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2 ***Rhizobium leguminosarum* symbiovar *viciae* strains are natural wheat endophytes and**
3 **can stimulate root development and colonization by arbuscular mycorrhizal fungi**

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36 **Summary**

- 37 • Although rhizobia establishing a nitrogen-fixing symbiosis with legumes are also known
38 to promote growth in non-legumes, studies on rhizobia association with wheat roots are
39 scarce.
- 40 • We searched for *Rhizobium leguminosarum* symbiovar *viciae* (*Rlv*) strains naturally
41 competent for wheat roots colonization. We isolated 20 strains and tested the ability of a
42 subset for wheat roots colonization when co-inoculated with other *Rlv*. We also measured
43 the effect of these strains on wheat root architecture and Arbuscular Mycorrhizal Fungal
44 (AMF) colonization.
- 45 • We found a low diversity of *Rlv* in wheat roots compared to that observed in the *Rlv*
46 species complex. Only a few strains, including those isolated from wheat roots, and one
47 strain isolated from pea nodules, were efficient to colonize wheat roots in co-inoculation
48 conditions. These strains had a high ability for endophytic colonization of wheat root and
49 were able to stimulate root development and AMF colonization in single strain inoculation
50 conditions.
- 51 • These results suggest that wheat is an alternative host for some *Rlv*; nevertheless, there is
52 a strong competition between *Rlv* strains for wheat root colonization. Furthermore, our
53 study suggests that the level of endophytic colonization is critical for *Rlv* ability to
54 promote wheat growth.

55 **Key words:** *Triticum aestivum* and *Triticum turgidum* (wheat), *Rhizobium leguminosarum*
56 symbiovar *viciae* (*Rlv*), Endophyte, Root development, Arbuscular Mycorrhiza (AM),
57 Competitiveness, Cooperation, Plant Growth-Promoting Rhizobacteria (PGPR).

58

59 **Introduction**

60 Plant roots interact with microorganisms that play key roles in their development,
61 nutrition and protection against pathogens (Ortíz-Castro *et al.*, 2009). Under the influence of
62 root exudates, microbes multiply in the rhizosphere, i.e. the soil portion in proximity to the
63 roots (Haichar *et al.*, 2014; Baetz & Martinoia, 2014; Hu *et al.*, 2018; Huang *et al.*, 2019).
64 Microorganisms inhabiting the rhizosphere are known to play important roles in plant
65 nutrition through various properties such as biological nitrogen fixation, phosphate
66 solubilization or siderophore secretion (de Souza *et al.*, 2015). Among the extremely abundant
67 diversity of soil/rhizospheric microorganisms, only a small fraction is able to colonize the
68 inner part of the plant root system. Several of these endophytic microbes have been shown to
69 offer important benefits to their hosts displaying Plant Growth-Promoting (PGP) activities or
70 protection against pathogens (Santoyo *et al.*, 2016). Mechanisms including: i) facilitation in
71 acquiring nutriment, ii) interference with plant hormone (auxin, cytokinin or ethylene)
72 homeostasis, iii) pathogen control via antibiosis (Santoyo *et al.*, 2016; Carrión *et al.*, 2019)
73 have been related to these beneficial activities.

74 Among the endophytic microorganisms, some, such as the Arbuscular Mycorrhizal
75 Fungi (AMF) interacting with most of plant species, have the ability to massively colonize
76 plant roots (Bonfante & Genre, 2010). This colonization relies on AMF recognition by plants
77 that is, at least in part, mediated by the perception of fungal LipoChitoOligosaccharide (LCO)
78 signals (Girardin *et al.*, 2019). Non-legume LCO receptors are poorly specific for LCO
79 structural variations (Buendia *et al.*, 2019a; Girardin *et al.*, 2019). Consistently, Arbuscular
80 Mycorrhiza (AM) display poor host-specificity as almost all AMF species are able to colonize
81 most of the terrestrial plants. The LCO perception machinery and the downstream signaling
82 pathway have been recruited in legumes to allow recognition of nitrogen fixing bacteria called
83 rhizobia that are accommodated in root organs called nodules (Girardin *et al.*, 2019). Rhizobia
84 also produce LCOs called Nod factors that are recognized by legume hosts (Gough &
85 Cullimore, 2011). Rhizobia are polyphyletic and belong to various bacterial genera. Indeed,
86 the Nod factor synthesis genes (*nod* genes) are frequently located on a plasmid (the symbiotic
87 plasmid), which can be horizontally transferred within or across rhizobia species (Boivin *et al.*,
88 2020a). In contrast with the AM, the rhizobium-legume symbiosis is generally host-
89 specific and symbiovars designate bacteria able to nodulate the same host(s). For example,
90 pea, faba bean, vetch and lentil plants are nodulated by *Rhizobium leguminosarum* bacteria of
91 the symbiovar (sv) *viciae* (*Rlv*), while clover is nodulated by *Rhizobium leguminosarum* sv.

92 *trifolii*. Other legumes such as alfalfa and soybean are nodulated by rhizobia from other
93 genera, *Ensifer* (formerly called *Sinorhizobium*) and *Bradyrhizobium* respectively. Nod factor
94 structural variations are associated to this host-specificity (Gough & Cullimore, 2011). In
95 addition, in natural soils, multiple rhizobia of the same symbiovar coexist but display
96 contrasted Competitiveness to Form Nodules (CFN) depending on their host genotype
97 (Boivin et al, 2020a; Boivin *et al.*, 2020b). Together with the host-specificity associated with
98 Nod factor specificity, CFN contribute to determine partner choices during the rhizobium-
99 legume symbiosis.

100 Several studies have shown that rhizobia can also interact with non-legumes. *Rlv* were
101 previously isolated from soils under wheat monoculture (Depret *et al.*, 2004) suggesting that
102 *Rlv* can be maintained in absence of rotation with compatible legumes. Rhizobia belonging to
103 various genera were isolated and/or shown to have PGP activities in rice (Biswas *et al.*, 2000;
104 Chaintreuil *et al.*, 2000; Peng *et al.*, 2002; Yanni & Dazzo, 2010). *R. leguminosarum* sv.
105 *trifolii* (isolated from legumes, rice or wheat) were also shown to colonize and have PGP
106 activities on wheat (Höflich *et al.*, 1995; Webster *et al.*, 1997; Hilali *et al.*, 2001; Yanni *et al.*,
107 2016). Although mechanisms involved in AM and rhizobial symbiosis establishment share
108 similarities, there is little information on the rhizobia-AMF interaction, particularly in wheat.
109 Nevertheless, recently Raklami and coworkers showed that rhizobia inoculation in open fields
110 increase wheat colonization by AMF and yield (Raklami *et al.*, 2019).

111 Here we performed a functional ecology study on the wheat-*Rlv* interaction. We
112 investigated whether: i) wheat is a natural host for *Rlv* strains, by isolating bacteria from
113 wheat grown in open fields in the southwest of France ii) there is partner choice in the wheat-
114 *Rlv* interaction, by co-inoculating strains representative of the known *Rlv* diversity iii) there is
115 diversity in *Rlv* for colonization and stimulation of responses in wheat roots, by measuring *Rlv*
116 epiphytic/endophytic colonization, root development and AMF colonization.

117

118 **Material and Methods**

119 **Wheat sampling from open fields**

120 Wheat plants (*Triticum aestivum* ssp. *aestivum* and *Triticum turgidum* ssp. *turgidum*) were
121 collected in the CREABio experimental station de la Hourre in Auch (southwest of France;
122 (43°37'17.7"N 0°34'20.6"E) in February 2014. Plants were collected in 2 experimental field
123 plots: LH1 under rotation with pea (*Pisum sativum*) and fertilized with 80 kg/ha of organic
124 fertilizer and LH7 under rotation with soybean (*Glycine max*) and fertilized with 100 kg/ha of
125 organic fertilizer. Both plots were characterized by a limestone clay soil with a pH of 8.1 and
126 8.4 for LH7 and LH1 respectively. Specific soil composition for the field plots are reported in
127 Fig. S1. Sixteen wheat varieties, 8 from LH1 and 8 from the LH7, were collected (Table S1).
128 Six plants per variety were sampled and transported in sterilized plastic bags.

