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Effect of symbiosis in the production of melon seedlings with arbuscular mycorrhizal fungi

Efecto de la simbiosis con hongos micorríticos arbusculares en la producción de plantines de melón

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ABSTRACT

This research was performed in a semi-controlled greenhouse of the "Estación Experimental Canchones", in which the evolution of the effect of the mycorrhizal fungi over the growth parameters, differentiation, biomass, stress indicators and biochemical indicators for the production of the horticultural seedlings; and the percentage of mycorrhization obtained was evaluated. Inodorous type honeydew melon was used as the plant model. The mycorrhizal fungus *Glomus intraradices* Schenk and Smith, commercially known as MYCOSYM TRITON, was used for the inoculation at the moment of sowing with doses of 0; 20; 40 and 80 spores per plant. A completely randomized design was used; analysis used a factorial analysis variance (ANOVA) with the a posteriori LSD, using the statistical software INFOSTAT and an alpha value of 0.05. The results obtained in the destructive sample on the 50th day after sowing showed that the treatment with 40 spores per plant produced maximum root biomass, low etiolation index, a high value of the proportion of dry root weight to dry shoot weight. However, the activity of both endogenous and induced enzymes of nitrate reductase measured in the leaves was unaffected. We conclude that the symbiotic association between the roots of the melon plants and the mycorrhizal species *Glomus intraradices* produced a greater root biomass and very favorable conditions for transplanting.

Key words: *Glomus intraradices,* arbuscular mycorrhiza, *Cucumis melo,* seedling.

RESUMEN

Esta investigación fue realizada en un invernadero semicontrolado de la Estación Experimental Canchones, donde se evaluó el efecto de los hongos micorríticos sobre los parámetros de crecimiento, diferenciación, biomasa, indicadores de estrés y bioquímicos para la producción de plántulas hortícolas, así como también el porcentaje de micorrización obtenido. El material vegetal utilizado fue melón cv. Honeydew tipo Inodorus. El hongo micorrítico utilizado fue Glomus intraradices *Schenk y Smith, nombre comercial MYCOSYM TRI-TON, el cual fue inoculado al momento de la siembra con las siguientes dosis 0; 20; 40 y 80 esporas por planta respectivamente. Se utilizó un diseño completamente aleatorizado, realizándose un análisis de varianza multifactorial (ANOVA) y para la separación de medias se empleó el test LSD, mediante el software estadístico INFOSTAT a un* $\alpha = 0,05$*. Los resultados obtenidos en el muestreo destructivo a los 50 días de siembra determinaron que el tratamiento inoculado con 40 esporas por planta presentó una mayor producción de biomasa radical, menor índice de ahilamiento y una mayor relación peso seco raíz-peso seco vástago; sin embargo, la actividad de la enzima nitrato reductasa endógena e inducida medida en las hojas no fue afectada. En esta investigación se concluye que la asociación simbiótica entre las raíces de las plántulas de melón y las micorrizas de la especie* Glomus intraradices *determinó una mayor producción de biomasa radicular y una condición más favorable para el trasplante.*

Palabras claves: Glomus intraradices*, micorrizas arbusculares*, Cucumis melo*, semilleros*.

Introduction

Microbiota plays an important role in agriculture since it contributes to soil fertility, improving soil structure and biodiversity and has a real effect on plant development (Avis *et al.,* 2008). Among these microorganisms are the symbiotic mycorrhizal fungi, which allow the plants to explore a greater useful surface of the soil by the production of external mycelia connected to the root system, increasing the absorption of nutrients and water, while the fungus receives carbonated compounds from photosynthesis which are necessary to complete its life cycle (Azcón-Aguilar y Barea, 1980; Harley y Smith, 1983; Pereira *et al.,* 1999).

One of the main effects of mycorrhizae is the improvement in the nutritional state of the plants, increasing the capture of phosphorus, calcium,

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copper, sulfur, zinc and iron; Smith *et al.* (2001) confirmed the absorption of two nitrogenous forms –N-NO_3^- and N-NH_4^+– from the soil solution and their transfer to the associated plants. Based on this information, the objective of this study was to evaluate the effect of arbuscular mycorrhizal fungi on the architecture, biomass production, stress tolerance and synthesis of nitrate reductase in seedlings of the Inodorus type honeydew melon.

