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Native Mycorrhizh-Forming Fungi Associated with Cultivated Forage Plants in the Central Valley of Catamarca, Argentina

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Abstract- Mycorrhizae are a symbiosis between the roots of some plants and certain soil fungi, where both participants in this association obtain benefits. The plant provides the fungus with carbohydrates, proteins, and lipids, and in turn, the fungus allows the plant to better uptake nutrients from the soil and water. The objective was to determine the existence of mycorrhizal associations between native fungi and forage crops in the Central Valley of Catamarca. Root colonization of Melilotus officinalis, Avena sativa, Hordeum vulgare, Secale cereale, Panicum maximum, and Cenchrus ciliaris from field crops in the Central Valley of Catamarcawas studied. Fungal infection or colonization was quantified using the line intersection method and the frequency of appearance of fungal structures. Endomycorrhizal structures of the arbuscular vesicle type were observed, with continuous mycorrhizal hyphae, some of them with rosary lipids inside and vesicles with fat globules. In addition, dark septate endophytic fungi were observed. The association of mycorrhizae is described in six forage species cultivated in the Central Valley of Catamarca and the co-occurrence of arbusculo-vesicular mycorrhizae and native dark septate endophytes.

Keywords: *oatmeal; barley; rye; gatton panic; buffalo-grass.*

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NATIVEMYCORRHIZHFORMINGFUNGIASSOCIATEDWITHCULTIVATEDFORAGEPLANTSINTHECENTRALVALLEYOFCATAMARCAARGENTINA

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Native Mycorrhizh-Forming Fungi Associated with Cultivated Forage Plants in the Central Valley of Catamarca, Argentina

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I. Introduction

Mycorrhizae are symbioses between plant roots and certain soil fungi. The term mycorrhiza describes the group made up of the absorption structures of plants and the fungal symbionts that colonize them in a distinctive way (Sánchez de Prager, 2007; Perez *et al.,* 2010), generating a structural and metabolic integration typical of a symbiosis. The absorption of nutrients is affected by these associations that also influence plant health, productivity, and the adaptation of plants to environmental conditions (Sánchez de Prager, 2007).

The plant provides the fungus with carbohydrates, proteins, and lipids, necessary for its development, and in turn, the fungus allows the plant to better capture water and nutrients from the soil, both macronutrients and micronutrients, especially those few mobile, such as phosphorus (Sánchez de Prager, 2007).

Endo mycorrhizae and ectomycorrhizae are two types of mycorrhizae that occur naturally (Sieverding, 1983; García *et al*., 2000; Biaus, 2017). Endomycorrhizae are more frequent in nature and are characterized by the colonization of cortical cells by a mycosymbiont, which lives between them and within them, inter and intracellularly (Montenegro Gómez *et al*., 2017). Among the different types of endomycorrhizal, the most widespread within plant species are vesicular-arbuscular mycorrhizae (VAM) or arbuscular mycorrhizae (AM) and some more specific forms such as orchidoids and ericoid (Sánchez de Prager, 2007; Montenegro Gómez *et al*., 2017).

Arbuscular mycorrhizal fungi (AMF) and vesicular-arbuscular fungi (HMVA) do not develop in pure culture media because they are not capable of growing in the absence of a host plant (Aguilar-Ulloa *et al*., 2016), so they are considered obligate symbionts, this biological condition that represents an obstacle to their massive propagation. These fungi are propagated using trap plants, which after a period of growth, the roots and soil are collected for use as inoculant of endomycorrhizal fungi (De la Rosa-Mera*et al.,* 2012).In recent decades, the study of these organisms has become important for apply them to the ground, as biofertilizers to increase the productivity in crops (Covacevich and Echeverria, 2010; Reyes Tena *et al.,*2015; Aguilar-Ulloa *et al*., 2016; Ordoñez-Castañeda *et al.,* 2021) and in phytoremediation, programs to correct contaminated soils (Pérez *et al*., 2021; Colombo *et al*., 2020; Quiroz-Mojica *et al*., 2021). The colonization of roots by endomycorrhizal fungi is the most used parameter as a quick indicator of the presence of mycorrhizal symbiosis (Covacevich and Echeverría, 2010).

Several plant species have been investigated as trap crops to produce mycorrhizae, to find plants that can be colonized and allow the rapid growth of mycorrhizae (Aguilar-Ulloa *et al*., 2016). The appropriate trap crop must be a fast-growing plant that adapts to the environmental conditions where it will grow, must be

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easily colonized by the mycorrhizal fungus, and produce many roots in a relatively short time (45-60 days) (Siqueira Martins *et al*., 2017).

