Impact of wheat genotype on its interactions with rhizobacteria : what influence of modern breeding on the relationships between plants and PGPR ?

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Impact du génotype de blé dans les interactions avec les rhizobactéries : quelle influence de la sélection variétale sur les relations entre plantes et bactéries phytostimulatrices ?

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Impact du génotype de blé dans les interactions avec les rhizobactéries : quelle influence de la sélection variétale sur les relations entre plantes et bactéries phytostimulatrices ?

Résumé

La domestication des plantes de grande culture comme le blé a abouti à l'apparition de nouvelles espèces plus simples à cultiver, puis la sélection moderne, plus contemporaine, a pris le relais. Depuis 1960, la grande majorité des variétés de blé sont des variétés naines (dues aux gènes *Rht*), présentant de nombreuses différences génétiques, morphologiques et physiologiques par rapport aux variétés plus anciennes. Ainsi, elles sont capables de mieux utiliser les apports d'engrais synthétiques utilisés en masse depuis le milieu du 20ème siècle, et présentent des rendements supérieurs. Cependant, peu d'intérêt a été porté au système racinaire et aux impacts sur les interactions avec les bactéries du sol. Pourtant, la main de l'Homme pourrait avoir rendu caduque les effets bénéfiques apportés par les PGPR (*Plant Growth-Promoting Rhizobacteria*) aux plantes, et les traits génétiques permettant les interactions entre plante et PGPR pourraient ne pas avoir été sélectionnés au sein des génotypes de variétés modernes. L'hypothèse testée dans cette thèse est que les PGPR interagiraient mieux avec des variétés anciennes qu'avec des variétés modernes. Pour cela nous avions à disposition un échantillonnage de 199 accessions de blé tendre (*Triticum aestivum aestivum*) représentatif des variétés de blés sélectionnées depuis le milieu du 19ème siècle. Par une approche de criblage, nous avons évalué *in vitro* la capacité de deux souches modèles PGPR, *Pseudomonas kilonensis* F113 et *Azospirillum brasilense* Sp245, à coloniser les racines de ces génotypes de blé, et à y exprimer des gènes impliqués dans des fonctions bénéfiques pour la croissance de la plante : l'opéron *phl* (synthèse de 2,4-diacétylphloroglucinol) pour la PGPR F113 et le gène *ppdC* (synthèse d'acide indole-3-acétique) pour Sp245. Par la suite nous avons testé si les résultats obtenus *in vitro* se traduisaient en une amélioration de la croissance du blé par une approche d'inoculation réalisée en sol, sous serre. Enfin, une étude aux champs a été menée pour analyser l'impact des génotypes de blé sur les bactéries indigènes du sol. Ces travaux ont montré (1) une meilleure aptitude de F113 et Sp245 à interagir avec les génotypes anciens *in vitro*, (2) de meilleurs effets phytostimulateurs suite à l'inoculation de PGPR chez les génotypes de blés ayant présenté de bons résultats lors du criblage et (3) un impact du génotype de blé sur les bactéries indigènes associées à ses racines, notamment entre génotypes anciens et génotypes modernes. En conclusion, même si certaines variétés modernes restent capables de bien interagir avec les PGPR, la sélection variétale a néanmoins eu globalement un impact significatif négatif sur les relations entre PGPR et plantes

Impact of wheat genotype on the interactions with rhizobacteria : which influence of modern breeding on the relationships between plants and PGPR ?

Abstract

Plant domestication led to the creation of new plant species better suited for agriculture, then modern breeding took the lead. Since 1960, the great majority of wheat modern varieties are dwarf varieties (because of *Rht* genes), showing several genetic, morphologic and physiologic differences compared to ancient varieties. Thus, they are more able to use synthetic fertilizers used in huge quantities since the mid-20th and show higher yield. However, few studies have been made regarding the impact of modern breeding on root systems and on interactions of crops with soil bacteria. Yet, these evolutions in agricultural practices could have reduced the beneficial effects brought by PGPR (Plant Growth-Promoting Rhizobacteria), and the genetic traits involved in these interactions between plant and PGPR may have not been selected in modern genotypes. Our work hypothesis in this thesis is that PGPR are more able to interact with ancient genotypes than modern ones. To test this hypothesis, we had a sampling of 199 bread wheat (*Triticum aestivum aestiuvm*) accessions representative of the wheat varieties selected since the mid-19th. Using an *in vitro* screening approach, we assessed the abilities of two PGPR model strains, *Pseudomonas kilonensis* F113 and *Azospirillum brasilense* Sp245, to colonize the roots of these genotypes and to express genes involved in plant-beneficial functions: *phl* operon (production of 2,4-diacetylphloroglucinol) for F113 and *ppdC* gene (production of indole-3-acetic acid) for Sp245. Then, we assessed whether the results obtained *in vitro* had a biological significance by measuring the amelioration of growth performance of wheat genotypes using a soil pot inoculation experiment under greenhouse. Finally, an in-field study was performed to analyze the impact of wheat genotypes on the indigenous bacterial communities. This work showed (1) a better ability of F113 and Sp245 to interact with ancient wheat genotypes than modern ones, (2) better growth performance improvements in wheat genotypes that showed good results during screening experiments and (3) an impact of wheat genotypes on indigenous bacterial communities, notably between ancient and modern genotypes. To conclude, even if some modern genotypes still are able to greatly interact with PGPR, modern breeding had overall a significant negative impact on the relations between plants and PGPR.

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Introduction générale

Diversité des organismes vivants et de leurs interactions biologiques

La diversité de la vie existant dans la nature est l'une des plus impressionnantes caractéristiques propres à notre planète. Les organismes vivants sont classés en taxons allant du domaine, qui inclut eucaryotes, bactéries et archées (Woese *et al.* 1990), à l'espèce (Mora *et al.* 2011), avec au sein même de l'espèce une diversité en souches souvent importante (Stackebrandt and Goebel 1994). Nombre de scientifiques ont voulu connaitre le nombre d'espèces existant à la surface de la Terre. Toutefois, si environ 1.8 millions de taxons ont à ce jour été décrits (Roskov *et al.* 2018) un grand pourcentage d'espèces reste à décrire et à ce jour aucun nombre totalisant l'intégralité des espèces sur Terre ne fait consensus, la plupart des estimations pouvant aller de 2 millions à 100 millions d'espèces (Larsen 2017). Concernant les microorganismes, il a même été récemment estimé que la Terre pourrait être le lieu de vie de 1 trillion (= 10^{12}) d'espèces (Locey and Lennon 2016). Une espèce se développe au sein d'un lieu de vie défini par des conditions physico-chimiques déterminées, et formant un biotope. Ce biotope est le théâtre d'interactions se déroulant entre les individus de différentes espèces au sein de la communauté. Ainsi, une gradation des interactions biologiques a été mise en évidence (Faust and Raes 2012). Cela inclut des interactions négatives (parasitisme, prédation, amensalisme), neutres ou positives pour l'un ou les 2 protagonistes (commensalisme, coopération, mutualisme ; **Fig 1**).

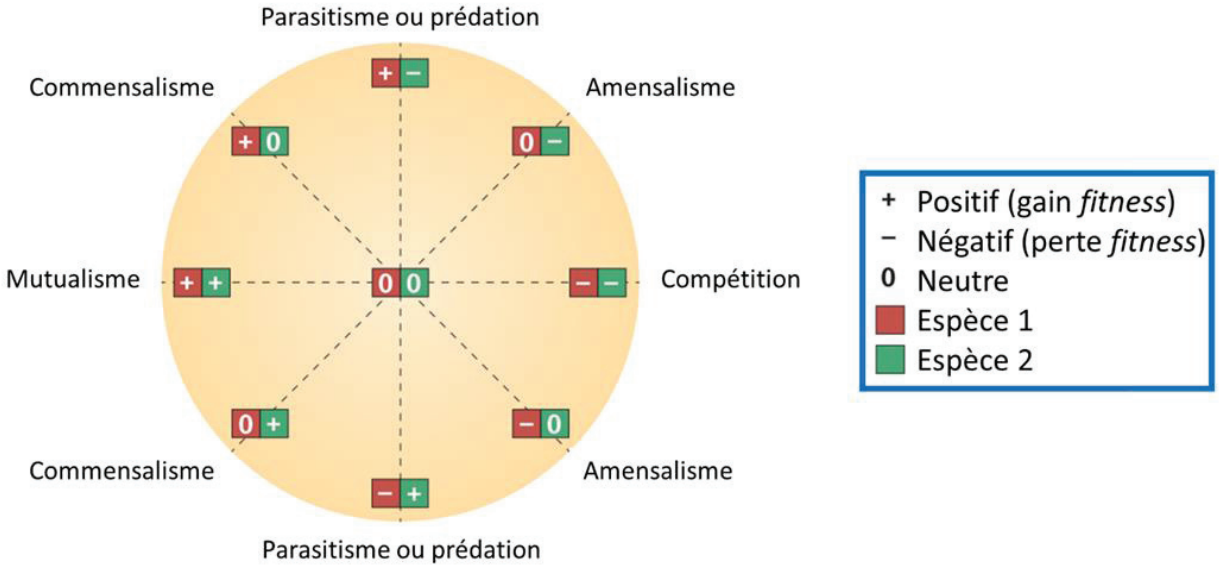

Fig 1 Résumé des interactions écologiques possibles entre 2 protagonistes d'espèces différentes. Pour chacune des interactions, 2 issues sont possibles pour chacun des protagonistes, soit neutre, soit un gain de valeur sélective (i.e. *fitness*), soit une perte de valeur sélective. Ces interactions sont susceptibles d'évoluer au cours de l'évolution de chacun des protagonistes, ou peuvent co-évoluer. D'après Faust et Raes 2012.

Les interactions entre les plantes et les microorganismes forment un domaine d'étude particulièrement riche. A l'instar du microbiote humain, qui regroupe de nombreuses espèces bactériennes tout en étant spécifique de son hôte (Tap *et al.* 2009), une plante présente un ensemble de microorganismes qui lui est associé, à la fois à sa surface et à l'intérieur, et l'association entre l'organisme–hôte et son microbiote forme l'holobionte (Vandenkoornhuyse *et al.* 2015) (**Fig 2**). Ainsi, si certains microorganismes sont considérés comme des phytopathogènes en raison de leur style de vie et des effets délétères qui en résultent sur la santé et le développement de leur plante-hôte (Sperschneider *et al.* 2015; Nowell *et al.* 2016), d'autres ont un style de vie conduisant à des bénéfices pour la santé et la croissance de la plante en participant à des fonctions comme la nutrition de la plante ou la résistance contre des phytopathogènes ou des stress abiotiques (Parnell *et al.* 2016; Finkel *et al.* 2017). Ces microorganismes bénéfiques vont ainsi être à l'origine d'une augmentation de la 'fitness' de leur plante-hôte, c'est-à-dire sa valeur sélective qui correspond à sa capacité à assurer une descendance (Kiers *et al.* 2002, 2010).

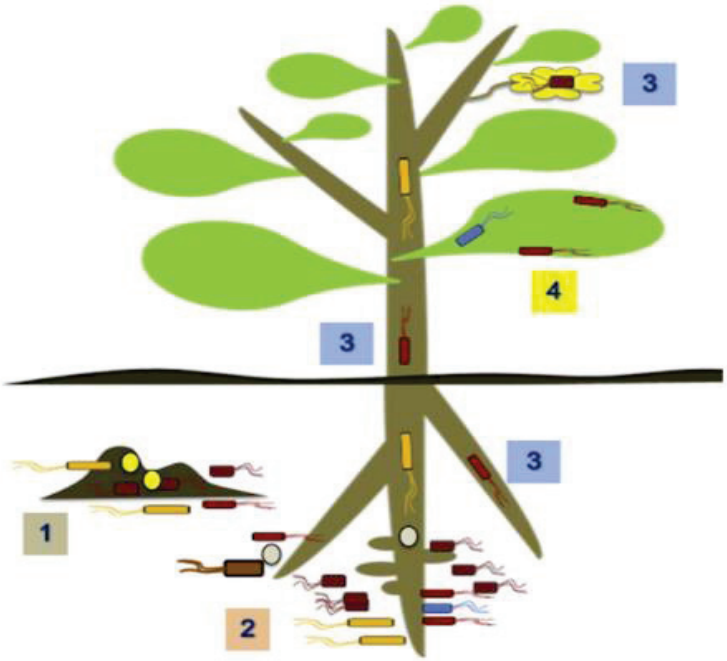

Fig 2 Le microbiote de la plante. En (1) on trouve le sol nu, en dehors de la zone d'influence des racines mais d'où les microorganismes peuvent avoir une certaine influence sur le développement de la plante. En (2) se trouve la rhizosphère, zone du sol où les microorganismes se trouvent en forte abondance et sous influence des racines. En (3) l'endosphère représente l'ensemble des tissus internes de la plante que les microorganismes endophytes peuvent coloniser. En (4) la phyllosphère représente les tissus foliaires en surface. L'ensemble du microbiote et de la plante forme l'holobionte. D'après Orozco-Mosqueda *et al.* 2018.

Parmi les microorganismes bénéfiques associés aux racines des plantes, on trouve notamment des champignons formant des mycorhizes arbusculaires, qui vont surtout assurer des fonctions de nutrition minérale, notamment pour l'acquisition de phosphore dans le sol, et recevoir en retour du carbone sous forme de photosynthétats (Jeffries *et al.* 2003). Les bactéries stimulatrices de la croissance des plantes ou *Plant*

Growth-Promoting Rhizobacteria (PGPR) sont des bactéries associées (coopération) aux racines de plantes et caractérisées par un style de vie qui profite à leur plante-hôte) (Vacheron *et al.* 2013; Puga-Freitas et Blouin 2015). Ces PGPR appartiennent à plusieurs genres dont *Pseudomonas* et *Azospirillum* et peuvent assurer diverses fonctions (Couillerot *et al.* 2011; Vacheron *et al.* 2016). Ainsi, les PGPR appartiennent à un ou plusieurs groupes fonctionnels tels que les diazotrophes qui vont contribuer à des fonctions de nutrition azotée de la plante par la fixation de l'azote atmosphérique (Bouffaud *et al.* 2016), les producteurs d'acide indole-3-acétique, un composé appartenant à la famille des auxines (hormone végétale ayant des propriétés de ramification racinaire) (Spaepen *et al.* 2007a), ou les producteurs de phloroglucinols, ces derniers représentant une classe biochimique de composés ayant des propriétés antimicrobiennes ainsi que des propriétés similaires à celles des auxines (Mazzola *et al.* 2004; Brazelton *et al.* 2008). Les bactéries appartenant à ces groupes fonctionnels sont caractérisées par la possession de gènes ou opérons codant des fonctions communes, respectivement *nifH* (fixation libre de l'azote atmosphérique), *ppdC* (une des voies connues pour la production d'auxine) et *phl* (production de 2,4-diacétylphloroglucinol) (Bruto *et al.* 2014; Lemanceau *et al.* 2017) pour ce qui est des exemples cités plus haut.

Il a été suggéré que les microorganismes du sol auraient joué un rôle indispensable depuis plusieurs millénaires dans la propagation des plantes sauvages sur terre (Heckman *et al.* 2001), et auraient coévolué avec elles notamment du fait des bénéfices mutuels apportés (Lemanceau *et al.* 2017; Blouin 2018). En effet, les interactions mutualistes ou coopératives se déroulant entre ces organismes microbiens et végétaux ont été comparées par certains auteurs à un marché biologique (en anglais *Biological Market Theory*, BMT), où les protagonistes proposent des commodités qui peuvent être des services ou des biens, et choisissent leur partenaire avec qui échanger (Noë and Kiers 2018). Il y a ainsi une compétition se déroulant entre les « vendeurs » qui proposent le même type de commodités et qui vont chercher à augmenter leur attractivité auprès des « acheteurs » en augmentant leur valeur d'échange de la commodité proposée, soit par la quantité soit par la qualité (Noë and Kiers 2018). Pour que la relation de mutualisme/coopération se prolonge entre les deux protagonistes, une coévolution de leurs besoins réciproques semble nécessaire.

Changements des pratiques agricoles et impacts sur les protagonistes des interactions coopératives plantes-PGPR

Au cours de l'évolution des génotypes de plantes sauvages, qui ne bénéficient d'aucune aide artificielle de la part de l'Homme, il est généralement admis que les microorganismes bénéfiques du sol et notamment les PGPR ont joué un rôle important dans leur développement (Bulgarelli *et al.* 2015; Pérez-Jaramillo *et al.* 2016, 2017, 2018) . Cependant, l'avènement de la domestication des plantes par l'homme, aboutissant à la création de nouvelles espèces de plantes plus simples à cultiver, puis la sélection variétale moderne ont induit un biais dans la sélection jusque-là naturelle de ces génotypes de plantes et pourraient avoir modifié les interactions

des plantes avec les PGPR (**Fig 3**). Deux raisons majeures pourraient expliquer cela, la première étant l'aménagement des parcelles utilisées pour l'agriculture, qui correspondent généralement à des zones de sol particulièrement fertiles pour les cultures et propices à leur développement, et l'utilisation massive d'intrants chimiques, surtout depuis 1960 (Baranski 2015; Schmidt *et al.* 2016). La deuxième raison est la sélection par l'homme de nouvelles variétés génétiquement stables et présentant des caractères aériens satisfaisants, ainsi que des critères de productivité et de qualité dans ces conditions de culture optimales, mais tout en négligeant globalement les effets de cette sélection sur les caractéristiques racinaires (Huffman and Evenson 2001; Brancourt-Hulmel *et al.* 2003; Brisson *et al.* 2010).

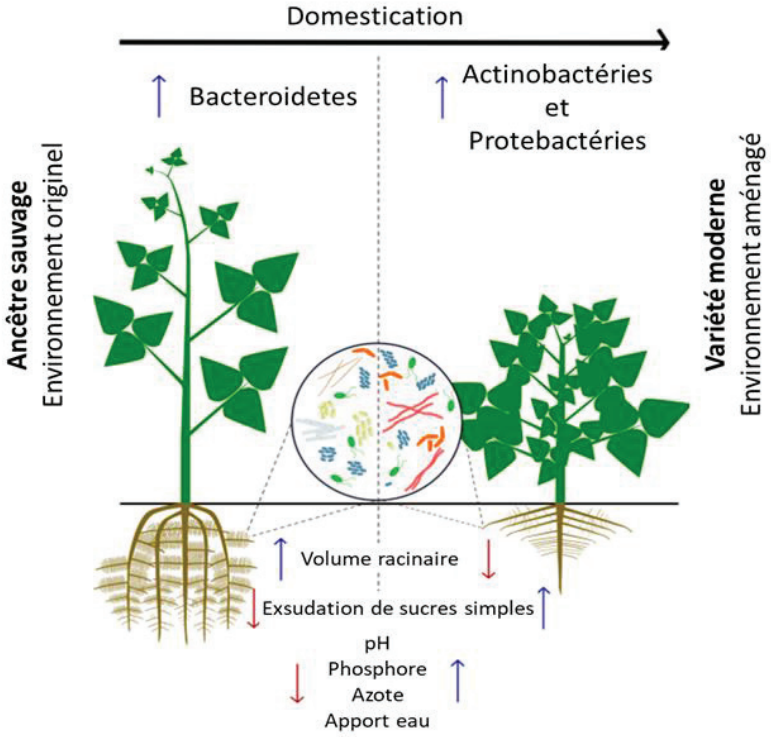

Fig 3 Impacts de la domestication sur le phénotype et la physiologie des plantes, et la diversité des rhizobactéries associées à ces plantes. Dans cette représentation en partie hypothétique, la morphologie aérienne et racinaire des plantes sauvages est sensiblement différente de celle des plantes modernes. La disponibilité des nutriments et de l'eau due à l'aménagement de l'environnement aurait conduit à des racines moins profondes et moins ramifiées chez les plantes modernes. D'après la littérature, les plantes modernes exsuderaient plus de sucres simples que les plantes sauvages. L'ensemble de ces changements aurait conduit à une modification des communautés bactériennes associées aux plantes, notamment une diminution des Bacteroidetes et une augmentation des Actinobactéries et Protéobactéries chez les plantes modernes par rapport aux plantes sauvages. D'après Pérez-Jaramillo *et al.* 2018.

L'aménagement du paysage agricole a certes eu un effet très bénéfique sur le rendement des plantes de grandes cultures, notamment le blé ou le maïs, mais a aussi induit un changement au sein des communautés microbiennes peuplant les sols. L'utilisation de fertilisants, principalement synthétiques, a favorisé le développement d'espèces bactériennes capables de métaboliser des sources de nutriments diverses et abondantes, dites copiotrophes, tandis que les espèces bactériennes se développant préférentiellement dans des milieux pauvres en nutriments, dites oligotrophes, ont été négativement impactées (Fierer *et al.* 2012; O'Brien *et al.* 2016). Certains groupes fonctionnels bactériens, comme les

bactéries dénitrifiantes, auraient été favorisés par l'apport d'azote en grande quantité (Geisseler and Scow 2014; van der Bom *et al.* 2018). Ces modifications au sein de la structure des communautés microbiennes dues à l'utilisation de fertilisants peuvent induire un déséquilibre menant à une réduction de la diversité microbienne dans les sols (Kavamura *et al.* 2018). De la même façon, l'usage de pesticides a un impact significatif sur les bactéries du sol, variant selon le type de pesticides utilisé (Jacobsen and Hjelmsø 2014). D'autres pratiques agricoles comme le labour, intensifié suite à l'ère de la mécanisation agricole, contribuent également à une modification de la composition microbienne des sols (Chávez-Romero *et al.* 2016; Dong *et al.* 2017). Ces changements vont ainsi avoir un impact immédiat sur la composition des communautés de bactéries associées aux plantes.

Au sein de la rhizosphère, qui est la zone du sol sous influence des racines vivantes, particulièrement riche en microorganismes (Hartmann *et al.* 2009), des interactions négatives (antagonisme, compétition, etc.) ont lieu entre microorganismes et notamment entre bactéries pour coloniser le système racinaire de la plante-hôte. Il a été montré que malgré leur abondance supérieure, la diversité des bactéries au sein de la rhizosphère d'une plante est en général plus faible que dans le sol nu (Marilley *et al.* 1998; Kowalchuk *et al.* 2002; Uroz *et al.* 2010). Cela s'explique par une sélection de microorganismes au sein de la communauté microbienne du sol environnant effectuée par la plante-hôte (Mavingui *et al.* 1992; Smalla *et al.* 2001; Bulgarelli *et al.* 2012) à travers sa morphologie et sa physiologie racinaire. L'exsudation (rhizodéposition), un processus pouvant être passif ou actif et consistant pour la plante à relâcher dans le sol des composés issus de la photosynthèse (Nguyen 2003; Bais *et al.* 2006), représente une source potentielle d'éléments nutritifs métabolisables pour les microorganismes et peut conduire à un chimiotactisme de la part de certaines espèces ou souches bactériennes (Oger *et al.* 2004). Les bactéries capables d'utiliser ces exsudats pour leur métabolisme pourront alors se développer (Haichar *et al.* 2008; López-Guerrero *et al.* 2013). Ainsi, les autres bactéries verront leur abondance relative drastiquement diminuée. Au niveau de l'architecture racinaire, la préférence de certaines PGPR pour des zones racinaires spécifiques a également été observée (Vande Broek *et al.* 1993; Gamalero *et al.* 2004). On sait que les conditions environnementales ont un impact significatif sur l'expression de ces caractères phénotypiques, ce qui peut conduire à moduler l'impact sur la plante de stress biotiques et abiotiques (Rengel 1997; Lynch 2007; Daneshbakhsh *et al.* 2013; Azarbad *et al.* 2018). Il est également vraisemblable que leur expression est dépendante du génotype de la plante, car des plantes d'une même espèce peuvent présenter différents profils d'exsudation ou d'architecture racinaire selon la variété, notamment entre variétés anciennes et modernes d'une même (Shaposhnikov *et al.* 2016; Beyer *et al.* 2018; Junaidi *et al.* 2018).

Hypothèse et objectifs de la thèse

Cependant, nous disposons encore de très peu d'information concernant les régions génétiques de la plante-hôte impliquées dans les interactions avec les PGPR. Ce manque d'information pourrait s'expliquer par le fait que jusqu'à maintenant, à notre connaissance les études portant sur les différences d'interaction entre génotypes de plantes et PGPR se sont limitées au maximum à l'utilisation de quelques dizaines d'accessions (Neiverth *et al.* 2014; do Amaral *et al.* 2016; Kazi *et al.* 2016). De plus, malgré son impact conséquent sur la morphologie et la physiologie des plantes, encore très peu d'informations existent sur l'impact de la sélection variétale moderne sur les interactions entre PGPR et génotypes de plante (Engelhard *et al.* 2000). Pourtant, l'aménagement du paysage agricole français et l'utilisation d'intrants chimiques en condition non-limitante pourraient avoir rendu caduques les effets bénéfiques des PGPR et l'on peut donc s'interroger sur la conservation des traits génétiques impliqués dans les relations entre plantes et PGPR chez les variétés modernes de plantes. Nous émettons ainsi l'hypothèse que les variétés modernes de plantes interagiraient moins bien que les variétés anciennes avec les PGPR, potentiellement à cause de la perte au cours de la sélection variétale de traits génétiques impliqués dans ces interactions.

Les objectifs de cette thèse sont donc de (1) comparer les capacités d'interactions de variétés anciennes et modernes de plantes avec des PGPR, (2) identifier chez la plante-hôte des régions génétiques impliquées dans les relations plantes-PGPR, et (3) comparer l'impact de différents génotypes anciens et modernes d'une même plante sur les bactéries indigènes du sol, notamment celles impliquées dans des fonctions bénéfiques pour la croissance des plantes.

Modèle d'étude végétal : le blé tendre

Nous avons décidé d'utiliser comme modèle végétal le blé tendre (*Triticum aestivum aestivum*), une plante de grande culture dont la production figure parmi les plus importantes dans le monde, notamment grâce à une sélection variétale moderne, fructueuse en termes d'augmentation du rendement, du fait notamment d'une meilleure utilisation de l'azote (principalement fourni sous la forme de fertilisants synthétiques) par les génotypes modernes (Brancourt-Hulmel *et al.* 2003; Cormier *et al.* 2014; Ruisi *et al.* 2015). Le blé se présente aujourd'hui sous la forme de milliers de variétés différentes, dont une très faible proportion est utilisée aujourd'hui dans les champs (en 2016 en France, 10 variétés couvraient environ 47 % des surfaces agricoles ; source FranceAgriMer2 2016 http://www.franceagrimer.fr/) (Goldringer *et al.* 2013). Afin d'avoir un échantillonnage représentatif des variétés de blés anciennes et modernes, nous avons utilisé 199 accessions de blé tendre: 196 ont été analysées pour leur capacité au champ à croître en condition de faible ou de forte fertilisation azotée (Bordes *et al.* 2008), 2 sont des variétés modernes de référence pour l'agriculture biologique (Hendrix et Skerzzo), et la dernière est une variété-population qui est le génotype de référence utilisé pour le séquençage du génome du blé tendre (Chinese Spring). Parmi ces 199 accessions, 78 étaient des génotypes anciens, incluant (1) 35 variétés-populations (en anglais *landraces*) qui présentent une

hétérogénéité génétique plus importante que les autres du fait de leur sélection massale, et (2) 43 variétés anciennes qui sont des lignées génétiquement plus pures que les variétés-populations. Enfin, 121 des génotypes étaient des variétés modernes sélectionnées après 1960, date à laquelle des gènes de nanisme (*Rht*) ont été introduits dans les nouvelles variétés de blé pour améliorer la tolérance à la verse, liée à l'utilisation de fertilisants (Berry *et al.* 2015).

Déroulement des travaux de thèse

Dans un premier temps, nous avons cherché à déterminer quels génotypes de blé présentaient de fortes/faibles interactions avec *Pseudomonas kilonensis* F113 *in vitro*, et quelles proportions de génotypes présentant de fortes interactions étaient retrouvées au sein des génotypes anciens et modernes. *P. kilonensis* F113 possède l'opéron *phl* et est ainsi capable de synthétiser du 2,4-diacétylphloroglucinol (DAPG), un composé antimicrobien aussi connu pour ses effets similaires à ceux des auxines (Brazelton *et al.* 2008), et a des effets bénéfiques sur la croissance de plusieurs espèces telles que l'arabette (Vacheron *et al.* 2016), le maïs (Walker *et al.* 2012) et le blé (Couillerot *et al.* 2011). Nous nous sommes focalisés sur les deux premières étapes des interactions entre PGPR et plantes, soit (1) la colonisation et (2) l'expression de gènes bactériens impliqués dans des fonctions bénéfiques pour la croissance des plantes (ici l'opéron *phl*). Toutefois, il est connu qu'un certain niveau de spécificité/affinité existe entre génotype de plante-hôte et souche de PGPR (Drogue *et al.* 2014a, b; Chamam *et al.* 2015), nous avons donc pris en compte le fait que les résultats obtenus avec F113 ne puissent sans doute pas être généralisables aux PGPR dans leur ensemble. Ainsi dans un deuxième temps, nous avons voulu savoir si les résultats obtenus avec F113 pouvaient montrer une certaine généricité, et avons donc réalisé une étude similaire avec *Azospirillum brasilense* Sp245, une PGPR connue pour sa faculté à fixer l'azote atmosphérique et à produire une auxine, l'acide indole-3-acétique (Vande Broek *et al.* 1999; Spaepen *et al.* 2007b), ainsi que pour ses effets bénéfiques sur la canne à sucre (Moutia *et al.* 2010) ou le blé (Creus *et al.* 2004; Kazi *et al.* 2016). Grâce au grand nombre de génotypes criblés, nous avons également pu identifier des régions génétiques chez le blé qui seraient impliquées dans les interactions avec ces deux PGPR, par une approche GWAS (pour *Genome-Wide Association Study*) menée par des partenaires du projet BacterBlé dans lequel s'inscrivent ces travaux de thèse (Jacques Le Gouis et collaborateurs, Unité GDEC, Clermont-Ferrand).

Si l'on suit la chronologie d'une interaction entre une plante-hôte et une PGPR, après la première et la deuxième étape citées précédemment, la troisième étape devrait être une modification phénotypique visible sur la plante-hôte, due à une amélioration des performances par la souche de PGPR. Ainsi, pour savoir si les résultats obtenus *in vitro* se traduisaient en de meilleures performances de la plante, nous avons sélectionné des génotypes anciens et modernes présentant des résultats contrastés *in vitro* et les avons inoculés avec F113 dans un premier temps, puis avec Sp245. Toutefois, il nous a paru également important de

ne pas négliger l'impact des conditions environnementales sur les interactions entre PGPR et plantes puisque (1) selon la théorie du BMT, il parait vraisemblable qu'en condition de stress la plante nécessite davantage les effets bénéfiques apportés par les PGPR, et (2) le comportement des variétés anciennes et modernes est différent selon les conditions de croissance. Ainsi, nous avons intégré un facteur environnemental dans cette expérience sous serre, consistant soit en des conditions optimales en termes d'eau et d'azote disponible, soit en un stress abiotique combinant sécheresse et déficit en azote.

Enfin, afin de connaitre l'impact de ces génotypes sélectionnés sur les populations bactériennes indigènes du sol et notamment les PGPR, nous les avons semés dans des stations expérimentales dans des parcelles avec des conditions optimales de croissance ou avec des conditions de stress abiotique et avons effectué des PCR quantitatives et du séquençage haut-débit afin de mesurer l'abondance et la diversité des bactéries indigènes associées à leurs racines.

Structure du document de thèse

Ce manuscrit est ainsi découpé en 5 grandes parties. La première (Partie 1) consiste en une synthèse bibliographique ayant pour objectif de réunir des informations permettant d'appréhender les impacts que pourraient avoir eu la sélection variétale par l'homme sur les interactions entre les plantes et les bactéries associées aux racines. Ainsi, on traitera (1) des étapes majeures de la domestication du blé et ses conséquences globales en termes de génétique, morphologie et physiologie, (2) de l'évolution de la sélection variétale du blé en France, (3) des conséquences de la sélection variétale sur la diversité génétique du blé, sa morphologie et sa physiologie racinaire, (4) des facteurs biotiques et abiotiques influençant les bactéries du sol et celles associées aux racines des plantes, et (5) de la relation étroite existant entre génotype de plantes et bactéries associées aux racines, notamment PGPR.

La seconde partie (Partie 2) traite de l'influence du génotype de blé sur la colonisation de F113 et l'expression de son opéron *phl*, ainsi que des améliorations de performance de la plante apportées par cette PGPR. Une comparaison entre génotypes anciens et modernes de blé a été menée parmi les 199 accessions de blés, ainsi qu'une comparaison des performances sous serre de génotypes ayant stimulé ou non la colonisation de F113 et l'expression de son opéron *phl* après inoculation avec F113.

La troisième partie (Partie 3) traite de l'influence du génotype de blé sur la colonisation de Sp245 et l'expression de son opéron *ppdC*, ainsi des améliorations de performance végétale entrainées par la PGPR. Une comparaison statistique avec les données obtenues pour F113 a été effectuée. Une analyse GWAS a également été menée pour identifier des régions génétiques chez le blé impliquées dans les interactions avec F113 et Sp245.

La Partie 4 traite de l'influence du génotype de blé sur les communautés bactériennes du sol et notamment les diazotrophes, les productrices d'AIA, les productrices d'ACC-désaminase et les productrices de

phloroglucinol. Une analyse de l'abondance de ces groupes fonctionnels a été menée par une approche qPCR, pour quantifier et comparer l'abondance de ces groupes tandis que la diversité bactérienne totale (gènes codant les ARNr 16S) et du groupe des diazotrophes (gènes *nifH*) ont été analysées par séquençage haut-débit. Une comparaison des génotypes anciens et modernes a été faite, ainsi que des génotypes ayant eu ou non des interactions fortes avec F113 et Sp245 *in vitro*.

Enfin, la dernière partie (Partie 5) correspond à une discussion générale de l'ensemble des résultats, et elle est accompagnée des perspectives sur lesquelles ces travaux de thèse pourraient déboucher.

Partie 1

Synthèse bibliographique

I - Impact of domestication on genome, morphology and physiology of wheat genotypes

In this part, the genetic evolution of the *Triticum* genus during the domestication process will be briefly reviewed. Then, the consequences on the morphology and the physiology of domesticated wheat and wheat relatives will be discussed. The end of this part will be focused on the culture of wheat right after the domestication and the emergence of landraces.

Evolution of wheat species and associated genetic shifts

Today, wheat is one of the most important staple foods and the second most produced cereal behind maize (USDA 2018). It regroups numerous *Triticum* species and sub-species, as the best-known *T. aestivum aestivum* (common wheat, or bread wheat) or *T. turgidum durum* (durum wheat). The domestication of wheat started about 10 000 years (Salamini *et al.* 2002; Faris 2014) ago in the Fertile Crescent in the Middle East, during the Neolithic revolution and supported the transition of human societies from hunter-gatherer lifestyle to an agrarian lifestyle (Bonjean 2001; Faris 2014). The ancestor of the modern bread wheat and durum wheat was the wild emmer, *T. turgidum dicoccoides,* a tetraploid form of wild wheat which came from the hybridation between the wild diploid wheat species *T. urartu* and a wild diploid relative *Aegilops speltoides* (Peng *et al.* 2011; Faris 2014) (**Fig 1**). Over time, the wild emmer was domesticated by humans, leading to the first subspecies *T. turgidum dicoccoidum*. Wild and domesticated emmer were distinct, as the last one showed a non-brittle rachis and bigger grains, more convenient to harvest and leading to bigger yield (Salamini *et al.* 2002; Peng *et al.* 2011). Several tetraploid domesticated wheat subspecies appeared then, such as *T. turgidum durum* (durum wheat), *T. turgidum polonicum* (Polish wheat) or *T. turgidum turgidum* (Poulard wheat), and are still used today. They are more convenient to harvest than domesticated emmer because of their free-threshing forms (Dubcovsky and Dvorak 2007; Gill *et al.* 2007). The evolution reached a new step afterwards with the apparition of hexaploid wheat species, *T. aestivum*, including g *T. aestivum aestivum*, *i.e.* the bread wheat (genome AABBDD, 6 x 7= 42 chromosomes, about 17 Gb), which represents the major part of the cultivated wheat today. It is suggested that bread wheat appeared following the hybridization between *T. turgidum dicoccoidum* (providing genomes AA and BB) and a wild diploid relative*, Aegilops tauschii* (providing genome DD) (Marcussen *et al.* 2014).

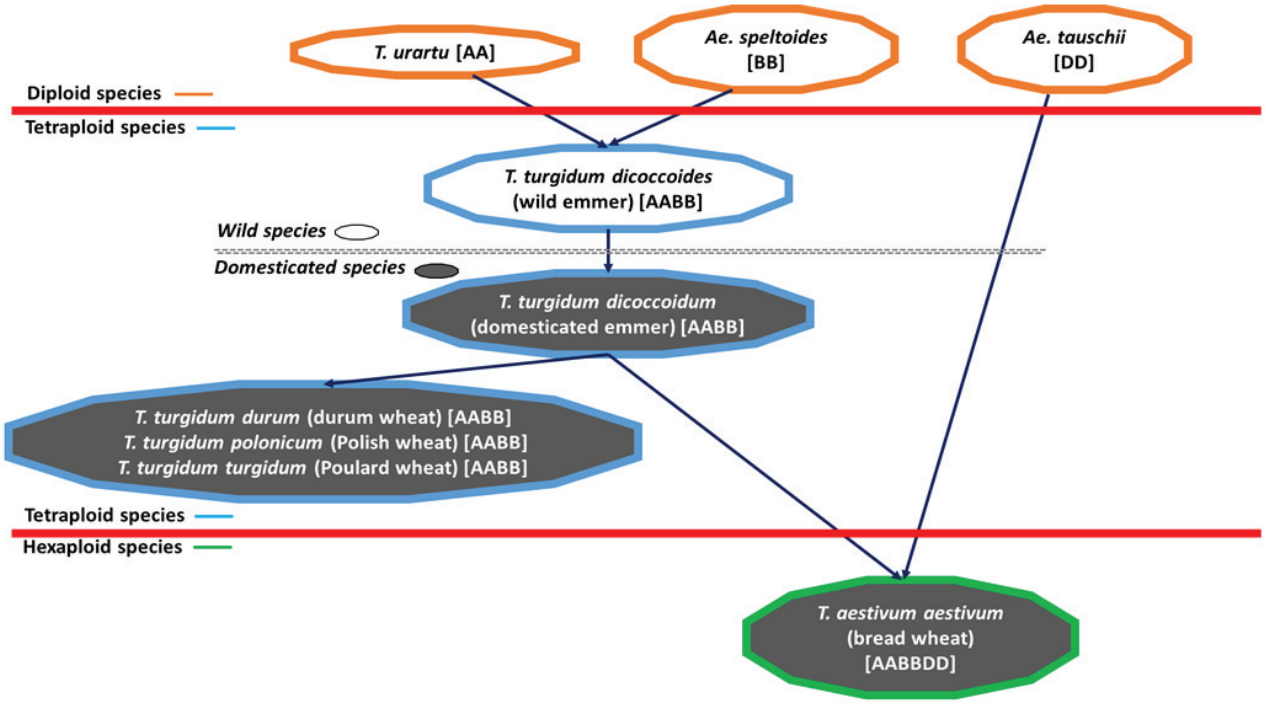

Fig 1 Major genetic evolution steps of the wheat species throughout domestication. Associated genomes are in brackets.

Besides, the domestication process allowed the humans to become farmers thanks to the domestication syndrome (*i.e.* the apparition of phenotypical traits as, for instance, non-brittle rachis), which led to an easier harvest and better yield, but it also caused a drop in the genetic diversity of wheat. Hence, the transition between *T. turgidum dicoccoides* and *T. turgidum dicoccum* was marked by a drop of 50-60 % of the genetic diversity (Haudry *et al.* 2007), which is one of the major diversity bottlenecks throughout the wheat evolution history. In addition, it has been hypothesized that the hybridization leading to hexaploidy caused gene loss, probably due to changes in the selective environment which favored the emergence of specific genes at the expense of genes involved in network which applies in "normal" condition (Berkman *et al.* 2013). The genetic erosion of the *Triticum* genus could be explained by genetic drift, which was the major actor in the domestication steps, but which affected only a small part of the wild wheat relative populations. But it also could be explained by the domestication syndrome, which led to highly favored (= selected) phenotypes by the farmers, and thus specific gene occurrence. For example, a gene named 0009733 involved in the response to auxins, a category of hormones known to enhance root formation, was more frequently found in domesticated wheat genotypes than wild ones, and the same observation was made in other cereals displaying genes involved in similar functions, such as the 09G0547100 gene from rice (Meyer and Purugganan 2013; Avni *et al.* 2017). The gene alleles involved in the non-brittle aspect of the rachis, a key wheat domestication trait, *Ttbtr1-A* and *Ttbtr1-B,* are another example of gene selection. These alleles are found on the chromosomes 3A and 3B of the domesticated tetraploid wheat, but not of the wild ones. They carry

mutations leading to a loss of function, which allow the non-brittle rachis, a phenotypical trait highly favored during the transition between wild emmer and domesticated emmer (Avni *et al.* 2017).

Morphological and physiological changes related to domestication

Morphological differences can be observed between wild and domesticated wheat species, and this is also the case for other cereals. Indeed, above-ground differences have been well documented, especially those resulting in easier and better harvest: non-brittle rachis, bigger grains and free-threshing form due to soft glumes (Salamini *et al.* 2002; Gill *et al.* 2007; Peng *et al.* 2011; Faris 2014) (**Fig 2**). Thus, it appeared that in order to raise yield, farmers favored plants presenting big spikelets but compact vegetative parts. This was striking when comparing the domesticated species of another *Poaceae,* maize, and its wild ancestor the teosinte, which presents clearly more tillered shoots (Gaudin *et al.* 2014). Wheat ancestors showed also longer shoots than durum or bread wheat (Gurcan *et al.* 2017; Pour-Aboughadareh *et al.* 2017). However, very little was investigated about underground traits. Given that the shoot/root ratio remains stable, at least in non-stressful environments (Bastow Wilson 1988; Feller *et al.* 2015), it is then not surprising that wild relatives of domesticated cereals show more root volume and biomass, like teosinte, which displays a significantly higher root volume than domesticated maize genotypes (Gaudin *et al.* 2014). Similar results have been shown regarding wheat, as wild wheat relatives show higher root biomass than domesticated wheat species (Waines and Ehdaie 2007; Pour-Aboughadareh *et al.* 2017; Ahmadi *et al.* 2018). However, there is a high heterogeneity at an inter- and infra-species level, and it appeared that some ancient species or subspecies present a root biomass lower than bread wheat or durum wheat (Akman 2017). Either way, it can be assumed that wild and domesticated wheat species display different root system morphologies.

In the same way that domestication shaped the genome and the morphology of crop plants such as wheat, it also impacted the physiology of the plants. Thus, Beleggia (2016) showed that transition from wild emmer to domesticated emmer was related to a significant decrease in saturated and unsaturated fatty acids in wheat kernels, and also that the transition from domesticated emmer to durum wheat was related to a significant change in amino acids abundance (notably a drop in alanine, valine, leucine, isoleucine, serine, and threonine concentrations) and sugar abundance (a drop in fructose and glucose concentrations). Moreover, the domestication led to a change in secondary metabolite profiles by the selection of crop genotypes with grains showing lower concentrations in components that could have a detrimental effect on human health or on taste (notably bitter components) (Meyer *et al.* 2012). It also led to a difference in phenolic compounds, notably flavonoids whose abundance and diversity dropped in grains of domesticated species (Cooper 2015; Şahin *et al.* 2017). Thus, it is not surprising to observe differences in terms of rhizodeposition contents, and mostly root exudation (*i.e.* the release of low weight molecular substances mostly derived from photosynthesis products). Ianucci (2017)observed that among wild emmer, domesticated emmer and durum

wheat, there were significant differences in exudate composition. For example, the transition between domesticated emmer and durum wheat was marked by a drop in fructose, galactose, mannose and glucose concentrations, but also mannitol and sorbitol. However, the difference is not always in favor of the older genotypes. For instance, durum wheat presented a bigger concentration of sucrose and policosanols (*i.e.* polymers of primary long-chain alcohols found in plant waxes) than wild or domesticated emmer.

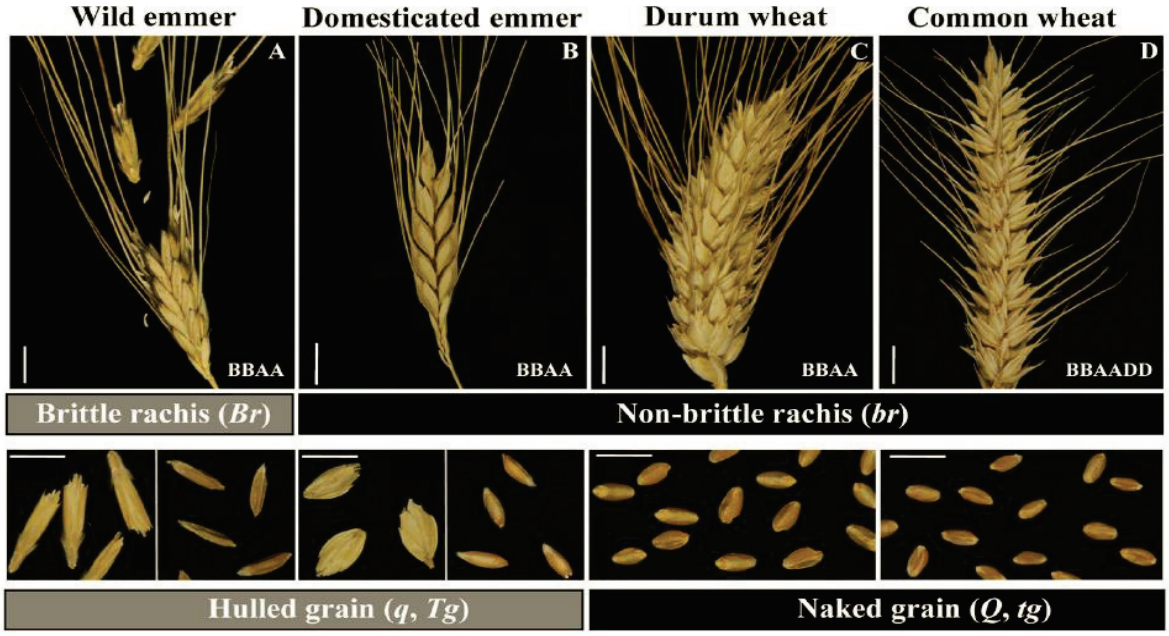

Fig 2 Wheat spikes morphology of modern wheat species and ancestral relatives. The figure shows (A) wild emmer (*T. turgidum dicoccoides*), (B) domesticated emmer (*T. turigdum dicoccoidum*), (C) durum wheat (*T. turgidum durum*) and (D) bread wheat (*T. aestivum aestivum*). Letters at the corner indicate the genome of each wheat. Gene symbols: *Br* = brittle rachis, *Tg* = tenacious glumes and *Q* = square head. Photos by C. Uauy. From Dubcovsky and Dvorak 2007.

Cultivation of wheat after its domestication: the landraces

Newly domesticated genotypes, notably *T. aestivum aestivum*, were disseminated throughout the world, crossing the Mediterranean Sea to spread to Greece, Spain or Italy, and the Danube valley to reach Austria or Germany, then northern Europe (Berkman *et al.* 2013; Faris 2014). Concurrently, they were also spread to Caucasus then Asia, following what we call today the “silk road”. Another road crosses a little further South, through Afghanistan and Pakistan, to northern India. Domesticated genotypes were also spread to Africa, essentially along the Nil, and also from southern Italy to Algeria (Bonjean 2001; Goldringer *et al.* 2013) (**Fig 3**). This dissemination in diverse environments required specific (local) adaptations to field conditions where wheat genotypes were cultivated, to handle high temperature, drought, high salt concentrations, or flooding (Bonjean 2001; Dwivedi *et al.* 2016) (**Fig 3**). Thus, farmers selected the plants that displayed the best development in their field, and used their grains to sow the next-year production (Kiszonas and Morris 2018).

This practice, which was also the same as the one allowing the creation of the different wheat species, was named the “selection massale” or mass selection (Bonnin *et al.* 2014). It led to the creation of particular wheat genotypes, called landraces, and referred to as “variétés populations” or “variétés paysannes” in French. They show great adaptation to local environmental conditions, but they are hardly ever used today because of their low yield compared to modern varieties (Bonneuil and Thomas 2012; Goldringer *et al.* 2013; Faris 2014). Generally, mixtures of different genotypes were grown in a same field, as a same unit of selection (a same landrace), which allowed to stabilize the yield by providing protection against disease and environmental hazards (Bonjean 2001; Feldman and Kislev 2007). However, it led to the emergence of competition, where taller individuals with large shoots, or individuals with better response against weeds and pathogens were favored over the others (Bonjean 2001; Feldman and Kislev 2007). Thus, the particularity of the landraces was their high genetic heterogeneity compared to fixed varieties selected since the end of the 18th century, due to their local adaptation. This led to an inter-genotype heterogeneity. Due to frequent spontaneous hybridizations in field, an infra-genotype heterogeneity also occurred (Feldman and Kislev 2007; Bonnin *et al.* 2014; Faris 2014).

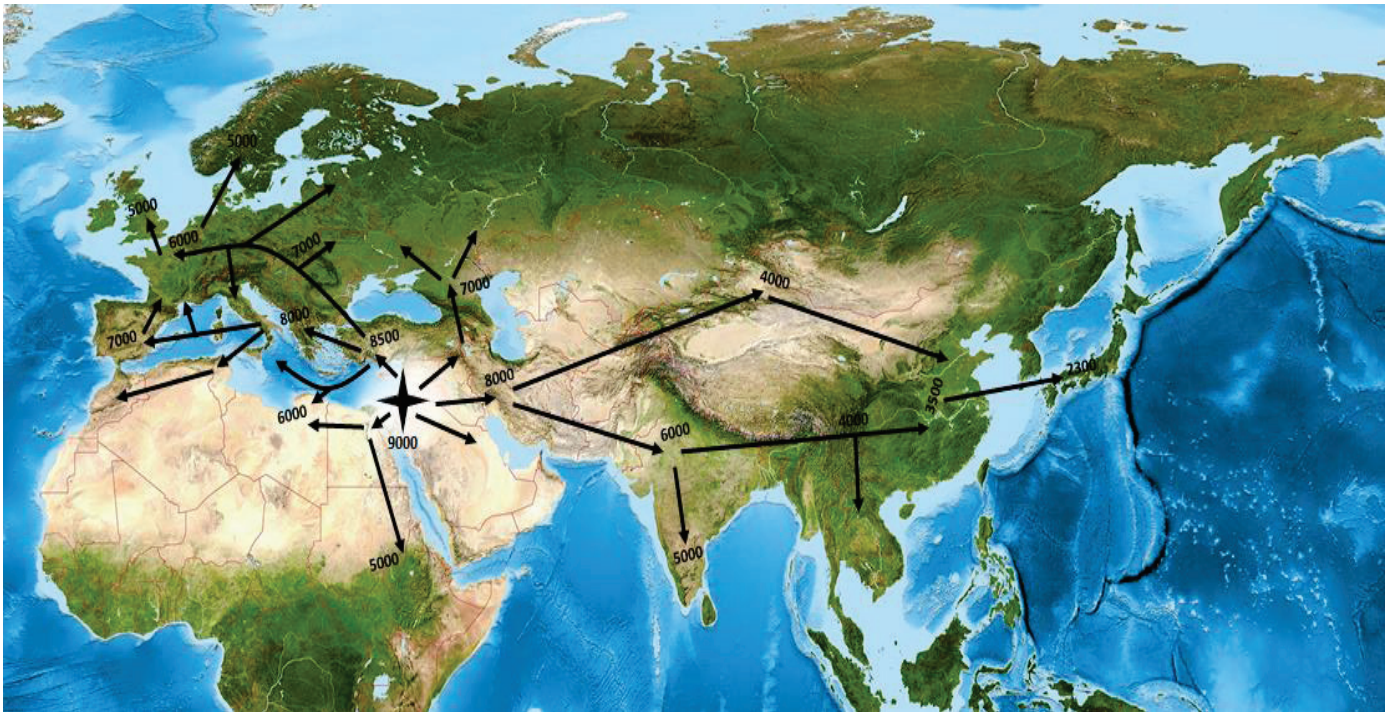

Fig 3 Spread steps of domesticated wheat species across the world and associated dates from today (adapted from Bonjean 2001)

II - A representative example of modern varietal selection: the emergence of French modern varieties

The modern breeding of wheat in France has been one of the most efficient breeding programs in the world, and it is very representative of the different steps in the genetic improvement of wheat genotypes that will give higher yield, better grain nutrient content, etc. First, we will review the emergence of old wheat varieties (*i.e.* the first pure line varieties), selected before 1960 and at the beginning of the massive use of agrochemicals. Then, we will see how the VAT ("Valeur Agronomique et Technologique") and DHS ("Distinction, Homogénéité et Stabilité") requirements have drifted the creation of new varieties towards quite homogenous genotypes showing high yield and great bakery quality. Finally, will be discussed how modern agricultural practices and molecular modifications have led to the emergence of modern wheat varieties.

