

EFFECT OF ARBUSCULAR MYCORRHIZAL FUNGI ON AN ECOLOGICAL CROP OF CHILI PEPPERS (*Capsicum annuum* L.)

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ABSTRACT

Mapuche farmers in southern Chile have been cultivating local ecotypes of chili pepper (*Capsicum annuum* L.), called locally “Cacho de cabra”, for many decades. It is used to make “merkén”, a condiment that is consumed locally and exported. This vegetable requires a nursery stage and can obtain nutritional benefits from symbiotic associations such as mycorrhizal fungi, achieving a better adaptation to transplanting. Arbuscular mycorrhizal fungi (AMF) are obligate biotrophes appearing in abundance in agroecosystems with conservation management. The aim of this study was to compare effectiveness of two AMF, a commercial mycorrhizal inoculant (IC, *Glomus intraradices*) and another native (IN, *Glomus claroideum*) with a control without inoculation (-I) on the production and quality of “Cacho de cabra”. At 45 days after sowing (DAS) transplanting was carried out and at 90 and 216 DAS fruit quality, fungal and edaphic parameters were evaluated. The harvest was at four stages. With IN inoculation plants and with greater foliar area were obtained. Also, precocity of fruit production was observed. The harvest started 49 days earlier and fresh weight was 177% higher than that of the control. Root colonization was low, showing significant differences between IN and IC, while a large number of spores was produced in the substrate. It was concluded that inoculation with native fungi decreased transplanting stress thus accelerating the maturation stage of plants and resulting in higher and better yield quality.

Key words: mycorrhiza, inoculant, biofertilizers, fruit quality, vegetables.

INTRODUCTION

Mycorrhiza is a symbiotic relationship between arbuscular mycorrhizal fungi (AMF) and the roots of the majority of vascular plants. Through extensive root external mycelia networks the fungi improve the capture of relatively immobile nutrients such as P (Souchie *et al.*, 2006), Cu and Zn, as well as improving water absorption (Augé, 2004), capturing at the same time C fixed as hexose from the apoplast of the root cortical cells (Douds *et al.*, 2005). However, not all AMF/plant combinations are compatible, with some fungi being more beneficial to a host and adapting themselves to determined edaphoclimatic

conditions, showing marked structural and functional differences among species and even among morphotypes of the same fungal species (Linderman and Davis, 2004). To achieve a satisfactory inoculation effect it is necessary to know the compatibility between a determined host and the AMF, in order to select the appropriate fungal strain for the specific plant cultivar (Rodríguez *et al.*, 2004).

AMF are important in ecological agriculture because of the benefits they provide to the majority of cultivars and the conservation of the environment by acting as biofertilizers, bioprotectors and biocontrol agents (Azcón-Aguilar *et al.*, 2002). In the framework of a sustainable agriculture, that includes a more respectful management of the environment based on the sustainable use of resources, and taking advantage of strategies that nature itself employs for its self-regulation, the soil is considered as an active element of the system, composed of inter-related physical, chemical and biological factors, the AMF forming part of a biological microcosm (Jaizme-Vega and Rodríguez-Romero, 2004). Consequently, the design of any agricultural production system should consider the use of these microsymbionts as inseparable components

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of agro-ecosystems to carry out diverse functions in association with plants, among others, acting as biological substitutes for mineral fertilizers. This last case does not mean not to fertilize, but rather that fertilization can be more efficient, saving important quantities of mineral fertilizers, together with better absorption of nutrients available in the soil.

Vegetable crops that at their start require a nursery stage can benefit from AMF inoculation, among these chili peppers (*Capsicum annuum* L.). Consequently the use of AMF is being incorporated into horticultural practices (Evans, 1997). However, the efficiency of commercial inoculants currently in the market is unknown, with the possibility of confusing its action with that of non-mycorrhizal additives (Von Alten *et al.*, 2002) that can increase plant growth and that is not due to mycorrhiza (Corkidi *et al.*, 2005).

