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# On-farm inoculants based on arbuscular mycorrhizal fungi on wheat performance

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**Abstract** – The objective of this work was to evaluate whether the use of on-farm inoculants based on arbuscular mycorrhizal fungi (AMF) interferes with the agronomic performance of wheat cultivars. The following treatments were applied to cultivars TBIO Calibre and TBIO Sossego: eight on-farm inoculants, i.e., *Acaulospora morrowiae*, *Cetraspora pellucida*, *Claroideoglomus etunicatum*, *Glomus intraradices*, *Rhizophagus clarus*, *Scutellospora heterogama*, and two mycorrhizal communities from native forest (NF) obtained in the Bom Princípio (BP) and in the Flores da Cunha (FC) Brazilian municipalities; and no inoculant (control). A randomized complete block experimental design was used. Mycorrhizal colonization, root morphology, and thousand grain weight were evaluated. The association between 'TBIO Sossego' and *S. heterogama* provided the greatest root volume, while that between 'TBIO Calibre' and *C. etunicatum* and between 'TBIO Sossego' and the BP NF community resulted in the best thousand-grain weight. The use of AMF, especially *C. etunicatum* and *S. heterogama*, enhances the development of the root system of wheat. 'TBIO Calibre' showed the greatest total length and quantity of very fine roots, while 'TBIO Sossego' developed a root system with the greatest surface area and quantity of thick roots. The use of on-farm inoculants affects the agronomic performance of wheat cultivars.

**Index terms**: *Triticum aestivum*, root system, sustainability, yield.

## Inoculantes *on farm* à base de fungos micorrízicos arbusculares no desempenho de trigo

**Resumo** – O objetivo deste trabalho foi avaliar se o uso de inoculantes *on farm* (no campo) à base de fungos micorrízicos arbusculares (FMA) interfere no desempenho agronômico de cultivares de trigo. Os seguintes tratamentos foram aplicados às cultivares TBIO Calibre e TBIO Sossego: oito inoculantes *on farm*, i.e., *Acaulospora morrowiae*, *Cetraspora pellucida*, *Claroideoglomus etunicatum*, *Glomus intraradices*, *Rhizophagus clarus*, *Scutellospora heterogama*, e duas comunidades micorrízicas de floresta nativa (NF) obtidas nos municípios brasileiros de Bom Princípio (BP) e Flores da Cunha (FC); e nenhum inoculante (controle). Utilizou-se o delineamento experimental de blocos ao acaso. A colonização micorrízica, a morfologia radicial e o peso de mil grãos foram avaliados. A associação entre 'TBIO Sossego' e *S. heterogama* proporciona maior volume radicial, enquanto a associação entre 'TBIO Calibre' e *C. etunicatum* e aquela entre 'TBIO Sossego' e a comunidade BP NF resulta no melhor peso de mil grãos. O uso de FMA, com destaque para *C. etunicatum* e *S. heterogama*, potencializa o desenvolvimento do sistema radicial do trigo. 'TBIO Calibre' apresentou maior comprimento total e maior quantidade de raízes muito finas, enquanto 'TBIO Sossego' desenvolveu um sistema radicial com maior área superficial e quantidade de raízes grossas. O uso de inoculantes *on-farm* afeta o desempenho agronômico de cultivares de trigo.

**Termos para indexação**: *Triticum aestivum*, sistema radicial, sustentabilidade, rendimento.

## Introduction

Wheat (*Triticum aestivum* L.) has become one of the most consumed crops by humanity because of the exponential growth in consumption and area occupied worldwide (Khalid et al., 2023). In Brazil, wheat is mostly grown in the south of the country, but production is already spreading to other areas, due to this crop great soil and climate adaptability. However, this increase in the number of cultivated areas does not negate the need to boost productivity to meet demand. Higher yields require greater use of inputs, which goes against the grain search by producers and consumers for a more sustainable end product (Mitura et al., 2023).

The excess use of chemical inputs in wheat cultivation – such as biocides and fertilizers – has led to the depletion of both this crop production chain and agroecosystems (Nilsson et al., 2023). As an alternative to the sustainability of triticulture, biotechnologies play a key role in an agroecological transition system, making it possible to improve the yield and quality of products and to reduce production and environmental costs. Among the biotechnologies that can be used for wheat cultivation are arbuscular mycorrhizal fungi (AMF). These microorganisms are naturally present in soils and establish a symbiotic relationship with most plants, including wheat (Ellouze et al., 2018). As reported for other crops, the association with AMF makes it possible to reduce the use of chemical inputs (Chiomento et al., 2022; Gómez-Leyva et al., 2023; Huang et al., 2023) and can function as a sustainable bio-tool for wheat management.

