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Melon growth-promoting rhizobacteria under saline stress

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ABSTRACT: The aim of this study was to evaluate melon growth promotion by fluorescent rhizobacteria of *Pseudomonas* spp. under saline stress. Soil samples were collected in agricultural properties of Juazeiro/BA, Petrolina/PE and Curaça/BA in the Submedium Region of the São Francisco Valley to conduct sampling of bacterial isolates. The obtained isolates were analyzed regarding cultural characteristics and tolerance to salinity in the following NaCl concentrations: 0; 250, 500, 750, 1000, 1250 and 1500 mM. Among the isolated bacteria, 20 were identified and used to evaluate growth promotion on melons in a protected environment. This experiment was conducted in a completely randomized 21 x 4 factorial design, with 20 bacterial isolates, one control without inoculation and four salinity levels (0.2, 2.0, 4.0 and 6.0 dS m^{-1}), and it was replicated seven times. A total of 147 bacterial isolates were obtained from soil samples with electric conductivity ranging from 0.8 to 60.4 dS m^{-1}. Cell growth was inhibited starting at 750 mM of "*in vitro*" saline concentration, with tolerance observed up to 1500 mM. *Pseudomonas* spp. isolates were capable of promoting melon growth under non-saline conditions and of mitigating the harmful effects of excessive concentrations of soluble salts in the growth substrate.

Key words: *Cucumis melon* L.; PGPR; *Pseudomonas* sp.; salinity

Rizobactérias promotoras de crescimento em melão sob estresse salino

RESUMO: Objetivou-se avaliar a promoção de crescimento de meloeiro por bactérias fluorescentes do gênero *Pseudomonas* spp., sob estresse salino. Os isolados bacterianos foram prospectados em amostras de solo coletadas em áreas agrícolas dos municípios Juazeiro/BA, Petrolina/PE e Curaça/BA no Submédio do Vale do São Francisco. Os isolados foram analisados quanto às características culturais e tolerância à salinidade nas concentrações de NaCl: 0; 250, 500, 750, 1000, 1250 e 1500mM. Dentre os isolados, 20 foram identificados e avaliados quanto à promoção de crescimento de melão, em ambiente protegido. O delineamento estatístico foi inteiramente casualizado, em arranjo fatorial 21 x 4, sendo 20 isolados e uma testemunha sem inoculação e 4 níveis de salinidade (0,2; 2,0; 4,0 e 6,0 dS m^{-1}), com 7 repetições. Foram obtidos 147 isolados bacterianos das amostras de solo com condutividade elétrica variando de 0,8 a 60,4 dS m^{-1}. O crescimento celular foi inibido a partir de 750mM de concentração salina "*in vitro*", com tolerância observada até 1500mM. Os isolados de *Pseudomonas* spp. foram capazes de promover crescimento de melão em condições não salinas e atenuar os efeitos deletérios da concentração excessiva de sais solúveis no substrato de crescimento.

Palavras-chave: *Cucumis melon* L.; RPCP; *Pseudomonas* sp.; salinidade

Introduction

Increased concentration of soluble salts near plant roots constitutes the main abiotic stress in global agriculture, especially in irrigated agriculture. Over 1,030,000 hectares of land are currently affected by salts, 9% of Earth surface land, of which 130,000 are in South America (FAO, 2015).

Salinity might directly or indirectly cause physiological changes to plant growth and development by reducing osmotic potential in soil solution, thus resulting in changes in ion homeostasis and increased levels of ethylene, which occurs throughout the plant and limits yield (Shabala & Munns, 2012). According to Banaei-Asl et al. (2016), these changes might be mitigated by the inoculation of some rhizobacteria species in plants cultivated under saline stress.

Some mechanisms, such as facilitating nutrient uptake, phosphate solubilization, production of siderophores, synthesis of growth hormones and enzymes (i.e. 1-aminocyclopropane-1-carboxylate deaminase - ACC deaminase), which might play a role in reducing or blocking ethylene synthesis in salt-stressed plants, are responsible for promoting plant growth under these conditions (Egamberdieva & Lutenberg, 2013). Among plant growth-promoting rhizobacteria (PGPR), studies have been conducted on fluorescent bacteria of the *Pseudomonas* genus due to the role they play in growth through mechanisms that enable plants to develop a higher resistance to saline stress in different cultures of economic importance. Studies developed with canola (Banaei-Asl et al., 2016), tomato (Ali et al., 2014; Cho et al., 2015), fava beans (*Vicia fava*) (Metwalil et al., 2015), and rice (Bal et al., 2013), among others, have shown that these plants have their growth promoted by these bacteria in saline environments. According to Rangaranjan et al. (2002), increased salinity selects more tolerant species of microorganisms and this adaptive ability of specific groups such as *Pseudomonas* spp. might enhance plant settlement and growth promotion in saline environments.

Therefore, the aim of this study was to evaluate growth promotion in melon under saline stress by inoculating fluorescent *Pseudomonas* spp.

Material and Methods

Isolating *Pseudomonas* spp. strains

Fluorescent *Pseudomonas* spp. strains were isolated from soil samples in crops of different irrigated production areas under different salinity conditions, located in the municipalities of Curaçá, Juazeiro, (BA), and Petrolina, (PE).

