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Developing novel techniques for efficient Arbuscular Mycorrhizal Fungi (AMF) Inoculum Production

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Abstract

The demand for AMF propagules has experienced a surge as a result of their capability to amplify growth and nutrient uptake. This demand has triggered the adoption of contemporary propagule cultivation techniques, as conventional AMF propagation methods often involve extensive labor and time requirements, creating difficulties for large-scale production. The adoption of bioreactors, for example, has become one of the most promising approaches for the substantial cultivation of AMF propagules. Bioreactors offer a promising approach for the large-scale production of AMF propagules, which boost nutrient absorption and plant development. They provide superior yield, cost-effectiveness, and scalability in contrast to conventional methods. Synthetic substrates also provide a regulated environment that enhances optimal growth and development. They can be adjusted to satisfy specific nutrient specifications and promote consistent spore production. Synthetic substrates enable researchers to manipulate concentration and timing, thereby increasing spore yields and preserving non-destructive yield. This approach enables the iterative extraction of spores from the same batch. The prioritization of nutrient composition optimization in bioreactors is crucial for supporting AMF propagule growth and reproduction in future research. For instance, understanding AMF's distinct nutrient requirements can lead to media that is nutrient-rich, increased yields of propagules, and improved interactions with plants. Furthermore, developments in synthetic substrate technology can produce substrates that replicate natural soil environments. This study focused on innovative materials and additives can amplify substrate fertility and stability. Likewise, the careful concentration and timing of application when optimizing growth regulators can have a substantial impact on the production of AMF spores. These advancements can contribute to the sustainable production of AMF propagules, thereby offering advantages to agriculture and ecological restoration initiatives.

Keywords: Arbuscular mycorrhizal fungi; AMF propagules; Bioreactors; AMF substrates; AMF regulators

1. Introduction

Arbuscular mycorrhizal fungi (AMF) are soil microorganisms capable of forming a symbiotic relationship characterized by the mutual transfer of nutrients within plant roots. These subterranean microbes reside in roots, establishing filamentous networks that assist in the mobilization of phosphorus and nitrogen, which would otherwise be unreachable [1,2]. Their elaborate mycelial structure assists in obtaining nutrients, thereby enabling them to furnish host plants with indispensable minerals that augment plant growth and development. In exchange, the fungi receive sugars generated by photosynthesis from the host plants. The adoption of AMF in agriculture has resulted in remarkable improvements in enhancing crop output and ecological sustainability [3,4]. By enhancing nutrient absorption efficiency, these fungi decrease reliance on chemical fertilizers, thereby mitigating environmental contamination resulting from excessive fertilizer application. In addition, AMF improve the water absorption capacity in plants, enhancing their ability to withstand drought conditions.

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Despite their potential benefits, current production methods of AMF are ineffectual and lack scalability for extensive agricultural implementation [5]. Additionally, conventional methods involve labor-intensive procedures such as manually collecting from natural ecosystems or cultivating in greenhouses using potted cultures. These approaches restrict the accessibility of AMF inoculants for farmers on a broader scope. To resolve this issue, new methods must be formulated to optimize the production efficiency of AMF inoculants. One encouraging method entails utilizing bioreactor-based cultivation systems to enable the controlled mass production of AMF spores [6]. These reactors offer ideal growth conditions for AMF while guaranteeing consistency and expandability. Additionally, progress in molecular biology techniques can assist in identifying particular strains of AMF that demonstrate superior characteristics like improved nutrient uptake or resilience to environmental pressures. This knowledge can be employed to engineer genetically modified AMF strains that showcase enhanced efficiency and adaptability for agricultural objectives.

1.1. AMF bioreactors

In the field of agriculture, the utilization of bioreactors has seen significant growth in the production of AMF inocula. They provide a controlled environment for the mass production of AMF, which are advantageous for establishing symbiotic relationships with plant roots, facilitating nutrient uptake, and improving plant growth [7]. With the growing demand for sustainable farming practices, the utilization of AMF inocula has become increasingly crucial in contemporary agricultural systems. The employment of AMF inocula provides various advantages in the field of agriculture. They improve the availability, productivity, and vitality of plants by extending root systems and enhancing absorption, while also strengthening soil structure and preventing erosion. On the other hand, bioreactors create optimal growth conditions for AMF, facilitating the production of significant quantities of inoculum for field application [8]. They exert exacting control over environmental parameters, encompassing pH, temperature, and nutrient composition, to secure a consistent and top-tier production of inocula. Different types of bioreactors are employed in the production process of AMF inocula. These comprise of stirred tank reactors, airlift reactors, and packed bed reactors [9]. Each type presents its own set of advantages and limitations, and the decision on which bioreactor to use is influenced by factors including scalability, cost-effectiveness, and the specific requirements of the cultivated AMF strain.

