Universidade Federal de São João del-Rei
Programa de Pós-Graduação em Bioengenharia

ISABELA FIGUEIREDO DE OLIVEIRA

Contribuição da colonização micorrízica e bacteriana na eficiência de aquisição de fósforo, produtividade e microbiota da rizosfera de plantas de sorgo

SETE LAGOAS
MINAS GERAIS – BRASIL
ABRIL DE 2024

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Tese submetida ao Programa de Pós-graduação em Bioengenharia da Universidade Federal de São João del-Rei como parte dos requisitos necessários para a obtenção do título de “Doctor Scientiae” (DS).

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“Que os vossos esforços desafiem as impossibilidades. Lembrai-vos de que as grandes coisas do homem foram conquistadas do que parecia impossível.”

(Charles Chaplin)

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RESUMO

Apesar da natureza altamente regulada e dinâmica tanto da colonização micorrízica quanto da colonização bacteriana, os fatores que influenciam a associação entre plantas de sorgo e microrganismos no microbioma da rizosfera ainda não são completamente compreendidos. Sendo assim, o objetivo deste trabalho foi investigar a contribuição da colonização micorrízica e bacteriana na eficiência de aquisição de fósforo (P), produtividade e microbiota da rizosfera de plantas de sorgo. No primeiro capítulo foram revisados os diversos papéis desempenhados pelos fungos micorrízicos arbusculares (FMA) em práticas agronômicas sustentáveis, incluindo a nutrição de plantas em solos com baixa disponibilidade de nutrientes, como o P. Já no segundo capítulo foram apresentadas as características e impactos da sorgoleona na comunidade microbiana do solo e examinado criticamente o seu papel alelopático. No terceito capítulo foi avaliado o efeito da sorgoleona na colonização micorrízica e na microbiota da rizosfera e seu papel na eficiência de aquisição de P e no crescimento e produção de plantas de sorgo. O genótipo P9401 de sorgo foi crescido em casa de vegetação sob baixo P, foram avaliadas a colonização micorrízica, a expressão de genes envolvidos no processo de colonização, a diversidade microbiana da rizosfera, a atividade enzimática e a morfologia radicular das plantas. Os resultados mostraram um aumento na colonização micorrízica, no peso seco e no teor de P em plantas de sorgo crescidas em casa de vegetação em resposta à adição de 20 µMol L^{-1} de sorgoleona. Além disso, os resultados da expressão gênica revelaram uma notável regulação positiva no gene *21G12*, associado a biossíntese da sorgoleona, juntamente com os genes *SbPT8*, *SbPT9*, *SbPT10* e *SbPT11*, ligados ao transporte de fosfato induzido por micorrizas. No quarto capítulo foi avaliado o efeito de doses do inoculante contendo cepas bacterianas solubilizadoras de fosfato e sua contribuição na colonização micorrízica, na produtividade e na microbiota da rizosfera de sorgo. O genótipo BRS373 de sorgo granífero foi cultivado em campo sob diferentes doses de BiomaPhos (0, 80, 100, 120 e 200 mL ha^{-1}) e de fosfato (0, 50 e

100%) foi avaliado seu efeito na produtividade, colonização micorrízica, a diversidade microbiana da rizosfera, a atividade enzimática e a arquitetura do sistema radicular das plantas de sorgo. Os resultados mostraram que o inoculante bacteriano solubilizador de fosfato na dose de 100 mL ha^{-1} aumenta significativamente a colonização micorrízica e a produtividade das plantas de sorgo, alterando a microbiota da rizosfera de plantas de sorgo cultivadas em campo. Além disso, com essa dose de inoculante bacteriano e 50% da adubação fosfatada recomendada, foi possível obter a mesma produtividade que com a dose total de adubação. Portanto, o uso combinado da inoculação de inoculante bacteriano solubilizador de fosfato, com genótipos eficientes e responsivos ao P, indica que é possível reduzir a quantidade de fertilizante fosfatado aplicado anualmente na cultura de sorgo, o que contribui de maneira significativa para a sustentabilidade de todo agroecossistema. O sucesso da associação entre plantas de sorgo e as comunidades bacteriana e de FMA da rizosfera parece ser resultado de seus efeitos diretos, indiretos ou de ambos, sendo a disponibilidade de P um fator preponderante para o sucesso desta associação.

Palavras-chave: Arquitetura radicular**;** BiomaPhos; diversidade genética; fungo micorrízico arbuscular; sorgoleona; *Sorghum bicolor*.

DE OLIVEIRA, Isabela Figueiredo (DS). Universidade Federal de São João del-Rei, April of 2024. Title: **Contribution of mycorrhizal and bacterial colonization to the efficiency of phosphorus acquisition, yield and rhizosphere microbiome of sorghum plants**. Advisor: Dra. Sylvia Morais de Sousa Tinôco. Co-advisor: Dra. Maria Lúcia Ferreira Simeone.

ABSTRACT

Despite the highly regulated and dynamic nature of both mycorrhizal and bacterial colonization, the factors that influence the association between sorghum plants and microorganisms in the rhizosphere microbiome are not yet completely understood. Therefore, the objective of this work was to investigate the contribution of mycorrhizal and bacterial colonization to the efficiency of phosphorus (P) acquisition, yield and rhizosphere microbiota of sorghum plants. In the first chapter, the various roles played by arbuscular mycorrhizal fungi (AMF) in sustainable agronomic practices were reviewed, including plant nutrition in soils with low nutrient availability, such as P. In the second chapter, the characteristics and impacts of sorgoleone were presented in the soil microbial community and critically examined its allelopathic role. In the third chapter, the effect of sorgoleone on mycorrhizal colonization and rhizosphere microbiota and its role in the efficiency of P acquisition and in the growth and production of sorghum plants was evaluated. The sorghum genotype P9401 was grown in a greenhouse under low P, mycorrhizal colonization, the expression of genes involved in the colonization process, the microbial diversity of the rhizosphere, the enzymatic activity and the root morphology of the plants were evaluated. The results showed an increase in mycorrhizal colonization, dry weight and P content in sorghum plants grown in a greenhouse in response to the addition of 20 µMol L^{-1} of sorgoleone. Furthermore, gene expression results revealed a notable upregulation in the *21G12* gene, associated with sorgoleone biosynthesis, along with the *SbPT8, SbPT9, SbPT10* and *SbPT11* genes, linked to mycorrhizal-induced phosphate transport. In the fourth chapter, the effect of doses of inoculant containing phosphate-solubilizing bacterial strains and their contribution to mycorrhizal colonization, productivity and microbiota of the sorghum rhizosphere was evaluated. The grain sorghum genotype BRS373 was cultivated in the field under different doses of BiomaPhos (0, 80, 100, 120 and 200 mL

ha^{-1}) and phosphate (0, 50 and 100%) and its effect on yield, mycorrhizal colonization, microbial diversity of the rhizosphere, enzymatic activity and root system architecture were evaluated. The results showed that the phosphate solubilizing bacterial inoculant at a dose of 100 mL ha^{-1} significantly increased mycorrhizal colonization and productivity of sorghum plants, altering the rhizosphere microbiota of sorghum plants grown in the field. Furthermore, with this dose of the bacterial inoculant and 50% of the recommended phosphate fertilizer, it was possible to obtain the same productivity as with the total dose of fertilizer. Therefore, the combined use of phosphate solubilizing bacterial inoculant, with efficient and P-responsive genotypes, indicates that it is possible to reduce the amount of phosphate fertilizer applied annually to sorghum crops, which significantly contributes to the sustainability of the entire agroecosystem. The success of the association between sorghum plants and the rhizosphere bacterial and AMF communities appears to be the result of their direct, indirect or both effects, with P availability being a preponderant factor for the success of this association.

Keywords: Arbuscular mycorrhizal fungi; BiomaPhos; genetic diversity; root architecture; sorgoleone; *Sorghum bicolor*.

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INTRODUÇÃO GERAL

Embora o Brasil apresente uma crescente produção de cereais, a maior parte dos solos tropicais possui baixa fertilidade natural. Contudo, o suprimento adequado de nutrientes é um fator imprescindível para o aumento da produtividade das culturas (Richardson et al. 2009; Lynch et al. 2019). Dentre os nutrientes essenciais, o fósforo (P) é um nutriente limitante ao crescimento vegetal, uma vez que desempenha um papel importante em praticamente todos os processos metabólicos da planta, incluindo a fotossíntese e a respiração (Nazir et al. 2018). Para a adaptação a ambientes sob baixa disponibilidade de P, as plantas, desenvolveram mecanismos morfológicos e fisiológicos que incluem a exsudação de compostos orgânicos, a plasticidade do sistema radicular e a associação com microrganismos da rizosfera, a fim de aumentar a absorção e utilização desse nutriente essencial (Lynch, 2011; Wen et al. 2019; De Oliveira et al. 2021).

As diferentes propriedades físico-químicas e biológicas de moléculas exsudadas na região da rizosfera, podem influenciar a maneira como plantas e microrganismos interagem. A comunicação entre plantas e microrganismos ocorre por meio de trocas de sinais que podem ser influenciadas por diversos fatores, incluindo as espécies e genótipos vegetais, o estágio de desenvolvimento da planta, a zona radicular, fatores ambientais, como a disponibilidade nutricional, e a presença de microrganismos (Walder et al. 2015; Mommer et al. 2016; Chagas et al. 2018; Lemanceau et al. 2018).

A elucidação do processo de colonização é importante a fim de compreender como os microrganismos, sejam fungos, como os fungos micorrízicos arbusculares (FMA), ou bactérias, como as promotoras de crescimento das plantas (BPCP), interagem com as plantas e se elas têm a habilidade de se estabelecerem no ambiente após a aplicação em campo como inoculantes microbianos. Há relatos na literatura que demonstram respostas distintas para diferentes culturas de plantas inoculadas com FMA (Walder et al. 2015; De Novais et al. 2017; Wen et al. 2019; Abdelhalim et al. 2019; Wang et al. 2021) ou com BPCP (Vidotti et al. 2019; Oliveira-Paiva et al. 2020; Yassue et al. 2022). Além disso, trabalhos relatam a interação de BPCP com FMA, havendo efeito sinérgico da co-inoculação, favorecendo o aproveitamento de nutrientes e crescimento de plantas de batata (Vosátka; Gryndler, 1999), trigo (Sala et al. 2007) milho e soja (Moreira et al. 2020; Oliveira-Paiva et al. 2020).

O papel da exsudação radicular para a colonização eficaz entre plantas e microrganismos foi estabelecido em várias espécies de plantas, incluindo milho, soja e sorgo (Yoneyama et al., 2013; Yoneyama et al. 2015; Borghi et al., 2016; Kobae, 2018; Abdelhalim et al. 2019; Oliveira et al. 2020; Sarr et al. 2021; Wang et al., 2021; Ortas et al., 2022), mas as respostas parecem ser complexas e não uniformes (espacial e temporalmente) (Wen et al. 2019). Em sorgo cultivado em campo e em casa de vegetação, estudos demonstram que a sorgoleona afeta a dinâmica da estrutura da comunidade microbiana do solo da rizosfera (De Oliveira et al. 2021; Sarr et al. 2021; Wang et al. 2021; Ortas et al. 2022), o que demonstra a necessidade de maiores investigações. Interessante destacar que o desempenho do sorgo associado à sua capacidade de resposta à colonização por microrganismos em termos de crescimento melhorado e/ou nutrição de P pode ser dependente do genótipo, sendo este um fator a mais a ser considerado.

A melhor compreensão da dinâmica das interações entre plantas e comunidades microbianas, bem como do papel da exsudação radicular nesse processo, pode fornecer informações potencialmente importantes, que podem melhorar a eficiência no uso desses microrganismos. Além disso, o entendimento mais acurado dos fatores que influenciam o estabelecimento da associação entre plantas e microrganismos na rizosfera pode contribuir para melhorar a produtividade das culturas (Wang et al. 2021; Ferreira et al. 2021), além de permitir o desenvolvimento de cultivares eficientes na aquisição de nutrientes (De Sousa et al. 2021).

Os mecanismos relacionados à absorção de nutrientes são importantes para a aquisição de qualquer nutriente, principalmente para aqueles que estão em baixa concentração na solução do solo ou que apresentam lentidão de transporte no solo, como o P. A eficiência na utilização de P pelas plantas é uma característica complexa e ainda pouco elucidada, que está associada a processos adaptativos moleculares e morfológicos, que incluem alterações na morfologia e arquitetura radicular e associações com microrganismos na rizosfera, que corresponde ao ambiente do solo que influencia profundamente a microbiota da planta (Niu et al. 2013; Malhotra et al. 2018; Barros et al. 2020).

Até o momento, existem trabalhos relacionados ao mecanismo de morfologia do sistema radicular para o desenvolvimento de cultivares de sorgo eficientes na aquisição de P (Hostetler et al. 2023). Entretanto, outros mecanismos, como a

associação com microrganismos, ou ainda a co-inoculação desses microrganismos, também pode desempenhar um papel significativo no desenvolvimento de sorgo em solos com baixa disponibilidade de P (Walder et al. 2015; Abdelhalim et al. 2019; Wen et al. 2019; De Oliveira et al. 2021; De Sousa et al. 2021; Sarr et al. 2021, Wang et al. 2021).

Sendo assim, no primeiro capítulo desta tese foram apresentados os diversos papéis desempenhados pelos FMA em práticas agronômicas sustentáveis, incluindo a nutrição de plantas em solos com baixa disponibilidade de nutrientes, como o P. Já no segundo capítulo foram apresentadas as características e impactos da sorgoleona na comunidade microbiana do solo e examinado criticamente o seu papel na alelopatia. Baseado nisso, no terceiro capítulo foi investigado o efeito da sorgoleona na colonização micorrízica e na microbiota da rizosfera e seu papel na melhoria da eficiência de aquisição de P e no crescimento de plantas de sorgo cultivadas em casa de vegetação. Já no capítulo quatro, foi avaliado o efeito de um inoculante contendo bactérias solubilizadoras de fosfato e sua contribuição na colonização micorrízica, na produtividade e na microbiota da rizosfera de sorgo cultivado em campo. Portanto, ao longo dos quatro capítulos desta tese foram investigados os efeitos da colonização por FMA e por BPCPs, além dos efeitos da presença de ambos os microrganismos na microbiota da rizosfera, uma vez que ambos afetam a composição e estrutura da comunidade um do outro. Esse trabalho procurou integrar diferentes aspectos que influenciam a dinâmica solo-planta-microrganismo em busca de uma maior eficiência no uso de P de forma econômica e ambientalmente sustentável.

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CAPÍTULO 1

ADVANCES IN AMF TECHNOLOGY FOR SUSTAINABLE AGRICULTURE

Volume 2 (Nutrient and Crop Management)

CHAPTER 18: Agronomic practices for optimizing the AMF abundance and diversity for sustainable food production

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Abstract

The effects of global climatic alterations and agricultural management practices have contributed to the soil fertility degradation. In this chapter, we focused on the role of the arbuscular mycorrhizal fungi (AMF) as an eco-friendly approach by symbiotically associating with crop plants. As a bioinoculant, AMF are especially involved in mineral nutrition, water absorption, biotic and abiotic stresses alleviation in plants. AMF also induce a synergistic effect on plant development and productivity because of beneficial interactions with other rhizosphere microorganisms. Regarding the impact of AMF on soil fertility, we discuss the functionality of AMF as beneficial microorganisms in agricultural sustainability and highlighted the effect of AMF inoculation on maize, sorghum and soybean crops. Mycorrhizal colonization can be stimulated by plant roots exudates, as strigolactones, among others. These compounds can help plants to adapt to different soil stresses, such as low P. Commercial products based on AMF are

available to farmers. These products contain mycorrhizal fungi of different species at high number of propagules that colonize plant roots increasing efficiency in nutrient and water absorption to optimize plant performance.

Keywords: bioinoculant; arbuscular mycorrhizal fungi, colonization; crops; exudates; low phosphorus

1 Introduction

The association between plants and arbuscular mycorrhizal fungi (AMF) is one of the most important symbiotic relationships between plants and microorganisms [1]. It represents a relevant mechanism to increase soil exploitation and nutrient acquisition [2,3]. In order to improve phosphorus (P) acquisition in crops, it is important to investigate the dynamics of mycorrhizal colonization in different conditions [4].

In this chapter, we will discuss how different agronomic practices effect AMF and how it can effect crop growth and yield. Arbuscular mycorrhizae play an important role in the growth and productivity of mycorrhizal plants compared to non-mycorrhizal plants, due to increased nutrient uptake [5]. However, mycorrhizal associations may eventually not be beneficial in terms of P nutrition for the plant. We will also discuss different factors that influence root colonization and plant benefits from AMF. Once, AMF and their associated bacterial communities are often specific to the soil type, historical land use, and specific crop plants species present [6].

Extensive research has been conducted to improve our understanding of how AMF deliver nutrients to their host. However, we need to improve our knowledge about the multifarious roles played by AMF in agriculture systems. The role of AMF in plant nutrition in soils under low nutrient availability is a research area with great potential for application in agriculture.

2 Conventional agronomic practices and their influence on soil management

The agriculture production system is composed of the set of cultivation components within a rural property, defined from the production factors (land, capital and labor) and interconnected by a management process. The ecological intensification of crop production systems seeks to maximize productivity, reducing

pressure on the opening of new areas destined for agriculture. These systems are classified as monoculture or isolated production, crop succession, rotation, intercropping or polyculture, and integrated system [7].

Soil management and preparation are practices that have a great influence on the degradation rates of organic matter provided by microbial activity. Among the factors and processes that affect the soil organic matter accumulation are mineralogy, moisture, pH, temperature and soil biology [8]. Soil management practices and nutrient input strongly affect the microbial community, thus changes in soil microbial characteristics are valuable tools to assess the impact of these factors on agricultural soils [9, 10].

In annual cropping systems, which typically contain monocultures of a single crop grown each year, one way to alter the diversity and function of rhizosphere-associated microorganisms is through selection of plant species and succession of crops grown in a rotation. In multi-year rotations, individual crop species are planted in a sequence throughout the growing seasons, and careful selection of crop identity, order, and number can be used to manage key plant-microorganism interactions [11, 12].

Incorporating additional crops into a continuous monoculture or two-year rotation can help manage and promote beneficial interactions that lead to improve the soil fertility through increased nutrient uptake [13]. In addition, shifts from monocultures or two-year rotations, including widespread maize-soybean rotation, to multi-year systems substantially reduces fertilizer use and soil greenhouse gas emissions, and improve air and water quality, without compromising economic or agronomic performance [14, 15].

Due to the complexity of the dynamics that regulate soil microbial communities, the mechanisms underlying the effects of crop rotation are still poorly known [16]. First, in an immediate legacy effect or earlier harvest effect, each individual plant species modulates soil and rhizosphere microorganisms through root exudates or signaling molecules, which recruit particular microbial populations in a species-specific manner [17]. These foregoing crop effects have been described for crop health and soil processes [18, 19], soil and rhizosphere microbial communities [20, 21, 22] and individual microbial rate [23, 24].

In general, in different crop rotation systems, the duration of the rotation and the plant species have a great influence on soil characteristics and crop benefits [20, 25].

Previous studies have shown that increasing the rotation of leguminous crops could release nutrients more quickly, leading to a higher nitrogen (N) and potassium (K) content in the plants and lower available phosphorus (P) content in the soil [26]. Most crops are symbiotic with AMF, so there are no reports of deleterious effects resulting from crop rotations when the different crops involved are all symbiotic with AMF [27]. However, there are cases of negative impacts on crop performance when AMF based crop plants are rotated with non-mycorrhizal crops, such as those of Brassicaceae, which are generally not symbiotic with AMF. These non-mycorrhizal crops can constrain AMF performance in rotations and interfere with their persistence over time [28]. However, the value of such crops in rotation should be weighed against potential negative impacts that result from these cropping combinations.

Fallow periods are part of some cropping cycles, but some studies have shown these practices could exert negative effects on AMF interactions with subsequent negative impacts on crop performance [29, 30]. Tillage results in an upheaval of soil layers, disrupting established mycelium networks in the soil, disturbing existing microbial communities, and impacting soil moisture and density. All of these factors will influence mycorrhizal communities found within soils, thus potentially influencing crop performance.

In the intercropped system, positive interactions increased the survival, growth and fitness of both crops [31]. Interspecific interactions in the rhizosphere improve N and P uptake in an intercropping system, which results in increased production compared to individual cultivation [32]. Essential factors to increase crop productivity include temperature, air humidity, soil conditions, such as pH, permeability, salinity, oxygenation and availability of nutrients, like P [33]. For the maximum use of nutrients, factors such as climate, soil, plants and their interactions should be considered, adapting them to the management of production systems.

Cover crops can increase nutrient utilization efficiency and reduce nutrient loss due to leaching and erosion. Covering the crop increases the total volume of roots in the agricultural system, increasing the surface area over which nutrients are absorbed and the total volume of plant root exudates [34]. Therefore, microbial consortia suitable for a given environment, soil conditions and association with crops that are efficient in nutrient absorption can guarantee soil fertility and agricultural productivity in the medium and long term [35]. Effects of crop tillage were an early focus for AMF function. Tillage disrupts extra-radical mycorrhiza, allowing for the possibility that in no-tillage

systems, plants may follow old root channels and potentially encounter more AMF propagules than plants growing in tilled soil [36]. AMF present in soils below typical tillage depths, deep-rooted crops, and deep-rooted cover crops can further improve access to AMF benefits [37]. Conservation tillage practices and the use of cover crops are promoted as best agricultural practices for P conservation. Less-intensive tillage is a viable strategy for enhancing root colonization by indigenous AMF across soil types and crop species [38]. Reduced tillage and winter cover cropping increased AMF colonization of summer crop roots by 30%, and suggest that farmers should seek optimal tillage and cover crop combinations [38]. Research in under-studied neotropical agroecosystems has recently shown that intensive tillage practices can negatively affect AMF functions [39].

There is a growing consensus regarding the importance that AMF plays in improving plant resilience and crop yield while also enhancing the functioning of soil microbial communities. However, heterogeneous practices across all scales complicate the successful integration of AMF in agroecological systems. AMF symbioses with crops are passive, or stimulated by incorporation of crop wastes in soil, soil inoculation with AMF spores, or the planting of inoculated of seeds. [40] suggest that AMF can have highest beneficial impacts in areas with low levels of agrochemical inputs.

Taken together, at least four critical elements, should be considered to enhance mycorrhizal symbiosis in agriculture systems: (1) use of mycorrhizal cover crops, especially during seasonal fallow; (2) inclusion of mycorrhizal crops in the rotation; (3) management practices that favor AMF such as reduced tillage and less use of agrochemicals; (4) inoculation with effective AMF isolates, especially when native AMF propagules are low or ineffective [41].

3 Arbuscular mycorrhizal fungi and its effects on crops

The N, P and K are the main nutrients needed for plant growth and development. Phosphorus is a limiting nutrient for plant growth, as it plays an important role in different plant metabolic processes [42, 43, 44]. The P availability for plants is generally higher in the surface soil layer, with reduced concentrations in the lower layers. Plants absorb only two soluble forms, the monobasic (H_2PO^{4-}) and the dibasic phosphates ions (HPO_4^{2-}) [45]. Despite being available in the soil in organic and inorganic forms,

and its total concentration in the soil is high, P is found predominantly (95-99%) in less soluble and non-absorbed forms by plants. Compared to other macronutrients, the concentration of inorganic P (Pi) in the soil solution is suboptimal for grain production [46]. Furthermore, this nutrient is transported to the roots by diffusion, a slow process [47, 48], creating a deficiency zone around the rhizosphere. The problem is further intensified by the fact that P can precipitate with calcium (Ca) in calcareous soils and with iron (Fe) and aluminum (Al) in acidic soils, reducing its solubility and availability to the plants [49].

The mechanisms related to the absorption of nutrients are important mainly for those that are in low concentration in the soil solution or that present slow transport in the soil, such as P. The efficiency in the use of P by the plants is a complex characteristic, which is associated with molecular and morphological adaptive processes, including changes in root architecture and associations with microorganisms in the rhizosphere [50, 51].

Mineral deposits are the main sources of P, which corresponds to 0.12% of the Earth's crust [52], constituting a finite source. The use of acidulated phosphate fertilizers, such as triple superphosphate (TSP), obtained from the acid treatment of phosphate rocks, is predominant in agricultural activities. However, the high cost of phosphate fertilization has aroused interest in natural phosphates, which have a lower cost per unit of P than soluble ones [53]. The natural phosphates result from the grinding of phosphate rock, which may or may not undergo physical concentration processes. The solubility of these fertilizers varies depending on the origin and degree of isomorphic ionic substitutions [54].

Soluble phosphates, quickly available to the plants show good results in different forms of application. Usually, natural phosphates are less efficient, especially in the year of application and in annual crops, which require P in a short period. When considering successive cycles of agricultural production with constant fertilization, it is verified that the performance of natural phosphates can match that of the most soluble sources. This is explained by the fact that P readily released from soluble fertilizers converts to less available forms, while natural phosphates are solubilized over time by different mechanisms, including the contribution of the soil microbiota [54, 55, 56]. AMF and their exoenzymes play pivotal roles in accessing, mobilizing, and transferring these resources in exchange for carbohydrates from plant partners.