To do so, the serial dilution technique was used by spreading the culture on a B medium surface (King et al., 1954). After the appearance of colonies, those that showed fluorescence under ultraviolet light (330 nm wave length) were selected and purified.

Cultural characterization of isolates

The cultural characterization of colonies that showed fluorescence was performed according to Fonseca et al. (2001), based on the observation of colonies developed after 48 hours of incubation in a solid B medium. The following characteristics were evaluated: size (2 mm, 1-25 mm, or punctiform), shape (round or irregular), edge (smooth or undulate), colony aspect (homogeneous or heterogeneous), color (yellow, light yellow, greenish yellow, orange, cream, or white), transparency (translucent or opaque), elevation (flat or absent), mucus (little, moderate, or much), and fluorescence (++ present or + relative). The results were converted into a binary matrix and analyzed using NTSYS.

Evaluating the tolerance of *Pseudomonas* spp. to salinity

Pseudomonas spp. isolates were tested in a liquid B medium supplemented with NaCl in the following concentrations: 0, 250, 500, 750, 1000, 1250, 1500 mM (electric conductivity in culture medium corresponding to 5.9, 26.5, 45.1, 60.3, 73.2, 84.7 and 94.4 dSm^{-1}, respectively), with four replicates. Control tubes were made by adding 20 µL to each tube to compare the readings with the inoculation of previously autoclaved isolates. After incubation at 28 ºC for 72 h, growth was evaluated using a Shimadzu spectrometer with wavelength of 580 nm.

Selection and identification of isolates

Twenty *Pseudomonas* spp. isolates were selected to be tested regarding melon growth promotion in saline substrates. Selection was performed considering *in vitro* tolerance to salinity, morphological group based on cultural characterization, and plant species from which bacteria were isolated to obtain higher diversity.

DNA was extracted from the 20 isolates using a Trizol® reactant according to the manufacturer's specifications. Bacteria were identified through the sequencing of an ITS 16S-23S rRNA (ITS1), using a pair of specific primers for *Pseudomonas* spp., namely fPs16S 5'-ACTGACACTGAGGTGCGAAAGCG-3' and rPs23S 5'-ACCG- TATGCGCTTCTTCACTTGACC-3', whose sequencing was performed using the Sanger method with an ABI PRISM 3700 DNA Analyzer (Applied Bio). The generated sequence tree according to Locatelli et al. (2002) were aligned and used to build a phylogenetic.

Melon growth promotion by *Pseudomonas* spp. in saline substrates

The experiment was conducted in a protected environment with 50% shading. The experiment was conducted in a completely randomized 21 x 4 factorial design, with 20 bacterial isolates, one control without inoculation, and four salinity levels (0.2, 2.0, 4.0 and 6.0 dS m^{-1}) with seven replicates. The experiment unit corresponded to an 800-mL pot, containing one plant each.

Fluvic Neosol substrate was used with the following chemical characteristics: pH in water 6.6, electric conductivity (EC) of 0.2 dSm^{-1}, O.M. 5.17 g kg^{-1}, Ca 1.2, Mg 0.7, K 0.24, H+Al 0.99, Na 0.02 $Cmol_c$ kg^{-1}, P 23.2, Cu 6.2, Fe 38.7, Mn 35.5, and Zn 5.1 mg d^{-3}. According to the soil analysis, mineral supplementation was performed with the following

macronutrients: N, from ammonium sulphate (248 mg dm^{-3}); single superphosphate (827 mg dm^{-3}); and potassium chloride (104 mg dm^{-3}), according to crop nutritional requirements (*Comissão Estadual de Fertilidade do Solo*, 1989).

An electric conductivity curve was built as a function of NaCl concentration based on the equation shown in Richards (1954) in order to obtain an approximate salinity of 4 and 6 dS m^{-1} in the substrate used. The soil salinization curve was adjusted using the linear equation (y = 0.5343x – 0.3655) with R^2 = 0.994, where X represents electric conductivity of a soil saturated extract (ECse). The blank sample corresponded to the electric conductivity of the irrigation water.

One microbiolized hybrid AF 6801 seed was packaged in each pot. To prepare the inoculate, a plate with solid B medium was scratched for each isolate and incubated for 48 hours at 28 °C. The inoculate received 10 ml of sterilized distilled water (SDW), colonies were scraped, and the bacterial suspension was transferred to an Erlenmeyer with 90 mL of SDW. After that, all suspensions were adjusted in a spectrophotometer (Shimadzu), DO_{580nm} = 300 nm, to obtain approximately 10^8 UFC mL^{-1}. For microbiolation, seeds were submerged in the bacterial solution for 12 hours and controls were submerged in sterilized distilled water (SDW) for the same period. Then, 1 mL of this solution was inoculated in each plant after sowing.

The irrigation rate was determined based on the method of weighing pots daily, and the water lost was replaced by evapotranspiration.

Plants were harvested after the beginning of flowering. After that, the following characteristics were evaluated: chlorophyll (CL), determined using a Minolta SPAD-502 chlorophyll meter; plant height (PH) given by the length of the shoot; leaf area (LA), measured using the QUANT software version 1.0.1. to analyze the image (Vale et al., 2003); and shoot dry matter (SDM). The results obtained were submitted to the normal distribution premise test, and then they were submitted to an analysis of variance comparing mean values using the Scott-Knott test ($p < 0.05$) through the Sisvar 4.6 program (Ferreira, 2003).

