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1 **Biopriming of maize germination by the plant growth-promoting**
2 **rhizobacterium *Azospirillum lipoferum* CRT1**

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18

19 Abbreviations : ABA, abscisic acid; PGPR, plant growth-promoting rizobacteria

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21 Declarations of interest : none

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24 No graphic requires color reproduction

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26

27 **Abstract**

28

29 Plant growth-promoting rhizobacteria (PGPR) naturally aid plant growth, development and
30 tolerance to stress. Yield increase by the commercial isolate *Azospirillum lipoferum* CRT1
31 was recently attributed to an enhanced sprouting success. In order to provide the first
32 biochemical and physiological analysis of sprouting enhancement by PGPR, seed germination
33 and metabolism were followed by time-lapse photography and GC/MS-based metabolomics,
34 respectively, after inoculating two differentially-responding maize cultivars with *A. lipoferum*
35 CRT1. Bacterial growth on the seeds and plantlet development were also determined.
36 Bacterial inoculation of the seeds of one cultivar led to a 6-8 h hastening of radicle
37 emergence, increased surface bacterial counts, lower contents of energetic primary
38 metabolites before radicle emergence and increased photosynthetic yield, and root surface
39 area, in 3-leaf plantlets. None of these changes were observed on the other maize cultivar that
40 rather accumulated greater levels of stress-related metabolites shortly after radicle emergence.
41 Bacterial counts and cell division-driven central root growth increased in parallel and
42 similarly on both cultivars. *A. lipoferum* CRT1 stimulated pre-germinating or defense events
43 in a cultivar-dependent manner in maize after rapid (less than 24 h) recognition with initially
44 resting seeds. This PGPR isolate therefore bears agronomic potential as a biopriming agent.

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48 *Key words:* *Azospirillum*, biopriming, germination, maize, PGPR, seed metabolism.

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50 1. Introduction

51

52 Over the past few decades, inoculation of seeds with plant growth-promoting
53 rhizobacteria (PGPR) has proved to be an effective and ecologically-friendly agro-
54 engineering practice to increase the durability of food production and limit its ecological
55 impact (Duhamel and Vandenkoornhuysse, 2013; Gupta et al., 2015). PGPR constitute a
56 diverse group of soil bacteria that naturally associate with plant roots for a host-specific
57 reciprocal benefice whereby PGPR have prime access to root exudates and plant host growth
58 and tolerance to stress are enhanced. A meta-analysis of 91 field and 175 pot studies
59 conducted between 1981 and 2008 on the inoculation of cereal seeds with *Azospirillum*
60 isolates has for example concluded to an average 10-15% increase in grain and forage yields
61 respectively (Veresoglou and Meneses, 2010) making of this innocuous PGPR genus a prime
62 target for agronomic application developments. The physiological and molecular mechanisms
63 leading to yield increases by *Azospirillum* have been the subject of numerous studies.

64 Most isolates of *Azospirillum* elicit lateral root growth under field and laboratory
65 conditions (Couillerot et al., 2012) to increase the host plant root system surface area and
66 therefore its capacity to prospect for soil minerals and water. Auxin production by some
67 *Azopsirillum* strains has been hypothesized to contribute to lateral root growth (Bruto et al.,
68 2014). Mineral nutrition can be further improved by the expression of the bacterial gene *nifH*
69 responsible for the ammonification of gaseous nitrogen (Brock et al., 1993) or by the release
70 of bacterial siderophores (Pedraza, 2015). Probably needed to sustain root growth,
71 photosynthetic activity is enhanced by *Azospirillum* inoculation (Rozier et al., 2016).
72 Although it is tempting to conclude that increased mineral nutrition and photosynthesis lead to
73 increased yield at harvest, no such direct link has been established and some studies have
74 challenged this simplistic explanation. Nitrogen fixation has for example been shown to be
75 too meager to justify yield increases (Orhan et al., 2006; Pérez-Montañaño et al., 2014) and co-
76 inoculating a strong nitrogen-fixing agent like *Azotobacter* did not further increase
77 *Azospirillum*-based yield enhancement of cereals (Veresoglou and Meneses, 2010).

78 A recent study conducted two consecutive years and four fields with contrasted soil or
79 farming practices suggested that improved maize sprouting by the commercial PGPR strain *A.*
80 *lipoferum* CRT1 was the main cause of yield increases. Indeed, no correlation was found
81 between yield enhancement and the ability of the bacterium to modify its host root-to-shoot
82 biomass ratio (via enhanced root growth) or photosynthetic potential (Rozier et al., 2017). The
83 content of primary metabolites involved in nitrogen or phosphorus metabolism were also not

84 modified in proportion to yield modifications. However, increased yield correlated with 6-leaf
85 plantlets being larger in all of their proportions, a phenomenon that was already visible on
86 plant radicle length under artificial growth conditions as early as 48 h *post*-sowing. Since
87 increased yield was most pronounced in fields where sprouting success was below 80%, these
88 authors hypothesized that accelerated plant development during sprouting allowed faster
89 access to lower, and more secure, soil layers to increase seedling survival under unfavorable
90 climate conditions and, as a consequence, mature plant density and final yield (Rozier et al.,
91 2017). Improved sprouting by PGPR was also observed by several independent investigations
92 (Raj et al., 2004; Cassán et al., 2009; Kaymak et al., 2009; Nezarat and Gholami, 2009;
93 Noumavo et al., 2013; Rozier et al., 2017). A 22.44% increase of maize sprouting was, for
94 example, observed after a co-inoculation with *Pseudomonas fluorescens* and *P. putida* and a
95 20.39% increase after *Azospirillum* spp. inoculation (Noumavo et al., 2013). Maize and
96 soybean sprouting success was also improved by *Bradyrhizobium japonicum* E109 and *A.*
97 *brasilense* Az39 inoculation (Cassán et al., 2009). Nevertheless, precise developmental steps
98 affected by PGPR during sprouting have not yet been determined and a link between seed
99 germination and the growth-promoting activity of PGPR remains to be established.

100 In order to provide the first detailed characterization of the impact of *Azospirillum* on
101 plant seed germination, radicle emergence and growth were followed by time-lapse
102 photography using the same commercial inoculant, *A. lipoferum* CRT1, as used by Rozier et
103 al., 2017. Seed primary metabolite and starch contents were estimated throughout the
104 germination process as well as bacterial development on the surface of the seeds. To ensure
105 that any modification in the germination processes was the consequence of the
106 phytostimulatory activity of the bacterial partner and not of the exogenous addition of a large
107 number of live bacteria on a seed, all experiments were conducted on two maize cultivars, one
108 that displayed sprouting promotion and one that displayed no phytostimulation at this stage.
109 The impact of *A. lipoferum* CRT1 inoculation on the root length and photosynthetic yield of
110 3-leaf maize plantlets was also estimated on these two maize genotypes.

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113 **2. Materials and methods**

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115 2.1. Bacterial culture

116

117 Pre-culture and culture conditions from a glycerol stock of the PGPR strain
118 *Azospirillum lipoferum* CRT1 (Fages and Mulard, 1988) were similar as in (Rozier et al.,
119 2016). The adequacy of the bacterial suspension was evaluated by PCR with the *A. lipoferum*
120 CRT1 specific primers F1676-Q1 (5'-ATCCCGGTGGACAAAGTGGA-3') and 1837-Q2
121 (5'-GGTGCTGAAGGTGGAGAACTG-3'). The proper mobility and the absence of
122 contaminants were checked by light microscopy. The final bacterial suspension was diluted to
123 a concentration of 1.10^7 cells/mL with sterile UP water and immediately used to inoculate
124 maize seeds.

125

126 2.2. Seed inoculation

127

128 Seeds of the maize cultivars FriedriXX (RAGT) and FuturiXX (RAGT) were
129 calibrated for weight and shape to homogenize their germination responses. The experimental
130 design involved four treatment conditions : cultivars FriedriXX or FuturiXX that were either
131 inoculated with *A. lipoferum* CRT1 or mock-inoculated with sterile UP water. Mock
132 treatments with water acted as controls to ensure that *A. lipoferum* and not another
133 microorganism originally present on the seeds was the initiator of the described effects. Each
134 treatment was carried in sterile 12x12 cm square Petri dishes containing 20 seeds each. Two
135 layers of Whatman paper (Chromatography paper, 3 mm Chr, Whatmann international Ltd
136 Maidstone, England) were pre-wetted with sterile UP water and placed at the bottom of the
137 Petri dishes. The seeds were laid (sown) on the wet papers. All seeds were similarly
138 inoculated with either *A. lipoferum* CRT1 or sterile UP water by three successive additions of
139 50 μ L deposited alternatively on the different sides of the seed at 1 h intervals. This procedure
140 allowed a complete covering of the seed with the treatment solution. It also prevented a
141 temporary drying of the surface of the seeds (and the freshly deposited bacteria) by the fast
142 imbibition of the seeds that was complete 3 h after the start of water contact. Petri dishes were
143 kept closed during the inoculation process.

