Symbiosis (2019) 78:229–240
https://doi.org/10.1007/s13199-019-00612-8

Wheat landraces with low mycorrhizing ability at field respond differently to inoculation with artificial or indigenous arbuscular mycorrhizal fungal communities

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Received: 12 October 2018 / Accepted: 27 February 2019 / Published online: 6 April 2019

Abstract
Conventional farming is not sustainable in a context of climate change and of dramatic reductions in natural resource stocks worldwide. A change of paradigm towards more sustainable farming is necessary, based on the preservation and management of ecosystem services. The soil is a reservoir of organisms beneficial for plant production. Among these are arbuscular mycorrhizal fungi. Nevertheless, the response of plants – especially cereal landraces – to mycorrhization, and the effect of domestication on the response to mycorrhization are controversial. In the present paper we investigated the response of four wheat landraces with a low mycorrhizogenous ability to inoculation with the indigenous arbuscular mycorrhizal fungi community or an artificial community in greenhouse and field conditions. We showed that the community of arbuscular mycorrhizal fungi can have an effect on yield, even in wheat landraces with a low mycorrhizogenous ability. We also highlighted the importance to properly choose the criteria (phenotypic criteria as root and shoot biomasses versus quality criteria as grain quality) used to measure this possible gain.

Keywords Arbuscular mycorrhiza · Sustainable farming · Landrace · Wheat · Crop production · Symbiosis

1 Introduction

The so-called conventional farming systems currently used by most farmers in developed countries are notably based on the consumption of large quantities of fertilizers and other synthetic chemical inputs. This practice causes severe pollution of water courses and arable soils (Johnson et al. 1991; Carpenter et al. 1998; Foley et al. 2005), that directly impacts biodiversity and human health (Tilman et al. 2001; ANSES 2016). Furthermore, high-input farming is the second most important emitter of greenhouse gases worldwide, and is therefore a major contributor to the current climate change context (IPCC 2014; FAO 2015). In addition, upcoming shortages in resources, e.g. in phosphate mining, are foreseeable (Cordell et al. 2008). Consequently, conventional farming is not sustainable in a context of climate change and of dramatic reductions in natural resource stocks worldwide. A change of paradigm, based on the preservation and management of ecosystem services provided by soil biodiversity, towards more sustainable farming is necessary (Altieri 1999). Such a change must have the primary objective to reach a coherent balance between resilience and productivity (Ulanowicz et al. 2009). The soil is not solely a physical substrate for plants; it is also a source of nutrients essential for plant growth, and the reservoir of organisms beneficial for plant production. Among these are arbuscular mycorrhizal fungi (AMF) and bacteria implicated in biological nitrogen fixation, phosphate solubilization, and biological control. Arbuscular mycorrhiza (AM) are an ancestral (Ordovician; Redecker et al. 2000) mutualistic symbiosis that concern most land plants. AMF from the *Glomeromycota* phylum colonize cortical root cells, forming highly branched structures called arbuscules (Smith and Read 2008). These are

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Electronic supplementary material The online version of this article (https://doi.org/10.1007/s13199-019-00612-8) contains supplementary material, which is available to authorized users.

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key interfaces for nutrient exchanges between the two symbiotic partners: the plant trades sugar produced from photosynthesis for minerals such as phosphorus. The characteristics of this symbiosis make AMF major players in plant nutrition. But AM is negatively impacted by tillage, the use of certain pesticides, and the use of inorganic mineral fertilizers, especially phosphate (Kabir 2005; Grant et al. 2005; Verbruggen et al. 2010; Säle et al. 2015). Thus, the implementation of farming practices favoring the ecosystem services supplied by AM would permit exploitation of AM within the framework of sustainable farming (Gianinazzi et al. 2010).

Hexaploid wheat (*Triticum aestivum*) appeared in the course of the domestication process ca. 10,000 years ago (Doebley et al. 2006). Before the development of modern varietal selection program that started in the rich countries in the 1950s, farmers used to practice local varietal selection essentially based on weight and open pollination, even though wheat is strongly autogamous. These varieties are labelled as 'landraces', they include morphologically similar, homozygous individuals, but intra-specific variability can still be found (Mollier 2017). Some farmer needs this variability so as to fit with the specific pedoclimatic conditions of the field as best as possible while maintaining a stable, good-quality production for bread-making. Wheat landraces, like all cereal crops, have kept their ability to establish symbiosis with AMF throughout evolution and varietal selection (Sawers et al. 2008). It was previously shown that the ability to establish mycorrhization and the effect of mycorrhization could vary in wheat according to landraces and/or modern varieties, the associated AMF, and the farming system (Azcón and Ocampo 1981; Manske 1990; Kapulnik and Kushnir 1991; Hetrick et al. 1992; Zhu et al. 2001). The current index aimed at assessing the response to mycorrhization estimates the impact of AM on dry shoot biomass (Hetrick et al. 1992; Janos 2007; Sawers et al. 2010). But the correlation between the supply of orthophosphates to the mycorrhizal plant via AMF and the effect of mycorrhizal symbiosis on the development of the mycorrhizal plant remains uncertain (Smith and Smith 2011). Moreover, the response of plants – especially cereal landraces – to mycorrhization, and in particular the effects of domestication on the response to mycorrhization are still controversial. For example, Sawers and collaborators (Sawers et al. 2010) suggested that the response to mycorrhization of landraces and modern varieties could be similar, but appears different in regard to the respective performances of these varieties when non-mycorrhized.