Results and Discussion

Isolation and cultural characterization

ECse of soil samples varied from 0.8 dS m^{-1} (non-saline soils) to 60.4 dS m^{-1} (saline soils), as shown in Table 1. A total of 147 isolates of fluorescent bacteria were obtained from the rhizosphere of the following plants: 38% banana (*Musa* ssp.), 38% melon (*C. melo*), 7% watermelon (*C. lanatus*), 6% rice (*O. sativa),* 4% passion fruit (*P. edulis*), and 7% *S. portulacastrum*.

Phenotypical differences were observed between isolates regarding the evaluated cultural characteristics. Figure 1 shows that two large groups (I and II) were formed at 30% of similarity. Group I was subdivided into two morphological groups, namely IA and IB; the first one was comprised of 15 specimens and the second was comprised of 22 specimens. Group II was also subdivided in two groups - IIA and IIB. Group IIA was represented by three isolates obtained from banana rhizosphere, and the formed group and the origin of isolates were related. Group IIB was comprised of 107 individuals. Based on the obtained data, there was no relation with the origin of isolates among groups IA, IB, and IIB, since these groups contained isolates derived from the rhizosphere of different cultures.

A total of 42 groups were formed at 80% similarity, presenting high cultural diversity of isolates. The phenotypical diversity of microorganisms selected might represent an adaptation, which depends on the nature and concentration of organic substrates exuded through plant roots, varying according to plant species and to the occurrence of abiotic stress such as salinity (Bassin et al., 2012). According to Delmo-Organo et al. (2017), there is an interplay of salinity level and plant variety as factors which affect the characteristics of the microbial community in the rhizosphere.

Evaluating the tolerance of *Pseudomonas* spp. to salinity

All *Pseudomonas* ssp. isolates grew at the concentration of 500 mM of NaCl in the medium, and there was a progressive inhibition of cell growth in higher concentrations, in which 5.4% of isolates tolerated up to 750 mM of NaCl, 53% tolerated up to 1000 mM, 35% tolerated to 1250 mM, and 6.2% of isolates tolerated a concentration of 1500 mM (table 2).

This inhibition of biological activity due to low osmotic potential in the substrate caused by salt leads to cell shrinkage, and consequently intracellular concentrations might reach toxic levels, thus inhibiting cell growth. The most tolerant isolates maintained their intracellular ion concentrations at low levels by accumulating ions in vacuoles, mainly Na^+ and Cl^- (Flowers et al., 2015).

Changes in tolerance to salt concentrations in the medium suggest natural selection and a likely ecological adaptability of the different isolates to the predominant environmental

Table 1. Chemical characteristics determined in soil samples collected at a 0-10cm depth.

Site	Crop	E.C.* (dS m^{-1})	pH H_2O	V (%)	K	Na^+	Ca	Mg	H + Al	P	ESP** (Mg kg^{-1})
					($cmol_c$ kg^{-1})						
Petrolina	Banana	1.59	7.46	93.44	1.75	0.09	7.55	2.29	0.82	173	0.72
Juazeiro	Melon	5.46	7.23	96.55	3.34	3.17	9.79	6.67	0.82	138	13.32
Curaçá	Watermelon	1.96	5.40	66.31	1.05	0.06	3.38	2.32	3.46	121	0.58
Juazeiro	Rice	7.41	7.41	94.98	3.18	0.14	9.48	2.7	0.82	129	0.86
Curaçá	Passion fruit	0.80	5.76	77.90	0.22	0.09	1.66	0.92	0.82	54	2.43
Petrolina	*Sesuvium portulacastrum*	24.3	6.62	99.05	2.34	25.4	43.3	14.7	0.82	128	29.31
Juazeiro	*Sesuvium portulacastrum*	60.4	6.25	95.89	1.11	5.33	8.22	4.46	0.82	64	26.7

* Electric Conductivity; **Exchangeable Sodium Percentage.

