HAL open science

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

Jordan Valente, Florence Gerin, Jacques Le Gouis, Yvan Moënne-loccoz, Claire Prigent–combaret

To cite this version:

Jordan Valente, Florence Gerin, Jacques Le Gouis, Yvan Moënne-loccoz, Claire Prigent–combaret. Ancient wheat varieties have a higher ability to interact with plant growth-promoting rhizobacteria. Plant, Cell and Environment, 2020, 43 (1), pp.246-260. 10.1111/pce.13652 . hal-02337059

HAL Id: hal-02337059

https://hal.science/hal-02337059v1

Submitted on 7 Dec 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Distributed under a Creative Commons Attribution 4.0 International License

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.

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

Jordan Valente[1], Florence Gerin[1], Jacques Le Gouis[2], Yvan Moënne-Loccoz[1], Claire Prigent-Combaret[1*]

[1] Univ Lyon, Université Claude Bernard Lyon 1, CNRS, INRA, VetAgro Sup, UMR5557 Ecologie Microbienne, 43 bd du 11 novembre 1918, F-69622 Villeurbanne, France

[2] GDEC, INRA, UCA, F-63000 Clermont-Ferrand, France

Running title: Wheat breeding and PGPR interaction

*Corresponding author: UMR CNRS 5557 Ecologie Microbienne, Université Lyon 1, 43 bd du 11 Novembre 1918, 69622 Villeurbanne cedex, France. Phone +33 4 72 43 13 49. E-mail: claire.prigent-combaret@univ-lyon1.fr

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.

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

Introduction

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

Modern breeding is typically carried out under optimal agronomic conditions, which is likely to limit the potential added-value resulting from beneficial plant-microbe interactions (Sasaki et al., 2010; Schmidt, Bowles, & Gaudin, 2016; Weese, Heath, Dentinger, & Lau, 2015). In the case of soil bacteria, this involves Plant Growth-Promoting Rhizobacteria (PGPR), which may stimulate root growth, improve nutrient uptake (Kumar, Maurya, & Raghuwanshi, 2014; Majeed, Kaleem Abbasi, Hameed, Imran, & Rahim, 2015), alleviate plant stress (Barnawal et al., 2017; Furlan et al., 2017; García et al., 2017; Pande, Ns, & Bodhankar, 2016) or protect the plants from pathogens (Díaz Herrera, Grossi, Zawoznik, & Groppa, 2016; Keshavarz-Tohid et al., 2017). These effects rely on various modes of action, such as increasing nutrient availability, modulating plant hormonal balance (Cassán, Vanderleyden, & Spaepen, 2014; Saleem, Arshad, Hussain, & Bhatti, 2007), and/or producing bioactive metabolites (Couillerot et al., 2011; Vacheron et al., 2018). Since these beneficial effects would be of less interest under optimum conditions, it could be that the ability of crop genotypes to interact with PGPR populations, which are naturally present in soil, was not selected for during modern breeding (Morgan, Bending, & White, 2005; Schmidt et al., 2016; Weese et al., 2015). This type of trait may even have been counter-selected in the case where it involves a cost for the plant. This hypothesis has been evoked on several occasions (Kiers, Palmer, Ives, Bruno, & Bronstein, 2010; Morgan et al., 2005; West, Kiers, Simms, & Denison, 2002), but little has been done to test it experimentally with PGPR. In the case of mycorrhizal fungi, some studies reported that modern breeding lead to reduced dependence on mycorrhizal symbiosis (Hetrick, Wilson, & Cox, 1992; Hetrick, Wilson, & Cox, 1993), while in a more recent meta-analysis it was concluded that new cultivars were more mycorrhiza-responsive when compared to ancestral genotypes (Lehman, Barto, Powell, & Rillig, 2012). Given these inconsistent results in the literature, whether or not modern plant breeding has modified the ability of crop plants 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 Skerzzo, 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 *att*Tn7::miniTn7-
154 Gm-P*tac-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*(P*phl-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*(P*phl-*
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 µL 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*(P*phl-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*(P*phl-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. kilonensi*s 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 µL 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 mCherr*y* 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 $MgSO_4$ 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-dm^3 pots containing 1.8 kg
240 of sieved non-sterile soil (loam, organic matter 5.5%, pH_{H_2O} 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 2.5×10^6 CFU, whereas seeds in the other
248 half received 200 µL of $MgSO_4$ 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 Tm 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 been 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 Khi² 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*(P*phl-egfp*) colonization ranged from 15 ± (SE) 3 for
300 genotype Hendrix to 165 ± 10 arbitrary units (AU) for D130-63 at one week (Fig. **1a**). F113-
301 *mCherry*(P*phl-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 ± [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*(P*phl-egfp*) colonization was significantly
315 higher ($P = 0.039$) for old varieties (62 ± [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*(P*phl-*
327 *egfp*) inoculation, at levels ranging between 79 ± (SE) 3 for genotype Adular and 1920 ± 313
328 AU for D130-63 (Fig. **1b**). *phl* expression in F113-*mCherry*(P*phl-egfp*) for the 77 ancient
329 genotypes was significantly higher overall (P = 0.01) (490 ± [SE] 25 AU) than for the 115
330 modern genotypes (429 ± 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 ± [SE] 24 vs 102 ± 12) and *phl* expression (1802 ± 251 vs 661 ± 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 × 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 × 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 (± 7.3) % vs -34.8 (± 2.6) % variation for root volume,
407 -18.5 (± 4.6) % vs -34.8 (± 5.8) % for fresh root biomass, +6.9 (± 12.0) % vs -19.9 (± 19.3) %
408 for dry root biomass, and -9.9 (± 0.8) % vs -10.8 (± 3.6) % for root diameter. For shoot
409 parameters, stress resulted in -40.2 (± 5.4) % vs -40.1 (± 1.5) % variation for fresh shoot
410 biomass, -32.5 (± 6.8) % vs -34.9 (± 2.2) % for dry shoot biomass and -14.8 (± 2.9) % vs -14.1
411 (± 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 *kilonensi*s 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

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

Acknowledgments

This work was funded by the ANR BacterBlé (ANR-14-CE19-0017). We thank the Center of Biological Resources of INRA GDEC for supply of wheat seeds, Biogemma for technical advice, the *Serre* and the *Centre Technologique des Microstructures* platforms of FR BioEnviS for technical support, and team members for help with wheat harvest.