Around 5000 ha of chili peppers are cultivated annually in Chile, from the north to the central south of the country. The Araucanía Region offers favorable growth conditions, especially for local ecotypes that small-scale Mapuche farmers have been growing for decades. They are used mainly in the production of “merkén”, an ancestral condiment made from ground, salted and smoked chili peppers. At a national level approximately 9000 t of pepper are marketed each year. The variety “Cacho de cabra” accounts for approximately 1500 t, of which 3% to 6% is destined to the production of “merkén”. Given the economic and cultural importance of this crop in the Araucanía Region, the objective of this study was to compare the effects of inoculation of two species of AMF, one commercial (*Glomus intraradices*) and the other native (*Glomus claroideum*), with a control without inoculation (-I), on the yield and quality of chili peppers grown under greenhouse conditions.

MATERIALS AND METHODS

Seed for bioassays was sown in January 2007 in greenhouses of the Universidad de La Frontera; at 45 days

after seeding (DAS) the seedlings were transplanted. The harvest was carried out in four stages (at 167, 189, 203 and 216 DAS), finishing in July of the same year.

Bioassay

For the seeding stage, 250 mL pots were used with an Ultisol soil from the area of Purén (38°40' S, 73°00' W). The soil had been used for cultivation of peppers by small-scale mapuche farmers for several years. The Ultisol was mixed with sand and vermiculite (ratio 7:2.5:0.5), vapor sterilized for 1 h for 3 consecutive days to eliminate native AMF (Table 1).

Two types of AMF inoculants were used: a native species, *G. claroideum* (IN), and the other a commercial, *G. intraradices* (IC). The equivalent of 20 mL of solid inoculants was added to each experimental unit, using the same substrate without inoculation as a control (-I). Two disinfected and pre-germinated pepper seeds from local ecotypes were planted in each pot and covered with the same substrate. A completely random design was used, with 40 replications, with a total of 120 experimental units that were kept under greenhouse conditions, with controlled lighting, temperature and humidity. At 45 DAS, plants of similar height and foliar area were transplanted with roots to plastic bags containing 5 L of sterile growth substrate. At this stage 16 replications per treatment were used, with a total of 48 plants. At the beginning of flowering (90 DAS) and fruiting (216 DAS) the agronomic variables and fungal and substrate chemical parameters were determined from eight replications of each treatment.

Agronomic variables. a) number of leaves; b) foliar area (cm²) using a digital image analyzer; c) plant fresh weight; d) root and foliar dry weight (leaves, stalks) using a air oven at 70 °C for 48 h until reaching a constant weight; e) shoot/root ratio (T/R); and f) root length, using the line intercept method (Newman, 1966).

Fungal parameters. a) AMF colonization percentage in the roots after trypan blue staining (Phillips and Hayman,

Table 1. Chemical parameters in an Ultisol under greenhouse conditions, inoculated with native (IN) and commercial (IC) arbuscular mycorrhizal fungi, and non-inoculated control (-I), in flowering and fruiting stage.

Parameters	Flowering			Fruiting		
	-I	IC	IN	-I	IC	IN
pH	5.65a	6.13a	6.38a	5.25a	5.28a	5.55a
MO, %	8.29a	9.30a	8.43a	7.83a	8.93a	9.22a
Available P, mg kg ⁻¹	3.05b	4.73ab	6.29a	2.60a	2.16ab	1.84b
P-ase, mg gsh ⁻¹	0.75a	0.71a	0.79a	1.51a	1.41a	1.29b

Different letters for each parameter in flowering and fruiting indicate differences according to the Tukey test (P < 0.05).

P-ase: phosphatase activity.

1970); b) fungal mycelia using the methodology for soils, through extraction with glycerin acid and subsequent quantification by the line intercept method (Rubio *et al.*, 2003); and c) number of spores by the wet sieving and sucrose gradient decanting method (Sieverding, 1991).

Chemical parameters and enzyme activity of the substrate. a) pH soil:water ratio (1:2.5); b) organic matter (OM, %) with acid digestion with dichromate (Walkley and Black, 1934); c) available P through extraction with NaHCO₃ 0.5 M at pH 8.5 (Olsen and Sommers, 1982), and soil acid phosphatase (E.C.3.1.3.2 orthophosphoricmonoester phosphohydrolase) was determined using *p*-nitrophenyl phosphate according to the procedure described by Tabatabai and Bremner (1969).

Plant height (cm), from the base to the apex, was measured periodically from 15 DAS until harvest. The harvest was carried out at four timings between 170 and 216 DAS, using visual appreciation, as well evaluations of fruit quality.