However, even when phosphate fertilizers are added to the soil, about 70-90% of these are quickly adsorbed and fixed in the soil matrix, which are not available to crop production [57]. This suggests that P fertilization alone is not a viable strategy to improve crop productivity in many P-deficient soils. Furthermore, the continued use of synthetic fertilizers eventually leads to toxicity and deterioration of soil quality, loss of rhizosphere microbiota diversity, leaching of toxic elements into the water resources, resulting in human health problems and loss of sustainability throughout the ecosystem [58]. The symbioses between plant roots and AMF function most efficiently in soil without external chemical inputs. Despite this, there may be a wide range of nutrient-supply rates under which AMF can mitigate nutrient losses from croplands where added fertilizers are not taken into biomass [59].

AMF are important soil microorganisms that form a non-pathogenic symbiotic association with the roots of most plant species. They are considered obligate biotrophs and, through symbiosis with plants, they can alter the physiology of the root system, causing changes in architecture, growth rate, length and longevity of roots [60].

Increased absorption of low-mobility nutrients, such as P, is considered the main nutritional benefit of arbuscular mycorrhizae. On average, a 1% of increment in the root absorption surface increases the P influx by more than 150% [61]. The association formed between AMF and plants contributes to better plant resistance to a variety of biotic and abiotic stresses in crops, thus contributing to greater agricultural productivity (Figure 1). AMF inoculation allows greater tolerance of host plants to water deficit, salinity, heavy metals, extreme temperatures [5], improvement of Zn nutrition [62] and increased resilience to pathogens. They also stimulate hormone production and increase chlorophyll levels in the plants. There is also an improvement in the absorption of N, since the fungi can accelerate the decomposition and acquire N directly from the organic material [3, 63].

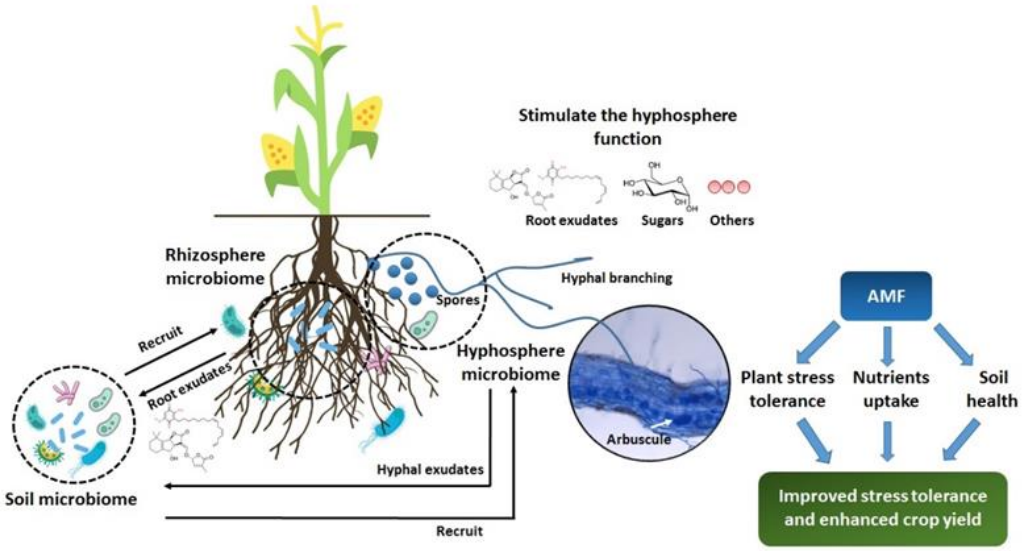

Figure 1. Schematic presentation of AMF influence on microbiome and plant growth.

Soil P availability is also increased since the extensive hyphal network of the AMF influences the physicochemical properties of the soil and contributes directly or indirectly to the release of inorganic phosphates available for absorption by plants [64]. Hyphosphere microbiome of AMF are key players in influencing the nutrient uptake efficiency of the mycorrhizal pathway. The hyphosphere is defined as the narrow region of soil around the hyphae where the physical, chemical, and biochemical conditions are different from the bulk soil due to the influence of hyphal exudates [65]. Similar to plant roots, AMF hyphae also recruit soil microbes in their hyphosphere [66] (Figure 1). However, many questions remain unclear in this highly complex belowground interaction [67].

The question regarding the need of farmers to modify management to enhance the abundance and diversity of AMF was recently reviewed [68]. The discussion was focused on field experiments that manipulated colonization by indigenous AMF and reported crop yield, or investigated community structure and diversity of AMF. One of the raised criticisms is that the yield benefits of maintaining a high abundance and diversity of AMF in agroecosystems are often overstated and more critical and long terms studies are necessary. They also discussed that mycorrhizal fungal community may be more resilient to many agricultural practices than often assumed. Some determinants of mycorrhizal growth response that should be considered are crop genotypes; the impact of resource balance in the field; and the complexity of

interactions among species of AMF in diverse communities and between AMF and soil microbes.

Despite the fact that there are inconsistencies, concerning the establishment of AMF in the field and eventual effectiveness in improving plant yield [64], there are studies that show positive effects of AMF in several crops. It is estimated that AMF enables a 20-fold increase in the contact surface between the plant and the soil, increasing the rate of P absorption per unit of root length [69]. AMF play an important role in the growth and productivity of mycorrhizal plants compared to non-mycorrhizal plants. However, since mycorrhizal symbiosis is a complex biological association, increased diversity of different elements is imperative to augment AMF community structure, which directly affects the diversity and productivity of plants [70, 71]. Increased fungal diversity in agroecosystems may enhance more ecological functions due to niche differentiation (complementarity effect) and facilitation as well as presence of a particular effective AMF species [72, 73].

3.1 Effect of arbuscular mycorrhizal fungi inoculation on maize and sorghum crops

In maize inoculated with *Rhizophagus irregularis*, phosphate uptake levels vary among plant genotypes [74].The effect of AMF on P assimilation by maize and its impact on plant growth and productivity cultivated in Ferralsol were evaluated by [75]. The authors observed that the weight, length, diameter, number of rows and grain weight of the ear were significantly higher in pots with plants inoculated with AMF. Furthermore, inoculated plants flowered earlier than non-inoculated plants and were also better colonized. The treatments without phosphate fertilizer inoculated with AMF resulted in small ears, but full of seeds. The P-free control without AMF resulted in empty ears. The authors concluded that the use of AMF could be an alternative to the use of phosphate.

In another study, it was evaluated that the impact of the symbiosis of five species of AMF isolated from soils in Benin (Africa) on maize productivity [76]. In this case, seed inoculation with AMF showed a significant effect on maize growth and yield. Treatments with *Glomus caledonius*, *Rhizophagus intraradices* and *Funneliformis geosporum*, in combination with 50% NPK and urea, increased maize grain productivity by 62.5%, compared to non-inoculated plants.

The symbiosis of plants and AMF, among other effects, can regulate the hydraulic properties of plant roots, promote water transport to the shoot and accelerate rehydration of plant leaves [77]. In addition to mitigating the effects of drought stress, AMF helps plants under salt stress, a serious problem for agricultural production since high concentrations of salts in the soil make it more difficult for plant roots to extract water, and high concentrations of salts inside the plant can be toxic. Salt leads to secondary oxidative stress in plants and plant antioxidant capacity could be increased by AMF [78].

It was investigated whether native AMF isolated from an arid saline environment can help maize plants to overcome the negative effects of salt stress better than AMF species from a culture collection [79]. The authors also investigated whether protection against oxidative stress is a mechanism used by native AMF, *R. intraradices*, *Claroideoglomus etunicatum* and *Septoglomus constrictum* to increase the host plants tolerance to salinity. The results showed that inoculation with three native AMF showed higher maize tolerance to saline environment, alleviating salt-induced oxidative stress and cell membrane damage comparing to non-mycorrhizal plants or plants inoculated with the collection AMF. The better performance of photosystem II and stomatal conductance of plants inoculated with native AMF may have contributed to this effect by reducing the generation of reactive oxygen species, evidenced by the lower accumulation of hydrogen peroxide in these plants.

Moreover, the inoculation of *Funneliformis mosseae*, *C. etunicatum* and *Acaulospora foveata* in maize caused upregulation of N levels, improved photosynthetic efficiency and water content, resulting in promotion of leaf area and shoot biomass [80].

The potential of AMF inoculation to mitigate the adverse effects of drought stress in maize grown in coal mine spoils for revegetation applications was investigated [81]. Their experiments aimed to evaluate the effects of inoculation with *R. intraradices* on growth, nutrient uptake, carbon:nitrogen:phosphorus (C:N:P) ratio and water status of maize under water stress conditions. Results suggested that *R. intraradices* inoculation played a positive role in drought tolerance and plant development in coal mine spoils, promoting nutrient uptake, adjusting C:N:P ratio, improving shoot fresh weight, water use efficiency, and accelerating the rehydration rate of plants after water stress.

The role of *Glomus versiforme* in amelioration of drought-induced effects on growth and physio-biochemical attributes in maize was also studied. AMF inoculation significantly ameliorated the deleterious effects of drought-induced oxidative damage, increased growth and photosynthesis by significantly improving chlorophyll content, mineral uptake and assimilation. AMF inoculation also increased the content of proline, sugars and free amino acids, assisting in maintaining the relative water content. In addition, AMF mediated up-regulation of the antioxidant system, contributed to maintenance of redox homeostasis, leading to protection of major metabolic pathways, including photosynthesis [60].

In other crops such as sorghum, the inoculation with AMF increases plant growth by presenting a significant enhance in shoot height, root length and dry weight [82, 83], in addition to greater nutrient absorption [84] and higher grain yield [85]. Inoculation with *Glomus mosseae* increased grain yield, biochemical content and bioactivity of sorghum plants grown under drought conditions. The inoculated seedlings showed higher superoxide dismutase, peroxidase, catalase, polyphenol oxidase, proline and glutathione activities compared to non-inoculated controls [86]. In addition, the inoculation of *Acaulospora mellea* in sweet sorghum genotypes under different NaCl concentrations improved plant biomass, mineral uptake and increased the activities of superoxide dismutase, peroxidase, catalase and soluble sugar content in leaves. AMF could help to alleviate the negative effects caused by salinity, and thus showed potential in biomass production of sweet sorghum in saline soil [87].

Sorghum inoculation with AMF (*Funneliformis* sp.) significantly increased plant growth and P uptake in saline and sodic soils, compared to treatment without inoculation [88]. The inoculation of sorghum plants with different taxa of AMF was more effective in increasing the tolerance of the plants to soils containing heavy metals than inoculum of a single species of fungus [89]. Thus, the best inoculum was a mixture of three AMF species (*Rhizophagus neocaledonicus*, *Pervetustus simplex*, *Scutellospora ovalis*) belonging to three different families of fungi that were able to reduce the translocation of potentially toxic metals to the aerial part of sorghum and, thus improving the suitability of the plants to these soils.

3.2 Effect of arbuscular mycorrhizal fungi inoculation on soybean crops

The benefits of AMF vary between species and the composition of fungal communities in the soil. In general, soybean is inoculated with the nitrogen fixing *Bradyrhizobium* bacteria and AMF [58]. The effects of AMF and other soil microorganisms in areas with different management practices (monoculture and crop rotation) on productivity parameters, protein content and quality of soybean oil were investigated [90]. The results indicated significant effects on seed quality and productivity. In general, the AMF promoted an increase in protein in the grains, and these effects were more pronounced in soils with crop rotation.

In soybean and cotton, AMF inoculation promoted a 20% increase in the root colonization [91] in addition to an increase of P and N content. Inoculated bean plants improved P uptake and productivity [92]. Moreover, the inoculation of soybean plants with AMF provides a potential alternative strategy for mitigating drought stress [93]. In this study, inoculation with *Rhizophagus clarus* mitigated the effects of water stress on the photosynthetic apparatus of “Anta82”, a drought-sensitive soybean genotype. In the genotype “Desafio”, which is moderately drought tolerant, the colonization by *R. clarus* increased the concentration of photosynthetic pigments and improved plant physiological performance and growth. In the other study, the effects of AMF species on the absorption and distribution of mineral nutrients in soybean cultivars under drought stress was investigated. Inoculation with *F. mosseae*, *Glomus hoi* and *R. intraradices* improved the plant nutrient uptake, fatty acid profile, efficiency of resource utilization, oil quality and stabilizing yield, hence reducing the production risks of crops grown under drought stress conditions [94].

Inoculation with AMF and *Bradyrhizobium* improved growth and productivity of soybeans under drought stress conditions [95, 96]. In addition, bacterial counts, levels of mycorrhizal colonization, and soil enzyme activities also increased in the rhizosphere soil of the inoculated plants. Biofertilizers improved soybean antioxidant system and its related genes that contribute to the reduction of oxidative damage induced by drought stress. There was also the accumulation of proline and the upregulation of their related metabolism genes in plants that can play a vital role as a stress signal influencing the adaptive responses of soybeans under drought stress [95].

The lead tolerance of soybean plants after AMF inoculation was also evaluated [97]. For this, soybeans were inoculated with three different AMF, *F. mosseae*, *C. etunicatum* and *Rhizophagus intraradices* and cultivated under different levels of lead. The authors observed that AMF treatments promoted greater plant growth, P uptake and lead accumulation in the roots, with less lead translocation from roots to shoots. Soybean plants inoculated with *R. intraradices* were considered more tolerant to lead toxicity and showed greater root colonization, biomass, P uptake and grain yield. The results of this study are essential for evaluating the use of AMF in promoting soybean growth and P uptake in soils contaminated by heavy metals.

Therefore, these studies indicate that AMF inoculation is an important tool for improving soybean productivity. It is believed that technology based on microbial inoculants will be increasingly incorporated into common practice in addition to increasing the productivity of agricultural systems. These innovations are important for sustainability, reduction the use of agricultural inputs and negative environmental impact of agriculture [98]. Thus, the selection of AMF and the production of inoculants, in terms of quality and quantity, are issues that need to be better explored, taking into account the different plant genotypes for their application in agriculture.

4 Stimulating factors of arbuscular mycorrhizal fungi

The different physical-chemical and biological properties of molecules exuded by plants in the rhizosphere can favor the growth and multiplication of certain groups of microorganisms, while they can inhibit others. Rhizosphere microbiome differs from cultivar to cultivar as differences in root exudation influence plant-microorganism interactions [99, 100].

Root exudation can determine not only which organisms will reside in the rhizosphere, but also generate physical and chemical benefits to plants [94]. In this context, the concentration of P in the soil is one of the main factors that affect AMF colonization [96]. Low P concentrations in the soil stimulate root exudation and subsequent mycorrhizal colonization, while high P concentrations can suppress exudation, which attenuates the level of signaling and negatively affects AMF root colonization and diversity [2, 3, 101, 102].

4.1 Plant root exudates

Under P-deficient conditions, several plant metabolites are exuded in the rhizosphere to ensure germination and growth of the AMF, whose concentration diminishes with increasing plant benefits from AMF colonization [63, 103, 104]. There is a considerable difference in the quantity and quality of these exudates produced by plant roots that indicate genetic diversity for their production [76, 105]. Among the compounds released by plants as strategies for adapting to low-P environments, there are organic acids, which have the ability to act by favoring the P-solubilization. Strigolactones are important signaling compounds from plants that affect the germination of the AMF spores and leads to the initiation of the plant–AMF symbiosis [63, 106, 107]. Apart from strigolactones, plant secondary metabolites, such as flavonoids, also act as chemical signals during the pre-symbiotic stage. Flavonoids are important for hyphal growth and their effect varies with their chemical composition [108]. Along with the plant metabolites, the AMF also produces certain chemical signals collectively termed as “myc-factors”, which initiate AMF colonization in plants [65]. The lipochitooligosaccharides (LCOs) released by AMF act as signals that induce the symbiotic-specific response in host plants, which in turn helps in the development of the symbiosis [109].

4.1.1 Strigolactones

Strigolactones are widely distributed in the plant kingdom. Strigolactones were initially characterized as germination stimulants for root parasitic plants and later as signaling factors that induce symbiosis with AMF [106]. Plants with impaired strigolactones production have a reduced capacity to be colonized by AMF [104].

Strigolactones, such as sorgolactone, sorgomol, orobanchol, 5-deoxystrigol, among others, belong to the class of lactones, and are derived from carotenoids [110]. They were isolated and identified in root exudates of several plant species, demonstrating the importance of strigolactones biosynthesis genes for the efficient formation of the appressorium and the consequent penetration of AMF hyphae into the roots, enabling the expansion of mycorrhizal colonization [96].

Strigolactones are synthesized in the chloroplast, and their biosynthesis involves two carotenoid-cleaving dioxygenases (CCD7 and CCD8), called HTD1 and

D10 in rice, a cytochrome P450 monooxygenase (MAX1) and an iron-binding β-carotene isomerase (D27), which functions upstream of CCD7 and CCD8, catalyzing the conversion of all-trans-β-carotene to 9-cis-β-carotene. The latter acts as a substrate for CCD7 to cleave cis-configured carotenoids into 9-cis-β-apo-10'-carotenal (C-27) and β-ionone (C-13). CCD8 then acts on the C-27 enzymatic cleavage product to form a compound called carlactone, which is an intermediate in the strigolactone biosynthesis pathway. Downstream of CCD8, the MAX1 gene (OsMAX1 in rice) acts, which, through rearrangements and modifications such as hydroxylation and oxidation, converts carlactone into functional strigolactones, such as 5-deoxystrigol, sorghum, sorgolactone, among others [111,112,113,114].

Numerous studies described the prominence of strigolactones and their derivatives in hormonal crosstalk in the development of root in many plant varieties [115, 116, 117, 118, 119]. The quality of root exudates is directly affected by host plant P acquisition [120]. Root exudates from plants grown under phosphate-limiting conditions are more active than those from plants with adequate nutrition [121]. Strigolactone production and exudation is increased under P deficiency, and even suppressed with addition phosphate fertilizers [63]. Indeed, in the case of red clover, phosphate deficiency significantly increases exudation of strigolactone/orobanchol [122]. In sorghum, not only P deficiency, but also N deficiency increases 5-desoxestrigol exudation [123], suggesting a regulation in the production and exudation of strigolactones in sorghum when there is nutritional deficiency [63].

Strigolactones have also been reported to play an essential role in plant communication in the rhizosphere region. The strigolactones signaling acts upstream of auxins in regulating lateral roots positioning, initiation, and elongation [116, 117, 118, 124]. However, their positive or adverse action depends on the condition of experiments and different plant species, respectively.

4.1.2 Sorgoleone

Sorgoleone is one of the predominant compounds in the sorghum root exudates [105,125]. It is synthesized in the cytoplasm of sorghum root hair cells and its biosynthesis involves fatty acid denaturases from *S. bicolor* (DES2 and DES3), alkyl resorcinol synthases (ARS1 and ARS2) and an O-methyl transferase (OMT3). In the final enzymatic step of the sorgoleone biosynthesis pathway, a cytochrome P450

enzyme, designated CYP71AM1, catalyzes the formation of dihydrosorgoleone, using 5-pentadecatrienyl resorcinol-3-methyl ether as a substrate. Once released into the rhizosphere as a chemically unstable hydroquinone, dihydrosorgoleone rapidly oxidizes to the active benzoquinone sorgoleone [126]. This hydrophobic compound, belonging to the class of quinones, was initially identified by [127], being related to the allelopathic potential of sorghum, which corresponds to the effect of inhibiting or stimulating plant growth [126].

The ability of sorgoleone to influence mycorrhizal colonization in sorghum plants was recently described [101, 102]. No statistically significant difference was observed in mycorrhizal colonization on indigenous soil when *R. clarus* was inoculated on P9401 genotype, low sorgoleone sorghum genotype, with no added sorgoleone [101]. When sorgoleone was added, however, mycorrhizal colonization significantly increased in a concentration-dependent way. There was a 20% and 40% increase in colonization with 10 and 20 µMol.L^{-1} of sorgoleone, respectively, compared with 5 µM sorgoleone and *R. clarus* inoculation. There was no significant increase in plant dry weight and P content when sorghum was inoculated with *R. clarus* without sorgoleone. However, there was a significant threefold increase in dry weight as well as P content when inoculated with *R. clarus* and 20 µMol.L^{-1} sorgoleone [101] (Figure 2).

Figure 2. Sorghum roots from genotype P9401 cultivated in low-P soil under greenhouse conditions for 40 days. A: Negative control (non-inoculated); B: *Rhizophagus clarus*; C: *R. clarus* + sorgoleone (5 µM); D: *R. clarus* + sorgoleone (10 µM); E: *R. clarus* + sorgoleone (20 µMol.L^{-1}).

Furthermore, sorgoleone influences the structure of the microbial community of sorghum in the soil close to the roots and rhizosphere, and modulate the growth of a wide range of different bacterial taxa in field and greenhouse experiments [100].

5 Interaction between arbuscular mycorrhizal fungi and plant growth promoting bacteria

Much of the studies on plant-AMF association have focused on understanding the beneficial effects of AMF on plant growth and development. However, to what extent the soil bacterial community influences this association remains unclear. Soil biodiversity constitutes an additional factor that can exert a significant and still unexplored effect on the colonization of plant roots by AMF [128], since under natural conditions, AMF are surrounded by complex microbial communities, which can modulate mycorrhizal symbiosis [129] (Figure 1).

The hyphosphere microbiome is unique compared with other microbial communities. The hyphosphere show less bacterial species richness but more cultivable bacterial species than the bulk soil [130]. Soil composition and background microbiome, as well as experimental methods, such as sampling of the hyphosphere, fungal species, or plant species influences the hyphosphere composition. For example, taxa from the Firmicutes responded positively to the fungus *Glomus hoi*, while taxa from the Actinobacteria and Comamonadaceae responded negatively to the same fungal species in the study by [131]. The hyphosphere microbiome of *R. irregularis* and G. versiforme were dominated by Proteobacteria, with fewer *Actinobacteria, Chloroflexi, Acidobacteria, Bacteroidetes* and *Fibrobacteres* [132]. More interestingly, when compared with the rhizosphere, it was found that bacterial numbers were greater in the rhizosphere than in the hyphosphere soil of three different AMF [133]. In this study, the taxa *Bacillus* and *Arthrobacter* were most frequently found in the hyphosphere while *Pseudomonas* dominated in rhizosphere soils, implying that the structure and biodiversity of the microbiome in the AMF hyphosphere were clearly separated from the bulk soil and rhizosphere. Furthermore, these studies suggest that different AMF species recruit distinct microbiomes [67].

Beneficial interactions between plant growth promoting bacteria (PGPB), plant roots and AMF can enhance the health of plant by improving its immune systems and thereby enhancing nutrient production [134]. PGPB comprise a group of

microorganisms that, through interactions with a host plant, can modulate plant metabolism, stimulating its growth and development [135,136]. PGPB can be associated with a large number of species of crops of commercial importance, such as maize, sorghum, soybean, wheat, rice and brachiaria, including the genera *Pseudomonas, Burkholderia, Bacillus, Bradyrhizobium, Rhizobium, Gluconacetobacter, Herbaspirillum* and *Azozpirillum* [137,138].

The symbiotic function of molecules, such as strigolactones, depends on the co-developed ability of the AMF partners to interpret them as signals [139]. Strigolactones have possible effects on microbes that are released into the region of rhizosphere in the soil around the root [140] (Figure 1). Strigolactones treatment has improved fungal metabolism, resulting in increased ATP production and division of mitochondria [141]. Pleiotropic effects of strigolactones affect root development as reported by [142]. Strigolactones also participate in the response of plants to nutrient deficiency by directing association with symbiotic organizations and by promoting root lengthening in addition to their role in the growth of roots and plant architecture in general. Moreover, it was compared the diversity of rhizosphere bacterial and fungal communities associated with wild-type *Arabidopsis thaliana* and a mutant impaired in the production of strigolactones due to a disruption of the *MORE AXILLARY GROWTH* 4 (*MAX*4) gene [143]. The authors showed that the plant's ability to produce strigolactone is significantly correlated with changes in the composition (beta diversity) of rhizosphere fungal but not bacterial communities.

Furthermore, studies reported that several bacterial taxa can exert beneficial and synergistic effects with AMF [144,145,146], the so-called mycorrhizal helper bacteria [126]. Bacteria belonging to the *Oxalobacteraceae* family, for example, have been reported to preferentially associate with mycorrhizal roots [147,148]. Members of this family promote AMF spore germination, hyphal growth, and root colonization of fungi of the genus *Rhizophagus* [145,149,150,151]. These studies suggest that the combination of fungi and bacteria can lead to interactions that may have a potential role in relieving stress and improving crop productivity [146]. A more accurate understanding of bacterial interactions and the factors that influence their establishment in the rhizosphere can improve the efficiency of PGPBs, favoring the solubilization of nutrients, such as phosphates.

6 Commercial inoculants based on arbuscular mycorrhizal fungi intended for soil management

Due to their beneficial nutritional effects on the yield of agricultural cultivars and forestry plantations and their environmental benefits, AMF should be considered as a soil management strategy aimed at sustainable agriculture, reducing the use of synthetic fertilizers inputs. However, the benefits provided by mycorrhizae have already been documented, the use of technology associated with inoculation with AMF should be better explored in the agricultural market [152,153] and associated concerns should be considered [154].