Author contribution

Conceptualization J.V., J.L.G., C.P.C. and Y.M.L.; Methodology, J.V., J.L.G., Y.M.L. and C.P.C.; Investigation, J.V., F.G., Y.M.L. and C.P.C.; Formal Analysis, J.V.; Writing – Original 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.; Funding Acquisition J.L.G., C.P.C. and Y.M.L.; Resources, J.L.G.

Conflict of interest

The authors declare that they have no conflict of interest

References

Andreote, F. D., Da Rocha, U. N., Araújo, W. L., Azevedo, J. L., & Van Overbeek, L. S. (2010). Effect of bacterial inoculation, plant genotype and developmental stage on root-associated and endophytic bacterial communities in potato (*Solanum tuberosum*). *Antonie van Leeuwenhoek*, *97*(4), 389–399. https://doi.org/10.1007/s10482-010-9421-9

Balfourier, F., Roussel, V., Strelchenko, P., Exbrayat-Vinson, F., Sourdille, P., Boutet, G., … Charmet, G. (2007). A worldwide bread wheat core collection arrayed in a 384-well plate. *Theoretical and Applied Genetics*, *114*(7), 1265–1275. https://doi.org/10.1007/s00122-007-0517-1

Baranski, M. R. (2015). Wide adaptation of Green Revolution wheat: International roots and the Indian context of a new plant breeding ideal, 1960-1970. *Studies in History and Philosophy of Science Part C*, *50*, 41–50. https://doi.org/10.1016/j.shpsc.2015.01.004

Barnawal, D., Bharti, N., Pandey, S. S., Pandey, A., Chanotiya, C. S., & Kalra, A. (2017). Plant growth-promoting rhizobacteria enhance wheat salt and drought stress tolerance by altering endogenous phytohormone levels and *TaCTR1/TaDREB2* expression. *Physiologia Plantarum*, *161*(4), 502–514. https://doi.org/10.1111/ppl.12614

Berry, P. M., Kendall, S., Rutterford, Z., Orford, S., & Griffiths, S. (2015). Historical analysis of the effects of breeding on the height of winter wheat (*Triticum aestivum*) and consequences for lodging. *Euphytica*, *203*(2), 375–383. https://doi.org/10.1007/s10681-014-1286-y

Beyer, S., Daba, S., Tyagi, P., Bockelman, H., Brown-Guedira, G., IWGSC, & Mohammadi, M. (2019). Loci and candidate genes controlling root traits in wheat seedlings — a wheat root GWAS. *Functional & Integrative Genomics*, *19*(1), 91–107. https://doi.org/10.1007/s10142-018-0630-z

Bonnin, I., Bonneuil, C., Goffaux, R., Montalent, P., & Goldringer, I. (2014). Explaining the decrease in the genetic diversity of wheat in France over the 20th century. *Agriculture, Ecosystems and Environment*, *195*, 183–192. https://doi.org/10.1016/j.agee.2014.06.003

Bordes, J., Branlard, G., Oury, F. X., Charmet, G., & Balfourier, F. (2008). Agronomic characteristics, grain quality and flour rheology of 372 bread wheats in a worldwide core

collection. *Journal of Cereal Science*, *48*(3), 569–579. https://doi.org/10.1016/j.jcs.2008.05.005

Borlaug, O. (1968). Wheat breeding and its impact on world food supply. In Proceedings of the Third International Wheat Genetics Symposium, pp. 1–36. Australian Academy of Science, Canberra

Borojevic, K., & Borojevic, K. (2005). The transfer and history of ''Reduced Height Genes'' (Rht) in wheat from Japan to Europe. *Journal of Heredity*, *96*(4), 455–459. doi:10.1093/jhered/esi06

Bouffaud, M. L., Kyselková, M., Gouesnard, B., Grundmann, G., Muller, D., & Moënne-Loccoz, Y. (2012). Is diversification history of maize influencing selection of soil bacteria by roots? *Molecular Ecology*, *21*(1), 195–206. https://doi.org/10.1111/j.1365-294X.2011.05359.x

Brazelton, J. N., Pfeufer, E. E., Sweat, T. A., McSpadden Gardener, B., & Coenen, C. (2008). 2,4-Diacetylphloroglucinol alters plant root development. *Molecular Plant-Microbe Interactions*, *21*(10), 1349–1358. https://doi.org/10.1094/MPMI-21-10-1349

Cao, P., Lu, C., & Yu, Z. (2018). Historical nitrogen fertilizer use in agricultural ecosystems of the contiguous United States during 1850-2015: Application rate, timing, and fertilizer types. *Earth System Science Data*, *10*(2), 969–984. https://doi.org/10.5194/essd-10-969-2018

Cassán, F., Vanderleyden, J., & Spaepen, S. (2014). Physiological and agronomical aspects of phytohormone production by model Plant-Growth-Promoting Rhizobacteria (PGPR) belonging to the genus *Azospirillum*. *Journal of Plant Growth Regulation*, *33*(2), 440–459. https://doi.org/10.1007/s00344-013-9362-4

Chamam, A., Wisniewski-Dyé, F., Comte, G., Bertrand, C., & Prigent-Combaret, C. (2015). Differential responses of *Oryza sativa* secondary metabolism to biotic interactions with cooperative, commensal and phytopathogenic bacteria. *Planta*, *242*(6), 1439–1452. https://doi.org/10.1007/s00425-015-2382-5

Costa, R., Gomes, N. C. M., Krögerrecklenfort, E., Opelt, K., Berg, G., & Smalla, K. (2007). *Pseudomonas* community structure and antagonistic potential in the rhizosphere: Insights gained by combining phylogenetic and functional gene-based analyses. *Environmental Microbiology*, *9*(9), 2260–2273. https://doi.org/10.1111/j.1462-2920.2007.01340.x