The successful operation of bioreactors for AMF inocula production demands thorough contemplation of multiple factors. Adequate aeration and agitation are crucial to facilitate fungal growth and mitigate constraints in mass transfer [10]. The selection of growth medium, addition of nutrients, and regulation of pH also have significant impacts on achieving optimal AMF biomass production. In order to maintain the efficacy of AMF inocula, quality control measures should be implemented throughout the production process. This involves the continuous monitoring of fungal growth, viability, and genetic stability. Moreover, the process of scaling production from small-scale lab bioreactors to large-scale industrial systems necessitates advanced engineering and optimization in order to uphold consistent product quality [11]. Upon production, AMF inocula can be utilized in agricultural fields via several means, such as seed coating, soil incorporation, or foliar spraying. Field trials have substantiated the advantageous effects of AMF inoculation on crop yield, disease resilience, and soil vitality. As the demand for sustainable farming practices grows, the prevalence of bioreactors in AMF inocula production is expected to increase.

1.2. AMF substrates

Synthetic substrates are intricately formulated growth substances that facilitate the proliferation and maturation of AMF inocula. These substrates provide a conducive environment for the colonization and establishment of AMF spores and mycelium within plant roots [12]. To ensure successful inoculation, a diverse range of synthetic substrates can be employed for AMF inocula. Vermiculite, a naturally occurring mineral, demonstrates exceptional water retention properties. It has the ability to retain moisture for extended periods, thereby ensuring the hydration and viability of the AMF inoculum [13]. Additionally, this mineral provides a stable physical structure for fungal colonization and the formation of symbiotic relationships with plant roots. On the other hand, Perlite, a lightweight volcanic glass, effectively enhances aeration and drainage. The permeable structure guarantees the delivery of oxygen to the roots while preventing water saturation. This substrate creates an optimal environment for AMF colonization and root development. Zeolite is another synthetic substrate that is commonly employed in AMF inocula. It is a substance with a crystal-like structure, composed of aluminosilicate and capable of ion exchange. According to [14], these substance can retain vital nutrients such as potassium and calcium ions and gradually release them to support plant growth. Peat, a substance formed through the decomposition of plant matter, offers an optimal habitat for AMF because of its ample organic material and moisture retention capabilities. This substance also function as a carbon source for the fungi, promoting their growth and activity. Notably, synthetic substrates provide several advantages over conventional inoculation methods, including but not limited to more effective spore dispersal, higher survival rates, and enhanced efficacy.

1.3. AMF regulators

Growth regulators, also referred to as phytohormones, are essential compounds that regulate various facets of growth and development. They function as chemical messengers, regulating processes such as cell division, differentiation, and elongation. AMF Inocula contains various growth regulators, including auxins [15]. They are renowned for promoting cell elongation and fostering root development. This is vital as it enables vertical expansion and nurtures a resilient root system. In addition, auxins play a role in establishing apical dominance, whereby the main shoot of a plant demonstrates increased growth compared to its side shoots. Another growth regulator found in AMF Inocula is cytokinins. They are acknowledged for their ability to stimulate cell division and shoot growth [16]. This encourages plants to thrive and generate an abundance of shoots. Cytokinins provide notable advantages for crops that necessitate high yields. Gibberellins are also utilized in the development of AMF Inocula. They play a pivotal role in regulating stem elongation, flower development, and fruit growth in plants. Gibberellic acid (GA3) is extensively used as a gibberellin variant in agriculture, facilitating seed germination and enhancing fruit size.