129 **Strain isolation and characterization**

130 To isolate *Rhizobium leguminosarum* symbiovar (sv) *viciae* (*Rlv*) strains, 2 methods were
131 employed: nodule-trapping method and direct root plating. Prior to bacterial isolation, wheat
132 roots of each variety were pooled and surface-sterilized. Roots were first rinsed in sterile
133 water to remove soil, and incubated 10 min in a 1.2 % sodium hypochlorite solution, 10 min
134 in a spiramycine 30 mg/ml solution and 1 min in 70% ethanol, followed by 3 rinses in sterile
135 water. Surface-sterilized roots were mixed in 5 ml of phi liquid medium (10 g/L bacto
136 peptone, 1 g/L Casimino acids and 1 g/L yeast extract) and root mixtures were stored in 50%
137 of glycerol at -80°C prior to bacterial isolation. For the nodule-trapping experiment pea
138 (*Pisum sativum*) and vetch (*Vicia sativa*) plantlets were used as specific hosts. Seeds were
139 sterilized and germinated as described in Methods S1. Seedlings were grown in 110 ml glass
140 tubes containing Farhæus agar medium (Catoira *et al.*, 2000). After 10 days, plantlets were
141 inoculated with wheat root homogenates and incubated for 4 weeks at 22°C. For each of the
142 16 wheat root homogenates, 3 plants of each legume host were inoculated. After 4 weeks,
143 plants were scored for the presence/absence of nodules (Table S1). Each nodule was collected
144 with a scalpel, homogenized and stored in 50% glycerol at -80°C prior to bacterial isolation.
145 Serial dilutions of nodule homogenates were spread on TY agar medium (16 g/L peptone, 10
146 g/L yeast extract and 5 g/L NaCl) supplemented with 2 mM CaCl₂. For direct isolation of
147 culturable bacteria, root homogenates were diluted and spread on TY agar medium
148 supplemented with 2 mM CaCl₂. All purified colonies were first stored in 30% of glycerol/TY
149 medium at -80°C. Bacteria isolated from nodules or by direct plating were tested for presence

150 of the *nodD* gene. We used the Y5 and Y6 primer set developed by Zeze *et al.*, (2001)
151 amplifying a 850bp *nodD* fragment from *Rlv*, *Rhizobium leguminosarum* sv. *trifolii* and
152 *Ensifer meliloti*. Strains showing an amplification were then used for *gyrB* amplification by
153 using primers and protocols already described (Martens *et al.*, 2008). Sequences (Table S2)
154 were trimmed and aligned with DAMBE version 6 (Xia, 2017) and neighbor-joining
155 phylogeny in comparison with other *Rhizobium* species (sequences retrieved from GenBank
156 <https://www.ncbi.nlm.nih.gov/genbank/>; Table S3), was inferred with MEGA 5 (Tamura *et*
157 *al.*, 2011) with *p*-distance model. Three strains isolated from wheat roots, FWPou15,
158 FWPou32 and FW Pou38 were selected for further analysis and their genomes were
159 sequenced and analyzed as described in Methods S2. Genomes are available in GenBank
160 under the accession numbers JACBGR000000000, JACBGQ000000000 and
161 JACBGP000000000, respectively. Phenotypic assays were performed on the 3 strains in
162 comparison with the A34 strain used as control (Götz *et al.*, 1985; Oono & Denison, 2010).

163 **Metabolic pathways and comparative genomics analyses**

164 A metabolic reconstruction in SBML format (Hucka *et al.*, 2003) was built for each strain
165 with the Carveme software (Machado *et al.*, 2018), a binary matrix containing the
166 presence/absence information for each metabolic reaction in each strain was created from
167 SBML files using ad-hoc Java programs. A correspondence analysis was performed with the
168 *dudi.coa* method of the R *ade4* package (Dray *et al.*, 2015). The correspondence analysis
169 result was then plotted using the *scatterD3* R package. Tryptophan metabolism was analyzed
170 with the *kbase* platform (Artkin *et al.*, 2018) and by using the RAST annotation pipeline
171 (Brettin *et al.*, 2015) and the Model Seed metabolic reconstruction pipeline (Henry *et al.*,
172 2010). Orthology search was performed with OrthoFinder with default settings (Emms &
173 Kelly, 2019). Functional category assignments and annotations of orthologous groups were
174 done by selecting a representative of each group and annotating with eggNOG-Mapper
175 (Huerta-Cepas *et al.*, 2017).

176 **Nodulation tests**

177 Three selected strains FWPou15, FWPou32 and FWPou38 and the A34 strain were tested for
178 their ability to nodulate pea, faba bean, vetch and clover plants. For pea and vetch plants,
179 germ-free seedlings (Methods S1) were placed into 110 ml glass tubes filled with attapulgit
180 and watered with 10 mL Farhæus liquid medium supplemented with 1 mM NH₄NO₃, and an
181 aluminum foil was placed around the bottom of the tube. Seedlings were incubated for one

182 week before inoculation with 2 ml of 10^8 /ml bacterial suspension of each strain (12 plant
183 replicates / plant species). For faba bean and clover plants, germ-free seedlings were
184 transferred in containers (250 ml of volume) filled with a mix of 50% perlite/50% sand and
185 humidified with 300 ml of sterilized water. Seedlings were inoculated by adding 150 ml of
186 10^7 /ml bacterial suspension and cultivated in a growth chamber (20°C and 16h/8h light/dark
187 period). Nodules were counted 6 weeks after inoculation. Microscopy analysis was performed
188 on 3 randomly selected nodules for each nodulated plant species (Methods S3) to confirm that
189 nodules were colonized by bacterial cells.

190 **Wheat root colonization in co-inoculation assays**

191 Seven-day old germ-free wheat seedlings (Methods S1) of the Energo variety, individually
192 grown in 110 ml glass tubes filled with 70 ml of Fahraeus agar medium (Fig. S2; Methods S4)
193 were inoculated with FWPou15 strain alone or with bacterial mixtures containing either the
194 FWPou15, FWPou32 or FWPou38 strains plus 22 strains of the *Rlv* core collection
195 representing the known genomic *Rlv* diversity (Table S3; Boivin *et al.*, 2020b). Each strain
196 present in the mixture was characterized by a unique 309 bp sequence of the *nodD* coding
197 region and were therefore easily unambiguously identified by DNA metabarcoding. Each
198 modality consisted in an inoculation of 6 plants; 3 independent replicates (3 temporal blocks)
199 were performed. In total 18 plants were inoculated with each bacterial mixtures. Each wheat
200 plantlet was co-inoculated with 2 ml of each mixture containing all bacterial strains at the
201 final $OD_{600nm} = 0.01$ (see Table S4 for initial OD_{600nm} of each strain) and incubated in a growth
202 chamber at 20°C and a light/dark period of 16h/8h. Roots were collected after 7 days. Half of
203 the roots were surface-sterilized using the following successive treatments: 1 min in pure
204 ethanol, sterilized water, 3 min in 1.2% sodium hypochlorite solution, 3 times in sterilized
205 water, 1 min in pure ethanol and 3 times in sterilized water. The other half was directly
206 analyzed without surface sterilization. For each temporal block, the plant roots of each
207 modality were pooled prior to DNA extraction with the DNeasy Plant Mini Kit following the
208 manufacturer's instructions (Table S4). To characterize the relative abundance of the *Rlv*
209 bacteria in the wheat roots, the 309 bp sequence of the *nodD* coding region was amplified by
210 PCR from the DNA extractions and the PCR products were sequenced by Illumina MiSeq
211 2x250 bp technology as described in Methods S5. Abundance of each strain was estimated by
212 the number of reads corresponding to its unique *nodD* sequence (Methods S5, Table S5).
213 Hierarchical clustering and heatmaps were built using the pheatmap R package (clustering
214 "maximum", method "wardD").

