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Use of plant growth-promoting rhizobacteria to ameliorate the performance of lentil under salinity

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Summary

In the current agricultural model, the increasing soil salinity, especially in arid and semiarid regions, causes environmental and economic losses. Inoculation of plant with growth-promoting rhizobacteria (PGPR) can be a sustainable strategy to increase plant abiotic stress tolerance mainly in the early vulnerable stage of their growth. The efficiency of PGPR inoculation was evaluated in three Italian lentil accessions and two Pakistan native varieties differing in salinity tolerance. *Pseudomonas putida* (6), *Pseudomonas fluorescens* (6K) and *Serratia ficaria* (W10) were used as bio-inoculants. Seedling growth was detected 16 days after NaCl treatments. Results showed that in absence of salinity, all strains increased differently the growth of lentils compared to the un-inoculated ones. Inoculum significantly increased the growth of the most salt sensitive in comparison to the most salt resistant varieties. 6 and 6K were the most effective-growth-inducers under salinity stress. A specificity between PGPR and lentil was evident. 6K mostly improved biomass and growth of the Italian accessions, while the strain 6 mostly affected the Pakistan landraces.

Keywords: Inoculation; lentil; plant growth promoting rhizobacteria; salinity stress

Introduction

The soil salinization constitutes one of the main processes responsible for the degradation and reduced productivity of agricultural lands in arid and semiarid regions (Ashraf and Sarwar, 2002; Munns, 2002). According to an estimate, 1128 million hectare of land are affected worldwide by salinity and sodicity (Wicke et al., 2011). Most of the legumes are sensitive to salinity and only few can grow on salt-affected soils (Ashraf and McNeilly, 2004; Morais et al., 2012). Lentil (*Lens culinaris* Medik.) is the 2nd most important grain legume widely cultivated in semi-arid regions of the world (Malik, 2005) throughout Indian sub-continent, Middle East, Northern Africa, Southern Europe, North and South America, Australia and Western Asia (Fikiru et al., 2007). The plants are grown for their small lens-shaped edible seeds, which are rich in protein, carbohydrates, calcium, phosphorus, iron and B vitamins. Lentil seeds also contain polyphenols, and antioxidants with free radical-scavenging abilities, useful in the prevention of many chronic diseases with consequent beneficial effects on human health (Dueñas et al., 2003). Lentil is considered a strategic component of the cropping systems in the Mediterranean areas. As the majority of legumes, lentil is salt sensitive and salinity is imposing serious limitations to its productivity, causing yield losses up to 50%. Kökten et al. (2010) showed a lentil yield reduction of 20% and 90-100% at an EC of 2 dS/m and 3 dS/m, respectively, suggesting that this crop should not even be grown under slightly saline conditions. Even if salinity affects plant growth at all stages of development, germination and seedling emergence represent the most vulnerable stages in plant life cycle because they are the first to be confronted with soil salinity (Kitajima and Fenner, 2000). It is widely demonstrated, in different crop species (Chikpea, barley, wheat, sunflower), that germination capacity decreases markedly increasing salinity (Panuccio et al., 2018; Kumar et al., 2012; Abdoli et al., 2013; Wu et al., 2015; Alom et al., 2016). Rapid, uniform and high germination percentage for legumes is a prerequisite for their successful stand establishment and yield (Demir and Ermis, 2003) and are considered the screening criteria to select salt tolerant genotypes. High salt concentrations affect plant metabolic processes leading to an increase in ethylene concentration that inhibits root elongation which in turn reduces plant growth and yield (Nadeem et al., 2010; Panuccio et al., 2014). The PGPR strains, containing ACC-deaminase, have the ability to reduce the negative impact of elevated level of ethylene by degrading it into ammonia and α-ketobutyrate (Glick et al., 1998). Numerous studies (Zahran et al., 2012; Sobti et al., 2015) showed that the lack of legume productivity in saline soil was due to the reduced survival and proliferation of PGPR in soil (Khosravi et al., 2010), with a consequent decline in exopolysaccharides and phytohormones that, binding Na^+, protect plants from the adverse effects of salinity (Upadhyay et al., 2011). On the basis of the above consideration, the development of salt-tolerant symbioses is considered an absolute priority to enable cultivation of leguminous crops in salt-affected soils. The aim of the current research was to relate the extent of a positive association between different strains and lentil varieties with the salt tolerance degree, to find an eco-sustainable and cheap method for improving germination and seedling establishment in salt condition, with the specific objective of extending lentil cultivation in saline and/or salinized area. In this study the efficiency of three salt resistant wheat-growth promoting rhizobacteria, in alleviating salt stress in five lentil varieties has been evaluated and compared by assessing early vegetative growth and phenotypic traits of the inoculated lentils in respect to the un-inoculated ones in saline and non-saline conditions. The existence of specificity between PGPR and lentil variety was also evaluated.

