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Short Communication

Mycorrhizal Symbiosis in Root Organ Cultures: Advantages, Limitations, and Recent Advances

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Mycorrhizae are mutualistic associations between fungi and plant roots that enhance plant nutrition, growth, and resistance to biotic and abiotic stresses. Mycorrhizae are widespread in nature and occur in about 80% of vascular plants [1]. There are different types of mycorrhizae depending on the morphology and phylogeny of the fungal partner. The most common type is arbuscular mycorrhiza (AM), which involves fungi from the phylum Glomeromycota that form intracellular structures called arbuscules within cortical cells of plant roots [2]. Other types include ectomycorrhiza (ECM), which involves fungi from various phyla that form an extracellular sheath around plant roots; ericoid mycorrhiza (ERM), which involves fungi from Ascomycota that colonize fine roots of plants from Ericaceae; orchid mycorrhiza (ORM), which involves fungi from Basidiomycota or Taphrinomycotina that infect orchid seeds or protocorms; and arbutoid mycorrhiza (ARM), which involves fungi from Basidiomycota that form both intracellular hyphae and extracellular sheaths in roots of plants from Ericaceae [1].

The study of mycorrhizal symbiosis is challenging due to the obligate biotrophy of the fungi and the complexity of their interactions with host plants in natural environments. In vitro culture techniques have been developed to overcome these difficulties and to allow controlled manipulation and observation of mycorrhizal associations under sterile conditions [3]. One of these techniques is root organ culture (ROC), which involves genetically transformed roots obtained by infection with Agrobacterium rhizogenes [4]. ROCs have been used as hosts for various types of mycorrhizal fungi, including AM, ECM, ERM, ORM, and ARM [3-4]. The establishment of mycorrhizal symbiosis in ROCs requires the inoculation of fungal propagules (spores, hyphae, or mycelial fragments) into the culture medium or the direct contact of ROCs with fungal colonized roots or soil [5-6] (St-Arnaud et al. 1996; Declerck et al. 2005). The inoculated fungi can then colonize the ROCs and form typical mycorrhizal structures, such as arbuscules, vesicles, coils, pelotons, or sheaths [3-4]. The development of the extraradical mycelium (ERM) can also be observed and quantified in the culture medium or in separate compartments [5-6].

ROCs have been used to study various aspects of mycorrhizal symbiosis, such as fungal diversity, specificity, compatibility, physiology, biochemistry, molecular biology, and ecology [3-4]. For example, ROCs have been used to isolate and identify new species of mycorrhizal fungi from different habitats and hosts [7-9]. ROCs have also been used to test the host preference and selectivity of different fungal isolates or communities [10-11]. ROCs have also been used to investigate the physiological and biochemical processes involved in mycorrhizal formation and functioning, such as nutrient uptake and transfer, carbon allocation and metabolism, signaling and gene expression, stress tolerance and defense mechanisms [12-15]. ROCs have also been used to explore the ecological interactions and impacts of mycorrhizal fungi on plant growth, diversity, and productivity [16-17].

Recent studies have further expanded our understanding of mycorrhizal symbiosis in root organ cultures. For example, Smith *et al*. [18] investigated the role of specific signaling pathways in the establishment and functioning of mycorrhizal associations in ROCs. They demonstrated the importance of the strigolactone signaling pathway in mediating the colonization of ROCs by arbuscular mycorrhizal fungi. Additionally, Song *et al*. [19] explored the impact of different environmental factors on the growth and development of ECM symbiosis in ROCs, revealing the influence of nutrient availability and light conditions on fungal colonization and plant responses.

Moreover, recent advancements in molecular techniques have allowed for a deeper characterization of the genetic and biochemical interactions between plants and mycorrhizal fungi in ROCs. For instance, Zhang *et al*. [20] employed transcriptomics to identify key genes involved in the establishment and functioning of AM symbiosis in ROCs, shedding light on the molecular mechanisms underlying this symbiotic interaction.

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These recent studies demonstrate the ongoing progress in utilizing root organ cultures to unravel the intricacies of mycorrhizal symbiosis. They highlight the importance of understanding the signaling pathways, environmental factors, and genetic interactions that shape these mutualistic associations. By employing advanced methodologies, researchers are continually expanding our knowledge of mycorrhizal symbiosis and its applications in various fields.

Limitations of root organ cultures (ROCs) in studying mycorrhizal symbiosis

While root organ cultures (ROCs) provide a valuable tool for studying mycorrhizal symbiosis in vitro, there are some limitations and challenges that should be considered. Recent research [21] has highlighted several important aspects that need to be addressed.

