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Balseiro-Romero, Maria; GKOREZIS, Panos; Kidd, Petra S.; Van Hamme, Jonathan; WEYENS, Nele; Monterroso, Carmen & VANGRONSVELD, Jaco (2017) Use of plant growth promoting bacterial strains to improve Cytisus striatus and Lupinus luteus development for potential application in phytoremediation. In: SCIENCE OF THE TOTAL ENVIRONMENT, 581, p. 676-688.

DOI: 10.1016/jscitotenv.2016.12.180
Handle: http://hdl.handle.net/1942/24126

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

María Balseiro-Romero [a,*], Panagiotis Gkorezis [b], Petra S. Kidd [c], Jonathan Van Hamme [d], Nele Weyens [b], Carmen Monterroso [a], Jaco Vangronsveld [b]

[a] Department of Soil Science and Agricultural Chemistry, University of Santiago de Compostela, Campus Vida, 15782 Santiago de Compostela, Spain

[b] Centre for Environmental Sciences, University of Hasselt, Agoralaan Building B, 3590 Diepenbeek, Belgium

[c] Instituto de Investigacións Agrobiolóxicas de Galicia (IIAG), Consejo Superior de Investigaciones Científicas (CSIC), 15780 Santiago de Compostela, Spain

[d] Department of Biological Sciences, Thompson Rivers University, Kamloops, Canada

[*] Corresponding author phone: +34-881816039; e-mail: maria.balseiro@usc.es

Abstract

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

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

Keywords

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

1. Introduction

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

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

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

nutrients through *e.g.* N_2 fixation and phosphate and iron solubilisation); organic contaminant degraders (lowering both contaminant phytotoxicity and evapotranspiration); phytostimulants (producing plant growth regulators and hormones, such as indoleacetic acid -IAA-, cytokinins and other auxins); stress controllers (by decreasing ethylene production through the synthesis of 1-aminocyclopropane-1-carboxylic acid deaminase -ACCD-); and as plant defence inducers against phytopathogens (by producing siderophores, antibiotics, or fungicidal compounds) (Becerra-Castro *et al.*, 2013a, 2013b; Compant *et al.*, 2010; Lugtenberg and Kamilova, 2009; McGuinness and Dowling, 2009; Weyens *et al.*, 2009a, 2009b; Zafar *et al.*, 2012).

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

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

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

2. Materials and methods

2.1. Bacterial strains

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

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

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

To prepare the bacterial inoculants, fresh cultures of the PGP strains were grown in 869 medium at 30 ºC (Mergeay *et al.*, 1985) for 1-2 days, harvested by centrifugation (3000 *g*, 15 min) and re-suspended in 10 mM $MgSO_4$ to an optical density of 1.0 at 660 nm (approximately 10^6 cells per mL). In addition to the individual strains (ER33, ER50, RP92, 12, 105 and 255), combinations of two strains were also tested: ER33+ER50, ER33+RP92, ER33+12, ER33+105, ER33+255, ER50+RP92, ER50+12, ER50+105, ER50+255, RP92+12, RP92+105, RP92+255, 12+105, 12+255 and 105+255. Pots were inoculated with 100 mL of a 1:10 dilution of the inoculants in half-strength Hoagland nutrient solution. For combinations, 5 mL of each bacterial suspension was added to the nutrient solution. Quadruplicate non-inoculated (NI) control pots were also prepared, and watered with 10 mM $MgSO_4$ diluted 1:10 with half-strength Hoagland solution. The first inoculation was carried out when seeding pots. The second inoculation was carried out when germination and early development of seedlings was observed in all pots. Throughout the experiment, pots were watered as required with 100 mL of half-strength Hoagland solution. Plants were grown under greenhouse conditions for 30 days for *L. luteus* and 60 days for *C. striatus*.

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

Samples of A and B horizon from an alumi-umbric Cambisol profile (HA and HB) collected in the surroundings of Santiago de Compostela (Galicia, NW Spain) were used in the experiment. Both samples were acid (pH in H_2O, 4.9 in HA and 5.1 in HB), showed a low cation exchange capacity (2.0 cmol(+) Kg^{-1} in HA and 1.2 cmol(+) Kg^{-1} in

HB) and sandy loam texture. The samples differed in their organic matter content (4.2 % in HA compared to < 0.5 % in soil HB) and their nutrient content, principally nitrogen (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 mg Kg^{-1} in HB). Soil samples were air-dried, sieved through a 2 mm mesh and mixed with sand at a 1:1 ratio (sand/soil), to improve the distribution of water in the pots, and the porosity of sieved soil samples.