144 Each assay was replicated successively three times with three independently-prepared
145 bacterial cultures (three biological replicates).

146 The seeds were not surface-sterilized before the experiment because the objective of
147 this study was to provide a rational explanation for field observations (Rozier et al., 2017)
148 where non-sterile seeds were used. Surface-sterilized seeds also generate plantlets that are
149 prone to wilting under artificial growth conditions, a phenomenon that is rare with non-
150 sterilized seeds. The absence of *A. lipoferum* CRT1 in the original batches of seeds used in

151 this study was confirmed by PCR with specific primers (see above section on bacterial
152 culture).

153

154 2.3. Follow-up of the germination process

155

156 For the four treatment conditions, two closed Petri dishes (two technical replicates)
157 containing 20 inoculated seeds each (total of $2 \times 20 = 40$ seeds per condition) were kept closed
158 in a water-saturated atmosphere at 23°C with dim natural light (5 555 Lux - $99 \mu\text{mol.m}^{-2}.\text{s}^{-1}$
159 Photosynthetic Photon Flux Density at mid-day) during the entire germination process. Every
160 2 h, a camera (Canon 550D) was activated by a remote controller coupled to an
161 intervalometer. A graduated ruler was laid next to the seeds to measure the length of the
162 radicles on the pictures.

163

164 2.4. Bacterial density measurement on the seeds

165

166 For the four treatment conditions, two closed Petri dishes (two technical replicates)
167 containing 20 inoculated seeds each (total of $2 \times 20 = 40$ seeds per condition) were maintained
168 in a water-saturated atmosphere at 23°C with dim natural light (5 555 Lux - $99 \mu\text{mol.m}^{-2}.\text{s}^{-1}$
169 Photosynthetic Photon Flux Density at mid-day) during the entire germination process. At 0
170 h, 24 h, 44 h and 72 h *post*-sowing, five seeds of each Petri dish were sampled and placed in
171 3 mL of a tryptone-NaCl solution (1 and 8.5 g/l respectively) in a 15 mL Falcon tube. Tubes
172 were shaken by a FastPrep System (FastPrep 24™ 5G, MP Biomedicals, Santa Ana, USA) for
173 40 s at 6.0 m/s. Serial 10-fold dilutions of the supernatants of the bacteria-inoculated seeds
174 were performed and 100 μL of the 10^{-2} - 10^{-4} dilutions were spread in duplicate on Red Congo
175 agar medium. For the mock-inoculated conditions, the undiluted supernatant was spread. The
176 CFU (colony-forming units) were counted, averaged among technical replicates (seeds
177 originating from the two Petri dishes having received the same treatment) and the results
178 expressed as CFU per seed. PCR were performed on random colonies using the same set of
179 primers as described above for bacterial culture characterization to ensure that the enumerated
180 colonies corresponded to *A. lipoferum* CRT1.

181

182 2.5. Starch and primary metabolite content measurements

183

184 For the four treatment conditions, two closed Petri dishes containing 20 inoculated
185 seeds each (total of 2x20=40 seeds per condition) were maintained in a water-saturated
186 atmosphere at 23°C with dim natural light (5 555 Lux - 99 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ Photosynthetic
187 Photon Flux Density at mid-day) during the entire germination process.

188 The dry weight (DW) of the seeds of each treatment condition was determined at the
189 inoculation step, and 44 h and 72 h after the start of the inoculation (*post-sowing*) by
190 weighting ten seeds after being milled and stored 24 h at 110°C.

191 Five maize seeds (five independent seeds treated with the same bacterial culture) of
192 each treatment condition were then harvested at sowing and 44 h, and 72 h, *post-sowing*.
193 Their radicles were removed if visible and the rest frozen in liquid nitrogen in order to halt
194 metabolism. Individual seeds were crushed in liquid nitrogen with a mortar and a pestle. From
195 the powder of each seed, 100 mg was used for the analysis of starch content and 80 mg for
196 primary metabolite analysis (list of annotated metabolites in Table S1).

197 Starch content was estimated with an enzyme assay kit for total starch measurement
198 (AA/AMG, Libios, Pontcharra sur Turdine, France) according to the manufacturer
199 instructions. Results were expressed as percent (w/w) starch per unit of seed dry weight (%
200 DW). The entire assay was replicated a second time (biological replicate) with an independent
201 bacterial cultures.

202 All primary metabolites listed in Table S1 were subjected to the same extraction and
203 derivatization steps and were analyzed simultaneously on each extract. Extraction,
204 derivatization and analyses were adapted from previously published protocols (Rozier et al.
205 2016; 2017). In short, eighty milligrams of each milled seed (n=5, 4 conditions) were
206 transferred into a 2 mL Eppendorf tube set on ice and mixed with 300 μL of cold methanol (-
207 20°C) and 7 μL of ribitol (1 mg/mL - Sigma-Aldrich, St Louis, USA) as internal standard.
208 The samples were then heated at 70°C during 15 min under stirring and cooled at room
209 temperature. Two hundred μL of CHCl_3 was added to each tube and the tubes vortexed and
210 incubated 5 min at 37°C under stirring before adding 400 μL of UP water. The samples were
211 vortexed and centrifuged 5 min at 14 000 rpm. Four hundred μL of the supernatants were
212 introduced in a new 1.5 mL Eppendorf tube and centrifuged again 5 min at 14 000 rpm. The
213 supernatants were dried 4 h in a Speed Vacuum (Centrivap Concentrator, Labconco, Kansas
214 city, USA) and stored in a desiccator until the derivatization step. A control sample that
215 lacked seed powder was constructed in parallel.

216 Just before derivatization, the samples were dried during 30 min in a Speed Vacuum.
217 They were then solubilized in 40 μL of methoxylaminehydrochloride (20 mg/mL in pyridine -

218 Macherey-Nagel, Düren, Germany), vortexed and incubated 1h30 at 30°C under stirring
219 before introducing 70 µL of MSTFA (N-methyl-N-trimethylsilyl-trifluoroacetamide -
220 Macherey-Nagel, Düren, Germany). The samples were vortexed, incubated 30 min at 37°C,
221 transferred into vials and immediately analyzed by GC-MS.

222 GC/MS analyses (Agilent 7890A and 7000A, Santa Clara, USA) were carried out as
223 described (Rozier et al., 2016). Metabolite annotations were performed with TagFinder
224 (Luedemann et al., 2008) and baseline correction of the chromatogram with MetAlign
225 (Lommen and Kools, 2012). Peak areas of each chromatogram were estimated with the
226 MassHunter software (Agilent, Santa Clara, USA). For every annotated compound, peak areas
227 were normalized with those of the internal standard and expressed as percent of the maximum
228 value among all conditions.

229 A total of 126 metabolites was annotated in all samples that encompassed the two
230 cultivars, three time-points and five repeats (list of annotated metabolites in Table S1). Based
231 on the large time distribution window of radicle emergence of the batches of seeds, three time
232 points were chosen to represent the metabolomes of resting seeds (at sowing - t=0 h) and
233 seeds at late pre-germination (first seeds showing radicle emergence - t=44 h) and early *post-*
234 germination (the radicle has emerged from all seeds - t=72 h) stages.

235

236 2.6. Physiological measurements on 3-leaf plantlets

237

238 Seeds of both cultivars that were either inoculated with *A. lipoferum* CRT1 or with UP
239 water (8 seeds for each of the four treatments conditions) as described above under 'Seed
240 inoculation' were then sown in pots (5 cm diameter and 18 cm in height) filled with a natural
241 soil from a commercial maize field (luvisoil with a clay-loam texture devoid of limestone and
242 consisting of 26.9% sand, 38.3% loam and 34.7% clay with 324.9 g of water per kg of soil at
243 field water holding capacity) and grown indoor as described (Rozier et al., 2016). Eleven days
244 later, the plantlets had reached the 3-leaf stage. The photosynthetic potential of the second
245 leaf from the base was measured with a portable photosynthesis yield analyzer (Mini-PAMII,
246 Walz, Germany) equipped with the clip holder 2035-B as described (Rozier et al., 2016). The
247 plants were then uprooted and their root system imaged with a scanner (Expression 1680,
248 Epson, Suwa, Japan). The cumulative root length was estimated with the WinRhizo software
249 (Regent Instruments Inc., Quebec, Canada) as described (Rozier et al., 2016).

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251 2.7. Statistical analyses

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All statistical analyses were carried out with the open source software R using the RVAidememoire, mixOmics and ade4 packages (downloaded on March 2016 - R Development Core Team 2008). Non-parametric two-by-two Wilcoxon mean comparison tests were conducted to assess significance levels of differences in metabolite content means between inoculated and mock-inoculated conditions for each cultivar. Significance levels were recorded at $p < 0.1$ (‘), $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***)).

3. Results

3.1. Impact of *A. lipoferum* CRT1 on seed germination

The first seeds of the maize cultivars FriedriXX and FuturiXX that started to germinate (marked by radicle emergence) did so 42 h and 46 h *post*-sowing respectively and all seeds of these lots of seeds had germinated 62 h and 78 h *post*-sowing, respectively (data not shown). Imbibition time was similar for both cultivars and was roughly 3 h, most water uptake taking place within the first hour (data not shown).

