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Non-volatile signals and redox mechanisms are required for the responses of Arabidopsis roots to *Pseudomonas oryzihabitans*

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19 **Abstract**

20 Soil bacteria promote plant growth and protect against environmental stresses, but the
21 mechanisms involved remain poorly characterized, particularly when there is no direct contact
22 between the roots and bacteria. Here, we explored the effects of *Pseudomonas oryzihabitans*
23 PGP01 on the root system architecture (RSA) in *Arabidopsis thaliana* seedlings. Significant
24 increases in lateral root (LR) density were observed when seedlings were grown in the presence
25 of *P. oryzihabitans*, as well as an increased abundance of transcripts associated with altered
26 nutrient transport and phytohormone responses. However, no bacterial transcripts were detected
27 on the root samples by RNAseq analysis, demonstrating that the bacteria do not colonize the roots.
28 Separating the agar containing bacteria from the seedlings prevented the bacteria-induced changes
29 in RSA. Bacteria-induced changes in RSA were absent from mutants defective in ethylene
30 response factor (ERF109), glutathione synthesis (*pad2-1, cad2-1, and rax1-1*) and in strigolactone
31 synthesis (*max3-9* and *max4-1*) or signalling (max2-3). However, the *P. oryzihabitans*-induced
32 changes in RSA were similar in the low ascorbate mutants (*vtc2-1* and *vtc2-2*) to the wild-type
33 controls. Taken together, these results demonstrate the importance of non-volatile signals and
34 redox mechanisms in the root architecture regulation that occurs following long-distance
35 perception of *P. oryzihabitans*.

36 **Keywords:** Ascorbate, ethylene-responsive transcription factor 109, glutathione, plant growth-
37 promoting rhizobacteria, *Pseudomonas oryzihabitans*, reactive oxygen species, root system
38 architecture.

39

40 **Introduction**

41 Plants live in harmony with soil microbiome communities, with whom they are in
42 constant chemical communication. Soil bacteria and fungi can influence plant growth and
43 performance, particularly through effects exerted at the seedling stage (Zhang et al., 2022). Plant
44 growth-promoting rhizobacteria (PGPR) are comprised of different orders of bacterial species.
45 They not only modulate plant growth and root system architecture (RSA) but they also trigger
46 host immune responses (Poitout et al., 2017; Shekhar et al., 2019). Soil-borne plant pathogens
47 can be controlled by the status of the soil microbiome, in what is known as 'disease-suppressive
48 soil effects', which rely heavily on competition for plant nutrients between the different
49 microorganisms (Schlatter et al., 2017). PGPR also produce compounds such as cyclic
50 lipopeptides, polyketides, and bacteriocins that can have a direct negative efect on soil pathogens
51 (Andric et al., 2021).

52 PGPR modulate RSA by regulating the production of phytohormones such as gibberellic
53 acid (GA), auxin [indole acetic acid (IAA)], abscisic acid, and salicylic acid (SA) (Yuhashi et al.,
54 2000; Poitout et al., 2017; Niu et al., 2018). Some PGPR species such as *Pseudomonas*
55 *aeruginosa*, *Klebsiella* spp., *Rhizobium* spp., and *Mesorhizobium* spp. secrete IAA and so directly
56 regulate RSA (Ahemad and Kibret, 2014). Such mutualistic interactions enhance the capacity of
57 roots to take up nutrients (Glick, 2012). PGPR also improve the solubilization of minerals such
58 as phosphorus, zinc, and potassium, and increase iron sequestration by siderophore production.
59 Several Rhizobium species secrete nitrogenases that improve the fixation of nitrogen in anaerobic
60 soils, as well as releasing organic acids to increase phosphorus uptake (Yanni et al. 2001).

61 The control of lateral root (LR) development involves a network of phytohormones that
62 includes auxin and strigolactones (SLs; Sharma et al., 2020). SLs inhibit branching (Kapulnik et
63 al., 2011; Rasmussen et al., 2012) and interact with other phytohormones, particularly auxins, to
64 control overall root morphology (Agusti et al., 2011; Ruyter-Spira et al., 2011; De Jong et al.,
65 2014). SLs also participate in the regulation of plant stress responses (Foo and Reid, 2013; De
66 Jong et al., 2014; Quain et al., 2014; Cooper et al., 2018). Crucially, they are important regulators
67 of plant–microbe interactions. For example, the SLs present in root exudates attract arbuscular
68 mycorrhizal fungi and they also stimulate the nodulation process in legumes (López-Ráez et al.,
69 2017).

70 Reactive oxygen species (ROS) are important components of the phytohormone
71 signalling pathways that control RSA (Manzano et al., 2014; Kong et al., 2018; Yamada et al.,
72 2020; Eljebbawi et al., 2021). For example, the control of ROS accumulation is an important
73 factor in the emergence of LR primordia and it also influences the number of pre-branch sites
74 (Orman-Ligeza et al., 2016). Transcription factors such as ethylene response factor (ERF)109

75 (also called redox-responsive transcription factor 1) are crucial regulators of the responses of RSA
76 to environmental cues through modulation of jasmonate (JA), ethylene, and ROS signalling (Cai
77 et al., 2014; Matsuo et al., 2015). While the effects of PGPR on plant morphology have been
78 extensively studied, little attention has as yet been paid to the roles of ROS and redox signalling
79 in plant–bacteria interactions, particularly when there is no direct contact between the roots and
80 bacteria.

81 The non-fermenting yellow-pigmented, Gram-negative, lactose- and oxidase-negative
82 rod-shaped bacterium, *Pseudomonas oryzihabitans* PGP01 (also known as *Chromobacterium*
83 *typhifavum* and *Flavimonas oryzihabitans*), is an opportunistic human pathogen. This saprophytic
84 bacterium has been isolated from a range of human wound and soft tissue infections, leading to
85 septicaemia, prosthetic valve endocarditis, and peritonitis. It also lives freely in soils as well as
86 on medical and other equipment (Keikha et al., 2019). In plants, *P. oryzihabitans* has been linked
87 to panicle blight in rice (Hou et al., 2020) and to stem and leaf rot in muskmelon (Li et al., 2021).
88 However, other studies have shown that *P. oryzihabitans* PGP01 can exert a positive effect on
89 root growth (Belimov et al., 2015; Cantabella et al., 2020). The aims of the present study were
90 firstly to determine the efects of P. oryzihabitans on RSA in A. thaliana, secondly to characterize
91 how perception of *P. oryzihabitans* alters the root transcriptome profile, and thirdly to determine
92 whether ROS-related mechanisms were involved in the responses of RSA to perception of the
93 presence of the bacterium.