Wheat is an important crop on a global scale. Therefore, finding ways to increase its productivity through the use of mycorrhizae is crucial to ensure food security (Huang et al., 2023). However, the availability of commercial inoculants based on AMF is restricted. Furthermore, this biotechnology is unknown to most producers. Despite this lack of inoculants and the dissemination of AMF to wheat growers, the literature reports that mycorrhization of wheat increased phosphorus absorption by up to 30% (Elliott et al., 2021). In addition, under salt stress, AMF improve carbon and nitrogen absorption, which has led to higher yields and better grain quality (Huang et al., 2023). In nonstressful environments, the effects of AMF inoculation on wheat should be understood, in association with the development of the root system and the crop's productive potential, in order to establish a sustainable management in the cultivation of this grain crop. Therefore, the hypothesis that wheat plants subjected to the inoculation of AMF perform better.

The objective of this work was to evaluate whether the use of on-farm inoculants based on AMF interferes with the agronomic performance of wheat cultivars. Our results may elucidate the benefits of arbuscular mycorrhizae in providing ecosystem services to ensure sustainable wheat production and improve the production chain of this crop.

## Materials and Methods

The plant material for the research consisted of two wheat cultivars developed by GDM Genética do Brasil S.A. and Biotrigo Genética Ltda. 'TBIO Calibre' has a super-early cycle and stands out for its high productivity, balanced resistance to the main crop diseases, low height and resistance to lodging. Its average thousand-seed weight (TSW) is 36 grams. 'TBIO Sossego' has a medium cycle, a medium to tall plant stature, and it is resistant to leaf and ear diseases. Its average TSW is 33 grams.

The experiment was carried out at Biotrigo Genética experimental area, in the municipality of Passo Fundo (28º15'41"S; 52º24'45"W, at 687 m altitude), in the state of Rio Grande do Sul (RS), Brazil. According to the Köppen-Geiger's climate classification, Passo Fundo is characterized by the Cfa climate type, with no defined dry season and hot summer (Alvares et al., 2022). The experiment was conducted from June (winter) to November (spring) 2021 in a 400 $m^2$ greenhouse with a semicircular roof. The galvanized steel structure was covered with low-density polyethylene film with an anti-ultraviolet additive and 150 µ thickness, and with an anti-aphid mesh in the sides.

The experiment was laid out in a randomized complete block design, with four replicates. Treatments were two cultivars ('TBIO Calibre' and 'TBIO Sossego') designed in a bifactorial arrangement, in the absence (control) and presence of eight on-farm inoculants [*Acaulospora morrowiae* Spain & N.C. Schenck, *Cetraspora pellucida* (T.H. Nicolson & N.C. Schenck) Oehl, F.A. Souza & Sieverd, *Claroideoglomus etunicatum* (W.N. Becker & Gerd.) C. Walker & A. Schüßler, *Glomus intraradices* N.C. Schenck & G.S. Smith, *Rhizophagus clarus* (T.H. Nicolson & N.C. Schenck) C. Walker & A. Schüssler, *Scutellospora* *heterogama* (T.H. Nicolson & Gerd.) C. Walker & F.E. Sanders, mycorrhizal community BP NF, and mycorrhizal community FC NF].

*A. morrowiae*, *C. pellucida*, *C. etunicatum*, *G. intraradices*, *R. clarus*, and *S. heterogama* were sourced from the International Culture Collection of Glomeromycota (CICG). The AMF communities used (Table 1) came from the trap crop of native forest (NF) soils collected in the municipalities of Bom Princípio (BP) (29º29'22"S; 51º21'12"W, at 37 m altitude) and Flores da Cunha (FC) (29º01'50"S; 51º11'30"W, at 710 m altitude), RS, Brazil (Chiomento et al., 2019).

The eight on-farm inoculants were obtained using the trap culture technique (Stutz & Morton, 1996), using sorghum [*Sorghum bicolor* (L.) Moench] as the host plant, and sterilized sand (120ºC, 20 min) as the substrate. After five months of growing sorghum, the inoculants were obtained and contained mycorrhizal spores, pieces of roots (trap culture), and sand (trap culture substrate).