The presence of *A. lipoferum* CRT1 on the seeds of FriedriXX hastened radicle emergence by 6-8 h, reducing by as much the times when the first and last seeds germinated. This is evidenced by a longer mean radicle length at times when no, or few, of the non-inoculated seeds had germinated (Fig. 1). This increase in radicle length was nevertheless short-lived and was only significant from 40 h to 52 h (less than 50% germination of non-inoculated seeds) *post*-sowing. It was maximum 44 h *post*-sowing (23-fold increase - $p < 0.002$, Wilcoxon non-parametric test). As the percent of non-inoculated seeds that had germinated neared 100% (62 h *post*-sowing) the mean radicle length of the two lots of seeds were similar.

To the opposite, bacterial inoculation had no effect on the timing of the emergence of the first radicle and on the mean radicle length of the maize cultivar FuturiXX (Fig. 1).

All of these results suggest that *A. lipoferum* CRT1 inoculation leads to an acceleration of radicle emergence in a cultivar-specific manner, and that it has no impact on radicle growth after its initial, rapid, protrusion from the seed.

285 3.2. Impact of *A. lipoferum* CRT1 on seed starch content

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287 Because starch degradation is a common *post*-germination marker, starch content was
288 measured during the first 72 h that followed sowing. No difference was seen between the non-
289 inoculated seeds of both cultivars at sowing ($69.97 \pm 5.37\%$ dry weight (DW) and $67.68 \pm$
290 4.77% DW, respectively) and contents remained constant up to 3 days *post*-sowing (Fig. 2).
291 Inoculation with *A. lipoferum* CRT1 had no significant impact on seed starch content ($p > 0.05$
292 – Wilcoxon test) of both cultivars.

293

294 3.3. Impact of *A. lipoferum* CRT1 on seed small metabolites

295

296 Unlike starch, many small metabolite contents are modified at each step of the *pre*-
297 and *post*-germination processes. A GC/MS-based metabolomic analysis of the seeds was
298 therefore performed in order to unveil the specific effects of *A. lipoferum* CRT1 on the seed
299 germination metabolism (see Table S1 for a list of annotated substances). A representation on
300 its first two axes of a principal component analysis (PCA) of the soluble metabolite contents
301 data matrix revealed that the metabolomes of the resting seeds (at sowing - $t=0$ h) of the two
302 maize cultivars were different (Fig. 3). Both were modified similarly by the germination
303 process 44 h and 72 h *post*-sowing since distribution ellipses (95% distribution estimated
304 from the variability among the five repeats) roughly moved in a similar direction on the PCA
305 plot to only gain partial overlap at 72 h. In agreement with a more precocious germination, the
306 metabolome of FriedriXX seeds was subject to more changes to the 44 h *post*-sowing.

307 Unsurprisingly, *A. lipoferum* CRT1 had no impact on the seed metabolomes at the
308 time of its deposition on the seeds (Fig. 3). Nevertheless, it impacted the metabolomes of the
309 seeds of both cultivars 44 h and 72 h *post*-inoculation. Ellipse separation (and hence
310 metabolome change) was the greatest for inoculated FriedriXX seeds between 0 h and 44 h
311 and was so weak for this inoculated cultivar between 44 h and 72 h that the metabolomes of
312 bacteria-inoculated seeds at 44 h, 72 h and of mock-inoculated seeds at 72 h were nearly
313 indistinguishable. Inoculation with *A. lipoferum* CRT1 therefore appeared to have hastened
314 germination-induced metabolic changes in FriedriXX. It, however, elicited changes that were
315 different from those induced by germination in the other cultivar, FuturiXX at 44 h and 72 h.
316 Ellipses of bacteria-inoculated seed metabolomes of this cultivar indeed did not follow the
317 same pattern of displacement by inoculation on the PCA plot as for FriedriXX.

318 In total, the contents of 67 substances were found to be significantly affected by
319 bacterial inoculation ($p < 0.1$ – Wilcoxon mean comparison test) (Table 1). In FriedriXX,
320 energy-related metabolites such as sugars and TCA cycle components were mostly affected
321 44 h after bacterial inoculation while mostly amino acid metabolism-related metabolites were
322 affected 72 h *post*-inoculation. The concentration of all of these metabolites was decreased by
323 bacterial inoculation. FuturiXX was subjected to fewer metabolic changes that were not
324 focused on any specific metabolic pathways. Most of them displayed concentration increases.
325 Noteworthy, three metabolites which contents were increased 72 h *post*-sowing/inoculation
326 were related to plant stress. These were the phytohormone abscisic acid (ABA), the key
327 precursor of phenolic phytoalexins, 4-hydroxycinnamic acid (*p*-coumaric acid), and the
328 microbial, non-plant, element N-acetylglucosamine.

329

330 3.4. Development of *A. lipoferum* CRT1 on maize seeds

331

332 In order to better understand the interaction between *A. lipoferum* CRT1 and its host
333 seed, its growth was estimated during the germination process of inoculated seeds of both
334 cultivars. The bacteria multiplied significantly on the surface of the seeds of both cultivars
335 during the 72 h that followed their inoculation on the seeds (Fig. 4). However, the timing of
336 the start of rapid bacterial division was different on both cultivars. On FuturiXX, it occurred
337 in parallel to radicle elongation. On FriedriXX, rapid bacterial development occurred a day
338 before radicle emerged of the first seeds and then increased more slowly to follow radicle
339 growth. As a consequence, CFU were greater on FriedriXX before radicle emergence ($t=24$ h)
340 and during early radicle emergence ($t=44$ h) than on FuturiXX. Bacterial density on the two
341 cultivars was no longer significantly different 72 h *post*-inoculation when all seeds had
342 germinated (Fig. 4).

343

344 3.5. Impact of *A. lipoferum* CRT1 on 3-leaf plantlets

345

346 To relate the observed germination effects to classically-measured effects of PGPR on
347 plants, modifications of the root system architecture and photosynthetic potential were
348 measured on 3-leaf plantlets of both cultivars. As shown on Fig. 5, *A. lipoferum* CRT1
349 inoculation of the seeds of FriedriXX led to a higher cumulative root length. It was not due to
350 longer central, or adventitious seminal roots but to increased lateral root number and growth
351 as previously described by numerous studies (Couillerot et al., 2012; Rozier et al., 2016). The

352 photosynthetic potential (Fv/Fm) was also increased in this cultivar. These two parameters
353 were unaffected in FuturiXX. Cumulative leaf area was not affected at this stage in both
354 cultivars (data not shown).

355

356 **4. Discussion**

357

358 It is well established that PGPR aid plants in various aspects of their development and
359 functioning (Vacheron et al., 2013) such as the growth of their supporting root system (Pérez-
360 Montañó et al., 2014), nitrogen, phosphorus and iron nutrition (Richardson et al., 2009),
361 photosystem functioning (Samaniego-Gómez et al., 2016) and protection to several stresses
362 such as drought (Vurukonda et al., 2016), cold (Kakar et al., 2016) and pathogen attack
363 (Beneduzi et al., 2012). A few reports have also shown that sprouting is positively affected by
364 several genera of PGPR such as *Pseudomonas*, *Azospirillum* and *Bradyrhizobium* (Raj et al.,
365 2004; Cassán et al., 2009; Kaymak et al., 2009; Nezarat and Gholami, 2009; Noumavo et al.,
366 2013; Rozier et al., 2017). Despite the potential impact of a higher germination rate on final
367 crop yield (Rozier et al., 2017), the present study is the first one to focus on the biochemical
368 and physiological mechanisms affected by PGPR during the first steps of the life of a plant.

369 Seed germination is a complex process that is terminated by the emergence of the
370 embryo radicle through the seed coat (Bewley, 1997). It is started by water imbibition that
371 increases seed weight in one quick step. This rise in internal water content will restore a
372 functional hydrophobic force to help cell membranes and proteins to reconfigure properly.
373 Mitochondria, and DNA, will be repaired to allow protein synthesis from stored and newly-
374 made RNAs. Germination inhibitors will be suppressed and small energetic substances will
375 diffuse throughout the seed to reach their usage targets. The radicle will then emerge thanks to
376 rapid cell elongation followed by a slower cell division-driven growth. Once the radicle
377 grows, seed weight increase resumes *via* active water intake. Internal polymeric energy stores
378 such as starch, proteins and lipids will only be consumed after germination (*i.e.* after radicle
379 emergence) to fuel seedling development (Bewley, 1997).