94 **Materials and methods**

95 **Plant material and growth conditions**

96 Seeds of the A. thaliana Columbia-0 (Col-0) wild-type (WT), the SL-deficient mutants
97 (max2-3, max3-9, and max4-1), the ascorbate-deficient (vtc2- 1 and vtc2-2) mutants, the
98 glutathione (GSH)-deficient (pad2-1, cad2-1, and rax1-1) mutants, a transformed line
99 overexpressing ERF109 (ov32), and a mutant line lacking a functional transcription factor
100 (erf109) were surface sterilized with 50% ethanol during 5 min, followed by three rinses with
101 sterile distilled water. Sterile seeds were cultured on 10 cm square Petri dishes containing half-
102 strength Murashige and Skoog medium (1/2 MS, pH 5.7), supplemented with 0.01% myo-
103 inositol, 0.05% MES, 1% sucrose, and 1% plant agar. Plates were stored at 4 °C in a dark room
104 for 2–4 d to synchronize germination. Seedlings were grown vertically in a controlled
105 environment cabinet at 22 °C with a 16 h photoperiod for 6 d.

106 **Inoculation of bacteria onto plates containing Arabidopsis seedlings**

107 The growth-promoting bacterium *P. oryzihabitans* strain PGP01 was obtained from the
108 IRTA Postharvest Plant Growth Promoter Microorganism (PGPM) Collection (Lleida, Catalonia,

109 Spain). Bacteria were grown in nutrient yeast dextrose agar (NYDA: nutrient broth, 8 g l^{-1}; yeast
110 extract, 5 g l^{-1}; dextrose, 10 g l^{-1}; and agar, 20 g l^{-1}) media for 48 h. Bacteria were applied to
111 plates containing 6-day-old Arabidopsis seedlings according to the method of Zamioudis et al.
112 (2013). Bacteria were collected in 10 mM MgSO4, and washed by centrifugation at 5000 *g* for
113 5 min. After resuspension in 10 mM MgSO4, the bacterial concentration was adjusted to 1×10^6
114 by measuring turbidity at 600 nm. Aliquots (50 µl) of bacteria were applied at a distance of 5 cm
115 from the root tip of 6-day-old Arabidopsis Col-0 seedlings. A concentration of 1×10^6 colony-
116 forming units (CFU) ml^{-1} was used to examine the effects of the presence of bacteria on root
117 architecture. For the experiments designed to determine whether volatile signals were involved in
118 root responses to *P. oryzihabitans*, 1 cm sections of the agar were removed from plates so as to
119 physically separate the agar containing seedlings from the agar containing bacteria, as illustrated
120 in Supplementary Fig. S1.

121 **Measurements of root architecture**

122 After 7 d of co-culture with bacteria, pictures of control and bacteria-treated plates were
123 taken, and different parameters such as primary root (PR) length, number of visible LRs, and
124 length of LRs were measured using ImageJ software. LR density was calculated by dividing the
125 number of LRs by the PR length for each root analysed, as described previously (Dubrovsky and
126 Forde, 2012). The LR density method provides a measure of the number of LRs per unit length
127 of PR and allows a comparison of LR formation in PRs with different elongation rates.

128 **RNAseq analysis**

129 The roots of Arabidopsis seedlings were harvested after 7 d growth in the absence or
130 presence of bacteria and immediately frozen in liquid nitrogen. Each biological replicate
131 contained roots from at least three plates, each of them with six seedlings. RNA was extracted
132 from frozen root samples using TRIreagent® (SigmaAldrich). RNA quality was checked by
133 Nanodrop, and RNA integrity was confirmed using a 0.8% agarose gel. RNAseq data were
134 analysed as described previously (De Simone et al., 2017).

135 **Statistical analysis**

136 All of the experiments were repeated at least three times. Data represent the mean ±SE of
137 the mean. Data from the experiments using Col-0 and bacteria were analysed by one-way
138 ANOVA and also by a pairwise t-test. A two-way ANOVA was also performed on the data from
139 studies on SL, ascorbate, and GSH mutants. Statistical signifcance was judged at the level $P <$
140 0.05, and Duncan's post-hoc test was used for the means separation when the differences were
141 significant using the IBM SPSS statistics 25 program.

142

143 ## Results

144 Previous studies have shown that the presence of *P. oryzihabitans* PGP01 induces
145 modifcations in *Pyrus* and *Prunus* rootstocks (Cantabella et al., 2020, 2021). The data presented
146 in Fig. 1 demonstrate that perception of *P. oryzihabitans* PGP01 also induces changes in RSA in
147 Arabidopsis. In these studies, *P. oryzihabitans* was placed on the same plates but not touching the
148 roots of the Arabidopsis seedlings (Fig. 1). Transcriptome profle comparisons of the roots of
149 seedlings grown on plates in the absence or presence of bacteria were measured 7 d after plating
150 (Fig. 2A; Supplementary Table S1; The RNAseq analysis revealed the absence of bacterial
151 transcripts from the roots of Arabidopsis plants (Supplementary Table S1 at JXB online). In total,
152 409 transcripts were increased in abundance in the roots grown in the presence of *P. oryzihabitans*
153 compared with those grown in the absence of bacteria, and 201 transcripts were less abundant
154 (Fig. 2B).

155 ### Root transcriptome responses to bacteria

156 A functional analysis of diferentially expressed genes (DEGs) in response to *P.*
157 *oryzihabitans* PGP01 (Fig. 3A) reveals Gene Ontology (GO) terms included are response to
158 absence of light (GO:0009646), xyloglucan metabolism (GO:0010411), cellular amino acid
159 metabolism (GO:00009063), carboxylic acid catabolism (GO:0046395), organic acid catabolism
160 (GO:0016054), and several terms related to hypoxia and decreased oxygen availability
161 (GO:0036294, GO:0070482, GO:0001666, GO:00771456, GO:0036293, and GO:0071453).
162 Other terms such as cellular response to chemical stimulus (GO:0051716, GO:00770887,
163 GO:0042221, and GO:0050896) and response to abiotic stress (GO:0033554, GO:0009628, and
164 GO:0006950) were present, as were terms related to the apoplast (GO:0048046) and
165 xyloglucan/xylotransferase activity (GO:0016762). The genes that were highly expressed in
166 response to *P. oryzihabitans* (Fig. 4A) include those encoding a guard cell enriched lipase called
167 GGL28 (GDSL-like), heat shock factor (HSF) A6b, high afnity K^+ transporter HAK5, and the
168 MYB transcription factor MYBL2 (Fig. 2A). Transcripts encoding ethylene response factor 2
169 (ERF2), ANACO29, and the related ERF/AP2 transcription factor family protein (RAP2.9) were
170 also increased in roots exposed to *P. oryzihabitans* (Fig. 4A). A small number of transcripts were
171 decreased in abundance in response to *P. oryzihabitans* (Fig. 4B). These include mRNAs
172 encoding UDP-glycosyltransferases (UGT91A1, UGT78D4, UGT84A1, and UGT78D1), as well
173 as transcripts encoding transparent testa (TT) 7, glutathione S-transferase (GST) 26, and
174 gibberellin 3-β-dioxygenase (GA3OX2; Fig. 4B). Further analysis of the most enriched GO terms
175 revealed that transcripts encoding some hormone-related proteins were more expressed in roots
176 exposed to *P. oryzihabitans* (Fig. 5C). These include ERF2, ERF107, DORMANCY/AUXIN
177 ASSOCIATED FAMILY PROTEIN 2 (DRM2), and KISS ME DEADLY 4 (KMD4) (Fig. 5A).