215 **Wheat root colonization in single strain inoculation assays**

216 To test the colonization level in single-inoculation conditions, experiments were performed as
217 described for co-inoculation assays except that plantlets were inoculated with 2 ml of each
218 bacterial suspension ($OD_{600nm} = 0.08$). The number of independent replicates (temporal
219 blocks) and the total number of plants analyzed for each strain and conditions are reported in
220 Table S6. Roots were excised 7 dpi and weighed (fresh weight). Bacterial isolation from roots
221 was performed by chopping the entire root system using a scalpel and by dilution plating on
222 TY plates with the addition of 2 mM $CaCl_2$. To estimate the endophytic growth wheat roots
223 were surface-sterilized as described for the co-inoculation assay. Plates were incubated 3 days
224 at 24°C before colony counting. The number of Colony-Forming Units (cfu) was normalized
225 by the root fresh weight.

226 **Wheat root colonization by the FWPou15 GFP-expressing strain**

227 Construction of the GFP expressing strain is described in Methods S6 and microscopy
228 analysis is detailed in Methods S3.

229 **Wheat root development assays**

230 Three-days old germ-free wheat seedlings (Methods S1) were placed on 20x20 cm plates
231 containing Fahraeus agar medium (Fig. S2, Methods S4). Plantlets were grown 4 days prior to
232 inoculation with 1 ml of bacterial suspension ($OD_{600nm} = 0.08$) of each strain. Control plants
233 were inoculated with 1 ml of sterilized water. The number of experiments (temporal blocks)
234 and plant replicates are shown in Table S7. Plants were grown at 20°C with a light/dark
235 period of 16h/8h. Roots were removed from the plates 12 dpi, washed with water to remove
236 traces of agar, and imaged with an Epson Expression 10000XL scanner. Total root length was
237 estimated by using WINRhizo software (Pouleur S, 1995) and the number of lateral roots was
238 counted manually on the scanned roots.

239 **Mycorrhiza assays**

240 Three-day old germ-free wheat seedlings (Methods S1) were transferred in 50 ml containers
241 filled with attapulgite supplemented with 20 ml of 1/2 modified Long Ashton liquid medium
242 (containing 7.5 μM NaH_2PO_4) and 200 *Rhizophagus irregularis* DAOM197198 spores
243 (purchased at Agronutrition, Carbonne, France) were inoculated on each plant. The number of
244 experiments (temporal blocks) and plant replicates are shown in Table S8. After 3 days at
245 23°C with a 16/8h light/dark regime, 1 ml of each bacterial suspension ($OD_{600nm} = 0.08$) was

246 inoculated and plants were further grown for 21 days. Roots were then washed and
247 permeabilized in a 10% KOH solution for 10 min at 95°C and stained with a 5% ink-vinegar
248 solution for 3 min at 95°C.

249 **Statistical analysis**

250 All the statistical analyses performed in the manuscript were carried out in the R environment.
251 Scripts used for the analyses and for the construction of the graphs are detailed in Methods
252 S7. Raw data that were used to run the statistical models are available in: i) Table S9 for the
253 relative abundances of the *R/v* strains in roots during the co-inoculation assays, ii) Table S10
254 for the numbers of cfu in the root colonization assays, iii) Table S11 for the total root lengths
255 and lateral root numbers obtained in the root development assays, iv) Table S12 for the
256 number of colonization sites in the mycorrhiza assays.

257

258 **Results**

259 ***Rhizobium leguminosarum* sv. *viciae* (*Rlv*) strains are associated with wheat roots**

260 In order to determine whether *Rlv* strains naturally interact with wheat, we collected wheat
261 roots from plants under rotation with pea or soybean in open fields located in the southwest of
262 France. We surface-sterilized and macerated them to extract bacterial endophytes. Several
263 wheat varieties belonging to 2 species (*Triticum aestivum* ssp. *aestivum* and *Triticum*
264 *turgidum* ssp. *turgidum*, Table S1) were compared. *Rlv* were isolated from these root samples
265 by two approaches. First, pea and vetch seedlings, which are known legume *Rlv* hosts, were
266 inoculated with the endophyte bacterial suspensions. The nodules formed were individually
267 collected and bacterial clones occupying these nodule were isolated (“nodule trapping”).
268 Second, surface-sterilized macerates from wheat roots were directly plated. Bacteria collected
269 by both methods were PCR screened by using primers that specifically amplify a fragment of
270 the *nodD* gene from *Rlv*, and a few other rhizobial species (Zeze *et al.*, 2001). We succeeded
271 in isolating 20 strains from plants in rotation with pea (Table S1). Phylogenetic analysis based
272 on a portion of the chromosomal *gyrB* gene demonstrated that these 20 strains cluster in 2
273 closely-related clades of the *R. leguminosarum* species complex (Fig. 1a). The first clade
274 includes the *Rlv* strains TOM, FRF1D12, Vaf10 and CCBAU83268 belonging to genospecies
275 gsF-1 (Boivin *et al.*, 2020a) and the second clade includes the *Rlv* strain SL16 belonging to
276 the genospecies gsF-2 (Boivin *et al.*, 2020a). Three strains (FWPou15, FWPou32 and
277 FWPou38) randomly selected from the most predominant clade, were further characterized.
278 Sequencing a large portion of the *nodD* coding region confirmed that they belong to *R.*
279 *leguminosarum* symbiovar *viciae* (Fig. S3). Phylogeny of the *nodD* gene suggested that the
280 symbiotic plasmid of these strains are closely related to that of the reference *Rlv* strain 3841
281 and the control strain A34 (Oono & Denison, 2010). The 3 strains (FWPou15, FWPou32 and
282 FWPou38) were found to nodulate pea, faba bean and common vetch but not clover plants
283 (Fig. 1b-1e, Fig. S4-S5), confirming that they belong to symbiovar *viciae*.

284 **Wheat-associated *Rlv* do not display specific genomic and metabolic signatures**

285 Whole genome sequencing was performed on FWPou15, FWPou32 and FWPou38. Genomes
286 of these strains were compared to genomes of *Rlv* isolated from legumes and representative of
287 the gsB, gsC, gsE and gsF-1/2 genospecies (Boivin *et al.*, 2020a). Sequences of the 3 strains
288 shared a ca. 100% Average Nucleotide Identity (ANI) and few SNPs were detected between
289 the strains. Genomic phylogeny confirmed that the 3 wheat strains cluster with the

290 representatives of genospecies gsF-1 (Fig. 2). Phylogeny on 11 concatenated *nod* genes
291 located on the symbiotic plasmid, showed that the 3 wheat strains cluster with the Nod group
292 B1 that includes the 3841 and FRF1D12 strains isolated from faba bean and pea respectively
293 (Fig. 3; Boivin *et al.*, 2020a). The genomes of strains FWPou15, FWPou32 and FWPou38
294 encode 301 orthologous genes, which are not shared with other *Rlv* representatives. A
295 majority of these genes (200/301) were not assigned to a specific Clusters of Orthologous
296 Groups (COG) functional category. We could not identify functions with an obvious link to
297 symbiotic or endophytic growth among the remaining genes. We further inferred and
298 compared the metabolic potential of the 3 wheat strains and 22 strains isolated from legume
299 nodules, representing the *Rlv* genetic diversity (Table S3, Boivin *et al.*, 2020b). We found that
300 all the strains shared most of the metabolic reactions (1,967 reactions, >87%; Fig. S6a) and
301 only 13% of the reactions were specific to a subset of strains. Correspondence Analysis (CA)
302 constructed on the presence/absence of the metabolic reactions, showed a hierarchical
303 clustering similar to that observed for the ANI (Fig. S6b; Fig. 2), in which the wheat isolates
304 group with the TOM strain. In conclusion, wheat-isolated *Rlv* do not display specific genomic
305 and metabolic signatures when compared with *Rlv* isolated from legume nodules.

