Microbial cooperation in the rhizosphere improves liquorice growth under salt stress

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Abstract

Liquorice (*Glycyrrhiza uralensis* Fisch.) is one of the most widely used plants in food production, and it can also be used as an herbal medicine or for reclamation of salt-affected soils. Under salt stress, inhibition of plant growth, nutrient acquisition and symbiotic interactions between the medicinal legume liquorice and rhizobia have been observed. We recently evaluated the interactions between rhizobia and root-colonizing *Pseudomonas* in liquorice grown in potting soil and observed increased plant biomass, nodule numbers and nitrogen content after combined inoculation compared to plants inoculated with *Mesorhizobium* alone. Several beneficial effects of microbes on plants have been reported; studies examining the interactions between symbiotic bacteria and root-colonizing *Pseudomonas* strains under natural saline soil conditions are important, especially in areas where a hindrance of nutrients and niches in the rhizosphere are

high. Here, we summarize our recent observations regarding the combined application of rhizobia and Pseudomonas on the growth and nutrient uptake of liquorice as well as the salt stress tolerance mechanisms of liquorice by a mutualistic interaction with microbes. Our observations indicate that microbes living in the rhizosphere of liquorice can form a mutualistic association and coordinate their involvement in plant adaptations to stress tolerance. These results support the development of combined inoculants for improving plant growth and the symbiotic performance of legumes under hostile conditions.

Introduction

Salt-affected arid lands negatively impact agricultural production and the livelihood of rural populations in many regions of the world, making them a major environmental problem. Phytoremediation of saline soils with nitrogen-fixing legumes such as liquorice has the potential to become a promising technique for the restoration of soil fertility because legumes can increase nitrogen content, improve organic matter quality, stimulate biological activity, and improve the water-holding capacity of soils 1. In regards to their medicinal use, liquorice roots have been used since ancient times for the treatment of lung diseases, cardiovascular ailments, gastrointestinal disease, and various infection illnesses 2,3,4. Essentially, salinity (i) inhibits root development, thus decreasing the ability of plants to absorb soil minerals, and (ii) affects rhizobia - host symbioses and the process of nodule initiation 5,6,7. Plants rely on their microbiome for specific traits and activities, including growth promotion, nutrient acquisition, induced systemic resistance, and tolerance to abiotic stress factors 8,9. Living in the rhizosphere or inside plants, microbes deliver metabolites that can be used as nutrients for partner organisms and are beneficial for plant growth 10. We recently showed that liquorice tolerates salt stress under conditions of 75 mM NaCl, but nodulation is inhibited. The disturbance in nodule formation was linked to a reduced population size and survival rate of rhizobia in the rhizosphere of liquorice. However, combined inoculation with P. extremorientalis and the symbiotic Mesorhizobium significantly enhanced plant growth, nutrient acquisition, and nodulation compared to inoculation with the rhizobial strain alone. Synergistic associations between root-colonizing beneficial bacteria and rhizobia are beneficial to plants by synthesizing biologically active compounds, such as phytohormones, antifungal compounds, osmoprotectants and siderophores, thus improving the availability of nutrients to plants and inducing a plant defense against various stress factors, including drought and salinity 11,12. Despite many reports on the growth, nutrition and physiology of liquorice, studies concerning the associated microbes in the rhizosphere, particularly the physiological interactions between the host and microbes under natural field conditions, are not yet understood [13]. This knowledge is important for our understanding of the relationship between root-colonizing bacteria, rhizobia and their hosts under hostile environments, such as salinization, and for developing the best management practices for restoring degraded lands. In this Addendum, we provide an addition to our observations on the beneficial synergistic effects of rhizobia and root-colonizing bacteria on *Glycyrrhiza uralensis*, their potential to improve the stress tolerance of the plant and their symbiotic performance under salt-affected arid field conditions.

Plant growth and nutrient acquisition

We recently evaluated the effect of dual inoculation of liquorice with the *Mesorhizobium* sp. strain NWXJ31 and root-colonizing *Pseudomonas extremorientalis* strain TSAU20 on the growth, nodulation and N uptake of salt-stressed liquorice grown in potting soil [14]. Here we report that also under open-field conditions in Uzbekistan, the growth and nutrient acquisition of liquorice responded positively to microbial inoculation in saline soil. The soil was selected from salt-affected fields with an electrical conductivity of 7.9 dS m^{-1}, Nt 0.01, P 1.2, Ct 0.25, Mg 21.0, Ca 63.5, K 6.2, Cl 0.1 g/kg. Bacterial inoculants were prepared, and the germinated seeds were inoculated by immersing seeds in cell suspensions, as described by Egamberdieva et al. [14]. The plants inoculated with *Mesorhizobium* sp. alone showed slower growth compared to co-inoculated plants. The shoot and root dry weights of the plants co-inoculated with *Mesorhizobium* sp. and *P. extremorientalis* were 27% higher compared to uninoculated plants (Fig. 1). A decline in plant growth regulators under salt stress has been reported [15], which has been shown to result in an inhibition of root growth, root hair formation and a disturbance of nutrient acquisition from soil. According to López-Bucio et al. 16, the modulation of the root system architecture by root-colonizing bacteria is related to the production of plant growth–regulating substances due to the extensive supply of substrates exuded from the roots. When colonizing the root system, the Pseudomonas strain produces the plant growth regulator auxin as well as fungal cell wall-degrading enzymes and may deliver metabolites directly to the rhizosphere of plants. The enlarged root system contains more available niches in the rhizosphere for the colonization by rhizobia 17.