We first studied the ability of a large number of wheat landraces to establish mycorrhizal symbioses with the indigenous AMF community in the field. We focused on the ability of these wheat landraces to establish mycorrhizal symbiosis at tillering (approx. 20 weeks after germination), because the early development of root colonization by AMF can influence the effect of AM on the host wheat development (Hay 1999).

In a second step, we investigated the response of 4 wheat landraces with a low mycorrhizogenous ability to inoculation with the indigenous AMF community or an artificial community in greenhouse and field conditions. Our objectives were to determine whether (i) root colonization evolved in the presence of a different AMF community, and (ii) the low response to mycorrhization of certain wheat landraces varied with the AMF community.

2 Materials and methods

2.1 Biological materials

2.1.1 Bread wheat (*Triticum aestivum*)

The 53 wheat landraces used in the present study are issued from the bank of Graines de Noé seed society (http://www.graines-de-noe.org).

2.1.2 Arbuscular mycorrhizal fungi

We used a commercial inoculum (150,000 propagules.kg^{-1}) of SYMBIVIT® arbuscular mycorrhizal fungi (AMF) provided by INOCULUM*plus* (RD31, 21,110 Bretenière - France). It contained the following six AMF species: *Rhizophagus irregularis* (BEG140), *Claroideoglomus claroideum* (BEG96), *Funneliformis mosseae* (BEG95), *Funneliformis geosporum* (BEG199), *Claroideoglomus etunicatum* (BEG92), and *Glomus microaggregatum* (BEG56). We used this group of AMF as an artificial community.

2.2 Selection of four wheat landraces with a low mycorrhizogenous ability in the presence of indigenous arbuscular mycorrhiza in the field

The trial was conducted in a plot located at the Technopole AgrOnov (N47°14′27.661 E5°6′50,414, 21,110 Bretenières - France). In the plot, the soil was covered by a natural meadow for 8 years prior to the implementation of the trial. An estimation of the mycorrhizal propagules through trap cultures (most probable number analysis; Porter 1979) on the experimental plot was performed prior to the experiment itself, and the average propagule concentration was 2000 propagules/l. Each of the 53 wheat landraces was sown in a 1.5-m^2 micro-plot in November of year n. Two hundred grams of seeds were broadcast-sown on four rows *per* micro-plot. In August of year n + 1, three individuals were randomly sampled at tillering, a key phenological stage of wheat. We determined the prevalence of AMF fungal structures (arbuscules, intraradicular hyphae) in the sampled roots by specific staining using the method of Phillips and Hayman (1970). At that stage, it was possible, depending on the landrace, to have from

0 to 3 plants exhibiting AMF structures (Online Resource 1). In order to use landraces with a reduced mycorrhizogenous ability while forming mycorrhiza in the early developmental stages, we randomly selected 3 wheat landraces exhibiting fungal structures in 1 plant out of the 3 plants sampled at the tillering stage: Blanc de Lorraine (BL), Blé Autrichien (BA), and Rouge de Roc (RR). We also decided to randomly pick up one landrace Blé de la Saône (BS) from the cluster that exhibited no fungal structures at the tillering stage. These four landraces were used for our field and greenhouse trials.

2.3 Analysis of the impact of mycorrhization on the production of four wheat landraces in the field

We biofumigated the same plot (at the Technopole AgrOnov) through mustard cultivation in September of year n + 1. Mustard was ground before seeding and then buried in the soil so as to benefit from the synthesis of volatile isothiocyanates through enzymatic hydrolysis of the glucosinolates present in the plant cells (Ploeg 2008). Isothiocyanates have biocide effects that decrease the indigenous mycorrhizal potential of the soil. In the spring of year n + 2, the plot was submitted to solarization: a transparent plastic tarpaulin was placed on it after abundant watering, and left there for 45 days. This technique destroys certain undesired organisms, especially pathogenic microorganisms, as well as the seeds of adventitious plants; it also decreases the mycorrhizogenous potential of the soil (Caussanel 1996). In November of year n + 2, each of the 4 selected landraces was sown on 8 1.5-m^2 micro plots randomly chosen in the plot. The 4 landraces were sown in a 1.5-m^2 micro-plot, where 200 g of seeds were broadcast-sown on four rows *per* micro-plot. A 5-cm deep furrow was dug in each row, and half of the micro-plots were inoculated with 300 g of SYMBIVIT® (45,000 propagules) *per* row, before sowing the seeds and closing the furrow. Harvest took place on August 1st of year n + 3.

2.4 Analysis of the responses of the four wheat landraces to mycorrhization in the greenhouse

Seeds of the 4 wheat landraces were disinfected in a 7% calcium hypochlorite solution for 5 min and then rinsed in deionized water. The seeds were vernalized in solid agar medium in sterile Petri dishes for 4 weeks at 4 °C, with a 12-h photoperiod. At the 3-leaf stage, 36 plantlets of each landrace were planted one per pot, containing soil taken from the first 20 cm of the plot used for screening the 53 wheat landraces in October of year n + 1 before biofumigation. The soil characteristics were as follows: sand 9%, silt 60%, clay 31%, available P (Olsen 1954) 56 μg/g, NO_3^- 7.7 μg/g, pH (H_2O) 7.1. The soil was first dried, sieved to 5 mm, and mixed with 25% of sterile gravel. Four types of treatment were applied: (i) the control (c), i.e. the substrate autoclaved at 180 °C for 6 h; (ii) the substrate inoculated with indigenous AMF community (i), i.e. the non-sterilized substrate; (iii) the substrate inoculated with the artificial AMF community (a), with 30 g of the product (4500 propagules) placed in the planting hole (to follow a similar inoculation procedure as the one used in the field experiments) with sterilized substrate; and (iv) the substrate inoculated with artificial and indigenous AMF communities (i + ac). Nine plants *per* landrace were used for each of the 4 treatments, and 3 plants *per* treatment were sampled at (i) the tillering stage (20 weeks after planting), (ii) the heading stage (30 weeks after planting), and (iii) ear maturity (39 weeks after planting).