The beginning of the scientific selection: the old varieties

At the end of the 18th century, the first private seed companies appeared, notably Vilmorin-Andrieux in 1774 in France. These private seed companies were the first to develop a more rigorous and scientific method to select wheat genotypes. At that time, public research structures, such as the "Institut national des Sciences et des Arts", the "Société d'Agriculture de France", or some years later the "Institut National Agronomique", focused more on chemistry than on biology and agronomy, and it is Louis de Vilmorin who presented at the end of the 1850s the new method of genealogical selection (Gayon and Zallen 1998; Bonjean 2001). It consisted to sow the wheat progeny separately and not as a mix to obtain pure genotypes, which is the opposite of the mass selection that leads to a high genetic heterogeneity (Gayon and Zallen 1998; Kiszonas and Morris 2018). The individual genotypes became the unit of selection, thus it was the early stages of the fixed varieties as we know them today (Bonnin *et al.* 2014). It marked the end of the spontaneous hybridizations occurring in the fields, but also of the competition between genotypes in the fields as each individual plant was genetically identical to the others. Vilmorin started then a scientific screening, doing cross-pollination of multiple pure varieties and choosing the ones showing particular agronomic characters of interest, such as disease resistance, heat and cold resistance, or even improved yield (Gayon and Zallen 1998; Bonneuil and Thomas 2012). However, the beginnings were slow and farmers showed very little interest for these new varieties and genetic-based selection methods, favoring their landraces except for the richest farmers. In the early 1930s was created the French official catalogue of varieties for wheat, which collected at that time the information on about 400 varieties (Bonneuil and Thomas 2012; Boulineau and Leclerc 2013; Bonnin *et al.* 2014). Then, the agronomy during the 1940s was marked by the rise of the Mendelian genetics, and once again, the private seed companies, and notably Vilmorin, were the first ones to select and breed

pure varieties, with the purpose to improve their progeny (Roussel *et al.* 2004; Bonneuil and Thomas 2012; Cavanagh *et al.* 2013). This scientific selection contributed to the decrease of genetic diversity.

The DHS and VAT requirements

In 1944, J. Bustarret, head of the department of plant genetics and improvements of the IRA ("Institut de Recherche Agronomique") stated the concept of agronomic variety, which is not a taxonomic rank but is based onto technical concerns. He defined three types of varieties, (1) the pure line variety (= the genealogical variety) whose individuals are genetically identical, (2) the clonal varieties, which concerns the species that can be vegetatively propagated and (3) the population variety, which is obtained by massal selection and presents genetic heterogeneity (Bonneuil and Thomas 2012). In the early 1950s, started the obligation to register varieties in the official catalogue of plant varieties in order to commercialize or trade them. To be registered in the catalogue, varieties must succeed regarding two types of strict evaluations (Labarthe *et al.* 2018). The first evaluation type is about the "Distinction, Homogénéité et Stabilité" *i.e.* the DHS testing, also known as DUS for "Distinctness, Uniformity and Stability" (Serpolay *et al.* 2011; Boulineau and Leclerc 2013). These three required features, still valid today to enter a variety in an official catalogue of varieties, are used to ensure the genetic - and of course the phenotypic - identity of a variety, and to prevent any genetic shift in the future (Doré and Varoquaux 2006; Leclerc 2009). Therefore, the landraces, whose great majority did not fulfill the requirements, were not allowed to be registered in the catalogue, contributing to their elimination in the fields (Leclerc 2009; Bonneuil and Thomas 2012; Bonnin *et al.* 2014). The second type of evaluation was about the "Valeurs Agronomique et Technologique" *i.e.* the VAT testing, also known as VCU for "Value for Cultivation and Use" (Serpolay *et al.* 2011; Boulineau and Leclerc 2013). For the wheat varieties, yield, baking strength and disease resistance were evaluated regarding the VAT (Cooper 2015; Beleggia *et al.* 2016; Kiszonas and Morris 2018). Only varieties that showed a progress compared to the older ones could be registered in the catalogue thanks to a scalable score system (Bonneuil and Thomas 2012). This decreased even more the number of landraces in use, because they generally showed lower yield and baking strength than registered varieties (Ceccarelli 1996; Serpolay *et al.* 2011; Konvalina *et al.* 2014). Over the years, VAT evaluations were then performed following a standardized procedure, which erased the impact of different agricultural practices and terroirs, and focused on the use of mechanization and of chemicals such as fertilizers and pesticides (Bonny 1993; Brancourt-Hulmel *et al.* 2003; Gervois *et al.* 2008; Bonnin *et al.* 2014; Cao *et al.* 2018). All in all, from the about 400 varieties that were registered in the catalogue in the 1930s, less than 150 varieties remained by the mid-1950s (Bonneuil and Thomas 2012; Bonnin *et al.* 2014).

Molecular modifications and agrochemicals to improve yield: the modern varieties

In 1960, appeared in France the semi-dwarf wheat varieties, which are more convenient to harvest. The genes involved in this phenotypic modification are the *Rht* genes, which came from Japanese wheat varieties (Borojevic and Borojevic 2005; Berry *et al.* 2015) and allowed the wheat varieties to support increasing concentrations of fertilizers without lodging (Berry *et al.* 2015). Indeed, between 1960 and 1990, more and more chemicals, notably chemical nitrogen fertilizers, were used (**Fig 4**). Associated with monoculture, seed densification constrained farmers to use more pesticides in fields, due to increased sensitivity of the crops to fungal diseases (Cao *et al.* 2015; Parker and Gilbert 2018). Between 1960s and 1980s, only about 20% of selected varieties were commercialized because catalogue registration requirements became more and more difficult to reach (Leclerc 2009; Bonneuil and Thomas 2012). At the end of the 1960s, no more than 80 varieties all in all were still registered in the catalogue. However, the yield of bread wheat was in constant augmentation, and increased from about 2000 kg/Ha in 1950 to about 5000 kg/Ha in 1980 (Brancourt-Hulmel *et al.* 2003; Brisson *et al.* 2010), which is an increase similar to the ones observed in other countries like in the USA (**Fig 5**), while concurrently the use of commercialized seeds for bread wheat increased from 4 to 50 % of the total seeds used.

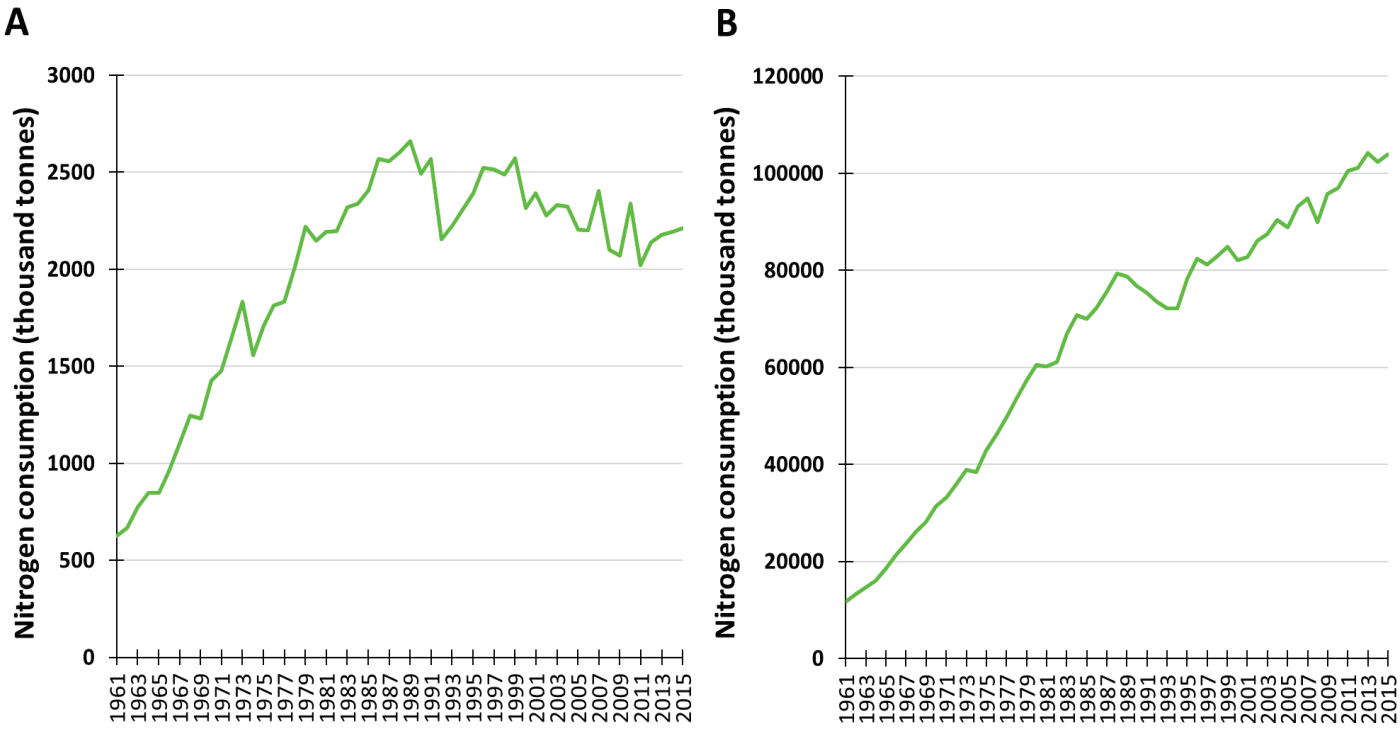

Fig 4 Nitrogen fertilizer consumption in (A) France and (B) world. The total quantity of used nitrogen per year between 1961 and 2015 is represented. Source : International fertilizer Association (IFA, https://www.fertilizer.org/)

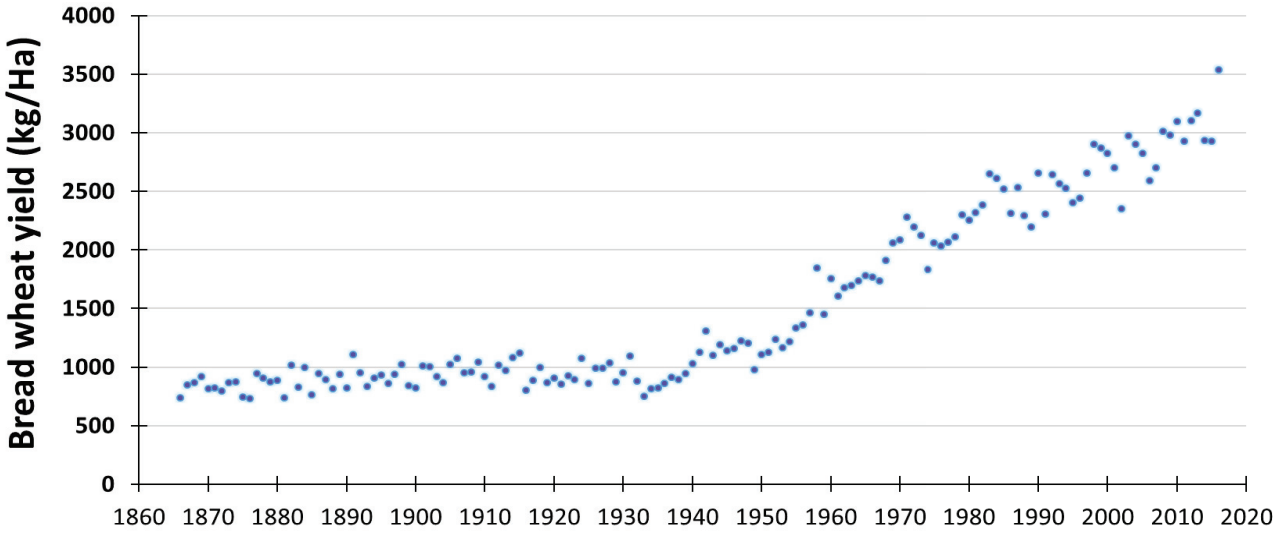

Fig 5 Evolution of wheat yield in USA between 1866 and 2016. Source : U.S. Department of Agriculture (USDA, https://www.usda.gov/)

The last decades of the 20th century marked the success of the use of biotechnologies applied to agronomy, with the possibility to transfer genes of interest (Doussinault *et al.* 2001). To create new wheat varieties, scientists crossed the species barrier (using radiations, X-ray or mutagenic substances) and succeeded to produce hybrids *i.e.* bread wheat (or durum wheat) with one or several gene(s) of interest coming from ancestral relatives (disease resistance gene(s) in general) (Vandam 1975; Doussinault *et al.* 2001; Doré and Varoquaux 2006). These genetically engineered varieties were used as tools by breeders and were bred with elite varieties to obtain "rustic varieties", which displayed correct yield even in presence of limited inputs (Vandam 1975; Doussinault 1983; Doussinault *et al.* 2001), like Renan (1989) which was the first French variety designed for organic agriculture, or its recent successors Hendrix and Skerzzo (2012). However, most of the rustic varieties were selected only for the resistance to particular disease at first, and were not fully adapted to organic agriculture because they still need chemical fertilization. Yet, the consumption of nitrogen fertilizers is slowly decreasing since 1990 in France, whereas it is still increasing throughout the world (Fig 4), notably in countries that were involved in the "Green revolution" (for example India, Pakistan or Brazil). However, concurrently to this decrease in nitrogen consumption in France, a stagnation of wheat yield was recorded (Brisson *et al.* 2010). Since 2014, in France the VATE (E for Environmental) evaluates the nitrogen use efficiency of the new varieties (Boulineau and Leclerc 2013; Labarthe *et al.* 2018). Today, about 300 bread wheat varieties are registered in the French catalogue of varieties, which now contains three different categories of crops: (1) varieties which succeeded VATE and DHS evaluations and thus can be commercialized in France, (2) varieties which succeeded only DHS evaluation and thus can be multiplied in France and are then exported out of the European Union, and (3) conservation varieties, which regroup landraces threatened by genetic erosion. However, no wheat variety has been registered as a conservation variety so far.

III - Consequences of modern wheat breeding on the genome, morphology and physiology of wheat

Because of the breeder's objectives regarding yield and quality, modern wheat breeding led to the apparition of modern wheat genotypes presenting significant changes compared to old genotypes. In this part, we will see that these changes occurred at multiple imbricated levels, starting by the genome, then the morphology, the physiology and finally the behavior of modern wheat genotypes in fields.

Drop in wheat genetic diversity

Among the bread wheat varieties, the genetic diversity is clustered in geographical groups, which have been identified by diverse genetic markers. First, a clustering based on about 40 polymorphic loci regrouped: (1) North-West Europe, (2) South-East Europe (and North America) (3) Mediterranean area and Oceanic countries, (4) Africa and South America, (5) Near-eastern and central Asia , (6) Far-eastern Asia (Balfourier *et al.* 2007). Another clustering analysis, based on DarT (Diversity Array Technology) markers, showed an alternative but close clustering: (1) North-West Europe, (2) East and South-East Europe along with North America, (3) diverse regions such as Africa, Mediterranean area, Central and South America or Australia, which can be considered as the CIMMYT group (the International Maize and Wheat Improvement Centre) and the ICARDA group (the International Centre for Agricultural Research in the Dry Areas), (4) Asia (including Caucasia and Middle-East) and (5) Nepal (Horvath *et al.* 2009) (**Fig 6**). It is worth noting that the genetic diversity is different according to the geographic group. For example, wheat varieties from western Europe show less genetic diversity than the ones from the Mediterranean group (Roussel *et al.* 2005). It could be due to the isolation and the specific climate (drought and temperature notably) of these countries compared to the countries of western Europe. The separation between western and eastern Europe groups could be explained either by the climatic differences caused by the separation made by the Alps and Carpathian mountains or by the chronology of the migration pathway of the domesticated wheat from the Middle-East to western Europe (Roussel *et al.* 2005). The separation between Europe and Asia groups can also be explained by the migration pathways of domesticated wheat from the Middle-East. The clustering of North American with Europe unveils the European origins of wheat varieties cultivated in the USA and Canada introduced during the 17th century. In the first clustering study, varieties from the Mediterranean and Oceanic groups clustered together, as were those from Africa and South-America, and varieties from all these countries have been clustered together as the CIMMYT-ICARDA group in the second clustering. This is consistent with the recent breeding methods of the CIMMYT and ICARDA, which are aiming at wheat varieties that can be cultivated under various stress conditions, such as drought or high temperature, notably present in South-America and Africa (Balfourier *et al.* 2007; Horvath *et al.* 2009). Finally, the isolated situation of the Nepalese group can be explained by its composition because only landraces are included in this group (Horvath *et al.* 2009).

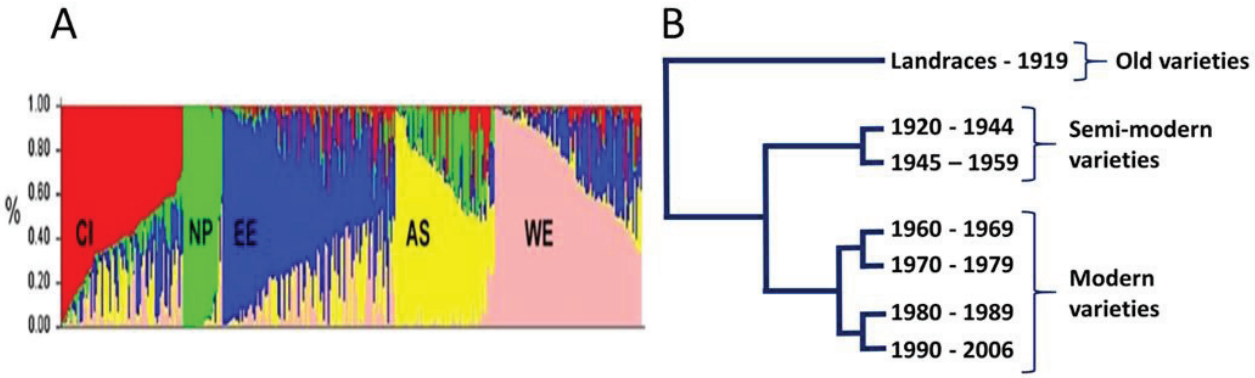

Fig 6 Genetic clustering of a collection of 372 accessions of bread wheat in (A) geographic groups or (B) temporal groups. In (A), each wheat accession is represented by a vertical line, which is split into K = 5 segments that represent the genetic belonging fractions (%) of the accession to five genetic clusters : CI = CIMMYT-ICARDA ; NP = Nepal ; EE = eastern Europe ; AS = Asia ; WE = western Europe. In (B), a dendogram was generated based on Nei genetic distance and three major clades can be distinguished : landraces and varieties selected before 1920 (named old varieties by the authors), varieties selected from 1920 to 1959 (named semi-modern varieties) and varieties selected since 1960 (named modern varieties). From Horvath *et al.* 2009

A temporal evolution of the genetic diversity between the mid-19th and the end of the 20th century was reported in the study of Roussel *et al.* 2005, who detected a decrease (around -18%) of the number of alleles for a selection of 40 polymorphic loci among 480 European wheat accessions. Another study of Roussel *et al.* (2004) concluded to the loss of about 25% of the genetic diversity between landraces and modern varieties among 559 French wheat accessions. One of the conclusions of these two studies was that over time the allelic composition of modern varieties was becoming more and more similar. Another one was the difference in clustering for genotypes selected before or after 1970, on the basis of Nei's distance matrix (Roussel *et al.* 2005). Thus, it appeared that the number of rare alleles increased from 1840 to 1930, but then decreased after the 1960s (Roussel *et al.* 2004, 2005). Horvath *et al.* (2009), using DarT markers, also showed this loss in allelic diversity between landraces and modern varieties and, using Nei's distance matrix, a clustering similar to that established by Roussel *et al.* (2005) was highlighted with three clusters regrouping (1) landraces and varieties selected before 1920, (2) varieties selected from 1920 to 1959 and (3) varieties selected since 1960 showing reduced diversity (**Fig 6**). In 2010, Bonnin *et al.* (2014) used a most integrative indicator than Nei's index, named H_T indicator, and concluded that a minimum loss of 50% of the bread wheat genetic diversity was observed in France during the last 100 years. In another study, Cavanagh *et al.* (2013) showed that a sub-sample of 134 landraces covered 99% of the genetic diversity of the 3000 wheat accessions used in their study.

Wheat genes selected by modern breeding

The loss of genetic diversity throughout modern breeding could be explained by some genes that were highly favored by breeders, leading to phenotypes able to success VAT testing. The evidence of modern selection of

peculiar genomic regions, such as the *Rht* genes (*i.e.* dwarfing phenotypes), *Ppd-B1* and *Vm1* (*i.e.* day-length insensitivity and flowering time) and resistance genes was observed in the study of Cavanagh *et al.* (2013).

The *Rht* genes regroup several genes leading to a dwarf or a semi-dwarf phenotype in wheat. They have been introduced because of the need to reduce lodging in modern wheat genotypes, due to the increase in size and biomass of the spike thanks to the massive fertilization in fields. These genes generally present two alleles, *RhtXa* being the height-increasing allele and *RhtXb* the dwarfing one. *Rht1* (= *Rht-B1*) and *Rht2* (= *Rht-D1*) probably are the most extensively used dwarfism genes in wheat breeding, but several more exist, the most recent being *Rht25* (Mo *et al.* 2018). The great majority of modern wheat varieties own at least one dwarfing allele, which explains the significant decrease of the mean height of wheat since 1960 (Berry *et al.* 2015).

Due to the economic cost resulting from wheat disease like Fusarium Head Blight (FHB) or Fusarium Seedling Blight (FSB) (both caused by fungi of the *Fusarium* complex), leaf or stem rust (caused by fungi from the *Puccinia* genus), take-all (*Gaeumannomyces graminis* var. *tritici*), breeders have tried to improve wheat resistance to these plant pathogens using conventional and markers-assisted breeding (Gupta *et al.* 2010). Multiple resistance genes and loci have been found in the last three decades, the major part in wild plant species and ancient wheat genotypes (Goutam *et al.* 2015; Kaur *et al.* 2018). Thus, numerous past studies have shown that wild grass species were an excellent pool of resistance genes, as were the ancient wheat relatives (Doussinault 1983; Friebe *et al.* 1996). For example, the modern varieties Renan, Hussar, Eureka, Rendezvous, Torfrida, Apache or Corsair (non-exhaustive list) are known to possess the gene clusters containing *Yr17*, *Lr37* and *Sr38*, which confer resistance to multiple rust diseases and plant pathogens, and were translocated from *Aegilops ventricosa* (Ambrozková *et al.* 2002), a wild relative of the wheat. Renan and Rendezvous also harbor the *Pch1* gene, conferring a resistance to eyespot disease, and so do the modern genotypes Audace, Clarus or Beduin (non-exhaustive list) (Dumalasová *et al.* 2015). More recently, studies tended to focus rather on wheat landraces which are, thanks to their high genetic diversity, a great source of disease resistance genes (Bansal *et al.* 2013; Qureshi *et al.* 2017; Winfield *et al.* 2018). Also, recent Genome Wide-Association Studies (GWAS) were made to detect genetic markers significantly involved in disease resistance. Hence, Juliana *et al.* (2018) detected several candidate genes for wheat disease resistance, which were predicted to encode LRR-like receptors, serine/threonine protein kinases, peroxidases, ABC transporters or cystein-rich receptors. Because of modern breeding, these resistance genes, which derived from ancient genotypes by natural selection, are now frequently found in modern wheat genotypes, as they are most of the time required to present a phenotype able to success the VAT testing.

It clearly appears that modern breeding caused an erosion of the genetic diversity and increased genetic homogeneity (Doussinault *et al.* 2001; Fu and Somers 2009; Bonneuil *et al.* 2012; Goldringer *et al.* 2013; Winfield *et al.* 2018). It can be suggested, then, that landraces and old varieties may harbor particular

genes of interest, like those involved in the interaction with plant-helper soil microbial communities, and these traits might be useful for future breeding (Dwivedi *et al.* 2016; Winfield *et al.* 2018). Thus, it could be interesting to point out morphological and physiological differences between modern varieties and ancient wheat genotypes (including landraces and old varieties selected before 1960) regarding the underground compartment, as the above-ground has already been extensively studied, while the underground part of plants has been globally neglected.

Impact of modern breeding on wheat root morphology

Due to the introduction of dwarfism genes in modern wheat varieties (**Fig 7**), a decrease of root system size would be expected because of a dynamic balance between roots and shoots (Bastow Wilson 1988; Feller *et al.* 2015), even if it is worth noting that this balance is not always respected and is influenced by environmental conditions and the plant growth stages (Siddique *et al.* 1990). Such a negative impact of the dwarfism genes on root morphology has been observed by several authors, who have shown that the *Rht1* gene has a significant negative impact on primary and lateral root length and leads to a significant decrease in root biomass (Laperche *et al.* 2006; Subira *et al.* 2016). Concurrently, using isogenic wheat lines, it has also been shown that the genes *Rht2, Rht8* and *Rht12* have a significant impact on root length (Bai *et al.* 2013). There is also a trend for wheat modern varieties to focus their energy (*i.e.* their photosynthates) on their reproductive parts, leading to bigger spikelets at the expense of the total biomass of the plant, and thus a better harvest index (Calderini *et al.* 1995; Guarda *et al.* 2004; Álvaro *et al.* 2008). It can be suggested that this energy partitioning is made at the expense of the root system of modern varieties, and it is admitted that between 15 and 50% of daily photosynthates are allocated to the roots for nutrient assimilation and growth, depending on plant species and the environment (Lynch *et al.* 2011). Thus, in the context of modern breeding, selecting genotypes with abundant root production may have been counterproductive, due to metabolic costs and the potential reduction of energy allocated to reproductive parts and so the yield.

The use of agrochemicals in huge quantity is thought to have driven recent root morphology evolution (Zhang *et al.* 2013; York *et al.* 2015) as it has been shown that the concentration of nutrients in soil can lead to a modulation of root morphology (López-Bucio *et al.* 2003; Lynch 2007). For example, in soil with low phosphorus (P) concentration, *i.e.* in no or low-input farming system, plants form highly branched root systems by the development of numerous lateral roots and longer root hairs, which allow a better P uptake in the topsoil, were most of the phosphorus sources originating from fertilizers are found (Lynch 2007; Bovill *et al.* 2013) (**Fig 6**). Also, it has been shown that in presence of a great P concentration, roots have short hairs (Horst *et al.* 1993). It has been shown that plants under phosphorus starvation show an accumulation of sugars in their shoot, and it results in a sugar-signalling cascades leading to a relocation of the carbon to the root and a modification of the root system architecture (Hammond and White 2008) (Hammond and White 2008). In a

similar way, Shangguan *et al.* (2004) observed that wheat root growth was negatively correlated to the available nitrogen, notably root length which was significantly reduced at high nitrogen concentration. The improvement of irrigation methods, leading to more available for plants, could also have an impact on the evolution of the root morphology. Indeed, plant genotypes showing deep root growth angle are able to better explore sub-surface soil, and thus to acquire water under drought condition, while it is not a useful trait in non-limiting water status (Ho *et al.* 2005; Manschadi *et al.* 2008). It is worth noting, however, that the impact of water deficiency on roots can lead to a reduction or an increase in root biomass depending on wheat genotype (Azarbad *et al.* 2018).

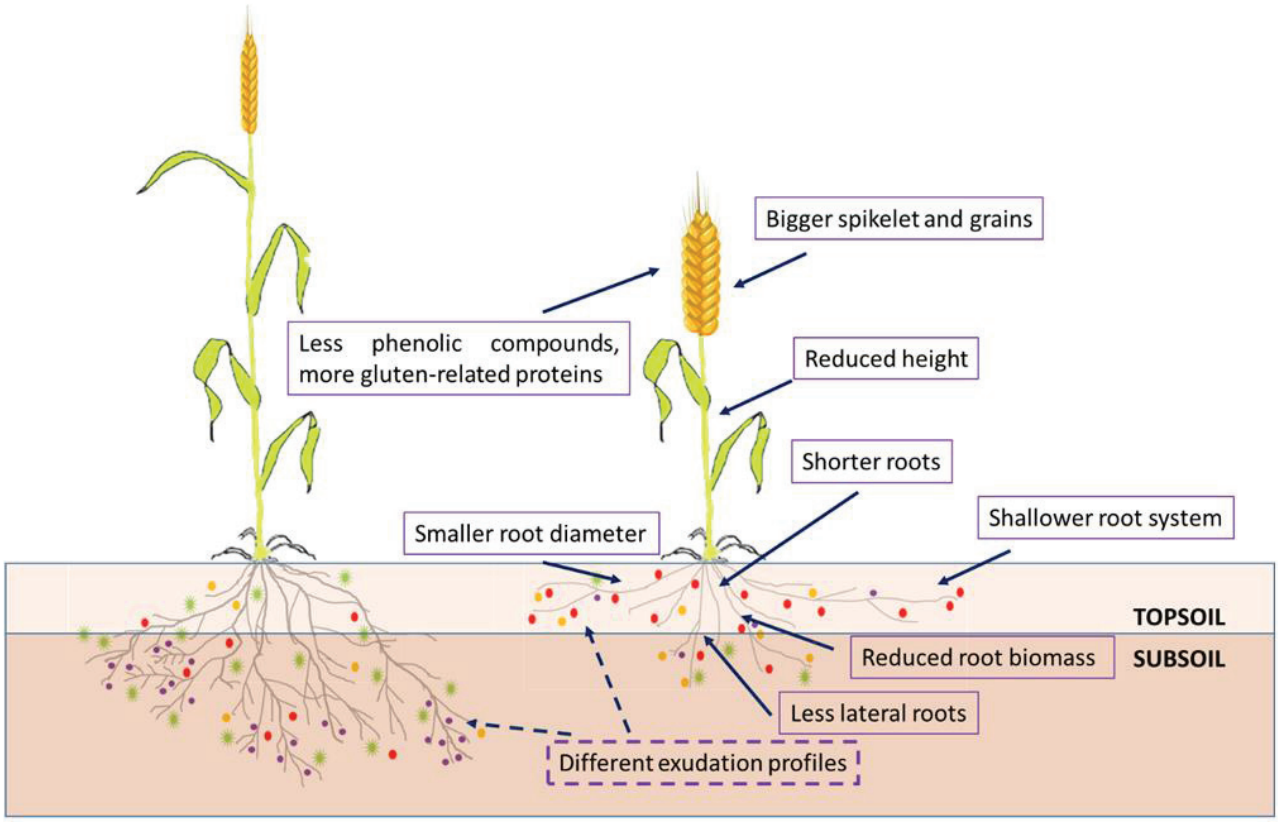

Fig 7 Described shifts in morphology and physiology of modern wheat varieties compared to old varieties. Regarding exudation profiles, more evidence are needed to be attested.

In accordance to these results, the authors who compared the root system of diverse varieties in the same growth conditions globally concluded on a root architecture difference between old and modern varieties, most of the time expressed by a more vigorous root system for the old varieties (**Fig 7**). For example, Horst *et al.* (1993), using a traditional wheat variety (Peragis) and a modern one (Cosir), observed that the traditional variety exhibited a bigger root system than the modern one at all development stages. Siddique *et al.* (1990) found that the old wheat variety Purple Straw exhibited one of the highest root dry biomasses among the 10 tested varieties, especially at late plant development stages. Shaposhnikov *et al.* (2016) showed

that Albosta, a wheat landrace, displayed a significantly higher mean root diameter when compared with two modern genotypes. Junaidi *et al.* (2018), using two modern and two old genotypes of bread wheat, observed that one of the old genotypes showed a significantly higher root length than the three others, and that the two old genotypes displayed higher root angle than the two modern ones. Accordingly, Beyer *et al.* (2018) found that, among 215 wheat accessions selected from 1814 to 2015, there was a significant negative correlation, at the seedling stage, between the year of release of the accessions and (1) their number of seminal roots, or (2) the length of branched roots.

Impact of modern breeding on wheat root physiology

Concurrently to root morphological changes, root physiological changes could be expected too as a result of modern breeding. A great number of studies focused on the metabolic differences occurring between ancient and modern varieties, at least indirectly, thanks to a comparison of biochemical compounds in grains. As mentioned above, modern breeding highly focused on yield, but also on quality of the grains (*i.e.* baking quality). The content in proteins involved in gluten production (notably gliadins and glutenins) in wheat grains was higher in modern wheat varieties than ancient varieties (**Fig 7**), which did not come as a surprise as gluten is one of the major baking quality traits, whose improvement was sought during modern selection (Morris and Sands 2006; Kiszonas and Morris 2018). Dinelli *et al.* (2009) pointed out differential phytochemical profiles among ten varieties, including ancient and modern ones, and especially a significant higher number of phenolic compounds in the grain of old varieties compared to modern ones. Di Loreto *et al.* (2018) showed similar results regarding phenolic compounds in 22 varieties. Interestingly, they even observed the presence of unique phenolic compounds in old varieties. Similarly, Gotti *et al.* (2018) concluded that the amounts of ferulic, hydroxybenzoic and vanillic acids in grains were significantly higher for old varieties than modern ones.

Thus, as it seems obvious that there are metabolic differences between old and modern wheat varieties, it is interesting to focus on the potential impact on root exudation, *i.e.* the release by roots of plant organic compounds such as secondary metabolites, which play a substantial role in rhizosphere interactions as we will see later (Heinrich and Hess 1985; Nguyen 2003; Bais *et al.* 2006). The major fraction of exudates is released through a passive process, and especially in meristematic regions (Darwent *et al.* 2003). However, there are also exudates that are actively released by the roots, using highly specific ATP-binding transporters (Loyola-Vargas *et al.* 2007; Badri and Vivanco 2009). Wheat exudate compounds correspond to a very broad range of molecules and ions, including sugars, organic acids, amino acids, or phenol substances (Vančura 1964; Heinrich and Hess 1985). These compounds can have diverse functions, such as for instance the alleviation of environmental stresses. For example, some wheat genotypes are able to exude phytosiderophores to alleviate micronutrient deficiency stress. Daneshbakhsn *et al.* (2013)showed a significant difference in the production of siderophores among three wheat genotypes, which correlates with their own resistance to Zn deficiency.

Rengel (1997) also showed that under Mn deficiency, some wheat genotypes are able to exude higher quantities of Mn reducers, thereby increasing Mn bioavailability. Exudates can also help to alleviate phosphorus starvation, as it has been shown that some organic acids exuded by wheat can increase bioavailability of phosphorus in soil, and are more exuded under P-starvation condition (Gahoonia *et al.* 1999; Hinsinger 2001). The nitrogen content in the soil may also impact the exudation process. Zhu *et al.* (2016) showed that an increase in N content can lead to a significant increase of the exudation rates for sugars and phenols, but a decrease for carboxylic acids. Similarly, it has been shown that N fertilization increases the soil C content derived from rice plant (Ge *et al.* 2014). Also, there is variability in the quantity and quality of exudates along the roots (Marschner 2011), thus it can be suggested that a modification of root morphology, as described above, might also influence root exudation. Finally, it is important to note that, as for root growth, root exudation has a metabolic cost for the plant (Nguyen 2003; Lynch 2007), notably at the expense of the reproductive parts. All in all, we could expect a substantial difference of exudation, regarding quality and/or quantity, between old and modern wheat genotypes (**Fig 7**). These differences have been observed with the landrace Albostan, which displayed significantly lower sugar (glucose and maltose) exudation than the modern genotype Karahan (Shaposhnikov *et al.* 2016). However, to our knowledge, there is a crucial lack of exudation profile comparisons between old and modern varieties. In this context, it is worth noting than in barley, the allelopathic activity has significantly decreased since 1890 (Bertholdsson 2004), which is interesting because the allelopathic activity can imply root exudation (Weston and Duke 2003).

Impact of modern breeding on wheat behavior in field

Due to the above described genetic, morphologic and physiological changes, modern wheat breeding globally resulted in the creation and selection of varieties that are able to benefit better from high level of fertilization (notably nitrogen) in field than ancient genotypes do, and that show great yield under this condition (Brancourt-Hulmel *et al.* 2003; Guarda *et al.* 2004; Ahrends *et al.* 2018). To assess these differences, Ahrends *et al.* (2018) used 15 wheat genotypes selected from 1895 to 2007 and a broad range of fertilization compounds, *i.e.* N, P, K and Ca in different combinations, either in synthetic form, or manure, or both. They showed that modern varieties had a higher maximum yield than old ones, in plots with high nutrient supply. However, in plots with no or low nutrient supply, there was no differences between modern and old varieties. It is consistent with the observations of other authors, who showed that when the conditions turned to be less optimal, *i.e.* low-input system, it often appeared that ancient wheat genotypes are less impacted than modern varieties by the reduction in synthetic fertilizers and can even increase their yield in some cases (Brancourt-Hulmel *et al.* 2003; Guarda *et al.* 2004; Junaidi *et al.* 2018). Thus, under condition of nutrient-deficiency, the genetic improvement of modern varieties turns to be greatly under-expressed (**Fig 8**).

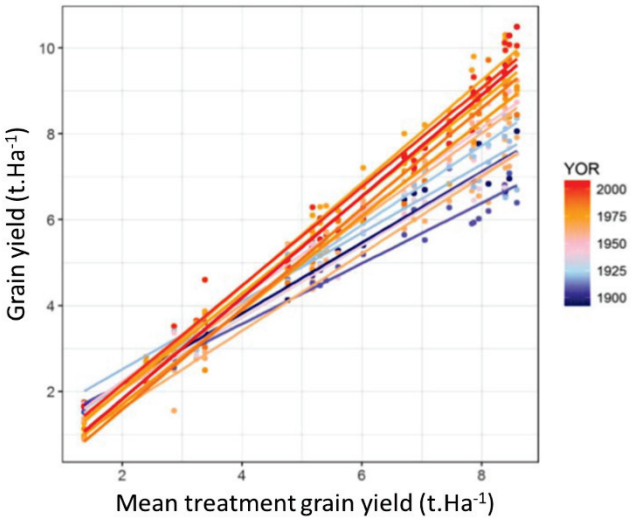

Fig 8 Linear regression fit between the mean grain yield of each variety (Y-axis) and the mean yield across all varieties (X-axis) of 15 wheat genotypes released from 1895 to 2007, grown under 24 different fertilization conditions (from no input to high-input). The regression slopes indicate a great responsiveness of modern varieties to long-term fertilization (at the right of the chart) but a weak tolerance to low nutrient supply (at the left of the chart). From Ahrends *et al.* 2018.

These differences of response to fertilizer application seem to be mainly due to a better nutrient use efficiency (NUE) from modern genotypes compared to ancient ones under non-limiting nitrogen condition (Ortiz-Monasterio R. *et al.* 1997; Guttieri *et al.* 2017). Better NUE leads to a better yield, which is in a big part due to higher nitrogen concentration in grains of modern genotypes compared to the grains of ancient ones, even if it is not always translated in a higher protein concentration (Guarda *et al.* 2004). However, Le Gouis *et al.* (2000) showed that in nitrogen-deficient condition, Cappelle, one of the two old varieties used in the study, had one of the five best nitrogen uptake efficiency under this condition among the 20 assessed varieties. Moreover, Cappelle was also the only genotype not showing nitrogen uptake efficiency changes under nitrogen-deficient condition. This suggests that ancient genotypes are better able to acquire rare nutrient resources in soil.

Ancient genotypes also seem to be less sensitive to other environmental variations. Indeed, it is now well-established that old varieties and landraces are a formidable genetic pool for the improvement of modern varieties to a broad range of abiotic stress resistances (Dwivedi *et al.* 2016). Thus, regarding wheat, it has been shown that the growth of ancient genotypes was less impacted than modern genotypes under drought (Fang *et al.* 2017). Moreover, another study comparing a landrace and a modern wheat varieties showed that the landrace did not show a drop in germination rate under saline stress, whereas modern variety did (Maucieri *et al.* 2018). It is also worth noting that pesticides also are needed to express the full genetic potential of modern varieties in field, as it has been shown that in presence of weeds in field, old varieties were less impacted and even tended to present a higher yield than modern varieties (Ruisi *et al.* 2015).

As we saw in Parts I and II, the selection of wheat varieties by humans led to dramatic changes in terms of plant morphology and physiology, both for above-ground and underground systems even though the latter has often been neglected in the analysis (Waines and Ehdaie 2007). However, another factor has been neglected, especially throughout modern breeding: the underground interactions with soil microbial community, notably bacteria.

IV –Abiotic and biotic factors influencing the composition of soil and plant root-associated bacteria

The great diversity and abundance of bacteria in soil can be subject to environmental disturbance. Moreover, plants are able to select their root-associated bacteria thanks to root features discussed above. Here we will discuss (1) the diversity of soil and plant root-associated bacteria, notably PGPR, (2) the impact of agricultural practices (such as the use of fertilizers, the use of pesticides and the tillage) on the composition of soil bacteria, and (3) the impact of biotic factors driven by plant root features on the composition of root-associated bacteria.

Diversity of soil and plant root-associated bacteria

It was estimated that between 2000 and 18000 distinct bacterial genomes can be found in one gram of soil (Dunbar *et al.* 2002). Thus, the soil houses a wide diversity of bacterial genera, species and subspecies that can assume a great diversity of functions (Tringe *et al.* 2005; Fierer and Jackson 2006). At the interface of the soil and the plant root system is the rhizosphere, which was defined by Hiltner (1904) as the root-surrounding soil influenced by rhizodeposition, which includes root exudates, mucilages and exfoliated cells (known as border cells) at the root cap (Nguyen 2003; Hartmann *et al.* 2009; López-Guerrero *et al.* 2013). The presence of a great abundance of carbon compounds due to rhizodeposition near the roots represents an opportunity for microorganisms, notably bacteria, which can catabolize them in the rhizosphere and use them as carbon and energy sources (Bais *et al.* 2006; Haichar *et al.* 2008; Philippot *et al.* 2013). The entire bacteria community in interaction with the root system (*i.e.* the root-associated bacteria) of the plant-host is called the root microbiota, and is directly involved in the health and growth of the plant (Torsvik and Øvreås 2002; Philippot *et al.* 2013; Puga-Freitas and Blouin 2015).

The gene pool harbored by the root-associated bacteria represents a new set of functions associated to the plant (Sánchez-Cañizares *et al.* 2017). These functions can be beneficial for the plant-host, and they are mostly carried out by Plant Growth-Promoting Rhizobacteria (PGPR ; **Fig 9**) (Höfte and Altier 2010; Vacheron *et al.* 2013; Bruto *et al.* 2014). PGPR includes strain belonging to diverse genera such as *Azospirillum, Azotobacter, Burkholderia, Enterobacter* or *Pseudomonas* (Proteobacteria phylum), *Bacillus* or *Paenibacillus* (Firmicutes phylum), *Streptomyces, Micromonospora* or *Microbispora* (Actinobacteria phylum) (Franco *et al.* 2007; Podile and Kishore 2007; Höfte and Altier 2010). For decades, scientists have made inoculation trials, in field or under simplified conditions, for the purpose of evaluating the use of PGPR to improve agricultural practices, especially in the last years because of the economic and environmental cost of conventional farming practices. Thus, diverse PGPR showed positive effects in field on the development and yield of crops like rice (Ashrafuzzaman *et al.* 2009; Shakeel *et al.* 2015), maize (Alves *et al.* 2014; Di Salvo *et al.* 2018), sugarcane (da Silva *et al.* 2017; Santos *et al.* 2018) or wheat (Naiman *et al.* 2009; Kumar *et al.* 2014; Karimi *et al.* 2018).

Moreover, it appeared that the use of PGPR inoculants can alleviate biotic and abiotic stress, such as drought (Creus *et al.* 2004; Furlan *et al.* 2017; García *et al.* 2017), heat (Abd El-Daim *et al.* 2014; Meena *et al.* 2015), saline stress (Upadhyay and Singh 2015; Barnawal *et al.* 2017), or phytopathogens (Fatima *et al.* 2009; Díaz Herrera *et al.* 2016; Oni *et al.* 2018). Some authors have suggested an engineering of the root microbiome, aiming to increase its positive effects on the plant (Bakker *et al.* 2012; Orozco-Mosqueda *et al.* 2018) for the purpose of decreasing the use of chemical inputs and stabilizing yield despite the emergence of stresses due to climate change(Brisson *et al.* 2010; Asseng *et al.* 2015). But the key to the success of such an engineering would probably be the focus on functional groups involved in benefits for plant growth.

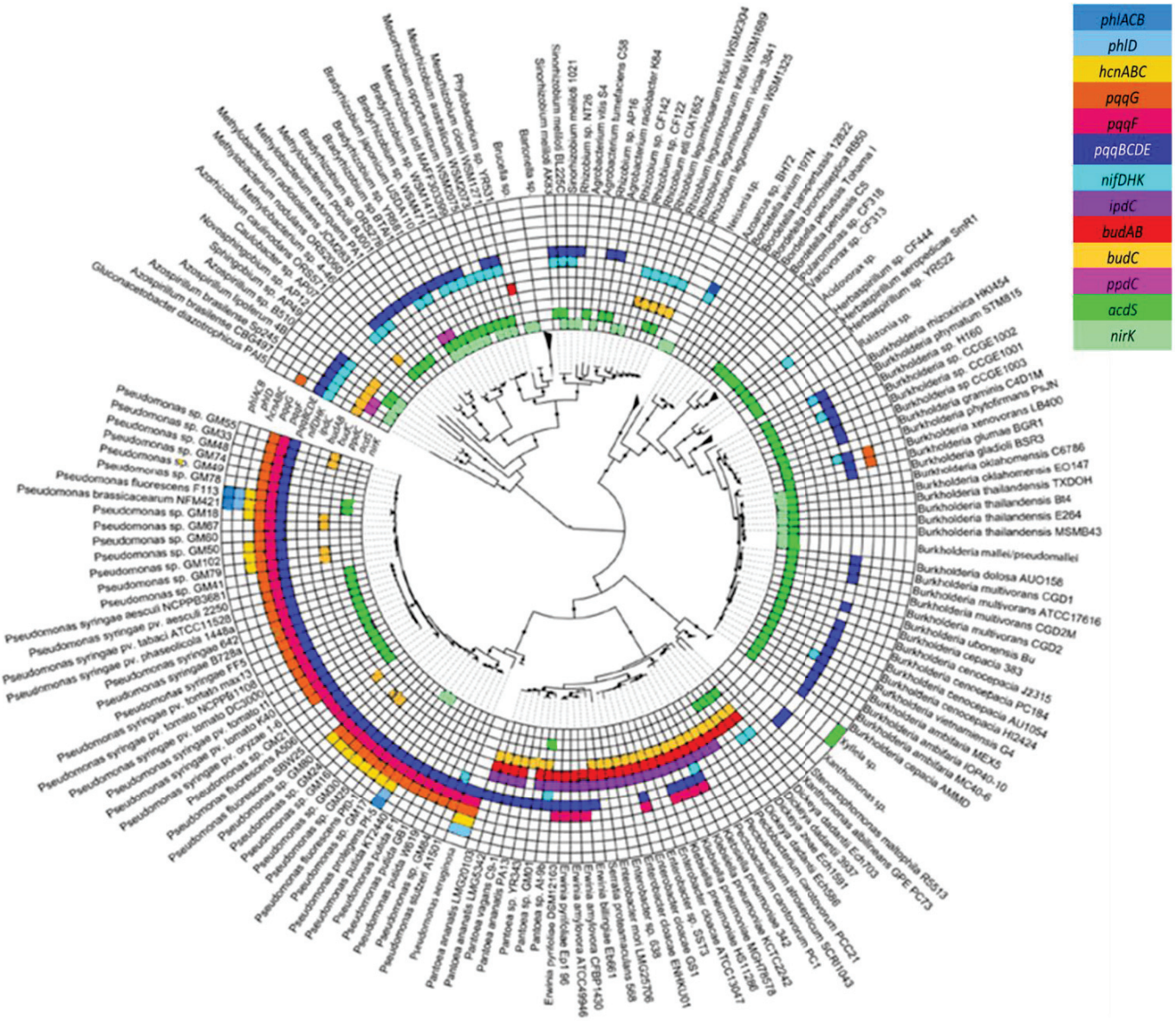

Fig 9 Phylogenetic distribution of genes along Proteobacteria phylogeny. The presence of a coloured square indicates the presence of a gene. For clarity, taxonomically coherent groups were collapsed. From Bruto *et al.* 2014.

The plant microbiota harbors bacteria that form functional groups, each corresponding to a group of bacteria able to perform a given function, typically because they share a similar set of functional genes (Torsvik and Øvreås 2002; Allison and Martiny 2008). We can cite for example the producers of 1-aminocyclopropane-1-carboxylate (ACC) deaminase, involved in the regulation of ethylene synthesis in plants by the deamination of its precursor ACC, which can help plants tolerate biotic and abiotic stresses (Glick *et al.* 2007; Saleem *et al.* 2007; Bouffaud *et al.* 2018; Safari *et al.* 2018). This group includes several well-known PGPR, such as *Pseudomonas kilonensis* F113 or *Azospirillum lipoferum* 4B, but also bacteria from the genera *Acidovorax*, *Burkholderia, Bradyrhizobium, Mesorhizobium or Rhizobium* and they all harbor the *acdS* gene (Prigent-Combaret *et al.* 2008; Vacheron *et al.* 2016; Bouffaud *et al.* 2018). Another well-known functional group is the group of nitrogen fixers, which has been known for several decades to contribute to the growth of the plant host and may increase crop yield (Kundu and Gaur 1980; Ozturk *et al.* 2003; Alves *et al.* 2014). The bacteria of this functional group harbor the *nifH* gene, and are part of genera such as *Rhizobium, Azospirillum, Bradyrhizobium, Burkholderia, Curvibacter* or *Halorhodospira* (Roesch *et al.* 2008; Bouffaud *et al.* 2016).

However, as we will see below, before applying their beneficial effects on their plant-host the root-associated bacteria will undergo a drastic selection process, first because of the variation of environmental conditions in soil, and second because of the direct influence of the host plant on the root microbiota thanks to its root morphology and physiology features.

Impact of soil factors and agricultural practices on the composition of the soil bacterial community

As mentioned before, to shape its root microbiota the plant is able to select bacterial strains from the surrounding soil, which indicates that the plant microbiota is directly linked to the bacterial community present in the soil (Mavingui *et al.* 1992; Smalla *et al.* 2001). Hence, it has been shown for *Arabidospsis thaliana* a close relationship between soil bacterial community and bacteria associated to its root system (Bulgarelli *et al.* 2012; Lundberg *et al.* 2012).

Many abiotic factors influence the composition of a soil bacterial community, like pH (Fierer and Jackson 2006), water content (Azarbad *et al.* 2018) or soil types, *i.e.* soils with different textures and history of management (Dong *et al.* 2017). O'Brien *et al.* (2016), performing their study in plots planted with a lowland variety of switchgrass, showed a surprising heterogeneity in soil community composition in soil plots, with considerable variation of relative abundance of bacterial taxa from soil samples which came from zones separated by only a few centimeters. Grundmann and Debouzie (2000), focusing on the functional group of nitrifiers (*i.e.* NH_4^+ and NO_2^- oxidizers), even showed a spatial structure of this group at the millimeter scale, in a sandy loam soil cultivated with maize. Similarly, a study of depth profile of microbial community showed different community structures between the surface and the subsurface samples (Chu *et al.* 2016). The

authors of this last study suggested that this difference could be due to a difference of ammonia content, which was higher in topsoil and to changes of the C:N ratio. Besides, it is interesting to see that many studies were made to evaluate the effect of fertilizer application (notably nitrogen) on microbial community, and if there was no consensus about the increase or the decrease of bacterial diversity, most of the authors agreed about a shift of community composition due to nitrogen fertilizer.

Many authors performing meta-analysis found that long-term (*i.e* several decades) fertilizer application led to a decrease of the microbial biomass and respiration rates (Treseder 2008; Liu and Greaver 2010), but it cannot be generalized as some authors also found opposite results (Geisseler and Scow 2014). This seems to imply that even if it has had an impact, fertilizer application alone cannot explain these changes in microbial abundance. Several hypotheses have been stated to explain the negative impact of fertilizer application, notably N fertilizer, on soil microorganism abundance, such as (1) the accumulation of recalcitrant compounds, unavailable for microbial growth, due to the reaction between soil organic matter and inorganic N, (2) micronutrient leaching due to acidification caused by the N input, (3) the reduction of the activity of lignolytic enzymes, which leads to the accumulation of lignin and binding of cellulose and other C sources that become unavailable to microbial communities able to catabolize the latter (Treseder 2008). In contrast, to explain the beneficial impact of fertilizer application on microbial abundance, it has been advanced that some functional groups, like the ammonium-oxidizing and other nitrifying bacteria, and also bacteria involved in denitrifying process, could benefit from the agricultural nitrogen inputs, which leads to an increase of their activity (Geisseler and Scow 2014; van der Bom *et al.* 2018). All in all, these changes in microbial abundance and functions probably mean a perturbation within the microbial community, causing benefits to some functional groups and, as a consequence, unbalanced competition, and therefore the decline of other functional groups.

This perturbation in microbial community is consistent with the observation of Kavamura *et al.* (2018) that inorganic N fertilization caused a drop of bacterial diversity in bulk soil and wheat rhizosphere. O'Brien *et al.* (2016) also showed a shift in microbial community composition between a nitrogen fertilized soil plot and an unfertilized one, and attributed this difference to the competitive advantage of copiotrophs over oligotrophs in an environment with more nitrogen. This hypothesis was also suggested in other studies to explain the particularly high richness of some bacterial phyla like the Actinobacteria or Proteobacteria in amended soils, which regroup members often described as copiotrophs, and possessing broad ability to catabolize several C sources, to the detriment of others phyla like Acidobacteria and Deltaproteobacteria, often described as oligotrophs (van der Bom *et al.* 2018, Fierer *et al.* 2012). It is important to note that these modifications of bacterial community composition are not only due to nitrogen fertilization. Thus, Beauregard *et al.* (2010) showed a substantial impact of a long-term phosphorus fertilization on bacterial community composition, even if it had no impact on bacterial richness. Allison and Martiny (2008), performing a meta-

analysis, reported that 84% of 38 studies showed that microbial community composition is sensitive to fertilization (including nitrogen, phosphorus and potassium fertilizers). However, they could not determine whether particular functional or taxonomic groups were more impacted than others. Besides this induced shift in bacterial composition, it is also worth noting that the massive use of mineral fertilizer can lead to the emergence of less-cooperative beneficial bacteria in rhizosphere. For example, Weese *et al.* (2015)showed that long-term inorganic N fertilization caused decreased mutualism between rhizobia and legumes, resulting in less positive effect of rhizobial strains on the growth of legume hosts. Thus, one can suggest that old and modern genotypes that have been selected at contrasted levels of fertilization may have interacted during their selection with different rhizobacterial communities, including PGPR.