These properties, either together or alone, may explain the capacity of the inoculant *P. extremorientalis* to improve root growth and nutrient acquisition. Generally, salt stress inhibits the acquisition of nutrients by plants, such as nitrogen (N), phosphorus (P), potassium (K), and magnesium (Mg), through an antagonistic relationship of sodium[18]. In our study, the combined inoculation of *Pseudomonas* with rhizobia resulted in higher N, P, K, and Mg contents in liquorice by 24, 19, 27, and 9%, respectively, indicating that increased nodulation and growth correlate with N acquisition (Fig. 2). Liu et al. [19] observed increased plant biomass and phosphorus concentrations in the leaves and roots of co-inoculated liquorice with AM fungi compared to uninoculated plants. In another study, dual inoculation with *Sinorhizobium ciceri* and phosphate-solubilizing *Pseudomonas* was reported to increase the concentration of P in chickpea [20]. The phosphate-solubilizing activity of *P. extremorientalis* TSAU20 was determined by the method of Sperber [21]; we found that the strain was able to solubilize phosphate, thus providing more phosphorus to liquorice.

Physiological parameters

Some reports have suggested that the mutualistic interaction between root-associated microbes modifies the physiological status of host plants and induced systemic tolerance (IST) in plants through elevated antioxidant responses 22.

Salt stress inhibits the production of photosynthetic pigments due to changes in the synthesis of chlorophyll-related proteins and therefore reduces plant growth and development 23. The chlorophyll contents in the leaves of liquorice as measured by the method of Richardson et al.24 showed that chlorophyll a and b were lower compared to plants inoculated with microbes (Table 1). Compared to single inoculation, the combination of P. extremorientalis TSAU20 and Mesorhizobium sp. NWXJ31 increased chlorophyll a and b content by 39 and 36%, respectively, which may be a mutual result of positive modifications in the plants. Enhanced chlorophyll synthesis as well photosynthetic electron transport due to Pseudomonas inoculation under water stress conditions has been reported for the medicinal plant Ocimum basilicum 25.

We also elucidated the salt stress defense and tolerance mechanisms of liquorice by the mutualistic interaction between Mesorhizobium sp. NWXJ31 and P. extremorinetalis TSAU20. The hydrogen peroxide (H_2O_2) content of leaf samples was measured as described by Mukherjee and Choudhuri 26. Uninoculated liquorice grown in saline soil contained a higher level of H_2O_2 in its leaves compared to inoculated plants. The enhanced membrane leakage by salt stress was reported to be correlated with H_2O_2 production, which causes a disturbance in cellular homeostasis 27. Plants co-inoculated with P. extremorientalis TSAU20 and Mesorhizobium sp. NWXJ31 showed a 35% reduction in H_2O_2 production, whereas single inoculation with NWXJ31 decreased H_2O_2 production only by 10% (Table 1). Our present observations support the mutualistic benefits of rhizobia and root-colonizing bacteria in protecting membrane lipids from peroxidation.

The antioxidant defense system in plants is important for counteracting salt stress-induced oxidative damage 22,23. The antioxidant enzymes in plant tissues, namely peroxidase (POD), catalase (CAT), superoxide dismutase (SOD) and glutathione reductase (GR), are also affected by bacterial inoculation under salt stress. The activities of SOD (EC 1.15.1.1) were determined by Giannopolitis and Ries 28; POD (EC 1.11.1.7) by procedures of Kar and Mishra 29; CAT (EC 1.11.1.6) as reported by Chance and Maehly 30; and GR (EC 1.6.4.2) as described by Carlberg and Mannervik 31. Antioxidant enzyme activities (SOD, POD, GR, and CAT) increased following the combined inoculation of liquorice with TSAU20 and NWXJ31 compared to single inoculation with NWXJ31 and uninoculated control plants. Dual inoculation led to significant increases in SOD and CAT activities (26% above untreated plants), whereas POD and GR activities were only slightly but not significantly improved. Similar observations have been reported for chickpea 32 and Indian mustard 27. It has been concluded that SOD reduces the formation of hydroxyl (OH-) radicals, and CAT mediates the quick removal of H2O2, thereby assisting in the normal functioning of membranes 33. An overview of the beneficial properties of PGPR on plant growth, symbiotic performance and stress tolerance is provided in Fig. 3.