Currently, only a few AMF genotypes are produced as bioinoculants and they are distributed globally. In the United States, since 2004, the company Mycorrhizal Applications is responsible for formulating several products based on AMF including MycoApply® Endo, MycoApply® Soluble MAXX and MycoApply® Injector Endo. In 2015, Mycorrhizal Applications became part of the Valent BioSciences group of companies, and all AMF-based products have an extensive global distribution network [152,153].

In Brazil, Novatero started the production of the first commercial product in 2018, called Rootella BR® and Rootella BR® Ultra, which are the only products to be commercialized on a large scale today. It is an AMF-based inoculant of the *R. intraradices* recommended for different crops, including soybean and maize. The agronomic validations showed an average increase of 54% of maize grain yield, and 25% for soybean grain yield, using the recommended fertilizer dosage [155].

In 2022, Sumitomo Chemical launched in the Brazilian market the inoculants MycoApply® EndoFuse® and MycoApply EndoMaxx®, based on four species of mycorrhizal fungi: *Glomus mosseae*, *G. aggregatum*, *G. intraradices* and *G. etunicatum*. EndoFuse® is used exclusively at seed treatment and EndoMaxx® can be used in the planting furrow, irrigation and drenching; and be mixed with different products, such as fertilizers and/or insecticides and fungicides. Company research indicates a productivity gain of 5% to 7% in soybeans and 4% to 5% in maize.

7 Conclusion

The current chapter provided an overview about the benefits of AMF symbiotic relationships, especially with maize, sorghum and soybean crops. The application of AMF causes a variation in their abundance, colonization, influence the rhizosphere and soil microbiota and functionality and play an important role in plant growth and productivity by alleviation of biotic and abiotic stresses. Encouragement of AMF usage is of immense importance for modern global agricultural systems for their consistent sustainability. The production of customized fungal inoculants by exploration of field parameters, identifying useful AMF strains, selection of genotypes with the highest AMF colonization potential, the impact of co-inoculation with other microorganisms, are some promising areas that need to be further understand and explored. It is also important to develop a “best practice” guideline regarding AMF inoculum selection and quality control.

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CAPÍTULO 2

Sorgoleone unveiled: exploring its biosynthesis, functional perspectives and applications

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Abstract: Sorghum's ability to exhibit allelopathy is linked to the secretion of lipophilic exudates from its roots. Sorgoleone, a member of the quinone class, constitutes a substantial part of these exudates. While studies typically focus on testing the exudate or crude extract, other compounds are also present, although in lesser quantities.

Initially suspected molecular target sites affected by sorgoleone include photosynthetic and mitochondrial electron transport processes, along with p-hydroxyphenylpyruvate dioxygenase. Despite acting as a Photosystem II inhibitor in isolated chloroplasts, its impact on overall photosynthesis remains uncertain. Proposed mechanisms suggest inhibition of root H^+-ATPase activity and water uptake, but questions persist regarding sorgoleone's absorption, transportation to the shoot, and entry into chloroplasts. The spatial separation between sorgoleone exudation and its presumed site of action presents a notable challenge. This review delves into the characteristics and effects of sorgoleone, critically assessing its role in allelopathy. Furthermore, it explores the role of sorgoleone in signaling to facilitate the establishment of a symbiotic relationship between plants and arbuscular mycorrhizal fungi, as well as its impact on the microbiota. However, key questions regarding its mode of action, specific activity and selectivity, bioactive concentration, persistence and release in the rhizosphere, as well as its absorption and translocation, remain to be fully elucidated.

Keywords: allelopathy; arbuscular mycorrhizal fungi; exudation; *Sorghum bicolor*; rhizosphere; root hair

1 Introduction

The allelopathic potential of sorghum is attributed to the release of phytotoxic lipophilic exudates from its roots. Sorgoleone, a compound found in the exudate produced by sorghum root hairs, is believed to play a role in the observed allelopathic effects. However, the majority of studies assessing the allelopathic potential of sorghum species typically test the exudate or crude extract. While sorgoleone makes up a significant portion of this extract, constituting up to 90%, the exudate also contains lipid benzoquinone, along with a resorcinol analogue, and several other compounds, albeit in much lower quantities (Netzly et al. 1988; Czarnota et al. 2003; Dayan et al. 2003; Erickson et al. 2001; Kagan et al. 2003).

The initial suspected molecular target sites affected by sorgoleone were the photosynthetic and mitochondrial electron transport processes (Rasmussen et al. 1992; Einhellig and Souza, 1992; Nimbal et al. 1997; Rimando et al. 1998), as well as the enzyme p-hydroxyphenylpyruvate dioxygenase (Meazza et al., 2002). Although sorgoleone acted as an inhibitor of PSII in isolated chloroplasts, the photosynthesis of 7 to 10-day-old plants did not seem to be affected (Hejl and Koster, 2004).

Subsequently, it was proposed that sorgoleone's mode of action involved inhibiting root H^+-ATPase activity and water uptake. However, a crucial aspect that remains to be clarified is the mechanism by which sorgoleone is absorbed by roots, transported to the shoot, and enters the chloroplast to inhibit PSII in the thylakoid membrane (Hejl and Koster, 2004).

Thus, while sorgoleone disrupts various physiological and biochemical processes in vitro, its primary mechanism of action in plants remains unclear. One significant challenge is the spatial separation between where sorgoleone is exuded (soil) and its presumed site of action (foliage) as a PSII inhibitor, a question that has not been adequately addressed so far. In this review, we will present the characteristics and impacts of sorgoleone and critically examine its role in allelopathy. However, there are essential questions that still need further clarification.

2 Chemical structure and discovery of sorgoleone

Sorgoleone, a constituent of sorghum exudate, falls within the quinone class and is known for its biological activity (Chang et al. 1986; Netzly et al. 1988; Weston and Czarnota, 2001; Weston et al. 2013; Pan et al. 2018). This secondary metabolite is exuded through the root hairs of sorghum roots (Jesudas et al. 2014; Weston and Czarnota, 2001; Weston et al. 2013; Wang et al. 2021).

Chemically, sorgoleone is a polyunsaturated fatty acid with a benzoquinone ring, specifically 2-hydroxy-5-methoxy-3-[(8-Z,11-Z)-8,11,14-pentadecatriene]-p-benzoquinone [CAS 105018-76-6] (Glab et al. 2017; Figure 1). Alongside sorgoleone, sorghum root exudate contains various structurally related lipophilic p-benzoquinones pre-sent in small quantities, as well as a comparable amount of non-quinoid lipid resorcinols. These compounds vary in the length or degree of saturation in the aliphatic side chain and in the substitution pattern of the quinone ring (Erickson et al. 2001; Kagan et al. 2003; Rimando et al. 2003; Dayan et al. 2009; Dayan et al. 2010; Pan et al. 2018).

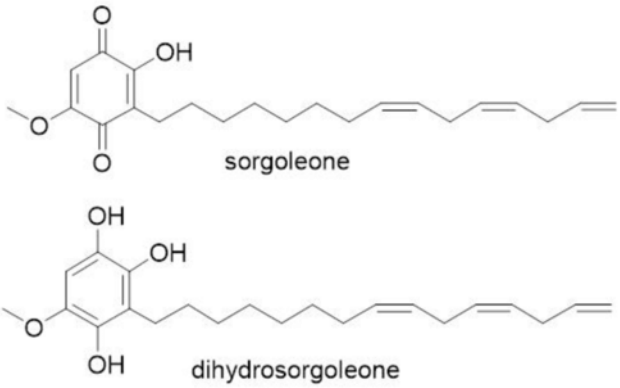

Figure 1. Chemical structures of (A) sorgoleone and (B) dihydroquinone of sorgoleone (Glab et al. 2017).

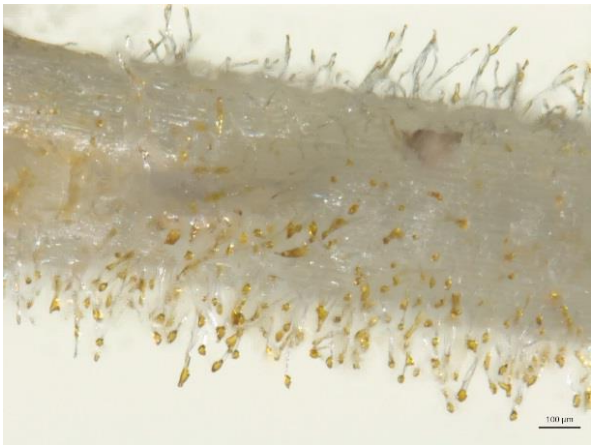

Figure 2. Sorgoleone droplets on root hairs of sorghum (CMSXS 206 B genotype) grown for seven days in the dark. Magnification 80x.

3 Sorgoleone biosynthesis

The exclusive site for the biosynthesis of the biologically active benzoquinone sorgoleone is the root hairs of sorghum (Baerson et al. 2008; Dayan et al. 2007; Pan et al. 2007; Yang et al. 2004; Weston and Czarnota, 2001). Within mature sorghum root hairs lies the complete genetic material and biochemical machinery necessary for the production of this bioactive benzoquinone (Czarnota et al. 2003; Dayan et al. 2007; Pan et al. 2007; Baerson et al. 2008). Root hairs, specialized cells abundant in mitochondria, rough endoplasmic reticulum, and vesicles, play a crucial role in sorgoleone synthesis (Weston and Czarnota, 2001). Sorgoleone can be visualized

through transmission electron microscopy as densely osmiophilic globules within the cytoplasm of root hair cells, deposited between the plasmalemma and the cell wall (Weston and Czarnota, 2001).

The process of sorgoleone biosynthesis has been clarified using retrobiosynthetic nuclear magnetic resonance (NMR) analysis (Fate and Lynn, 1996; Dayan et al. 2003). The exudate production from root hairs remains consistent regardless of the root development stage (Dayan, 2006), resulting in an accumulation of up to 20 µg of exudate per milligram of root dry weight (Dayan et al. 2009; Weston and Czarnota, 2001). The production of sorgoleone can be halted and then restarted after gently rinsing the roots with water. However, although the biosynthesis of lipid benzoquinones and resorcinols is a dynamic process (Dayan et al. 2009), the regulatory mechanism governing root exudate production remains unknown.

The expression of enzymes involved in sorgoleone biosynthesis is induced in a specific root zone, indicating that the secretion is developmentally regulated. Preceding the peak of sorgoleone biosynthesis and secretion, there is an accumulation of internal vesicles, suggesting that these vesicles are involved in precursor storage rather than secretion (Maharjan et al. 2023). Plant hormones play a role in regulating sorgoleone production; for instance, abscisic acid (ABA) was found to increase sorgoleone production, while gibberellic acid (GA) decreased it (Bais et al., 2006). This indicates that ABA and GA are part of the signaling pathway that regulates sorgoleone production. Moreover, sorgoleone production was observed to be higher in plants grown under conditions of drought stress or nutrient deficiency (Sarr et al. 2021; Wang et al., 2021; Oliveira et al. 2020; Wen et al. 2019; Abdel-halim et al. 2019). This suggests that environ-mental stresses can trigger sorgoleone production as a defense mechanism.

Understanding the regulatory mechanism of sorgoleone production holds significant value for various reasons. Firstly, it could facilitate the development of novel approaches to manipulate sorgoleone production in sorghum plants, enabling enhancement or reduction of sorgoleone levels. This modulation could be instrumental in bolstering sorghum's resistance against pests and diseases or amplifying its allelopathic effects. Secondly, it may shed light on the regulation of other root exudates, offering opportunities to optimize plant nutrition and enhance overall crop productivity. Sorgoleone exudation is subject to variation based on sorghum genotype and specific cultivation conditions. The optimal temperature range for sorgoleone production is

typically between 25 and 35 ºC, with significantly reduced production observed at lower temperatures, potentially dropping by as much as 95% (Dayan, 2006). Additionally, the presence of other plants appears to stimulate sorgoleone production (Dayan, 2006).

The biosynthetic pathway leading to the formation of sorgoleone involves several key enzymatic steps. It begins with the production of an alkylresorcinole intermediate, *SbARS2*, which utilizes a fatty acyl-CoA starter unit generated from consecutively catalyzed palmitoleoyl-CoA by *S. bicolor* fatty acid denaturases, *SbDES2* and *SbDES3*. This intermediate undergoes methylation by O-methyltransferase *SbOMT3*. In the final step of the sorgoleone biosynthesis pathway, a cytochrome P450 enzyme (*CYP71AM1*) catalyzes the formation of dihydrosorgoleone, using 5-pentadecatrienyl resorcinol-3-methyl ether as a substrate. Once released into the soil from the rhizosphere as a chemically unstable hydroquinone, dihydrosorgoleone undergoes auto-oxidation in vivo to produce sorgoleone, a more stable benzoquinone (Pan et al. 2021; Pan et al. 2018). All identified and functionally characterized genes (*SbDES*2, *SbDES*3, *SbARS*2, *SbOMT*3, *CYP71AM*1) in this process encode enzymes that facilitate every biosynthetic step leading to the production of dihydrosorgoleone, the precursor of sorgoleone (Pan et al. 2021; Pan et al. 2018; Figure 3). However, the regulation of genes in this pathway remains incompletely understood. A recent question has emerged regarding whether the simultaneous expression of all genes associated with the sorgoleone biosynthesis pathway could produce dihydrosorgoleone, the precursor of sorgoleone. Additionally, there is interest in understanding how heterologous host cells would respond physiologically to the synthesized compound and how the expression of the host gene would be affected (Pan et al. 2021).

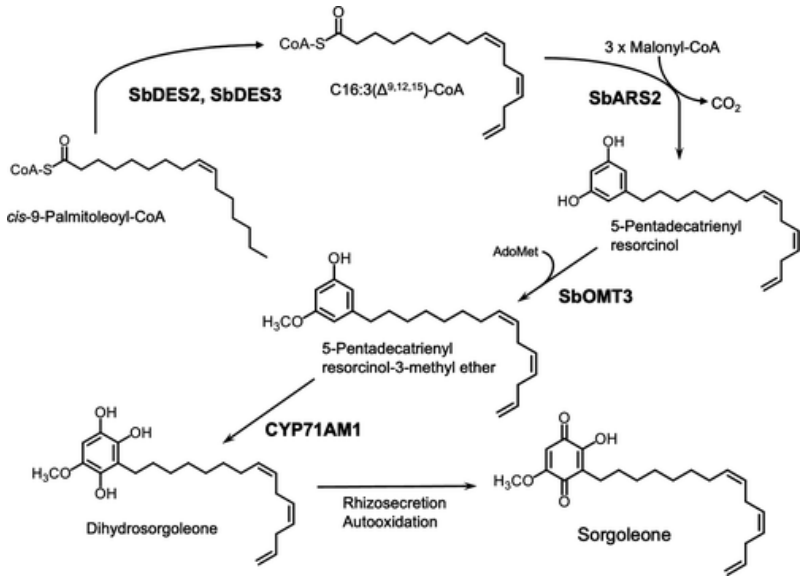

Figure 3. Sorgoleone is synthesized through a series of enzymatic reactions, commencing with palmitoleoyl-CoA. The process leads to the formation of dihydrosorgoleone, which, when released into the soil, undergoes auto-oxidation to yield sorgo-leone, a more stable benzoquinone. The key enzymes involved are cytochrome P450 (CYP71AM1), alkylresorcinol synthase (SbARS), fatty acid desaturase (SbDES), and O-methyltransferase (SbOMT) (Pan et al. 2021).

4 Stimulatory effect of water extracts of sorghum (Sorgaab)

Sorgoleone, categorized as a phytotoxin, represents a class of biodegradable and environmentally safe molecules with potential for enhancing crops in both controlled and field conditions. Research examining weed control using water extracts of sorghum (also know as Sorgaab) has shown a significant increase in crop yield post-treatment with plant-released phytotoxins (Cheema et al., 2001, 2003; Ashraf and Akhlaq, 2007; Jamil et al., 2009; Khan et al., 2015). This increase may stem from effective weed control, reducing competition between weeds and crops, or from hormetic growth stimulation in crop plants. Consequently, applying phytotoxins during vulnerable weed stages, aligning with optimal crop growth periods, could offer both crop protection and enhancement. Abbas et al. (2017) suggested that the optimal timing for weed control coincides with peak of crop growth stimulation.

While few studies have explored the stimulatory responses of plant-released phytotoxins, research on the inhibitory effects of various plant phytotoxins indicates that the response of tested plants depends on the type of phytotoxins involved (Manandhar et al., 2007; Farooq et al., 2011a,b). The extent of growth inhibition varied

among toxic compounds, with some showing no significant impact on growth (Abbas et al., 2017). In a study by Kamran et al. (2016), a 3% water extract of sorghum, maize, rice, and moringa, applied alone or in combination, was used to enhance maize growth. Results revealed that the phytotoxins released by these crops differed in their ability to initiate stimulatory responses in morphological and yield traits. The variance in the potential of plant phytotoxins to induce crop stimulation (hormesis) likely relates to the mechanisms underlying the production of growth stimulants.

Timing of plant phytotoxin application is crucial for achieving increased harvest yields. Early application may not sustain stimulatory responses over an extended period. Utilizing plant-released phytotoxins as growth promoters is preferable to herbicide hormesis due to their environmental safety, biodegradability, and absence of residual effects on food quality (Petroski and Stanley, 2009). Exogenous application of plant-released phytotoxins via foliar spray can effectively enhance crop growth directly or indirectly. For instance, Jahangeer (2011) demonstrated that a foliar spray containing 3% aqueous extracts of moringa, sorghum, and brassica increased maize grain yield by 52%, 42%, and 42%, respectively.

5 Allelopathy effect of sorghum root extract

In recent years, extensive research has been dedicated to exploring the allelopathic effects of sorghum root extract, with a particular emphasis on sorgoleone (Einhellig and Souza, 1992; Nimbal et al. 1997; Weston and Czarnota, 2001; Erickson et al. 2001; Kagan et al. 2003; Rimando et al. 2003; Dayan et al. 2009; Dayan et al. 2010; Uddin et al. 2010; Uddin et al. 2014; Sabahie et al. 2014; Pan et al. 2018; Campos et al. 2020; Besançon et al. 2020; Naby and Ali, 2021; Tibugari et al. 2021; Gomes et al. 2023).

Allelopathy refers to the production and release of one or more biologically active compounds, termed allelochemicals, which affect the growth, survival, and reproduction of other organisms (Pan et al., 2018). Evidence suggests that allelopathic activity results from the collective action of mixtures of allelochemicals rather than a single allelochemical. For example, pure artemisinin demonstrates less inhibition of redroot pigweed growth compared to an annual wormwood leaf extract (Lydon et al. 1997).

Allelochemicals have been identified in economically significant cereals like wheat, rice, and sorghum (Bertin et al. 2003; Duke, 2003; Inderjit and Duke, 2003). In sorghum leaves, phenolic compounds serve as the primary allelochemicals. Although Vaughan and Ord (1991) initially expressed skepticism regarding the allelopathic activity of phenolic acids in nature, subsequent studies have demonstrated that, under appropriate concentrations and conditions, certain phenolic acids can indeed be considered allelochemicals (Blum,1995; Blum, 1996; Inderjit, 1996; Blum et al. 1999; Dalton, 1999).

The allelopathic properties of sorghum have been a subject of study for many years. Among the initial investigations into the biologically active elements of sorghum root exudates, it was revealed that these exudates hindered the growth of lettuce seedlings (*Lactuca sativa*) as well as invasive weed species (Netzly and Butler, 1986). The primary component of these exudates, sorgoleone, has been pinpointed, constituting approximately 40% to 90% (w/w) in various sorghum accessions (Nimbal et al. 1997; Weston and Czarnota, 2001; Dayan et al. 2009).

Three sorghum varieties, namely Dhlakama, Shirikure, and Macia, exhibit autotoxicity in sorghum. The extent of toxicity varies based on the genotype (Tibugari et al. 2021). However, in Tibugari et al (2021) study, the researchers utilized the total sorghum exudate instead of purified sorgoleone. Another research using a formulation containing 4.6% of sorgoleone demonstrated its effectiveness in inhibiting weed growth (Uddin et al. 2014). Sorghum root extract displays significant inhibitory effects on various plant species, including *Abutilon theophrasti*, *Datura stramonium*, *Amaranthus retroflexus*, *Setaria viridis*, *Digitaria sanguinalis*, and *Echinochloa crusgalli* (Einhellig and Souza, 1992), as well as on caruru germination rate and percentage (Sabahie et al. 2014), early development of canola plants (Campos et al. 2020) and weed growth in wheat fields (Naby and Ali, 2021). These findings highlight that sorghum exudate exhibits biological activity even at very low concentrations, suggesting a significant contribution to sorghum allelopathy.

The impact of allelopathic effects on crops succeeding sorghum cultivation or under sorghum straw, and the optimal duration of fallow straw before introducing a new crop, has been a subject of prolonged study. In a greenhouse study, the allelopathic effects on soybean establishment and biomass production were evaluated at various time intervals between sweet sorghum harvest and soybean sowing (Silva et al. 2016). Differences were observed in both culture establishment and biomass; however, while

the authors attributed these effects to sorgoleone, no subsequent research was conducted to conclusively establish sorgoleone as the causative agent.

In another greenhouse experiment, two sweet sorghum cultivars (BRS 506 and BRS 511) were utilized to investigate the optimal timing for sowing soybeans after sorghum (Garcia and Sutier, 2016). The analyses were conducted at 65 days after emergence and 15 days after plant cutting, assessing sorgoleone levels. The findings indicated that a nine-day interval between sorghum management and soybean sowing was sufficient to mitigate the negative effects. Soybeans sown on the day of sorghum management exhibited symptoms of leaf bleaching at stage V2, which were absent in soybeans sown 15 days after management.

In a separate greenhouse study, Olibone et al (2006) investigated the impact on early soybean growth and root system development in the presence of guinea sorghum and forage residues. The presence of guinea sorghum straw led to a reduction in soil base saturation, and both types of straw hindered soybean growth.

In field conditions, Biesdorf (2017) explored the allelopathic impact of sorghum on soybeans planted at various intervals after sorghum harvesting, and its influence on the infesting plant community. The study revealed that sorghum cultivation influenced the phytosociology and reduced weed incidence. Regarding soybeans, planting within 40 days after sorghum harvest had a negative impact on initial development, although it did not affect overall productivity. In a related study, Paixão (2019) investigated the allelopathic effect of sorghum regrowth on succeeding soybean crops at different intervals after sorghum desiccation in the field. The analysis did not observe significant alterations in the evaluated parameters. The research concluded that soybean development was enhanced in the presence of regrowth straw, possibly due to in-creased nutrient availability, indicating the absence of a toxic allelopathic effect under the experimental conditions.

In sorghum-cultivated areas, a substantial amount of plant material remains after harvest, especially with no-tillage and minimum tillage practices. This straw can serve as an effective tool for weed control. However, it may also have a detrimental impact on the establishment and growth of succeeding crops. Allelopathic chemical substances identified in sorghum include chlorogenic acid, m-coumaric acid, and caffeic acid *(*Cheema et al. *2009)*; p-hydroxybenzoic acid, p-coumaric acid, ferulic acid, vanillic acid, serpidric acid, and p-hydroxybenzaldehyde (Sene et al. 2020); and gallic

acid, p-coumaric acid, and syringic acid (Alsaadawi and Dayan, 2009), as well as p-hydroxybenzoic acid (Alsaadawi et al. 2007).

Notably, the allelopathic effect may not solely be attributed to sorgoleone. Sorgoleone is a phenolic compound exhibiting allelochemical properties, but sorghum also synthesizes other chemicals that inhibit the growth of neighboring plants (Shehzad et al. 2020). Further studies focusing on the absorption of substances released by sorghum straw and their mechanisms of action are crucial to comprehend their behavior. Understanding how these substances are diluted, incorporated into the soil volume, and their concentration is vital, as the intensity of allelopathic effects is contingent on the concentration of allelochemicals.

6 Differences in exudation and composition of sorghum extracts among genotypes

In recent years, research has been focused on understanding the variability in extract production among sorghum genotypes, exploring the composition of these extracts, and delving into the biochemical interactions between the compounds. The hydrophobic crude root extracts from 41 sorghum genotypes were quantified, revealing a variability ranging from 0.35 to 2.98 mg per 100 rootlets (Trezzi et al. 2005). Within this range, researchers selected five genotypes, purified sorgoleone using column chromatography, and confirmed its presence through H-NMR analysis. Chromatograms showed five distinct peaks with varying areas, with sorgoleone being the most predominant compound, constituting 73 to 82% of the total extract.