Couillerot, O., Bouffaud, M. L., Baudoin, E., Muller, D., Caballero-Mellado, J., & Moënne-Loccoz, Y. (2010). Development of a real-time PCR method to quantify the PGPR strain *Azospirillum lipoferum* CRT1 on maize seedlings. *Soil Biology and Biochemistry*, *42*(12), 2298–2305. https://doi.org/10.1016/j.soilbio.2010.09.003

613 Couillerot, O., Combes-Meynet, E., Pothier, J. F., Bellvert, F., Challita, E., Poirier, M. A., …
614 Prigent-Combaret, C. (2011). The role of the antimicrobial compound 2,4-
615 diacetylphloroglucinol in the impact of biocontrol *Pseudomonas fluorescens* F113 on
616 *Azospirillum brasilense* phytostimulators. *Microbiology*, *157*(6), 1694–1705.
617 https://doi.org/10.1099/mic.0.043943-0

618 De Werra, P., Baehler, E., Huser, A., Keel, C., & Maurhofer, M. (2008). Detection of plant-
619 modulated alterations in antifungal gene expression in *Pseudomonas fluorescens* CHA0
620 on roots by flow cytometry. *Applied and Environmental Microbiology*, *74*(5), 1339–1349.
621 https://doi.org/10.1128/AEM.02126-07

622 Den Herder, G., Van Isterdael, G., Beeckman, T., & De Smet, I. (2010). The roots of a new
623 green revolution. *Trends in Plant Science*, *15*(11), 600–607.
624 https://doi.org/10.1016/j.tplants.2010.08.009

625 Di Loreto, A., Bosi, S., Montero, L., Bregola, V., Marotti, I., Sferrazza, R. E., … Cifuentes, A.
626 (2018). Determination of phenolic compounds in ancient and modern durum wheat
627 genotypes. *Electrophoresis*, *39*(15), 2001–2010. https://doi.org/10.1002/elps.201700439

628 Di Salvo, L. P., Ferrando, L., Fernández-Scavino, A., & García de Salamone, I. E. (2018).
629 Microorganisms reveal what plants do not: wheat growth and rhizosphere microbial
630 communities after *Azospirillum brasilense* inoculation and nitrogen fertilization under field
631 conditions. *Plant and Soil*, *424*(1–2), 405–417. https://doi.org/10.1007/s11104-017-3548-
632 7

633 Díaz Herrera, S., Grossi, C., Zawoznik, M., & Groppa, M. D. (2016). Wheat seeds harbour
634 bacterial endophytes with potential as plant growth promoters and biocontrol agents of
635 *Fusarium graminearum*. *Microbiological Research*, *186–187*, 37–43.
636 https://doi.org/10.1016/j.micres.2016.03.002

637 Drogue, B., Sanguin, H., Chamam, A., Mozar, M., Llauro, C., Panaud, O., … Wisniewski-Dyé,
638 F. (2014). Plant root transcriptome profiling reveals a strain-dependent response during
639 *Azospirillum*-rice cooperation. *Frontiers in Plant Science*, *5*(November), 1–14.
640 https://doi.org/10.3389/fpls.2014.00607

641 Duffy, B. K., Défago, G., & Duffy, B. K. (1999). Environmental factors modulating antibiotic and
642 siderophore biosynthesis by *Pseudomonas fluorescens* biocontrol strains. *Applied and*
643 *Environmental Microbiology*, *65*(6), 2429–2438

644 El Zemrany, H., Czarnes, S., Hallett, P. D., Alamercery, S., Bally, R., & Jocteur Monrozier, L.
645 (2007). Early changes in root characteristics of maize (*Zea mays*) following seed
646 inoculation with the PGPR *Azospirillum lipoferum* CRT1. *Plant and Soil*, *291*(1-2), 109–

118

Engelhard, M., Hurek, T., & Reinhold-Hurek, B. (2000). Preferential occurrence of diazotrophic endophytes, *Azoarcus* spp., in wild rice species and land races of *Oryza sativa* in comparison with modern races. *Environmental Microbiology*, *2*(2), 131–141. https://doi.org/10.1046/j.1462-2920.2000.00078.x

Esmail, R. M., Eldessouky, S. E. I., Mahfouz, S. A., & El-Demardash, I. S. (2016). Evaluation of new bread wheat lines (Triticum aestivum L.) under normal and water stress conditions. *International Journal of ChemTech Research*, *9*(05), 89–99.

Furlan, F., Saatkamp, K., Volpiano, C. G., De Assis Franco, F., Dos Santos, M. F., Vendruscolo, E. C. G., … Da Costa, A. C. T. (2017). Plant growth-promoting bacteria effect in withstanding drought in wheat cultivars. *Scientia Agraria*, *18*(2), 104–113. https://doi.org/10.5380/rsa.v18i2.51385

García de Salamone, I. E., Hynes, R. K., & Nelson, L. M. (2001). Cytokinin production by plant growth promoting rhizobacteria and selected mutants. *Canadian Journal of Microbiology*, *47*(5), 404–411. https://doi.org/10.1139/cjm-47-5-404

García, J. E., Maroniche, G., Creus, C., Suárez-Rodríguez, R., Ramirez-Trujillo, J. A., & Groppa, M. D. (2017). *In vitro* PGPR properties and osmotic tolerance of different *Azospirillum* native strains and their effects on growth of maize under drought stress. *Microbiological Research*, *202*(April), 21–29. https://doi.org/10.1016/j.micres.2017.04.007

Gerland, P., Raftery, A. E., Ševčíková, H., Li, N., Gu, D., Spoorenberg, T., … Wilmoth, J. (2014). World population stabilization unlikely this century. *Science*, *346*(6206), 234 LP – 237. Retrieved from http://science.sciencemag.org/content/346/6206/234.abstract

Germida, J. J., & Siciliano, S. D. (2001). Taxonomic diversity of bacteria associated with the roots of modern, recent and ancient wheat cultivars. *Biology and Fertility of Soils*, *33*(5), 410–415. https://doi.org/10.1007/s003740100343