Materials and Methods

The research was performed in a semi-controlled greenhouse of the Canchones Experimental Station. The trial used cv. Orange Flesh of Inodorus type honeydew melon, called "melon tuna" in Chile, and included a period of 50 days after sowing. At planting, the mycorrhizal fungus *Glomus intraradices* was inoculated using the commercial MYCOSYM Tri-Ton MYCOSYM International AG (Switzerland), which contains latent spores at a concentration of 150 spores per gram. Seedlings were grown in thermoforming plastic trays with 72 cells with a capacity of 43 cc each, on a table 40 cm above the ground. The substrate used was a mixture of peat:Perlite 70:30; seeds were sown homogeneously at a depth of 1 cm. From the emergence of the cotyledons to the first leaf plants were irrigated with water. After the formation of the first leaf we applied a 1/3 concentration of fertirrigation, after the second leaf 2/3, and after the formation of the second leaf with the complete concentration (Table 1). The nutritive solution was adjusted to a pH of 6.0-6.5; electrical conductivity of 1.6 dS m^{-1}, equivalent to a osmotic potential (Ψs) of –0,067 Mpa. pH and E.C. were measured every three days to adjust the frequency and time of irrigation.

Inoculation was performed together with sowing. We evaluated four doses (4 treatments); 0; 20; 40 and 80 spores $plant^{-1}$, using each tray as a treatment; each cells was a pseudo-replicate (72 cells). We took destructive samples of five plants of each treatment 50 days after sowing. We determined the percentage of mycorrhization of the roots using the method of Phillips and Hayman (1970) with some modifications, and calculated the degree of mycorrhization using the method of Trouvelot (Trouvelot *et al*., 1986). We evaluated the physical parameters tissue differentiation, production of dry biomass (leaf, stem and root), stem diameter, plant height, etiolation index and leaf surface, and the stress indicators specific leaf surface (SLF) and the ratio root dry weight to stem dry weight (RDW/SDW).

The biochemical parameters evaluated were the enzymatic activities of endogenous and induced nitrate reductase. For endogenous activity we used the method of Bar-Akiva *et al.* (1970), adapted by Valenzuela *et al.* (1987) and determined induced activity using the method of Bar-Akiva and Sternbaum in 1966, modified by Bar-Akiva *et al.*, (1970) and adapted by Valenzuela *et al.* (1987). Color intensity was measured at 540 nm in a SPECTRONIC model GENESYS 2 spectrophotometer. A calibration curve was constructed in the range of 0 to 4 µM, using $NaNO^{-2}$ 1 mM; the results were expressed in µM $NO^{-2} * g^{-1}pf\ h^{-1}$.

The experimental design was completely randomized. Analysis employed factorial ANOVA with LSD a posteriori tests, using the software INFOSTAT and $\alpha = 0.05$. The number of leaves was log transformed using $\log_{10}(X+1)$; percentage values were transformed with the Arcsine Transformation.

Results and Discussion

Percentage of mycorrhization

Inoculation with different doses of *G. intraradices* spores in melon seedlings produced significant differences in the percentage of mycorrhization (Figure 1). The greatest percentage was observed with 40 spores $plant^{-1}$ (T_2, 49.8%), followed by T_3 (80 spores $plant^{-1}$) with 36.3% and T_1 (20 spores $plant^{-1}$) with 16.9%. The mycorrhization response

Table 1. Fertirrigation solution (meq L^{-1}) used in honeydew melon (Inodorus) seedlings.

Solutions	$H_2PO_4^-$	NO_3^-	SO_4^-	K^+	Ca^{2+}	Mg^{2+}	NH_4^+
DN[1]	1.25	7.50	2.50	3.25	5.00	2.50	0.5

[1] Steiner modified (Casas, 2005); solution adjusted for concentrations of Cl^- y Na^+ present in salty irrigation water.

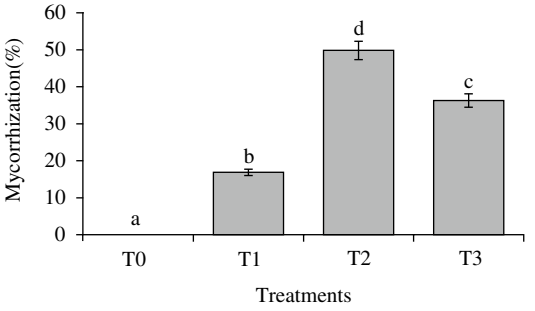

Figure 1. Percent of mycorrhization in roots of Inodorus var. Honeydew melon 50 days after inoculation with *G. intraradices*. Different letters indicate significant differences (LSD, $p < 0.05$).

with different doses of *G. intraradices* reached a maximum and then decreased (Figure 2), indicating that there is an optimum concentration of *G. intraradices* near 40 spores plant^{-1}, and that greater concentrations do not increase the percentage of mycorrhization. Similar results were obtained in tomato cv. Mara by Mujica and Medina (2008), in which there was no further increase after doubling the dose of *Glomus mosseae* and *Glomus Hoi-like*. Anaya *et al.* (2009) also obtained the best response in tomato seedlings inoculated with EcoMic (*Glomus fasciculatum*) at a medium dose.

