Chapter

Exploring the Potential of *Serendipita indica* in Agriculture

Adolfo Posada, Rodrigo Patiño and Dagoberto Castro

Abstract

This document discusses the characteristics and applications of the facultative endophytic fungus *Serendipita indica*, emphasizing its colonization of plant roots, enhancement of nutrient absorption, and promotion of plant growth under stressful conditions. *S. indica* can grow axenically on synthetic growth media and its symbiotic association with various plant species is highlighted. The production of chlamydospores, which aid in plant resistance and survival, is described. Additionally, the document outlines methods for cultivating *S. indica*, including its propagation in liquid media and inoculation of in vitro *Mentha spicata* plantlets. Experimental results demonstrate the significant impact of *S. indica* on plant survival and biomass. Furthermore, the fungus's role in enhancing plant tolerance to pathogens and environmental stresses is discussed, highlighting its potential as a sustainable solution for improving plant development and agricultural productivity.

Keywords: endophytic root fungus, biotic stress, plant biostimulant, chlamydospores, *Mentha spicata*

1. Introduction

Serendipita indica is a facultative endophytic fungus belonging to the order Basidiomycetes. This fungus colonizes the roots of both monocotyledonous and dicotyledonous plants [1]. According to Varma et al. [2], *S. indica* shares many similarities with arbuscular mycorrhizal fungi (AMF), but unlike them, it can grow axenically in synthetic media. Colonization occurs in the root zone of plants and begins with the germination of chlamydospores, followed by the formation of a hyphal network in and within the root.

The hyphae branch out and continue to develop by penetrating the subepidermal layers of the root, with maximum colonization occurring in the zone of cellular differentiation [3]. Once the fungus is inside the plant, a symbiotic association develops.

Colonization by *S. indica* increases nutrient absorption, enabling plant survival under unfavorable abiotic conditions, such as drought, sudden temperature changes, and salinity stress. Additionally, it confers systemic resistance to toxins, heavy metal ions, and pathogenic organisms; it stimulates seed development and production [4, 5].

The mode of action of *S. indica* involves interactions with phytohormones, metabolites, photosynthates, and gene regulation. Assimilation of macronutrients, such as phosphorus, potassium, nitrogen, magnesium, and sulfur has been demonstrated in crops such as *Poncirus trifoliata*, *Triticum aestivum*, *Brassica napus*, *Oryza sativa, Panicum miliaceum,* and *Arabidopsis thaliana*, among others [6–8].

S. indica promotes the production of various solutes in plants, such as proline, sucrose, polyols, glycine, and betaine, as well as antioxidant enzymes like catalase, ascorbate peroxidase, and superoxide dismutase, protecting various types of stress [9].

Similarly, colonization by *S. indica* in plant roots has been shown to enhance tolerance to bacterial pathogens, viruses, and fungal diseases. Some authors have demonstrated its action as a biocontrol agent against pathogens such as black spots in cabbage caused by *Alternaria brassicicola* [10], onion leaf blight caused by *Stemphylium* [11], and *Rhizoctonia solani* in tomato [12].

The purpose of this paper was to evaluate the production of the fungus *S. indica* in axenic nutrient media and to assess its effect on *M. spicata* plants inoculated under in vitro conditions.

2. Production of *S. indica* in synthetic culture media

The growth of this microorganism in synthetic culture media forms colonies that are white and velvety, with rapid radial growth. The mycelium is hyaline, with cylindrical hyphae having very thin walls typical of the fungus (**Figure 1A**). Spores in the shape of a pear, known as chlamydospores, are produced from these hyphae (**Figure 1B**).

2.1 Scale propagation of *S. indica* production

Commercial-scale propagation can be carried out in agitated liquid media at 110 rpm and 30°C (**Figure 2A**), and after 10–12 days, structures in the form of rounded cellular aggregates are formed (**Figure 2B**). Microscopic observations reveal mycelium and the beginning of chlamydospore formation (**Figure 2C**), and finally, spine-like structures form from which spores differentiate (**Figure 2D**). The concentration of chlamydospores was determined with the aid of a Neubauer chamber and was estimated at 1×10^6.

An important aspect is the production of chlamydospores, which correspond to enlarged vegetative cells with thick walls, exhibiting varied shapes and condensed cytoplasm, formed within hyphae or at the tips of hyphae. They have been observed in the three main clades of the fungal kingdom, particularly in the order of basidiomycetes [13].