Gopal, M., & Gupta, A. (2016). Microbiome selection could spur next-generation plant breeding strategies. *Frontiers in Microbiology*, *7*(December), 1–10. https://doi.org/10.3389/fmicb.2016.01971

Gotti, R., Amadesi, E., Fiori, J., Bosi, S., Bregola, V., Marotti, I., & Dinelli, G. (2018). Differentiation of modern and ancient varieties of common wheat by quantitative capillary electrophoretic profile of phenolic acids. *Journal of Chromatography A*, *1532*, 208–215. https://doi.org/10.1016/j.chroma.2017.11.058

Hetrick, B. A. D., Wilson, G. W. T., & Cox, T. S. (1992). Mycorrhizal dependence of modern

wheat varieties, landraces, and ancestors. *Canadian Journal of Botany*, *70*(10), 2032-2040. https://doi.org/10.1139/b92-253

Hetrick, B. A. D., Wilson, G. W. T., & Cox, T. S. (1993). Mycorrhizal dependence of modern wheat varieties, landraces, and ancestors: a synthesis. *Canadian Journal of Botany*, *71*(3), 512-518. https://doi.org/10.1139/b93-056

Horst, W. J., Abdou, M., & Wiesler, F. (1993). Genotypic differences in phosphorus efficiency of wheat. *Plant and Soil*, *155–156*(1), 293–296. https://doi.org/10.1007/BF00025040

Huffman, W. E., & Evenson, R. E. (2001). Structural and productivity change in US agriculture, 1950-1982. *Agricultural Economics*, *24*(2), 127–147. https://doi.org/10.1016/S0169-5150(00)00065-7

Iannucci, A., Fragasso, M., Beleggia, R., Nigro, F., & Papa, R. (2017). Evolution of the crop rhizosphere: Impact of domestication on root exudates in tetraploid wheat (*Triticum turgidum* L.). *Frontiers in Plant Science*, *8*(December). https://doi.org/10.3389/fpls.2017.02124

Kazi, N., Deaker, R., Wilson, N., Muhammad, K., & Trethowan, R. (2016). The response of wheat genotypes to inoculation with *Azospirillum brasilense* in the field. *Field Crops Research*, *196*, 368–378. https://doi.org/10.1016/j.fcr.2016.07.012

KC, S., & Lutz, W. (2017). The human core of the shared socioeconomic pathways: Population scenarios by age, sex and level of education for all countries to 2100. *Global Environmental Change*, *42*, 181–192. https://doi.org/10.1016/j.gloenvcha.2014.06.004

Keel, C., Schnider, U., Maurhofer, M., Voisard, C., Laville, J., Burger, U., … Défago, G. (1992). Suppression of root disease by *Pseudomonas fluorescens* CHA0: Importance of the bacterial secondary metabolite 2,4-diacetylphloroglucinol. *Molecular Plant-Microbe Interactions*, *5*, 4–13. https://doi.org/10.1094/MPMI-5-004

Keshavarz-Tohid, V., Taheri, P., Muller, D., Prigent-Combaret, C., Vacheron, J., Taghavi, S. M., … Moënne-Loccoz, Y. (2017). Phylogenetic diversity and antagonistic traits of root and rhizosphere pseudomonads of bean from Iran for controlling *Rhizoctonia solani*. *Research in Microbiology*, *168*(8), 760–772. https://doi.org/10.1016/j.resmic.2017.08.002

Khakwani, A. A., Dennett, M. D., & Munir, M. (2011). Drought tolerance screening of wheat varieties by inducing water stress conditions. *Songklanakarin Journal of Science and Technology*, *33*(2), 135–142. https://doi.org/10.1016/j.fcr.2007.04.007

Kiers, E., Palmer, T. M., Ives, A. R., Bruno, J. F., & Bronstein, J. L. (2010). Mutualisms in a changing world: An evolutionary perspective. *Ecology Letters*, *13*(12), 1459–1474. https://doi.org/10.1111/j.1461-0248.2010.01538.x

Koch, B., Jensen, L. E., & Nybroe, O. (2001). A panel of Tn*7*-based vectors for insertion of the *gfp* marker gene or for delivery of cloned DNA into Gram-negative bacteria at a neutral chromosomal site. *Journal of Microbiological Methods*, *45*(3), 187–195. https://doi.org/10.1016/S0167-7012(01)00246-9

Kumar, A., Maurya, B. R., & Raghuwanshi, R. (2014). Isolation and characterization of PGPR and their effect on growth, yield and nutrient content in wheat (*Triticum aestivum* L.). *Biocatalysis and Agricultural Biotechnology*, *3*(4), 121–128. https://doi.org/10.1016/j.bcab.2014.08.003

Landa, B. B., Mavrodi, O. V, Raaijmakers, J. M., McSpadden Gardener, B. B., Thomashow, L. S., & Weller, D. M. (2002). Differential ability of genotypes of *Pseudomonas fluorescens* strains to colonize the roots of pea plants. *Applied and Environmental Microbiology*, *68*(7), 3226–3237. https://doi.org/10.1128/AEM.68.7.3226

Lehman, A., Barto, E. K., Powell, J. R., & Rillig, M. C. (2012). Mycorrhizal responsiveness trends in annual crop plants and their wild relatives—a meta-analysis on studies from 1981 to 2010. *Plant and Soil*, *355*, 231–250. https://doi.org/10.1007/s11104-011-1095-1

Lumpkin, T.A. (2015). How a gene from Japan revolutionized the world of wheat: CIMMYT's quest for combining genes to mitigate threats to global food security. In Advances in wheat genetics: from genome to field. (eds. Y. Ogihara, S. Takumi, H. Handa), pp 13-20. Springer, Tokyo

Majeed, A., Kaleem Abbasi, M., Hameed, S., Imran, A., & Rahim, N. (2015). Isolation and characterization of plant growth-promoting rhizobacteria from wheat rhizosphere and their effect on plant growth promotion. *Frontiers in Microbiology*, *6*(March), 1–10. https://doi.org/10.3389/fmicb.2015.00198

Marx, C. J., & Lidstrom, M. E. (2002). Broad-host-range *cre-lox* system for antibiotic marker recycling in Gram-negative bacteria. *BioTechniques*, *33*(5), 1062–1067