Materials and methods

Plant material

In this study we analyzed two salt sensitive lentils (Masoor 85, MAS; Punjab Masoor 2009, PUN) native to, and cultivated in Pakistan, two Italian accessions 'Ustica' (UST), and Castelluccio (CAST) moderate salt stress tolerant landraces, and a Canadian commercial variety salt sensitive Eston (EST) (Sidari et al., 2007, 2008; Muscolo et al., 2014, 2015). Each lentil variety was stored at 20 ± 1 °C and 95% R.U., and selected for size and homogeneity before using. The lentil varieties: Masoor 85 (MAS) and Punjab Masoor 2009 (PUN), were purchased from Pulses Section, Ayub Agriculture Research Institute, Faisalabad, Pakistan. Ustica was collected in the homonymous island close to Sicily (Southern Italy) and Castelluccio was collected in Umbria (Central Italy).

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Strain selection

The PGPR strains (6, 6K and W10) used in this study have been previously and comprehensively evaluated in axenic and field conditions for their growth promoting activities in wheat under salinity. These strains were shown to have ACC-deaminase activity, production of exopolysaccharides, auxin production, P solubilization and root colonization ability (NADEEM et al., 2010).

Growth pouch assay

A growth pouch assay was conducted under axenic conditions at three salinity levels (0, 4 and 8 dS m^{-1}) to check the responses of five different lentil varieties to the inoculation with PGPR strains (6, 6K and W10). For each strain, inoculum was prepared in flasks using Luria Bertani (LB) medium without agar. Each flask containing 125 mL of broth was inoculated with a selected strain of bacteria and incubated in a shaking incubator (100 rpm) for 72 h at 28 ± 1 °C. Before seed inoculation, an optical density of 0.5 at 535 nm was achieved by getting cell pallets through centrifugation of the bacterial culture and making dilutions in saline solution to maintain uniform cell density (10^8-10^9 colony forming units (CFU) mL^{-1}). Lentil seeds of each variety were surface sterilized by momentarily dipping them in 95% ethanol and then in 5% sodium hypochlorite solution for 3-4 minutes and a subsequent 5-6 washings with sterilized distilled water. Three surface sterilized pre-germinated seeds of each variety were dipped into the inocula for 10 minutes and placed in autoclaved growth pouches (MEGA International, West St. Paul, Minnesota, USA). Sterilized LB broth was used for the control treatment. The whole procedure of inoculation and sowing was executed in a laminar flow hood to eliminate or reduce the chances of contamination. Salinity levels of 4 dS m^{-1} and 8 dS m^{-1} were maintained using NaCl in sterilized half strength Hoagland solution (HOAGLAND and ARNON, 1950). Each treatment was replicated thrice following completely randomized design. Six days after sowing, salinity treatments were applied by irrigating seedlings with saline Hoagland (1/2 strength) solution. In the growth chamber, suitable temperature was between 25-30 °C with 10 h light (275 µmol m^{-2} s^{-1}) and 14 h dark. Root and shoot length, fresh and dry biomass, and chlorophyll content were recorded at 16 days after salinity treatment.

Chlorophyll assay

The chlorophyll contents were determined by using photosynthesis measuring system/SPAD meter (SPAD-502 plus, Konica, Minolta Inc.). Seedlings were harvested and root weight was recorded.

Statistical analysis

Data of three replicates were analyzed by using one-way ANOVA and mean comparisons were made with Tukey's test ($p<0.05$). All data were analyzed using SYSTAT 13.0 software (SPSS Inc.). Two-way ANOVA was performed to analyze the effects of both salinity and strains and their interaction on shoot and root length, shoot and root fresh and dry biomass and chlorophyll content. All analyses were conducted using SYSTAT 13 for Windows. Significant effects were determined by $p = 0.05$.

Results

In absence of salinity, for all lentil varieties, root and shoot fresh weights (Tab. 1, 2) of inoculated seedlings were significantly higher than un-inoculated ones (controls). However, the degree of lentil growth, depended on the PGPR used for the inoculum. In absence of salinity, UST increased its root and shoot fresh biomass when inoculated with W10, while CAS when treated with 6K. In EST, MAS and PUN varieties, the strains that improved shoot biomass were different from those that influenced root growth (Tab. 1, 2). In EST, shoot biomass was increased by the strain 6, while root growth by the strain W10. Shoot biomass of MAS increased with W10 while root growth enhanced with 6 and 6k strains. In PUN, shoots were mostly increased by 6K, while roots were enhanced by 6. Under 4 dS m^{-1} salinity, 6 and 6K increased the shoot biomass of all lentil varieties except for Eston, while W10 mostly improved the root biomass only of CAST, EST and MAS compared to the other treatments. At 8 dS m^{-1}, the inoculation significantly increased the growth of lentils except for PUN in which the negative effect of salinity was only slightly reduced by the inocula in comparison to the controls (Tab. 1, 2). In absence of salinity, the strains behaved differently in respect to the type of lentil, organ and phenotypic traits. All strains increased shoot elongation in CAST, EST and MAS (Fig. 1). All strains, except W10 enhanced shoot length in PUN. Only