Quantification of crude extract production from 50 sorghum accessions revealed a variation between 1.88 and 8.55 mg per gram of fresh root mass (Franco et al. 2011). The analysis exhibited three to ten peaks with retention times falling within 1.3 to 17.5 minutes. Sorgoleone stood out with the highest absorption peak and a retention time between 8.4 and 8.7 minutes, constituting 60.3 to 89.9% of the crude extracts. Notably, 68% of the evaluated genotypes showed production ranging from 1.0 to 2.0 mg per gram of fresh root mass (Franco et al. 2011). However, the production of sorgoleone exhibits variance based on the cultivar studied. May et al (2016) reported this variance, where crude extract production ranged from 5.03 mg g^{-1} to 10.60 mg g^{-1} and sorgoleone production ranged from 32.64 mg g^{-1} to 615.31 mg g $^{-1}$, depending on the

cultivar. Uddin et al (2009) also observed a range of sorgoleone production between 0.41 µg $^{-1}$ and 6.98 µg $^{-1}$ of fresh root mass.

Several other works observed variations in the production of sorgoleone (Hess et al. 1992; Ferreira et al. 1999; Rodrigues et al. 2001) with values ranging from 11 to 32 mg 100 rootlets^{-1}. Nimbal et al (1997) found differences of 96% between the highest and lowest sorgoleone content per root dry mass, among the 25 sorghum genotypes evaluated. An important piece of information observed in all these works is that the variation in the amount of extract and sorgoleone produced did not have a direct relationship with the sorghum quality (biomass, saccharine, grain), indicating that this trait is dependent both by genetic and environment interactions.

In recent research, the quantification of three sorghum root extracts using high-performance liquid chromatography (HPLC) revealed a production range of 43.36 to 67.8 mg per gram of root dry mass, with sorgoleone production ranging from 20.56 to 28.67 mg per gram of fresh root mass (Gomes et al. 2023). Interestingly, genotypes with longer and denser root hairs were found to exude more and produce higher amounts of sorgoleone. The chromatographic profiles displayed five to six distinct substances with varying peaks and areas. Moreover, when allelopathy evaluations were conducted using extracts with standardized sorgoleone amounts; different results were observed, suggesting that other compounds within the extract play a significant role in influencing the outcomes (Gomes et al. 2023).

The majority of studies assessing the allelopathic potential of sorghum species utilize sorghum extract, often attributing its effects to sorgoleone, which can constitute up to approximately 90% of these extracts. However, gaining a deeper understanding of the specific effects of sorgoleone compared to the whole extract is essential for assessing allelopathic effects accurately. Another crucial aspect is identifying the compounds present in sorghum root extracts. Weston et al (2013) conducted a chemical analysis using HPLC on extracts from two sorghum cultivars and identified several phenolic compounds, including protocatecheic, hydroxybenzoic, vanillic, syringic, p-coumaric, ferulic, and sinapic acids, along with four unidentified compounds. However, further research is needed to comprehend the relationship between extract production, composition, and their allelopathic effects.

7 Potential allelopathic effect of sorgoleone

The allelopathic activity of substances can be elucidated through various modes of action and by targeting multiple molecular components affected by these allelochemicals. An example is observed in a freshwater microphyte called *Myriophyllum spicatum* (Haloragaceae), which releases thelymagrandin II, a compound with algaecidal and cyanobactericidal properties (Gross, 1999). Leu et al (2002) further elucidated that thelymagrandin II operates through two distinct modes of action: inhibition of Photosystem II and inhibition of microalgal exoenzyme formation, demonstrating a combined action involving these pathways. Understanding such multifaceted modes of action is critical in comprehending the mechanisms underlying allelopathic effects (Leu et al. 2002).

The suggested allelopathic activity of sorgoleone is primarily linked to its ability to inhibit photosynthesis in higher plant systems. It achieves this by competing for the plastoquinone binding site in Photosystem II, a key component of the photosynthetic machinery (Dayan et al. 2003). Moreover, sorgoleone has been shown to hinder electron trans-fer reactions integral to mitochondrial respiration and to inhibit the enzyme p-hydroxyphenylpyruvate dioxygenase, a crucial enzyme for plastoquinone synthesis (Rasmussen et al. 1992).

The activity of sorgoleone can be attributed to both direct and indirect effects, given its multiple modes of action (Netzly and Butler, 1986; Einhellig and Souza, 1992; Nimbal et al. 1997; Rimando et al. 1998; Czarnota et al. 2001; Bertin et al. 2003; Duke, 2003). Studies on the translocation of carbon-14-labeled sorgoleone in velvet plants (*Abutilon theophrasti*) suggested uncertainty regarding whether sorgoleone exuded from sorghum roots is absorbed and translocated to its foliage, where it should enter the chloroplast and inhibit Photosystem II (Dayan et al. 2009). Experiments in this domain revealed that the inhibitory activity of sorgoleone on photosynthesis is strongly contingent on the age of the leaf tissue, inhibiting photosynthesis in germinating seedlings but not in older plants. Sorgoleone's mode of action may involve inhibiting photosynthesis in young seedlings while also affecting other molecular targets in older plants (Dayan et al. 2009).

Apart from sorgoleone, sorghum root extract contains smaller analogues with various substitutions in the quinone moiety and different configurations of carbon atoms and double bonds in the aliphatic side chain. Through high-performance

chromatography coupled with mass spectrometry and 1H nuclear magnetic resonance, researchers have isolated sorgoleone and three other quinone compounds. These compounds share the p-quinone fraction with sorgoleone but exhibit distinctions in the number of double bonds or carbon atoms in the aliphatic side chain. These compounds are collectively termed sorgoleones and include sorgoleone-358, sorgoleone-360 (with two double bonds in the C15 side chain), sorgoleone-362 (with a double bond in the C15 side chain), and sorgoleone-386 (with three double bonds in a C17 side chain) (Netzly and Butler, 1986).

Examination of the sorghum root extract using gas chromatography coupled with mass spectrometry reveals peaks in the chromatogram with molecular ions not only at 358, as expected for sorgoleone, but also at 359, 360, 362, 363, 364, 365, and 366. This indicates the presence of sorgoleone-like compounds with varying degrees of unsaturation in the side chain (Erickson et al. 2001). These findings support the notion that these analogues of sorgolene may collectively contribute to the overall allelopathic effects observed in sorghum.

It was hypothesized that the biosynthesis of sorgoleone and related compounds is the outcome of the convergence of two pathways: the fatty acid biosynthetic pathway for creating the aliphatic tail and the activity of polyketide synthase-type enzymes for forming the quinone fraction of the molecule (Dayan et al. 2003). Although this convergence of pathways has been confirmed for aflatoxin biosynthesis in fungi, similar examples in plants have not been fully clarified, relying on assumptions that have not been conclusively demonstrated for these compounds (Dayan et al. 2003). For sorgoleone, exogenous acetate labeled with isotopes is integrated into the quinone head instead of the tail (Fate and Lynn, 1996*)*. This finding implies that the allelopathic effect attributed to sorgoleone might be specifically linked to the quinone fraction of sorgoleone. In an investigation involving the synthesis and assessment of eight resorcinol lipid derivatives and ten quinones with varying side chain sizes identified from sorghum root extracts, the results revealed that quinones possess phytotoxicity, while resorcinolic lipids do not. This suggests that different fractions constituting the chemical structure of the sorgoleone molecule can exhibit distinct effects (Mizuno et al. 2010).

8 Influence of sorgoleone on the soil microbial community

Plants employ a diverse array of mechanisms to absorb and transport bioactive compounds to the rhizosphere (Wen et al. 2019). The rhizosphere is a critical zone where extensive microorganism-plant-soil interactions take place, encompassing both agricultural and natural systems (Wang et al. 2021). The unique physical, chemical, and biological properties of molecules exuded into the rhizosphere can influence the growth and proliferation of specific groups of microorganisms while inhibiting others. Microbial populations, given their versatile metabolic capabilities, can exhibit variability from one cultivar to another due to differences in root exudation and significant metabolic modifications, influencing the interactions between plants and microorganisms. Even with the same isolate and plant genotype, alterations in the plant's exudation pattern can lead to different interactions. Root exudation not only determines the microbial community residing in the rhizosphere but also confers physical and chemical advantages to plants, such as those observed in sorghum (Vejan et al. 2016; Wen et al. 2019; Liu et al. 2017).

As an avenue to understand how sorghum and its root microbiome may be connected through root exudates. Oda et al (2023) identified the molecular determinants of microbial sorgoleone degradation and the distribution of this trait among microbes. They isolated and studied from sorghum-associated soils, three bacterial strains classified as *Acinetobacter*, *Burkholderia*, and *Pseudomonas* species that grow with sorgoleone as a sole carbon and energy source.

In a recent study involving five *Bacillus* isolates, it was observed that sorgoleone strongly inhibited the growth of three isolates, while stimulating the growth of two. Among the inhibited isolates, two were identified as *B. safenensis* and one as *B. cereus*. On the other hand, the two isolates that showed growth stimulation were classified as *B. flexus* (Wang et al. 2021).

Additionally, the role of root exudation as a signaling mechanism for establishing effective symbiosis between plants and arbuscular mycorrhizal fungi (AMF) has been established in various plant species, including maize, soybean, and sorghum (Yoneyama et al. 2015; Kobae, 2018; Abdelhalim et al. 2019). Recent research has demonstrated sorgoleone's ability to influence mycorrhizal colonization in sorghum plants (Oliveira et al. 2020; Sarr et al. 2021). Furthermore, sorgoleone has shown potential to significantly enhance plant biomass and phosphorus (P) content in

mycorrhizal plants compared to non-mycorrhizal ones, especially when grown under low P (Oliveira et al. 2020; Sarr et al. 2021). Importantly, sorgoleone has been found to affect the microbial community structure in the sorghum rhizosphere soil (Wang et al. 2021; Ortas et al. 2022).

The concentration of P in the soil significantly influences AMF colonization (Kobae, 2018). This is due to the impact on root exudation of fungal signaling compounds, a key aspect of the symbiotic response. Low-P concentrations in the soil promote increased root exudation and subsequent mycorrhizal colonization. Conversely, high-P concentrations suppress exudation, reducing the level of plant-fungus signaling during symbiosis and hindering colonization (Chiu and Paszkowski, 2019). In cases of P deficiency, plants deficient in root exudate biosynthesis exhibit lower levels of colonization (MacLean et al. 2017; Lanfranco et al. 2018). When soil P availability is abundant and comparable to what a non-mycorrhizal plant can absorb, rendering the fungus an energy burden for the plant with no additional nutritional benefit in terms of P absorption, it can lead to a depressive effect on plant development in the presence of AMF (Pedersen et al. 1996; Siqueira et al. 1998; De Novais et al. 2014).

9 Final considerations and perspectives

Since its discovery, sorgoleone has emerged as a pivotal secondary metabolite with extensive applications. Understanding the hormesis of this plant-released phytotoxin is crucial for comprehending its role in microorganism-plant-soil interactions. It is crucial to focus efforts on identifying compounds within sorghum root extract and understanding the chemical characteristics of sorgoleone-related analogs found in the extract. The practical application of post-emergence sorgoleone through leaf spraying, a common field practice, presents challenges. There is a lack of evidence demonstrating the translocation of sorgoleone from leaves to roots, where it is subsequently exuded into the soil. Exploring genes and functional genomics becomes imperative to unravel the role of trans*criptional modulation of genes* and regulatory factors involved in sorgoleone biosynthesis. This foundational work is essential before advancing technologies aimed at reducing dependence on synthetic herbicides. Ongoing research in this field holds promise in unveiling the intricacies of sorgoleone's biosynthesis and function, with potential applications in agriculture and ecology. This

continual exploration contributes to a deeper understanding of plant biology, enabling us to leverage it to enhance agricultural productivity.

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CAPÍTULO 3

Sorgoleone enhances mycorrhizal colonization in sorghum

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Abstract

Keywords: P uptake; Plant growth; *Sorghum bicolor; Striga* resistant genotype

1 Introduction

The symbiotic relationship between plants and arbuscular mycorrhizal fungi (AMF) as one of the most crucial associations between plants and microorganisms (Vejan et al. 2016). This relationship serves as a significant mechanism for enhancing soil exploitation and improving nutrient acquisition efficiency (Wen et al. 2019; Abdelhalim et al. 2019; Sarr et al. 2021; Wang et al. 2021). Understanding the dynamics of mycorrhizal colonization and the growth conditions influencing the symbiotic uptake of phosphate is essential for enhancing phosphorus nutrition in crops (Kobae, 2018). Exploring AMF's role in plant nutrition within low-nutrient soils holds considerable promise for practical applications in agriculture. This is particularly

significant due to AMF's capacity to mitigate the impact of both biotic and abiotic stresses on agriculturally important crops (De Sousa et al. 2021).

Phosphorus (P) is a vital macronutrient, commonly encountered in suboptimal concentrations in tropical soils (Lanfranco et al. 2018). The limited availability of P in the soil stands out as a primary factor impeding the growth and development of plants (Lynch, 2011; Jarvie, Flaten 2015; Lynch, 2019; Bindraban, Pandey 2020). Plants like sorghum have evolved morphological and physiological mechanisms to adapt to low P availability. These adaptations include root system plasticity, exudation of organic compounds, and association with AMF. These strategies aim to enhance the absorption and utilization of P (Wen et al. 2019; Oliveira et al. 2020).

While existing studies have primarily focused on the mechanism of root system morphology for developing cultivars efficient in P acquisition, other mechanisms, such as associations with microorganisms, could also significantly contribute to sorghum development in low-P soils (Wen et al. 2019; De Sousa et al. 2021; Oliveira et al. 2020).

The distinctive physical, chemical, and biological properties of molecules released in the rhizosphere, including sorgoleone (Chang et al. 1986), can shape the interactions between plants and microorganisms (Vejan et al. 2016; Liu et al. 2018; Oliveira et al. 2020; Sarr et al. 2021; Wang et al. 2021; Ortas et al. 2022). Understanding the interplay between plant root exudates and the diverse soil microbial communities holds the potential to offer valuable insights for enhancing crop sustainability and yield (Oliveira et al. 2020; Sarr et al. 2021; Wang et al. 2021; Ortas et al. 2022).

In our previous study, we demonstrated for the first time that sorgoleone influences AMF colonization (Oliveira et al., 2020). In conditions of low P, P9401 inoculated with *Rhizophagus clarus*, without sorgoleone, exhibited a 15% mycorrhizal colonization. However, with the addition of 20 µMol.L^{-1} sorgoleone, the mycorrhizal colonization increased significantly to 83%. This finding suggests that sorgoleone enhances mycorrhizal colonization, P content, and dry weight in P9401 (Oliveira et al., 2020). Nonetheless, the mechanism behind this enhancement was unclear, whether increased sorgoleone directly stimulates AMF or improves P availability, leading to enhanced plant growth and, subsequently, indirect stimulation of root colonization. Building upon this, Sarr et al. (2020) demonstrated that a sorghum genotype releasing high amounts of sorgoleone per gram of dry root exhibits greater root and shoot biomass than lines producing medium and low levels of sorgoleone during the early

growth stage. Thus, the current study aimed to investigate the effect of sorgoleone on mycorrhizal colonization and rhizosphere microbiota and its role in improving P acquisition efficiency and the overall growth of sorghum plants.

2 Materials and methods

2.1 Production and purification of sorgoleone

The sorghum BR007B genotype from Embrapa Maize and Sorghum Breeding Program, was used to produce sorgoleone for the greenhouse experiment. The seeds underwent a disinfection process using 5% (v/v) sodium hypochlorite for 5 minutes, followed by washing with deionized water. Subsequently, the seeds were transferred to filter paper moistened with deionized water. A second layer of filter paper, also moistened, covered the seeds. The trays were then placed in a germination chamber, maintaining an average temperature of 25 ºC for seven days in darkness. Following germination and growth, sorghum roots were separated from shoots and weighed to determine fresh weight. For extraction, the roots were immersed in a solution of dichloromethane and acetic acid for 5 minutes. The resulting solution underwent filtration, and the solvent was evaporated in a rotary evaporator at 40 ºC. Subsequently, the roots were subjected to a forced air circulation oven at 65 ºC until a constant weight was attained, allowing for the determination of dry mass.

The crude sorgoleone extract was applied onto preparative plates measuring 20 x 20 cm with silica F254 and aluminum backing. This application was carried out using a hexane-isopropanol mixture (9:1 v/v). Subsequently, the sorgoleone (RF=0.35) band was carefully scraped off the plates and eluted with dichloromethane. The eluted sample was concentrated through a rotary evaporator and then stored at 4 ºC. The identification of sorgoleone was accomplished by comparing it with a standard retention time, as established by Czarnota et al. in 2003, utilizing High-Performance Liquid Chromatography (HPLC) with a Watters model Alliance 2695, PDA detector 2998, and a column Xbridge C18 (150 mm x 4.6 mm x 3.5 µm). During the chromatographic run, the mobile phase consisted of acetonitrile (75%) and an aqueous acetic acid solution (2.5% v/v). All solvents utilized were of HPLC grade. The flow rate during the chromatographic run was maintained at 1.0 $mL.min^{-1}$, with an injection volume of 20 µL. Elution was monitored at a wavelength of 280 nm at room temperature. To establish the calibration curve (ranging from 0.015 to 0.125 mg mL^{-1}),

a purified sorgoleone standard with a purity of 97.75% was used. Identifying the sorgoleone peak involved analyzing the UV absorption spectrum and comparing it with the retention time of the purified standard.

2.2 AMF and sorgoleone inoculation of the P9401 sorghum genotype under greenhouse conditions

Sorghum P9401 genotype seeds (Satish et al. 2012) were germinated in paper rolls within a growth chamber. After four days, the seedlings were transplanted into pots filled with 2 kg of red-latosol soil by with a very clayey texture and a pH of 6.0. The soil originated from the Cerrado area at the Embrapa Maize and Sorghum Experimental Station in Sete Lagoas, Minas Gerais, Brazil (19º 28' S, 44º 15' W, at an altitude of 732 m above sea level). Subsamples from the 0-40 cm depth were collected and composited. The soil parameters, measured following Embrapa (1997) guidelines, were as follows: potential acidity (H+Al) = 4.36; calcium (Ca) = 1.78 mg dm-3; magnesium (Mg) = 0.35 mg dm^{-3}; cation exchange capacity = 6.6 cmolc dm^{-3}; Mehlich 1 extractable P = 2.8 mg dm^{-3}; potassium (K) = 44.3 mg dm^{-3}; base saturation = 33.9 % and organic matter (OM) = 3.49 g kg^{-1}. To enhance soil properties, dolomite lime (2 g kg^{-1}) and gypsum (0.5 g kg^{-1}) were supplemented, and super triple phosphate was added to achieve a final P concentration of 5 mg dm^3. The soil was left non-sterilized and retained its indigenous AMF community.

The experiment involved eight treatments distributed in a completely randomized design, consisting of 0 and 500 spores of *Rhizophagus clarus* with 0, 20, 40 and 80 µMol L^{-1} sorgoleone. The inoculation with the mycorrhizal fungus (*R. clarus*) comprised a mixture of soil, grass root fragments, and 500 spores of *R. clarus* per plant, as detailed in previous studies (Dayan et al. 2009; Brunetto et al. 2019). Each treatment involved two plants per pot, and three pots were employed per treatment.

The sorgoleone solution, prepared in ethanol, was applied by adding 200 µL of each concentration once into the planting hole of each seedling. This was done concurrently with the inoculation of *R. clarus*. Only the ethanol solvent was applied for control treatments, serving as the control conditions (Besserer et al. 2006).

Forty-five days post-inoculation, the shoots were harvested, and the roots of the two plants were jointly removed from the pots and thoroughly washed to determine root and shoot dry weight, root morphology, P content, and AMF colonization. The reported values represent the average of the two plants per pot.

Roots were separated from the shoot, scanned and analyzed with the software WinRHIZO v. 4.0 (Regent Systems, Canada) to measure traits related to root morphology, such as total root length (L), average root diameter (D), total root surface area (SA), and surface area of roots with diameters between 0–1 mm (SA1), 1–2 mm (SA2) and larger than 2 mm (SA3) (cm2) (Sousa et al. 2012). Roots and shoots were individually dried at 65 °C in a forced-air oven until a constant weight was achieved, allowing for the determination of dry weight. For P content analysis, root and shoot tissues were ground using a Wiley mill and underwent nitric perchloric acid digestion at Laboratório de Análises Ambientais e Agrícolas (LABRAS, Monte Carmelo, MG - Brazil) (Malavolta et al. 1997). The P content was calculated by multiplying the dry weight of shoots and roots by their respective P concentrations.

2.3 Qualitative and quantitative analysis of mycorrhizal colonization

A small (~1 g) portion of the fine fresh roots from the sorghum plants was placed into conical tubes filled with 70% ethanol and stored in a refrigerator at 4 ºC for 3 days. The remaining roots were dried to determine root dry weight and P content. Subsequently, the roots were washed with deionized water and clarified in a potassium hydroxide solution (KOH 10%) overnight at room temperature. The next day, the roots underwent a wash and were immersed in hydrochloric acid (HCl 0.3 $Mol.L^{-1}$) for 30 minutes at room temperature. Following the acid treatment, the HCl was removed, and the roots were stained with trypan blue for 30 minutes at room temperature. The stained roots were then transferred to conical tubes containing an acidified glycerol solution (1:1 glycerol and 0.3 $Mol.L^{-1}$ HCl). Mycorrhizal colonization was quantified using the gridline intersect method described by Paszkowski et al. (2006), with modifications. Total colonization was determined by assessing the proportion of intersections displaying specific fungal structures, such as vesicles or arbuscules. This assessment was conducted under an Axio Zoom V16 (Zeiss) stereoscope at 20-fold magnification, covering 100 intersection points per root sample.

The mycorrhizal colonization results underwent analysis of variance (ANOVA), and the means of treatments were compared using Tukey's test ($p < 0.05$). The statistical analysis was performed using the software Sisvar version 5.6. To facilitate statistical analysis, the mycorrhizal colonization percentages were normalized through an arcsine-square root transformation before being subjected to the statistical procedures.

2.4 Gene Expression

Total RNA was isolated from fine root tissues using the SV Total RNA Isolation System kit (Promega Corporation, Madison, WI, USA) according to the manufacturer's instructions. Total RNA was used for cDNA synthesis using the High-Capacity cDNA Reverse Transcription kit (Applied Biosystems, Foster City, CA, USA), following the manufacturer's instructions. Transcripts were quantified by quantitative real-time PCR (qPCR-RT), using SYBR Green technology with the ABI Prism 7500 Fast system (Applied Biosystems, Foster City, CA, USA). The relative gene expression (RQ) was calculated using the $2^{-\Delta\Delta CT}$ method (Schmittgen; Livak, 2008).

The expression profile of the genes *CYP71AM1* (Pan et al. 2018), *Sb02g009880*, *Sb06g002560*, *Sb06g002540* and *Sb03g029970* (Walder et al. 2014), *SbAM3* and *RiEF* (Table S1) was assessed in the roots of the P9401 sorghum genotype with 0 and 20 µMol L^{-1} sorgoleone, as described above.

Table S1. Primers used for gene expression analysis by quantitative real-time PCR (q-PCR).

Description	Gene	Primer	Sequence (5'-3')
Cytochrome P450 enzyme involved in biosynthesis of sorgoleone	*CYP71AM1*	*21G12*	Fwd - AAGATCCAAGGCTACCATGTGC Rev - AACGTTGGCGACGACTATTG
AMF colonization marker	*AM3*	*SbAM3*	Fwd - GGCAGCAACAAGGCTAATTC Rev - ACCCTTGTGACGGAGAACAC
Rhizophagus irregulares Elongation factor	*RiEF*	----------	Fwd - TGTTGCTTTCGTCCCAATATC Rev - GGTTTATCGGTAGGTCGAG
AMF-induced Pi transporter	*Sb02g009880*	*SbPT8*	Fwd - GCAGCGAGGCCAATGAGACT Rev - TTGGCTCCGGTAGGAAGCAG
AMF-induced Pi transporter	*Sb06g002560*	*SbPT9*	Fwd - GAGGACGAGCCGTTCAAGAG Rev - CGCGACGGAGAAGAAGTACC
AMF-induced Pi transporter	*Sb06g002540*	*SbPT10*	Fwd - CACCATGTGCTGGTTACTTC Rev -GATAATCGCCTGAGTACGTG
AMF-induced Pi transporter	*Sb03g029970*	*SbPT11*	Fwd - CGTGGTTCCTTCTGGACATA Rev - TCTCGAACACCTCCTTGAGT

2.5 Diversity and composition of microbial taxa in the rhizosphere

2.5.1 Soil DNA extraction

Total DNA extraction from 0.45 g of rhizospheric soil samples was conducted using the DNeasy PowerSoil Pro Kit from Qiagen (USA), following the manufacturer's guidelines. Subsequently, the extracted DNA was suspended in 50 µL of solution C6 buffer. DNA was quantified using a Nanodrop® spectrophotometer (Thermo Fisher Scientific, USA), and the concentration was adjusted to 5.0 ng.µL^{-1} through dilution.

2.5.2 16S and *28S* rRNA gene amplification

Fragments of the 16S rRNA gene were amplified using the 8F-FAM primer, which was fluorescence-labeled at the 5' end (5'-AGAGTTGATCCTGGCTCAG-3') (Liu et al. 1997; LaMontagne et al. 2001), along with the 1492R primer (5'-TACGGTACCTTGTACGACTT-3') (Turner et al. 1999). The PCR reaction comprised of 15 ng of DNA, each primer at a concentration of 5.0 ng. µL-1, reaction buffer at 1X, $MgCl_2$ at 2.5 mMol.L-1, dNTPs at 2.5 mMol.L-1 each, and 2.5 U of Taq DNA polymerase (Promega GoTaq® G2 Flexi DNA Polymerase), in a final volume of 50 µL.

