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1 **Use of plant growth promoting bacterial strains to improve *Cytisus striatus***
2 **and *Lupinus luteus* development for potential application in**
3 **phytoremediation**

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16 **Abstract**

17 Plant growth-promoting (PGP) bacterial strains possess different mechanisms to
18 improve plant development under common environmental stresses, and are therefore
19 often used as inoculants in soil phytoremediation processes. The aims of the present work
20 were to study the effects of a collection of plant-growth promoting bacterial strains on
21 plant development, antioxidant enzyme activities and nutritional status of *Cytisus striatus*
22 and/or *Lupinus luteus* plants a) growing in perlite under non-stress conditions and b)
23 growing in diesel-contaminated soil. For this, two greenhouse experiments were
24 designed. Firstly, *C. striatus* and *L. luteus* plants were grown from seeds in perlite, and
25 periodically inoculated with 6 PGP strains, either individually or in pairs. Secondly, *L.*
26 *luteus* seedlings were grown in the A and B horizon of a Cambisol contaminated with
27 1.25% (w/w) of diesel and inoculated with best PGP inoculant selected from the first
28 experiment. The results indicated that the PGP strains tested in perlite significantly
29 improved plant growth. Combination treatments provoked better growth of *L. luteus* than
30 the respective individual strains, while individual inoculation treatments were more
31 effective for *C. striatus*. *L. luteus* growth in diesel-contaminated soil was significantly
32 improved in the presence of PGP strains, presenting a 2-fold or higher increase in plant
33 biomass. Inoculants did not provoke significant changes in plant nutritional status, with
34 the exception of a subset of siderophore-producing and P-solubilising bacterial strains
35 that resulted in significantly modification of Fe or P concentrations in leaf tissues.
36 Inoculants did not cause significant changes in enzyme activities in perlite experiments,
37 however they significantly reduced oxidative stress in contaminated soils suggesting an
38 improvement in plant tolerance to diesel. Some strains were applied to non-host plants,
39 indicating a non-specific performance of their plant growth promotion. The use of PGP

40 strains in phytoremediation may help plants to overcome contaminant and other soil
41 stresses, increasing phytoremediation efficiency.

42 **Keywords**

43 plant growth promoting bacteria; pot inoculation; phytoremediation; oxidative stress-
44 related enzymes; nutritional status

45 **1. Introduction**

46 Remediation of contaminated soils has historically been performed using civil-
47 engineering based methods, which often present high economic and environmental costs,
48 due to soil excavation and removal, application of chemicals, such as solvents or
49 surfactants, and application of high pressure hot water or air. These disadvantages
50 encouraged researchers to develop more environmental-friendly and cost-effective
51 remediation technologies (Afzal *et al.*, 2014).

52 Phytoremediation is defined as the use of green plants and associated
53 microorganisms to remove, contain or render harmless potentially toxic substances such
54 as heavy metals, organic contaminants (*e.g.* pesticides or fuel-derived compounds) and
55 nutrients (Chaney *et al.*, 1997; Kidd *et al.*, 2015; Pilon-Smits, 2005; Salt *et al.*, 1998;
56 Schnoor *et al.*, 1995). Microbe-assisted phytoremediation has emerged as a sustainable
57 soil clean-up technology with reduced soil disturbance, low maintenance, and overall low
58 costs.

59 For phytoremediation to be successful, some important constraints must be
60 considered such as achieving proper plant development, contaminant phytotoxicity, and
61 contaminant bioavailability (Vangronsveld *et al.*, 2009). Inoculation with plant-
62 associated bacteria can be applied to overcome these limitations. For example,
63 endophytic microorganisms with the ability to metabolize a contaminant can lessen
64 phytotoxicity and evapotranspiration of organic contaminants (Weyens *et al.*, 2010);
65 further, some microbes can produce biosurfactants, organic acids and siderophores which
66 can modify organic contaminants and trace element bioavailability (Bordoloi and
67 Konwar, 2009; Weyens *et al.*, 2009a). Plant growth promoting bacteria can also enhance
68 plant development by acting as biofertilisers (increasing the availability of essential

69 nutrients through *e.g.* N₂ fixation and phosphate and iron solubilisation); organic
70 contaminant degraders (lowering both contaminant phytotoxicity and
71 evapotranspiration); phytostimulants (producing plant growth regulators and hormones,
72 such as indoleacetic acid -IAA-, cytokinins and other auxins); stress controllers (by
73 decreasing ethylene production through the synthesis of 1-aminocyclopropane-1-
74 carboxylic acid deaminase -ACCD-); and as plant defence inducers against
75 phytopathogens (by producing siderophores, antibiotics, or fungicidal compounds)
76 (Becerra-Castro *et al.*, 2013a, 2013b; Compant *et al.*, 2010; Lugtenberg and Kamilova,
77 2009; McGuinness and Dowling, 2009; Weyens *et al.*, 2009a, 2009b; Zafar *et al.*, 2012).

78 Adequate plant development is of critical importance in phytoremediation as
79 contaminants can substantially affect plant growth, limiting remediation outcomes (Afzal
80 *et al.*, 2014). In the case of rhizoremediation (phytoremediation in the rhizosphere), an
81 extensive root system is required to achieve adequate development of microbial
82 communities (Yousaf *et al.*, 2010). In the case of *in planta* degradation, a process
83 normally associated with endophytes, and phytoextraction (contaminant bioaccumulation
84 in plant tissues), strong plant development is required. In this sense, the use of PGP
85 inoculants in phytoremediation has been recognized as being beneficial, as PGP
86 microorganisms can enhance plant development under contaminant stress conditions
87 (Wani *et al.*, 2007). The use of a combination of PGP bacterial strains may have
88 beneficial effects on plant growth, as they could induce a more significant effect than a
89 PGP bacterial strain alone.

90 The aim of the present study was to investigate the effects of a collection of PGP
91 bacterial inoculants (individually or in combinations) on the development, nutritional
92 status and antioxidant-related enzyme activities of two plants species (*Cytisus striatus* L.

93 and *Lupinus luteus* L.) under no stress conditions (grown in perlite under greenhouse
94 conditions and watered with nutritive solution). Since the PGP bacterial strains were
95 isolated from *C. striatus* and *Populus deltoides* x (*trichocarpa* x *deltoides*), the results were
96 also used to elucidate if the PGP strains had a broad host plant range. Additionally, *L.*
97 *luteus* plants were grown in soil samples contaminated with 1.25% (w/w) of diesel and
98 inoculated with the best inoculant selected from perlite experiments, to evaluate the
99 performance of PGP strains under contaminant stress conditions. These results may be
100 used as decision tool to choose the best PGP treatment for enhancing plant development
101 in phytoremediation procedures.

102 **2. Materials and methods**

103 **2.1. Bacterial strains**

104 Six bacterial strains isolated from contaminated sites were used for plant inoculation
105 (Table 1). Strains ER33, ER50 and RP92 were previously isolated from hairy-fruited broom
106 (*Cytisus striatus*) growing in a lindane-contaminated soil (Porriño, Spain) (Becerra-Castro *et*
107 *al.*, 2011). Both ER33 and ER50 are root endophytes, and RP92 was isolated from the
108 rhizoplane of this plant species. Strains 12, 105 and 255 were previously isolated from hybrid
109 poplar (*Populus deltoides* x (*trichocarpa* x *deltoides*) cv. Grimminge) growing in a diesel-
110 contaminated site (Genk, Belgium) (Gkorezis, 2014). Strain 12 was isolated from the
111 rhizosphere soil and strain 105 is a root endophyte. Strain 255 was isolated from bulk soil at
112 the same location. Some plant growth promoting properties of the bacterial strains determined
113 by Becerra-Castro *et al.* (2011) and Gkorezis (2014) are presented in Table 1.

114 **2.2. Pot experiment in perlite and inoculation of *Cytisus striatus* and *Lupinus luteus*** 115 **seeds**

116 Seeds of *Cytisus striatus* L. and *Lupinus luteus* L. were surface-sterilized with 2.5%
117 NaClO + Tween 80 (10 min) and rinsed in sterile tap water. Quadruplicate polypropylene pots
118 were filled with perlite, and four *C. striatus* or three *L. luteus* seeds were placed in each pot, at
119 1 cm depth.