2.5 Evaluation of the production (quantity and quality) parameters of the four wheat landraces in the field

At harvest, all seeds of each micro-plot were collected and mixed. Then 300 seeds *per* micro-plot were randomly sampled; 100 were used to measure the germination rate (8 days at 4 °C on water agar), 100 to determine seed viability following the method developed by Association of Official Seed Analysts of North America et al. (1970), and 100 for P content determination. To determine seed viability, seeds were soaked in water overnight and then longitudinally cut in two, so that the embryo was visible in profile. Then it was placed in contact with 2 ml of a 0.25% triphenyl tetrazolium (TTC) solution prepared in phosphate buffer (50 mM, pH 7.4). The reaction was stopped by replacing the TTC solution by water. In case of embryonic metabolic activity, TTC was reduced and a red TTC-formazan compound was formed. The color intensity was determined under a binocular microscope, based on Grabe's color scale (1970). The red color therefore indicated metabolic activity by the embryo, but also its intensity and the potential of the seed to germinate: the more intense the color, the higher the potential. The seed phosphorus content was analyzed by the SADEF 'Agronomie et Environnement' laboratory (Pôle d'Aspach, Rue de la Station F-68700 Aspach-le-Bas) using Inductively Coupled Plasma - Atomic Emission Spectrometry, ICP-AES) (Internal method MA7-16 V rev3 / IF04–18 rev1).

2.6 Evaluation of the mycorrhization, development, and production parameters of the four wheat landraces in the greenhouse

Three plants *per* treatment were randomly selected at each phenological stage (tillering, heading, ear maturity). A representative sample of the root system (around 100 mg) of each plant was taken, washed, and stained according to the method of Phillips and Hayman (1970). The following mycorrhization parameters were calculated: mycorrhization frequency (F%), mycorrhization intensity (M%), and the arbuscules richness

(A%) in the roots as defined by Trouvelot et al. (1986). Plants were sampled at the three phenological stages of wheat to determine aerial and root fresh biomass values. At the end of the cultivation period, the seed number and the seed weight *per* plant were evaluated, together with their filling rate (mean weight of one seed *per* plant).

2.7 Statistical analyses

All statistical analyses were performed using R software (R Core team 2018) and the following packages: car (Fox and Weisberg 2011), coin (Hothorn et al. 2008), emmean (Lenth and Lenth 2018) and vegan (Oksanen et al. 2018). Experiments were designed according to three factors: the developmental stages (tillering, heading, ear maturity), the landrace (BA, BS, RR, BL) and the treatment (in the greenhouse: (c), (i), (a), (i + a); in the field: inoculated wheat vs. non-inoculated wheat). Developmental stages were used as a fixed factor. We determined the level of accuracy of the mean values for each parameter using the Bootstrap method, which makes it possible to calculate the 95% confidence intervals of the mean values. The data were first analyzed with factorial ANOVA to determine the significance of main factor effects and interactions. However, at least one *assumption* for the use of ANOVA, namely the assumption of homogeneity of variance, was never met, except for the germination rate parameter. Consequently, we applied the Kruskal-Wallis non-parametric test, followed by Dunn's test with Bonferroni correction.

General linear models (GLMs) were developed to assess the influence of both landrace and treatment factors on each parameter. The resulting models were analyzed by pairwise comparisons of the estimated marginal means using the emmean package and the pair function adjusted with Tukey's test.

Non-metric multidimensional scalings (nMDS) were constructed:

- in greenhouse conditions by using the following parameters: the three mycorrhization parameters (F%, M%, A%), the root and aerial biomass values, and the seed number and the seed weight *per* plant (only at ear maturity),
- in field conditions by using the following parameters: the seed number and the seed weight *per* plant, seed viability, the germination rate, and the seed P content.

The quality of the nMDS, i.e. the proper ordination of distances, is deduced from the calculation of stress, which is a global index of the grouping of points around the Shepard's diagram curve; stress is considered as acceptable when it is <0.2. Moreover, we implemented Adonis function (non-parametric MANOVA) with 999 permutations to determine possible significant differences among groups. We checked the Adonis prerequisite of homogeneity of group dispersion using a permutation test equivalent to an ANOVA. We chose permutational nMDS because the dataset was small, did not follow theoretical distributions, and included extreme values. Once structuring groups were determined within the nMDS representation, we used the simper function in the vegan package to determine the contribution of each parameter between two groups using Bray-Curtis dissimilarities.