380 The time-lapse measurements of radicle growth and the metabolomic analyses of this
381 study both agree to indicate that the commercial PGPR strain, *A. lipoferum* CRT1, speeds pre-
382 germination events culminating in radicle elongation in a cultivar-specific manner and that it
383 does not promote radicle growth *post*-germination *via* increased cell division. For one of the
384 two cultivars, radicle emergence could indeed be hastened by 6-8 h in parallel to an
385 accelerated consumption of small energetic substance. Effects of the bacteria on the seed

386 metabolism were then meager once radicle had emerged, a time when root growth was similar
387 between PGPR-inoculated and non-inoculated seedlings and when starch degradation had not
388 yet started. As such, the PGPR strain acted as a bio-priming agent. If germination success was
389 close to 100% under the artificial conditions used in this study, it can drop significantly under
390 field conditions due to the unpredictable nature of weather conditions. Under such
391 circumstances, cereal seed priming is routinely conducted through a short storage at high
392 temperature and humidity or micronutrient application to hasten the germination of all seeds
393 and secure sprouting (Perera and Cantliffe, 1994; Mirshekari, 2012). The results of this study
394 confirm earlier suggestions (Kaymak, 2010; Reddy, 2012) that PGPR are a valuable
395 alternative to the canonical energy-demanding physical treatment of seeds.

396 Narrow, cultivar level, host-specificity is a common characteristic of the expression of
397 the phytostimulatory action of PGPR on their hosts (Walker et al., 2011; Chamam et al., 2013;
398 Drogue et al., 2014). This study reveals that hastened germination, increased production, and
399 growth, of lateral roots and improved functioning of the photosynthetic apparatus followed
400 the same pattern of cultivar specificity. Although a correlation between these apparently
401 unrelated physiological effects cannot be established without the testing of a large cohort of
402 maize genotypes, two similarities can be noticed between the growth responses of maize
403 seeds and 3-leaf plantlets to *A. lipoferum* CRT1. In both cases, cell division-mediated central
404 root growth was unaffected by bacterial inoculation so that increased root surface resulted
405 from either root cell elongation at germination or lateral root growth in plantlets. The
406 metabolomes of bacterially-inoculated seeds (this study) and plantlet saps (Rozier et al., 2016)
407 both revealed a decrease in glucose content in parallel to growth stimulation. If glucose was
408 the only common hexose to have a decreased concentration in 3-leaf plantlet sap (Rozier et
409 al., 2016), it was not the case in pre-germinating seeds where simple sugar content lowering
410 was less specific. In the later case, energetic metabolite content lowering may be the
411 consequence of an accelerated germination process. But it may also be its cause. It may
412 indeed also result from the observed more abundant bacterial growth on the surface of
413 growth-stimulated seeds a day prior to germination. It is also increasingly recognized that
414 common hexoses, and especially glucose, do not only serve as a reservoir of energy in plants
415 and that they initiate a cell signaling cascade that modulates the functioning of several plant
416 growth hormones including auxins (Gibson, 2004). Glucose, for example, impacts auxin-
417 regulated gene transcription to modulate root growth and development in *Arabidopsis* (Mishra
418 et al., 2009). Simple hexoses also act as germination inhibitors in maize (Dekkers and
419 Smeekens, 2018) by repressing the biosynthesis of auxins (LeClere et al., 2010). As glucose

420 exerts a product feedback inhibitory action on photosystem functioning, it is possible that
421 glucose may be a central regulator, and coordinator, of *A. lipoferum* CRT1 action in plants
422 from the lifting of dormancy to vegetative development. It may also explain why this isolate
423 generates auxin-type phenotypes in its hosts despite not having *ipdC* (C. Prigent-Combaret
424 pers. comm.), a key gene for auxin biosynthesis in other *Azospirillum* (Bruto et al., 2014).

425 The absence of a germination response of the maize cultivar FuturiXX to *A. lipoferum*
426 CRT1 does not signify an absence of response of this cultivar to the presence of the bacteria.
427 The rise in content of three substances in the seed metabolome 72 h *post*-inoculation indeed
428 suggests that the germinating seeds developed a defense response towards the PGPR. One of
429 them was abscisic acid (ABA), a substance up-regulating defense responses (Vishwakarma et
430 al., 2017) and inhibiting germination (Dekkers and Smeekens, 2018). In agreement, 4-
431 hydroxycinnamic acid, a central biosynthetic precursor of the phenolic defenses (Weisshaar
432 and Jenkins, 1998) also exhibited a higher content as well as the microbial-specific
433 component N-acetylglucosamine that is a central building block of Gram-positive bacterial
434 and fungal cell walls (Scheffers and Pinho, 2005) and of Gram-negative bacterial membrane
435 associated lipopolysaccharides (Gronow and Brade, 2001). The presence of this substance in
436 the inoculated seed extracts suggests some level of microbial degradation by plant defense
437 lytic enzymes present in cereal seeds (Roberts and Selitrennikoff, 1988; Grover, 2012). As the
438 seeds used in this study were not sterile, it is impossible to know whether it originated from
439 inoculated *A. lipoferum* CRT1 or previously present microorganisms. An analysis of the seed
440 transcriptome is now required to complement the present metabolomic analysis and determine
441 the extent of the defense response developed by the host germinating seed, a defense response
442 that, according to the results of this study, does not prevent *A. lipoferum* CRT1 development
443 but may be such that it impairs the induction of a growth response at all steps of the plant
444 development.

445 The nature of signaling substances is, to the present day, unknown between
446 *Azospirillum* and its hosts. The results of this study, however, suggest that they are at play as
447 early as 24 h after the contact between *A. lipoferum* CRT1 and resting maize seeds. They also
448 induced a unique pattern of cultivar-specific responses that either led to rapid bacterial growth
449 and accelerated radicle emergence or to delayed bacterial development and the upgrading of
450 seedling defenses.

451

452 **Competing interests**

453

454 The authors declare that they have no competing interests.

455

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457

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463

464

465 **References**

466

467 Beneduzi, A., Ambrosini, A., Passaglia, L.M.P., 2012. Plant growth-promoting rhizobacteria
468 (PGPR): Their potential as antagonists and biocontrol agents. *Genet. Mol. Biol.* 35,
469 1044–1051. <https://doi.org/10.1590/s1415-47572012000600020>

470 Bewley, J., 1997. Seed Germination and Dormancy. *Plant Cell* 9, 1055–1066.
471 <https://doi.org/10.1105/tpc.9.7.1055>

472 Broek, A.V., Michiels, J., van Gool, A., Vanderleyden, J., 1993. Spatial-temporal colonization
473 patterns of *Azospirillum brasilense* on the wheat root surface and expression of the
474 bacterial *nifH* gene during association. *Mol. Plant-Microbe In.* 6, 592–600.
475 <https://doi.org/10.1094/mpmi-6-592>

476 Bruto, M., Prigent-Combaret, C., Muller, D., Moëgne-Loccoz, Y., 2014. Analysis of genes
477 contributing to plant-beneficial functions in plant growth-promoting rhizobacteria and
478 related Proteobacteria. *Sci. Rep.* 4, e6261. <https://doi.org/10.1038/srep06261>

479 Cassán, F., Perrig, D., Sgroj, V., Masciarelli, O., Penna, C., Luna, V., 2009. *Azospirillum*
480 *brasilense* Az39 and *Bradyrhizobium japonicum* E109, inoculated singly or in
481 combination, promote seed germination and early seedling growth in corn (*Zea mays*
482 L.) and soybean (*Glycine max* L.). *Eur. J. Soil Biol.* 45, 28–35.
483 <https://doi.org/10.1016/j.ejsobi.2008.08.005>

484 Chamam, A., Sanguin, H., Bellvert, F., Meiffren, G., Comte, G., Wisniewski-Dyé, F.,
485 Bertrand, C., Prigent-Combaret, C., 2013. Plant secondary metabolite profiling
486 evidences strain-dependent effect in the *Azospirillum–Oryza sativa* association.
487 *Phytochemistry* 87, 65–77. <https://doi.org/10.1016/j.phytochem.2012.11.009>

488 Couillerot, O., Ramírez-Trujillo, A., Walker, V., von Felten, A., Jansa, J., Maurhofer, M.,
489 Défago, G., Prigent-Combaret, C., Comte, G., Caballero-Mellado, J., Moëgne-Loccoz,
490 Y., 2012. Comparison of prominent *Azospirillum* strains in *Azospirillum–*
491 *Pseudomonas–Glomus* consortia for promotion of maize growth. *Appl. Microbiol.*
492 *Biot.* 97, 4639–4649. <https://doi.org/10.1007/s00253-012-4249-z>

493 Dekkers, B.J., Smeekens, S.C., 2018. Sugar and abscisic acid regulation of germination and
494 transition to seedling growth. *Annu. Plant Rev.* 305–327.
495 <https://doi.org/10.1002/9781119312994.apr0286>

496 Drogue, B., Sanguin, H., Chamam, A., Mozar, M., Llauro, C., Panaud, O., Prigent-Combaret,
497 C., Picault, N., Wisniewski-Dyé, F., 2014. Plant root transcriptome profiling reveals a

498 strain-dependent response during *Azospirillum*-rice cooperation. *Front. Plant Sci.* 5,
499 e607. <https://doi.org/10.3389/fpls.2014.00607>

500 Duhamel, M., Vandenkoornhuyse, P., 2013. Sustainable agriculture: possible trajectories from
501 mutualistic symbiosis and plant neodomestication. *Trends Plant Sci.* 18, 597–600.
502 <https://doi.org/10.1016/j.tplants.2013.08.010>