Fruit parameters. a) number and fresh and dry weight (g), length and diameter (mm) of the fruit; b) length and diameter of the peduncle (mm); c) total titlable acid (TTA) determined from ground pulp with a potentiometer until the final point 8.2 and expressed as a percentage of anhydride citric acid 100 mL⁻¹ of juice (Tressler and Joslyn, 1961); d) pH, with a potentiometer in drops of ground pulp; e) total soluble solids (TSS) with a refractometer (°Brix), this commercial index considers all dissolved solids as sucrose; f) maturity index, which is the ratio between sugar and acid (% °Brix/% citric acid) that expresses the sum of the citric, malic, oxalic and tartaric acids; g) ascorbic acid content (AA) by volumetry using the Tillman method based on the reduction of 2,6 dichlorophenol indophenol (Schmidt-Hebbel, 1981).

Statistical analysis

The data obtained were submitted to the Shapiro-Wilk (1965) normality test and then an ANOVA of one factor was carried out using the Tukey *a posteriori* multiple range means separation test ($P < 0.05$). The Shapiro-Wilk test was used for data normalization for measurements of plant height, submitting them to repeated ANDEVA measurements over time, verifying sphericity through the Mauchly test, with correction of degrees of freedom by Greenhouse-Geisser test (1959). Finally the Tukey *a posteriori* multiple range means separation test was applied ($P < 0.05$). Lineal Pearson correlations were carried out among some agronomic, fungal and substrate variables ($P < 0.05$). SPSS software for Windows version 15.0 was used for processing the information.

RESULTS AND DISCUSSION

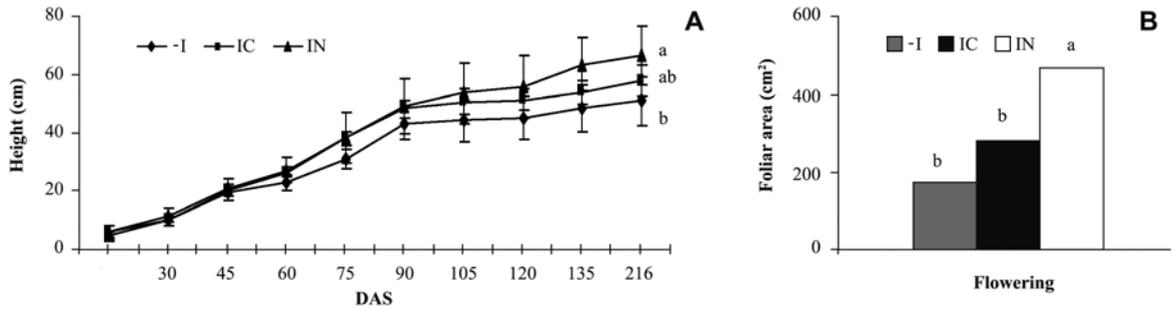
Of the chemical soil parameters measured at the flowering and fruiting stages (Table 1), soil pH did not present significant differences among the treatments, but was important for the development of fungal propagules given that it was significantly related to the number of spores in the soil ($r = 0.52$; $P < 0.05$). In this respect, Friberg (2001) reported that pH influences the capacity of some AMF species to colonize roots. A low quantity of available P was found in the substrate at flowering, which decreased even more at fruiting because no nutritive solution was added. Ultisols are characterized by a low availability of P and in agricultural soils P is potentially available for plants depending on the soil-root environment, such as phosphatase enzymes (Borie *et al.*, 1989). This parameter presented differences at fruiting, with the control and the IC treatment showing higher contents. On the other hand, high P content in the soil can inhibit the formation of AM symbiosis owing to a reduction in the development of external mycelia.

Mycorrhizal inoculation increased the growth rate of the plant measured as height, which in the IN treatment increased rapidly until 90 DAS (Figure 1A), while in the control and *G. intraradices* treatments plant height remained almost constant until fruiting. Significant differences were found at 216 DAS in the measurements between IN and (-), the IN treatments registering the greatest height, with an average of 66.5 cm, 15.2% higher than IC (57.7 cm) and 30.8% higher than the control (50.8 cm). These findings correspond to literature reports with other plants (Ouahmane *et al.*, 2007).