Wang *et al*. (2010), obtained results similar to those reported here with treatment T_2, they inoculated seedlings of melon cv. 901 and 908 with 10 g of substrate containing spores of different mycorrhizae, obtaining after 45 days 39.89% for *Glomus versiforme* and 51.75% for *Glomus mosseae* (cv. 901) and 46.38% for *G. versiforme* and 57.08% for *G. mosseae* (cv. 908); there were different percentages of mycorrhization between both fungal species and melon cultivars. Contrasting results were reported by Huang *et al.* (2011), who found 70% mycorrhization in seedlings of cv. Zhongmi 3 melon inoculated with *G. intraradices*, and Martínez-Medina *et al*. (2011), who reported only 8% mycorrhization of seedlings of cv. Giotto inoculated with *Glomus intraradices*.

The highest percentage obtained in this study inoculating cv. Honeydew melon with *Glomus intraradices* was 49% (T_2), which shows that even different cultivars of a species have different degrees of mycorrhization when inoculated with the same fungal species. This agrees with the results of Wang *et al.* (2010), indicating that the success of plant symbiosis is based on the adequate establishment, development and extension of symbiotic mycorrhizal; this depends on the fungal species and its genetic characteristics, the environment in which it was isolated and its affinity with the plant species

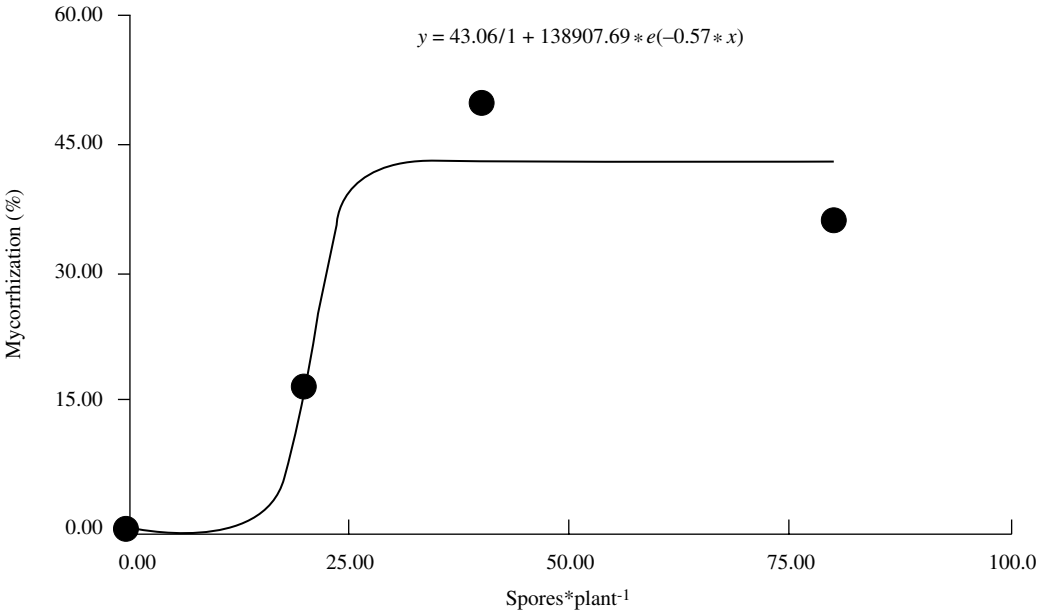

Figure 2. Effect of the dose of spores of *G. intraradices* on the percentage of mycorrhizal colonization in roots of melon Inodorus var. Honeydew 50 days after sowing.

and/or cultivar. Other factors which influence symbiosis include the physiology of the plants and the properties of their roots (Brundrett, 2002, Drew *et al.* 2006); these authors reported that the mycorrhizal colonization process is determined by the capacity of soil exploration, which is greater in *Glomus mosseae* than in *G. intraradices.*

Growth, differentiation and biochemical parameters

Biomass production

Inoculation with *G. intraradices* did not affect the production of leaf biomass, stem or total biomass (Table 2). The main effect was produced in the production of dry root biomass; the differences among treatments were highly significant ($p = 0.0001$); treatment T_2 had the greatest dry weight with 0.21 g, 31.25% and 75% greater than T_3 and T_1, respectively, and 133% greater than the control. The differences in the production of total dry mass were not significant.

