**Siderophore-producing rhizobacteria:
empowering plants to cope with iron limitation in salt-
affected soils**

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Manuscripts

1 **Siderophore-Producing Rhizobacteria:**
2 **Empowering Plants to Cope with Iron Limitation in Salt-Affected Soils**

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15 Originality-Significance Statement

Iron (Fe) bioavailability to plants is reduced in saline soils although the exact mechanisms underlying this effect are still not completely understood. Siderophore-expressing rhizobacteria may represent a promising alternative to chemical fertilizers by tackling at once salt-stress effects and Fe limitation in saline soils

21 Summary

In addition to draught, plants growing in arid soils face two major challenges: high salinity and iron (Fe) deficiency. Salinity attenuates growth, affects plant physiology and causes nutrient imbalance which is, in fact, one of the major consequences of saline stress. Fe is a micro-nutrient essential for plant development. It is required for several metalloenzymes involved in photosynthesis and respiration and Fe-deficiency is associated to chlorosis and low crop productivity. The role of microbial siderophores in Fe supply to plants is well documented as well as the effect of plant growth promoting rhizobacteria (PGPR) on the

mitigation of saline stress in crop cultures. However, the dual effect of siderophore-producing PGPR both on salt-stress and on Fe limitation is still poorly explored.

This review provides a critical perspective on the combined effect of Fe limitation and soil salinization as challenges to modern agriculture and intends to summarize some indirect evidence that argue in favour of siderophore-producing PGPR as bio-fertilization agents in salinized soils. Recent developments as well as future perspectives on the use of PGPR are discussed as clues to sustainable agriculture practices, in the context of present and future climate change scenarios.

Key Words: aridity, biofertilizers, Fe limitation, plant growth-promoting rhizobacteria (PGPR), saline stress, siderophores.

INTRODUCTION

Soil salinization may be caused by natural processes (primary salinization) or human activities (secondary salinization). Salt-water intrusion and wind-born salt deposition on land are two major causes of natural salinization of the soil in addition to the weathering of primary rock minerals (Daliakopoulos *et al.*, 2016). However, in cultivated lands the most common origin of salts is the circulating water (Aragüés *et al.*, 2014; Wang *et al.*, 2015), whereas in urban areas inadequate drainage systems and the use of deicing mixtures also contribute to the development of saline and sodic soils (Nikiforova *et al.*, 2014).

Fe is a micronutrient, essential for almost all living organisms and its availability is often limited. The mechanisms involved in Fe dynamics under saline conditions and the precise regulatory elements of this interaction are still poorly understood. Salinity decreases the solubility of trace elements, like Fe (Chen *et al.*, 2012), and recent studies suggest that salinity correlates negatively with Fe availability to plants (Abbas *et al.*, 2015; Li *et al.*, 2016; Purohit *et al.*, 2016).

In this review we address the effect of soil salinity on plant growth, the relation between

57 saline stress and Fe limitation and the role of bacterial and plant siderophores, emphasizing
58 the potential use of siderophore-producing bacteria as a mean to alleviate Fe deficiency in
59 saline soils.

60

61 **EFFECTS OF SOIL SALINITY ON PLANT GROWTH**

62 The nature and magnitude of the effects of salinity depend on the sensitivity of plants to salt.
63 In general, cereals (rice, maize), forages and horticultural crops (tomatoes, lettuce, onions,
64 potatoes) are very susceptible to salinity (Shannon and Grieve, 1998; Paul and Lade, 2014).
65 The response of plants to increased salinity occurs in two phases: the first is a result of the
66 osmotic effect and the second is a salt-specific effect (Fig. 1).

67

68 **Fig 1** Schematic representation of the plant responses to saline-alkaline stress (osmotic
69 adjustment and ionic effect).

70

71 The first phase happens within minutes after the exposure to salt. It is related to osmotic
72 changes outside the root, diminishing the ability of the plant to absorb water. The second

phase, that may actually overlap the first, is a slower response that involves the accumulation of ions in the tissues, leading to ion toxicity (Munns *et al.*, 1995; Negrão *et al.*, 2017). Elevated salinity leads to structural damage and metabolic impairment that will ultimately cause an overall reduction in crop productivity (Meng *et al.*, 2017). Germination and seedling growth are significantly retarded under salt-stress conditions, even for halophytes (Ameixa *et al.*, 2016; Konuşkan *et al.*, 2017), and plant maturation is delayed (Becker *et al.*, 2017). Plants growing under salt-stress conditions tend to be shorter and have fewer and smaller leaves and a lighter coloration because of a decrease in chlorophyll content (Taïbi *et al.*, 2016; Acosta-Motos *et al.*, 2017). Root meristems and root length are reduced with an overall decrease in below-ground biomass (Liu *et al.*, 2015). Protein synthesis, photosynthesis and several enzymes are inhibited and organelle function is impaired (Dubey, 1999; Pessarakli, 2014). Salt exposure induces nutrient imbalances (Grattan and Grieve, 1998). Ionic strength in the rhizosphere may negatively affect the intake of water and solutes (Abbasi *et al.*, 2016; Ma *et al.*, 2017) and the competition between Na^+ and Cl^- (major ions in saline/sodic soils) and other ions like Mg^{2+} and NO_3^- , reduces nutrient availability (Reich *et al.*, 2017). Although soil salinity induces detrimental physiological, biochemical and morphological effects, some improvements on the nutritional value of edible plants, mainly in terms of antioxidant enzymes, phenolic compounds, carotenoids, compatible osmolytes and minerals, have been reported (Kim *et al.*, 2008; López-Berenguer *et al.*, 2009; Petropoulos *et al.*, 2017).

SALINE STRESS AND Fe LIMITATION

Fe is the second most common metal in the Earth's crust. In biological systems, Fe has an essential role in a large number of proteins, such as oxygen carriers (haemoglobin, myoglobin), activators of molecular oxygen (cytochrome P450, cytochrome oxydases), electron transporters, Fe-S enzymes, mononuclear Fe proteins (e.g. dioxygenases, hydroxylases), dinuclear Fe proteins (carboxylases), di-Fe proteins and major Fe transport and storage proteins (e.g. transferrin, transferrin receptor ferritins) (Cassat and Skaar, 2013; Cohen, 2014; Bogdan *et al.*, 2016). It acts as a catalyst in chlorophyll synthesis (Hu *et al.*,

101 2017) and in enzymatic processes involving oxygen (peroxidase, catalase), hydrogen
102 (hydrogenases), nitrogen (nitrogenases) and electron transfer processes in the respiratory and
103 photosynthetic electron chains (cytochromes and Fe-sulphur proteins) (Sandmann, 1985;
104 Enthaler *et al.*, 2008; Masepohl, 2017) and is essential for DNA and RNA synthesis and
105 repair processes (Puig *et al.*, 2017).

106 Although widely available in nature, Fe is not always accessible to plants and it often limits
107 biological productivity of plants (Briat *et al.*, 2015; Falkowski *et al.*, 2017). Fe can exist in
108 aqueous solution in two readily inter-convertible states: Fe^{2+} and Fe^{3+} . In soils, the balance
109 between the two forms is influenced by pH, aeration, organic matter (OM) content and
110 salinity. In the presence of oxygen and neutral pH, a rapid oxidation occurs and the ion Fe^{2+} is
111 oxidized to Fe^{3+} , which leads to the formation of insoluble and potentially biologically
112 inaccessible Fe^{3+} oxides and hydroxides (Colombo *et al.*, 2014). In aerated acid soils, when
113 microbial activity is high, oxygen levels decrease and Fe^{3+} hydroxides transform into more
114 soluble forms of Fe (Masalha *et al.*, 2000). In alkaline conditions, high soil pH reduces the
115 solubility of Fe in the soil solution, which causes symptoms of Fe chlorosis in plants (Chen
116 and Barak, 1982).

117 Organic matter combines with Fe, reducing chemical fixation and precipitation of Fe as Fe^{3+}
118 hydroxide. This process results in higher concentrations of available Fe for root absorption
119 (Lindsay, 1991). OM also has an indirect effect on Fe bioavailability by stimulating microbial
120 activity and consequently decreasing the concentration of oxygen and lowering pH.
121 Ultimately, it creates conditions that are less favourable to the formation of insoluble oxides
122 and hydroxides (Colombo *et al.*, 2014). The release of reducing and chelating agents by
123 bacteria in the rhizosphere also increases Fe^{2+} availability to plants (Aguado-Santacruz *et al.*,
124 2012; Mimmo *et al.*, 2014).