178 Several transcripts associated with hypoxia responses (Fig. 5B) and nutrient acquisition and
179 transport (Fig. 5C) were also increased in roots exposed to *P. oryzihabitans* PGP01.,

180 **Root responses to bacteria in lines with modifed expression of ERF109**

181 To analyse the role of ERF109 in root responses to *P. oryzihabitans*, RSA was compared
182 in WT Arabidopsis seedlings, a transformed line overexpressing ERF109 (*ov32*), and a mutant
183 line lacking a functional transcription factor (*erf109*; Fig. 6). The presence of bacteria increased
184 LR density only in the WT (Fig. 7). LR density was not changed by perception of the bacteria in
185 the *ov32* plants or the *erf109* mutants (Fig. 7).

186 **Root responses to bacteria in ascorbate-deficient mutants**

187 Two independent lines of ascorbate-deficient, vitamin C (*vtc2*) mutants were used to
188 analyse the role of this low molecular weight antioxidant buffer in root responses to *P.*
189 *oryzihabitans* (Fig. 8). LR densities were similar in all genotypes in the absence of bacteria (Fig.
190 8B). Moreover, the presence of *P. oryzihabitans* significantly increased LR density in all
191 genotypes (Fig. 8).

192 **Root responses to bacteria in glutathione-deficient mutants**

193 Three independent lines of GSH-defcient mutants [phytoalexin-defcient 2 (*pad2-1*), the
194 cadmium-sensitive 2 (*cad2-1*), and the regulator of APX2-1 (rax1-1)], which accumulate less
195 glutathione (~30%) than the WT (Schnaubelt et al., 2015) were used to analyse the role of the low
196 molecular weight antioxidant in root responses to *P. oryzihabitans*. The PRs of all genotypes were
197 not signifcantly changed by the presence of *P. oryzihabitans* (Fig. 9A). Moreover, the presence
198 of *P. oryzihabitans* significantly increased LR density in the WT roots but not in those of the
199 *cad2-1*, *pad2-1*, and *rax1-1* mutants (Fig. 9B).

200 **Root responses to bacteria in SL-defcient mutants**

201 The presence of bacteria increased LR density only in the WT. LR density was not
202 changed by perception of the bacteria in mutants defective in SL synthesis or SL signalling (Fig.
203 10B). LR density was decreased in the WT in the presence of the synthetic SL GR24 but increased
204 in the presence of GR24 and bacteria (Fig. 10D). In contrast, LR density was not significantly
205 increased in the presence of GR24 and bacteria in any of the SL mutant lines (Fig. 10D).
206 Moreover, bacteria-induced decreases in LR density were observed in the presence of GR24 in
207 the roots of the max 4-1 mutants (Fig. 10D).

208 **Root system architecture responses to P. oryzihabitans do not appear to be**
209 **triggered by volatile signals**

210 To test whether volatile signals were involved in the interactions between *P.*
211 *oryzihabitans* and Arabidopsis roots, 1 cm sections of the agar were removed from the plates.
212 Thus, the agar containing seedlings was physically separated from the agar containing bacteria
213 (Supplementary Fig. S1 at JXB online). PR lengths (Fig. 11A) and LR densities (Fig. 11B) were
214 similar in seedlings separated by a 1 cm gap in the agar (Control), separated from seedlings grown
215 in the presence of *P. oryzihabitans* (Plants and bacteria), or separated from agar on which *P.*
216 *oryzihabitans* was grown (Plants/bacteria).

217 **Discussion**

218 RSA undergoes fine tuning in response to cues from the soil microbiome (Hodge et al.,
219 2009; Ruiz Herrera et al., 2015). For example, the presence of PGPR modifes RSA and primes
220 plant defences against pathogens and herbivores through induced systemic resistance responses
221 (Pieterse et al., 2014; Rashid et al., 2017; Veselova et al., 2019). The data presented here
222 demonstrate that remodelling of the root transcriptome and RSA occurs upon perception of *P.*
223 *oryzihabitans*, without direct contact between the bacteria and the roots. However, root cap-
224 derived signals from the soil microbiome were found to be important in the regulation of RSA
225 (Crombez et al., 2020). The root responses to *P. oryzihabitans* reported here involve subtle
226 transcriptome remodelling and require SLs and redox signalling through GSH and ERF109, but
227 not ascorbate. The RSA response was lost once the agar containing the seedlings was physically
228 separated from that containing the bacteria, suggesting that volatile signals are not important
229 drivers of root remodelling.

230 Considerable genetic variation in the ability of Arabidopsis accessions to benefit from
231 root associations with *P. simiae* has been reported (Wintermans et al., 2016). *Pseudomonas*
232 species deploy a range of signals that modulate root development, including the secretion of
233 phytohormones such as IAA and other small molecules, and the release of volatile organic
234 compounds (VOCs; Zamioudis et al., 2013). For example, *P. fuorescens* SS101 promotes plant
235 growth through the release of 13-tetradecadien-1-ol, 2-butanone, and 2-methyl-n-1-tridecene
236 (Park et al., 2015) while *P. putida* and *P. fuorescens* produce cyclodipeptides such as cyclo(l-Pro-
237 l-Val), cyclo(l-Pro-lPhe), and cyclo(l-Pro-l-Tyr), which modulate the expression of auxin-
238 responsive genes in roots (Ortiz-Castro et al., 2020). *P. oryzihabitans* PGP01 is able to produce
239 IAA, when supplied with appropriate substrates (Cantabella et al., 2021). Like other Pseudomonas
240 strains, *P. oryzihabitans* PGP01 triggers auxin-dependent root developmental programmes
241 including abundant LR formation (Ortiz-Castro et al., 2011, 2020; Zamioudis et al., 2013). The
242 data presented here suggest that non-volatile signals are essential for the control root responses to
243 *P. oryzihabitans* PGP01.

244 While volatile signals do not appear to be important in the control of RSA by *P.*
245 *oryzihabitans* PGP01, the transcriptome signature reveals a role for ethylene signalling, which
246 regulates auxin transport and the frequency of LR formation (Xu et al., 2020). Transcripts
247 encoding ERF2 and the related ERF/AP2 transcription factor family protein (RAP2.9) were more
248 abundant in roots exposed to *P. oryzihabitans*. These transcription factors play crucial roles in
249 immunity, regulating multiple SA, JA, and ROS signalling pathways (Yang et al., 2021). Ethylene
250 also stimulates the expression of senescence-associated genes such as ANACO29 (Kim et al.,
251 2015), which is also highly expressed in roots exposed to *P. oryzihabitans*. Ethylene promotes
252 the homeostasis of Na^{+}/K^{+}, nutrients, and ROS to enhance plant tolerance to salinity (Tao et al.,
253 2015).

