INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
13–1464/2015/17–1–119–126
http://www.fspublishers.org

Full Length Article

Influence of Arbuscular Mycorrhizal Fungi and Nitrogen Concentrations on C*arica papaya* Plant Growth

Quiñones-Aguilar Evangelina Esmeralda[1], Rincón-Enríquez, Gabriel[1], Hernández-Cuevas[1], Laura Verónica[2] and López-Pérez Luis[3*]

[1]*Unidad de Biotecnología Vegetal, Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco (CIATEJ), Av. Normalistas 800, Colinas de la Normal, 44270, Jalisco, México*

[2]*Laboratorio de Micorrizas, Centro de Investigación en Ciencias Biológicas, Universidad Autónoma de Tlaxcala, 90122, Tlaxcala, México*

[3]*Instituto de Investigaciones Agropecuarias y Forestales (IIAF), Km. 9.5, Carr. Morelia-Zinapecuaro, 58880, Tarímbaro, Michoacán, México*

[*]For correspondence: lexquilax@yahoo.com.mx

Abstract

Although the *Carica papaya* plant is a mycotrophic species that requires high nitrogen (N) concentration to obtain high yield, the effect of interaction between inoculums and nitrogen doses has not been studied thoroughly for this species. In this work we evaluated the effect of interaction between five inoculums (a native inoculum denominated Agua Dulce; commercial inoculum containing *Glomus* spp*; Glomus claroideum; Acaoulospora spinosa* and without inoculum) and four N concentrations (0, 0.5, 1.5 or 4.0 mM of N) on *C. papaya* cv 'Maradol' plant growth. Plants treated with different inoculums of AM fungi showed significant differences (P<0.05) in plant growth. The Agua Dulce inoculum obtained 65.23 cm^2 of leaf area, 10.17 g of total fresh weight and 56.67% of root colonization percentage. Also, the plant growth was affected by N concentration supply, concentrations up to 1.5 mM increased the growth and concentrations of 4 mM of N decreased the plant growth and the root colonization percentage. In regards to the interaction between factors, the Agua Dulce treatment inoculum and 1.5 mM N recorded the major values in the growth variables on *C. papaya* plants. The fact that we have obtained better results with Agua Dulce inoculum could be due to the diversity of AMF in this inoculum that promotes the root growth as shown by the increased volume and root length. This would allow increased N uptake by the plant, which was reflected, in higher growth. In conclusion, the use of Agua Dulce (native inoculum) and 1.5 mM N may be recommended to obtain good quality plants at the nursery.

Keywords: Mycorrhizal native; *Glomus claroideum; Acaoulospora spinosa;* Ammonium; Papaya

Introduction

Carica papayas like all plants require nitrogen (N) in order to grow and develop. It is well known that N is necessary for plant growth, because this element is essential for the synthesis of proteins, nucleic acids, co-enzymes and many products and by-products of secondary metabolism (Marschner, 1998). Although N is present in the soil as a complex mixture of organic and inorganic compounds, ammonium and nitrate are the main sources for nutrition for most of higher plants species (Williams and Miller, 2001). The concentration of these two ions varies a lot in the soil solution; therefore, the plants have developed strategies to increase their capacity for N mobilization. One strategy is the symbiotic association with arbuscular mycorrhizal fungi (AMF) (Pérez-Tienda *et al*., 2012). The AM fungi is known to be a major microbial component of plants. This symbiosis, play a crucial role on several aspects of plant physiology such as mineral nutrition, resistance to thermal and water stress, induce resistance against diseases, improve soil structure, etc. (Gianinazzi *et al*., 1990). On the other hand, there are studies that show as the extra radical mycelia of AM fungi are able to take up and assimilate NH_4^+ (Frey and Schüepp, 1993; Johansen *et al*., 1996), NO_3^- and amino acids (Tobar *et al*., 1994; Johansen *et al.*, 1996; Hawkins *et al.,* 2000; Hodge *et al.,* 2001). This nitrogen is translocating to the intraradical mycelium and then released to the host plants (Govindarajulu *et al.,* 2005). Although AM fungi are able to take up both NO_3^- and NH_4^+, a clear preference for NH_4^+ has been demonstrated (Toussaint *et al.*, 2004). This is explained, at least partially, by the extra energy the fungus must expend in reducing NO_3^- to NH_4^+ before it can be incorporated into organic compounds (Marzluf, 1996). Also, there is evidence that AM fungi have the potential to take up and transfer significant amounts of N to the host plant (Karagiannidis *et al.,* 2007; Miransari, 2011; Pérez-Tienda *et al*., 2012; Smith and Smith, 2012; Ngwene *et al.,* 2013), in case AM fungi hyphae has access to N sources that are