125 Salinity has an additive effect on Fe deficiency in plants and chlorosis related with Fe-
126 deficiency is enhanced (Nenova, 2008; Abbas *et al.*, 2015). Factorial experiments
127 demonstrated that plant growth and chlorophyll content are significantly more affected by the
128 combination of salt-stress and Fe deprivation than by a single factor (Rabhi *et al.*, 2007).

129 Negative effects of salinity on Fe acquisition have been demonstrated in several salt-sensitive
130 plants, namely barley (Yousfi *et al.*, 2007), maize (Turan *et al.*, 2010), rice (Li *et al.*, 2016),
131 mustard (Chakraborty *et al.*, 2016) and wild species of annual medics (Ferchichi *et al.*, 2016).
132 Salt-stress effects were attenuated and plant growth could be stimulated by experimentally
133 supplying supplemental Fe to roots or leaves (El Fouly *et al.*, 2010; Heidari and Sarani, 2012;
134 Torabian *et al.*, 2017; Mozafari and Ghaderi, 2018)
135 It has been proposed that Fe limitation may develop from a down-regulation of Fe
136 transporters in response to salinity (Cotsaftis *et al.*, 2011). However, the analysis of root
137 proteome of salt-sensitive genotypes of barley and rice showed that proteins involved in Fe
138 uptake were expressed at a higher level upon exposure to increased salinity or sodicity
139 (Witzel *et al.*, 2009; Li *et al.*, 2016).
140 The reduced Fe bioavailability under saline conditions has also been attributed to an
141 inhibition of some proton pumps that will attenuate the acidification of the surrounding
142 medium in relation to what is observed under Fe-limitation in non-saline conditions, (Rabhi *et*
143 *al.*, 2007). However, the effect of Fe-deficiency and salinity on proton pumps is still
144 controversial. Experiments with two rice genotypes demonstrated a significant reduction in
145 the pH of the growth medium after exposure to sodic-alkaline stress (10 mM Na₂CO₃ and 40
146 mM NaCl), indicating that depending on the chemical context, genes related with
147 acidification may actually be overexpressed as a response to salt stress (Li *et al.*, 2016).
148 Therefore, although being a well-known effect, salt-enhanced Fe limitation is probably the
149 results of a complex network of chemical and biological processes that are not yet fully
150 understood.

151

152 **SALINITY AND Fe MOBILIZATION IN THE RHIZOSPHERE**

153 In terrestrial ecosystems, plant root activity creates a microenvironment (rhizosphere) with
154 different physical, chemical and biological characteristics from the bulk soil which extends
155 from a few μm to several cm and even m, depending on the root morphology, soil
156 characteristics, microbial and fungal symbiosis, among other factors (Coleman *et al.*, 2017;

157 Pausch and Kuzyakov, 2018). Rather than being defined by its size or shape, the rhizosphere
158 consists of a gradient in chemical, biological and physical properties of the sediments under
159 the influence of roots (McNear Jr, 2013).

160 Plant root exudates are directly and indirectly involved in Fe mobilization in the rhizosphere
161 (Mimmo et al., 2014). Large amounts of low-molecular-weight primary (sugars, amino acids
162 and organic acids) and secondary (phenolics, flavonoids and terpenoids) metabolites and
163 some inorganic molecules (CO₂ and H₂O, among others) are released into the rhizosphere
164 (Gargallo-Garriga *et al.*, 2018). These compounds are directly involved in processes of
165 dissolution, reduction and complexation of Fe and also provide an important source of
166 substrates for rhizospheric microorganisms whose activity will affect the biological Fe
167 mobilization in soils (Fig. 2). The release of protons and organic acids by microbial
168 respiration, nitrification and phosphate solubilization activities in the rhizosphere causes the
169 solubilization of iron-containing minerals (Malhi *et al.*, 1998; Posada *et al.*, 2013).

170

171

172 **Fig 2** Mechanisms of biological Fe mobilization in soils. Reduction involves the production
173 of reductases and phenolic acids. Siderophores (microorganisms), phytosiderophores (plants)

are involved in Fe chelation. Protonation occurs via release of protons or organic acids to the rhizosphere.

Once released into the environment, under aerobic and neutral pH conditions, Fe^{2+} is readily oxidized to Fe^{3+} and tends to complex with organic molecules or precipitate as oxides, hydroxide or other insoluble forms. Reductases and phenolic compounds with reducing capacity, allow the dissociation and reduction of complexed Fe^{3+} making it available for transport into the cells.

Salinity can affect the composition of root exudates and, by extension, Fe-DOC interactions. In experiments with *Acacia* exposed to saline stress, increased expression of antioxidant enzymes (SOD and CAT) and enhanced release of organic acids (tartaric acid and citric acid) and H^+ were observed (Abbas *et al.*, 2015). Higher concentrations of organic acids in root exudates were also reported in tomato, in response to saline conditions (Chen and Lin, 2010). The excretion of organic acids into the rhizosphere improves Fe uptake by plants (Abadía *et al.*, 2002) and it may also reduce the harmful effects of soil salinity and sodicity by dissolving calcite and allowing the leaching of Na^+ below the effective rooting depth (Qadir *et al.*, 2005). The dynamics of root-derived DOC and Fe availability in the rhizosphere are affected by salinity and sodicity in different ways. Salinity can cause an increase in DOC concentrations in soil by reducing microbial respiration rates. Sodicity associated with low electrical conductivity may also cause an increase in DOC concentrations by enhancing organic matter solubilization, without having, however, a significant effect on microbial respiration (Mavi *et al.*, 2012). This effect, associated with coarse sediment texture can lead to DOC leaching and further soil deterioration (Mavi *et al.*, 2012).

By affecting microbial activity and microbe-plant interactions in the rhizosphere, salinity may also have an impact on the availability of Fe to plants. Some studies suggest that soil microorganisms can regulate Fe acquisition in plants via a signaling process (Hindt and Guerinot, 2012) in which hormones, such as auxin (Chen *et al.*, 2010), ethylene (García *et al.*, 2011) and cytokinins (Séguéla *et al.*, 2008), function as signaling elements. Symbiotic

interactions within the rhizosphere microhabitat, like rhizobium nodulation and mycorrhizal fungal infection, stimulate the production of Fe^{3+} chelators and protons and ultimately enhance plant Fe uptake capacity (Jin *et al.*, 2013).

205

206 **SIDEROPHORES – OVERCOMING Fe LIMITATION**

Siderophores and phytosiderophores are low-molecular weight (<1500 Da) Fe^{3+} -specific chelating agents, which form complexes with free Fe and transport it into the cell by interacting with membrane receptors (Johnstone and Nolan, 2015; Saha *et al.*, 2016). The strong binding between Fe^{3+} and siderophore protects the complex against hydrolysis and enzymatic degradation in the environment (Winkelmann, 2007).

The mechanisms of Fe acquisition differ between organisms (Fig. 3). In plants, these mechanisms are referred as strategies I and II. In Strategy I, operated by some dicotyledonous and non-graminaceous monocotyledonous, H^+ and other secondary metabolites are released from the roots, liberating Fe^{3+} from the negatively charged particles in the soil, and triggering the Fe chelate reductase. The Fe^{3+} chelate reductase FRO2 reduces Fe^{3+} to Fe^{2+} , which is finally translocated into the intracellular compartment of epidermis cells by the Fe regulated transporter (Kim and Guerinot, 2007; Tsai and Schmidt, 2017). More recently, the role of root-derived iron-mobilizing compounds was also highlighted. Fe-deficient *Arabidopsis* releases high amounts of phenolic compounds into the growth media (Rodríguez-Celma *et al.*, 2013). The reduction of Fe^{3+} by coumarin and flavin and the production of complexes that will be taken up by the root have been proposed as important strategies of Fe scavenging in plants (Sisó - Terraza *et al.*, 2016; Curie and Mari, 2017; Tsai and Schmidt, 2017). Gramineous monocotyledonous use Strategy II in which Fe^{3+} binds to phytosiderophores that are excreted to the rhizosphere via the phytosiderophore efflux transporter TOM1 (Kobayashi and Nishizawa, 2012). Phytosiderophores act as high-affinity chelators and the complex Fe^{3+} -PS is then transported into the cell by YSL-like proteins (YS1 in maize) that are efficient transporters for phytosiderophore-chelated Fe (Nozoye *et al.*, 2011).