Soil samples were spiked with diesel, purchased in a local gasoline station, at approximately 1.25 % (*w/w*). The spiked soils were kept in closed recipients and stabilised at 4 ºC for at least 2 weeks before preparing the pots. Polypropylene pots were filled, with approximately 300 g of spiked or uncontaminated soil. One-week-old lupine seedlings were transferred to each pot, and left to stabilise for 1 week before inoculation with the best inoculant in perlite experiment (RP92+105). Inoculants were prepared as described in perlite experiments using sterile distilled water for dilution, and they were added directly to the pots around the seedlings. Non-inoculated (NI) pots were also prepared, and watered with 10 mM $MgSO_4$ 1:10 diluted with distilled water. The first inoculation was carried out 1 week after preparing the pots with the seedlings, and a second inoculation was carried out 2 weeks after the first inoculation. Plants were watered with distilled water as required and were grown under greenhouse conditions for 30 days from the first inoculation. Six pot replicates were prepared for each inoculation treatment (NI, or PGP), each soil (HA or HB), and either diesel-contaminated or uncontaminated.

2.4. Germination and morphological plant responses

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

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

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

2.5. Determination of plant nutritive status

Plant nutritive status was determined in *C. striatus* and *L. luteus* leaves of 3 selected replicates of each inoculation treatment from perlite and soil experiments. Powdered leaves (approximately 0.1 g) were digested in 65% HNO_3:37% HCl mixture (2:1) to a maximum temperature of 130 ºC until achieving complete digestion of the tissues. Concentrations of macro- and micro-nutrients, including Ca, Cu, Fe, K, Mg, Mn, P, and Zn were determined by inductively coupled plasma coupled to optical emission spectrometry (ICP-OES) (Vista Pro; Varian Inc.).

2.6. Determination of antioxidant enzymatic activities

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

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

2.7. Statistical analysis

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

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

3. Results

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

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

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

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

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

For *L. luteus*, the inoculation of combined PGP strains generally lead to an increased plant growth, particularly for shoots (Appendix, Table A.2), in comparison to plants 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.*

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

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

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

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

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

3.3. Plant growth, nutritional status and stress-related enzyme activities in diesel-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

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

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

4. Discussion

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

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

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

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

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

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

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

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

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

On the other hand, an increase in antioxidant enzyme activities was observed when plants were submitted to oxidative stressful conditions, in this case, diesel contamination (Figure 3d, e and f). Abiotic stresses usually lead to the overproduction of reactive oxygen species in plant tissues. Among the antioxidant enzymes measured in these experiments, APOD, GPOD, GR and CAT are involved in the decomposition of H_2O_2 to H_2O or O_2, and SOD catalyses the dismutation of superoxide (O_2^{-2}) to H_2O_2 or O_2 (Gill and Tuteja, 2010). GPOD, GR and CAT were those enzymes presenting the most significant decrease in activity in the presence of PGP inoculations, indicating that the stress conditions held in soil experiments probably

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

5. Conclusions

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

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

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

Acknowledgements

This research was supported by ERDF Interreg Sudoe Program (PhytoSUDOE-SOE1/P5/E0189); by the Galician government (Agrupación Estratéxica CRETUS; AGRU2015/02); and by the University of Hasselt (BOF project 06G02 and Methusalem project 08M03VGRJ).