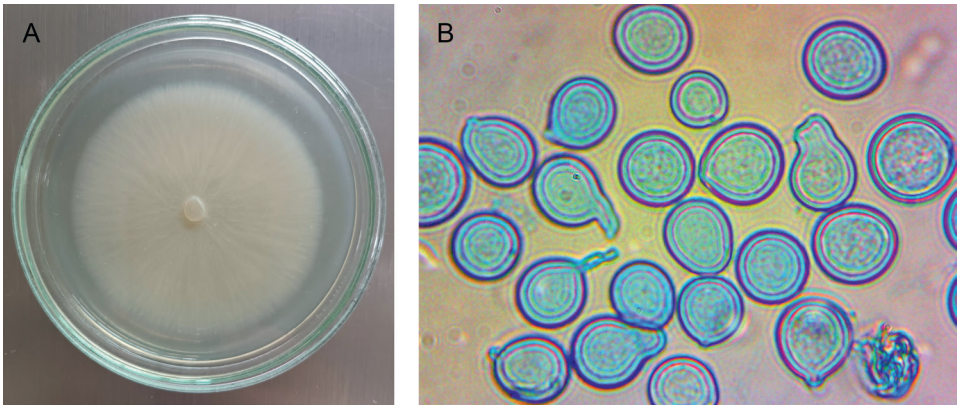

Figure 1.
Morphological characteristics of Serendipita indica*. A. Development of a colony of* S. indica *in a semisolid medium. B. Formation of chlamydospores in liquid media (photographs by Bioquirama S.A.S.).*

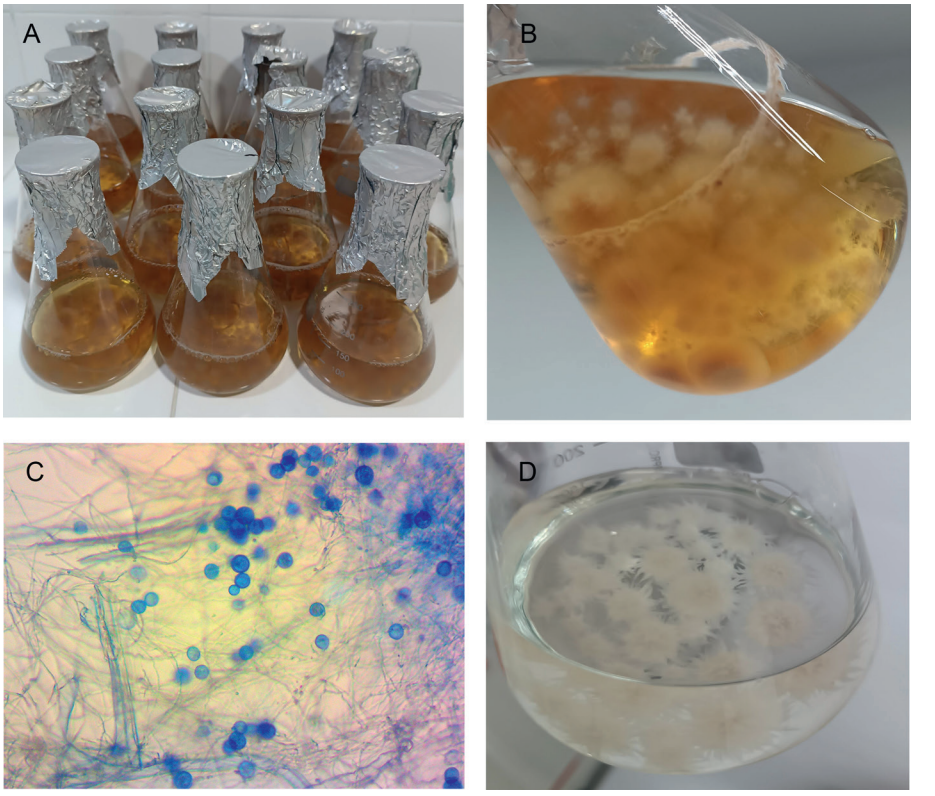

Figure 2.
Production scaling of Serendipita indica *in liquid media. A. Cultivation of* S. indica *mycelium in liquid medium under agitation. B. Formation of cellular aggregates of mycelium. C. Microscopic observation of the mycelium. D. Formation of spine-like structures. (Photographs by Bioquirama S.A.S.).*

Among the biological functions of chlamydospores, their resistance to dehydration stands out. They are produced within plant roots during drought and are transported in fragments. They germinate when they encounter favorable conditions of moisture and temperature.