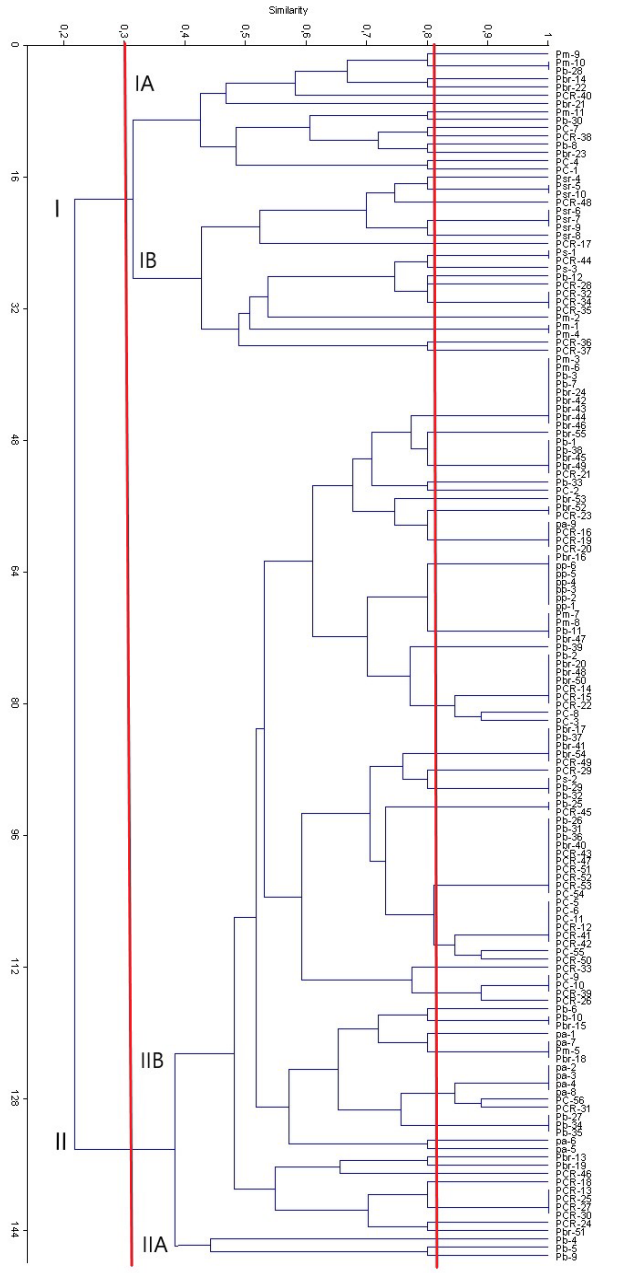

Figure 1. Dendrogram built using the UPGMA method and the Jaccard coefficient based on the cultural characterization of 147 fluorescent *Pseudomonas spp*. isolates isolated from salinized soils and soils under salinization.

conditions and of the types of solutes excreted by plant roots in the ecosystem where selection was performed (Upadhyay et al., 2009).

Identification of Isolates

The twenty (20) selected isolates are described in Table 3. Among them, 13 were related to the Pseudomonaceae family, belonging to species of the *Pseudomonas* (*P. putida, P. mendocina and P. stutzeri*) genus, with similarities between 97 and 99% (Figure 2). Among the other isolates, PC4, PCR23, and PCR29 showed 82 to 80% and 99% identity with *Pseudomonas* spp., respectively. PCR30 and PCR33 showed 95% identity with *P. putida* and *P. putida* S16, respectively. PB33 and PCR38 isolates were identical; however, it was not possible to identify them.

Table 3. Bacterial isolates of the fluorescent group of *Pseudomonas* spp. selected for the *in vivo* experiment in protected environment related to electric conductivity of the soil of origin, tolerance interval to *in vitro* salinity, and morphological group.

Isolates	Origin	Soil ECse (dS m^{-1})	*In vitro* tolerance to NaCl (mM)	Morphological group
PP-1	passion fruit	0.8	1000	I B
PA-6	rice	5.41	1250	I B
PS-2	*S. portulacastrum*	24.3	1500	I B
PSR-8	*S. portulacastrum*	60.2	1000	II A
PM-1	watermelon	1.77	750	II B
PM-6	watermelon	1.51	1000	I B
PM-8	watermelon	2.44	1000	I B
PB-5	banana	1.3	750	I A
PB-32	banana	1.83	1500	I B
PB-33	banana	1.83	1500	I B
PBR-15	banana	2.66	1000	I B
PBR-40	banana	1.9	1250	I B
PC-4	melon	7.7	750	I B
PCR-23	melon	11	1000	I B
PCR-29	melon	6.9	1500	I B
PCR-28	melon	6.9	1500	II A
PCR-30	melon	6.9	1250	I B
PCR-33	melon	11	1500	I B
PCR-37	melon	6.9	750	II A
PCR-38	melon	6.9	1000	II B

Melon growth promotion by *Pseudomonas* spp. in saline substrates

Salinity negatively affected melon growth, regardless of the biological treatment and of the level of saline stress. The

Table 2. Tolerance of fluorescent *Pseudomonas* spp. isolates cultivated in B medium at different concentrations of NaCl related to crops and ECse of collected soil samples.

Host plant	Soil ECse (dS m^{-1})	Concentration of NaCl in the medium (mM)			
		750 (60.3 dSm^{-1})	1000 (73.2 dS m^{-1})	1250 (84.7 dS m^{-1})	1500 (94.4 dS m^{-1})
Passion fruit (C)*	0.80		6		
Rice (J)	7.41		3	6	
Watermelon (C)	1.96	2	5	3	1
S. portulacastrum (P)	24.4		2		
S. portulacastrum (J)	60.4		7		1
Banana (P)	1.59	4	37	10	4
Melon (J)	5.46	2	18	33	3
	Total	8	78	52	9

*(C) – Curaçá; (J) Juazeiro; (P) Petrolina.