To cite this paper: Quiñones-Aguilar, E.E, R.E. Gabriel, H.C.L. Verónica and L.P. Luis, 2015. Influence of arbuscular mycorrhizal fungi and nitrogen concentrations on *Carica papaya* plant growth. *Int. J. Agric. Biol*., 17: 119–126

not accessible to roots (Frey and Schüepp, 1993; Hodge, 2003; Leigh *et al.,* 2009). McFarland *et al.* (2010) indicated that more than 50% of plant N requirement was supplied by mycorrhizal association. The effect of interaction between AM fungi and N, already been studied in other crops as lettuce (Tobar *et al.,* 1994), cowpea (Ngwene *et al.,* 2013), carrot (Pérez-Tienda *et al*., 2012), strawberry (Castellanos-Morales *et al.,* 2010, 2012; Salgado-Barreiro *et al.,* 2012), grapevine (Karagiannidis *et al.,* 2007) among others (Miransari, 2011). However, for *C. papaya* not been studied yet. The aim of this work was to study the effect that both AM fungi and N concentration and their interaction, on growth of *C. papaya* plants. Based in previous results from our laboratory we hypothesized that plants mycorrhizal supply with low N concentrations may increase growth compared with non-inoculate plants or inoculate with high N concentrations.

Materials and Methods

Experimental Conditions and Design

The experiment was conducted in the greenhouse at the Instituto de Investigaciones Agropecuarias y Forestales, Universidad Michoacána de San Nicolás de Hidalgo in Morelia Michoacán, México (lat. 19°45’95’’N, long. 101°09’16’’, alt. 1900 m) under natural conditions of light, temperature, and humidity. The 5×4 factorial experiment was designed with five mycorrhizal inoculums: a commercial containing *Glomus* spp (G); a native inoculum denominated Agua Dulce (AD) consortium isolated from rhizospheric soil of *Agave cupreata* plantations in Michoacán, México; *Glomus claroideum* (Gc); *Acaoulospora spinosa* (Ac) (these two strains kindly provided by M.C. Laura Verónica Hernández-Cuevas of Universidad de Tlaxcala, México) and non-inoculum combined with four nitrogen concentrations: 0,0.5, 1.0 and 4.0 mM $(NH_4)_2SO_4$. Thus, there were twenty treatment combinations arranged in a randomized complete block design, which was duplicated ten times.

Plant Material and AM Fungi Inoculum

Seeds of *C. papaya* cv. ‘Maradol’ were germinated on sterilized sand (8 h at 120ºC and 1.055 kg cm^{-2} of pressure), when seedlings showed similar growth and had two real leaves, these were transplanted into black plastic bags and 2 kg of sand was used as growth medium. The sand had the following physicochemical properties: sandy loam texture, pH, 6.63; OM,1.2%; P, 13 ppm; Ca^{2+}, 218 ppm; Mg^{2+}, 147 ppm; NH_4^+, 5 ppm. Two kilograms of autoclaved substrate was dispensed into each pot. For inoculation a hole was made in the center of the pot and was added an amount of 50 g of soil-based inoculum (containing 80 spores), so perfectly covering the roots of transplants. In the non-inoculated plants, the inoculation was made with 50 g of sterilized sand.

Irrigation Solution

Concentrations of 0, 0.5, 1.5 and 4 mM of nitrogen as ammonium sulfate were applied with the irrigation water, these were prepared from a stock solution of 1 M $(NH_4)_2SO_4$. Treatments with higher concentration (1.5 and 4 mM) they were watered with deionized water every 15 days to avoid the accumulation of salts. The pots were watered as required to keep the substrate moist at all time.