The amplification protocol for the 16S rRNA gene involved an initial denaturation step at 95 °C for 2 minutes, followed by 30 cycles at 95 °C for 1 minute, 58 °C for 1 minute, and 72 °C for 1 minute. The reaction was concluded with a final extension at 72 °C for 5 minutes. A nested-PCR approach was employed to amplify the 28S rRNA gene from AMF. In the initial reaction, primers LR1 (5'-GCATATCAATAAGCGGAGGA-3') (Van Tuinen et al. 1998; Trouvelot et al. 1999) and FLR2 (5'-GTCGTTTAAAGCCATTACGTC-3') (Trouvelot et al. 1999) were used. The reaction consisted of 15 ng of DNA, each primer at a concentration of 5.0 ng.µL^{-1}, reaction buffer at 1X, $MgCl_2$ at 2.5 mMol.L-1, dNTPs at 2.5 mMol.L-1 each, and 2.5 U of Taq DNA polymerase (Promega GoTaq® G2 Flexi DNA Polymerase), in a final volume of 50 µL. For the second PCR, 15 ng of the product from the first reaction was used. The primers FLR3 was labeled with FAM (5'-TTGAAAGGGAAACGATTGAAGT-3') (Gollotte et al, 2004) and FLR4 was labeled with HEX (5'-TACGTCAACATCCTTAACGAA-3') (Gollotte et al, 2004) at a final concentration of 5.0 ng.µL^{-1} each. The reaction also included 1X reaction buffer, $MgCl_2$ at 2.5 mMol.L-1, dNTPs at 2.5 mMol.L-1, and 2.5 U of Taq DNA polymerase (Promega GoTaq® G2 Flexi DNA Polymerase), in a total volume of 50 µL. The amplifications for AMF were

performed with an initial denaturation at 95 °C for 2 minutes, followed by 30 cycles at 95 °C for 1 minute, 58 °C for 1 minute, 72 °C for 1 minute, and a final extension at 72 °C for 5 minutes. Subsequently, 1 µL of the PCR products was stained with GelRed (Biotium, Hayward, California, USA) and analyzed in 1% (w/v) agarose gel electrophoresis using 1Kb Plus DNA Ladder (Life Technologies, USA). The amplified DNA was confirmed using a transilluminator under ultraviolet light and photographed using the L-PIX Image EX equipment (Loccus Biotecnologia, Brazil).

2.5.3 T-RFLP analysis

The amplified fragments underwent digestion with the restriction enzymes *Alu*I *Hae*III e *Hpa*II (Invitrogen, USA). To assess DNA fragments, 2 µL of the digestion product was combined with 9.8 µL of deionized formamide (Applied Biosystems, USA) and 0.2 µL of ROX500 standard (Applied Biosystems). The PCR-digested products were resolved through capillary electrophoresis using the Genetic Analyzer 3500XL (Applied Biosystems, USA) with GeneMapper 5.0 software (Applied Biosystems, USA). Terminal Restriction Fragment (T-RF) peaks, falling within 30 to 500 bp range with fluorescence intensities exceeding 40 fluorescence units (peak height), were considered for profile analysis. The T-Rex program was employed to align different samples and generate consensus profiles from two parallel runs of each sample. For data creation, T-RFs with ≥1% relative abundance (individual peak area divided by the sum of all peak areas) were considered. The relative abundances of the applied microbial species were determined by the average T-RF size values resulting from digestions with the restriction enzymes. Past software (Hammer; Harper, 2001) was used to calculate the similarity between fragment sizes, and then the diversity profile of bacteria and AMF was evaluated by non-metric multidimensional scaling (NMDS) based on the Bray-Curtis distance matrix. To test whether the sample groups had significantly different mean, the one-way similarity analysis test (ANOSIM) was used with a significance level of 95% ($p \leq 0.05$).

2.5.4 Bacterial community identification

The T-RFs were compared by aligning the observed fragment lengths with the predicted lengths resulting from three enzymes. This comparison was carried out using *Microbial Community Analysis* III - MiCA 3 (http://mica.ibest.uidaho.edu/). For

taxonomic classification, information was retrieved from NCBI using the Taxonomy Status tool (https://www.ncbi.nlm.nih.gov/Taxonomy/TaxIdentifier/tax_identifier.cgi).

2.6 Phosphatases activities

The methods employed for assessing phosphatase enzyme activities were detailed per Tabatabai (1994). The procedures encompass the extraction and quantitative determination of micrograms of p-nitrophenol released during soil incubation with either p-nitrophenyl phosphate or bis-p-nitrophenyl phosphate. This incubation occurs in a modified universal buffer adjusted to pH 6.5 and 11 for acid and alkaline phosphatase, respectively. The enzymatic reactions were halted by adding $CaCl_2$ and NaOH, and the resulting solutions were subjected to centrifugation at 8,000 rpm for 5 minutes. Subsequently, the supernatant was utilized for colorimetric measurements at 400 nm. Enzyme activities were assessed in triplicate rhizosphere soil samples.

2.7 Data statistical analysis

The analysis of variance (ANOVA) for root morphology traits, dry weight, P content, mycorrhizal colonization, gene expression, and soil enzymatic activity was conducted using the "ExpDes.pt" package (Ferreira et al. 2021) within the R software. The Tukey test was employed for statistical comparisons of means, with a significance level of 95% ($p \leq 0.05$). Principal Component Analysis (PCA) was conducted with the "factoextra" package (Kassambara; Mundt, 2020) in the R software.

For the relative abundance analysis of bacterial community phyla resulting from taxonomic identification via T-RFLP analysis, ggplot2 (Wickham, 2016) and reshape2 (Wickham, 2007) packages in the R software were utilized. ANOVA was conducted for the evaluated treatments using the "ExpDes.pt" package (Ferreira et al. 2021), and for statistical comparisons of means, the LSD test was employed with a significance level of 95% ($p \leq 0.05$).

3 Results

3.1 The concentration of 20 µMol L^{-1} sorgoleone promotes increased mycorrhization, P content and plant biomass

The analysis of variance for root morphology, plant biomass, P content, and AMF colonization revealed significant variability in the analyzed traits (Table 1). A significant enhancement in plant length, total surface area, plant biomass, and shoot, root, and total P content was observed in the presence of the fungus *R. clarus* and with 20 µMol L^{-1} sorgoleone (Table 1). This suggests that the addition of sorgoleone at this concentration led to a significant increase in the percentage of mycorrhization. However, no significant increase in mycorrhization was observed with the addition of other concentrations of sorgoleone (40 and 80 µMol L^{-1}) (Table 1).

Table 1. Analysis of Variance (ANOVA) for the means of morphophysiological and mycorrhization characteristics of the sorghum genotype P9401 cultivated with different concentrations of sorgoleone (SGL), in the presence (Myc+) and absence (Myc-) of the arbuscular mycorrhizal fungus (AMF) *Rhizophagus clarus*, under low phosphorus (P) conditions.

Traits	**AMF**	**SGL (µMol.L^{-1})**			
		0	**20**	**40**	**80**
L (cm)	Myc+	2472.57 ABa	3360.02Aa	2930.90 Aa	1355.48Ba
	Myc-	1769.02 Aa	2082.23Ab	2830.16 Aa	1683.63 Aa
SA (cm²)	Myc+	557.71 Aa	636.14 Aa	484.39 Aa	214.84 Ba
	Myc-	347.40 Ab	360.20 Ab	385.97 Aa	216.76 Aa
D (mm)	Myc+	0.370 Aba	0.376 ABa	0.311 Ba	0.509 Aa
	Myc-	0.313 Aa	0.272 Aa	0.388 Aa	0.301 Ab
SA1 (cm²)	Myc+	195.47 ABa	268.42 Aa	233.40 Aa	115.03 Ba
	Myc-	136.23 Aa	162.21 Ab	218.70 Aa	135.95 Aa
SA2 (cm²)	Myc+	108.96 ABa	129.58 Aa	96.15 ABa	56.19 Ba
	Myc-	68.685 Ab	74.89 Ab	70.73 Aa	38.50 Aa
SA3 (cm²)	Myc+	163.04 Aa	149.99 Aa	90.15 ABa	21.00 Ba
	Myc-	92.33 Ab	75.36 Ab	45.21 Aa	15.78 Aa
RDW (g)	Myc+	0.87 Aa	0.92 Aa	0.62 Aa	0.19 Aa
	Myc-	0.44 Aa	0.46 Aa	0.91 Aa	0.08 Aa

SDW (g)	Myc+	1.36 Aba	1.72 Aa	1.37 ABa	0.60 Ba
	Myc-	1.20 Aa	0.84 Ab	0.75 Ab	1.11 Aa
TDW (g)	Myc+	2.23 Aba	2.64 Aa	1.99 ABa	0.79 Ba
	Myc-	1.65 Aa	1.30 Ab	1.66 Aa	1.19 Aa
MYC (%)	Myc+	26.0 Ba	67.0 Aa	39.5 ABa	20.0 ABa
	Myc-	16.0 Ba	24.0 Bb	33.0 Aa	18.0ABa
RPCont (g)	Myc+	0.345 Aa	1.01 Aa	0.49 Aa	0.26 Aa
	Myc-	0.25 Aa	0.21 Ab	0.38 Aa	0.28 Aa
SPCont (g)	Myc+	0.30 Aa	1.05 Aa	0.31 Aa	0.51Aa
	Myc-	0.12 Aa	0.10 Ab	0.41 Aa	0.51 Aa
TPCont (g)	Myc+	0.65 Aa	2.06 Aa	0.80 Aa	0.319 Aa
	Myc-	0.37 Aa	0.31 Ab	0.795 Aa	0.318 Aa
Soil_PCont (g)	Myc+	13.50 Aa	11.27 Aa	13.45 Aa	8.60 Aa
	Myc-	12.65 Aa	11.15 Aa	15.95 Aa	10.50 Aa

*Means followed by the same capital letters indicate non-significant differences between sorgoleone concentrations, and identical lower-case letters indicate non-significant differences between the mycorrhization condition, by Tukey's test at 5% probability. Legend: total root length (L), total root surface (SA), average root diameter (D), surface area of roots with diameters between 0 and 1 (SA1), 1 and 2 (SA2) and 2 to 4, 5 mm (SA3), root, shoot and total dry weight (RDW, SDW and TDW), quantitative analysis of AMF colonization (MYC) and root, shoot, total and soil P content (RPCont, SPCont, TPCont and Soil_PCont).

In PCA analysis, the first and second principal components (PC1 and PC2) explained 62.9 and 23.5%, respectively, totaling 86.4% of the variation within treatments (Figure 1). The first principal component (PC1) had positive eigenvector coefficients for all variables (Table S2). The second principal component (PC2) was mainly composed of mycorrization (TableS1). PCA analysis was able to differentiate treatments based on the selected traits. Treatments with AMF were in the left quadrants, while those without AMF inoculation were on the right. The treatments with 20 and 40 µMol.L^{-1} of sorgoleone were in the upper quadrant and without sorgoleone in the lower quadrant. Considering the results (Table 1) and comparing them with their position on the scatterplot, we observed that 20 µMol.L^{-1} of sorgoleone presented higher mycorrhization, dry weight and total root surface than the other treatments (Figure 1).

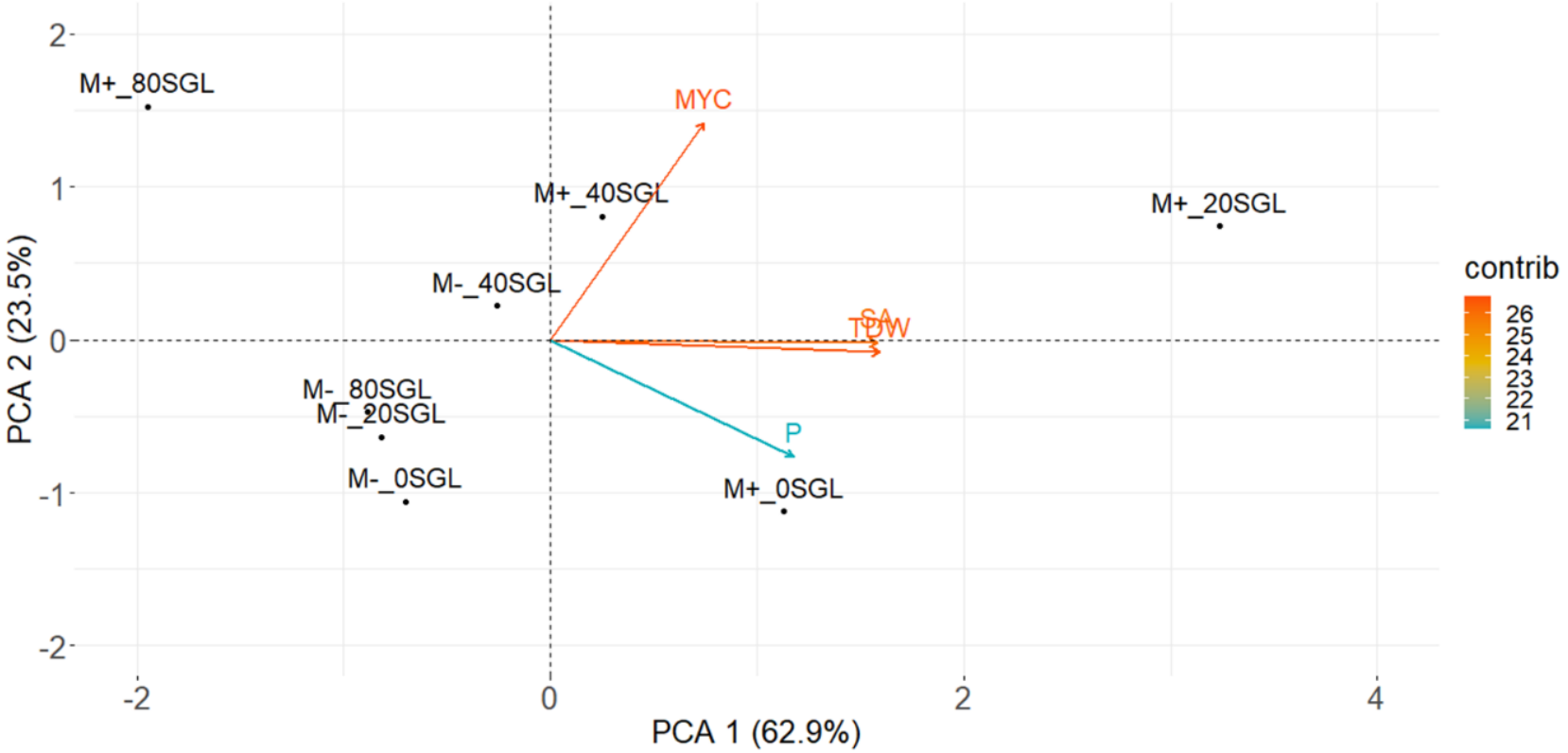

Figure 1. Principal component analysis (PCA) for sorghum plants grown in a greenhouse under low P with 0, 20, 40 and 80 µMol L^{-1} of sorgoleone, and with and without inoculation of *Rhizophagus clarus*.SA: total surface area; TDW: total dry weight; P: total P content; MYC: mycorrhization.

Table S2. Principal Component Analysis (PCA) for root, total dry weight, P content and mycorrhization traits. Eingenvectors, eigenvalues and the cumulative proportion of total variance (%) explained are shown for each principal component (PC).

Trait	PC1	PC2
SA (cm^2)	0.95	-0.010
TDW (g)	0.96	-0.047
MYC (%)	0.45	0.852
P (g)	0.71	-0.459
Eigenvalue	2.52	0.94
Cumulative Variance (%)	62.9	86.4

SA: total surface area; TDW: total dry weight; MYC: mycorrhization; P: total P content.

We selected 0 and 20 µMol L^{-1} sorgoleone treatments for further examination based on the morphophysiological analysis. The gene expression results revealed a notable upregulation in the *21G12* gene, associated with sorgoleone biosynthesis, along with *SbPT8*, *SbPT9*, *SbPT10*, and *SbPT11* genes, linked to phosphate transport induced by mycorrhiza (Figure 2). Conversely, there was no observed increase in expression for the *SbAM3* and *RiEF* genes, which served as markers for AMF colonization (Figure 2).

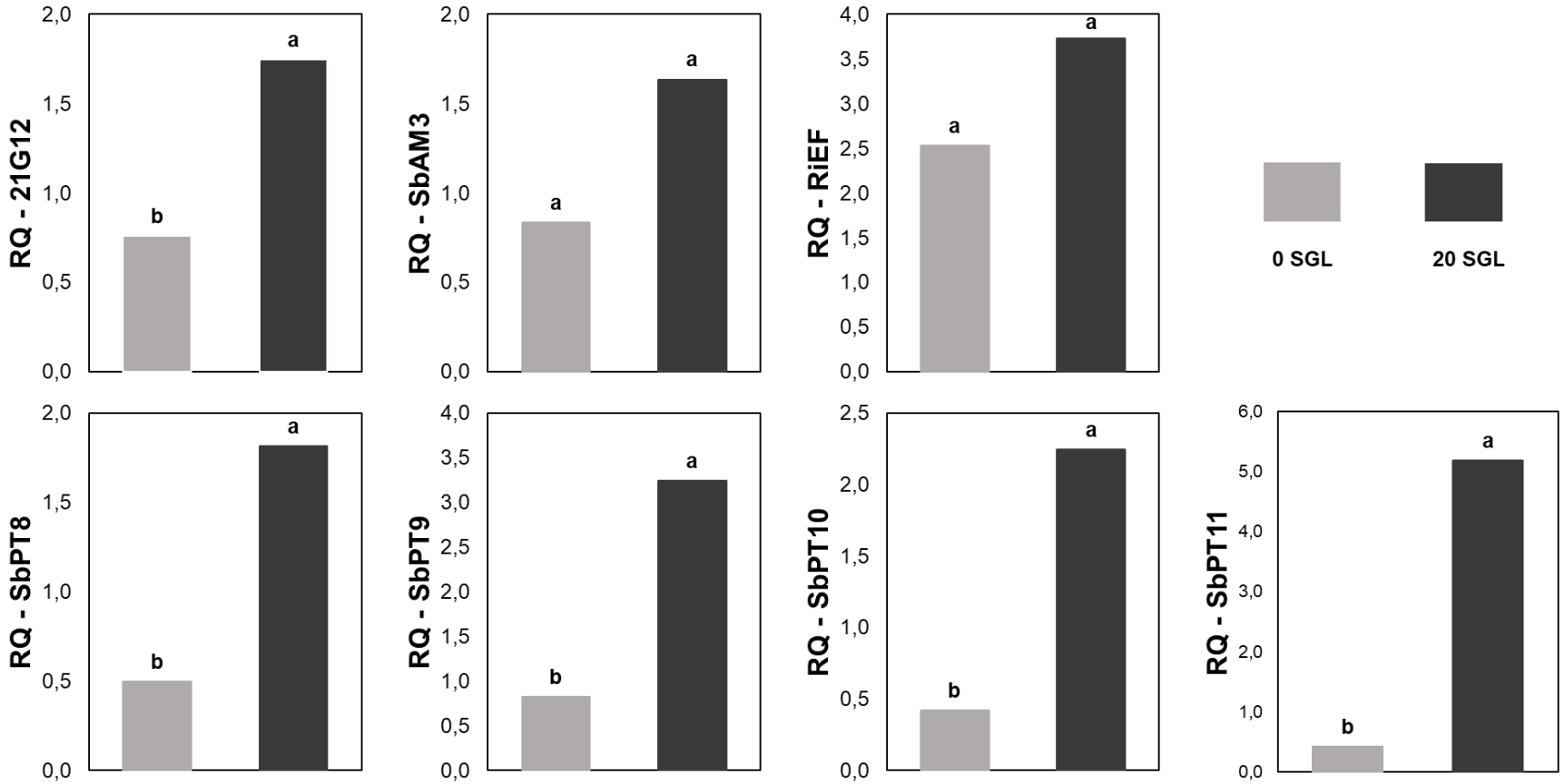

Figure 2. Expression of genes *21G12*, *SbAM3*, *RiEF*, *SbPT8*, *SbPT9*, *SbPT10* and *SbPT11* on sorghum plants grown in a greenhouse under low P with 0 and 20 µMol L^{-1} of sorgoleone, and with and without inoculation of *Rhizophagus clarus* after 45 days.

The non-metric multidimensional scaling (NMDS) analysis, employing the Bray-Curtis distance matrix and the ANOSIM test with a significance level of 95% ($p \leq 0.05$), was conducted to assess the genetic diversity profile of the microbial community in the rhizosphere of sorghum cultivated in a greenhouse under low P. The analysis indicated that the bacterial community remained unchanged with the introduction of sorgoleone (Figure 3A; $p=0.2252$). However, a significant difference ($p=0.0001$) was detected in the genetic diversity profile of the AMF community, indicating dissimilarity between treatments with and without *R.clarus* inoculation (Figure 3B). There was no significant increase in the enzymatic activity of acid and alkaline phosphatases. Moreover, no statistically significant distinction was observed in the relative abundance of bacterial phyla (Figure 3C). The most abundant phylum in the four treatments were *Bacillota, Pseudomonadota* and *Actinomycetota* (Figure 3C).

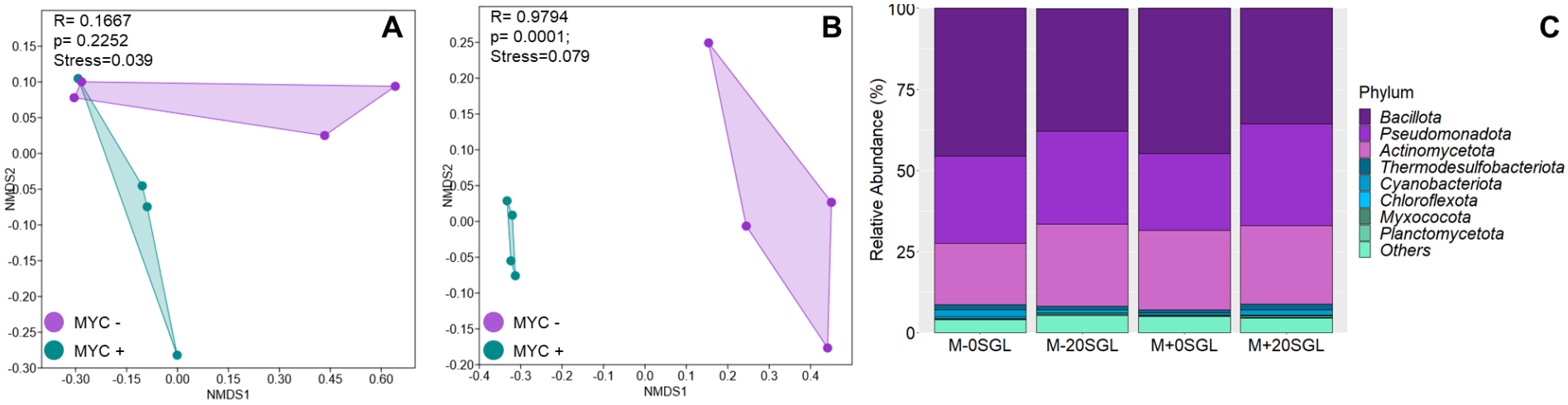

Figure 3. Profile based on non-metric multidimensional scaling (NMDS) using the Bray-Curtis distance matrix to analyze the genetic diversity of the bacterial community (A) and AMF (B), and relative abundance of the phyla of the bacterial community (C) from rhizosphere soil of sorghum grown in a greenhouse under low P with and without inoculation of *R. clarus* and with 20 µMol L^{-1} of sorgoleone.

4 Discussion

In a previous study by Oliveira et al. (2020), different concentrations of sorgoleone (5, 10, and 20 µMol L^{-1}) were tested in the sorghum genotype P9401. This genotype is known for its resistance to *Striga*, an obligate parasitic plant prevalent in regions of Africa and Asia, which commonly infests various crops, including sorghum (Hess et al. 1992; Rich et al. 2004; Gobena; Rich, 2017; Mohemed; Fradin, 2018). Additionally, P9401 exhibits low root exudation, resulting in minimal stimulation of *Striga* seed germination (Gobena; Rich, 2017; Mohemed; Fradin, 2018). In this study, we used higher sorgoleone concentrations and tested all sorgoleone concentrations with and without AMF. Our results confirmed that 20 µMol L^{-1} of sorgoleone has the potential to enhance mycorrhization, plant biomass, and P content in mycorrhizal plants compared to non-mycorrhizal plants cultivated under low P. These findings corroborate with and expand upon those observed by Oliveira et al. (2020), where a notable increase in mycorrhization percentage and plant biomass was observed in greenhouse-grown plants in the presence of *R. clarus* fungus and with the addition of 20 µMol L^{-1} sorgoleone under P deficiency. Moreover, in sorghum plants, inoculation with AMF has been documented to enhance plant growth, exhibiting significant increases in shoot height, root length (Geo et al. 2018), and dry weight (Nakmee et al. 2016). Furthermore, AMF inoculation contributes to greater nutrient absorption (Nasr et al. 2013; Sarr et al. 2021; Wang et al. 2021) and higher grain productivity (Watts-Williams et al. 2022).