Mazzola, M., Funnell, D. L., & Raaijmakers, J. M. (2004). Wheat cultivar-specific selection of 2,4-diacetylphloroglucinol-producing fluorescent *Pseudomonas* species from resident soil populations. *Microbial Ecology*, *48*(3), 338–348. https://doi.org/10.1007/s00248-003-1067-y

Morgan, J. A. W., Bending, G. D., & White, P. J. (2005). Biological costs and benefits to plant-microbe interactions in the rhizosphere. *Journal of Experimental Botany*, *56*(417), 1729–1739. https://doi.org/10.1093/jxb/eri205

Neiverth, A., Delai, S., Garcia, D. M., Saatkamp, K., de Souza, E. M., Pedrosa, F. de O., … da Costa, A. C. T. (2014). Performance of different wheat genotypes inoculated with the plant

749 growth promoting bacterium *Herbaspirillum seropedicae*. *European Journal of Soil*
750 *Biology*, *64*, 1–5. https://doi.org/10.1016/j.ejsobi.2014.07.001

751 Notz, R., Maurhofer, M., Schnider-Keel, U., Duffy, B., Haas, D., & Défago, G. (2001). Biotic
752 factors affecting expression of the 2,4-diacetylphloroglucinol biosynthesis gene *phlA* in
753 *Pseudomonas fluorescens* biocontrol strain CHA0 in the rhizosphere. *Phytopathology*,
754 *91*(9), 873–881. https://doi.org/10.1094/PHYTO.2001.91.9.873

755 Pande, A. M., Ns, K., & Bodhankar, M. G. (2016). Effect of PGPR with ACC- deaminase activity
756 on growth performance of wheat cultivated under stress conditions. *International Journal*
757 *of Applied Research*, *2*(1), 723–726

758 Paulin, M. M., Novinscak, A., St-Arnaud, M., Goyer, C., Decoste, N. J., Privé, J. P., … Filion,
759 M. (2009). Transcriptional activity of antifungal metabolite-encoding genes *phlD* and
760 *hcnBC* in *Pseudomonas* spp. using qRT-PCR. *FEMS Microbiology Ecology*, *68*(2), 212–
761 222. https://doi.org/10.1111/j.1574-6941.2009.00669.x

762 Peng, J., Richards, D. E., Hartley, N. M., Murphy, G. P., Devos, K. M., Flintham, J. E., …,
763 Harberd, N. P. (1999). 'Green revolution' genes encode mutant gibberellin response
764 modulators. *Nature*, *400*(6741), 256-261. https://doi.org/10.1038/22307

765 Pérez-Jaramillo, J. E., Carrion, V. J., Bosse, M., Ferrao, L. F. V, de Hollander, M., Garcia, A.
766 A. F., … Raaijmakers, J. M. (2017). Linking rhizosphere microbiome composition of wild
767 and domesticated *Phaseolus vulgaris* to genotypic and root phenotypic traits. *ISME*
768 *Journal*, *11*, 2244–2257. https://doi.org/10.1038/ismej.2017.85

769 Plessis, A., Ravel, C., Bordes, J., Balfourier, F., & Martre, P. (2013). Association study of wheat
770 grain protein composition reveals that gliadin and glutenin composition are trans-
771 regulated by different chromosome regions. *Journal of Experimental Botany*, *64*(12),
772 3627–3644. https://doi.org/10.1093/jxb/ert188

773 Pour-Aboughadareh, A., Ahmadi, J., Mehrabi, A., Moghaddam, M., & Etminan, A. (2017).
774 Evaluation of diversity in wild relatives of wheat. *Journal of Agricultural Science and*
775 *Technology*, *19*(4), 943–956

776 Ramshini, H., Mirzazadeh, T., Moghaddam, M. E., & Amiri, R. (2016). Comparison of old and
777 new wheat cultivars in Iran by measuring germination related traits, osmotic tolerance and
778 ISSR diversity. *Physiology and Molecular Biology of Plants*, *22*(3), 391–398.
779 https://doi.org/10.1007/s12298-016-0372-0

780 Rochat, L., Péchy-Tarr, M., Baehler, E., Maurhofer, M., & Keel, C. (2010). Combination of
781 fluorescent reporters for simultaneous monitoring of root colonization and antifungal gene
782 expression by a biocontrol pseudomonad on cereals with flow cytometry. *Molecular Plant-*

Microbe Interactions, *23*(7), 949–961. https://doi.org/10.1094/MPMI-23-7-0949

Roussel, V., Leisova, L., Exbrayat, F., Stehno, Z., & Balfourier, F. (2005). SSR allelic diversity changes in 480 European bread wheat varieties released from 1840 to 2000. *Theoretical and Applied Genetics*, *111*(1), 162–170. https://doi.org/10.1007/s00122-005-2014-8

Saleem, M., Arshad, M., Hussain, S., & Bhatti, A. S. (2007). Perspective of plant growth promoting rhizobacteria (PGPR) containing ACC deaminase in stress agriculture. *Journal of Industrial Microbiology and Biotechnology*, *34*(10), 635–648. https://doi.org/10.1007/s10295-007-0240-6

Sasaki, K., Ikeda, S., Eda, S., Mitsui, H., Hanzawa, E., Kisara, C., … Sato, T. (2010). Impact of plant genotype and nitrogen level on rice growth response to inoculation with *Azospirillum* sp. strain B510 under paddy field conditions. *Soil Science and Plant Nutrition*, *56*(4), 636–644. https://doi.org/10.1111/j.1747-0765.2010.00499.x

Schmidt, J. E., Bowles, T. M., & Gaudin, A. C. M. (2016). Using ancient traits to convert soil health into crop yield: Impact of selection on maize root and rhizosphere function. *Frontiers in Plant Science*, *7*(March), 1–11. https://doi.org/10.3389/fpls.2016.00373

