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Could effective microorganisms improve tolerance of UCB1 pistachio to salinity?

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Abstract

Salinity is one of the most important limiting factors for plant growth which can reduce its undesirable effects through stress modulators. In order to investigate the effect of effective microorganisms (EM) on morphological indices and activity of some enzymes in UCB1 rootstock pistachio under salinity stress, a factorial experiment was conducted as a randomized complete blocks design. Factors included were salinity stress at four levels (0.7, 5, 10 and 13.6 dS m$^{-1)}$ and EM at two levels (0, 1%) with three replications. Treatments were applied for three months on plants. The results showed that increasing salinity decreased seedling height, leaf number, stem and root fresh and dry weight, but the activity of (CAT), peroxidase (POD) and ascorbate peroxidase (AXP) enzymes increased compared to control treatment, whereas EM application showed that concentration of 1% of this fertilizer increased the measured morphological indices and decreased the activity of CAT, POD and AXP enzymes. Interaction effect between salinity and EM was significant only on morphological indices. According to the results, it can be stated that application of EM could reduce adverse effects of salinity stress on the UCB1 rootstock.

Keyword: beneficial microorganisms, enzyme activity, morphology, saline water

Introduction

Pistachio it is one of the most important horticultural crops of Iran which widely cultivated in Kerman, Yazd and Khorasan province. Most of Iran's pistachio orchards are located in arid and semi-arid climates. Salinity is a main limiting factor for crop production in these areas. Salinity stress has been reported to affected many plant growth characteristics, mineral elements, physiological and biochemical factors and enzymes activity (Parida & Das, 2005; Kamiab et al., 2012). Karimi et al. (2012), Saadatmand et al. (2007) and Picchioni et al. (1990) reported that salinity decrease growth indices in pistachio rootstocks.

The UCB1 rootstock introduce in the University of California-Berkeley, from a controlled cross between a *Pistacia atlantica* (female) and *Pistacia integerrima* (male). From characteristics of this rootstock include is high vegetative growth rate, resistance to disease Verticillium, early yield, moderate tolerance to cold and salinity (Ferguson et al., 2005).

The meaning of EM (effective microorganisms) is based on the inoculation of mixed cultures of effective microorganisms into the soil where creates it favorable environment to plants growth (Higa &Wididana., 1991). The major species existing in EM consist photosynthetic bacteria, yeasts, lactic acid bacteria, fermenting fungi and actinomycetes (Higa, 2000). The various species of organisms in EM complement each other and have a useful interaction with the roots of plants in the soil (Sun et al., 2014).

The use of EM in agriculture has many benefits, including: improve soil structure, accelerate the decomposition of organic matter in the soil, reduce environmental pollution, increase plant photosynthetic capacity, enhances nutrient uptake, increase the yield and quality of the crops (Javaid & Bajwa, 2011; Joshi et

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al., 2019). Shokouhian et al. (2013) indicated that the application of effective microorganisms cause increasing plant growth, leaf number, leaf area, leaf fresh and dry weight and nitrogen, Phosphorus and potassium contents in leaves of almonds in water stress conditions .Also, the results of Aref-Poorian et al. (2014) on pomegranate (*Punica granatum* L.) showed the measured parameters include main stem height, shoot fresh weight, root dry weight, root length, relative water content of leaf, chlorophyll index and potassium content were significantly higher in EM treatment than the no EM treatment in salty conditions. Application of EM as anti-salinity agent was effective to reduce the adverse effect of salinity on yield and fruit quality of date palm cultivars as compared to the control treatment (El-Khawaga, 2013).

The aim of present study was to evaluate the effect of EM biofertilizer on morphological parameters and activity of antioxidant enzymes in leaves UCB1 pistachio rootstock under salinity stress.