Conclusion

As shown in our previous study, the combined inoculation of liquorice with P. extremorientalis TSAU20 and Mesorhizobium sp. mitigated salt stress and increased nitrogen acquisition as well as nodule numbers compared with single-inoculated liquorice. Our observations in this study indicate that root-colonizing Pseudomonas and symbiotic Mesorhizobium strains are both involved in promoting specific tolerance mechanisms in response to salt stress. Mutualistic interactions between microbes in the rhizosphere enhanced the activities of antioxidant enzymes SOD and CAT, thereby preventing reactive oxygen species (ROS)-induced oxidative damage. Furthermore, we showed enhanced plant growth, nutrient acquisition and the production of photosynthetic pigments of liquorice by combined inoculation under saline soil conditions. In conclusion, we suggest that the application of an efficient consortium of root-colonizing bacteria and rhizobia in the cultivation of liquorice under hostile environmental conditions may help farmers increase plant production in a sustainable way.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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Table 1. Effect of microbial inoculants on chlorophyll contents and hydrogen peroxide levels, as well as superoxide dismutase, peroxidase, glutathione reductase, and catalase activities in *Glycyrrhiza uralensis*. Values are shown as the mean ± S.E. (n = 5). Columns marked with an asterisk differed significantly from uninoculated plants at P < 0.05 (Student's *t* test).

Treatment	Chlorophyll *a*		Hydrogen	Superoxide dismutase (SOD)	Peroxidase (POD)	Glutathione reductase (GR)	Catalase (CAT)
Control	1.52 ± 0.61	0.52 ± 0.05	1.23 ± 0.21	12.12 ± 0.92	4.32± 0.34	60.3 ± 1.72	20.2 ± 0.72
NWXJ31	1.75 ± 0.42	0.63 ± 0.03	1.20 ± 0.42	13.0 ± 1.02	4.71± 0.73	64.4 ± 2.01	19.4 ± 1.02
NWXJ31+TSA U20	2.12 ± 0.70*	0.78 ± 0.04*	0.80 ± 0.11	15.23 ± 0.82*	5.0 ± 0.62	65.3 ± 1.52	25.6 ± 1.91*

Chlorophyll a, b (mg/g fresh weight); Hydrogen peroxide (µM/g fresh weight); Superoxide dismutase (EU/mg protein); Peroxidase (EU/mg protein); Glutathione reductase (EU/mg protein); and Catalase (EU/mg protein).

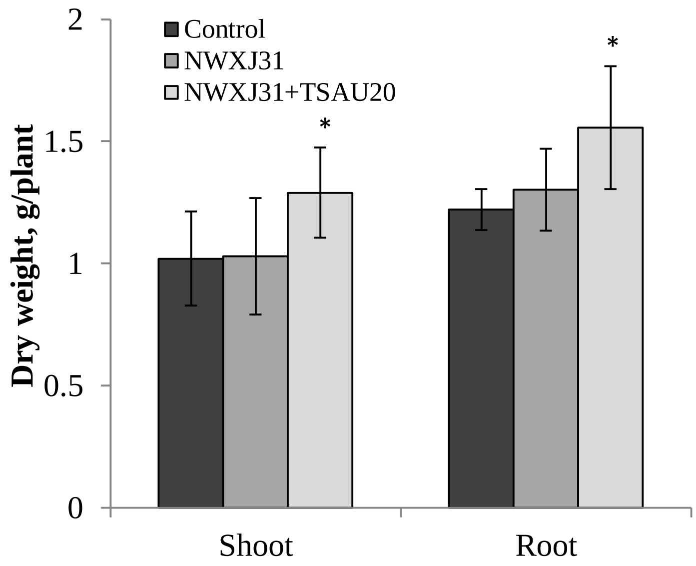

Figure 1. Effect of *P. extremorientalis* TSAU20 and *Mezorhizobium* sp. NWXJ31 inoculation on liquorice dry weight. Plants were grown under an open-field condition for three months in pots with saline soil. Columns represent the means of four plants (n = 5), with error bars showing standard deviation. Columns marked with an asterisk differed significantly from uninoculated plants at P < 0.05 (Student's *t* test).