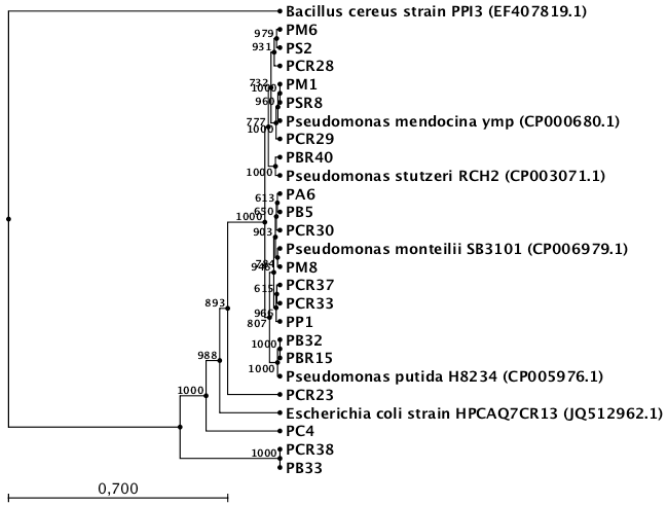

Figure 2. Phylogeny mounted with sequences generated by ITS sequencing typical of *Pseudomonas* spp. using the UPGMA method. Sequences of PM1/PSR8, PB32/PBR15, and PCR38/PB33 are genetically identical. PCR38/PB33 could not be identified.

inoculation of bacteria, regardless of the level of salinity in the soil, stimulated melon plant growth. There was significant interaction ($p \leq 0.05$) between isolated bacterial factors and salinity levels, and inoculation with fluorescent *Pseudomonas* spp. at the different levels of salinity induced a higher tolerance of melon plants to salinity compared to the control without bacteria (Tables 4 and 5).

The plants inoculated with fluorescent *Pseudomonas* spp. isolates evaluated in the salinity intervals showed an average increase in LA, CL, SDM and PH ranging from 11 to 35, 6 to 17, 8 to 37, and 10 to 37%, respectively, compared to non-inoculated plants. These results showed that the inoculation of isolates in melon plants mitigated the negative effects of salinity (Tables 4 and 5).

Increased saline concentration in soil solution progressively decreased the foliar area in melons, regardless of bacterial isolates (Table 4); however, inoculation with all *Pseudomonas* spp. isolates provided statistically higher results than those of the control, regardless of salinity, with a higher increase for *P. putida* PCR-28, PCR-30, PM-8, PBR-15, PA-6 isolates; *P. stutzeri* PBR-40 isolate, and *Pseudomonas sp.* PCR-29 and PC-4 isolates.

Except for *P. putida* PBR-15 and *P. mendocina* PSR-8 isolates, statistically higher LA results in ECse of 0.2 dS m^{-1} were observed for all isolates, with maximum increase in LA of 39% (*P. putida* PM-8 isolate) compared to the control. Under ECse of 2 dS m^{-1}, 17 isolates showed statistically higher results than the control; however, a higher LA value was observed for *P. putida* PBR-15, PA-6, PCR-30 and PB-5 isolates, with an increase of 58 to 79% compared to the control. In ECse of 4 dS m^{-1}, *P. putida* PBR-15, PA-6, and PCR-28 isolates, *P. stutzeri*

Table 4. Leaf area and chlorophyll of melons under different salinity levels and inoculation of fluorescent *Pseudomonas* spp. isolates.

Isolates	Leaf area (cm^2)				ECse	Chlorophyll (SPAD)				
	0.2	2.0	4.0	6.0	Mean	0.2	2.0	4.0	6.0	Mean
Control	417 c	264 c	243 b	224 b	287 d	23.7 c	20.8 b	21.0 b	23.2 c	22.1 c
PSR-8	345 d	333 b	295 b	304 a	319 c	24.0 c	22.2 a	22.5 b	23.7 c	23.1 c
PB-33	466 b	259 c	317 b	241 b	321 c	25.1 c	19.6 b	22.3 b	24.1 c	22.8 c
PCR-33	474 b	376 b	268 b	183 b	325 c	26.1 c	22.7 a	21.4 b	25.3 b	23.9 b
PS-2	483 b	273 c	249 b	312 a	328 c	26.7 b	19.2 b	20.0 b	24.4 c	22.6 c
PM-6	486 b	377 b	282 b	225 b	343 b	25.7 c	22.1 a	21.3 b	22.1 c	22.8 c
PCR-38	493 b	384 b	270 b	252 b	350 b	26.4 b	22.4 a	20.9 b	26.2 b	24.0 b
PP-1	493 b	333 b	282 b	311 a	355 b	26.8 b	21.8 a	22.8 a	24.2 c	23.9 b
PB-32	452 b	389 b	298 b	292 a	358 b	25.0 c	23.2 a	22.1 b	23.5 c	23.4 b
PM-1	474 b	360 b	310 b	299 a	361 b	25.5 c	21.8 a	20.6 b	25.1 b	23.3 c
PCR-37	485 b	388 b	290 b	280 a	361 b	24.2 c	22.2 a	21.9 b	27.4 a	23.9 b
PCR-23	506 b	365 b	309 b	295 a	369 b	26.4 b	20.9 b	22.5 b	23.4 c	23.3 c
PB-5	457 b	423 a	305 b	313 a	374 a	25.3 c	24.8 a	23.6 a	28.4 a	25.5 a
PCR-28	448 b	384 b	363 a	308 a	376 a	25.5 c	22.7 a	24.6 a	26.5 b	24.8 a
PCR-30	449 b	475 a	302 b	292 a	379 a	23.7 c	23.4 a	22.9 a	24.9 c	23.7 b
PM-8	581 a	394 b	301 b	224 b	381 a	26.3 b	22.6 a	24.0 a	24.4 c	24.3 a
PCR-29	89 b	392 b	349 a	298 a	382 a	27.1 b	23.2 a	21.9 b	23.6 c	23.9 b
PC-4	469 b	389 b	343 a	333 a	384 a	23.8 c	22.8 a	22.3 b	23.6 c	23.1 c
PBR-40	503 b	379 b	340 a	318 a	385 a	24.9 a	22.9 a	23.8 a	25.6 b	24.9 a
PA-6	474 b	419 a	365 a	289 a	387 a	29.1 a	23.1 a	21.9 b	25.7 b	24.9 a
PBR-15	422 c	460 a	368 a	298 a	387 a	29.1 a	24.0 a	24.7 a	25.6 b	25.9 a
Mean	470 a	370 b	308 c	282 d		25.8 a	22.3 c	22.3 c	24.8 b	
Significance factors										
Isolates			**					**		
Salinity			**					**		
Interaction			**					**		
C. V. (%)			15.79					8.3		

