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Bibliographic review

New challenges in the production of inoculants from arbuscular mycorrhizal fungi in Cuba

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ABSTRACT

Arbuscular mycorrhizal fungi (AMF) are an edaphic microorganisms group that establish symbiosis with numerous plant species of agricultural interest. Production of mycorrhizal inoculants has experienced an increase since the last decade, with the emergence of products in various formulations aimed at guaranteeing their insertion in the different crop technologies. However, practical experiences with the use of these formulations have shown that there is no universal carrier and that the choice of inoculants depends on the characteristics of the crops and the conditions for their management. Cuban's experience with the mycorrhizal symbiosis's management, using solid formulations, has provided significant results over more than 20 years of sustained research and represents a current model to be considered in the implementation of new studies related with the inclusion of these symbionts in current agricultural practices. The 21st century marked a new stage in the improvement of inoculants based on these fungi with the obtaining of a liquid formulation with a view to diversify the forms of application of these symbionts, from their inclusion in agricultural systems through fertigation. In the present review aspects related to the technologies for the reproduction of fungal propagules, the inoculants productions, as well as the potentialities of the AMF inoculation in liquid support will be addressed.

Key words: arbuscular mycorrhizae, inoculation, diversification, agriculture

INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) represent a group of edaphic microorganisms that establish symbiosis with numerous plant species of agricultural interest [1]. Among the main benefits of this interaction are: the direct effects on mineral nutrition; especially in the absorption of macro and micronutrients [2,3], the induction of tolerance against conditions of biotic (eg phytopathogenic) [4] and abiotic (eg drought and salinity) [5,6], their participation in phytoremediation processes [7,8] and their contribution to the stability of soil aggregates [9].

The inoculation of the plants has been the most generalized mechanism for the inclusion of these symbionts in the agricultural processes [10], which caused an increase in the production of mycorrhizal inoculants, from the emergence of new companies and chains of distributors that guided its strategies to obtain products in various formulations (solids and liquids) [11,12]. However, practical experiences with the use of these formulations showed that there is no universal carrier [10] and some reports suggest that the choice of inoculants depends on the characteristics of the crops and the conditions for their management [13].

Research related to the management of mycorrhizal symbiosis in Cuba, based on the inoculation of solid formulations (EcoMic®), established the criteria for its application in correspondence with the edaphic environment, which contributed to establishing a strain recommendation system Efficient by soil type [14] and under suboptimal nutrient supply conditions to that required by non-mycorrhized plants [15]. However, the success achieved in our country with the traditional solid formulation, it was necessary to obtain a new inoculant, in liquid formulation, that would allow the application of AMF through fertigation systems, as a way to diversify the use of these microorganisms to more current production technologies and reduce the volumes of product to be handled [16].

Fertigation is an advanced technology that optimizes the consumption of water and nutrients based on the phenological stages of the crop [17]. Currently, its implementation in our country is enhanced, as part of the agroindustrial policy reflected in the guidelines of 156, 157 and 158 of the Communist Party of Cuba [18].

For this reason, in this review some aspects related to the technologies for the reproduction of fungal propagules involved in obtaining inoculants will be addressed, as well as the background related to the management of AMF in liquid support.

Arbuscular mycorrhizal fungi

Mycorrhizae are mutualistic associations that are established between certain soil fungi and the roots of most vascular plants with varied morphological patterns [19], but the most common is arbuscular mycorrhizal symbiosis, represented in more than 74 % of plants terrestrial, which demonstrates that the essential genetic characters for their association are similar in a large part of the plant kingdom [20]. Some fossil evidence demonstrated the existence of this type of association for more than 450 million years in the tissues of the first land plants [21].

From the morphological characters of the spores, about 244 species of AMF have been described [22], however, it is estimated that this fungal diversity can range from 341 to 1600 species considering the sequence of the ribosomal DNA [23,24]. Recently the fungi forming this type of mycorrhizae were reclassified to the *Phylum Mucoromycota* and the *Glomeromycotina* class [25].

The character of obligated symbionts of HMAs is given because they cannot complete their life cycle in the absence of a host root [19]. Among the factors that explain this condition, the metabolism or the absorption of carbon in the presimbiotic stage is indicated, since extra-radical hyphae are unable to absorb carbohydrates [26]. HMA receives carbon from the photosynthesis of the host plant and mycelium growth is promoted by forming an extensive network of hyphae in the soil that transport water and nutrients to the internal compartments of the plant [27]. Some studies have estimated that between 4 and 17 % of photosynthetic carbon is consumed by the fungus for its metabolism [28].

Among the main benefits that this symbiosis confers on the host plants are the increase in the absorption of water and nutrients [10,29], which favors the establishment and survival of the plants [30,31]; the tolerance of plant species against conditions of biotic (phytopathogenic) and abiotic (drought and salinity) stress [32]; the increase in nutrient availability for plants [33], which allows a rational use of chemical fertilizers [34]; In addition to its contribution to the stability of soil aggregates [35] and its effect on ecological restoration [34].