3 Results

3.1 Evaluation of the mycorrhization, development, and production parameters of the four wheat landraces in the greenhouse

3.1.1 Mycorrhization rate

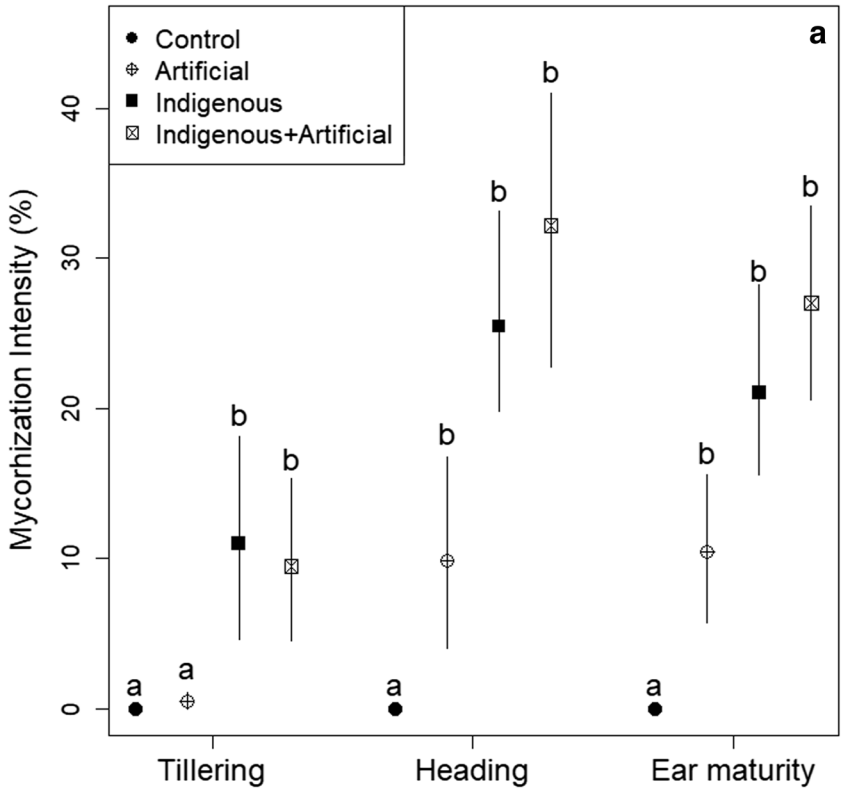
In the greenhouse conditions, no difference among the landraces was evidenced to account for the 2-factorial design of the experiment (data not showed). Yet, we decided to pool the data across all four landraces to test if the wheat landraces were significantly structured in groups according to the M%, and A% mycorrhization parameters were null for the non-inoculated (c) wheat whatever its developmental stage, as expected (Fig. 1). It should be noted that the values for A% for artificial (a)-inoculated wheat were close to zero for each developmental stage (Fig. 1b).

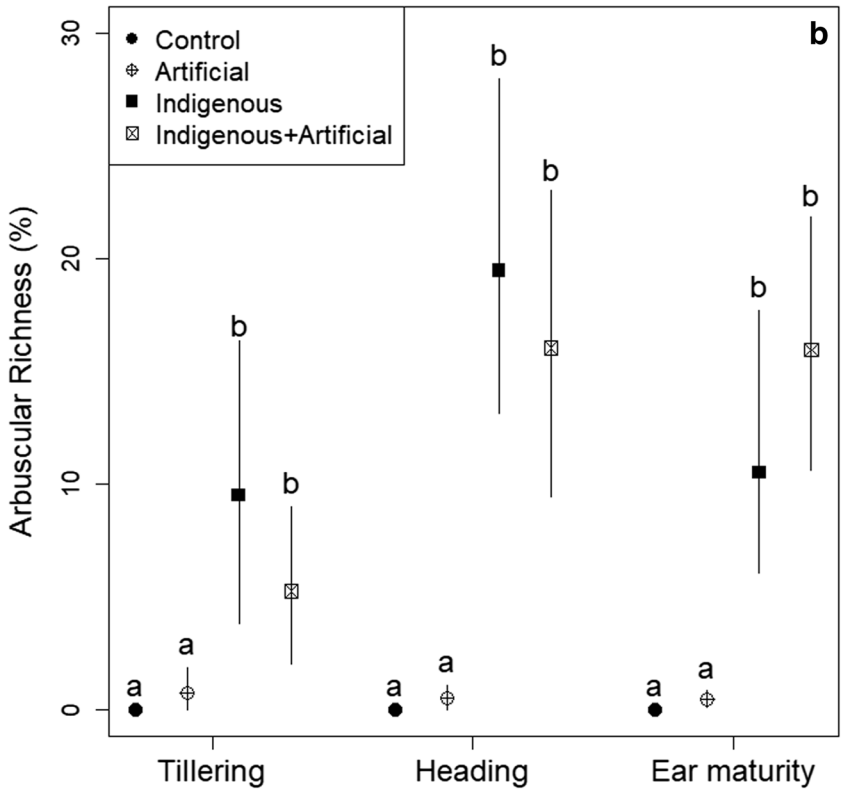
At tillering, the values of the three parameters measured in indigenous (i)- and (i + a)-inoculated wheat differed significantly from those measured in (c)- and (a)-inoculated wheat (Fig. 1; Online Resource 2; Online Resource 5; Online Resource 6). There were more fungal structures and especially arbuscules in (i)- and (i + a)-inoculated wheats than in (a)-inoculated wheat (Fig. 1).