229

230 **Fig 3** Strategy I and Strategy II Fe acquisition mechanisms in plants (simplified). Strategy I231 plants acidify the rhizosphere by releasing protons in order to liberate Fe³⁺ ions from the232 negatively charged particles in the soil. FRO2, an Fe reductase, reduces Fe³⁺ to Fe²⁺. Finally,233 IRT1 (Iron Regulated Transporter) moves Fe²⁺ into the epidermis cells. Strategy II plants234 excrete phytosiderophores (PS) to the rhizosphere via TOM1. The complex Fe³⁺-PS is then

235 transported into the cell by the YS1 membrane protein (in maize) or YSL (in other grasses).

236

237 Additionally, plants take advantage of siderophores secreted by microorganisms as an

238 alternative or complementary strategy for Fe uptake (Shirley *et al.*, 2011). Although the

239 mechanisms are still not completely understood, Fe uptake may occur by ligand exchange

240 between microbial siderophores and phytosiderophores or by direct uptake of Fe³⁺

241 siderophores (Loper and Buyer, 1991). Therefore, the production of siderophores by

242 rhizosphere bacteria is actually valued as plant-growth promoting trait (Souza *et al.*, 2015).

243 Laboratory assays have demonstrated an increase in plant growth as result of inoculation with

244 siderophore-producing bacteria (Gobelak and Hiller, 2017; Shameer and Prasad, 2018)

245 which constitutes direct evidence of the role of microbial siderophores in enhancing plant

development. Other observations provide indirect evidence that plants suffering from Fe limitation may actually modulate the composition of root exudates to favour the development of siderophore-producing microbial populations. Phenolic root exudates of Fe-deficient red clover plants induced an increase in the relative abundance of siderophore producing bacteria in soil communities, when compared to phenolic-free soil (Jin *et al.*, 2010).

Since their discovery, in the 1950s, more than 500 siderophores produced by bacteria and fungi have been identified (Ali and Vidhale, 2013; Neilands, 2014; Comensoli *et al.*, 2017).

The Siderophore Database contains detailed information on the chemical structure and producing organisms of more than 260 siderophores of microbial origin (http://bertrandsamuel.free.fr/siderophore_base/siderophores.php, accession date 2018-08-24).

Siderophores produced by bacteria are assigned to three main categories, hydroxamates, catecholates, and carboxylates, depending on the Fe-chelating group (Schalk and Mislin, 2017). Gram-positive and Gram-negative bacteria operate different mechanisms of siderophore-mediated Fe uptake (Fig. 4). In Gram-negative bacteria, Fe transport involves siderophores and Fe^{3+} -siderophore specific receptors integrated in the outer membrane (Outer Membrane Transporters, OMTs). Fe^{3+} -siderophore complexes directly bind to free OMTs or exchange with Fe-free siderophores that are already bound, moving the complex through the periplasm into the cytoplasm using the TonB-ExbBD protein complex, periplasmic binding proteins (SBPs), and a siderophore-permease-ATPase system (Faraldo-Gómez and Sansom, 2003; Klebba, 2016). As the outer membrane is not present in Gram-positive bacteria, they lack siderophore-binding OMTs. SBP lipoproteins anchored to the cell membrane, bind extracellular Fe-siderophore complexes and translocate them into the intracellular compartment through a siderophore-permease-ATPase system, similar to the SBP-permease-ATPase system in Gram-negative bacteria (Fukushima *et al.*, 2014).

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271

272

273 **Fig 4** Fe uptake mechanism in Gram-negative and Gram-positive bacteria. In Gram-negative
 274 bacteria the OMT (outer membrane transporter) binds Fe³⁺-siderophores complexes, moving
 275 the complex to the periplasm through the TonB-ExbBD protein complex. Later it binds to the
 276 periplasmic binding protein, crossing the peptidoglycan layer and is delivered to a
 277 siderophore-permease-ATPase system in the cytoplasmic membrane that will release it into
 278 the cytoplasm. In Gram-positive bacteria, SBP lipoproteins, anchored to the cell membrane,
 279 will bind extracellular Fe-siderophores and import them by a siderophore-permease-ATPase
 280 system.

281

282 **USING SIDEROPHORE-PRODUCING RHIZOBACTERIA TO MITIGATE OF Fe**
283 **DEFICIENCY IN SALINE SOILS**

284 The rhizosphere microenvironment is characterized by dense, rich and active bacterial
285 communities (Oliveira *et al.*, 2012; Lewicka-Rataj *et al.*, 2018). Plants and bacteria withdraw
286 mutual benefits from this association. In general, plant benefit from enhanced access to
287 nutrients (e.g. N₂ fixation, phosphate solubilization, Fe transport), phytohormones (e.g. IAA),
288 stress-mitigation (e.g. ACC deaminase) and biocontrol effects (e.g. HCN) and bacteria are
289 favoured by root-derived carbon sources and oxygen (Oliveira *et al.*, 2015; Pii *et al.*, 2015;
290 Hassani *et al.*, 2018). However, rhizosphere bacterial communities may also suffer with
291 antibacterial and antifungal exudates released by the roots (Bais *et al.*, 2005; Nóbrega *et al.*,
292 2005; Baetz and Martinoia, 2014) and with antagonistic relations between rhizosphere
293 microbial populations (Ciancio *et al.*, 2016). Biotic relations between plants and
294 microorganisms expressing antibacterial or antifungal activity represent a promising
295 alternative to hazardous chemical pesticides (Liu *et al.*, 2018) and fertilizers (Gunes *et al.*,
296 2015) currently used in agriculture.

297 Bacteria that have the particular ability to promote plant growth are designated as plant
298 growth-promoting bacteria (PGPB), or rhizobacteria (PGPR) in the particular case of
299 soil/rhizosphere isolates. It is now well established that PGPB enhance productivity and
300 tolerance to environmental stress and improve nutritional, phytochemical and biological
301 properties in crop plants (Miransari, 2014; Etesami and Maheshwari, 2018).

302 Either salinization or Fe limitation represent stress factors *per se*. Successful attempts of using
303 PGPB to mitigate the negative effects of each of these factors have been reported. Inoculation
304 with PGPB, namely halotolerant strains, reduces saline stress effects, stimulates plant growth
305 in different cultivated glycophytes (Ali *et al.*, 2014; Nabti *et al.*, 2015; Yang *et al.*, 2016;
306 Hahm *et al.*, 2017) and also attenuates the negative effect of high salinity on the germination
307 of seeds of crop halophytes (Rueda-Puente *et al.*, 2007). Several studies provide experimental
308 evidence of a wide range of beneficial effects of PGPB in crop plants. Positive effects (Table
309 1) include enhanced growth (increased shoot and root biomass and length), elevated

310 photosynthetic rate (elevated chlorophyll content, increased stomatal conductance), increased
311 stress resistance (antioxidant indicators improved, such as carotenoids content and CAT and
312 SOD activity; increased expression of ACC deaminase), higher levels of phytohormones (*e.g.*
313 IAA), accumulation of compatible osmolytes (improvement in osmotic stress reaction),
314 inhibition in Na⁺ uptake and improved nutrient uptake (*e.g.* phosphate solubilization, nitrogen
315 fixation, potassium and Fe acquisition).

316

317 Table 1
318 Positive effects of PGPB on salt-stressed plants

Positive effects	PGPB	Plant	Reference
germination, root surface, N ₂ fixation	<i>Aureobacterium spp.</i> <i>Cellulomonas spp.</i>	Wild lupine	(Gutiérrez Mañero <i>et al.</i> , 2003)
stomatal conductance	<i>Bacillus sp. and Glomus sp.</i>	Lettuce	(Vivas <i>et al.</i> , 2003)
rhizospheric soil aggregation around roots, dry matter yield of roots and shoots, Na ⁺ exclusion	<i>Bacillus sp.</i> <i>Aeromonas hydrophila</i> <i>Aeromonas caviae</i>	Wheat	(Ashraf <i>et al.</i> , 2004)
salt tolerance, ACC deaminase	<i>Pseudomonas putida</i>	Canola	(Cheng <i>et al.</i> , 2007)
salt tolerance, ACC deaminase	<i>Pseudomonas fluorescens</i>	Groundnut plants	(Saravanakumar and Samiyappan, 2007)
growth, N ₂ fixation	<i>Chryseobacterium balustinum</i> <i>Rhizobium tropici</i>	Common bean Soybean	(Estevez <i>et al.</i> , 2009)
growth; proline content	<i>Rhizobium</i> -like	Soybean	(Naz <i>et al.</i> , 2009)
root and shoot length, ACC deaminase, IAA, phosphate solubilization, siderophores	<i>Pseudomonas aeruginosa</i> <i>P. fluorescens</i> <i>P. stutzeri</i>	Tomato	(Tank and Saraf, 2010)