Sowing took place in June 2021, in pots (15 L) filled with soil and kept in a greenhouse. The soil used to fill the pots was taken from the Biotrigo Genética experimental area. The soil was not autoclaved or sieved. The predominant soil in the area is classified as a Latossolo Vermelho distrófico, according of Brazilian Soil Classification System (Santos et al., 2018), which corresponds to Oxisol. The soil chemical characterization was carried out according to the methodology described in Brasil (2014). The soil presented the following characteristics: pH $H_2O$, 4.9; SMP index (Shoemaker-Mac'Lean-Pratt's index), 5.4; soil organic matter, 1.9 dag $kg^{-1}$; P, 3.60 mg $dm^{-3}$; K, 55.0 mg $dm^{-3}$; Ca, 1.39 $cmol_c$ $dm^{-3}$; Mg, 1.08 $cmol_c$ $dm^{-3}$; Al, 2.5 $cmol_c$ $dm^{-3}$; H+Al, 8.7 $cmol_c$ $dm^{-3}$; CEC (T), 11.3 $cmol_c$ $dm^{-3}$; Al saturation (m), 49%; base saturation (V), 23%; K saturation, 1.2%; S, 17.4 mg $dm^{-3}$; B, 0.3 mg $dm^{-3}$ Zn, 2.18 mg $dm^{-3}$; Mn, 35.1 mg $dm^{-3}$; Cu, 3.44 mg $dm^{-3}$; and clay, 700 g $kg^{-1}$.

Fifteen seed were added to each pot. For the treatments inoculated with AMF, 30 g of on-farm inoculant were added when the cultivars were sown in the pots.

No temperature or relative humidity control was carried out in the greenhouse. Using a thermometer, the average monthly temperatures inside the greenhouse showed the following values: 13.50ºC (June); 13.12ºC (July); 17.50ºC (August); 19.17ºC (September); 19.21ºC (October); and 22.43ºC (November).

The irrigation used in the experiment was localized by means of drip lines in an automated system. The nutrient solutions (De Nardi et al., 2024) supplied to the plants were given weekly.

To verify AMF infective capacity, portions of plant roots were prepared (Phillips & Hayman, 1970), and percentage of mycorrhizal colonization (MC) was determined by the following equation (Trouvelot et al., 1986): MC (%) = (TNFWMR/ TNF) × 100, where TNFWMR is the total number of fragments with mycorrhizal roots and TNF is the total number of fragments.

**Table 1.** Arbuscular mycorrhizal fungi (AMF) communities identified in native forest (NF) soils collected in the municipalities of Bom Princípio and Flores da Cunha, in the state of Rio Grande do Sul (RS), Brazil, 2019.

| Municipality | Acronym | Mycorrhizal communities[(1)] |
|---|---|---|
| Bom Princípio | BP NF | *Acaulospora* aff. *scrobiculata*; *Acaulospora colossica* P.A. Schultz, Bever & J.B. Morton; *Acaulospora scrobiculata* Trappe; *Acaulospora* sp.; *Acaulospora* sp.1 (*Entrophospora infrequens* like); *Acaulospora* sp.2 (*excavata* like); *Ambispora leptoticha* (N.C. Schenck & G.S. Sm.) R.J. Bills & J.B. Morton; *Claroideoglomus* aff. *luteum*; *Claroideoglomus claroideum* (N.C. Schenck & G.S. Sm.) C. Walker & A. Schüßler; *Claroideoglomus etunicatum*; *Funneliformis mosseae* (T.H. Nicolson & Gerd.) C. Walker & A. Schüßler; *Glomus microaggregatum* Koske, Gemma & Olexia; *Glomus* sp1; *Glomus* sp2 |
| Flores da Cunha | FC NF | *Acaulospora colossica*; *Acaulospora koskei* Blaszk.; *Acaulospora lacunosa* J.B. Morton; *Acaulospora mellea* Spain & Schenck; *Acaulospora tuberculata* Janos & Trappe; *Claroideoglomus claroideum*; *Dentiscutata savannicola* (R.A. Herrera & Ferrer); *Funneliformis mosseae*; *Glomus* aff. *manihotis*; *Racocetra* sp.; *Racocetra verrucosa* (Koske & C. Walker) Oehl, F.A. Souza & Sieverd |

[(1)]Classification of Glomeromycota by Redecker et al. (2013).


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The root system morphology was assessed by collecting plant roots and washing them in water to remove soil fragments. The roots were scanned and the images obtained were analyzed using WinRHIZO software (Chiomento et al., 2021). The attributes evaluated were total root length (TRL, cm), surface area (SA, $cm^2$), and root volume (RV, $cm^3$). The roots were grouped by the software into different diameter classes in relation to their total length (Böhm, 1979), as follows: very thin roots (VTR, Ø < 0.5 mm); fine roots (FR, Ø from 0.5 to 2.0 mm); and thick roots (TR, Ø > 2.0 mm).

To assess the thousand-grain weight (TGW), seed were counted in series of a hundred and, then, a calculation was made to arrive at the TGW (Brasil, 2009).

Data were subjected to the analysis of variance, and the means of the treatments were compared using the Tukey's test, at a 5% probability, using RStudio (R Core Team, 2020). Multivariate analysis was also carried out, using the principal component analysis (PCA) by the "factoextra" package in RStudio (R Core Team, 2020). This was done after standardizing the attributes, in which each one had a mean of 0 and a variance of 1. As there was no relevant information, eigenvalues below 1 were disregarded from the analysis.