1.4. Search strategy

The study employed a comprehensive selection of diverse English publications covering the period from 2007 to 2023. The investigation required the division of the research topic into various search terms, including "AMF Inoculum," "bioreactors and AMF production," "synthetic substrates and AMF production," and "growth regulators and AMF production." After conducting the search, a total of 49 journal articles were discovered, with 3 duplicates eliminated, leaving a final count of 46 unique articles. The summaries of all these journals were subjected to thorough scrutiny, and relevant papers were identified for further evaluation. The study set its criteria for inclusion and exclusion primarily by considering a time limit of 16 years. Upon meeting this requirement, a comprehensive analysis of 43 journal articles was carried out to ascertain their relevance to the research topic. At this point, 1 of these journals was discarded given the study's emphasis on peer-reviewed articles above all else. A detailed examination of 42 peer-reviewed journals was carried out to determine if they meet the criteria for inclusion in this analysis. Two of these journals were dismissed as they did not provide any valuable information for keyword identification. Thus, the research approach resulted in the identification of 40 publications utilized in this review (Figure 1).

2. Novel Techniques for Efficient AMF Inoculum Production: Literature from previous studies

2.1. The use of bioreactors for mass production of AMF propagules

In the agricultural sector, the utilization of arbuscular mycorrhizal (AM) fungi has received significant attention because of their ability to stimulate plant growth and facilitate nutrient absorption. Despite this, the mass production of these biotrophic fungi has encountered multiple hurdles. A study conducted by Akhtar and Abdullah illuminates these obstacles and examines various techniques that have been developed to surmount them. One significant obstacle in mass production is the inherent dependency of AM fungi, restricting their survival to plant root symbiosis [17]. This interdependence makes it arduous to cultivate them beyond their native environment. Besides, early species identification presents another challenge owing to the large number of AM fungal species with morphological characteristics that closely resemble each other. In an effort to address these challenges, researchers have utilized in-vitro cultivation approaches like hydroponic systems and root organ culture. These techniques offer a controlled setting for the cultivation of AM fungi, ensuring the preservation of their propagule quality and consistency. Hydroponic systems entail growing plants in a solution filled with nutrients, without the use of soil, resulting in enhanced root accessibility and fostering AM fungal colonization [17] [Table 1]. On the other hand, root organ culture involves isolating plant roots from soil and growing them in artificial media supplemented with necessary nutrients. Regardless of these advancements, further research is required to optimize mass production techniques for AM fungi. By overcoming the challenges associated with their obligate nature and early species identification, we can unlock the full potential of these beneficial fungi in improving agricultural practices and sustainable crop production.

In the investigation carried out by Ellatif et al., the consequences of different phenolic substances on the development of the AMF *Gigaspora gigantea* in cultured tomato root organs were analyzed. The scientists utilized eight distinct substances, specifically cinnamic acid, dehydrated catechin, protocatechuic acid, ferulic acid, tannic acid, coumarin, esculetin, and catechol [18]. The findings of the research unveiled that dehydrated catechin had a noteworthy influence on the development of *Gigaspora gigantea*. This substance was discovered to boost the growth of AMF. This discovery is significant as AMF play a vital role in enhancing nutrient absorption and stimulating plant growth. The other phenolic compounds tested did not show a similar effect on AMF growth. This suggests that catechin anhydrous may have unique properties that stimulate AMF development. Additional investigation is required to comprehend the mechanisms underlying this occurrence

