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Modern breeding had a negative impact on the ability of wheat to interact with beneficial root-colonizing bacteria termed PGPR, but this capacity has been maintained in a minority of modern cultivars. Breeding strategies should give further consideration to below-ground symbiotic interactions.

1 **Ancient wheat varieties have a higher ability to interact with plant growth-promoting**
2 **rhizobacteria**

3

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12 Running title: Wheat breeding and PGPR interaction

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35

Abstract

Plant interactions with Plant Growth-Promoting Rhizobacteria (PGPR) are highly dependent on plant genotype. Modern plant breeding has largely sought to improve crop performance, but with little focus on the optimization of plant × PGPR interactions. The interactions of the model PGPR strain *Pseudomonas kilonensis* F113 were therefore compared in 199 ancient and modern wheat genotypes. A reporter system, in which F113 colonization and expression of 2,4-diacetylphloroglucinol biosynthetic genes (*phl*) were measured on roots was used to quantify F113 × wheat interactions under gnotobiotic conditions. Thereafter, eight wheat accessions that differed in their ability to interact with F113 were inoculated with F113 and grown in greenhouse in the absence or presence of stress. F113 colonization was linked to improved stress tolerance. Moreover, F113 colonization and *phl* expression were higher overall on ancient genotypes than modern genotypes. F113 colonization improved wheat performance in the four genotypes that showed the highest level of *phl* expression compared to the four genotypes in which *phl* expression was lowest. Taken together, these data suggest that recent wheat breeding strategies have had a negative impact on the ability of the plants to interact with PGPR.

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Keywords : Breeding - DAPG - Fluorescence - Genotypes - PGPR - Phytostimulation - *Pseudomonas* - Wheat

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66 Introduction

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68 Agriculture productivity is a major issue, as world population may reach about 9 billion people
69 in 2050 (Gerland et al., 2014; KC & Lutz, 2017), needing enhanced crop yields in the coming
70 decades. Significant yield improvement was already achieved during the second half of the
71 20th century, based on the use of chemical inputs including pesticides and mineral fertilizers
72 (Cao, Lu, & Yu, 2018; Huffman & Evenson, 2001), drainage/irrigation, and modern breeding
73 to select crop genotypes efficient at taking full advantage of farming inputs (Baranski, 2015;
74 York, Galindo-Castañeda, Schussler, & Lynch, 2015). However, the consequences of these
75 farming conditions on the interactions between plant roots and the associated bacterial
76 community are not well documented (Engelhard, Hurek, & Reinhold-Hurek, 2000; Germida &
77 Siciliano, 2001; Pérez-Jaramillo et al., 2017).

78 Modern breeding is typically carried out under optimal agronomic conditions, which is
79 likely to limit the potential added-value resulting from beneficial plant-microbe interactions
80 (Sasaki et al., 2010; Schmidt, Bowles, & Gaudin, 2016; Weese, Heath, Dentinger, & Lau,
81 2015). In the case of soil bacteria, this involves Plant Growth-Promoting Rhizobacteria
82 (PGPR), which may stimulate root growth, improve nutrient uptake (Kumar, Maurya, &
83 Raghuwanshi, 2014; Majeed, Kaleem Abbasi, Hameed, Imran, & Rahim, 2015), alleviate plant
84 stress (Barnawal et al., 2017; Furlan et al., 2017; García et al., 2017; Pande, Ns, & Bodhankar,
85 2016) or protect the plants from pathogens (Díaz Herrera, Grossi, Zawoznik, & Groppa, 2016;
86 Keshavarz-Tohid et al., 2017). These effects rely on various modes of action, such as
87 increasing nutrient availability, modulating plant hormonal balance (Cassán, Vanderleyden, &
88 Spaepen, 2014; Saleem, Arshad, Hussain, & Bhatti, 2007), and/or producing bioactive
89 metabolites (Couillerot et al., 2011; Vacheron et al., 2018). Since these beneficial effects would
90 be of less interest under optimum conditions, it could be that the ability of crop genotypes to
91 interact with PGPR populations, which are naturally present in soil, was not selected for during
92 modern breeding (Morgan, Bending, & White, 2005; Schmidt et al., 2016; Weese et al., 2015).
93 This type of trait may even have been counter-selected in the case where it involves a cost for
94 the plant. This hypothesis has been evoked on several occasions (Kiers, Palmer, Ives, Bruno,
95 & Bronstein, 2010; Morgan et al., 2005; West, Kiers, Simms, & Denison, 2002), but little has
96 been done to test it experimentally with PGPR. In the case of mycorrhizal fungi, some studies
97 reported that modern breeding lead to reduced dependence on mycorrhizal symbiosis (Hetrick,
98 Wilson, & Cox, 1992; Hetrick, Wilson, & Cox, 1993), while in a more recent meta-analysis it
99 was concluded that new cultivars were more mycorrhiza-responsive when compared to
100 ancestral genotypes (Lehman, Barto, Powell, & Rillig, 2012). Given these inconsistent results
101 in the literature, whether or not modern plant breeding has modified the ability of crop plants
102 to interact with plant-beneficial microorganisms remains an open question. If the ability of

103 modern genotypes to interact with PGPR was partially or progressively lost, it could provide a
104 basis to understand why the magnitude of PGPR effects differs from one variety to the next
105 (Furlan et al., 2017; Kazi, Deaker, Wilson, Muhammad, & Trethowan, 2016; Neiverth et al.,
106 2014; Vacheron et al., 2016). It would also mean that ancient genotypes could represent a
107 source of PGPR interaction traits, which may be of potential value for future PGPR-based
108 breeding (Schmidt et al., 2016; Wei & Jousset, 2017).

109 The first objective of this work was to test the hypothesis that PGPR interaction ability
110 had not been favored during modern breeding. The second objective was to compare, under
111 stress conditions, PGPR inoculation effects on the growth of wheat genotypes of different
112 PGPR interaction abilities. To address these objectives, a collection of 199 bread wheat
113 accessions, representing world-wide crop genetic diversity and including both ancient
114 genotypes and modern genotypes, was screened for the ability to interact with the model
115 PGPR strain *Pseudomonas kilonensis* F113. This strain produces 2,4-diacetylphloroglucinol
116 (DAPG), an antimicrobial compound at high concentration (Schnider-Keel et al., 2000;
117 Shanahan, O'Sullivan, Simpson, Glennon, & O'Gara, 1992) but with auxinic-type root-
118 branching properties at lower concentration (Brazelton, Pfeufer, Sweat, McSpadden Gardener,
119 & Coenen, 2008; Vacheron et al., 2018). The pseudomonad stimulates growth of *Arabidopsis*
120 (Vacheron et al., 2018), maize (Walker et al., 2012) and wheat (Couillerot et al., 2011).
121 Importantly, bacteria closely related to *P. kilonensis* F113 seem widespread in arable soils
122 (Costa et al., 2007; Landa et al., 2002; Mazzola, Funnell, & Raaijmakers, 2004). An innovative
123 screening method of double fluorescent tagging was developed to measure root colonization
124 by *P. kilonensis* F113 and expression of the DAPG genes *phl*. Uneven fluorescence levels
125 were obtained depending on the wheat genotypes, modern genotypes interacting less with the
126 PGPR overall. Then, to evaluate the relevance of the screening, wheat accessions showing
127 contrasted results during the screening (including both ancient and modern genotypes) were
128 selected to compare their response to F113 inoculation, under conditions of water stress and
129 nutrient deprivation.