Response Variables Evaluated

Every 20 days after being transplanted (DAT) the height and shoot diameter were recorded until the end of the experiment. After 100 days of being transplanted the plants were harvested and separated into root, shoot and leaves. The fresh weights, length and volume root and leaf area were recorded. A representative sample (about 5 g) of fresh roots were collected and stored in a solution of formol-acetic acid-alcohol until analysis. Root samples were cleared with 10% KOH, stained with ink and vinegar as described by Vierheilig *et al.* (1998) and microscopically (Motic, AE31) examined for AM colonization. The percentage was evaluated by a modified intersection method (Mcgonigle *et al*., 1990). The chlorophyll concentration in leaves was determined according to Barnes *et al.* (1992). The last fully expanded leaves, were cut into small pieces (0.1 g) and placed in a vial containing 5 mL dimethyl sulfoxide (DMSO) saturated with calcium carbonate. The vials were incubated at 60°C for 4 hours. The extracts were transferred to UV-Vis cells and the absorbance of the extract was taken at 648.2 and 664.9 nm in a UV-Vis spectrophotometer (Lamdba 40, Perkin-Elmer) and the concentrations of chlorophyll A and B were calculated according to Barnes *et al.* (1992).

Statistical Analysis

Experimental data were analyzed with SPSS 14.0 statistical program (SPSS Inc., Chicago, IL, USA). Two-way ANOVA was performed considering AM fungi and nitrogen concentrations as independent factors. Differences between individual means were compared by Tukey's multiple comparison tests and were considered significant at $P < 0.05$.

Results

Mycorrhizal Colonization

The inoculum factors and nitrogen concentration were significant ($P \leq 0.001$ and $P \leq 0.05$, respectively). Papaya plants inoculated showed high colonization percentages (33% on average) compared to non-inoculated. The largest percentage was obtained with the Agua Dulce inoculum (56.67%), which was 42% greater than the average of other inoculums (Fig. 1). The colonization percentages were affected by the nitrogen concentration, doses of 0 to

1.5 mM didn't show significant difference and averaged 27%; for 4 mM of N concentration the colonization (17.73%) decreased 1.5 times (Fig. 2). In regards to the interaction inoculum and N, the treatment of Agua Dulce consortium with 1.5 mM of N reached the highest colonization percentage with 71.67% (Table 1).

Growth Parameters

The effect of nitrogen concentration, inoculum and their interaction on *C. papaya* growth parameters is shown in Table 1 and Fig. 3 and 4. The factors and their interaction were statistically significant in all variables measured (P<0.05). For the inoculums, Agua Dulce inoculum (native), obtained the highest values in LFW (4.21 g), RFW (5.96 g), LA (65.23 cm^2), RL (19.54 cm) and RV (6.03 cm^3).The other inoculums (*Acaulospora spinosa*, *Glomus* spp and *Glomus claroideum*) were statistically equal to the treatment without AMF or in some cases lower as in RV. The increase in plant growth and dry biomass in the inoculum of Agua Dulce depended of nitrogen concentration in the irrigation solution. This factor caused an increase in LFW, RFW, LA and RV as dose increased to 1.5 mM, at higher concentration (4 mM) the values decreased. RL showed an opposite effect, lower value to higher nitrogen concentrations. The treatment that increased the plant growth was Agua Dulce inoculum with 1.5 mM of N concentration, which obtained the higher values of all evaluated variables (Table 1).

Height dynamics is shown in Fig. 3. Papaya plants inoculated and non-inoculated increased their height during the experiment; however, this increase was affected by both the inoculum and N concentration. Lower heights were obtained with concentrations of 0 and 4 mM of N and *Glomus* spp, *Glomus claroideum* and *Acaulospora spinosa*. In general there was a decrease in the plant´s height when the concentration of N was 4 mM, except in Agua Dulce inoculum. During the first 40 DAT no significant difference exist in height between treatments, but from this date Agua Dulce inoculum with 1.5 and 4 mM of N, increased steadily up to height until the end of the experiment and on average obtained 12.72 cm. *Acaulospora spinosa* with 4 mM of N showed lower heights of plants during the experiment obtaining at the end (100 days) 4.82 cm. Plants inoculated with *Glomus claroideum*, *Acaulospora spinosa* and *Glomus* spp increased its height when the N concentration was 1.5 mM, however not exceeded the plants inoculated with Agua Dulce inoculum (Fig. 3).