120 To prepare the bacterial inoculants, fresh cultures of the PGP strains were grown in 869
121 medium at 30 °C (Mergeay *et al.*, 1985) for 1-2 days, harvested by centrifugation (3000 g, 15
122 min) and re-suspended in 10 mM MgSO₄ to an optical density of 1.0 at 660 nm
123 (approximately 10⁶ cells per mL). In addition to the individual strains (ER33, ER50, RP92,
124 12, 105 and 255), combinations of two strains were also tested: ER33+ER50, ER33+RP92,
125 ER33+12, ER33+105, ER33+255, ER50+RP92, ER50+12, ER50+105, ER50+255, RP92+12,
126 RP92+105, RP92+255, 12+105, 12+255 and 105+255. Pots were inoculated with 100 mL of a
127 1:10 dilution of the inoculants in half-strength Hoagland nutrient solution. For combinations,
128 5 mL of each bacterial suspension was added to the nutrient solution. Quadruplicate non-
129 inoculated (NI) control pots were also prepared, and watered with 10 mM MgSO₄ diluted
130 1:10 with half-strength Hoagland solution. The first inoculation was carried out when seeding
131 pots. The second inoculation was carried out when germination and early development of
132 seedlings was observed in all pots. Throughout the experiment, pots were watered as required
133 with 100 mL of half-strength Hoagland solution. Plants were grown under greenhouse
134 conditions for 30 days for *L. luteus* and 60 days for *C. striatus*.

135 **2.3. Pot experiment in contaminated soil samples using selected PGP inoculants**

136 Samples of A and B horizon from an alumi-umbric Cambisol profile (HA and HB)
137 collected in the surroundings of Santiago de Compostela (Galicia, NW Spain) were used
138 in the experiment. Both samples were acid (pH in H₂O, 4.9 in HA and 5.1 in HB),
139 showed a low cation exchange capacity (2.0 cmol(+) Kg⁻¹ in HA and 1.2 cmol(+) Kg⁻¹ in

140 HB) and sandy loam texture. The samples differed in their organic matter content (4.2 %
141 in HA compared to < 0.5 % in soil HB) and their nutrient content, principally nitrogen
142 (2.89 g Kg⁻¹ in HA and 1.10 g Kg⁻¹ in HB) and magnesium (16.8 mg Kg⁻¹ in HA and 4.8
143 mg Kg⁻¹ in HB). Soil samples were air-dried, sieved through a 2 mm mesh and mixed
144 with sand at a 1:1 ratio (sand/soil), to improve the distribution of water in the pots, and
145 the porosity of sieved soil samples.

146 Soil samples were spiked with diesel, purchased in a local gasoline station, at
147 approximately 1.25 % (w/w). The spiked soils were kept in closed recipients and
148 stabilised at 4 °C for at least 2 weeks before preparing the pots. Polypropylene pots were
149 filled, with approximately 300 g of spiked or uncontaminated soil. One-week-old lupine
150 seedlings were transferred to each pot, and left to stabilise for 1 week before inoculation
151 with the best inoculant in perlite experiment (RP92+105). Inoculants were prepared as
152 described in perlite experiments using sterile distilled water for dilution, and they were
153 added directly to the pots around the seedlings. Non-inoculated (NI) pots were also
154 prepared, and watered with 10 mM MgSO₄ 1:10 diluted with distilled water. The first
155 inoculation was carried out 1 week after preparing the pots with the seedlings, and a
156 second inoculation was carried out 2 weeks after the first inoculation. Plants were
157 watered with distilled water as required and were grown under greenhouse conditions for
158 30 days from the first inoculation. Six pot replicates were prepared for each inoculation
159 treatment (NI, or PGP), each soil (HA or HB), and either diesel-contaminated or
160 uncontaminated.

161 ***2.4. Germination and morphological plant responses***

162 At the end of the pot experiments, plants were harvested and roots and shoots were
163 separated, washed in deionised water, and fresh weight and length were determined. The

164 plant material was oven-dried at 45 °C, until no weight change was observed any more, in
165 order to determine dry weight. Emerged seed numbers were recorded in perlite experiments to
166 calculate germination and survival indices (as percentage of the total number of sowed seeds
167 in the replicate pots).

168 Other plant growth indices were calculated: seedling vigour index (SVI= (mean shoot
169 length + mean root length) x germination percentage); and specific shoot and root lengths
170 (SSL or SRL= shoot or root length per unit biomass) (Calvelo Pereira *et al.*, 2010).

171 **2.5. Determination of plant nutritive status**

172 Plant nutritive status was determined in *C. striatus* and *L. luteus* leaves of 3 selected
173 replicates of each inoculation treatment from perlite and soil experiments. Powdered leaves
174 (approximately 0.1 g) were digested in 65% HNO₃:37% HCl mixture (2:1) to a maximum
175 temperature of 130 °C until achieving complete digestion of the tissues. Concentrations of
176 macro- and micro-nutrients, including Ca, Cu, Fe, K, Mg, Mn, P, and Zn were determined by
177 inductively coupled plasma coupled to optical emission spectrometry (ICP-OES) (Vista Pro;
178 Varian Inc.).

179 **2.6. Determination of antioxidant enzymatic activities**

180 The activity of stress-related enzymes involved in antioxidative defence was determined
181 in *C. striatus* plants grown in perlite and *L. luteus* plants grown in contaminated soil. During
182 harvest, leaf and root samples were taken from selected replicates of non-inoculated and
183 inoculated plants and snap-frozen in liquid nitrogen before storing them at -80°C. These
184 samples were homogenized in ice-cold 0.1 M Tris-HCl buffer (pH 7.8) containing 1 mM
185 EDTA, 1 mM dithiotreitol and 4% insoluble polyvinylpyrrolidone (1 ml buffer per 100 mg
186 fresh weight). The homogenate was centrifuged for 10 min at 20000 g at 4°C. Superoxide
187 dismutase (SOD, EC 1.15.1.1), ascorbate peroxidase (APOD, EC 1.11.1.11), guaiacol

188 peroxidase (GPOD, EC 1.11.1.7), glutathione reductase (GR, EC 1.6.4.2), and catalase (CAT,
189 EC 1.11.1.6) activities (in units or miliunits of activity per gram of fresh weight, U or mU g
190 FW⁻¹) were determined spectrophotometrically in the supernatant at 25 °C as markers for
191 oxidative stress (Vangrosnveld and Clijsters, 1994). CAT, GR and GPOD activities were
192 determined at 240, 340 and 436 nm, respectively according to Bergmeyer *et al.* (1974).
193 APOD activity was measured at 298 nm according to Gerbling *et al.* (1984). Analysis of SOD
194 activity was based on the inhibition of cytochrome c at 550 nm following the method
195 described by McCord and Fridovich (1969).

196 **2.7. Statistical analysis**

197 PASW Statistics software (Version 20.0.0; IBM SPSS Statistics Inc.) was used to analyze
198 the data.

199 Univariate ANOVA, with Tukey *post hoc* analysis, was performed to assess the
200 significant differences between PGP inoculation treatments and NI controls, and between
201 individual and combined treatments. The same test was used to compare the antioxidant
202 enzyme activities and nutrient contents of inoculated and non-inoculated plants. Bivariate
203 Pearson correlations were performed between all growth parameters, nutrient
204 concentrations and enzyme activities. Student *t*-tests were performed to assess the
205 differences between NI and PGP treatments in contaminated soil experiments.

206 **3. Results**

207 **3.1. Plant growth responses to PGP inoculation in perlite pot experiment**

208 Shoot lengths of *C. striatus* plants inoculated with PGP strains (Figure 1a) were
209 significantly higher than for non-inoculated (NI) controls (with $p < 0.01$ in most cases)
210 except for 105, ER33+RP92, ER33+255, ER50+105, ER50+255 and RP92+105.
211 Significant growth promoting bacterial strains induced elongation values which generally

212 reached the double of the NI control plants. The increases in shoot biomass observed for
213 plants inoculated with PGP strains were significant, ranging from 2- (RP92+105) to 5-
214 fold (12 and RP92+255) times higher in comparison to shoots of NI control plants
215 (Figure 1b). The effect of PGP inoculants on the roots of *C. striatus* was not as distinct.
216 Roots were only significantly longer when strains 12, 105, ER50+12, RP92+12,
217 RP92+105 and 12+105 were used as inoculants (Figure 1a); root weight was significantly
218 higher when RP92+255 and 12+105 were used (Figure 1b).

219 The effects of PGP strains on *L. luteus* elongation were not as pronounced as those
220 observed for *C. striatus* (Figure 2a). Significant differences in shoot elongation (not root
221 elongation), in comparison to NI controls, were found for inoculants other than ER33,
222 ER50, 255, ER33+255 and ER59+RP92. Lupine dry weight was only enhanced
223 significantly with inoculants RP92+12, RP92+105 and 12+105, for both shoots and roots.

224 In general, the combinations of two strains did not significantly improve the growth
225 of *C. striatus* over and above that achieved by individually inoculated strains (Appendix,
226 Table A.1). The greatest differences were found for ER50+RP92 and 255+RP92, which
227 resulted in a 71% and 84 % increase of *C. striatus* root weight with respect to plants
228 inoculated with only ER50 and 255, respectively. In some cases, negative effects of PGP
229 strain combinations over individual strains were observed for shoot weight. For example,
230 ER33+ER50 compared to ER33 (68% reduction) and ER50 (63% reduction) alone,
231 RP92+105 compared to RP92 (54% reduction) and to 105 (61% reduction) alone, and 12
232 +ER50 compared to 12 (58% reduction) alone (Appendix, Table A.1).