Tab. 1: Shoot fresh weight (mg $plant^{-1}$) (n = 3) in inoculated lentils Ustica (UST), Castelluccio (CAS), Eston (EST), Masoor 85 (MAS), and Punjab Masoor 2009 (PUN) with the different strains (6, 6K, W10) in respect to the un-inoculated one (control), in absence (0 dS m^{-1}) and in presence of NaCl (4 and 8 dS m^{-1})

		Shoot fresh biomass				
NaCl (dS m^{-1})	**Strain**	**UST**	**CAS**	**EST**	**MAS**	**PUN**
0	**CTR**	123.3 ± 0.3c	146.7 ± 0.2d	126.7 ± 0.2c	83.3 ± 0.5c	76.7 ± 0.2d
	6	150.0 ± 0.3b	186.7 ± 0.5b	156.7 ± 0.5a	103.3 ± 0.2b	100.0 ± 0.3c
	6K	146.7 ± 0.1b	193.3 ± 0.3a	143.3 ± 0.3b	106.7 ± 0.3b	130.0 ± 0.3a
	W10	156.7 ± 0.3a	153.3 ± 0.2c	130.0 ± 0.1c	110.0 ± 0.1a	116.7 ± 0.2b
4	**CTR**	101.7 ± 0.6d	100.0 ± 0.3d	126.7 ± 0.1c	73.3 ± 0.3c	66.7 ± 0.2c
	6	106.7 ± 0.2c	140.0 ± 0.1b	200.0 ± 0.2a	136.7 ± 0.4a	100.0 ± 0.1b
	6K	166.7 ± 0.7a	156.7 ± 0.4a	150.0 ± 0.b	110.0 ± 0.4b	123.3 ± 0.2a
	W10	146.7 ± 0.3b	120.0 ± 0.1c	196.7 ± 0.2a	133.3 ± 0.3a	96.7 ± 0.2b
8	**CTR**	76.7 ± 0.3d	83.3 ± 0.3 c	93.3 ± 0.2d	66.7 ± 0.3d	63.3 ± 0.1b
	6	143.3 ± 0.3b	160.0 ± 0.4a	146.7 ± 0.3c	80.0 ± 0.1c	73.3 ± 0.2a
	6K	136.7 ± 0.3c	160.0 ± 0.1a	210.0 ± 0.1a	123.3 ± 0.2a	66.7 ± 0.3b
	W10	153.3 ± 0.2a	150.0 ± 0.3b	156.7 ± 0.2b	90.0 ± 0.2b	63.3 ± 0.2b

Values are the means of three experiments ± SE. Different letters indicate significant differences ($P \leq 0.05$) among different strain treatments within the same cultivar and at the same salt concentration.

Tab. 2: Root fresh biomass (mg plant^{-1}) (n = 3) in inoculated lentils Ustica (UST), Castelluccio (CAS), Eston (EST) Masoor 85 (MAS) and Punjab Masoor 2009 (PUN) with the different strains (6, 6K, W10) in respect to the un-inoculated one (control), in absence (0 dS m^{-1}) and in presence of NaCl salinity (4 and 8 dS m^{-1}).

		Root fresh biomass				
NaCl (dS m^{-1})	**Strain**	**UST**	**CAS**	**EST**	**MAS**	**PUN**
0	**Control**	50.0 ± 0.1d	39.7 ± 0.2c	20.0 ± 0.1c	26.7 ± 0.5c	30.0 ± 0.1c
	6	60.0 ± 0.2c	83.3 ± 0.3b	33.3 ± 0.3b	53.3 ± 0.1a	93.3 ± 0.4a
	6K	73.3 ± 0.3b	103.3 ± 0.5a	36.7 ± 0.1b	53.3 ± 0.5a	80.0 ± 0.1b
	W10	79.7 ± 0.4a	80.0 ± 0.1b	53.3 ± 0.5a	33.3 ± 0.1b	80.0 ± 0.2b
4	**Control**	49.3 ± 0.5c	26.7 ± 0.2d	20.0 ± 0.1d	16.7 ± 0.1d	13.3 ± 0.3d
	6	49.7 ± 0.40c	63.3 ± 0.5c	74.0 ± 0.2c	103.3 ± 0.6b	40.0 ± 0.2c
	6K	103.3 ± 0.2a	110.0 ± 0.2b	83.3 ± 0.3b	63.3 ± 0.5c	103.3 ± 0.6a
	W10	80.0 ± 0.1b	116.7 ± 0.1a	113.3 ± 0.3a	166.7 ± 0.2a	90.0 ± 0.2b
8	**Control**	30.0 ± 0.1d	14.0 ± 0.2d	16.7 ± 0.4d	13.3 ± 0.3c	13.3 ± 0.3c
	6	93.3 ± 0.2a	133.3 ± 0.5a	60.0 ± 0.2c	146.7 ± 0.1a	16.7 ± 0.4b
	6K	60.0 ± 0.1c	120.0 ± 0.2b	116.7 ± 0.2a	136.7 ± 0.2b	13.3 ± 0.6c
	W10	77.0 ± 0.1b	113.3 ± 0.6c	93.3 ± 0.3b	133.3 ± 0.6b	19.7 ± 0.3a