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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

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

Table A.3. Leaf nutrient concentrations of selected *C. striatus* replicates grown in perlite (indicated as the mean ± standard deviation; *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 ± 2.5	12.1 ± 0.9	4.8 ± 0.1	3.5 ± 0.5	315.8 ± 36.4	193.3 ± 18.5	41.8 ± 5.2	6.6 ± 0.3
ER33	37.7 ± 1.7	12.1 ± 4.2	4.6 ± 1.2	3.1 ± 0.7	309.9 ± 64.0	208.7 ± 70.4	64.0 ± 6.1**	13.3 ± 4.9**
ER50	35.4 ± 1.2	12.2 ± 1.6	4.6 ± 0.6	3.6 ± 0.3	305.7 ± 19.6	167.4 ± 23.4	46.6 ± 3.7	7.1 ± 0.5
RP92	38.2 ± 2.3	13.1 ± 1.2	5.2 ± 0.2	3.7 ± 0.4	210.2 ± 45.7	160.7 ± 24.7	49.4 ± 5.3	7.5 ± 0.8
12	37.4 ± 1.5	11.1 ± 1.4	4.6 ± 0.6	4.1 ± 0.2	301.1 ± 14.4	211.5 ± 25.3	57.2 ± 5.6	8.2 ± 0.8
105	39.0 ± 7.2	12.3 ± 0.8	5.1 ± 0.1	2.9 ± 0.2	371.2 ± 73.9	122.1 ± 30.3	49.6 ± 15.2	6.5 ± 1.2
255	38.7 ± 3.0	12.7 ± 0.9	4.7 ± 0.2	3.5 ± 0.1	262.8 ± 15.3	174.4 ± 41.2	47.5 ± 5.2	8.8 ± 1.0
ER33+ER92	37.2 ± 2.6	10.7 ± 0.7	4.3 ± 0.2	3.5 ± 0.2	285.4 ± 20.0	198.5 ± 31.5	56.4 ± 3.9	8.7 ± 0.6
ER33+12	40.9 ± 2.9	10.0 ± 0.9	4.4 ± 0.3	3.6 ± 0.3	262.0 ± 18.3	148.8 ± 23.6	69.4 ± 4.9**	10.4 ± 0.7
ER33+105	41.8 ± 2.5	10.7 ± 1.0	4.4 ± 0.6	4.5 ± 0.3	337.0 ± 23.6	182.1 ± 28.9	58.8 ± 4.1	9.2 ± 0.6
ER33+255	41.5 ± 2.9	9.3 ± 0.7	3.9 ± 0.3	3.8 ± 0.3	295.0 ± 20.7	150.1 ± 23.8	58.3 ± 4.1	11.3 ± 0.8**
ER50+RP92	37.9 ± 4.9	13.4 ± 0.1	5.3 ± 0.1	3.5 ± 0.1	272.1 ± 39.8	105.5 ± 23.3	60.9 ± 8.1	6.6 ± 0.5
ER50+12	35.3 ± 1.9	12.0 ± 0.5	4.5 ± 0.2	3.2 ± 0.3	221.1 ± 8.6	120.2 ± 11.9	52.3 ± 6.6	6.5 ± 0.4
ER50+105	38.0 ± 0.8	12.2 ± 1.3	4.5 ± 0.2	6.0 ± 0.6**	335.4 ± 28.8	177.9 ± 36.4	44.3 ± 8.2	7.2 ± 0.8
ER50+255	39.3 ± 2.1	10.5 ± 0.6	3.9 ± 0.4	4.3 ± 0.9	271.8 ± 14.0	133.8 ± 21.6	34.4 ± 3.3	7.5 ± 1.4
RP92+12	40.4 ± 1.3	10.6 ± 1.1	4.7 ± 0.1	4.6 ± 0.2	330.3 ± 9.0	170.0 ± 19.4	67.9 ± 9.5**	7.6 ± 0.6
RP92+105	39.1 ± 1.7	12.3 ± 1.9	4.8 ± 0.6	3.5 ± 0.6	279.3 ± 10.5	99.6 ± 10.7	35.8 ± 5.7	7.6 ± 1.8
RP92+255	40.1 ± 4.7	12.4 ± 1.6	4.6 ± 0.3	4.2 ± 0.3	296.1 ± 61.6	110.4 ± 5.9**	34.9 ± 2.2	7.1 ± 0.9
12+105	37.7 ± 3.4	12.4 ± 1.6	5.2 ± 0.5	3.7 ± 0.5	315.2 ± 27.1	105.2 ± 15.3**	39.7 ± 7.4	7.4 ± 1.2
12+255	40.6 ± 3.5	12.6 ± 2.7	4.7 ± 0.8	3.8 ± 0.5	283.3 ± 32.5	101.0 ± 13.1**	36.4 ± 3.3	6.2 ± 0.5
105+255	39.1 ± 2.1	12.4 ± 1.6	4.7 ± 0.8	4.0 ± 0.3	263.4 ± 13.2	95.2 ± 2.1**	51.5 ± 9.3	6.8 ± 0.9

