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Occurrence of symbiotic fungi and rhizospheric phosphate solubilization in weeds

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ABSTRACT. Studies on the ecology of the organisms involved in the production process are necessary for the development of sustainable agriculture, and sustainability is currently closely linked to the profitability of production. The objective of this study was to verify the occurrence of arbuscular mycorrhizal fungi in weeds infesting Brazilian crops and to evaluate the inorganic phosphate solubilization potential of the associated microbiota. A total of 36 weed species were evaluated for the occurrence of mycorrhizae; of these, 11 were selected to evaluate their potential for total and relative phosphate solubilization. All of the species demonstrated mycorrhizal colonization, including a member of the Brassicaceae family, which is usually assumed to be non-mycorrhizal. In most of the species, morphological types of arbuscular and coiled hyphae were observed, with the coiled hyphae being the most common in the grasses. Dark septate endophytic fungi were observed in most of the plants. The weeds presented different potentials for P solubilization in the rhizosphere; *Amaranthus retroflexus*, *Bidens pilosa* and *Leonotis nepetaefolia* showed high values of relative phosphate solubilization. This is the first report on the mycorrhizae and phosphate solubilization activity in weeds in Brazil.

Keywords: arbuscular mycorrhiza, dark septate endophytes, *Sinapis arvensis* L., microbial biomass, phosphate solubilizers.

Ocorrência de fungos simbiontes e solubilização rizosférica de fosfato em plantas daninhas

RESUMO. Estudos sobre a ecologia de organismos envolvidos no processo de produção são necessários para o desenvolvimento de uma agricultura sustentável, e hoje, a sustentabilidade está intimamente ligada à rentabilidade de produção. Objetivou-se com este trabalho verificar a ocorrência de fungos micorrízicos arbusculares em plantas daninhas de lavouras brasileiras e avaliar o potencial de solubilização de fosfato inorgânico da microbiota associada. 36 espécies de plantas daninhas foram avaliadas quanto à ocorrência de micorrizas, e 11 foram selecionadas para avaliação do potencial de solubilização total e relativa de fosfato. Todas as espécies apresentaram colonização por micorrizas, inclusive um exemplar da família Brassicaceae, geralmente enquadrada como não micorrizada. Na maioria das espécies, os tipos morfológicos de arbúsculos e enovelados de hifas foram observados, sendo os enovelados mais comuns entre as gramíneas. Fungos endofíticos do tipo dark septate foram visualizados na maioria das plantas. As plantas daninhas apresentaram distintos potenciais de solubilização de P na rizosfera. *Amaranthus retroflexus*, *Bidens pilosa* e *Leonotis nepetaefolia* apresentaram elevados valores de solubilização relativa de fosfato. Este é o primeiro relato de micorrizas e da atividade de solubilização de fosfato em plantas daninhas no Brasil.

Palavras-chave: micorriza arbuscular, endofíticos dark septate, *Sinapis arvensis* L., biomassa microbiana, solubilizadores de fosfato.

Introduction

Agricultural ecosystem sustainability is as desirable as high productivity and profitability. According to Kitamura (2003), the future of agriculture demands innovations in conventional intensive practices and, consequently, more responsibility with regard to the environment. However, sustainable techniques in agricultural production must be based on studies on the ecology of the populations involved. It is noteworthy that crops suffer interference from many organisms and that greater importance has been given to weeds, which present a high aggressiveness that is often conditioned by the high soil-use capacity (RADOSEVICH et al., 1997). This fact has attracted special interest with regard to the many processes occurring at the soil/root interface, and many of these processes are unknown due to the intense participation of several edaphic organisms, other than plants, in competition (ASAEDA et al., 2011; LI et al., 2007).

In respect of crops, weed competitive advantage in the field may result from the interaction with different groups of soil microorganisms (REINHART; CALLAWAY, 2006). The interaction between plants and two groups of fungi, Arbuscular Mycorrhizal Fungi (AMFs) and Dark Septate Endophytes (DSEs), provide more efficient use of the available resources in plants (ZAK et al., 2003). AMFs provide plants with a higher absorption of nutrients and water and protection against pathogens and toxic elements in the soil (HERRMAN et al., 2004). AMFs are strongly affected by their host plants, and this association has been classified as vital in the structuring among plant species (WARDLE, 2002). Although many attributes of DSEs are similar to those of mycorrhizae, their function has not yet been clarified and demands further study (LINGFEI et al., 2005). Detmann et al. (2008) report a high frequency of DSEs in the plants of the Brazilian savannah, revealing the adaptive character of these fungi to adverse conditions.