Schnider-Keel, U., Seematter, A., Maurhofer, M., Blumer, C., Duffy, B., Gigot-Bonnefoy, C., … Keel, C. (2000). Autoinduction of 2, 4-diacetylphloroglucinol biosynthesis in the biocontrol agent *Pseudomonas fluorescens* CHA0 and repression by the bacterial metabolites salicylate and pyoluteorin. *Journal of Bacteriology*, *182*(5), 1215–1225. Retrieved from http://jb.asm.org/content/182/5/1215.short

Shanahan, P., O'Sullivan, D. J., Simpson, P., Glennon, J. D., & O'Gara, F. (1992). Isolation of 2,4-diacetylphloroglucinol from a fluorescent pseudomonad and investigation of physiological parameters influencing its production. *Applied and Environmental Microbiology*, *58*(1), 353–358

Shaposhnikov, A., Morgounov, A., Akin, B., Makarova, N., Belimov, A., & Tikhonovich, I. (2016). Comparative characteristics of root systems and root exudation of synthetic, landrace and modern wheat varieties. *Agricultural Biology*, *51*(1), 68–78. https://doi.org/10.15389/agrobiology.2016.1.68rus

Siddique, K. H. M., Belford, R. K., & Tennant, D. (1990). Root:shoot ratios of old and modern, tall and semi-dwarf wheats in a mediterranean environment. *Plant and Soil*, *121*(1), 89–98. https://doi.org/10.1007/BF00013101

Subira, J., Ammar, K., Álvaro, F., García del Moral, L. F., Dreisigacker, S., & Royo, C. (2016). Changes in durum wheat root and aerial biomass caused by the introduction of the *Rht-B1b* dwarfing allele and their effects on yield formation. *Plant and Soil*, *403*(1–2), 291–

817 304. https://doi.org/10.1007/s11104-015-2781-1

818 Thomas, P., & Sekhar, A. C. (2016). Effects due to rhizospheric soil application of an
819 antagonistic bacterial endophyte on native bacterial community and its survival in soil: A
820 case study with *Pseudomonas aeruginosa* from banana. *Frontiers in Microbiology*,
821 *7*(April), 1–16. https://doi.org/10.3389/fmicb.2016.00493

822 Vacheron, J., Combes-Meynet, E., Walker, V., Gouesnard, B., Muller, D., Moënne-Loccoz, Y.,
823 & Prigent-Combaret, C. (2016). Expression on roots and contribution to maize
824 phytostimulation of 1-aminocyclopropane-1-decarboxylate deaminase gene *acdS* in
825 *Pseudomonas fluorescens* F113. *Plant and Soil*, *407*(1–2), 187–202.
826 https://doi.org/10.1007/s11104-016-2907-0

827 Vacheron, J., Desbrosses, G., Renoud, S., Padilla, R., Walker, V., Muller, D., & Prigent-
828 Combaret, C. (2018). Differential contribution of plant-beneficial functions from
829 *Pseudomonas kilonensis* F113 to root system architecture alterations in *Arabidopsis*
830 *thaliana* and *Zea mays*. *Molecular Plant-Microbe Interactions*, *31*(2), 212–223.
831 https://doi.org/10.1094/MPMI-07-17-0185-R

832 Von Felten, A., Défago, G., & Maurhofer, M. (2010). Quantification of *Pseudomonas*
833 *fluorescens* strains F113, CHA0 and Pf153 in the rhizosphere of maize by strain-specific
834 real-time PCR unaffected by the variability of DNA extraction efficiency. *Journal of*
835 *Microbiological Methods*, *81*(2), 108–115. https://doi.org/10.1016/j.mimet.2010.02.003

836 Walker, V., Bruto, M., Bellvert, F., Bally, R., Muller, D., Prigent-Combaret, C., … Comte, G.
837 (2013). Unexpected phytostimulatory behavior for *Escherichia coli* and *Agrobacterium*
838 *tumefaciens* model strains. *Molecular Plant-Microbe Interactions*, *26*(5), 495–502.
839 https://doi.org/10.1094/MPMI-12-12-0298-R

840 Walker, V., Couillerot, O., von Felten, A., Bellvert, F., Jansa, J., Maurhofer, M., … Comte, G.
841 (2012). Variation of secondary metabolite levels in maize seedling roots induced by
842 inoculation with *Azospirillum, Pseudomonas* and *Glomus* consortium under field
843 conditions. *Plant and Soil*, *356*(1–2), 151–163. https://doi.org/10.1007/s11104-011-0960-
844 2

845 Walsh, U. F., Moënne-Loccoz, Y., Tichy, H. V., Gardner, A., Corkery, D. M., Lorkhe, S., &
846 O'Gara, F. (2003). Residual impact of the biocontrol inoculant *Pseudomonas fluorescens*
847 F113 on the resident population of rhizobia nodulating a red clover rotation crop. *Microbial*
848 *Ecology*, *45*(2), 145–155. https://doi.org/10.1007/s00248-002-2026-8

849 Weese, D. J., Heath, K. D., Dentinger, B. T. M., & Lau, J. A. (2015). Long-term nitrogen addition
850 causes the evolution of less-cooperative mutualists. *Evolution*, *69*(3), 631–642.

https://doi.org/10.1111/evo.12594

Wei, Z., & Jousset, A. (2017). Plant breeding goes microbial. *Trends in Plant Science*, *22*(7), 555–558. https://doi.org/10.1016/j.tplants.2017.05.009

West, S. A., Kiers, E. T., Simms, E. L., & Denison, R. F. (2002). Sanctions and mutualism stability: Why do rhizobia fix nitrogen? *Proceedings of the Royal Society B: Biological Sciences*, *269*(1492), 685–694. https://doi.org/10.1098/rspb.2001.1878

Winfield, M. O., Allen, A. M., Wilkinson, P. A., Burridge, A. J., Barker, G. L. A., Coghill, J., … Edwards, K. J. (2018). High-density genotyping of the A.E. Watkins Collection of hexaploid landraces identifies a large molecular diversity compared to elite bread wheat. *Plant Biotechnology Journal*, *16*(1), 165–175. https://doi.org/10.1111/pbi.12757

Wissuwa, M., Mazzola, M., & Picard, C. (2009). Novel approaches in plant breeding for rhizosphere-related traits. *Plant and Soil*, *321*(1–2), 409–430. https://doi.org/10.1007/s11104-008-9693-2

