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Evaluation of the incidence of endophytic fungi in leaves, stems and roots of bean plants in the State of Paraná

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Abstract

The present study aimed to isolate endophilic fungi from plant tissues (leaves, stems and roots) of beans, in addition to identifying them at the genus level. The experiment was conducted at IDR-Londrina. Bean samples were collected at the Londrina Research Station and at UENP-Bandeirantes. The leaves, stems and roots were separated, fragments of 5 mm diameter were cut and disinfested. They were later transferred to BDA culture medium. The plates were incubated at room temperature for seven days for growth of the microorganisms present. The presence and absence, colonization rate (TC) for each tissue and gender identification of fungi were evaluated. The experiment was conducted in a completely randomized design, with five replications. Forty-eight endophilic isolates were found, 13 in root, 17 in stem and 18 in leaf. CT ranged from 17-100% (leaf), 17-83% (stem) and 16-100% (root). The genus *Alternaria* sp. and *Cladosporium* sp. have greater specificity with the leaf and stem, *Penicillium* sp. root and stem. The genera *Fusarium* sp. and *Aspergillus* sp. are found in all organs, *Curvularia* sp., *Nigrospora* sp. and *Thichoderma* sp. demonstrated leaf specificity, *Macrophomina* sp. the root, and *Colletotrichum* sp. to the stem. Thus, the identified fungi present potential for future studies in their application in the biocontrol of various plant diseases.
Keywords: seeds pathology; common bean; micology; sanity.

Avaliação da incidência de fungos endofíticos em folhas, caules e raízes de plantas de feijão no Estado do Paraná

Resumo

O presente estudo teve como objetivo realizar o isolamento de fungos endofíticos dos tecidos vegetais (folhas, caules e raízes) de feijão, além de identifica-los a nível de gênero. O experimento foi conduzido no IDR-Londrina. Amostras de feijão foram coletadas na Estação de Pesquisa de Londrina e na UENP-Bandeirantes. Foram separadas as folhas, caules e raízes, cortados fragmentos de 5 mm diâmetro e desinfestados. Posteriormente foram transferidos para meio de cultura BDA. As placas foram incubadas à temperatura ambiente por sete dias, para crescimento dos microrganismos presentes. Foram avaliados a presença e ausência, taxa de colonização (TC) para cada tecido e identificação a nível de gênero dos fungos. O experimento foi conduzido em delineamento inteiramente casualizado, com cinco repetições. Encontrou-se 48 isolados endofíticos, sendo 13 em raiz, 17 em caule e 18 em folha. A TC variou de 17-100% (folha), de 17-83% (caule) e 16-100% (raiz). O gênero *Alternaria* sp. e *Cladosporium* sp. apresentam especificidade maior com a folha e caule, o *Penicillium* sp. com raiz e caule. Os gêneros *Fusarium* sp. e *Aspergillus* sp. são encontrados em todos os órgãos, *Curvularia* sp., *Nigrospora* sp. e *Thichoderma* sp. demonstraram especificidade em folha, *Macrophomina* sp. a raiz, e *Colletotrichum* sp. ao caule. Dessa forma, os fungos identificados apresentam-se com potencial para futuros estudos em sua aplicação no biocontrole de diversas doenças de plantas.
Palavras-chave: patologia de sementes; feijão comum; micologia; sanidade.

Introduction

The bean crop (*Phaseolus vulgaris* L.) is an herbaceous plant with an annual cycle, grown worldwide on approximately 30 million hectares (DEPEC, 2017).

This legume is of great economic and nutritional importance, as it is an excellent source of protein. In addition, it has great social importance, as it provides an income and food option for small rural producers, who most often produce the seeds for their cultivation, which are known as saved seeds (CABRAL *et al.,* 2011).

Brazil is the world's largest producer and consumer of beans (SNA, 2017). According to data from Conab 2018/2019, Brazilian production was 672.3 thousand tons (CONAB, 2019). The crop stands out in Paraná's agriculture, occupying fourth place in planted area, contributing approximately 22% of the national total (SALVADOR, 2012).

The crop has a wide edaphoclimatic adaptation, which allows it to be cultivated throughout the year at different times, as a rule, sowing is concentrated in three seasons: "water" or first season, "dry" or second season and "autumn-winter” or third season (POSSE *et al.,* 2010).