Mycorrhization of melon seedlings with *Glomus intraradices* produced a descending movement of photosynthates, coinciding with Wu *et al.* (2006) who reported that the mycorrhizal fungus generated a basipetal movement of photosynthates towards the roots. This movement is generated principally by the interchange of nutrients between the fungus and the plant; the hyphae of the fungus increase the effective root system and provide the plant with nutrients outside the depletion zone, while the plant transports carbohydrates towards the root which provide a substrate for the growth and development of the fungus. It has been observed that the greater the level of mycorrhization, the greater the fungal demands for substrate, producing greater movement of photosynthates.

The mycorrhization of the seedlings stimulated root growth; there was 4% more accumulation of dry material compared to the control, coinciding with the results of González-Monterrubio *et al.* (2005), who reported a significant increase of 59.2% in dry root biomass of *Opuntia streptacantha* seedlings inoculated with mycorrhizal fungi, and with those Huang *et al.* (2011), who obtained a significant increase by inoculating melon seedlings.

Mycorrhization produced more vigorous plants after transplanting, confirming the proposal for Tessi (1991) and Hoyos (1996), who proposed that greater accumulation of dry material in seedlings makes them more resistant to transplant. Tessi (1991) suggested that a 1% increase in seedling dry material increases the percentage of rooting by 30%, while Oseni *et al.* (2010), proposed that an increase in root weight in plants with mycorrhizae produces a positive correlation with nutrient absorption and a better post-transplant response, expressed as greater plant growth.

Parameters of growth and differentiation

The only significant difference in leaf differentiation and growth parameters was in the etiolation index ($p = 0.0340$). The melon seedlings inoculated with 40 spores plant^{-1} had a more equilibrated distribution between height and diameter (IA: 25.89) compared to seedlings inoculated with 20 and 80 spores plant^{-1} and the control, which had 6%, 26% and 52% more disequilibrium, respectively. Although there were no significant differences in the majority

Table 2. Production of dry biomass in Inodorus var. Honeydew melon seedlings 50 days after sowing, inoculated with *G. intraradices*.

Treatments	Parameters			
	LDW (g)	SDW (g)	RDW (g)	TDW (g)
T0	0.47a	0.2a	0.09a	0.76a
T1	0.33a	0.16a	0.12b	0.61a
T2	0.49a	0.17a	0.21d	0.87a
T3	0.41a	0.18a	0.16c	0.75a
p	ns	ns	0.0001	ns

LDW: leaf dry weight; SDW: stem dry weight; RDW: Roots dry weight; TDW: total dry weight. ns: not significant. Means in the same column with different letters indicate significant differences (LSD, $p < 0.05$).

Table 3. Growth parameters in seedlings of melon Inodorus var. Honeydew at 50 days of planting, inoculated with *G. intraradices*.

Treatments	Parameters				
	SD (cm)	PH (cm)	EI	LS (cm^2)	Nº de hojas
T0	0.43a	16.76a	39.46b	80.1a	5.6a
T1	0.45a	12.28a	27.49a	62.89a	5.6a
T2	0.53a	13.32a	25.89a	82.71a	6.0a
T3	0.46a	14.72a	32.62ab	72.55a	5.2a
p	ns	ns	0.0340	ns	ns

SD: Stem diameter; PH: Plant height; EI: Etiolation index (PH/SD); LS: Leaf surface; ns: not significant. Means in the same column with different letters indicate significant differences (LSD, $p < 0$ .05).

of the parameters measured, the plants inoculated with 40 spores*plant^{-1} had the best response in the parameters of growth and differentiation, except for plant height (Table 3).