Values are the means of three experiments ± SE. Different letters indicate significant differences ($P \leq 0.05$) among different strain treatments within the same cultivar and at the same salt concentration.

6K and W10 stimulated the growth of shoots in UST (Fig. 1). Under salinity, the inocula increased lentil shoot length compared to the respective controls. At 4 dS m^{-1} 6K and W10 induced the best shoot elongation in UST and CAST, 6 and 6K increased shoot length in PUN, all strains increased shoot length in MAS and EST. At 8 dS m^{-1} all strains increased shoot length in all the varieties in respect to the un-inoculated controls.

The effects of strains on root apparatus depended on lentil variety, data evidenced that in absence of salinity only the strain W10 increased root length in UST and CAST accessions. Conversely, in presence of salinity all the strains increased root elongation in all the lentils in respect to the own controls, but at different extent (Fig. 1). In short, our results highlighted that in the absence of salinity, all strains increased the shoot length more than root length compared to the un-inoculated lentils, except for UST, where both root and shoot lengths were likewise improved. At 8 dS m^{-1}, all strains improved root length. 6 and 6K induced the greatest root elongation in CAST and PUN, while W10 positively affected EST and UST root length.

Two-way ANOVA showed that salinity and strains individually or in combination, significantly affected lentil growth (Tab. 3). Shoot length was mainly affected by salinity in UST, CAST and MAS, while root length was more influenced by strains, except for MAS, that was influenced similarly by salinity and strain (F-ratios). Shoot and root biomass of lentils were mostly affected by strains except for PUN. In short, our results evidenced a specificity between strains and lentils showing that the effects of salt and strain were differentiated and species-specific. The most promising strains for lentil growth in saline conditions were in the following order 6> 6K>W10.

At original EC, inoculation increased dry weights (DW) in respect to all the un-inoculated lentils (Fig. 2). In presence of salinity, the effect of inoculum was different and depended on the type of strain used and on lentil accession. The DW of the inoculated UST seedlings, under the highest salt condition did not change compared to that of their respective controls at original EC. In CAST, under salinity, all inocula induced the highest increase in biomass if compared to the inoculated at original EC. A similar trend was observed in salt affected MAS but with a minor increment in biomass. In Eston, the seedlings inoculated with the strains 6 and w10 showed the highest dry weight at 4 dS m^{-1}, while at 8 dS m^{-1} only the strain 6k was able to induce a further dry weight increase. A different behavior was observed for PUN, in which salinity decreased the biomass of all inoculated seedlings.

Root mass ratio (RMR) increased in presence of the inocula but at different extent depending on the affinity of the inoculum with a specific lentil cultivar. RMR increased in UST and MAS when inoculated with 6 and W10, in CAST and EST when treated with 6K and W10, in PUN with 6K and W10 but only at a salinity of 4 dS/m. Conversely to RMR, shoot mass ratio (SMR) decreased in increasing salinity in all lentils and in presence of all the inocula. W10 appeared as the strain the most transversally effective on all the lentils (data not shown).

Two way Anova (Tab. 4) evidenced that root and shoot dry weight in CAST, UST and EST was mainly influenced by strains (F-ratios). Conversely, PUN shoot and root dry weights were mainly affected by salinity. In MAS, root dry weight was influenced by salt, while shoot dry weight was more affected by strain.

Strains positively influenced also chlorophyll content (Fig. 3). The maximum increase in chlorophyll, in the absence of salinity, was observed in CAST followed by EST and UST, in response to the inoculation with 6K. Under 4 dS m^{-1} salinity, 6K significantly increased chlorophyll amount in UST and PUN compared to the respective un-inoculated seedlings. The maximum chlorophyll contents were recorded at 8 dS m^{-1} in PUN and EST, inoculated with W10 and 6K, respectively. At the highest salinity (8 dS m^{-1}) also the strain 6 was able to significantly increase the chlorophyll content in all lentil varieties compared to the respective controls. Chlorophyll, in all the lentils were more influenced by strains than salinity (F-ratios). In all lentils the combined effect of strain and salinity was less significant than the two factors individually considered, except in PUN (Fig. 3).