The association established by AMF is not specific, which allows the same fungus to colonizes different plant species to generate symbiosis (Aguilar-Ulloa *et al*., 2016). Also, there is a preference that certain AMF colonize and spread better in certain plant species (Covacevich and Echeverría, 2010). Furthermore, using the same fungal species is probably not optimal for all crops. For this reason, it is convenient to evaluate the MVA in each trap crop species.

Because the interactions between different VMAs with soil microorganisms are complex, it is necessary to determine their behavior in the field under the growth conditions of each crop. The use of native MVA is recommended due to its adaptation to prevailing conditions, avoiding ecological risks associated with introducing exotic species (Sánchez de Prager, 2007).

The objective was to determine the existence of mycorrhizal associations between autochthonous soil fungi and forage crops planted in the Central Valley of Catamarca and to evaluate the biological aspects of the interactions found.

II. Materials and Methods

Colonization of mycorrhizae in plant roots of six commonly cultivated forage species in the region was studied. The sowings were carried out in the locality of Miraflores, Department of Capayán, in the Central Valley of Catamarca, in plots of native forest or with agricultural history without application of mycorrhizal fungi. The species evaluated were: clover (*Melilotus officinalis* L.), oats (*Avena sativa* L.), barley (*Hordeum vulgare* L.), rye (*Secale cereale* L.), Gatton panic (*Panicum maximum* Jacq.), and buffel grass (*Cenchrus ciliaris* L.).

In the laboratory, the roots of each of the collected plants were extracted and washed with running water. The thinnest were selected, those that were clarified and stained following the methodology of Phillips and Hayman (1970) to determine fungal colonization and detection of mycorrhizal structures. Staining was performed with Gueguén's triple dye solution, allowing fungi to stain proteins blue simultaneously, starch violet, fats red (Sarasola and Rocca, 1975), and glycogen in mahogany (Verna and Herrero, 1952).

In each specimen, the percentage of fungal colonization by MVA and the percentages of the content of arbuscules (A) and vesicles (V) were quantified by the line intersection method of Giovannetti and Mosse (1980), and the frequency of appearance of A and V (Covacevich *et al*., 2001). For each specimen, 15 segments of the colored roots were taken, and distributed randomly on a grid slide. By means of microscopic observation (10x and 40x), the presence-absence of mycorrhizal structures (A and V) was recorded in the horizontal and vertical intersections between roots and grid lines. Three repetitions of each species were performed, quantifying at least 100 intersections per preparation to later calculate the frequency of mycorrhizal infection, according to Giovannetti and Mosse (1980).

$$\text{MGiovannetti (\%)} = \text{No. SI x 100 / No. SO}$$

Where:

SI: number of infected segments (hyphae + arbuscules + vesicles) and

SO: numberof total segments observed (hyphae + arbuscules + vesicles + no infection).

Also, the percentage of hyphae of dark septateendophytes (ESO) and the percentage of microsclerotia were recorded.

III. Results and Discussion

In the six analyzed forage species, typical structures of endomycorrhizal fungi of the MVA type (hyphae, arbuscules, and vesicles), and of ESO fungi (with septate, melanized hyphae, and numerous microsclerotia) were observed.

In all species there were continuous thin and thick hyphae, with intracellular and intercellular growth and some of them with lipids in a rosary inside.

In clover, arbuscular distributed throughout the bark were observed, involved in the bidirectional transfer of nutrients (Smith and Read, 1997). Numerous vesicles of diverse morphology (spherical, oval, tapered) were also observed in this legume, so it is inferred that the roots are colonized by various species or genera of native HMVA. Vesicles with light blue (saccule) and red (single or multiple globules) colorations were observed. These structures are related to the carbon storage of in the form of lipids and fatty acids. For this reason, vesicles are defined as reserve organs of the fungal symbiont (Sieverding, 1983).