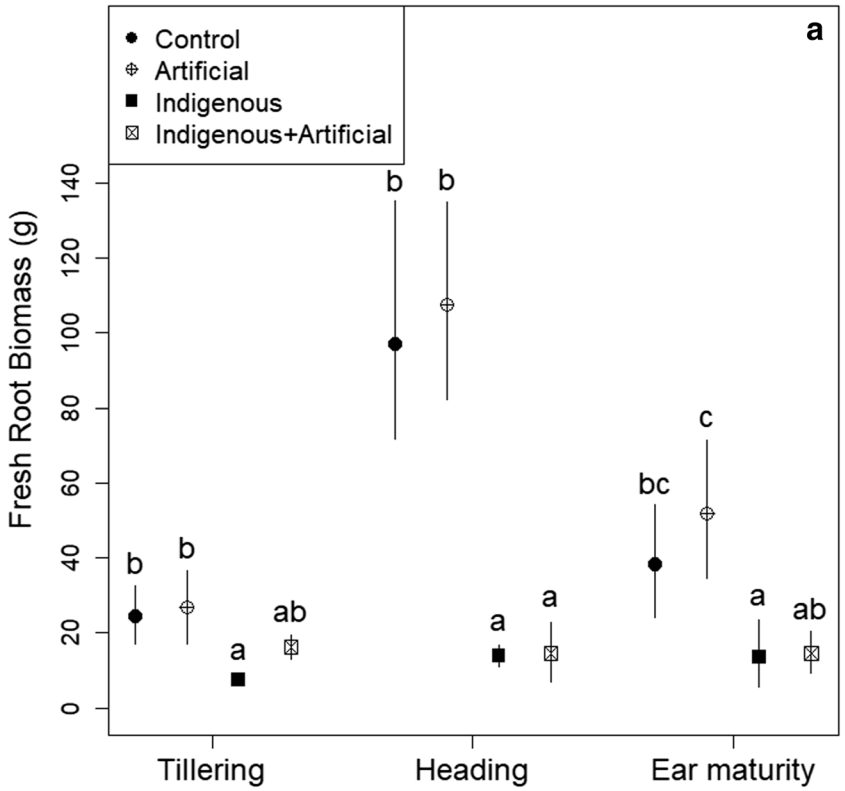
At heading, mycorrhization did not differ significantly following treatments with (i), (a), and (i + a) (Fig. 1; Online Resource 6).

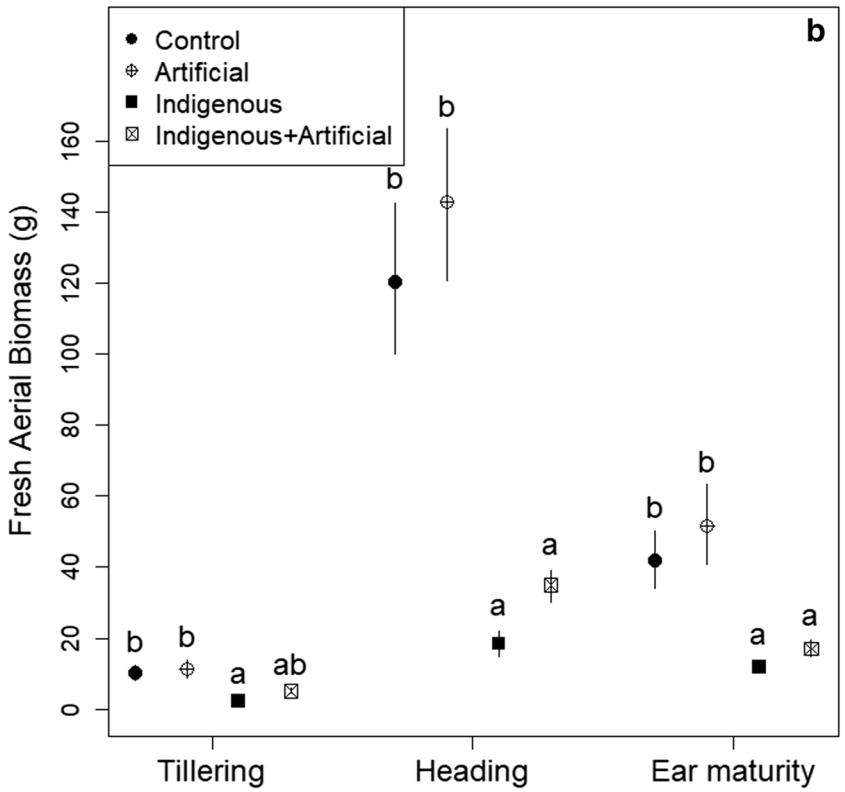
At ear maturity, F%, M%, and A% values were similar to the heading stage values whatever the treatment (Fig. 1; Online Resource 6).

Fig. 1 Evaluation of the mycorrhization parameters in greenhouse conditions: time course of **a** the mean mycorrhization intensity (M%) and **b** mean arbuscular richness (A%) (%) with a 95% confidence interval. Mycorrhization values are given according to the treatment type for the 4 landraces taken together: indigenous (i), artificial (a), indigenous + artificial (i + a), and non-treated (c). Mean comparisons are treated separately for each developmental stage (tillering, heading, ear maturity). Different lowercase letters above the bars indicate significant differences ($P < 0.05$) among the inoculated treatments according to Dunn's test with Bonferroni correction. $n = 12$


a
Control
Artificial
Indigenous
Indigenous+Artificial
Mycorhization Intensity (%)
0
10
20
30
40
a
a
b
b
a
b
b
a
b
b
Tillering
Heading
Ear maturity


b
Control
Artificial
Indigenous
Indigenous+Artificial
Arbuscular Richness (%)
0
10
20
30
a
a
b
b
a
a
b
b
a
a
b
b
Tillering
Heading
Ear maturity


a
Control
Artificial
Indigenous
Indigenous+Artificial
Fresh Root Biomass (g)
0
20
40
60
80
100
120
140
b
b
a
ab
b
b
a
a
bc
c
a
ab
Tillering
Heading
Ear maturity


b
Control
Artificial
Indigenous
Indigenous+Artificial
Fresh Aerial Biomass (g)
0
20
40
60
80
100
120
140
160
b
b
a
ab
b
b
a
a
b
b
a
a
Tillering
Heading
Ear maturity