2.2 Plant material

Microplants shoots of *Mentha spicata* were developed in an MS medium [14], supplemented with 3% sucrose and 0.5 mg/L benzyladenine in a semisolid medium, and were used as source materials. Rooting of plantlets was conducted under completely aseptic conditions in a culture medium composed of MS mineral salts added with 3% sucrose, 0.5 mg/L indole butyric acid, and 0.7% agar-agar. The internodes were planted in nine plastic boxes, each containing 15 plantlets, and incubated at 28°C with a photoperiod of 12 hours light, using fluorescent light lamps FFF 80 μmol m^{-2} s^{-1}. Thirty days after sowing, plantlets formed roots.

2.3 In vitro inoculation of *S. indica* in *Mentha spicata* shoots

The inoculum was prepared from a suspension of *S. indica* spores in sterile water in 2-mL microtubes. Spore concentration was determined using a Neubauer chamber.

Inoculations with the microorganisms consisted of adding a suspension of 2 mL of *S. indica* to the in vitro plantlets under aseptic conditions at a final concentration of 1.0×10^5 spores/mL.

2.4 Evaluation of the effect of microplants biotization on survival during the acclimatization phase and dry biomass

Using the aforementioned methodology, in vitro rooted plantlets were inoculated with *S. indica* and left to incubate for 10 days (**Figure 3A**). Then microplants were transferred to a humid chamber and planted in seedling trays with peat moss, previously sterilized with autoclave for 3 hours at 121°C (**Figure 3B**). The plants were kept for a period of 30 days, after which measurements of survival and growth of the seedlings were made. A randomized trial with 45 plants per treatment was conducted, where the treatments corresponded to plants biotized with *S. indica* and a control group of plantlets without endophyte. For the determination of dry mass, five plants were randomly selected from each treatment. The entire plants were weighed on an analytical balance (MF) and then placed in an oven with a forced draft of air at 65°C until constant weight.

2.5 Assessment of survival and dry mass of hardened *M. spicata* seedlings

According to the results shown in **Figure 4A**, significant differences were observed regarding the survival of seedlings during the acclimatization process. Seedlings inoculated with *S. indica* exhibited a 90.5% survival rate, whereas control plants had a 76% survival rate. Similarly, the dry mass of plants inoculated with *S. indica* averaged 0.55 g/plant, while control plants averaged 0.37 g/plant (**Figure 4B**).

Figure 3.
Inoculation and acclimatization process of M. spicata *plants. A. In vitro inoculation of plants during the rooting phase with* S. indica *fungus. B. Acclimatization of plants produced in vitro in seed trays.*

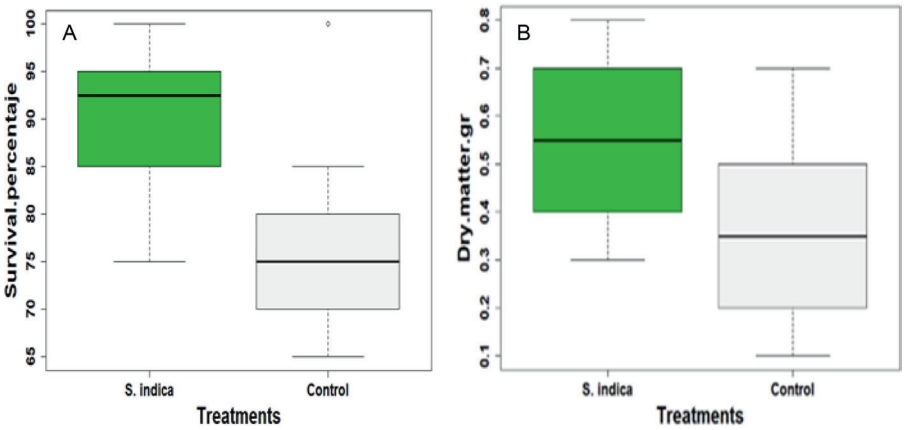

Figure 4.
Response of Mentha spicata *plants to in vitro inoculation with* Serendipita indica*. A. Percentage of survival of* M. spicata *seedlings. B. Dry biomass of 30-day-old seedlings. Significant differences were found between the means of the treatments (P < 0.05; Tukey HSD).*

Ref. [15] Indicated that *S. indica* is a microorganism that assists plants in growth, nutrient absorption, and environmental stress tolerance when in coexistence with them. Inoculated plants respond positively to *S. indica* through various mechanisms, including improved root development and plant growth, which may explain the results obtained with *M. spicata* [16]. Additionally, it enhances water and nutrient absorption [17].