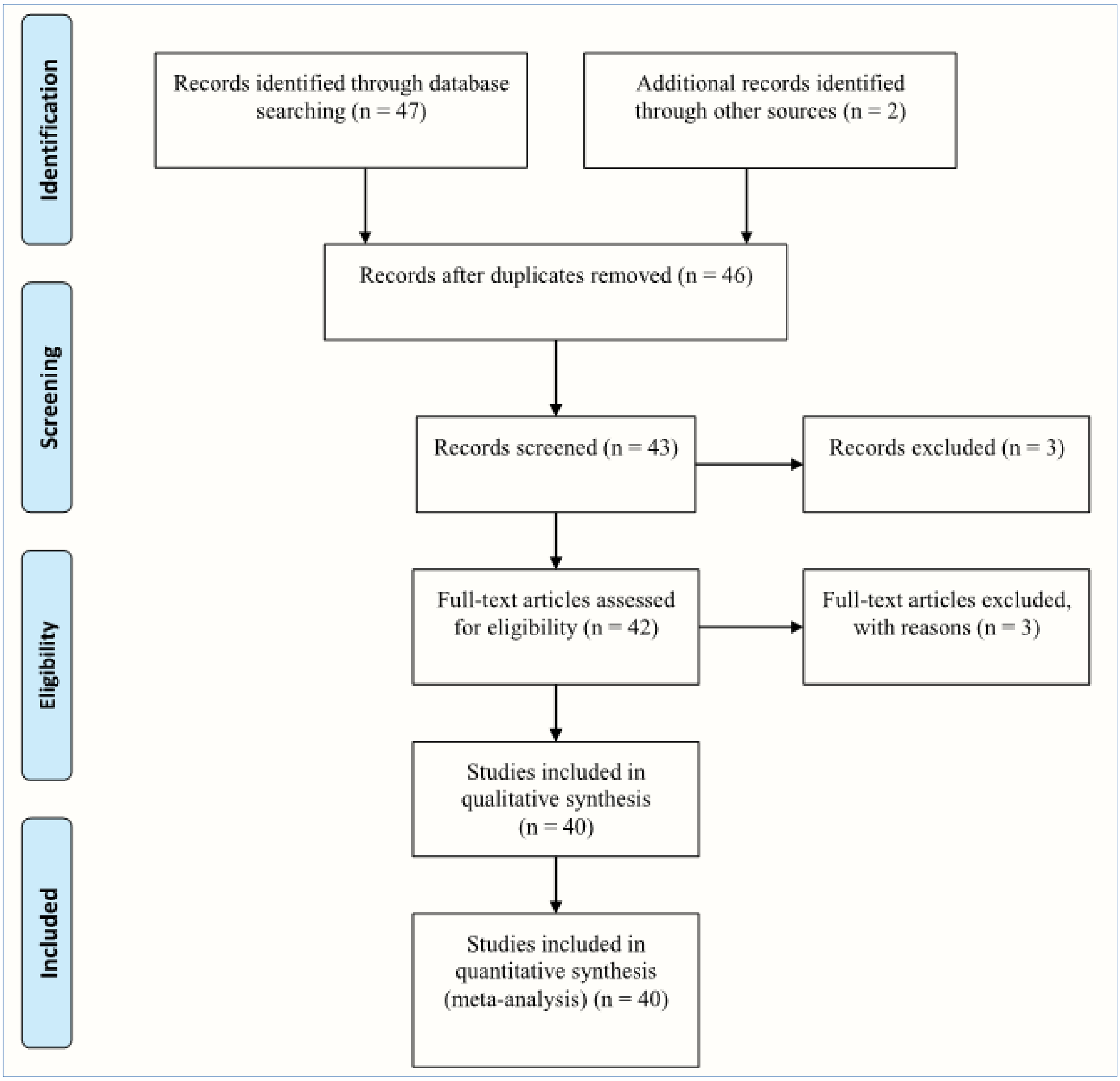

Figure 1 PRISMA diagram showing how papers were selected, excluded and included

and delve into its prospective implementations in agriculture [18]. In general, this examination contributes to our comprehension of how distinct phenolic substances can impact AMF development. It highlights the potential benefits of using specific compounds like catechin anhydrous to enhance plant-microbe interactions and improve crop productivity.

In a recent study conducted by Ceballos et al., a commercially-produced in vitro inoculum of *Rhizophagus irregularis* was tested in Colombia, with the objective of enhancing cassava yields. The results of this study emphasize the significance of investigating alternative methods to enhance crop productivity [19]. Cassava serves as a fundamental dietary component for millions of individuals globally, especially in developing nations where ensuring food stability is a critical concern. Hence, any attempts to amplify cassava yields are vital for ensuring a satisfactory food stock. While the use of mass-produced inoculum did not yield economic benefits in this study, the potential advantages of genetic manipulation cannot be ignored. By manipulating the genes of *R. irregularis,* scientists may be able to enhance its symbiotic relationship with cassava plants and promote more efficient nutrient uptake. Genetic manipulation has demonstrated potential in diverse areas like agriculture [Table 1]. It has been successfully employed to breed crops with resistance to diseases and enhance nutritional value [19]. Therefore, applying this technique to *R. irregularis* holds great potential for enhancing plant growth and increasing cassava yields. Overall, although Ceballos et al.'s study showed that a commercially produced inoculum did not provide a higher return on investment compared to traditional cultivation techniques for cassava production, it paves the way for further investigation into the genetic manipulation of *R. irregularis*. This approach has the potential to result in substantial enhancements in plant growth and ultimately aid in addressing global food security challenges.