◀ **Fig. 2** Evaluation of the development parameters in greenhouse conditions: time course of **a** the mean root biomass and **b** the mean aerial biomass (g) with a 95% confidence interval. Biomass values are given according to the treatment type for the four landraces taken together: indigenous (i), artificial (a), indigenous + artificial (i + a), and non-treated (c). Mean comparisons are treated separately for each developmental stage (tillering, heading ear maturity). Different lowercase letters above the bars indicate significant differences ($P < 0.05$) among the inoculated treatments according to Dunn's test with Bonferroni correction. $n = 12$

3.1.2 Total biomass

The (a)-inoculated wheats did not significantly differ in total biomass (aerial biomass + root biomass) from non-treated (c) wheat whatever the developmental stage (Fig. 2; Online Resource 5; Online Resource 7). Similarly, the total biomass values of (i)- and (i + a)-inoculated wheats did not significantly differ similar whatever the developmental stage of the wheats (Fig. 2; Online Resource 7). However, total biomass was significantly higher in (a)-inoculated and non-treated (c) wheats than in (i)- and (i + a)-inoculated wheats whatever the developmental stage, except (i) at the tillering stage when aerial and root biomass values did not differ between (i + a)-inoculated wheats on the one hand and both (a)-inoculated and non-treated (c) wheats on the other hand, and (ii) at the heading stage when root biomass values did not differ between (i + a)-inoculated wheats and non-treated (c) wheats (Fig. 2; Online Resource 7).

3.1.3 Yield

We estimated yield based on number of seeds *per* plant and seed weight *per* plant parameters. These parameters were significantly lower for (i)- and (i + a)-inoculated wheats as compared to non-treated (c) and (a)-inoculated wheats (Online Resource 3; Online Resource 5; Online Resource 7).

3.1.4 Multivariate analysis of the impact of inoculation of four wheat landraces with arbuscular mycorrhizal fungi in the greenhouse

Graphical representation of the data by nMDS according to the wheat landrace did not show any structuring into groups whatever the developmental stage (data not showed). Yet, when all landraces were taken together, the analysis revealed that the wheat landraces were significantly structured in groups according to the treatment factor at tillering (Online Resource 4A; Online Resource 8), as shown by previous Kruskal Wallis analyses. Indeed, the non-treated (c) and (a)-inoculated wheats differed significantly from the (i)- and (i + a)-inoculated wheats, mainly due to the mycorrhization parameters F%, M%, and A%, whereas the contributions of aerial biomass and root biomass to explain data structuring at the tillering stage were low (Online Resource 9). Furthermore, for the other two developmental stages (heading and ear maturity), the data were similarly structured in the graph according to the treatment factor, except treatments (c) and (a) that might appear as distinct (Online Resource 4B; Online Resource 4C); but the absence of an equivalence of variance dispersion of the groups precluded statistical verification (Online Resource 8).

3.2 Analysis of the impact of mycorrhization on the quantitative and qualitative production of the four wheat landraces in the field

3.2.1 Seed quality

The statistical analyses of the GLM results showed that the landrace factor had no effect on the germination rate (data not showed); conversely, the germination rate was significantly influenced by the treatment factor for all landraces, except BA (no significant difference) (Online Resource 11A). We decided to group all landraces together to analyse the impact of the treatment factor on the germination rate (Fig. 3); the germination rate was significantly higher for inoculated wheat as compared to non-inoculated wheat (Online Resource 10;

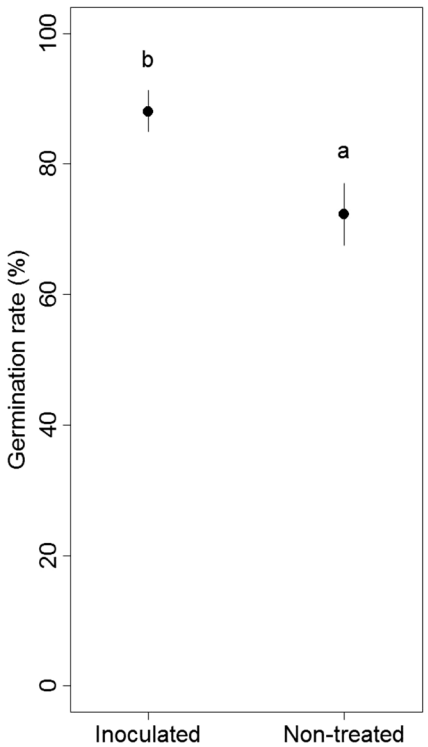


Fig. 3 Evaluation of the germination rate (%) of wheat seeds depending on the 'treatment' factor in field conditions. Germination rate values are given according to the treatment type for the four landraces taken together, inoculated and non-inoculated. The two lowercase letters above the bars indicate significant differences ($\alpha = 0.05$, P value = 0.02) among the inoculated treatments according to Dunn's test with Bonferroni correction. $n = 4$