Material and Methods

In order to investigate the role of EM in reducing the damages caused by the use of saline water in UCB1 rootstock pistachio, a pot experiment was carried out in one the areas Khorasan Razavi Province as one of the main important pistachio production provinces in Iran. The environmental conditions during the experimental period were as follow: average temperature (27.6 °C), mean relative humidity (27 %), average rainfall (3.9 mm) and evaporation value (531.35 mm). UCB1 tissue culture rootstocks were prepared and transferred to pots with the span diameter of 30 cm and the height of 40 cm containing soil composition the specified in Table 1 .The experiment was conducted as a factorial arrangement in a randomized complete block design with three replications. The experimental treatments included two levels of EM (0 and 1%) and four salinity levels (0.7 (control), 5, 10 and 13.6 dS m^{-1}). Salinity stress was applied from June, after the complete establishment of the plants for three months. Plants that were more uniform in size were selected for treatment. The height of the plants at the beginning of treatment was about 35 cm. The used EM was a mixture of beneficial microorganisms. The main microbial species are included lactic acid bacteria (*Lactobacillus plantarum, Lactobacillus casei* and *Streptococcus lactis*), photosynthetic bacteria (*Rhodopseudomonas palustris* and *Rhodobacter spaeroides*), yeasts (*Saccharomyces cerevisiae* and *Candida utilis*), actinomycetes (*Streptomyces albus* and *Streptomyces griseus*) and fermenting fungi (*Aspergillus oryzae* and *Mucor hiemalis*). To determine the salinity concentrations, saline water with a salinity of 13.6 dS m^{-1} with chemical characteristics (Na^{+} = 99; Mg^{2+} = 12; Ca^{2+} = 25.5; Cl^{-} = 88; So_4^{2-} = 47.2; Hco_3^{-} = 1 meq L^{-1}) was prepared from agricultural well and two other levels salinity were diluted from that water. In order to prevent osmotic shock of plants, salinity levels were gradually applied. At each time of irrigation, 1% EM solution was prepared and given to 50% of the pots with irrigation water. Also, salinity stress at different levels concurrently with EM treatment was used to irrigate the plants. The EC measurement of the drain pots was carried out regularly. In the end of experiment, the following parameters were measured.

Table1. Physical and chemical characteristics of the soil used in the experiment.

Physical attributes	Values	Chemical attributes	Values
Clay (%)	23	PH	7.9
Silt (%)	64	EC (ds m^{-1})	2.15
Sand (%)	13	Organic C (%)	0.56
Soil texture	Silt loam	N (%)	0.04
FC (%)	21	P (mmol dm^{-3})	0.28
SP (%)	39.5	K (mmol dm^{-3}	7.5

FC = Field capacity; SP = Saturation percentage

Growth parameters

Growth parameters including plant height, root length, leaf number, fresh and dry weight of stems and roots were measured. Plant height and root length was measured using ruler. Fresh and dry weight of stems and roots were measured by a sensitive scale. To drying, the samples were placed in an oven at 70 ° C.

Measurement of enzymes

Activity of three enzymes of catalase, peroxidase and ascorbate peroxidase was measured by the methods Chance & Maehly (1955) and Nakano & Asada (1981).

Statistical Analysis

Statistical analysis was carried out using SAS statistical software. Duncan's multiple-range test was used to compare the means.

Results and Discussion

Growth parameters

The effect of salinity levels on plant height, leaf number, root length, fresh and dry weight of the stems and roots were significant ($P \leq 0.01$). The effect of EM treatment was significant on plant height, number of leaves, fresh and dry weight of stems and roots ($P \leq 0.01$). Also, interactions between salinity levels and EM was significant on plant height, root length, leaf number, fresh and dry weight of stems ($p \leq 0.01$) and fresh and dry weight of the roots ($p \leq 0.05$).

The results showed that the maximum of plant height (77.46 cm), root length (49.96 cm), stem fresh weight (58.87 g), stem dry weight (27.18 g), root fresh weight (28.27 g), root dry weight (11.42 g) and number of leaf (18.66 units) in plants treated with EM and irrigation with control level (0.7 dS m^{-1}) was obtained, while at high salinity level (13.6 dS/m) and without the use of EM, the lowest amount of plant height (41.2 cm), root length (23.13 cm), stem fresh weight (22.77 g), stem dry weight (10.88 g), root fresh weight (10.84 g) and number of leaf (0.66 units) were observed (Figure1). Application of EM at down salinity levels increased growth parameters compared to non-EM treatment (Figure1). Adverse effects of salinity on vegetative parameters of pistachio have been reported (Karimi et al., 2009; Shahriaripour et al., 2010; Razavi Nasab et al., 2014). Salinity stress limits plant growth through osmotic and toxic effects and upsetting the nutrient balance. In salinity conditions, the amount and activity of growth hormones such as auxin, gibberellin and cytokinin and other growth stimulants are reduced, while growth inhibitors such as abscisic acid increase, which generally these changes reduce plant growth (Javid et al., 2011). Neumann (1997) reported that salinity caused to reduce root growth and its capacity to water uptake and essential nutrients. The reduction in stem dry weight with increasing the salinity can be related to reducing the number of leaves, smaller leaf area and thus reducing the photosynthesis.