A high level of fungal colonization was determined in the six forage plants, obtaining the highest values of mycorrhizal colonization (MC) in clover and barley roots (Table 1). The highest frequency of appearance of arbuscules (FAA) was observed in clover, while the lowest number of arbuscules was determined in oats. The highest frequency of vesicle appearance (FAV) was also observed in clover, with average values of 20% (Table 1). These results coincide with the statements of Covacevich and Echeverría (2010) that indicate that there is a preference for certain AMF to better colonize certain plant species.The more signicant colonization in clover is explained by the fact that Rhizobios-arbuscular mycorrhizae (AM) associations occur in legumes that act synergistically in infection, mineral nutrition, and plant growth (Fitter & Garbaye, 1994; Barea, 1997). The main effect of AM in

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enhancing rhizobia, activity is through a generalized stimulation of plant nutrition, some more localized effects may occur at the root (Melo de Miranda *et al*., 2008; Spagnoletti *et al*., 2021).

Table 1: Fungal colonization in forage species from the Central Valley of Catamarca

Species	Mycorrhizal Fungi			Fungi ESO
	MC[1] (%)	FAA[2] (%)	FAV[3] (%)	FAM[4] (%)
Clover	89	47	20	15
Barley	89	17	15	15
Rye	83	27,7	16	13
Buffalograss	76,5	20	8	2
gatton panic	75,8	32	9,8	1,7
Oatmeal	75	10	12	4

[1](MC): Mycorrhizal colonization. [2](FAA) Frequency of appearance of arbuscules. [3](FAV): Frequency of appearance of vesicles. [4](FAM): Frequency of appearance of microsclerotia of dark septate fungi.

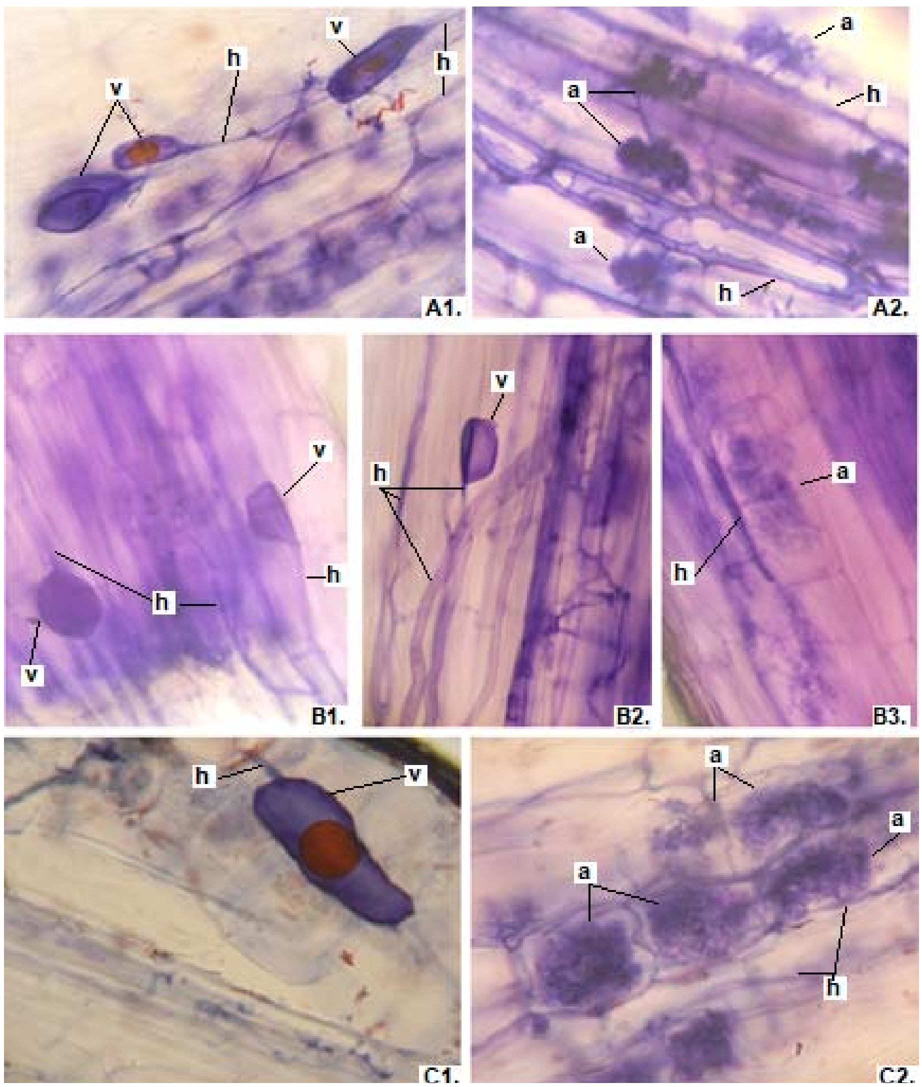

Figure 1: Photomicrographs of mycorrhizal structures in roots of forage species. (A: Clover; B: Barley; C: Rye). (a: Arbuscle; h: hyphae; v: Vesicle).(Magnification: 40x)