130

131

132 **Materials and Methods**

133

134 **Panel of wheat accessions**

135 A total of 199 accessions of wheat (*Triticum aestivum*) were used (Supporting information
136 Table S1). They included (i) the 196CC core-collection (Plessis, Ravel, Bordes, Balfourier, &
137 Martre, 2013) sub-sampled from the INRA worldwide bread wheat collection of 372 accessions
138 set up by Balfourier *et al.* (2007), based on field evaluation data (Bordes, Branlard, Oury,
139 Charmet, & Balfourier, 2008), geographic origin and registration date, (ii) the two reference

140 (modern) varieties Hendrix and Skerzso, and (iii) the genome-sequenced reference (landrace)
141 Chinese Spring. These accessions were chosen to maximize genetic diversity, as indicated by
142 representative genome-wide molecular markers (Balfourier et al., 2007), and they originated
143 from 38 different countries of six continents. Among them, 7 presented major germination
144 problems on agar plate and were removed from the panel, leaving a total of 192 wheat
145 genotypes. The remaining 192 genotypes included 77 ancient genotypes (i.e. 35 landraces,
146 plus 42 old varieties from the 19th century to 1960), as well as 115 modern genotypes (i.e.
147 developed after 1960 and the introduction of *Rht-B1b* (*Rht1*) and *Rht-D1b* (*Rht2*) alleles from
148 the Japanese variety Norin 10 that conferred reduced gibberellin response; Borlaug, 1968;
149 Borojevic, & Borojevic, 2005; Peng et al., 1999).

150

151 **Bacterial strain construction and growth conditions**

152 For the screening, we performed chromosomal insertion (Koch, Jensen, & Nybroe, 2001) into
153 *P. kilonensis* F113 (previously classified as *P. fluorescens*) of the construct *attTn7::miniTn7-*
154 *Gm-Ptac-mCherry* for constitutive expression of reporter gene *mCherry* (Rochat, Péchy-Tarr,
155 Baehler, Maurhofer, & Keel, 2010). The gentamicin-resistance cassette was then replaced with
156 a kanamycin-resistance cassette by homologous recombination, using plasmid pCM184 (Marx
157 & Lidstrom, 2002), and proper replacement was verified using PCR and restriction enzymes
158 (not shown). Finally, the derivative *P. kilonensis* F113-*mCherry*(*Pphl-egfp*) was obtained by
159 electroporation (Vacheron et al., 2018) of the gentamicin-resistance plasmid pOT1e carrying
160 a copy of the promoter of the *phl* operon fused to the promoterless reporter gene *egfp*,
161 constructed as described previously (Vacheron et al., 2016).

162 The inoculum for the screening was prepared by growing strain F113-*mCherry*(*Pphl-*
163 *egfp*) for 24 h in Minimal Medium (MM) broth (García de Salamone, Hynes, & Nelson, 2001)
164 supplemented with gentamycin (25 µg/mL) and kanamycin (50 µg/mL), at 27°C with shaking
165 (180 rpm). For the greenhouse experiment, the wild-type *P. kilonensis* F113 was grown for
166 24 h in MM broth without antibiotics, at 27°C, with shaking (180 rpm).

167

168 **Wheat inoculation in the screening experiment**

169 Wheat seeds were surface-disinfected by consecutive immersion for 1 min in 70% ethanol,
170 then for 40 min with shaking (180 rpm) in sodium hypochlorite solution (Na_2CO_3 0.1 g, NaCl
171 3 g and NaOH 0.15 g in 100 mL distilled water) supplemented with 10% commercial bleach
172 (containing 9.6% of active chlorine) and 0.01% of Tween 20%. They were washed three times
173 (5 min each) with sterile water, and soaked 60 min in a last bath of sterile water. For pre-
174 germination, disinfected seeds were placed on plates containing sterile agar for plant culture
175 at 8 g/L (Agar A7921; Sigma-Aldrich, Saint-Quentin Fallavier, France) and incubated in the
176 dark for 24 h at 27°C. The one-day-old seedlings were then transferred in 120 × 120 mm

177 square Petri dishes containing 50 mL of sterile agar for plant culture (3 seedlings per dish). No
178 nutrient solution was added. The seedlings were inoculated with 50 μ L of cell suspension
179 containing 5×10^7 CFU (OD_{600} adjusted to 8.0, giving 10^9 CFU/mL) from an overnight culture
180 of F113-*mCherry(Pphl-egfp)*, whose cells had been washed and resuspended in $MgSO_4$ 10
181 mM. For each of the 192 wheat genotypes, 3 seeds were not inoculated and 3 others were
182 inoculated with F113-*mCherry(Pphl-egfp)*. The inoculated seedlings and the controls were
183 placed for seven days in a growth chamber at 21°C, with a 16/8 h day/night cycle and 60%
184 hygrometry.

185

186 **Root sampling and bacterial fluorescence measurements**

187 To evaluate the ability of each wheat accession to interact with *P. kilonensis* F113, we
188 developed a simplified screening method to enable large-scale, robust comparison of the 192
189 accessions, based on measurements of bacterial fluorescence. After seven days of growth,
190 the plantlets were removed from agar, each root system was cut in 1-cm fragments and all
191 fragments from a same plant were introduced into a 15-mL Falcon tube containing 3 mL of
192 $MgSO_4$ 10 mM and three steel beads (5 mm diameter). The roots were ground for 1 min (twice)
193 with a FastPrep-24 Classic Instrument (MPbiomedicals, Santa Ana, CA) at maximum speed
194 (6 m/s) and room temperature. The biggest plant debris were pelleted by centrifugation for 2
195 min at 1,500 rpm, and 200 μ L of each supernatant were transferred in black 96-well plates with
196 a clear bottom. The fluorescence intensities of supernatants were then measured with an
197 Infinite M200 pro microplate reader (Tecan, Männedorf, Switzerland). EGFP level was
198 recorded using an excitation wavelength of 488 nm and an emission wavelength of 530 nm,
199 and mCherry level using an excitation wavelength of 587 nm and an emission wavelength of
200 661 nm. The ratio between green and red fluorescence intensities was computed and used as
201 a proxy for the *phl* induction rate. F113 colonization, *phl* expression and *phl* induction rate from
202 each individual inoculated plant were used to compute the means for each wheat genotype,
203 after subtracting the mean fluorescence intensity of the three corresponding non-inoculated
204 plantlets.

205

206 **Screening validation and confocal microscopy observations**

207 To confirm the screening results, an alternative fluorescence experiment was performed using
208 20 genotypes that had shown contrasted fluorescence intensities on their roots during the
209 screening (Table 1) and twice as many replicates. To select these genotypes, a PGPR
210 interaction score was calculated for each genotype as the sum of its ranks (i.e. the worst rank
211 was 1 and the best one 192) for F113 colonization, for *phl* expression and for *phl* induction
212 rate. Seed surface-disinfection and seedling inoculation were done as described above to
213 obtain six plants per genotype. After seven days of growth, each root system was cut in 1-cm

214 fragments and all fragments from a same plant were introduced into a 2-mL Eppendorf tube
215 containing 1.5 mL of MgSO₄ 10 mM (without steel beads). The roots were shaken for 2 × 5
216 min with a TissueLyser (Qiagen, Hilden, Germany) at maximum speed (30 Hz) and room
217 temperature. Then, the fluorescence of each supernatant was measured, as described above.
218 Root biomass was also determined.

219 To verify that spectrofluorimeter measurements were truly related to the
220 colonization/expression of the fluorescent F113 derivative, the same 20 genotypes were
221 inoculated (two plantlets per genotype) and grown as described above. For each plant, two
222 root fragments were observed with a confocal microscope (Carl Zeiss, Le Pecq, France), using
223 an excitation laser light of 488 nm and an emission filter of 504-555 nm for EGFP green
224 fluorescence, and an excitation laser light of 561 nm and an emission filter 570-636 nm for
225 mCherry red fluorescence, with the same gains for all samples.

226

227 **Soil pot experiment**

228 A greenhouse experiment was performed with eight wheat genotypes giving contrasted
229 screening results, i.e. Coronation, Concurrent (ancient genotypes), Amifort and D130-63
230 (modern genotypes), which showed good F113 interaction results, as well as Jaszaji TF,
231 Odesskaya16 (ancient genotypes), Danubia and Hendrix (modern genotypes), which showed
232 weak interaction results (Table 1). For each genotype, plants from seven pots were inoculated
233 with F113 and subjected to abiotic stress, whereas plants from seven other pots were
234 inoculated with F113 but not subjected to stress. For non-inoculated controls, plants from
235 seven pots were subjected or not to stress for each genotype. Thus, the whole experiment
236 contained 224 pots.