A condition that exposes the plant to several unfavorable factors, such as diseases and pests, which are a major obstacle to agriculture and can cause significant damage to crops, which justifies the use of control measures (BARBOSA; GONZAGA, 2012).

Among the control practices, we can highlight biological control, which is nothing more than a phenomenon that occurs spontaneously in nature, based on the regulation of the numbers of plants and microorganisms by natural enemies (MENEZES, 2003).

Farmers are leaving chemical control aside and adopting this practice, due to its economic viability, some even out of conscience, as it is an ecological method and less harmful to the environment (BARBOSA; GONZAGA, 2012).

Among the biocontrol methods, we can highlight the use of endophytic fungi as a strategy for disease control. These microorganisms are a diverse group of ascomycetes due to their asymptomatic behavior in plants (JALGAONWALA *et al.,* 2011).

Microorganisms characterized as endophotic encompass fungi and bacteria that colonize and inhabit the interior of plant species asymptomatically (AZEVEDO, 2014).

These microorganisms infect the plant through wounds and natural openings (AZEVEDO, 1998; SANTOS, 2011). Some examples of means of entry are the breakage that happens in the roots when they develop, open stomata and even injuries caused by another pathogen or insect, some are even transmitted via seed (AZEVEDO, 1998; SANTOS, 2011).

Pathogens have a lot of variety in terms of genus or species in the same plant, and in different organs, however, they can be found in different times and places, according to the phenological variation (AZEVEDO, 1998).

Endophilic fungi are distributed among different organs and tissues of plants and are associated with leaves, branches, stems, roots (FELBER *et al.,* 2016) and floral structures such as ovaries, anthers and amys (PORRAS-ALFARO; BAYMAN, 2011).

Endophytic fungi may be present in plant species from various regions (SILVA, 2014) and from all categories, and are found inhabiting briophytes, pteraphytes, gymnosperms and angiosperms (ZHANG *et al.,* 2013; OLMO-RUIZ; ARNOLD, 2014; SOCA-CHAFRE *et al.,* 2011; IMPULLITTI; MALVICK, 2013) and may be generalists or specific sits host (FERNANDES, 2015).

Among the six fungi most frequently isolated endophytically include: *Colletotrichum gloeosporioides*, *Cladosporium* Link, *Phomopsis* (Sacc.) Bubák., *Fusarium* Link:Fr. and *Xylaria* L. (ARAÚJO *et al*., 2001; MARIANO *et al.,* 1997; PEREIRA *et al.,* 1999; PHOTITA *et al.,* 2001).

Several cases of disease control with endophytic fungi have been reported, as already said, these microorganisms protect the plant against pathogen attacks. Endophytic fungi play an important role in agriculture, and have been increasingly used as biocontrol agents of pests and diseases and because they positively influence plant growth by the production of phytoregulators (AFZAL *et al.,* 2014).

Studies carried out show the positive effect in the control of some diseases in beans. In an in vitro test experiment, they showed that the endophytic fungi *Trichoderma viride* and *T. tomentosum* inhibited the mycelial growth of *C. lindemuthianum* pathogen causing anthracnose, the main fungal disease of beans (CHRISTMANN, 2019).

The isolation of fungi that are endophytic inhabitants in plants, without being pathogenic, has been studied for possible applications in the control of pathogens in the culture itself. They colonize a virtually unexplored ecological habitat and their secondary metabolites are particularly active, possibly due to metabolic interactions with their hosts.

In this context, the objective was to carry out the isolation of endophytic fungi from plant tissues (leaves, stems and roots) of beans, in addition to identifying them for future studies.

Material and methods

Isolation of pathogens in plant tissues

The test was conducted at the Seed Pathology Laboratory of the Rural Development Institute of Paraná - IAPAR-EMATER, under laboratory conditions in the city of Londrina, Paraná-Brazil.

Bean plants of the cultivars IPR Tangará and IPR Curió were collected at the Londrina experimental station and in an organic experimental field at UENP (Universidade Estadual do Norte Pioneiro), in Bandeirantes. Five plants were randomly collected in the areas when they presented pre-flowering phenological stage (R5). All plants were visibly saly.