Beside fertilization, other agricultural practices have impacted the soil community, starting by the use of pesticides. Indeed, pesticides can have a negative effect on the growth on bacteria such as the diuron and linuron on Acidobacteria or the metam-sodium on gram negative bacteria, and also on bacterial functions such as the dazomet on nitrification (Jacobsen and Hjelmsø 2014). In contrast, it has also been shown that successive use of pesticides can lead to the selection of microorganisms able to degrade these active compounds, increasing their population in soil treated with these compounds (Lancaster *et al.* 2010; Bælum *et al.* 2012).

The tillage also has a significant impact on bacterial community. Tillage destabilizes the soil structure and can release nutrients contents and organic materials from aggregates and make them available for microorganisms. Tillage also increases the dioxygen availability for soil microorganisms (Chávez-Romero *et al.* 2016). Thus, Dong *et al.* (2017) showed a significant increase of bacterial diversity in soil without tillage treatment, and several studies showed that soil bacterial communities were different between soils with conventional tillage and soils with no tillage (Yin *et al.* 2010; Sengupta and Dick 2015; Chávez-Romero *et al.* 2016; Dong *et al.* 2017).

Consistently with results discussed above, Hartmann *et al.* (2015) showed an overall difference of microbial community composition between field plots under organic management (low-input) and fields under conventional management (high-input ; **Fig 10**). This differences are still not fully understood, as it involves the modification of a highly complex community network, even it is obviously linked to the increase of mineral and energy sources in soil, which could favor some bacterial functional groups over others (Hartmann *et al.* 2015; O'Brien *et al.* 2016; van der Bom *et al.* 2018). The acidification that can result from the use of mineral nitrogen fertilizers containing notably ammonium can also play a crucial role, as it has been shown that bacterial diversity is higher in neutral soils than in acidic soils (Fierer and Jackson 2006). Besides, it is well known that the increase in nutrient sources could change root physiology, and notably root exudation, which is a process highly involved in shaping the root microbiome (Zhu *et al.* 2016).

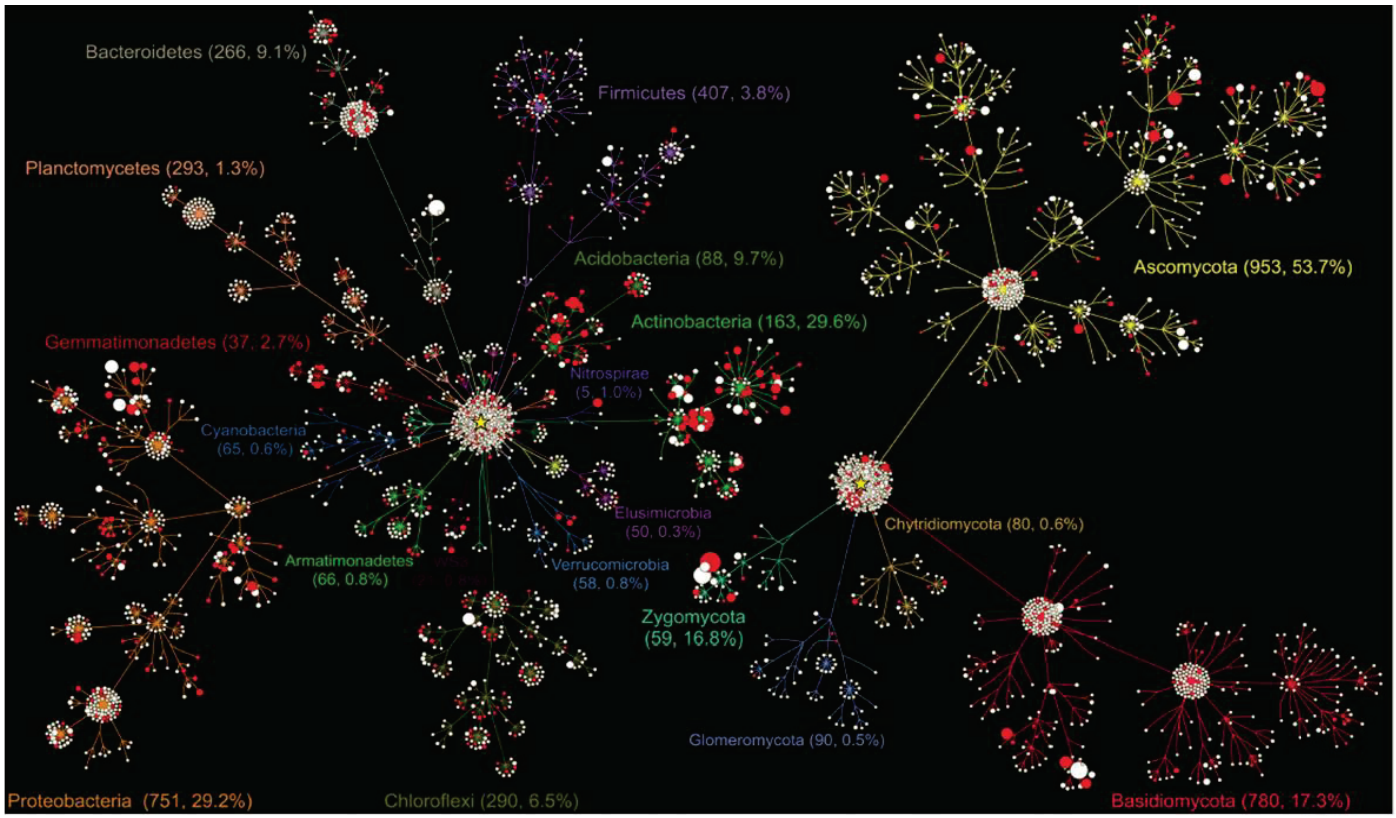

Fig 10 Taxonomic dendograms of detected fungal and bacterial communities in soil among conventionnal and organic farming systems, showing the OTU distribution across the distinct taxonomic branches (colour coded by phylum). Nodes represent OTUs and their sizes correspond to their relative abundances among the total microbial community. Lines between nodes represent the taxonomic path, from bacteria or fungi taxonomic roots indicated by a yellow star to OTU level (which were placed at the level of the lowest possible assignement). Most abundant phyla are labelled with the number and relative abundance of their associated OTUs. Red nodes are OTUs that significantly differed among conventionnal and organic farming systems, white nodes are insensitive OTUs. From Hartmann et al. 2015

Impact of root physiology and morphology on the composition and activity of the root-associated bacteria

Soil bacteria are considered overall as oligotrophic organisms, because of the low carbon concentration available in bulk soil (Zelenev *et al.* 2005). Yet, to shape its root microbiome, the host plant is able to recruit specific bacterial strains and to house them. Several exudate's compounds, such as carbohydrates, amino or organic acids, can attract by chemotaxis bacteria from the surrounding soil to the root system where they will proliferate (Benizri *et al.* 2001; Baudoin *et al.* 2003). For example, in the study of Heimrich and Hess (1985), focused on wheat exuded metabolites, sucrose was the sugars showing the highest chemotactic activity on *A. lipoferum* Sp108 while mannitose and xylose were sugars with the least chemotactic activity. Among amino acids, glutamine had the highest and threonine the lowest chemotactic activity towards this same bacterial strain. However, it is commonly known that bacterial species have different abilities to use and compete for substrate (López-Guerrero *et al.* 2013), and only bacteria strains able to catabolize these exudates and other

rhizodeposits will proliferate in an efficient way and colonize the root system in a long-term way, whereas other strains will suffer from this competition. Oger *et al.* (2004) showed for example that in the rhizosphere of engineered lotus plants overproducing opines, a greater abundance of bacteria able to catabolize opines were found, whereas the abundance of other bacterial species were not positively affected. Haichar *et al.* (2008), using a SIP method, were able to determine what bacterial populations are able to assimilate the exudates of wheat, maize, rape and barrel clover. In another hand, it is important to note that plants can also exude metabolites with antimicrobial properties, in a defense purpose against fungal or bacterial pathogens (Baetz and Martinoia 2014), like hydroxamic acids, which can be used as siderophores (Saha *et al.* 2016)and thus inhibit bacterial growth, or phenylpropanoids (notably cinnamic acid and hydroxycinnamic acids) which can inhibit fungal growth (Taofiq *et al.* 2017). Thus, the root bacterial community is strongly dependent on composition of the root exudation. Phenolic compounds notably have been shown to strongly influence the composition of the bacterial community, either acting as specific substrates or signaling molecules (Badri *et al.* 2013; Lundberg and Teixeira 2018). Several studies using purified exudates metabolites found in diverse plant species, such as maize, pine tree, soybean (Baudoin *et al.* 2003; Shi *et al.* 2011; Guo *et al.* 2017) also showed a significant influence on the bacterial community composition. Consistently, a recent study of Hu *et al.* (2018)who used maize mutants deficient in the production of benzoxazinoids showed that this class of secondary metabolites exuded by cereals such as wheat or maize significantly influence the root microbiome.

Besides their effect on the composition of the root microbiota, plant metabolites also have an effect on the expression of bacterial genes. A study of de Werra *et al.* (2011)showed that a great number of plant metabolites impacts negatively or positively the expression of bacterial genes known to be involved in the production of antimicrobial compounds, notably *phlA* (coding for 2,4-diacetylphloroglucinol [DAPG]). Tryptophan plays an important role in production of indole-3- acetic acid (IAA), an auxin hormone known to have root-ramification properties. Tryptophan is indeed the main precursor for IAA biosynthesis, thus it is necessary that the plant host exudes tryptophan to induce the expression of bacterial genes involved in the IAA production (Spaepen and Vanderleyden 2011). Exudates also impact the genes involved in the molecular communication between bacteria, named quorum-sensing (QS). This communication system is involved in many interactions between plant and bacteria, such as biofilm formation or bacterial functions with positive effect on plant growth, and uses molecules named *N*-acyl-homoserine lactones (AHL) produced by LuxI synthase, which will be recognized by LuxR receptors. However, some AHL can only be produced thanks to plant exudates, such as *p*-coumarate, used as a precursor by *Rhodopseudomonas plustris* to produce AHL (Schaefer *et al.* 2008). Besides, some plant metabolites can bind some bacterial LurxR receptors, such as the rosmarinic acid, which mimics microbial AHL and binds to the *Pseudomonas aeruginosa* RhlR QS receptor (Corral-Lugo *et al.* 2016). Thus, the presence of this plant exudate lead to the differential expression of 138 bacterial genes in *P. aeruginosa*, demonstrating the major effect that a plant exudate can have on bacterial

gene expression (Fernández *et al.* 2018). Finally, it is also known that exudation quantity and quality depend on the root zone, which is likely to influence the spatial colonization pattern of bacteria (Nguyen 2003; Razavi *et al.* 2016). This can explain the preference of some bacterial communities or some bacterial strains for particular root zones (Benizri *et al.* 2001; Kawasaki *et al.* 2016), such as bacteria from the *Azospirillum* genus, generally found in larger abundance in root hairs (Vande Broek *et al.* 1993).

Plant roots can impact their microbiota by their morphology. First because exudation is depending on root zone, therefore a root system with large root hair zones should show an exudation profile different from a root system with few root hairs. Second because as it had been mentioned above, bacterial community composition is heterogeneous in space, sometimes at the cm-scale (Chu *et al.* 2016; O'Brien *et al.* 2016), therefore an extended root system should be in contact with a broader range of bacteria than a restricted root system. Thus, the interaction between soil bacterial community and plant may depend on the root system architecture of the plant.

Finally, the last mean for plants to influence their root microbiome that we will mention below is the type of receptors at their root surface. To interact with each other, plants and bacteria need mutual recognition. In a first time, bacteria are recognized by plant as alien organisms, and thus will activate the defense system of the plant. The microbial molecules involved in this immune signaling are called MAMPs, for Microbe-Associated Molecular Patterns, and are found at the surface of a broad range of microorganisms, both beneficial and pathogens, and recognized by specific plant receptors called PRRs (for Pattern Recognition Receptors ; **Fig 11**) (Pel and Pieterse 2013). For example, the flagellin is a well-known MAMPs and is recognized by the FLS2 gene, generally involved in recognition of phytopathogens, which encodes a LRR-receptor at the surface of the plant cells coupled with an intracellular serine-threonine kinase domain, leading to an immune response when activated (Gómez-Gómez and Boller 2000). However, *Mesorhizobium loti,* known to have a symbiotic lifestyle with *Lotus japonicus*, is able to dodge this receptor because of divergent peptide structure of its flagellin, and thus doesn't initiate any immune response from *L. japonicus* (Lopez-Gomez *et al.* 2012). In a similar way, PGPR are able to use phase variation, switching to another morphology and altering or differentially expressing surface molecules in a reversible way, and this may contribute to minimize the immune reaction from the host plant (Pieterse *et al.* 2014). For example, *Pseudomonas brassicacearum* is able to switch from a phase I (low amount of flagellins) to a phase II (significantly higher amount of flagellins), and its localization on the roots depends on its current phase (Achouak *et al.* 2004). It has also been shown that PGPR can either secrete molecules able to suppress immune system of the host plant, such as particular LPS or EPS (which are bacterial membrane components), or interfere with hormone–signaling pathways which are involved in immune reaction (Pieterse *et al.* 2014). However, there are also situations where PGPR are detected by plant receptors because of their MAMPs and initiate an immune response from the host plant (called Induces systemic resistance ISR), beneficial to stimulate plant defenses against forthcoming pathogen

attacks, but without being warded off, suggesting a continuous molecular dialog between the PGPR and the plant and a great coordination of this molecular dialog (Van Wees *et al.* 2008). Finally it is interesting to note that even if they are generally involved in detection of pathogens, LRR-receptor can also be specifically involved in the interaction between the host plant and beneficial bacteria. Thus, Vinagre *et al.* (2006)showed that SHR5, a gene encoding a LRR-receptor, is involved is the specific beneficial interaction with diazotrophs. Indeed, the authors observed that in sugarcane associated with diazotrophs, the SHR5 gene has a significantly lower expression than in sugarcane not associated with diazotrophs, whereas it was not expressed when sugarcane is inoculated with pathogens.

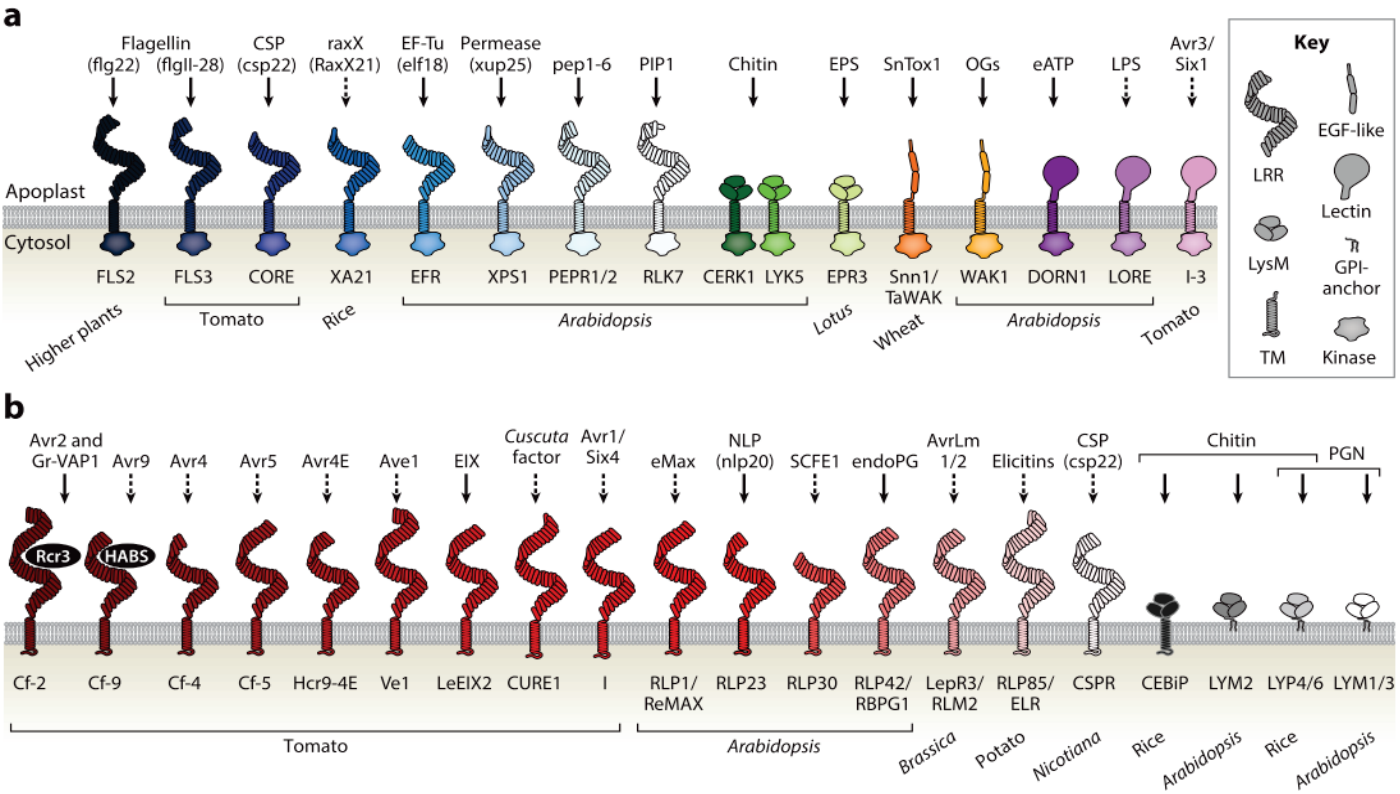

Fig 11 Non-exhaustive list of proven and potential plant pattern receptors (PRRs) and their known ligands/agonists. (a) Receptors kinases (Rks), (b) Receptor-like proteins (RLPs). Solid arrows indicate demonstrated direct binding while dashed arrows indicate a current lack of evidences for direct binding. EGF = epidermal growth factor ; EIX = ethlylene-inducing xylanase ; EPS = extracellular polysaccharides ; GPI = glycophosphatidylinositol ; LPS = lipopolysaccharide ; LRR = Leucine-rich repeat ; OGs = Oligogalacturonides ; PGN = peptidoglycan ; TM = transmembrane. From Boutrot and Zipfel 2017

Looking at this whole set of plant parameters, which can influence the interaction between plant and soil bacteria, it is not surprising that the root bacterial community is less diverse than that of the bulk soil (Tkacz *et al.* 2015). Consistently, rhizosphere bacterial community composition is different from the bulk soil, including in the case of the wheat rhizosphere (Fan *et al.* 2017). Even more, as it will be discussed next, there is an existing specificity of interaction between the plant host genotype and the associated rhizobacteria.

V – The close relationship between the plant-host genotype and its root-associated bacteria

We saw in the previous part that the composition of the root-associated bacterial community can be modulated by plant root features. Consistently, it appears that root-associated bacteria are directly under the influence of the plant-host genotype. In this part, we will discuss the influence of plant-host variety on root-associated bacteria, and make a focus on the specificity of interaction between plant genotypes and PGPR.

Influence of plant-host variety on root-associated bacteria

At the level of the whole microbiota, it has been found a significant correlation between the phylogenetic distances between *Poaceae* species (including teosinte, maize, wheat and sorghum) and the genetics distance between associated rhizobacterial communities (Bouffaud *et al.* 2014). This result suggests that the evolution history of the plant host has significantly influenced the composition of its microbiota. Given the high variability of plant features between genotypes of a same species discussed above in the text, numbers of authors have highlighted that bacterial abundance and/or community composition depend on cultivar-host genotype, and to some extent the way these cultivars were selected. Gomes *et al.* (2018)showed that the composition of total bacterial communities associated to maize genotypes with contrasting phosphorus (P) use efficiencies was different under high P-input condition (**Fig 12**), which suggests that the breeding method to obtain these cultivars has led to gain or loss of genetic traits involved in interaction with rhizobacteria. Emmett *et al.* (2018)used 12 maize varieties released from 1936 to 2011 and showed that maize identity explained a portion (*i.e.* 7-20%) of the total rhizobacteria diversity, especially at the anthesis stage. Moreover, they showed a difference of bacterial community composition between genotypes released before 1960 (selected under low N-input condition) and genotypes released from 1960 to 1980 (selected under high N-input condition), but surprisingly no more difference between genotypes released before 1960 and genotypes released after 1980. The authors attributed the bacterial community composition shifts regarding the genotypes released between 1960 and 1980 to a commercial breeding program which would have induced a change in plant features associated to interactions with rhizobacteria.

Regarding wheat, a recent study of Mahooney *et al.* (Mahoney *et al.* 2017)established that 95% of the rhizosphere bacterial samples from 9 wheat genotypes presented a core-microbiome consisting of 962 OTUs including 146 genera. Yet, there was a significant impact of the wheat genotype on the relative abundance of OTUs, and 24 OTUs were found to be specifically enriched or depleted in the rhizosphere of a wheat genotype compared to the others. This suggest that despite a set of genes shared by distinct wheat genotypes, there are several genes that are specifically harbored by some wheat genotypes, hence causing microbial community changes. In their study, Azarbad *et al.* (2018)used four wheat genotypes selected for their performance under low or high level of precipitation and showed that the abundance of total bacteria

associated to the roots of each genotype presented significant differences, but without satisfying matching with their drought susceptibility. Yang *et al.* (2018)performed a DGGE analysis on rhizosphere bacterial communities from different wheat varieties and observed bands that are not common to all wheat genotypes, consistent with a difference of bacterial diversity between the genotypes. Notably, they observed that Shannong 129, a wheat genotype that have been selected in the area of the experiment field of their study, was the only one of the seven used bread wheat genotypes not displaying a particular band common to all other genotypes. It could be explained by a particular microbiota harbored by this genotype due to its local adaptation to the environment. A particular interest can be addressed to the study of Germida and Siciliano (2001), showing a significant difference of culturable bacterial community composition between a modern and an old variety. Interestingly, their results showed that this difference is not the same, depending on the studied compartment. Thus, if there was few differences regarding the number of isolates from the rhizosphere of the two genotypes, authors observed that a higher number of culturable isolates were found in the root-interior of the modern genotype, compared to the ancient genotype (Germida and Siciliano 2001).

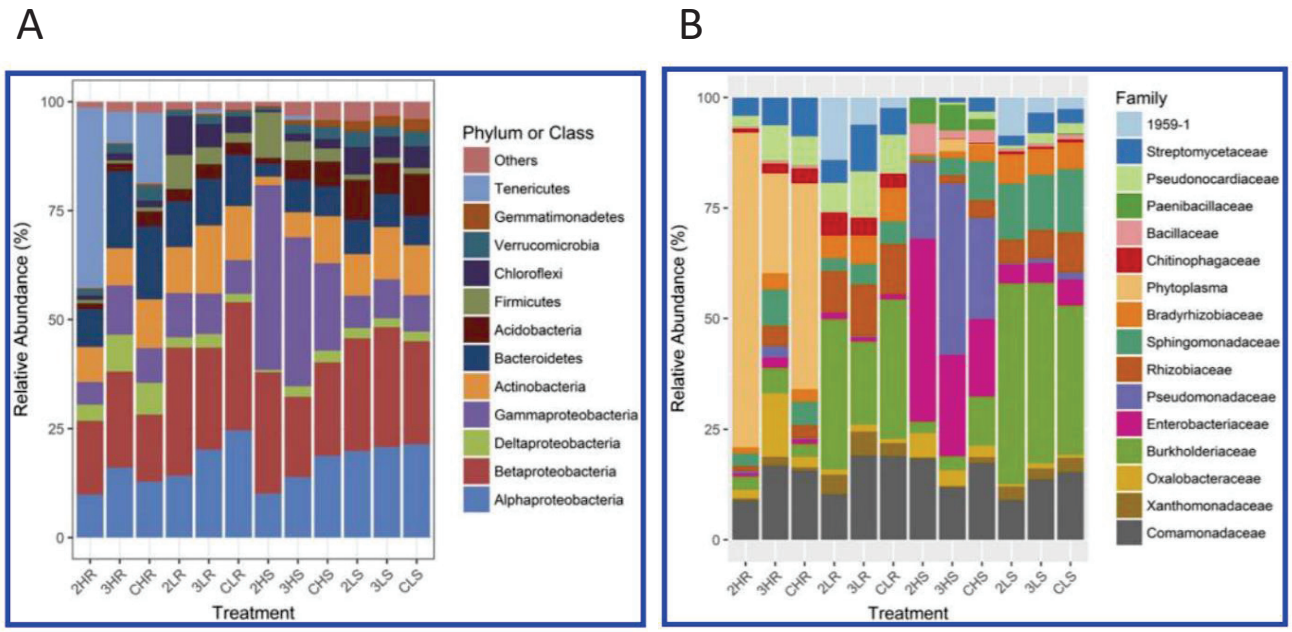

Fig 12 Relative abundances of bacterial phyla (A) or families (B) in the rhizosphere or the roots of two maize showing contrasting phosphorus use efficiency and an F1 cross between them, under condition of high P supply or low P supply. Treatment codes : 2 = genotype L22 (P-inefficient), 3 = L3 (P-efficient), C = genotype L3xL22 ; H = high P level, L = low P level ; S = rhizosphere samples, R = root samples. From Gomes *et al.* 2018

Specificity of interactions between plant genotypes and PGPR

It has been shown that plant-host genotype has an impact on specific functional groups known to have positive effects on plants. For example, Bouffaud *et al.* (2016) using a range of *Poaceae*, showed that the abundance of the group of the diazotrophs and their *nifH* expression was differentially affected regarding the associated plant species. These results were consistent with those of Knauth *et al.* (2005), which showed using T-RFLP

analysis on transcripts that different sets of indigenous OTUs expressed their *nifH* gene depending on the rice genotype that they colonized. Notably, they showed that landraces presented pools of expressed *nifH* genes highly different from modern cultivars. A study of Engelhard *et al.* (2000)determined that indigenous *Azoarcus* spp. that are known to be diazotrophic were preferentially established in the roots of wild rice species and landraces than in the roots of modern rice genotypes (*i.e.* established in 75% of wild species, 80% of landraces and only 33% of modern rice genotypes). The composition of the functional group of ACC deaminase producers was also differentially affected by *Poaceae* species and a significant correlation between the phylogenetic distance between the used *Poaceae* and that of the active ACC deaminase producers was found (a feature that was not found when considering the diazotrophs ; Bouffaud *et al.* 2018). Similarly, Stromberger *et al.* (2017)showed that the number of distinct culturable ACC deaminase bacteria depend on the genotype of four wheat cultivars and consistently, by performing pyrosequencing, that the composition of this functional group is related to the associated wheat genotype. Similar observations were done regarding the DAPG producers by Mazzola *et al.* (2004) who demonstrated that wheat cultivars specifically select different indigenous strains harboring the *phl*D gene and showed differences in terms of abundance of DAPG producers and number of distinct isolates. Interestingly, it has been shown that a wheat genotype displaying greater allelopathic activity than the others harbored a significantly higher number of culturable bacteria on its roots, and notably cellulose decomposers, diazotrophs and nitrifying bacteria (Zuo *et al.* 2014). We can also note that different cultivars of wheat can also present contrasted ability to recruit zinc solubilizing bacteria (Shakeel *et al.* 2015).

Inoculation studies have highlighted that the differences of interaction between PGPR and plant genotype can be observed at different steps of the cooperative interaction, such as the colonization, the induction of bacterial genes by the plant-host or the chemical communication between the protagonists. Inoculation of two endophytes (*i.e. Paenibacillus* spp. E119 and *Methylobacterium mesophilicum* SR1.6/6) on three potato cultivars led to different colonization levels inside stem base: one month after inoculation, the cultivar Karnico was more than ten-fold less colonized than the other ones by the SR1.6/6 strain (Andreote *et al.* 2010). In this context, it is interesting to note that Karnico was chosen for its high resistance to disease, and the existence of a defense mechanism toward the endophytic strain SR1.6/6 higher for Karnico than the two other cultivars was thus suggested by the authors. At the transcriptional level, a cross inoculation between 2 rice genotypes, Cigalon and Nipponbare, and 2 Azospirillum strains, B510 (isolated from the inner parts of Nipponbare shoot) and 4B (isolated from the roots of Cigalon) showed that rice-host genes and inoculated strains genes are expressed in a specific way, depending on the plant-bacteria combination (Drogue *et al.* 2014a, b). Using the strain 4B and the rice genotypes Cigalon and Nipponbare, Chamam *et al.* (2015) highlighted then that 4B induces much metabolic changes in Cigalon rather than Nippobare. Thus, it is likely that a co-evolution process might have occurred between a cultivar and members of its root microbiota.

Besides the difference in terms of PGPR community associated to the roots of distinct plant genotypes of a same species, and the specificity of interaction between inoculated plant genotype and inoculant strain discussed above, it has been shown that a same PGPR strain can have different impacts on the performance of a plant, depending on its genotype. Using 21 *Herbaspirillum* inoculants and two maize genotypes, Alves *et al.* (2014) showed contrasted amelioration of growth performance depending on the association between the maize genotypes and the *Herbaspirillum* strains. Similar observations were made by Furlan *et al.* (2017) using two wheat genotypes and either *Herbaspirillum* or *Azospirillum* inoculants, and it can be noted that surprisingly, the cultivar CD 120, which responds better to the inoculants, is a modern genotype with Mexican origin whereas Frontana, which responds to a lesser extent to the inoculant, is an old genotype. However, a previous study using this same cultivar CD 120 and another modern cultivar, CD 108, also showed that CD 120 had good response to *Herbaspirillum* inoculant while CD 108 did not respond to the inoculation, in an even lesser extent than Frontana previously mentioned (Neiverth *et al.* 2014) (**Fig 13**). Kazi *et al.* (2016) used *Azospirillum brasilense* strains (Sp245, Sp7 and Sp7-S, a spontaneous mutant of Sp7 that can only colonize cracks where lateral roots emerge whereas Sp7 can colonize the whole root surface) to inoculate five wheat genotypes, and demonstrated that (1) Sp245 inoculation led to an overall better response from most of wheat genotypes than Sp7 and Sp7-S, (2) Sp7 led to an overall better response from most of wheat genotypes than Sp7-S and (3) a differential response between genotypes in the presence of Sp245, Sp7 or Sp7-S.

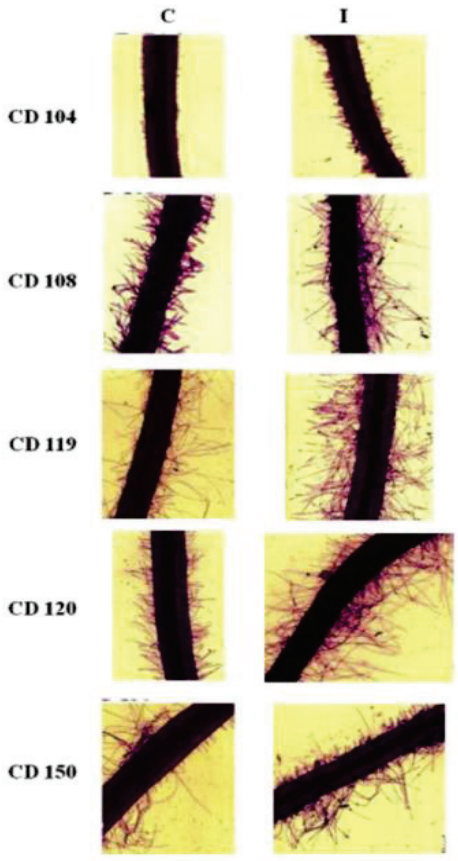

Fig 13 Microscopic images of root hairs of five modern wheat cultivars inoculated by *Herbaspirillum seropedicae* SmR1 (I) or not (C) at seven days after inoculation. A significant difference of response is observed, especially between CD 108 and CD 120. From Neiverth *et al.* 2014.

Conclusion

We saw that many genetic, morphologic and physiologic features have been modified during the transition between wild wheat relatives and domesticated wheat. Then, the modernization of agriculture, notably with the use of agrochemicals, led to the selection of genotypes with higher yield. This modern breeding was made without considering the interaction between roots and soil microorganisms, whereas we saw that environmental conditions significantly impact soil bacterial communities. Moreover, the modern breeding ended in the selection of modern varieties with root morphologic and physiologic features contrasting with old varieties. We saw that numbers of these features are involved in the interaction between roots and PGPR, leading to a close relationship between plant-host genotype and root-associated bacteria, notably PGPR. However, at our knowledge the studies comparing the ability of modern genotypes and ancient genotypes of a same species to interact with PGPR are very rare and used a very restricted number of plant genotypes. Thus, this work has been implemented to address this gap. Yet, in the current agricultural context, the understanding of the interactions between plant and PGPR to drive breeding programs in an eco-friendly and sustainable way are indispensable.

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Partie 2

Modern breeding and the ability of wheat to interact with the PGPR *Pseudomonas kilonensis* F113

Preamble

The use of Plant Growth-Promoting Rhizobacteria (PGPR) in agriculture is a promising solution in light of the negative environmental impacts and economic issues caused by the use of synthetic fertilizers [1, 2] . However, the performance of crops inoculated by PGPR shows a high variability, which it is still poorly understood [3]. In this context, several previous studies showed that a PGPR strain can have contrasted effects on different plant genotypes, notably on different varieties of a same plant species [4, 5]. Several authors suggested that modern breeding could have disturbed the interaction between plants and PGPR because of the use of artificial conditions (notably synthetic fertilizers) to select the modern varieties [6, 7]. Indeed, the modern varieties used today in field do not to display the same performances than older varieties, which can be explained by the different physiologic and morphologic features exhibited by these different categories of genotypes [8–10]. Therefore, we think it is relevant to enquire about the difference in PGPR interaction ability of different genotypes of a same plant species, which have been selected throughout the history of plant breeding. This question has been raised, in the case of bread wheat, during the course of the 'Bacterblé' ANR project (2015-2019) coordinated by Y. Moënne-Loccoz, by first comparing the colonization ability and expression of a phytostimulation-relevant gene by a model PGPR of the *Pseudomonas* genus under *in vitro* conditions.

In this context, in collaboration with Jacques Le Gouis from the INRA GDEC, a collection of 196 genotypes (kindly provided by the 'Centre de Ressources Biologiques (CRB) des Céréales à paille') representative of modern breeding since the 19th century was used in this work [11]. It includes (1) modern genotypes, which are pure line varieties selected after 1960 and the introduction of dwarfism genes to support higher yield thanks to non-limiting fertilization conditions, (2) a first category of ancient genotypes, the old varieties, which are pure line varieties selected before 1960 and (3) a second category of ancient genotypes, the landraces, which were for the most part selected before 1920 and are not pure line varieties (therefore present a high infra-genotype genetic heterogeneity). To this collection were added 2 reference modern genotypes that are extensively used in organic agriculture, Hendrix and Skerzzo, and the landrace Chinese Spring that is the reference genotype for bread wheat sequencing.

The model PGPR *Pseudomonas kilonensis* F113 was used to assess the effect of the collection of plant genotypes on the F113 colonization and the expression of *phl*, a bacterial operon involved in the biosynthesis of 2,4-diacetylphloroglucinol (*i.e.* an antimicrobial compound with plant auxinic effect [12]). For this purpose, I constructed a fluorescent derivative of the strain, expressing a constitutive red fluorescent fusion (F113 colonization) and an inducible green fluorescent fusion (*phl* expression). I developed a simplified method to screen the collection of wheat accessions and, with the help of a technician, screened the interactions abilities of 192 genotypes (7 showed gemination problems during the process) with F113 with enough sensitivity to avoid issues caused by autofluorescence (*i.e.* fluorescence from roots).

Then, to determine if these differences of interactions under *in vitro* conditions can be related to different responses of genotypes in terms of plant growth performance when inoculated by F113, I selected 10 wheat genotypes. These selection was made according to (1) their contrasted stimulation impact on F113 colonization and gene expression but also on another PGPR *Azospirillum brasilense* Sp245 (cf. following Part n°3), and (2) their availability at the CRB of INRA GDEC. This selection includes 4 ancient and 6 modern wheat genotypes. I inoculated these 10 genotypes with F113 under greenhouse, and to assess the impact of environmental conditions on the plant-PGPR interactions, a combination of drought and nutrient deficiency was applied on half of the pots. After a regular watering every two days, the plants in the 280 pots were harvested one month later with the help of the Rhizosphere team members, and nine features were assessed: this comprises different plant architecture parameters, fresh and dry root and shoot biomasses and also the F113 population levels assessed by a qPCR method [13]. I analyzed the results and performed all the statistical analyses. Results of this work are presented in a manuscript that will be submitted in November 2018.

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Modern breeding and the ability of wheat to interact with the PGPR *Pseudomonas kilonensis* F113

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Abstract

Plant genotype is a key factor influencing interactions with bacteria, including Plant Growth-Promoting Rhizobacteria (PGPR). Crop breeding has largely modified crop performance, but its impact on beneficial plant-microbe interactions is poorly understood. Since modern breeding has been carried out mostly under optimal agronomic conditions, it is likely that PGPR effects have not been selected for. Here, we tested the hypothesis that ancient crop genotypes have better PGPR interaction ability than modern genotypes using 199 wheat accessions (192 germinated) representing worldwide wheat diversity, including ancient and modern genotypes, and the PGPR *Pseudomonas kilonensis* F113. Using a reporter system approach developed to quantify F113 colonization and expression of *phl* (coding 2,4-diacetylphloroglucinol) on roots under gnotobiotic conditions, we showed that both F113 colonization and *phl* expression were more effective overall on ancient genotypes than modern genotypes. Ten contrasted wheat accessions (F113-stimulating or not) were then inoculated with *P. kilonensis* F113 and grown in non-sterile soil in the greenhouse, under optimum or nitrogen/drought stress conditions. Under stress, inoculation improved wheat performance for 4 of 6 F113-stimulating genotypes but none of the 4 non F113-stimulating genotypes. This screening of unprecedented scale shows that modern breeding has had a negative impact on PGPR interaction ability, even though this capacity has been maintained in certain modern cultivars.

Introduction

Agriculture productivity is a major issue since the world population may reach about 9 billion people in 2050 [1, 2], 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 [3, 4]), drainage/irrigation, and modern breeding to select crop genotypes efficient at taking full advantage of farming inputs [5, 6]. However, the consequences on the interactions between plant roots and the associated bacterial community are not well documented [7,8,9].

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 [10,11,12]. In the case of soil bacteria, this involves Plant Growth-Promoting Rhizobacteria (PGPR), which may stimulate root growth, improve nutrient uptake [13, 14], alleviate plant stress [15,16,17,18] or protect plant from pathogens [19, 20]. These effects rely on various modes of action, such as increasing nutrient availability, modulating plant hormonal balance [21, 22], and/or producing bioactive metabolites [23, 24]. Since these beneficial effects would be of less interest under optimum conditions, it can be thought that the ability of crop genotypes to interact with PGPR populations naturally present in soil was not selected for during modern breeding [11, 12, 25]. 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 [25,26,27], but little has been done to test it experimentally. If it were true, it could provide a basis to understand why the magnitude of PGPR effects differs from one variety to the next [16,28,29,30]. It would also mean that ancient genotypes could present a source of PGPR interaction traits, of potential value for future PGPR-based breeding [11, 31].

The objective of this work was to test the hypothesis that PGPR interaction ability had not been favored during modern breeding, by assessing whether or not this trait was less prevalent in modern crop genotypes than in ancient ones. First, a collection of 199 bread wheat accessions, representing world-wide crop genetic diversity and including both ancient and modern genotypes, was screened for the ability to interact with the model PGPR strain *Pseudomonas kilonensis* F113. *P. kilonensis* F113 produces 2,4-diacetylphloroglucinol (DAPG), an antimicrobial compound at high concentration [32, 33] but with auxinic-type root-branching properties at lower concentration [24, 34]. The pseudomonad stimulates growth of Arabidopsis [24], maize [35] and wheat [23]. Importantly, bacteria closely related to *P. kilonensis* F113 seem widespread in arable soils [36,37,38]. A new screening method of double fluorescent tagging was developed to measure root colonization by *P. kilonensis* F113 and expression of the DAPG genes *phl*. Then, 10 wheat accessions showing contrasted results during the screening and including ancient and modern genotypes were compared for their ability to respond to F113 inoculation under optimum or stress conditions, in a soil pot experiment.

Materials and methods

Panel of wheat accessions

A total of 199 accessions of wheat (*Triticum aestivum*) were used (**Supplementary Table S1**). They included (i) the 196CC core-collection [39] sub-sampled from the INRA worldwide bread wheat collection of 372 accessions set up by Balfourier *et al.* [40], based on field evaluation data [41], geographic origin and registration date, as well as (ii) the two reference (modern) varieties Hendrix and Skerzzo and (iii) the genome-sequenced reference (landrace) Chinese Spring. These accessions were chosen to maximize genetic diversity, as indicated by representative genome-wide molecular markers [39], and originated from 38 different countries of six continents. Among them, 7 presented major germination problems on agar plate and were removed from the panel, leaving a total of 192 wheat genotypes. The 192 genotypes included 77 ancient genotypes (*i.e.* 35 landraces, plus 42 old varieties from the 19th century to 1960), as well as 115 modern genotypes (*i.e.* developed after 1960).

Bacterial strain construction and growth conditions

For the screening, we performed chromosomal insertion [42] into *P. kilonensis* F113 of the construct *att*Tn*7::*miniTn*7*-Gm-P*tac-mCherry* for constitutive expression of reporter gene *mCherry* [43]. The gentamicin-resistance cassette was then replaced with a kanamycin-resistance cassette by homologous recombination, using plasmid pCM184 [44], and proper replacement was verified using PCR and restriction enzymes (not shown). Finally, the derivative *P. kilonensis* F113-*mCherry*(P*phl-egfp*) was obtained by electroporation [24] of the gentamicin-resistance plasmid pOT1e carrying a copy of the promoter of the *phl* operon fused to the promoterless reporter gene *egfp*, constructed as described by Vacheron *et al.* [30].

The inoculum for the screening was prepared by growing strain F113-*mCherry*(P*phl-egfp*) for 24 h in Minimal Medium (MM) broth [45] supplemented with gentamycin (25 µg/mL) and kanamycin (50 µg/mL), at 27°C with shaking (180 rpm). For the greenhouse experiment, the wild-type *P. kilonensis* F113 was grown for 24 h in MM broth without antibiotics, at 27°C, with shaking (180 rpm).

Wheat inoculation in the screening experiment

Wheat seeds were surface-disinfected by consecutive immersion for 1 min in 70% ethanol, then for 40 min with shaking (180 rpm) in sodium hypochlorite solution (Na_2CO_3 0.1 g, NaCl 3 g and NaOH 0.15 g in 100 mL distilled water) supplemented with 10% commercial bleach (containing 9.6% of active chlorine) and 0.01% of Tween 20%. They were washed three times (5 min each) with sterile water, and soaked 60 min in a last bath of sterile water. For pre-germination, disinfected seeds were placed on plates containing sterile agar for plant culture at 8 g/L (Agar A7921; Sigma-Aldrich, Saint-Quentin Fallavier, France) and incubated in the dark for 24 h at 27 °C. The one-day-old seedlings were then transferred in 120 × 120 mm square Petri dishes containing 50

mL of sterile agar for plant culture (3 seedlings per dish). No nutrient solution was added. Then, seedlings were inoculated with 50 µL of cell suspension containing 5×10^7 CFU (OD_{600} adjusted to 8.0, giving 10^9 CFU/mL) from an overnight culture of F113-*mCherry*(P*phl-egfp*), whose cells had been washed and resuspended in $MgSO_4$ 10 mM. For each of the 192 wheat genotypes, 3 seeds were non-inoculated and 3 others were inoculated with F113-*mCherry*(P*phl-egfp*). The inoculated seedlings and the controls were placed in plant growth chamber for seven days at 21°C, with a 16/8 h day/night cycle and 60% hygrometry.

Root sampling and bacterial fluorescence measurements

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

Screening validation and confocal microscopy observations

To confirm fluorescence results, another experiment was performed using 20 genotypes which showed contrasted fluorescence intensities on their roots during the screening (**Supplementary Table S2**) and twice as many replicates. To select these genotypes, a PGPR interaction score was calculated for each genotype as the sum of its ranks (*i.e.* the worst rank was 1 and the best one 192) for F113 colonization, for *phl* expression and for *phl* induction rate. Seed surface-disinfection and seedling inoculation were done as described above to obtain six plants per genotype. After seven days of growth, a different method of fluorescence measurement was used, after cutting each root system in 1-cm fragments and introducing all fragments from a same plant into a 2-mL Eppendorf tube containing 1.5 mL of $MgSO_4$ 10 mM (without steel beads). The roots were shaken for 2 × 5 min with a TissueLyser (Qiagen, Hilden, Germany) at maximum speed (30 Hz) and room

temperature. Then, the fluorescence of each supernatant was measured as described above. Root biomass was also determined.

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

Soil pot experiment

A greenhouse experiment was performed with 10 wheat genotypes giving contrasted screening results, *i.e.* Coronation, Concurrent (ancient genotypes), Amifort, ATUT-II, D130-63 and DI-276 (modern genotypes), which showed good interaction results, and Jaszaji TF, Odesskaya16 (ancient genotypes), Danubia and Hendrix (modern genotypes), which showed weak results (**Supplementary Table S2**). Plants were subjected (or not) to combined water and nutrient deficiencies and inoculated (or not) with *P. kilonensis* F113, making 2 × 2 treatment combinations, and the effect on wheat growth was monitored.

On the first day, wheat seeds were surface-disinfected by stirring 40 min in sodium hypochlorite solution (see above), washed three times 5 min with sterile water, and soaked in a last bath of sterile water for 60 min. They were put in 2-dm^3 pots containing 1.8 kg of sieved non-sterile soil (loam, organic matter 5.5%, pH_{H_2O} 6.0) taken from the topsoil of an arable luvisol located at La Côte Saint-André (France). At first, the soil was watered every two days to maintain a water content of 22% w/w. Each pot received 4 seeds, and the number of plants was reduced to 3 at seven days and then 2 seven days later. In half the pots, each seed was inoculated with 200 µL of a cell suspension of *P. kilonensis* F113 (obtained as described above) containing 2.5 × 10^6 CFU, whereas seeds in the other half received 200 µL of $MgSO_4$ 10 mM each.

The experiment was run with 7 replicates for each soil treatment x wheat genotype combination, and the 280 pots were placed in a greenhouse (randomized block design), with a 16/8 h day/night cycle at respectively 24 °C/20 °C and 40 %/60 % hygrometry. At 14 days, a combined stress of water and nutrient deficiencies was applied to half the non-inoculated pots and half the inoculated pots. To this end, soil was left to dry to 12 % w/w until the end of the experiment in the stress condition only, and a NPK nutrient solution (Plant-Prod 20-20-20; Plantproducts, Leamington, ON) was used to bring 16 mg N/plant (8 mg on days 14 and 21) in the non-stress condition only.

At the four-leaf stage, all the plants were harvested. In each pot, one plantlet was used to evaluate plant growth (*i.e.* 7 plants/treatment), after separating root systems from shoots and carefully washing them with water. Shoot height and biomass were measured. Fresh and dry (105°C overnight) root biomass were

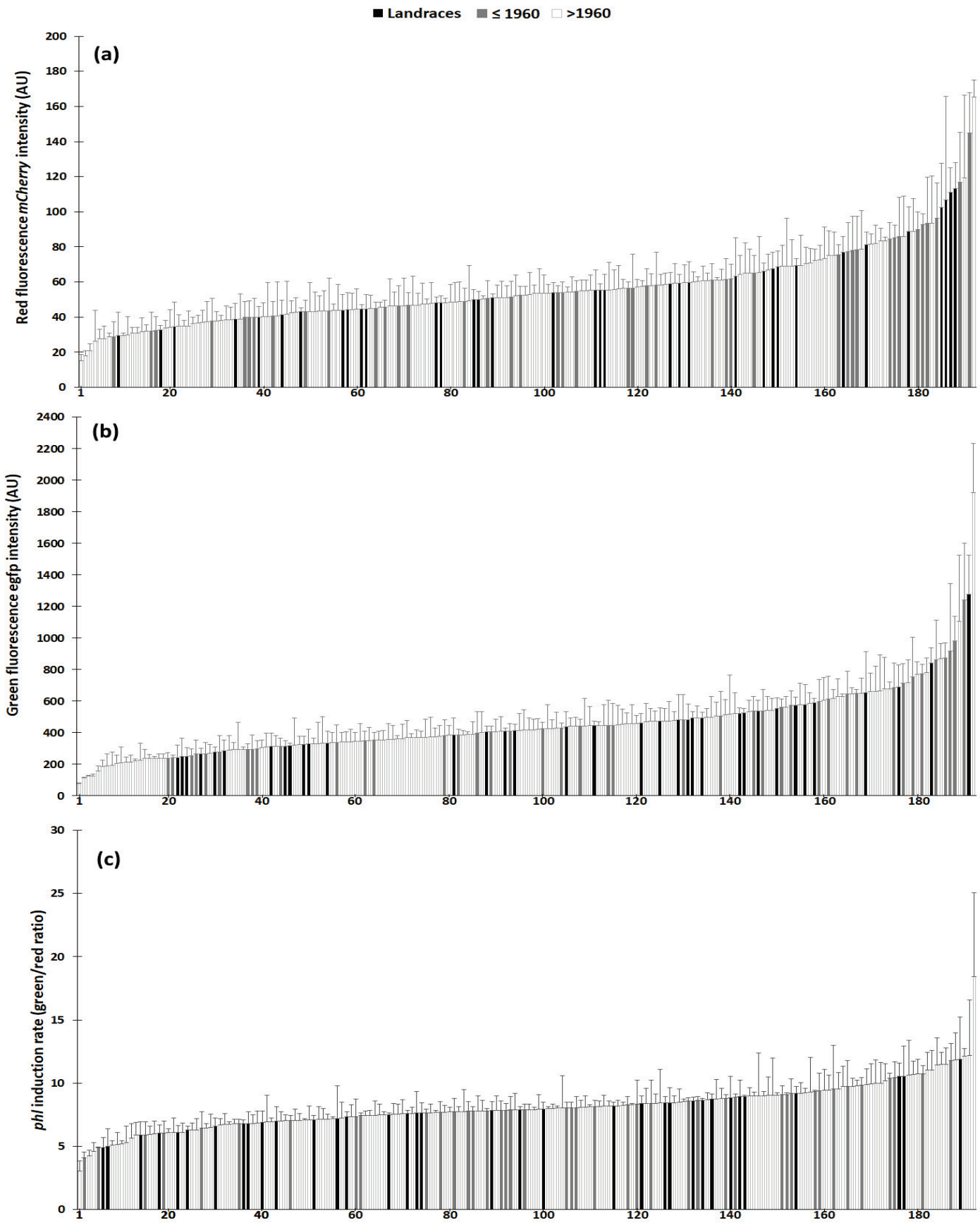

Fig. 1 Root colonization and *phl* expression of *P. kilonensis* F113-*mCherry*(P*phl*-*egfp*) on 192 individual wheat genotypes corresponding to 115 modern genotypes (> 1960) and 77 ancient genotypes (including 42 old varieties [≤ 1960] and 35 landraces). Red fluorescence (root colonization) is shown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

determined, along with root system architecture (number, length, diameter, surface and volume of roots) using WinRhizo image analysis (Regent Instruments, Nepean, ON). The other plantlet of each pot was used to quantify F113 colonization (5 plants/treatment; see below).

Real-time PCR assessment of inoculant colonization

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

P. kilonensis F113 was assessed in rhizosphere soil by real-time PCR, as described by Von Felten *et al.* [46], using a LC-480 LightCycler and the LightCycler 480 SYBR Green I Master mix (Roche Applied Science, Indianapolis, IN). Melting curve calculation and determination of Tm values were performed using the Lightcycler Software (Roche Applied Science). The C_T values obtained were normalized using the plasmid Apa9 as internal standard, as described by Couillerot *et al.* [47] and Von Felten *et al.* [46], in order to standardize DNA extraction efficiencies between rhizosphere samples. The primers amplify a specific region of the F113 strain. In our experiment, the detection limit was 10 copies of the target sequence per reaction. The efficiency was higher than 86% and the error rate lower than 2%. Quantities were expressed per g of dry rhizosphere soil and per root system.

Statistics

Standard errors (SE) were used to show data variability. As screening and quantitative PCR data could not been normalized, comparisons between multiple treatments were made using Kruskal-Wallis rank-sum tests followed by Conover-Iman tests for pairwise comparisons, and comparisons between only two treatments were made using Wilcoxon rank-sum tests. Comparison of proportions were made using Khi² test. For the greenhouse experiment, the plant data were normalized using a box-cox transformation and comparisons were made using multiple-factors ANOVA followed with Fisher's LSD tests for pairwise comparisons. All analyses were carried out at $P < 0.05$, using Xlstat software v2018.4 (Addinsoft, Bordeaux, France).