At flowering, the foliar area of the plants inoculated with *G. claroideum* increased significantly, by 160% more than the control and 59% more than in the *G. intraradices* treatment (Figura 1B).

According to Román (2003) the leave number and size in a pepper crop play an important role in that they determine future yield. The pepper plants inoculated with *G. claroideum* at 33 and 50 DAS had the highest number of leaves (4.4 and 5.6 leaves plant⁻¹, respectively) followed by the control (4.2 and 5.3 leaves plant⁻¹), while the IC treatment showed the lowest number (3.9 and 4.9 leaves plant⁻¹). These results coincide with what was reported by Román (2003) with pepper plants grown in greenhouses.

At flowering, the dry weight of the plants inoculated with the two morphotypes (Figure 2A) was significantly greater than that of the plants without inoculation, which indicates a better supporting of the transplanting stress. The weight of the IC plants increased by 60%, while the IN plants increased by 116% in comparison to the control. At harvest, the IN showed even greater differences, with a 217% increase in comparison to the control. These effects



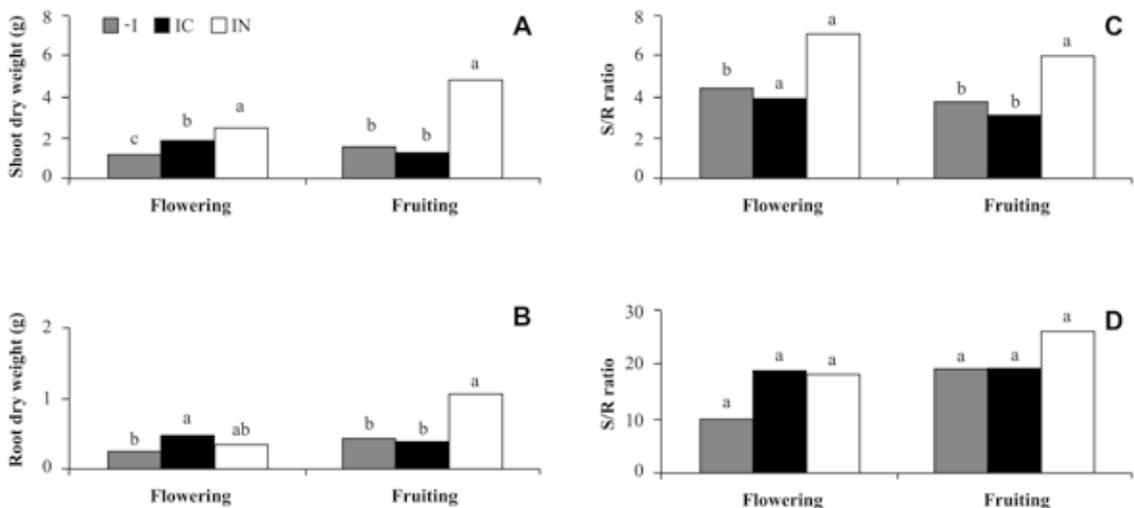
Different letters in each parameter indicate significant differences according to the Tukey test ($P < 0.05$).

Figure 1. Effect of inoculation with native (IN) and commercial (IC) arbuscular mycorrhizal fungi and non-inoculated control (-I) in an Ultisol under greenhouse conditions: A) plant height over time (days after sowing, DAS) and B) foliar area at flowering.

are greater than those reported by Waterer and Coltman (1989) and Gaur *et al.* (1998), who, working with pepper plants, obtained yield increases of 112% in a soil inoculated with *G. intraradices*. On the other hand, at physiological maturity the plants inoculated with IC decreased notably in weight. Having significantly increased the vegetative growth of the plant, the native inoculants, composed of *G. claroideum*, showed great compatibility with the local ecotype of our chili pepper. According to Davies *et al.* (2000) it is important to select native and adapted AMF strains to achieve a higher yield.