254 The perception of *P. oryzihabitans* caused changes to the root transcriptome even though
255 there was no direct colonization or physical contact between the organisms except through the
256 agar. The genes that were most highly expressed in response to *P. oryzihabitans* include mRNAs
257 encoding GDSL28 and HSFA6b. HSFA6b plays a pivotal role in pant responses to abscisic acid
258 and in thermotolerance (Huang et al., 2016) as well as ROS accumulation and the expression of
259 antioxidant genes (Wenjing et al., 2020). Other transcripts that were increased in abundance
260 include DRM2, which is important in plant defence responses (Roy et al., 2020), and KMD4,
261 which targets type-B ARR proteins for degradation and is required for cytokinin responses
262 through control of transcription factors (Kim et al., 2013).

263 Transcripts encoding enzymes and proteins involved in plant responses to hypoxia, such
264 as unknown proteins 26 and 32, were increased in roots exposed to *P. oryzihabitans* (Fig. 5B).
265 Severe oxygen depletion can suppress LR formation (Shukla et al., 2019; Pedersen et al., 2021).
266 The uptake of oxygen in respiration by the bacteria may contribute to some of the observed
267 metabolic adaptations in the transcriptome signature (Pucciariello and Perata, 2021). Other genes
268 that were highly expressed in the presence of bacteria encode proteins that are involved in nutrient
269 acquisition and transport. For example, the levels of transcripts encoding several root hair-specific
270 proteins including RHS7, RH17, and RH18, and a number of transporters such as the sucrose
271 transporter SUC2, the POLYOL/ monosaccharide transporter PMT6, the boron transporter 1
272 BOR1, the aluminium-activated malate transporter ALMT1, and the zinc transporter 3 precursor
273 ZIP3 were higher in roots in the presence of *P. oryzihabitans*. Similarly, the levels of transcripts
274 encoding HAK5 that is required for plant growth and K+ acquisition particularly under saline
275 conditions (Nieves-Cordones et al., 2010) were significantly higher in the roots exposed to *P.*
276 *oryzihabitans*, as were transcripts encoding the MYB transcription factor MYBL2, which is a key
277 negative regulator of anthocyanin biosynthesis in response to changes in sucrose availability
278 (Dubos et al., 2008).

279 The expression of genes encoding UDP-glycosyltransferases UGT91A1, UGT78D4, 280 UGT84A1, and UGT78D1, as well as those encoding transparent testa TT7 and GST26, which 281 play an important role in regulating the availability of secondary metabolites, was lower in 282 bacteria-exposed roots. Similarly, transcripts encoding GA3OX, which catalyses the conversion 283 of precursor GAs to their bioactive forms during vegetative growth (Mitchum et al. 2006), were 284 significantly lower in the roots exposed to *P. oryzihabitans*.

285 Targeted ROS production is crucial to the hormone-dependent regulation of RSA 286 (Eljebbawi et al., 2021). For example, hydrogen peroxide is required for brassinosteroid-mediated 287 cell division in the root quiescent centre and for seedling development (Tian et al., 2018). The 288 data presented here provide evidence that ROS signalling is important in RSA responses to *P.* 289 *oryzihabitans*. For example, while levels of ERF109 transcripts were not changed in the roots 290 exposed to bacteria, the *P. oryzihabitans*-induced changes in RSA were absent from the erf109 291 mutants. ERF109 is involved in the amplifcation of ROS signalling and systemic transmission of 292 ROS signals in response to biotic and abiotic stresses (Bahieldin et al., 2016), as well as in the 293 JA-dependent regulation of RSA (Xu et al., 2020).

294 The *P. oryzihabitans*-induced changes in RSA were similar in the *vtc* mutants that are 295 defficient in the low molecular weight antioxidant ascorbate (Foyer et al. 2020) and the WT 296 plants. This finding demonstrates that changes in total antioxidant capacity alone are not 297 important in plant–bacteria interaction. The vtc mutants have modified phytohormone signalling 298 pathways (Kerchev et al., 2013; Caviglia et al., 2018) but these changes do not influence the 299 responses of RSA to *P. oryzihabitans*. In contrast, the *P. oryzihabitans*-induced changes in LR 300 density were absent from the *cad2-1*, *pad2-1*, and *rax1-1* mutants, indicating that GSH-mediated 301 redox regulation is important in root responses to the bacterium. GSH is essential for root 302 development (Passaia et al., 2014, Ehrary et al., 2020). The GSH-deficient rootmeristemless1 303 (*rml1*) mutant is unable to develop roots because of impaired root apical meristem functions 304 (Vernoux et al., 2000). The glutathione reductase-deficient *miao* mutants also show poor root 305 growth (Yu et al., 2013). Mutants lacking glutathione peroxidases have modified root phenotypes 306 (Passaia et al., 2014). Crucially, glutaredoxins (GRXs) such as GRXS8 and GRXS17 are involved 307 in the regulation of RSA (Ehrary et al., 2020; Martins et al., 2020). GSH enhances the sensitivity 308 of roots to auxin (Pasternak et al., 2020) and is required for the conversion of indole butyric acid 309 (IBA) to IAA (Trujillo-Hernandez et al., 2020). The data presented here demonstrate that the root 310 GSH pool is essential for the facilitation of bacteria-driven changes in RSA.

311 The GSH pool is involved in the SL-dependent control of RSA through the MAX2 protein 312 (Marquez-Garcia et al., 2014). SLs are important in rhizosphere communication (Bouwmeester 313 et al., 2007) and are required for plant responses to nutrient defciencies (Shindo et al., 2020).

314 They are required for the initiation of symbiotic interactions with arbuscular mycorrhizal fungi,
315 when nutrients are limiting (Akiyama et al., 2005; Aliche et al., 2020). The bacteria-induced
316 increases in LR density were absent from mutants that are defective in SL synthesis or signalling,
317 demonstrating the essential role of these phytohormones in plant–bacteria interactions.

318 In summary, evidence is presented showing that the root system of A. thaliana seedlings
319 is changed in the presence of *P. oryzihabitans* PGP01 in a manner that suggests that this bacterium
320 functions as a PGPR. Moreover, the observed changes in the root transcript profle are due to
321 increases in mRNAs encoding proteins involved in mineral nutrition and phytohormone
322 signalling but not defence or immune responses. Crucially, the data show that the long-distance
323 perception of *P. oryzihabitans* PGP01 is sufficient to modulate RSA. ERF109, SLs, and GSH are
324 key components required for the bacteria-mediated control of RSA. These findings demonstrate
325 that SL and redox signalling are important factors in root responses to *P. oryzihabitans*, but
326 changes in antioxidant capacity alone do not influence this process.

327 **Supplementary data**

328 The following supplementary data are available at JXB online. Fig. S1. Representative
329 images of wild-type Arabidopsis seedlings that were separated by a 1 cm gap in the agar, by a
330 1 cm gap from seedlings growing in the presence of P. oryzihabitans, or separated from agar on
331 which P. oryzihabitans was grown. Table S1. Bacteria-induced changes in diferentially expressed
332 genes in Arabidopsis thaliana roots.

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338 **Author contributions**

339 DC, CHF, RD-S, and NT: project development; DC, CHF, and BK: design and
340 performing experiments and data analysis; DC and BK: preparing the figures; DC and CHF:
341 writing; all other authors read and contributed to previous versions and approved the final version.