2.2. The use of synthetic substrates for AMF cultivation

The study conducted by Coelho et al. aimed to enhance the production of arbuscular mycorrhizal fungi (AMF) by employing diverse organic substrates including vermicompost, coir dust, tropstrato, sand, and vermiculite. The findings of this research revealed that the system of inoculum production using sand and vermiculite combined with 10% vermicompost favored the production of infective inoculum of *A. longula* with the fungus benefiting the growth of corn plants [13]. Among the various substrates examined, the combination of sand and vermiculite with 10% vermicompost demonstrated the highest efficacy in enhancing AMF production. This combination yielded a well-balanced nutrient composition and optimal physical characteristics for fungal colonization [13]. Furthermore, the presence of *A. longula* in the inoculum significantly improved corn plant growth [Table 1]. The fungus facilitated the uptake of nutrients from the soil, resulting in enhanced plant vigor and productivity. Overall, this study underscores the importance of maximizing AMF production through the utilization of organic substrates, specifically vermicompost. The findings indicate that the use of sand and vermiculite, combined with 10% vermicompost, can improve infective inoculum production of A. longula, ultimately resulting in enhanced corn plant growth. Future studies should concentrate on examining different organic substrates and their potential influence on AMF production in order to enhance agricultural productivity.

A study carried out by Hu et al. has illuminated the beneficial impacts of utilizing absorptive substances like perlite, vermiculite, and biochar on AMF colonization in constructed wetlands (CWs). It was unveiled that the implementation of absorptive substances in CWs establishes an optimal habitat for AMF colonization. These substances offer a permeable framework that permits enhanced water retention and nutrient accessibility, which are vital for AMF development [20]. Moreover, they possess favorable surface area-to-volume ratios, enabling them to proficiently adsorb pharmaceuticals and personal care products (PPCPs) from wastewater. Once AMF has taken root, plants serve as conduits for the transportation of PPCPs from the root system to their aerial tissues. This process is facilitated by mycorrhizal hyphae, which form a network connecting roots with surrounding soil particles. Through this network, PPCPs can be transported from contaminated soil or water into plant tissues. The positive effects observed in this study highlight the potential of using adsorptive substrates to enhance both wastewater treatment efficiency and phytoremediation capabilities [20]. By promoting AMF colonization, these substrates contribute to the removal of harmful substances from wastewater while simultaneously improving plant health and growth. Overall, [20]'s study demonstrates that the application of adsorptive substrates in CWs positively affects AMF colonization.

The experiment carried out by Azimi et al. strives to explore the effects of zeolite and AMF on the growth of plants, with a specific focus on *A. desertorum*. The outcomes obtained from this investigation demonstrated noteworthy advancements in various aspects of plant growth. The experiment integrated the utilization of mycorrhiza, zeolite, and superabsorbent in the soil [21]. The researchers observed that these additives positively influenced the speed of plant establishment, weight of dry roots, height of plants, biomass above the ground, and overall biomass. Concerning the speed of plant establishment, mycorrhiza was found to enhance it by 50%, while zeolite and superabsorbent increased it by 42% and 25% respectively [21] [Table 1]. The study also found that incorporating mycorrhiza, zeolite, and superabsorbent into *A. desertorum* plants significantly improved their initial development and survival. Zeolite increased root weight by 72% compared to mycorrhiza (62%), suggesting it aids in root growth and nutrient absorption. Plant height also increased with both mycorrhiza (44%), indicating vertical growth. Aboveground dry biomass increased by 51% with mycorrhiza, while zeolite increased by 61% [21]. Both mycorrhiza and zeolite significantly improved total dry matter biomass, indicating overall improvement in plant growth characteristics. These findings have implications for enhancing plant establishment rates, especially in arid environments like deserts.

In the study conducted by M. Papafotiou et al., it was revealed that gardenia plant growth was significantly enhanced when inoculated with arbuscular mycorrhizal fungi under reduced phosphate fertilization. This finding holds significant importance within the realm of commercial gardenia cultivation, where a commonly utilized substrate consists of high-peat composition (95:5). One of the main advantages identified in this study was the formation of a comprehensive network of roots in gardenia plants [22]. The arbuscular mycorrhizal fungi played a critical role in facilitating root growth and branching, thereby optimizing nutrient uptake and overall plant robustness. This is of particular significance in high-peat substrates, which tend to have low levels of essential nutrients such as phosphorus. By facilitating root growth, the fungi mitigated the limited phosphorus availability and ensured gardenia plants obtained sufficient nutrients. Additionally, the study also revealed that the introduction of arbuscular mycorrhizal fungi resulted in a more resilient plant canopy. The augmented root growth facilitated more efficient water and nutrient absorption, resulting in enhanced shoot growth and overall plant vigor [22]. This finding holds considerable significance for the commercial cultivation of gardenia, as it indicates that the inclusion of arbuscular mycorrhizal fungi in cultivation practices can bolster both crop yield and quality. In conclusion, [22]'s study emphasizes the beneficial effects of arbuscular mycorrhizal fungi on gardenia plants when phosphate fertilization is reduced. The use of these beneficial fungi has been observed to result in the promotion of extensive root systems and enhanced plant canopy robustness, particularly in high-peat substrates typically employed in commercial gardenia cultivation. These findings present valuable insights for growers who are striving to optimize their cultivation practices and enhance overall crop productivity.