Table 1

(cont.)

root and shoot biomass, chlorophyll, carotenoids, protein, IAA, ionic balance	<i>Bacillus pumilus</i> <i>Halomonas</i> sp. <i>Arthrobacter</i> sp.	Wheat	(Tiwari <i>et al.</i> , 2011)
root length, dry weight	<i>Hallobacillus</i> spp. <i>Bacillus halodenitrificans</i>	Wheat	(Ramadoss <i>et al.</i> , 2013)
shoot biomass, proline, K ⁺ , CAT and SOD activity, Na ⁺ exclusion	<i>Pseudomonas fluorescens</i>	Common bean	(Younesi and Moradi, 2014)
seed yield, dry biomass, plant height, leaf area, relative water content, chlorophyll, carotenoids, stomatal conductance, transpiration, salt tolerance	<i>Enterobacter cloacae</i> <i>Bacillus drentensis</i>	Mung bean	(Mahmood <i>et al.</i> , 2016)
phenols and polyphenols	<i>Azospirillum brasilense</i>	White clover	(Khalid <i>et al.</i> , 2017)

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Bacterial siderophores have higher affinity to Fe than phytosiderophores, and are able to remove Fe from Fe³⁺-phytosiderophores complexes (Aguado-Santacruz *et al.*, 2012). Therefore, inoculation with siderophore-expressing PGPB has been tested as a strategy to overcome the problem of Fe limitation. The effects may involve a microbe-mediated induction of plant genes associated with biochemical responses to Fe limitation (Zhang *et al.*, 2009) or the direct action of microbial siderophores. Experiments in which plants were grown under either sterile or non-sterile conditions demonstrated that microbial activity was decisive for Fe accumulation in roots and to the transport of Fe to the leaves, and confirm microbial siderophores as relevant players in the process of Fe supply in maize, sunflower and cucumber (Masalha *et al.*, 2000; de Santiago *et al.*, 2013; Pii *et al.*, 2015). Siderophore-producing endophytic *Streptomyces* sp. increased root and shoot biomass in rice by supplying plants with sequestered Fe (Rungin *et al.*, 2012). The growth promoting effect of *Pseudomonas fluorescens* has been associated to a competitive sequestration of Fe by siderophore production (Kloepper *et al.*, 1980) and the growth of *Arabidopsis thaliana* under Fe deficiency, was enhanced in presence of pyoverdine (Trapet *et al.*, 2016). As a preventive approach, the effect may even be induced before exposure to stress. Inoculation of cucumber with siderophore-producing *Azospirillum brasilense* triggered mechanisms of response to Fe deficiency, even in Fe sufficient conditions, pre-conditioning the plants to better resist to Fe limitation (Pii *et al.*, 2016).

Salinity-related Fe deficiency in soils can be regarded as a multi-stress condition adding some complexity to the problem of soil degradation. There are examples of a cross-effect of microbial siderophores on salt-tolerance of several plant models, namely corn, wheat and mung bean (Kavamura *et al.*, 2013; Ramadoss *et al.*, 2013; Singh *et al.*, 2015; Mahmood *et al.*, 2016). However, the exact involvement of siderophores, as a sole plant-growth promoting trait, in the overall process is not yet established. Features like ACC deaminase, IAA production, exopolysaccharide and osmolyte production have been more extensively studied in relation with their plant growth promoting ability and most commonly, tested PGPB strains or consortia express several plant growth promoting traits (Ashraf *et al.*, 2004; Martínez *et al.*,

2015; Orhan, 2016; Li and Jiang, 2017), which precludes the discrimination of the individual contribution of each of these traits. An increase in root Fe chelate reductase activity was observed in barley and tomato plants exposed to Fe deficiency conditions, after inoculation with bacterial strains capable of producing high levels of siderophores, but expressing also IAA and phosphate solubilization activity (Scagliola *et al.*, 2016). Therefore, the evidence that bacterial siderophores actually contribute to relief Fe limitation in saline soils is still indirect.

Strikingly, although the positive effects of siderophore-producing PGPB on either Fe accumulation or saline stress in edible or cultivated plants have been unequivocally demonstrated (Freitas *et al.*, 2015; Hahm *et al.*, 2017; Sarkar *et al.*, 2018), specific studies addressing the effect of siderophore-producing PGPB on the combined effect of the two stress factors, to which plants growing in saline soils are likely to be exposed, have not been conducted. There is, however, indirect evidence that leads to the hypothesis of a dual positive effect of siderophore-producing PGPB on saline stress and Fe supply to plants. A siderophore-producing *Tricoderma* spp. promoted cucumber growth in saline conditions by alleviating both salinity stress and Fe deficiency (Qi and Zhao, 2013).

CONCLUSIONS

Salt-affected soils represent a considerable proportion of the total arable soil worldwide and this situation may aggravate as a consequence of the future scenarios of climate change and sea-level rise. Salinization alters soil chemistry and makes micronutrients like Fe, less accessible to plants. This represents a challenge for agriculture for which innovative and sustainable alternatives are urgently needed.

PGPB represent a promising alternative to traditional farming practices particularly in the perspective of reducing the necessity of fertilizers and chemical pesticides. Although there is still the need for the scientific demonstration of the direct beneficial effect of siderophore-producing PGPB on Fe acquisition and plant growth in saline soils, indirect evidences support

the perspective of siderophore-producing PGPB as promising tool to improve crop productivity in saline, arid and Fe deficient soils.

380

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388 **CONFLICT OF INTEREST**

The authors have no affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript.

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394 **REFERENCES**

Abadía J, López-Millán A-F, Rombolà A, Abadía A. 2002. Organic acids and Fe deficiency: A review. *Plant Soil*. **241**: 75-86.

Abbas G, Saqib M, Akhtar J. 2015. Interactive effects of salinity and iron deficiency on different rice genotypes. *J Plant Nutr Soil Sci*. **178**: 306-311.

Abbasi H, Jamil M, Haq A, Ali S, Ahmad R, Malik Z. 2016. Salt stress manifestation on plants, mechanism of salt tolerance and potassium role in alleviating it: A review. *Žemdirbystė (Agriculture)*. **103**: 229-238.

Acosta-Motos J R, Ortuño M F, Bernal-Vicente A, Diaz-Vivancos P, Sanchez-Blanco M J, Hernandez J A. 2017. Plant responses to salt stress: Adaptive mechanisms. *Agronomy*. **7**: 18.

- 405 Aguado-Santacruz G A, Moreno-Gomez B, Jimenez-Francisco B, Garcia-Moya E,
406 Preciado-Ortiz R E. 2012. Impact of the microbial siderophores and
407 phytosiderophores on the iron assimilation by plants: A synthesis. *Revista*
408 *Fitotecnica Mexicana*. **35**: 9-21.
- 409 Ali S, Charles T C, Glick B R. 2014. Amelioration of high salinity stress damage by
410 plant growth-promoting bacterial endophytes that contain acc deaminase.
411 *Plant Physiol Biochem*. **80**: 160-167.
- 412 Ali S S, Vidhale N. 2013. Bacterial siderophore and their application: A review. *Int*
413 *J Curr Microbiol Appl Sci*. **2**: 303-312.
- 414 Ameixa O M C C, Marques B, Fernandes V S, Soares A M V M, Calado R, Lillebø A I.
415 2016. Dimorphic seeds of *salicornia ramosissima* display contrasting
416 germination responses under different salinities. *Ecol Eng*. **87**: 120-123.
- 417 Aragüés R, Medina E, Martínez-Cob A, Faci J. 2014. Effects of deficit irrigation
418 strategies on soil salinization and sodification in a semiarid drip-irrigated
419 peach orchard. *Agric Water Manage*. **142**: 1-9.
- 420 Ashraf M, Hasnain S, Berge O, Mahmood T. 2004. Inoculating wheat seedlings
421 with exopolysaccharide-producing bacteria restricts sodium uptake and
422 stimulates plant growth under salt stress. *Biol Fertility Soils*. **40**: 157-162.
- 423 Baetz U, Martinoia E. 2014. Root exudates: The hidden part of plant defense.
424 *Trends Plant Sci*. **19**: 90-98.
- 425 Bais H P, Prithiviraj B, Jha A K, Ausubel F M, Vivanco J M. 2005. Mediation of
426 pathogen resistance by exudation of antimicrobials from roots. *Nature*.
427 **434**: 217.
- 428 Becker V I, Goessling J W, Duarte B, Caçador I, Liu F, Rosenqvist E, Jacobsen S-E.
429 2017. Combined effects of soil salinity and high temperature on