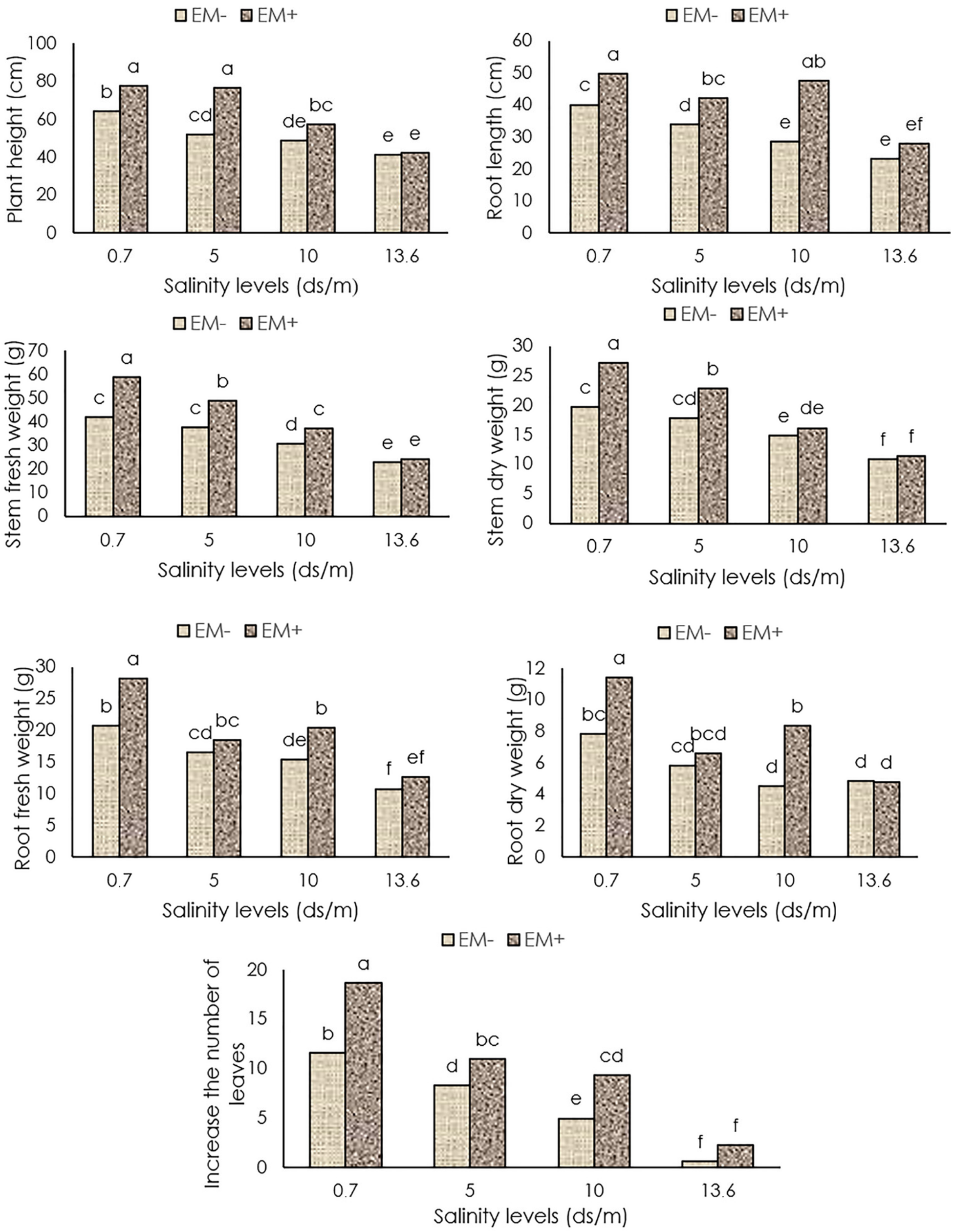

Figure1. Interaction effect of salinity and EM on plant height, root length, leaf number, fresh and dry weight of the roots and stems of UCB1 rootstock.