The dynamics of diameter increase of plants is shown in Fig. 4. In our experiment, the shoot was affected by the inoculum and nitrogen concentration (P <0.0000), it showed a dynamic as that observed in height of the plant. At 40 days after inoculation, treatments of Agua Dulce inoculum with 1.5 and 4 mM of N increased significantly their diameter until the end of the experiment compared with others treatments. At 100 days Agua Dulce with 1.5 mM of N had the highest shoots with 6.86 mm. The lowest shoots were obtained in *Glomus* spp with 4 mM of N and non-inoculated plants with 1.5 mM being 3 times smaller compared to larger diameter reached (Fig. 4).

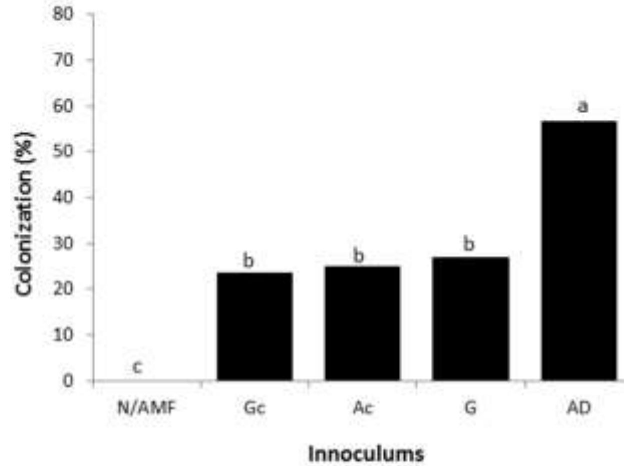

Fig. 1: Percentage of root colonization on *Carica papaya* plants with different inoculums at 100 days after inoculation. Values are average of twenty dates independently the N concentration. Columns with the same letter are statistically equals according to Tukey test (P < 0.05). N/AMF, without inoculum; Gc, *Glomus claroideum*; Ac, *Acaulospora spinosa*; G, *Glomus* spp; AD, Agua Dulce

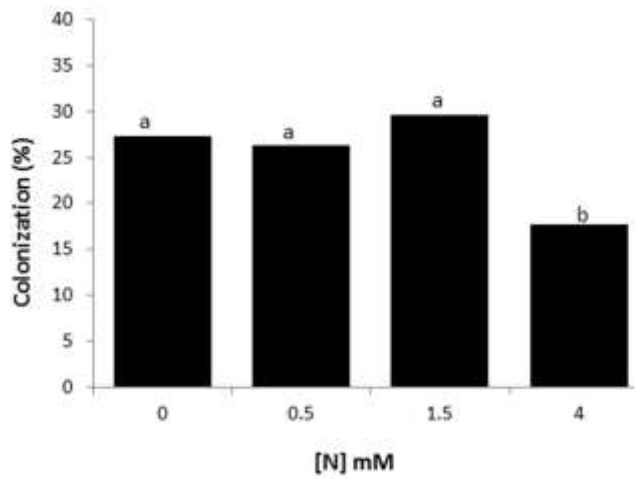

Fig. 2: Percentage of root colonization on *Carica papaya* plants with different N concentrations at 100 days after inoculation. Values are average of twenty dates independently the inoculum. Columns with the same letter are statistically equals according to Tukey test (P < 0.05)

Chlorophyll Concentration

For chlorophyll, the variance analysis showed a significant effect of the factors evaluated and interaction (Table 1).

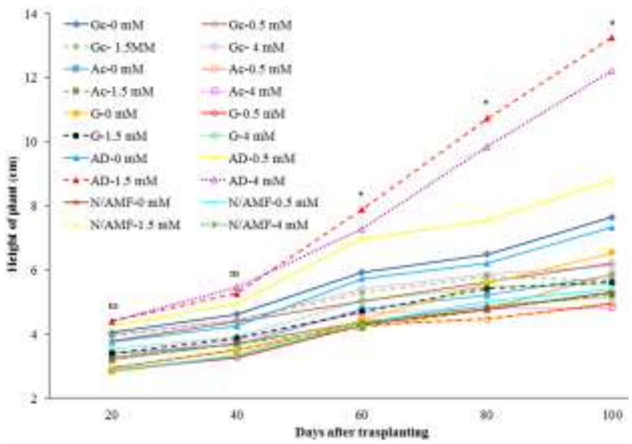

Fig. 3: Effect of interaction between AM fungi inoculums and N concentration on height of *Carica papaya* plants. Gc (*Glomus claroideum*); Ac (*Acaulospora spinosa*); G (*Glomus* spp); AD (Agua Dulce); N/AMF (without inoculum); ns and * indicate not significant or significant at P < 0.05, between treatments in this date