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

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

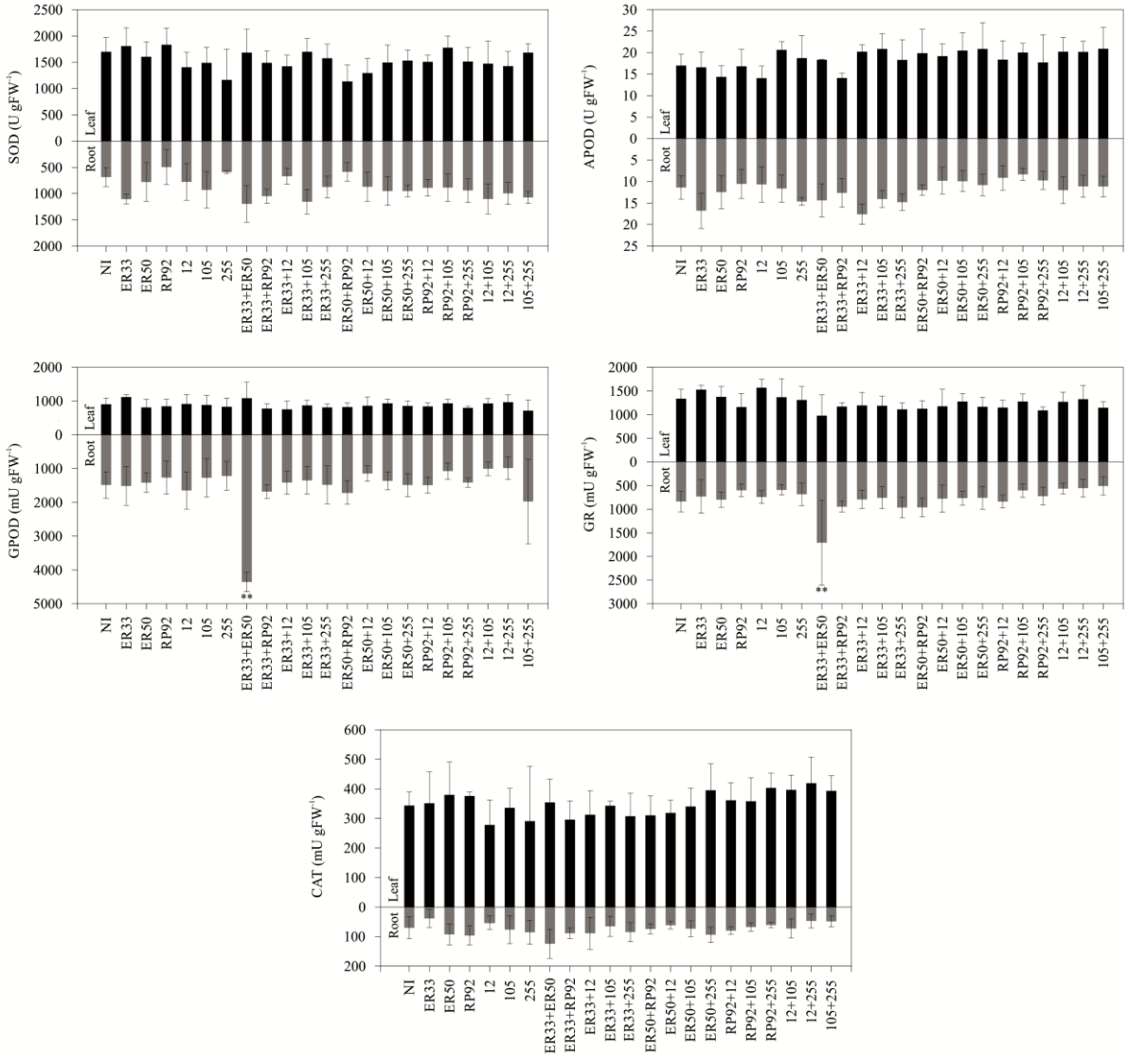


Table 1. PGP characteristics of bacterial strains used in the experiment.