Results

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

Under the gnotobiotic conditions for the screening of the 192 wheat genotypes, *mCherry* fluorescence resulting from F113-*mCherry*(P*phl-egfp*) colonization ranged from 15 ± (SE) 3 for Hendrix to 165 ± 10 arbitrary

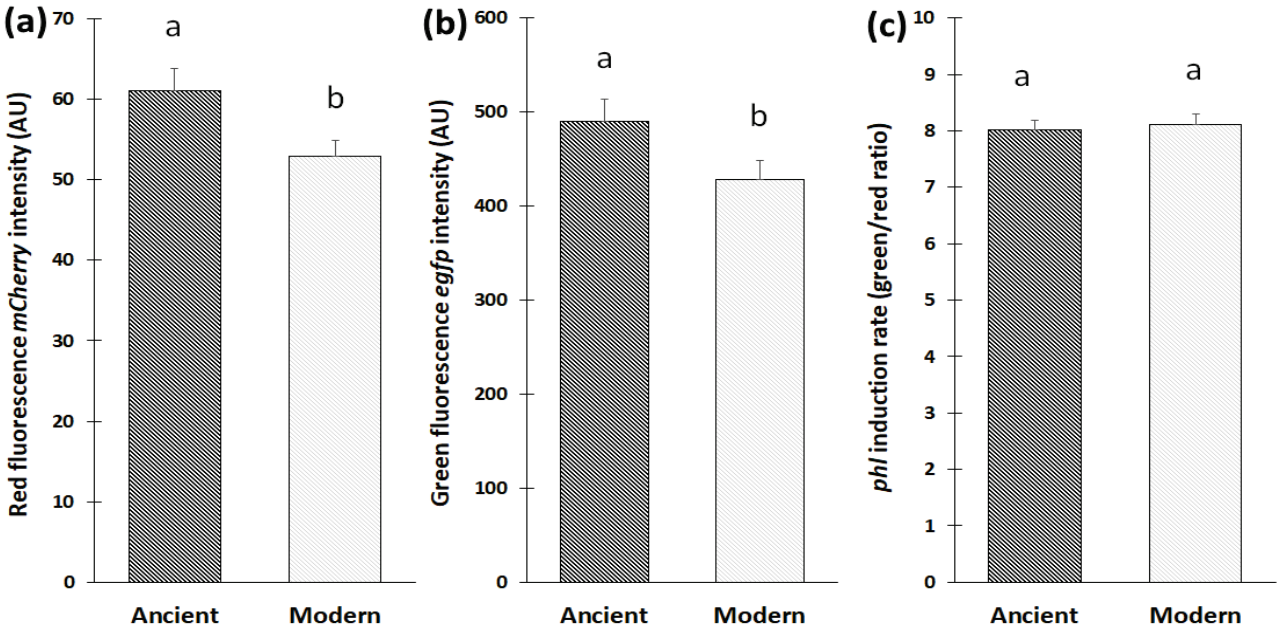

Fig. 2 Root colonization and *phl* expression of *P. kilonensis* F113-*mCherry*(P*phl-egfp*) for modern (n = 115) and ancient wheat genotypes (n = 77). Red fluorescence (root colonization) is shown (**a**), green fluorescence (*phl* expression) (**b**), and the *phl* induction rate (red fluorescence:green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

Table 1 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, $P < 0.05$).

units (AU) for D130-63 at one week (Fig. 1a). F113-*mCherry*(P*phl-egfp*) colonization was higher for ancient genotypes than modern genotypes, based on two criteria. First, colonization was significantly higher (P = 0.022) for ancient genotypes overall (61 ± [SE] 3 AU, n = 77) compared with modern genotypes (53 ± 2 AU, n = 115) (Fig. 2a). Second, the 25 genotypes presenting the best F113 colonization corresponded to 15 of 77 ancient genotypes (*i.e.* 19.5%) vs only 10 of 115 modern genotypes (*i.e.* 8.7%) (**Table 1**). Conversely, 6 of the ancient genotypes (7.8%) vs as many as 19 of the modern genotypes (16.5%) were part of the 25 least colonized wheat genotypes. Similar trends were observed when considering the 50 best and 50 worst colonized genotypes. Higher colonization of ancient genotypes is also indicated by the distribution of red fluorescence classes, which showed positive differences in frequency (frequency of modern genotypes subtracted from frequency of ancient genotypes) among the five categories above 80 AU (Fig. 3a,c).

When distinguishing between landraces and old varieties (< 1960) within the ancient genotype category, it appeared that (i) F113-*mCherry*(P*phl-egfp*) colonization was significantly higher (P = 0.039) for old varieties (62 ± [SE] 4 AU) than modern genotypes (53 ± 2 AU), landraces being in intermediate position (60 ± 4 AU ; Supplementary Figure S1), and (ii) there were non-significant trends towards higher proportions of landraces (17.1%) and old varieties (21.4%) than modern genotypes (only 8.7%) among the 25 best colonized genotypes and lower proportions of landraces (8.6%) and old varieties (7.1%) than modern genotypes (as much as 21.7%) among the 25 worse colonized genotypes (**Supplementary Table S3**). This is also indicated by positive differences in frequencies for both landraces and old varieties (vs modern genotypes) among the five classes above 80 AU (Supplementary Figure S2).

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

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

In addition, the 25 genotypes with the best *phl* expression corresponded to 13 of the 77 ancient genotypes (*i.e.* 16.9%) and 12 of the 115 modern genotypes (*i.e.* 10.4%), and the difference was statistically significant when considering the 50 genotypes showing the best *phl* expression, which included 27 of the 77 ancient genotypes (35.1%) vs only 23 of the 115 modern genotypes (20%). Conversely, there was also a trend (not significant) for a lower prevalence of ancient genotypes (6 of 77, *i.e.* 7.8%) than modern genotypes (19 of 115, *i.e.* 16.5%) in the 25 genotypes presenting the worst *phl* expression (**Table 1**). Higher *phl* expression on ancient genotypes is also indicated by the distribution of green fluorescence categories, which showed positive differences in frequency (frequency of modern genotypes subtracted from frequency of ancient genotype) among the four classes above 700 AU (Fig. 3b,d).

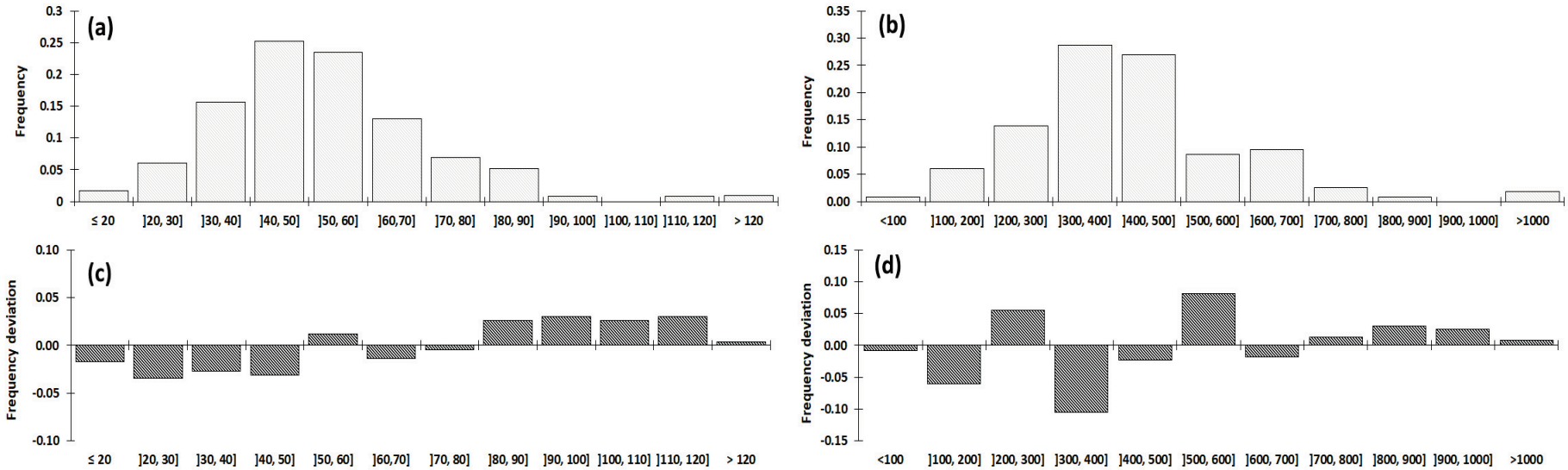

Fig. 3 Frequency distribution of root colonization (red fluorescence, expressed as arbitrary units [AU]) and *phl* expression data (green fluorescence) of *P. kilonensis* F113-*mCherry*(P*phl-egfp*) for wheat genotypes. The frequency distribution of modern wheat genotypes (n = 115) is shown for root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

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

Screening results were validated by alternative fluorescence methodology and confocal microscopy

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

Confocal microscopy observations confirmed spectrofluorimeter data, in that F113-stimulating genotypes showed more prevalent and larger F113 biofilms formed of cells expressing the red and/or the green fluorescence on roots, in comparison with non F113-stimulating genotypes (Supplementary Figure S3).

F113 colonized roots of all 10 selected genotypes in soil

In non-sterile soil, *P. kilonensis* F113 was recovered by quantitative PCR from inoculated wheat at 2.5×10^5 to 4.2×10^6 copies per root system (Supplementary Figure S4), whereas it was below the detection limit (3.5×10^4 copies per root system) in non-inoculated plants. The differences between (i) optimum and stress conditions, (ii) F113-stimulating and non F113-stimulating groups of genotypes, (iii) ancient and modern groups of genotypes, or (iv) individual wheat genotypes were not statistically significant. Similar findings were made when expressing results per g of dry rhizosphere soil (data not shown).

F113 inoculation improved growth of certain wheat genotypes

Three-factor ANOVA (**Supplementary Table S4**) indicated that F113 inoculation, overall, enhanced root volume (+13.9 %, $P < 0.01$), root diameter (+3.7 %, $P < 0.001$), root number (+13.5 %, $P < 0.001$) and dry root biomass (+12.4 %, $P < 0.001$), with a significant inoculation × genotype interaction for root number and dry root biomass.

Under optimum condition, the overall inoculation benefits for F113-stimulating vs non F113-stimulating genotypes were +30.8 ± 21.4 % vs +17.1 ± 29.1 % for dry root biomass, +20.6 ± 11.0 % vs +18.4 ± 12.2 % for root number, +17.9 ± 8.3 % vs +13.0 ± 11.8 % for root volume, and +3.9 ± 3.3 % vs +3.2 ± 1.8 % for

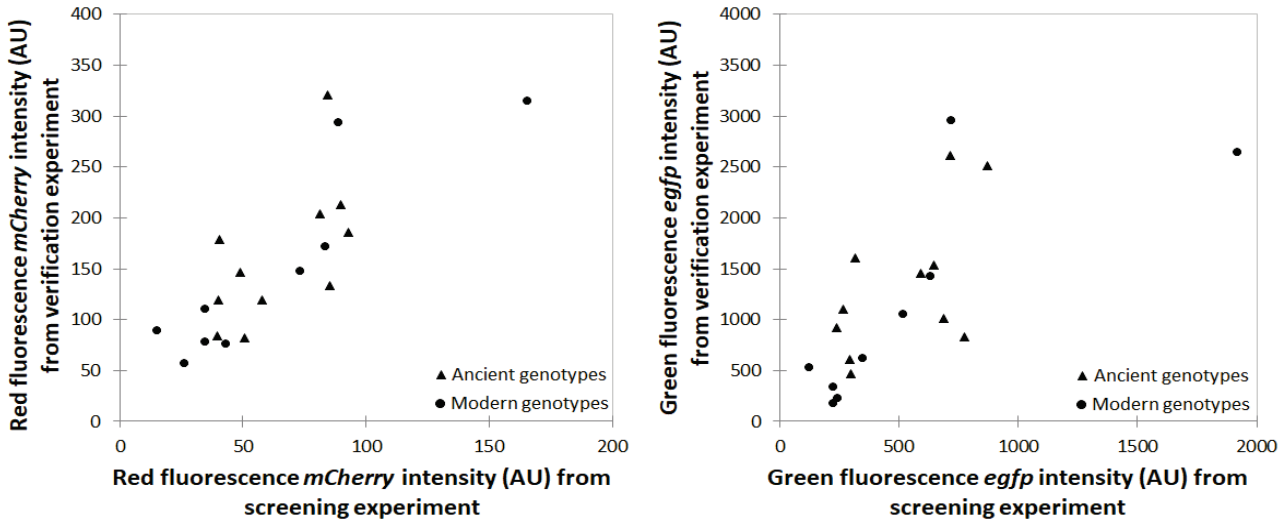

Fig. 4 Correlation analysis of root colonization data (red fluorescence, expressed as arbitrary units [AU]) (**a**) and *phl* expression data (green fluorescence) (**b**) of *P. kilonensis* F113-*mCherry*(P*phl*-*egfp*) obtained in the screening experiment (X axis) and the verification experiment (Y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

root diameter (Fig. 5a). Inoculation resulted in higher dry root biomass of F113-stimulating genotypes Coronation (+95.8%, $P < 0.01$) and D130-63 (+92.3%, $P < 0.001$) and non F113-stimulating genotype Odesskaya16 (+103.4%, $P < 0.001$) **(Supplementary Table S5**).

Under stress, the overall inoculation benefits for F113-stimulating vs non F113-stimulating genotypes were respectively +37.5 ± (SE) 24.2 % vs +3.7 ± 5.1 % for dry root biomass, +31.8 ± 27.3 % vs +12.1 ± 7.0 % for root number, +20.3 ± 18.1 % vs +12.6 ± 2.7 % for root volume, and +4.2 ± 2.4 % vs +2.8 ± 2.8 % for root diameter (Fig. 5b). Inoculation of F113-stimulating genotypes enhanced root volume for Coronation (+81.3%, $P < 0.05$), root diameter for Concurrent (+11.7%, $P < 0.01$), root number and root dry biomass for Coronation (respectively +102.3%, $P < 0.001$ and +84.6%, $P < 0.01$), D130-63 (+101.7%, $P < 0.001$ and +106.1%, $P < 0.001$) and ATUT-II (+67.8%, $P < 0.05$ and +79.3%, $P < 0.01$), but reduced root number (-50.4%, $P < 0.001$) and root dry biomass (-35.3%, $P < 0.05$) for Amifort, whereas inoculation of non F113-stimulating genotypes had no effect (**Supplementary Table S5**).

Impact of stress on non-inoculated wheat genotypes

As *P. kilonensis* F113 was below detection limit in non-inoculated pots (see above), a two-factor ANOVA was performed on the dataset from non-inoculated plants, on the basis that genotype response to stress integrated the possible contribution of resident plant-beneficial microorganisms (*i.e.* other than F113). Stress had a significant negative impact on every plant parameter except root length and root number, with a significant stress × genotype interaction for root diameter and dry root biomass (**Supplementary Table S6**).

Overall, the impact of stress on F113-stimulating vs non F113-stimulating genotypes on root parameters was respectively -22.8 ± (SE) 7.6 % vs -34.8 ± 2.6 % for root volume, -20.6 ± 5.2 % vs -34.8 ± 5.8 % for fresh root biomass and -1.5 ± 10.0 % vs -19.9 ± 19.3 % for dry root biomass, and -12 ± 2.8 % vs -10.8 ± 3.6 % for root diameter. For shoot parameters, it was -43.3 ± 4.0 % vs -40.1 ± 1.5 % for fresh shoot biomass, -36.1 ± 4.7 % vs -34.9 ± 2.2 % for dry shoot biomass and -15.2 ± 1.8 vs -14.1 ± 1.3 for shoot height (Fig. 5c). When considering the relative impact of stress, *i.e.* by computing the stress response index [(replicate performance under stress – mean performance under optimum condition) / mean performance under optimum condition], it appeared that the top five performing genotypes included 4 of 6 F113-stimulating genotypes and 1 of 4 non F113-stimulating genotypes for root length, root number, and dry root biomass, and 5 of 6 F113-stimulating genotypes for root volume.

At the scale of individual F113-stimulating genotypes, the impact of stress was significant on root volume (for ATUT-II), root diameter (for all but DI276), fresh shoot biomass and shoot height (for all but Coronation), and dry shoot biomass (for Concurrent, Amifort, ATUT-II and DI-276) (**Supplementary Table S7**). For individual non F113-stimulating genotypes, the effect of stress was significant on root volume, fresh and

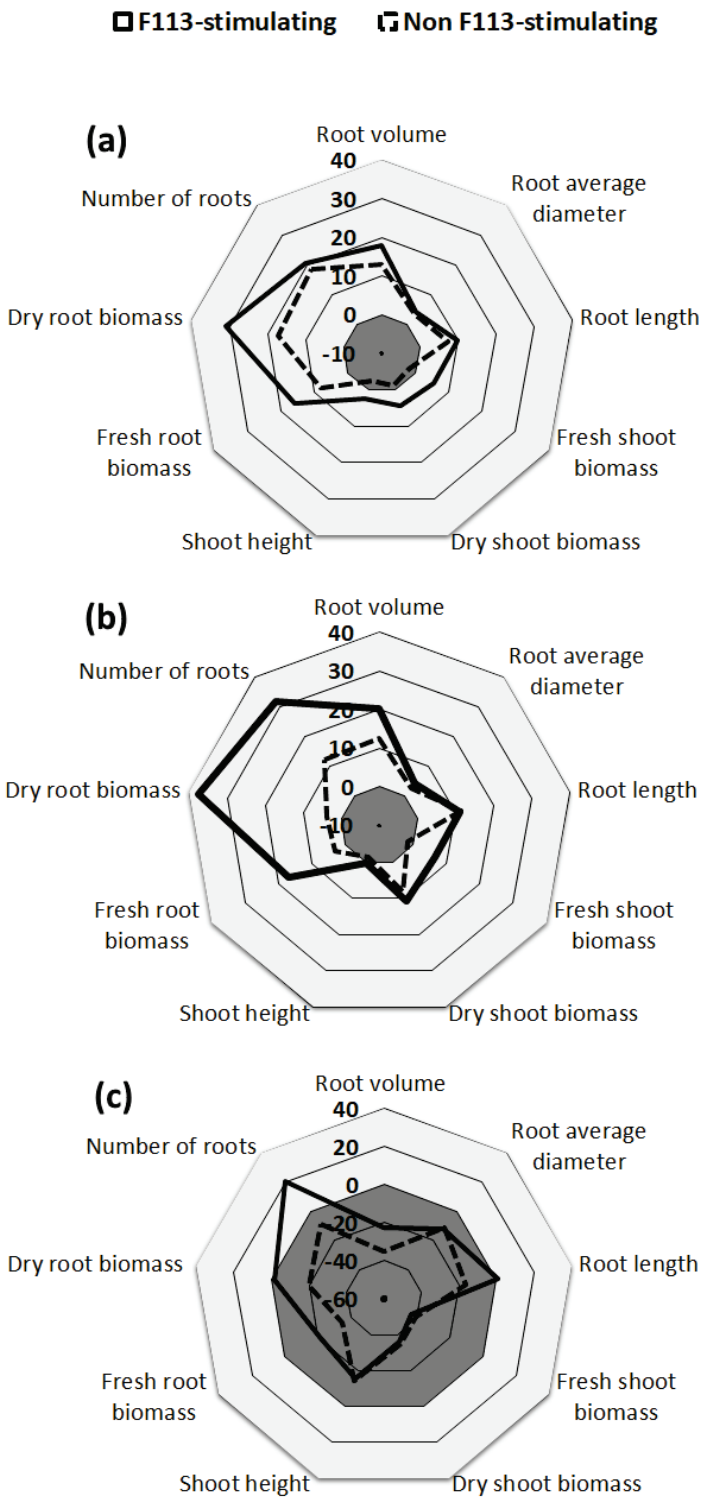

Fig. 5 Relative impact of seed inoculation with *P. kilonensis* F113 on the growth of six F113-stimulating wheat genotypes and four non F113-stimulating genotypes under optimum (**a**) or combined stress condition (**b**), and relative impact of stress on performance of non-inoculated plants (**c**). For each of the nine plant parameters investigated, the relative impacts were computed as (inoculated – non-inoculated)/non-inoculated [in **a,b**], and (stress – optimum)/optimum [in **c**]. The differences were not significant at $P < 0.05$.

dry root biomass (for Hendrix and Danubia), root diameter (for all but Danubia), fresh shoot biomass and shoot height (for all four genotypes), and dry shoot biomass (for Jaszaji TF and Hendrix).

Discussion

Crop breeding has resulted in the development of thousands of genotypes, which show contrasted agro-morphological and metabolic features [6, 48,49,50,51,52,53], as well as stress responses [54,55,56]. Crop genotypes can also differ in their ability to recruit soil bacteria [57], induce gene expression in rhizobacteria [30, 58], and respond to PGPR inoculation[10, 30]. The impact of breeding on PGPR-crop interactions is an important issue, but it has been little studied, and when so using a very small number of genotypes when considering the extent of crop diversity [16, 28, 29].

Here, we compared wheat genotypes resulting from modern breeding efforts (*i.e.* post-1960) with ancient wheat genotypes, based on their ability to interact with the model PGPR *P. kilonensi*s F113, which was a relevant approach since (i) this type of bacterium may occur naturally in various types of cultivated soils [36,37,38], and (ii) strain F113 can interact with different crop species and cultivars, but with differences from one crop cultivar to the next [30]. To cope with the extent of wheat diversity, almost 200 genotypes representative of this diversity were considered, which signifies an unprecedented scale for this type of work. To obtain robust screening results, we had to rely on a simplified experimental design, which proved effective to compare wheat genotypes (but which could not integrate the complexity of the rhizosphere ecosystem). It required the development of a novel methodology, based on the use of reporter gene fusions and the assessment of autofluorescent proteins. The results of the screening were validated for a subset of 20 genotypes, based both on an alternative fluorescence measurement protocol (and twice as many replicates) and direct confocal microscopy observations. Importantly, the approach that was followed enabled to monitor both colonization and gene expression of the inoculant on roots.

Screening results showed that root colonization by F113 was higher for ancient genotypes than modern ones. This type of finding was not made in the pot experiment, as the difference in F113 root colonization between the 10 wheat genotypes was not significant. This discrepancy may result from the contrasts in growth matrix (mineral soil vs agar), wheat development stage [59], and/or the presence of an indigenous microbiota in the soil [24, 60]. As for root colonization, *phl* expression was higher for ancient genotypes than modern ones. There was a strong correlation between root colonization and *phl* expression, and indeed the ratio between both (*i.e. phl* induction ratio) did not fluctuate as much between genotypes, pointing to a rather constitutive *phl* expression in F113 on most wheat genotypes, which is consistent with previous observations about the regulation of *phl* expression in (other) *Pseudomonas* strains [32, 61]. It might be explained by (i) ecological conditions on roots during the short duration of the screening assay, and/or (ii) the production by a majority of wheat genotypes of similar key exudate compounds controlling *phl* expression

[33, 62, 63]. Here again, however, higher *phl* induction rates (along with rather high root colonization) were more prevalent with ancient genotypes than modern ones.

In this work, the screening focused on bacterial root colonization and gene induction. To determine whether PGPR interaction ability could translate into enhanced plant performance, a greenhouse pot experiment was carried out with six F113-stimulating genotypes and four non F113-stimulating genotypes (as determined in the gnotobiotic screening). Contrasted phytostimulation effects between wheat genotypes may be expected based on previous studies on plant × bacteria interactions [28]. Indeed, inoculation with *P. kilonensis* F113 enhanced plant performance for 2 of 6 F113-stimulating genotypes and 1 of 4 non F113-stimulating genotypes under optimum condition, versus as many as 4 of 6 F113-stimulating genotypes but none of the 4 non F113-stimulating genotypes under stress (**Supplementary Table S5**). Differences in interaction specificity/affinity between plant genotypes and inoculated bacteria might entail differences in inoculant survival (which was not the case here) or inoculant effects on host transcriptional and metabolic profiles [64, 65]. It had been reported that DAPG could have phytotoxic effect on roots at high concentration [34, 66], but inoculation did not have any negative effect on the 10 genotypes in optimum condition. F113 inoculation may also trigger changes in the root microbial community [67], but it is unknown whether this impact could be plant genotype-dependent (although it is likely [68]). Here, in the absence of inoculation, the top performing genotypes in terms of stress response index included mainly F113-stimulating genotypes, which raises the possibility that other (resident) plant-beneficial microorganisms could have contributed to stress alleviation. In maize, cultivar PR37Y15 responded to various PGPR inoculants (as well as the PGPR *P. kilonensis* F113 and mycorrhizal fungi [35]), whereas cultivar DK315 did not respond [69].

Our results suggest that the search for high-yield wheat cultivars under agronomically- optimized condition, which caused genetic diversity loss [70,71,72], did not favor the maintenance of plant genes promoting PGPR interactions. Modern genotypes are mostly dwarf or semi-dwarf varieties because of the introduction of dwarfism genes *Rht* in 1960 [73, 74], and they may differ from ancient genotypes in terms of metabolic composition [50, 51, 75, 76] and root architecture [49, 51, 52, 77, 78], 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 with *P. kilonensis* F113, while (ii) interaction effectiveness had been maintained in a significant proportion of modern wheat genotypes. On one hand, breeding strategies may need to be reassessed to give further consideration to roots and below-ground processes [79], including the interactions with beneficial microorganisms [80, 81]. 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 interaction specificities of other types of PGPR as well as beneficial fungi.

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Conflict of interest

The authors declare that they have no conflict of interest

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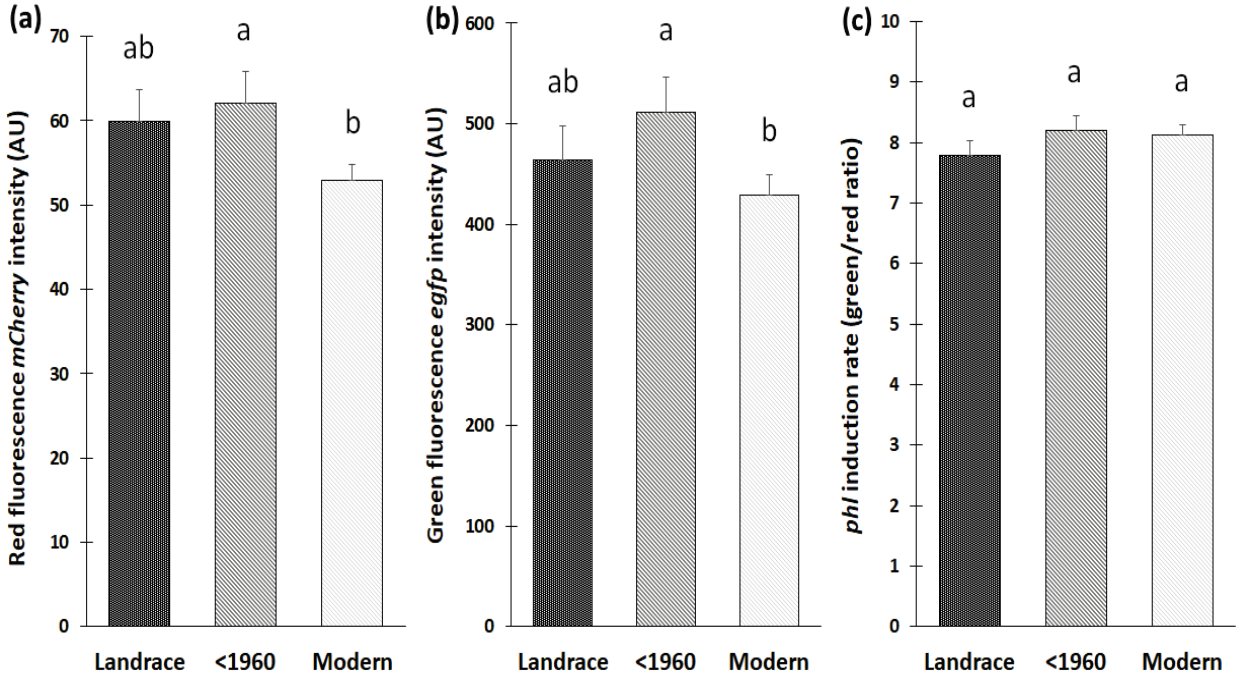
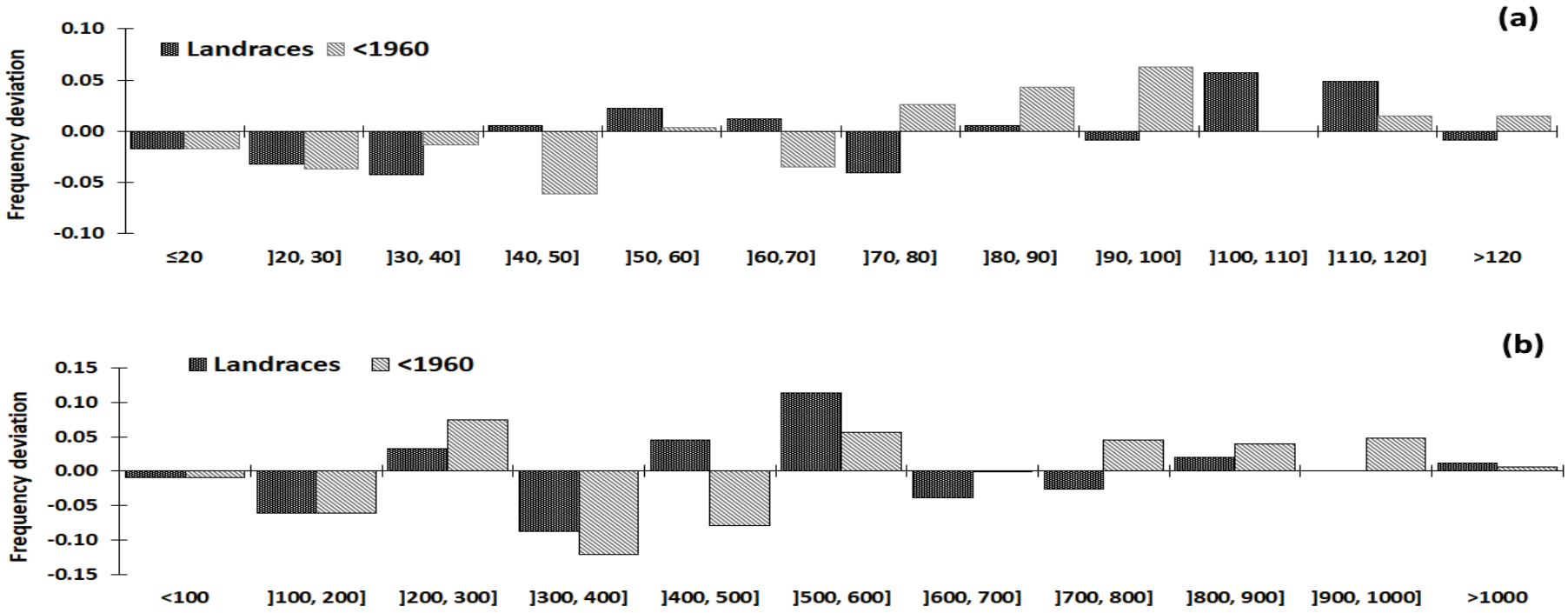
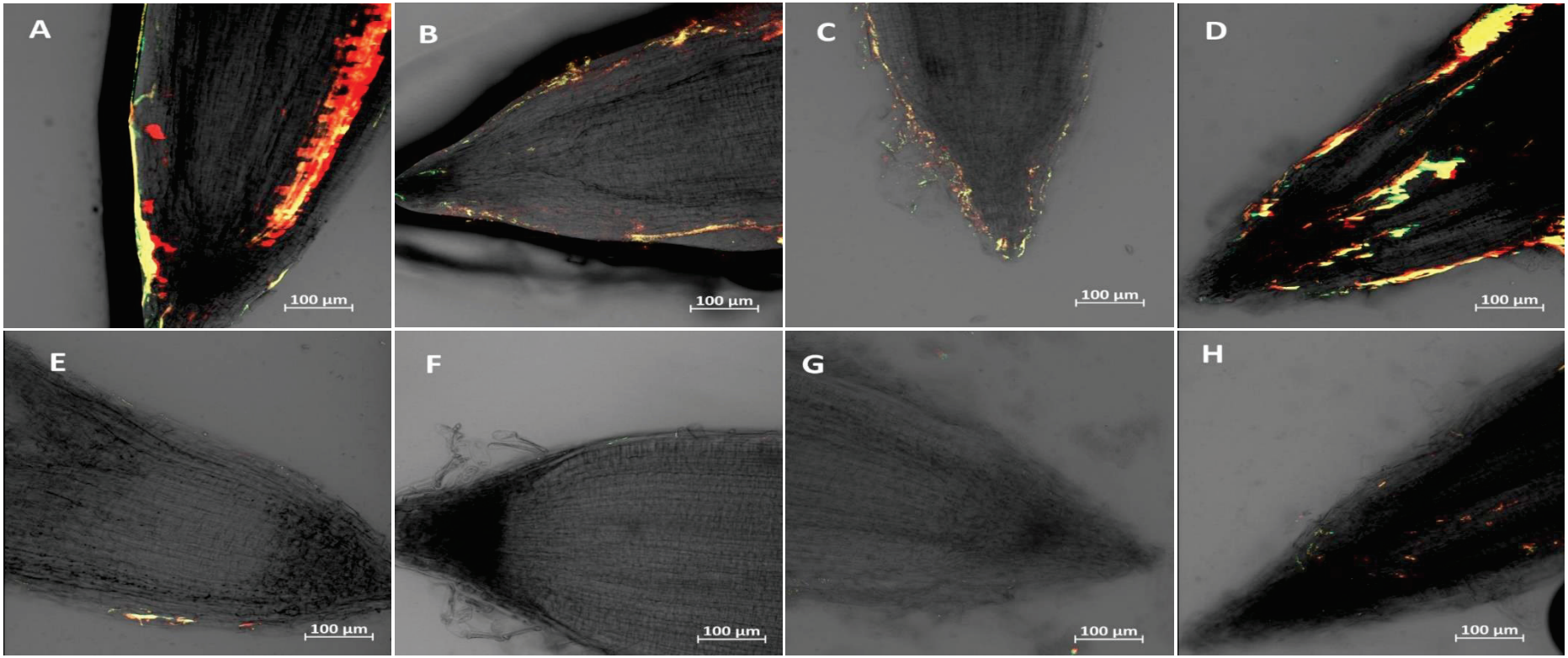
Supplementary Information



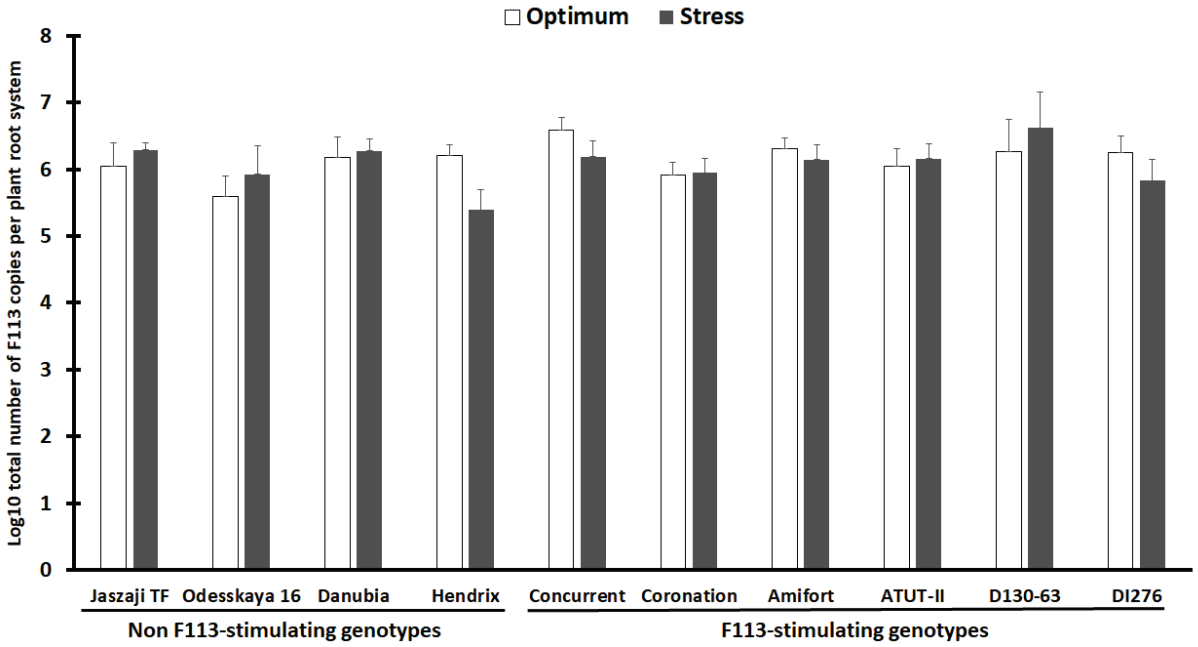
Supplementary Figure S1 Root colonization and *phl* expression of *P. kilonensis* F113-*mCherry*(P*phl*-*egfp*) for modern wheat genotypes (> 1960 ; n = 115) and ancient genotypes (which included old varieties [≤ 1960 ; n = 42] and landraces [n = 35]). Red fluorescence (root colonization) is shown (**a**), green fluorescence (*phl* expression) (**b**), and the *phl* induction rate (red fluorescence:green fluorescence ratio) (**c**). Fluorescence is expressed as arbitrary units (AU) and data are presented as means (computed from individual genotype data) with standard errors. Statistical differences between the three wheat categories are shown using letters a and b (Kruskal-Wallis tests, $P < 0.05$).



Supplementary Figure S2 Relative frequency distribution of root colonization (red fluorescence, expressed as arbitrary units [AU]) and *phl* expression data (green fluorescence) of *P. kilonensis* F113-*mCherry*(P*phl*-*egfp*) for ancient wheat genotypes, in relation to the data obtained for modern wheat genotypes (> 1960 ; n = 115). The deviation recorded for ancient genotypes, *i.e.* old varieties (≤ 1960 ; n = 42) and landraces (n = 35), was computed by subtracting the frequencies (number of genotypes in each of the fluorescence classes divided by the total number of genotypes) obtained for the 115 modern genotypes from the corresponding frequencies for the old varieties or landraces in each of the fluorescence classes, both for root colonization data (**a**) and *phl* expression data (**b**).



Supplementary Figure S3 Root colonization by *P. kilonensis* F113-mCherry(P*phl*-egfp) and *phl* expression at the surface of the apex of eight genotypes (selected from the 20 genotypes studied) used for the validation experiment. Photographs were taken using a confocal microscope with an excitation laser light of 488 nm and an emission filter of 504-555 nm for EGFP green fluorescence, and an excitation laser light of 561 nm and an emission filter 570-636 nm for mCherry red fluorescence. The same gains were used for all samples. Root-colonizing cells not expressing *phl* are red, root-colonizing cells with strong *phl* expression (but with insufficient gain for red fluorescence) appear in green, and root-colonizing cells expressing *phl* are yellow. Large numbers of mainly red or yellow cells were obtained for genotypes Blé de Redon (Landrace) (A), Coronation (≤ 1960) (B), Concurrent (≤ 1960) (C) and D130-63 (> 1960) (D), which had shown high red and green fluorescence values in the screening experiment, whereas fluorescent cells were sparse for genotypes Orlandi (≤ 1960) (E), Orfield (> 1960) (F), Hendrix (> 1960) (G) and Jaszaji TF (≤ 1960) (H), which had shown low red and green fluorescence values in the screening experiment.



Supplementary Figure S4 Root colonization by *P. kilonensis* F113 at one month after seed inoculation of six F113-stimulating and four non F113-stimulating wheat genotypes grown in non-sterile soil under greenhouse conditions. Plants were exposed to optimal conditions or combined stress conditions of low N and water availability. Quantitative PCR data were log-transformed and are presented as means with standard errors (n = 5). The effect of wheat genotype or optimal/stress conditions was not statistically different (Kruskal-Wallis test, $P < 0.05$).

Supplementary Table S1 List of the 192 wheat genotypes used in the study and their rankings in the screening experiment. These 192 genotypes correspond to the accessions that germinated among the 196 genotypes of the 196CC core-collection sub-sampled from the 372CC collection set up by Balfourier *et al.* [39], plus the two reference modern lines Hendrix and Skerzzo and the reference landrace Chinese Spring. The genotypes are listed according to their ERGE code in the French National Cereal Genetic Resources database. For each of the three rankings done in the screening experiment, the worst rank was 1 and the best rank 192. The total score was obtained by summing the three ranks.

ERGE Code	Genotype	Geographic origin	Genotype category	F113 colonization rank	*phl* expression rank	*phl* induction rate rank	Total score
7	(95-13*BEZOSTAIA)3-3	France	> 1960	157	157	119	433
19	CH01193	Switzerland	> 1960	26	91	191	308
92	11IWSWSN14	USA	> 1960	40	69	163	272
177	DI15	France	> 1960	158	166	145	469
234	DI182-9	France	> 1960	59	102	173	334
236	DI185	France	> 1960	6	19	160	185
338	DI276	France	> 1960	179	178	114	471
347	2838-39	Bulgaria	> 1960	25	29	59	113
386	DI330	France	> 1960	142	139	98	379
419	DI37-12-2	France	> 1960	170	182	164	516
421	3716-1	Bulgaria	> 1960	14	48	183	245
477	DI50-12	France	> 1960	56	76	130	262
524	60293-	The Netherlands	> 1960	134	116	57	307
537	CH62022	Switzerland	> 1960	116	119	146	381
546	664-258-18	Bulgaria	> 1960	68	65	79	212
748	A4	Afghanistan	> 1960	72	67	122	261
794	ADMONTER	Austria	≤ 1960	163	151	61	375
797	ADULAR	Germany	> 1960	2	1	4	7
833	AKADARUMA	Japan	≤ 1960	95	100	111	306
871	ALMA	France	≤ 1960	71	79	93	243
901	AMIFORT	France	> 1960	160	141	49	350
957	ARAWA	Australia or New Zealand	≤ 1960	64	64	91	219
983	ARGENT	UK or Ireland	> 1960	92	80	68	240
1005	ARKAS	Germany	> 1960	144	118	54	316
1032	ARROMANCHES	France	> 1960	80	98	148	326
1044	ARTOIS-DESPREZ	France	≤ 1960	66	89	141	296
1080	ATUT II	Austria	> 1960	172	164	76	412
1182	BAIONETTE I	Italy	≤ 1960	182	86	2	270
1192	BALKAN	Croatia	> 1960	153	124	45	322
1232	BARBU DU FINISTERE	France	Landrace	131	121	100	352
1236	BARBU DU TRONCHET	France	Landrace	44	32	36	112

1281	BEL ET BON	France	≤ 1960	37	62	151	250
1288	BELLIEI 590	Hungary	≤ 1960	22	18	52	92
1321	BENNI	USA	> 1960	128	126	103	357
1332	BERZATACA	Finland	> 1960	81	109	125	315
1357	BIRGITTA	Sweden	> 1960	152	140	44	336
1400	BLANC PRECOCE	Switzerland	Landrace	39	24	18	81
1402	BLASON	France	> 1960	67	75	129	271
1417	BLE D'OR	France	≤ 1960	54	56	120	230
1429	BLE DE HAIE	France	Landrace	111	131	136	378
1446	BLE DE MARAT BARBU	France	Landrace	154	132	51	337
1498	BLE DU ROUSSILLON	France	Landrace	62	50	43	155
1529	BLONDYNKA	Poland	≤ 1960	189	190	175	554
1531	BLUEBOY	USA	> 1960	19	12	25	56
1747	114/62	Austria	> 1960	156	149	77	382
1768	CANDEAL DE AREVALO	Spain	Landrace	178	154	30	362
1885	CENAD 512	Romania	≤ 1960	122	175	187	484
1899	CEREALOR	France	> 1960	138	99	38	275
1957	CF3003-2-7-4-4-3	France	> 1960	10	3	3	16
1974	CF4563-1-5-3-2-5	France	> 1960	51	17	17	85
2025	CH73052	Switzerland	> 1960	114	106	157	377
2135	CHINESE SPRING	China	Landrace	102	125	140	367
2145	CHITLANG	Nepal	Landrace	149	46	6	201
2153	CHORTANDINKA	Central Asia	> 1960	84	35	9	128
2169	CHYAKSILA EPI NON VELU	Nepal	Landrace	21	22	7	50
2308	COMPTON	USA	> 1960	117	137	138	392
2345	CORSODOR	France	> 1960	32	57	147	236
2364	CP4	France	> 1960	30	101	178	309
2399	D130-63	Poland	> 1960	192	192	186	570
2424	DANUBIA	Czech Republic	> 1960	24	61	171	256
2438	DAVIDOC	France	> 1960	94	160	185	439
2475	DETENICKA CERVENA	Czech Republic	Landrace	57	81	127	265
2489	DI6402-34-2-4	France	> 1960	133	127	83	343
2491	DI6404-19-15	France	> 1960	12	72	190	274
2507	DI7003-1-12	France	> 1960	177	162	94	433
2508	DI7005-113-3	France	> 1960	53	2	1	56
2526	DI7202-103	France	> 1960	99	63	32	194

2534	DI7210-15-11	France	> 1960	161	171	135	467
2536	DI7215-100	France	> 1960	13	5	8	26
2573	DIANA	Poland	> 1960	90	95	90	275
2574	DIANA II	Czech Republic	> 1960	96	66	31	193
2606	DNEPROVSKAIA	Ukraine	> 1960	91	78	62	231
2626	DONG-FANG-HONG-NO3	China	> 1960	50	117	188	355
2644	DRAGON-FRA	France	> 1960	109	112	116	337
2650	DRAVA	Croatia	> 1960	106	103	86	295
2802	ESPOIR	France	≤ 1960	181	167	46	394
2991	FERRUGINEUM	Russia	≤ 1960	119	114	88	321
3050	FLAMURA 85	Romania	> 1960	101	120	137	358
3070	FLINT	USA	Landrace	58	42	40	140
3278	GELPA	France	> 1960	7	16	117	140
3299	GH126	France	> 1960	121	84	34	239
3342	GK SZOKE	Hungary	> 1960	171	185	180	536
3366	GODOLLOI 15	Hungary	≤ 1960	103	93	78	274
3406	GRANIT	Russia	> 1960	126	144	150	420
3414	GRENIER	France	> 1960	74	70	80	224
3485	H93-70	Spain	> 1960	70	113	172	355
3617	HIVERNAL	France	> 1960	43	7	11	61
3753	IAS 1	Brazil	≤ 1960	93	82	84	259
3896	JANGO	France	≤ 1960	136	153	158	447
3912	JASZSAGI TF	Hungary	≤ 1960	88	37	19	144
3970	JUFY II	Belgium	≤ 1960	104	104	105	313
3991	K1898-9/L200-6	Bulgaria	> 1960	105	51	23	179
4036	KATYIL	Australia or New Zealand	> 1960	159	152	99	410
4105	KID	France	> 1960	31	173	192	396
4111	KIRAC 66	Turkey	> 1960	35	33	109	177
4157	KOLBEN 3	Sweden	Landrace	77	92	132	301
4187	KRAKA	Norway or Denmark	> 1960	143	136	82	361
4194	KRELOF 3	France	≤ 1960	69	115	153	337
4300	LESZYNSKA WCZESNA	Poland	≤ 1960	124	138	139	401
4324	LITTLE CLUB	USA	Landrace	89	146	176	411
4343	LONTOI	Finland	> 1960	100	71	29	200
4525	MALGORZATKA UDYCKA	Poland	≤ 1960	49	21	15	85
4664	MASTER	UK or Ireland	> 1960	5	9	65	79

4670	MATRADERECSKEITF	Hungary	> 1960	130	43	10	183
4838	MINTURK	USA	≤ 1960	166	184	181	531
4947	MOTTIN	France	Landrace	129	111	67	307
4991	MV MA	Hungary	> 1960	98	85	53	236
5293	NOUGAT	France	> 1960	79	36	20	135
5401	NZ(81)P43	Australia or New Zealand	> 1960	115	107	113	335
5421	ODESSA EXPSTA20722	Portugal	> 1960	146	87	16	249
5438	ODESSKAYA 16	Ukraine	≤ 1960	83	20	5	108
5448	OGOSTA	Bulgaria	> 1960	190	189	156	535
5501	ORLANDI	Italy	≤ 1960	38	26	27	91
5536	OULIANOWSKA	Russia	> 1960	46	41	47	134
5552	P. DE BROLLON	Spain	Landrace	185	150	14	349
5558	P4523-80	Austria	> 1960	28	52	104	184
5773	POILU DU TARN	France	≤ 1960	36	39	81	156
6027	RECITAL	France	> 1960	4	14	184	202
6086	RENAN	France	> 1960	65	60	70	195
6191	RINGOT 2	France	≤ 1960	17	28	162	207
6308	ROUGE D'ALTKIRCH	France	Landrace	48	27	22	97
6318	ROUGE DE MARCHISSY	Switzerland	Landrace	186	169	56	411
6529	SEU SEUN 27	China	≤ 1960	8	25	159	192
6740	STRUBES DICKKOPF	Germany	≤ 1960	176	187	165	528
6922	TF6	France	≤ 1960	145	147	107	399
6986	TOM THUMB	USA	> 1960	11	8	41	60
7011	TOUZELLE-BLANCHE-BARBUE	France	Landrace	141	129	71	341
7085	TURDA 81-77	Romania	> 1960	20	10	12	42
7092	TYLER	USA	> 1960	155	168	170	493
7117	US(59)34	USA	> 1960	125	163	179	467
7279	VALDOR	France	≤ 1960	29	38	106	173
7490	VPM V1-1-2-4R2-3-8-3-2	France	> 1960	15	6	13	34
7585	WATTINES	France	> 1960	76	96	128	300
7848	RONGOTEA	Australia or New Zealand	> 1960	3	15	182	200
7968	BLE DANOIS	France	Landrace	150	143	89	382
7973	BORDEAUX 113	France	Landrace	188	191	189	568
7988	CREPIN A	France	≤ 1960	184	179	60	423
8011	INSTITUT 1802	France	≤ 1960	167	159	75	401
8048	RALET	France	Landrace	127	105	73	305

8051	BLE BARBU DE MUROL	France	Landrace	187	183	74	444
8058	ZANDA	Belgium	≤ 1960	107	130	123	360
8073	CORONATION	Canada	≤ 1960	174	186	174	534
8079	KITCHENER	Canada	≤ 1960	118	108	85	311
8097	STANLEY	Canada	≤ 1960	139	161	168	468
8165	NAVARRO150	Spain	> 1960	82	110	144	336
8170	WS-13 CARDENO 34/45	Spain	> 1960	151	133	55	339
8194	NEELKANT	Syria	> 1960	173	180	155	508
8197	SANUNU	Syria	> 1960	148	170	167	485
8227	NISHIKAZE KOMUGI	Japan	> 1960	73	122	169	364
8254	CADENZA	France	> 1960	135	135	112	382
8276	CARIBO	Germany	> 1960	183	172	48	403
8287	DC147U	France	> 1960	27	11	21	59
8289	TM7MB1-1	France	> 1960	123	123	108	354
9024	GENESIS	France	> 1960	75	83	110	268
9077	NON PLUS EXTRA	Austria	Landrace	164	176	142	482
9087	PRINCE LEOPOLD	Belgium	≤ 1960	180	177	92	449
13210	SOLARIS	Czech Republic	> 1960	168	155	72	395
13282	ANATOLIE2	France	≤ 1960	16	31	131	178
13292	CONCURRENT	France	≤ 1960	175	181	152	508
13310	FRUH-WEIZEN	Germany	Landrace	18	30	126	174
13436	FONDARD CRESPIN	France	≤ 1960	191	188	35	414
13445	VOLT	Hungary	> 1960	47	34	42	123
13461	BEHERT	France	> 1960	97	68	33	198
13471	ORNICAR	France	> 1960	45	47	39	131
13481	APACHE	France	> 1960	87	77	64	228
13494	BELLOVAC	France	> 1960	140	145	133	418
13500	ORFIELD	France	> 1960	23	13	28	64
13502	PALIO	France	> 1960	55	73	124	252
13792	CENTURK	USA	> 1960	132	74	26	232
13870	TALISMAN	France	> 1960	108	148	166	422
14000	ROKYCANSKA SAMETKA	Czech Republic	Landrace	147	142	95	384
14011	HANA	Czech Republic	> 1960	110	97	66	273
15606	BLE DE REDON BLANC BARBU 1 1	France	Landrace	113	156	177	446
15658	BLE DE REDON BLANC 1/2 LACHE 1 1	France	Landrace	85	88	115	288
15710	BLE DE REDON GLUMES VELUES 1	France	Landrace	169	158	58	385

15950	AS68VM4-3-2/TJB636 13	France	> 1960	52	58	101	211
15954	ASVM4/BEAUCHAMP 81B13	France	> 1960	63	55	63	181
20074	MIRLEBEN	Ukraine	> 1960	60	59	97	216
20224	FANTASIYA-ODESSKAYA	Ukraine	> 1960	162	174	161	497
20276	EQUINOX	UK or Ireland	> 1960	41	53	69	163
20366	SKERZZO	France	> 1960	33	40	102	175
20384	DI9234-11-15	France	> 1960	120	90	50	260
24031	KRASNAYA	Canada	Landrace	112	134	143	389
24058	SARI-BUGDA	Caucasia	Landrace	34	54	134	222
24066	CROISEMENT 268	Switzerland	≤ 1960	42	44	96	182
24075	SPIN, 121-VAR.12/536	Pakistan	≤ 1960	165	165	118	448
24089	TAU-BUGDA	Caucasia	Landrace	9	23	121	153
24108	ALBIDUM 12	Russia	> 1960	137	128	87	352
24193	LANDRACE	Caucasia	Landrace	61	94	154	309
24196	ARABUGDASI	Caucasia	Landrace	86	45	24	155
24210	LAMMAS	UK or Ireland	Landrace	78	49	37	164
28978	HENDRIX	France	> 1960	1	4	149	154

Supplementary Table S2 The 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 10 genotypes in bold were used for the greenhouse experiment. The total score was the sum of the three genotype rankings 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

Supplementary Table S3 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 genotypes, *i.e.* landraces (n = 35) and old varieties (n = 42), and the modern genotypes (n = 115).

	Landraces	Old varieties (≤ 1960)	Modern genotypes (> 1960)
Root colonization by F113			
25 best genotypes	17.1 %	21.4 %	8.7 %
50 best genotypes	31.4 %	33.3 %	21.7 %
50 worst genotypes	20.0 %	21.4 %	29.6 %
25 worst genotypes	8.6 %	7.1 %	16.5 %
***phl* expression in F113**			
25 best genotypes	11.4 %	21.4 %	10.4 %
50 best genotypes	28.6 %	40.5 %	20 %
50 worst genotypes	31.4 %	23.8 %	25.2 %
25 worst genotypes	8.6 %	7.1 %	16.5 %

Supplementary Table S4 Three-way ANOVA results performed on the raw values of the whole dataset of the greenhouse pot experiment for the nine plant parameters investigated.