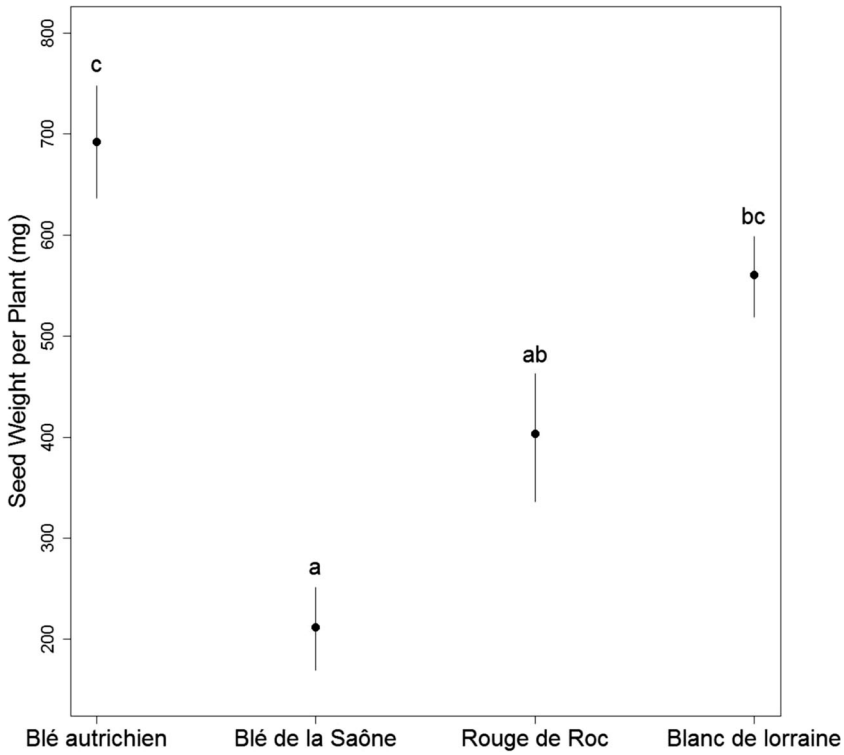


Fig. 4 Evaluation of the seed weights *per* plant (mg) depending on the 4 landraces Blé autrichien, Blé de Saône, Rouge de Roc and Blanc de Lorraine with a 95% confidence interval in field conditions. The mean seed weights of the wheat inoculated with the artificial product and of the non-inoculated wheat were grouped. Different lowercase letters above the bars indicate significant differences ($P < 0.05$) among the inoculated treatments according to Dunn's test with Bonferroni correction. $n = 6$

Online Resource 11B). In addition, the statistical analyses of the GLM models showed that there was no interaction between the landrace and treatment factors (data not showed).

The statistical analyses did not reveal any significant differences in terms of seed viability or phosphorus content, nor did it show any significant differences according to the wheat landrace or the treatment effect (data not shown).

3.2.2 Wheat yield in seed weight per plant

Seed weight *per* plant did not differ significantly among the inoculated treatments (data not shown).

In contrast, we noted a significant landrace factor effect on the seed weight *per* plant (Fig. 4; Online Resource 10; Online Resource 12). Seed weight *per* plant was higher in BA than in BS and RR; the seed weight *per* plant of BL was also higher than the seed weight per plant of BS.

4 Discussion

4.1 Responses in greenhouse conditions

We did not find any difference among the wheat landraces, as the four landraces exhibited similar mycorrhization profiles. However, we found variability within landraces, possibly explained by the fact that wheat landraces display greater morphological differences than wheats issued from fixed pure lines (Mollier 2017).

Inoculation with the indigenous AMF community (ec) blocked wheat development relatively to the biomass and the yield of (i) (i)-inoculated wheat as compared to control (c) wheat, and (ii) (i + a)-inoculated wheat as compared to (a)-inoculated wheat (Fig. 2). However, this conclusion has to be balanced in regard to the fact that AMF are only a part of the field soil microbe community. Nevertheless the mycorrhization rate of the 4 wheat landraces with low mycorrhizing ability was improved in the presence of the indigenous (i) and mixed (i + a) AMF communities at the tillering. It is also worthwhile to note that wheats inoculated with indigenous and indigenous+artificial communities displayed low biomass and high root colonization (with arbuscules), whereas wheats inoculated with only the artificial community and non-inoculated wheats displayed both high biomass and low root colonization. This apparent contradiction between yield and mycorrhization levels shows that the blocked wheat development could be due to either (i) the other components of the field soil microbe community, or (ii) that colonization of the roots of certain wheat landraces by AMF is not necessarily beneficial as suggested by Hetrick et al. (1992). The same idea was demonstrated for a given plant species colonized by different AMF strains (Fernández et al. 2014).

The response to mycorrhization varied according to the AMF community ranging from no effect on the plant and the absence of arbuscules in the presence of the artificial community, to a negative impact and the presence of arbuscules in the presence of the indigenous community. This variation illustrates (i) the variability of the responses of wheat landraces

to mycorrhization, as in other plant species such as *Bromus erectus* Huds., *Festuca ovina* L., and *Hieracium pilosella* L. (van der Heijden et al. 1998), and (ii) the possibility for the mycorrhization index to be negative in the case of symbiosis established with the indigenous AMF community (Moora 2014). Moreover, the results of a study conducted by Klironomos (2003) indicate that plant growth responses to mycorrhizal inoculation within an ecosystem can range from highly parasitic to highly mutualistic. Besides, the observed near-complete absence of development suggests that the soil used as an indigenous inoculum may have contained microorganisms pathogenic for our wheat landraces. As a result, the carbon resources of the infected wheat may have been allocated to defense and/or diverted by the pathogen instead of being allocated to growth (Jones and Dangl 2006).