233 For *L. luteus*, the inoculation of combined PGP strains generally lead to an increased
234 plant growth, particularly for shoots (Appendix, Table A.2), in comparison to plants
235 inoculated with single strains. The greatest differences were found for bacterial combinations

236 that included strain 255, which stimulated up to 48% increases in shoot elongation and 62%
237 increases in shoot weight in comparison to plants inoculated with strain 255 alone ($p<0.01$).
238 Root weight significantly increased (by up to 76%) with combinations that included strain 12
239 in comparison to strain 12 inoculated alone. Also negative effects of PGP combinations in
240 comparison to individual PGP strains were observed, but these differences were not
241 significant except in some cases, including 105+ER33, which resulted in a 38% reduction in
242 shoot weight ($p<0.01$) and a 35% reduction in root weight in comparison to inoculation with
243 the individual strain 105 treatment.

244 In some cases, inoculation improved germination of *C. striatus* (e.g. up to 85% with
245 RP92+255), compared to 65% germination of NI controls (Table 2); however, significantly
246 lower germination rates (38%), were observed for inoculations that included strain ER33:
247 ER33, ER33+50 and ER33+RP92. Other growth indices, such as seedling vigour indices
248 (SVI), and shoot and root specific lengths (SSL and SRL), were generally higher than those
249 for NI control plants. SVI of PGP inoculated plants varied from 10.7 (ER33) to 27.7
250 (RP92+255), being ER50, RP92, 12, 255, ER50+12, ER50+255, RP92+105, 12+105 and
251 12+255 the inoculations which doubled SVI values of control plants. Specific shoot and root
252 lengths (SSL and SRL) were higher in plants inoculated with PGP strains than in NI control
253 plants. This was observed for the shoots of plants inoculated with ER33+ER50, ER33+12 and
254 ER50+12, and for the roots of plants inoculated with 105, 255, ER33+105, RP90+105,
255 RP92+255 and 12+255. *L. luteus* showed better germination rates (73%-100%) than *C.*
256 *striatus* (38%-85%), and all inoculation treatments increased the germination rates (75%-
257 100%) in comparison to NI seeds (73%) (Table 3). Seedling vigour was better for PGP
258 inoculated plants of both species, while in contrast to *C. striatus*, SSL and SRL of *L.*

259 *luteus* plants inoculated with PGP strains were generally lower than those of NI control
260 plants, especially for RP92+105 and 12+105 treatments.

261 **3.2. Plant nutritive status and stress-related enzyme activities in perlite pot experiment**

262 The concentration of nutrients in leaves of *C. striatus* and *L. luteus* was determined in
263 order to assess plant nutritional status (Appendix, Tables A.3 and A.4). In general, nutrient
264 concentrations were within reference sufficiency ranges (Kalra, 1998), with the exception of
265 Mn in *C. striatus* and Zn in *L. luteus*, which were slightly above the normal range in some
266 inoculation treatments. Nutrient concentrations did not significantly vary between plant
267 species, except for Mn, which was approximately an order of magnitude higher in *C. striatus*,
268 and Zn concentrations, which were approximately 2 times higher in *L. luteus*.

269 In general, inoculation treatments did not have a significant effect on the nutrient content
270 of *C. striatus* or *L. luteus* leaves in comparison to NI controls, with some exceptions: *e.g.*
271 phosphorous concentrations in *C. striatus* leaves were significantly increased in the presence
272 of ER50+105 strains, and in *L. luteus* leaves in the presence of ER33+ER50 and 12+255, and
273 concentrations of iron increased significantly in *L. luteus* leaves inoculated with ER50+105.

274 The activities of some enzymes involved in defence against oxidative stress (SOD,
275 APOD, GPOD, GR and CAT) were determined in the leaves and the roots of *C. striatus*
276 plants growing in perlite, in order to determine if PGP inoculation caused any stress to plants
277 (Appendix, Figure A.1). In general, inoculation with the PGP strains, individually or in
278 combinations, did not affect the activities of stress-related enzymes except for the roots
279 inoculated with ER33+ER50, where significantly increased activities of GR and GPOD
280 ($p < 0.01$) were observed.

281 **3.3. Plant growth, nutritional status and stress-related enzyme activities in diesel-** 282 **contaminated soil inoculated with PGP strains**

283 The combination of RP92+105 strains was used for inoculation in greenhouse
284 experiments with diesel-contaminated soils due to the excellent results obtained in perlite
285 experiments: shoot and root weight of *L. luteus* plants inoculated with RP92+105 increased
286 by 50 % compared to the NI control (Figure 2). This plant was selected for contaminated-soil
287 experiments due to its fast growth and contaminant tolerance (Balseiro-Romero *et al.*, 2016;
288 Weyens *et al.*, 2010).

289 Contamination of NI soil with diesel provoked a highly significant decrease in *L.*
290 *luteus* growth ($p<0.01$) with regard to uncontaminated soils. Inoculation with selected
291 PGP bacterial strains (RP92+105) provoked a significant increase in plant shoot and root
292 biomass in both contaminated soil samples, almost reaching a similar plant development
293 to that in uncontaminated soil, and this effect was especially significant for the roots
294 ($p<0.01$) (Figure 3a): root biomass of PGP inoculated plants developed in contaminated
295 HA and HB was 3-fold higher than NI controls. In uncontaminated HB soil, PGP strains
296 also provoked a positive effect on root growth (PGP inoculation provoked a 2.5-fold
297 increase) ($p<0.01$), while in uncontaminated HA no effect of PGP was appreciated. The
298 effect of PGP inoculation in contaminated soils was more significant on plant biomass
299 than on plant length, resulting in a decrease of specific shoot and root lengths (SSL and
300 SRL) respectively by 1.5 and 3-fold (Figure 3b).

301 Plant nutritional status (as leaf nutrient concentrations) was not significantly
302 improved in PGP inoculated soils compared to NI soils, with the notable exceptions of
303 copper (leaf concentrations significantly increased with PGP inoculation by 1.5-fold in
304 contaminated HB sample ($p<0.05$)) (data not shown) and iron (Figure 3c). Leaf iron
305 concentrations increased in the presence of PGP inoculants under contaminant stress

306 conditions in both soil samples, and this was especially accused for plants grown in HB
307 soil sample which increased by 8-fold with regard to NI plants ($p<0.01$).

308 Generally, the activities of antioxidant enzymes measured (SOD, APOD, GPOD, GR,
309 and CAT) in leaf tissues were very similar in uncontaminated soils for both inoculation
310 treatments (NI and PGP), and their activity increased in NI contaminated samples. The
311 enzymes presenting the most significant differences are represented in Figure 3d, e and f
312 (respectively GPOD, GR and CAT). The presence of PGP strains in contaminated soils
313 provoked significant decreases in enzymatic activities to similar or lower levels than in
314 uncontaminated soil samples. The decrease in enzyme activities was more significant in
315 contaminated HB than in contaminated HA. GPOD was the enzyme whose activity
316 reflected a more drastic stress drop in contaminated soils in the presence of PGP
317 inoculants: PGP inoculation provoked a 2-fold decrease of GPOD activity in
318 contaminated HA ($p<0.05$) and a 7.5-fold decrease in contaminated HB ($p<0.01$)
319 compared to NI soils.

320 **4. Discussion**

321 Inoculation of plants using bacterial strains with plant growth promoting properties
322 has been reported: (a) to improve the performance of plants under contaminant stress
323 conditions in phytoremediation experiments (Aung *et al.*, 2015; Becerra-Castro *et al.*,
324 2013a; Das *et al.*, 2014; Ma *et al.*, 2015; Tara *et al.*, 2014); (b) as biological fertilizers
325 (de Oliveira *et al.*, 2006; Rueda-Puente *et al.*, 2010); (c) to alleviate environmental
326 stresses (such as nutrient deficiency, salinity, water stress, ambient temperature) (Ali *et al.*,
327 2014; Egamberdiyeva and Höflich, 2003; Grichko and Glick, 2001; Mayak *et al.*,
328 2004; Pii *et al.*, 2015); and (d) as biocontrol agents of plant diseases (Compant *et al.*,
329 2005; Zhang *et al.*, 2010).