* * Significant at 1% of probability using the F test.
Means followed by the same lowercase letters within each salinity level evaluated in the column for isolates and lowercase letters on the row do not differ from each other using Scott-Knott test at 5 %.

Table 5. Effects of fluorescent *Pseudomonas* spp. isolates in shoot dry matter and melon height under different salinity levels.

Isolates	Shoot dry matter (g)				ECse	Plant height (cm)				
	0.2	2.0	4.0	6.0	Mean	0.2	2.0	4.0	6.0	Mean
Control	2.40 c	1.78 c	1.52 b	1.50 b	1.80 c	61.1 b	42.5 c	39.8 b	32.2 b	43.9 c
PSR-8	2.00 d	1.70 c	1.95 b	1.60 b	1.81 c	56.4 b	48.4 b	45.2 b	48.0 a	48.5 b
PB-33	2.50 c	1.81 c	2.07 b	1.54 b	1.98 b	6.4 b	41.5 b	47.8 b	44.1 a	51.0 b
PCR-33	2.80 b	2.35 b	1.92 b	1.42 b	2.12 b	74.1 a	58.8 a	42.5 b	34.0 b	52.3 b
PS-2	2.75 b	1.81 c	1.56 b	1.69 b	1.95 b	66.4 b	44.2 b	37.0 b	51.8 a	49.8 b
PM-6	2.82 b	2.46 b	1.82 b	1.73 b	2.21 a	72.1 a	58.2 a	42.0 b	37.0 b	52.3 b
PCR-38	3.05 a	2.62 a	1.78 b	1.50 b	2.24 a	68.7 b	54.8 a	37.5 b	44.2 a	51.8 b
PP-1	3.08 a	2.29 b	1.85 b	2.19 a	2.35 a	76.0 a	48.0 b	42.7 b	44.5 a	52.8 b
PB-32	2.19 d	2.45 b	2.06 b	1.62 b	2.08 b	67.0 b	62.8 a	50.8 a	50.0 a	57.6 a
PM-1	3.03 a	2.30 b	2.02 b	1.92 a	2.32 a	75.7 a	55.8 a	47.7 b	53.5 a	58.2 a
PCR-37	2.54 c	2.49 b	1.93 b	1.52 b	2.12 b	68.2 b	56.8 a	47.5 b	48.1 a	54.9 a
PCR-23	3.26 a	2.36 b	1.95 b	1.93 a	2.38 a	78.1 a	57.2 a	46.0 b	47.4 a	57.2 a
PB-5	2.56 c	2.93 a	1.94 b	1.69 b	2.28 a	75.1 a	55.4 a	45.5 b	47.4 a	55.8 a
PCR-28	2.67 b	2.16 b	2.29 a	2.27 a	2.35 a	68.8 b	49.5 b	52.1 a	42.4 b	53.2 b
PCR-30	2.67 b	2.98 a	2.10 a	1.50 b	2.31 a	66.8 b	63.1 a	49.1 a	49.4 a	57.1 a
PM-8	3.22 a	2.62 a	1.94 b	1.37 b	2.29 a	73.8 a	53.5 a	44.5 b	38.4 b	53.1 b
PCR-29	2.95 a	2.48 b	2.24 a	1.84 a	2.38 a	65.0 b	55.1 a	53.1 a	51.2 a	56.1 a
PC-4	2.80 b	2.58 a	2.31 a	2.22 a	2.48 a	68.7 b	56.4 a	56.7 a	55.0 a	59.2 a
PBR-40	3.01 a	2.22 b	2.27 a	1.98 a	2.37 a	72.7 a	48.8 b	54.2 a	54.2 a	57.5 a
PA-6	2.47 c	2.58 a	2.30 a	1.43 b	2.19 a	69.5 b	59.0 a	55.1 a	45.8 a	57.3 a
PBR-15	2.03 d	2.68 a	2.42 a	1.51 b	2.16 a	60.7 b	63.2 a	54.8 a	47.4 a	56.1 a
Mean	2.70 a	2.35 b	2.02 c	1.71 d		69.1 a	53.5 b	47.4 c	46.6 c	
Significance factors										
Isolates			**					**		
Salinity			**					**		
Interaction			**					**		
C. V. (%)			15.20					15.94		

* * Significant at 1% of probability using the F test.
Means followed by the same lowercase letters within each salinity level evaluated in the column for isolates and lowercase letters on the row do not differ from each other using Scott-Knott test at 5 %.

PBR-40 and PC-4 isolates promoted a statistically significant increase of 41 to 51% in LA compared to the control. In ECse of 6.0 dS m^{-1}, 15 isolates promoted statistically significant increases, ranging from 25 to 48%.