Methodologies used in the reproduction of fungal propagules. Production of inoculants based on HMA

The management of mycorrhizal symbiosis via inoculation has been the most widespread mechanism for the inclusion of AMF in current agricultural practices, corresponding to the characteristics of crop technologies and requirements of each plant species (36-38).

In this sense, the production of mycorrhizal inoculants experienced a rise (40 %) since the last decade in the European continents (United Kingdom, Czech Republic, Germany, Switzerland, Spain and France), American (United States, Canada, Chile, Peru, Mexico, Colombia, Ecuador and Guatemala) and Asian (China and Japan) (10).

Similarly, an increase in the registration of mycorrhizal inoculants (15 %) and in the creation of new companies and distributor chains (11) that identified their opportunities in sectors for nursery production, horticulture and ornamental plants (39,40), as well as in phytoremediation in soils with high heavy metal contents (41).

Other research indicated that the selection and reproduction of HMA species constitutes a decisive stage in the process of formulating the inoculants (41) and highlighted the direct participation of international collections [INVAM (http://invam.wvu.edu) , IBG (http://www.kent.ac.uk/bio/beg) or GINCO (http://www.mycorrhiza.be/ginco-bel/)] in the isolation, characterization and multiplication of strains, based on criteria related to their functioning (colonization capacity and spore germination rate) (22).

Recently, the use of new techniques in some developed countries (Canada, China, Germany and Belgium) has allowed the incorporation of molecular methods, based on ribosomal DNA gene sequences (rDNA) and PCR techniques with specific primers, such as tool that allows characterizing and evaluating the behavior of inoculated AMF species (42,43).

HMAs require a host to complete their life cycle, so that the cultivation system from the use of trap plants is recognized as a classic method and the most widespread in its reproduction (19). Various materials can be used for this purpose (example: soil, peat, perlite, vermiculite, sand, clay, calcined clay) or combinations of some of them (10,11,44,45).

Some criteria indicate that through this technique inoculants are obtained that can be applied from the sowing of the crop and at the stage of transplantation, but it has a disadvantage that they require sterilization of the substrate to be used, in addition to being usually heavy and difficult to transport (1.46).

In other techniques such as the hydroponic system, rootlets of previously colonized plants are used that come into contact with a nutrient solution in constant motion. This method has the disadvantage that a pH control of the solution must be carried out and they also require the application of nutritive solutions with a certain systematicity [11,40].

These systems have been refined to reach more complex technologies that involve in vitro culture methods for the reproduction of AMF from the use of transformed roots that are colonized and subcultured in controlled environments [46]. Through this methodology, a homogeneous material free of phytopathogens is obtained, but it is appropriate to point out that they are highly specialized techniques [1].

On the other hand, some research projects have focused on improving the methods of reproduction of AMF towards the search for new alternatives that guarantee the gradual release of microorganisms to the soil after inoculation [47,48]. In this sense, the immobilization of structures (spores, small segments of hyphae or colonized roots) using the encapsulation technique, by means of gelling carriers or other similar substances, represents a step forward for the management of new formulations for agricultural purposes [49,50].

HMA reproduction methods define, in most cases, the type of carrier to be used in the formulation of mycorrhizal inoculants [11]. Some research points to the non-existence of a universal carrier and, in turn, highlights that the one selected for this purpose must [51]:

- Provide a suitable microenvironment for the microorganism.
- To possess adequate physical and chemical properties that guarantee the survival and stability of fungal structures.
- Ensure its compatibility with new agricultural technologies (machinery) [51].

With regard to the composition of the inoculants, practical experience indicates that in many of the experiments, regardless of the conditions being evaluated and the proposed objectives, the application of a single species is carried out, highlighting *Rhizoglomus irregularulare* and *Funneliformis mosseae* [10,52]. Aspects related to their ability to colonize various groups of plant species, their easy and rapid multiplication, their resistance to long conservation periods and their wide distribution in various ecosystems, make these species suitable symbionts to be used in the formulation of inoculants [2,3].

Some reports indicate that one of the strategies adopted by some companies to enhance the commercialization of their formulations in the international market, is to obtain mixed inoculants (composed of several species of AMF) or by other microorganisms (plant growth promoting bacteria) [13,40].

The mycorrhizal inoculants that are currently marketed can be composed of spores, colonized root fragments and hyphae or by the combination of these propagules [(53)] and are found in solid, liquid or gels support [(11,40,54)].

General aspects of liquid inoculants

Biofertilizers in liquid support are aqueous suspensions (they can be composed of crop broth, mineral or organic oils, or polymers) [(52)] that have gained popularity in recent decades for their easy handling and their synchronization with the new technologies associated with current agricultural practices [(41)].