330 As observed by other authors (Adam and Duncan, 2002; Calvelo Pereira *et al.*, 2010;
331 Sytar *et al.*, 2013) and in our previous experiments (Balseiro-Romero and Monterroso,
332 2015), contamination stresses can provoke significant inhibitions of germination, growth,
333 seedling vigour, SRL and SSL of exposed plants. Therefore, the application of PGP
334 strains in such conditions, as occurs in phytoremediation experiments, can be beneficial
335 for overcoming these constraints and improving plant performance in contaminated
336 environments.

337 *C. striatus* and *L. luteus* have been previously used in phytoremediation research
338 studies (Balseiro-Romero *et al.*, 2016; Barac *et al.*, 2004; Becerra-Castro *et al.*, 2013a;
339 Gutiérrez-Ginés *et al.*, 2014; Weyens *et al.*, 2010). These plants are moderately
340 contaminant-tolerant leguminous crops with extensive shoot and root systems, desirable
341 characteristics for phytoremediation species. In this study, the annual *L. luteus* was
342 observed to have a faster growth rate (length and weight data on day 30; Figure 1) and
343 developed more biomass (according to SSL and SRL) than *C. striatus* plants. While
344 slower growing, *C. striatus*, a woody perennial, also developed vigorously (length and
345 weight data on day 60; Figure 2). Selection of plants species for phytoremediation
346 depends on biomass growth characteristics, contaminant tolerance, the time required to
347 achieve adequate soil clean-up, and the remediation goals. For example, phytoextraction
348 of trace elements (commonly termed as heavy metals) requires fast growing high biomass
349 producing plants with effective accumulation of contaminants in the aerial biomass that
350 is easy to harvest (Vangronsveld *et al.*, 2009), while rhizodegradation of organic
351 contaminants is more effective with non-harvestable plants with extensive root systems
352 that stay healthy during the remediation process. As such, perennial or annual plants
353 should be chosen according to these specific requirements.

354 The bacterial strains used in this study were positive for several plant growth
355 promoting characteristics, *i.e.* siderophore production, phosphate solubilisation, and IAA,
356 ACCD and organic acid production (Table 1), potentially enhancing plant biomass
357 production and facilitating bacterial colonization. Some studies suggested that PGP
358 bacteria that most effectively protect plants against a wide range of stresses produce both
359 IAA and ACCD (Glick, 2012). In addition, bacteria possessing ACCD genes may be
360 more effective in association with many rhizobial strains (Glick, 2014).

361 In this study, inoculation of *C. striatus* and *L. luteus* plants with PGP bacterial strains
362 generally improved plant performance in terms of germination, seedling vigour, and plant
363 growth in general, and this effect was also appreciated under contaminant stress
364 conditions in soil.

365 Generally, germination of *C. striatus* was not significantly improved by the presence
366 of PGP strains in perlite experiments (Table 2): in some cases, germination even
367 decreased or increased less than 10% in the presence of PGP strains. However, *L. luteus*
368 germination was substantially enhanced by inoculations (Table 3), even reaching 100%
369 germination with some inoculants. *L. luteus* seeds are larger than those of *C. striatus*, and
370 therefore they are expected to present a better germination performance (larger seeds also
371 contain more internal nutritional reserves and stored energy) (Clark *et al.*, 2004), as was
372 observed for NI controls.

373 In general, under non-stress conditions (perlite experiments, watered with nutrient
374 solution) all PGP inoculation treatments provoked an increase in plant growth, especially
375 of the shoots (Figures 1 and 2). Under contaminant stress conditions (contaminated soil
376 experiments), inoculation of *L. luteus* with the selected PGP combination RP92+105
377 provoked a significant increase in plant growth with regard to NI soils, indicating that

378 PGP inoculants were also exerting their effect on plant growth under stressful conditions
379 (Figure 3). In addition to contamination, HB sample presents naturally stressful
380 conditions (absence of organic matter and lower nutrient content than HA). In
381 accordance, PGP strains provoked a positive effect on root growth in uncontaminated
382 HB, while on uncontaminated HA the effect of PGP was not appreciated.

383 In perlite experiments, in terms of plant length and weight, individual inocula
384 generally resulted in better performance than the combinations for *C. striatus*, while
385 combinations provoked better plant growth promotion of *L. luteus* plants (Appendix,
386 Tables A.1 and A.2). This probably reflected a competence in root tissue colonization.
387 During harvest, it was observed that *C. striatus* plants possessed a lower root surface (*i.e.*
388 roots were thinner) and roots were shorter than those of *L. luteus*, although in terms of
389 dry weight there was no difference between both species.

390 *C. striatus* SSL and SRL indices were significantly higher than those of *L. luteus* in
391 NI perlite, indicating that naturally *C. striatus* plants grew proportionally more in length
392 than in biomass compared to *L. luteus* plants (Tables 2 and 3). This was also visually
393 observed, as *C. striatus* plants developed thinner shoots and fewer leaves with lower
394 foliar surface than those of *L. luteus*. Inoculation of plants with PGP strains enhanced
395 these differences with regard to NI control plants, and relatively longer plants of *C.*
396 *striatus* (SSL and SRL were generally higher than NI plants) and heavier plants of *L.*
397 *luteus* (SSL and SRL were generally lower than NI plants) were developed, translated
398 into a more branched root system (Bhattacharyya and Jha, 2012). This indicates that PGP
399 strains provoked different growth responses in both plant species. This effect was also
400 observed in contaminated-soil experiments: *L. luteus* plants inoculated with PGP
401 presented significantly lower SRL and SSL indices than NI plants, reflecting that PGP

402 inoculation provoked a more significant development of plant biomass over elongation
403 also in soil.

404 Generally, the nutrient status of PGP inoculated plants was not significantly modified
405 with regard to NI control plants in both the non-stressful perlite environment and
406 contaminated-soils. In perlite experiments (Appendix, Tables A.3 and A.4), remarkable
407 exceptions were ER50+105 and 12+255 combinations, which possess P-solubilising
408 properties, and significantly increased leaf phosphorous concentrations in *C. striatus* and *L.*
409 *luteus*, respectively; and the joint inoculation of strains ER50 and siderophore-producing 105,
410 which also improved iron concentration in *L. luteus* leaves. This slight influence of PGP
411 inoculation on plant nutrition could be due to the favourable experimental conditions: plants
412 were grown in perlite and periodically watered with Hoagland nutrient solution. Under
413 contaminant-stress conditions in soil experiments, inoculation of *L. luteus* with siderophore-
414 producers RP92 and 105 provoked a significant increase in leaf iron concentration with regard
415 to non-inoculated contaminated soils, and even compared to uncontaminated soils (Figure 3c).
416 This effect was especially significant for HB. In this type of soil horizon (where stress
417 conditions were higher), there are more potential sources of free iron (oxyhydroxides) than in
418 HA, which could be solubilized in the presence of siderophores and more easily uptaken by
419 plants and translocated to leaves. For other nutrients, as occurred in perlite, leaf
420 concentrations were not generally influenced by PGP inoculation. Therefore, nutrient-
421 solubilising properties of PGP strains seemed to have a meaningless influence on the
422 observed plant growth improvement in PGP inoculated experiments (perlite and soil).
423 Therefore, other PGP mechanisms (including production of phytohormones, ethylene
424 production suppression, defence against pathogens, *etc.*) may be exerting also significant
425 influences on plant development enhancement. In general, inoculation of *C. striatus* with PGP

426 strains under non-stressful conditions in perlite experiments did not provoke any additional
427 oxidative stress to the plants (Appendix, Figure 1.A). This plant was selected for these
428 measurements in order to compare the effect of PGP strains isolated from this species (ER33,
429 ER50 and RP92 were isolated from *C. striatus* tissues or rhizosphere) and other plant species
430 (12, 105 and 255 were isolated from *P. deltoides* tissues or rhizosphere). Apart from
431 morphological aspects, the determination of the activities of oxidative stress-related enzymes
432 may be useful to verify whether the PGP strains are host specific or non-specific, by assessing
433 stress in plant tissues. These results suggested that the PGP strains used are non-host
434 specific colonizers, and they were not causing any negative effect to plant activity, since
435 they did not provoke any oxidative stress and they all promoted *C. striatus* plant growth
436 despite being isolated from different plant species. Analogously, Ma *et al.* (2011) found
437 that PGP endophytes isolated from *Alyssum serpyllifolium*, induced growth promotion of
438 *Brassica juncea* and improved the Ni phytoextraction performance. This is of particular
439 interest for promoting plant growth in phytoremediation (Ma *et al.*, 2011), and perhaps as
440 an approach to replace chemical fertilizers in organic agriculture (Glick, 2014; Khan *et*
441 *al.*, 2012).