The findings of this study also revealed an upregulation in the expression of a genes involved in the sorgoleone biosynthesis pathway (*21G12*) and phosphate transporters induced by AMF (*SbPT8*, *SbPT9*, *SbPT10*, and *SbPT11*). While the importance of root exudation for effective symbiosis between plants and AMF have been established across various plant species such as maize, soybean, and sorghum (Yoneyama et al. 2013; Yoneyama et al. 2015; Borghi et al. 2016; Kobae, 2018; Abdelhalim et al. 2019; Oliveira et al. 2020; Sarr et al. 2021; Wang et al. 2021; Ortas et al. 2022), this study reaffirms sorgoleone's role as a signaling mechanism for symbiosis with AMF and subsequent increase in P uptake.

It is crucial to acknowledge the increase in root surface area facilitated by hyphae, as observed in this study. The expanded volume and extension of soil

explored by fungal hyphae are instrumental in enhancing nutrient absorption, particularly P. Hyphae can extend beyond the nutrient-depleted zone that forms around absorbing roots. In this zone, there is a reduction in concentration, especially of P, which has a slower transport rate in the soil solution and limited availability compared to the plant's demand. While roots rapidly absorb phosphate ions in their proximity, the soil solution struggles to balance the concentration in this region due to slow transport and low P concentration. Thus, AMF plays a crucial role in P absorption by extending beyond the depletion zone and exploring a larger soil volume in their search for nutrients (De Novais et al. 2014).

Although sorghum varieties with higher sorgoleone exudation tend to develop a denser mycorrhizal network in rhizosphere soils (MacLean et al. 2017; Lanfranco et al. 2018; Sarr et al. 2021), mycorrhizal colonization appears to be suppressed with more than 20 μM L^{-1} sorgoleone. The plant roots incur an energy cost with the exudation of signaling compounds for spore germination and branching of fungal hyphae in the rhizosphere during pre-symbiotic recognition. Additionally, in plant-mycorrhizal symbiosis, both symbionts are capable of detecting variations in the resources provided by each other, allowing them to adjust their resource allocation to strike a balance between costs and benefits under varying P conditions (Johri et al. 2015; Wen et al. 2019). Therefore, it is conceivable that 20 μMol L^{-1} represents the optimal concentration for plant-AMF signaling, consistent with the sensitivity expected for a plant flag (Gomez-Roldan et al. 2008).

Moreover, a cost-benefit relationship exists associated with the diverse mechanisms employed by plants to transport bioactive compounds to the rhizosphere (Wen et al. 2019). An illustrative example of this relationship is the impact of soil P availability on mycorrhizal colonization (Kobae, 2018). Low soil P concentrations stimulate increased root exudation and subsequent mycorrhizal colonization. Conversely, high P concentrations suppress exudation, diminishing the level of plant-fungus signaling during symbiosis and impeding colonization (Chiu and Paszkowski, 2019). In essence, when soil P availability is ample and comparable to what a non-mycorrhizal plant can absorb, the fungus imposes an energetic cost on the plant without providing any additional nutritional benefit regarding P absorption. This scenario can lead to a depressive effect on plant development in the presence of AMF (Siqueira et al. 1998; De Novais et al. 2014).

The analogy also extends to the observation that no significant difference was noted in the activity of acid and alkaline phosphatases. These enzymes are linked to P remobilization in plants, and higher enzyme activity is typically associated with low cellular phosphate levels (Richardson et al. 2000; Baldwin et al. 2001; Bozzo et al. 2006). Since AMF promotes increased phosphate uptake, there is no incentive for plant roots to invest in secreting phosphatases. Hyphae prove to be much more efficient in creating a plant-soil contact surface for nutrient capture, with substantially lower carbon (energy) expenditure than roots. Consequently, it is more efficient for the plant to invest in mycorrhiza than in root production (De Novais et al. 2014).

Furthermore, in a greenhouse, no alteration was observed in the bacterial communities of rhizosphere soil with the addition of sorgoleone. However, studies indicate that sorgoleone does influence the dynamics of microbial community structure in rhizosphere soil of field-grown sorghum (Wang et al. 2021; Ortas et al. 2022), highlighting the necessity for further investigation. Notably, sorghum's performance in the field concerning its response to AMF colonization in terms of improved growth and/or P nutrition may be genotype-dependent, adding another factor to consider.

Despite the highly regulated and dynamic nature of mycorrhizal colonization, the factors influencing sorgoleone's mode of action, bioactive concentration, persistence, release in the rhizosphere, as well as its absorption and translocation, remain incompletely understood (Dayan et al. 2006; Tresseder, 2013; Pan et al. 2018; Pan et al. 2021; Wang et al. 2021). Further studies are warranted to explore the effects of bioactive molecules on plants, given that the root exudation of fungal signaling compounds is a fundamental aspect of the symbiotic response. This understanding not only sheds light on the regulation of sorgoleone production but also offers opportunities to manipulate crop levels to enhance agricultural productivity.

5 Conclusion

Consistent with previous observations, sorgoleone impacted mycorrhizal colonization. This study showed that an increase in mycorrhizal colonization, plant dry weight, and P content in sorghum was a response to added sorgoleone, which may have resulted from its direct effect, indirect effect, or both.

Furthemore, there is an upregulation in the expression of a genes involved in the sorgoleone biosynthesis pathway (*21G12*) and phosphate transporters induced by

AMF (*SbPT8*, *SbPT9*, *SbPT10*, and *SbPT11*), reaffirms sorgoleone's role as a signaling mechanism for symbiosis with AMF and subsequent increase in P uptake.

These findings provide additional insight into how altering these root exudation processes in plants may provide practical approaches for enhancing stress tolerance of the P and increasing agroecosystem productivity.

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The authors declare that they have no conflict of interest.

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CAPÍTULO 4

Efeito da inoculação de diferentes doses de inoculante contendo cepas bacterianas solubilizadoras de fosfato na produção, arquitetura radicular e microbiota da rizosfera do sorgo

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1 Introdução

Grande parte dos estudos sobre associação entre plantas e microrganismos tem se concentrado em entender os efeitos isolados dos microrganismos para o crescimento e desenvolvimento das plantas. Contudo, até que ponto os microrganimos isolados podem afetar a composição e a estrutura de outras comunidades ainda não está claro (Schlemper et al. 2018).

Há relatos na literatura que demonstram que a interação de bactérias promotoras de crescimento de plantas (BPCP) com fungos micorrízicos arbusculares (FMA) tem efeito sinérgico, favorecendo o uso de nutrientes e crescimento de plantas de batata (Vosátka; Gryndler, 1999), trigo (Sala et al. 2007) milho e soja (Moreira et al. 2020; Oliveira-Paiva et al. 2020). Porém, os efeitos que a inoculação bacteriana provoca na colonização micorrízica, bem como na microbiota da rizosfera das plantas, ainda foram pouco explorados.

O entendimento mais acurado das interações bacterianas e dos fatores que influenciam seu estabelecimento na comunidade microbiana da rizosfera, podem melhorar a eficiência das BPCP. Estudos demonstram que o uso de inoculantes contendo BPCP solubilizadoras de fosfato tem aumentado significativamente o fósforo disponível e a sua absorção pelas plantas (Oliveira-Paiva et al., 2020).

Recentemente, foi desenvolvido pela Embrapa Milho e Sorgo em parceria com a empresa Bioma um inoculante comercial solubilizador de fosfato, chamado BiomaPhos® (https://www.bioma.ind.br/product/bioma-phos). Este produto é formulado com cepas de *Bacillus subtilis* (CNPMS B2084) e *Priestia megaterium* (CNPMS B119), sendo a primeira tecnologia para a solubilização de fosfato do Brasil, que converte formas insolúveis de P em forma solúvel (Etesami; Maheshwari, 2018; Tabassum et al. 2017), deixando disponível para a absorção e a assimilação pela planta (Oliveira-Paiva et al. 2020; de Sousa et al. 2021).

As BPCP, como as que estão presentes no BiomaPhos, podem se associar com grande número de espécies de cereais e gramíneas forrageiras de importância comercial, tais como trigo, milho e braquiária, beneficiando o crescimento e o desenvolvimento das plantas (Hungria et al. 2010; Videira et al. 2012; de Sousa et al. 2021). Em milho, essa tecnologia é capaz de aumentar a absorção de P pelas plantas e consequentemente aumentar a produtividade em até 12% (Oliveira-Paiva et al. 2020; Paiva et al. 2020). No entanto, não há informação sobre seu efeito em sorgo. Baseado nisso, o objetivo desse trabalho foi avaliar o efeito de diferentes doses do inoculante bacteriano BiomaPhos e sua contribuição na colonização micorrízica, na produtividade e na microbiota da rizosfera de sorgo cultivado em campo.

2 Metodologia

2.1 Delineamento experimental

Os experimentos de campo foram conduzidos em duas safras, durante os períodos de 2021/2022 e 2022/2023, no campo experimental da Embrapa Milho e Sorgo (latitude 19° 47' Sul, longitude 44° 25' Oeste), localizada em Sete Lagoas, MG, com Latossolo Vermelho com textura argilosa.

O delineamento experimental foi estabelecido em blocos casualizados, com 4 repetições para cada um dos tratamentos, que consistiram em: T1: controle (sem adubação fosfatada e sem inoculação das sementes); T2: 50 % de adubação fosfatada e sem inoculação das sementes; T3: 100 % de adubação fosfatada e sem inoculação das sementes; T4 a T7: 50% de adubação fosfatada e inoculação nas doses de 80, 100, 120 e 200 mL $^{-1}$ do inoculante microbiano comercial BiomaPhos (Bioma). A inoculação ocorreu via semente de sorgo granífero, com adição de

Biomafix, na dose de 0,5 g para cada 10 mL de inoculante bacteriano, e adição de BiomaPhos para cada um dos tratamentos (80, 100, 120 e 200 mL ha^{-1}) realizada tendo como base de cálculo a recomendação de 10 mL do inoculante bacteriano para cada 1 kg de semente tratada. Ambos foram misturados em saco plástico, fechando a boca do saco e sacodindo bem para homogeneizar. Em seguida, o saco foi aberto e foi aguardada a secagem das sementes por cerca de 30 minutos ao ar livre. As sementes então foram plantadas, respeitando o prazo máximo de 24 h após a inoculação.

Os híbridos de sorgo granífero BRS373 foram semeados manualmente, com espaçamento entre linhas de 0,50 m e densidade de 12 plantas por metro linear. O genótipo BRS373 é um híbrido de sorgo granífero da Embrapa Milho e Sorgo desenvolvido especialmente para os plantios em sistemas de sucessão à soja. Temciclo superprecoce, baixa suscetibilidade a micotoxinas, tolerância ao alumínio tóxico no solo e ao estresse hídrico e alta produtividade na segunda safra (Embrapa Milho e Sorgo, 2016).

Na adubação da semeadura foi aplicada mecanicamente no sulco de plantio uma mistura de 400 kg ha^{-1} de 08-28-16 para o tratamento com 100% de P_2O_5 (T3), e uma mistura de 200 kg ha^{-1} de 08-28-16 para os tratamentos com 50% de P_2O_5 (T2, T4, T5, T6 e T7). As doses de fósforo (0%, 50% e 100% da recomendação para o local) foram aplicadas manualmente nos sulcos previamente construídos, utilizando superfosfato triplo como fertilizante. A adubação de cobertura foi realizada no estágio de desenvolvimento vegetativo de 5 a 6 folhas (30 - 35 dias após a semeadura), com aplicação na superfície do solo, nas entrelinhas do sorgo, de 250 kg de uréia 42%. O controle de plantas daninhas foi aplicado pós-emergência (30 dias) com o herbicida atrasina na dose de 4,0 L ha^{-1}. A irrigação do experimento foi feita alternando-se os dias.

As panículas foram coletas e realizada a trilhagem e pesagem dos grãos para o cálculo de produtividade. Para análise da concentração de P, os grãos foram triturados em moinho Wiley e submetidos à digestão com ácido nítrico perclórico no Laboratório de Análises Ambientais e Agrícolas (LABRAS, Monte Carmelo/MG, Brasil) (Malavolta et al. 1997).

2.2 Diversidade e composição de táxons microbianos na rizosfera

2.2.1 Extração de DNA do solo

A extração total de DNA de 0,45 g de amostras de solo rizosférico foi realizada utilizando o kit DNeasy PowerSoil Pro da Qiagen (EUA), seguindo as orientações do fabricante. O DNA extraído foi subsequentemente ressuspendido em 50 µL de tampão Solução C6. A quantificação do DNA foi realizada utilizando espectrofotômetro Nanodrop® (Thermo Fisher Scientific, EUA), e a concentração foi ajustada para 5,0 ng μL^{-1}.

2.2.2 Amplificação do gene 16S e 28S rRNA

Fragmentos do gene *16S* rRNA foram amplificados usando o primer 8F-FAM, que foi marcado com fluorescência na extremidade 5' (5'-AGAGTTGATCCTGGCTCAG-3') (Liu et al. 1997; LaMontagne et al. 2001), juntamente com o primer 1492R (5'-TACGGTACCTTGTACGACTT-3') (Turner et al. 1999). A reação de PCR consistiu em 15 ng de DNA, cada primer na concentração de 5,0 ng μL^{-1}, tampão de reação a 1X, $MgCl_2$ a 2,5 mM, dNTPs a 2,5 mM cada e 2,5 U de Taq DNA polimerase (Promega GoTaq® G2 Flexi DNA Polymerase), em uma reação com volume final de 50 µL O protocolo de amplificação para o gene 16S rRNA envolveu uma etapa inicial de desnaturação a 95 °C por 2 minutos, seguida de 30 ciclos a 95°C por 1 minuto, 58 °C por 1 minuto e 72 °C por 1 minuto. A reação foi concluída com uma extensão final a 72°C por 5 minutos.

Para a amplificação de fragmentos do gene *28S* rRNA de fungos micorrízicos arbusculares (FMA), foi empregada uma abordagem nested-PCR. Na reação inicial foram utilizados os oligonucleotídeos LR1 (5'-GCATATCAATAAGCGGAGGA-3') (Van Tuinen et al. 1998; Trouvelot et al. 1999) e FLR2 (5'-GTCGTTTAAAGCCATTACGTC-3') (Trouvelot et al. 1999). A reação consistiu em 15 ng de DNA, cada oligonucleotídeo na concentração de 5,0 ng µL-1, tampão de reação a 1X, $MgCl_2$ a 2,5 mM, dNTPs a 2,5 mM cada e 2,5 U de Taq DNA polimerase (Promega GoTaq® G2 Flexi DNA Polymerase), em uma reação com volume final de 50 µL Para a segunda PCR foram utilizados 15 ng do produto da primeira reação, e os oligonucleotídeos FLR3 (5'-[6FAM] TTGAAAGGGAAACGATT-3') (Gollotte et al, 2004) e FLR4 (5'[HEX]TACGTCAACATCCTTAACGAA-3') (Gollotte et al, 2004) na concentração de 5,0 ng µL-1. A reação também incluiu tampão de reação 1X, $MgCl_2$ a 2,5 mM, dNTPs

a 2,5 mM e 2,5 U de Taq DNA polimerase (Promega GoTaq® G2 Flexi DNA Polymerase), em um volume final de 50 µL. As amplificações para FMA foram realizadas com desnaturação inicial a 95 °C por 2 minutos, seguida de 30 ciclos a 95 °C por 1 minuto, 58 °C por 1 minuto, 72 °C por 1 minuto e extensão final a 72 °C por 5 minutos.

Posteriormente, 1 µL dos produtos de PCR foram corados com GelRed (Biotium, Hayward, Califórnia, EUA) e analisados por eletroforese em gel de agarose a 1% (p/v), usando 1Kb Plus DNA Ladder (Life Technologies, EUA). O DNA amplificado foi confirmado em transiluminador sob luz ultravioleta e fotografado no equipamento L-PIX Image EX (Loccus Biotecnologia, Brasil).

2.2.3 Análise T-RFLP

Os fragmentos amplificados foram digeridos com as enzimas de restrição *Alu*I *Hae*III e *Hpa*II (Invitrogen, EUA). Para a digestão foram utilizados 10 µL do produto de PCR, 2 µL do tampão da enzima 10 X e 1 µL da enzima 10 U μL^{-1}, incubados a 37 °C por 3 horas.

Para avaliar os fragmentos de DNA, 2 µL do produto de digestão foram combinados com 9,8 µL de formamida deionizada (Applied Biosystems, EUA) e 0,2 µL do padrão ROX500 (Applied Biosystems). Os produtos digeridos por PCR foram avaliados por meio de eletroforese capilar utilizando o Genetic Analyzer 3500XL (Applied Biosystems, EUA) com software GeneMapper 5.0 (Applied Biosystems, EUA). Os picos do Fragmento de Restrição Terminal (T-RF) com tamanho entre 30 a 500 pb e com intensidade de fluorescência superior a 40 unidades (altura do pico), foram considerados para análise de perfil de diversidade genética das comunidades bacteriana e de FMA da rizosfera de sorgo.

O software Past (Hammer; Harper, 2001) foi utilizado para calcular a similaridade entre os tamanhos dos fragmentos, e então o perfil de diversidade de bactérias e FMA foi avaliado por escala multidimensional não métrica (NMDS) baseada na matriz de distância de Bray-Curtis. Para testar se os grupos de amostras possuíam diferenças médias significativamente diferentes, foi utilizado o teste de análise de similaridade *one-way* (ANOSIM) com nível de significância de 95% ($p \leq 0,05$).

2.2.4 Identificação taxonômica da comunidade bacteriana

A identificação taxonômica dos fragmentos obtidos pela análise de T-RFLP foi realizada a partir da ferramenta online Microbial Community Analysis – MiCA III (http://mica.ibest.uidaho. edu/pat.php). A classificação taxonômica foi realizada no National Center for Biotechnology Information (NCBI) usando a ferramenta online Taxonomy disponível em http://www.ncbi.nlm.nih.gov/Taxonomy/TaxIdentifier/tax_identifier.cgi.

2.3 Análise da arquitetura do sistema radicular

O sistema radicular de três plantas de sorgo de cada tratamento foi coletado ao acaso nas fileiras mais externas do experimento em campo, conforme descrito pela metodologia Shovelomics (Trachsel et al. 2011). As raízes foram lavadas e a parte mais robusta dos sistemas radiculares foi fotografada segundo Bucksch et al. (2014) e as imagens obtidas foram introduzidas na plataforma online *Digital Imaging of Root Traits* (DIRT) 5.2 (Das et al. 2015) modificada, que analisa as raízes e realiza estimativas de características da arquitetura radicular (Campolino et al., 2023).

2.4 Análise da atividade enzimática

Os métodos empregados para avaliação da atividade das enzimas fosfatase ácida e alcalina foram detalhados por Tabatabai (1994). Os procedimentos abrangem a extração e a determinação quantitativa de microgramas de p-nitrofenol liberados durante a incubação do solo com p-nitrofenil fosfato ou bis-p-nitrofenil fosfato. Esta incubação ocorre num tampão universal modificado ajustado a pH 6,5 e 11 para fosfatase ácida e alcalina, respectivamente. As reações enzimáticas foram interrompidas pela adição de $CaCl_2$ e NaOH, e as soluções resultantes foram submetidas à centrifugação a 8.000 rpm por 5 minutos. Posteriormente, o sobrenadante foi utilizado para medições colorimétricas. As atividades enzimáticas foram avaliadas em amostras de solo da rizosfera em triplicata.

2.5 Avaliação qualitativa e quantitativa da colonização micorrízica

Uma pequena porção (~1 g) das raízes finas frescas das plantas de sorgo foi colocada em tubos cônicos preenchidos com etanol 70% e armazenada em geladeira a 4 ºC por 3 dias. As raízes restantes foram secas para determinar o peso seco das raízes e o teor de P. Posteriormente, as raízes foram lavadas com água deionizada e clarificadas em solução de hidróxido de potássio (KOH 10%) durante a noite em temperatura ambiente. No dia seguinte, as raízes foram lavadas e imersas em ácido clorídrico (HCl 0,3 Mol L^{-1}) por 30 minutos em temperatura ambiente. Após o tratamento ácido, o HCl foi removido e as raízes foram coradas com azul de metileno por 30 minutos em temperatura ambiente. As raízes coradas foram então transferidas para tubos cônicos contendo solução acidificada de glicerol (1:1 glicerol e 0,3 Mol L^{-1} HCl). A colonização micorrízica foi quantificada usando o método de intersecção de linhas de grade conforme descrito por Paszkowski et al. (2006), com modificações. A colonização total foi determinada avaliando a proporção de interseções apresentando estruturas fúngicas específicas, como vesículas ou arbúsculos. Esta avaliação foi realizada sob um estereoscópio Axio Zoom V16 (Zeiss) com ampliação de 20 vezes, cobrindo 100 pontos de intersecção por amostra de raiz.

Os resultados da colonização micorrízica foram submetidos à análise de variância (ANOVA) e as médias dos tratamentos foram comparadas pelo teste de Tukey ($p \leq 0,05$). A análise estatística foi realizada por meio do software Sisvar. Para facilitar a análise estatística, as porcentagens de colonização micorrízica foram normalizadas através de uma transformação arco-seno-raiz quadrada antes de serem submetidas aos procedimentos estatísticos.

2.6 Análise estatística dos dados

A análise de variância (ANOVA) acerca das características atividade enzimática, conteúdo de P, colonização micorrízica e produtividade foi realizada utilizando o pacote "ExpDes.pt" (Ferreira et al. 2021) no software R. Para as comparações estatísticas das médias foi utilizado o teste LSD para produtividade e o teste Tukey para as demais características, com nível de significância de 90% ($p \leq 0,10$).

A produtividade de grãos foi estimada com base no peso de grãos da parcela, e o percentual de aumento da produtividade foi calculado subtraindo a produtividade do tratamento controle dos demais tratamentos, dividido pela produtividade do tratamento controle.

Para as características do sistema radicular de sorgo estimadas pela metodologia Shovelomics/DIRT, foi inicialmente realizada uma ANOVA pelo teste de Tukey com nível de significância de 95% ($p \leq 0,05$), pelo programa estatístico Sisvar. Em seguida, foi realizada uma filtragem dos dados e seleção de características radiculares que apresentaram um coeficiente de variação (CV) inferior ou igual a 20% (≤ 20%), para maior confiabilidade dos dados.

A análise de abundância relativa dos filos da comunidade bacteriana, resultante da identificação taxonômica via análise T-RFLP, foi realizada utilizando os pacotes ggplot2 (Wickham, 2016) e reshape2 (Wickham, 2007), no software R. Os índices de diversidade de Simpson, Shannon e Chao foram calculados no software Past (Hammer; Harper 2001). Foi realizada ANOVA para os tratamentos avaliados, utilizando o pacote “ExpDes.pt” (Ferreira et al. 2021), e para as comparações estatísticas das médias foi usado o teste LSD com nível de significância de 90% ($p \leq 0,10$).

Foi realizada uma Análise de Componentes Principais (PCA) para as características que apresentaram diferença estatística significativa entre os tratamentos, sendo elas: conteúdo de P do solo (P_Solo), colonização micorrízica (MYC), produtividade (PROD), atividade enzimática da fosfatase ácida (F_Ácida) e densidade de raízes laterais (LT_MED_DIA), que é uma característica de arquitetura radicular de sorgo avaliada pela metodologia Shovelomics/DIRT, com o pacote “factoextra” (Kassambara; Mundt, 2020) no software R.

3 Resultados

3.1 Resposta da inoculação de diferentes doses de BiomaPhos e de fosfato na atividade enzimática, teor de fósforo, colonização micorrízica e produtividade de sorgo cultivado em campo

Para a primeira safra (2021/2022), houve um aumento significativo da atividade da fosfatase ácida apenas comparando o tratamento com 100% de P_2O_5 sem

inoculação de BiomaPhos (tratamento 3) com o tratamento com 50% de P_2O_5 e 200 mL ha^{-1} de BiomaPhos (tratamento 7). Para os demais tratamentos e para a fosfatase alcalina não houve diferença. Quanto ao fósforo, para os grãos não foram observadas diferenças significativas para a concentração de P. Já para o teor de P no solo, o tratamento com 100% de P_2O_5 sem inoculação de BiomaPhos (tratamento 3) e o tratamento com 50% de P_2O_5 e 100 mL ha^{-1} de BiomaPhos (tratamento 5) foram significativamente diferentes do tratamento sem adição de P e sem inoculação de BiomaPhos (tratamento 1 - controle). Com relação a micorrização, houve aumento significativo no tratamento contendo 50% de P_2O_5 e 200 mL ha^{-1} do inoculante microbiano (tratamento 7), quando comparado aos demais tratamentos (Tabela 1)

Além disso, comparando os tratamentos sem inoculação de BiomaPhos, a produtividade foi maior no tratamento com 100% de P_2O_5 (tratamento 3), do que no tratamento com 50% de P_2O_5 (tratamento 2). Entretanto, a inoculação de BiomaPhos nas doses de 100 e 120 mL ha^{-1} com 50% de adubação fosfatada (tratamentos 5 e 6, respectivamente) mostrou a mesma significância estatística do tratamento sem inoculação de BiomaPhos com 100% de adubação fosfatada (tratamento 3). Isto é, a inoculação de BiomaPhos nas doses de 100 e 120 mL ha^{-1} permitiu alcançar a mesma produtividade do tratamento com 100% de P_2O_5, porém utilizando metade da adição de fertilizante fosfatado. O tratamento com a maior dose de BiomaPhos inoculada (tratamento 7) não refletiu em maior produtividade (Tabela 1).