237 On the first day, wheat seeds were surface-disinfected by stirring them 40 min in
238 sodium hypochlorite solution (see above), washed three times 5 min with sterile water, and
239 soaked in a last bath of sterile water for 60 min. They were put in 2-dm³ pots containing 1.8 kg
240 of sieved non-sterile soil (loam, organic matter 5.5%, pH_{H₂O} 6.0) taken from the topsoil of an
241 arable luvisol located at La Côte Saint-André (France). At first, the soil was watered every two
242 days to maintain a water content of 22% w/w (corresponding to 78% of the water holding
243 capacity ; El Zemrany et al., 2007). Water content was adjusted by weighing the pots every
244 two days and adding the volume of water that had been lost. Each pot received four seeds,
245 and the number of plants was reduced to three at seven days and then two seven days later.
246 In half the pots, each seed was inoculated with 200 µL of a cell suspension of *P. kilonensis*
247 F113 (obtained as described above) containing 2.5 × 10⁶ CFU, whereas seeds in the other
248 half received 200 µL of MgSO₄ 10 mM each.

249 The experiment was run in a greenhouse (randomized block design), with a 16/8 h
250 day/night cycle at respectively 24/20°C and 40/60% relative humidity. At 14 days, a combined

251 stress of water and nutrient deficiencies was applied to half the non-inoculated pots and half
252 the inoculated pots. To this end, soil was left to dry to 12 % w/w water content (corresponding
253 to 43% water holding capacity) until the end of the experiment in the stress conditions only,
254 and a NPK nutrient solution (Plant-Prod 20-20-20; Plantproducts, Leamington, ON) was used
255 to bring 16 mg N/plant (8 mg each on days 14 and 21) in the non-stress conditions only.

256 At the four-leaf stage (i.e. one-month post-inoculation), all the plants were harvested.
257 In each pot, one plantlet was used to evaluate plant growth (i.e. with 7 plants/treatment), after
258 separating root systems from shoots and carefully washing them with water. Shoot height and
259 biomass were measured. Fresh and dry (105°C overnight) root biomass were determined,
260 along with root system architecture (number, length, diameter, surface and volume of roots)
261 using WinRhizo image analysis (Regent Instruments, Nepean, ON). The other plantlets were
262 sampled to quantify F113 colonization (using 5 plants/treatment; see below).

263

264 **Real-time PCR assessment of inoculant colonization**

265 Root systems were shaken to detach non-adhering soil. They were introduced each into a 50-
266 mL Falcon tube, which was shaken for 15 min, and flash-frozen in liquid nitrogen. The root
267 systems were removed. The soil was pelleted by centrifugation (30 min at 5,100 *g* and room
268 temperature), lyophilized for 48 h, weighed, and conserved at -20°C. DNA extraction was
269 performed on 300 mg of lyophilized rhizosphere soil transferred into Lysing Matrix E tubes from
270 the FastDNA Spin Kit (MPbiomedicals), according to the manufacturer's instructions, and DNA
271 concentration adjusted to 5 ng/μL for each sample.

272 *P. kilonensis* F113 was assessed in rhizosphere soil by real-time PCR, as described
273 by Von Felten *et al.* (Von Felten, Défago, & Maurhofer, 2010), using a LC-480 LightCycler and
274 the LightCycler 480 SYBR Green I Master mix (Roche Applied Science, Indianapolis, IN).
275 Melting curve calculation and determination of T_m values were performed using the Lightcycler
276 Software (Roche Applied Science). The C_T values obtained were normalized using the plasmid
277 Apa9 as internal standard, as described by Couillerot *et al.* (2010) and Von Felten *et al.* (2010),
278 in order to standardize DNA extraction efficiencies between rhizosphere samples. The primers
279 amplify a specific region of the F113 strain. In our experiment, the detection limit was 10 copies
280 of the target sequence per reaction. The efficiency was higher than 86% and the error rate
281 lower than 2%. Quantities were expressed per g of dry rhizosphere soil and per root system.

282

283 **Statistics**

284 Standard errors (SE) were used to show data variability. As screening and quantitative PCR
285 data could not be normalized, comparisons between multiple treatments were made using
286 Kruskal-Wallis rank-sum tests followed by Conover-Iman tests for pairwise comparisons, and
287 comparisons between only two treatments were done using Wilcoxon rank-sum tests.

288 Comparison of proportions were made using χ^2 test. For the greenhouse experiment, plant
289 data were normalized using a box-cox transformation and comparisons were made using
290 multiple-factors ANOVA followed with Fisher's LSD tests for pairwise comparisons. All
291 analyses were carried out at $P < 0.05$, using Xlstat software v2018.4 (Addinsoft, Bordeaux,
292 France).

293

294

295 **Results**

296

297 **Root colonization by *P. kilonensis* F113 was higher on ancient wheat genotypes**

298 Under the gnotobiotic conditions used for the screening of the 192 wheat genotypes, *mCherry*
299 fluorescence resulting from F113-*mCherry*(*Pphl-egfp*) colonization ranged from $15 \pm$ (SE) 3 for
300 genotype Hendrix to 165 ± 10 arbitrary units (AU) for D130-63 at one week (Fig. **1a**). F113-
301 *mCherry*(*Pphl-egfp*) colonization was higher for ancient genotypes than modern genotypes,
302 based on the two following criteria. First, colonization was significantly higher ($P = 0.022$) for
303 ancient genotypes overall ($61 \pm$ [SE] 3 AU, $n = 77$) compared with modern genotypes (53 ± 2
304 AU, $n = 115$) (Fig. **2a**). Second, the 25 genotypes presenting the best F113 colonization levels
305 corresponded to 15 of 77 ancient genotypes (i.e. 19.5%) vs only 10 of 115 modern genotypes
306 (i.e. 8.7%) (Table **2**). Conversely, 6 of the ancient genotypes (7.8%) vs as many as 19 of the
307 modern genotypes (16.5%) were part of the 25 least colonized wheat genotypes. Similar trends
308 were observed when considering the 50 best and 50 worst colonized genotypes. Higher
309 colonization of ancient genotypes by F113 was also indicated by the distribution of red
310 fluorescence classes, which showed positive differences in frequency (i.e., the frequency of
311 modern genotypes subtracted from the frequency of ancient genotypes) among the five
312 fluorescence classes above 80 AU (Fig. **3a,c**).

313 When distinguishing between landraces and old varieties (< 1960) within the ancient
314 genotype category, it appeared that F113-*mCherry*(*Pphl-egfp*) colonization was significantly
315 higher ($P = 0.039$) for old varieties ($62 \pm$ [SE] 4 AU) than for modern genotypes (53 ± 2 AU),
316 landraces being in an intermediate position (60 ± 4 AU ; Supporting information Fig. S1). In
317 addition, there were trends (not significant at $P < 0.05$) towards (i) higher proportions of
318 landraces (17.1%) and old varieties (21.4%) than modern genotypes (only 8.7%) among the
319 25 best colonized genotypes, and (ii) lower proportions of landraces (8.6%) and old varieties
320 (7.1%) than modern genotypes (as much as 21.7%) among the 25 worst colonized genotypes
321 (Supporting information Table S2). This is also indicated by positive differences in frequencies
322 for both landraces and old varieties (vs modern genotypes) among the five fluorescence
323 classes above 80 AU (Supporting information Fig. S2).

324

325 ***phl* expression of *P. kilonensis* F113 on roots was higher on ancient wheat genotypes**

326 All 192 genotypes showed *egfp* fluorescence (i.e. *phl* expression) upon F113-*mCherry*(*Pphl-egfp*)
 327 inoculation, at levels ranging between $79 \pm$ (SE) 3 for genotype Adular and $1920 \pm$ 313
 328 AU for D130-63 (Fig. **1b**). *phl* expression in F113-*mCherry*(*Pphl-egfp*) for the 77 ancient
 329 genotypes was significantly higher overall ($P = 0.01$) ($490 \pm$ [SE] 25 AU) than for the 115
 330 modern genotypes ($429 \pm$ 20 AU) (Fig. **2b**).