2.3. The use of growth regulators on AMF spore production

Recent studies conducted by Liao et al. have illuminated the pivotal function fulfilled by phytohormones such as strigolactones, gibberellic acids, and auxin in the regulation of arbuscular mycorrhizal (AM) symbiosis. According to [23]'s investigation, strigolactones (SLs) have a substantial impact on enhancing fungal growth in AM symbiosis. Plant roots secrete SLs, which act as chemical signals to draw fungal hyphae towards the root system. This interaction results in the development of specialized structures known as arbuscules, which enhance nutrient exchange between the plant and fungus. Additionally, it was determined that gibberellic acids (GAs) can alter arbuscule formation through Della proteins. GAs are acknowledged for their significant role in advancing plant growth and development. According to [23]'s study, GAs control arbuscule formation by regulating Della proteins, which serve as negative regulators of this process. Moreover, auxin was recognized as an additional significant factor in the regulation of AM symbiosis. Auxin is a versatile plant hormone that participates in numerous physiological processes in plants. It was observed that the build-up of auxin is vital for the successful establishment of AM fungi in roots. Overall, [23]'s study underscores the significance of phytohormones such as strigolactones, gibberellic acids, and auxin in governing various phases of AM symbiosis. Acquiring knowledge about these molecular mechanisms can yield valuable insights for enhancing crop productivity by optimizing nutrient uptake through mycorrhizal associations.

In the study conducted by Christoph Stephan Schmidt et al. on Miscanthus × giganteus, the researchers sought to examine the impacts of symbiotic fungi and a growth regulator called thidiazuron (TDZ) on plant growth [Table 1]. The outcomes of their research demonstrated that despite the limited effect on plant growth, these factors did alter the levels of phytohormones in the leaves [16]. The discoveries from this investigation highlight the importance of further investigating the interplay between vegetation and microorganisms. Understanding the influence of these tiny organisms on vegetation growth and development is crucial for the advancement of agricultural methods and the optimization of harvest efficiency. Through the examination of the intricate dynamics between vegetation and microorganisms, researchers can unveil groundbreaking strategies to foster the interactions between plants and microbes for sustainable agriculture. Symbiotic fungi have a crucial function in nutrient absorption by establishing mycorrhizal associations with plant roots. These associations improve the absorption of nutrients, especially phosphorus, which is frequently scarce in soil [16]. Cytokinins, on the other hand, play a crucial role as phytohormones in multiple physiological processes like cell division, shoot initiation, and leaf senescence. While no significant impacts on plant growth were found, the study showcased the impact of symbiotic fungi and TDZ on phytohormone levels in leaves. This implies that these factors might indirectly impact plant physiology. Overall, [16]'s study highlights the importance of further examining the intricate interplay between plants and microbes in order to devise novel strategies for improving crop productivity and environmental sustainability.

A study by Foo et al. provided insights into the impact of gibberellin-deficient Na-1 mutants on AMF colonization in pea roots. The researchers ascertained that these mutants showcased a noteworthy rise in AMF in contrast to wild-type plants [24] [Table 1]. This finding implies that gibberellins are essential for regulating mycorrhizal colonization. However, when mutant La Cry-s was introduced in combination with GA3, the colonization was reversed, suggesting that GA3 can counteract the effects of Na-1 mutants. Additionally, changes in mycorrhizal colonization genes and expression were seen during the duration of this study. This implies an intricate interaction between plant hormones and gene regulation during AMF colonization. This research offers pivotal insights into the complex molecular mechanisms that govern plant-fungal interactions, presenting substantial implications for agriculture and strategies to enhance crop improvement [24]. In summary, the 2013 study by Foo et al. emphasizes the importance of gibberellins in governing AMF colonization in pea roots. The findings enrich our understanding of plant-fungal interactions and set the stage for additional research on enhancing crop productivity by manipulating these mutually beneficial relationships.