Já para a segunda safra (2022/2023), houve novamente um aumento significativo da atividade da fosfatase ácida apenas comparando o tratamento com 100% de P_2O_5 sem inoculação de BiomaPhos (tratamento 3) ao tratamento com 50% de P_2O_5 e 200 mL ha^{-1} de BiomaPhos (tratamento 7). Para os demais tratamentos e para a fosfatase alcalina não houve diferença. Também não foram observadas diferenças significativas para o teor de P no solo e concentração de P nos grãos, bem como para a micorrização entre os tratamentos avaliados (Tabela 1).

Com relação a produtividade, diferente do que ocorreu na primeira safra, não houve diferença estatística significativa quando comparados os tratamentos sem inoculação de BiomaPhos e com 50% e 100% de P_2O_5 (tratamentos 2 e 3, respectivamente). Além disso, para os tratamentos com inoculação de BiomaPhos nas doses de 80, 100 e 120 mL ha^{-1} e 50% de adubação fosfatada (tratamentos 4, 5 e 6, respectivamente), a produtividade foi significativamente maior que no tratamento sem inoculação de BiomaPhos e 100% de adubação fosfatata (tratamento 3). Nesse caso

a inoculação de BiomaPhos nas doses de 80, 100 e 120 mL ha^{-1} superou a produtividade do tratamento com 100% de P_2O_5, utilizando metade da adição de fertilizante fosfatado. Novamente o tratamento com a maior dose de BiomaPhos inoculada (tratamento 7) não refletiu em maior produtividade, tal como na primeira safra (Tabela 1).

Ainda com relação ao incremento de produtividade, foram comparados os tratamentos que receberam adubação fosfatada com 50% de P_2O_5, e diferentes inoculações de BiomaPhos (tratamentos 2, 4, 5, 6 e 7) para as duas safras (Tabela 2). Comparando o tratamento 2, onde não houve inoculação de BiomaPhos, com os tratamentos 4, 5, 6 e 7, onde houve adição de 80, 100, 120 e 200 mL ha^{-1} do inoculante microbiano, respectivamente, é possível observar respostas semelhantes para as duas safras. Na primeira safra houve aumento significativo da produtividade com a adição de 100 mL ha^{-1} e 120 mL ha^{-1} de BiomaPhos, com incremento de 20%, quando comparado ao tratamento 2 (Tabela 2). E na segunda safra, a adição de 100 mL ha^{-1} de BiomaPhos, promoveu aumento significativo da produtividade, com incremento de 40%, quando comparado ao tratamento 2 (Tabela 2).

Tabela 1. ANOVA para produtividade, teor de P no solo e concentração de P nos grãos, micorrização e atividade enzimática do genótipo de sorgo BRS373 inoculado com diferentes doses de BiomaPhos (0, 80, 100, 120 e 200 mL ha^{-1}) e doses de fertilização fosfatada (0, 50 e 100% da adubação recomendada) no campo experimental da Embrapa Milho e Sorgo nas safras 2021/2022 e 2022/2023.

Tratamento	PROD		P_Grãos		MYC		P_Solo		F_Ácida		F_Alcalina	
	kg ha^{-1}		g kg^{-1}		(%)		g kg^{-1}		($\mu g\ g^{-1}$)		($\mu g\ g^{-1}$)	
	2021/2022	**2022/2023**	**2021/2022**	**2022/2023**	**2021/2022**	**2022/2023**	**2021/2022**	**2022/2023**	**2021/2022**	**2022/2023**	**2021/2022**	**2022/2023**
1- 0 BiomaPhos e 0% P_2O_5	2575,00 d	1766,67 c	1,47 a	2,03 a	18 c	21 a	2,83 c	49,23 a	559,36 ab	1078,37 ab	268,18 a	660,84 a
2- 0 BiomaPhos e 50% P_2O_5	6341,67 b	4675,00 ab	1,60 a	2,40 a	34 b	29 a	15,37 abc	41,33 a	647,20 ab	1109,13 ab	318,37 a	530,30 a
3- 0 BiomaPhos e 100% P_2O_5	7900,00 a	3575,00 bc	1,63 a	2,40 a	39 ab	28 a	31,13 a	48,03 a	720,19 a	963,56 ab	283,34 a	546,04 a
4- 80 BiomaPhos e 50% P_2O_5	6658,33 b	6075,00 a	1,73 a	2,43 a	38 ab	28 a	903 bc	29,50 a	569,93 ab	987,17 ab	282,87 a	495,61 a
5- 100 BiomaPhos e 50% P_2O_5	7633,33 ab	6600,00 a	1,80 a	2,56 a	35 ab	38 a	24,37 ab	39,57 a	539,21 ab	1355,19 a	223,65 a	458,77 a
6- 120 BiomaPhos e 50% P_2O_5	7637,50 ab	5650,00 a	1,80 a	2,40 a	36 ab	41 a	14,43 bc	45,77 a	494,96 ab	924,26 ab	168,23 a	583,52 a
7- 200 BiomaPhos e 50% P_2O_5	4716,67 c	4575,00 ab	1,87 a	2,13 a	50 a	37 a	6,53 c	37,87 a	463,26 b	769,68 b	206,89 a	444,47 a
CV (%)	7,82	12,89	16,24	10,76	8,73	16,72	44,53	29,99	21,31	20,89	35,23	28,58

*Médias seguidas por letras iguais não diferem para teste de Tukey a 10% de probabilidade. Produtividade de grãos (PROD); concentração de P dos grãos (P_Grãos); micorrização (MYC); teor de P do solo (P_Solo) e atividade enzimática de fosfatase ácida (F_Ácida) e fosfatase alcalina (F_Alcalina).

Tabela 2. Produtividade (kg ha^{-1}) e percentual de aumento da produtividade (%) de sorgo cultivado em campo sob diferentes doses de BiomaPhos (0, 80, 100, 120 e 200 mL ha^{-1}) e adubação fosfatada com 50% de P_20_5, nas safras 2021/2022 e 2022/2023.

Tratamento	PROD (kg ha^{-1})		Incremento da produtividade (%)	
	2021/2022	**2022/2023**	**2021/2022**	**2022/2023**
2- 0 BiomaPhos e 50% P_2O_5	6341,7 b	4675,0 bc	--	--
4- 80 BiomaPhos e 50% P_2O_5	6658,3 ab	6075,0 ab	5	29
5- 100 BiomaPhos e 50% P_2O_5	7633,3 a	6600,0 a	20	40
6- 120 BiomaPhos e 50% P_2O_5	7637,5 a	5650,0 abc	20	21
7- 200 BiomaPhos e 50% P_2O_5	4716,7	4575,0 c	<0	<0
CV (%)	7,67	12,89	--	--

*Médias seguidas por letras iguais não diferem para teste de Tukey a 10% de probabilidade. PROD: produtividade de grãos.

3.2 Avaliação da arquitetura radicular de sorgo pela metodologia Shovelomics/DIRT

Para a primeira safra, houve aumento significativo do diâmetro médio da ponta da raiz (TD_MED) e da densidade de raízes laterais (LT_MED_DIA) para o tratamento com 120 mL de BiomaPhos e 50% de adubação fosfatada (tratamento 6), em comparação com o tratamento sem adição de BiomaPhos e 50% de adubação fosfatada (tratamento 2) (Tabela 3; Figura 1). Na segunda safra, não houve diferença estatística significativa entre nenhuma das características avaliadas (Tabela 4).

Tabela 3. Características estimadas pela metodologia Shovelomics/DIRT para o genótipo de sorgo BRS373 inoculado com diferentes doses de BiomaPhos (0, 80, 100, 120 e 200 mL ha^{-1}) e doses de fertilização fosfatada (0, 50 e 100% da adubação recomendada) no campo experimental da Embrapa Milho e Sorgo na safra 2021/2022.

Tratamento	AREA	TD_AVG	WIDTH_ MED	WIDTH_ MAX	D10	SKL_WIDHT	ANG_TOP	AVG_DENSIT	TD_MED	LT_MED_DIA	LT_AVG_DIA
	(cm^2)	(cm)	(cm)	(cm)	(cm)	(cm)	(º)	(g cm^{-3})	(cm)	(mm)	(mm)
0 BiomaPhos e 0 P	3472,57 a	0,3547 a	51,62 a	145,51 a	0,28 a	173,63 ab	34,88 a	1,92 ab	0,3398 b	0,3398 b	0,3490 ab
0 BiomaPhos e 50 P	3284,46 a	0,3541 a	60,19 a	119,62 a	0,30 a	145,73 ab	34,25 a	2,27 a	0,3400 b	0,3400 b	0,3456 b
0 BiomaPhos e 100 P	3930,68 a	0,3649 a	67,59 a	157,25 a	0,25 a	181,16 a	50,39 a	1,37 b	0,3399 b	0,3399 b	0,3618 ab
80 BiomaPhos e 50 P	3976,07 a	0,3549 a	55,12 a	128,46 a	0,22 a	153,39 ab	46,92 a	1,34 b	0,3400 b	0,3400 b	0,3537 ab
100 BiomaPhos e 50 P	3259,26 a	0,3542 a	60,91 a	134,24 a	0,23 a	154,74 ab	53,00 a	1,31 b	0,3403 ab	0,3403 ab	0,3470 b
120 BiomaPhos e 50 P	3438,06 a	0,3658 a	75,54 a	121,20 a	0,18 a	134,13 b	62,08 a	1,15 b	0,3417 a	0,3417 a	0,3670 a
200 BiomaPhos e 50 P	3964,49 a	0,3604 a	61,74 a	132,33 a	0,25 a	159,22 ab	39,25 a	1,62 ab	0,3405 ab	0,3405 ab	0,3475 b
CV (%)	25,99	1,44	16,24	11,52	27,59	10,43	29,23	20,66	0,18	0,18	2,08

*Médias seguidas por letras iguais não diferem para teste de Tukey a 10% de probabilidade. Legenda: área projetada da raíz (AREA), diâmetro da ponta da raiz principal (TD_AVG), largura média do sistema radicular (WIDTH_MED), largura máxima do sistema radicular (WIDTH_MAX), percentual de largura acumulada a 10% de profundidade (D10), largura calculada do eixo medial do sistema radicular (SKL_WIDTH), ângulo do topo da raiz (ANG_TOP), densidade média da raiz (AVG_DENSITY), diâmetro médio da ponta da raiz (TD_MED) e densidade de raízes laterais (LT_MED_DIA).

Tabela 4. Características estimadas pela metodologia Shovelomics/DIRT para o genótipo de sorgo BRS373 inoculado com diferentes doses de BiomaPhos (0, 80, 100, 120 e 200 mL ha^{-1}) e doses de fertilização fosfatada (0, 50 e 100% da adubação recomendada) no campo experimental da Embrapa Milho e Sorgo na safra 2022/2023.

Tratamento	AREA	TD_AVG	WIDTH_ MED	WIDTH_ MAX	D10	SKL_WIDHT	ANG_TOP	AVG_DENSIT	TD_MED	LT_MED_DIA	LT_AVG_DIA
	(cm^2)	(cm)	(cm)	(cm)	(cm)	(cm)	(º)	(g cm^{-3})	(cm)	(mm)	(mm)
0 BiomaPhos e 0 P	3094,858 a	0,2398 a	51,57 a	121,74 a	0,245 a	138,11 a	45,30 a	1,70 a	0,22 a	0,22 a	0,24 a
0 BiomaPhos e 50 P	4429,641 a	0,2709 a	70,13 a	139,41 a	0,21 a	166,56 a	52,77 a	1,53 a	0,25 a	0,25 a	0,27 a
0 BiomaPhos e 100 P	4760,585 a	0,2769 a	75,07 a	161,15 a	0,225 a	183,92 a	52,90 a	1,54 a	0,25 a	0,25 a	0,27 a
80 BiomaPhos e 50 P	4368,908 a	0,2751 a	64,87 a	146,97 a	0,22 a	162,70 a	52,54 a	1,75 a	0,25 a	0,26 a	0,28 a
100 BiomaPhos e 50 P	3585,672 a	0,2732 a	59,935 a	124,12 a	0,21 a	150,65 a	53,40 a	1,46 a	0,25 a	0,28 a	0,30 a
120 BiomaPhos e 50 P	3112,238 a	0,2637 a	58,84 a	116,14 a	0,22 a	131,72 a	50,58 a	1,52 a	0,24 a	0,25 a	0,28 a
200 BiomaPhos e 50 P	4074,225 a	0,2763 a	71,63 a	154,34 a	0,20 a	175,60 a	55,27 a	1,33 a	0,25 a	0,25 a	0,27 a
CV (%)	21,37	10,17	20,62	19,19	12,29	20,19	13,35	17,64	10,12	14,13	14,33

*Médias seguidas por letras iguais não diferem para teste de Tukey a 10% de probabilidade. Legenda: área projetada da raíz (AREA), diâmetro da ponta da raiz principal (TD_AVG), largura média do sistema radicular (WIDTH_MED), largura máxima do sistema radicular (WIDTH_MAX), percentual de largura acumulada a 10% de profundidade (D10), largura

calculada do eixo medial do sistema radicular (SKL_WIDTH), ângulo do topo da raiz (ANG_TOP), densidade média da raiz (AVG_DENSITY), diâmetro médio da ponta da raiz (TD_MED) e densidade de raízes laterais (LT_MED_DIA).

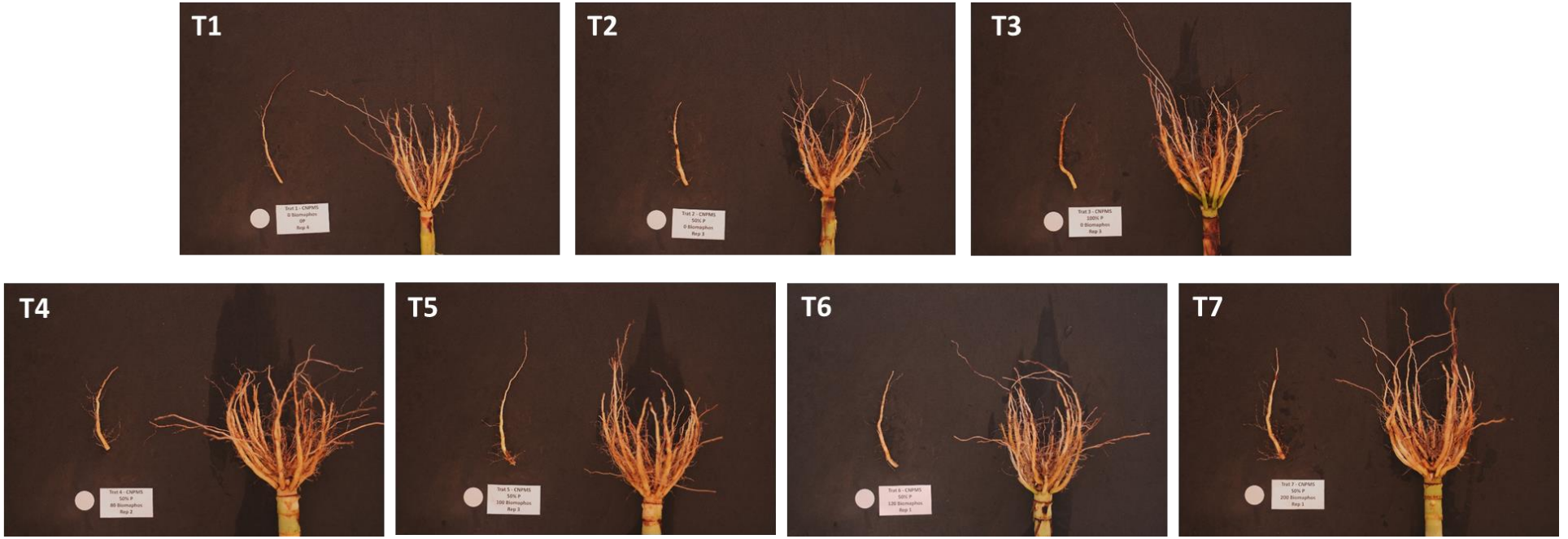

Figura 1. Raízes do genótipo de sorgo BRS373 inoculado com diferentes doses de BiomaPhos (0, 80, 100, 120 e 200 mL ha^{-1}) e doses de fertilização fosfatada (0, 50 e 100% da adubação recomendada) no campo experimental da Embrapa Milho e Sorgo coletadas durante o período de florescimento na safra 2021-2022. T1 - Controle (sem adubação fosfatada e sem inoculação das sementes); T2 - 50 % de adubação fosfatada e sem inoculação das sementes; T3 - 100 % de adubação fosfatada e sem inoculação das sementes; T4 - 50 % de adubação fosfatada e inoculação das sementes com o inoculante BiomaPhos na dose de 80 mL para 200.000 sementes ha^{-1}; T5 - 50 % de adubação fosfatada e inoculação das sementes com o inoculante BiomaPhos na dose de 100 mL para 200.000 sementes ha^{-1}; T6 - 50 % de adubação fosfatada e inoculação das sementes com o inoculante BiomaPhos na dose de 120 mL para 200.000 sementes ha^{-1}; T7- 50 % de adubação fosfatada e inoculação das sementes com o inoculante BiomaPhos na dose de 200 mL para 200.000 sementes ha^{-1}.

3.3 Impacto da comunidade microbiana da rizosfera de sorgo cultivado em campo sob diferentes doses de BiomaPhos e de fosfato

Na primeira safra, comparando os tratamentos inoculados com as doses 80, 100, 120 e 200 mL ha^{-1} de BiomaPhos (tratamentos 4, 5, 6 e 7, respectivamente) com o tratamento sem adição de BiomaPhos (tratamento 2), todos com e 50% de adubação fosfatada, o teste ANOSIM indicou que houve diferença significativa entre a comunidade bacteriana das amostras com e sem BiomaPhos (R=0,2754; p= 0,0019) (Figura 2A). Analisando apenas os tratamentos com 0%, 50% e 100% de adubação fosfatada e sem adição de BiomaPhos (tratamentos 1, 2 e 3, respectivamente), o teste indicou que houve diferença significativa na diversidade bacteriana das amostras para a diferentes doses de P_20_5 adicionadas (R=0,7708; p=0,0006) (Figura 2B).

Além disso, não houve diferença significativa na diversidade de FMA, tanto para os tratamentos inoculados com as diferentes doses de BiomaPhos e 50% de adubação fosfatada quanto para os tratamentos com as diferentes doses de P_2O_5 sem adição de BiomaPhos (p>0,05).

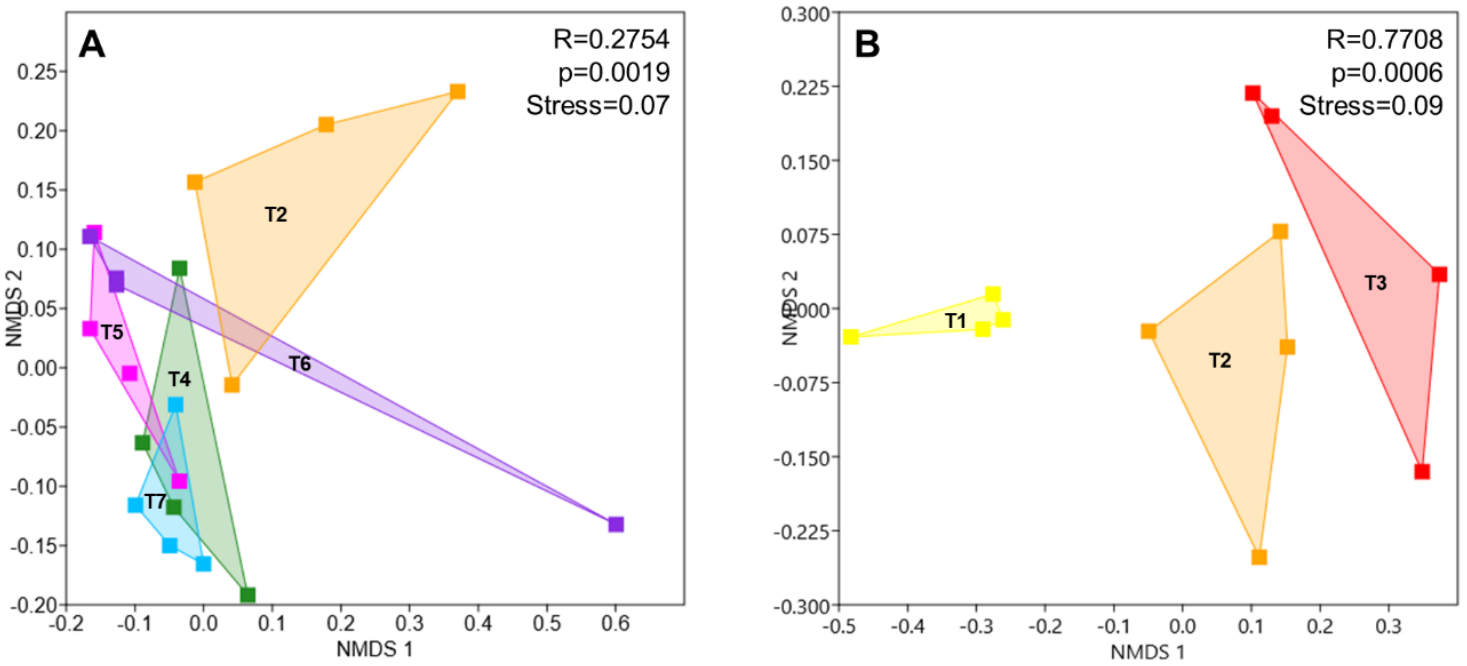

Figura 2. Perfil NMDS de diversidade genética da comunidade bacteriana da rizosfera do sorgo para as diferentes inoculações de BiomaPhos (0, 80, 100, 120 e 200 mL ha^{-1}) na dose de 50% de P_2O_5 (A) e para as diferentes doses de P (0, 50 e 100%) sem inoculante BiomaPhos (B), cultivado na Embrapa Milho e Sorgo, na safra 2021-2022. T1 - 0 % de adubação fosfatada; sem inoculação BiomaPhos (controle); T2 - 50 % de adubação fosfatada; sem inoculação BiomaPhos; T3 - 100 % de adubação fosfatada; sem inoculação BiomaPhos; T4 - 50 % de adubação fosfatada; com inoculação BiomaPhos na dose de 80 mL ha^{-1}; T5 - 50 % de adubação fosfatada; com inoculação BiomaPhos na dose de 100 mL ha^{-1}; T6 - 50 % de adubação fosfatada; com inoculação BiomaPhos na dose de 120 mL ha^{-1}; T7 - 50 % de adubação fosfatada; com inoculação BiomaPhos na dose de 200 mL ha^{-1}.

Na segunda safra, comparando os tratamentos inoculados com as doses 80, 100, 120 e 200 mL ha^{-1} de BiomaPhos (tratamentos 4, 5, 6 e 7, respectivamente) com o tratamento sem adição de BiomaPhos (tratamento 2), todos com e 50% de adubação fosfatada, o teste ANOSIM indicou que houve diferença significativa entre a comunidade bacteriana das amostras com e sem BiomaPhos (R= 0,4479; p=0,0001) (Figura 3A). Analisando apenas os tratamentos com 0%, 50% e 100% de adubação fosfatada e sem adição de BiomaPhos (tratamentos 1, 2 e 3, respectivamente), o teste indicou que houve diferença significativa na diversidade bacteriana das amostras para a diferentes doses de P_20_5 adicionadas (R=0,1829; p=0,0405) (Figura 3B).

Além disso, não houve diferença significativa na diversidade de FMA, tanto para os tratamentos inoculados com as diferentes doses de BiomaPhos e 50% de adubação fosfatada quanto para os tratamentos com as diferentes doses de P_2O_5 sem adição de BiomaPhos (p>0,05).