York, L. M., Galindo-Castañeda, T., Schussler, J. R., & Lynch, J. P. (2015). Evolution of US maize (*Zea mays* L.) root architectural and anatomical phenes over the past 100 years corresponds to increased tolerance of nitrogen stress. *Journal of Experimental Botany*, *66*(8), 2347–2358. https://doi.org/10.1093/jxb/erv074

Zhang, F. L., Niu, X. K., Zhang, Y. M., Xie, R. Z., Liu, X., Li, S. K., & Gao, S. J. (2013). Studies on the root characteristics of maize varieties of different eras. *Journal of Integrative Agriculture*, *12*(3), 426–435. https://doi.org/10.1016/S2095-3119(13)60243-9

872 **Figure legends**

873

874 **Fig. 1** Root colonization and *phl* expression of *P. kilonensis* F113-*mCherry*(P*phl-egfp*) on 192
875 individual wheat genotypes corresponding to 115 modern genotypes (> 1960) and 77 ancient
876 genotypes (including 42 old varieties [≤ 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*(P*phl-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*(P*phl-*
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*(P*phl*-*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(P*phl*-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
***phl* 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

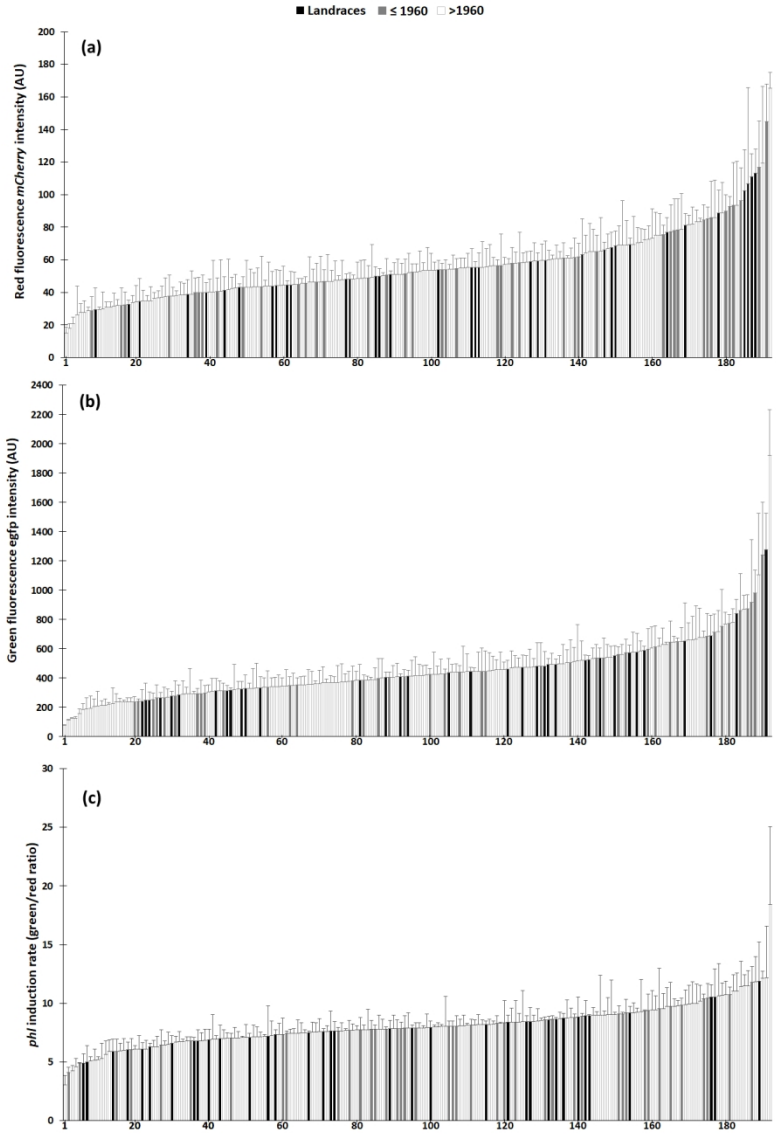

Figure 1

353x542mm (96 x 96 DPI)

Fig. 2

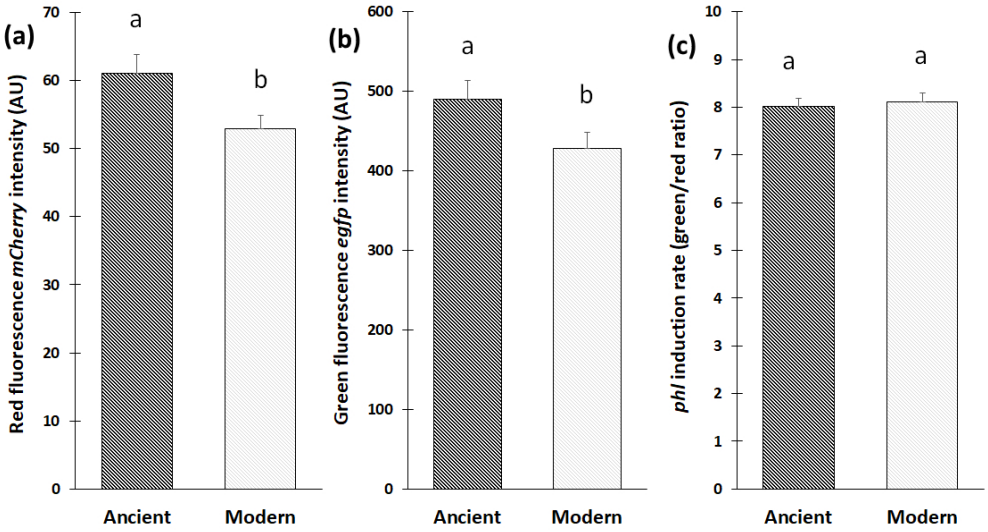

Figure 2

296x177mm (96 x 96 DPI)