306 ***Rlv* strains colonize wheat roots with different degrees of success in co-inoculation assays**

307 We then tested whether there is diversity among the *Rlv* complex species for wheat root
308 colonization ability. For this, we compared the colonization success of wheat isolates with
309 that of strains isolated from legume nodules in a co-inoculation experiment. We inoculated in
310 gnotobiotic conditions, plantlets of the *T. aestivum* Energo variety with 3 mixtures containing
311 an equal concentration of 23 strains, each mixture consisting of the 22 strains representing the
312 genetic diversity of *Rlv* (Table S3) and either FWPou15, FWPou32 or FWPou38. In each
313 replicate, plantlets were also inoculated with FWPou15 alone to verify the efficacy and
314 specificity in detecting the *Rlv* strains. We quantified the relative abundance of each strain 7
315 days post inoculation (dpi) by Illumina MiSeq sequencing of a *nodD* gene fragment (Methods
316 S5, Boivin *et al.*, 2020b) on DNA extracted after surface-sterilization of the roots (treatment
317 S) or without root surface-sterilization (treatment NS). In the control plants inoculated with
318 FWPou15 alone, on average 95% of the total reads corresponded to this bacterium (Fig. S5)
319 confirming its ability to colonize wheat roots and suggesting little contamination in the
320 experiment. In the co-inoculated plants, thirteen strains were either not detected (no reads,
321 CZP3H6, GD25 and CCBAU03058, Table S5) or had a relative abundance lower than 1%
322 (BLR195, CCBAU10279, FRP3E11, GB51, GLR2, GLR17, P1NP2K, SL16, TOM,

323 UPM1134; Fig. 4, Table S5), suggesting there are either not able to colonize wheat roots or
324 poor competitors. The other strains including ten strains isolated from legumes as well as the
325 wheat strains were detected both in NS and S roots, revealing wheat root epiphytic and
326 endophytic colonization abilities are widely distributed among the *Rlv* diversity. However,
327 strong differences in the relative abundance of these strains were observed. A generalized
328 mixed-linear model (*glmm*) on the relative abundances of the strains in the 3 mixtures showed
329 a significant effect of the ‘*strain*’ factor, but not of the ‘*sterilization*’ factor, on the
330 colonization success (Table 1). The IAUb11 strain isolated from pea nodules was significantly
331 more abundant than all the other strains in both root compartments (Table S13), with a mean
332 relative abundance of 63% and 45% in NS and S roots, respectively (Fig. 4, Table S5). The
333 wheat strains FWPou32 and FWPou38 showed intermediate colonization abilities, with a
334 mean relative abundance of 13 and 4% in NS roots and 19 and 22 % in S roots (Fig. 4, Table
335 S5). Significant differences in their abundance compared to other strains (except IAUb11)
336 were only found in the S roots (Table S13). Interestingly, the strain TOM, belonging to the
337 same gsF-1 genospecies as the wheat strains, had very low ability to colonize wheat roots in
338 this assay (read abundance <1%) suggesting that the genospecies does not predict wheat
339 colonization ability.

340 The colonization success of each *Rlv* strain in the mixtures is globally equivalent in S and NS
341 roots, suggesting that epiphytic colonization success drives the endophytic colonization
342 success. However, the strains FWPou32 and particularly FWPou38 were more successful in
343 colonizing S roots than NS roots, suggesting a stronger endophytic ability compared to most
344 of the *Rlv* strains isolated from legumes.

345 ***Rlv* strains are wheat root endophytes**

346 We then quantified the endophytic and epiphytic wheat root colonization abilities of a few
347 individual strains. We selected the strains IAUb11, FWPou38 and FWPou15 displaying
348 respectively the high, intermediate and low degree of colonization in co-inoculation
349 conditions. We included in the analysis the commonly used *Rlv* strain A34 as a control. We
350 performed single strain inoculations in gnotobiotic conditions on two *T. aestivum* varieties,
351 Energo and Numeric, and colonization was assessed by measuring cfu of strains isolated from
352 wheat roots 7 dpi and following surface-sterilization (treatment S) or not (NS) of the wheat
353 root system.

354 A linear-mixed model (*lmm*) on the cfu /mg of root fresh weight showed a significant effect of
355 the ‘sterilization’ and ‘strains’ factors (Table 2). No significant effect was observed for the
356 ‘variety’ factor, however, significant nested ‘sterilization × variety’ factor and ‘strain ×
357 variety’ effects were observed (Table 2).

358 All strains colonized the root surface of the 2 wheat varieties (10^6 to 10^7 cfu/mg, Fig. 5) and
359 no significant difference was observed between strains and between the wheat varieties in the
360 NS roots (Table S14). By contrast, bacteria differed in their endophytic colonization abilities.
361 FWPou38 and IAUb11 were the most efficient in both varieties (10^3 to 10^4 cfu/mg), while
362 A34 was the least efficient root endophyte (10^2 cfu/mg, Fig.5) on Numeric. FWPou15, which
363 behaved similarly to A34 on the Energo variety, displayed an intermediate endophytic
364 colonization ability on Numeric (10^3 cfu/mg, Fig. 5, Table S14).

365 To confirm the epiphytic and the endophytic capacities of *Rlv* through microscopy
366 observation, we constructed a GFP-tagged version of FWPou15 and observed its *in vitro* root
367 colonization on the cultivar Energo 7 dpi. Confocal microscopy showed that FWPou15
368 massively colonized the surface of wheat roots (Fig. 5c). A few bacteria were also observed
369 inside the roots, at least around the outer cortical cells (Fig. 5d), confirming their endophytic
370 colonization ability.

371 ***Rlv* can stimulate wheat root development**

372 We then tested whether *Rlv* strains show differences in their ability to induce plant responses.
373 We first investigated whether *Rlv* strains have different effects on root architecture. We
374 inoculated A34, FWPou15, FWPou38 or IAUb11 on both Energo and Numeric varieties in
375 gnotobiotic conditions, measured the number of lateral roots and the total root length at 12
376 dpi. *lmm* on both variables showed significant effects of the ‘strain’ and ‘variety’ factors as
377 well as a significant nested ‘strain x variety’ effect (Table 3). Despite that for each wheat
378 variety the total root lengths and the lateral root numbers were correlated ($R^2 \sim 0.7$, Fig. S7),
379 these traits responded differently to inoculation (Fig. 6). FWPou38 and IAUb11 induced a
380 significant increase in the lateral root number (on average + 35% each; Fig. 6d, Table S15),
381 while FWPou15 induced a weak but significant effect on the total root length (on average +12
382 %; Fig. 6a, Table S15) in the Energo variety. In contrast, FWPou15 induced a significant
383 increase of both total root length (on average +29%; Fig. 6a, Table S15) and lateral root
384 number (on average +52%; Fig. 6b, Table S15) in the Numeric variety; while FWPou38
385 induced a significant, although weak, decrease in the total root length (on average -18 %; Fig.

386 6d, Table S11). Inoculation by A34 did not affect root architecture in either wheat variety.
387 Interestingly, on the Energo variety, strains with the highest level of endophytic colonization
388 (FWPou38 and IAUb11) showed the strongest effect on wheat root architecture. FWPou15
389 displayed a root-growth activity mostly on the Numeric variety in which it showed a higher
390 level of endophytic colonization compared to the Energo variety (Fig. 4).

391 ***Rlv* can enhance colonization of wheat roots by arbuscular mycorrhizal fungi**

392 We then investigated whether *Rlv* strains have different effects on wheat root colonization by
393 AMF. For this, we co-inoculated Energo and Numeric roots with the *Rhizophagus irregularis*
394 isolate DAOM197198 and one of the 4 *Rlv* strains (FWPou15, FWPou38, IAUb11 or A34).
395 We measured the number of AMF colonization sites 24 dpi and compared it to wheat plantlets
396 inoculated only with *R. irregularis*.

397 *Imm* showed a significant effect of the ‘strain’ factor only on the Energo variety (Table 4).
398 More specifically, when compared to the control, inoculation with the FWPou38 and IAUb11
399 strains resulted in significantly more fungal colonization sites (on average +55% and +31%
400 respectively; Fig. 7b; Table S16). Similarly, roots inoculated with either FWPou38 or IAUb11
401 displayed significantly more fungal colonization sites than roots inoculated with A34 (on
402 average +77% and +50% respectively; Table S16). Only FWPou38 induced significantly
403 more fungal colonization sites than FWPou15 (on average +33%; Table S16). On the
404 Numeric variety, inoculation with any of *Rlv* strain did not result in a significant change in the
405 number of fungal colonization sites (Fig. 7c and d; Table S16) reinforcing the influence of the
406 wheat genotype on the response induced by *Rlv*. Taken together, these results suggest that the
407 *Rlv* strains stimulating AMF colonization in wheat roots are those able to stimulate the lateral
408 root number.