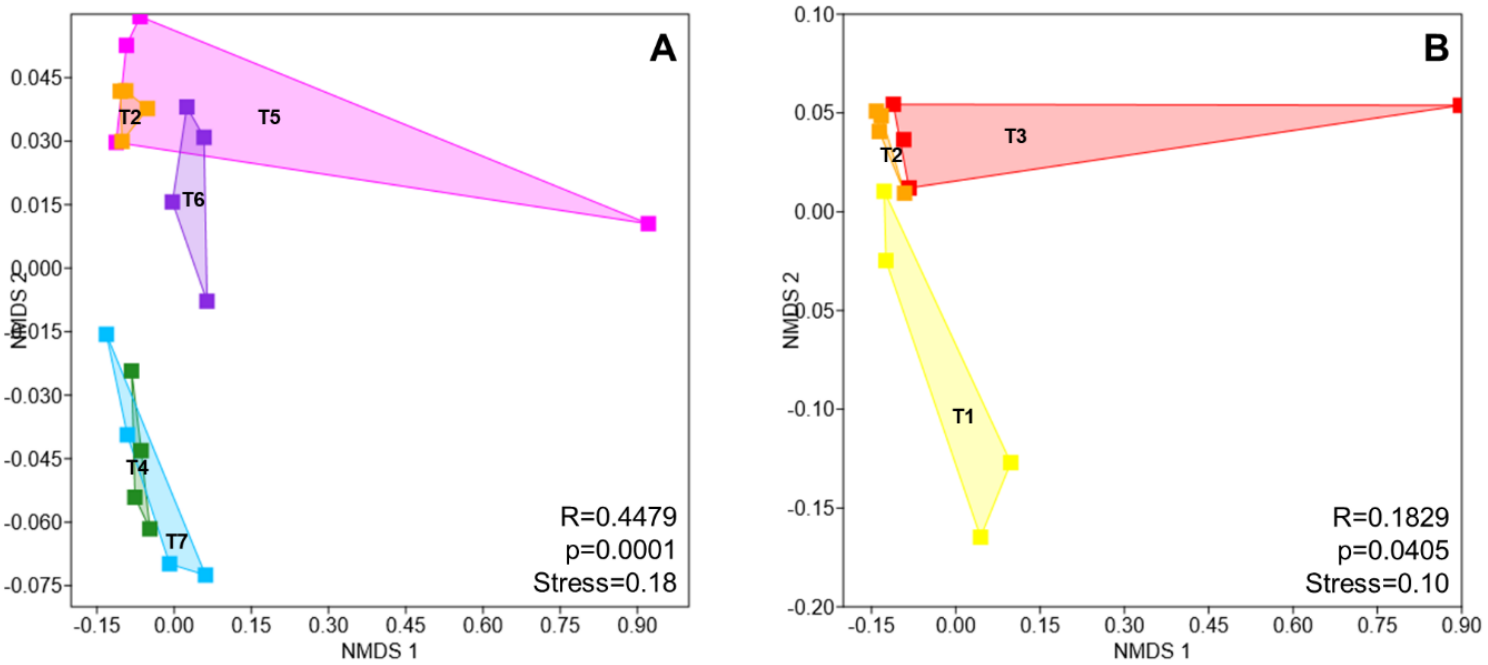

Figura 3. Perfil NMDS de diversidade genética da comunidade bacteriana da rizosfera do sorgo para as diferentes inoculações de BiomaPhos (0, 80, 100, 120 e 200 mL) na dose de 50% de P_2O_5 (A) e para as diferentes doses de P_2O_5 (0, 50 e 100%) sem inoculante BiomaPhos (B), cultivado na Embrapa Milho e Sorgo, na safra 2022-2023. T1 - 0 % de adubação fosfatada; sem inoculação BiomaPhos (controle); T2 - 50 % de adubação fosfatada; sem inoculação BiomaPhos; T3 - 100 % de adubação fosfatada; sem inoculação BiomaPhos; T4 - 50 % de adubação fosfatada; com inoculação BiomaPhos na dose de 80 mL ha^{-1}; T5 - 50 % de adubação fosfatada; com inoculação BiomaPhos na dose de 100 mL ha^{-1}; T6 - 50 % de adubação fosfatada; com inoculação BiomaPhos na dose de 120 mL ha^{-1}; T7 - 50 % de adubação fosfatada; com inoculação BiomaPhos na dose de 200 mL ha^{-1}.

Para as duas safras foi observada diferença estatística significativa para a diversidade da comunidade bacteriana (p≤0,10) entre os tratamentos, sendo os filos *Actinomycetota*, *Bacillota* e *Pseudomonadota* os de maior abundância relativa. (Figuras 4 e 5). Os índices de diversidade de Shannon, Simpson e Chao não apresentaram diferenças estatísticas significativas entre os tratamentos (p>0,05).

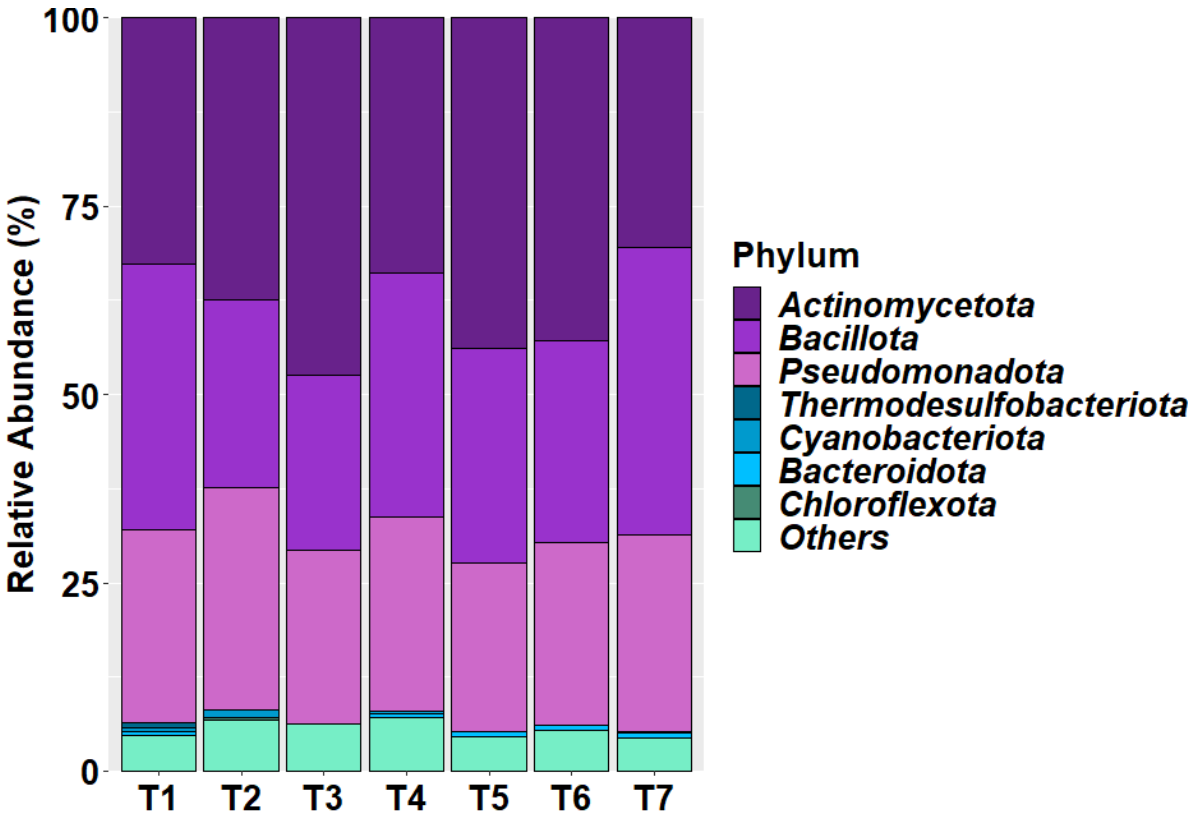

Figura 4. Abundância relativa dos filos da comunidade bacteriana do solo rizosférico de sorgo cultivado na área experimental da Embrapa Milho e Sorgo, sob diferentes doses de BiomaPhos e de fosfato, durante a safra 2021-2022. T1 - 0 % de adubação fosfatada; sem inoculação BiomaPhos (controle); T2 - 50 % de adubação fosfatada; sem inoculação BiomaPhos; T3 - 100 % de adubação fosfatada; sem inoculação BiomaPhos; T4 - 50 % de adubação fosfatada; com inoculação BiomaPhos na dose de 80 mL ha^{-1}; T5 - 50 % de adubação fosfatada; com inoculação BiomaPhos na dose de 100 mL ha^{-1}; T6 - 50 % de adubação fosfatada; com inoculação BiomaPhos na dose de 120 mL ha^{-1}; T7 - 50 % de adubação fosfatada; com inoculação BiomaPhos na dose de 200 mL ha^{-1}.

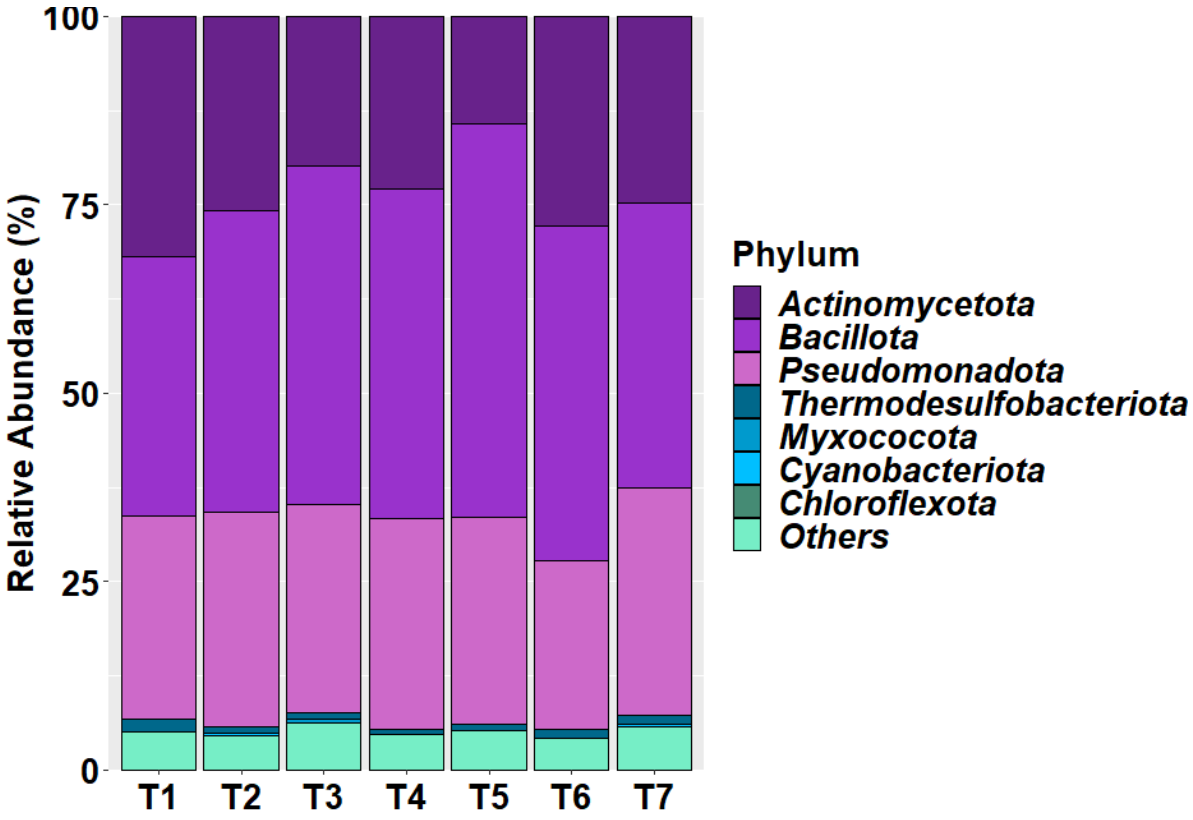

Figura 5. Abundância relativa dos filos da comunidade bacteriana do solo rizosférico de sorgo cultivado na área experimental da Embrapa Milho e Sorgo, sob diferentes doses de BiomaPhos e de fosfato, durante a safra 2021-2022. T1 - 0 % de adubação fosfatada; sem inoculação BiomaPhos (controle); T2 - 50 % de adubação fosfatada; sem inoculação BiomaPhos; T3 - 100 % de adubação fosfatada; sem inoculação BiomaPhos; T4 - 50 % de adubação fosfatada; com inoculação BiomaPhos na dose de 80 mL ha^{-1}; T5 - 50 % de adubação fosfatada; com inoculação BiomaPhos na dose de 100 mL ha^{-1}; T6 - 50 % de adubação fosfatada; com inoculação BiomaPhos na dose de 120 mL ha^{-1}; T7 - 50 % de adubação fosfatada; com inoculação BiomaPhos na dose de 200 mL ha^{-1}.

Com base nos resultados obtidos na primeira safra (2021/2022) foi realizada uma Análise de Componentes Principais (PCA) (Figura 6; Tabela 5). O primeiro componente principal (CP1) explicou 46,4% da variabilidade, enquanto o segundo componente principal (CP2) explicou 36,4%, somando um total de 82,8% (Tabela 5). O CP1 teve coeficientes positivos para todas as características, sendo explicado por todas elas, enquanto o CP2 teve coeficiente negativo para conteúdo de fósforo do solo e atividade enzimática da fosfatase ácida (Tabela 5).

No quadrante 1 da PCA estão os tratamentos com adição de 100 e 120 mL ha^{-1} de BiomaPhos e 50% de adubação fosfatada (tratamentos 5 e 6, respectivamente).

A variação entre os tratamentos foi explicada pelas características produtividade (PROD), colonização micorrízica (MYC) e densidade de raízes laterais (LT_MED_DIA), sendo a produtividade a característica de maior contribuição para os tratamentos. Esses tratamentos mostraram impacto positivo na produtividade, em relação ao tratamento sem inoculação e mesma dose de adubação fosfatada. Em contraste, no quadrante 3 estão os tratamentos controle (tratamento 1) e com adição de 80 mL ha^{-1} de BiomPhos e 50% de adubação fosfatada (tratamento 4). Já no quadrante 4 estão os tratamentos com 50% e 100% de adubação fosfatada sem inoculação de BiomaPhos (tratamentos 2 e 3, respectivamente). A variação entre os tratamentos foi explicada pelas características conteúdo de P do solo (P_Solo), e atividade enzimática da fosfatase ácida (F_Ácida), ambas características apresentando contribuição similar para os tratamentos. Em contraste, no quadrante 2 ficou o tratamento 7, que contem a maior dose de BiomaPhos.

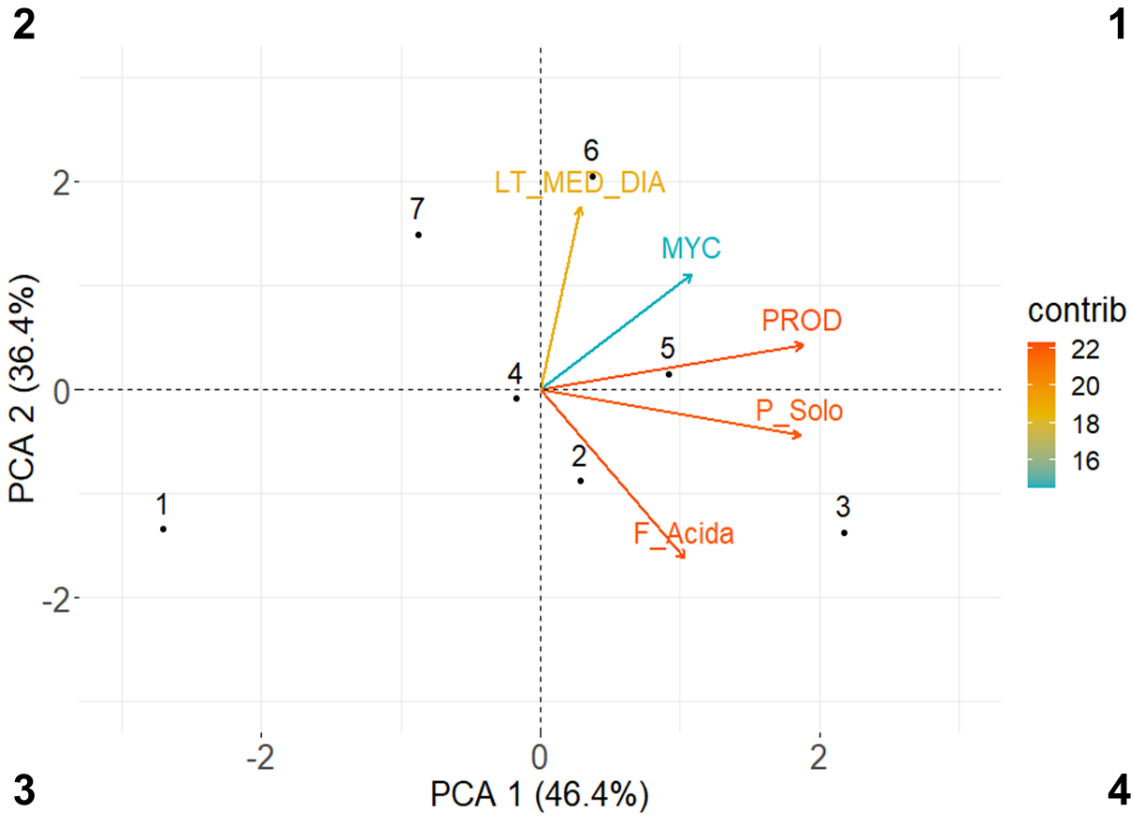

Figura 6. Análise de componentes principais (PCA) para plantas de sorgo cultivadas em campo sob diferentes doses de BiomaPhos e de fosfato, durante a safra 2021/2022. PROD: Produtividade de grãos; P_Solo: teor de P do solo; MYC: micorrização; F_Ácida: atividade enzimática de fosfatase ácida; LT_MED_DIA: densidade de raízes laterais.

Tabela 5. Análise de Componentes Principais (PCA) para características de colonização micorrízica, conteúdo de P, produtividade, atividade enzimática e arquitetura do sistema radicular de sorgo. Autovetores, autovalores e a proporção cumulativa da variância total (%) explicada são mostrados para cada componente principal (CP).

Característica	CP1	CP2
PROD (kg ha^{-1})	0.94	0.21
P_Solo (g)	0.93	-0.22
MYC (%)	0.54	0.55
F_Ácida (μg)	0.52	-0.80
LT_MED_DIA (mm)	0.14	0.87
Autovalor	2.31	1.81
Variância acumulada	46.4	82.7

PROD: Produtividade de grãos; P_Solo: teor de P do solo; MYC: micorrização; F_Ácida: atividade enzimática de fosfatase ácida; LT_MED_DIA: densidade de raízes laterais.

4 Discussão

Os resultados obtidos indicaram que 100 mL ha^{-1} de Biomaphos promoveu aumento significativo da produtividade, uma vez que foi possível com metade da adubação fosfatada obter a mesma produtividade que com a dose cheia de adubação. Ou seja, o uso combinado da inoculação de inoculante bacteriano solubilizador de fosfato, com genótipos eficientes e responsivos ao P, indica que é possível reduzir a quantidade de fertilizante fosfatado aplicado anualmente na cultura de sorgo, o que contribui de maneira significativa para a sustentabilidade de todo agroecossistema.

Embora a disponibilidade de P exerça forte influência no solo para a colonização micorrízica (Kobae, 2018; Abdelhalim et al. 2019), nas duas safras, o percentual de adubação fosfatada (50% e 100%) não interferiu na micorrização. Contudo, um fator importante a se considerar é que há variabilidade genética entre as linhagens de sorgo granífero quanto às eficiências de absorção, de utilização e de uso do P, e quanto à responsividade ao nutriente (Rodrigues et al. 2014).

A aquisição de P é favorecida por mudanças na arquitetura e na morfologia do sistema radicular, uma vez que a disponibilidade de P para plantas é geralmente maior na camada superficial do solo, estando em baixas concentrações nas camadas inferiores (Hufnagel *et al.* 2014; Erel *et al.* 2017). Porém, as adaptações radiculares

desenvolvidas pelas culturas dependem do tipo de solo, do tempo de amostragem, da espécie da planta e do genótipo dentro de uma espécie (Erel et al. 2017; Lynch, 2019; Liu, 2021). Além disso, com a adição do inoculante bacteriano, é possível que o potencial produtivo máximo das plantas tenha sido atingido, não havendo necessidade de gasto metabólico da planta para maior expansão do sistema radicular durante seu desenvolvimento.

Na primeira safra (2021/2022), a inoculação de BiomaPhos interferiu significativamente na colonização micorrízica. O inoculante bacteriano BiomaPhos promove o aumento das raízes finas das plantas (Oliveira-Paiva et al. 2020; de Sousa et al. 2020), que consequentemente pode promover maior comprimento de hifa (Van der Heijden et al. 2016). Estudos relatam que vários táxons bacterianos podem exercer efeitos benéficos e sinérgicos com FMA (Vosátka; Gryndler, 1999; Sala et al. 2007; Van der Heijden et al. 2016; Moreira et al. 2020), as chamadas bactérias auxiliares de micorriza (Frey-Klett et al. 2007). Bactérias pertencentes à família *Oxalobacteraceae*, por exemplo, foram relatadas como associadas preferencialmente com raízes micorrízicas (Offre et al. 2007; Offre et al. 2008). Além disso, os membros dessa família mostraram promover a germinação de esporos de FMA, crescimento de hifas, e colonização radicular de fungos do gênero *Rhizophagus* (Toljander et al. 2006; Pivato et al. 2009; Scheublin et al. 2010; Van der Heijden et al. 2016;).

Na segunda safra (2022/2023), a concentração de P no solo foi maior quando comparada a primeira safra, o que refletiu em não ser observado aumento da colonização micorrízica. A concentração de P pode aumentar ao longo dos anos de plantio, devido aos processos físico-químicos e à atividade microbiana da rizosfera. Baixas concentrações de P no solo promovem aumento da exsudação radicular e subsequente colonização micorrízica. Por outro lado, concentrações elevadas de P suprimem a exsudação, reduzindo o nível de sinalização planta-fungo durante a simbiose e dificultando a colonização (Chiu e Paszkowski, 2019). O mesmo vale para a atividade enzimática. As fosfatases estão associadas à remobilização de P nas plantas, e uma maior atividade dessas enzimas tem sido associada a baixos teores celulares de fosfato (Richardson et al. 2000; Baldwin, et al. 2001; Bozzo et al. 2006).

A estrutura, a função e a composição da comunidade microbiana da rizosfera são influenciadas por vários fatores em resposta a adição de inoculantes bacterianos, incluindo o tipo de solo, a disponibilidade de nutrientes, o estágio de crescimento da planta, o genótipo da planta e a composição microbiana do solo (Schlemper et al.

2018). Neste cenário, alguns microrganismos são predominantes em amostras de solos fertilizados em relação a solos não fertilizados, como é o caso das Proteobactérias, das Actinobactérias e das Firmicutes (Trabelsi et al. 2017). Neste trabalho, para o sorgo cultivado sob diferentes doses de BiomaPhos e de fosfato, os filos *Pseudomonadota, Actinomycetota* e *Bacillota* foram os de maior abundância relativa para as duas safras.

A abundância destes grupos de microrganismos é frequentemente relacionada com a ciclagem de P no solo (Trabelsi et al. 2017). No entanto, microorganismos do solo não estão apenas envolvidos na ciclagem de nutrientes, sendo capazes de alterar a microbiota geral da rizosfera, dada a natureza interconectada das comunidades microbianas com as propriedades físicas, químicas e biológicas do solo (Schlemper et al. 2017; Kari et al. 2019). Além disso, é possível que a colonização micorrízica tenha interferido na estrutura da comunidade bacteriana da rizosfera, uma vez que ambas as comunidades podem afetar a composição e a estrutura umas das outras (Schlemper et al. 2018).

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CONCLUSÃO GERAL

Apesar da natureza altamente regulada e dinâmica tanto da colonização micorrízica quanto da colonização bacteriana, os fatores que influenciam a associação entre plantas de sorgo e microrganismos na microbiota do solo ainda não são completamente compreendidos. Grande parte dos estudos enfatiza a necessidade de considerar fatores como a espécie e o genótipo da planta, o estágio de desenvolvimento da planta, a zona radicular e o tipo de solo. No entanto, fatores como a presença de exsudatos radiculares, a adição de inoculantes bacterianos e a disponibilidade de P, merece a mesma atenção, dada a sua importância no processo de interação microbiana na rizosfera.

Este trabalho evidenciou um aumento na colonização micorrízica, no peso seco e no teor de P em plantas de sorgo cultivadas em casa de vegetação em resposta à adição de sorgoleona. Além disso, este trabalho mostrou que a adição do inoculante bacteriano BiomaPhos interferiu significativamente na colonização micorrízica, na microbiota da rizosfera e na produtividade das plantas de sorgo cultivadas em campo. Para ambos os casos, o sucesso da associação entre plantas de sorgo e as comunidades bacteriana e de FMA da rizosfera parece ser resultado de seus efeitos diretos, indiretos ou de ambos, sendo a disponibilidade de P é um fator preponderante para o sucesso desta associação.

Os resultados deste trabalho reforçam a necessidade de entender os efeitos das moléculas bioativas, como a sorgoleona, e dos inoculantes bacterianos solubilizador de fosfato, como o BiomaPhos, nas plantas, uma vez que se mostram como aspectos fundamentais da interação planta-solo-microrganismos. Este entendimento oferece oportunidades para manipular o uso de genótipos para diferentes nichos de mercado, seja para melhorar a eficiência de aquisição de P, ou para modular as relações morfofisiológicas e da microbiota da rizosfera de sorgo, objetivando aumentar a produtividade agrícola. Isso corrobora com uma visão mais inclusiva da biodiversidade, e avança no conhecimento das distintas facetas pelas quais a biodiversidade promove a multifuncionalidade do agroecossistema.