The shoot/root ratio (S/R) was higher at flowering than at fruiting, a stage when the plants show evident signs of senescence, such as the fall of old leaves, that

consequently reduce the quantity of dry matter. The production of biomass is linked to the phenology of the plant, mainly during flowering and fruiting. In these phases the plant invests similar quantities of photoassimilates for the production of fruit and the vegetative part; at the beginning of fruiting, vegetative growth is limited, with the fruit presenting the highest growth rates. At these two phenological stages, the IN treatment had the highest S/R ratio, followed by -I and IC (Figure 2C). A high S/R ratio reflects a high degree of mycorrhizal effectiveness (Tobar *et al.*, 1999), *G. claroideum* being more effective to promote growth of pepper plants than *G. intraradices*. In comparison to the non-inoculated control, the plants colonized by native AMF improved their vigor, robustness,



Different letters in each parameter indicate significant differences according to the Tukey test ($P < 0.05$).

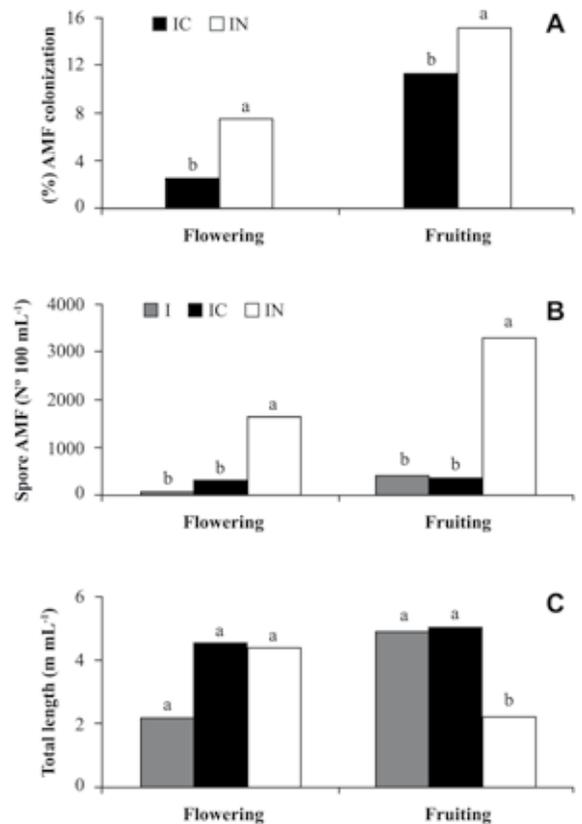
Figure 2. Pepper crop inoculated with native (IN) and commercial (IC) arbuscular mycorrhizal fungi and non-inoculated control (-I) in an Ultisol under greenhouse conditions. A) Shoot dry weight; B) root dry weight; C) shoot/root ratio; and D) root length, in flowering and fruiting.

development and consequently, their yield more. This beneficial effect of endomycorrhiza on horticultural crops was also reported by Aguilera-Gómez *et al.* (1999) in peppers inoculated with *G. intraradices*, obtaining a higher number of leaves, foliar area, shoot and yield. Root length was not different between the two inoculated treatments, but with the control at flowering, which did not exceed 10 m (Figure 2D). The capacity of the seedlings to overcome the transplanting shock depends on the capacity of the roots to support structural and functional changes, to the absorption of water and nutrients as well as the capacity of regeneration of new roots (Montaño-Mata and Núñez, 2003).

At 90 DAS, in the two inoculated treatments the colonization of the pepper plant roots was low in comparison to other vegetable crops in the region (Rubio *et al.*, 1994; 1997), fluctuating between 2.4% and 7.4%. Colonization with *G. claroideum* was significantly greater than with *G. intraradices*, differences that disappeared at physiological maturity (Figure 3A). High infectiveness does not always guarantee an improvement in plant growth; thus, beneficial responses have been reported with only 0.4% of AMF colonization (Carpio *et al.*, 2005).

At flowering and fruiting IN plant at the highest number of AMF spores, with significant differences in comparison to the IC treatment and the control (Figure 3B). At harvest, the density of spores of *G. claroideum* in the substrate increased by 950% in comparison to the *G. intraradices* treatment. Producing fungus-plant compatibility avoids the problem of “parasitism” in the development of the symbioses (Rodríguez *et al.*, 2004). There were a low number of spores in the control at 90 DAS, probably because of the presence of mycorrhizal propagules that resisted the sterilizing conditions of the substrate, thus being subsequently reproduced by the presence of a compatible host.