Two morphological types of symbiosis between AMFs and plants have been distinguished—Arum and Paris—as first described by Gallaud (1905). The former is characterized by the distribution of hyphae throughout the cortex, extracellularly, spreading along the apoplast, and the formation of arbuscules. In contrast, the Paris-type is characterized by the extensive development of intracellular mycelia, forming coils that may or may not be interspersed with arbuscular hyphae. Yamato (2004) showed the importance of defining the main factor acting on their morphologies, as the functional differences between the two groups are not known with certainty.

In the rhizosphere environment, a portion of the microbial biomass excretes organic acids that dissolve rock phosphate or phosphate precipitated in the soil with Al, Ca, and Fe by the chelation of the cations that accompany the phosphate anion, thus releasing P (KUCEY, 1983). The activity of these phosphate-solubilizing microorganisms is affected by the soil management and vegetation (NAHAS et al., 1994; SOUCHIE et al., 2005). Thus, Reis et al. (2008) proposed the use of a quotient to evaluate the efficiency of the phosphate-solubilization activity per microbial biomass unit, defined as the relative inorganic phosphate solubilization (RIPS). The quotient is calculated by dividing the amount of inorganic P released by the units of microbial biomass in the rhizosphere. The RIPS allows the identification of plants that host microbiota that are more efficient in promoting phosphate solubilization in the rhizosphere.

The elucidation of these symbiotic associations and their role in the interactions of competition between weeds and crops may contribute to the development of more sustainable management practices. Thus, the present work aimed to (1) verify whether arbuscular mycorrhizal fungi and dark septate endophytic fungi are present in weeds and (2) determine whether the rhizosphere microbiota of the evaluated weeds presented differences in their microbial phosphate-solubilization potential.

Material and methods

In January and February of 2009, we collected 36 species of plants belonging to 14 botanical families, which are considered weeds for the most important Brazilian agricultural crops. The plants were collected during full flowering, which is considered to be the stage when increased symbiotic activity occurs (SMITH; READ, 1997). The 0.8 ha collection site, 650 meters above sea level, was located in the city of Viçosa, Minas Gerais State, Brazil, at lat 20° 46' 00" S and long 42° 52' 04" W. This site had not been subjected to the application of pesticides in the six years prior to the collection. A square shovel was used to collect the plants to preserve the soil mass adhered to the roots, and the sample was placed in 5.0 L buckets and transported to the laboratory for botanical identification. At least three plants per species were collected when found isolated, i.e., without the interference of other plants.

After the botanical identification, the roots were separated from the soil and washed in running water. The samples of roots were grouped according to their species and sectioned into fragments of 1.0 to 2.0 cm. A sample of 1.0 g of the fragments was preserved in formaldehyde:ethanol:acetic acid (FAA) solution with a 5:90:5 ratio. The fragments were washed and clarified with 10% KOH and subjected to trypan blue staining. The observation of fungal structures was performed according to Giovannetti and Mosse (1980). The root fragments were observed in Petri dishes using a binocular stereoscope with a magnification of 40x. Thus, the most characteristic fragments were selected for the preparation of slides for observation using an optical microscope (Olympus BX50). The fungal structures were photographed with a Qcolor 3 Olympus digital camera using the QCapture Pro 6.0.0.412 software system. The presence of mycorrhizae was assessed by the observation of their structures, such as arbuscules, vesicles, spores and hyphae, and the occurrence of DSEs was determined by the observation of microesclerotia.