- 430 photosynthesis and growth of quinoa plants (*Chenopodium quinoa*). *Funct*
431 *Plant Biol.* **44**: 665-678.
- 432 Bogdan A R, Miyazawa M, Hashimoto K, Tsuji Y. 2016. Regulators of iron
433 homeostasis: New players in metabolism, cell death, and disease. *Trends*
434 *Biochem Sci.* **41**: 274-286.
- 435 Briat J-F, Dubos C, Gaymard F. 2015. Iron nutrition, biomass production, and
436 plant product quality. *Trends Plant Sci.* **20**: 33-40.
- 437 Cassat J E, Skaar E P. 2013. Iron in infection and immunity. *Cell host & microbe*.
438 **13**: 509-519.
- 439 Chakraborty K, Sairam R, Bhaduri D. 2016. Effects of different levels of soil
440 salinity on yield attributes, accumulation of nitrogen, and micronutrients
441 in brassica spp. *J Plant Nutr.* **39**: 1026-1037.
- 442 Chen J-H, Lin Y-H. 2010. Sodium chloride causes variation in organic acids and
443 proteins in tomato root. *African Journal of Biotechnology.* **9**: 8161-8167.
- 444 Chen W, Chang A, Page A. 2012. Agricultural salinity assessment and
445 management.
- 446 Chen W W, Yang J L, Qin C, Jin C W, Mo J H, Ye T, Zheng S J. 2010. Nitric oxide acts
447 downstream of auxin to trigger root ferric-chelate reductase activity in
448 response to iron deficiency in *Arabidopsis thaliana*. *Plant Physiol.* pp.
449 110.161109.
- 450 Chen Y, Barak P. 1982. *Advances in agronomy*. Elsevier.
- 451 Cheng Z, Park E, Glick B R. 2007. 1-aminocyclopropane-1-carboxylate deaminase
452 from *Pseudomonas putida* UW4 facilitates the growth of canola in the
453 presence of salt. *Can J Microbiol.* **53**: 912-918.

- 454 Ciancio A, Pieterse C M, Mercado-Blanco J. 2016. Harnessing useful rhizosphere
455 microorganisms for pathogen and pest biocontrol. *Frontiers in*
456 *microbiology*. **7**: 1620.
- 457 Cohen G N. 2014. Microbial biochemistry. Springer.
- 458 Coleman D C, Callaham M A, Crossley Jr D. 2017. Fundamentals of soil ecology.
459 Academic press.
- 460 Colombo C, Palumbo G, He J-Z, Pinton R, Cesco S. 2014. Review on iron
461 availability in soil: Interaction of Fe minerals, plants, and microbes. *J Soils*
462 *Sed.* **14**: 538-548.
- 463 Comensoli L, Bindschedler S, Junier P, Joseph E. 2017. Adv appl microbiol.
464 Elsevier.
- 465 Cotsaftis O, Plett D, Johnson A A, Walia H, Wilson C, Ismail A M, Close T J, Tester
466 M, Baumann U. 2011. Root-specific transcript profiling of contrasting rice
467 genotypes in response to salinity stress. *Molecular Plant*. **4**: 25-41.
- 468 Curie C, Mari S. 2017. New routes for plant iron mining. *New Phytol.* **214**: 521-
469 525.
- 470 Daliakopoulos I N, Tsanis I K, Koutroulis A, Kourgialas N N, Varouchakis A E,
471 Karatzas G P, Ritsema C J. 2016. The threat of soil salinity: A european
472 scale review. *Sci Total Environ.* **573**: 727-739.
- 473 de Santiago A, García-López A M, Quintero J M, Avilés M, Delgado A. 2013. Effect
474 of trichoderma asperellum strain t34 and glucose addition on iron
475 nutrition in cucumber grown on calcareous soils. *Soil Biology and*
476 *Biochemistry.* **57**: 598-605.
- 477 Dubey R. 1999. Handbook of plant and crop stress. 2nd. Marcel Dekker, New
478 York.

- 479 El Fouly M M, Mobarak Z M, Salama Z A. 2010. Improving tolerance of faba bean
480 during early growth stages to salinity through micronutrients foliar spray.
481 *Notulae Scientia Biologicae*. **2**: 98.
- 482 Enthaler S, Junge K, Beller M. 2008. Sustainable metal catalysis with iron: From
483 rust to a rising star? *Angew Chem Int Ed*. **47**: 3317-3321.
- 484 Estevez J, Dardanelli M, Megías M, Rodríguez-Navarro D. 2009. Symbiotic
485 performance of common bean and soybean co-inoculated with rhizobia
486 and chryseobacterium balustinum aur9 under moderate saline conditions.
487 *Symbiosis*. **49**: 29-36.
- 488 Etesami H, Maheshwari D K. 2018. Use of plant growth promoting rhizobacteria
489 (pgprs) with multiple plant growth promoting traits in stress agriculture:
490 Action mechanisms and future prospects. *Ecotoxicol Environ Saf*. **156**:
491 225-246.
- 492 Falkowski P G, Lin H, Gorbunov M Y. 2017. What limits photosynthetic energy
493 conversion efficiency in nature? Lessons from the oceans. *Phil Trans R Soc*
494 *B*. **372**: 20160376.
- 495 Faraldo-Gómez J D, Sansom M S. 2003. Acquisition of siderophores in gram-
496 negative bacteria. *Nature reviews Molecular cell biology*. **4**: 105.
- 497 Ferchichi S, El Khouni A, Zorrig W, Atia A, Rabhi M, Gharsalli M, Abdelly C. 2016.
498 Nutrient uptake and use efficiencies in medicago ciliaris under salinity. *J*
499 *Plant Nutr*. **39**: 932-941.
- 500 Freitas M A, Medeiros F H V, Carvalho S P, Guilherme L R G, Teixeira W D, Zhang
501 H, Paré P W. 2015. Augmenting iron accumulation in cassava by the
502 beneficial soil bacterium bacillus subtilis (gbo3). *Frontiers in Plant Science*.
503 **6**: 596.

- 504 Fukushima T, Allred B E, Raymond K N. 2014. Direct evidence of iron uptake by
505 the gram-positive siderophore-shuttle mechanism without iron reduction.
506 *ACS chemical biology*. **9**: 2092-2100.
- 507 García M J, Suárez V, Romera F J, Alcántara E, Pérez-Vicente R. 2011. A new
508 model involving ethylene, nitric oxide and fe to explain the regulation of
509 fe-acquisition genes in strategy i plants. *Plant Physiol Biochem*. **49**: 537-
510 544.
- 511 Gargallo-Garriga A, Preece C, Sardans J, Oravec M, Urban O, Peñuelas J. 2018.
512 Root exudate metabolomes change under drought and show limited
513 capacity for recovery. *Scientific reports*. **8**: 12696.
- 514 Grattan S, Grieve C. 1998. Salinity-mineral nutrient relations in horticultural
515 crops. *Scientia horticulturae*. **78**: 127-157.
- 516 Grobelak A, Hiller J. 2017. Bacterial siderophores promote plant growth:
517 Screening of catechol and hydroxamate siderophores. *International*
518 *journal of phytoremediation*. **19**: 825-833.
- 519 Gunes A, Karagoz K, Turan M, Kotan R, Yildirim E, Cakmakci R, Sahin F. 2015.
520 Fertilizer efficiency of some plant growth promoting rhizobacteria for
521 plant growth. *Research journal of soil biology*. **7**: 28-45.
- 522 Gutiérrez Mañero F J, Probanza A, Ramos B, Colón Flores J J, Lucas García J A.
523 2003. Effects of culture filtrates of rhizobacteria isolated from wild lupine
524 on germination, growth, and biological nitrogen fixation of lupine
525 seedlings. *J Plant Nutr*. **26**: 1101-1115.
- 526 Hahm M-S, Son J-S, Hwang Y-J, Kwon D-K, Ghim S-Y. 2017. Alleviation of salt
527 stress in pepper (*capsicum annum* l.) plants by plant growth-promoting
528 rhizobacteria. *J Microbiol Biotechnol*. **27**: 1790.