503 Fages, J, Mulard, D., 1988. Isolement de bactéries rhizosphériques et effet de leur inoculation
504 en pots chez *Zea mays*. *Agronomie* 8, 309–314.
505 <https://doi.org/10.1051/agro:19880405>

506 Gibson, S.I., 2004. Sugar and phytohormone response pathways: navigating a signalling
507 network. *J. Exp. Bot.* 55, 253–264. <https://doi.org/10.1093/jxb/erh048>

508 Gupta, G., Parihar, S. S., Ahirwar, N. K., Snehi, S. K., Singh, V., 2015. Plant growth
509 promoting rhizobacteria (PGPR): current and future prospects for development of
510 sustainable agriculture. *J. Microbial Biochem. Technol.* 7, 96–102.
511 <https://doi.org/10.4172/1948-5948.1000188>

512 Gronow, S., Brade, H., 2001. Invited review: lipopolysaccharide biosynthesis: which steps do
513 bacteria need to survive?. *J. Endotoxin Res.* 7, 3–23.
514 <https://doi.org/10.1177/09680519010070010301>

515 Grover, A., 2012. Plant chitinases: genetic diversity and physiological roles. *Crit. Rev. Plant*
516 *Sci.* 31, 57–73. <https://doi.org/10.1080/07352689.2011.616043>

517 Kakar, K.U., Ren, X.-L., Nawaz, Z., Cui, Z.-Q., Li, B., Xie, G.-L., Hassan, M.A., Ali, E., Sun,
518 G.-C., 2016. A consortium of rhizobacterial strains and biochemical growth elicitors
519 improve cold and drought stress tolerance in rice (*Oryza sativa* L.). *Plant Biol.* 18,
520 471–483. <https://doi.org/10.1111/plb.12427>

521 Kaymak, H.C., 2010. Potential of PGPR in agricultural innovations, in: Maheshwari, D.K.
522 (Ed.), *Plant growth and health promoting bacteria*, Microbiology Monographs.
523 Springer, Berlin, Heidelberg, pp. 45–79.

524 Kaymak, H.Ç., Güvenç, İ., Yarali, F., Dönmez, M.F., 2009. The effects of bio-priming with
525 PGPR on germination of radish (*Raphanus sativus* L.) seeds under saline conditions.
526 *Turk. J. Agric. For.* 33, 173–179. <https://doi.org/10.3906/tar-0806-3>

527 LeClere, S., Schmelz, E.A., Chourey, P.S., 2010. Sugar levels regulate tryptophan-dependent
528 auxin biosynthesis in developing maize kernels. *Plant Physiol.* 153, 306–318.
529 <https://doi.org/10.1104/pp.110.155226>

530 Lommen, A., Kools, H.J., 2012. MetAlign 3.0: performance enhancement by efficient use of
531 advances in computer hardware. *Metabolomics* 8, 719–726.
532 <https://doi.org/10.1007/s11306-011-0369-1>

533 Luedemann, A., Strassburg, K., Erban, A., Kopka, J., 2008. TagFinder for the quantitative
534 analysis of gas chromatography—mass spectrometry (GC-MS)-based metabolite
535 profiling experiments. *Bioinformatics* 24, 732–737.
536 <https://doi.org/10.1093/bioinformatics/btn023>

537 Mirshekari, B., 2012. Seed priming with iron and boron enhances germination and yield of
538 dill (*Anethum graveolens*). *Turk. J. Agric. For.* 36, 27–33. [https://doi.org/10.3906/tar-](https://doi.org/10.3906/tar-1007-966)
539 [1007-966](https://doi.org/10.3906/tar-1007-966)

540 Mishra, B.S., Singh, M., Aggrawal, P., Laxmi, A., 2009. Glucose and auxin signaling
541 interaction in controlling *Arabidopsis thaliana* seedlings root growth and
542 development. *PloS one* 4, e4502. <https://doi.org/10.1371/journal.pone.0004502>

543 Nezarat, S., Gholami, A., 2009. Screening plant growth promoting rhizobacteria for
544 improving seed germination, seedling growth and yield of maize. *Pak. J. Biol. Sci.* 12,
545 26–32. <https://doi.org/10.3923/pjbs.2009.26.32>

546 Noumavo, P.A., Kochoni, E., Didagbé, Y.O., Adjanohoun, A., Allagbé, M., Sikirou, R.,
547 Gachomo, E.W., Kotchoni, S.O., Baba-Moussa, L., 2013. Effect of different plant
548 growth promoting rhizobacteria on maize seed germination and seedling development.
549 *Am. J. Plant Sci.* 4, 1013–1021. <https://doi.org/10.4236/ajps.2013.45125>

550 Orhan, E., Esitken, A., Ercisli, S., Turan, M., Sahin, F., 2006. Effects of plant growth
551 promoting rhizobacteria (PGPR) on yield, growth and nutrient contents in organically
552 growing raspberry. *Sci. Hortic.* 111, 38–43.
553 <https://doi.org/10.1016/j.scienta.2006.09.002>

554 Pedraza, R.O., 2015. Siderophores production by *Azospirillum*: biological importance,
555 assessing methods and biocontrol activity, in: Cassan, F.D., Okon, Y., Creus, C.M.
556 (Eds.), *Handbook for Azospirillum, technical issues and protocols*. Springer, Cham,
557 pp. 251–262.

558 Parera, C.A., Cantliffe, D.J., 1994. Presowing seed priming. *Hort. rev.* 16, 109–141.
559 <https://doi.org/10.1002/9780470650561.ch4>

560 Pérez-Montaña, F., Alías-Villegas, C., Bellogín, R.A., del Cerro, P., Espuny, M.R., Jiménez-
561 Guerrero, I., López-Baena, F.J., Ollero, F.J., Cubo, T., 2014. Plant growth promotion
562 in cereal and leguminous agricultural important plants: From microorganism

563 capacities to crop production. *Microbiol. Res.* 169, 325–336.
564 <https://doi.org/10.1016/j.micres.2013.09.011>

565 Raj, S.N., Shetty, N.P., Shetty, H.S., 2004. Seed bio-priming with *Pseudomonas fluorescens*
566 isolates enhances growth of pearl millet plants and induces resistance against downy
567 mildew. *Int. J. Pest Manage.* 50, 41–48.
568 <https://doi.org/10.1080/09670870310001626365>

569 Reddy, P.P., 2012. Bio-priming of seeds, in: Reddy, P.P. (Ed), *Recent advances in crop*
570 *protection*. Springer, New Delhi, pp. 83–90.

571 Richardson, A.E., Barea, J.-M., McNeill, A.M., Prigent-Combaret, C., 2009. Acquisition of
572 phosphorus and nitrogen in the rhizosphere and plant growth promotion by
573 microorganisms. *Plant Soil* 321, 305–339. <https://doi.org/10.1007/s11104-009-9895-2>

574 Roberts, W.K., Selitrennikoff, C.P., 1988. Plant and bacterial chitinases differ in antifungal
575 activity. *Microbiology* 134, 169–176. <https://doi.org/10.1099/00221287-134-1-169>

576 Rozier, C., Erban, A., Hamzaoui, J., Prigent-Combaret, C., Comte, G., Kopka, J., Czarnes, S.,
577 Legendre, L., 2016. Xylem sap metabolite profile changes during phytostimulation of
578 maize by the plant growth-promoting rhizobacterium, *Azospirillum lipoferum* CRT1.
579 *Metabolomics (Los Angel.)* 6, e2. <https://doi.org/10.4172/2153-0769.1000182>

580 Rozier, C., Hamzaoui, J., Lemoine, D., Czarnes, S., Legendre L., 2017. Field-based
581 assessment of the mechanism of maize yield enhancement by *Azospirillum lipoferum*
582 CRT1. *Sci. Rep.* 7, e7416. <https://doi.org/10.1038/s41598-017-07929-8>

583 Samaniego-Gómez, B.Y., Garruña, R., Tun-Suárez, J.M., Kantun-Can, J., Reyes-Ramírez, A.,
584 Cervantes-Díaz, L., 2016. *Bacillus* spp. inoculation improves photosystem II
585 efficiency and enhances photosynthesis in pepper plants. *Chil. J. Agri. Res.* 76, 409–
586 416. <https://doi.org/10.4067/s0718-58392016000400003>

587 Scheffers, D.J., Pinho, M.G., 2005. Bacterial cell wall synthesis: new insights from
588 localization studies. *Microbiol. Mol. Biol. R.* 69, 585–607.
589 <https://doi.org/10.1128/membr.69.4.585-607.2005>

590 Vacheron, J., Desbrosses, G., Bouffaud, M.-L., Touraine, B., Moëne-Loccoz, Y., Muller, D.,
591 Legendre, L., Wisniewski-Dyé, F., Prigent-Combaret, C., 2013. Plant growth-
592 promoting rhizobacteria and root system functioning. *Front. Plant Sci.* 4, e356.
593 <https://doi.org/10.3389/fpls.2013.00356>