EM shown beneficial effects on growth parameters of plants .The results presented are in agreement with findings by Aref-Pourian et al. (2014) on pomegranate, Glinicki et al. (2011) on strawberry and Eissa (2003) on plum. EM increased the growth of *Albizia saman* seedlings in nursery (Khan et al., 2006).

Application of EM in the pigweed plant (*Amaranthus dubians*) increased the shoot height, stem diameter, leaf area, leaf number, leaf and root fresh and dry weights. Increasing the number of leaves leads to increased photosynthetic activity in plants. Furthermore, EM increased chlorophyll a and b content in plants (Muthaura et al., 2010). Also, Lim et al. (1999) reported that effect of EM is due to increasing the number of beneficial microorganisms in the soil and increasing the content of available nutrients. Hormones produced by effective microorganisms such as auxin and cytokinin stimulated root activity (Yamada & Xu, 2001; Martin et al., 1989), this increased the levels of root hairs, absorption nutrients from the soil and thereby enhance growth of roots and shoots (Jagnow et al., 1991). Some PGPR (plant growth promoting rhizobacterial) can reduce stress-induced ethylene through the mechanism of production of the enzyme ACC deaminase, thereby increasing plant growth under saline conditions (Mayak et al., 2004).

It seems that the microorganisms present in EM could with these methods reduction the adverse effects of salinity stress on plant growth.

Activity of Antioxidant Enzymes

The effect of salinity levels and EM on the activity of catalase (CAT), peroxidase (POD) and ascorbate peroxidase (APX) enzymes was significant ($P \leq 0.01$). Interaction effect between salinity and EM on the activity of CAT, POD and APX enzymes was not significant. Salinity stress caused a significant increase in the activity of CAT, POD, APX enzymes, while application EM significantly decreased activity of CAT, POD, APX enzymes (Figure 2).

When plants are exposed to salinity stress, the morphological, physiological and biochemical responses of plants change (Amirjani, 2010). Such responses include changes in morphology, photosynthesis, water relations, non-enzymatic and enzymatic components (Acosta-Motos et al., 2017). Our findings are in agreement with results of (Raoufi et al., 2020; Bagheri et al., 2019; Tavallali et al., 2010) on pistachio, (Sorkheh et al., 2012) on Wild almonds (Bharti et al., 2014) on Mentha and (Kang et al., 2014) on cucumber that reported antioxidant enzymes activity increased with increasing salinity.

Reactive oxygen species (ROS) are produced in both stress and non-stress conditions, but under stress conditions the balance between production and elimination is disturbed (Karuppanapandian et al., 2011). When ROS increases in response to saline stress, plants for confronting with ROS use an effective antioxidant defense system include non-enzymatic and enzymatic components (Demiral & Turkan, 2005). Enzymatic antioxidants include superoxide dismutase (SOD), peroxidase (POX), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR) that detoxify ROS (Chawla et al., 2012).

EM treatment decreased the activity of CAT, POD and APX enzymes in plant leaves (Figure2). The results presented here are in agreement with decreased activity of CAT and POD enzymes in Barley Inoculation with *Azospirillum brasilense* under salt stress (Omar et al., 2009), reduced activity of antioxidant enzymes in wheat (Upadhyay et al., 2012) and paddy (Jha & Subramanian., 2014)l under salinity stress by PGPR , decreased activity of CAT and POD enzymes in Cucumber (Kang et al., 2014) and effect of Bio-chemical fertilizer on CAT and POD enzymes activity in leaves of wheat under drought stress (Jawad et al., 2015). Also, EM application significantly reduced the activities of diamine oxidase and polyamine oxidase in common bean (*Phaseolus vulgaris* L.) plants grown under saline conditions (Talaat, 2015). Probably, effective microorganisms due to water relationships and better nutrition of plants have been able to reduce the effect of stress on the plant and make the plant in better condition and consequently, the plant the amount of antioxidant enzymes (CAT, POD and AXP) produced less.

Conclusions

Increasing level of salinity, the plant height, number of leaves, fresh and dry weight of stems and roots reduced and CAT, POD and AXP enzymes activity in UCB1 rootstock increased.

The application of EM increased growth parameters and decreased CAT, POD and AXP enzymes activity in UCB1 rootstock under salinity stress. Use of effective microorganisms is one of the new solutions to reduce the negative effects of environmental stresses such as salinity. Adding effective microorganisms to the soil increases the diversity of soil microorganisms and not only improves nutrient availability to the plant, stimulates plant growth by producing hormones such as cytokinins, gibberellins, etc. The EM combination reduce the effect of stress on the plant and make the plant in a better condition and consequently, the plant produces less antioxidant enzymes. Based on the current results, the use of EM can reduce adverse effects of salinity stress on the UCB1 rootstock.