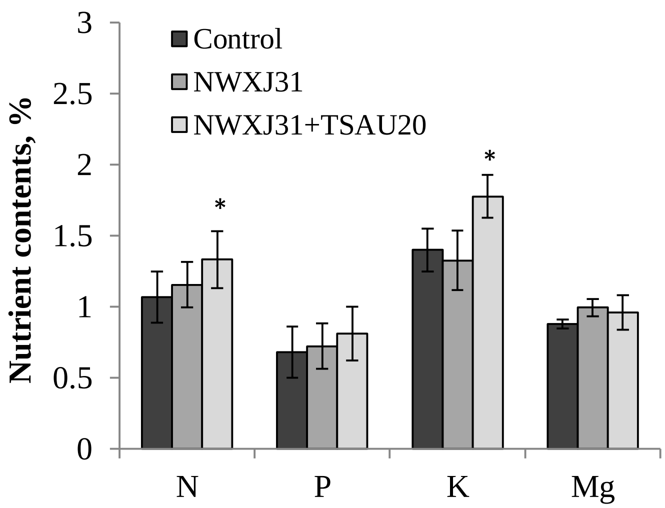

Figure 2. Effect of *P. extremorientalis* TSAU20 and *Mezorhizobium* sp. NWXJ31 inoculation on nitrogen (N), phosphorus (P), potassium (P), and magnesium (Mg) contents in liquorice. Plants were grown in open-field conditions for three months in pots with saline soil. Columns represent the means of four plants (n = 5), with error bars showing standard deviation. Columns marked with an asterisk differed significantly from uninoculated plants at $P < 0.05$ (Student's *t* test).

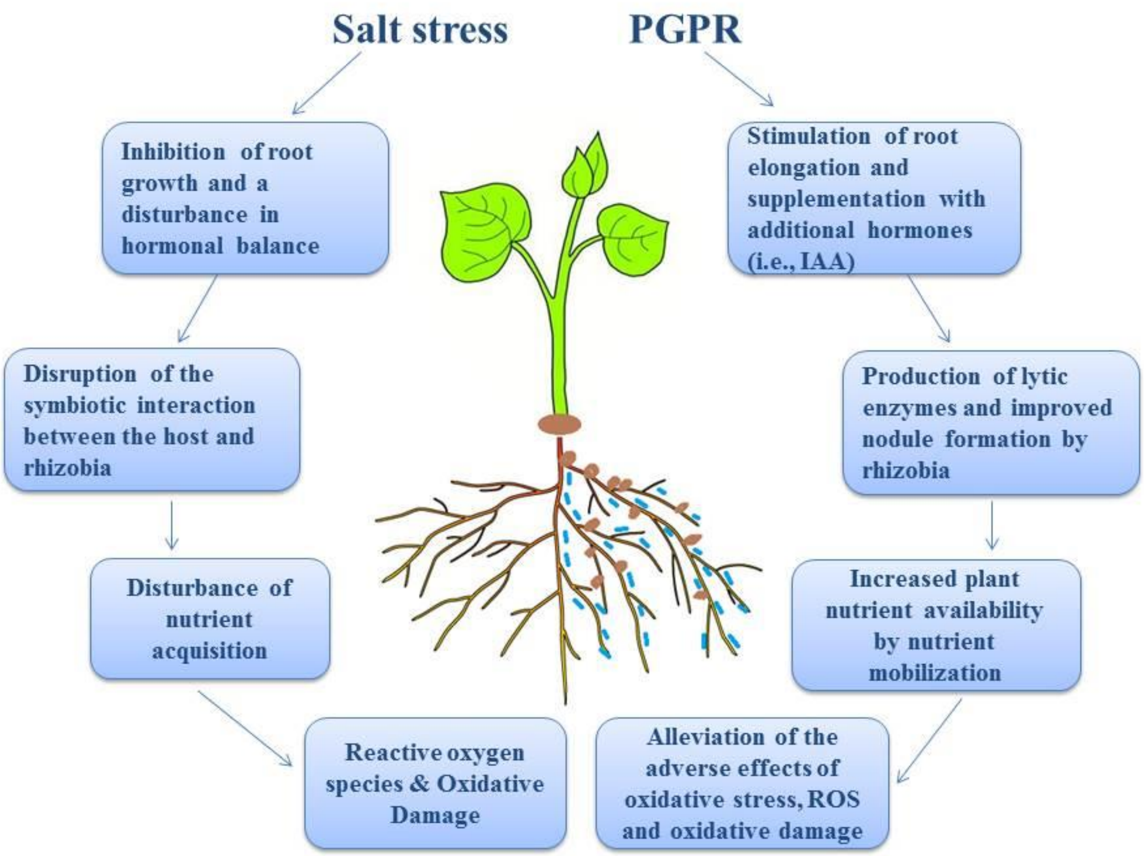

Figure 3. Schematic illustration of the main effects of plant growth-promoting rhizobacteria (PGPR) on the growth and stress tolerance of plants. The root-colonizing PGPR strain stimulates root growth, nutrient uptake and nodule formation through supplying additional phytohormones, mobilizes minerals and alleviates the adverse effects of oxidative stress. IAA: indole-3 acetic acid, ROS: reactive oxygen species.