## Results and Discussion

The analysis of variance showed that there was an interactive effect between inoculants and wheat cultivars for the attributes MC, RV, and TGW. In isolation, the cultivar factor influenced the attributes TRL, SA, VTR, and TR, while the inoculant factor had effect on the attributes TRL, SA, VTR, FR, and TR. The AMF structures observed on the roots were hyphae, vesicles, and arbuscules (Figure 1).

The addition of on-farm mycorrhizal inoculants to the soil significantly increased the colonization of the roots of 'TBIO Calibre' and 'TBIO Sossego' (Table 2). Different fungal species, related to the same plant symbiont through strategies unique to each fungus, were influenced by the compatibility and efficiency of the symbiotic system (Barbosa et al., 2019). The first stages of the mycorrhizal association are controlled by the strigolactone released by the plants. Afterwards, the AMFs release lipochito-oligosaccharides to penetrate the plant roots. Colonization begins with the penetration of hyphae (Figure 1 A) into the cortical tissue, with the possibility of intraradicular vesicle formation (Figure 1 B). Inside the plant cells, the fungus branches out into structures called arbuscules (Figure 1 C), which are responsible for the bidirectional flow between symbionts. As arbuscules are wrapped in the periarbuscular membrane, these microorganisms are not phytopathogenic (Gutjahr, 2014).

The highest percentages of MC (>75%) occurred in 'TBIO Calibre' plants inoculated with *C. etunicatum*, *G. intraradices*, *R. clarus*, and the BP NF community (Table 2). In 'TBIO Sossego', this result was observed in plants inoculated with *G. intraradices*, *S. heterogama*, and the FC NF community. The association between 'TBIO Sossego' and *S. heterogama* provided the highest RV. The best TWG was reported in the interaction

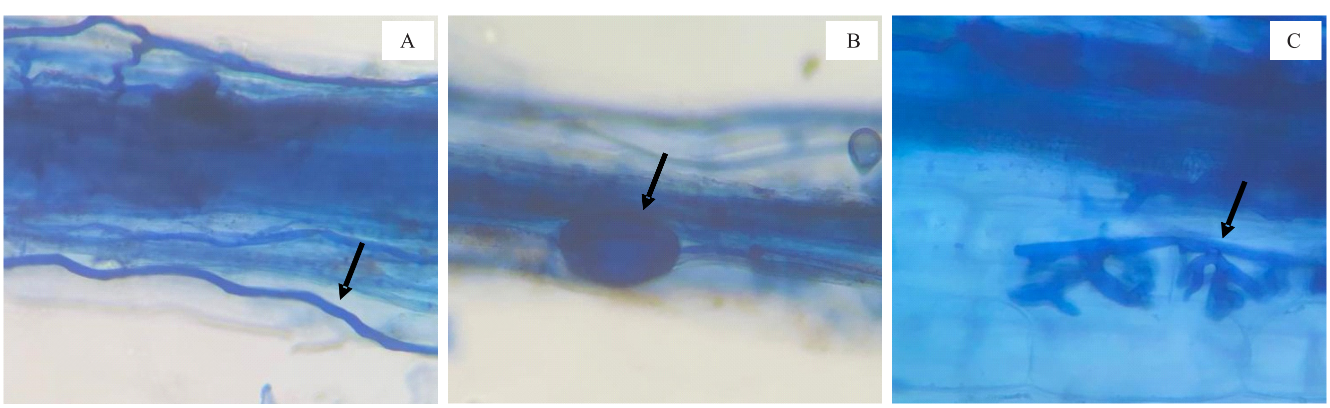


**Figure 1.** Arbuscular mycorrhizal fungi (AMF) structures visualized in wheat (*Triticum aestivum*) roots referring to: A, hyphae; B, vesicles; and C, arbuscules. Observation under an optical microscope with 400x magnification.

between 'TBIO Calibre' and *C. etunicatum* (51.55 g), and between 'TBIO Sossego' and BP NF community (41.90 g).

In general, the use of on-farm inoculants enhanced the development of the wheat root system, regardless of the cultivars subjected to them (Table 3). We also observed that *C. etunicatum* and *S. heterogama* intensified the root SA. 'TBIO Calibre' showed higher TRL and VTR, while 'TBIO Sossego' gave rise to a root system with higher SA and TR.