Table 1 Summary of results from previous studies on novel techniques for efficient AMF inoculum production

Novel Techniques for Efficient AMF Inoculum Production	Findings from Previous Studies	Reference
The use of bioreactors for mass production of AMF propagules	It is arduous to cultivate AMF beyond their native environment	[17]
	Dehydrated catechin had a noteworthy influence in boosting the growth of AMF	[18]
	Commercially produced AMF inoculum did not provide a higher return on investment compared to traditional techniques	[19]
The use of synthetic substrates for AMF cultivation	Inoculum production using sand and vermiculite combined with 10% vermicompost favored the production of AMF	[13]
	Perlite, vermiculite, and biochar enhance AMF colonization in constructed wetlands	[20]
	The utilization of zeolite, and superabsorbent enhance AMF functionality in the soil	[21]
	Gardenia plant growth is enhanced when inoculated with AMF under reduced phosphate fertilization	[22]
The use of growth regulators on AMF spore production	Strigolactones (SLs) have a substantial impact on enhancing fungal growth in AM symbiosis	[23]
	Thidiazuron (TDZ) indirectly enhance the process of AMF symbiosis	[16]
	Gibberellin-deficient Na-1 mutants enhance AMF colonization	[24]
	Strigolactones are instrumental in establishing symbiotic relationships between leguminous plants and nitrogen-fixing bacteria	[25]
	Microbe-derived hormones contribute to AMF microbiome assembly	[26]

Faizan's study highlights the significant role of plant hormones, specifically strigolactones (SLs), in plant metabolism and growth, derived from carotenoids, crucial for plant development and physiology. SLs play a crucial part in shaping plant architecture. They control shoot branching through the suppression of axillary bud growth, thereby dictating the plant's overall form and architecture [25]. Furthermore, SLs play a crucial role in photomorphogenesis, which pertains to the light-induced developmental alterations in plants. They regulate processes such as leaf expansion and chlorophyll accumulation. These phytohormones also have a function in the processes of seed germination and nodulation. They promote the germination of seeds by hindering seed dormancy and facilitating the emergence of the radicle. Concerning nodulation, SLs are instrumental in establishing symbiotic relationships between leguminous plants and nitrogen-fixing bacteria. According to [25], these phytohormones also have a crucial part to play in plants' reactions to abiotic factors, such as drought, salinity, and nutrient availability. They assist plants in adapting to unfavorable environmental conditions by controlling stomatal closure, stimulating root growth, and facilitating the uptake of nutrients. Another fascinating discovery from this study is that SLs stimulate hyphal branching in arbuscular mycorrhizal fungi (AMF). This establishes a symbiotic relationship between plants and AMF through which nutrient exchange can occur [25]. In summary, the study by Faizan et al. emphasizes the significance of strigolactones in regulating plant metabolism and growth. Acquiring knowledge about the roles of strigolactones can provide valuable insights into plant development and have potential implications for improving crop productivity in the future.

A recent investigation carried out by Eichmann and colleagues illuminates the function of hormones in constructing plant microbiomes, ultimately impacting the structure of these communities. Hormones, originating from both plants and microbes, contribute to the variety of plants and the development of healthy ecosystems. They mold plant microbiomes, which are crucial for acquiring nutrients, resisting diseases, and maintaining overall plant well-being [26]. Eichmann et al.'s research uncovers the crucial role hormones play in shaping these microbiomes. Hormones generated by plants can allure specific microorganisms or deter harmful ones. For instance, certain secretions from roots release compounds that serve as signals for beneficial bacteria to inhabit the rhizosphere. Conversely, some hormones impede pathogenic microbes from establishing themselves in plant tissues. Furthermore, microbe-derived hormones also contribute to microbiome assembly. Some bacteria produce auxins or cytokinins that promote root growth and enhance nutrient uptake by plants. These hormone-producing microbes establish symbiotic relationships with plants, benefiting both parties involved [26]. The versatility of these hormones allows for the creation of diverse habitats within ecosystems. Different types of plants release unique combinations and concentrations of hormones into their surroundings, attracting specific microbial communities tailored to their needs. According to [26], this diversity promotes overall ecosystem health by increasing resilience against pathogens and enhancing nutrient cycling. Overall, Eichmann et al.'s study highlights the significant role played by hormones in assembling plant microbiomes. The interaction between plant- and microbe-derived hormones creates diverse habitats that support a wide range of species and promote ecosystem resilience. Understanding these mechanisms is essential for developing sustainable agricultural practices and preserving biodiversity in our ever-changing world.