331 In addition, the 25 genotypes with the best *phl* expression corresponded to 13 of the
 332 77 ancient genotypes (i.e. 16.9%) vs only 12 of the 115 modern genotypes (i.e. 10.4%), and
 333 the difference was statistically significant when considering the 50 genotypes showing the best
 334 *phl* expression, which included 27 of the 77 ancient genotypes (35.1%) vs only 23 of the 115
 335 modern genotypes (20%). Conversely, there was also a trend (not significant at $P < 0.05$) for
 336 a lower prevalence of ancient genotypes (6 of 77, i.e. 7.8%) than modern genotypes (19 of
 337 115, i.e. 16.5%) in the 25 genotypes presenting the worst *phl* expression (Table **2**). Higher *phl*
 338 expression on ancient genotypes is also indicated by the distribution of green fluorescence
 339 categories, which showed positive differences in frequency (i.e., the frequency of modern
 340 genotypes subtracted from the frequency of ancient genotypes) among the four fluorescence
 341 classes above 700 AU (Fig. **3b,d**).

342 Spearman's correlation between F113 root colonization and *phl* expression was
 343 significant ($P < 0.001$, $r = 0.85$, $n = 192$). Accordingly, the level of *phl* induction (i.e., the green
 344 fluorescence:red fluorescence ratio) was rather similar for a majority of wheat genotypes (Fig.
 345 **1c**), without any statistical difference between the ancient (8.0 ± 0.2) and modern (8.1 ± 0.2)
 346 genotype categories (Fig. **2c**).

347

348 **Screening results were validated by alternative fluorescence methodology and confocal** 349 **microscopy**

350 When the screening was repeated with 20 contrasted genotypes and an alternative
 351 fluorescence measurement methodology, the group of 10 genotypes effective at interacting
 352 with F113 (hereafter referred to as F113-stimulating genotypes) displayed higher root
 353 colonization ($210 \pm$ [SE] 24 vs $102 \pm$ 12) and *phl* expression ($1802 \pm$ 251 vs $661 \pm$ 139)
 354 compared with the group of 10 ineffective genotypes (i.e. non F113-stimulating). In both
 355 screenings, red fluorescence and green fluorescence of the 10 F113-stimulating genotypes
 356 were respectively about twice and three times as high as for the non F113-stimulating
 357 genotypes. Correlation was significant between the two screenings, both for red ($P < 0.001$, r
 358 $= 0.80$, $n = 20$) and green fluorescence levels ($P < 0.001$, $r = 0.78$, $n = 20$) (Fig. **4**).

359 Confocal microscopy observations confirmed spectrofluorimeter data, in that F113-
 360 stimulating genotypes showed more prevalent and larger F113 biofilms formed of cells

361 expressing the red and/or the green fluorescence on roots, in comparison with non F113-
362 stimulating genotypes (Fig. 5).

363

364 **F113 colonized roots of all eight selected genotypes in soil**

365 In non-sterile soil, *P. kilonensis* F113 was recovered by quantitative PCR from inoculated
366 wheat at 2.5×10^5 to 4.2×10^6 copies per root system (Supporting information Fig. S3),
367 whereas it was below the detection limit (i.e. 3.5×10^4 copies per root system) for non-
368 inoculated plants. The differences between (i) optimum and stress conditions, (ii) F113-
369 stimulating and non F113-stimulating groups of genotypes, (iii) ancient and modern groups of
370 genotypes, or (iv) individual wheat genotypes were not statistically significant. Similar findings
371 were made when expressing results per g of dry rhizosphere soil (data not shown).

372

373 **F113 inoculation improved growth of certain wheat genotypes**

374 Three-factor ANOVA (Supporting information Table S2) indicated that F113 inoculation,
375 overall, enhanced root volume (+14.8 %, $P < 0.01$), root diameter (+3.5 %, $P < 0.001$), root
376 number (+14.9 %, $P < 0.001$) and dry root biomass (+16.3 %, $P < 0.001$), with a significant
377 inoculation \times genotype interaction for root number and dry root biomass.

378 Under optimum conditions, the overall inoculation benefits for F113-stimulating vs non
379 F113-stimulating genotypes were higher for all measured parameters when the means [or the
380 medians] of the two groups were compared, with for instance +47.2 % [+57.7 %] vs +17.1 %
381 [-5.6 %] variation for dry root biomass, +27.8 % [+26.1 %] vs +18.4 % [+18.1 %] for root
382 number, +23.2 % [+21.5 %] vs +13.0 % [+14.3 %] for root volume, and +4.6 % [+5.7 %] vs
383 +3.2 % [+3.7 %] for root diameter (Fig. 6a). Inoculation resulted in higher dry root biomass of
384 the two F113-stimulating genotypes Coronation (+95.8%, $P < 0.01$) and D130-63 (+92.3%, P
385 < 0.001) and the non F113-stimulating genotype Odesskaya16 (+103.4%, $P < 0.001$) (Table
386 3).

387 Under stress, the overall inoculation benefits for F113-stimulating vs non F113-
388 stimulating genotypes were also more important for all measured parameters, with for instance
389 means enhanced by +36.8 % [and medians enhanced by +38,3 %] vs -1.4 % [+2.0 %] for dry
390 root biomass, +35.0 % [+44.0 %] vs +12.1 % [+14.1 %] for root number, +23.3 % [+23.7 %] vs
391 +12.6 % [+13.2 %] for root volume, and +3.9 % [+4.4 %] vs +2.8 % [+2.9 %] for root diameter
392 (Fig. 6b). Inoculation of F113-stimulating genotypes enhanced root volume for Coronation
393 (+81.3%, $P < 0.05$), root diameter for Concurrent (+11.7%, $P < 0.01$), root number and root dry
394 biomass for Coronation (respectively +102.3%, $P < 0.001$ and +84.6%, $P < 0.01$) and D130-
395 63 (+101.7%, $P < 0.001$ and +106.1%, $P < 0.001$), but it reduced root number (-50.4%, $P <$
396 0.001) and root dry biomass (-35.3%, $P < 0.05$) for Amifort, whereas inoculation of non F113-
397 stimulating genotypes had no effect (Table 3).

398 **Impact of stress on non-inoculated wheat genotypes**

399 As *P. kilonensis* F113 was below detection limit in non-inoculated pots (see above), a two-
400 factor ANOVA was performed on the dataset from non-inoculated plants, on the basis that
401 genotype response to stress integrated also the possible contribution of resident plant-
402 beneficial microorganisms (i.e. other than F113). Stress had a significant negative impact on
403 every plant parameter except root length and root number, with a significant stress \times genotype
404 interaction for root diameter and dry root biomass (Supporting information Table S4).

405 Overall, the impact of stress on F113-stimulating vs non F113-stimulating genotypes
406 on root parameters was respectively $-17.6 (\pm 7.3) \%$ vs $-34.8 (\pm 2.6) \%$ variation for root volume,
407 $-18.5 (\pm 4.6) \%$ vs $-34.8 (\pm 5.8) \%$ for fresh root biomass, $+6.9 (\pm 12.0) \%$ vs $-19.9 (\pm 19.3) \%$
408 for dry root biomass, and $-9.9 (\pm 0.8) \%$ vs $-10.8 (\pm 3.6) \%$ for root diameter. For shoot
409 parameters, stress resulted in $-40.2 (\pm 5.4) \%$ vs $-40.1 (\pm 1.5) \%$ variation for fresh shoot
410 biomass, $-32.5 (\pm 6.8) \%$ vs $-34.9 (\pm 2.2) \%$ for dry shoot biomass and $-14.8 (\pm 2.9) \%$ vs -14.1
411 $(\pm 1.3) \%$ for shoot height (Fig. 6c). When considering the relative impact of stress, i.e. by
412 computing the stress response index [(replicate performance under stress – mean
413 performance under optimum conditions) / mean performance under optimum conditions], it
414 appeared that the top five performing genotypes under stress conditions included all 4 F113-
415 stimulating genotypes versus only 1 of 4 non F113-stimulating genotypes for root length, root
416 number, dry root biomass and root volume.