**Table 2.** Association between arbuscular mycorrhizal fungi (AMF) and wheat (*Triticum aestivum*) cultivars in relation to mycorrhizal colonization (MC), root volume (RV), and thousand-grain weight (TGW), in the municipality of Passo Fundo, of Rio Grande do Sul state (RS), Brazil, 2021(1).

| Inoculant(2) | Mycorrhizal colonization (%) | | Root volume ($cm^3$) | | Thousand-grain weight (g) | |
|---|---|---|---|---|---|---|
| | 'TBIO Calibre' | 'TBIO Sossego' | 'TBIO Calibre' | 'TBIO Sossego' | 'TBIO Calibre' | 'TBIO Sossego' |
| Control | 10.00±0.89Ab | 12.50±0.93Ab | 02.35±0.01Ab | 04.12±0.03Ac | 32.20±1.19Ac | 31.25±1.17Bd |
| *Acaulospora morrowiae* | 75.00±2.73Aa | 70.00±2.49Aa | 05.20±0.03Bab | 11.48±0.65Abc | 38.35±1.26Abc | 39.45±1.96Aab |
| *Cetraspora pellucida* | 75.00±2.76Aa | 75.00±2.27Aa | 08.87±0.04Bab | 13.28±0.11Aabc | 41.75±1.38Ab | 36.35±1.63Abc |
| *Claroideoglomus etunicatum* | 80.00±3.17Aa | 62.50±2.17Ba | 09.19±0.15 Bab | 23.28±0.21Aab | 51.55±1.65Aa | 33.95±1.36Bcd |
| *Glomus intraradices* | 77.50±3.04 Aa | 82.50±3.19Aa | 06.70±0.04Bab | 21.19±0.31Aab | 41.30±1.62Ab | 37.05±1.51Bbc |
| *Rhizophagus clarus* | 77.50±2.98 Aa | 62.50±3.02Ba | 10.05±0.12Aab | 15.65±0.21Aabc | 38.20±1.19Abc | 38.05±1.94Aabc |
| *Scutellospora heterogama* | 72.50±2.52 Aa | 85.00±2.85Aa | 13.06±0.72Ba | 24.71±0.31Aa | 36.55±1.27Abc | 36.15±1.73Abc |
| BP NF community | 80.00±3.18 Aa | 67.50±2.81Aa | 07.34±0.04Bab | 14.01±0.37Aabc | 41.05±1.84Ab | 41.90±1.38Aa |
| FC NF community | 62.50±2.94 Aa | 82.50±2.94 Aa | 06.90±0.02 Aab | 12.17±0.15 Abc | 37.50±1.95Abc | 37.80±1.45Aabc |
| Mean | 67.22 | | 11.64 | | 38.35 | |
| Coefficient of variation (%) | 15.59 | | 27.25 | | 7.75 | |

(1)Means followed by equal uppercase letters, in the rows, and lowercase letters, in the column, do not differ by the Tukey's test, at 5% probability. Data presented as mean ± standard deviation. (2)Control, absence of on-farm inoculants; BP NF community, mycorrhizal community formed by fourteen species, from the municipality of Bom Princípio; FC NF community, mycorrhizal community formed by eleven species, from the municipality of Flores da Cunha.

**Table 3.** Root system – total root length (TRL), surface area (SA), very thin roots (VTR), fine roots (FR), and thick roots (TR) of wheat (*Triticum aestivum*) cultivars in the absence and presence of mycorrhizal inoculants, in the municipality of Passo Fundo, Rio Grande do Sul state (RS), Brazil, 2021(1).

| Inoculant(2) | TRL (cm) | SA ($cm^2$) | VTR (cm) | FR (cm) | TR (cm) |
|---|---|---|---|---|---|
| Control | 205.56±33.83b | 090.44±18.43c | 115.81±36.78b | 066.68±16.06b | 22.98±1.29b |
| *Acaulospora morrowiae* | 999.97±48.97a | 296.67±24.47ab | 706.50±23.49a | 225.79±26.79a | 69.43±2.13a |
| *Cetraspora pellucida* | 927.41±65.73a | 343.90±31.19ab | 623.37±29.74a | 226.43±41.11a | 77.18±3.25a |
| *Claroideoglomus etunicatum* | 916.72±76.18a | 406.47±38.52a | 563.59±46.15a | 254.07±29.47a | 98.72±4.68a |
| *Glomus intraradices* | 907.79±30.23a | 374.36±29.93ab | 608.19±47.91a | 214.01±28.33a | 85.19±3.17a |
| *Rhizophagus clarus* | 828.18±67.72a | 342.70±22.19ab | 558.90±50.03a | 198.51±36.95a | 70.37±1.98a |
| *Scutellospora heterogama* | 751.72±51.14a | 403.08±40.58a | 480.41±35.17a | 201.28±40.11a | 69.63±2.05a |
| BP NF community | 765.83±57.97a | 307.81±21.72ab | 512.10±29.72a | 186.86±29.96a | 66.53±2.16a |
| FC NF community | 636.41±50.86a | 263.03±34.79b | 418.90±31.13a | 158.68±32.67a | 58.50±1.87a |
| Cultivar | | | | | |
| TBIO Calibre | 869.93±63.29a | 281.78±40.04b | 613.57±54.12a | 197.36±31.29ns | 58.58±3.18b |
| TBIO Sossego | 672.69±53.75b | 346.77±37.38a | 405.93±48.63b | 187.60±30.14 | 78.87±2.76a |
| Mean | 771.31 | 314.27 | 509.76 | 192.48 | 68.72 |
| Coefficient of variation (%) | 25.01 | 23.94 | 28.77 | 26.98 | 27.16 |