342 **Confict of interest**

343 The authors have no conflicts to declare.

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352 data from this article are available at the BioProject database
353 (www.ncbi.nlm.nih.gov/bioproject/868724) under the accession number PRJNA868724.

354

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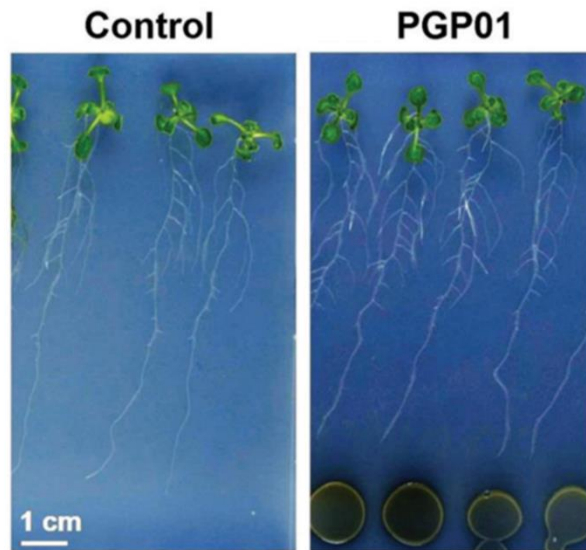
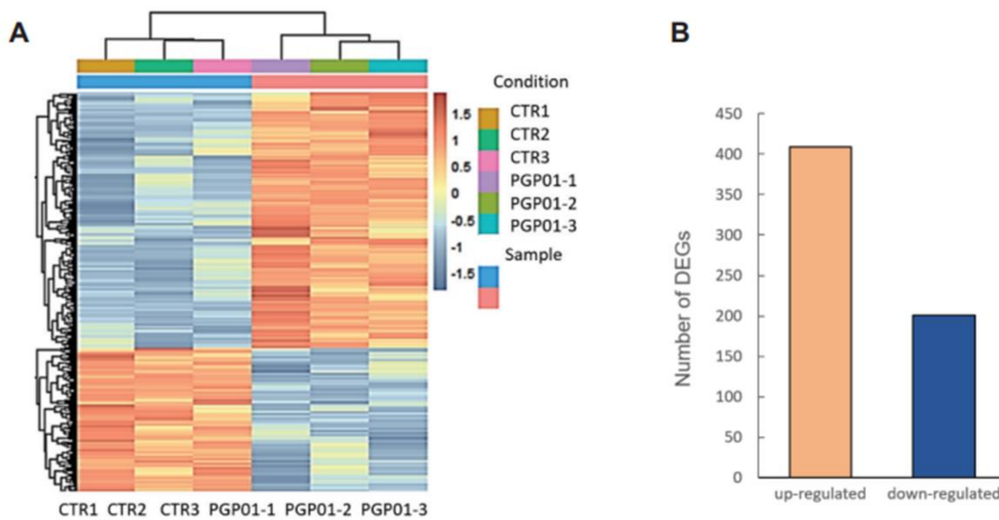
580

581 **FIGURES**



582

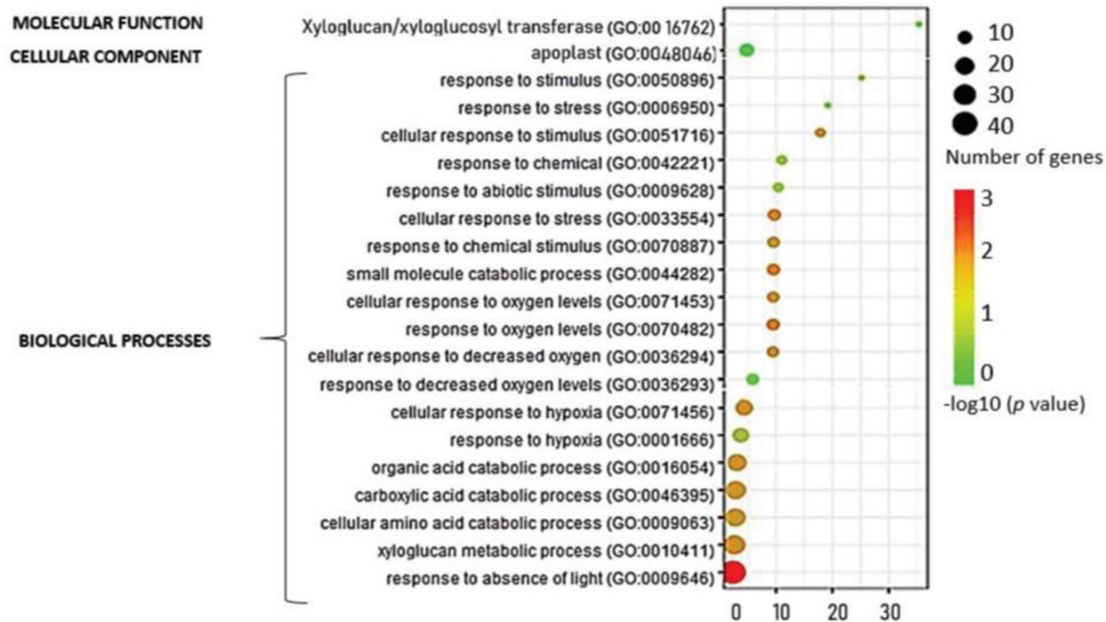
583 **Fig. 1.** Representative images of wild-type Arabidopsis seedlings that had been grown for 6 d in the absence
584 of P. oryzihabitans and then for a further 7 d in either the absence (control) or the presence of bacteria
585 (PGP01).



586

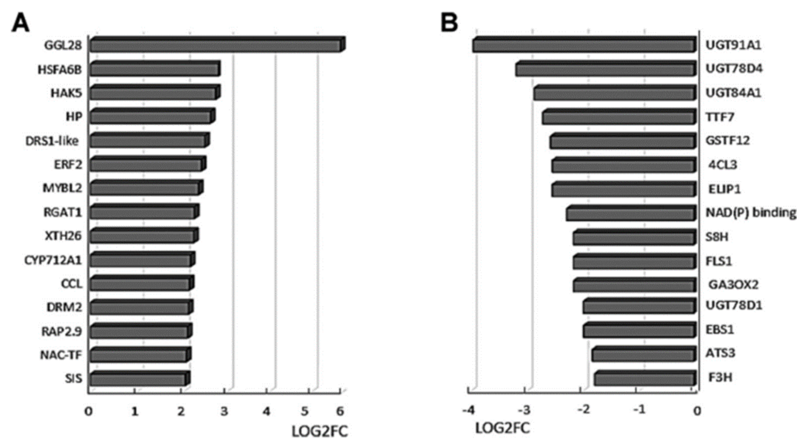
587 **Fig. 2.** Differentially expressed transcripts in the roots of the wild type (A) and number of transcripts
588 signifcantly increased and decreased (B). Seedlings had been grown for 6 d in the absence of *P.*
589 *oryzihabitans* and then for a further 7 d in either the absence or presence of bacteria.