		Root volume (cm^3)	Fresh root biomass (mg)	Dry root biomass (mg)	Number of roots	Root average diameter (mm)	Root length (cm)	Fresh shoot biomass (mg)	Dry shoot biomass (mg)	Shoot height (cm)
R^2		0.365	0.433	0.548	0.356	0.564	0.317	0.672	0.565	0.765
F		3.357	4.462	7.089	3.233	7.566	2.718	11.953	7.699	19.011
Pr > F		< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Stress	F	41.039	45.524	19.699	2.215	173.427	2.288	369.602	154.220	302.751
	P-value	< 0.0001	< 0.0001	< 0.0001	0.138	< 0.0001	0.132	< 0.0001	< 0.0001	< 0.0001
Inoculation	F	7.712	2.261	10.029	8.730	13.618	2.305	0.627	1.878	0.006
	P-value	0.005	0.134	0.002	0.003	0.000	0.130	0.429	0.173	0.941
Genotype	F	4.610	6.561	11.053	3.317	5.230	4.919	6.388	5.320	44.794
	P-value	< 0.0001	< 0.0001	< 0.0001	0.001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Stress x Inoculation	F	0.159	0.000	0.153	0.042	0.219	0.019	0.196	1.057	0.012
	P-value	0.691	0.987	0.696	0.837	0.641	0.890	0.658	0.305	0.911
Stress x Genotype	F	1.968	2.050	4.064	3.374	3.334	2.330	1.537	1.378	2.104
	P-value	0.044	0.035	< 0.0001	0.001	0.001	0.016	0.136	0.226	0.030
Inoculation x Genotype	F	1.450	4.374	10.527	4.158	1.363	2.924	0.668	0.813	1.133
	P-value	0.168	< 0.0001	< 0.0001	< 0.0001	0.206	0.003	0.738	0.562	0.340
Stress x Inoculation x Genotype	F	0.997	0.924	1.369	1.881	1.474	1.090	0.997	1.088	0.725
	P-value	0.444	0.505	0.203	0.056	0.158	0.371	0.443	0.372	0.686

Supplementary Table S5 Relative impact of seed inoculation with *P. kilonensis* F113 on the growth of ten wheat genotypes under optimum or combined stress conditions. Concurrent, Coronation, Amifort, ATUT-II, D130-63 and DI276 are F113-stimulating genotypes, 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%
	ATUT-II	-4.8%	-0.4%	16.9%	26.1%	-8.8%	20.2%	1.2%	-1.8%	3.0%
	D130-63	50.6%	119.8%	**92.3% *****	41.9%	-1.4%	46.1%	18.4%	ND	4.6%
	DI276	19.7%	-17.0%	-20.7%	-13.9%	13.8%	-6.9%	-7.2%	-4.8%	-2.1%
	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%
	ATUT-II	45.1%	49.9%	**84.6% ****	**67.8% ***	2.1%	37.0%	19.1%	15.7%	0.3%
	D130-63	46.3%	47.3%	**106.1% *****	**101.7% *****	6.8%	30.5%	-4.8%	ND	-2.7%
	DI276	-16.7%	-28.6%	-6.7%	-17.2%	7.8%	-26.6%	9.1%	10.8%	-1.2%
	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%

Supplementary Table S6 Two-way ANOVA results performed on the raw values for non-inoculated plants of the greenhouse pot experiment for the nine plant parameters investigated.

		Root volume (cm^3)	Fresh root biomass (mg)	Dry root biomass (mg)	Number of roots	Root average diameter (mm)	Root length (cm)	Fresh shoot biomass (mg)	Dry shoot biomass (mg)	Shoot height (cm)
R^2		0.314	0.442	0.566	0.380	0.579	0.358	0.667	0.598	0.760
F		2.816	4.874	8.034	3.779	8.460	3.430	12.337	6.447	19.489
Pr > F		0.0003	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Genotype	F	3.266	6.772	12.700	6.171	4.823	5.828	3.885	4.251	23.964
	P-value	0.001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.000	0.001	< 0.0001
Stress	F	17.254	23.227	8.031	1.586	92.271	0.982	189.782	89.162	154.143
	P-value	< 0.0001	< 0.0001	0.005	0.210	< 0.0001	0.324	< 0.0001	< 0.0001	< 0.0001
Genotype x Stress	F	0.735	0.872	3.238	1.629	2.889	1.291	1.416	1.718	1.188
	P-value	0.676	0.552	0.002	0.115	0.004	0.249	0.189	0.127	0.309

Supplementary Table S7 Relative impact of combined stress on the growth of ten non-inoculated wheat genotypes. Concurrent, Coronation, Amifort, ATUT-II, D130-63 and DI276 are F113-stimulating genotypes, 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 (stress – optimum)/optimum. For each genotype, significant differences between stressed 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
Concurrent	-39.3%	-18.0%	-0.4%	14.9%	**-11.3% *****	-5.1%	**-46.5% *****	**-36.4% *****	**-16.6% *****
Coronation	-12.1%	-7.0%	20.8%	57.4%	**-8.2% ***	6.3%	-26.1%	-17.3%	-6.5%
Amifort	-10.4%	-29.7%	30.8%	55.2%	**-8.9% ***	6.9%	**-50.4% *****	**-43.7% *****	**-19.6% *****
ATUT-II	**-53.4% ****	-40.6%	-32.5%	-8.0%	**-25.5% *****	-9.5%	**-52.8% *****	**-46.7% *****	**-15.2% *****
D130-63	-8.4%	-19.4%	-23.6%	7.3%	**-11.3% ****	8.2%	**-37.8% *****	ND	**-16.7% *****
DI276	-13.4%	-9.1%	-4.1%	-3.8%	-6.8%	-1.1%	**-46.4% *****	**-36.5% *****	**-16.7% *****
Jaszaji TF	-30.0%	-34.3%	-23.5%	-16.3%	**-11.0% ****	-12.9%	**-41.5% *****	**-37.9% *****	**-16.0% *****
Odesskaya 16	-30.9%	-18.6%	35.9%	12.4%	**-20.1% *****	12.7%	**-38.1% *****	ND	**-16.2% *****
Danubia	**-38.9% ***	**-44.4% ****	**-47.9% *****	4.8%	-3.0%	-34.9%	**-44.9% *****	ND	**-13.5% *****
Hendrix	**-39.5% ***	**-42.0% ***	**-44.1% ****	-34.8%	**-9.2% ***	-29.8%	**-39.2% *****	**-31.8% *****	**-10.6% ****

Partie 3

Symbiotic variations among wheat genotypes and detection of quantitative trait loci for interaction with two contrasted proteobacterial PGPR strains

Preamble

In the previous experimental chapter, we observed a broad range of effects of wheat genotypes on F113 colonization and *phl* expression, and a significant difference between ancient and modern genotypes, the latter being overall less efficient to interact with F113. Furthermore, we measured a better impact of F113 inoculation on plant performance of wheat genotypes able to efficiently stimulate F113 than non F113-stimulating wheat genotypes. All in all, we suggested a negative impact of modern breeding on the interaction of wheat with F113, which could be related to morphologic or physiologic shifts. However, it is known that there is a specificity of interaction between the host plant and PGPR strain [1–3], and it can be expected that different results could be obtained when implementing the screening of the 199 accessions with another PGPR strain. Thus, to conclude overall that modern breeding has had a negative impact on the interaction between wheat and PGPR, it is relevant to use another PGPR with other characteristics and plant-beneficial functions than F113 to assess its interaction with wheat genotypes selected from the 19th to nowadays.

We chose the PGPR *Azospirillum brasilense* Sp245, which belongs to another subdivision of Proteobacteria than F113 (alpha-Proteobacteria for Sp245 *vs* gamma-Proteobacteria for F113) and have auxinic effect on the roots of its host plant thanks to its *ppdC* gene [4]. However, during a preliminary experiment, I observed that despite being fused to a strong promoter P*tac* the use of *egfp/cfp/yfp* genes coding for fluorescent proteins was not sensitive enough to discriminate genotypes because of a low differential between fluorescences due to Sp245 and to the root autofluorescence. Thus, I changed of reporter gene and used the *gus* reporter gene system [5], which displays higher sensitivity thanks to the high fluorescence intensity emitted by the product of the hydrolysis of 4-methylumbelliferyl-β-D-glucuronide by the GUS activity and the weak autofluorescence from roots at the wavelength used to assess sample fluorescence. We also changed the inoculation condition *in vitro,* and used glass tubes rather than Petri dish to grow wheat plantlets in presence of Sp245, because of the microaerophilic lifestyle of this PGPR, and also because originally we wanted also to assess the expression of the *nifH* gene (involved in nitrogen fixation), which was unsuccessful. Therefore, using this novel procedure we were then able to assess Sp245 colonization on wheat genotypes, and measure the expression of the *ppdC* gene.

Also, the performance under greenhouse of the same 10 genotypes used in the precedent part of this manuscript for F113, which have been chosen because of their contrasted results during the screening with both PGPR, were measured after an inoculation with Sp245. Therefore, I searched whether modern genotypes also show lower abilities to interact with Sp245 than the ancient genotypes as observed with F113, and determined the proportion of genotypes able to greatly interact with both PGPR or with only one of the two, after having performing all the statistical analyses.

Thanks to the two screening results with Sp245 and F113, wheat genetic regions that may be involved in the interactions between plants and PGPR were then searched by the team of Jacques Le Gouis (GDEC) by

implementing a Genome-Wide Association Study. The latter approach has been made in the purpose of potentially improving breeding methods in future, by identifying genetic markers that might be involved in the efficient interaction of plant with bacteria having positive effects on plant growth.

Results of this work are presented in a manuscript that is in preparation.

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Symbiotic variations among wheat genotypes and detection of quantitative trait loci for interaction with two contrasted proteobacterial PGPR strains

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Running title: Wheat genotypes and PGPR interaction

Abstract

Crop varieties differ in their ability to interact with Plant Growth-Promoting Rhizobacteria (PGPR). Recently, the screening of 198 bread wheat accessions showed that root colonization by the PGPR *Pseudomonas kilonensis* F113 and expression of 2,4-diacetylphloroglucinol genes *phl* were higher on ancient than on modern genotypes, and the accessions most effective at PGPR interaction were identified (Valente *et al. submitted*). This data can be used to search Quantitative Trait Loci (QTL) for PGPR interaction ability, but whether the same wheat accessions would be the most effective with all types of PGPR remains to be shown. To address these issues, first the same screening was carried out with a very different type of PGPR. It confirmed that ancient genotypes were more effective overall than modern genotypes for root colonization by *Azospirillum brasilense* Sp245 and expression of the phenylpyruvate decarboxylase gene *ppdC* (for synthesis of the auxin indole-3-acetic acid), but the most effective accessions were not the same as for *P. kilonensis* F113. Second, in non-sterile soil under nitrogen/drought stress conditions, *A. brasilense* Sp245 improved wheat performance for 3 of 6 PGPR-stimulating genotypes and none of the 4 non PGPR-stimulating genotypes, but phytostimulation results differed from those of *P. kilonensis* F113. Third, a genome-wide association approach was implemented to identify genomic regions potentially implicated in the interaction of wheat with *A. brasilense* Sp245. While no region was involved in root colonization, 21 regions spread on 12 wheat chromosomes were identified for *ppdC* expression and *ppdC* induction rate. The molecular markers provide the possibility to increase the frequency of favorable alleles and improve the capacity of modern genotypes to interact with Sp245 and perhaps other *A. brasilense* strains also.

Introduction

Symbiotic interactions with Plant Growth-Promoting Rhizobacteria (PGPR) are important for plant growth and health [1, 2]. These PGPR, especially from the genera *Azospirillum*, *Pseudomonas*, *Herbaspirillum* or *Bacillus*, benefit plants via biological nitrogen fixation, phosphorus solubilization, production of phytohormones or antimicrobial compounds, and/or by eliciting systemic resistance pathways [3–5].

Plant response to PGPR inoculation can vary depending on environmental/agronomic conditions, PGPR features and plant host genotype [6–9]. For the latter, differences may be expected between plant species but also plant varieties within species, in relation to particular root system architectures, root surface properties and rhizodeposition (root exudation) patterns, which can impact root colonization by PGPR and their gene expression patterns [10–15]. Thus, many studies have shown the effect of plant genotype on bacterial colonization of roots and the composition of the rhizosphere bacterial community, including for bacterial taxa known to include PGPR strains [16–20]. A few studies have also evidenced differential bacterial gene expression according to plant host genotype, notably genes involved in plant-beneficial functions such as *acdS* or *nifH* [9, 21, 22].

In the case of crops, key events determining current variety properties include domestication [23–26] and in the second half of last century the advent of modern breeding, which typically aims at developing high-yield cultivars able to value farming inputs, *i.e.* under conditions close to agronomic optimum [27–29]. Modern cultivars may differ from ancient plant genotypes in terms of root exudation because of differences in physiology and metabolic composition of plant tissues [30–32], root architecture [32–34] and disease resistance [35–37], which might lead to particular rhizobacterial community composition [17, 20, 38–41]. Indeed, interaction analysis of 198 bread wheat accessions with the 2,4-diacetylphloroglucinol (DAPG) producing PGPR *Pseudomonas kilonensis* F113 showed that the abilities for PGPR root colonization and expression of the DAPG genes *phl* on roots were higher overall on ancient wheat genotypes than modern genotypes, even though the capacity for PGPR interaction was maintained in certain modern cultivars [42]. We hypothesize that this type of approach could be relevant to implement a genome-wide association study to identify relevant Quantitative Trait Loci (QTL) corresponding to PGPR interaction ability, as implemented to detect for example wheat genomic regions involved in nitrogen use efficiency or *Fusarium* head blight resistance [43, 44]. However, *P. kilonensis* F113 is a particular type of PGPR in terms of taxonomy (γ-Proteobacteria), bioactive metabolites produced (siderophore, hydrogen cyanide and DAPG) and key enzymatic functions (ACC deaminase), as well as resulting plant-beneficial effects (phytoprotection from pathogens and auxinic phytostimulation) [9, 45, 46]. Whether similar interaction abilities would be observed with a very different type of PGPR, such as the nitrogen-fixing, auxin-producing α-proteobacterium *Azospirillum brasilense* Sp245, is unknown.

The objective of the present study was to assess whether the model PGPR *A. brasilense* Sp245 interacted with the same range of wheat genotypes than *P. kilonensis* F113, especially when considering modern vs ancient wheat genotypes, and to implement a genome-wide association approach to explore genomic fragments potentially implicated in wheat-PGPR interactions. To this end, a collection of 198 accessions of bread wheat and a gnotobiotic screening protocol derived from Valente *et al.* [42] were used to quantify root colonization by *A. brasilense* Sp245 and expression of the phenylpyruvate decarboxylase gene *ppdC* (for synthesis of the auxin indole-3-acetic acid ; [47]) by the PGPR on roots, based on fluorimetric monitoring of the reporter gene *gusA*. A greenhouse experiment was then conducted, using a selection of wheat genotypes stimulating or not Sp245 in the screening, to compare their ability to benefit from Sp245 (as well as F113) inoculation in soil under optimum or stress condition. Finally, a genome-wide association study was performed to identify wheat chromosome regions shared by accessions interacting well with *A. brasilense* Sp245.

Material and Methods

Plant material and bacterial strains

This study was carried out using the collection of 199 bread wheat (*Triticum aestivum*) accessions described in Valente *et al.* [42], which includes 35 landraces, 43 old varieties (≤ 1960) and 121 modern genotypes (> 1960). However, 12 genotypes showed poor germination and the analysis was done with 33 landraces, 40 old varieties (≤ 1960) and 114 modern genotypes (> 1960) (**Supplementary Table S1**).

For the *in vitro* screening experiment, two plasmidic derivatives of *Azospirillum brasilense* Sp245 (kindly provided by the Centre of Microbial and Plant Genetics, University of Leuven, Belgium) were used. The first derivative Sp245(pFAJ31.2) contains a plasmidic fusion between an un-characterized constitute promoter and the reporter gene *gusA*, and the second derivative Sp245(pFAJ64) a fusion between the *ppdC* promoter and *gusA* [15, 48]. To prepare the inoculum, the two derivatives were grown separately in Nitrogen-Free Broth [49] supplemented with LBm (final concentration 2.5%) and tetracycline (final concentration 10 µg/mL) for 24 h at 27°C, with shaking at 180 rpm.

For the greenhouse pot experiment, the wild-type *A. brasilense* Sp245 was used and grown the same way as described above (but without adding tetracycline).

Inoculation and plant growth conditions in the screening experiment

Wheats seeds were surface-disinfected by successive immersions in 70% ethanol for 1 min then in sodium hypochlorite solution (Na_2CO_3 0.1 g, NaCl 3 g and NaOH 0.15 g in 100 mL distilled water supplemented with 10% commercial bleach containing 9.6% of active chlorine and 0.01% of Tween 20) for 40 min at 180 rpm, followed by rinsing three times (5 min each, with strong manual agitation) in sterile water and a final a last

bath in sterile water for 60 min (adapted from Pothier *et al.* [50]). Seeds were then transferred on plates containing sterile agar for plant culture (Agar A7921; Sigma-Aldrich, Saint-Quentin Fallavier, France) at 8 g/L, which were incubated for 24 h in the dark at 27°C. The one-day-old seedlings were placed each in a sterile 50-mL glass tube (Ø 19.3 × 200 mm) containing 20 mL of sterile semi-solid agar for plant culture at 2 g/L. Then, three seeds of each genotype were inoculated with 100 µL of cell suspension of Sp245(pFAJ31.2) and three others with 100 µL of cell suspension Sp245(pFAJ64). Each 100 µL bacterial suspension contained 4×10^7 cells previously washed in sterile 10 mM $MgSO_4$ solution. Controls received 100 µL of 10 mM $MgSO_4$ solution per seed. The tubes were placed in a growth chamber for seven days at 21°C, with a 16/8 hours day/night cycle and 60% hygrometry.

Root sampling and GUS assays

For the screening experiment, 4-MethylUmbelliferyl-β-D-Glucuronide (MUG) was used as a substrate for GUS activity, which can be quantified by spectrofluorimetry of the product 4-methylumbelliferone (4-MU) [51]. The GUS activity from Sp245(pFAJ31.2) was measured to estimate Sp245 colonization level on roots, while the GUS activity from Sp245(pFAJ64) was used to evaluate *ppdC* expression in Sp245 cells. At seven days of growth, plants were removed from the tubes and each root system was cut off in 1 cm fragments and put in a 2 mL Eppendorf tube. Then, 1.5 mL of an extraction solution for GUS assay (*i.e.* phosphate buffer 50 mM at pH 7, EDTA 10 mM, Sarkosyl 0.1%, Triton X-100 0.1%, 2-mercaptoethanol 1mM) supplemented with MUG at 0.35 mg/mL was added to each tube. The tubes were strongly shaken 5 min using a Vortex and put for 4 h in the dark at 37°C. After the incubation, 200 µL of supernatant from each tube were transferred in the wells of black 96-well plates with clear bottom. The GUS activity was measured using an Infinite M200 pro microplate reader (Tecan, Männedorf, Switzerland), with an excitation at 365 nm and an emission at 455 nm to quantify fluorescence intensity from 4-methylumbelliferone (cleaved MUG). For each wheat genotype, the mean fluorescence intensity of non-inoculated plants was subtracted from data of inoculated plants. Because of high intra-genotype variability, median data were used rather than the means.

Greenhouse experiment

Ten genotypes differing in their ability to interact with *A. brasilense* Sp245 in vitro were selected from the 198 accessions to compare plant growth promotion effects of *A. brasilense* Sp245 in non-sterile soil under greenhouse pot conditions. It included four modern genotypes (Amifort, ATUT-II, D130-63 and DI276) and two old varieties (Concurrent and Coronation) with effective interactions in vitro with Sp245, as well as two modern genotypes (Danubia and Hendrix) and two old varieties (Jaszaji TF and Odesskaya 16) not effective in their interactions with Sp245.

Wheat seeds were surface-disinfected in sodium hypochlorite, as described above (but without using ethanol). They were put in pots containing 1700 g of sieved non-sterile soil taken from the topsoil of a luvisol at La Côte Saint-André (France), with a soil water content of 22% w/w. Each seed was then inoculated with 200 µL of cell suspension of *A. brasilense* Sp245 containing 2×10^7 cells or received 200 µL of $MgSO_4$ 10 mM (in the controls). At day 14, a combined stress was applied for half the plants, by stopping watering until water content was 12% w/w and maintaining this level until the end of the experiment and not adding any nutrient solution, whereas for optimum conditions soil water content was maintained at 22% w/w and a NPK nutrient solution (Plant-Prod 20-20-20; Plantproducts, Leamington, ON) was used to bring 8 mg N per plant on day 14 and on day 21. Eight plants were grown per genotype for each of the four inoculated/control × stress/optimum treatment combinations (randomized block design, with two plants per pot), and the experiment was carried out in a greenhouse with a 16/8 h day/night cycle at respectively 24°C/20°C and 40%/60% hygrometry.

At day 28, plants were removed from the soil and each system root was carefully cut off and washed. The root architecture was assessed for each plant using WinRhizo image analysis software (Regent Instruments, Nepean, ON). In addition, each root system and shoot were separately weighted when fresh and dry (after 48 h at 105°C).

Genotyping data

One hundred and eighty accessions were genotyped with an Affymetrix SNP array [52]. Among the 423,385 single-nucleotide polymorphism (SNP) markers, 31,340 are labelled as Off-Target Variant (OTV) markers. Along the SNP alleles and eventually missing data (NA), they show a null allele (OTV) with no amplification. As suggested by Didion *et al.* [53], they can be used to detect presence/absence variations (PAV). In order to take them into account, the OTV markers were split into two markers: a SNP (OTV were transformed into NA) and an OTV (encoded with A for the presence of the fragment, and T for the absence of the fragment). Before data cleaning, there were so 454,725 markers in the matrix.

Heterozygous genotypes were replaced by NA. Markers monomorphic (114,562 markers) or with NA percentage superior to 10% (30,124 markers) were deleted from the matrix. Imputation of NA was then performed with the Beagle v4.1 software [54] using standard parameters. SNP without physical position (16,985 markers) on Chinese Spring RefSeqv1.0 [55] were first removed because the Beagle software cannot impute unmapped markers. After imputation, markers with a Minor Allele Frequency (MAF) inferior to 5% (53,358 markers) were deleted from the matrix. In the end, there were 239,696 SNP among which 230,574 are public and have been used for Genome Wide Association Study (GWAS).

Genome wide association study

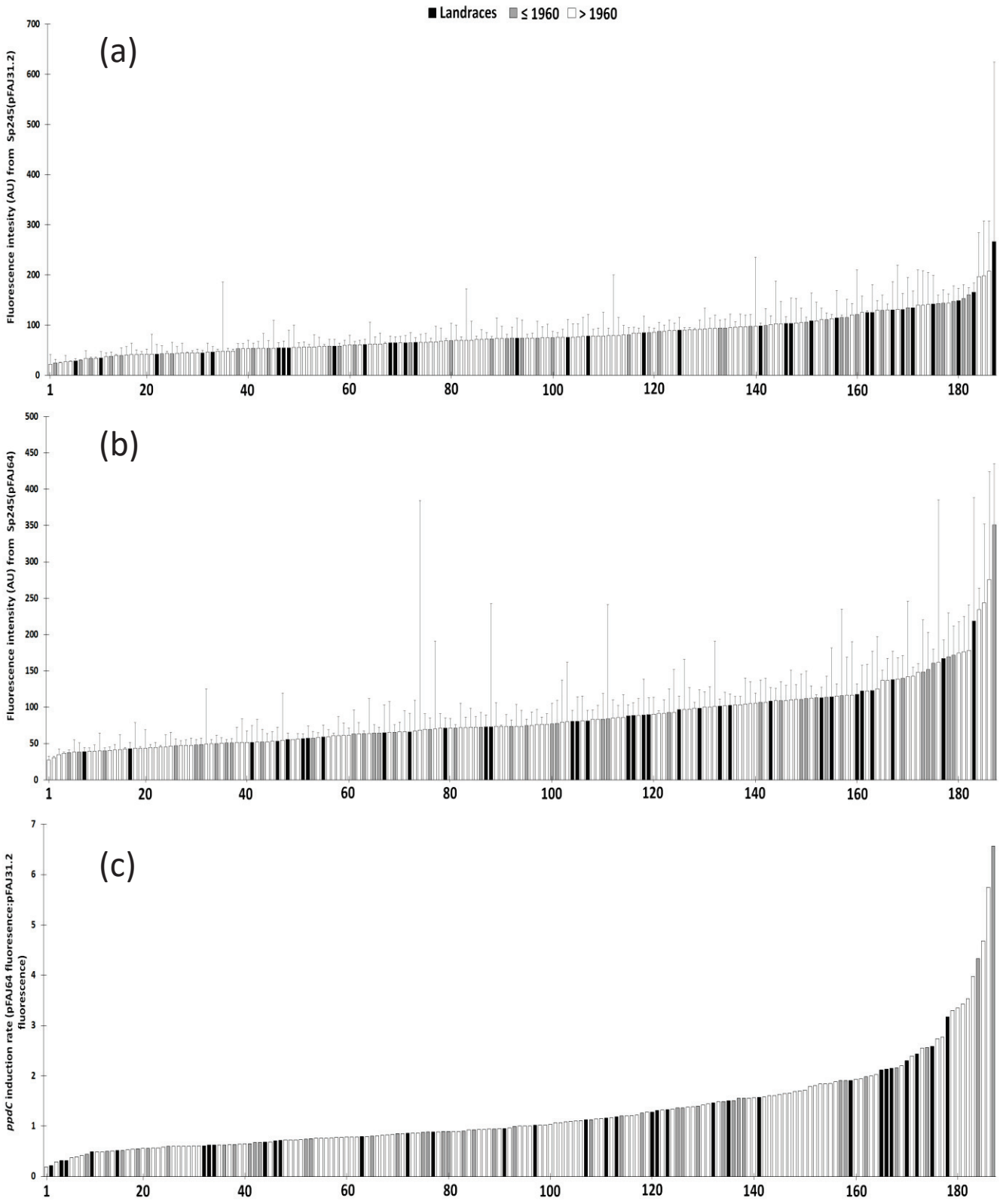

Fig. 1 Colonization of *A. brasilense* Sp245(pFAJ31.2) and *ppdC* expression of Sp245(pFAJ64) on the roots of 187 individual wheat genotypes corresponding to 114 modern (> 1960) and 73 ancient wheat genotypes (including 33 landraces and 40 old varieties [≤ 1960]). Fluorescence from 4-MU is shown in (a) for the pFAJ31.2 plasmid (root colonization) and in (b) for the pFAJ64 plasmid (*ppdC* expression), whereas the *ppdC* induction rate (pFAJ64 fluorescence:pFAJ31.2 fluorescence ratio) is given in (c). Fluorescence is expressed as arbitrary units (AU) and data are presented as means (n = 3) with standard errors. Corresponding ranking of the 187 genotypes is in **Supplementary Table 1**.

GWAS was performed with R package GenABEL [56] and more specifically with the "polygenic" (Thompson *et al.* 1990) and the "mmscore" functions [57]. The model used for GWAS was:

$$[1]\ Y = \mu + X\beta + G + E$$

Where Y is the vector of phenotypic values, μ the overall mean, X the vector of SNP scores, β the additive effect of the SNP, and G and E are the random polygenic and residual effects. As proposed by Rincent *et al.* [58], the random polygenic effect was modelled with a genomic kinship matrix derived from all SNP except those on the chromosome being tested. In order (i) to remove redundant SNP (and consequently, shorten calculation time) and (ii) not to allocate too much weight to SNP in LD, in particular SNP located around centromeres, the matrix was pruned before computing. For each chromosome, the Linkage Disequilibrium (LD) of each pair of SNP within 600 kb was estimated and if the r^2 was superior to 0.9, one SNP of the pair was removed. The kinship matrix was then calculated as proposed by VanRaden [59].

To control for the effect of multiple testing, the number of independent tests was calculated as in Li and Ji [60] and a $-\log_{10}(P)$ of 5 was then considered to identify significant marker-trait associations. To define the number of chromosomal regions, significant markers were clustered by average distance on LD using a cut-off at (1-"critical LD"). Critical LD was estimated as the 99.9th percentile of LD distribution assessed on 21,000 randomly chosen pairs of unlinked loci (mapped on different chromosomes). A Khi^2 was used to test the hypothesis that three accessions classes (landraces, ≤ 1960, > 1960) have the same marker alleles proportions.

Statistics

Absolute deviations (AD) and standard errors (SE) were used to display data fluctuations when using median and mean data, respectively. Comparisons between screening data were performed using Kruskal-Wallis rank-sum tests followed by Conover-Iman tests for pairwise comparisons, as data could not been normalized. When only two treatments, comparisons were carried out using Wilcoxon rank-sum tests. Comparisons of multiple and pairwise proportions were done using Khi^2 tests and Z-tests, respectively. Plant data were normalized using optimizing box-cox transformation, and compared using multiple-factors ANOVA followed by Fisher's LSD tests. The analyses were done using Xlstat software v2018.4 (Addinsoft, Bordeaux, France) at $P < 0.05$ level.

Results

Sp245 colonization of wheat in the screening experiment

The analysis of GUS activity related to root colonization by *A. brasilense* Sp245(pFAJ31.2) gave median fluorescence intensities that ranged from 22 ± AD 19 to 266 ± 358 arbitrary units (AU) depending on wheat genotype (**Fig. 1**). Data for modern genotypes, old varieties and landraces were 76 ± SE 3, 84 ± 6 and 92 ± 8

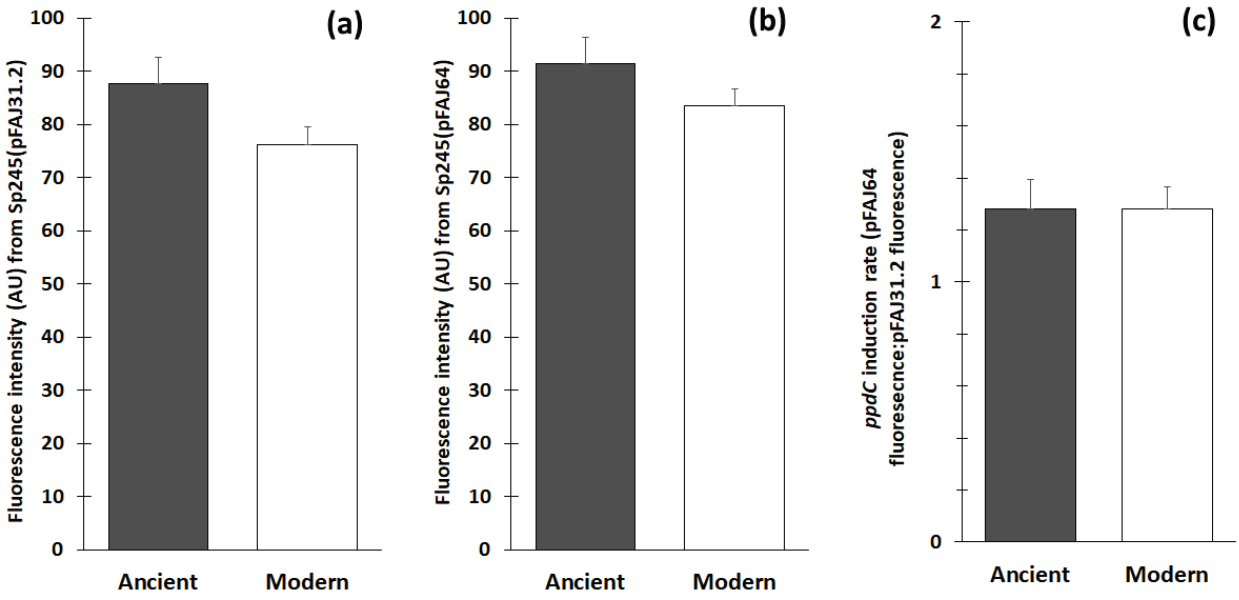

Fig. 2 Colonization of *A. brasilense* Sp245(pFAJ31.2) and *ppdC* expression of Sp245(pFAJ64) on the roots of modern (n = 114) and ancient wheat genotypes (n = 73). Fluorescence from 4-MU is shown in (a) for the pFAJ31.2 plasmid (root colonization) and in (b) for the pFAJ64 plasmid (*ppdC* expression), whereas the *ppdC* induction rate (pFAJ64 fluorescence:pFAJ31.2 fluorescence ratio) is given in (c). Fluorescence is expressed as arbitrary units (AU) and data are presented as means (computed from individual genotype data) with standard errors. There was no significant difference at $P < 0.05$.

Table 1 Percentages of wheat genotypes showing the highest and lowest values of root colonization by *A. brasilense* Sp245(pFAJ31.2) or *ppdC* expression in root-colonizing Sp245(pFAJ64) among the ancient (n = 73) and modern genotypes (n = 114).

	Ancient	Modern
Root colonization by Sp245		
25 best genotypes	20.5 % a†	8.8 % b
50 best genotypes	38.4 % a	19.3 % b
50 worst genotypes	24.7 %	28.1 %
25 worst genotypes	13.7 %	13.2 %
ppdC expression in Sp245		
25 best genotypes	15.1 %	12.3 %
50 best genotypes	31.5 %	23.8 %
50 worst genotypes	20.6 %	30.7 %
25 worst genotypes	6.8 % a	17.5 % b

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

AU (**Supplementary Figure 1**), respectively, but the significance level was only *P* = 0.07 when comparing the 114 modern genotypes with all 73 ancient genotypes (including old varieties and landraces) (**Fig. 2**). However, 20.5% and 38.4% of the ancient genotypes were among the 25 and the 50 most colonized genotypes, respectively, versus only 8.7% and 19.3% of the modern genotypes (both at *P* < 0.05) (**Table 1A, Supplementary Table S2**).

ppdC expression on wheat in the screening experiment

For *ppdC* expression analysis, the median fluorescence intensities varied from 27 ± AD 5 to 351 ± 84 AU between genotypes (**Fig. 1B**). Differences were not significant when comparing the 114 modern (83 ± SE 4 AU) vs the 73 ancient genotypes (91 ± 6 AU) (**Fig. 2**). There was a significant difference when comparing the prevalence of ancient vs modern genotypes among the 25 least colonized genotypes (respectively 6.8% vs 17.4%, *P* < 0.05), but not among the most colonized ones (**Table 1, Supplementary Table S2**). The correlation between Sp245 colonization and *ppdC* expression data (n = 190) was significant (*P* < 0.05) but weak (Spearman's r = 0.19).

The *ppdC* induction rate (*i.e.* the median fluorescence intensity in the Sp245(pFAJ6) treatment divided by the median fluorescence intensity in the Sp245(pFAJ31.2) treatment) ranged from 0.19 to 6.57 depending on the genotype (**Fig. 1**). There was no difference in *ppdC* induction rate between the 114 modern (1.28 ± 0.08) and the 73 ancient genotypes (1.28 ± 0.11) (**Table 1**).

Impact of stress on wheat genotypes of different interaction abilities

A genotype × stress two-factor ANOVA performed on the dataset from non-inoculated plants showed that stress had a significant impact (*P* < 0.001) on every plant parameters (except root length and number of roots), with a significant interaction between genotype and stress for root diameter (*P* < 0.05). Depending on genotype, the effect of stress was significant on 2 to 5 of the 8 plant parameters studied (**Supplementary Table S3**). Overall, the impact of stress on the six genotypes effective at interacting with Sp245 (hereafter referred to as Sp245-stimulating genotypes) vs the four genotypes ineffective at interacting with Sp245 (hereafter referred to as non Sp245-stimulating genotypes) was -33.4 ± (SE) 3.9 % vs -46.0 ± 8.1 % for root volume, -9.4 ± 4.4 % vs -12.6 ± 5.4 % for root diameter, -26.6 ± 2.3 % vs -34.4 ± 9.3 % for fresh root biomass, -21.4 ± 1.3 % vs -30.3 ± 9.8 % for dry root biomass, -47.8 ± 2.6 % vs -35.5 ± 6.1 % for fresh shoot biomass, and -46.6 ± 3.5 % vs -36.9 ± 6.8 % for dry shoot biomass (**Fig. 3**), but none of these differences was significant at *P* < 0.05 level.

Stimulation effects of A. brasilense Sp245 on wheat genotypes of different interaction abilities

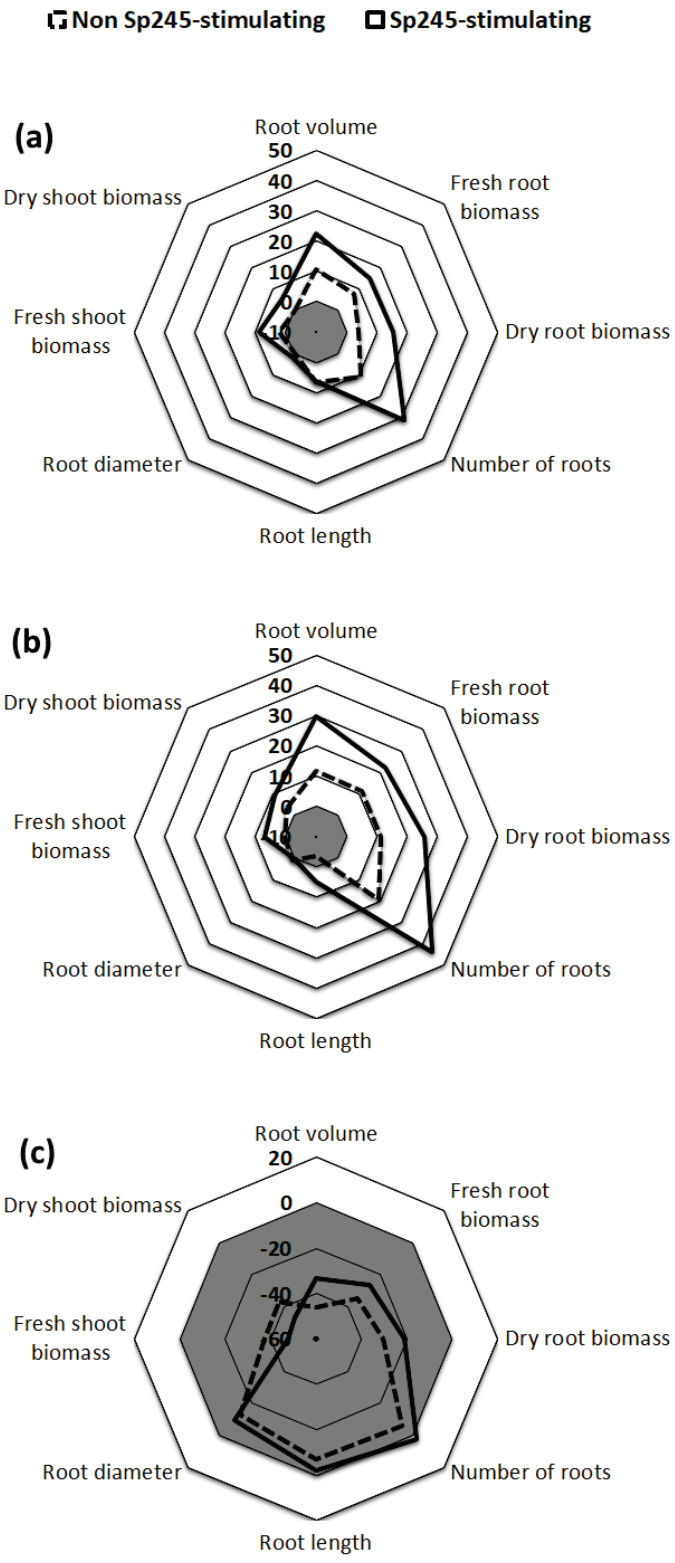

Fig. 3 Relative impact of seed inoculation with *A. brasilense* Sp245 on the growth of four non Sp245-stimulating and six Sp245-stimulating wheat genotypes under optimum (a) or combined stress condition (b), and relative impact of stress on performance of non-inoculated plants (c). The relative impacts were computed as (inoculated – non-inoculated)/non-inoculated [in a,b], and (stress – optimum)/optimum [in c] for each of the eight plant parameters investigated. There was no significant differences at $P < 0.05$.

The results of three-factor (genotype × stress × inoculation) ANOVA showed that Sp245 inoculation had a significant effect on root volume ($P < 0.001$), number of roots ($P < 0.001$), fresh ($P < 0.01$) and dry root biomass ($P < 0.001$).

Under optimum condition, inoculation led to a significant increase in root volume (+61.9%), the number of roots (+94.6%) and dry root biomass (+38.9%) of Sp245-stimulating genotype Concurrent, whereas positive effects on other genotypes were not significant (**Supplementary Table S4**). Overall, plant parameters in inoculated treatments for Sp245-stimulating vs non Sp245-stimulating genotypes were + 22.5 ± (SE) 9.5 % vs + 10.7 ± 3.5 % for root volume, + 31.1 ± 15.1 % vs 11.0 ± 4.1 % for the number of roots, + 14.8 ± 4.0 % vs 7.5 ± 3.7 % for fresh root biomass, and + 15.4 ± 4.9 % vs 4.2 ± 5.6 % for dry root biomass in comparison with the controls (**Fig. 3**).

Under stress, Sp245 inoculation had a significant positive effect on 3 of 6 Sp245-stimulating genotypes when considering the root volume of Concurrent (+75.8%) and Amifort (+49.6%), the number of roots of Concurrent (+80.6%), Amifort (+86.3%) and Coronation (+88.3%), the fresh root biomass of Concurrent (+54.2%) and Amifort (+43.2%) and the dry root biomass of Concurrent (+57.0%) and Amifort (+52.2%), but no significant effect on any of the 4 non Sp245-stimulating genotypes (**Supplementary Table S4**). Thus, in comparison with the non-inoculated controls, growth parameters upon inoculation of Sp245-stimulating vs non Sp245-stimulating genotypes were + 29.6 ± (SE) 13.4 % vs + 11.8 ± 2.3 % for root volume, + 44.1 ± 19.7 % vs + 19.4 ± 6.3 % for the number of roots, + 22.1 ± 10.0 % vs 11.3 ± 3.1 % for fresh root biomass, and + 25.6 ± 11.3 % vs + 11.6 ± 3.4 % for dry root biomass (**Fig. 3**).

Wheat stimulation of A. brasilense Sp245 vs Pseudomonas kilonensis F113

When the data in Valente *et al.* [42] on the interaction of the same wheat genotypes with *P. kilonensis* F113 were included, the correlation between mCherry red fluorescence intensity (F113 colonization) and 4-MU fluorescence intensity (Sp245 colonization) was not significant, regardless of whether ancient (n = 73), modern (n = 114) or all 187 genotypes were considered. Among the 50 genotypes most colonized by *P. kilonensis* F113 and the 50 most colonized by *A. brasilense* Sp245 (making 86 genotypes in total, of which 23 landraces, 21 old varieties and 42 modern genotypes), only 14 of them (*i.e.* 16.3%) were common to both lists. These 14 genotypes included 3 of 23 landraces (13.0%), 7 of 21 old varieties (33.3%) and 4 of 42 modern genotypes (9.5%) among the 86 most colonized genotypes, and only the difference between old varieties and modern genotypes was significant ($P < 0.05$) (**Table 2**). In contrast, for the 50 least colonized genotypes by Sp245 and/or F113 (85 in total), the 15 genotypes common to the Sp245 and F113 lists included 0 of 14 landraces, 2 of 17 old varieties (11.8%) and 13 of 54 modern genotypes (24.1%), and only the difference between landraces and modern genotypes was significant ($P < 0.05$) (**Table 2**).

Table 2 Percentages of wheat genotypes showing the highest and lowest values of root colonization by *P. kilonensis* F113, by *A. brasilense* Sp245 or by both PGPR strains among the landraces (n = 23 for most colonized and n = 14 for least colonized), old varieties (≤ 1960, n = 21 for most colonized and n = 17 for least colonized) and modern genotypes (> 1960, n = 42 for most colonized and n = 54 for least colonized), based on the 50 genotypes most or least colonized by F113 and/or the 50 genotypes most or least colonized by Sp245.

	Landraces	Old varieties (≤ 1960)	Modern genotypes (> 1960)
Genotypes most colonized by Sp245 and/or F113			
All 86 genotypes	23/23 (100%)	21/21 (100%)	42/42 (100%)
36 genotypes most colonized by Sp245 only	11/23 (47.8%)	7/21 (33.3%)	18/42 (47.6%)
36 genotypes most colonized by F113 only	9/23 (39.1%)	7/21 (33.3%)	20/42 (42.9%)
14 genotypes most colonized by Sp245 and F113	3/23 (13.0%) ab†	7/21 (33.3%) b	4/42 (9.5%) a
Genotypes least colonized by Sp245 and/or F113			
All 85 genotypes	14/14 (100%)	17/17 (100%)	54/54 (100%)
35 genotypes least colonized by Sp245 only	8/14 (57.1%)	8/17 (47.1%)	19/54 (35.2%)
35 genotypes least colonized by F113 only	6/14 (42.9%)	7/17 (41.2%)	22/54 (40.7%)
15 genotypes least colonized by Sp245 and F113	0/14 (0%) a	2/17 (11.8%) ab	13/54 (24.1%) b

†For each row, significant differences between landraces, old varieties and modern genotypes are indicated by letters a and b (Z-tests carried out on numbers of genotypes, $P < 0.05$).

Table 3 Percentages of wheat genotypes showing the highest and lowest values of gene expression in root-colonizing PGPR, when considering *phl* in *P. kilonensis* F113, *ppdC* in *A. brasilense* Sp245, or both, among the landraces (n = 18 for highest expressions and n = 14 for lowest expressions), old varieties (≤ 1960, n = 24 for highest expressions and n = 16 for lowest expressions) and modern genotypes (> 1960, n = 46 for highest expressions and n = 57 for lowest expressions), based on the 50 genotypes showing the highest or lowest *phl* expression and/or the 50 genotypes showing the highest or lowest *ppdC* expression.

	Landraces	Old varieties (≤ 1960)	Modern genotypes (> 1960)
Genotypes showing the highest ppdC and/or phl expression in root-colonizing PGPR			
All 88 genotypes	18/18	24/24	46/46
38 genotypes with high *ppdC* expression only	7/18 (38.9%)	7/24 (29.2%)	24/46 (52.2%)
38 genotypes with high *phl* expression only	9/18 (50.0%)	10/24 (41.7%)	19/46 (41.3%)
12 genotypes with high *ppdC* and *phl* expression	2/18 (11.1%) ab†	7/24 (29.2%) b	3/46 (6.5%) a
Genotypes showing the lowest ppdC and/or phl expression in root-colonizing PGPR			
All 87 genotypes	14/14 (100%)	16/16 (100%)	57/57 (100%)
37 genotypes with low *ppdC* expression only	4/14 (28.6%)	6/16 (37.5%)	27/57 (47.4%)
37 genotypes with low *phl* expression only	9/14 (64.2%) a	4/16 (25.0%) b	24/57 (42.1%) ab
13 genotypes with low *ppdC* and *phl* expression	1/14 (7.1%) a	6/16 (37.5%) b	6/57 (10.5%) a

†For each row, significant differences between landraces, old varieties and modern genotypes are indicated by letters a and b (Z-tests carried out on numbers of genotypes, $P < 0.05$).

The correlation between EGFP green fluorescence (*phl* expression) and 4-methylumbelliferone fluorescence intensity (*ppdC* expression) was not significant when assessing 73 ancient, 114 modern or all 187 genotypes. Among the 50 genotypes showing the highest *phl* expression and the 50 with the highest *ppdC* expression (making 88 genotypes in total, of which 18 landraces, 24 old varieties and 46 modern genotypes), only 12 of them (*i.e.* 13.6%) were present in both lists. These 12 genotypes included 2 of 18 landraces (11.1%), 7 of 24 old varieties (29.2%) and 3 of 46 modern genotypes (6.5%), and only the difference between old varieties and modern genotypes was significant ($P < 0.05$). Among the 50 genotypes showing the lowest expression of *ppdC* or *phl* (87 in total), there were 13 genotypes common to the *ppdC* and *phl* lists, which included 1 of 14 landraces (7.1%), 6 of 16 old varieties (37.5%) and 6 of 57 modern genotypes (10.5%). The differences between old varieties and (i) modern genotypes or (ii) landraces were significant ($P < 0.05$) (**Table 3**).

Genome-wide association study

GWAS was conducted on root colonization by *A. brasilense* Sp245, *ppdC* expression and *ppdC* induction rate (considering the means and the medians) with 230 574 SNP (**Fig. 4**). Using a $-\log_{10}(P)$ superior to 5 led to the identification of 173 significant marker-trait associations distributed on 12 chromosomes (**Table 4**). No significant association was observed for root colonization. After clustering the 161 markers involved, we identified 10 regions for *ppdC* expression in Sp245, six for *ppdC* induction rate and five common to the two traits. In all cases the minor allele increased the value of the trait. The SNP with the largest effect is in group 20 on chromosome 6B for *ppdC* expression (+49 AU for the minor allele) and in group 13 on chromosome 3B for *ppdC* induction rate (+1.12 for the minor allele). Twelve markers showed a highly significant ($P < 0.01$) difference for allele proportion between the three accession classes (landraces, ≤ 1960, > 1960). This concerned four of the five markers in group 4 on chromosome 1B, the marker in group 6 on chromosome 2B, four of the 60 markers in group 7 on chromosome 2B, one of the three markers in group 8 on chromosome 2B, the marker in group 11 on chromosome 3A and the marker of group 14 on chromosome 4A. Except for group 6 on chromosome 2B, the minor allele frequency decreased from landraces to modern genotypes (registered after 1960).

Discussion

Successful interaction of PGPR with plants involves root colonization, expression of key bacterial genes, and implementation of phytostimulation effects. In this work, the screening of wheat accessions was carried out by focusing on the first two stages, whereas the ecological relevance of the findings was assessed by investigating the third interaction stage, on a range of Sp245-stimulating and non Sp245-stimulating wheat

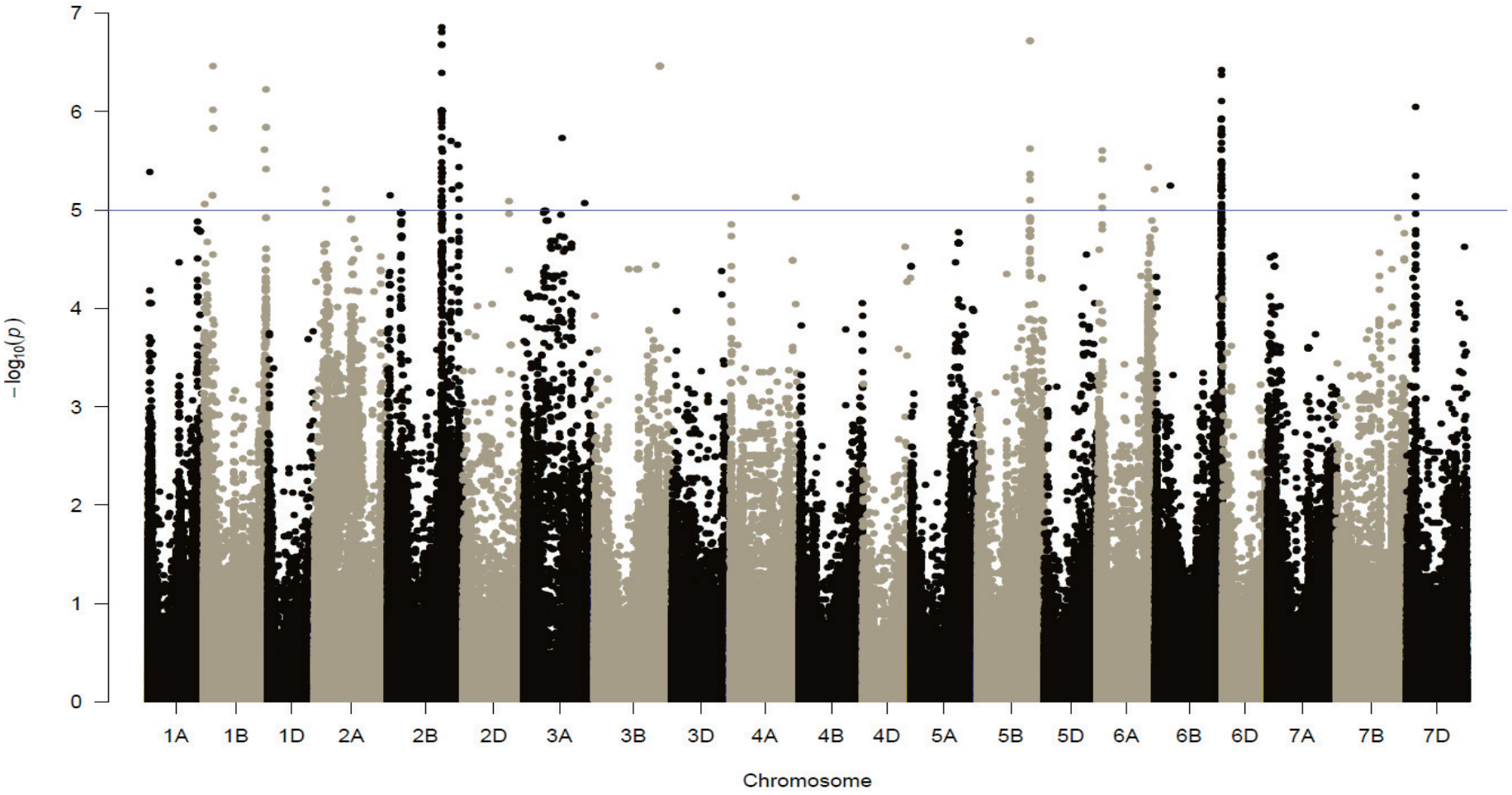

Fig. 4 Manhattan plot for GWAS conducted on 180 bread wheat accessions for *A. brasilense* Sp245 root colonization, *ppdC* expression and *ppdC* induction rate (considering the means and the medians) with 230,574 SNP markers. The $-\log_{10}(P)$ values are plotted against their respective physical positions on each chromosome of the Chinese Spring RefSeqv1.0. A $-\log_{10}(P)$ of 5 was considered to identify significant marker-trait associations.

genotypes. For this screening, the reporter gene *gusA* was preferred to *egfp* or *mCherry* genes, as the latter were not sensitive enough for effective quantification of root colonization or *ppdC* expression on roots.