442 On the other hand, an increase in antioxidant enzyme activities was observed when plants
443 were submitted to oxidative stressful conditions, in this case, diesel contamination (Figure 3d,
444 e and f). Abiotic stresses usually lead to the overproduction of reactive oxygen species in
445 plant tissues. Among the antioxidant enzymes measured in these experiments, APOD, GPOD,
446 GR and CAT are involved in the decomposition of H₂O₂ to H₂O or O₂, and SOD catalyses the
447 dismutation of superoxide (O₂⁻²) to H₂O₂ or O₂ (Gill and Tuteja, 2010). GPOD, GR and CAT
448 were those enzymes presenting the most significant decrease in activity in the presence of
449 PGP inoculations, indicating that the stress conditions held in soil experiments probably

450 caused an increase in hydrogen peroxide production. PGP inoculation provoked a significant
451 decrease in enzymatic activities or decrease in plant oxidative stress, which could be
452 translated into a better tolerance to soil contamination (also observed by Xun *et al.* (2015)),
453 which was also reflected by better plant growth. The enzymatic activity decrease was
454 especially significant in contaminated HB, where plants were doubly stressed due to diesel
455 contamination and to the adverse soil properties for plant growth compared to HA soil.

456 **5. Conclusions**

457 Inoculation of *C. striatus* and *L. luteus* with PGP bacterial strains under non-stress
458 conditions generally improved the performance of plant viability in terms of germination,
459 seedling vigour and biomass production, and did neither provoke increases in oxidative
460 activity nor modifications in plant nutrient content. Inoculation of the best PGP treatment
461 in diesel-contaminated soil samples provoked a significant improvement in *L. luteus*
462 development as well as a decrease in oxidative stress, probably due an increase in plant
463 diesel-tolerance and adaptation to soil conditions, mediated by diverse PGP mechanisms.

464 Within our results, the PGP strains used in our experiments could be inoculated in
465 phytoremediation experiments to enhance plant development under contaminant stress
466 conditions and, since some of them were non-host specific, they could be also used to
467 promote growth of other phytoremediation species.

468 Based on the results, further investigations will be performed to determine the
469 mechanisms involved in plant growth promotion of each specific bacteria-plant
470 association, as well as the effectiveness of plant tissues colonization of the inoculants.
471 The performance of plants and bacterial inoculants should also be studied under
472 contamination stress in greenhouse and field conditions.

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662

663 **APPENDIX**

664 **Table A.1.** Shoot and root length and dry weight of *C. striatus* plants inoculated with
665 combinations of PGP bacteria, normalized to respective treatments with individual strains.
666 Significant differences with individual strains are indicated with asterisks: * $p < 0.05$ and
667 ** $p < 0.01$.

Individual	Combination	Shoot length	Root length	Shoot weight	Root weight
ER33	ER33+ER50	1.22	0.81	**0.32	0.48
	ER33+RP92	0.95	0.64	0.92	0.55
	ER33+12	1.27	1.08	0.87	0.78
	ER33+105	1.34	1.08	1.03	0.86
	ER33+255	0.88	0.94	0.71	0.73
ER50	ER50+ER33	1.18	0.64	*0.37	0.69
	ER50+RP92	1.14	0.88	1.10	1.71
	ER50+12	1.03	1.08	0.65	1.13
	ER50+105	0.81	0.94	0.79	1.33
	ER50+255	0.85	1.05	1.01	1.07
RP92	RP92+ER33	0.89	**0.53	0.90	0.54
	RP92+ER50	1.10	0.92	0.92	1.14
	RP92+12	0.98	1.15	0.73	0.89
	RP92+105	0.82	1.27	**0.46	0.79
	RP92+255	1.04	1.05	1.26	1.33
12	12+ER33	1.06	0.70	0.67	0.63
	12+ER50	0.89	0.88	**0.42	0.63
	12+RP92	0.87	0.89	**0.57	0.74
	12+105	0.90	0.93	0.79	1.11
	12+255	1.01	0.87	0.86	0.89
105	105+ER33	1.36	*0.59	0.87	0.70
	105+ER50	0.85	*0.65	**0.57	0.75
	105+RP92	0.88	0.84	**0.39	0.67
	105+12	1.10	0.80	0.86	1.12
	105+255	1.18	**0.56	0.68	0.65
255	255+ER33	0.82	0.70	0.78	0.98
	255+ER50	0.82	0.99	0.95	0.99
	255+RP92	1.03	0.94	1.40	**1.84
	255+12	1.13	1.01	1.24	1.48
	255+105	1.09	0.76	0.88	1.07

668

669

670 **Table A.2.** Shoot and root length and dry weight of *L. luteus* plants inoculated with
671 combinations of PGP bacteria, normalized to respective treatments with individual strains.
672 Significant differences with individual strains are indicated with asterisks: * p <0.05 and
673 ** p <0.01.

Individual	Combination	Shoot length	Root length	Shoot weight	Root weight
ER33	ER33+ER50	1.16	1.08	0.85	0.97
	ER33+RP92	1.17	1.05	0.83	1.06
	ER33+12	**1.28	1.18	0.99	1.32
	ER33+105	1.16	1.20	0.78	0.72
	ER33+255	1.05	0.98	0.81	0.81
ER50	ER50+ER33	1.11	0.84	0.99	0.98
	ER50+RP92	0.97	0.90	1.20	1.02
	ER50+12	1.10	1.04	1.21	1.10
	ER50+105	1.15	1.05	1.15	1.08
	ER50+255	1.07	0.83	1.02	0.92
RP92	RP92+ER33	1.04	0.82	0.73	0.84
	RP92+ER50	0.90	0.89	0.90	0.80
	RP92+12	1.05	1.04	1.12	1.16
	RP92+105	1.21	1.08	1.25	1.28
	RP92+255	1.01	0.78	0.71	0.78
12	12+ER33	*1.16	0.95	1.03	1.37
	12+ER50	1.04	1.07	1.08	1.12
	12+RP92	1.07	1.08	**1.33	**1.52
	12+105	**1.24	0.98	**1.48	**1.76
	12+255	**1.22	1.03	1.22	1.35
105	105+ER33	1.02	0.96	**0.62	0.65
	105+ER50	1.05	1.06	0.78	0.95
	105+RP92	1.19	1.11	1.13	1.45
	105+12	1.19	0.96	1.13	1.52
	105+255	0.97	0.92	0.74	1.02
255	255+ER33	1.15	0.88	1.12	0.93
	255+ER50	**1.23	0.95	1.22	1.04
	255+RP92	*1.24	0.90	1.13	1.13
	255+12	**1.48	1.14	**1.62	1.50
	255+105	*1.22	1.03	1.28	1.30

674 **Table A.3.** Leaf nutrient concentrations of selected *C. striatus* replicates grown in perlite (indicated as the mean \pm standard deviation;
 675 $n=3$). Significant differences with non-inoculated control (NI) are indicated with asterisks: * $p<0.05$ and ** $p<0.01$.