594 Veresoglou, S.D., Meneses, G., 2010. Impact of inoculation with *Azospirillum* spp. on growth
595 properties and seed yield of wheat: a meta-analysis of studies in the ISI Web of

596 Science from 1981 to 2008. *Plant Soil* 337, 469–480. <https://doi.org/10.1007/s11104->
597 010-0543-7

598 Vishwakarma, K., Upadhyay, N., Kumar, N., Yadav, G., Singh, J., Mishra, R.K., Kumar, V.,
599 Verma, R., Upadhyay, R.G., Pandey, M., Sharma, S., 2017. Abscisic acid signaling and
600 abiotic stress tolerance in plants: a review on current knowledge and future prospect.
601 *Front. Plant Sci.* 8, e161. <https://doi.org/10.3389/fpls.2017.00161>

602 Vurukonda, S.S.K.P., Vardharajula, S., Shrivastava, M., SkZ, A., 2016. Enhancement of
603 drought stress tolerance in crops by plant growth promoting rhizobacteria. *Microbiol.*
604 *Res.* 184, 13–24. <https://doi.org/10.1016/j.micres.2015.12.003>

605 Walker, V., Bertrand, C., Bellvert, F., Moënne-Loccoz, Y., Bally, R., Comte, G., 2011. Host
606 plant secondary metabolite profiling shows a complex, strain-dependent response of
607 maize to plant growth-promoting rhizobacteria of the genus *Azospirillum*. *New Phytol.*
608 189, 494–506. <https://doi.org/10.1111/j.1469-8137.2010.03484.x>

609 Weisshaar, B., Jenkins, G.I., 1998. Phenylpropanoid biosynthesis and its regulation. *Curr.*
610 *Opin. Plant Biol.* 1, 251–257. [https://doi.org/10.1016/s1369-5266\(98\)80113-1](https://doi.org/10.1016/s1369-5266(98)80113-1)
611
612

613 **Figure legends**

614

615 **Fig. 1.** Impact of *A. lipoferum* CRT1 inoculation on maize radicle length. Two maize
616 cultivars, FuturiXX (balls) and FriedriXX (squares) were mock-inoculated (open symbols) or
617 inoculated (full symbols) with *A. lipoferum* CRT1. Mean radicle length +/- SD was calculated
618 on a lot of n=20 seeds. Wilcoxon non-parametric test between inoculated and mock-
619 inoculated seeds were conducted at each time point (* p<0.05; ‘ p<0.1). Similar results were
620 obtained during two additional independent biological replicates conducted with independent
621 bacterial cultures

622

623 **Fig. 2.** Impact of *A. lipoferum* CRT1 on maize seed starch content. Maize cultivars FuturiXX
624 and FriedriXX were mock-inoculated (white bars) or inoculated (black bars) with *A.*
625 *lipoferum* CRT1. The starch content of the seeds was estimated at 0 h, 44 h and 72 h post-
626 sowing and was expressed as % of dry matter (DW). No significant differences were found at
627 p<0.05 by using one-way ANOVA and two-by-two Wilcoxon non-parametric mean
628 comparison tests (n=5). Similar results were obtained with a separate biological replicate
629 generated with an independent bacterial cultures

630

631 **Fig. 3.** Principal component analysis (PCA) plot of maize seed metabolite contents. Cultivars
632 FuturiXX (Futu - balls) and FriedriXX (Frie - squares) were either mock-inoculated (NI -
633 empty symbols) or inoculated (I - filled symbols) with *A. lipoferum* CRT1 and analyzed for
634 metabolite content (n=5) at sowing (t0 - lightest grey) or at 44 h (t44 - medium grey) and 72 h
635 (t72 darkest grey) post-sowing

636

637 **Fig. 4.** Colony forming units (CFU) of *A. lipoferum* CRT1 on germinating maize seeds.
638 Microorganisms adhering to the seeds of cultivars FriedriXX (squares) and FuturiXX (balls)
639 were detached and the CFU of *A. lipoferum* CRT1 counted on a selective solid medium. Data
640 represent means +/- SE (5-seed extracts and 2 counts). Significance levels of the differences
641 between the two cultivars were assessed at each time point with the Wilcoxon mean
642 comparison test (***, p<0.001; **, p<0.01). Similar results were obtained with two biological
643 replicates made with independent bacterial cultures

644

645 **Fig. 5.** Modification of cumulative root length (a) and photosynthetic potential (b) of 3-leaf
646 maize plantlets inoculated with *A. lipoferum* CRT1. The length of the central, seminal and
647 lateral roots was summed. The maximum quantum efficiency of photosystem II (Fv/Fm) of
648 the second leaf from the bottom was used as an estimate of the youngest mature leaf
649 photosynthetic efficiency. FuturiXX and FriedriXX were either mock-inoculated (white bars)
650 or *A. lipoferum* CRT1-inoculated (black bars). Significance levels of the impact of the
651 bacterium were assessed with the Wilcoxon mean comparison test (***, p<0.001; *, p<0.05)

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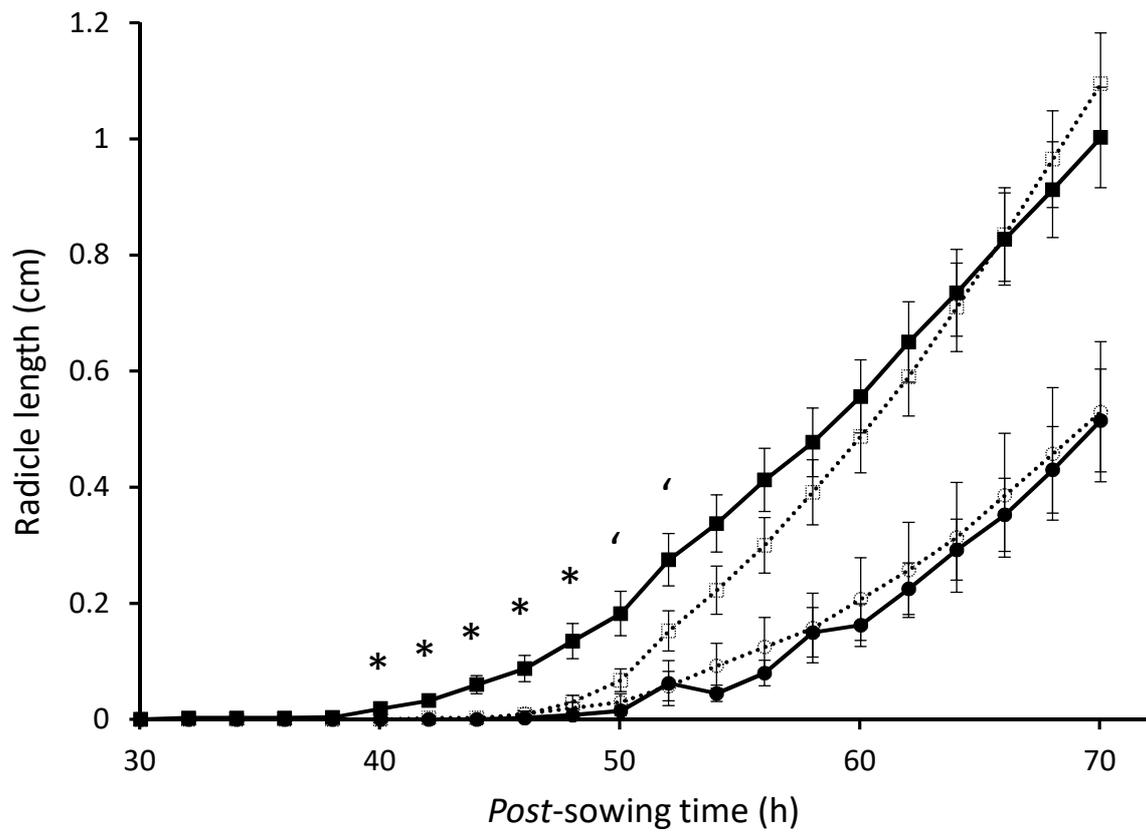
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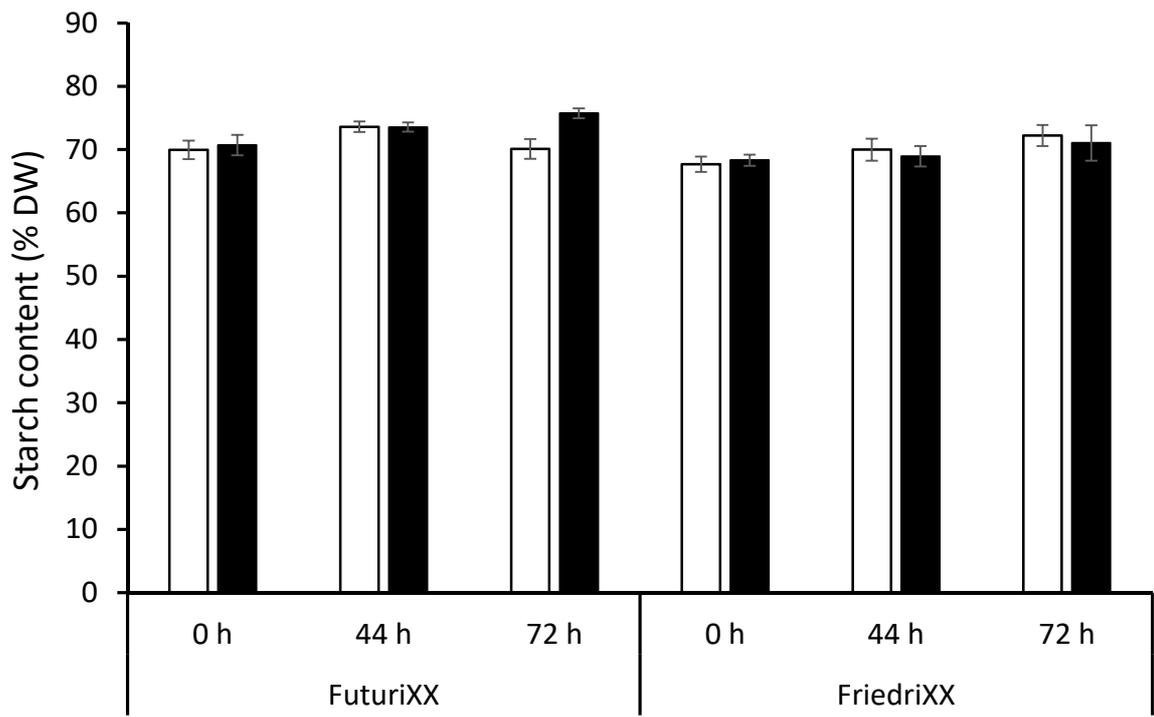
658 **Supporting information**