Root colonization by *A. brasilense* Sp245 varied largely between replicates, which could be due to its particular root colonization pattern on wheat, making very local cell aggregates especially near root tips [13, 15, 45]. Root colonization depended also on wheat genotype, some genotypes displaying 10 fold higher bacterial fluorescence than others, which is reminiscent of findings made with *P. kilonensis* F113 [42]. Spearman's correlation between fresh root biomass and Sp245(pFAJ31.2) fluorescence intensity was significant ($P < 0.05$, n = 187) but weak (r = 0.24), which suggests that the impact of root system size on Sp245 colonization was marginal. As for *P. kilonensis* F113, the prevalence of ancient genotypes (especially landraces) among the most colonized genotypes was higher than that of modern genotypes, pointing to adverse effects of modern breeding on PGPR recruitment ability. Yet, the genotypes most (or least) colonized by *A. brasilense* Sp245 were largely different from those most (or least) colonized by *P. kilonensis* F113, indicating differences in partnership affinity.

As for root colonization, *ppdC* expression varied between replicates and between wheat genotypes. IAA biosynthesis is modulated by compounds exudated by roots or present on root cells [14], whose presence or level may vary depending on plant genotype [23, 31, 32, 61, 62]. This screening can be compared to the one performed with *P. kilonensis* F113 et *phl* genes, as both DAPG and IAA act as auxinic signals stimulating root system branching and root growth [14, 63]. In contrast to *phl* expression, there was no indication that modern breeding had had any negative effect on the ability of resulting wheat genotypes to stimulate *ppdC* expression. This is consistent with the literature, in that *Azospirillum* strains were shown to produce auxins on roots of modern wheat cultivars, thereby enhancing the number of root hairs and lateral roots [64, 65].

When considering the implementation of phytostimulation effects by *A. brasilense* Sp245 and *P. kilonensis* F113, using the same selection of six Sp245/F113 stimulating genotypes and four genotypes stimulating neither Sp245 nor F113 in vitro, it appeared that the occurrence of phytostimulation depended on the interaction between wheat genotype, PGPR strain and environmental (stress) conditions. It was observed for 3 of 6 stimulating genotypes (Coronation and D130-63 for F113, vs Concurrent for Sp245) and 1 of 4 non-stimulating genotypes (Odesskaya 16 for F113) under optimum condition, in comparison with 5 of 6 stimulating genotypes (Concurrent and Coronation for both F113 and Sp245, Atut-II and D130-63 for F113, Amifort for Sp245) and 0 of 4 non-stimulating genotypes under stress ([42] ; **Supplementary Table S5**). Differences in root colonization, gene expression and plant-beneficial effects due to plant genotype have been documented for *P. kilonensis* F113 [9, 42]. Such differences are also well documented for *A. brasilense* Sp245 [66, 67] and other *Azospirillum* strains [68–70]. Here, it appeared that the two PGPR displayed different affinity profiles towards wheat genotypes, which suggests that different genotypes in a same soil may select and rely upon different assortments of PGPR populations.

Table 4 Chromosomal regions identified after GWAS on 180 bread wheat accessions for *A. brasilense* Sp245 colonization, *ppdC* expression and *ppdC* induction rate (considering the means and the medians) with 230,574 SNP markers. For each region, the chromosome assignment, the number of significant markers, the maximum -$\log_{10}(P)$ value, the position of the region on the Chinese Spring RefSeqv1.0 and the trait concerned are indicated. A –$\log_{10}(P)$ of 5 was considered to identify significant marker-trait associations.

Region	Chromosome	Nb markers	-$\log_{10}(P)$	End (bp)	Start (bp)	Trait
1	1A	1	5.4	20008947	20008947	Induction
2	1B	1	5.6	12806570	12806570	Expression
3	1B	7	6.5	97215708	106409911	Expression, Induction
4	1B	5	6.2	650955077	667519412	Expression
5	2A	2	5.2	120681877	123202999	Expression
6	2B	1	5.1	23006356	23006356	Expression
7	2B	60	6.8	572314013	582645061	Expression
8	2B	3	5.7	674029409	742692170	Expression, Induction
9	2B	5	5.2	757386253	759178940	Induction
10	2D	1	5.1	490565246	490565246	Expression
11	3A	1	5.7	405321848	405321848	Expression
12	3A	1	5.1	644202467	644202467	Expression
13	3B	5	6.5	695562054	695962596	Induction
14	4A	1	5.1	699885775	699885775	Expression
15	5B	4	6.7	560451114	562280657	Expression, Induction
16	6A	5	5.6	51947725	51992351	Expression, Induction
17	6A	1	5.4	540840475	540840475	Induction
18	6A	1	5.2	609172967	609172967	Induction
19	6B	1	5.2	159410401	159410401	Induction
20	6B	51	6.4	703206711	705314123	Expression, Induction
21	7D	4	6.0	93028858	93555477	Expression

Twenty-one wheat genomic regions were associated to *A. brasilense* Sp245 interaction. However, no region was identified for root colonization. This may be due to the large variability between replicates that limited the possibility to detect small effect associations. Indeed, using a different strain (DMS 7030) and the Opata × synthetic mapping population, Dıaz De Leon *et al.* [71] were able to detect one major QTL on chromosome 1A for the adhesion of *A. brasilense* cells to wheat roots. We identified 21 regions involved in the induction rate and expression of *ppdC* in Sp245. Most of the regions are located on genomes A and B, which is not surprising as it is well known that wheat genome D is less polymorphic and so less covered with SNP markers [52]. In addition, two regions (one on chromosome 2B and the other on 6B) gathered most of the significant markers. This last region comprised the SNP with the largest effect on *ppdC* expression, which could make of this region a priority for further genetic studies. Interestingly, nine markers showed different proportions of alleles between the three classes of accessions (landraces, ≤ 1960, > 1960). In 12 cases, the frequency of the allele increasing *ppdC* expression was lower in modern cultivars than in landraces. Moreover, although some modern genotypes showed high expression levels, none carried the favorable allele for all the genomic regions identified. Provided these genomic regions are not linked to unfavorable alleles for other agronomic traits, this opens the possibility to use the molecular markers to increase the frequency of favorable alleles and improve the capacity of modern genotypes to interact with *A. brasilense* Sp245, and perhaps also other *A. brasilense* or *Azospirillum* PGPR strains if the alleles are also relevant at these scales.

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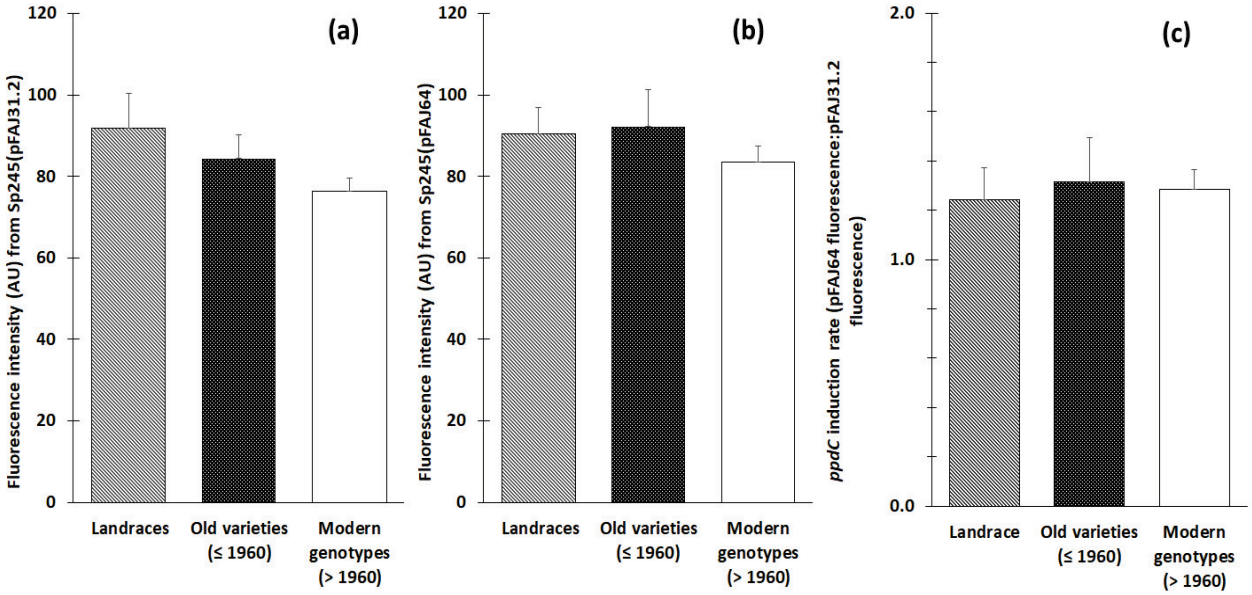
Supplementary information



Supplementary Figure S1 Colonization of *A. brasilense* Sp245(pFAJ31.2) and *ppdC* expression in Sp245(pFAJ64) on the roots of landraces (n = 34), old varieties (≤ 1960, n = 40) and modern genotypes (>1960, n = 114). Fluorescence from 4-MU is shown in (a) for the pFAJ31.2 plasmid (root colonization) and in (b) for the pFAJ64 plasmid (*ppdC* expression), whereas the *ppdC* induction rate (pFAJ64 fluorescence:pFAJ31.2 fluorescence ratio) is given in (c), Fluorescence is expressed as arbitrary units (AU) and data are presented as means (computed from individual genotype data) with standard errors. There was no significant difference at $P < 0.05$.

Supplementary Table S1 List of the 187 wheat genotypes used in the study and their rankings in the screening experiment. These 187 genotypes correspond to the accessions that germinated among the 187 genotypes of the 196CC core-collection sub-sampled from the 372CC collection set up by Balfourier *et al.* [39], plus the reference landrace Chinese Spring and the two reference modern lines Hendrix and Skerzzo. The genotypes are listed according to their ERGE code in the French National Cereal Genetic Resources database. For each of the three rankings done in the screening experiment, the worst rank was 1 and the best rank 187. The total score was obtained by summing the three ranks.

ERGE Code	Genotype	Geographic origin	Genotype category	Sp245 colonization rank	*ppdC* expression rank	*ppdC* induction rate rank	Total score
7	(95-13*BEZOSTAIA)3-3	France	> 1960	55	40	82	177
19	CH01193	Switzerland	> 1960	113	122	110	345
92	11IWSWSN14	USA	> 1960	29	80	143	252
177	DI15	France	> 1960	71	28	51	150
234	DI182-9	France	> 1960	60	158	160	378
236	DI185	France	> 1960	86	60	74	220
338	DI276	France	> 1960	89	185	180	454
347	2838-39	Bulgaria	> 1960	34	71	127	232
386	DI330	France	> 1960	5	38	154	197
419	DI37-12-2	France	> 1960	140	99	60	299
421	3716-1	Bulgaria	> 1960	10	74	164	248
477	DI50-12	France	> 1960	8	146	179	333
524	60293-	The Netherlands	> 1960	109	97	92	298
537	CH62022	Switzerland	> 1960	129	171	140	440
546	664-258-18	Bulgaria	> 1960	83	78	98	259
748	A4	Afghanistan	> 1960	98	128	122	348
794	ADMONTER	Austria	≤ 1960	38	81	125	244
797	ADULAR	Germany	> 1960	148	168	124	440
833	AKADARUMA	Japan	≤ 1960	158	179	134	471
871	ALMA	France	≤ 1960	9	69	162	240
901	AMIFORT	France	> 1960	131	180	156	467
957	ARAWA	Australia or New Zealand	≤ 1960	176	92	16	284
983	ARGENT	UK or Ireland	> 1960	174	113	29	316
1005	ARKAS	Germany	> 1960	178	114	26	318
1032	ARROMANCHES	France	> 1960	102	108	104	314
1044	ARTOIS-DESPREZ	France	≤ 1960	15	95	157	267
1080	ATUT II	Austria	> 1960	159	151	86	396
1192	BALKAN	Croatia	> 1960	45	127	152	324
1232	BARBU DU FINISTERE	France	Landrace	6	118	178	302
1236	BARBU DU TRONCHET	France	Landrace	107	46	44	197
1281	BEL ET BON	France	≤ 1960	121	66	52	239
1288	BELLIEI 590	Hungary	≤ 1960	142	61	38	241
1321	BENNI	USA	> 1960	101	109	106	316
1332	BERZATACA	Finland	> 1960	51	23	66	140
1357	BIRGITTA	Sweden	> 1960	136	54	30	220
1400	BLANC PRECOCE	Switzerland	Landrace	63	104	121	288
1402	BLASON	France	> 1960	78	42	56	176
1417	BLE D'OR	France	≤ 1960	115	30	25	170
1429	BLE DE HAIE	France	Landrace	11	8	107	126
1446	BLE DE MARAT BARBU	France	Landrace	70	133	141	344
1498	BLE DU ROUSSILLON	France	Landrace	151	72	32	255
1529	BLONDYNKA	Poland	≤ 1960	134	111	79	324
1531	BLUEBOY	USA	> 1960	166	148	71	385
1747	114/62	Austria	> 1960	106	27	31	164
1768	CANDEAL DE AREVALO	Spain	Landrace	73	167	165	405
1885	CENAD 512	Romania	≤ 1960	92	149	136	377
1899	CEREALOR	France	> 1960	52	50	95	197
1957	CF3003-2-7-4-4-3	France	> 1960	14	85	155	254

1974	CF4563-1-5-3-2-5	France	> 1960	135	94	58	287
2025	CH73052	Switzerland	> 1960	123	24	14	161
2135	CHINESE SPRING	China	Landrace	141	155	111	407
2145	CHITLANG	Nepal	Landrace	46	160	167	373
2153	CHORTANDINKA	Central Asia	> 1960	120	144	119	383
2169	CHYAKSILA EPI NON VELU	Nepal	Landrace	103	67	72	242
2308	COMPTON	USA	> 1960	108	15	17	140
2345	CORSODOR	France	> 1960	18	2	54	74
2364	CP4	France	> 1960	85	62	78	225
2399	D130-63	Poland	> 1960	97	166	153	416
2424	DANUBIA	Czech Republic	> 1960	104	18	23	145
2438	DAVIDOC	France	> 1960	26	77	144	247
2475	DETENICKA CERVENA	Czech Republic	Landrace	68	125	135	328
2489	DI6402-34-2-4	France	> 1960	67	98	117	282
2491	DI6404-19-15	France	> 1960	36	33	100	169
2507	DI7003-1-12	France	> 1960	112	25	24	161
2508	DI7005-113-3	France	> 1960	59	124	139	322
2526	DI7202-103	France	> 1960	77	184	182	443
2534	DI7210-15-11	France	> 1960	53	131	151	335
2536	DI7215-100	France	> 1960	186	136	12	334
2573	DIANA	Poland	> 1960	35	186	186	407
2574	DIANA II	Czech Republic	> 1960	105	82	87	274
2606	DNEPROVSKAIA	Ukraine	> 1960	21	83	150	254
2626	DONG-FANG-HONG-NO3	China	> 1960	1	93	181	275
2644	DRAGON-FRA	France	> 1960	3	130	183	316
2650	DRAVA	Croatia	> 1960	137	139	103	379
2683	E108	France	≤ 1960	ND	ND	ND	ND
2698	EBRO	Spain	> 1960	173	132	48	353
2802	ESPOIR	France	≤ 1960	170	178	118	466
2991	FERRUGINEUM	Russia	≤ 1960	44	31	83	158
3050	FLAMURA 85	Romania	> 1960	114	70	67	251
3070	FLINT	USA	Landrace	163	119	47	329
3278	GELPA	France	> 1960	87	84	96	267
3299	GH126	France	> 1960	65	9	37	111
3342	GK SZOKE	Hungary	> 1960	110	73	73	256
3366	GODOLLOI 15	Hungary	≤ 1960	ND	ND	ND	ND
3406	GRANIT	Russia	> 1960	155	58	18	231
3414	GRENIER	France	> 1960	50	170	173	393
3485	H93-70	Spain	> 1960	4	164	185	353
3617	HIVERNAL	France	> 1960	43	103	133	279
3753	IAS 1	Brazil	≤ 1960	96	110	108	314
3896	JANGO	France	≤ 1960	7	53	158	218
3912	JASZSAGI TF	Hungary	≤ 1960	25	12	85	122
3970	JUFY II	Belgium	≤ 1960	91	65	76	232
3991	K1898-9/L200-6	Bulgaria	> 1960	172	140	55	367
4036	KATYIL	Australia or New Zealand	> 1960	19	3	69	91
4105	KID	France	> 1960	12	11	101	124
4111	KIRAC 66	Turkey	> 1960	122	64	50	236
4157	KOLBEN 3	Sweden	Landrace	ND	ND	ND	ND
4187	KRAKA	Norway or Denmark	> 1960	145	176	142	463
4194	KRELOF 3	France	≤ 1960	133	152	114	399
4300	LESZYNSKA WCZESNA	Poland	≤ 1960	150	76	41	267
4324	LITTLE CLUB	USA	Landrace	69	52	77	198
4343	LONTOI	Finland	> 1960	168	32	6	206
4525	MALGORZATKA UDYCKA	Poland	≤ 1960	119	26	19	164
4664	MASTER	UK or Ireland	> 1960	144	121	81	346
4670	MATRADERECSKEITF	Hungary	> 1960	54	22	61	137

4838	MINTURK	USA	≤ 1960	100	157	137	394
4947	MOTTIN	France	Landrace	147	161	113	421
4991	MV MA	Hungary	> 1960	127	172	145	444
5293	NOUGAT	France	> 1960	28	162	177	367
5401	NZ(81)P43	Australia or New Zealand	> 1960	185	165	45	395
5421	ODESSA EXPSTA20722	Portugal	> 1960	62	10	40	112
5438	ODESSKAYA 16	Ukraine	≤ 1960	23	7	80	110
5448	OGOSTA	Bulgaria	> 1960	132	90	62	284
5501	ORLANDI	Italy	≤ 1960	13	5	93	111
5536	OULIANOWSKA	Russia	> 1960	116	29	22	167
5552	P. DE BROLLON	Spain	Landrace	22	105	159	286
5558	P4523-80	Austria	> 1960	124	138	112	374
5773	POILU DU TARN	France	≤ 1960	61	37	70	168
6027	RECITAL	France	> 1960	32	20	88	140
6086	RENAN	France	> 1960	99	182	171	452
6191	RINGOT 2	France	≤ 1960	2	141	184	327
6308	ROUGE D'ALTKIRCH	France	Landrace	125	79	63	267
6318	ROUGE DE MARCHISSY	Switzerland	Landrace	33	153	172	358
6529	SEU SEUN 27	China	≤ 1960	157	175	129	461
6740	STRUBES DICKKOPF	Germany	≤ 1960	181	100	13	294
6922	TF6	France	≤ 1960	139	101	65	305
6986	TOM THUMB	USA	> 1960	66	117	130	313
7011	TOUZELLE-BLANCHE-BARBUE	France	Landrace	93	143	132	368
7085	TURDA 81-77	Romania	> 1960	161	96	27	284
7092	TYLER	USA	> 1960	20	35	115	170
7117	US(59)34	USA	> 1960	24	44	116	184
7166	US(62)P66	Colombia	> 1960	ND	ND	ND	ND
7279	VALDOR	France	≤ 1960	58	173	174	405
7490	VPM V1-1-2-4R2-3-8-3-2	France	> 1960	81	102	109	292
7585	WATTINES	France	> 1960	153	112	57	322
7848	RONGOTEA	Australia or New Zealand	> 1960	31	89	146	266
7968	BLE DANOIS	France	Landrace	30	135	170	335
7973	BORDEAUX 113	France	Landrace	47	48	97	192
7988	CREPIN A	France	≤ 1960	165	86	21	272
8011	INSTITUT 1802	France	≤ 1960	57	36	75	168
8048	RALET	France	Landrace	183	41	4	228
8051	BLE BARBU DE MUROL	France	Landrace	162	116	46	324
8058	ZANDA	Belgium	≤ 1960	160	154	89	403
8073	CORONATION	Canada	≤ 1960	72	169	168	409
8079	KITCHENER	Canada	≤ 1960	88	150	138	376
8097	STANLEY	Canada	≤ 1960	179	123	36	338
8165	NAVARRO150	Spain	> 1960	40	120	149	309
8170	WS-13 CARDENO 34/45	Spain	> 1960	ND	ND	ND	ND
8194	NEELKANT	Syria	> 1960	64	134	147	345
8197	SANUNU	Syria	> 1960	76	47	68	191
8227	NISHIKAZE KOMUGI	Japan	> 1960	39	156	169	364
8254	CADENZA	France	> 1960	143	137	99	379
8276	CARIBO	Germany	> 1960	17	56	131	204
8287	DC147U	France	> 1960	42	142	163	347
8289	TM7MB1-1	France	> 1960	74	16	39	129
9024	GENESIS	France	> 1960	ND	ND	ND	ND
9077	NON PLUS EXTRA	Austria	Landrace	187	51	2	240
9087	PRINCE LEOPOLD	Belgium	≤ 1960	154	174	126	454
13210	SOLARIS	Czech Republic	> 1960	49	57	105	211
13282	ANATOLIE2	France	≤ 1960	177	63	9	249
13292	CONCURRENT	France	≤ 1960	182	145	43	370
13310	FRUH-WEIZEN	Germany	Landrace	ND	ND	ND	ND

13436	FONDARD CRESPIN	France	≤ 1960	94	34	42	170
13445	VOLT	Hungary	> 1960	16	147	176	339
13454	SPONSOR	France	> 1960	ND	ND	ND	ND
13461	BEHERT	France	> 1960	95	21	28	144
13471	ORNICAR	France	> 1960	184	4	1	189
13481	APACHE	France	> 1960	128	181	161	470
13494	BELLOVAC	France	> 1960	79	91	102	272
13500	ORFIELD	France	> 1960	130	39	20	189
13502	PALIO	France	> 1960	27	13	84	124
13792	CENTURK	USA	> 1960	37	106	148	291
13861	AUGUSTE	France	> 1960	117	159	128	404
13870	TALISMAN	France	> 1960	111	59	59	229
14000	ROKYCANSKA SAMETKA	Czech Republic	Landrace	48	88	123	259
14011	HANA	Czech Republic	> 1960	90	75	90	255
15606	BLE DE REDON BLANC BARBU 1 1	France	Landrace	156	55	15	226
15658	BLE DE REDON BLANC 1/2 LACHE 1 1	France	Landrace	118	183	175	476
15710	BLE DE REDON GLUMES VELUES 1	France	Landrace	146	129	91	366
15950	AS68VM4-3-2/TJB636 13	France	> 1960	82	19	35	136
15954	ASVM4/BEAUCHAMP 81B13	France	> 1960	126	68	49	243
20074	MIRLEBEN	Ukraine	> 1960	164	6	3	173
20224	FANTASIYA-ODESSKAYA	Ukraine	> 1960	ND	ND	ND	ND
20276	EQUINOX	UK or Ireland	> 1960	152	45	11	208
20366	SKERZZO	France	> 1960	149	14	7	170
20384	DI9234-11-15	France	> 1960	75	1	8	84
20417	HAMAC	The Netherlands	> 1960	ND	ND	ND	ND
24031	KRASNAYA	Canada	Landrace	180	87	10	277
24058	SARI-BUGDA	Caucasia	Landrace	175	115	33	323
24066	CROISEMENT 268	Switzerland	≤ 1960	80	43	53	176
24075	SPIN, 121-VAR.12/536	Pakistan	≤ 1960	41	187	187	415
24089	TAU-BUGDA	Caucasia	Landrace	167	107	34	308
24108	ALBIDUM 12	Russia	> 1960	138	126	94	358
24193	LANDRACE	Caucasia	Landrace	171	17	5	193
24196	ARABUGDASI	Caucasia	Landrace	169	177	120	466
24210	LAMMAS	UK or Ireland	Landrace	56	163	166	385
28978	HENDRIX	France	> 1960	84	49	64	197

Supplementary Table S2 Percentages of wheat genotypes showing the highest and lowest values of root colonization by *A. brasilense* Sp245 or *ppdC* expression in root-colonizing Sp245 among the landraces (n = 33), the old varieties (≤ 1960, n = 40) and modern genotypes (>1960, n = 114).

	Landraces	Old varieties (≤ 1960)	Modern genotypes (> 1960)
Root colonization by Sp245			
25 best genotypes	24.2 %	17.5 %	8.8 %
50 best genotypes	42.4 % a[†]	35.0 % ab	19.3 % b
50 worst genotypes	24.2 %	25.0 %	28.1 %
25 worst genotypes	9.1 %	17.5 %	13.2 %
ppdC expression in Sp245			
25 best genotypes	12.1 %	17.5 %	12.3 %
50 best genotypes	27.3 %	35.0 %	23.8 %
50 worst genotypes	15.2 %	25.0 %	30.7 %
25 worst genotypes	6.1 % a	7.5 %	17.5 %

Supplementary Table S3 Relative impact of combined stress on the growth of ten non-inoculated wheat genotypes. Concurrent, Coronation, Amifort, ATUT-II, D130-63 and DI276 are Sp245-stimulating genotypes, Jaszaji TF, Odesskaya 16, Danubia and Hendrix are non Sp245-stimulating genotypes. For each of the eight plant parameters investigated, the relative impacts were computed as (stress – optimum)/optimum. For each genotype, significant differences between stressed 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 (cm^3)	**Fresh root biomass (mg)**	**Dry root biomass (mg)**	**Number of roots**	**Root average diameter (mm)**	**Root length (cm)**	**Fresh shoot biomass (mg)**	**Dry shoot biomass (mg)**
Concurrent	-32.4%	-32.5%	-22.5%	22.6%	-10.3%	20.2%	**-48.8% *****	**-49.4% *****
Coronation	-24.6%	-29.1%	-24.5%	19.7%	**-20.1% *****	-16.4%	**-36.8% ***	-31.3%
Amifort	-20.0%	-16.5%	-21.9%	4.5%	**-19.3% *****	-1.3%	**-54.7% *****	**-56.6% *****
ATUT-II	**-45.5% ****	-24.9%	-18.7%	-1.0%	**-11.9% ***	-50.1%	**-52.0% *****	**-51.1% *****
D130-63	**-39.0% ***	-26.5%	-16.4%	4.0%	8.1%	18.0%	**-49.4% *****	**-43.3% ****
DI276	-39.0%	**-30.1% ***	-24.2%	-34.3%	-2.8%	18.5%	**-45.1% *****	**-47.8% *****
Jaszaji TF	**-47.1% ****	-25.3%	-18.6%	-8.2%	**-23.7% *****	2.1%	**-47.9% *****	**-45.1% *****
Odesskaya 16	-22.9%	-13.7%	-26.1%	2.3%	2.2%	25.4%	**-40.1% ***	**-42.3% ***
Danubia	**-54.8% ****	**-43.7% ****	-17.4%	-13.1%	**-13.4% ***	-17.6%	**-42.3% ****	**-43.6% ****
Hendrix	**-59.0% ****	**-55.1% *****	**-59.2% *****	-6.1%	**-15.5% ***	-37.3%	-19.92%	-16.%

Supplementary Table S4 Relative impact of seed inoculation with *A. brasilense* Sp245 on the growth of ten wheat genotypes under optimum or combined stress conditions. Concurrent, Coronation, Amifort, ATUT-II, D130-63 and DI276 are Sp245-stimulating genotypes, Jaszaji TF, Odesskaya 16, Danubia and Hendrix are non Sp245-stimulating genotypes. For each of the eight 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 (cm^3)	Fresh root biomass (mg)	Dry root biomass (mg)	Number of roots	Root average diameter (mm)	Root length (cm)	Fresh shoot biomass (mg)	Dry shoot biomass (mg)
Optimum	Concurrent	**61.9% ***	32.4%	**38.9% ***	**94.6% ****	4.9%	12.0%	2.9%	3.53%
	Coronation	10.3%	10.1%	8.6%	21.1%	2.5%	1.9%	8.0%	4.4%
	Amifort	37.2%	16.6%	16.6%	48.6%	-0.5%	4.7%	1.0%	-4.4%
	ATUT-II	13.4%	12.2%	7.9%	29.4%	0.6%	4.1%	12.9%	11.8%
	D130-63	-1.7%	3.2%	6.9%	0.8%	1.5%	4.3%	14.8%	9.9%
	DI276	13.9%	14.6%	13.5%	-7.7%	-1.5%	11.5%	13.2%	7.7%
	Jaszaji TF	4.3%	3.0%	-4.4%	1.1%	-4.0%	8.4%	2.1%	-3.8%
	Odesskaya 16	18.6%	17.8%	16.6%	21.3%	2.9%	4.0%	-2.4%	-1.0%
	Danubia	14.4%	7.7%	10.8%	11.6%	2.3%	4.0%	8.6%	10.2%
	Hendrix	5.5%	1.6%	-6.2%	10.2%	-2.3%	9.8%	0.0%	-4.9%
Stress	Concurrent	**75.8% ***	**54.2% ***	**57.0% ****	**80.6% ***	-2.5%	15.4%	9.2%	9.9%
	Coronation	38.4%	19.9%	25.9%	**88.3% ****	2.1%	8.8%	13.5%	15.6%
	Amifort	**49.6% ***	**43.2% ***	**52.2% ***	**86.3% ****	7.9%	14.5%	6.4%	6.3%
	ATUT-II	24.6%	20.9%	28.4%	29.0%	0.7%	7.8%	6.7%	6.9%
	D130-63	7.3%	10.0%	6.8%	6.4%	-5.4%	5.1%	6.9%	16.1%
	DI276	-18.1%	-14.9%	-16.4%	-26.1%	-1.7%	-22.0%	-0.9%	0.9%
	Jaszaji TF	12.4%	13.7%	7.5%	11.1%	0.2%	1.1%	-5.8%	0.6%
	Odesskaya 16	17.8%	17.8%	21.5%	37.4%	-3.5%	1.9%	5.9%	9.3%
	Danubia	12.2%	10.7%	10.2%	18.9%	2.4%	-14.9%	2.4%	3.1%
	Hendrix	4.8%	3.2%	7.1%	10.2%	5.0%	-3.3%	-1.1%	1.7%

Supplementary Table S5 Detection of genetic markers in wheat genome involved in interactions with Sp245. The group number identifies the QTL where the markers are located. Trait indicated the type of results linked to the genetic markers (*ppdC* expression or *ppdC* induction rate). The position corresponds to the physical position of the markers on wheat chromosomes.

Group	Trait	Marker	Chr	Position	A	B	NoMeasured	NbA	NbB	effB	se_effB	p_Marker	$-\log_{10}(p)$	Khi²
1	Sp245.Ind.Moy	AX.89366874_OTV	1A	20008947	A	T	174	164	10	0,91	0,1978	4,10E-06	5,39	0,0127
2	Sp245.Exp.Med	AX.89451818	1B	12806570	T	A	175	145	30	20,66	4,6487	8,82E-06	5,05	0,0666
3	Sp245.Exp.Med	AX.89346256	1B	99685230	C	T	176	164	12	33,94	6,9310	9,75E-07	6,01	0,0462
3	Sp245.Exp.Med	AX.89652072	1B	97345788	T	A	176	162	14	28,98	6,4564	7,16E-06	5,14	0,0714
3	Sp245.Exp.Med	AX.89653443	1B	97215708	G	A	176	162	14	28,98	6,4564	7,16E-06	5,14	0,0714
3	Sp245.Exp.Med	AX.89367916	1B	102353273	T	G	176	163	13	32,14	6,6795	1,49E-06	5,83	0,0906
3	Sp245.Exp.Med	AX.89496064	1B	102397199	A	G	176	163	13	32,14	6,6795	1,49E-06	5,83	0,0906
3	Sp245.Exp.Med	AX.89335073	1B	100287819	C	A	175	163	12	35,33	6,9325	3,47E-07	6,46	0,1887
3	Sp245.Ind.Moy	AX.89482827	1B	106409911	T	G	173	165	8	1,01	0,2199	4,69E-06	5,33	0,6539
4	Sp245.Exp.Med	AX.89373743	1B	667502640	A	G	176	141	35	21,08	4,3769	1,46E-06	5,84	0,0020
4	Sp245.Exp.Med	AX.89475040	1B	667519412	A	G	176	141	35	21,08	4,3769	1,46E-06	5,84	0,0020
4	Sp245.Exp.Med	AX.89340071	1B	667518845	G	C	176	142	34	22,08	4,4251	6,05E-07	6,22	0,0066
4	Sp245.Exp.Med	AX.89415492	1B	667515405	G	C	176	144	32	20,92	4,5295	3,87E-06	5,41	0,0117
4	Sp245.Exp.Med	AX.89462719	1B	650955077	C	T	176	167	9	37,38	7,9310	2,43E-06	5,61	0,1020
5	Sp245.Exp.Med	AX.89757505	2A	123202999	A	G	176	167	9	35,28	7,9310	8,66E-06	5,06	0,6496
5	Sp245.Exp.Med	AX.89715338	2A	120681877	C	T	176	168	8	37,88	8,3871	6,30E-06	5,20	0,9160
6	Sp245.Exp.Med	AX.89544417_OTV	2B	23006356	A	T	176	161	15	28,08	6,2568	7,20E-06	5,14	0,0041
7	Sp245.Exp.Med	AX.89429798	2B	575235683	C	T	176	164	12	33,53	6,9310	1,31E-06	5,88	0,0005
7	Sp245.Exp.Med	AX.89582408	2B	573199626	A	G	176	161	15	30,38	6,2568	1,20E-06	5,92	0,0031
7	Sp245.Exp.Med	AX.89588842	2B	572557343	C	A	176	167	9	40,17	7,9310	4,08E-07	6,39	0,0063
7	Sp245.Exp.Med	AX.89434650	2B	575339365	C	T	176	167	9	41,75	7,9310	1,41E-07	6,85	0,0171
7	Sp245.Exp.Med	AX.89540301	2B	575934051	C	T	173	127	46	19,04	3,9884	1,81E-06	5,74	0,0188
7	Sp245.Exp.Med	AX.89374062	2B	575165223	A	G	176	130	46	18,39	3,9761	3,76E-06	5,42	0,0205
7	Sp245.Exp.Med	AX.89416120	2B	575147373	A	G	176	130	46	18,39	3,9761	3,76E-06	5,42	0,0205
7	Sp245.Exp.Med	AX.89701142	2B	575148227	A	G	176	130	46	18,39	3,9761	3,76E-06	5,42	0,0205
7	Sp245.Exp.Med	AX.89384765	2B	575147497	T	A	176	166	10	36,92	7,5467	9,96E-07	6,00	0,0213
7	Sp245.Exp.Med	AX.89386011	2B	574043333	G	C	176	166	10	36,92	7,5467	9,96E-07	6,00	0,0213
7	Sp245.Exp.Med	AX.89387691	2B	575706535	G	C	176	166	10	36,92	7,5467	9,96E-07	6,00	0,0213
7	Sp245.Exp.Med	AX.89392471	2B	573593898	C	G	176	166	10	36,92	7,5467	9,96E-07	6,00	0,0213
7	Sp245.Exp.Med	AX.89400900	2B	572622617	C	T	176	166	10	36,92	7,5467	9,96E-07	6,00	0,0213
7	Sp245.Exp.Med	AX.89457236	2B	575706596	C	T	176	166	10	36,92	7,5467	9,96E-07	6,00	0,0213
7	Sp245.Exp.Med	AX.89465706	2B	575147405	C	T	176	166	10	36,92	7,5467	9,96E-07	6,00	0,0213
7	Sp245.Exp.Med	AX.89472541	2B	572789475	T	C	176	166	10	36,92	7,5467	9,96E-07	6,00	0,0213
7	Sp245.Exp.Med	AX.89479386	2B	574713488	C	T	176	166	10	36,92	7,5467	9,96E-07	6,00	0,0213
7	Sp245.Exp.Med	AX.89536611	2B	573672989	C	A	176	166	10	36,92	7,5467	9,96E-07	6,00	0,0213
7	Sp245.Exp.Med	AX.89543231	2B	573720522	C	T	176	166	10	36,92	7,5467	9,96E-07	6,00	0,0213
7	Sp245.Exp.Med	AX.89558788	2B	572321667	C	T	176	166	10	36,92	7,5467	9,96E-07	6,00	0,0213
7	Sp245.Exp.Med	AX.89604763	2B	573219613	G	A	176	166	10	36,92	7,5467	9,96E-07	6,00	0,0213
7	Sp245.Exp.Med	AX.89625338	2B	573052357	A	T	176	166	10	36,92	7,5467	9,96E-07	6,00	0,0213
7	Sp245.Exp.Med	AX.89635740	2B	574042114	G	T	176	166	10	36,92	7,5467	9,96E-07	6,00	0,0213
7	Sp245.Exp.Med	AX.89655965	2B	572322661	G	C	176	166	10	36,92	7,5467	9,96E-07	6,00	0,0213
7	Sp245.Exp.Med	AX.89712625	2B	573688492	C	A	176	166	10	36,92	7,5467	9,96E-07	6,00	0,0213

7	Sp245.Exp.Med	AX.89716141	2B	573205718	T	C	176	166	10	36,92	7,5467	9,96E-07	6,00	0,0213
7	Sp245.Exp.Med	AX.89764839	2B	574712159	C	A	176	166	10	36,92	7,5467	9,96E-07	6,00	0,0213
7	Sp245.Exp.Med	AX.89521523	2B	575706667	C	T	175	165	10	36,95	7,5480	9,84E-07	6,01	0,0224
7	Sp245.Exp.Med	AX.89335163	2B	575837447	G	A	174	138	36	22,74	4,3375	1,58E-07	6,80	0,0224
7	Sp245.Exp.Med	AX.89508647	2B	573688461	T	C	174	164	10	36,82	7,5493	1,08E-06	5,97	0,0235
7	Sp245.Exp.Med	AX.89464190	2B	574716544	G	A	176	167	9	36,15	7,9310	5,18E-06	5,29	0,0245
7	Sp245.Exp.Med	AX.89608961	2B	572586442	C	T	176	167	9	36,15	7,9310	5,18E-06	5,29	0,0245
7	Sp245.Exp.Med	AX.89656849	2B	573203394	A	G	176	167	9	41,17	7,9310	2,09E-07	6,68	0,0245
7	Sp245.Exp.Med	AX.89735345	2B	572670087	C	A	176	167	9	41,17	7,9310	2,09E-07	6,68	0,0245
7	Sp245.Exp.Med	AX.89576079	2B	574041955	T	C	176	129	47	18,15	3,9488	4,27E-06	5,37	0,0312
7	Sp245.Exp.Med	AX.89419933	2B	575174058	C	T	173	129	44	18,83	4,0463	3,27E-06	5,49	0,0320
7	Sp245.Exp.Med	AX.89472819	2B	575930503	C	G	176	130	46	17,84	3,9761	7,27E-06	5,14	0,0337
7	Sp245.Exp.Med	AX.89746318	2B	575864365	A	G	176	136	40	18,82	4,1688	6,34E-06	5,20	0,0399
7	Sp245.Exp.Med	AX.89385117	2B	575865205	T	C	175	135	40	18,68	4,1723	7,55E-06	5,12	0,0432
7	Sp245.Exp.Med	AX.89435742	2B	573210497	C	T	176	128	48	17,41	3,9227	9,11E-06	5,04	0,0461
7	Sp245.Exp.Med	AX.89451317	2B	573205653	C	T	176	128	48	17,41	3,9227	9,11E-06	5,04	0,0461
7	Sp245.Exp.Med	AX.89474485	2B	573205674	C	T	176	128	48	17,41	3,9227	9,11E-06	5,04	0,0461
7	Sp245.Exp.Med	AX.89546023	2B	573207780	T	G	176	128	48	17,41	3,9227	9,11E-06	5,04	0,0461
7	Sp245.Exp.Med	AX.89701067	2B	572314013	T	C	176	136	40	20,09	4,1688	1,44E-06	5,84	0,0632
7	Sp245.Exp.Med	AX.89338777	2B	574710599	A	G	176	127	49	17,38	3,8977	8,19E-06	5,09	0,0662
7	Sp245.Exp.Med	AX.89343018	2B	575149623	T	C	176	127	49	17,38	3,8977	8,19E-06	5,09	0,0662
7	Sp245.Exp.Med	AX.89345560	2B	574693828	T	C	176	127	49	17,38	3,8977	8,19E-06	5,09	0,0662
7	Sp245.Exp.Med	AX.89351473	2B	575377838	T	C	176	127	49	17,38	3,8977	8,19E-06	5,09	0,0662
7	Sp245.Exp.Med	AX.89417406	2B	574713427	C	T	176	127	49	17,38	3,8977	8,19E-06	5,09	0,0662
7	Sp245.Exp.Med	AX.89426922	2B	575171812	A	G	176	127	49	17,38	3,8977	8,19E-06	5,09	0,0662
7	Sp245.Exp.Med	AX.89477474	2B	574715971	A	G	176	127	49	17,38	3,8977	8,19E-06	5,09	0,0662
7	Sp245.Exp.Med	AX.89584393	2B	574712834	A	G	176	127	49	17,38	3,8977	8,19E-06	5,09	0,0662
7	Sp245.Exp.Med	AX.89589714	2B	575149606	A	T	176	127	49	17,38	3,8977	8,19E-06	5,09	0,0662
7	Sp245.Exp.Med	AX.89593657	2B	575385248	A	G	176	127	49	17,38	3,8977	8,19E-06	5,09	0,0662
7	Sp245.Exp.Med	AX.89626581	2B	575115755	C	A	176	127	49	17,38	3,8977	8,19E-06	5,09	0,0662
7	Sp245.Exp.Med	AX.89743901	2B	575176667	G	C	176	127	49	17,38	3,8977	8,19E-06	5,09	0,0662
7	Sp245.Exp.Med	AX.89698767	2B	575933438	T	C	176	153	23	24,44	5,1832	2,41E-06	5,62	0,0912
7	Sp245.Exp.Med	AX.89719394	2B	575877955	T	G	176	127	49	17,86	3,8977	4,62E-06	5,34	0,0998
7	Sp245.Exp.Med	AX.89711832	2B	582645061	G	A	176	164	12	32,61	6,9310	2,55E-06	5,59	0,3777
7	Sp245.Exp.Med	AX.89497267	2B	581582604	T	C	176	163	13	30,71	6,6795	4,27E-06	5,37	0,5379
8	Sp245.Exp.Med	AX.89622005	2B	688374113	C	T	176	168	8	37,88	8,3871	6,30E-06	5,20	0,0095
8	Sp245.Exp.Med	AX.89479632	2B	742692170	G	C	176	154	22	25,01	5,2825	2,20E-06	5,66	0,4508
8	Sp245.Ind.Moy	AX.89668550	2B	674029409	G	T	174	165	9	0,99	0,2079	2,00E-06	5,70	0,6496
9	Sp245.Ind.Moy	AX.89757265	2B	759178940	C	T	174	162	12	0,84	0,1817	3,73E-06	5,43	0,2448
9	Sp245.Ind.Moy	AX.89511582	2B	757386253	T	C	174	162	12	0,81	0,1817	7,85E-06	5,10	0,8877
9	Sp245.Ind.Moy	AX.89431594	2B	757452859	C	T	174	163	11	0,86	0,1892	5,71E-06	5,24	0,9574
9	Sp245.Ind.Moy	AX.89441916	2B	758112233	C	T	174	163	11	0,86	0,1892	5,71E-06	5,24	0,9574
9	Sp245.Ind.Moy	AX.89526336	2B	758556708	G	A	174	163	11	0,86	0,1892	5,71E-06	5,24	0,9574
10	Sp245.Exp.Med	AX.89388511	2D	490565246	G	A	176	127	49	17,38	3,8977	8,19E-06	5,09	0,0662
11	Sp245.Exp.Med	AX.89467602	3A	405321848	A	G	176	152	24	24,26	5,0908	1,88E-06	5,73	0,0218
12	Sp245.Exp.Med	AX.89352119	3A	644202467	A	G	176	161	15	27,85	6,2568	8,52E-06	5,07	0,6946
13	Sp245.Ind.Moy	AX.89343547	3B	695562054	G	A	174	166	8	1,12	0,2199	3,48E-07	6,46	0,8638
13	Sp245.Ind.Moy	AX.89536337	3B	695589555	G	C	174	166	8	1,12	0,2199	3,48E-07	6,46	0,8638

13	Sp245.Ind.Moy	AX.89548989	3B	695592578	C	A	174	166	8	1,12	0,2199	3,48E-07	6,46	0,8638
13	Sp245.Ind.Moy	AX.89648213	3B	695589428	G	A	174	166	8	1,12	0,2199	3,48E-07	6,46	0,8638
13	Sp245.Ind.Moy	AX.89710913	3B	695962596	A	G	174	166	8	1,12	0,2199	3,48E-07	6,46	0,8638
14	Sp245.Exp.Med	AX.89500210	4A	699885775	G	A	175	147	28	21,42	4,7790	7,41E-06	5,13	0,0019
15	Sp245.Exp.Med	AX.89625435	5B	560510249	C	T	176	160	16	27,76	6,0770	4,91E-06	5,31	0,5118
15	Sp245.Ind.Med	AX.89625435	5B	560510249	C	T	174	158	16	0,56	0,1196	2,38E-06	5,62	0,5118
15	Sp245.Ind.Med	AX.89467143	5B	560451114	T	C	174	157	17	0,52	0,1164	7,97E-06	5,10	0,5848
15	Sp245.Ind.Med	AX.89525713	5B	562280657	G	C	174	151	23	0,47	0,1021	4,31E-06	5,37	0,7741
15	Sp245.Exp.Med	AX.89746797	5B	560744181	G	A	176	162	14	33,62	6,4564	1,92E-07	6,72	0,9718
15	Sp245.Ind.Med	AX.89746797	5B	560744181	G	A	174	160	14	0,66	0,1271	1,94E-07	6,71	0,9718
16	Sp245.Exp.Med	AX.89673138	6A	51992351	A	G	176	156	20	25,90	5,5047	2,54E-06	5,60	0,0233
16	Sp245.Exp.Med	AX.89531749	6A	51953289	C	G	176	157	19	26,27	5,6297	3,06E-06	5,51	0,0394
16	Sp245.Exp.Med	AX.89730699	6A	51951693	T	C	176	157	19	26,27	5,6297	3,06E-06	5,51	0,0394
16	Sp245.Exp.Med	AX.89734201	6A	51951424	C	A	176	157	19	26,27	5,6297	3,06E-06	5,51	0,0394
16	Sp245.Exp.Med	AX.89488865	6A	51947725	C	T	176	142	34	19,58	4,4251	9,70E-06	5,01	0,8022
16	Sp245.Ind.Med	AX.89488865	6A	51947725	C	T	174	142	32	0,40	0,0892	7,23E-06	5,14	0,8022
17	Sp245.Ind.Moy	AX.89586813	6A	540840475	C	T	174	162	12	0,84	0,1817	3,74E-06	5,43	0,4327
18	Sp245.Ind.Moy	AX.89708135	6A	609172967	G	A	174	160	14	0,77	0,1693	6,18E-06	5,21	0,5158
19	Sp245.Ind.Moy	AX.89361492	6B	159410401	G	A	174	163	11	0,86	0,1892	5,71E-06	5,24	0,9574
20	Sp245.Exp.Med	AX.89772533	6B	704303112	A	G	176	151	25	23,29	5,0044	3,24E-06	5,49	0,0350
20	Sp245.Exp.Med	AX.89698906	6B	704507398	C	T	176	146	30	21,45	4,6459	3,91E-06	5,41	0,1764
20	Sp245.Exp.Med	AX.89447957	6B	704505286	A	G	176	154	22	26,83	5,2825	3,81E-07	6,42	0,1796
20	Sp245.Ind.Moy	AX.89410503	6B	704504279	C	T	174	161	13	0,79	0,1751	5,68E-06	5,25	0,2027
20	Sp245.Ind.Moy	AX.89503405	6B	704504339	T	C	173	160	13	0,79	0,1752	5,70E-06	5,24	0,2128
20	Sp245.Exp.Med	AX.89585079	6B	704153437	C	T	176	156	20	24,87	5,5047	6,25E-06	5,20	0,2370
20	Sp245.Exp.Med	AX.89438241	6B	704188924	G	C	176	167	9	38,07	7,9310	1,58E-06	5,80	0,3121
20	Sp245.Exp.Moy	AX.89438241	6B	704188924	G	C	176	167	9	49,34	10,9991	7,28E-06	5,14	0,3121
20	Sp245.Ind.Moy	AX.89438241	6B	704188924	G	C	174	165	9	0,95	0,2079	4,77E-06	5,32	0,3121
20	Sp245.Ind.Med	AX.89667199	6B	704860511	C	T	174	159	15	0,56	0,1232	5,72E-06	5,24	0,4507
20	Sp245.Ind.Moy	AX.89667199	6B	704860511	C	T	174	159	15	0,79	0,1641	1,49E-06	5,83	0,4507
20	Sp245.Ind.Med	AX.89405827	6B	704964327	G	A	174	159	15	0,57	0,1232	3,50E-06	5,46	0,4926
20	Sp245.Ind.Moy	AX.89405827	6B	704964327	G	A	174	159	15	0,80	0,1641	1,20E-06	5,92	0,4926
20	Sp245.Ind.Med	AX.89590209	6B	704859756	A	T	174	159	15	0,55	0,1232	8,99E-06	5,05	0,4926
20	Sp245.Ind.Moy	AX.89590209	6B	704859756	A	T	174	159	15	0,78	0,1641	2,08E-06	5,68	0,4926
20	Sp245.Ind.Moy	AX.89573171	6B	704703913	A	T	174	161	13	0,83	0,1751	2,44E-06	5,61	0,5146
20	Sp245.Ind.Moy	AX.89668441	6B	703206711	G	A	174	161	13	0,83	0,1751	2,44E-06	5,61	0,5146
20	Sp245.Exp.Med	AX.89712251	6B	704884186	T	C	176	163	13	30,66	6,6795	4,42E-06	5,35	0,5146
20	Sp245.Exp.Med	AX.89761693	6B	705138687	T	C	176	163	13	30,66	6,6795	4,42E-06	5,35	0,5146
20	Sp245.Ind.Moy	AX.89321148	6B	704888102	T	A	174	160	14	0,77	0,1693	6,18E-06	5,21	0,5158
20	Sp245.Ind.Moy	AX.89357867	6B	705281356	A	G	174	160	14	0,77	0,1693	6,18E-06	5,21	0,5158
20	Sp245.Ind.Moy	AX.89358001	6B	704750791	G	T	174	160	14	0,77	0,1693	6,18E-06	5,21	0,5158
20	Sp245.Ind.Moy	AX.89379712	6B	704884025	C	A	174	160	14	0,77	0,1693	6,18E-06	5,21	0,5158
20	Sp245.Ind.Moy	AX.89393793	6B	703297869	C	T	174	160	14	0,77	0,1693	6,18E-06	5,21	0,5158
20	Sp245.Ind.Moy	AX.89411948	6B	704885912	A	G	174	160	14	0,77	0,1693	6,18E-06	5,21	0,5158
20	Sp245.Ind.Moy	AX.89421975	6B	704945102	T	C	174	160	14	0,77	0,1693	6,18E-06	5,21	0,5158
20	Sp245.Ind.Moy	AX.89428743	6B	704551062	C	T	174	160	14	0,77	0,1693	6,18E-06	5,21	0,5158
20	Sp245.Ind.Moy	AX.89471662	6B	703833358	C	T	174	160	14	0,77	0,1693	6,18E-06	5,21	0,5158
20	Sp245.Ind.Moy	AX.89474943	6B	704789532	G	A	174	160	14	0,77	0,1693	6,18E-06	5,21	0,5158

20	Sp245.Ind.Moy	AX.89490161	6B	703217330	T	C	174	160	14	0,77	0,1693	6,18E-06	5,21	0,5158
20	Sp245.Ind.Moy	AX.89496681	6B	704948594	G	A	174	160	14	0,77	0,1693	6,18E-06	5,21	0,5158
20	Sp245.Ind.Moy	AX.89502932	6B	704304337	T	C	174	160	14	0,77	0,1693	6,18E-06	5,21	0,5158
20	Sp245.Ind.Moy	AX.89509555	6B	704948613	T	A	174	160	14	0,77	0,1693	6,18E-06	5,21	0,5158
20	Sp245.Ind.Moy	AX.89526184	6B	704964066	C	T	174	160	14	0,77	0,1693	6,18E-06	5,21	0,5158
20	Sp245.Ind.Moy	AX.89550611	6B	704885557	T	C	174	160	14	0,77	0,1693	6,18E-06	5,21	0,5158
20	Sp245.Ind.Moy	AX.89570443	6B	704506835	T	C	174	160	14	0,77	0,1693	6,18E-06	5,21	0,5158
20	Sp245.Ind.Moy	AX.89587148	6B	704882333	C	G	174	160	14	0,77	0,1693	6,18E-06	5,21	0,5158
20	Sp245.Ind.Moy	AX.89627592	6B	704881961	C	G	174	160	14	0,77	0,1693	6,18E-06	5,21	0,5158
20	Sp245.Ind.Moy	AX.89683104	6B	704651610	G	A	174	160	14	0,77	0,1693	6,18E-06	5,21	0,5158
20	Sp245.Ind.Moy	AX.89725357	6B	704302682	A	G	174	160	14	0,77	0,1693	6,18E-06	5,21	0,5158
20	Sp245.Ind.Moy	AX.89733441	6B	704975136	G	A	174	160	14	0,77	0,1693	6,18E-06	5,21	0,5158
20	Sp245.Ind.Moy	AX.89735767	6B	704948263	C	T	174	160	14	0,77	0,1693	6,18E-06	5,21	0,5158
20	Sp245.Ind.Moy	AX.89739667	6B	704153649	G	A	174	160	14	0,77	0,1693	6,18E-06	5,21	0,5158
20	Sp245.Ind.Moy	AX.89745923	6B	703284282	G	A	174	160	14	0,77	0,1693	6,18E-06	5,21	0,5158
20	Sp245.Exp.Med	AX.89414544	6B	704188952	T	C	176	157	19	27,80	5,6297	7,86E-07	6,10	0,5723
20	Sp245.Exp.Moy	AX.89414544	6B	704188952	T	C	176	157	19	34,69	7,8074	8,84E-06	5,05	0,5723
20	Sp245.Exp.Med	AX.89354527	6B	704882809	C	T	176	163	13	31,94	6,6795	1,74E-06	5,76	0,6120
20	Sp245.Ind.Moy	AX.89354527	6B	704882809	C	T	174	161	13	0,84	0,1751	1,70E-06	5,77	0,6120
20	Sp245.Exp.Med	AX.89426825	6B	704787297	C	T	176	163	13	31,94	6,6795	1,74E-06	5,76	0,6120
20	Sp245.Ind.Moy	AX.89426825	6B	704787297	C	T	174	161	13	0,84	0,1751	1,70E-06	5,77	0,6120
20	Sp245.Ind.Moy	AX.89438186	6B	705311811	G	A	174	156	18	0,67	0,1512	9,44E-06	5,03	0,6588
20	Sp245.Exp.Med	AX.89331766	6B	704302490	G	C	176	161	15	29,07	6,2568	3,39E-06	5,47	0,8336
20	Sp245.Exp.Med	AX.89524992	6B	704303434	A	G	176	161	15	29,07	6,2568	3,39E-06	5,47	0,8336
20	Sp245.Exp.Med	AX.89580771	6B	704175509	A	G	176	161	15	29,07	6,2568	3,39E-06	5,47	0,8336
20	Sp245.Exp.Med	AX.89622911	6B	704303477	A	G	176	161	15	29,07	6,2568	3,39E-06	5,47	0,8336
20	Sp245.Exp.Med	AX.89499199	6B	704303414	C	T	176	158	18	29,16	5,7656	4,25E-07	6,37	0,8539
20	Sp245.Exp.Moy	AX.89499199	6B	704303414	C	T	176	158	18	36,41	7,9960	5,28E-06	5,28	0,8539
20	Sp245.Ind.Moy	AX.89314150	6B	705314123	G	C	174	155	19	0,67	0,1476	4,88E-06	5,31	0,8704
20	Sp245.Exp.Med	AX.89448288	6B	704302054	A	T	176	160	16	27,43	6,0770	6,39E-06	5,19	0,9266
20	Sp245.Exp.Med	AX.89635801	6B	703285922	G	T	176	161	15	30,37	6,2568	1,21E-06	5,92	0,9284
21	Sp245.Exp.Med	AX.89512638	7D	93555477	T	C	176	160	16	27,24	6,0770	7,37E-06	5,13	0,3385
21	Sp245.Exp.Med	AX.89644216	7D	93028858	A	G	176	160	16	27,24	6,0770	7,37E-06	5,13	0,3385
21	Sp245.Exp.Med	AX.89536477	7D	93553937	A	T	176	161	15	30,72	6,2568	9,13E-07	6,04	0,5848
21	Sp245.Exp.Med	AX.89622716	7D	93045033	T	A	176	160	16	27,87	6,0770	4,51E-06	5,35	0,5848

Partie 4

Field analysis of rhizosphere interactions of wheat genotypes stimulating the PGPR *Pseudomonas kilonensis* F113 and *Azospirillum brasilense* Sp245 with microbial functional groups relevant for plant growth

Preamble

In realistic conditions, the use of PGPR inoculant in field can show inconsistent results, notably because of competition with indigenous soil bacteria and due to abiotic soil features [1, 2]. Therefore, the use of crop varieties able to greatly interact with indigenous PGPR, in field, seems to be the best alternative. The previous results of these thesis already showed wheat genotypes with contrasted ability to interact with *Pseudomonas kilonensis* F113 and *Azospirillum brasilense* Sp245. Thus, it could be expected that these genotypes will be associated to different bacterial communities in field, notably regarding the genera *Azospirillum* and *Pseudomonas*. Moreover, if these genotypes present contrasted abilities to interact with PGPR, they should be associated with contrasted abundance and/or diversity of bacteria associated to functional groups involved in positive effect on plant growth.