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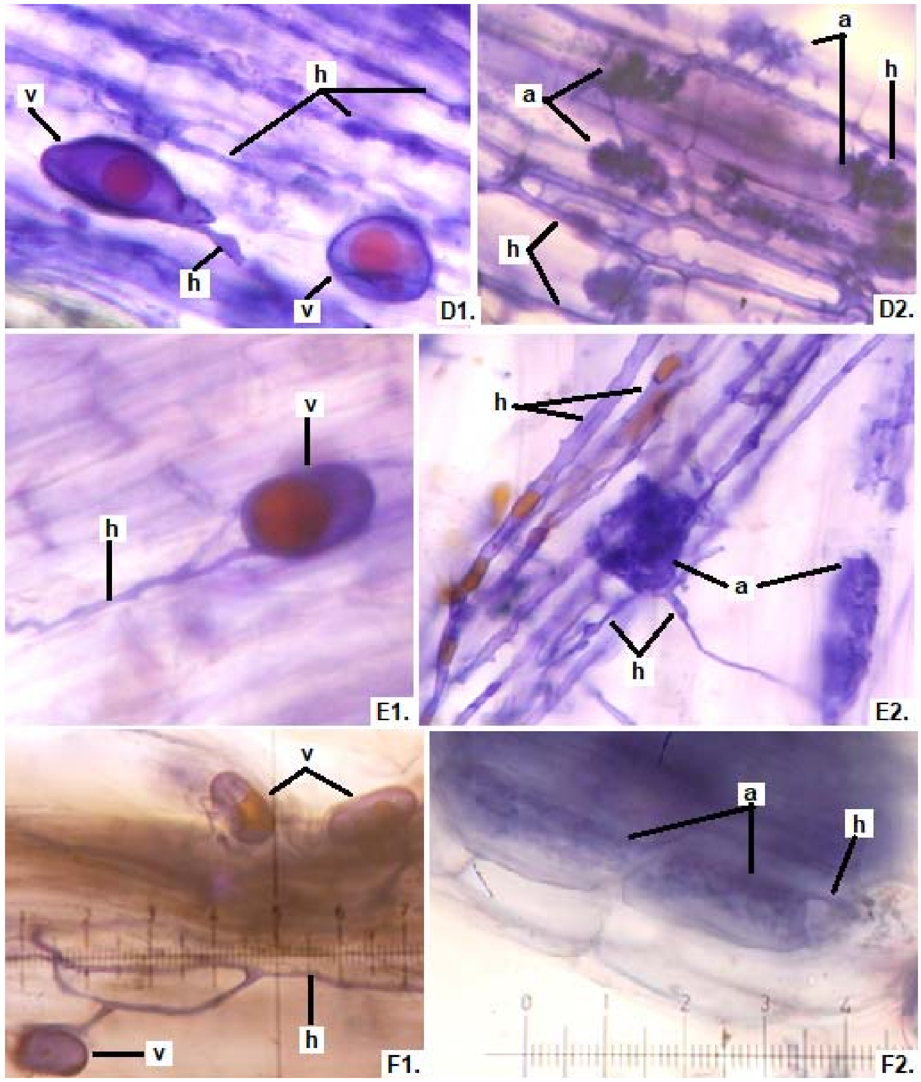

Figure 2: Microphotographs of mycorrhizal structures in roots of forage species. (D: Oats; E: Buffel Grass and F: Gatton Panic). (a: Arbuscle; h: hyphae; v: Vesicle). (Magnification: 40x)

In addition, in all the forage species analyzed, another type of hyphae was observed: septate, melanized, and with numerous microsclerotia of the kind ESO fungi (Peterson *et al*., 2004). In clover, barley, and rye roots, similar values of FAM of ESO (between 15 and 13%) were determined (Table 1).