409

410 **Discussion**

411 **Wheat might participate in shaping *Rlv* populations in agronomical soils**

412 *Rhizobium leguminosarum* sv. *viciae* strains are known to nodulate legume hosts such as pea
413 and faba bean and have PGP activities on wheat. Here we show that wheat is an alternative
414 natural host for *Rlv*. Yet, in our study we only isolated *Rlv* strains from root samples of wheat
415 plants cultivated in rotation with pea plants (*Rlv* host), while we did not succeed in isolating
416 *Rlv* from wheat plants in rotation with soybean (non-*Rlv* host). This suggests an enrichment of
417 *Rlv* in wheat roots through rotation with a legume host. On the other hand, a previous study
418 described *Rlv* strains in soils under wheat monoculture (Depret *et al.*, 2004). Although the *Rlv*
419 species complex had been currently described to contain 5 genospecies, only members of two
420 of them (gsF-1 and gsF-2) were identified among the 20 wheat isolated strains. The limited
421 diversity of *Rlv* found in our wheat samples might be explained through three non-exclusive
422 hypotheses. Firstly, the sampling we performed was not saturated. Secondly, we cannot
423 exclude that soils in which wheat have been grown were deprived of other *Rlv* genospecies.
424 However, this is not in accordance with previous results showing a high *Rlv* diversity in
425 European soils including in the southwest of France (Boivin *et al.*, 2020a). Thirdly, the low
426 *Rlv* diversity is related to partner choice in wheat-*Rlv* interactions. Nevertheless, through co-
427 inoculation assays, we demonstrated that *Rlv* strains isolated from legume plants and
428 belonging to other genospecies (i.e. IAUb11, gsE) can efficiently colonize wheat roots while
429 other members of gsF-1/2 genospecies were not efficient wheat root colonizers suggesting
430 that wheat partner choice is not related to *Rlv* genospecies. However, the co-inoculation
431 assays resulted in a reduced diversity associated with the wheat roots compared to the
432 inoculum, supporting the hypothesis of partner choice. Such partner choice was also observed
433 in the legume-*Rlv* interaction (Boivin *et al.*, 2020b). Interestingly, the pattern of colonization
434 success we found for the *Rlv* strains in the wheat roots was different to that observed with the
435 same 22 *Rlv* for colonization in various legumes during nodulation (Fig. 8; Boivin *et al.*,
436 2020b), suggesting that *Rlv* strains might carry different competitiveness abilities to colonize
437 legume nodules, wheat roots or other putative hosts. By consequence, multiplicity of plant
438 hosts in fields can participate in maintaining *Rlv* diversity in soils. Field campaigns at a
439 broader geographical scale to investigate the diversity of *Rlv* in wheat roots, legume nodules
440 and in the surrounding soils, should help to validate these hypotheses.

441 **Different mechanisms could influence *Rlv* competitiveness for endophytic and epiphytic**
442 **wheat root colonization**

443 The colonization success of a given strain could be due to its ability to colonize the host
444 and/or to compete with other microorganisms. Although we have not tested individually all
445 the *Rlv* strains used in the co-inoculation assays, we have not found any difference for
446 epiphytic growth between the 4 strains we have tested individually. This suggests that the
447 differences observed for the epiphytic growth in the co-inoculation assay are due to
448 differences in competitiveness. Competition for nutrient resources is the most common
449 mechanism underlying variations in bacterial root colonization, as strains/species carrying
450 resource-specific pathways are often those able to rapidly grow and dominate niches (Simons
451 *et al.*, 1996; Pieterse *et al.*, 2014; Yang *et al.*, 2019). Other hypotheses to explain different
452 bacterial abilities to compete for root colonization include antagonism *via* the production of
453 secondary metabolites (Weller *et al.*, 2002). However, the minor differences found in this
454 study among the *Rlv* strains in term of metabolic pathways (Fig. S6) did not correlate with the
455 differences observed for wheat root colonization in the co-inoculation assays. Comparative
456 genomics revealed that a cluster of 4 genes annotated as a putative sulfide/taurine transporter
457 was only found in the genomes of the strains FWPou15, FWPou32, FWPou38 and IAUb11
458 and not in the 21 other strains (gene identifiers in strain IAUb11: RLVIAUB1145.7 -
459 RLVIAUB1145.10). It is possible that this transporter is involved in competitiveness of the
460 FWPou32, FWPou38 and IAUb11 strains by facilitating export of sulfate or sulfite
461 (Weinitschke *et al.*, 2007).

462 Overall, in the co-inoculation assays performed in this study, the competitiveness pattern for
463 epiphytic colonization is similar to the pattern found for endophytic colonization. This
464 indicates that *Rlv* multiplication on the root surface is an important factor underlying the
465 endophytic success of *Rlv* strains. Nevertheless, mechanisms underlying competitiveness of
466 the *Rlv* strains in colonizing the inner part of wheat roots might be different to those
467 regulating the epiphytic growth. First, we found differences for endophytic colonization
468 between the strains we tested individually. Secondly, the endophytic/epiphytic colonization
469 ratio were higher for wheat strains FWPou32 and FWPou38 than for the pea strain IAUb11.
470 The ability of strains to escape recognition by host and to avoid plant defenses might also
471 account for the ability to multiply inside the roots (Pieterse *et al.*, 2014). A combination of

472 mechanisms might thus modulate the ability of *Rlv* strains to endophytically colonize wheat
473 roots and explain adaptation of some *Rlv* strains to wheat.

474 Surprisingly, while almost clonal, FWPou15 and FWPou38 strains showed contrasted ability
475 to colonize wheat roots. Based on short read Illumina genome sequencing, we found only one
476 non-synonymous mutation between FWPou15 and FWPou38 strains, which occurs in a gene
477 encoding a DNA polymerase IV involved in DNA repair. It is known in bacteria, that in
478 response to stress DNA polymerase can synthesize error-containing DNA leading to the
479 formation of genetic variants and different phenotypes of the progeny (Foster, 2005). This
480 process called phenotypic plasticity also leads to bacterial cells showing different responses
481 when inoculated in a plant host (i.e. loss of pathogenicity or decrease of epiphytic growth;
482 Bartoli *et al.*, 2015). Further experiments are required to determine whether this mutation,
483 genome re-arrangements and/or epigenetic modifications are responsible for these phenotypic
484 differences. Moreover, these variants represent a promising material to decipher the molecular
485 mechanisms controlling the ability of *Rlv* strains to colonize wheat roots.

486 **Common molecular mechanisms might be involved in the stimulation of wheat root** 487 **development and AMF colonization**

488 Several bacteria displaying PGP activity, including rhizobia, have been shown to enhance
489 wheat interaction with AMF (Germida & Walley, 1996; Russo *et al.*, 2005; Pivato *et al.*,
490 2009). Soil microorganisms can modulate the auxin-dependent root developmental program
491 leading to lateral root formation in both monocots and dicots (Contreras-Cornejo *et al.*, 2009;
492 Pieterse *et al.*, 2014). Indeed many PGP bacteria, including rhizobia, produce auxins
493 (Camerini *et al.*, 2008; Spaepen & Vanderleyden, 2011; Boivin *et al.*, 2016). The effect of *Rlv*
494 on wheat roots development could be attributed to auxin produced by *Rlv* strains. In support
495 of this hypothesis, key enzymes of 3 of the known bacterial auxin synthesis pathways using
496 tryptophan as substrate, the indole acetamide, the indole pyruvate and the tryptamine
497 pathways (Spaepen & Vanderleyden, 2011), are encoded in the FWPou15, FWPou38 and
498 IAUb11 genomes (Fig. S8). Alternatively, rhizobial LCOs can also affect root development
499 both in legumes and non-legumes through the regulation of the plant auxin homeostasis
500 (Herrbach *et al.*, 2017; Buendia *et al.*, 2019a). Stimulation of AMF colonization by *Rlv* can be
501 indirectly favored by the increase in the number of lateral roots, preferential sites for AMF
502 colonization (Gutjahr *et al.*, 2013). LCOs and auxin have been also both shown to stimulate
503 AMF colonization in legumes and non-legumes plants (Maillet *et al.*, 2011; Etemadi *et al.*,

504 2014; Buendia *et al.*, 2019b) and may also directly affect AM. Effects of exogenous
505 application of either auxin or LCO on root development are dependent on their concentration
506 (Herrbach *et al.*, 2017; Buendia *et al.*, 2019a). Different levels of auxin and/or LCO
507 production could, at least partially, explain the difference between *Rlv* strains on root
508 development and AMF colonization.