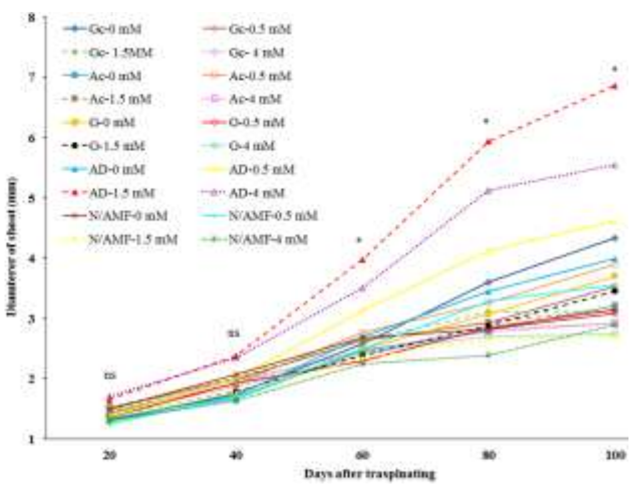

Fig. 4: Effect of interaction between AM fungi inoculums and N concentration on shoot diameter of *Carica papaya* plants. Gc (*Glomus claroideum*); Ac (*Acaulospora spinosa*); G (*Glomus* spp); AD (Agua Dulce); N/AMF (without inoculum); ns and * indicate not significant or significant at P < 0.05, between treatments in this date

To inoculum factor, *Glomus claroideum* and *Acaulospora spinosa* decreased the chlorophylls concentration, while *Glomus* spp and Agua Dulce inoculums had values statistically equal to non-inoculated plants. However Agua Dulce inoculum obtained the higher values of chlorophyll a, b and total (Table 1). In regards to nitrogen concentration, the chlorophylls increased their values when increasing nitrogen concentration. For chlorophyll a, *Glomus* spp with 1.5 mM of N obtained the highest value, for chlorophyll b and total, the higher content was obtained in the treatment of Agua Dulce with 4 mM (Table 1).

Discussion

In this experiment, we found fungal structures in roots of plants treated with AM fungi, regardless of nitrogen level in the irrigation solution, which reaffirms the mentioned by Khade *et al.* (2002) and Khade and Rodrigues (2008a, b; 2009). They mentioned that *C. papaya* is highly dependent to mycorrhizal. The values found in the root colonization percentage (25%) when the plants were treated with monospecific inoculum (*Glomus claroideum*, *Acualospora spinosa* and *Glomus* spp) were similar to the ones reported by Constantino *et al.* (2011) on papaya plants inoculated with *Glomus intraradices* and López-Moctezuma *et al.* (2005) using a consortium of *Gl. albidum*, *Gl. claroides* and *Gl. daphanum*. In the case that the percentage obtained by the native inoculum, these were similar to the ones reported by Quiñones-Aguilar *et al.* (2012) working with papaya plants inoculated with a consortium of *Glomus* denominated ZAC 19. The fact that we found a higher percentage of roots colonized in Agua Dulce inoculum could be due to this contained other species of AM fungi. Burleigh *et al.* (2002) indicated that the association plant fungi might be more or less beneficial in the plant growth depending, of the plant and fungi species. In Sunrise and Tainung, two papaya commercial varieties the inoculated with *Gigaspora margarita* induced higher dry matter production that *Glomus clarum* under the same growth conditions (Trindale *et al.*, 2001). Sharda *et al.* (2010) reported significant variations in root colonization levels on different papaya varieties inoculated with native mycorrhizal fungi, indicating that there was a preference the fungi by a particular papaya variety.

