significantly increased compared to unvaccinated plants under the same stress conditions (Gururani et al., 2013). Application of PGPR increased the activity of certain of antioxidative enzymes in rice combined with the stresses of NaCl and high boron (Khan et al., 2016). Produced $\text{H}_2\text{O}_2$ is separated from water and oxygen by the effect of CAT enzyme activity (Lata and Prasad, 2011). Decreased CAT activity under stress conditions has been reported in other plants, such as sunflower; this reduction is parallel to a rise in the $\text{H}_2\text{O}_2$ content (Younesi and Moradi, 2014). Our results show that CAT activity is $2.13 \text{ mmol g}^{-1}\text{ f.w }/\text{min}^{-1}$ in Control-S2, which has the highest amount of $\text{H}_2\text{O}_2$, while CAT activity is $2.86 \text{ mmol g}^{-1}\text{ f.w }/\text{min}^{-1}$ in B4-S2, and while the amount of $\text{H}_2\text{O}_2$ is $6.85 \text{ ìmol g}^{-1}\text{ f.w}$. In some studies, such as in our study, PGPR strains caused a higher increase in antioxidant enzymes in response to severe salinity (Younesi and Moradi, 2014).

Means with the same letter are not significantly different at P<0.05 when compared by Duncan. **Fig 1:** (a-e). PGPR effect on $\text{H}_2\text{O}_2$ (a), MDA (b), APX (c), CAT (d) and SOD (e) activity of chickpea leaves.
significantly inhibits the increase in MDA content, typically stimulated by salt stress.

CONCLUSION
Our results establish a direct relationship that exists between salt stress-induced oxidative damage and the efficiency of the PGPR to regulate the plant antioxidant enzymes. PGPRs, which we have used to reduce the harmful effects of salt stress on chickpea plant, all have had a positive impact on this harm prevention. The results of this study showed that PGPR may serve as a useful tool for alleviating salt stress in chickpea plant.

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