509 **Levels of endophytic colonization rather than epiphytic colonization might be critical for**
510 ***Rlv* ability to induce plant responses**

511 Both co-inoculation and single strain inoculation assays showed that FWPou38 and IAUb11
512 are efficient wheat endophytic colonizers with $\sim 10^4$ cfu/mg of root tissues on both Energo and
513 Numeric varieties. The bacterial population sizes found in wheat roots for these strains are
514 similar to those previously reported for PGP rhizobia colonizing rice roots (Chaintreuil *et al.*,
515 2000; Mitra *et al.*, 2016). Both strains can increase the number of lateral roots and stimulate
516 AMF colonization in Energo. This was not observed on the Numeric variety, suggesting that
517 bacterial effects are plant genotype dependent. The effect of wheat genotype on the
518 colonization by PGP rhizobacteria has been described, for example for bacteria of the genus
519 *Pseudomonas* (Valente *et al.*, 2020). Here, we show that beside the colonization level, host
520 responses also vary depending on the plant genotype. Interestingly, FWPou15, had a
521 stimulation activity on Numeric root architecture in which it is an intermediate endophytic
522 colonizer with $\sim 10^3$ cfu/mg, while not in Energo in which it is a poor endophytic colonizer
523 with $\sim 10^2$ cfu/mg. Similarly, the poorest endophytic colonizer in both Energo and Numeric,
524 A34, did not induce any root architecture or AMF colonization responses. In this regard, we
525 can hypothesize that *Rlv* strains with the best endophytic colonization ability were able to
526 reach an endophytic population size sufficient for production of auxins, LCOs or other PGP
527 molecules at concentrations required for stimulating lateral root development and AMF
528 colonization. Further analysis with a larger number of strains should establish whether there is
529 a correlation between endophytic colonization ability and stimulation of plant responses and
530 its genotype-genotype dependency.

531 In conclusion, our study suggest that *Rlv* competitiveness and level of endophytic colonization
532 are critical for potential PGP activities. These novel concepts will help in understanding
533 and/or design microbial consortia based on beneficial bacteria for improving wheat
534 yield/quality in a context of reducing the use of inputs.

535

536

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548

549 **Author Contributions**

550 Conceptualization of the project: CB, SB, BL, ML and CMB. Experimental design: CB, SB,
551 BL and ML. Field sampling and microbiology: CG and CB. Wheat phenotyping: CB, VG and
552 MG. Molecular analysis: CB and MG. Statistical Analysis: CB. Microscopy: MM and MCA.
553 Bio-informatic analysis: SB, AC and LC. Manuscript writing: CB, SB, BL, ML and CMB.

554

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722 **Figure Legends**

723 **Fig. 1. Functional *Rhizobium leguminosarum* sv. *viciae* (*Rlv*) are associated with wheat**

724 **roots.** (a) Neighbor-Joining tree based on a portion of the chromosomal *gyrB* gene from the
725 20 wheat-isolated strains (indicated in blue), a set of *Rlv* strains representative of the diversity
726 of the species complex (Boivin *et al.*, 2020b), and the strain A34. *Ensifer meliloti* was used as
727 outgroup. The *Rlv* strains isolated from wheat roots cluster with strains from genospecies gsF-
728 1 (TOM, FRF1D12, Vaf10, CCBAU83268) or gsF-2 (SL16). (b-g) Sections of pea nodules
729 colonized by (b) FWPou15, (c) FWPou32, (d) FWPou38 and (e) A34. Scale bars correspond
730 to 100 μ m.

731 **Fig. 2. Selected *Rlv* strains for functional analyses belong to genospecies gsF-1.**

732 Hierarchical clustering and heat map based on the Average Nucleotide Identity (ANI) values
733 between each couple of the 3 wheat strains, FWPou15, FWPou32 and FWPou38 (indicated in
734 blue), and a set of *Rlv* strains representative of the diversity of the species complex (Boivin *et*
735 *al.*, 2020b). Genospecies (gs) have been defined using an ANI threshold of 95%. Star
736 indicates phylogenetically distant isolates that do not cluster with any of the genospecies.

737 **Fig. 3. Selected *Rlv* strains for functional analyses belong to the Nod group B1.**

738 Neighbor-
739 Joining tree based on concatenated *nodABCDEFGHIJLMN* gene sequences of the 3 wheat
740 strains, FWPou15, FWPou32 and FWPou38 (indicated in blue), and *Rlv* strains representative
741 of the Nod groups (Boivin *et al.*, 2020b). WSM1689 (*R. leguminosarum* symbiovar *trifolii*)
and *E. meliloti* 1021 were used as outgroups.

742 **Fig. 4. *Rlv* strains colonize wheat roots with varying degrees of success in co-inoculation**

743 **assays.** (a-c) Least-Squares-Means (lsmeans) of the *Rlv* strain abundance in wheat roots when
744 co-inoculated in an *in vitro* assay. Co-inoculation of 22 *Rlv* strains representing the diversity
745 of *Rlv* (Table S3) together with the wheat strains FWPou15 (a), FWPou32 (b) or FWPou38
746 (c). Each mixture was inoculated in a total of 18 wheat plant root systems in 3 independent
747 replicates. Total DNA was extracted from pools of 3 roots systems, with or without surface-
748 sterilization, at 7 dpi, and a *nodD* amplicon was sequenced by MiSeq Illumina. Mean
749 abundance was inferred by normalizing the number of reads corresponding to each strain with
750 the total number of reads obtained in the run. Dark grey bars indicate results obtained from
751 surface-sterilized (S) roots and light grey bars indicate results obtained from non-sterilized
752 (NS) roots. The S and NS treatments allow estimating respectively the relative endophytic and
753 epiphytic abundance of each inoculated strain since endophytic bacteria are in negligible

754 amounts compared to epiphytic bacteria. Three *Rlv* strains GD25, CCBAU03058 and
755 CZP3H6 were not detected by MiSeq sequencing, thus, they are not reported in the barplots.

756 **Fig. 5. *Rlv* strains are wheat root endophytes.** (a-b) Barplots representing the endophytic
757 (surface-sterilized: S; dark grey bars) and epiphytic (non-sterilized: NS; light grey bars)
758 bacterial abundance in roots of the wheat varieties Energo (a) or Numeric (b). Strain name is
759 reported in the *x*-axis. Least-Squares-Means (lsmeans) and standard deviation of bacterial
760 abundance 7 dpi is expressed as log₁₀ of cfu (colony-forming units)/mg of root fresh weight.
761 Total number of plants analyzed (≥ 6 for NS treatment and 12 for S treatment, in at least 2
762 independent replicates) are indicated in Table S6. Significant differences were found in the
763 surface-sterilized roots. *P* values (corrected for FDR) ≤ 0.05 are indicated by *, ≤ 0.005 by **
764 and ≤ 0.0005 by *** (See Table S14). (c-d) Confocal microscope images of Energo wheat
765 roots inoculated with the FWPou15 GFP-tagged strain. 3D reconstruction of a cleared root
766 fragment (c). Root median focal plan (central) and transversal section reconstructions (bottom
767 and right) of a root fragment in absence of clearing (d). Green fluorescence corresponds to
768 FWPou15-GFP. Red and blue fluorescence correspond to root cell wall auto fluorescence.
769 Arrows indicate the presence of endophytic bacteria between epidermal and cortical cells.
770 Scale bars corresponds to 20 μ m.

771 **Fig. 6. *Rlv* can stimulate wheat root development in a genotype-dependent manner.**
772 Boxplots representing the variation in wheat total root length (a, c; cm) and lateral root
773 number (b, d) 12 dpi in 2 wheat varieties (Energo and Numeric) inoculated with the strains
774 A34 or FWPou15 (a, b), FWPou38 or IAUb11 (c, d). Controls (ctr) are non-inoculated plants.
775 Total numbers of plants analyzed (≥ 30 , in at least 2 independent replicates) are indicated in
776 Table S7. *P* values (corrected for FDR) ≤ 0.05 are indicated by *, ≤ 0.005 by ** and ≤ 0.0005
777 by *** (See Table S15).