Discussion

Because of the contemporary increase in world population and saline lands, nowadays it is of primary importance to maintain soil productivity for a sustainable agriculture mostly in developing countries. Microbes and legumes through symbiosis and synergistic co-evolution have a great potential for improving productivity playing a key role in inducing abiotic stress resistance in plants (Paredes and Lebeis, 2016; Rosenberg and Rosenberg, 2016; Agler et al., 2016).

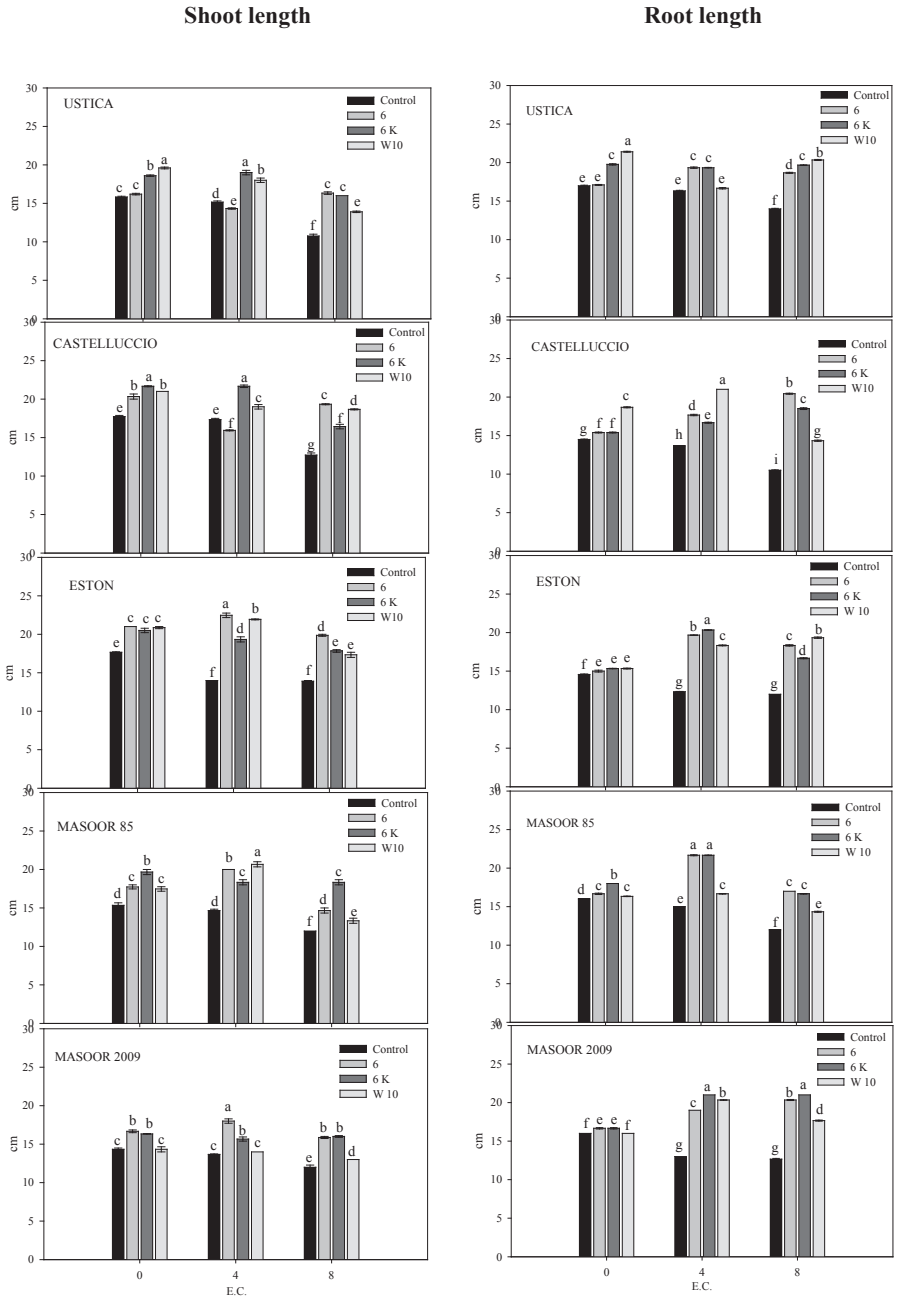

Fig. 1: Shoot and root length (cm) of Ustica (UST), Castelluccio (CAST), Eston (EST), Masoor 85 (MAS) and Punjab (PUN) lentils, 16 days after the inoculation with 6, 6K and W10 strains under different NaCl salinity concentrations (0, 4 and 8 dS m^{-1}). Bars represent the mean ± SE. Different letters indicate significant differences ($P \leq 0.05$)