417 At the scale of individual F113-stimulating genotypes, the impact of stress was
418 significant on root diameter (for all 8 genotypes), fresh shoot biomass and shoot height (for all
419 genotypes but Coronation), and dry shoot biomass (for Concurrent and Amifort) (Supporting
420 information Table S5). For individual non F113-stimulating genotypes, the effect of stress was
421 significant on root volume, fresh and dry root biomass (for Hendrix and Danubia), root diameter
422 (for all but Danubia), fresh shoot biomass and shoot height (for all four genotypes), and dry
423 shoot biomass (for Jaszaji TF and Hendrix).

424

425

426 **Discussion**

427

428 Crop breeding has resulted in the development of thousands of genotypes, which show
429 contrasted agro-morphological and metabolic features (Berry, Kendall, Rutterford, Orford, &
430 Griffiths, 2015; Beyer, Daba, Tyagi, Bockelman, & Brown-Guedira, 2018; Gotti et al., 2018;
431 Shaposhnikov et al., 2016; Siddique, Belford, & Tennant, 1990; York et al., 2015; Zhang et al.,
432 2013), as well as contrasted stress response patterns (Esmail, Eldessouky, Mahfouz, & El-
433 Demardash, 2016; Khakwani, Dennett, & Munir, 2011; Ramshini, Mirzazadeh, Moghaddam, &
434 Amiri, 2016). Crop genotypes can also differ in their ability to recruit soil bacteria (Bouffaud et

435 al., 2012), induce gene expression in rhizobacteria (Notz et al., 2001; Vacheron et al., 2016),
436 and respond to PGPR inoculation (Sasaki et al., 2010; Vacheron et al., 2016). The impact of
437 breeding on PGPR × crop interactions is an important issue, but there have been very few
438 studies on this. When investigated, it was done by using a very small number of genotypes
439 considering the extent of crop diversity (Furlan et al., 2017; Kazi et al., 2016; Neiverth et al.,
440 2014).

441 Here, we compared wheat genotypes resulting from modern breeding efforts (i.e. post-
442 1960) with ancient wheat genotypes, based on their ability to interact with the model PGPR *P.*
443 *kilonensis* F113. This approach was relevant since (i) this type of bacterium may occur
444 naturally in various types of cultivated soils (Costa et al., 2007; Landa et al., 2002; Mazzola et
445 al., 2004), and (ii) strain F113 can interact with different crop species and cultivars, but with
446 differences from one crop cultivar to the next (Vacheron et al., 2016). To cope with the extent
447 of wheat diversity, almost 200 genotypes representative of this diversity were considered,
448 which signifies an unprecedented scale for this type of work. To obtain robust screening
449 results, we had to rely on a simplified experimental design, which proved effective to compare
450 wheat genotypes (but which could not integrate the complexity of the rhizosphere ecosystem).
451 It required the development of a novel methodology, based on the use of reporter gene fusions
452 and the assessment of autofluorescent proteins. Importantly, this approach enabled the
453 monitoring of both colonization and gene expression of the inoculant on roots. The results of
454 the screening were validated using a subset of 20 genotypes, based on an alternative
455 fluorescence measurement protocol (and twice as many replicates) as well as direct confocal
456 microscopy observations.

457 Screening results showed that root colonization by F113 was higher on ancient
458 genotypes than modern ones. This type of finding was not found in the pot experiment, as the
459 difference in F113 root colonization between the 8 wheat genotypes was not significant. This
460 might be due to the small number of genotypes that could be compared in the pot experiment.
461 The discrepancy may result also from the contrasts in growth matrix (mineral soil vs agar),
462 wheat development stage (Di Salvo, Ferrando, Fernández-Scavino, & García de Salamone,
463 2018), and/or presence in the soil only of an indigenous microbiota (Thomas & Sekhar, 2016;
464 Vacheron et al., 2018). As for root colonization, *phl* expression was higher for ancient
465 genotypes than modern ones. There was a strong correlation between root colonization and
466 *phl* expression, and indeed the ratio between both measurements (i.e. *phl* induction ratio) did
467 not fluctuate as much between genotypes, pointing to a rather constitutive *phl* expression in
468 F113 on most wheat genotypes. This is consistent with previous observations on the regulation
469 of *phl* expression in (other) *Pseudomonas* strains (Paulin et al., 2009; Schnider-Keel et al.,
470 2000). It might be explained by (i) ecological conditions on roots during the short duration of
471 the screening assay, and/or (ii) the production by a majority of wheat genotypes of similar key

472 exudate compounds controlling *phl* expression (De Werra, Baehler, Huser, Keel, & Maurhofer,
473 2008; Duffy, Défago, & Duffy, 1999; Shanahan et al., 1992).

474 In this work, the screening focused on bacterial root colonization and gene induction.
475 To determine whether PGPR interaction ability could translate into enhanced plant
476 performance, a greenhouse pot experiment was carried out with four F113-stimulating
477 genotypes and four non F113-stimulating genotypes, whose status had been determined in
478 the gnotobiotic screening. Contrasted phytostimulation effects between wheat genotypes may
479 be expected based on (i) previous studies on plant × bacteria interactions (Kazi et al., 2016),
480 and (ii) the ability of DAPG (like auxins) to increase root system branching (Brazelton et al.,
481 2008). Indeed, inoculation with *P. kilonensis* F113 enhanced plant performance for 2 of 4 F113-
482 stimulating genotypes and 1 of 4 non F113-stimulating genotypes under optimum conditions,
483 versus as many as 4 of 4 F113-stimulating genotypes but none of the 4 non F113-stimulating
484 genotypes under stress (Table 3). This shows the ability of F113 to enhance growth of certain
485 host genotypes under stress conditions, as previously described for maize (Vacheron et al.,
486 2016), and strongly suggests an impact of stress on wheat × F113 interactions. Differences in
487 interaction specificity/affinity between plant genotypes and inoculated bacteria might entail
488 differences in inoculant survival (which was not the case here) or in inoculant effects on host
489 transcriptional and metabolic profiles (Chamam, Wisniewski-Dyé, Comte, Bertrand, & Prigent-
490 Combaret, 2015; Drogue et al., 2014). It had been reported that DAPG could have phytotoxic
491 effect on roots at high concentration (Brazelton et al., 2008; Keel et al., 1992), but this was
492 unlikely as inoculation did not have any negative effect on the 8 genotypes under optimum
493 conditions. F113 inoculation may also trigger changes in the root microbial community (Walsh
494 et al., 2003), but it is unknown whether this impact could be plant genotype-dependent,
495 although it is likely to be the case (Andreote, Da Rocha, Araújo, Azevedo, & Van Overbeek,
496 2010). Here, in the absence of inoculation, the top performing genotypes in terms of stress
497 response index included mainly F113-stimulating genotypes, which raises the possibility that
498 other (resident) plant-beneficial microorganisms could have contributed to stress alleviation. In
499 maize, cultivar PR37Y15 responded to the PGPR *P. kilonensis* F113 as well as to various
500 PGPR inoculants (Walker et al., 2012), whereas cultivar DK315 did not respond (Walker et al.,
501 2013).