590



591

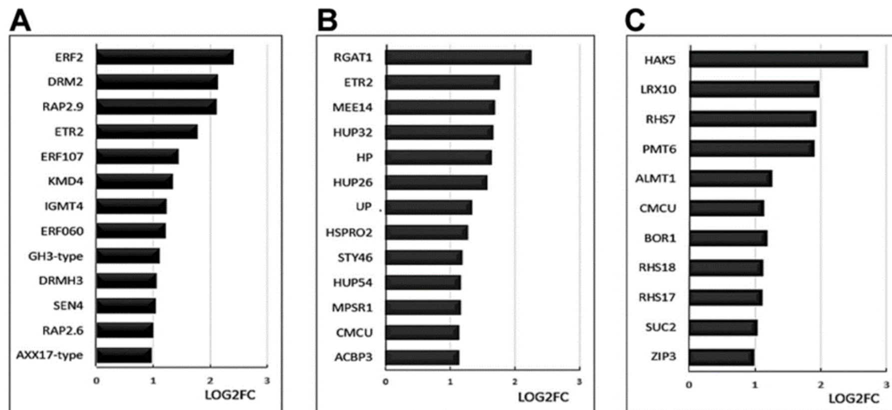
592 **Fig. 3.** Gene Ontology (GO) analysis showing the biological processes involved in root responses to *P.*
593 *oryzihabitans.*



594

595 **Fig. 4.** Transcripts that were most increased (A) or decreased in abundance (B) in response to the presence
596 of *P. oryzihabitans* PGP01. (A) AT5G45950 (GGL28, GDSL-motif esterase/acetyltransferase/lipase);
597 AT3G22830 (HSFA6B, heat stress transcription factor A-6b), AT4G13420 (HAK5, potassium channel);
598 AT2G39980 (HP, hypotetical unknown protein); AT5G28610 (DRS1-like, ATP-dependent RNA helicase);
599 AT5G47220 (ERF2, ethylene response factor 2); AT1G71030 (MYBL2, myb family transcription factor);
600 AT1G19530 (RGAT1, RGA target 1); AT4G28850 (XTH26, xyloglucan endotransglucosylase 26);
601 AT2G42250 (CYP12A1, cytochrome P450); AT3G26740 (CCL, circadian control of mRNA stability);
602 AT2G33830 (DRM2, dormancy/auxin associated protein 2); AT4G06746 (RAP2.9, ERF/AP2 transcription
603 factor family); AT1G69490 (NAC-TF, transcription factor); AT5G02020 (SIS, salt-induced serine rich).
604 (B) AT2G22590 (UGT91A1, UDP-glucosyltransferase 91A1); AT5G17040 (UGT78D4, UDP-

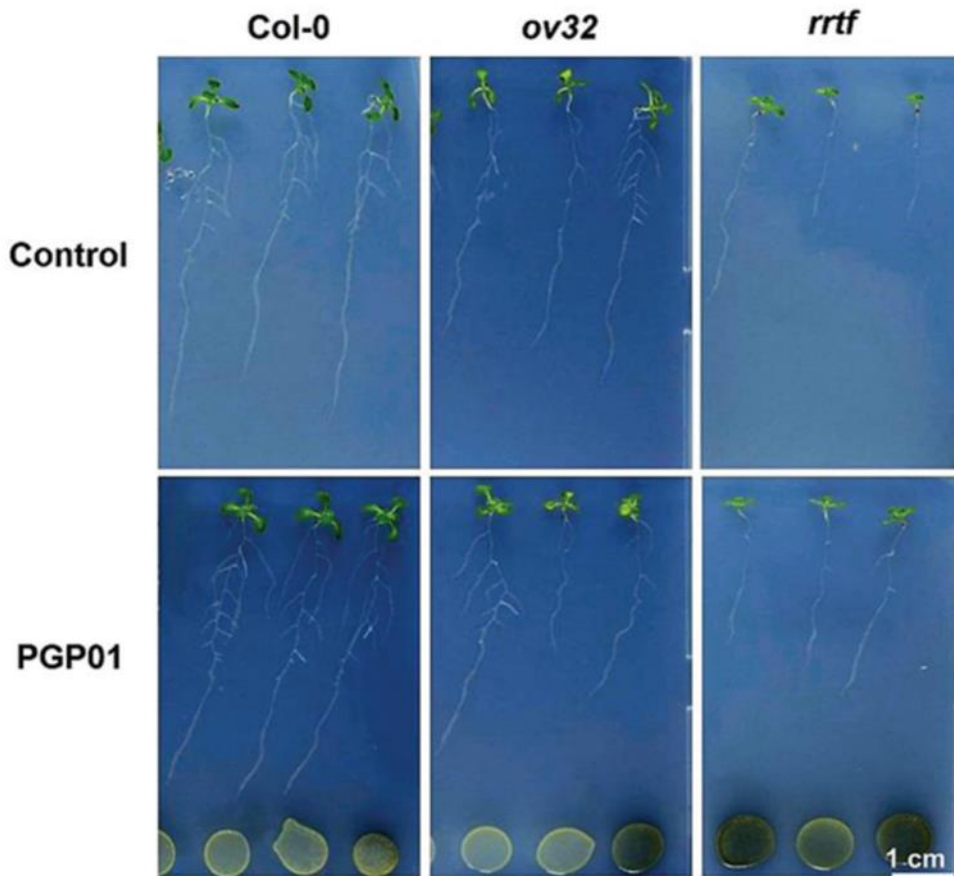
605 glucosyltransferase 78D4); AT4G15480 (UGT84A1, UDP-glucosyltransferase 84A19); AT5G07990
606 (TT7, favonoid 3′ hydroxylase activity); AT5G17220 (GSTF12, glutathione S-transferase 12); AT1G65060
607 (4CL, 4-coumarate:CoA ligase); AT3G22840 (ELIP1, early light inducible 1); AT2G23910 [NAD(P)
608 binding, Rossmannfold superfamily]; AT3G12900 (S8H, scopoletin 8 hydrolase); AT5G08640 (FLS1,
609 favonol synthase 1); AT1G80340 (GA3OX2, gibberellin 3-oxidase 2); AT1G30530 (UGT78D1, UDP-
610 glucosyl transferase 78D1); AT4G17680 (EBS1, exclusivly sensitive to bicarbonate 1); AT5G62210
611 (ATS3, embryo-specifc protein 3); AT3G51240 (F3H, favanone 3-hydroxylase).