The color of the fruit originates from the plastids contained in the mesocarp, which are green at physiological immaturity because of the presence of chlorophyll, and in the mature stage change to red. In this last stage the chlorophyll and the anthocyanin degrade and the chloroplasts are transformed into chromoplasts, which contain carotenoids that are responsible for the final yellow-red color (Popovsky and Paran, 2000; Méndez *et al.*, 2004). The velocity of fruit development or the anthesis-maturation time (Gómez, 2000) was different for the two AMF inoculants. The treatment that showed greater precocity of production was IN, with the first harvest at 167 DAS and with 64% of the fruit matured, while the control had only 18% of fruit matured, while in IC the fruit, still remained immature. At 189 DAS the same percentage of fruits had matured in both inoculated treatments (18%) finalizing the phenological



Different letters in each parameter indicate significant differences according to the Tukey test ($P < 0.05$).

Figure 3. Fungal parameters of pepper crop after inoculation with native (IN) and commercial (IC) arbuscular mycorrhizal fungi (AMF) and non-inoculated control (-I) in an Ultisol under greenhouse conditions. A) Percentage of AMF colonization; B) spore number; and C) total hyphae length at flowering

stage for the IN treatment at 203 DAS, while the last harvest for the other treatments was concluded at 216 DAS. The inoculation with IN shortened the maturation process by 22 days, providing an attractive advantage for the cultivation of this vegetable. This acceleration in the velocity of development is possibly achieved because of the compatibility between *G. claroideum* and the local ecotype of chili pepper that grew in an Ultisol (Table 2). Thus, *G. claroideum* had a beneficial effect for the plants and although it proved not to be very infective, its behavior was very effective, given the high correlation obtained between fruit weight and plant weight ($r = 0.79$; $n = 24$; $P < 0.05$). These results show the importance of achieving a better association between a determined AMF strain and the ecotype of pepper, in order to improve and optimize the production process.

Table 2. Quality parameters of “Cacho de cabra” chili pepper fruits in an Ultisol under greenhouse conditions inoculated with native (IN) and commercial (IC) arbuscular mycorrhizal fungi and non-inoculated control (-I).

Parameters of quality of the fruit	Treatments		
	-I	IC	IN
N° fruit per plant	1.0b	1.0b	2.3a
Fresh weight, g	4.9b	3.6b	13.5a
Dry weight, g	0.7b	0.4b	2.3a
Length, mm	75.8b	96.2b	158.2a
Widest diameter, mm	13.5b	10.6b	18.0a
Peduncle diameter, mm	4.5b	4.4b	6.5a
Peduncle length, mm	32.5a	32.5a	36.0a
Ascorbic acid, g citric acid 100 mL ⁻¹	319.0a	298.0ab	294.0b
pH	5.33ab	5.69a	5.19b
Total titratable acidity (TTA)	20.0a	15.6a	22.8a
Total soluble solids, °Brix	11.8a	9.7a	11.5a
Maturity ratio, °Brix/% TTA	0.5a	0.7a	0.5a

Different letters in each parameter indicate significant differences according to the Tukey test ($P < 0.05$).

The parameter evaluated for fruit quality, such as weight, length, diameter and peduncle diameter, were significantly higher in the IN treatment, while no differences were found between the IC treatment and the control (Table 2). Fresh weight of fruits in IN increased in comparison to the control and the IC by 177% and 272%, and the length by 86% and 75%, respectively. After transplanting, measurements taken at flowering showed differences among the IN, IC and the control in terms of the quantity of mycorrhizal propagules. According to what was reported by Román (2003) AMF colonization in pepper plants cultivated in greenhouses enhanced the number and fresh weight of fruits. The increase in fruit weight was greater in our experiment than that reported by Mena-Violante *et al.* (2006) in *Capsicum annum* cv. “San Luis”, where the inoculated treatments reached increases of 25% in comparison to the control without inoculants.