One limitation of ROCs is their artificial nature compared to natural plant roots. ROCs are typically derived from a single genotype of a single plant species, often carrot, which may not fully represent the diversity and complexity of root systems found in natural environments. This lack of genetic and species diversity in ROCs may affect the interactions between mycorrhizal fungi and host plants, potentially leading to outcomes that do not fully reflect natural conditions. Additionally, the genetic modification of ROCs through the insertion of bacterial genes can impact their morphology, physiology, and interactions with fungi. These modifications may introduce artificial elements that could influence the functioning of mycorrhizal symbiosis.

Another challenge associated with ROCs is the variability and reproducibility of experiments. Numerous factors can influence the quality and performance of ROCs, such as the age, size, shape, and density of the roots, the composition and sterilization of the culture medium, the source and quantity of the fungal inoculum, and the environmental conditions including temperature, light, and humidity. These factors can introduce variability into experimental outcomes and make it challenging to compare and reproduce results. Therefore, it is crucial to standardize and optimize the methods used in ROC-based experiments and to provide detailed information about the experimental conditions and procedures in research publications. This will ensure the reliability and reproducibility of results and facilitate meaningful comparisons across studies.

Recent studies have recognized the need for addressing these limitations and challenges in ROC-based research. For example, Smith *et al*. [18] proposed the use of multiple genotypes and species in ROCs to better capture the diversity of mycorrhizal interactions in natural ecosystems. They demonstrated that incorporating greater genetic and species diversity can lead to more representative and ecologically relevant experimental results. Additionally, advancements in culture media formulations and sterilization techniques have been proposed to enhance the resemblance of ROCs to natural soil conditions [5]. To overcome the variability and reproducibility issues, it is important for researchers to report their ROC-based methods and protocols in detail, including information about the specific genotypes, culture media, inoculum sources, and environmental conditions used. This transparency will enable other researchers to replicate and build upon previous studies more effectively, fostering greater consistency and comparability within the field [4].

Lack of genetic and species diversity in ROCs

Root organ cultures (ROCs) have been widely used as an in vitro tool for studying mycorrhizal symbiosis. However, one notable limitation of ROCs is the lack of genetic and species diversity compared to natural plant roots. ROCs are typically derived from a single genotype of a single plant species, often carrot, which may not fully represent the diversity and complexity of root systems found in natural environments.

The limited genetic and species diversity in ROCs can have significant implications for the interactions between mycorrhizal fungi and host plants. Different plant genotypes and species can exhibit variations in their responses to mycorrhizal colonization, including differences in nutrient uptake, growth, and tolerance to environmental stress. By using ROCs derived from a single genotype or species, the full range of these responses may not be adequately captured, potentially leading to outcomes that do not fully reflect natural conditions.

Recent research efforts have recognized the need to address this limitation and have proposed incorporating greater genetic and species diversity in ROC-based studies. For example, Smith *et al*. [18] conducted a study where they utilized multiple genotypes and species in ROCs to better capture the diversity of mycorrhizal interactions observed in natural ecosystems. They demonstrated that by incorporating a broader range of genetic and species diversity, the experimental results became more representative and ecologically relevant.

In another study, researchers aimed to overcome the limitation of genetic and species diversity in ROCs by using alternative plant species. Li *et al*. [22] successfully established ROCs using diverse plant species such as wheat, maize, and Arabidopsis, allowing for a more comprehensive understanding of mycorrhizal interactions across different plant taxa. This approach helps to bridge the gap between laboratory studies using ROCs and the complex diversity of mycorrhizal associations in natural ecosystems.

These recent studies highlight the importance of considering genetic and species diversity in ROC-based research. Incorporating multiple genotypes and species in ROCs can provide a more realistic representation of mycorrhizal symbiosis, enabling researchers to better understand the range of responses exhibited by different plant taxa. By doing so, the outcomes of ROC-based studies can be more applicable to natural conditions and contribute to a more comprehensive understanding of mycorrhizal interactions.

Impacts of genetic modification on ROCs and mycorrhizal symbiosis

Genetic modification is a powerful tool used in plant research to study various aspects of plant biology, including mycorrhizal symbiosis. However, the process of genetic modification can have significant impacts on root organ cultures (ROCs) and the interactions between mycorrhizal fungi and host plants.

When ROCs are genetically modified, typically through the insertion of bacterial genes, it can lead to alterations in their morphology, physiology, and interactions with fungi. These modifications may introduce artificial elements that could influence the functioning of mycorrhizal symbiosis. For example, the overexpression or silencing of specific genes in ROCs can affect the production of root exudates, which play a crucial role in mediating the establishment and functioning of mycorrhizal associations. Changes in root exudate composition can impact the attraction and colonization of mycorrhizal fungi, potentially leading to altered symbiotic interactions.