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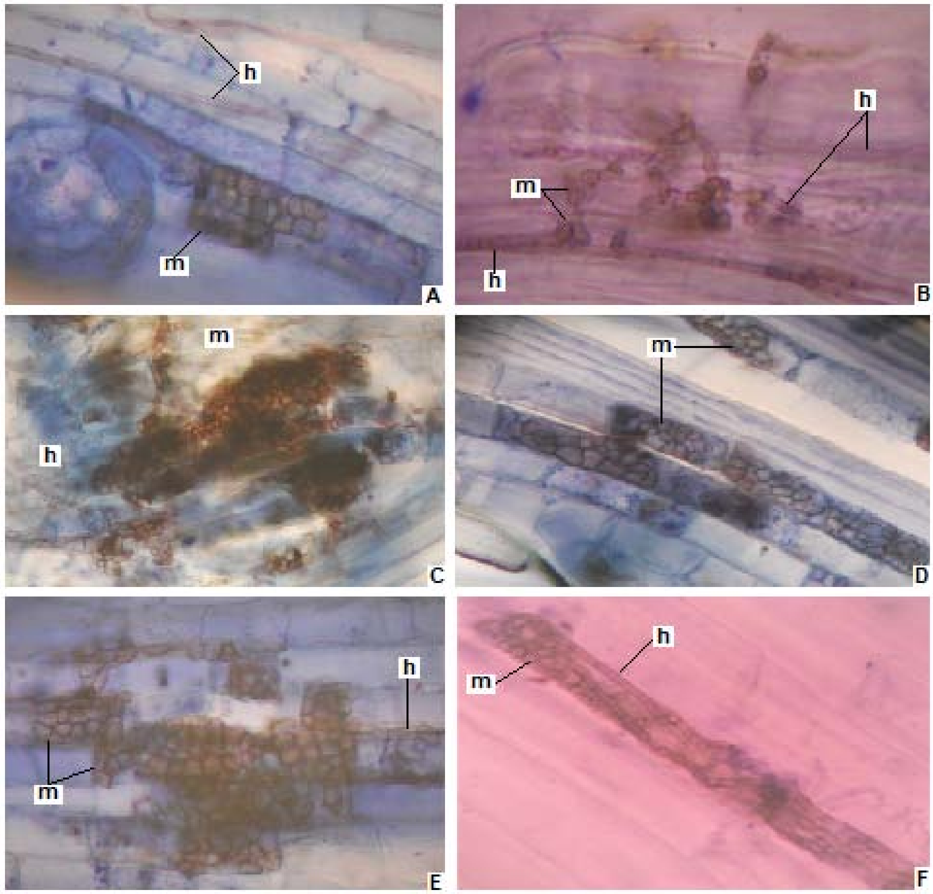

Figure 3: Photomicrographs of dark septate fungal structures on roots of forage species. (A: Clover; B: Barley; C: Rye; D: Oats; E: Buffel Grass and F: Gatton Panic). (m: Microsclerosia; h: hypha). (Magnification: 40x).

In all the plants studied, their roots were simultaneously colonized by both endophytes, MVA and ESO hyphae, also detecting the presence of vesicles, arbuscules typical of VA mycorrhizae, and ESO microsclerotia. This was also observed in other plants including ferns, mono, and dicots that are simultaneously colonized by MVA and ESO. (Urcelay *et al*., 2005; Lugo *et al*., 2011; Lizárraga *et al*., 2015; Di Barbaro *et al*., 2017).

Therefore, MVA and other fungal structures developed in all the forage species studied. This coincidence could be due to the fact that all these crops were carried out in contiguous lots with identical soil characteristics and environmental conditions, and because the fungal colonization developed from the native microflora, with the ability to associate and generate MVA, which is consistent with what expressed by Aguilar -Ulloa *et al.* (2016) where they explain that the same fungus can colonize different plant species to generate symbiosis.

These mycorrhizal-forming fungi can be considered as potential constituents of biofertilizers. Diaz Franco *et al.* (2019) achieved the reduction of inorganic fertilization through the inoculation of FAM in the sorghum crop. The higher yield of sorghum obtained allows considering the inclusion of FAM as a viable practice that guarantees greater profitability, as well as the agroecological conservation of production systems.

IV. Conclusion

The association of mycorrhizae in six forage species cultivated in the Central Valley of Catamarca and the co-occurrence of vesicular-arbuscular mycorrhizae and native dark septate endophytes is described.

High levels of mycorrhizal colonization were obtained in all the evaluated forage species. The suitability of *Melilotus officinalis* as a trap species is highlighted as it is easily colonized by mycorrhizal fungi and generates rapid mycorrhizal growth.

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