In the laboratory, the collected plants were separated into leaves, stems and roots, washed with soap in running water and placed to dry on sterilized filter paper.

To isolate the pathogens, ten discs of 5 mm diameter of the leaf tissue and ten fragments of 5 mm length of the stem and root were removed. Discs and fragments were disinfested in 70% alcohol for 30 seconds and 1.5% sodium hypochlorite (commercial product with 2%) for 1 minute, washed in sterilized distilled water to remove excess product.

After disinfestation, the discs and fragments were transferred to Petri dishes containing Potato-Dextrose-Agar (BDA) medium with streptomycin sulfate antibiotic. The plates were kept in B.O.D at a temperature of 25 ± 1 ºC and a 12/12 hour light and dark photoperiod.

Fungal colonies that showed distinct staining and growth characteristics in culture medium were observed under a microscope for differentiation.

The evaluations took place seven days after incubation, determining the colonization rate (CT) in the leaf tissue, stem and root (PETRINI *et al*., 1992), where:

$$\text{TC: } \frac{\text{total number of segments}}{\text{total number of segments}} \times 100 = (\%)$$

Subsequently, the identified colonies were subcultured in PDA medium and after growth were preserved in Castellani (CASTELLANI, 1939), storage at ± 4 ºC.

Gender-level identification of endophytic fungi

To identify the isolated endophytic fungi at the genus level, colony disks of each fungus were subcultured in PDA medium, the plates incubated in B.O.D at 25 ºC for seven days.

Identification evaluations were carried out by the characteristics of color, shape of fungal colonies and spores.

The experiment was carried out in a completely randomized design (DIC) with five replications, and the experimental unit consisted of a plate with six leaf, stem and root discs.

After tabulation, the data were subjected to analysis of variance and mean, based on the colonization rate, with subsequent application of the Tukey mean comparison test ($p < 0.05$).

Results and Discussion

Colonization rate in plant tissues

Forty-eight endophytic fungi were found from leaf, stem and root tissues. There was a 50% colonization rate of endophytic fungi in the root, followed by the leaf tissue, with 47.03%, and the stem with 32.96%.

There was no significant difference in the colonization rate between the different tissues of the bean plants. Colonization rates ranged from 17 to 100% for leaves, 17 to 83% for stem and 16.9 to 100% for roots.

Magalhães *et al.* (2008), in their studies, found a significant difference in the rate of colonization of tissues such as seed, leaf and stem, taking into account that it is a perennial, non-cultivated plant.

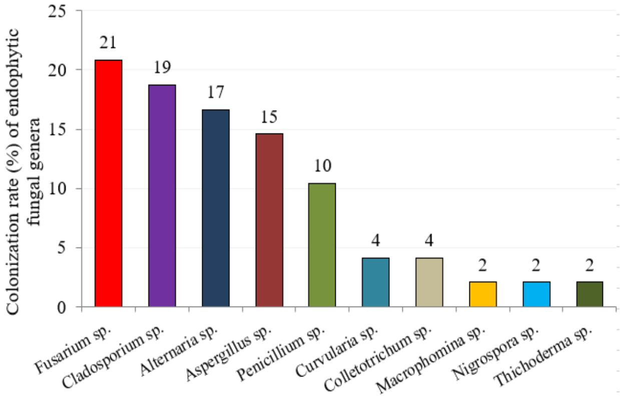
On the other hand, studies carried out with pine cone and soursop showed a significant difference between the rate of colonization of endophytic fungi in leaves, stem and roots in a sample, different from soursop where no difference was observed between the tissues evaluated (SILVA *et al.,* 2006).

According to Gamboa *et al.* (2003), the rate of colonization by endophytic fungi is high (+-600), and the number of endophytic fungi found in beans was low (48), a result also obtained by Magalhães *et al.* (2008).

Forty-eight fungi were found, 46 of which were identified at the genus level: *Fusarium* sp. (20.83%), *Cladosporium* sp. (18.75%), *Alternaria* sp. (16.67%), *Aspergillus* sp. (14.58%), *Penicillium* sp. (10.42%), *Curvularia* sp. (4.17%), *Macrophomina* sp.

(2.08%), *Nigrospora* sp. (2.08%) and *Thichoderma* sp. (2.08%) (Figure 1).