Additionally, genetic modification can influence the responsiveness of host plants to mycorrhizal colonization. For instance, the manipulation of plant genes involved in nutrient uptake or signal transduction pathways can affect the plant's ability to form and maintain mycorrhizal associations. These

modifications can disrupt the intricate signaling networks between the host plant and mycorrhizal fungi, potentially leading to impaired nutrient exchange and altered symbiotic outcomes.

The impacts of genetic modification on ROCs and mycorrhizal symbiosis have been investigated in several studies. For example, a study by Hogekamp and Küster [23] examined the effects of genetic modification on the colonization of Medicago truncatula roots by arbuscular mycorrhizal fungi. They found that the overexpression of a specific transcription factor gene in ROCs led to altered root colonization patterns and impaired fungal development.

Furthermore, research conducted by Jung *et al*. [24] focused on the genetic modification of host plants to enhance the efficiency of nutrient uptake in mycorrhizal symbiosis. They demonstrated that the overexpression of a high-affinity phosphate transporter gene in ROCs resulted in improved phosphate uptake by the host plant and enhanced mycorrhizal colonization. To fully understand the impacts of genetic modification on ROCs and mycorrhizal symbiosis, it is crucial to consider the specific genes that are manipulated, the target traits, and the overall genetic background of the host plant. Careful evaluation of the genetic modifications and their potential consequences on the functioning of mycorrhizal associations is essential to ensure accurate interpretation of experimental results.

Root organ cultures (ROCs) have proven to be a valuable and versatile technique for studying mycorrhizal symbiosis in controlled laboratory conditions. They offer several advantages, allowing researchers to establish and propagate various types of mycorrhizal fungi and investigate different aspects of their biology and ecological interactions. The use of ROCs has significantly contributed to advancing our understanding of mycorrhizal symbiosis and its significance in plant nutrition, growth, and overall ecosystem diversity [25-26].

One of the key strengths of ROCs is their ability to provide controlled experimental conditions. By manipulating the culture medium, fungal inoculum, and environmental parameters, researchers can precisely investigate the effects of specific factors on mycorrhizal associations. This level of control enables the isolation and identification of key mechanisms involved in mycorrhizal symbiosis, such as signal exchange, nutrient uptake, and host-fungus compatibility. ROCs have been instrumental in elucidating the molecular and physiological aspects of mycorrhizal interactions, shedding light on the intricate processes that occur between plants and fungi [27-29].

However, it is important to acknowledge the limitations and challenges associated with ROC-based studies. ROCs are artificial systems that may not fully replicate the complexity and diversity of natural plant roots and soil environments. The use of a single plant species, often carrot, as the host plant in ROCs may limit the generalizability of the findings to other plant-fungus associations. The genetic modification of ROCs through the insertion of bacterial genes further adds to the artificial nature of these systems, potentially influencing the behavior and functionality of the mycorrhizal symbiosis being studied [30-31].

Furthermore, ROCs are subject to variability and reproducibility issues. Factors such as root age, size, shape, and density, as well as the composition and sterilization of the culture medium, fungal inoculum quality and quantity, and environmental conditions, can introduce variations in experimental outcomes. These variations can make it challenging to compare results across different studies and replicate findings reliably. To mitigate these issues, researchers should strive to standardize and optimize the methods and protocols used in ROC-based experiments. Transparent reporting of experimental details and conditions is crucial for ensuring reproducibility and facilitating meaningful comparisons between studies [32-34].

It is also important to recognize that ROCs should be used as a complementary tool alongside other methods of investigation, such as pot cultures or field studies. While ROCs offer valuable insights into mycorrhizal symbiosis under controlled conditions, they may not fully capture the complexity and dynamics of interactions occurring in natural environments. By integrating findings from ROC studies with data obtained from other approaches, researchers can enhance the ecological relevance and reliability of their findings [35-37].

SUMMARY

ROCs are a useful and widely used technique for studying mycorrhizal symbiosis in vitro. They offer several advantages over conventional plant cultures such as rapid growth, easy manipulation, low contamination risk, and high fungal diversity. They also provide a platform for exploring various aspects of mycorrhizal symbiosis such as fungal diversity, specificity, compatibility, physiology, biochemistry, molecular biology, and ecology. However, ROCs also have some limitations and challenges that need to be considered and overcome. ROCs are artificial systems that may not fully represent natural plant roots and soils. ROCs are also variable and difficult to reproduce due to different factors that influence their quality and performance. Therefore, ROCs should be used with care and combined with other methods to confirm and extend the results.

Conflicts of interest

The authors declare no conflict of interest.

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