- 529 Hahm M, Son J, Hwang Y, Kwon D, Ghim S. 2017. Alleviation of salt stress in
530 pepper (*capsicum annum* l.) plants by plant growth-promoting
531 rhizobacteria. *J Microbiol Biotechnol*.
- 532 Hassani M A, Durán P, Hacquard S. 2018. Microbial interactions within the plant
533 holobiont. *Microbiome*. **6**: 58.
- 534 Heidari M, Sarani S. 2012. Growth, biochemical components and ion content of
535 chamomile (*matricaria chamomilla* l.) under salinity stress and iron
536 deficiency. *Journal of the Saudi Society of Agricultural Sciences*. **11**: 37-42.
- 537 Hindt M N, Guerinot M L. 2012. Getting a sense for signals: Regulation of the
538 plant iron deficiency response. *Biochimica et Biophysica Acta (BBA)-*
539 *Molecular Cell Research*. **1823**: 1521-1530.
- 540 Hu X, Page M T, Sumida A, Tanaka A, Terry M J, Tanaka R. 2017. The iron-sulfur
541 cluster biosynthesis protein sufb is required for chlorophyll synthesis, but
542 not phytochrome signaling. *The Plant Journal*. **89**: 1184-1194.
- 543 Jin C W, Li G X, Yu X H, Zheng S J. 2010. Plant fe status affects the composition of
544 siderophore-secreting microbes in the rhizosphere. *Ann Bot-London*. **105**:
545 835-841.
- 546 Jin C W, Ye Y Q, Zheng S J. 2013. An underground tale: Contribution of microbial
547 activity to plant iron acquisition via ecological processes. *Ann Bot-London*.
548 **113**: 7-18.
- 549 Johnstone T C, Nolan E M. 2015. Beyond iron: Non-classical biological functions
550 of bacterial siderophores. *Dalton T*. **44**: 6320-6339.
- 551 Kavamura V N, Santos S N, da Silva J L, Parma M M, Ávila L A, Visconti A, Zucchi T
552 D, Taketani R G, Andreote F D, de Melo I S. 2013. Screening of brazilian

- 553 cacti rhizobacteria for plant growth promotion under drought. *Microbiol*
554 *Res.* **168**: 183-191.
- 555 Khalid M, Bilal M, Hassani D, Iqbal H M, Wang H, Huang D. 2017. Mitigation of salt
556 stress in white clover (*trifolium repens*) by *azospirillum brasilense* and
557 its inoculation effect. *Botanical studies.* **58**: 5.
- 558 Kim H-J, Fonseca J M, Choi J-H, Kubota C, Kwon D Y. 2008. Salt in irrigation water
559 affects the nutritional and visual properties of romaine lettuce (*lactuca*
560 *sativa* l.). *J Agric Food Chem.* **56**: 3772-3776.
- 561 Kim S A, Guerinot M L. 2007. Mining iron: Iron uptake and transport in plants.
562 *FEBS Lett.* **581**: 2273-2280.
- 563 Klebba P E. 2016. Roset model of tonb action in gram-negative bacterial iron
564 acquisition. *J Bacteriol.* **198**: 1013-1021.
- 565 Kloepper J W, Leong J, Teintze M, Schroth M N. 1980. Enhanced plant growth by
566 siderophores produced by plant growth-promoting rhizobacteria. *Nature.*
567 **286**: 885.
- 568 Kobayashi T, Nishizawa N K. 2012. Iron uptake, translocation, and regulation in
569 higher plants. *Annu Rev Plant Biol.* **63**: 131-152.
- 570 Konuşkan Ö, Gözübenli H, Atış İ, Atak M. 2017. Effects of salinity stress on
571 emergence and seedling growth parameters of some maize genotypes
572 (*zea mays* l.). *Turkish Journal of Agriculture-Food Science and Technology.*
573 **5**: 1668-1672.
- 574 Lewicka-Rataj K, Świątecki A, Górniak D. 2018. The effect of *lobelia dortmanna* l.
575 On the structure and bacterial activity of the rhizosphere. *Aquat Bot.* **145**:
576 10-20.

- 577 Li H, Jiang X. 2017. Inoculation with plant growth-promoting bacteria (pgpb)
578 improves salt tolerance of maize seedling. *Russ J Plant Physiol.* **64**: 235-
579 241.
- 580 Li Q, Yang A, Zhang W-H. 2016. Efficient acquisition of iron confers greater
581 tolerance to saline-alkaline stress in rice (*oryza sativa* l.). *J Exp Bot.*
582 *erw407*.
- 583 Lindsay W. 1991. Iron nutrition and interactions in plants. Springer.
- 584 Liu K, McInroy J A, Hu C-H, Kloepper J W. 2018. Mixtures of plant-growth-
585 promoting rhizobacteria enhance biological control of multiple plant
586 diseases and plant-growth promotion in the presence of pathogens. *Plant*
587 *Dis.* **102**: 67-72.
- 588 Liu W, Li R-J, Han T-T, Cai W, Fu Z-W, Lu Y-T. 2015. Salt stress reduces root
589 meristem size by nitric oxide-mediated modulation of auxin accumulation
590 and signaling in arabidopsis. *Plant Physiol.* **168**: 343-356.
- 591 Loper J E, Buyer J S. 1991. Siderophores in microbial interactions on plant
592 surfaces. *Mol Plant-Microbe Interact.* **4**: 5-13.
- 593 López-Berenguer C, Martínez-Ballesta M a d C, Moreno D A, Carvajal M, García-
594 Viguera C. 2009. Growing hardier crops for better health: Salinity
595 tolerance and the nutritional value of broccoli. *J Agric Food Chem.* **57**:
596 572-578.
- 597 Ma J, Lv C, Hao P, Yuan Z, Wang Y, Shen W, Xu C, Lv C, Chen G, Gao Z. 2017. Sub-
598 cellular distribution of nutrient elements and photosynthesis
599 performance in *oryza sativa* l. Seedlings under salt stress. *Pak J Bot.* **49**:
600 405-411.

- 601 Mahmood S, Daur I, Al-Solaimani S G, Ahmad S, Madkour M H, Yasir M, Hirt H, Ali
602 S, Ali Z. 2016. Plant growth promoting rhizobacteria and silicon
603 synergistically enhance salinity tolerance of mung bean. *Frontiers in plant*
604 *science*. **7**: 876.
- 605 Mahmood S, Daur I, Al-Solaimani S G, Ahmad S, Madkour M H, Yasir M, Hirt H, Ali
606 S, Ali Z. 2016. Plant growth promoting rhizobacteria and silicon
607 synergistically enhance salinity tolerance of mung bean. *Frontiers in plant*
608 *science*. **7**: Article 876.
- 609 Malhi S, Nyborg M, Harapiak J. 1998. Effects of long-term n fertilizer-induced
610 acidification and liming on micronutrients in soil and in bromegrass hay.
611 *Soil and Tillage Research*. **48**: 91-101.
- 612 Martínez R, Espejo A, Sierra M, Ortiz-Bernad I, Correa D, Bedmar E, López-Jurado
613 M, Porres J M. 2015. Co-inoculation of halomonas maura and ensifer
614 meliloti to improve alfalfa yield in saline soils. *Appl Soil Ecol*. **87**: 81-86.
- 615 Masalha J, Kosegarten H, Elmaci Ö, Mengel K. 2000. The central role of microbial
616 activity for iron acquisition in maize and sunflower. *Biol Fertility Soils*. **30**:
617 433-439.
- 618 Masepohl B. 2017. Modern topics in the phototrophic prokaryotes: Metabolism,
619 bioenergetics, and omics. Springer, Cham
- 620 Mavi M S, Marschner P, Chittleborough D J, Cox J W, Sanderman J. 2012. Salinity
621 and sodicity affect soil respiration and dissolved organic matter dynamics
622 differentially in soils varying in texture. *Soil biology and biochemistry*. **45**:
623 8-13.