In this experiment the inoculation with *G. intraradices* did not improve yield or quality of chili peppers. The majority of commercial inoculants contain *G. intraradices*, which has been considered as a “super strain” and “generalist” because it is highly infective for a wide range of cultivars (Vosatka and Dodd, 2002). It may be that, in this study the differences obtained in response between the two inoculants were caused by the adaptability of *G. claroideum* to local conditions. Sensoy *et al.* (2007) observed that in eight pepper genotypes inoculated with *G. intraradices* and *Gigaspora margarita*, five cultivars had greater dry weight while three obtained yields similar to plants without inoculation. There is some evidence that the AMF strains are positive influencing plant growth

under the same condition as those from where they were originally isolated (Vosatka and Dodd, 2002).

During the maturation process the fruit pulp had high sugar content, due to starch production that transforms into sugars; nevertheless, the native and commercial AMF inoculations did not produce differences in the fruit in relation to the control. Another parameter, the TAA, is a sensitive indicator of quality during the transport and storing of the vegetables because it is highly vulnerable to chemical and enzymatic oxidation and solubility in water (Martins and Silva, 2004), fluctuated between 294 and 319 g 100 mL⁻¹, with differences between IN and the control. With regard to the TTA, which is a property perceptible by consumers, no differences among the treatments were found. The low pH of the fruits originates from organic acids formed in tissue vacuoles (González *et al.*, 2001) and fluctuated between 5.19 and 5.69. Fruit of the treatments inoculated with native AMF were significantly more acidic, so that consumer would prefer them.

At flowering higher yields of the plants were obtained with IN, related to greater AMF colonization in the roots which increased substantially at fruiting, achieving larger fruit, higher weight and better quality than that obtained with IC. The results show the positive effect in the growth and development of local ecotypes of chili peppers, as well as the quality of the fruit when an appropriate selection is made of the mycorrhizal fungus for inoculation, corroborating what was reported by Llonín and Medina (2002). From these results it appears necessary to carry out another tests comparing native AMF prior to mass inoculum production for inoculation of vegetable crops that require nursery or greenhouse cultivation.

CONCLUSIONS

Under the test conditions, the inoculation of a substrate with a native AMF inoculant, *Glomus claroideum*, in comparison to a commercial inoculant, *Glomus intraradices*, positively affected the production of “Cacho de cabra” chili pepper, resulting in more vigorous plants with a higher foliar area and shoot/root ratio, which accelerated fruiting and improved quality, and led to greater quantity of fungal propagules in the soil. These results once again show the importance of using native local and autochthonous AMF strains as biofertilizers that are better adapted to the regional edaphoclimatic conditions and can therefore be expected to be of greater nutritional benefit to regional plants varieties which are often grown in organic farming systems.

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RESUMEN

Efecto de los hongos micorrícicos arbusculares en un cultivo ecológico de ají (*Capsicum annuum* L.) cacho de cabra. En el sur de Chile, agricultores mapuches han cultivado durante décadas ecotipos locales de ají (*Capsicum annuum* L.) “Cacho de cabra” para elaborar “merkén”, producto con reconocidas ventajas en el mercado internacional. Esta hortaliza requiere etapa de almácigo, pudiendo beneficiarse nutricionalmente con la asociación simbiótica del tipo micorrizas, logrando una mejor adaptación al trasplante. Los hongos micorrícicos arbusculares son biótrosfos obligados abundantes en agrosistemas con manejo conservacionista. El objetivo de este estudio fue comparar, en invernadero, el efecto de la inoculación de dos hongos, uno comercial, *Glomus intraradices* (IC), y otro nativo, *Glomus claroideum* (IN), con un testigo sin inoculación (-I) sobre la producción y calidad de ají “Cacho de cabra”. A los 45 días después de la siembra (DDS) se realizó el trasplante y a los 90 y 216 DAS se evaluaron parámetros de calidad del fruto, fúngicos y edáficos. La cosecha fue escalonada en cuatro etapas. Con la inoculación de IN se obtuvieron plantas de mayor altura y área foliar que mostraron mayor velocidad de desarrollo del fruto adelantándose la cosecha en 49 días y con aumentos en el peso fresco de 177% en relación al control. La colonización en las raíces de ají fue baja con diferencias significativas entre IN e IC, mientras que una gran cantidad de

esporas permaneció en el sustrato. Se concluye que la inoculación con hongos nativos disminuyó el estrés del trasplante acelerando la maduración del ají con aumento de producción y calidad.

Palabras clave: micorriza, inoculante, biofertilizantes, calidad fruto, hortalizas.

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