(1)Means followed by equal letters in the column do not differ significantly by the Tukey's test, at 5% probability. Data presented as mean ± standard deviation. (2)Control, absence of on-farm inoculants; BP NF community, mycorrhizal community formed by fourteen species from the municipality of Bom Princípio; FC NF community, mycorrhizal community formed by eleven species from the municipality of Flores da Cunha. nsNonsignificant.

Despite the high rate of mycorrhizal colonization of roots (Table 2), for mycorrhization to be efficient and contribute positively to sustainable food production systems, it should provide a measurable benefit to the plant host (Elliott et al., 2021), such as higher yields (Table 2) and greater root development (Table 3). During the association process, the lipochito-oligosaccharide released by the AMF induces the activation of “symbiosis genes” in the plant, for the recognition between partners, which also allows of the formation of the hyphae network (Gutjahr, 2014) and changes in the root architecture (Chiomento et al., 2021). These changes include greater length, area, and volume, as we observed in our work (Tables 2 and 3).

The root system morphology of the wheat cultivars subjected to the absence or presence of AMF was recorded using the WinRHIZO software coupled with a scanner (Figure 2). A more profuse root system, associated with the network of hyphae, provides the host with greater efficiency in capturing water and nutrients, which also explains our yield results (Table 2). In addition to making the root system more profuse, AMF provide the increase of very thin roots, so that the mycelial tissue itself allows roots to reach regions that are difficult to access (Kuyper & Jansa, 2023). In addition to the greater length, the diameter of the hyphae varies from 2 to 20 µm and that of the roots reaches values of over 300 µm. With their reduced diameter, hyphae are much more efficient at increasing the surface area of contact with the soil than the plant own roots. The hyphae length can reach more than 500 m, while roots can reach one meter (Hawkins et al., 2023). Thus, plants “invest” in the association with AMF instead of investing in root growth, which would consume more energy and would not be as efficient in acquiring nutrients (Trentin et al., 2022).

In the study of the pattern of cultivars for mycorrhizal colonization, root system morphology, and yield, 'TBIO Calibre' and 'TBIO Sossego' formed two contrasting groups in relation to the AMF inoculants (Figure 3).