According to phytosociological relevance in the area, 11 of the species were selected for the evaluation of the phosphate-solubilization potential of the rhizosphere microbiota and were used to determine the microbial biomass carbon in compliance with Mueller-Dombois and Ellenberg (1974). To estimate the solubilization potential, 1.0 g of rhizosphere soil (fresh weight) was transferred to a test tube containing NBRIP liquid medium, pH 6.8-7.0, composed of the following (g L^{-1}): glucose, 10; $Ca_3(PO_4)_2$, 5; $MgCl_2.6H_2O$, 0.5; $MgSO_4.7H_2O$, 0,25; KCl, 0.2; and $(NH_4)_2SO_4$, 0.1 (NAUTIYAL, 1999). After the incubation of the samples for 15 days at 27°C, the liquid phase was centrifuged at 5,008 x g for 20 minutes, and the quantity of inorganic P was determined in the supernatant by the colorimetric method of vitamin C, according to Braga and Defelipo (1974).

To estimate the microbial biomass, the method described by Vance et al. (1987), as modified by Islam and Weil (1998), was used. The relative solubilization quotient was determined by dividing the value of the solubilized P by the microbial biomass.

Where appropriate, experiments were performed with four replicates. The data were subjected to an analysis of variance, and the means were grouped by Scott-Knott test at a 5% probability.

Results and discussion

The colonization by arbuscular mycorrhizal fungi was observed for all 36 species, as characterized by the presence of vesicles, hyphae, arbuscules, hyphal coils and fungal spores. Moreover, auxiliary vesicles in the roots of *Eupatorium urticaefolium* and the spores of *Panicum maximum* were observed. Hyphal coils were more frequently observed in the root system of the species evaluated, compared to arbuscules, which were not observed among the grasses. In *Sinapis arvensis* L., a species of the Brassicaceae family, vesicles, hyphae, arbuscules and Arum-type mycorrhizal colonization were observed. The presence of DSEs was observed in 33% of the analyzed plant species, belonging to the following families: Amaranthaceae, Asteraceae, Convolvulaceae, Labiatae, Malvaceae, Solanaceae and Verbenaceae (Figure 1 and Table 1).