Interestingly, we found and confirmed that seed inoculation with PGPR improved salinity resistance in lentils, highlighting that salt-tolerant species of lentils had reduced responsiveness to rhizobia, both in survival and growth. In fact, lentils showing higher levels of tolerance benefitted less from rhizobia inoculation than those with lower salt-tolerance.. These results were in agreement with previous findings of GABALLAH and GOMAA (2005), reporting that the performance of two fava bean varieties improved after bacterial inoculation in different way. The growth improvement of inoculated lentils was due to the effect of PGPR which having ACC-deaminase activity mitigated the inhibitory effects of salinity on root growth, by lowering the ethylene concentration in plant as previous demonstrated by NADEEN et al. (2010). It is well known that ethylene inhibits cell expansion in both roots and shoots acting by means of cross-talk with the growth hormone auxin (VANSTRAELEN and BENKOVÁ, 2012). Our results evidenced a better root growth in inoculated seedlings under salinity stress compared to the un-inoculated control, confirming that the PGPR contrasted the negative effects of ethylene as demonstrated by NADEEN et al. (2010) that used the same our strains. GLICK et al. (2007) demonstrated that inoculation was able to confer major stress resistance to numerous plants, because of the over-production of exopolysaccharides that protected the plant root from sodium (Na) toxicity by decreasing its availability. ILANGUMARAN and SMITH (2017) in a recent review elucidated the positive impact of exopolysaccharides produced by PGPR in enhancing plant growth in saline environment, suggesting that bacteria promoted plant growth via their growth promoting traits and highlighting that the beneficial effects of PGPR involved key physiological processes such as nutrient up-

Tab. 3: Analysis of Variance of the effects of salinity and strains on shoot and root length (cm plant^{-1}) and shoot and root biomass (mg plant^{-1}) of Ustica (UST), Castelluccio (CAST), Eston (EST), Masoor 85 (MAS) and Punjab Masoor 2009 (PUN) lentils.

	Shoot length	Root length	Shoot biomass	Root biomass	
UST					
Salinity	426.6 ***	198.4 ***	2378.4 ***	2059.3 ***	F-ratio
Strains	336.5 ***	2157.3 ***	12811.6 ***	48785.0 ***	
Salinity × Strains	103.2 ***	700.7 ***	2852.2 ***	21125.2 ***	
	0.995	0.998	1.000	1.000	R
CAS					
Salinity	308.3 ***	391.9 ***	6286.8 ***	8335.0 ***	F-ratio
Strains	260.6 ***	4618.8 ***	28574.3 ***	92142.7 ***	
Salinity × Strains	109.5 ***	1439.5 ***	8378.3 ***	10583.3 ***	
	0.994	0.999	1.000	1.000	R
EST					
Salinity	218.1 ***	1612.6 ***	16696.7 ***	67120.8 ***	F-ratio
Strains	510.1 ***	3775.8 ***	37129.9 ***	105785.3 ***	
Salinity × Strains	44.9 ***	930.8 ***	18140.8 ***	15412.8 ***	
	0.999	0.999	1.000	1.000	R
MAS					
Salinity	201.8 ***	8005.3 ***	2619.8 ***	89068.8 ***	F-ratio
Strains	153.7 ***	7810.5 ***	13987.0 ***	100553.7 ***	
Salinity × Strains	34.2 ***	1205.3 ***	7909.2 ***	29643.9 ***	
	0.989	0.999	1.000	1.000	R
PUN					
Salinity	48.1 ***	1603.3 ***	5036.1 ***	105114.8 ***	F-ratio
Strains	232.4 ***	6794.4 ***	2187.9 ***	41732.1 ***	
Salinity × Strains	12.5 ***	1607.8 ***	543.5 ***	17919.3 ***	
	0.986	0.999	1.000	1.000	R

take, photosynthesis, and source-sink relationships that promoted a significant plant growth and development. Our results perfectly agree with the above assertions showing that PGPR affected chlorophyll content and/or root growth in a selective way and in respect to lentil variety and salinity level. This results highlighted a specificity between strain and lentil variety. The most promising strains for lentil growth in saline conditions were in the following order 6> 6K> W10. The results obtained showed that the Italian local lentils, in absence and also in presence of salinity, had a biomass double than the Pakistan accessions, confirming a major productivity also under stress condition. The Pakistan varieties, when inoculated, resulted more responsive to treatment showing an increase in biomass in percentage twice higher than that of its own non-inoculated control.