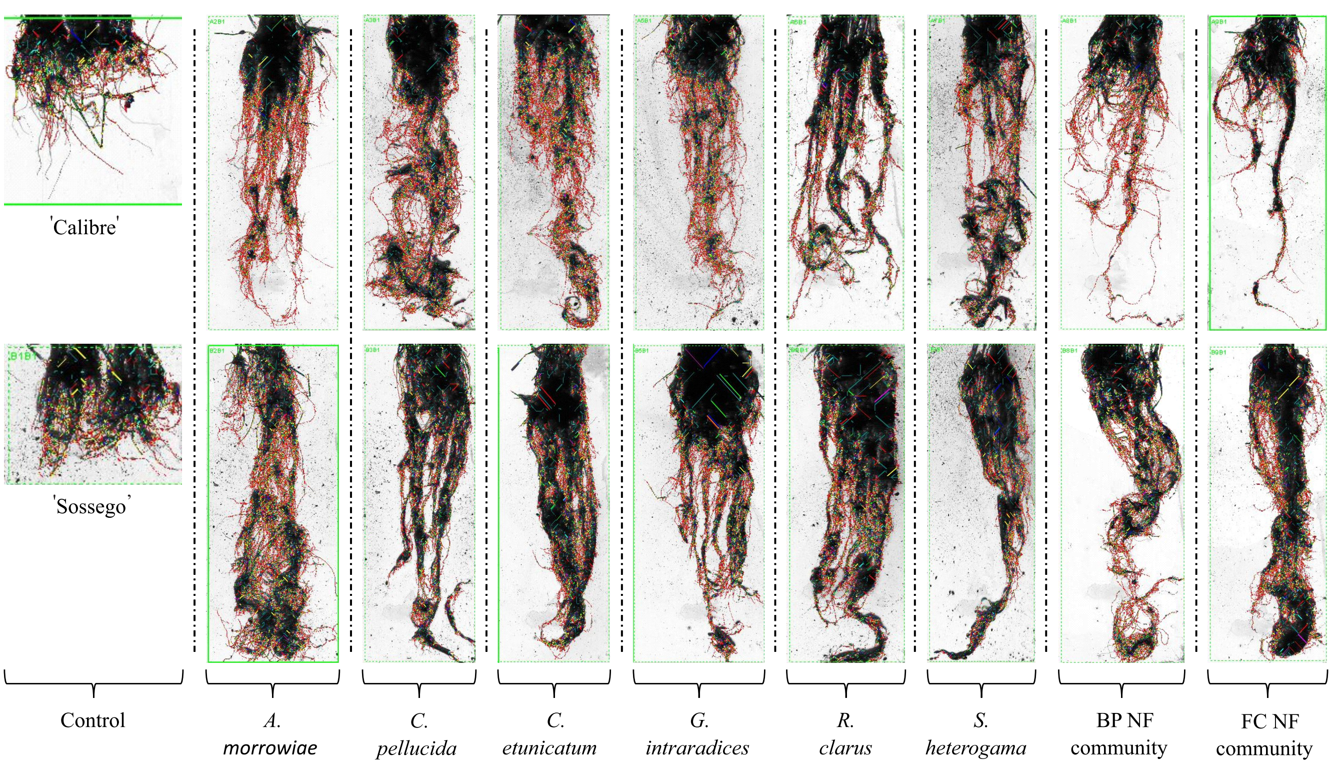


**Figure 2.** Effect of mycorrhization on the morphology of the root system of wheat (*Triticum aestivum*) cultivars (TBIO Calibre and TBIO Sossego) by *Acutelospora morrowiae*; *Cetraspora pellucida*; *Claroideoglomus etunicatum*; *Glomus intraradices*; *Rhizophagus clarus*; *Scutellospora heterogama*; BP NF, micorrhizal community from Bom Princípio, RS, Brazil; and FC NF, micorrhizal community from Flores da Cunha, RS, Brazil.

For both cultivars, the eight inoculants were grouped together. The control formed an isolated group, which confirmed the results of the univariate analysis (Tables 2 and 3).

For 'TBIO Calibre', the first two PCs had a cumulative proportion of 77.6%, in which the first PC explained 66.4% of the total variance, and the second PC explained 11.2% (Figure 3). For 'TBIO Sossego', the first two PCs had a cumulative proportion of 77.5%, in which the first PC explained 59.6% of the total variance, and the second PC explained 17.9%.

Arbuscular mycorrhizae are capable of modifying the physiology and environment of the plant host, in ways that can intensify the nutrient absorption (Elliott et al., 2021). As a result of a greater nutrient input provided by hyphae (Figure 1 A), together with the robustness of the root system (Tables 2 and 3), the plants were able to improve their yield (Table 2). Duan et al. (2021) reported the positive effects of AMF on wheat plant growth, productivity, and soil quality.

Although wheat plants have been not subjected to any stressor directly, we should point out that the soil used is considered to be nutritionally poor, with high acidity, and a large amount of aluminum. Even in these soil conditions, which would be limiting for wheat in the field without any anthropogenic intervention, mycorrhizae significantly improved the growth, development, and plant yield (Table 2). Several factors can influence the effectiveness of arbuscular mycorrhizae, such as host, fungal species, and soil environment (Chiomento et al., 2019). However, edaphic factors are the main environmental filters of AMF action (Jansa et al., 2014), especially pH (Bainard et al., 2015). Commonly, increases of pH and P, in the