The results obtained from the symbiosis of *S. indica* with *M. spicata* included an increase in dry mass, which, according to Nanda et al. [18], this fungus aids in enhancing chlorophyll contents and photosynthetic rates, thereby improving plant development.

2.6 Determination of root colonization of *M. spicata* by *S. indica*

Thirty days after transplanting to seedling germination trays, root samples were taken, and staining was performed according to the techniques described by Phillip and Hayman [19]. Roots were washed with water and cut into 1.0 cm fragments, then placed in a 10% KOH solution for 15 minutes. Subsequently, root segments were neutralized with 1 N HCl and thoroughly washed with water. Staining was conducted with a 0.05% trypan blue solution for 12 hours and then mounted in lactophenol for microscopic observations. Fragments were placed in a hemocytometer for analysis under a microscope. Colonization was evaluated using the method of Giovannetti and Mose [20]. The percentage of colonization was calculated as follows:

$$\text{Percentage of Colonization} = \left(\begin{array}{l}\text{Number of Colonized Segments /}\\ \text{Total Number of Observed Segments}\end{array}\right)^{*} 100 \quad (1)$$

2.7 Colonization by *S. indica*

According to the results shown in **Table 1**, the colonization percentage was 70%.

Microscopic studies of *M. spicata* roots treated with *S. indica* revealed extensive inter- and intracellular root colonization with chlamydospores (**Figure 5A** and **B**). Hifal colonization was observed on the root surface and in the intercellular and intracellular spaces of the root cortex. Chlamydospores were found in both single

Treatment	Colonization percentage (%)
Plants inoculated with *S. indica*	70
Control (non-inoculated plants)	0

Table 1.
Colonization of S. indica *in* M. spicata *roots.*

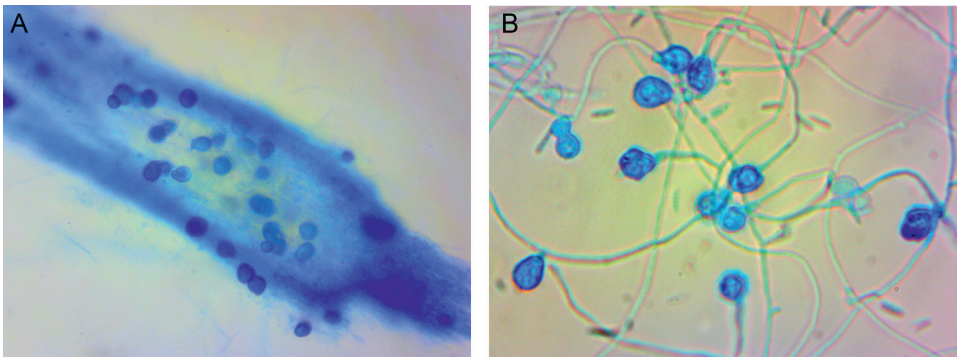

Figure 5.
Spores of S. indica *were observed under a conventional microscope. A. Root of* M. spicata *with inter- and intracellular spores. B. Spores in areas adjacent to the root zone.*

and double spore forms. The spore shapes varied from round to ovoid, with some exhibiting the typical pear-shaped structure.

3. Conclusions

The endophytic fungus *S. indica* can be cultivated on a large scale in liquid culture media at concentrations of 1.0 x 10^6 spores/mL, making it a viable option for enhancing plant development. One advantage is that chlamydospores are produced, which serve as resistance structures that associate with roots, positively impacting plant development and physiology.

Inoculating *M. spicata* seedlings under in vitro conditions with *S. indica* revealed its endophytic nature, with a 70% root colonization rate. Plants treated with the endophyte exhibited a survival rate of 90.5% and a higher percentage of dry biomass, which correlates with improved plant development.

S. indica shows promise as an endophytic microorganism, providing plants with protection against both biotic and abiotic stresses by regulating various processes. These include the synthesis of antioxidant substances, osmolytes, secondary metabolites, and phytohormones related to defense mechanisms. Consequently, it plays a crucial role in enhancing global food security by positively influencing plant development, production, and quality. As a result, it presents itself as an environmentally sustainable and economically viable solution.

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Conflict of interest

The authors declare no conflict of interest.

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