Regarding growth response of papaya plants inoculated with AM fungi, there are reports that demonstrate a beneficial effect (Constantino *et al.*, 2010, 2011; Vázquez-Hernández *et al.*, 2011; Quiñones-Aguilar *et al.*, 2012). In our work, plants inoculated with the inoculum Agua Dulce showed higher growth compared to plants without inoculum and mycorrhizal with monospecific inoculum (Ac, Gc and G). We recorded an increase of 400% on average in the LFW, RFW, LA and 150% for the RL respect to non-mycorrhizal plants (Table 1). Similar responses were found by our working group in papaya plants inoculated with different native consortiums isolated from rhizosphere of *Agave cupreata* plantations from Michoacán, Mexico. These consortiums promoted the growth of papaya plants compared with plants non-inoculated (unpublished data). The positive effect shown by the native inoculum on papaya plants growth may be explained by the fact that the fungi promoted the root growth (showed in the increase on volume and length root of this treatment) and the extraradical mycelium are acting together to have a higher exploration surface, nitrogen uptake and nutrients supplied by the growth medium. Another reason may be the fact that the native inoculum contained other AM fungi species or too likely other

Table 1: Effect of AM fungi, N concentration and their interaction on *Carica papaya* plants growth at 100 days after inoculation

Factor	LFW	RFW	LA	RL	RV	MC	CA	CB	TC
AMF		(g)	(cm^2)	(cm)	(cm^3)	%		$\mu g.g^{-1}$	
Gc	0.74 bc	0.94 b	6.72 d	9.94 b	0.76 a	23.56 b	1.39 ab	0.42 a	1.76 ab
Ac	0.77 c	1.16 b	9.73 cd	11.17 b	1.20 ab	25.11 b	1.20 a	0.36 a	1.56 a
G	0.94 bc	1.22 b	11.21 bc	11.87 b	1.10 ab	27.08 b	1.50 b	0.49 ab	2.00 bc
AD	4.21 a	5.96 a	65.23 a	19.54 a	6.03 c	56.67 c	1.48 b	0.73 b	2.20 c
N/AMF	0.91 b	1.42 b	18.81 b	12.61 b	1.47 b	0.00 a	1.45 b	0.48 ab	1.93 bc
N (mM)									
0	1.19 a	2.10 a	14.34 a	16.42 a	2.03 b	27.33 ab	1.14 a	0.32 a	1.42 a
0.5	1.24 ab	2.20 a	16.67 ab	15.37 ab	2.11 ab	26.33 ab	1.48 ab	0.48 b	1.97 b
1.5	1.95 b	2.65 ab	28.37 ab	11.55 b	2.69 ab	29.6 b	1.52 b	0.56 b	2.08 b
4	1.66 b	1.610 b	29.47 b	8.41 c	1.78 a	17.73 a	1.47 b	0.61 b	2.09 b
AMF x N									
Gc-0	0.87 a	1.71 a	8.18 abc	13.53 abcd	1.58 ab	26.67 ab	1.13 ab	0.35 a	1.30 a
Gc-0.5	0.58 a	0.79 a	4.58 a	12.02 abcd	0.90 b	30.00 ab	1.38 abcd	0.32 ab	1.69 ab
Gc- 1.5	0.91 a	0.70 a	9.14 ab	7.30 abc	0.36 abc	26.00 ab	1.32 abcd	0.42 abc	1.73 ab
Gc- 4.0	0.59 a	0.56 abc	4.99 a	6.90 ab	0.20 a	12.00 a	1.73 bcd	0.58 abcd	2.31 ab
Ac-0	1.06 a	1.76 a	12.64 abc	16.37 bcd	1.92 bc	26.67 ab	0.90 a	0.24 a	1.14 a
Ac-0.5	1.07 a	1.93 ab	16.57 abc	16.33 abcd	1.37 abc	26.67 ab	1.43 abcd	0.46 abcd	1.89 ab
Ac-1.5	0.54 a	0.68 a	5.31 a	7.78 abc	0.50 abc	22.00 ab	1.19 abcd	0.36 abc	1.55 ab
Ac-4.0	0.40 ab	0.28 abc	4.39 abc	4.20 a	1.00 abc	0.00 a	1.28 abcd	0.38 abcd	1.66 ab
G-0	1.24 a	1.97 a	14.93 abc	18.05 bcd	1.82 abc	30.00 ab	1.17 abcd	0.32 ab	1.49 a
G-0.5	0.80 a	1.17 a	9.69 abc	12.42 abcd	0.77 ab	25.00 ab	1.46 abcd	0.45 abc	1.91 ab
G-1.5	0.73 a	1.02 a	8.41 ab	10.33 abcd	1.02 ab	28.33 ab	1.92 d	0.73 bcd	2.65 b
G-4.0	0.97 a	0.71 a	11.82 abc	6.70 ab	0.78 abc	25.00 ab	1.46 abcd	0.47 abcd	1.93 ab
AD-0	1.68 a	2.85 ab	23.43 abcd	18.10 bcd	2.83 abc	53.33 bc	1.11 abc	0.29 ab	1.40 a
AD-0.5	2.72 ab	5.44 abc	39.37 bcd	22.87 cd	5.53 bc	50.00 bc	1.47 abcd	0.59 abcd	2.06 ab
AD-1.5	6.71 c	9.81 c	109.20 d	23.57 d	9.55 c	71.67 c	1.77 cd	0.88 cd	2.65 b
AD-4.0	5.71 bc	5.74 bc	88.92 cd	13.63 abcd	6.20 abc	51.67 bc	1.56 abcd	1.14 d	2.70 ab
N/AMF-0	1.08 a	2.21 a	12.51 abc	16.03 bcd	2.00 abc	0.00 a	1.38 abcd	0.40 abc	1.78 ab
N/AMF -0.5	1.05 a	1.66 a	13.14 abc	15.02 abcd	1.98 abc	0.00 a	1.68 abcd	0.60 abcd	2.29 ab
N/AMF -1.5	0.86 a	1.04 a	9.83 abc	8.78 abcd	1.15 ab	0.00 a	1.39 abcd	0.44 abc	1.83 ab
N/AMF -4.0	0.63 a	0.77 a	7.77 ab	10.60 abcd	0.73 abc	0.00 a	1.34 abcd	0.48 abcd	1.83 ab
Significances									
AMF	_***	_***	_***	_***	_***	_***	_**	_***	_***
N	_***	_***	_***	_***	_***	_*	_***	_***	_***
AMF x N	_***	_***	_***	_***	_*	ns	_*	_**	_**