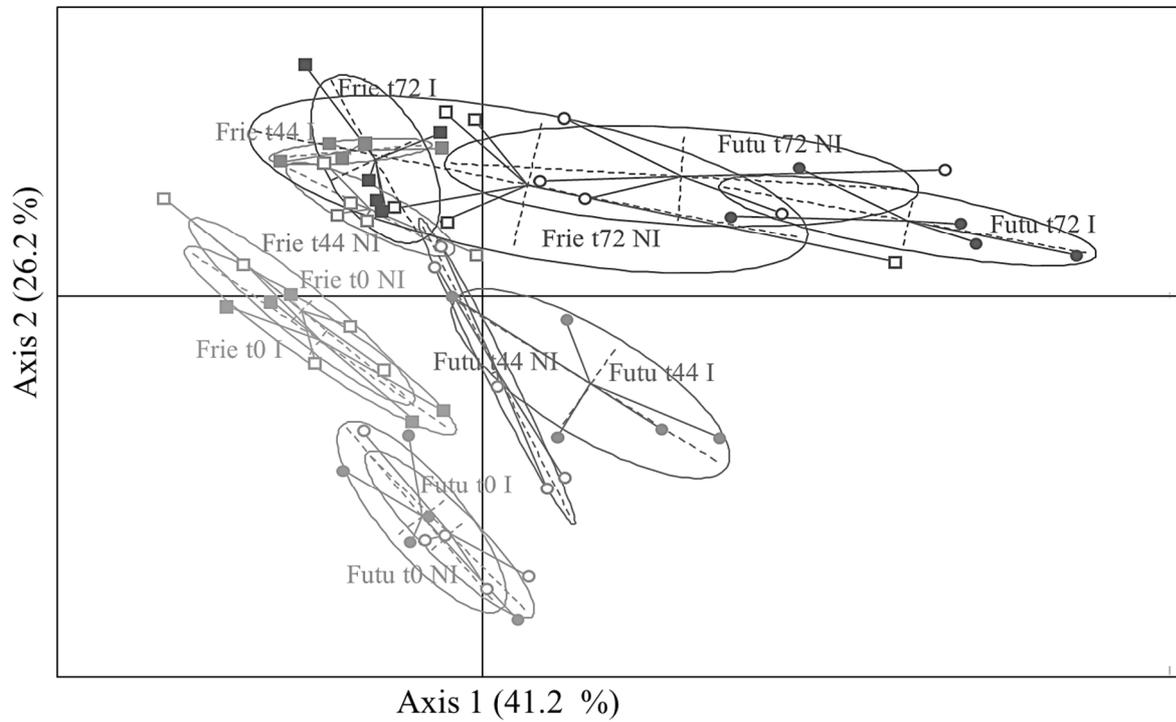
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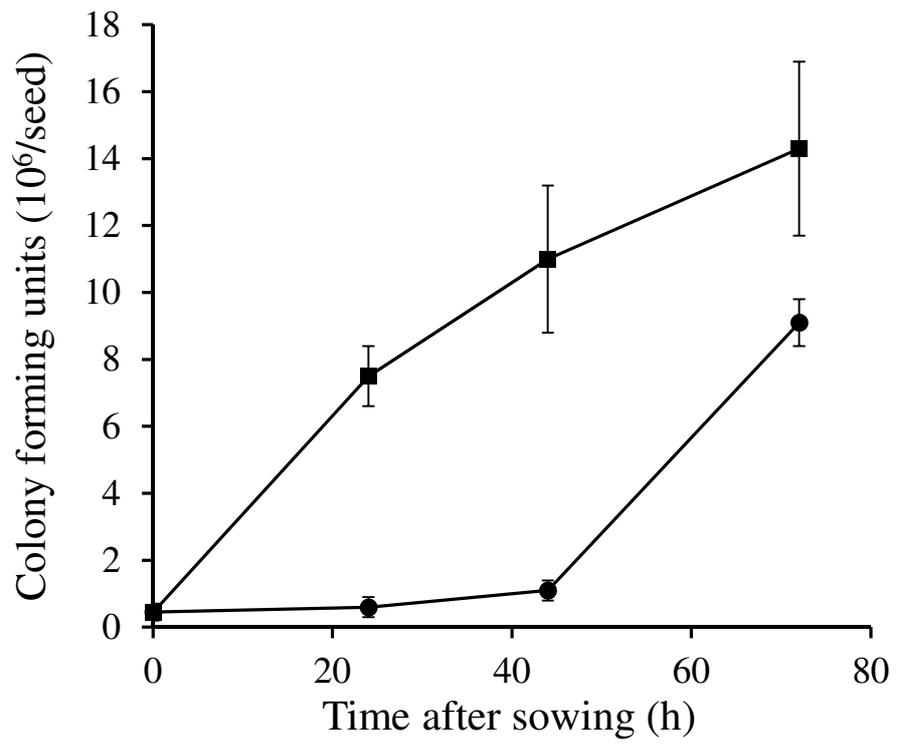
660 **Table S1** List of annotated substances

661









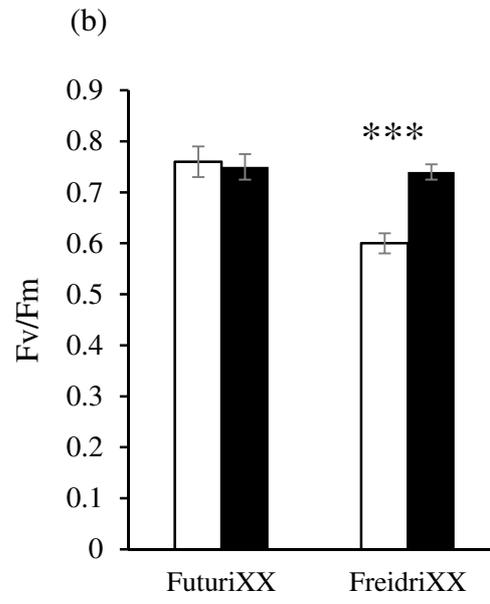
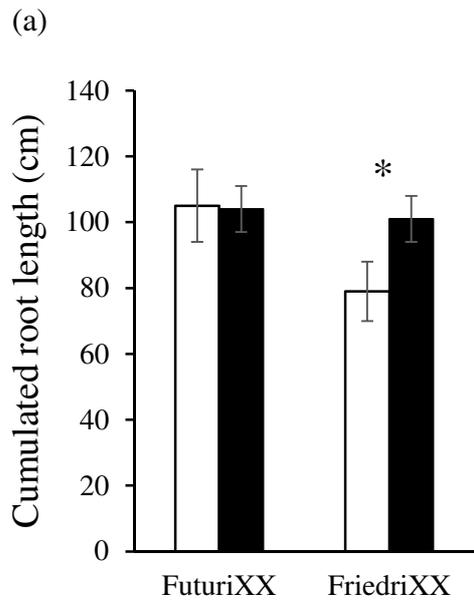


Table 1Metabolite content changes in maize seeds after inoculation with *A. lipoferum* CRT1.