A decrease in leaf area due to increased soil salinity has been observed in several crops, as reported by Machado & Serralheiro (2017), since plants submitted to salinity show limitation in cell expansion through increased osmotic pressure in the environment and consequent cell plasmolysis, thus affecting the division and elongation phases (Hu & Schmidhalter, 2008).

Important physiological processes, including elongation and cell division, as well as responses to light, are controlled by plant hormones such as Indol Acetic Acid (IAA), Gibberellins and Cytokinins (Almeida & Rodrigues, 2016), and growth regulator producing-bacteria might interact with the plants and show potential to interfere in any one of these processes. Thus, factors that influence hormone balance, as well as nutritional regulation in the plant might be related to a higher tolerance to osmotic stress (Nadeem et al., 2014), which might explain the fact that several bacterial isolates provided statistically higher results of leaf area in salinized substrates.

Results presented in Table 4 show that there was a decrease in chlorophyll content in the plants under saline stress compared to the control, regardless of bacterial inoculation; however, inoculation of bacterial isolates significantly increased chlorophyll content (measured in SPAD) in melons. Eight isolates caused a significant increase under ECse of 0.2 dS m^{-1}, with maximum values of 21% observed in plants that received *P. putida* PBR-15 and PA-6 isolates compared to the non-inoculated control. Under ECse of 2 dS m^{-1}, 17 isolates caused statistically higher results than the control, with increases of 5 to 19%. Under ECse of 4 dS m^{1}, 17 isolates caused statistically higher results than the control, with increases from 9 to 17%. In ECse of 6 dS m^{-1}, the inoculation of nine isolates caused increased chlorophyll content in melons, with an increase of 18 to 22% observed for *P. putida* PCR-37 and PB-5 isolates compared to the control. Additionally, plants inoculated with 14 bacterial isolates were observed to show statistically higher chlorophyll content than the control, with a higher increase with *P. monteilii* PM-8 isolate, *P. putida* PA-6, PCR-28, PB-5, PBR-15 isolates, and *P. stutzeri* PBR-40 isolate, regardless of salinity levels. These results corroborate Ferreira et al. (2018), who reported that corn crops inoculated with *B. subtilis* presented better tolerance to salts and showed higher chlorophyll content than non-inoculated plants.

According to Machado & Serralheiro (2017), salinity might induce both the inhibition in absorption of essential elements and the accumulation of toxic ions, which in turn might inhibit photosynthesis and protein synthesis, inactivate enzymes, and cause damage to chloroplasts and other organelles. However, increased chlorophyll contents might be justified by the positive effect of bacteria on nutritional regulation of plants under saline stress. Studies conducted by Kang et al. (2016)

showed that in addition to chlorophyll content in cucumber, there was increased potassium and phosphorus contents in plants submitted to saline stress under the effect of PGPRs compared to non-inoculated controls. In addition, fluorescent bacteria of genus *Pseudomonas* spp. provide ferric ion (Fe^{3+}) to plants as they produce siderophores, thus indirectly causing plant growth (Nadeem et al., 2014). The solubility of this ion might be very low and insufficient to meet the nutritional needs of the plant; although it is frequently the most required micronutrient, it is not part of the chlorophyll molecule, but participates in the synthesis of a catalytic group of oxyreducer enzymes, going from ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}) and vice-versa, playing an important role in electron transfer in photosynthesis and respiration (Dudeja et al., 1997).

Shoot dry matter (SDM) decreased progressively with increased soil ECse (table 5), regardless of bacterial inoculation. However, for melons inoculated with 13 *Pseudomonas* spp. isolates, under ECse of 0.2 dS m^{-1}, a statistically higher performance in SDM accumulation was observed, with a higher increase observed with *Pseudomonas* sp. PCR-29 and PCR-23 isolates, *P. stutzeri* PBR-40 isolate, *P. mendocina* PM-1 isolate, *P. putida* PP-1 and *P. monteilii* PM-8 isolates, and PCR-38 isolate, with values ranging from 23 to 35% compared to the control. *P. mendocina* PSR-8 isolate, and *Pseudomonas sp*. PB-32 and PBR-15 isolates showed statistically lower results than those of the control.

Under ECse of 2 dS m^{-1}, the inoculation of 17 isolates provided a higher performance than that of the control, with higher increase observed in plants that received *P. putida* PA-6, PM-8, PBR-15, PB-5, PCR-30 isolates, *Pseudomonas* sp. PC-4 isolate, and the unidentified PCR-38 isolate, with increase of 44 to 64% compared to the control. In ECse of 4 dS m^{-1}, the inoculation of *P. putida* PCR-30, PBR-15, PCR-28, PA-6 isolates, *Pseudomonas* sp. PC-4, and PCR-29 isolates, and *P. stutzeri* PBR-40 isolate resulted in an increase from 38% to 59% compared to the control. Under ECse of 6 dS m^{-1}, the inoculation of *Pseudomonas* sp. PC-4, PCR-29 and PCR-23 isolates, *P. mendocina* PM-1 isolate, *P. stutzeri* PBR-40 isolate, and *P. putida* PCR-28 isolate caused a statistically significant increase compared to the control. On average, 19 isolates caused statistically higher results compared to those of the control regarding shoot dry matter production, except for *P. mendocina* PSR-8 isolate.