- 624 Mavi M S, Sanderman J, Chittleborough D J, Cox J W, Marschner P. 2012. Sorption
625 of dissolved organic matter in salt-affected soils: Effect of salinity, sodicity
626 and texture. *Sci Total Environ.* **435-436**: 337-344.
- 627 McNear Jr D H. 2013. The rhizosphere-roots, soil and everything in between.
628 *Nature Education Knowledge.* **4**: 1.
- 629 Meng R, Saade S, Kurtek S, Berger B, Brien C, Pillen K, Tester M, Sun Y. 2017.
630 Growth curve registration for evaluating salinity tolerance in barley. *Plant*
631 *methods.* **13**: 18.
- 632 Mimmo T, Del Buono D, Terzano R, Tomasi N, Vigani G, Crecchio C, Pinton R,
633 Zocchi G, Cesco S. 2014. Rhizospheric organic compounds in the soil-
634 microorganism-plant system: Their role in iron availability. *Eur J Soil Sci.*
635 **65**: 629-642.
- 636 Miransari M. 2014. Plant growth promoting rhizobacteria. *J Plant Nutr.* **37**: 2227-
637 2235.
- 638 Mozafari A-a, Ghaderi N. 2018. Grape response to salinity stress and role of iron
639 nanoparticle and potassium silicate to mitigate salt induced damage
640 under in vitro conditions. *Physiol Mol Biol Plants.* **24**: 25-35.
- 641 Munns R, Schachtman D, Condon A. 1995. The significance of a two-phase growth
642 response to salinity in wheat and barley. *Funct Plant Biol.* **22**: 561-569.
- 643 Nabti E, Schmid M, Hartmann A. 2015. Halophiles. Springer.
- 644 Naz I, Bano A, Ul-Hassan T. 2009. Isolation of phytohormones producing plant
645 growth promoting rhizobacteria from weeds growing in khewra salt
646 range, pakistan and their implication in providing salt tolerance to glycine
647 max l. *African Journal of Biotechnology.* **8**: 5762-5766.

- 648 Negrao S, Schmöckel S, Tester M. 2017. Evaluating physiological responses of
649 plants to salinity stress. *Ann Bot-London*. **119**: 1-11.
- 650 Neilands J B. 2014. Microbial iron metabolism: A comprehensive treatise.
651 Academic Press.
- 652 Nenova V. 2008. Growth and mineral concentrations of pea plants under
653 different salinity levels and iron supply. *Gen Appl Plant Physiol*. **34**: 189-
654 202.
- 655 Nikiforova E, Kasimov N, Kosheleva N. 2014. Long-term dynamics of the
656 anthropogenic salinization of soils in moscow (by the example of the
657 eastern district). *Eurasian Soil Sci*. **47**: 203.
- 658 Nóbrega F, Santos I, Cunha M D, Carvalho A, Gomes V. 2005. Antimicrobial
659 proteins from cowpea root exudates: Inhibitory activity against fusarium
660 oxysporum and purification of a chitinase-like protein. *Plant Soil*. **272**:
661 223-232.
- 662 Nozoye T, Nagasaka S, Kobayashi T, Takahashi M, Sato Y, Sato Y, Uozumi N,
663 Nakanishi H, Nishizawa N K. 2011. Phytosiderophore efflux transporters
664 are crucial for iron acquisition in graminaceous plants. *J Biol Chem*. **286**:
665 5446-5454.
- 666 Oliveira V, Gomes N C, Almeida A, Silva A M, Silva H, Cunha Â. 2015. Microbe-
667 assisted phytoremediation of hydrocarbons in estuarine environments.
668 *Microbial ecology*. **69**: 1-12.
- 669 Oliveira V, Santos A L, Aguiar C, Santos L, Salvador Â C, Gomes N C M, Silva H,
670 Rocha S M, Almeida A, Cunha Â. 2012. Prokaryotes in salt marsh
671 sediments of ria de aveiro: Effects of halophyte vegetation on abundance
672 and diversity. *Estuarine, Coastal and Shelf Science*. **110**.

- Orhan F. 2016. Alleviation of salt stress by halotolerant and halophilic plant growth-promoting bacteria in wheat (*triticum aestivum*). *Braz J Microbiol.* **47**: 621-627.
- Paul D, Lade H. 2014. Plant-growth-promoting rhizobacteria to improve crop growth in saline soils: A review. *Agronomy for sustainable development.* **34**: 737-752.
- Pausch J, Kuzyakov Y. 2018. Carbon input by roots into the soil: Quantification of rhizodeposition from root to ecosystem scale. *Global Change Biology.* **24**: 1-12.
- Pessarakli M. 2014. Handbook of plant and crop physiology. CRC Press, New York.
- Petropoulos S A, Levizou E, Ntatsi G, Fernandes Â, Petrotos K, Akoumianakis K, Barros L, Ferreira I C. 2017. Salinity effect on nutritional value, chemical composition and bioactive compounds content of *cichorium spinosum* l. *Food chemistry.* **214**: 129-136.
- Pii Y, Marastoni L, Springeth C, Fontanella M C, Beone G M, Cesco S, Mimmo T. 2016. Modulation of fe acquisition process by *azospirillum brasilense* in cucumber plants. *Environ Exp Bot.* **130**: 216-225.
- Pii Y, Mimmo T, Tomasi N, Terzano R, Cesco S, Crecchio C. 2015. Microbial interactions in the rhizosphere: Beneficial influences of plant growth-promoting rhizobacteria on nutrient acquisition process. A review. *Biol Fertility Soils.* **51**: 403-415.
- Pii Y, Penn A, Terzano R, Crecchio C, Mimmo T, Cesco S. 2015. Plant-microorganism-soil interactions influence the fe availability in the rhizosphere of cucumber plants. *Plant Physiol Biochem.* **87**: 45-52.

- 698 Posada R H, Heredia-Abarca G, Sieverding E, Sánchez de Prager M. 2013.
699 Solubilization of iron and calcium phosphates by soil fungi isolated from
700 coffee plantations. *Archives of Agronomy and Soil Science*. **59**: 185-196.
- 701 Puig S, Ramos-Alonso L, Romero A M, Martínez-Pastor M T. 2017. The elemental
702 role of iron in DNA synthesis and repair. *Metallomics*. **9**: 1483-1500.
- 703 Purohit D, Sankararamasubramanian H M, Kumar Pal A, Kumar Parida A. 2016.
704 Identification and characterization of a novel iron deficiency and salt
705 stress responsive transcription factor idef1 in porteresia coarctata. *Biol*
706 *Plant*. **60**: 469-481.
- 707 Qadir M, Noble A D, Oster J, Schubert S, Ghafoor A. 2005. Driving forces for
708 sodium removal during phytoremediation of calcareous sodic and saline-
709 sodic soils: A review. *Soil Use and Management*. **21**: 173-180.
- 710 Qi W, Zhao L. 2013. Study of the siderophore - producing trichoderma
711 asperellum q1 on cucumber growth promotion under salt stress. *J Basic*
712 *Microbiol*. **53**: 355-364.
- 713 Rabhi M, Barhoumi Z, Ksouri R, Abdelly C, Gharsalli M. 2007. Interactive effects
714 of salinity and iron deficiency in medicago ciliaris. *C R Biol*. **330**: 779-788.
- 715 Ramadoss D, Lakkineni V K, Bose P, Ali S, Annapurna K. 2013. Mitigation of salt
716 stress in wheat seedlings by halotolerant bacteria isolated from saline
717 habitats. *SpringerPlus*. **2**: 6.
- 718 Reich M, Aghajanzadeh T, Helm J, Parmar S, Hawkesford M J, De Kok L J. 2017.
719 Chloride and sulfate salinity differently affect biomass, mineral nutrient
720 composition and expression of sulfate transport and assimilation genes in
721 brassica rapa. *Plant Soil*. **411**: 319-332.

- Rodríguez-Celma J, Lin W-D, Fu G-M, Abadía J, López-Millán A-F, Schmidt W. 2013. Mutually exclusive alterations in secondary metabolism are critical for the uptake of insoluble iron compounds by arabidopsis and medicago truncatula. *Plant Physiol.* pp. 113.220426.
- Rueda-Puente E O, García-Hernández J L, Preciado-Rangel P, Murillo-Amador B, Tarazón-Herrera M A, Flores-Hernández A, Holguin-Peña J, Aybar A N, Barrón Hoyos J M, Weimers D, Mwandemele O, Kaaya G, Mayoral J L, Troyo-Diéguez E. 2007. Germination of salicornia bigelovii ecotypes under sstressing cconditions of temperature and salinity and ameliorative effects of plant growth-promoting bacteria. *Journal of Agronomy and Crop Science.* **193**: 167-176.
- Rungin S, Indananda C, Suttiviriya P, Kruasuwan W, Jaemsang R, Thamchaipenet A. 2012. Plant growth enhancing effects by a siderophore-producing endophytic streptomycete isolated from a thai jasmine rice plant (oryza sativa l. Cv. Kdml105). *Antonie Van Leeuwenhoek.* **102**: 463-472.
- Saha M, Sarkar S, Sarkar B, Sharma B K, Bhattacharjee S, Tribedi P. 2016. Microbial siderophores and their potential applications: A review. *Environmental Science and Pollution Research.* **23**: 3984-3999.
- Sandmann G. 1985. Consequences of iron deficiency on photosynthetic and respiratory electron transport in blue-green algae. *Photosynthesis Res.* **6**: 261-271.
- Saravanakumar D, Samiyappan R. 2007. Acc deaminase from pseudomonas fluorescens mediated saline resistance in groundnut (arachis hypogea) plants. *J Appl Microbiol.* **102**: 1283-1292.