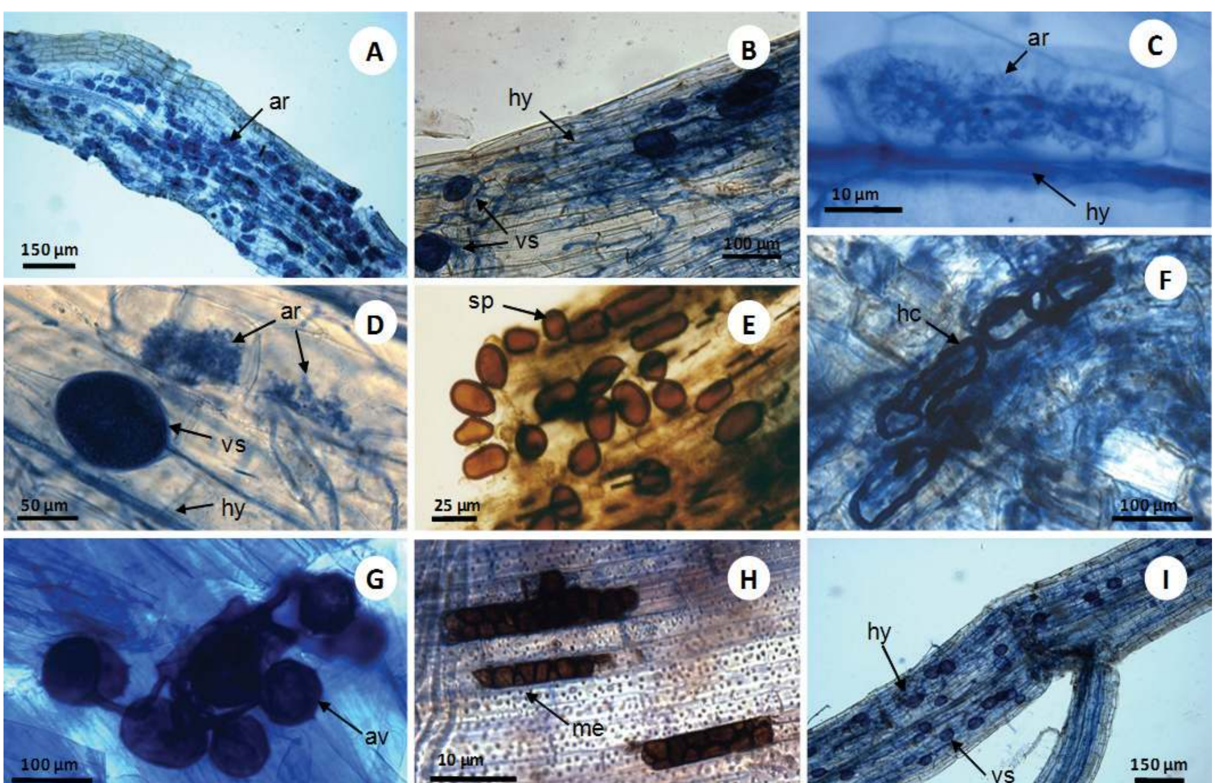

Figure 1. Arbuscular mycorrhizal and dark septate endophytic fungi in roots of weeds in Brazil. A - Arbuscule (ar) in *Amaranthus retroflexus*; B - Vesicle (vs) and hyphae (hy) in *Sinapis arvensis*; C - *Leonurus sibiricus*; D - *Leonotis nepetaefolia*; E - Spores (sp.) in *Panicum maximum*; F - Hyphal coils (hc) in *Sorghum arundinaceum*; G - Auxiliary vesicles (av) in *Eupatorium urticaefolium*; H - Microesclerotia (me) in *Bidens pilosa* and I - *Conyza bonariensis*.

Table 1. Mycorrhizal structures and fungi of the type dark septate (DSE) in the root system of weeds of different botanic families, collected in the Municipality of Viçosa, Minas Gerais State, Brazil.

Family	Species	Mycorrhizal structures		DSE
		Arbuscule	Hyphal coils	
Asteraceae	*Ageratum conyzoides* (L).	Yes	Yes	N.O*
	Artemisia verlotorum Lamotte	Yes	Yes	N. O.
	Bidens pilosa (L).	Yes	Yes	N. O.
	Blainvillea rhomboidea Cass.	Yes	Yes	Yes
	Conyza bonariensis (L).	Yes	Yes	Yes
	Emilia fosbergii Nicolson	Yes	Yes	N. O.
	Eupatorium urticaefolium Reichard	Yes	Yes	N. O.
	Galinsoga parviflora Cav.	Yes	Yes	N. O.
	Porophyllum ruderale (Jacq.) Cass.	Yes	Yes	N. O.
	Siegesbeckia orientalis (L.)	Yes	Yes	N. O.
	Sphagneticola trilobata (L.)	Yes	Yes	N. O.
	Vernonia polyanthes Less.	Yes	Yes	N. O.
Amaranthaceae	*Amaranthus retroflexus* (L.)	Yes	Yes	Yes
	Amaranthus spinosus (L.)	Yes	Yes	Yes
Gramineae	*Andropogon bicornis* (L.)	N. O.	Yes	N. O.
	Cynodon dactylon (L.)	N. O.	Yes	N. O.
	Imperata brasiliensis Trin.	N. O.	Yes	N. O.
	Panicum maximum Jacq.	N. O.	Yes	N. O.
	Paspalum conspersum Schrad.	N. O.	Yes	N. O.
	Sorghum arundinaceu (Willd.) Stapf.	N. O.	Yes	N. O.
Euphorbiaceae	*Chamaesyce hirta* (L.)	Yes	Yes	N. O.
	Chamaesyce hyssopifolia (L.)	Yes	Yes	N. O.
	Euphorbia heterophylla (L.)	Yes	Yes	N. O.
Commelinaceae	*Commelina benghalensis* (L.)	Yes	N. O.	N. O.
Lythraceae	*Cuphea aperta* Koehe	N. O.	Yes	N. O.
Convolvulaceae	*Ipomoea grandifolia* (Dammer) O'Don	Yes	Yes	Yes
Verbenaceae	*Lantana camara* (L.)	Yes	Yes	Yes
Labiatae	*Leonotis nepetaefolia* (L.) R. Br.	Yes	N. O.	Yes
	Leonurus sibiricus (L.)	Yes	N. O.	Yes
Urticaceae	*Pilea microphylla* (L.)	Yes	Yes	N. O.
Malvaceae	*Sida rhombifolia* (L.)	Yes	Yes	Yes
	Sida santaremensis Monteiro	Yes	Yes	Yes
Brassicaceae	*Sinapis arvensis* (L.)	Yes	N. O.	N. O.
Solanaceae	*Solanum americanum* Mill.	Yes	Yes	Yes
	Solanum lycocarpum St. Hill	N. O.	Yes	Yes
Sapindaceae	*Cardiospermum halicacabum* (L.)	N. O.	Yes	N. O.
	* N.O – Non-Observed			