502 Our results suggest that the search for high-yield wheat cultivars under agronomically-
503 optimized conditions, which caused genetic diversity loss (Bonnin, Bonneuil, Goffaux,
504 Montalent, & Goldringer, 2014; Roussel, Leisova, Exbrayat, Stehno, & Balfourier, 2005;
505 Winfield et al., 2018), did not favor the maintenance of plant genes promoting PGPR
506 interactions. Modern genotypes are mostly dwarf or semi-dwarf varieties because of the
507 introduction of dwarfism *Rht-B1b* and *Rht-D1b* alleles in the 1960's, by CIMMYT (International
508 Maize and Wheat Improvement Center) in Mexico and thereafter during wheat breeding

509 programs in other parts of the world (Borlaug, 1968; Lumpkin, 2015; Subira et al., 2016). These
510 genes confer a reduced response to gibberellic acid. This prevents stem lodging that arose in
511 response to the increase in size and biomass of the spike, because of the massive fertilization
512 of fields implemented after the Second World War (Berry et al., 2015; Borojevic, & Borojevic,
513 2005). Modern genotypes may differ from ancient genotypes in terms of metabolic composition
514 (Di Loreto et al., 2018; Gotti et al., 2018; Iannucci, Fragasso, Beleggia, Nigro, & Papa, 2017;
515 Shaposhnikov et al., 2016) and root architecture (Beyer et al., 2018; Horst, Abdou, & Wiesler,
516 1993; Pour-Aboughadareh, Ahmadi, Mehrabi, Moghaddam, & Etminan, 2017; Shaposhnikov
517 et al., 2016; Siddique et al., 1990), which is likely to affect plant × PGPR interactions. Against
518 this background, however, results also revealed that (i) many ancient genotypes were not
519 effective for interaction (especially colonization) with *P. kilonensis* F113, while (ii) interaction
520 effectiveness with F113 had been maintained in a significant proportion of modern wheat
521 genotypes. Yet, this work was performed with one *Pseudomonas* model PGPR only, and it
522 would be valuable to check, in further studies, whether these genotypes would respond in the
523 same way to other PGPR species or to plant-beneficial fungi. On one hand, breeding strategies
524 may need to be reassessed to give further consideration to roots and below-ground processes
525 (Den Herder *et al.*, 2010), including the interactions with beneficial microorganisms (Gopal &
526 Gupta, 2016; Wissuwa, Mazzola, & Picard, 2009). On the other hand, it may be wise to identify
527 PGPR-friendly genotypes among the current and future high-yield wheat cultivars available to
528 farmers, and the current approach could be expanded to take into account the interaction
529 specificities of other types of PGPR as well as beneficial fungi.

530

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536

537

538 **Author contribution**

539 Conceptualization J.V., J.L.G., C.P.C. and Y.M.L.; Methodology, J.V., J.L.G., Y.M.L. and
540 C.P.C.; Investigation, J.V., F.G., Y.M.L. and C.P.C.; Formal Analysis, J.V.; Writing – Original
541 Draft, J.V., Y.M.L. and C.P.C.; Writing – Review & Editing, J.V., J.L.G., Y.M.L. and C.P.C.;
542 Funding Acquisition J.L.G., C.P.C. and Y.M.L.; Resources, J.L.G.

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545 **Conflict of interest**

546 The authors declare that they have no conflict of interest

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872 Figure legends

873

874 **Fig. 1** Root colonization and *phl* expression of *P. kilonensis* F113-*mCherry*(*Pphl-egfp*) on 192
875 individual wheat genotypes corresponding to 115 modern genotypes (> 1960) and 77 ancient
876 genotypes (including 42 old varieties [\leq 1960] and 35 landraces). Red fluorescence (root
877 colonization) is shown in (a), green fluorescence (*phl* expression) in (b), and the *phl* induction
878 rate (green fluorescence:red fluorescence ratio) in (c). Fluorescence is expressed as arbitrary
879 units (AU) and data are presented as means with standard errors ($n = 3$). The ranking of the
880 192 genotypes is indicated in Supporting information Table S1.

881

882 **Fig. 2** Root colonization and *phl* expression of *P. kilonensis* F113-*mCherry*(*Pphl-egfp*) for
883 modern ($n = 115$) and ancient wheat genotypes ($n = 77$). Red fluorescence (root colonization)
884 is shown in (a), green fluorescence (*phl* expression) in (b), and the *phl* induction rate (red
885 fluorescence:green fluorescence ratio) in (c). Fluorescence is expressed as arbitrary units
886 (AU) and data are presented as means (computed from individual genotype data) with
887 standard errors. Statistical differences between the two wheat categories are shown using
888 letters a and b (Wilcoxon tests, $P < 0.05$).

889

890 **Fig. 3** Frequency distribution of root colonization (red fluorescence, expressed as arbitrary
891 units [AU]) and *phl* expression data (green fluorescence) of *P. kilonensis* F113-*mCherry*(*Pphl-*
892 *egfp*) for wheat genotypes. The frequency distribution of modern wheat genotypes ($n = 115$) is
893 shown for root colonization data (a) and for *phl* expression data (b), and the frequencies were
894 computed as the number of observations (i.e. of genotypes) in each of the 12 classes (red
895 fluorescence) or 11 classes (red fluorescence) of fluorescence intensity divided by the total
896 number of observations (i.e. 115). The deviation for the 77 ancient genotypes was computed
897 by subtracting the frequencies for the 115 modern genotypes from the corresponding
898 frequencies for the 77 ancient genotypes in each of the fluorescence classes, both for root
899 colonization data (c) and *phl* expression data (d).

900

901 **Fig. 4** Correlation analysis of root colonization data (red fluorescence, expressed as arbitrary
902 units [AU]) (a) and *phl* expression data (green fluorescence) (b) of *P. kilonensis* F113-
903 *mCherry(Pphl-egfp)* obtained in the screening experiment (X axis) and the verification
904 experiment (Y axis) for 20 wheat genotypes. Spearman correlation coefficients were $r = 0.80$
905 in (a) and $r = 0.78$ in (b) (both with $P < 0.001$).

906

907 **Fig. 5** Root colonization by *P. kilonensis* F113-*mCherry(Pphl-egfp)* and *phl* expression at the
908 surface of the apex of eight wheat genotypes (selected from the 20 genotypes studied) used
909 for the validation experiment. Photographs were taken using a confocal microscope with an
910 excitation laser light of 488 nm and an emission filter of 504-555 nm for EGFP green
911 fluorescence, and an excitation laser light of 561 nm and an emission filter 570-636 nm for
912 *mCherry* red fluorescence. The same gains were used for all samples. Root-colonizing cells
913 not expressing *phl* are red, root-colonizing cells with strong *phl* expression (but with insufficient
914 gain for red fluorescence) appear in green, and root-colonizing cells expressing *phl* are yellow.
915 Large numbers of mainly red or yellow cells were obtained for genotypes Blé de Redon
916 (Landrace) (A), Coronation (≤ 1960) (B), Concurrent (≤ 1960) (C) and D130-63 (> 1960) (D),
917 which had shown high red and green fluorescence values in the screening experiment,
918 whereas fluorescent cells were sparse for genotypes Orlandi (≤ 1960) (E), Orfield (> 1960) (F),
919 Hendrix (> 1960) (G) and Jaszaji TF (≤ 1960) (H), which had shown low red and green
920 fluorescence values in the screening experiment.

921

922 **Fig. 6** Relative impact of seed inoculation with *P. kilonensis* F113 on the growth of four F113-
923 stimulating wheat genotypes and four non F113-stimulating genotypes under optimum (a) or
924 combined stress conditions (b), and relative impact of stress on performance of non-inoculated
925 plants (c). For each of the nine plant parameters investigated, the relative impacts were
926 computed as (inoculated – non-inoculated)/non-inoculated [in a,b], and (stress –
927 optimum)/optimum [in c]. The differences were not significant at $P < 0.05$ level.

Table 1 List of wheat genotypes used for the validation experiment in vitro and the greenhouse experiment in soil. All 20 genotypes were used for validation, whereas only the 8 genotypes in bold print were used for the greenhouse experiment. The total score indicated was the sum of the three genotype rankings, i.e. for F113 colonization, *phl* expression and *phl* induction rate. For each ranking, the worst rank (lower value measured) was 1 and the best rank (higher value measured) was 192.