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

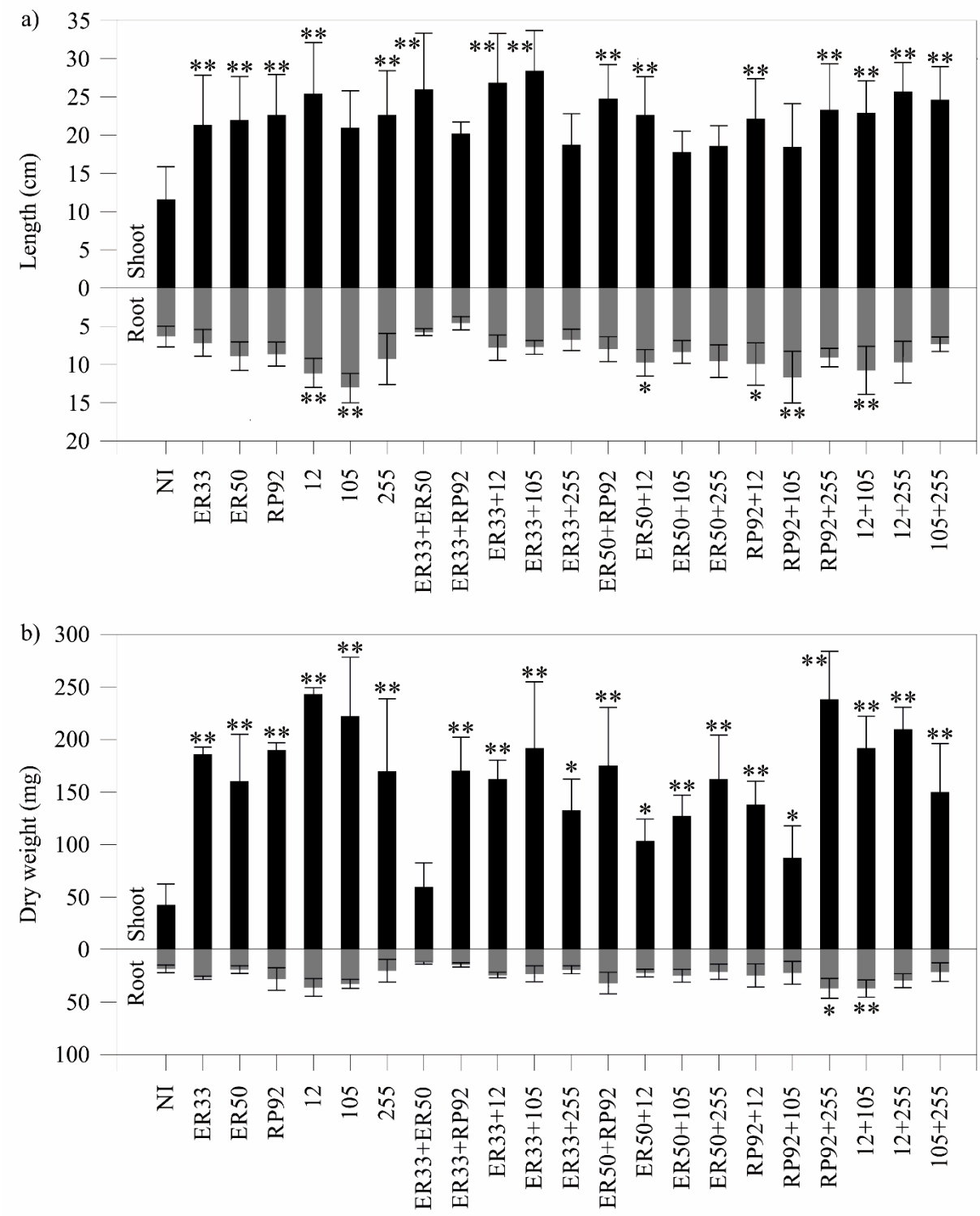

a)
Length (cm)
Shoot
Root
b)
Dry weight (mg)
Shoot
Root
NI
ER33
ER50
RP92
12
105
255
ER33+ER50
ER33+RP92
ER33+12
ER33+105
ER33+255
ER50+RP92
ER50+12
ER50+105
ER50+255
RP92+12
RP92+105
RP92+255
12+105
12+255
105+255