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According to Smith and Smith (1997), the Arum arbuscular mycorrhizae are found in most angiosperms, whereas the Paris type is prevalent in gymnosperms. Environmental factors, such as temperature, light intensity and soil moisture, also determine the morphological type of the arbuscular mycorrhizae (CAVAGNARO et al., 2001). The results of Yamato and Iwasaki (2002) showed that the Arum type prevails in pioneer herbaceous plants and that the Paris-type occurs more frequently in herbaceous understory plants. In the present work, the Arum type was not observed in the grasses and only the botanic classification probably defined the colonization type, as the plants were found in the same environment.

In the literature, it has been reported that mycorrhizal association is rare or absent in certain botanical families, such as Brassicaceae, Proteaceae and Cyperaceae (LAMONT, 2003; SOUZA et al., 2006). However, the presence of arbuscular mycorrhizae in plants of the Proteaceae family in soil with high contents of nickel and low contents of phosphorus indicates that the botanical factor alone does not determine colonization (BOULET; LAMBERS, 2005). Similarly, Regvar et al. (2003) report the presence of hyphae, vesicles, coils and arbuscules in the roots of *Thlaspi praecox, Thlaspi caerulescens* and *Thlaspi montanum*, which belong to the Brassicaceae family. Carneiro et al. (2001) highlight mycorrhizal colonization in Brassicaceae (*Brassica* sp.) after the inoculation of spores or only by natural mycorrhizal colonization in an environment contaminated with heavy metals, indicating the importance of the environment in the process of the colonization of plants by AMFs. In the present work, the occurrence of AMFs associated with the roots of *S. arvensis* L. is reported for the first time under Brazilian conditions, which indicates the need of a more comprehensive survey on the occurrence of arbuscular mycorrhizae in weeds grouped in botanical families that are considered, thus far, to be non-mycorrhizal.

More than 600 plant species can associate with dark, septate, endophytic fungi, and their occurrence is related to abiotic factors, such as low humidity in the environment and the day length (BARROW; AALTONEN, 2001). Li and Guan (2007) reported a close relationship between DSEs and AMFs, suggesting competition or even cooperation between them. Confirming the association of the studied weeds with EDS, the biggest difficulty in the presentation of our results can be explained by the presence of certain components in the cell walls of those fungi that hinder their visualization in some plant species (BARROW; AALTONEN, 2001).

The evaluation of the plant's ability to solubilize phosphorus in the soil demonstrated that *Bidens pilosa*, *Amaranthus retroflexus* and *Leonotis nepetaefolia* presented higher potentials for solubilizing phosphate when compared to the other weed species (Figure 2). However, it must be stressed that, for all of the other species evaluated, the solubilization potential values were higher in the rhizosphere soil compared with the non-rhizosphere soil (Figure 2). *L. nepetaefolia*, *B. pilosa*, and *A. retroflexus* presented the best phosphate-solubilization potential (Figure 3) in relation to the rhizosphere biomass (relative solubilization).

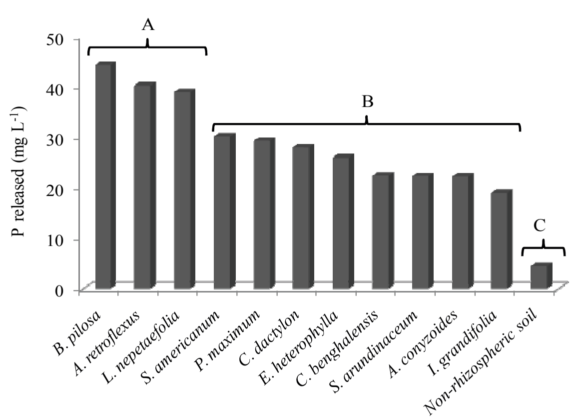

Figure 2. Phosphorus (P) solubilized after rhizospheric soil incubation with weeds in NBRIP medium. Averages followed by the same letter do not differ by the grouping criterion of Scott Knott ($p > 0.05$ of error probability).