The effects of AM have a genetic basis that at least partly explains the variability in mycorrhizogenous ability in plants of the same species (Hetrick et al. 1992). In our trials, the absence of positive effects of AM on the yield of wheat landraces inoculated with the artificial AM community indicates that the AMF communities involved may not have been compatible with our four wheat landraces in terms of beneficial effects. The discrepancy between these greenhouse results and the field results obtained when using the artificial community, could be due to the fact that in the field the "artificial AM community" is only a part of the soil microbe community, which would then result in a different compatibility/incompatibility result. This demonstrates that care must be taken in transferring lessons from the greenhouse to the field (Rowe et al. 2007), especially when considering that AMF represent only a reduced part of soil microbe community.

The components of wheat yield appear as early as the tillering stage (Hay 1999), therefore the late mycorrhization of (a)-inoculated wheat could explain the absence of positive effects of symbiosis (Singh et al. 2012); the absence of arbuscules, i.e. the main site of nutrient exchanges, in the roots of (a)-inoculated wheat can also explain this observation, even though other nutrient exchange interfaces can exist inside AM (Helber et al. 2011). Moreover, we evaluated the benefits of AM solely based on the biomass produced by the 4 wheat landraces. Whereas the development of AMF inside the roots, even in the absence of arbuscules, indicates a probable transfer of carbon components by the host plant due to the obligate biotroph character of AMF. Furthermore, AM induce other benefits that we did not measure, such as improved tolerance to certain biotic and abiotic stresses (Smith and Read 2008; Gianinazzi et al. 2010).

4.2 Responses of wheat landraces in field conditions

In contrast to our greenhouse results, the analysis of the mean seed weight *per* plant revealed that our wheats grouped according to the landrace factor whatever the treatment. This difference may be due to the impact of environmental factors in the field such as biotic factors and abiotic factors, especially the soil nutrient status (Johnson et al. 1997). Nevertheless, these environmental factors do not appear to have influenced the impact of the treatment factor: in field conditions as well as in greenhouse conditions, no significant difference in seed weight *per* plant emerged between the artificial-AMF-inoculated wheat and the control wheat.

If we compare the landraces with one another, BA and BL were the most productive, and the best adapted to the pedoclimatic context of the field. BS was the only landrace infected by stinking smut (common bunt) of wheat, caused by *Tilletia caries* and characterized by so-called bunted grains that contain a large number of spores and emit a characteristic putrid smell. This probably impacted the yield: the mean number of seeds *per* plant was 3-fold lower in BS than in BA and BL. Besides, apart from phytochemical treatments, the only technical solutions available to control this pathogen are a rapid emergence of the plantlets after sowing and varietal tolerance. As the main two vectors are the seeds and the soil, BS is not adapted for the field where the trial was carried out.

At least two studies have shown no effect of mycorrhization on the germination rate of wild wheat seeds, although the seed P content increased (Heppell et al. 1998; Nuortila et al. 2004). By contrast, in our study mycorrhization improved the germination rate of wheat landrace seeds without modifying their P content. Although the P content was not modified, we cannot definitively conclude that AM had no effect on the seed P uptake: P is taken up via two independent pathways in mycorrhizal plants, i.e. via the direct pathway through the roots and via the partner fungus through its extra-radicular hyphae (Sawers et al. 2008; Smith and Smith 2011). Thus, the seeds of mycorrhizal wheat could display the same P content, but taken up via an at least partly different pathway. (Casieri et al. 2013; Drain et al. 2017).

5 Conclusions and perspectives

In summary, the community of arbuscular mycorrhizal fungi can have an effect on yield, even in wheat landraces with a low mycorrhizogenous ability. But it is essential to properly choose the criteria (phenotypic criteria as root and shoot biomass versus quality criteria as grain quality) used to measure this possible gain.

It would be of interest to develop and use fungal taxonomic and functional markers (Simon et al. 1992; Walder et al. 2016) and plant functional markers (measuring the expression levels of phosphate transporter genes in mycorrhizal roots; Glassop et al. 2005; Gamper et al. 2010; Casieri et al. 2013; Drain et al. 2017) for future assessments of the intensity of nutrient exchanges inside AM, using the genome sequences of wheat (IWGSC 2018) and of the AMF *Rhizophagus irregularis*

DAOM 197198 (Tisserant et al. 2013). Even though, one of the major key of sustainable agriculture is to promote services provided by beneficial organisms such as AMF (Douds et al. 1993; Sawers et al. 2008; Gianinazzi et al. 2010), specific criteria highlighting positive effects from such interactions are not always considered for varietal selection (Wortman et al. 2013; Leiser et al. 2016).

Landraces are often presented as a plausible solution for sustainable agriculture (Wolfe et al. 2008), especially as they are believed to better respond to mycorrhization than the modern varieties selected in high-input conditions after the 1950s (Manske 1990; Hetrick et al. 1992; Zhu et al. 2001; Sawers et al. 2018). However, these conclusions are controversial because of the index used to determine the mycorrhizal response (Sawers et al. 2010). Incidentally, it would be interesting to pursue future comparative analyses by using modern varieties and studying more parameters of the impact of AM on the quality of the production of wheat landraces and even transformed products such as bread (Rillig et al. 2018).

Acknowledgements The authors are very grateful to Graines de Noé seed society for providing landrace seeds as well as to B. Ronot for his accurate advices. OEO is thankful to Agence nationale des Bourses du Gabon for the supporting grant number 043827. The authors wish to thank D. Redecker, D. van Tuinen, R. Thomson and G. Adeux whose comments improved the manuscript. Finally, the authors also wish to thank A. Buchwalter for English proofreading.

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