Although the differences in number of leaves, leaf surface and stem diameter were not significant, mycorrhization appeared to stimulate the growth of these parameters. An increase in stem diameter was observed in plants of *Poncirus trifoliata* (Wu *et al.,* 2010), *Cucumis melo* (Huang *et al.,* 2011), *Leucaena leucocephala* (Flores-Bello *et al.,* 2008), *Lycopersicum esculentum* (He *et al.,* 2010) and *Calocedrus decurrens* (Amaranthus and Steinfeld, 2005) when inoculated with mycorrhizae. Preciado *et al.* (2002) suggested that stem diameter is a good indicator of plant vigor, since it reflects directly the accumulation of photosynthates which after transplantation can be translocated to the demand sites. This was confirmed by Ortiz *et al.* (2009), who indicated that a thicker stem implies greater phloem area and thus more efficient transport and a greater reserve capacity of photosynthates. Mycorrhization generated a lower etiolation index, favoring better plant architecture; these characteristics are desirable to obtain seedlings with a better capacity to support transplant (Preciado *et al.* 2002).

Indicators of stress

The stress indicator most sensitive to mycorrhizal activity was the relation RDW/SDW and not SLF, since the former considers the behavior of the root where the symbiotic action occurs. Plants inoculated with *Glomus intraradices* had less stress than those not inoculated, since they had a greater RDW/SDW. The plants inoculated with 40 spores plant^{-1} showed less stress (0.33) than those inoculated with 20 or 80 spores*plant^{-1}, due to greater root weight (Table 4).

Our results are coincident with those obtained by Tobar *et al.* (1994) in lettuce, Meddad-Hamza *et al.* (2010) in micropropagated olives, Lee and Kim (2004) in cucumber and Oseni *et al* (2010) in tomato; these authors all reported an increase in the ratio RDW/SDW in plants with mycorrhizae, showing a high degree of efficiency of these fungi. This indicates that seedlings with mycorrhizae had a better value for this stress indicator and a better condition to support transplant, since they produced plants with better equilibrium between plant height and stem diameter.

Enzymatic activity of nitrate reductase

The enzymatic activity of nitrate reductase in leaves was not affected by the activity of *Glomus intraradices* in the inoculated plants (Table 5). All

Table 4. Indicators of stress in melon seedlings Inodorus var. Honeydew inoculated with *G. intraradices* 50 days after sowing.

Treatments	Parameters	
	SLS	RDW/SDW)
T0	184.84a	0.14a
T1	194.31a	0.26b
T2	171.09a	0.33c
T3	176.54a	0.27b
p	ns	0.0001

SLS: Specific leaf surface (LS/LDW); R (LDW/SDW): (Root dry weight/Stem dry weight); ns: not significant. Means in the same column with different letters indicate significant differences (LSD, $p < 0.05$).

Figure 3. Comparison between the plants without inoculation (left) and with treatment T_2 (right).

Table 5. Nitrate reductase activity in seedlings of melon Inodorus var. Honeydew inoculated with *G. intraradices* at 50 days of sowing.

Treatments	Parameters		
	NRE	NRI	NRI/NRE
	(µM de NO_{2-} $g^{-1}pf\ h^{-1}$.)	(µM de NO_{2-} $g^{-1}pf\ h^{-1}$.)	
T0	1.52a	1.53a	1.01a
T1	1.47a	1.59a	1.18a
T2	1.29a	1.58a	1.24a
T3	1.12a	1.54a	1.39a
p	ns	ns	ns

NRE: Endogenous Nitrate Reductase; NRI: Induced Nitrate Reductasa. ns: not significant. Means in the same column with different letters indicate significant differences (LSD, $p < 0\ .05$).

treatments received 7.5 meq L^{-1} NO_3^{-1} in the fertirrigation solution; however, there was a better NRI/NRE ratio (Induced Nitrate Reductasa/Endogenous Nitrate Reductase) (T_1:18; T_2:24 y T_3:39%), which indicates a greater availability of NO_3^{-1} in the leaf tissue which may be reduced by the enzyme nitrogen reductase compared to the control. This indicates a certain level of abiotic stress present in seedlings with mycorrhizae, and concurs with the proposal of Tobar *et al.* (1994). The greater availability of NO_3^{-1} in plants with mycorrhizae is explained because part of the nitrate was absorbed in the soil by the external mycelia of the fungi and reduced (Bago *et al.* 1996 y Johansen *et al.* 1996), allowing the