Effects on decreased dry matter, vigor, and height of melon seedlings submitted to saline stress were observed by Sarabi et al. (2016). These effects are associated to disturbances in homeostasis and nutrient distribution, which deactivate the transport of electrons from the photosynthetic and respiratory apparatus (Munns & Gilliam, 2015).

As observed in the present study, other authors have reported mitigation in reduced plant growth under saline stress, with the inoculation of PGPR in several crops (Karlidag et al., 2013; Bacilio et al., 2016; Pereira et al., 2016; Egamberdieva et al., 2017). Shoot growth in pumpkin seedlings observed by Yildirim et al. (2006) was related to reduced absorption of Na^+ and increased absorption of K^+ by plants compared to the control treatment under saline stress. The increase in the K^+/Na^+ ratio might have contributed to the higher tolerance of melons to salinity.

The increase in salinity levels negatively affected melon height, regardless of bacterial inoculation (Table 5). The inoculation of the 20 bacterial isolates caused an increase in plant height compared to non-inoculated plants, regardless of salinity. For inoculated plants, under the conditions of ECse of 0.2 dS m^{-1}, PM-6, PBR-40, PM-8, PCR-33, PB-5, PM-1, PP-1, and PCR-23 isolates caused statistically significant increases, from 18 to 28%, in PH compared to the control. Under ECse of 2 dS m^{-1}, 14 isolates showed higher performance than the non-inoculated control, with a higher increase observed with *P. putida* PCR-30 and PBR-15 isolates, and *Pseudomonas* sp. PB-32 isolate, with values ranging from 38 to 48% compared to the control. Under the electric conductivity of 4 dS m^{-1} in the substrate, the inoculation of *P. putida* PCR-30, PBR-15, PCR-28, PA-6 isolates, *Pseudomonas* spp. PB-32, PCR-29 and PC-4 isolates, and *P. stutzeri* PBR-40 isolate caused increased melon plant height. The same occurred with ECse of 6 dS m^{-1}; the inoculation of 20 isolates resulted in a higher performance compared to the non-inoculated control.

Decreased plant height due to increased soil salinity might be a secondary effect of increased ethylene levels in the plant. Aside from the mechanisms discussed previously through which PGPR promote plant growth in saline environments, we highlight the decreased levels of ethylene due to the action of an enzyme on 1-aminocyclopropane-1-carboxilate (ACC) (Ali et al., 2014). The ACC deaminase enzyme, produced by some bacteria, hydrolyzes ACC (an immediate precursor of ethylene in plants), ammonia and alpha-ketobutyrate, thus decreasing the levels of endogenous ethylene in the plants.

Overall, the results in this study showed that microorganisms, either in their individual aspects and/or through their interactions with melon, mitigated the negative effects of salinity on the analyzed variables, with emphasis on isolates PBR-40 (*P. Stutzeri*) and PCR-28 (*P. Putida*), which showed higher agronomic performance of all variables analyzed under salinity conditions. The PGPR, a metabolically and functionally diverse group of soil-inhabiting bacteria, exhibit multiple mechanisms that promote crop growth (Vimal et al., 2017). They can affect plants in several different ways, depending on the biotic and abiotic conditions that are exposed, and it is often difficult to attribute all changes to a specific bacterial mechanism. While PGPB can provide some protection against the inhibitory effects of salt stress (i.e. by promoting plant growth), they may also alter plant gene expression so that the plant is less likely to succumb to these stresses (Forni et al. 2017).

It is important to highlight the effect observed in melons inoculated with PSR8, obtained from rhizospheric soil samples of *S. portulacastrum*, highly salinized (electric conductivity of approximately 60 dS m^{-1} in the saturation extract at 25 °C). In these plants, values of all analyzed variables were lower or statistically similar to the control from non-saline soil. However, LA and PH variables in ECse of 6dS m^{-1} and chlorophyll content with ECse of 2 dS m^{-1} showed lower magnitude of performance loss in melons (less than 20%) with increased salinity. These

results indicate that the mechanisms which were developed for absorption, transport, and use of mineral nutrients from saline substrates might not be the most efficient or effective under non- saline conditions for the abovementioned isolate. According to Nadeem et al. (2014), the same mechanisms through which PGPR caused plant growth might also be harmful in certain situations, i.e. the production of excess auxins which might inhibit root development. This necessary balance between the factors for plant growth promotion to be effective must be elucidated. Thus, further in-depth studies on underlying physiological and molecular mechanisms related to the interaction plant-microorganisms in saline soils might provide subsidies to optimize the agronomic application of these microorganisms (Dood & Pérez-Alfocea, 2012).

It is also worth noting that the specificity between the origin of isolates and levels of salinity tolerated *in vitro* in the present study was not observed in melon growth promotion in salinized soils.

Conclusions

The isolates obtained were tolerant to high concentrations of salts; however, with reduced populations in higher doses.

Inoculated isolates are capable of promoting growth in melons under non-saline and saline conditions.

Plants inoculated with PBR-40 (*P. stutzeri*) and PCR-28 (*P. putida*) showed a better agronomic performance in all analyzed variables when exposed to saline substrate conditions, mitigating harmful effects of excessive concentrations of soluble salts in the growth substrate.

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