3. Potential for future research

3.1. Enhancing nutrient composition of bioreactors

The production of AMF propagules through bioreactors presents a hopeful resolution for widespread agricultural cultivation and implementation. One essential element future studies can explore is the fine-tuning of nutrient concentrations in bioreactors. The growth and development of AMF are highly reliant on essential nutrients like carbon, nitrogen, and phosphorus [27,28,29]. Through precise adjustments of their concentrations, it is possible to ensure ideal conditions for attaining the maximum propagule yield. Another aspect worth investigating is the rate of aeration in bioreactors. Adequate oxygen is essential for enhancing AMF growth through facilitating respiration [30,31]. By precisely adjusting aeration rates, researchers can create an ideal environment for AMF propagation. Furthermore, the examination of pH levels in bioreactors is crucial in the pursuit of maximum output. This is a result of the fact that different AMF species favor specific pH levels. Thus, the maintenance of an appropriate pH range is predicted to have a substantial impact on AMF growth and reproduction. These advancements will support sustainable agricultural practices and enhance our understanding of utilizing AMF to increase crop productivity and soil health.

3.2. Improving the structure and composition of synthetic substrates

As researchers explore the intricacies of AMF colonization, it becomes essential to investigate the influences of different nutrient compositions, physical structures, and supplementation strategies on AMF growth and sporulation in synthetic substrates. An area that necessitates additional examination is the impact of nutrient compositions on AMF development. By adjusting the concentrations of vital nutrients like phosphorus, nitrogen, and potassium, researchers can identify the most advantageous combinations for AMF growth. This knowledge can then be utilized to optimize synthetic substrate formulations for commercial production. Furthermore, the examination of the physical properties of synthetic substrates is crucial for comprehending their impact on AMF colonization. Factors such as particle size, porosity, and water retention capacity significantly influence fungal hyphal penetration and root colonization [32,33,34]. Through the analysis of these parameters, researchers can generate substrates that promote the establishment of AMF. In addition, it is important to assess supplementation strategies that can boost AMF growth and sporulation in synthetic substrates. It is conceivable that the integration of organic matter or beneficial microorganisms can increase fungal activity and enhance the quality of the inoculant. Finally, it is crucial to evaluate the long-term stability and effectiveness of AMF inoculants derived from synthetic substrates to ensure their efficient application in agriculture. Building knowledge about the sustained performance of these inoculants will secure their efficacy in enhancing plant health and productivity.

3.3. Optimization of growth regulators

The crucial role of AMF in plant growth and nutrient assimilation underscores their significance in the domain of sustainable agriculture. Despite this, their commercial production is currently limited due to low spore yields [35,36,37]. To overcome this obstacle, it is crucial to explore the various growth regulators that impact spore production in particular AMF species. By undertaking a comprehensive analysis of a broader selection of growth regulators, scientists can ascertain specific regulators that magnify spore production. This knowledge can then be applied to devise targeted strategies for optimizing AMF inoculum production. For instance, if a particular growth regulator is discovered to enhance spore yields in a designated AMF species, it could be employed as a supplementary component in commercial cultivation. Moreover, [38,39,40] indicate that a deep understanding of the underlying mechanisms governing spore regulation is indispensable for maximizing AMF inoculum production. By identifying genetic and biochemical pathways implicated in spore formation, it is possible to manipulate these mechanisms to increase spore yields. This could involve gene manipulation or the administration of specific compounds to induce spore formation. In general, gaining comprehension of the core mechanisms of spore regulation will empower researchers to devise focused strategies for improving AMF inoculum production.

4. Conclusion

The enhancement of AMF inoculant production's efficiency and scalability has immense potential for widespread use in agriculture. Applying bioreactor systems, synthetic substrates, and growth regulators can elevate AMF propagule production and quality. However, further study is needed to refine these techniques, understand their underlying mechanisms, and ensure their long-term viability and effectiveness. Investing in innovative strategies for efficient AMF inoculum production can improve nutrient availability and crop productivity, thereby contributing to sustainable agriculture.

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