778 **Fig. 7. *Rlv* can stimulate wheat root colonization by AMF in a genotype-dependent
779 manner.** (a-c) Boxplots representing the variability in the number of colonization sites by the
780 AMF *R. irregularis* isolate DAOM197198, 24 dpi, when inoculated alone (ctr) or in
781 combination with *Rlv* strains. Two wheat varieties, Energo (a, b) and Numeric (c, d) were co-
782 inoculated with the strains A34 or FWPou15 (a, c), FWPou38 or IAUb11 (b, d). Total
783 numbers of plants analyzed (≥ 31 , in at least 2 independent replicates) are indicated in Table
784 S8. *P* values (corrected for FDR) ≤ 0.005 are indicated by ** and ≤ 0.0005 by *** (See Table
785 S16). Significant differences, not represented on the boxplots, were also found in the Energo

786 variety between A34 vs FWPou38 ($P < .0001$) or IAUb11 ($P = 0.0007$), and FWPou15 vs
787 FWPou38 ($P = 0.0062$).

788 **Fig. 8. Success of wheat and legume plant colonization in the *Rlv* co-inoculation assays.**

789 Hierarchical clustering and heatmap based on the relative abundance values (\log_2) of each *Rlv*
790 found in the wheat roots and legume nodules during co-inoculation assays (Table S2 and
791 Boivin *et al.*, 2020b). FWPou38 or 3841 strains were used in the co-inoculation assays in
792 wheat or legumes respectively as they are undistinguishable by Illumina *nodD* sequencing.
793 Wheat cv. 'Energó', Lentil cv. 'Rosana', Pea cv. 'Kayanne', and Fababean cv. 'Diva'.

794

795 **Supporting information**

796 Fig. S1. Soil composition of field plots in which wheat varieties were sampled.

797 Fig. S2. Images of wheat plantlets grown in gnotobiotic conditions.

798 Fig. S3. *Rlv* Neighbor-Joining tree based on a portion of the *nodD* gene.

799 Fig. S4. Nodule sections obtained from vetch plants inoculated with *Rlv* strains isolated from
800 wheat roots.

801 Fig. S5. Nodulated faba bean root systems inoculated with *Rlv* strains isolated from wheat
802 roots.

803 Fig. S6. *Rlv* metabolic pathway reconstructions.

804 Fig. S7. Correlation between the total root length and the lateral root number measured in
805 wheat plantlets inoculated by *Rlv* strains.

806 Fig. S8. Metabolic reactions of bacterial tryptophan metabolism found in the FWPou15,
807 FWPou38 and IAUb11 strains.

808 Table S1. List of the wheat varieties sampled and the *Rlv* strains isolated.

809 Table S2. *gyrB* sequences obtained for strains isolated from wheat roots and the A34 strain.

810 Table S3. List of the *Rlv* strains used in the co-inoculation assays.

811 Table S4. Quality information on the co-inoculation assays.

812 Table S5. Results of the co-inoculation assays.

813 Table S6. Experimental design for the root colonization in single strain inoculation assays.

814 Table S7. Experimental design for the root development assays.

815 Table S8. Experimental design for the mycorrhiza assays.

816 Table S9. Relative abundances (from linear-mixed model) of the *Rlv* strains in roots during
817 the co-inoculation assays.

818 Table S10. Numbers of colony-forming units (cfu) in the single strain inoculation assays.

819 Table S11. Total root lengths and lateral root numbers in the root development assays.

820 Table S12. Number of colonization sites in the mycorrhiza assays.

- 821 Table S13. Statistical analysis of the co-inoculation assays.
- 822 Table S14. Statistical analysis of the in the single strain inoculation assays.
- 823 Table S15. Statistical analysis of the root development assays.
- 824 Table S16. Statistical analysis of the mycorrhiza assays.
- 825 Supplementary methods (S1-S9).
- 826

827 **Tables**

828 **Table 1.** Analysis of Deviance on the variation of the strain relative abundance inferred from the total
 829 number of reads obtained by Illumina MiSeq sequencing of a *nodD* fragment in the co-inoculation
 830 assays. Chisq: value of the type II Wald chi squared, Df: degree of freedoms. Significant results
 831 obtained after FDR correction are reported in bold.

| Factors | FWPou15 mix | | | FWPou32 mix | | | FWPou38 mix | | |
|--------------------|-------------|----|------------------|-------------|----|------------------|-------------|----|------------------|
| | Chisq | Df | P value | Chisq | Df | P value | Chisq | Df | P value |
| Sterilization | 0.08 | 1 | 0.7775 | 0.0012 | 1 | 0.945 | 0.0012 | 1 | 0.9727 |
| Strain | 545.25 | 19 | <2e-16 | 482.07 | 19 | <2e-16 | 605.93 | 19 | <2e-16 |
| Treatment : Strain | 27.05 | 19 | 0.1035 | 26.57 | 19 | 0.1151 | 24.83 | 19 | 0.1662 |

832 **Table 2.** Analysis of Deviance on the variation of bacterial colony-forming units (cfu) / mg of wheat
 833 roots obtained after inoculation with the A34, FWPou15, FWPou38 or IAUb11 *Rlv* strains. Chisq:
 834 value of the type II Wald chi squared, Df: degree of freedoms. Significant results obtained after FDR
 835 correction are reported in bold.

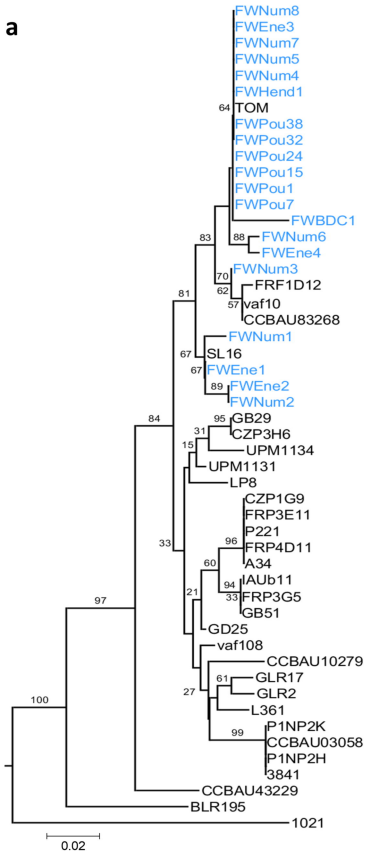
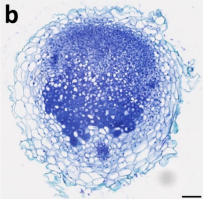
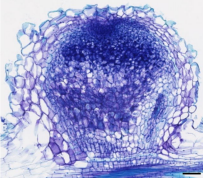
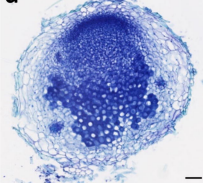
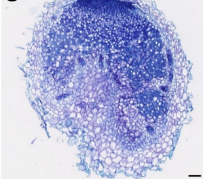
| Energic and Numeric | | | |
|-------------------------|-----------|----|-----------------|
| Factors | Chisq | Df | P value |
| Sterilization | 1121.6401 | 1 | 2.20E-16 |
| Variety | 0.0047 | 1 | 0.97533 |
| Strain | 53.9235 | 3 | 1.17E-11 |
| Sterilization : Variety | 4.2279 | 1 | 0.03976 |
| Sterilization : Strain | 32.7774 | 3 | 3.59E-07 |
| Variety : Strain | 9.8637 | 3 | 0.01976 |