Reference is made in the literature that some advantages of these formulations may be related to: (I) the high concentration (cells or microorganisms) which allows optimizing their applications [(55)], (II) the incorporation of additives (sucrose or glycerol) that favor the survival of microorganisms and extend their viability [(50)] and (III) the adjustment with current agricultural systems (compatibility with agricultural implements or fertigation systems) which facilitates their application under production conditions [(12)].

Background related to the application of AMF in liquid support. Practical experiences

The Cuban experience with the management of mycorrhizal symbiosis, using solid formulations, has provided significant results over more than 20 years of sustained research and represents a current model to consider in the implementation of new studies related to the inclusion of these symbionts in current agricultural practices.

In this sense, with the commercial inoculant EcoMic®, obtained at the National Institute of Agricultural Sciences (INCA), several experiments were conducted on different crops of agricultural importance [such as soybeans (*Glycine max* L.), sorghum (*Sorghum bicolor* L .), rice (*Oryza sativa* L.), corn (*Zea mays* L.), beans (*Phaseolus vulgaris* L.), sunflower (*Helianthus annuus* L.), cassava (*Manihot esculenta* Crantz), wheat (*Triticum aestivum* L.) and vegetables, etc.], demonstrating significant increases in yields between 15 and 40 % depending on the plant species [(56)].

Similarly, with these investigations some criteria were established for the management of mycorrhizal symbiosis in correspondence with the edaphic environment, based on the recommendation of efficient strains by edaphic condition [57]. Likewise, other contributions focused on the effective integration of mycorrhizal symbiosis, via inoculation of efficient strains, in the presence of nutrient supply systems [56] and other production technologies such as coffee tree posture management [58], green manures [59], grassland [60] and banana in vitro plants [61].

But the 21st century marked a new stage in the improvement of inoculants based on these fungi in Cuba with the obtaining of a liquid formulation LicoMic®, whose main objective was to diversify the ways of application of these symbionts from their inclusion in the systems agricultural through fertigation [16].

Initially, the effect of Glomus fasciculatum application on solid and liquid support on lettuce (*Lactuca sativa* L.), cucumber (*Cucumis sativus* L.) and tomato (*Solanum lycopersicum* L.) crops was evaluated and both formulations increased mycorrhizal efficiency (25 %), extraction of mineral nutrients and crop yields under the experimental conditions studied [62]. Subsequently, the viability and germinative capacity of spores of two Glomus species in liquid support was evaluated and stability was obtained greater than eight months in fungal propagules, with colonization percentages (25-28 %) that demonstrated the functioning of both species [63].

In other studies, the efficiency of different mycorrhizal inoculants (EcoMic® and LicoMic®) in tomato plants (*S. lycopersicum* L.) was compared and despite the variations in mycorrhizal colonization marked by the stages of crop development, the fungal values (50 %) in the fruiting stage in the presence of liquid inoculation [64].

Similarly, the effect of the application of two mycorrhizal inoculants (liquid and solid) in the cultivation of durum wheat (Triticum durum L.) under field conditions was evaluated and the positive action of both formulations on the variables related to Mycorrhizal function, nutritional status and crop yield [65].

In other investigations, the effect of Glomus hoi liquid support inoculation on the growth and production of rice (Oryza sativa L. 'INCA-LP5') under salinity conditions was evaluated and the effectiveness of said inoculant in stimulating growth was demonstrated, development and crop production [66].

Subsequently, other trials were conducted to study the protective effect of *Glomus iranicum var tenuihypharum* inoculated in liquid support in durillo plants (*Viburnum tinus*) treated with wastewater and an efficient colonization was found in the roots of the crop with significant increases in the levels of Glomalin and carbon in the soil [67].

Other authors verified the functional viability of *Glomus cubense* in liquid support under controlled conditions and demonstrated that said inoculant retained its stability and favored growth indicators in sorghum plants (10 %) (*S. bicolor* L. Moench) [68].

Other investigations were carried out to know the effect of different doses of a mycorrhizal inoculant in liquid support (75, 150 and 300 spores per plant) in the variables of growth, water relations and efficiency in the use of nutrients from tomato plants (*S. lycopersicum* L.). The results showed that symbiosis was favored with any of the applied doses, with increases in fungal variables, dry biomass content, water relations and better efficiency in nutrient utilization compared to non-mycorrhized plants [69].

CONCLUSIONS

- With the results previously, it was shown that the inoculation of AMF in liquid formulation induced positive responses in mycorrhized plants, however, it is important to highlight that the studies developed so far with this inoculant do not vary the criteria for selection of established strains with solid inoculum, which favors the management of symbiosis for different agroecosystems.
- These aspects suggest that the management of AMF in liquid formulation can be effective as well as the solid inoculant and allows establishing new avenues for the management of these symbionts, with a view to diversifying their forms of application through fertigation systems and favoring Agricultural productions
- Therefore, the use of AMF in liquid formulation can become an agricultural practice that faces new challenges and approaches in the management of these symbionts in the face of the different crop technologies in force in the Cuban agricultural model.

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