The results of the inoculation of model PGPR in gnotobiotic experiment and under greenhouse conditions on the roots of a collection of 199 wheat genotypes evidence a negative impact of modern breeding on the interactions between crops and PGPR. Thus, one can also suggest that modern genotypes may have different root-associated bacteria than ancient genotypes, and therefore that ancient and modern genotypes sown in a same field may display different associated bacterial community.

To challenge these two hypotheses, four genotypes, including two modern F113/Sp245-stimulating genotypes and two modern non F113/Sp245-stimulating genotypes have been sown in an experimental field near Montpellier. In another site, near Clermont-Ferrand, eight genotypes, including the same genotypes and also two ancient F113/Sp245-stimulating genotypes and two ancient non F113/Sp245-stimulating genotypes has been sown. To assess the impact of environmental conditions, a combined stress (drought and nutrient-deficiency) has been applied for half of the plots, since it has been showed that nitrogen and water content can impact the soil bacterial communities [3, 4] and that artificial condition could lead to the selection of less mutualistic bacteria [5].

I contributed to the two harvests of plants in March (tillering stage) and May (flowering stage) in Clermont-Ferrand. Then, the abundance of four bacterial functional groups known to be involved in amelioration of plant performance, *i.e.* diazotrophs [6], 1-aminocyclopropane-1-carboxylic acid [ACC]-deaminase producers [7], phloroglucinol producers [8] and indole-3-acetic acid [IAA] producers [9], has been assessed by quantitative PCR with the help of a technician student that I supervised. I also tested several RNA extraction procedures in order to perform transcriptional studies, but none procedure succeeded to extract enough RNA quantity in a reproducible way.

Furthermore, at Clermont-Ferrand, the composition of two of these bacterial functional groups (diazotrophs and ACC-deaminase producers) as well as the composition of the total bacterial community (*rrs* gene) associated to the rhizosphere of the eight genotypes at the flowering stage has been analyzed using

high throughput sequencing. I participate to the analyses of sequencing results with the help of Danis Abrouk (LEM's iBio platform).

Results of this work are presented in a manuscript that is in preparation.

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Field analysis of rhizosphere interactions of wheat genotypes stimulating the PGPR *Pseudomonas kilonensis* F113 and *Azospirillum brasilense* Sp245 with microbial functional groups relevant for plant growth

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Running title: Wheat genotypes and indigenous PGPR

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Abstract

Taking advantage of Plant Growth-Promoting Rhizobacteria (PGPR) naturally occurring in fields is a promising alternative to the use of synthetic fertilizers. In this study, bread wheat genotypes previously selected based on their ability to stimulate or not the PGPR *Pseudomonas kilonensis* F113 and *Azospirillum brasilense* Sp245 were compared in two fields and under contrasted agronomic conditions, to test the hypothesis that F113/Sp245 stimulating genotypes would select higher numbers or diversity of indigenous bacteria important for plant growth, in comparison with non F113/Sp245 stimulating genotypes. Quantitative PCR analysis of diazotrophs (harboring *nifH* gene), 2,4-diacetylphloroglucinol producers (*phlD*), bacteria with ACC deaminase potential (*acdS*) or IAA producers via the indole pyruvate pathway (*ppdC*) in the rhizosphere did not evidence any significant difference between both types of wheat genotypes. A similar conclusion was made with Illumina sequencing of diazotrophs and the total bacterial community carried out at flowering in one field. In contrast, differences in microbial diversity were found when considering the same genotypes in terms of their modern vs ancient status, or the impact of agronomic conditions.

Introduction

Certain microorganisms isolated from the soil can enhance crop performance once used as inoculants [1–3]. This includes Plant Growth-Promoting Rhizobacteria (PGPR), which colonize the rhizosphere and/or roots and have been extensively studied for their ability to enhance plant development and/or nutrition [4], alleviate abiotic stress such as heat and drought [5–7], and protect plants from pathogens [8, 9].

PGPR inoculation may lead to inconsistent results [10, 11], which can be due to ineffective pairing of crop × PGPR [12–14]. This interaction specificity/affinity is substantiated by genotype-dependent molecular effects between partners [14–17]. Accordingly, different varieties of a same plant species may select different bacterial communities or functional groups in their rhizosphere [18–20]. Thus, it appears that to be reproducible, the use of PGPR inoculants in the field would require the use of appropriate crop genotype(s), able to interact with these particular PGPR strains. Against this background, it appears that certain crop genotypes are able to interact successfully with a wide range of PGPR, whereas others are poorly effective even when tested with different types of PGPR [14].

Recently, using about 200 bread wheat (*Triticum aestivum*) accessions, subsampled from the worldwide INRA collection of Balfourier *et al.* [21], we have found wheat genotype-dependent differences in root colonization of the model PGPR *Pseudomonas kilonensis* F113 and *Azospirillum brasilense* Sp245, and in expression of respectively their genes *phl* (2,4-diacetylphloroglucinol synthesis) and *ppdC* (auxin synthesis) [22, 23]. As the collection included both ancient and modern wheat genotypes, the investigations also showed that the ability to interact with these PGPR was more prevalent among ancient than modern wheat genotypes, even though some of the latter were quite effective. It suggested that modern breeding, which (i) is typically carried out under agronomic conditions artificially fulfilling plant requirements [24] and (ii) tends to focus on fertilizer acquisition and disease resistance [25], may not have favored the genotypes with high affinity with indigenous symbiotic bacteria (Engelhard *et al.* 2000; Kiers *et al.* 2007) [26, 27]. However, these studies targeted only two PGPR strains and two phytostimulation functions, which is very limited when considering the diversity of PGPR strains and phytostimulation functions [8, 28, 29]. In addition, even though the corresponding taxa can be readily evidenced in farm soils [30, 31], strains present in a given soil probably differ from strains F113 and Sp245. Therefore, it questions the ecological relevance of the results of Valente *et al.* [22, 23] in terms of the interaction of wheat with indigenous PGPR populations.

The objective of this work was to assess whether wheat genotypes stimulating the PGPR *P. kilonensis* F113 and *A. brasilense* Sp245 differed in their ability to interact with indigenous microorganisms harboring phytostimulation-relevant genes, when compared with wheat genotypes stimulating neither F113 nor Sp245. To this end, we grew four F113/Sp245-stimulating genotypes (two ancient and two ancient genotypes) and four non F113/Sp245-stimulating genotypes (two ancient and two ancient genotypes) in one field site (Crouël), as well as two F113/Sp245-stimulating and two non F113/Sp245-stimulating genotypes (all four ancient) in

another field site (Mauguio). Monitoring of indigenous microbial populations relevant for plant growth focused on two functional groups to which *P. kilonensis* F113 belongs, *i.e.* (i) the producers of the root-branching signal 2,4-diacetylphloroglucinol [32, 33], and (ii) the consumers of 1-aminocyclopropane-1-carboxylate (ACC), which interfere with ethylene plant metabolism [34, 35], as well as two functional groups to which *A. brasilense* Sp245 belongs, *i.e.* the producers of the phytohormone indole-3-acetic acid (IAA) via the phenylpyruvate decarboxylase of the indole pyruvic acid pathway [36], and the nitrogen fixers [18]. The abundance of the four microbial functional groups was investigated at tillering and flowering, based on quantitative PCR of the corresponding marker genes *phlD*, *acdS, ppdC* and *nifH.* This was completed by Illumina sequencing of the members of one of the groups (nitrogen fixers) as well as the total bacterial community (using the 16S rRNA gene *rrs*) at flowering in Crouël.

Material and methods

Field experiments

The first experiment was conducted at the Pheno3C phenotyping field platform of INRA GDEC in Crouël, near Clermont-Ferrand (France) between November 2017 and May 2018. There were four blocks, and in each block half the plots were located in an area equipped with a mobile shelter that was deployed from March to May 2018 each time it rained (drought conditions) and did not receive N fertilizer, whereas the other half was located in another area nearby and received rain and irrigation water and N fertilizers. In each of these eight areas, the different varieties were randomly distributed as small plots.

The second field experiment was carried out at INRA Domaine de Melgueil in Mauguio, near Montpellier (France) between November 2017 and May 2018. There were three blocks, and in one half of each block the plots received only rain water and no N fertilizer while in the other half the plots received both rain and irrigation water and N fertilizers. In each half of a given block, the different varieties were randomly distributed as small plots.

Wheat accessions

The Crouël experiment was run with four accessions stimulating the PGPR *P. kilonensis* F113 and *A. brasilense* Sp245 in vitro (referred to as F113/Sp245-stimulating), *i.e.* the two ancient varieties (selected before 1960) Concurrent and Coronation and the two modern varieties (selected after 1960) Amifort and D130-63, as well as four accessions not stimulating the two PGPR in vitro (referred to as non F113/Sp245-stimulating), *i.e.* the two ancient varieties Jaszaji TF and Odesskaya 16 and the two modern varieties Danubia and Hendrix (**Table 1**). The Mauguio experiment only included the four modern varieties, *i.e.* Amifort and D130-63 (F113/Sp245-stimulating) and Danubia and Hendrix (non F113/Sp245-stimulating).

Rhizosphere samplings

In both experiments, two samplings were carried out, *i.e.* at the tillering stage (March 14th in Crouël and March 9th in Mauguio; before nitrogen fertilizers were added) and at the flowering (May 18th in Crouël and May 7th in Mauguio). For the March samplings, only the plots under optimum conditions were sampled because nitrogen/drought stress conditions had not started yet. At each sampling, two plants were taken per plot studied (*i.e.* eight and six plants per condition and per variety in Crouël and Mauguio, respectively). They were unearthed (from the first 20-30 cm) and shaken to remove non-adherent soil, then three tillers from each plant were severed and carefully wrapped to determine fresh biomass, whereas root fragments (and their tightly-adhering soil; *i.e.* rhizosphere soil) taken from near-surface, intermediate and deeper parts of the root systems were placed into 50-mL Falcon tubes. Two bulk soil samples were collected at the same depths, near each series of plots (making eight and six bulk soil samples per condition in Crouël and Mauguio, respectively). Rhizosphere and bulk soil samples were flash-frozen in liquid nitrogen at the field sites, lyophilized for 48 hours at -50°C and stored at -20°C. Rhizosphere soil was separated from the roots by vigorous shaking of tubes for 5 min with a Vortex, and the residual root fragments were removed and discarded.

DNA extraction from soil

DNA in 300 mg of each lyophilized bulk soil or rhizosphere sample was extracted using the FastDNA Spin kit (MP Biomedicals, Santa Ana, CA) and eluted in 100 µL of pre-heated DES solution according to the manufacturer's guideline. DNA concentration was assessed using the Picrogreen kit (Thermo Fisher Scientific, Waltham, MA) and diluted 10-fold in PCR-grade water.

Real-time PCR assessments of microbial functional groups

The size of four microbial functional groups was assessed using quantitative PCR methods, using the eight DNA extracts available per genotype × condition combination and primers acdSF5/acdSR8 [34] for *acdS* (ACC deaminase producers), polF/polR [37] for *nifH* (N fixers), B2BF/B2BR3 [38] for *phlD* (2,4-diacetylphloroglucinol producers) and F3ppdC/R3ppdC (Oudot, Moënne-Loccoz and Muller, unpublished) for *ppdC* (IAA producers). Briefly, the reaction mix was composed of PCR-grade water (4 µL for *acdS*, 6 µL for *nifH*, 5.4 µL for *phlD* and 5.3 µL for *ppdC*), the primers (respectively 2 µL, 1 µL, 1 µL and 1 µL of each, for a final concentration of 1 µM, 0.5 µM, 0.5 µM and 0.5 µM), 10 µL of SYBR Green I Master mix (Roche Applied Science, Penzberg, Germany), and also 0.6 µL of DMSO for *phlD* and *ppdC*, and 0.1 µL of T4g32 protein (final quantity 0.5 µg) for *ppdC*. For each reaction, 2 µL of previously diluted DNA were used. The PCR was done using the following program : 10 min at 95°C, then 50 cycles of (i) 94°C for 15 s (*acdS*), 95°C for 15 s (*nifH* and *ppdC*), 94°C for 30 s (*phlD*), (ii) 66°C for 15 s (*acdS*), 64°C for 15 s (*nifH*), 67°C for 7 s (*phlD*), 63°C for 15 s (*ppdC*), and (iii) 72°C for 15 s (*acdS* and *phl*D), 72°C for 10 sec (*nifH* and *ppdC*). The Light-Cycler Software (Roche Applied Science) was used to

perform melting curve calculation and Tm determination besides the real-time PCR quantification. The data were expressed as number of gene copies per g of dry soil and log10 transformed.

Illumina sequencing

Sequencing was performed on samples of the Crouël experiment at the flowering stage, using six DNA extracts available (*i.e.* from three of four blocks) per genotype × condition combination. Illumina MiSeq sequencing (2 x 300 bp for *rrs* and *nifH*) was carried out by Molecular Research DNA laboratory (Shallowater, TX). Primers 341F/785R [39] were used for the V3/V4 variable region of *rrs* genes and primers polF/polR for *nifH*, with a barcode on each forward primer. A 28-cycle PCR (5 cycles implemented on PCR products) was performed with HotStarTaq Plus Master Mix (Qiagen, Valencia, CA): 94°C for 3 min, then 28 cycles of 94°C for 30 s, 53°C for 40 s and 72°C for 1 min, and a final elongation step at 72°C for 5 min. Amplification success and relative band intensity of the PCR products were checked on 2% agarose gel. Based on their DNA concentrations and molecular weight, multiple samples were pooled together in equal proportions. A purification of the pooled samples was performed using calibrated Ampure XP beads, in order to use them to prepare a DNA library following Illumina TruSeq DNA library preparation protocol.

Analysis of Illumina sequences

The analysis pipeline of Molecular Research DNA was used to process sequence data. Sequences were depleted of barcodes, discarded when < 300 bp or with ambiguous base calls, and denoised. Operational taxonomic units (OTUs) were defined (clustered at 3% divergence, *i.e.* 97% similarity), and singletons and chimera were removed. Taxonomic classification of final OTUs was processed using BLASTN against a curated database derived from NCBI (www.ncbi.nlm.nih.gov), Greengenes (DeSantis *et al.* 2006) and RDPII (http://rdp.cme.msu.edu). Taxa represented by an average of less than 10 sequences per million in the six samples were removed from the list. Venn diagrams were generated to depict differences in OTU distribution, based on OTUs found in ≥ 50% of a given condition or genotype category but totally absent from the other condition or genotype category, as well as identify common OTUs, based on OTUs found in ≥ 50% of the samples.

Statistics

Quantitative PCR data for *acdS* and *nifH* genes were normalized using a box-cox transformation and compared using one-way (at tillering) or two-way (genotype × optimum/stress) ANOVA followed by pairwise comparisons with Benjamini-Hochberg correction. Quantitative PCR data for *phlD* and *ppdC* could not been normalized and were compared using non-parametric Kruskal-Wallis rank sum tests followed by Conor-Iman

Table 1 Wheat genotypes used in this study. Some of these genotypes stimulated the PGPR *Pseudomonas kilonensis* F113 and *Azospirillum brasilense* Sp245 *in vitro*, whereas the others did not. They include ancient genotypes (*i.e.* selected before 1960) or modern genotypes (*i.e.* selected after 1960)

ERGE code	Wheat genotype	Stimulation of F113 and Sp245 *in vitro*	Genotype category
901	Amifort	Yes	> 1960 (modern)
2399	D130-63	Yes	> 1960 (modern)
2424	Danubia	No	> 1960 (modern)
28978	Hendrix	No	> 1960 (modern)
8073	Coronation	Yes	≤ 1960 (ancient)
13292	Concurrent	Yes	≤ 1960 (ancient)
3912	Jaszaji TF	No	≤ 1960 (ancient)
5438	Odesskaya 16	No	≤ 1960 (ancient)

tests for pairwise comparisons associated with Bonferroni correction. Statistical analyses of quantitative PCR data were performed using Xlstat software v2018.4 (Addinsoft, Bordeaux, France) at $P < 0.05$ level.

Comparison of bacterial community composition between genotypes or conditions was carried out by Between-Class Analysis (BCA), using ADE4 R and ggplot2 packages [39]. Class significance was assessed with Monte-Carlo test using 1000 permutations, and the 20 genera contributing most to genotype or condition differentiation were identified. In addition, a differential analysis based on a pairwise comparison approach between genotypes and between conditions was performed to identify OTUs whose abundance significantly differed. For each gene sequenced, Shannon and Chao diversity indices were computed. Statistical analyses of sequencing data were performed using R v3.1.3, at $P < 0.01$ for the differential analysis and $P < 0.05$ otherwise.

Results

Abundance of acdS microorganisms

The number of *acdS* copies was higher in the rhizosphere than bulk soil at tillering in Crouël ($P < 0.01$) and at both samplings in Mauguio ($P < 0.01$) (**Table 2**). At tillering, there was a significant impact of wheat genotype ($P < 0.001$) in Crouël, with lower and higher *acdS* numbers for respectively the ancient genotypes Concurrent (F113/Sp245-stimulating) and Odesskaya 16 (non F113/Sp245-stimulating) than all other genotypes ($P < 0.05$). In Mauguio also, the number of *acdS* copies at tillering was significantly affected by wheat genotype ($P < 0.01$), but with small differences (*i.e.* well below 0.3 log) between genotypes. At flowering, the effect of wheat genotype was significant in Crouël ($P < 0.001$) and Mauguio ($P < 0.01$), and the effect of stress was not significant. In Crouël, *acdS* levels were lower for Hendrix under optimum conditions than for Concurrent and Coronation (under optimum conditions), Odesskaya 16 (under both conditions), and Danubia (under stress). In Mauguio, *acdS* levels were lower for D130-63 than for Amifort under optimum condition, and Danubia under stress.

Abundance of nifH microorganisms

The number of *nifH* copies was higher in the rhizosphere than bulk soil at tillering in Crouël ($P < 0.01$) except for Concurrent, and at tillering ($P < 0.01$) except for Amifort and flowering ($P < 0.05$) in Mauguio (**Table 2**). At tillering, there was a significant impact of wheat genotype on the abundance of *nifH* bacteria in Crouël ($P < 0.001$, with values lower for Concurrent than the seven others) and Mauguio ($P < 0.01$, with values lower for Amifort than Danubia). At flowering, there was a significant impact of stress ($P < 0.001$) but not wheat genotype in Crouël, whereas there was a significant impact of wheat genotype ($P < 0.001$, yet differences between genotypes were biologically marginal) but not of stress in Mauguio.

Table 2 Size of four microbial functional groups in bulk soil and the rhizosphere of wheat genotypes grown in Crouël and Mauguio (Mauguio) at the tillering (T) and flowering (F) stage, under optimum or stress conditions. Data are expressed as mean ± standard error (n = 8 for Crouël and n = 6 for Mauguio) of the $\log_{10}$-number of copies of genes *acdS* (ACC deaminase producers), *nifH* (N fixers), *phlD* (2,4-diacetylphloroglucinol producers) and *ppdC* (IAA producers) per g.

	Concurrent		Coronation		Jaszaji TF		Odesskaya16		Amifort		D130-63		Danubia		Hendrix		Bulk soil	
	Optimum	Stress	Optimum	Stress	Optimum	Stress	Optimum	Stress	Optimum	Stress	Optimum	Stress	Optimum	Stress	Optimum	Stress	Optimum	Stress
acdS																		
Crouël - T	6.83 ± 0.03 b[i]		7.06 ± 0.05 cd		7.08 ± 0.03 cd		7.21 ± 0.04 e		6.99 ± 0.02 c		7.07 ± 0.03 cd		7.08 ± 0.02 cd		7.11 ± 0.03 d		6.51 ± 0.06 a	
Crouël - F	7.15 ± 0.07 cd	7.13 ± 0.04 bcd	7.19 ± 0.05 d	7.07 ± 0.03 abcd	7.09 ± 0.06 abcd	7.08 ± 0.06 abcd	7.23 ± 0.04 d	7.17 ± 0.06 cd	7.00 ± 0.05 abcd	7.02 ± 0.03 abcd	7.06 ± 0.06 abcd	6.98 ± 0.04 abcd	7.10 ± 0.05 abcd	7.19 ± 0.10 d	6.86 ± 0.06 ab	7.03 ± 0.03 abcd	6.82 ± 0.12 a	6.91 ± 0.11 abc
Mauguio - T									7.27 ± 0.02 b		7.49 ± 0.04 c		7.46 ± 0.03 c		7.30 ± 0.08 b		6.73 ± 0.06 a	
Mauguio - F									7.85 ± 0.06 d	7.44 ± 0.33 bcd	7.46 ± 0.08 bc	7.41 ± 0.06 b	7.73 ± 0.07 cd	7.73 ± 0.08 cd	7.53 ± 0.11 bc	7.51 ± 0.12 bc	6.86 ± 0.09 a	6.70 ± 0.12 a
nifH																		
Crouël - T	5.80 ± 0.04 a		6.36 ± 0.10 b		6.40 ± 0.05 b		6.32 ± 0.04 b		6.37 ± 0.04 b		6.38 ± 0.05 b		6.39 ± 0.05 b		6.43 ± 0.05 b		5.91 ± 0.06 a	
Crouël - F	6.15 ± 0.04	6.23 ± 0.02	6.04 ± 0.17	6.28 ± 0.06	6.23 ± 0.04	6.22 ± 0.04	6.13 ± 0.07	6.35 ± 0.03	6.17 ± 0.04	6.23 ± 0.05	6.19 ± 0.05	6.23 ± 0.05	6.08 ± 0.05	6.17 ± 0.05	6.05 ± 0.09	6.14 ± 0.05	6.05 ± 0.09	6.14 ± 0.12
Mauguio - T									6.49 ± 0.09 ab		6.60 ± 0.26 bc		6.85 ± 0.02 c		6.68 ± 0.03 bc		6.41 ± 0.06 a	
Mauguio - F									6.76 ± 0.09 bc	6.59 ± 0.07 b	6.69 ± 0.07 bc	6.78 ± 0.03 bc	6.79 ± 0.05 bc	6.66 ± 0.05 bc	6.64 ± 0.07 bc	6.81 ± 0.05 c	6.30 ± 0.04 a	6.16 ± 0.12 a
phlD																		
Crouël - T	3.40 ± 0.34		4.41 ± 0.79		3.57 ± 0.40		3.96 ± 0.41		3.71 ± 0.36		3.26 ± 0.33		2.94 ± 0.26		3.01 ± 0.29		2.98 ± 0.23	
Crouël - F	4.65 ± 0.28 abcd	5.76 ± 0.20 d	4.56 ± 0.28 abcd	5.64 ± 0.24 cd	5.57 ± 0.38 ab	4.09 ± 0.23 cd	4.6 ± 0.27 abcd	5.64 ± 0.40 cd	3.82 ± 0.20 a	4.94 ± 0.25 abcd	4.31 ± 0.22 abc	5.28 ± 0.24 bcd	4.29 ± 0.21 abc	4.84 ± 0.41 abcd	4.39 ± 0.26 abc	5.34 ± 0.16 bcd	4.17 ± 0.27 ab	4.83 ± 0.34 abcd
Mauguio - T									3.37 ± 0.51		3.99 ± 0.29		3.11 ± 0.37		2.75 ± 0.20		3.21 ± 0.47	
Mauguio - F									4.80 ± 0.38	4.20 ± 0.24	4.02 ± 0.23	4.36 ± 0.34	4.48 ± 0.25	4.12 ± 0.26	4.66 ± 0.47	4.16 ± 0.29	4.58 ± 0.33	3.69 ± 0.17
ppdC																		
Crouël - T	7.60 ± 0.02 a		7.90 ± 0.05 cd		7.95 ± 0.01 cd		7.94 ± 0.02 cd		7.92 ± 0.01 bc		7.96 ± 0.01 cd		7.98 ± 0.01 d		7.95 ± 0.03 cd		7.71 ± 0.05 ab	
Crouël - F	7.87 ± 0.02 abc	7.86 ± 0.03 abc	7.90 ± 0.03 c	7.89 ± 0.02 abc	7.86 ± 0.02 abc	7.89 ± 0.02 abc	7.87 ± 0.02 abc	7.85 ± 0.03 abc	7.91 ± 0.03 c	7.89 ± 0.01 c	7.88 ± 0.02 abc	7.83 ± 0.03 abc	7.96 ± 0.08 abc	7.68 ± 0.05 a	7.83 ± 0.03 abc	7.84 ± 0.01 abc	7.75 ± 0.03 ab	7.93 ± 0.08 abc
Mauguio - T									7.29 ± 0.03 a		7.50 ± 0.04 bc		7.52 ± 0.01 c		7.41 ± 0.05 ab		7.42 ± 0.02 ab	
Mauguio - F									7.46 ± 0.04 ab	7.50 ± 0.04 ab	7.31 ± 0.03 a	7.37 ± 0.05 ab	7.56 ± 0.03 ab	7.51 ± 0.05 ab	7.46 ± 0.04 ab	7.44 ± 0.04 ab	7.71 ± 0.16 ab	7.88 ± 0.16 b

[i]For each row, significant differences between data are indicated by different letters (Benjamini-Hochberg for *acdS* and *nifH* genes, Conover-Iman with Bonferroni correction for *phlD* and *ppdC* genes).

Abundance of ppdC microorganisms

The difference in number of *ppdC* copies between rhizosphere and bulk soil did not differ, except that this number was marginally higher in the rhizosphere at tillering in Crouël ($P < 0.05$), except for Concurrent and Amifort (**Table 2**). At tillering, there was a significant impact of wheat genotype on the abundance of *ppdC* microorganisms in Crouël ($P < 0.001$, with values lower for Concurrent than the seven others) and Mauguio ($P < 0.01$, but with biologically-negligible differences between genotypes). At flowering, there was a significant impact of wheat genotype ($P < 0.001$, yet with minor differences between genotypes) but not stress in Crouël, and no difference at all in Mauguio.

Abundance of phlD microorganisms

The difference in number of *phlD* copies between rhizosphere and bulk soil was not significant, regardless of the site (Crouël or Mauguio) and sampling (tillering or flowering) (**Table 2**). However, there was a significant impact of stress in Crouël ($P < 0.001$, evidenced for Jaszaji TF) and Mauguio ($P < 0.05$) at flowering.

Distribution of rrs-based OTUs in the total bacterial community

Venn diagram analysis was carried out with 7140 of 33144 OTUs found in ≥ 50% of the samples of a given category under optimum condition (and 8284 of 33408 OTUs under stress). It showed that 256 OTUs (including 3 *Pseudomonas* OTUs) and 346 OTUs (including 5 *Pseudomonas* OTUs and 1 *Azospirillum* OTU) were associated only with respectively F113/Sp245-stimulating and non F113/Sp245-stimulating genotypes under optimum condition (**Fig 1A**). These figures were respectively 278 OTUs (including 3 *Pseudomonas* OTUs and 1 *Azospirillum* OTU) and 151 OTUs (including 2 *Pseudomonas* OTUs) under stress (**Fig 1B**). Under optimum condition, 975 OTUs were associated with bulk soil only (1895 OTUs under stress), and there were 4602 OTUs common to bulk soil, F113/Sp245-stimulating and non F113/Sp245-stimulating genotypes (4946 OTUs under stress). When considering genotype origin, respectively 307 vs 404 OTUs (231 vs 198 OTUs under stress; **Fig 2B**) were associated only with ancient vs modern genotypes (**Fig 2A**).

rrs-based composition of the total bacterial community

The F113/Sp245-stimulating vs non F113/Sp245-stimulating status of genotypes did not influence significantly the composition of the rhizobacterial community according to BCA, neither under optimum nor stress condition (**Fig 3A**). However, the ancient vs modern genotype status had a significant effect, both under optimum ($P \leq 0.01$) and stress conditions ($P \leq 0.05$) (**Fig 4A**). BCA also indicated a significant impact of optimum vs stress conditions on the rhizobacterial community ($P \leq 0.001$) (**Fig 3A**). This significant impact of environmental conditions was also detected when considering ancient genotypes only ($P \leq 0.001$) or modern genotypes only ($P \leq 0.001$) (**Fig 4A**).

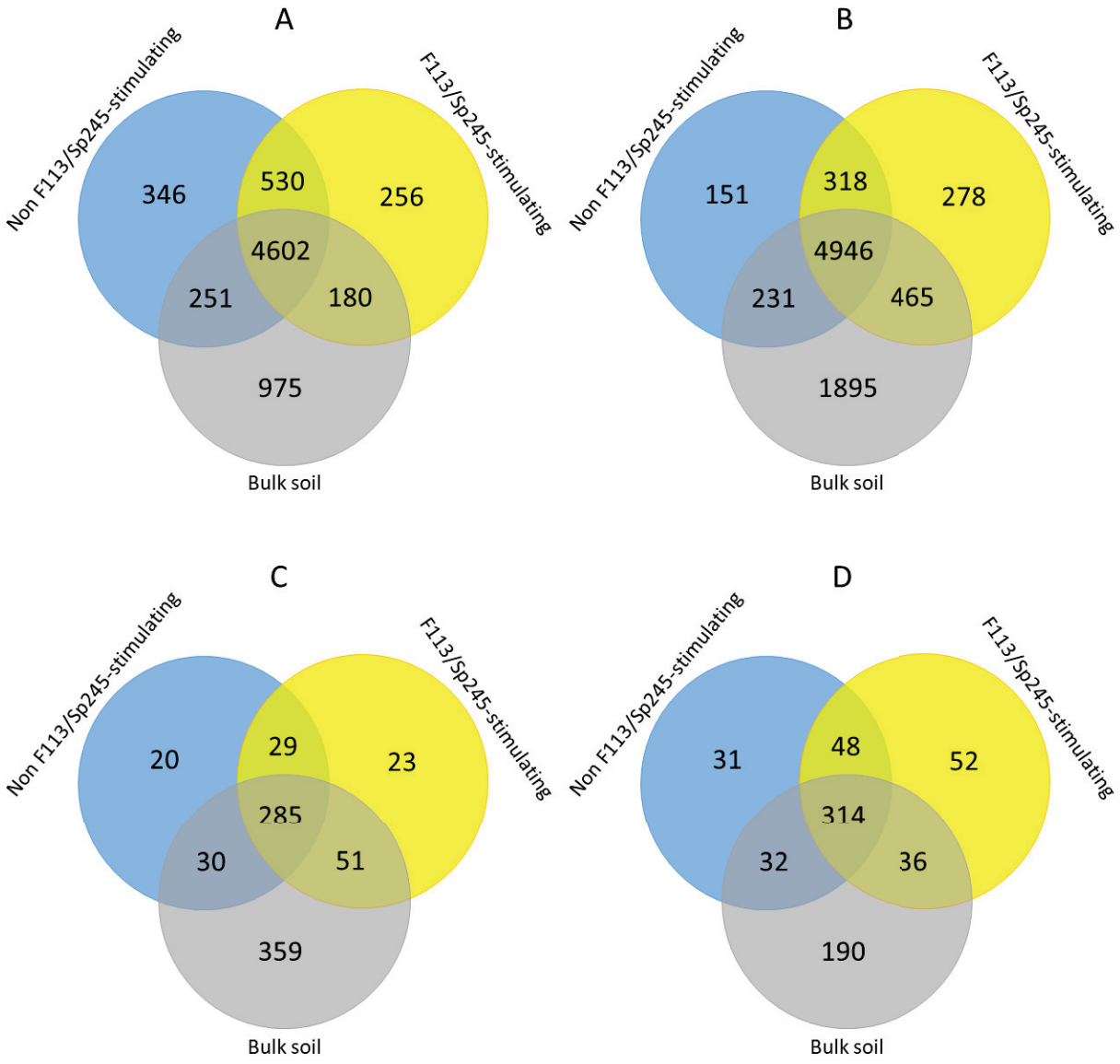

Fig 1 Venn diagrams for the total bacterial community (based on *rrs* OTUs) and the diazotroph community (based on *nifH* OTUs) of F113/Sp245-stimulating genotypes and non F113/Sp245-stimulating genotypes. OTUs were considered exclusive when found in ≥ 50% of a given condition or genotype category but totally absent from the other condition or genotype category, whereas common OTUs were found in ≥ 50% of the samples. OTUs from the total bacterial community are represented in A (optimum condition) and B (stress condition), and OTUs from the diazotroph community in C (optimum condition) and D (stress condition).

Under optimum condition, *Leucobacter, Agrococcus, Frigoribacterium, Devosia, Bosea, Jeotgalibacillus, Arenimonas, Haloferula, Thermincola* and *Rhizobium* were the 10 genera contributing most to the differentiation of ancient genotypes, vs *Holophaga, Moorella, Acidobacterium, Cupriavidus, Steroidobacter, Dehalococcoides, Thermoanaerobacter, Thermovum, Thiorhodospira* and *Thioalkalimicrobium* for modern genotypes. Under stress condition, *Massilia, Oxalobacter, Burkholderia, Pseudomonas, Syntrophomonas, Halobacillus, Duganella, Rhodanobacter, Candidatus Phytoplasma* and *Dyadobacter* were the 10 genera contributing most to the differentiation of ancient genotypes, vs *Geodermatophilus, Ideonella, Aminobacter, Emticicia, Azoarcus, Haliea, Iaceyella, Asanoa, Niastella* and *Christensenella* for modern genotypes.

Pairwise comparisons (all at $P \leq 0.01$ or better) identified very few genera differing in abundance between individual wheat genotypes under optimum condition (2 genera at the most), whereas between 13 (for Danubia, a modern genotype) and 59 genera (for Odesskaya 16, an ancient genotype) differed when comparing bulk soil with the rhizosphere of a wheat genotype (**Supplementary Table S1**). The same observation was made under stress condition, with at best 3 genera discriminating between two individual genotypes (*i.e.* Amifort, a modern genotype and Jaszaji TF, an ancient one), whereas between 24 (for D130-63, modern genotype) and 65 (for Hendrix, modern genotype) genera displayed different abundance levels between bulk soil and the rhizosphere of a wheat genotype (**Supplementary Table S1**). When considered together, the F113/Sp245-stimulating genotypes did not differ significantly from the non F113/Sp245-stimulating genotypes, nor did modern genotypes differ from ancient genotypes. However, pairwise-comparison differential analysis identified as many as 237 genera associated to either optimum or stress condition ($P \leq 0.01$). This included the *Pseudomonas* ($P \leq 0.001$) and *Azospirillum* genera ($P \leq 0.01$), which were significantly more abundant (respectively +56% and +11%) under stress condition, but whose prevalence did not differ when comparing F113/Sp245-stimulating vs non F113/Sp245-stimulating genotypes or modern vs ancient genotypes.

Distribution of nifH-based OTUs in the diazotroph community

Venn diagram assessment was performed using 703 of 11905 OTUs found in ≥ 50% of the samples of a given category under optimum condition (and 797 of 11671 OTUs under stress). It indicated that 52 and 31 OTUs (none *Pseudomonas* or *Azospirillum*) were associated only with respectively F113/Sp245-stimulating and non F113/Sp245-stimulating genotypes (**Fig 1C**). Under stress, the data were respectively 23 and 20 OTUs (of which no *Pseudomonas* or *Azospirillum* OTU ; **Fig 1D)**. Under optimum condition, 190 OTUs were associated with bulk soil only (vs 359 OTUs under stress), and 314 OTUs were common to bulk soil, F113/Sp245-stimulating and non F113/Sp245-stimulating genotypes (vs 285 OTUs under stress). When considering genotype origin,

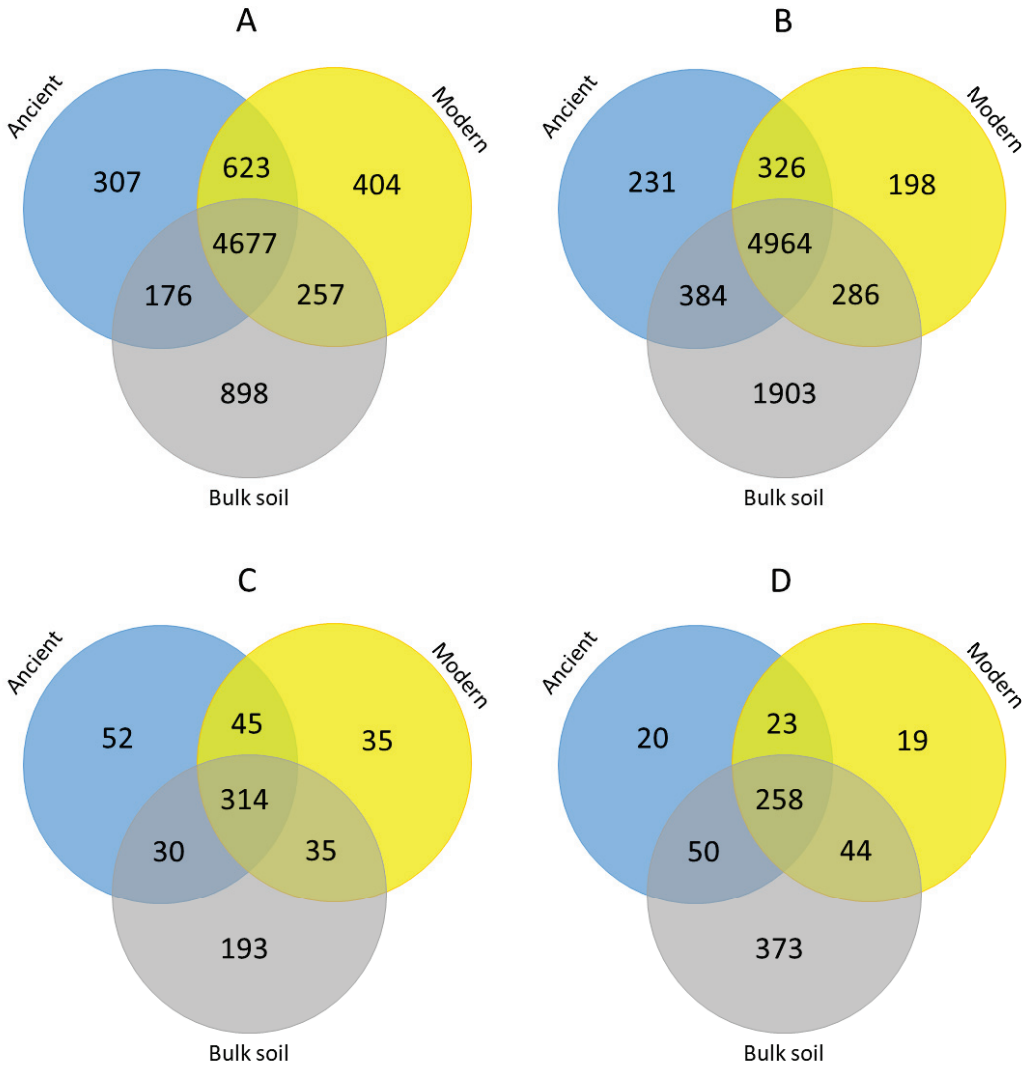

Fig 2 Venn diagrams for the total bacterial community (based on *rrs* OTUs) and the diazotroph community (based on *nifH* OTUs) of the ancient and modern genotypes. OTUs were considered exclusive when found in ≥ 50% of a given condition or genotype category but totally absent from the other condition or genotype category, whereas common OTUs were found in ≥ 50% of the samples. OTUs from the total bacterial community are represented in A (optimum condition) and B (stress condition), and OTUs from the diazotroph community in C (optimum condition) and D (stress condition).

respectively 52 vs 35 OTUs (20 vs 19 OTUs under stress) were associated only with ancient vs modern genotypes (**Fig 2 C, D**).

nifH-based composition of the diazotroph community

BCA indicated that the F113/Sp245-stimulating vs non F113/Sp245-stimulating status of the wheat genotypes did not have a significant effect on the composition of the diazotroph community, regardless of the optimum or stress conditions (**Fig 3B**). In contrast, the impact of ancient vs modern genotype status was significant, but only under optimum condition ($P \leq 0.05$) (**Fig 4B**). BCA also showed a significant impact of optimum vs stress conditions on the diazotroph community ($P \leq 0.001$) (**Fig 3B**). This significant impact of environmental conditions was also detected when considering ancient genotypes only ($P \leq 0.001$) or modern genotypes only ($P \leq 0.001$) (**Fig 4B**).

BCA analysis identified the genera *Cyanothece, Methylosinus, Cellulosilyticum, Knoellian Marinobacter, Pseudomonas, Candidatus Accumulibacter, Azoshydromonas, Ensifer* and *Sinorhizobium* as diazotroph community members contributing most to the differentiation of ancient genotypes under optimum condition, vs *Nocardiopsis, Propionibacterium, Dietzia, Aggregatibacter, Methylocapsa, Crocosphaera, Desulfurispirillum, Hydrogenophaga, Photorhabus* and *Brachybacterium* for modern genotypes. Under stress condition, the most differentiating genera were *Aurantimonas, Ralstonia, Mesorhizobium, Thiobacillus, Nocardioides, Advenella, Streptosporangium, Thiorhodospira, Exiguobacterium* and *Chloroherpeton* for ancient genotypes, vs *Phascolarctobacterium, Clostridium, Aeromonas, Brachymonas, Ruegeria, Janibacter, Ammonifex, Actinoplanes, Aclicycliphilus* and *Yersinia* for modern genotypes.

Under optimum condition, differential analysis identified 1 (for D130-63, Concurrent, Hendrix and Coronation) to 5 genera (for Danubia) whose abundance differed between wheat rhizosphere and bulk soil (with often a decrease compared to bulk soil ; **Supplementary Table S2**). When the pairwise comparisons were made between individual wheat genotypes, there were up to 4 genera (*i.e.* for the modern genotype Danubia vs the ancient genotype Jaszaji TF) differing significantly in abundance. Under stress condition, we found between 0 (for Concurrent, ancient genotype) and 4 genera (for Danubia, modern genotype) showing a different abundance between wheat rhizosphere and bulk soil (higher in the rhizosphere for 6 genotypes; **Supplementary Table S2**). When comparing between wheat, up to 4 genera differed in abundance (*i.e.* between the two ancient/modern genotypes Odesskaya 16/Hendrix and Danubia/Coronation). When considered together, the F113/Sp245-stimulating genotypes differed from the non F113/Sp245-stimulating genotypes based on a single genus only, both under optimum (*Desulfurivibrio*) as well as stress condition (*Treponema*). Modern genotypes differed from ancient genotypes under optimum conditions, based on two genera (*Treponema* and *Dehalococcoides*). Pairwise-comparison differential analysis identified as many as 7 genera (none *Pseudomonas* or *Azospirillum*) associated to either optimum or stress condition ($P \leq 0.001$).

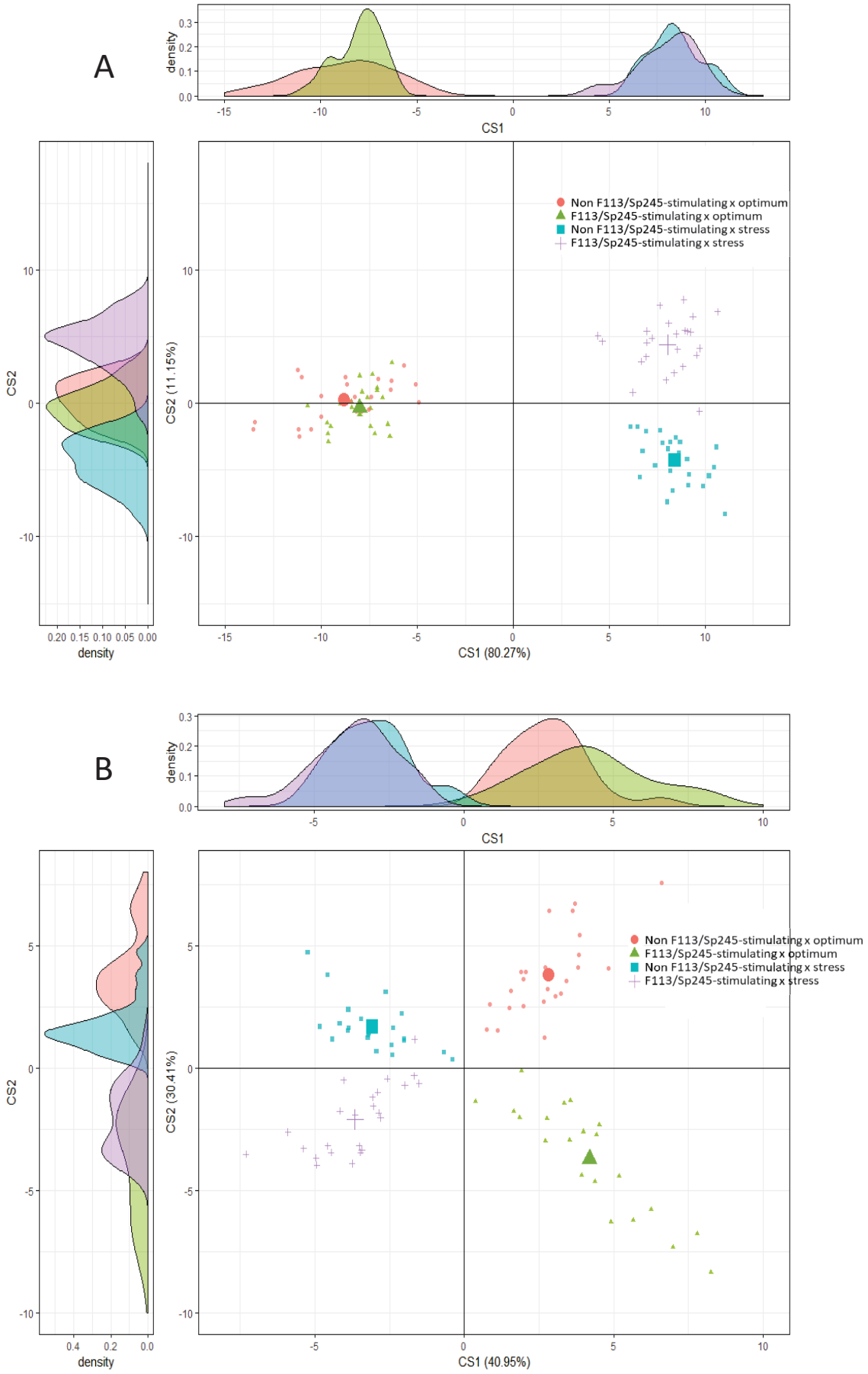

Fig 3 Differences in composition of the total bacterial community (A) and the diazotroph community (B) between F113/Sp245-stimulating genotypes and non F113/Sp245-stimulating wheat genotypes, as indicated by between-class analysis (BCA). Results are shown for the F113/Sp245-stimulating genotypes under optimum (green triangles) or stress condition (pink crosses) and the non F113/Sp245-stimulating genotypes under optimum (red circles) or stress condition (blue squares), and the four bigger symbols represent the barycentres for the corresponding treatments. Curves at the left and on the top represent respectively the sample distribution on the Y and X axes.

Discussion

Soil can host a great diversity of bacteria belonging to diverse functional groups, *i.e.* groups of species and strains involved in a same ecological function [28]. This work focused on four functional groups that are important for plant growth, *i.e.* the diazotrophs (using the marker gene *nifH* ; [18]), the producers of ACC deaminase (*acdS* ; [34]), the producers of 2,4-diacetylphloroglucinol (*phl* ; [33]) and the producers of IAA (which can be synthesized by different pathways [36, 40], and here we focused on the indole pyruvic acid pathway via the phenylpyruvate decarboxylase enzyme).

Out of the four functional groups, the diazotrophs are the most extensively studied so far, because of their particular ecological importance and their effects on diverse plant species [41, 42]. There is variability in this functional group when considering the high diversity of diazotrophs in soil and on/in plant roots [43, 44] and nitrogen fixation efficacy, which depends in part on the composition of the functional group [45], making this group a great candidate for high-throughput sequencing. Furthermore, it has been suggested that their prevalence and activity on plant roots could have been impacted by plant evolution [18], especially since the use of nitrogen fertilizers in a non-limiting way [27, 46]. Microorganisms with *acdS* gene also present significant diversity among bacteria [47, 48] and fungi [49], and they have been well studied for their alleviation of abiotic stress in plants [50]. Recently, it has been shown that their beneficial effect could depend on plant cultivar [13]. The producers of 2,4-diacetylphloroglucinol represent a minority of soil bacteria, which belong mainly to the *Pseudomonas* genus [51, 52], but they are well-known for their beneficial effects on plant health and growth [32, 53, 54] as well as their effective selection by wheat genotypes [33, 55]. Methods to monitor the abundance of these three functional groups in field were already available, but to our knowledge this is the first time that these methods were combined to compare different plant genotypes. Moreover, we used a new method developed by Oudot *et al.* (unpublished) to quantify bacteria harboring the *ppdC* gene, implicated in IAA biosynthesis. Auxin producers have been estimated to represent about 80% of culturable rhizosphere bacteria [56]. They have a great importance for plant growth through the direct root-branching effect of auxin on roots [56] but also effects (probably plant-mediated) on the rhizosphere community [57].