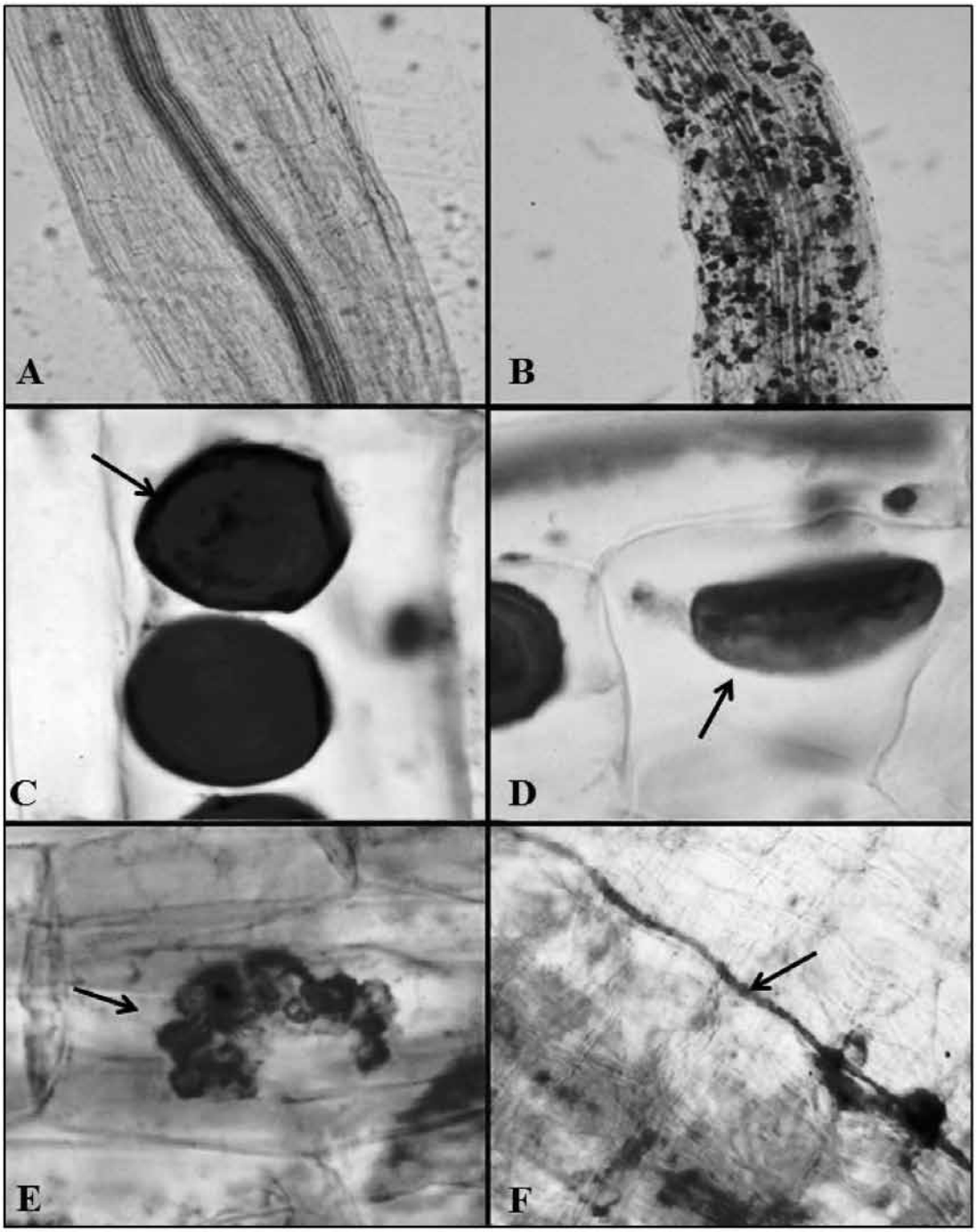

Figure 4. Fungal structures of *Glomus intraradices* colonizing the roots, staining with Tripan blue. A: Non-mycorrhized roots T_0 (40X); B: Mycorrhized roots T_2 (40X); C: Spores (100X); D: Vesicles (100X); E: Arubuscles (100X); F: Intraradical mycelium (100X).

formation of intermediate molecules of the nitrogen cycle which are transferred to the plant for protein synthesis (Tilak and Dwivedi 1990, Subramanian and Charest 1998).

Conclusions

The inoculation at sowing with *Glomus intraradices* produced more equilibrated seedlings of

honeydew melon, with a greater root development than non-inoculated plants, a lower etiolation index and greater availability of nitrates in the leaves for reduction by nitrate reductase. The dose of 40 spores*plant^{-1} produced the greatest percentage of mycorrhization (49%), a lower level of stress due to greater production of root biomass and 24% more nitrogen available for reduction in the leaves.

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