Conclusion

In short, the inoculum with selected wheat-promoting PGPR capable of tolerating saline conditions represents a good practice for improving the adaptation of lentils to saline environment. Such strains could be very useful for promoting growth of other legumes by itself or in combination with other strains. Additionally, the identification of PGPR efficient on both cereal and legumes, may favor a successful use of lentils in crop rotation with maize and other cereals, representing a winning strategy to improve or maintain soil fertility under saline conditions with a number of agronomic, economic and environmental benefits compared to monoculture cropping. Salt-tolerant-PGPR represents a promising way for sustainable lentil production in saline environment.

Author contribution

AM and ZZ designed the research; SM and SMN conducted the experiments; AM and MRP analyzed the data and wrote the paper. All authors have read and approved the final manuscript.

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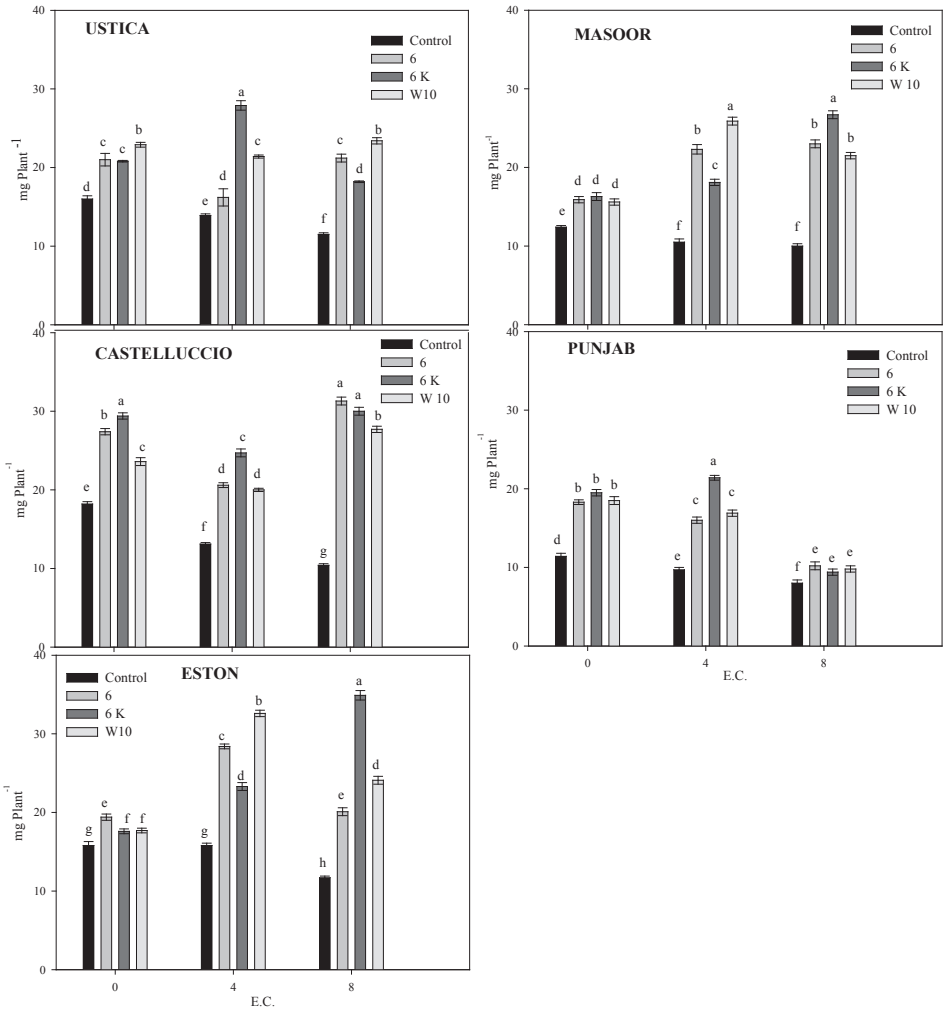

Fig. 2: Total dry weight of Ustica (UST), Castelluccio (CAST), Eston (EST), Masoor 85 (MAS) and Punjab (PUN) lentils, 16 days after the inoculation with 6, 6K and W10 strains in presence of different NaCl salinity concentrations (0, 4 and 8 dS m^{-1}). Bars represent the mean ± SE. Different letters indicate significant differences ($P \leq 0.05$)

Tab. 4: Analysis of Variance of the effects of salinity and strains on shoot and root dry weight (D.W.) of Ustica (UST), Castelluccio (CAST), Eston (EST), Masoor 85 (MAS) and Punjab Masoor 2009 (PUN) lentils.