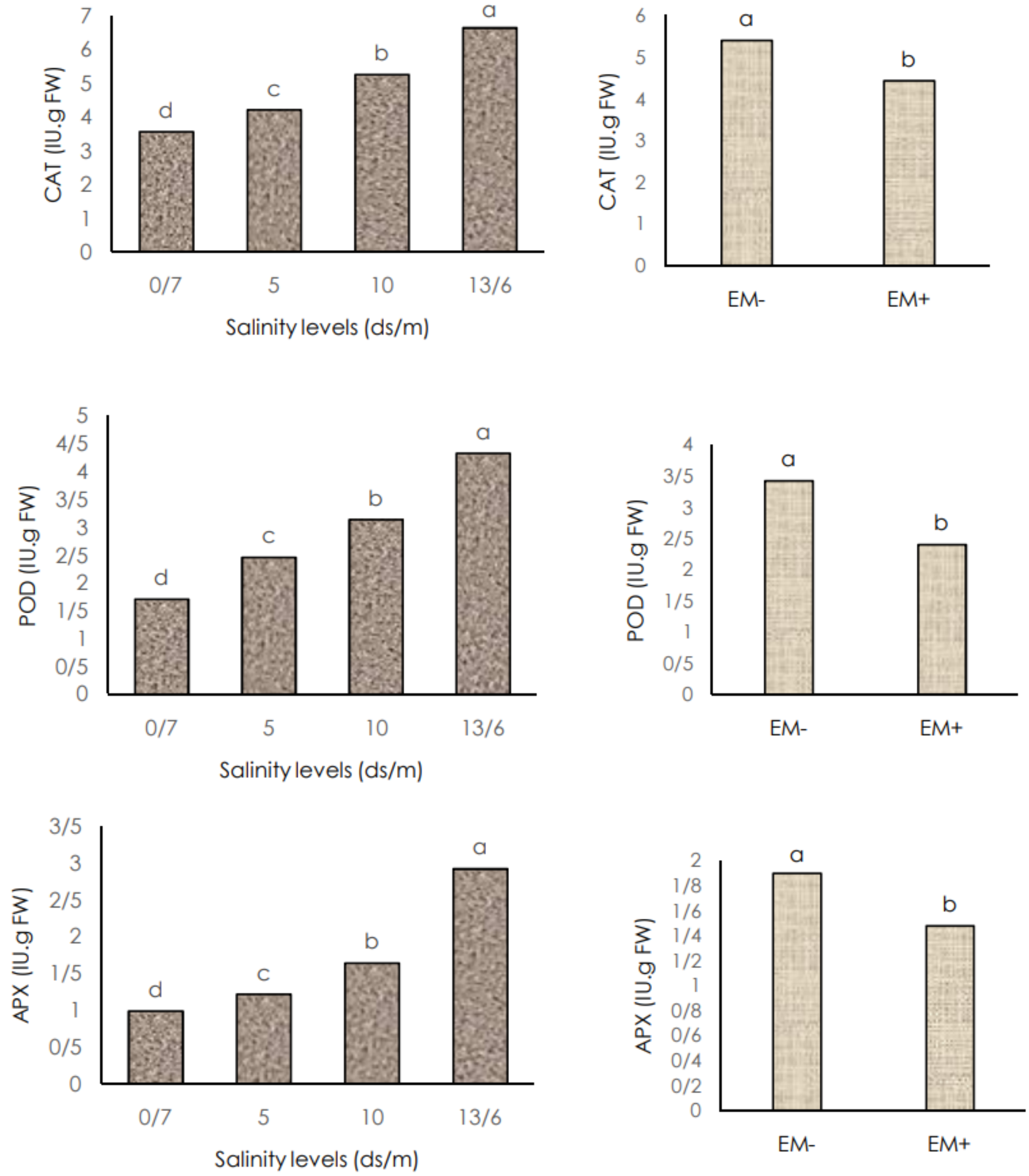

Figure2. Effect of salinity levels and EM on activity of catalase (CAT), peroxidase (POD) and ascorbate peroxidase (APX) of UCB1 rootstock.

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