Inoculant	K (g/kg)	Ca (g/kg)	Mg (g/kg)	P (g/kg)	Mn (mg/kg)	Fe (mg/kg)	Zn (mg/kg)	Cu (mg/kg)
NI	39.8 \pm 2.5	12.1 \pm 0.9	4.8 \pm 0.1	3.5 \pm 0.5	315.8 \pm 36.4	193.3 \pm 18.5	41.8 \pm 5.2	6.6 \pm 0.3
ER33	37.7 \pm 1.7	12.1 \pm 4.2	4.6 \pm 1.2	3.1 \pm 0.7	309.9 \pm 64.0	208.7 \pm 70.4	64.0 \pm 6.1**	13.3 \pm 4.9**
ER50	35.4 \pm 1.2	12.2 \pm 1.6	4.6 \pm 0.6	3.6 \pm 0.3	305.7 \pm 19.6	167.4 \pm 23.4	46.6 \pm 3.7	7.1 \pm 0.5
RP92	38.2 \pm 2.3	13.1 \pm 1.2	5.2 \pm 0.2	3.7 \pm 0.4	210.2 \pm 45.7	160.7 \pm 24.7	49.4 \pm 5.3	7.5 \pm 0.8
12	37.4 \pm 1.5	11.1 \pm 1.4	4.6 \pm 0.6	4.1 \pm 0.2	301.1 \pm 14.4	211.5 \pm 25.3	57.2 \pm 5.6	8.2 \pm 0.8
105	39.0 \pm 7.2	12.3 \pm 0.8	5.1 \pm 0.1	2.9 \pm 0.2	371.2 \pm 73.9	122.1 \pm 30.3	49.6 \pm 15.2	6.5 \pm 1.2
255	38.7 \pm 3.0	12.7 \pm 0.9	4.7 \pm 0.2	3.5 \pm 0.1	262.8 \pm 15.3	174.4 \pm 41.2	47.5 \pm 5.2	8.8 \pm 1.0
ER33+ER92	37.2 \pm 2.6	10.7 \pm 0.7	4.3 \pm 0.2	3.5 \pm 0.2	285.4 \pm 20.0	198.5 \pm 31.5	56.4 \pm 3.9	8.7 \pm 0.6
ER33+12	40.9 \pm 2.9	10.0 \pm 0.9	4.4 \pm 0.3	3.6 \pm 0.3	262.0 \pm 18.3	148.8 \pm 23.6	69.4 \pm 4.9**	10.4 \pm 0.7
ER33+105	41.8 \pm 2.5	10.7 \pm 1.0	4.4 \pm 0.6	4.5 \pm 0.3	337.0 \pm 23.6	182.1 \pm 28.9	58.8 \pm 4.1	9.2 \pm 0.6
ER33+255	41.5 \pm 2.9	9.3 \pm 0.7	3.9 \pm 0.3	3.8 \pm 0.3	295.0 \pm 20.7	150.1 \pm 23.8	58.3 \pm 4.1	11.3 \pm 0.8**
ER50+RP92	37.9 \pm 4.9	13.4 \pm 0.1	5.3 \pm 0.1	3.5 \pm 0.1	272.1 \pm 39.8	105.5 \pm 23.3	60.9 \pm 8.1	6.6 \pm 0.5
ER50+12	35.3 \pm 1.9	12.0 \pm 0.5	4.5 \pm 0.2	3.2 \pm 0.3	221.1 \pm 8.6	120.2 \pm 11.9	52.3 \pm 6.6	6.5 \pm 0.4
ER50+105	38.0 \pm 0.8	12.2 \pm 1.3	4.5 \pm 0.2	6.0 \pm 0.6**	335.4 \pm 28.8	177.9 \pm 36.4	44.3 \pm 8.2	7.2 \pm 0.8
ER50+255	39.3 \pm 2.1	10.5 \pm 0.6	3.9 \pm 0.4	4.3 \pm 0.9	271.8 \pm 14.0	133.8 \pm 21.6	34.4 \pm 3.3	7.5 \pm 1.4
RP92+12	40.4 \pm 1.3	10.6 \pm 1.1	4.7 \pm 0.1	4.6 \pm 0.2	330.3 \pm 9.0	170.0 \pm 19.4	67.9 \pm 9.5**	7.6 \pm 0.6
RP92+105	39.1 \pm 1.7	12.3 \pm 1.9	4.8 \pm 0.6	3.5 \pm 0.6	279.3 \pm 10.5	99.6 \pm 10.7	35.8 \pm 5.7	7.6 \pm 1.8
RP92+255	40.1 \pm 4.7	12.4 \pm 1.6	4.6 \pm 0.3	4.2 \pm 0.3	296.1 \pm 61.6	110.4 \pm 5.9**	34.9 \pm 2.2	7.1 \pm 0.9
12+105	37.7 \pm 3.4	12.4 \pm 1.6	5.2 \pm 0.5	3.7 \pm 0.5	315.2 \pm 27.1	105.2 \pm 15.3**	39.7 \pm 7.4	7.4 \pm 1.2
12+255	40.6 \pm 3.5	12.6 \pm 2.7	4.7 \pm 0.8	3.8 \pm 0.5	283.3 \pm 32.5	101.0 \pm 13.1**	36.4 \pm 3.3	6.2 \pm 0.5
105+255	39.1 \pm 2.1	12.4 \pm 1.6	4.7 \pm 0.8	4.0 \pm 0.3	263.4 \pm 13.2	95.2 \pm 2.1**	51.5 \pm 9.3	6.8 \pm 0.9

676 ER33+ER50 was not processed due to the lack of leaf biomass

677 **Table A.4.** Leaf nutrient concentrations of selected *L. luteus* replicates grown in perlite (indicated as the mean \pm standard deviation;
 678 $n=3$). Significant differences with non-inoculated control (NI) are indicated with asterisks: * $p<0.05$ and ** $p<0.01$.

Inoculant	K (g/kg)	Ca (g/kg)	Mg (g/kg)	P (g/kg)	Mn (mg/kg)	Fe (mg/kg)	Zn (mg/kg)	Cu (mg/kg)
NI	± 2.6	8.4 \pm 0.5	5.8 \pm 0.6	8.9 \pm 0.9	37.2 \pm 8.6	91.8 \pm 2.0	83.8 \pm 5.5	10.4 \pm 1.5
ER33	44.6 \pm 4.9	8.7 \pm 0.5	6.1 \pm 0.6	9.5 \pm 0.1	36.0 \pm 5.9	89.6 \pm 17.8	97.5 \pm 10.7	10.0 \pm 0.1
ER50	44.1 \pm 4.0	10.1 \pm 1.0	6.1 \pm 0.6	9.6 \pm 0.4	37.2 \pm 9.9	101.1 \pm 2.5	124.5 \pm 8.0*	11.6 \pm 0.1
RP92	43.6 \pm 2.8	9.0 \pm 1.1	6.0 \pm 0.1	10.9 \pm 0.8	45.9 \pm 7.2	110.7 \pm 12.8	91.6 \pm 5.2	12.9 \pm 1.8
12	45.9 \pm 3.6	9.1 \pm 0.8	6.0 \pm 0.5	10.7 \pm 0.4	41.8 \pm 5.0	100.7 \pm 7.8	100.5 \pm 3.9	14.3 \pm 2.4
105	43.8 \pm 3.2	8.6 \pm 0.7	6.2 \pm 0.3	11.3 \pm 0.2	34.1 \pm 3.3	98.3 \pm 16.3	107.3 \pm 3.9	10.1 \pm 1.3
255	41.5 \pm 6.0	9.5 \pm 0.8	6.9 \pm 0.3	12.2 \pm 0.4	36.1 \pm 4.1	100.1 \pm 7.5	125.5 \pm 19.1**	17.3 \pm 0.7*
ER33+ER50	31.1 \pm 2.6	8.5 \pm 1.0	5.9 \pm 0.4	12.9 \pm 1.2**	39.6 \pm 9.4	123.4 \pm 9.5	135.3 \pm 10.9**	23.3 \pm 4.7**
ER33+ER92	40.3 \pm 3.4	9.4 \pm 0.9	6.2 \pm 0.9	11.7 \pm 0.1	56.9 \pm 5.2	99.5 \pm 1.8	128.0 \pm 18.7*	11.9 \pm 1.9
ER33+12	38.8 \pm 2.7	8.1 \pm 0.5	4.9 \pm 0.3	10.0 \pm 0.2	53.2 \pm 3.4	108.8 \pm 6.8	95.8 \pm 5.8	13.1 \pm 0.2
ER33+105	34.7 \pm 2.6	8.1 \pm 1.3	6.0 \pm 0.5	12.1 \pm 0.8	56.0 \pm 6.2	92.4 \pm 10.6	121.5 \pm 1.5	11.0 \pm 1.1
ER33+255	41.3 \pm 2.3	9.2 \pm 1.6	6.0 \pm 0.7	11.6 \pm 1.0	37.8 \pm 6.7	94.0 \pm 10.1	95.8 \pm 4.5	9.9 \pm 0.3
ER50+RP92	36.4 \pm 3.4	9.5 \pm 0.6	6.1 \pm 0.7	10.5 \pm 1.2	44.9 \pm 6.6	111.3 \pm 11.2	99.1 \pm 7.1	11.3 \pm 1.5
ER50+12	44.2 \pm 2.8	9.1 \pm 1.5	5.7 \pm 0.3	11.1 \pm 0.6	42.3 \pm 3.6	126.3 \pm 2.5	91.7 \pm 10.6	10.8 \pm 0.5
ER50+105	40.1 \pm 4.2	8.5 \pm 0.3	6.0 \pm 0.4	10.9 \pm 0.7	38.3 \pm 4.3	161.6 \pm 31.2**	100.2 \pm 4.9	10.6 \pm 2.9
ER50+255	44.0 \pm 6.6	8.4 \pm 0.8	5.7 \pm 0.8	11.1 \pm 1.2	38.3 \pm 1.2	122.8 \pm 17.2	95.8 \pm 4.6	11.8 \pm 1.0
RP92+12	43.7 \pm 3.2	11.4 \pm 0.8	6.1 \pm 0.3	9.9 \pm 1.2	50.0 \pm 6.0	98.6 \pm 10.3	91.1 \pm 18.0	9.8 \pm 1.0
RP92+105	35.3 \pm 5.3	9.7 \pm 1.1	5.9 \pm 1.5	10.6 \pm 2.2	52.9 \pm 8.7	117.2 \pm 14.3	105.3 \pm 7.6	11.4 \pm 1.9
RP92+255	37.5 \pm 6.0	9.2 \pm 0.6	6.7 \pm 1.0	11.2 \pm 0.9	43.1 \pm 4.4	102.3 \pm 9.0	116.9 \pm 10.6	8.7 \pm 1.3
12+105	40.2 \pm 2.9	9.5 \pm 0.9	5.7 \pm 0.7	10.5 \pm 1.1	52.8 \pm 4.9	118.2 \pm 19.5	93.1 \pm 12.9	12.5 \pm 0.5
12+255	38.2 \pm 4.4	8.2 \pm 1.6	6.0 \pm 0.3	13.1 \pm 0.6**	35.7 \pm 4.7	93.0 \pm 14.7	117.8 \pm 18.9	9.8 \pm 2.3
105+255	38.6 \pm 8.5	8.1 \pm 0.9	5.7 \pm 0.4	10.8 \pm 0.7	33.5 \pm 6.9	94.7 \pm 18.8	112.5 \pm 10.4	12.3 \pm 1.1