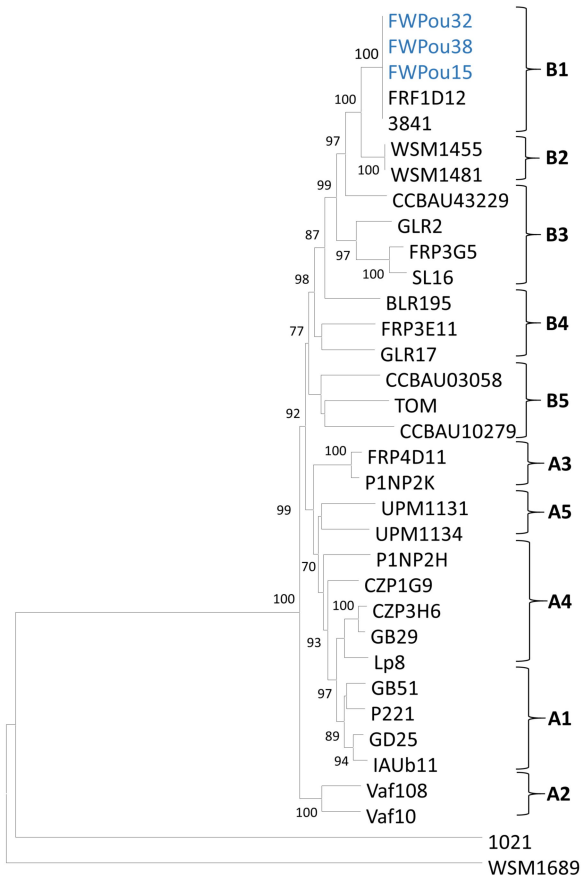
Table 3. Analysis of Deviance on the variation of the total root length and number of lateral roots obtained after inoculation with the A34, FWPou15, FWPou38 or IAUb11 *Rlv* strains. Analysis was performed on two datasets: A34-FWPou15 and IAUb11-FWPou38, as values for controls (plantlets inoculated with water) were different in the two datasets. Chisq: value of the type II Wald chi squared, Df: degree of freedoms. Significant results obtained after FDR correction are reported in bold.

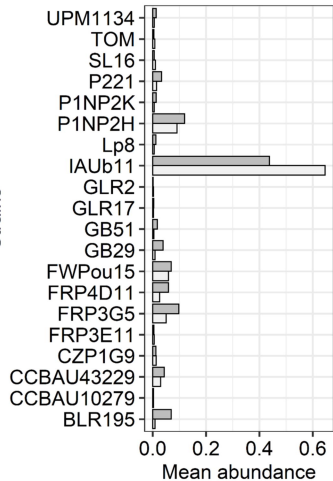
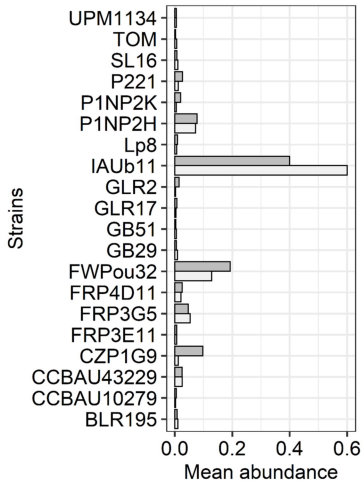
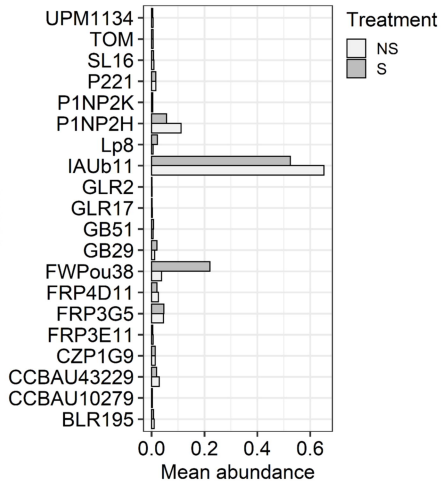
| Factors | Energó & Numeric | | | | | | Energó & Numeric | | | | | | |
|------------------|-------------------|----|-----------------|------------------|----|---------------------|-------------------|--------|----|------------------|---------|----|---------------------|
| | Total root length | | | N° lateral roots | | | Total root length | | | N° lateral roots | | | |
| | A34 & FWPou15 | | | | | | IAUb11 & FWPou38 | | | | | | |
| | Chisq | Df | <i>P</i> value | Chisq | Df | <i>P</i> value | Factors | Chisq | Df | <i>P</i> value | Chisq | Df | <i>P</i> value |
| Strain | 27.897 | 2 | 8.76E-07 | 24.119 | 2 | 5.7910E-06 | Strain | 1.6147 | 2 | 0.446028 | 11.8746 | 2 | 0.002639 |
| Variety | 23.397 | 1 | 1.32E-06 | 230.02 | 1 | < 2.2e-16 | Variety | 47.047 | 1 | 6.93E-12 | 128.79 | 1 | < 2.2e-16 |
| Strain : Variety | 7.2163 | 2 | 0.0271 | 21.132 | 2 | 2.58E-05 | Strain : Variety | 10.218 | 2 | 0.006041 | 7.5968 | 2 | 2.24E-02 |

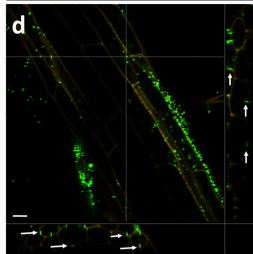
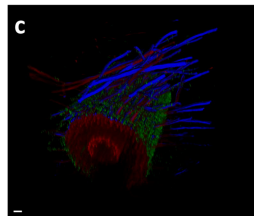
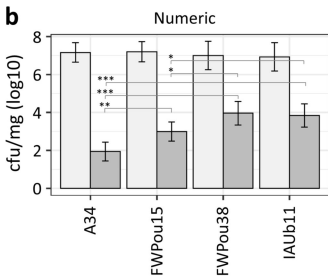
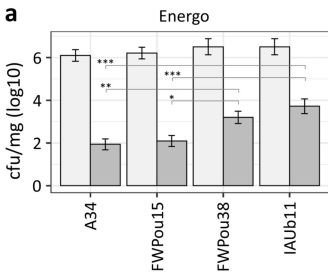
Table 4. Analysis of Deviance on the variation of the number of AMF colonization sites after inoculation with the A34, FWPou15, FWPou38 or IAUb11 *R/v* strains. Chisq: value of the type II Wald chi squared; Df: degrees of freedom. Strain refers to wheat roots inoculated with the four *R/v* strains or sterilized water (control). Significant result is reported in bold corresponding to P-value after FDR correction.

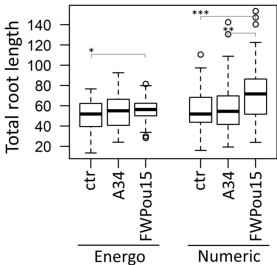
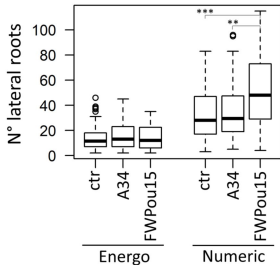
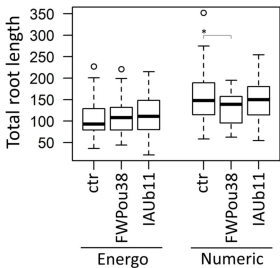
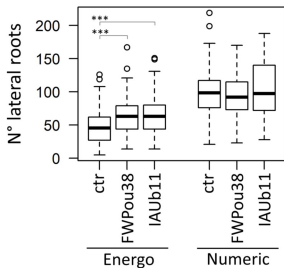
| Factor | Energo | | | Numeric | | |
|--------|--------|----|-----------------|---------|----|----------------|
| | Chisq | Df | <i>P</i> value | Chisq | Df | <i>P</i> value |
| Strain | 60.733 | 4 | 2.04E-12 | 3.5748 | 2 | 0.1674 |

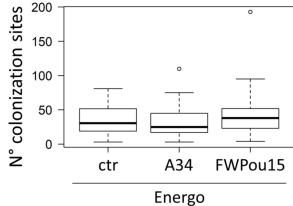
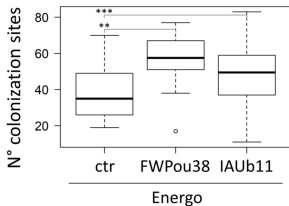
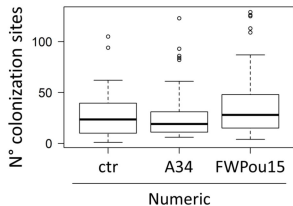
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