Global ranking	Genotype	Genotype category	Total score
Among the 1-20% best genotypes	D130-63	> 1960	570
	Coronation	≤ 1960	534
	Concurrent	≤ 1960	508
	Cenad 512	≤ 1960	484
	DI276	> 1960	471
	Prince Leopold	≤ 1960	449
	ATUT-II	> 1960	412
Among the 20-40% best genotypes	Espoir	≤ 1960	394
	Blé de Redon glumes velues	Landrace	385
	Amifort	> 1960	350
Among the 20-40% worst genotypes	Danubia	> 1960	256
	Recital	> 1960	202
	Croisement 268	≤ 1960	182
Among the 1-20% worst genotypes	Poilu du Tarn	≤ 1960	156
	Hendrix	> 1960	154
	Jaszaji TF	≤ 1960	144
	Odesskaya 16	≤ 1960	108
	Orlandi	≤ 1960	91
	Malgorzatka udycka	≤ 1960	85
Orfield	> 1960	64	

Table 2 Percentages of wheat genotypes showing the highest and lowest values of root colonization by *P. kilonensis* F113 or *phl* expression in root-colonizing F113 among the ancient (n = 77) and modern genotypes (n = 115).

	Ancient genotypes	Modern genotypes
Root colonization by F113		
25 best genotypes	19.5 % a [†]	8.7 % b
50 best genotypes	32.5 % a	21.7 % a
50 worst genotypes	20.8 % a	29.6 % a
25 worst genotypes	7.8 % a	16.5 % a
<i>phl</i> expression in F113		
25 best genotypes	16.9 % a	10.4 % a
50 best genotypes	35.1 % a	20.0 % b
50 worst genotypes	27.3 % a	25.2 % a
25 worst genotypes	7.8 % a	16.5 % a

[†]For each row, significant differences between ancient and modern genotypes are indicated by letters a and b (χ^2 tests carried out on numbers of genotypes, $P < 0.05$).

Table 3 Relative impact of seed inoculation with *P. kilonensis* F113 on the growth of eight wheat genotypes under optimum or combined stress conditions. Concurrent, Coronation, Amifort and D130-63 are F113-stimulating genotypes, whereas Jaszaji TF, Odesskaya 16, Danubia and Hendrix are non F113-stimulating genotypes. For each of the nine plant parameters investigated, the relative impacts were computed as (inoculated – non-inoculated)/non-inoculated. For each genotype, significant differences between inoculated plants and controls are indicated in bold. Statistical level is indicated with * ($P \leq 0.05$), ** ($P \leq 0.01$) or *** ($P \leq 0.001$), based on ANOVA and Fisher's LSD tests on raw plant values.

		Root volume	Fresh root biomass	Dry root biomass	Number of Roots	Root average diameter	Root length	Fresh shoot biomass	Dry shoot biomass	Shoot height
Optimum	Concurrent	-0.9%	-23.0%	-22.6%	0.3%	3.2%	-6.6%	-9.9%	-10.1%	0.2%
	Coronation	28.2%	20.5%	95.8% **	58.8%	8.1%	7.8%	30.8%	37.3%	7.6%
	Amifort	14.8%	-4.6%	23.1%	10.2%	8.3%	-0.7%	-0.5%	1.5%	0.8%
	D130-63	50.6%	119.8%	92.3% ***	41.9%	-1.4%	46.1%	18.4%	ND	4.6%
	Jaszaji TF	-15.7%	-20.0%	-23.8%	-10.0%	3.3%	-20.2%	-12.8%	-10.0%	-7.6%
	Odesskaya 16	39.1%	61.9%	103.4% ***	10.1%	-1.6%	45.3%	1.5%	ND	-1.3%
	Danubia	5.6%	-11.6%	-2.7%	26.0%	7.1%	-7.4%	-1.4%	ND	0.1%
	Hendrix	23.0%	2.0%	-8.5%	47.5%	4.1%	11.7%	4.5%	7.9%	-0.7%
Stress	Concurrent	1.0%	-5.7%	-2.8%	-13.7%	11.7% **	-19.3%	0.5%	5.1%	-4.0%
	Coronation	81.3% *	65.8%	79.3% **	102.3% ***	1.9%	71.7%	5.9%	12.4%	4.2%
	Amifort	-35.5%	-27.2%	-35.3% *	-50.4% ***	-4.9%	-26.3%	9.1%	9.5%	5.4%
	D130-63	46.3%	47.3%	106.1% ***	101.7% ***	6.8%	30.5%	-4.8%	ND	-2.7%
	Jaszaji TF	16.5%	4.9%	-26.0%	19.8%	4.7%	8.6%	8.3%	17.9%	0.5%
	Odesskaya 16	9.9%	6.4%	6.9%	8.4%	1.1%	11.8%	-5.5%	ND	-1.7%
	Danubia	6.4%	-3.4%	16.7%	-5.9%	7.8%	-3.0%	-4.1%	ND	-2.7%
	Hendrix	17.5%	4.6%	-3.0%	26.0%	-2.6%	28.6%	-4.2%	-1.8%	-1.7%

Fig. 1

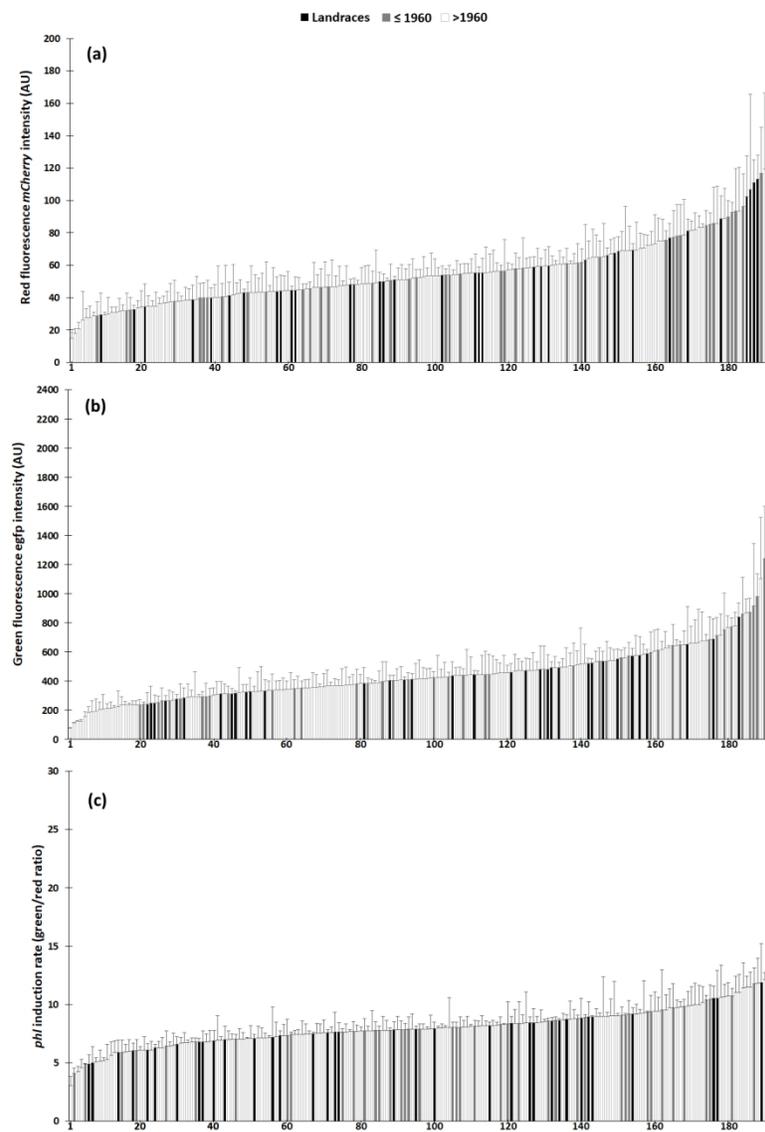


Figure 1

353x542mm (96 x 96 DPI)