680 **Figure A.1.** Activities of antioxidant enzymes superoxide dismutase (SOD), ascorbate
681 peroxidase (APOD), catalase (CAT), glutathione reductase (GR), and guaiacol peroxidase
682 (GPOD) (in units or miliunits of activity per gram of leaf fresh weight, U or mU g FW⁻¹)
683 in shoots and roots of *C. striatus* grown in perlite inoculated with the PGP strains,
684 individually or in combinations (indicated as the mean ± standard deviation; *n*=6).
685 Significant differences with non-inoculated control (NI) are indicated with asterisks:
686 **p*<0.05 and ***p*<0.01.

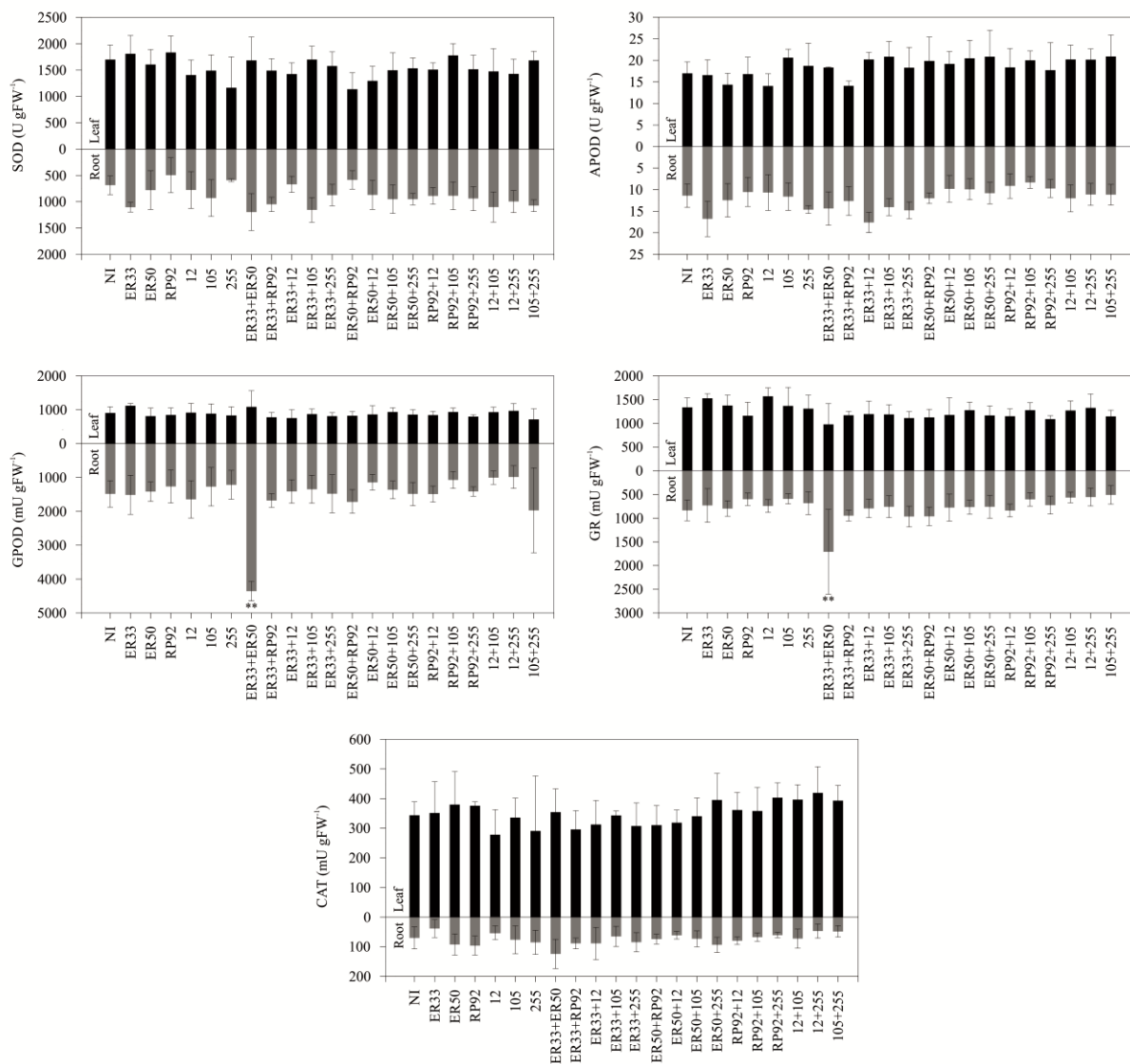


Table 1. PGP characteristics of bacterial strains used in the experiment.

Isolate	Sd[†]	P[‡]	IAA[§]	ACCD[#]	Organic acids^{††}	Reference
ER33 <i>Bradyrhizobium japonicum</i>	-	-	+	-	+	Becerra-Castro <i>et al.</i> (2011)
ER50 <i>Rhizobium pisi</i>	-	+	+	-	+	Becerra-Castro <i>et al.</i> (2011)
RP92 <i>Streptomyces costaricanus</i>	+	-	+	-	+	Becerra-Castro <i>et al.</i> (2011)
12 <i>Pseudomonas sp.</i>	+	+	+	+	+	Gkorezis (2014)
105 <i>Pantoea ananatis</i>	+	+	+	+	+	Gkorezis (2014)
255 <i>Bacillus licheniformis</i>	+	+	-	+	-	Gkorezis (2014)

[†] Siderophore producer. Determined following Schwyn and Neilands (1987).

[‡] Phosphate solubiliser. Determined following Nautiyal (1999).

[§] Indoleacetic acid (IAA) producer. Determined following a method modified from Sheng *et al.* (2008).

[#] 1-aminocyclopropane-1-carboxylate deaminase (ACCD) producer. Determined following Belimov *et al.* (2005).

^{††} Determined following Cunningham and Kniack (1992).

Table 2. Germination indices, seedling vigour indices and shoot and root specific lengths of *C. striatus* for the different inoculation treatments.

Inoculum	Germination index[†] (%)	SVI[‡] (cm)	SSL[§] (mm g⁻¹)	SRL[#] (mm g⁻¹)
CONTROL	65	11.5	239.7	326.3
ER33	38	10.7	379.0	446.2
ER50	80	24.9	360.8	525.1
RP92	80	25.1	323.8	431.2
12	70	25.8	296.5	547.8
105	56	19.2	229.1	605.6
255	81	26.4	273.8	632.8
ER33+ER50	38	11.9	430.9	450.0
ER33+RP92	38	9.3	324.5	409.4
ER33+12	56	19.6	456.5	504.6
ER33+105	56	20.4	370.1	638.4
ER33+255	56	14.4	376.4	484.3
ER50+RP92	56	18.5	300.3	397.2
ER50+12	70	22.8	405.2	544.9
ER50+105	75	19.8	287.2	452.2
ER50+255	85	24.1	216.2	540.8
RP92+12	65	21.0	339.8	537.9
RP92+105	70	20.7	261.0	660.0
RP92+255	85	27.7	215.6	600.0
12+105	80	26.8	249.2	438.9
12+255	70	24.9	325.9	602.2
105+255	70	22.5	282.0	403.6

[†] Germination index, total seeds emerged at the end of the experiment from total seeds sown (in percentage)

[‡] Seedling vigour index, (mean shoot length + mean root length) x germination percentage

[§] Specific shoot length, shoot length per unit weight

[#] Specific root length; root length per unit weight

Table 3. Germination indices, seedling vigour indices and shoot and root specific lengths of *L. luteus* for the different inoculation treatments.