612

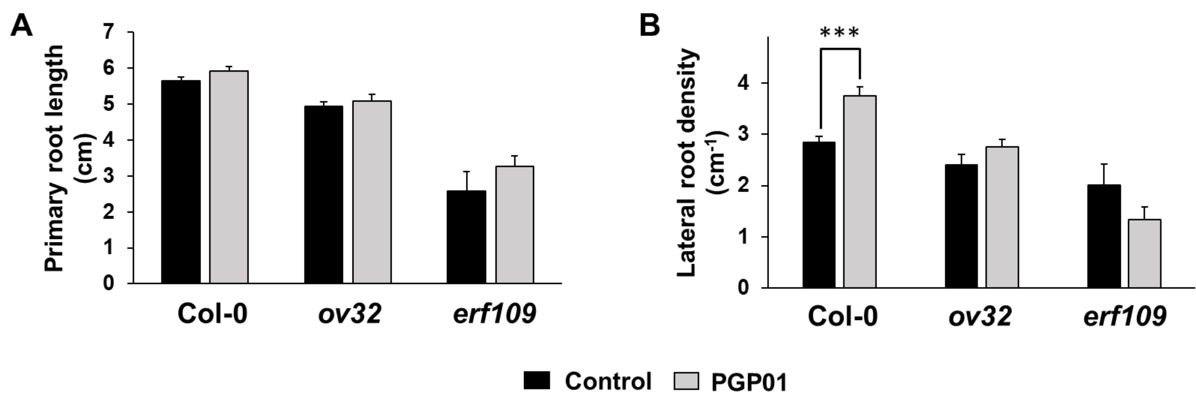
613 **Fig. 5.** Subsets of transcripts involved in (A) phytohormone signalling, (B) hypoxia, and (C) nutrient status
614 that were increased in abundance in the presence of *P. oryzihabitans* PGP01. (A) Responses to hormones:
615 AT5G47220 (ERF2, ethylene-responsive transcription factor 2); AT2G33830 (DRM2, dormancy/auxin
616 associated protein 2); AT4G06746 (RAP2.9, ethylene responsive RAP2.9); AT3G23150 (ETR2, ethylene
617 response 2); AT5G61590 (ETR107, ethylene responsive transcription factor 107); AT3G59940 (KMD4,
618 kiss me deadly 4, controls cytokinin signalling); AT1G21130 (IGMT4, indole glucosinolate-O-
619 methyltransferase 4); AT4G39780 (ERF060, ethylene responsive factor 1); AT1G48690 (GH3-type, auxin
620 responsive GH3-type protein); AT1G56220 (DRMH3, dormancy-associated protein homologue 3),
621 AT4G30270 (SEN4, senescence 4, brassinosteroid response); AT1G43160 (RAP2.6, ethylene responsive
622 factor RAP2.6). (B) Responses to hypoxia: AT1G19530 (RGAT1, RGA Target 1); AT3G23150 (ETR2;
623 ethylene response 2); AT2G15890 (MEE14, maternal effect embryo arrest 14); AT1G33055 (HUP32,
624 hypoxia response protein 32); AT5G65207 (HP, hypothetical protein responsive to hypoxia); AT3G10020
625 (HUP26, hypoxia response protein 26); AT1G10140 (UP, uncharacterized protein responsive to hypoxia);
626 AT2G40000 (HSPRO2, orthologue sugar beet HSPRO2); AT4G38470 (STY46, serine/threonine kinase);
627 AT4G27450 (HUP54, hypoxia response protein 54); AT1G26800 (MPSR1, misfolded protein sensing ring
628 E3 ligase); AT5G66650 (CMCU, chloroplast-localized mitochondrial calcium uniporter); AT4G24230
629 (ACBP3, acyl-CoA-binding domain 3). (C) Transport facilitation and root growth: AT4G13420 (HAK5,
630 potassium channel transporter 5); AT1G54970 (RHS7, root hair specifc 7, ethylene regulated); AT4G36670
631 (PMT6, POLYOL/monosaccharide transporter 6); AT5G17860 (CCX4, cation/calcium exchanger);
632 AT1G08430 (ALMT1, aluminium activated malate transporter); AT5G66650 (CMCU, chloroplast-
633 localized mitochondrial calcium uniporter 3); AT2G47160 (BOR1, boron transporter 1); AT5G22410

634 (RHS18, root hair specifc 18); AT4G38390 (RHS17, root hair specifc 17); AT1G22710 (SUC2, sucrose
635 protein symporter 2); AT2G32270 (ZIP3, zinc transporter 3 precursor).



636

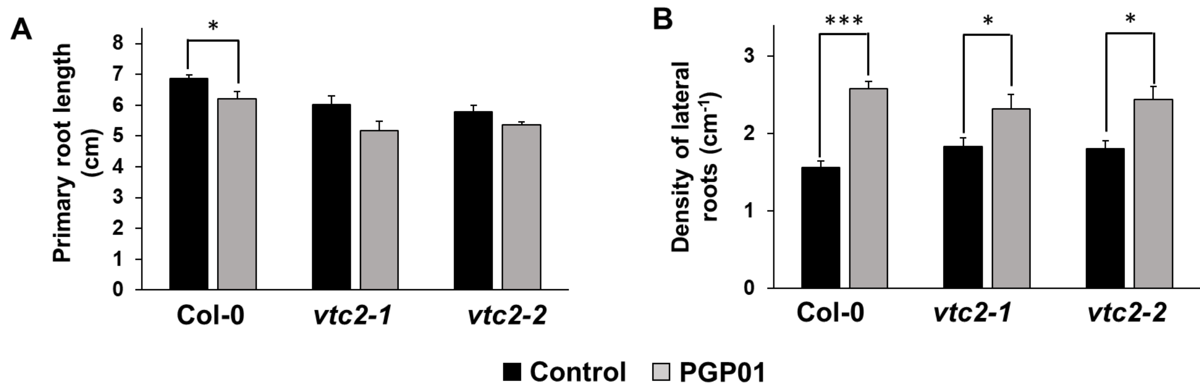
637 **Fig. 6.** Representative images of wild-type Arabidopsis seedlings, seedlings overexpressing ERF109
638 (ov32), and erf109 mutants. Seedlings had been grown for 6 d in the absence of *P. oryzihabitans* and then
639 for a further 7 d in either the absence (control) or the presence of bacteria (PGP01).



640

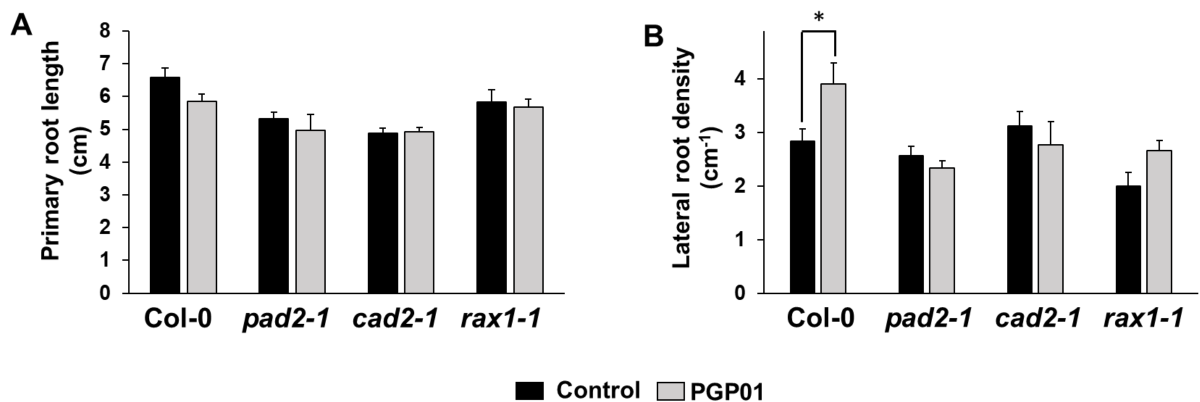
641 **Fig. 7**. The effect of the presence of *P. oryzihabitans* PGP01 on primary root length (A) and lateral root
642 density (B) in wild-type A. thaliana, a transgenic line overexpressing redox-responsive transcription factor

643 1 (ov32), and a erf109 mutant line. Samples of bacterial inoculum was placed 5 cm away for the tips of the
644 primary roots of 6-day-old seedlings that had been grown on agar plates. Root parameters were measured
645 7 d after inoculation. Data show the mean ±SE of three independent biological samples. Asterisks indicate
646 signifcant differences according to t-test (P<0.05).