Fig. 2

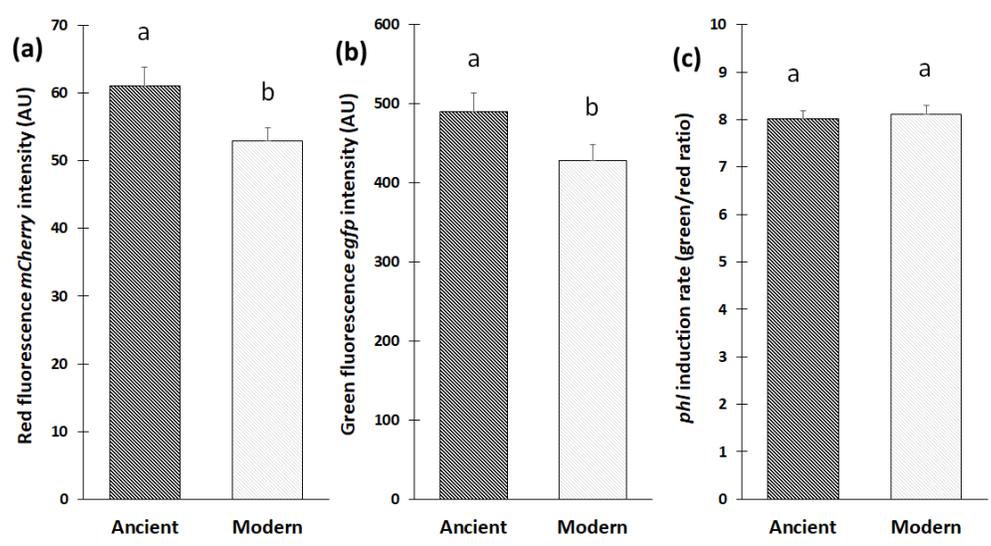


Figure 2

296x177mm (96 x 96 DPI)

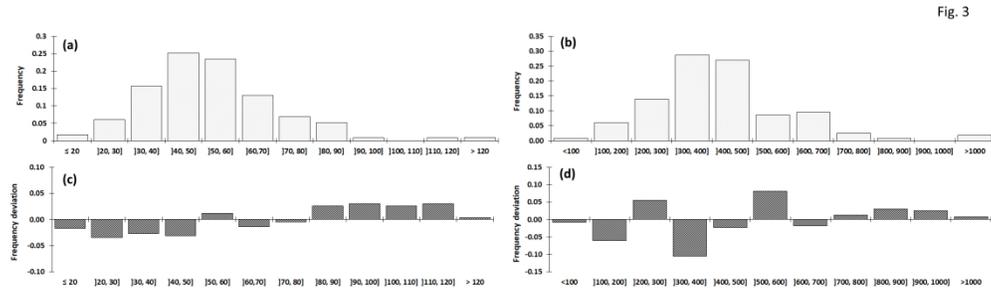


Figure 3

605x177mm (96 x 96 DPI)

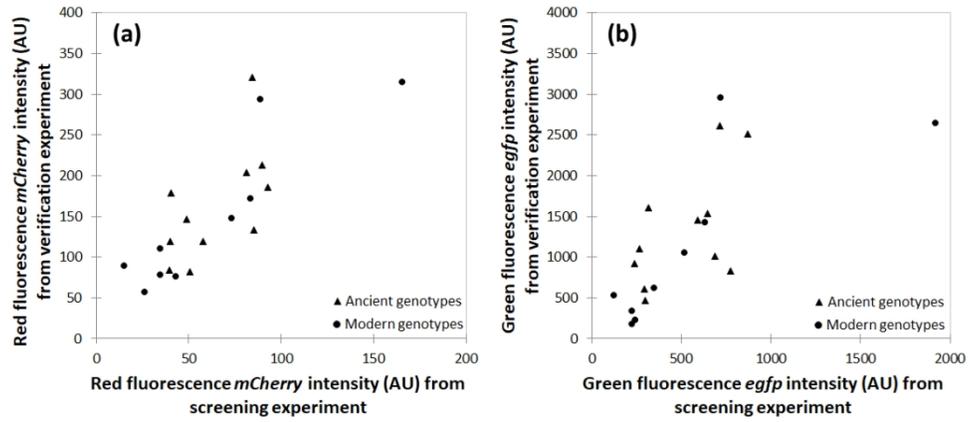


Fig. 4

Figure 4

320x158mm (96 x 96 DPI)

Fig. 5

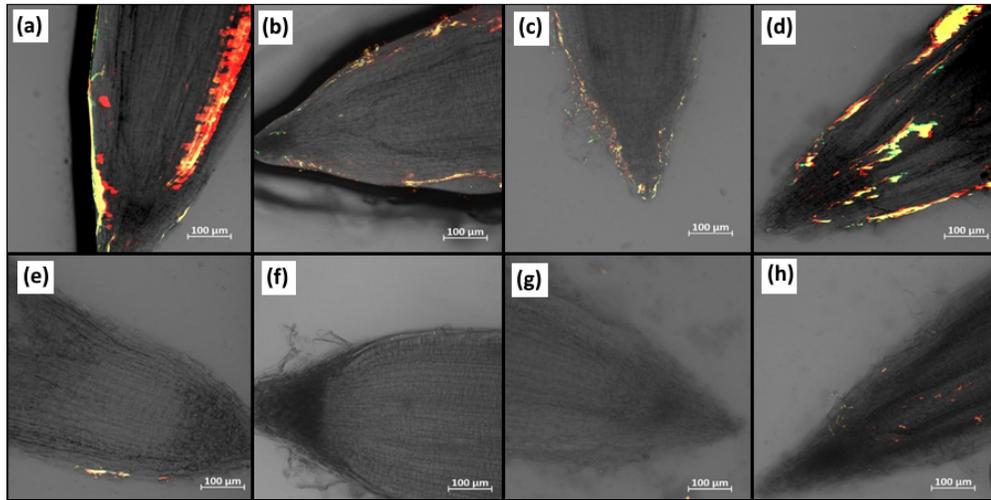


Figure 5

239x126mm (96 x 96 DPI)

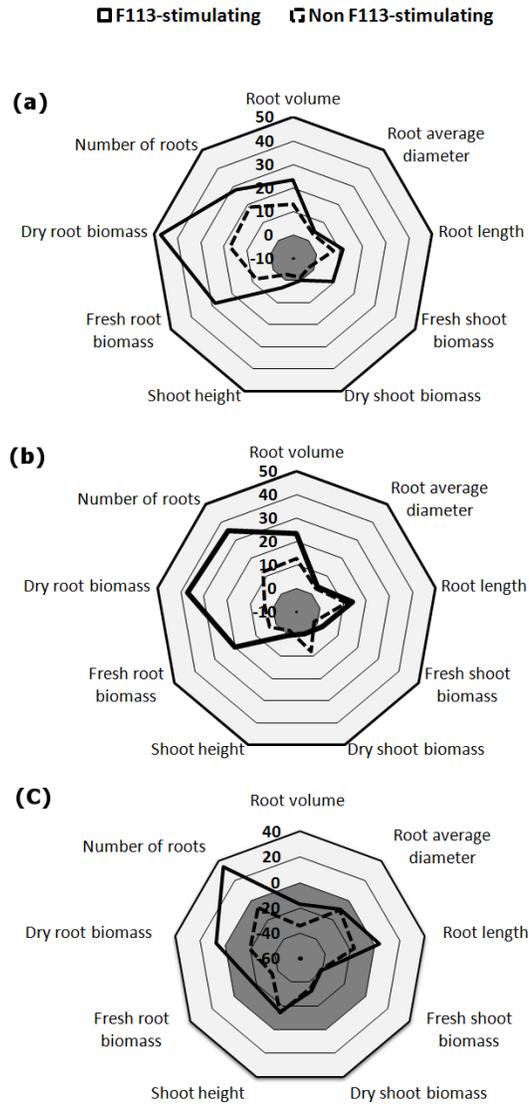


Fig. 6

Figure 6

182x363mm (96 x 96 DPI)