It is known that environmental conditions strongly impact the morphology and physiology of the plants [58–60], and can lead to inconsistent results when plants are inoculated with PGPR [61–63]. Thus, we implemented two types of environmental conditions in fields, *i.e.* agronomically-optimal growth conditions for wheat (*i.e.* with nitrogen fertilization and irrigation) or combined stress conditions (*i.e.* drought and nitrogen-starvation). The environmental conditions significantly impacted on the abundance of 2,4-diacetylphloroglucinol producers, as well as the abundance and diversity of the diazotrophs. Mineral fertilization is known to have a significant impact on the total rhizobacterial community of wheat [64], but whether this impact is indirect, through plant response to stress, or direct is uncertain. Yet, we suggest that in this study the effect of environmental conditions on bacterial communities was both indirect and direct

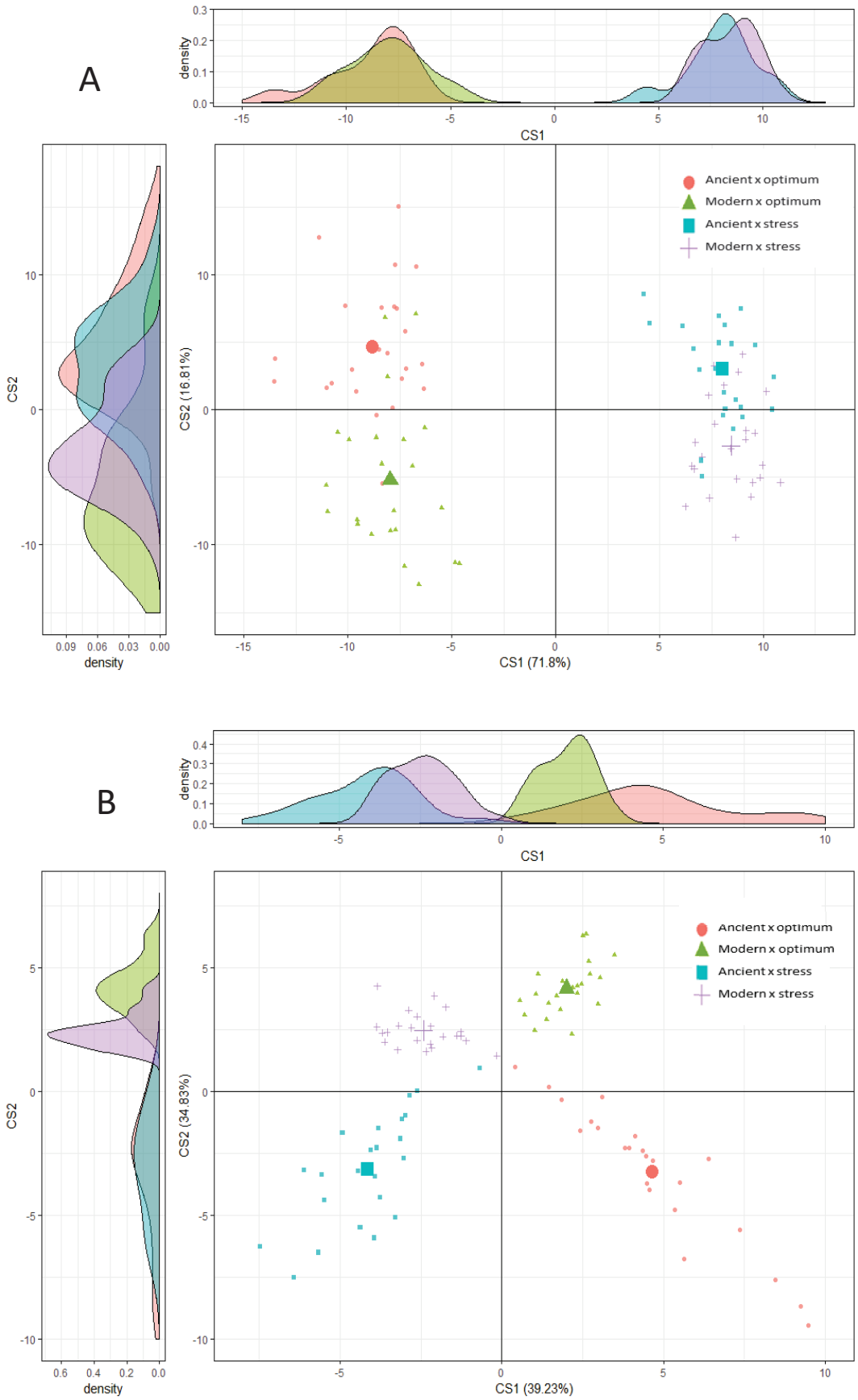

Fig 4 Differences in composition of the total bacterial community (A) and the diazotroph community (B) between modern and ancient wheat genotypes, as indicated by between-class analysis (BCA). Results are shown for the modern genotypes under optimum (green triangles) or stress condition (pink crosses) and the ancient genotypes under optimum (red circles) or stress condition (blue squares), and the four bigger symbols represent the barycentres for the corresponding treatments. Curves at the left and on the top represent respectively the sample distribution on the Y and X axes.

because of (1) the lack of significant difference in abundance of the four groups when comparing bulk soil conditions, whereas there were differences between rhizospheres, and (ii) the shift in diazotroph and total bacterial community compositions when comparing bulk soil conditions (not shown).

In this work, we used bread wheat genotypes that had shown contrasted interactions with *P. kilonensis* F113 and *A. brasilense* Sp245 in previous studies [22, 23]. The different genotypes displayed significant differences between them regarding the abundance of *acdS*, *nifH* and *ppdC* genes, which is consistent with genotype-dependent selection of root-associated bacteria. However, wheat genotypes stimulating both *P. kilonensis* F113 and *A. brasilense* Sp245 did not differ significantly from non-stimulating genotypes in their ability to interact with indigenous microorganisms harboring phytostimulation-relevant genes, regardless of field site, sampling stage, optimum/stress condition and functional group. Similarly, the diversity analysis did not show any difference between stimulating and non-stimulating genotypes. In particular, there was no difference either when focusing on the *Pseudomonas* and *Azospirillum* genera, despite the added-value of inoculation with *P. kilonensis* F113 or *A. brasilense* Sp245 on growth of F113/Sp245-stimulating genotypes [22, 23]. Overall, results suggest that these genotypes do not necessarily have a particular relation with PGPR strains other than the two model strains used to screen wheat genotypes.

Against this background, significant differences were found between ancient and modern wheat genotypes regarding the composition of their associated diazotrophs. In rhizosphere samples, several *nifH*-based OTUs were found only with ancient genotypes, and others only with modern genotypes. Thus, the distinction between ancient vs modern genotypes is ecologically meaningful when considering the recruitment of diazotrophs. In soybean, ancient genotypes are more effective than modern ones for selection of high-performance nitrogen-fixing partners [27]. Mutualistic benefits can be expected if they exceed the resources necessary to maintain the interaction [65], and modern breeding performed under favorable conditions is likely to have neglected the importance of microbial benefits [66, 67]. Therefore, it would be interesting to assess whether ancient wheat genotypes select more effective diazotrophs than modern counterparts. At a larger scale, differences in total rhizobacterial community composition between ancient and modern genotypes have been evidenced in the case of wheat (only culturable bacteria [19]), maize [68] and barley [69], but typically by comparing very limited numbers of genotypes (maximum 3). Accordingly, we also showed differences regarding the composition of the total rhizobacterial community of the ancient and modern wheat genotypes, and the appraisal of the corresponding taxa suggests that differences in rhizosphere ecology are not restricted to bacteria with plant-beneficial potential.

The lack of difference between F113/Sp245-stimulating genotypes and non F113/Sp245-stimulating genotypes showed the limit of the screening procedure of Valente *et al.* [22, 23] to target the plant-beneficial wheat microbiome in its totality. Only two types of PGPR had been considered, and the screening was carried out in the absence of soil, so alternative methodologies are needed to identify crop genotypes interacting

efficiently with indigenous PGPR. This would allow a better use of ancient genotypes in breeding to enhance the ability of future varieties to benefit from indigenous PGPR populations. In the current context of agriculture, looking for an alternative to the use of fertilizers while maintaining yield remains a priority.

Acknowledgements

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Supplementary Table S1 Number of bacterial genera showing different abundance (based on *rrs* sequences ; Benjamini-Hochberg correction, $P < 0.01$) in pairwise comparisons of individual genotypes (or with bulk soil). Symbols + and – refer respectively to an increase or a decrease in the number of genera compared to the genotype indicated in the column.

			Ancient genotypes				Modern genotypes				Bulk soil
			F113/Sp245-stimulating		Non F113/Sp245-stimulating		F113/Sp245-stimulating		NonF113/Sp245-stimulating		
			Concurrent	Coronation	Jaszaji TF	Odesskaya 16	Amifort	D130-63	Danubia	Hendrix	
	OPTIMUM										
Ancient genotypes	F113/Sp245-stimulating	Concurrent		0	0	0	0	1 (-)	0	0	34 [24 (+) 10 (-)]
		Coronation	0		2 [1 (-) 1 (+)]	0	2 (-)	0	0	2 [1(-) 1(+)]	51 [33 (+) 18 (-)]
	Non F113/Sp245-stimulating	Jaszaji TF	0	2 [1 (+) 1 (-)]		2 (+)	0	0	0	0	39 [32 (+) 7 (-)]
		Odesskaya 16	0	0	2 (-)		0	1 (-)	0	0	59 [38 (+) 21 (-)]
Modern genotypes	F113/Sp245-stimulating	Amifort	0	2 (+)	0	0		1 (+)	0	0	20 [16 (-) 4 (+)]
		D130-63	1 (+)	0	0	1 (+)	1 (-)		0	0	43 [33 (+) 10 (-)]
	Non F113/Sp245-stimulating	Danubia	0	0	0	0	0	0		0	13 [10 (+) 3 (-)]
		Hendrix	0	2 [1 (+) 1(-)]	0	0	0	0	0		26 [21 (+) 5 (-)]
		Bulk soil	34 [24 (-) 10 (+)]	51 [33 (-) 18 (+)]	39 [32 (-) 7 (+)]	59 [38 (-) 21 (+)]	20 [16 (+) 4 (-)]	43 [33 (-) 10 (+)]	13 [10 (-) 3 (+)]	26 [21 (-) 5 (+)]	
	STRESS										
Ancient genotypes	F113/Sp245-stimulating	Concurrent		0	1 (-)	0	0	0	0	0	45 [28 (-) 17 (+)]
		Coronation	0		0	0	1 (+)	1 (-)	1 (-)	0	52 [28 (-) 24 (+)]
	Non F113/Sp245-stimulating	Jaszaji TF	1 (+)	0		0	3 (+)	1 (+)	2 (+)	0	64 [34 (+) 30 (-)]
		Odesskaya 16	0	0	0		1 (-)	0	0	0	56 [34 (-) 22 (+)]
Modern genotypes	F113/Sp245-stimulating	Amifort	0	1 (-)	3 (-)	1 (+)		0	0	0	49 [25 (+) 24 (-)]
		D130-63	0	1 (+)	1 (-)	0	0		0	0	24 [15 (-) 9 (+)]
	Non F113/Sp245-stimulating	Danubia	0	1 (+)	2 (+)	0	0	0		0	58 [36 (-) 22 (+)]
		Hendrix	0	0	0	0	0	0	0		65 [38 (-) 27 (+)]
		Bulk soil	45 [28 (+) 17 (-)]	52 [28 (+) 24 (-)]	64 [34 (-) 30 (+)]	56 [34 (+) 22 (-)]	49 [25 (-) 24 (+)]	24 [15 (+) 9 (-)]	58[36 (+) 22 (-)]	65 [38 (+) 27 (-)]	

60-69
50-59
40-49
30-39
20-29
10-19
1-9
0

Supplementary Table S2 Number of diazotroph genera showing different abundance (based on *nifH* sequences ; Benjamini-Hochberg correction, $P < 0.01$) in pairwise comparisons of individual genotypes (or with bulk soil). Symbols + and – refer respectively to an increase or a decrease in the number of genera compared to the genotype indicated in the column.

			Ancient genotypes				Modern genotypes				Bulk soil
			F113/Sp245-stimulating		Non F113/Sp245-stimulating		F113/Sp245-stimulating		NonF113/Sp245-stimulating		
			Concurrent	Coronation	Jaszaji TF	Odesskaya 16	Amifort	D130-63	Danubia	Hendrix	
	OPTIMUM										
Ancient genotypes	F113/Sp245-stimulating	Concurrent		0	1 (+)	1 (+)	1 (-)	0	1 (-)	0	1 (+)
		Coronation	0		0	2 (-)	2 [1 (-) 1 (+)]	0	2 [1 (-) 1 (+)]	0	1 (+)
	Non F113/Sp245-stimulating	Jaszaji TF	1 (-)	0		3 [2 (-) 1 (+)]	3 [2 (-) 1 (+)]	0	4 [2 (-) 2 (+)]	1 (-)	2 [1 (-) 1 (+)]
		Odesskaya 16	1 (-)	2 (+)	3 [2 (+) 1 (-)]		3 [2 (+) 1 (-)]	2 (-)	3 [2 (-) 1 (+)]	2 (+)	3 [2 (-) 1 (+)]
Modern genotypes	F113/Sp245-stimulating	Amifort	1 (+)	2 [1 (+) 1 (-)]	3 [2 (+) 1 (-)]	3 [2 (-) 1 (+)]		1 (+)	2 [1 (-) 1 (+)]	1 (+)	3 [2 (-) 1 (+)]
		D130-63	0	0	0	2 (+)	1 (-)		2 [1 (-) 1 (+)]	0	1 (+)
	Non F113/Sp245-stimulating	Danubia	1 (+)	2 [1 (+) 1 (-)]	4 [2 (+) 2 (-)]	3 [2 (+) 1 (-)]	2 [1 (+) 1 (-)]	2 [1 (+) 1 (-)]		2 [1 (-) 1 (+)]	5 [3 (+) 2 (-)]
		Hendrix	0	0	1 (+)	2 (-)	1 (-)	0	2 [1 (+) 1 (-)]		1 (+)
		Bulk soil	1 (-)	1 (-)	2 [1 (+) 1 (-)]	[2 (+) 1 (-)]	3 [2 (+) 1 (-)]	1 (-)	5 [3 (-) 2 (+)]	1 (-)	
	STRESS										
Ancient genotypes	F113/Sp245-stimulating	Concurrent		0	1 (+)	2 (+)	1 (-)	2 (-)	3 (-)	0	0
		Coronation	0		0	3 [2 (+) 1 (-)]	1 (-)	2 [1 (-) 1 (+)]	4 [2 (-) 2 (+)]	0	3 (-)
	Non F113/Sp245-stimulating	Jaszaji TF	1 (-)	0		2 (-)	0	1 (+)	1 (+)	0	1 (-)
		Odesskaya 16	2 (-)	3 [2 (-) 1 (+)]	2 (+)		2 [1 (-) 1 (+)]	3 [2 (-) 1 (+)]	2 [1 (-) 1 (+)]	4 [3 (+) 1 (-)]	2 (-)
Modern genotypes	F113/Sp245-stimulating	Amifort	1 (+)	1 (+)	0	2 [1 (+) 1 (-)]		0	2 [1 (-) 1 (+)]	2 [1 (-) 1 (+)]	1 (+)
		D130-63	2 (+)	2 [1 (+) 1 (-)]	1 (-)	3 [2 (+) 1 (-)]	0		1 (+)	2 [1 (+) 1 (-)]	2 (-)
	Non F113/Sp245-stimulating	Danubia	3 (+)	4 [2 (+) 2 (-)]	1 (-)	2 [1 (+) 1 (-)]	2 [1 (+) 1 (-)]	1 (-)		1 (-)	4 (-)
		Hendrix	0	0	0	4 [3 (-) 1 (+)]	2 [1 (+) 1 (-)]	2 [1 (-) 1 (+)]	1 (+)		1 (-)
		Bulk soil	0	3 (+)	1 (+)	2 (+)	1 (-)	2 (+)	4 (+)	1 (+)	

Partie 5

Discussion générale

Contexte de l'étude et questionnements

Dans un contexte de développement durable, les interactions bénéfiques de mutualisme et de coopération entre plantes et microorganismes sont particulièrement étudiées pour les potentielles applications qui pourraient en découler dans le domaine de l'agriculture (Gupta *et al.* 2015; da Silva *et al.* 2017). Ces travaux de thèse se sont focalisés sur les interactions bénéfiques existant entre plantes et rhizobactéries phytostimulatrices, *i.e.* des bactéries de la rhizosphère assurant des fonctions ayant des effets bénéfiques sur la croissance des plantes (Vacheron *et al.* 2013; Puga-Freitas and Blouin 2015) ainsi que leur santé (Höfte and Altier 2010). Ces bactéries appelées PGPR (pour *« Plant Growth-Promoting Rhizobacteria* ») vivent dans la rhizosphère d'une plante-hôte, et peuvent montrer des préférences pour coloniser certaines zones racinaires (Vande Broek *et al.* 1993) ou métaboliser certains exsudats libérés par la plante (López-Guerrero *et al.* 2013). De par leur style de vie, les PGPR vont donc être naturellement sensibles au génotype de leur plante-hôte, puisque ce dernier détermine le profil d'exsudation racinaire (Gobena *et al.* 2017; Ziegler *et al.* 2017), les mécanismes de défense (Gómez-Gómez and Boller 2000), les propriétés des surfaces racinaires (Achouak *et al.* 1994) et la morphologie du système racinaire (Pace *et al.* 2015; Han *et al.* 2016), que les bactéries percevront et auxquels elles répondront ou non Ainsi, il a été mis en évidence que certains génotypes d'une même espèce de plante profitaient mieux que d'autres de l'inoculation de souches de PGPR, suggérant des différences entre ces génotypes dans le pilotage des processus qui conduisent à l'expression d'un effet phytostimulateur par une PGPR (Kazi *et al.* 2016; Furlan *et al.* 2017). Toutefois, peu d'explications ont été avancées concernant la raison pour laquelle certains génotypes semblent ne pas posséder le matériel génétique nécessaire à de bonnes interactions avec les PGPR, alors que d'autres l'ont.

La sélection variétale moderne, qui a conduit au développement de génotypes présentant de meilleurs rendements en condition d'intrants chimiques non-limitants (Brancourt-Hulmel *et al.* 2003), a conduit à la sélection de variétés présentant des traits morphologiques et physiologiques bien différents des variétés plus anciennes (Berry *et al.* 2015; Shaposhnikov *et al.* 2016; Beyer *et al.* 2018). On pourrait ainsi penser que les schémas de sélection variétale moderne appliqués au champ auraient conduit à la sélection de variétés n'interagissant pas avec les PGPR de façon similaire que les variétés anciennes (Kiers *et al.* 2002, 2010). Cependant, à notre connaissance, les différences d'interaction entre variétés anciennes et modernes d'une même espèce avec des PGPR ont été très peu étudiées (Engelhard *et al.* 2000; Kiers *et al.* 2007), ce qui a motivé ces travaux de thèse.

Le modèle d'étude que nous avons choisi est le blé tendre (*Triticum aestivum aestivum*). Nous avions à disposition une collection de 196 accessions de blé tendre, représentative de la diversité génétique du blé tendre depuis le milieu du 19ème siècle (Plessis *et al.* 2013), fournie par nos partenaires de l'INRA GDEC. A cela, il a été ajouté 2 variétés modernes, Hendrix et Skerzzo, sélectionnées pour l'agriculture biologique, et 1 variété-population, Chinese Spring, utilisée comme référence pour le séquençage du blé tendre.

Notre approche lors de ces travaux a été dans un premier temps de cribler les 199 accessions à notre disposition, pour leur capacité (dans des conditions gnotobiotiques) à être colonisées par des PGPR modèles et à induire certains de leurs gènes impliqués dans des fonctions de phytostimulation, ce qui représente le premier et deuxième stade des interactions entre plantes et PGPR. Pour cela nous avons utilisé les bactéries *P. kilonensis* F113 et *A. brasilense* Sp245, deux protéobactéries capables de coloniser les racines de nombreuses espèces végétales comme le maïs (Vacheron *et al.* 2016), la tomate (Molina-Favero *et al.* 2008), l'arabette (Vacheron *et al.* 2016), la betterave (Shanahan *et al.* 1992), mais aussi le blé (Couillerot *et al.* 2011; Ramirez-Mata *et al.* 2018). *P. kilonensis* F113 possède un opéron *phl*, codant pour la production de DAPG (Cronin *et al.* 1997), dont l'expression sur les racines des différents génotypes a été mesurée. Il est à noter qu'au moins 2 systèmes de régulation post-transcriptionelle ont été mis en évidence pour l'opéron *phl,* un chez *Pseudomonas brassicacearum* NFM421 et un autre chez *Pseudomonas fluorescens* 2P24 (Lalaouna *et al.* 2012; Zhang *et al.* 2014), ainsi l'expression de l'opéron *phl* mesurée dans notre étude pourrait ne pas refléter exactement le niveau de DAPG effectivement produit par F113 dans la rhizosphère des différents génotypes de blé comparés. Concernant *A. brasilense* Sp245, c'est l'expression de son gène *ppdC*, codant pour la production d'AIA (Spaepen *et al.* 2007b), qui a été mesurée.

Par la suite, des variétés montrant des résultats contrastés lors du criblage ont été sélectionnées et mises en pots, sous serre. Une évaluation de leur performance suite à une inoculation, soit avec F113, soit avec Sp245, a été réalisée un mois après. Les 2 bactéries ayant des effets de stimulation de la rhizogenèse chez les plantes via la synthèse respectivement de DAPG et d'AIA (Brazelton *et al.* 2008; Spaepen *et al.* 2008), nous voulions savoir si les résultats obtenus *in vitro* se traduisaient par des effets rhizogènes, en présence des souches modèles, plus ou moins marqués sur la dizaine de génotypes de blés sélectionnés, ce qui représente le dernier niveau des interactions entre plantes et PGPR.

Enfin, une dernière approche a été de semer au champ des variétés montrant des résultats contrastés lors du criblage pour connaitre leur comportement vis-à-vis de bactéries indigènes et notamment de PGPR. Pour cela, nous avons quantifié dans leur rhizosphère l'abondance de quatre groupes fonctionnels : les diazotrophes, possédant le gène *nifH* (Bouffaud *et al.* 2016), les producteurs d'ACC désaminase, possédant le gène *acdS* (Bouffaud *et al.* 2018), les producteurs de DAPG, possédant le gène *phlD* (Mazzola *et al.* 2004) et les producteurs d'acide indole-3-acétique possédant le gène *ppdC* (Spaepen *et al.* 2007b). Nous avons également évalué la diversité du groupe fonctionnel *nifH* ainsi que la diversité bactérienne totale de la rhizosphère des génotypes de blé pour l'un des champs.

Des niveaux d'interaction entre PGPR modèles et génotypes modernes réduits comparativement au cas des génotypes anciens

Les résultats obtenus dans les parties 2 et 3 de ce manuscrit de thèse montrent que les génotypes de blé tendre modernes (sélectionnés après 1960) utilisés dans notre étude présentent dans leur ensemble de moins bonnes interactions avec F113 et Sp245 que les génotypes sélectionnés avant 1960. Cela pourrait s'expliquer par les différences morphologiques et physiologiques observées entre les variétés modernes et les variétés anciennes de blé (Shaposhnikov *et al.* 2016; Beyer *et al.* 2018). Ces différences portent en effet sur des caractéristiques de la plante impliquées dans les interactions avec les bactéries du sol, que ce soit au niveau de la colonisation ou de l'expression de gènes bactériens, comme cela a été décrit dans la synthèse bibliographique. On peut tout de même noter qu'une part non négligeable des variétés modernes présente de bonnes interactions avec F113 et Sp245, ce qui montre que les traits génétiques impliqués dans ces interactions sont toujours présents au sein des variétés modernes, mais que leur fréquence serait plus faible qu'au sein des génotypes anciens.

L'introduction de certains gènes *Rht* après 1960 (Berry *et al.* 2015) pourrait avoir entrainé, via des effets d'interactions, des modifications au sein du génome de certaines variétés porteuses de ces gènes de nanisme, et conduire à des phénotypes modernes ne présentant pas les caractères physiologiques et morphologiques nécessaires à de bonnes interactions avec les PGPR. Il a par exemple été montré un impact négatif de la présence de gènes *Rht* sur la taille des racines (Subira *et al.* 2016). Etant donné que la qualité et la quantité des exsudats dépendent de l'architecture racinaire (Nguyen 2003; Tückmantel *et al.* 2017), on peut également imaginer que la présence de gènes *Rht* pourrait être corrélée à une modification des profils d'exsudation du blé via son impact sur l'architecture racinaire, ce qui à notre connaissance n'a pas été évalué dans la littérature. Ainsi, il serait intéressant de comparer les profils d'exsudats racinaires ou à défaut les extraits racinaires de génotypes modernes de blé dans lesquels le gène *Rht* aurait été muté, à ceux des génotypes parentaux afin d'évaluer l'impact de la présence de ce gène sur les profils d'exsudation et les conséquences potentielles sur les interactions avec des PGPR. L'introduction de gènes de résistance (Ambrozková *et al.* 2002; Goutam *et al.* 2015) pourrait également avoir eu un impact sur les interactions entre variétés modernes et PGPR. En effet, les phytopathogènes possèdent des molécules à leur surface appelées « *Microbe-Associated Molecular Patterns* » (MAMPs) (Boutrot and Zipfel 2017) qui sont reconnues par la plante par des récepteurs appelés « *Pattern Recognition Receptors* » (PRRs), mais ces récepteurs peuvent aussi servir à détecter des bactéries bénéfiques (Vinagre *et al.* 2006) ce qui peut également entrainer une stimulation de la défense de la plante (Van Wees *et al.* 2008). Cela suggère qu'une amélioration de la résistance vis-à-vis de pathogènes, passant par des modifications ou des transferts entre génotypes des gènes codant ces PRRs - ce qui a été le cas pour de nombreux génotypes modernes de plantes (Boutrot et Zipfel 2017) - pourrait avoir entrainé une sensibilité diminuée vis-à-vis d'autres bactéries, notamment des PGPR. Ce type de mécanisme de résistance étant souvent monogénique (Nelson *et al.* 2018), une étude utilisant (1) des

variétés de plantes mutées pour ces gènes de résistance ou ne les possédant pas et (2) des PGPR modèles pourrait être envisagée pour tester cette hypothèse.

Dans le cadre du projet BacterBlé, dans lequel se sont intégrés ces travaux de thèse, une étude transcriptomique portant sur une sélection restreinte de génotypes présentant des résultats contrastés lors des criblages avec F113 et Sp245, et qui ont été inoculés séparément par ces mêmes bactéries, est en cours et pourrait notamment permettre de déterminer si des gènes codant pour des PRRs sont plus ou moins exprimés selon le génotype de blé, et si cette expression est spécifique d'une souche de bactérie ou non.

Il est aussi connu que la résistance de plantes vis-à-vis de pathogènes, mais aussi d'adventices, est notamment due à l'exsudation de composés particuliers ayant des propriétés biocides ou biostatiques (Bais *et al.* 2006; Gobena *et al.* 2017). La sélection de variétés résistantes aux stress biotiques pourrait ainsi avoir abouti à la sélection de variétés modernes présentant ce type de profils d'exsudation et qui auraient des difficultés à établir de bonnes interactions avec les PGPR. Dans le cadre du projet BacterBlé, une étude métabolomique est également en cours sur une sélection restreinte de génotypes présentant des résultats contrastés lors des criblages avec F113 et Sp245. Cela pourrait permettre d'établir des hypothèses en ce qui concerne l'occurrence et la concentration de composés connus pour leurs effets antimicrobiens dans les exsudats de variétés modernes. Cette même approche pourrait également permettre de repérer des profils d'exsudation spécifiques aux variétés anciennes, y compris des composés dont l'occurrence aurait diminué au sein des variétés modernes, ce qui pourrait être lié aux différences d'interaction avec les PGPR (Bais *et al.* 2006; de Werra *et al.* 2011).

Il est important de noter que la Partie 3 du manuscrit montre que les variétés présentant de bonnes interactions avec F113 n'étaient pas forcément les mêmes que celles présentant de bonnes interactions avec Sp245. Au contraire, le nombre de variétés présentant de bonnes ou de mauvaises interactions avec les deux PGPR ne représentent qu'une petite proportion de variétés. Cela souligne un certain degré de spécificité d'interaction existant entre le blé et les PGPR, comme cela a pu être montré chez le maïs (Walker *et al.* 2012, 2013) et chez le riz (Drogue *et al.* 2014a, b; Chamam *et al.* 2015), probablement dû à des traits de vie et modes d'action différents entre les deux bactéries. Il serait ainsi intéressant de tester d'autres souches modèles, afin de savoir si les résultats observés sont généralisables à d'autres PGPR, la méthode de criblage nécessitant probablement quelques ajustements mais pouvant être adaptée à d'autres PGPR exprimant des fusions avec des gènes rapporteurs, ainsi qu'éventuellement à d'autres modèles de plantes.

Des communautés bactériennes associées aux racines de variétés anciennes distinctes de celles associées aux racines de variétés modernes

Dans la Partie 4 du manuscrit, nous avons voulu savoir si la modification potentielle des conditions dans lesquelles les bactéries associées aux racines croissent (*i.e.* modification de l'architecture racinaire et profils d'exsudation des variétés sélectionnées au cours de la sélection variétale) a entrainé un changement au niveau

des communautés bactériennes associées aux racines des variétés modernes par rapport à celles associées aux racines de variété anciennes.

Les résultats ont montré que s'il y avait une différence d'abondance des groupes fonctionnels associés aux gènes *acdS, nifH, phlD* et *ppdC* entre génotypes, il n'y a pas de différence quand on considère le statut ancien ou moderne de ces génotypes dans leur ensemble. D'un point de vue évolutif, ce résultat peut paraitre inattendu. Avant tout, il faut prendre en compte les conditions dans lesquelles ont été sélectionnées les variétés modernes de blé, *i.e.* en condition de fertilisation azotée, et avec une meilleure utilisation des fertilisants par ces variétés. Ces conditions sont susceptibles d'avoir rendu caduques les effets bénéfiques apportés par les PGPR (Kiers *et al.* 2010; Kazemi *et al.* 2018). Or, les interactions de mutualisme ou de coopération ne sont intéressantes pour la plante uniquement dans le cas où les bénéfices qu'elle reçoit de la part de ses symbiotes sont supérieurs à l'investissement produit pour maintenir cette interaction (Morgan *et al.* 2005). Ainsi, il pourrait y avoir eu une optimisation génétique des variétés modernes au cours de leur sélection successive dans un environnement hautement favorable, résultant en une perte des traits génétiques (Albalat and Cañestro 2016) impliqués dans les interactions avec les PGPR. Cette perte peut signifier une diminution des interactions avec les PGPR due à la perte de caractères phénotypiques essentiels aux interactions avec les rhizobactéries comme discuté dans le paragraphe précédent. Toutefois, une autre hypothèse émerge de certaines études. Selon leurs auteurs, il est possible que l'aménagement de conditions hautement favorables à la croissance des variétés modernes ait entrainé une diminution des sanctions réalisées par les variétés modernes envers leurs partenaires mutualistes (West *et al.* 2002, 2007). Les variétés modernes auraient en effet moins besoin de sélectionner les partenaires les plus efficaces possibles puisque contrairement aux variétés anciennes, leurs besoins sont globalement comblés par la main de l'Homme (Kiers *et al.* 2010). Dans ce cas-là, on pourrait ne pas avoir de diminution de l'abondance de PGPR associées aux racines des variétés modernes, mais plutôt s'attendre à une diminution de leur diversité et/ou de leur activité. En effet, il a été montré que l'apport d'azote dans le sol conduisait à la sélection par les légumineuses de bactéries du genre *Rhizobium* moins efficaces à fixer l'azote (Weese *et al.* 2015). Chez le soja, des résultats suggèrent que les variétés modernes seraient moins aptes que les variétés anciennes à sélectionner des diazotrophes efficaces (Kiers *et al.* 2007). Ces observations sont cohérentes avec les résultats obtenus dans la partie 4 de ce manuscrit, montrant une absence de différence d'abondance des groupes fonctionnels étudiés mais une différence de composition des communautés bactériennes totales et des diazotrophes associées aux racines des génotypes anciens et à celles des génotypes modernes.

En effet, s'il n'y pas de différence en terme de diversité des communautés bactériennes (Partie 4, données non montrées) dans leur ensemble, la composition de la communauté des diazotrophes associée aux racines de génotypes anciens est différente de celle associée aux racines de génotypes modernes, ce qui, en accord avec les résultats mentionnés ci-dessus, tend à démontrer l'existence de bactéries diazotrophes

préférentiellement sélectionnées par les génotypes anciens. Il serait intéressant de comparer la composition des communautés d'autres groupes fonctionnels comme les producteurs d'ACC désaminase ou les producteurs d'AIA, afin de voir si une sélection préférentielle par la plante de certains taxa assurant ces fonctions pourrait s'y opérer, et faire des études de co-occurrence pour déterminer si certains membres de ces différents groupes fonctionnels sont co-sélectionnés par les différents génotypes de blé.

De façon générale, les genres contribuant le plus à la composition de la communauté bactérienne totale associée aux racines de génotypes anciens ne sont pas les mêmes que pour les génotypes modernes. Des différences de composition microbienne entre génotypes anciens et modernes d'une même espèce avaient déjà été observées pour du blé (Germida and Siciliano 2001), du maïs (Johnston-Monje *et al.* 2014) et de l'orge (Bulgarelli *et al.* 2015), mais à chaque fois en comparant un nombre plus restreint de génotypes que dans ce manuscrit. Ainsi cette étude corrobore pleinement ces précédentes observations.

Par la suite, il serait intéressant de mener une étude portant davantage sur l'expression des gènes ciblés (*i.e. nifH, acdS, phlD* et *ppdC*) au sein des taxons présents dans les groupes fonctionnels dont l'abondance a été quantifiée dans cette étude, ce qui permettrait notamment de valider ou réfuter l'hypothèse d'une sélection de bactéries diazotrophes plus efficaces par les génotypes anciens. Toutefois, ce genre d'étude est complexe du fait de l'extraction d'ARN bactériens à partir d'échantillons de sol, et malgré les différentes tentatives que j'ai réalisées en utilisant divers protocoles, à ce jour nos essais n'ont pas permis d'extraire, en quantité suffisante, et de façon reproductible les ARNs pour mener à bien ce type d'étude.

Une bonne adéquation du niveau d'interaction blé-PGPR in vitro avec la réponse des génotypes de blé à une inoculation en pot mais pas avec celle du microbiote racinaire associé

Les résultats des parties 2 et 3 de ce manuscrit montrent que les génotypes de blé sélectionnés pour être inoculés avec F113 puis Sp245 sous serre ont globalement répondu à ces inoculations de manière cohérente avec leurs niveaux d'interaction observés *in vitro*. En effet, 5 sur 6 des génotypes qui présentaient de fortes interactions et seulement 1 sur 4 des génotypes qui présentaient de faibles interactions avec F113 et Sp245 *in vitro* ont vu au moins un des paramètres mesurés sur leurs systèmes racinaires, impacté de façon significative par l'ajout d'une des PGPR modèles. Ainsi, malgré une forte variabilité infra et inter-génotype, le gain moyen de performance des génotypes stimulant F113 et Sp245 tendait à être plus élevé que celui des autres génotypes. Cela démontre qu'une approche *in vitro* n'est pas dénuée d'intérêt lorsque l'on s'intéresse à des questions écologiques, et peut permettre d'effectuer une première sélection robuste avant d'effectuer des études qui peuvent être difficilement menées d'un point de vue pratique sur un grand nombre d'individus. Il peut toutefois être intéressant de noter que cette adéquation entre résultats *in vitro* et en pots est particulièrement valable quand des conditions de stress étaient appliquées. En effet, les variétés présentant de bonnes interactions avec F113 et Sp245 *in vitro* ont montré une amélioration de leur performance après

inoculation plus importante en condition de stress, ce qui n'était pas le cas des autres variétés. Cela montre que les interactions entre blé et PGPR sont influencées par les variations d'environnement, et suggère que l'apport de nutriments dans le sol dans les conditions optimums en pots pourrait avoir perturbé les interactions entre PGPR modèles et blé, comme cela a pu être montré pour d'autres couples plantes-PGPR (Ozturk *et al.* 2003; Sasaki *et al.* 2010; Romero-Perdomo *et al.* 2017). Ces observations sont toutefois en contradiction avec celles de Nguyen *et al.* (2018) qui ont observé une diminution des effets bénéfiques par des PGPR sur le blé dans des conditions sans fertilisation. Cependant, leur étude s'est penchée sur un seul génotype de blé, ce qui explique probablement les différences observées avec notre étude.

De façon opposée, nous déplorons le manque de lien entre les résultats obtenus *in vitro* dans les Parties 2 et 3 et les résultats obtenus au champ dans la Partie 4. En effet, nous nous attendions à une différence d'abondance des groupes fonctionnels associés aux gènes *acdS, nifH, ppdC* et *phlD* dans la rhizosphère des génotypes ayant présenté de faibles interactions avec F113 et Sp245 par rapport aux génotypes ayant eu de fortes interactions avec F113 et Sp245, puisque ces deux PGPR modèles recouvrent les quatre groupes fonctionnels étudiés (*i.e. acdS* et *phlD* pour *P. kilonensis* F113 et *nifH* et *ppdC* pour *A. brasilense* Sp245). Or cette différence n'a pas été observée. Nous n'avons également pas pu mettre en évidence de différence de composition entre les communautés bactériennes indigènes associées aux racines de génotypes ayant présenté de faibles interactions avec F113 et Sp245 et celles associées aux racines de génotypes ayant eu de fortes interactions avec F113 et Sp245. Le fait qu'il n'y ait pas non plus de différences entre ces deux catégories de génotypes concernant la présence des genres *Pseudomonas* et *Azospirillum*, dans les communautés, montre les limites d'une étude *in vitro.* Ainsi, les génotypes de blé comparés dans cette étude, quand ils ne sont pas inoculés, vont sélectionner préférentiellement des genres bactériens autres que *Pseudomonas* et *Azospirillum* et le choix d'une distinction sur le critère d'interaction avec F113 et Sp245 *in vitro* s'avère à la lumière de ces résultats ne pas avoir été le plus judicieux.

Détection de régions génétiques impliquées dans les interactions entre le blé tendre et A. brasilense Sp245

Dans la partie 3 de ce manuscrit, une approche de génétique d'association (*Genome Wide Association Study* ; GWAS) a été menée par nos partenaires de l'INRA GDEC. Ce genre d'approche dans un contexte d'interaction entre plante et PGPR avait déjà été mené, par Diaz De Leon *et al.* (2015) qui ont mis en évidence 6 QTL (« *Quantitative Trait Loci* ») chez le blé qui seraient impliqués dans l'adhésion d'*A. brasilense* aux racines, dont un QTL majeur localisé sur le chromosome 1A. Dans notre étude, cette approche n'a pas permis de mettre en évidence de régions génétiques associées à la colonisation par Sp245 chez le blé tendre. Cela pourrait s'expliquer par une variabilité infra-génotype dans nos résultats trop forts pour détecter de faibles effets d'association. En dehors de la possibilité de résultats trop variables pour être exploités par GWAS, cette

absence de détection pourrait suggérer que les régions génétiques du blé impliquées pourraient se trouver dans des zones du génome du blé qui n'ont pas été sélectionnées pour faire partie de l'analyse (exactement 79.465 SNPs, voir Partie 3). L'approche GWAS a par contre permis de détecter 21 régions génétiques impliquées dans l'induction par la plante de l'expression du gène bactérien *ppdC* chez *A. brasilense* Sp245. Concernant F113, seulement une région a été détectée par GWAS à ce jour, impliquée dans la colonisation (données non montrées). Le faible nombre de régions détectées dans le cas de F113 par rapport à Sp245 pourrait s'expliquer de la même façon qu'en ce qui concerne le manque de régions détectées concernant la colonisation par Sp245, ou bien par l'utilisation de gènes rapporteurs différents : *gusA* pour Sp245 contre *egfp* et *mCherry* pour F113, ce qui conduit à des méthodes de mesure d'expression qui n'ont pas les mêmes niveaux de sensibilité. Par la suite, les gènes présents (déjà caractérisés) ou hypothétiquement présents (putatifs) dans ces régions mises en avant vont être recherchés. Cela pourrait permettre d'établir une liste de gènes codant des fonctions déjà décrites ou non pour être impliquées dans les interactions entre plantes et PGPR. Chez le blé, ce type d'approche a été très utilisé pour la recherche de gènes de résistance (Goutam *et al.* 2015), mais aussi pour la recherche de gènes impliqués dans le contrôle de l'architecture racinaire (Beyer *et al.* 2018) ou l'utilisation de l'azote (Cormier *et al.* 2014). Concernant l'induction de *ppdC*, les candidats pourraient être des gènes impliqués dans la production d'auxines ou de tryptophane, des composés connus respectivement pour induire ou être nécessaires à l'expression de *ppdC* (Spaepen *et al.* 2008; Spaepen and Vanderleyden 2011). Toutefois, une étude par approche mutationnelle sera nécessaire pour valider l'implication de certains de ces gènes dans l'induction du gène *ppdC*.

Vers une sélection variétale écoresponsable et durable ?

Pour satisfaire aux besoins grandissants de la population humaine au cours du 20ème siècle, les plantes de grande culture ont fait l'objet de multiples études portant sur le gain de rendement, de qualité des grains et de résistance aux maladies (Doussinault *et al.* 2001; Berry *et al.* 2015; Mefleh *et al.* 2018). Ces années de Recherche ont été concluantes puisque tout au long du 20ème, et tout particulièrement depuis 1960, le rendement des cultures a fortement augmenté (Huffman and Evenson 2001; Brancourt-Hulmel *et al.* 2003; Guarda *et al.* 2004). Toutefois, depuis le début du 21ème siècle, une stagnation des rendements du blé, notamment, a été notée (Brisson *et al.* 2010), et des prévisions indiquent même une diminution des rendements due à l'augmentation des températures sur Terre (Asseng *et al.* 2015). De nombreux auteurs suggèrent que les futures améliorations relatives au rendements des plantes, passant principalement par une stabilisation de ce rendement malgré des conditions environnementales fluctuantes et une réduction de l'utilisation de fertilisants chimiques, nécessitent une meilleure compréhension de la composante souterraine des plantes et notamment des interactions entre racines et rhizobactéries (Wissuwa *et al.* 2009; Herder *et al.* 2010; Bakker *et al.* 2012; Wei and Jousset 2017) et entre racines et champignons mycorhiziens à arbuscules

(Lynch 2007; Jacott *et al.* 2017; Verzeaux *et al.* 2017). En effet, depuis 1960 et l'aménagement des sols agricoles passant par la mécanisation (labour), l'irrigation, les fertilisants minéraux et les pesticides, les coopérations entre microorganismes et plantes semblent avoir laissé place à des 'coopérations' Homme-plantes artificielles. Ce genre de relation, reposant sur des ressources limitées et non-renouvelables (les engrais synthétiques) et ayant un impact écologique qui pourrait bientôt devenir plus préjudiciable à l'Homme que ce qu'il ne lui apporte (Tilman 1999; Bodirsky *et al.* 2014), ne pourra pas durer dans le temps.

Les plantes sont naturellement associées à des communautés microbiennes indigènes comprenant des souches pouvant virtuellement assurer les mêmes fonctions que les fertilisants (Kumar *et al.* 2014; Karimi *et al.* 2018) ou les pesticides (Perneel *et al.* 2008; Díaz Herrera *et al.* 2016; Oni *et al.* 2018) et qui peuvent permettre de stabiliser le développement de la plante dans des conditions de stress abiotiques comme la sécheresse (García *et al.* 2017). Ainsi, la sélection variétale du futur pourrait se concentrer sur l'exploitation de ces communautés indigènes (Wissuwa *et al.* 2009; Bakker *et al.* 2012). Ce genre de valorisation des microorganismes indigènes a déjà été mis en place plus ou moins consciemment, avec succès. C'est par exemple le cas de la rotation légumineuses-céréales qui permet aux céréales de profiter de l'apport d'azote dans le sol dû au recrutement de diazotrophes par les légumineuses (van Vugt *et al.* 2018; Zemek *et al.* 2018). Au Brésil, la sélection de la canne à sucre se fait depuis des décennies dans des conditions de faibles intrants tout en sélectionnant les individus présentant les meilleurs rendements. Cela a abouti à la sélection de variétés présentant à la fois de meilleurs rendements que les variétés anciennes, mais aussi de meilleurs niveaux de fixation biologique d'azote au sein de leur rhizosphère (Vinagre *et al.* 2006). Cela pourrait être dû à une co-évolution entre canne à sucre et diazotrophes, notamment au niveau de récepteurs de la plante capables de reconnaitre les diazotrophes (Vinagre *et al.* 2006). D'autre part, la sélection moderne, se focalisant sur la résistance aux maladies, pourraient déjà avoir sélectionné des variétés capables de recruter efficacement des microorganismes luttant contre les phytopathogènes du sol (Mendes *et al.* 2018). Toutefois, ce dernier point peut être sujet à débat puisqu'une trop forte proportion d'individus antagonistes pourrait significativement impacter les populations sensibles de microorganismes assurant d'autres fonctions bénéfiques pour la plante (Walsh *et al.* 2003; Winding *et al.* 2004).

Pour une valorisation efficace de ces communautés indigènes, il semble indispensable de (1) limiter drastiquement l'utilisation d'intrants chimiques qui pourraient avoir masqué comme nous l'avons vu précédemment les bénéfices apportés par les microorganismes lors du processus de sélection variétale, (2) introduire dans les processus de croisements des variétés caractérisées au préalable pour leurs meilleures interactions avec les microorganismes phytobénéfiques, notamment des variétés anciennes présentant des traits génétiques potentiellement perdus chez les variétés modernes, ce qui permettrait également de limiter l'érosion de la diversité génétique chez les plantes cultivées (Kazemi *et al.* 2018) et (3) utiliser des variétés adaptées localement à chaque sol puisque le type de sol va avoir une influence significative sur la composition

de la communauté microbienne présente (**Fig 1**). Concernant ce dernier point, la sélection participative apparait comme une nouvelle méthode particulièrement prometteuse (Desclaux 2006).

Les analyses de la composition microbienne de différents groupes fonctionnels connus pour leur effet positif sur la nutrition des plantes par séquençage haut-débit, pourraient permettre de mettre en évidence le microbiome cœur (*i.e.* l'ensemble des microorganismes associée à tous les échantillons d'un groupe défini à l'avance) (Vandenkoornhuyse *et al.* 2015; Gopal and Gupta 2016) associé aux variétés présentant les meilleurs comportements dans des conditions avec de faibles apports de nutriments. La comparaison de ce microbiome cœur à celui de variétés présentant de plus faible rendement dans ces mêmes conditions pourrait permettre d'identifier les microorganismes potentiels qui peuvent agir ensemble de façon positive sur la plante. C'est vraisemblablement au sein de ce microbiome cœur que devraient se trouver les individus contribuant le plus au bon développement de l'espèce végétale ciblée. Les espèces/souches au sein de ce microbiome cœur, isolables *in vitro,* pourraient ensuite être utilisées dans une approche de criblage à l'image de celle réalisé dans les parties 2 et 3 de ce manuscrit, afin de déterminer des QTL puis des gènes associés aux interactions entre la plante-hôte et ces microorganismes bénéfiques. Par la suite, ces gènes pourraient être ciblés dans les programmes de sélection variétale. En complément de cette approche, des consortiums synthétiques reproduisant le microbiome cœur évoqué précédemment pourraient être utilisés dans de nouvelles zones de culture ou des zones surexploitées devenues trop pauvres pour y cultiver des plantes, afin de (re)fertiliser le sol de façon efficace (Wubs *et al.* 2016) (**Fig 1**).

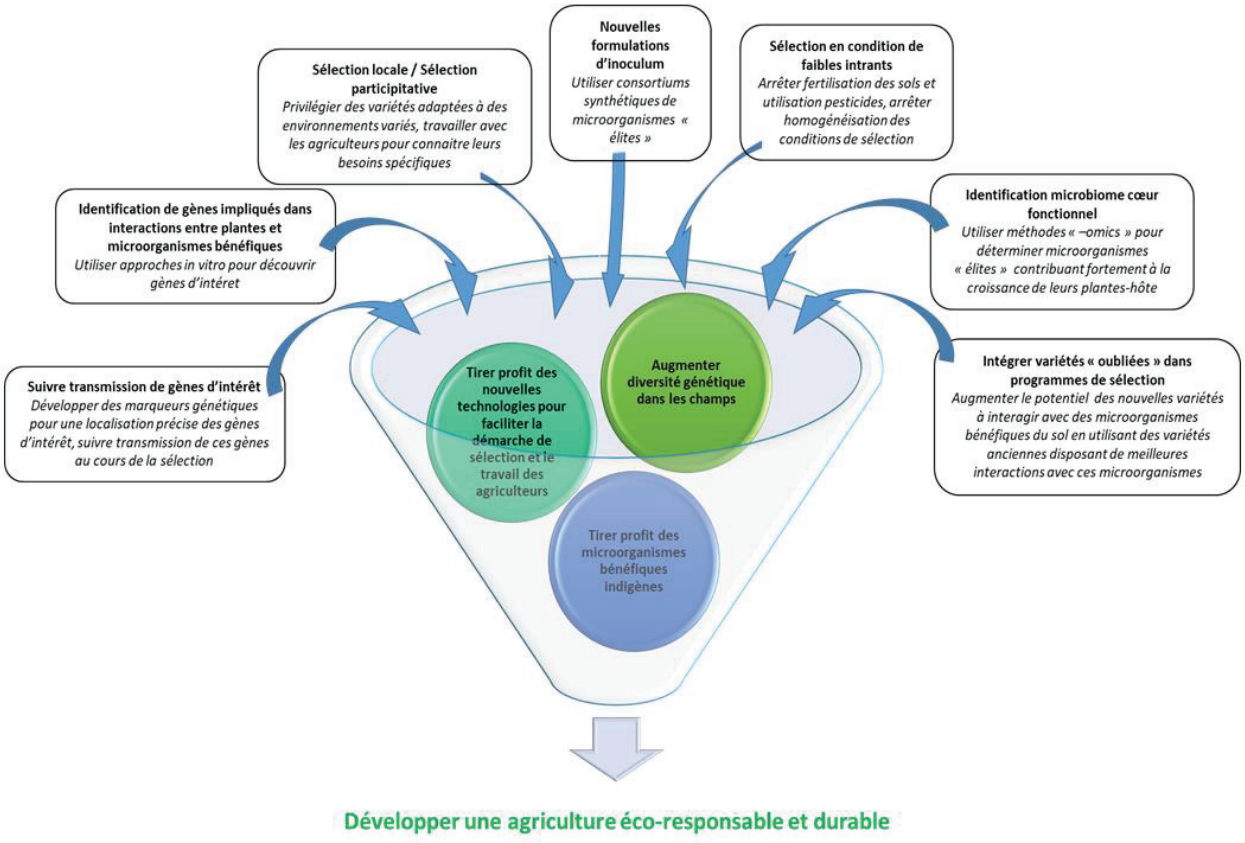

Fig 1 Pistes et outils pour développer une agriculture d'avenir, durable d'un point de vue écologique et économique.

Conclusion

Ces travaux de thèse avaient pour but de comparer les capacités d'interaction de génotypes végétaux anciens (sélectionnés avant 1960) et modernes (sélectionnés après 1960) avec des PGPR. Les résultats ont globalement montré une perte de la capacité des variétés anciennes à interagir avec les PGPR modèles utilisées, mais aussi un changement au sein des communautés bactériennes associées aux racines de génotypes modernes comparativement aux génotypes anciens.

Nous avons montré qu'au sein d'une liste de 199 génotypes de blé tendre, il existait une diversité des niveaux d'interaction des génotypes de blé avec la PGPR *P. kilonensis* F113 *in vitro*, et une adéquation des résultats du criblage *in vitro* avec les différences d'amélioration de performance observées, en serre, suite à l'inoculation de F113. Au sein de cette liste de 199 génotypes, plusieurs génotypes modernes présentaient de bonnes interactions avec F113, mais ce sont globalement les génotypes anciens, incluant les variétés-populations ainsi que les vieilles variétés pures sélectionnées avant 1960, qui interagissaient mieux avec la PGPR *P. kilonensis* F113.

En utilisant une autre PGPR, *A. brasilense* Sp245, nous avons montré qu'au sein des 199 génotypes de blé, une faible proportion seulement était capable de stimuler à la fois F113 et Sp245. Toutefois, de la même façon que pour F113, ce sont les génotypes anciens qui présentaient de meilleures interactions avec Sp245, même si la différence entre génotypes anciens et modernes était moins marquée que pour la souche F113. Les résultats du criblage avec Sp245 ont également fait l'objet d'une approche GWAS qui a permis de mettre en évidence 21 régions génétiques chez le blé impliquées dans l'induction de l'expression du gène *ppdC* chez Sp245. Des analyses transcriptomiques en cours permettront d'identifier les gènes candidats de blé impliqués dans les interactions avec les PGPR. Il sera alors possible d'établir s'ils appartiennent aux régions identifiées par l'approche GWAS.

Enfin, nous avons mis en évidence une différence d'abondance des groupes fonctionnels des diazotrophes, des producteurs d'ACC désaminase et des producteurs d'AIA (via le gène *ppdC*) entre les génotypes de blé. Toutefois, les génotypes modernes et anciens n'ont pas présenté de différences d'abondance. Ils ont par contre montré une différence au niveau de la composition de la communauté bactérienne totale et des diazotrophes leur étant associées, ainsi que la présence d'OTU spécifiques à leurs rhizosphères respectives. Les OTU spécifiquement présentes dans la rhizosphère des variétés anciennes *vs* modernes pourraient être isolées afin de déterminer si certaines pourraient améliorer la croissance du blé.

Ces résultats pourraient se poursuivre par des études transcriptomiques et métabolomiques pour mieux caractériser les différences entre génotypes anciens et modernes de plantes et mieux comprendre les interactions entre génotype de plantes et PGPR. D'un point de vue appliqué, ces résultats pourraient servir à repenser la façon dont la sélection variétale moderne est pratiquée afin d'accorder plus d'importance aux interactions avec les microorganismes bénéfiques pour la croissance des plantes.

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