Fig. 3

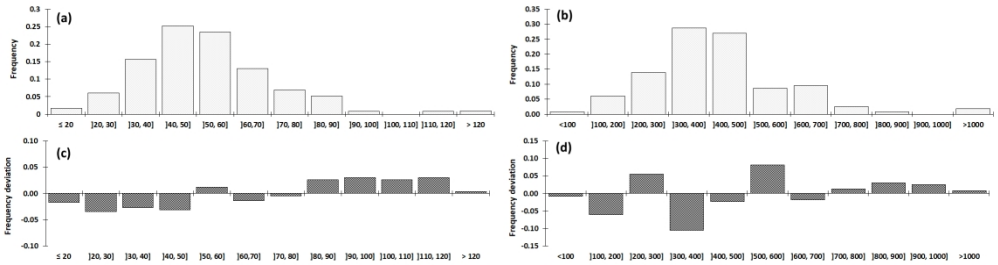

Figure 3

605x177mm (96 x 96 DPI)

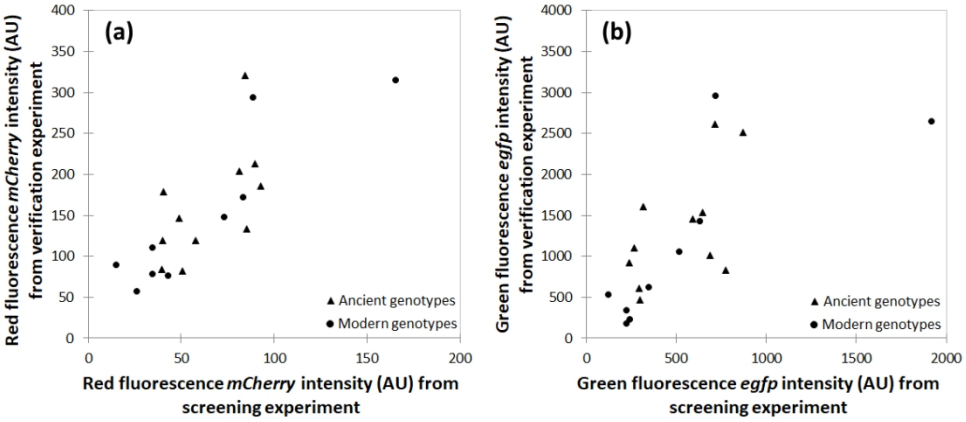

Fig. 4

Figure 4

320x158mm (96 x 96 DPI)

Fig. 5

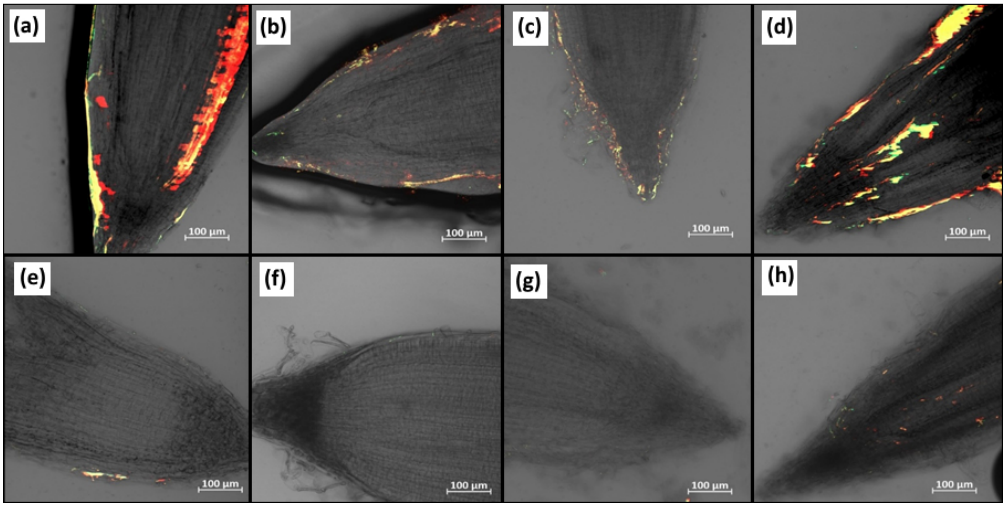

Figure 5

239x126mm (96 x 96 DPI)

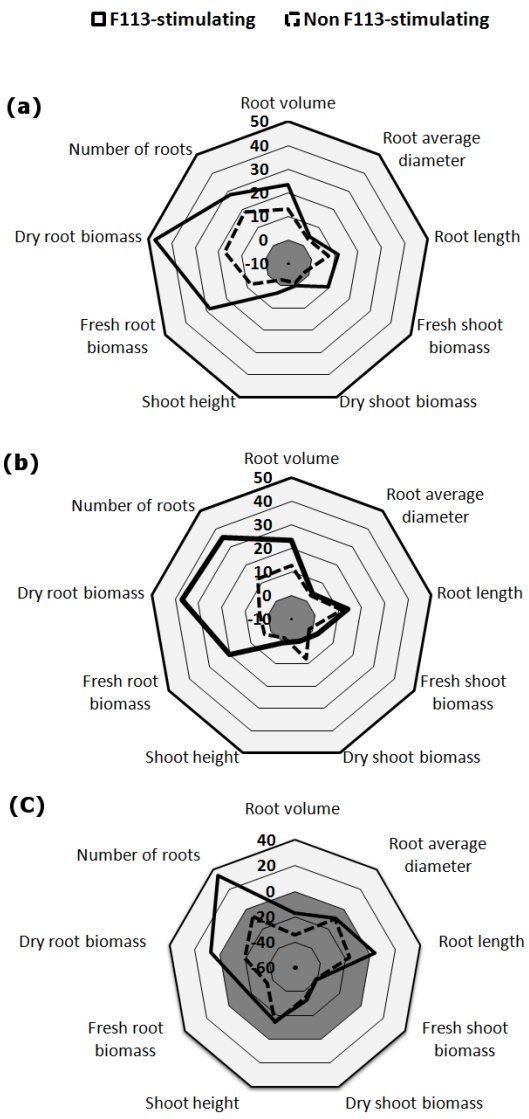

Fig. 6

Figure 6

182x363mm (96 x 96 DPI)