LFW, leaf fresh weight; RFW, root fresh weight; LA, leaf area; RL, root length; RV, root volume; MC, mycorrhizal colonization; CA, CB and TC, chlorophyll a, b and total respectively. Gc (*Glomus claroideum*), Ac (*Acaulospora spinosa*), G (*Glomus* spp), AD (Agua Dulce) and N/AMF (without mycorrhizal inoculum).Values in columns with the same letters are statistically equals according to Tukey test ($P \leq 0.05$). _*** ($P \leq 0.001$); _** ($P \leq 0.01$); _* ($P < 0.05$); ns, not significant

microorganisms such as plant growth-promoting rhizobacteria (PGPR); they might act together to promote plant growth. There is evidence that the combined interactions between AM fungi and PGPR may increase the plant growth (Artursson *et al.*, 2006). López-Moctezuma *et al.* (2005) reported that papaya plants favored its growth when they were inoculated with AM fungi complex and *Bacillus pumilis*. Constantino *et al.* (2010) reported a significant increase on shoot diameter and fresh and dry biomass on papaya seedling inoculated with *Gl. intraradices* and *Azospirillum brasilense*, compared with non-treated plants. In another work with papaya seedlings inoculated with *Azotobacter chroococcum* and *Gl. intraradices*, there was a significant increase on dry biomass of inoculated plants respect to non-treated (Constantino *et al.*, 2011). This positive effect might occur in the treatments with the inoculum Agua Dulce, however would be unlikely to occur in plants without AM fungi or in treatments with monospecific inoculums because the growth medium was previously sterilized. In the case of the treatments where monospecific inoculum were used, despite obtaining a colonization of 25% and that this does not reflect on plant growth increase in general, might be due to the specificity of the plant to AM fungi species. It has been demonstrated that the response of the plant to mycorrhizal depends on several factors, including the host plant and AM fungi species (Khade and Rodrigues, 2008a, b). In works with papaya, favorable results were founded on plant growth using *Gl. intraradices, Gl. mosseae* and *Gl. Diaphanum* (López-Moctezuma *et al.*, 2005; Constantino *et al.*, 2011; Quiñones-Aguilar *et al.*, 2012). Another possible explanation may be because these plants didn't have a higher volume of explorations by roots in the growth medium and low nutrient concentration in this mainly phosphorus, reason why these plants obtained similar or less growth than in plants without AM Fungi. Smith and Smith (2012), suggested that low or no effect of AM fungi on plant growth was due to a deficiency of phosphorus on plants, by a reduction in the levels of absorption by the roots and may not be sufficiently compensated by the low percentage of colonized roots (Grace *et al.,* 2009). This would be likely to occur in the case of treatments without mycorrhizal. In regards to the effect of AM fungi in the chlorophyll concentration, Baslam *et al.* (2013) and Li *et al.* (2013), mentioned that the effect of mycorrhizal symbiosis on chlorophyll contents depend on the host plant, leaf type and AM fungi species. Díaz and Garza (2006) reported increases in the chlorophyll content in three genotypes of *Cenchrus ciliaris* L. inoculated with *Gl. intraradices*. Similar results were reported in *Capsicum annum* plants inoculated with *Rhizophagus intraradices* under greenhouse conditions (Díaz *et al.,* 2013). In our study we did not detect changes in chlorophyll concentration between the different inoculums, this may be explained in part by the type of leaf sampled in this case, it was the last leaf