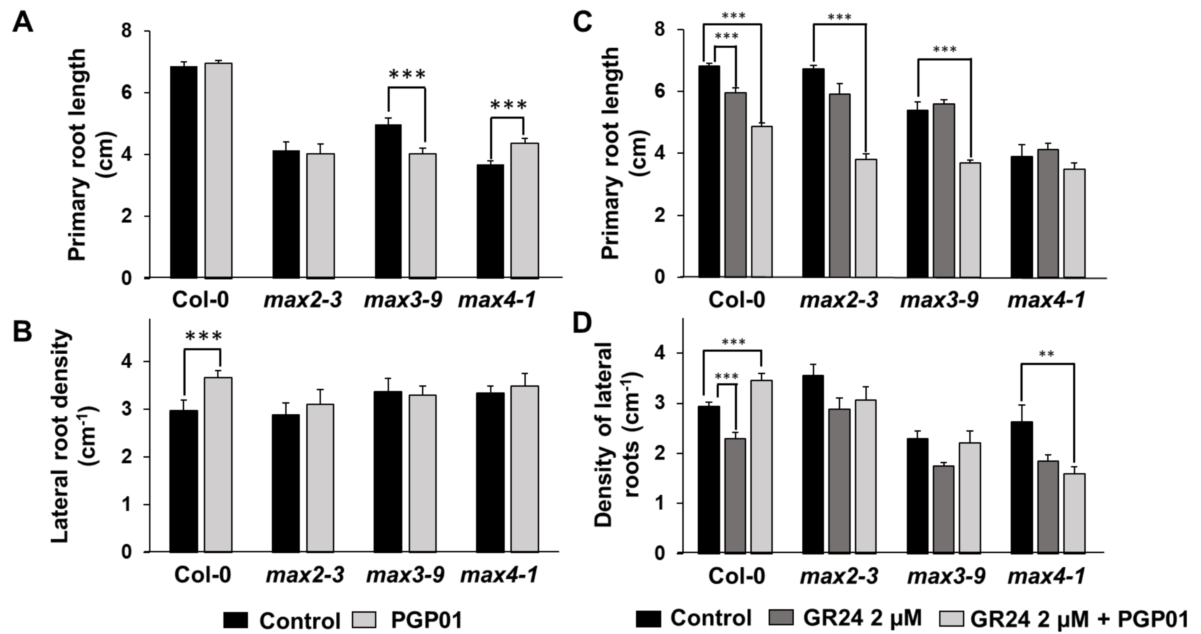
647

648 **Fig. 8.** The effect of the presence of *P. oryzihabitans* PGP01 on primary root length (A) and lateral root
649 density (B) in wild-type *A. thaliana* and mutants that are defective in ascorbate (vtc2-1 and vtc2-2). Samples
650 of bacterial inoculum were placed 5 cm away for the tips of the primary roots of 6-day-old seedlings that
651 had been grown on agar plates. Root parameters were measured 7 d after inoculation. Data show the mean
652 ±SE of three independent biological samples. Asterisks indicate signifcant differences according to t-test
653 (P<0.05).



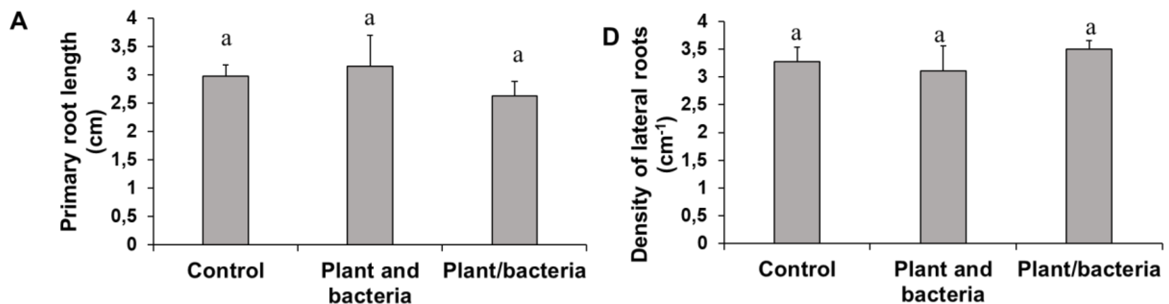
654

655 **Fig. 9.** The effect of the presence of *P. oryzihabitans* PGP01 on primary root length (A) and lateral root
656 density (B) in wild-type *A. thaliana* and mutants that are defective in glutathione (cad2-1, pad2-1, and rax1-
657 1). Samples of bacterial inoculum were placed 5 cm away for the tips of the primary roots of 6-day-old
658 seedlings that had been grown on agar plates. Root parameters were measured 7 d after inoculation. Data
659 show the mean ±SE of three independent biological samples. Asterisks indicate signifcant differences
660 according to t-test (P<0.05).



661

662 **Fig. 10.** The effect of the presence of *P. oryzihabitans* PGP01 on primary root length (A) and lateral root
663 density (B) in wild-type *A. thaliana* and mutants that are defective in SL synthesis (max3-9 and max4-1)
664 and signalling (max2-3). Samples of bacterial inoculum were placed 5 cm away for the tips of the primary
665 roots of 6-day-old seedlings that had been grown on agar plates. Root parameters were measured 7 d after
666 inoculation. Data show the mean ±SE of three independent biological samples. Asterisks indicate signifcant
667 differences according to t-test ($P<0.05$).



668

669 **Fig. 11.** The effect of removal of the agar between *P. oryzihabitans* PGP01 and Arabidopsis seedings.
670 Arabidopsis seedlings were either separated by a 1 cm gap in the agar (Control), separated by a 1 cm gap
671 from seedlings grown in the presence of *P. oryzihabitans* (Plants and PGP01), or separated from agar on
672 which *P. oryzihabitans* was grown (Plants/PGP01). Seedlings were grown for 6 d in the absence of *P.*
673 *oryzihabitans* and then for a further 7 d in either the absence or presence of bacteria. Primary root length
674 (A) and lateral root density (B).

675