- 747 Sarkar A, Ghosh P K, Pramanik K, Mitra S, Soren T, Pandey S, Mondal M H, Maiti T
748 K. 2018. A halotolerant enterobacter sp. Displaying acc deaminase activity
749 promotes rice seedling growth under salt stress. *Res Microbiol.* **169**: 20-
750 32.
- 751 Scagliola M, Pii Y, Mimmo T, Cesco S, Ricciuti P, Crecchio C. 2016.
752 Characterization of plant growth promoting traits of bacterial isolates
753 from the rhizosphere of barley (*hordeum vulgare* l.) and tomato (*solanum*
754 *lycopersicon* l.) grown under fe sufficiency and deficiency. *Plant Physiol*
755 *Biochem.* **107**: 187-196.
- 756 Schalk I J, Mislin G L A. 2017. Bacterial iron uptake pathways: Gates for the
757 import of bactericide compounds. *Journal of Medicinal Chemistry.* **60**:
758 4573-4576.
- 759 Séguéla M, Briat J F, Vert G, Curie C. 2008. Cytokinins negatively regulate the root
760 iron uptake machinery in arabidopsis through a growth - dependent
761 pathway. *The Plant Journal.* **55**: 289-300.
- 762 Shameer S, Prasad T. 2018. Plant growth promoting rhizobacteria for sustainable
763 agricultural practices with special reference to biotic and abiotic stresses.
764 *Plant Growth Regulation.* 1-13.
- 765 Shannon M, Grieve C. 1998. Tolerance of vegetable crops to salinity. *Scientia*
766 *horticulturae.* **78**: 5-38.
- 767 Shirley M, Avoscan L, Bernaud E, Vansuyt G, Lemanceau P. 2011. Comparison of
768 iron acquisition from fe-pyoverdine by strategy i and strategy ii plants.
769 *Botany.* **89**: 731-735.

- 770 Singh R P, Jha P, Jha P N. 2015. The plant-growth-promoting bacterium *klebsiella*
771 sp. Sbp-8 confers induced systemic tolerance in wheat (*triticum*
772 *aestivum*) under salt stress. *J Plant Physiol.* **184**: 57-67.
- 773 Sisó - Terraza P, Rios J J, Abadía J, Abadía A, Álvarez - Fernández A. 2016. Flavins
774 secreted by roots of iron - deficient *beta vulgaris* enable mining of ferric
775 oxide via reductive mechanisms. *New Phytol.* **209**: 733-745.
- 776 Souza R d, Ambrosini A, Passaglia L M. 2015. Plant growth-promoting bacteria as
777 inoculants in agricultural soils. *Genet Mol Biol.* **38**: 401-419.
- 778 Taïbi K, Taïbi F, Ait Abderrahim L, Ennajah A, Belkhodja M, Mulet J M. 2016.
779 Effect of salt stress on growth, chlorophyll content, lipid peroxidation and
780 antioxidant defence systems in *phaseolus vulgaris* l. *S Afr J Bot.* **105**: 306-
781 312.
- 782 Tank N, Saraf M. 2010. Salinity-resistant plant growth promoting rhizobacteria
783 ameliorates sodium chloride stress on tomato plants. *Journal of Plant*
784 *Interactions.* **5**: 51-58.
- 785 Tiwari S, Singh P, Tiwari R, Meena K K, Yandigeri M, Singh D P, Arora D K. 2011.
786 Salt-tolerant rhizobacteria-mediated induced tolerance in wheat (*triticum*
787 *aestivum*) and chemical diversity in rhizosphere enhance plant growth.
788 *Biol Fertility Soils.* **47**: 907.
- 789 Torabian S, Zahedi M, Khoshgoftar A H. 2017. Effects of foliar spray of nano-
790 particles of FeSO_4 on the growth and ion content of sunflower under saline
791 condition. *J Plant Nutr.* **40**: 615-623.
- 792 Trapet P, Avoscan L, Klinguer A, Pateyron S, Citerne S, Chervin C, Mazurier S,
793 Lemanceau P, Wendehenne D, Besson-Bard A. 2016. The *pseudomonas*
794 *fluorescens* siderophore pyoverdine weakens *arabidopsis thaliana*

- 795 defense in favour of growth in iron-deficient conditions. *Plant Physiol.*
796 **171**: 675-693.
- 797 Tsai H H, Schmidt W. 2017. Mobilization of iron by plant-borne coumarins.
798 *Trends Plant Sci.* **22**: 538-548.
- 799 Turan M A, Elkarim A H A, Taban S. 2010. Effect of salt stress on growth and ion
800 distribution and accumulation in shoot and root of maize plant. *African*
801 *Journal of Agricultural Research.* **5**: 584-588.
- 802 Vivas A, Marulanda A, Ruiz-Lozano J M, Barea J M, Azcón R. 2003. Influence of a
803 bacillus sp. On physiological activities of two arbuscular mycorrhizal
804 fungi and on plant responses to peg-induced drought stress. *Mycorrhiza.*
805 **13**: 249-256.
- 806 Wang X, Yang J, Liu G, Yao R, Yu S. 2015. Impact of irrigation volume and water
807 salinity on winter wheat productivity and soil salinity distribution. *Agric*
808 *Water Manage.* **149**: 44-54.
- 809 Winkelmann G. 2007. Ecology of siderophores with special reference to the fungi.
810 *BioMetals.* **20**: 379.
- 811 Witzel K, Weidner A, Surabhi G-K, Börner A, Mock H-P. 2009. Salt stress-induced
812 alterations in the root proteome of barley genotypes with contrasting
813 response towards salinity. *J Exp Bot.* **60**: 3545-3557.
- 814 Yang A, Akhtar S S, Iqbal S, Amjad M, Naveed M, Zahir Z, Jacobsen S-E. 2016.
815 Enhancing salt tolerance in quinoa by halotolerant bacterial inoculation.
816 *Funct Plant Biol.* **43**: 632-642.
- 817 Younesi O, Moradi A. 2014. Effects of plant growth-promoting rhizobacterium
818 (pgpr) and arbuscular mycorrhizal fungus (amf) on antioxidant enzyme

819 activities in salt-stressed bean (*phaseolus vulgaris* l.). *Agriculture*. **60**: 10-
820 21.

821 Yousfi S, Mahmoudi H, Abdelly C, Gharsalli M. 2007. Effect of salt on physiological
822 responses of barley to iron deficiency. *Plant Physiol Biochem*. **45**: 309-314.

823 Zhang H, Sun Y, Xie X, Kim M S, Dowd S E, Paré P W. 2009. A soil bacterium
824 regulates plant acquisition of iron via deficiency - inducible mechanisms.
825 *The Plant Journal*. **58**: 568-577.

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827

salinity stress

↓ osmotic potential

↓ water uptake

↑ oxidative stress

↑ ionic stress

↑ salt toxicity

↑ pH stress

↑ nutrient stress

osmolyte biosynthesis

antioxidants

Intracellular compartmentation

extrusion

organic acids

H⁺

↑ osmotic adjustment

salt ions

secretion

plant response

Strategy I and Strategy II Fe acquisition mechanisms in plants (simplified). Strategy I plants acidify the rhizosphere by releasing protons in order to liberate Fe^{3+} ions from the negatively charged particles in the soil. FRO2, an Fe reductase, reduces Fe^{3+} to Fe^{2+} . Finally, IRT1 (Iron Regulated Transporter) moves Fe^{2+} into the epidermis cells. Strategy II plants excrete phytosiderophores (PS) to the rhizosphere via TOM1. The complex Fe^{3+} -PS is then transported into the cell by the YS1 membrane protein (in maize) or YSL (in other grasses).

Fig 4 Iron uptake mechanism in Gram-negative and Gram-positive bacteria. In Gram-negative bacteria the OMT (outer membrane transporter) binds Fe (III)-siderophores complexes, moving the complex to the periplasm through the TonB-ExbBD protein complex. Later it binds to the periplasmic binding protein, crossing the peptidoglycan layer and is delivered to a siderophore-permease-ATPase system in the cytoplasmic membrane that will release it into the cytoplasm. In Gram-positive bacteria, SBP lipoproteins, anchored to the cell membrane, will bind extracellular Fe-siderophores and import them by a siderophore-permease-ATPase system.