Figure 2

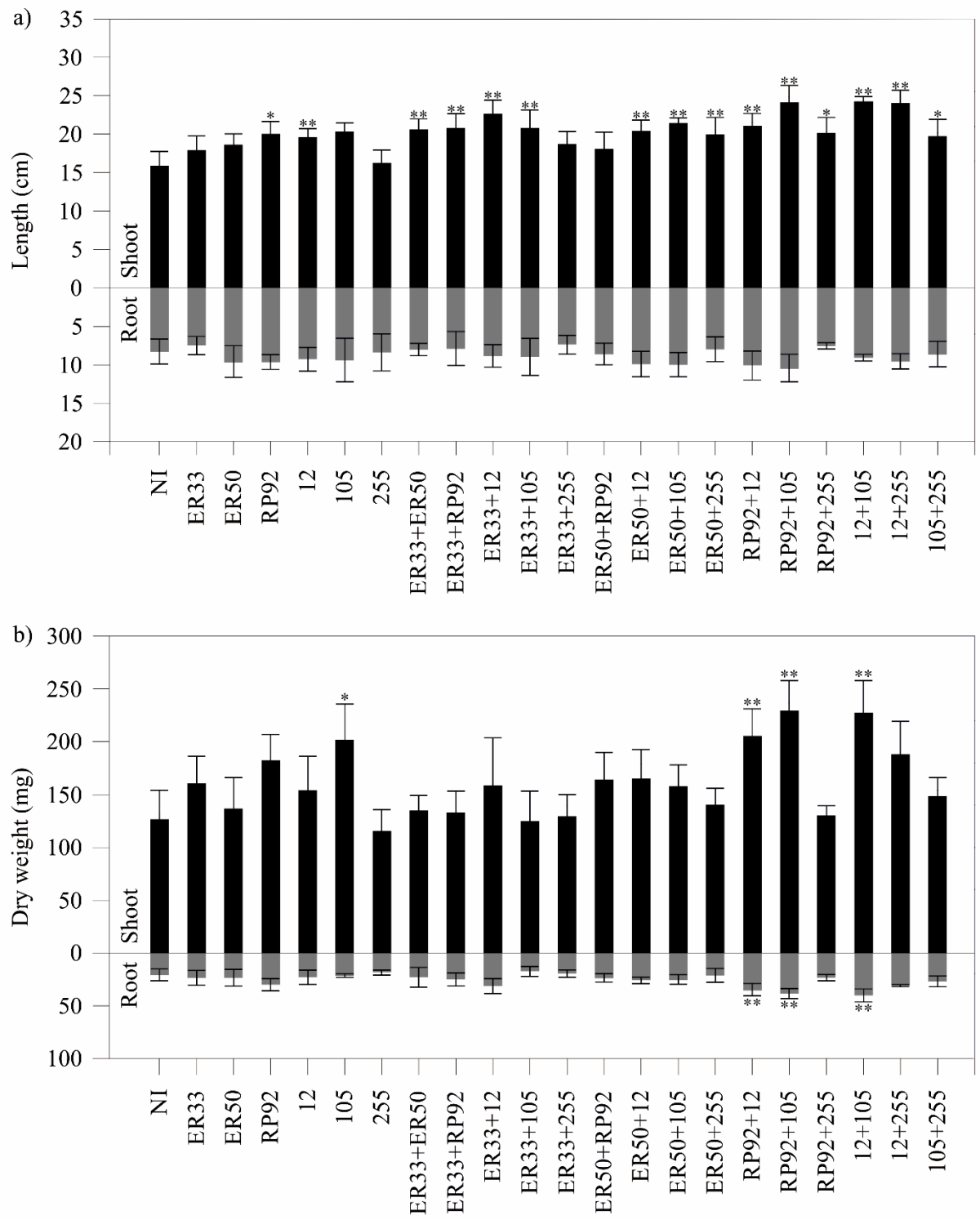
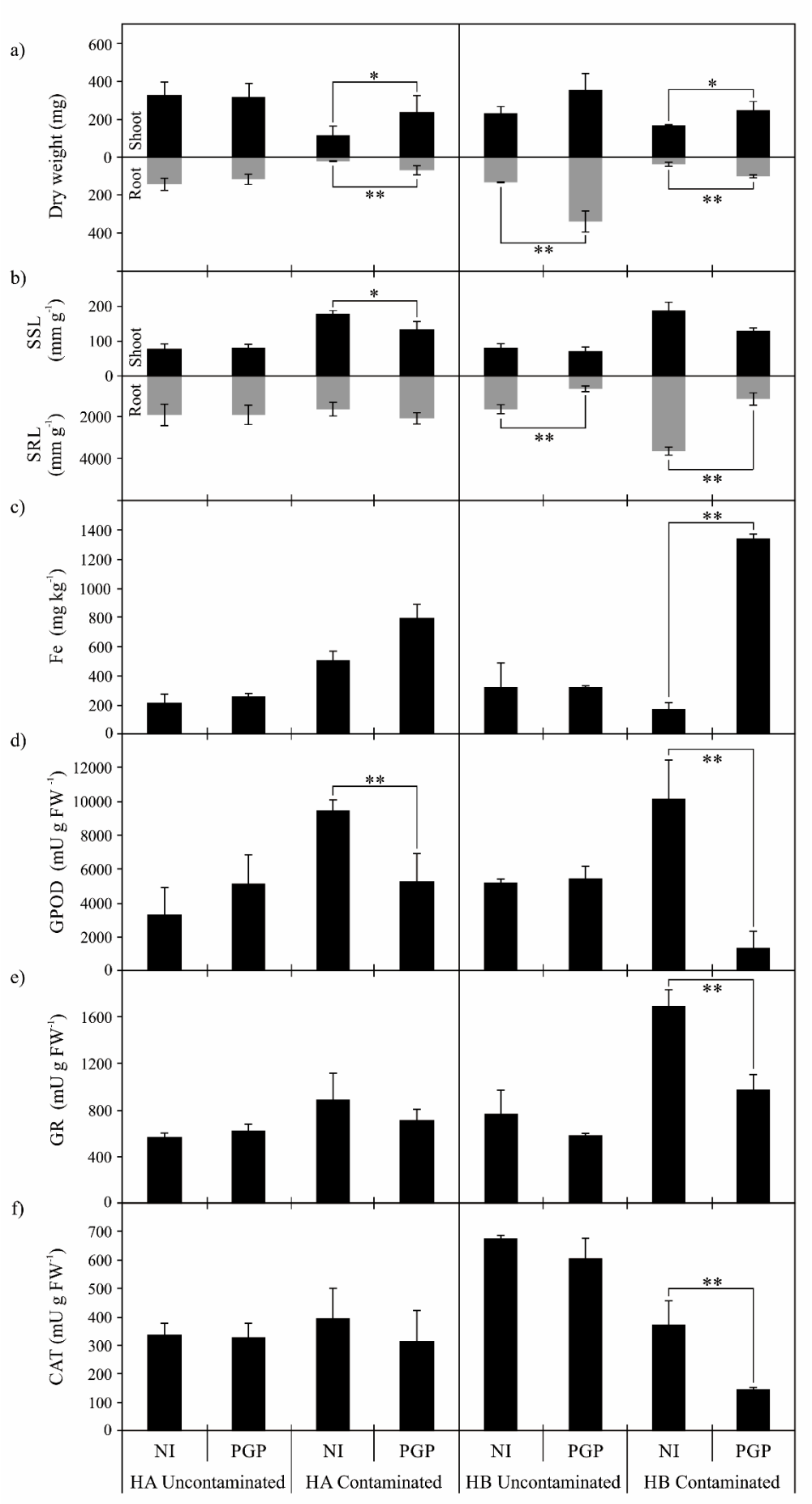


Figure 3


a)
Dry weight (mg)
Shoot
Root
b)
SSL (mm g-1)
SRL (mm g-1)
c)
Fe (mg kg-1)
d)
GPOD (mU g FW-1)
e)
GR (mU g FW-1)
f)
CAT (mU g FW-1)
NI
PGP
HA Uncontaminated
HA Contaminated
HB Uncontaminated
HB Contaminated