Metabolite ^a	FuturiXX ^b		FriedriXX ^b	
	44 h	72 h	44 h	72 h
<i>Glycolysis-TCA and related</i>				
A105001-Lactic acid	63.7±14.9	-73.8±42.9	93.5±15.5	-70.0±3.6 *
A123004-Glyceraldehyde	36.5±14.7	28.9±2.3 '	5.5±1.4	-31.9±2.0 '
A176014-Aconitic acid, trans-	17.3±9.6	36.2±2.2 '	19.1±8.6	-58.8±14.4
A182003-Isocitric acid	17.7±4.6	40.8±3.9 '	16.8±6.6	-62.5±18.9
A134001-Succinic acid	0.1±2.5	25.8±10.3	-45.2±1.6 *	-44.5±13.1
A137001-Fumaric acid	13.9±4.3	35.9±9.1	-69.6±2.3 *	-24.8±8.4
<i>Sugars and related</i>				
A167011-Ribose	-8.5±7.1	48.5±15.2	-23.5±0.1 *	-0.6±0.1
A199002-Galactonic acid	6.0±3.3	17.4±13.2	-47.6±0.3 *	-1.0±0.2
A186001-Gulonic acid, 2-oxo-	-3.8±2.1	10.7±0.1 *	-44.2±1.7 *	-4.8±2.2
A189002-Glucose	7.8±11.0	9.3±1.3 '	-40.7±1.8 *	-4.5±6.7
A189001-Mannose	24.4±9.2	26.5±2.1 '	-50.7±2.6 *	-2.5±1.9
A233002-Glucose-6-phosphate	13.1±4.1	44.9±2.0 *	-31.3±0.7 *	-18.1±4.1
A188001-Galactose	-93.2±68.1	-45.7±15.3	-54.5±0.6 *	-10.4±3.7
A188004-Fructose	-95.6±48.8	-42.8±17.4	-66.2±3.5 *	16.8±5.2
A198006-Glucopyranose, D-	2.3±4.1	42.8±10.2	-28.5±0.2 *	-13.1±3.1
A267008- <i>beta</i> -D-Fructofuranosyl-(2.1)- <i>beta</i> -D-fructofuranose	-10.6±7.3	9.9±6.2	-17.8±0.4 *	-6.2±0.6
A211003-Ribose-5-phosphate	13.6±4.2	24.1±6.1	-22.8±1.7 '	40.5±7.1
A269006-Cellobiose, D-	-1.4±0.3	9.6±1.1 '	-16.8±0.9 '	-5.9±1.4
A179001-Arabinonic acid	8.8±9.5	35.2±2.7 '	22.4±6.5	-52.7±11.4
A231001-Mannose-6-phosphate	85.4±14.7	58.0±2.3 '	24.6±1	-45.9±9.4
A193004-Glucuronic acid	NA	38.2±1.7 '	NA	NA
A287001-Isomaltose	32.1±9.2	54.0±5.8 '	NA	-47.6±9.8
A172002-Rhamnose	-39.1±0.5 *	-26.1±0.4 *	-5.9±1.9	-7.8±3
A204001-Galactaric acid	79.0±3.1 '	21.3±9.1	74.7±20.1	-41±10.1
<i>Amino acids and related</i>				
A164007-Asparagine	-97.8±16.7	42.4±16.2	-47.3±2.0 *	-72.7±10.0
A118002-Isobutanoic acid, 2-amino-	2.1±0.9	20.8±8.1	-32.8±2.1 '	-22.5±6.1
A192003-Lysine	-88.9±22.9	-14.2±6.1	-62.7±3.2 '	24.1±7.1
A194002-Tyrosine	-5.1±4.9	46.3±14.7	-61.3±4.9 '	22.1±6.9
A140001-Threonine	91.3±26.2	73.4±18.7	-21.9±6.5	-60.6±3.5 *
A133001-Glycine	30.0±11.4	44.8±10.0	0.8±0.2	-46.4±0.4 *
A153002-Pyroglutamic acid	28.6±6.6 '	35.9±2.9 *	5.7±5.7	-34.9±2.2 *
A129002-Leucine	9.6±3.6	41.9±0.6 *	33.8±8.7	-57.4±3.8 '
A122001-Valine	17.2±9.1	50.4±4.2 '	-11.4±3.1	-33.7±5.5
A142007-Methionine	-20.7±5.9	57.6±5.1 '	-23.8±7.8	-80.2±13.9
A145025-Glycinamide	19.6±8.3	31.8±1.2 '	-6.8±2.2	32.1±8.2
A164001-Phenylalanine	46.0±9.6	37.7±2.5 '	33.0±7.3	-41.5±9.5
<i>Polyols</i>				
A149002-Threitol	-5.6±12.4	42.4±13.9	-33.7±1.2 *	-15.6±15.7
A150002-Erythritol	-1.3±1.8	0.5±1.5	-35.1±1.0 *	-18.8±4.2
A194001-Galactitol	25.4±4.9	20.7±6.1	-57.0±2.9 '	28.9±8.3
A193002-Mannitol	12.3±3.9	-30.2±12.6	-64.9±3.8 '	27.5±7.5
A171001-Xylitol	-3.3±1.1	14.6±0.4 *	-1.5±0.9	-0.5±0.1
<i>Nucleotides and related</i>				
A136001-Uracil	46.8±10.5	94.5±10.6	-39.8±2.1 '	-89.3±3.6 '
A249007-Thymidine	-17.9±9.1	16.9±14.1	NA	-28.9±1.4 '
<i>Hormones and polyamines</i>				
A175002-Putrescine	83.8±10.6	18.1±4.2	11.6±6.2	-19.4±2.1 '

A228007-Abscisic acid	16.6±8.2	48.8±2.7 *	21.9±7.2	-29.5±10.2
<i>Phenolic compounds</i>				
A210001-Ferulic acid, trans-	NA	14.6±0.1	-41.9±2.9 '	-9.2±6.1
A199001-Caffeic acid, cis-	35.8±10.2	44.1±0.1	-45.6±1.1 '	-7.3±3.1
A180006-Cinnamic acid, 4-hydroxy-	-34.5±9.7	-42.5±0.2 *	-3.9±1.5	-65.5±2.2 '
A178004-Vanillic acid	-7.7±3.1	66.9±3.2 '	-15.7±7.3	-15.1±3.2
A185001-Quinic acid	-94.0±13.1	8.6±10.0	-20.9±0.8 '	40.3±10.0
<i>Others</i>				
A122003-Malonic acid	-60.8±6.7 '	-15.7±6.3	-27.6±7.2	37.2±7.6
A129001-Phosphoric acid	23.7±0.4 *	21.4±6.7	4.5±6.7	-25.0±12.4
A135003-Glyceric acid	98.9±2.6 *	17.6±9.4	-2.2±0.9	-58.0±2.7 *
A106002-Glycolic acid	3.9±1.1	72.7±2.2 '	1.5±0.7	-37.3±0.1 *
A143001-Glutaric acid	20.3±8.1	26.3±0.1 '	-7.4±2.1	-30.8±0.3
A195002-Ascorbic acid	NA	35.7±0.8 '	NA	-30.8±6.7
A145012-Isobutanoic acid, 3-amino-	-98.5±21.2	30.7±1.6 *	-27.3±7.9	16.6±5.1
A203003-NA	NA	11.9±2.1 '	-41.2±1.9 *	6.6±3.1
A140003-NA	14.1±2.3	31.7±1.2	-70.3±2.3 *	-33.1±12.9
A155004-NA	-2.7±34.9	29.1±35.0	-88.0±4.1 *	-94.0±22.0
A237001-NA	25.6±8.1	19.8±0.1	32.4±3.7 '	-56.8±0.8 *
A230001-NA	16.4±6.1	57.5±4.5 '	-22.3±5.4	10.7±10.0
A234001-NA	50.6±18.0	87.5±3.7 '	NA	NA
A254003-NA	8.4±18.3	45.7±4.2 '	-32.5±8.4	-49.6±12.4
A143003-NA	25.1±9.5	34.1±2.7 '	-11.1±7.8	-3.2±1.0
A189019-NA	16.3±4.3	39.5±3.9 '	15.7±3.3	-59.0±6.8
<i>Non-plant substances</i>				
A207013-Glucosamine, N-acetyl-	20.6±0.4	20.6±0.4 *	-12.0±0.1	-23.3±0.3

^a Metabolites were identified by comparison to a standard. They are grouped according to their metabolite class (KEGG - <https://www.genome.jp/kegg/>). Compound names are preceded by their code in the Golm Metabolome Database (<http://gmd.mpimgolm.mpg.de/>) and they are ordered by increasing code number within each metabolite class. Unidentified metabolites are named according to their code in the Golm Metabolome Database followed by the NA label.

^b Ratios of mean content values of inoculated over mock-inoculated seeds of maize cultivars FuturiXX and FriedriXX at 44 h and 72 h *post*-sowing are listed and expressed as percentage values $((I - NI)/NI * 100)$ (*mean* ± percentage relative standard error). Wilcoxon non-parametric tests were conducted for each metabolite to compare inoculated and non-inoculated plants mean values (n=5 - bold lettering - * p < 0.05; ' p < 0.1). Only substances displaying significant content changes in at least one condition are listed. NA indicates that the metabolite was detected in less than two of the five replicate samples.