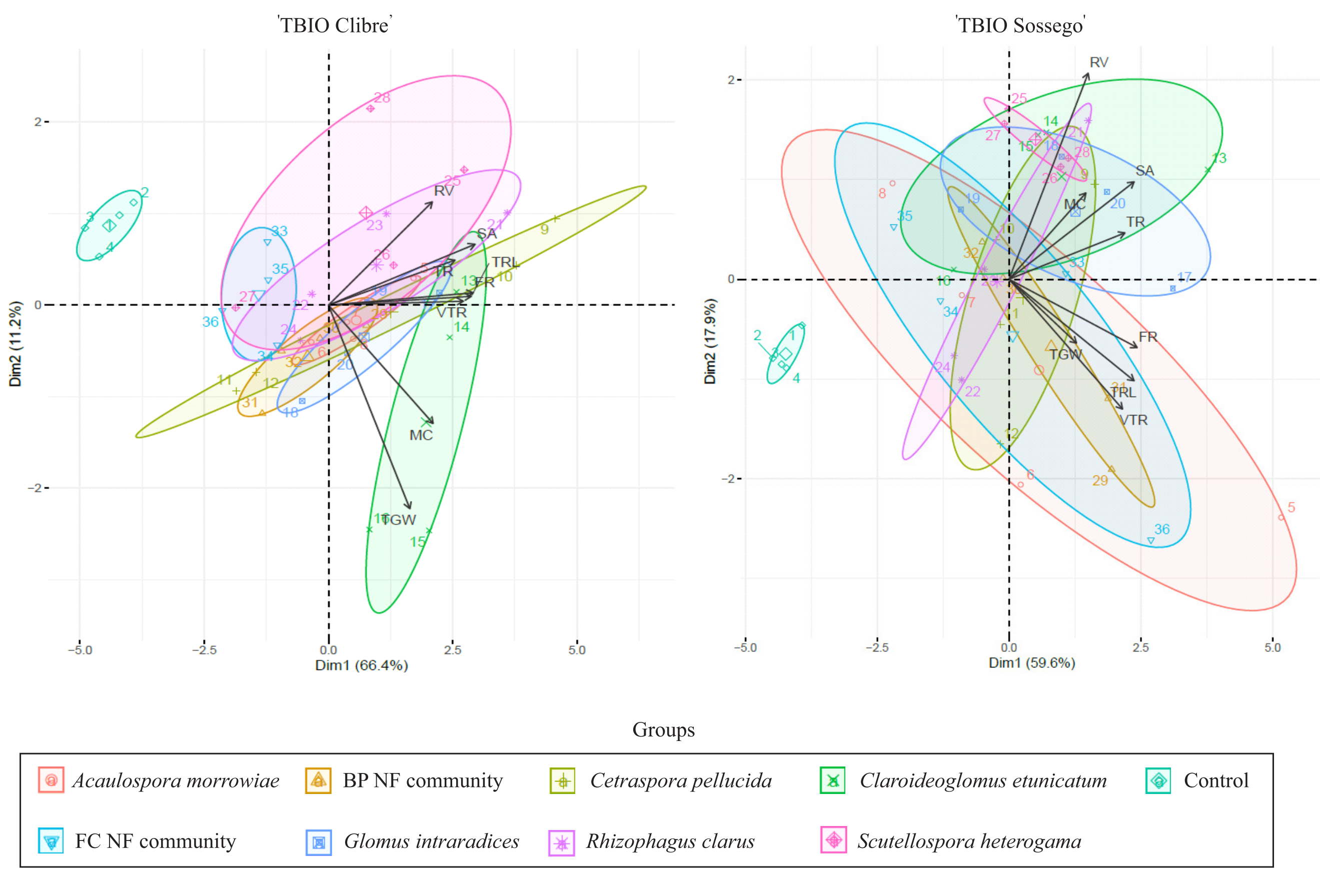


**Figure 3.** Analysis of principal components of wheat (*Triticum aestivum*) cultivars in relation to the absence and presence of arbuscular mycorrhizal fungi (AMF) inoculants. Control: absence of on-farm inoculants. BP NF: mycorrhizal community formed by fourteen species, from the municipality of Bom Princípio, RS, Brazil; FC NF: mycorrhizal community formed by eleven species, from the municipality of Flores da Cunha, RS, Brazil.


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plant growth medium, may decrease the efficiency of AMF (Trentin et al., 2022). Thus, because the acidic and low P availability of the soil used in our study, a great efficiency of the symbiosis with wheat was evidenced.

The use of agricultural practices that favor or stimulate the growth and abundance of soil microbial species, such as AMF, can reduce the negative impacts on agroecosystems, increase crop yields, and contribute to sustainability on a global scale (Chiomento et al., 2024). Our findings indicate that on-farm mycorrhizal inoculants have a potential, such as a biotool, to initiate transitions to management systems that are less offensive to the environment. Through symbiosis, it is possible to reduce the dependence on the large-scale use of chemical inputs during wheat cultivation, especially synthetic fertilizers whose raw materials are nonrenewable resources. These findings could have important practical ramifications because they show the potential of applying AMF to wheat with a view to its sustainable cultivation.

## Conclusions

1. The use of on-farm inoculants based on arbuscular mycorrhizal fungi affects the agronomic performance of wheat (*Triticum aestivum*) cultivars.

2. Regardless of the cultivars studied, the use of on-farm inoculants – especially the fungal species *Claroideoglomus etunicatum* and *Scutellospora heterogama* – enhances the development of wheat root system.

3. The combination of 'TBIO Sossego' and *Scutellospora heterogama* improves the development of the root system.

4. The combination of 'TBIO Calibre' with *Claroideoglomus etunicatum*, and of 'TBIO Sossego' with BP NF community improves the thousand-grain weight.

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