UST		CAST		EST		MAS		PUN	
Root D.W.	F-ratio	Root D.W.	F-ratio	Root D.W.	F-ratio	Root D.W.	F-ratio	Root D.W.	F-ratio
Salt	7.56 **	Salt	585.9 ***	Salt	542.9 ***	Salt	1369.7 ***	Salt	517.1 ***
Strain	75.9 ***	Strain	569.3 ***	Strain	655.2 ***	Strain	965.5 ***	Strain	245.7 ***
Salt × Strain	25.0 ***	Salt × Strain	123.5 ***	Salt × Strain	226.2 ***	Salt × Strain	327.4 ***	Salt × Strain	108. 9 ***
Shoot D.W.		Shoot D.W.		Shoot D.W.		Shoot D.W.		Shoot D.W.	
Salt	7.98 **	Salt	542.3 ***	Salt	255.5 ***	Salt	127.4 ***	Salt	788.8 ***
Strain	187.1 ***	Strain	602.7 ***	Strain	428.7 ***	Strain	228.9 ***	Strain	276.8 ***
Salt × Strain	81.5 ***	Salt × Strain	47.6 ***	Salt × Strain	212.4 ***	Salt × Strain	46.4 ***	Salt × Strain	43.4 ***

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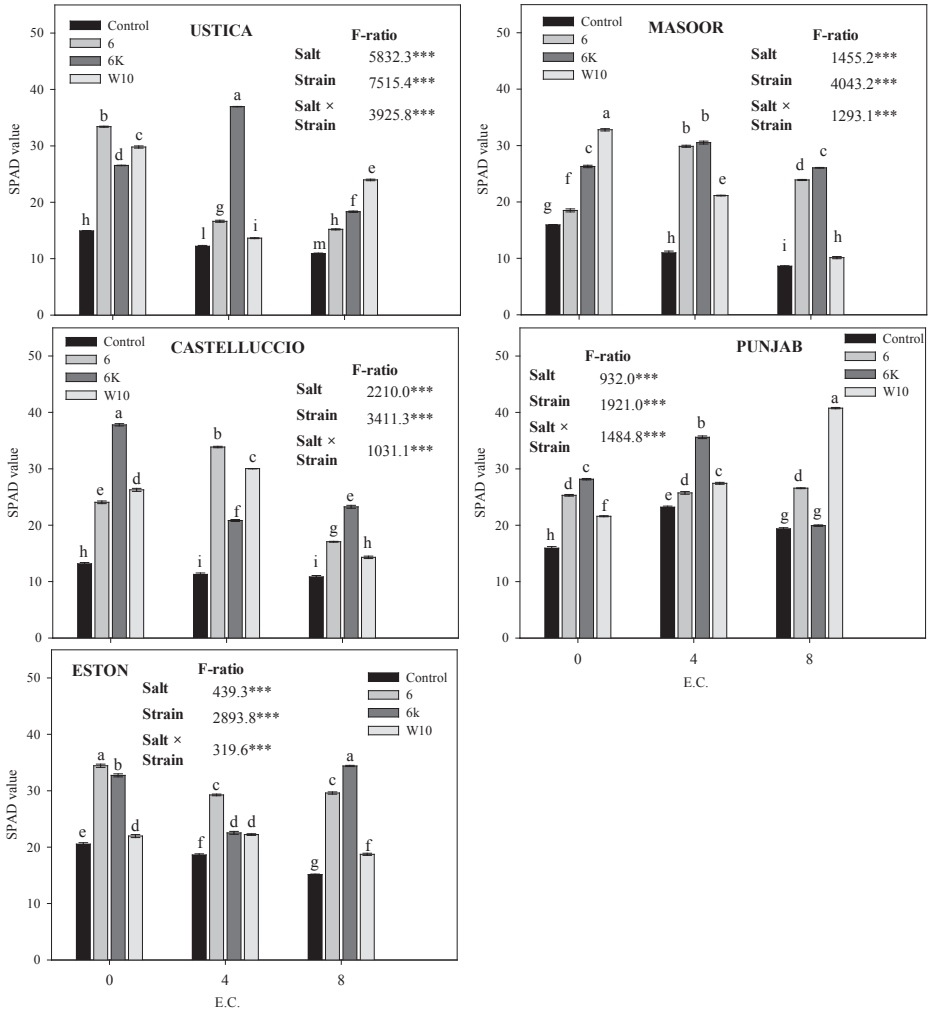

Fig. 3: Chlorophyll contents (SPAD value) detected in leaves of Ustica (UST), Castelluccio (CAST), Eston (EST), Masoor 85 (MAS) and Punjab (PUN) lentils, 16 days after the inoculation with 6, 6K and W10 strains in presence of different NaCl salinity concentrations (0, 4 and 8 dS m^{-1}). Bars represent the mean ± SE. Different letters indicate significant differences ($P \leq 0.05$)

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