Figure 1. Incidence of endophytic fungal genera on the colonization of plant tissues of collected bean plants.



Some endophotic fungi may promote the growth of plant species (JABER; ENKERLI, 2016). The improvement in plant growth from the presence of endophytic fungi that aid in plant growth can happen through the synthesis of phytomoniums and/or by tolerance to abiotic stresses according to Khan *et al.* (2015).

Many endophilic fungi have been isolated from plants of economic interest and some studies involving the relationship with plant growth have achieved good results (SOUZA; SANTOS, 2017).

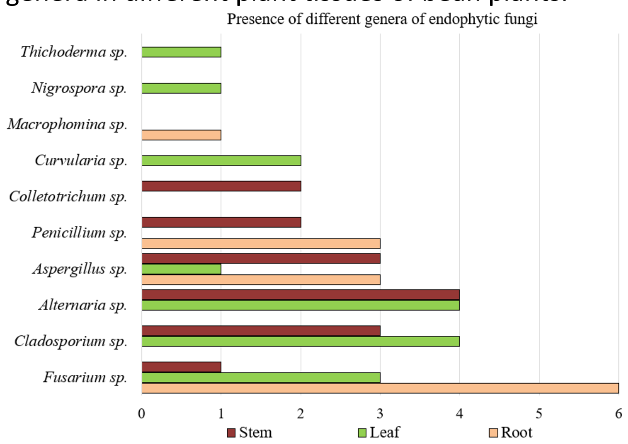
An example is the fungus species *Fusarium* sp. It has the capacity to promote plant growth in several plant species, produce gibereline that promoting teratogenic changes in hosts (OMOJASOLA; ADEJORO, 2018). Even knowing that some fungal species of the genus *Fusarium* sp. being responsible for causing diseases in plants of economic interest, are still responsible for the production of many beneficial compounds that indemrest the growth of phytopathogens that cause diseases in agricultural species (NASCIMENTO, 2015).

Regarding the specificity of colonization per sampled organ, it was observed that the genera *Fusarium* sp. and *Aspergillus* sp. they are generalist, as they were found in all sampled tissues (Figure 2).

They showed specification to organs sampled organs, the genus *Alternaria* sp. and *Cladosporium* sp., with the leaf and stem, and the genus *Penicillium* sp. with root and stem, the genera *Curvularia* sp., *Nigrospora* sp. and *Thichoderma* sp. demonstrated leaf specificity, the genus *Macrophomina* sp. the root, and the genus *Colletotrichum* sp. to the stem (Figure 2).

Similar results were described by Magalhães *et al.* (2008) who obtained two genera of generalist endophytic fungi and others demonstrated a certain specificity for certain plant organs.

Figure 2. Presence and absence of endophytic fungi genera in different plant tissues of bean plants.



In general, the fungi of the genera identified are described as phytopathogens, but in some cases, they were isolated as endophytic, not causing apparent damage in the plant species (BERNARDI-WENZEL *et al.,* 2012).

Amatuzzi (2014) identified 13 genera of endophytic fungi in strawberry leaves, the most important being *Cladosporium* sp., *Aspergillus* sp., *Nigrospora* sp., *Fusarium* sp., *Trichoderma* sp., *Alternaria* sp., *Penicillium* sp., *Phoma* sp. and *Biopolaris* sp. which were used in biological control tests of crown caterpillar (*Duponchelia fovealis*).

After in vitro antagonism tests, Louzada *et al.* (2009) identify the endophilic fungus *Curvularia* sp. as an antagonist of the phytopathogenic fungus *Fusarium* sp., inhibiting its growth significantly. Positive results for in vitro testing are essential for the development of biocontrol techniques in field.

Conclusions

Forty-eight isolates were obtained, 24 fungi from the root part, 23 from the stem and 15 from the leaf, where 46 of them were identified at the genus level.

There is no difference between colonization rates in each tissue analyzed.

The most frequent genera of endophilic fungi in this study were *Colletotrichum* sp., *Fusarium* sp., *Alternaria* sp., *Aspergillus* sp., *Cladosporium* sp., *Penicillium* sp.

The *Fusarium* sp. and *Aspergillus* sp. were considered generalists, as they were found in all vegetative parts sampled.

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