Inoculum	Germination index[†] (%)	SVI[‡] (cm)	SSL[§] (mm g⁻¹)	SRL[#] (mm g⁻¹)
CONTROL	73	17.7	117.2	244.7
ER33	100	25.2	100.5	208.0
ER50	92	25.7	114.2	239.2
RP92	83	24.7	96.8	215.4
12	92	26.4	109.9	234.1
105	75	22.3	91.7	201.4
255	92	22.5	107.3	221.3
ER33+ER50	92	26.2	112.4	209.4
ER33+RP92	75	21.5	112.9	201.6
ER33+12	92	28.9	103.2	207.9
ER33+105	83	24.7	112.9	269.4
ER33+255	92	23.8	114.4	264.7
ER50+RP92	92	24.4	104.1	216.9
ER50+12	100	30.3	108.9	236.2
ER50+105	100	31.3	114.0	224.6
ER50+255	100	27.9	115.5	200.4
RP92+12	100	31.1	96.0	193.1
RP92+105	75	25.9	84.8	146.6
RP92+255	83	23.0	107.8	193.5
12+105	100	33.3	83.6	144.0
12+255	92	30.7	92.2	179.2
105+255	100	28.3	94.6	172.8

[†] Germination index, total seeds emerged at the end of the experiment from total seeds sown (in percentage)

[‡] Seedling vigour index, (mean shoot length + mean root length) x germination percentage

[§] Specific shoot length, shoot length per unit weight

[#] Specific root length; root length per unit weight

Commented [MB1]: Table 4 and 5 are now included in Appendix as Tables A.3 and A.4

Figure 1

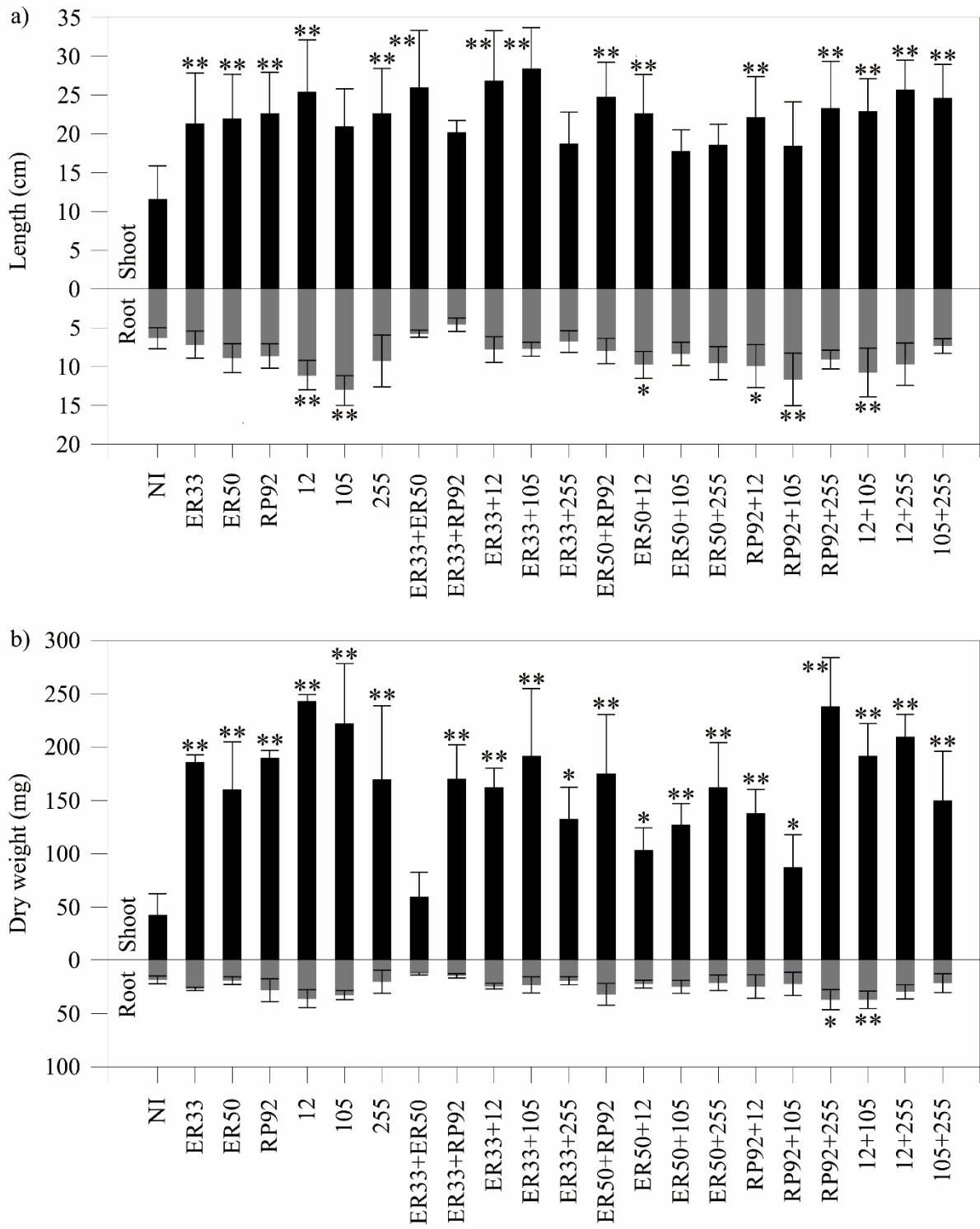


Figure 2

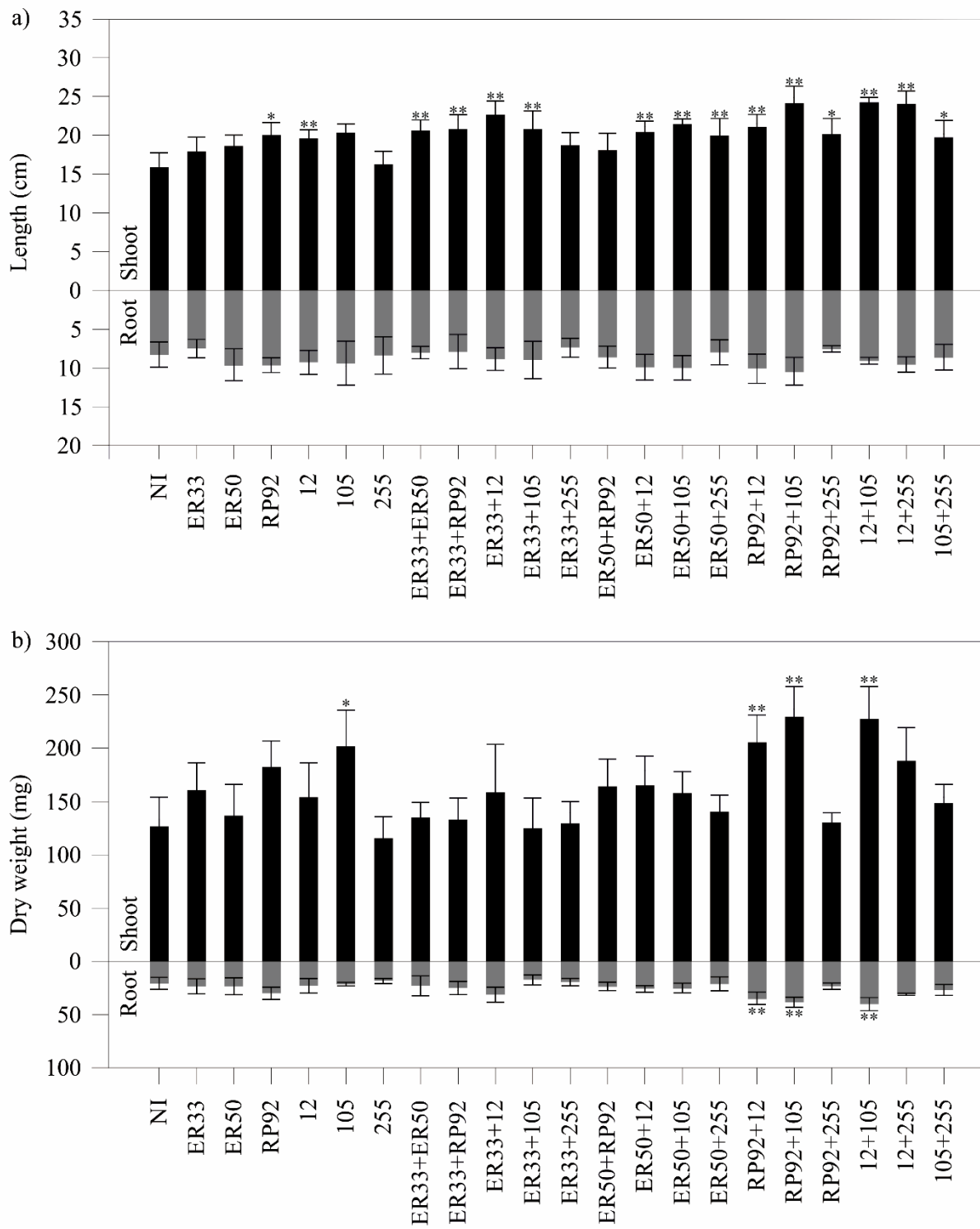


Figure 3