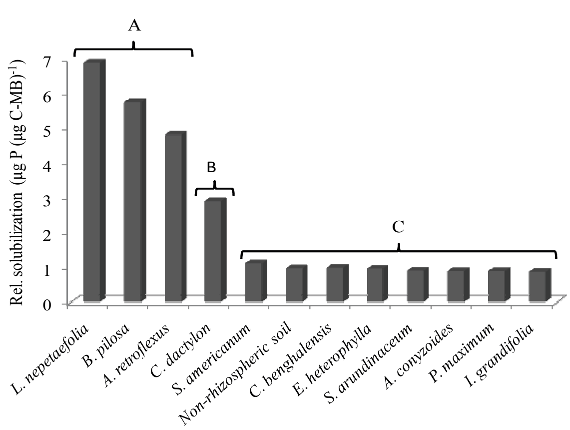

Figure 3. Relative solubilization of $Ca_3(PO_4)_2$ (Pi released by carbon from the microbial biomass) after the incubation of the rhizospheric soil with weeds in NBRIP medium. Averages followed by the same letter do not differ by the grouping criterion of Scott Knott ($p > 0.05$ of probability of error).

Weeds have different capacities for nutrient absorption, depending on the availability of the elements; soils with high phosphorus contents favor weeds, to the detriment of crops (TOMASO, 1995). Part of this nutrient-absorption capacity is due to the presence of microorganisms associated with the rhizosphere. Santos et al. (2012) reported that the accumulation of macronutrients, mainly phosphorus, was impaired in *B. pilosa* in a fumigated substrate using an assay to assess the accumulation of macro- and micronutrients in the tissues of several weeds after substrate fumigation. The fumigation may have inhibited the growth of microorganisms in the substrate, thus hindering the absorption of nutrients by *B. pilosa*. Therefore, it can be inferred that plants with a high phosphate-solubilization potential due to the association with soil microorganisms should be considered for use in integrated pest management programs in crops, as they operate more efficiently in nutrient cycling, especially phosphorus cycling, which is a limiting element in tropical-soil agriculture.

Ronchi et al. (2003) compared the extraction of phosphorus by coffee plants and *Bidens pilosa* and found that this weed (*B. pilosa*) extracted ten times more phosphorus from the soil than the coffee crop. Our present results suggest a significant contribution of the rhizosphere microbiota to the supply of phosphorus for weeds and in their competitive potential with relation to cultivated plants. However, because mycorrhizal fungi do not colonize all weed species, some weeds may have their activity affected by the presence of mycorrhizae. This fact is evidenced in a study by Rinaudo et al. (2010) who found that the species *Amaranthus retroflexus*, *Chenopodium album*, *Digitaria sanguinalis*, *Echinochloa crus-galli*, *Setaria viridis* and *Sinapis arvensis* are hindered by the presence of *Glomus mosseae*, *Glomus coronatum* and *Glomus intraradices* mycorrhizal fungi.

The relative solubilization index allows inferences about the intrinsic characteristics of a species in relation to other species. In this work, *L. nepetaefolia*, *B. pilosa* and *A. retroflexus* presented associated microbiota that promoted higher values of phosphate-solubilization potential, suggesting better plant development and the higher accumulation of phosphorus.

Conclusion

All of the evaluated species presented colonization by arbuscular mycorrhizal fungi, a symbiosis that has advantages for weeds, including the better access to nutrients, water and protection against soil-borne pathogens. Under Brazilian conditions, these associations have not been previously documented. Furthermore, a greater ability to extract phosphorus and a greater efficiency in competing with the crops for soil resources was verified in the weeds that had higher potentials of microbial phosphate solubilization.

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