fully extended and assume that this presented the highest photosynthetic activity and does not change the concentration of these pigments. Furthermore, its known that nitrogen is an essential nutrient for the growth and development of both plants (Marschner, 1998; Chapin *et al.,* 2004) and soil microorganisms (Demoling *et al.,* 2007) and that the effect of AM fungi on the host plant will depend of the dose and nitrogen source (Miransari, 2011; Pérez-Tienda *et al.,* 2012; Ngwene *et al.,* 2013). It has been demonstrated that under the same N concentrations, the ion nitrate decreases the mycorrhizal activity compared to ammonium (Valentine and Kleinert, 2006; Ngwene *et al.,* 2010) and that plants have preference for this ion (Smith and Smith, 2012). In our work, all growth variables evaluated were affected by the N concentration in the irrigation solution. Regarding mycorrhizal colonization, in this case we found out that plants with highest colonization percentage (Agua Dulce inoculum) and irrigate with 4 mM of N, increased 28% the percentage with respect to lower concentrations (from 0 to 1.5 mM). There are reports of an apparent negative effect of ion ammonium respect to nitrate in the root colonization percentage (Karagiannidis *et al.,* 2007; Salgado-Barreiro *et al.,* 2012; Ngwene *et al.,* 2013). However, this negative effect on the root colonization is mainly due to the increase in nitrogen concentration (Valentine and Kleinert, 2006; Ngwene *et al.,* 2010). Salgado-Barreiro *et al.* (2012) working with strawberry plants cv. Camino Real inoculated with *Gl. intraradices* found a significant decreased in the colonization percentage when the concentration of NO_3^- or NH_4^+ was increased in the irrigation solution. The possible reasons for this reduction may be due to the decrease in pH of rhizosphere by ammonium uptake (Habte, 1999; Rohyadi *et al.,* 2004), a decrease in the distribution of carbohydrates by the competition between both plant and fungal growth, the assimilation of ammonium by root, the fungi growth (Raven and Smith, 1976) or by amelioration in the phosphorus nutritional status plant as result of ammonium uptake (Johnson *et al.,* 1984). Above growth variables we found that when the N concentration of irrigation solution increase until 1.5 mM the higher plant growth was recorded. The N is one of the most important macronutrients that affect significantly the plant growth (Marschner, 1998; Miransari, 2011), it was expected that the plants would grow bigger to higher N irrigation solution supply. However, high concentration of this nutrient may produce negative effects on plant growth due to changes in the pH or an osmotic effect by the accumulation of salts in the growth medium. Today, the importance of AM fungi for plant N nutrition is a topic of controversy (Ngwene *et al.,* 2013). Some authors have mentioned that the potential N uptake and transport rates of roots are much higher than those of hyphae so that in soil with high mineral N supply, the contribution of AM fungi to shoot N content is likely to be small compared with uptake by the roots (Hawkins *et al.*, 2000; Hodge, 2003). However, the case may be different when AM fungi hyphae have access to N sources that are not accessible to roots (Frey and Schüepp, 1993; Hodge, 2003; Leigh *et al.,* 2009). There are works that have studied the effect of the interactions between AM fungi inoculation and different N concentrations and ionic form on plant growth. Salgado-Barreiro *et al.* (2012) inoculated *Fragaria x ananassa* D. Plants with *Gl. intraradices* and irrigated with doses of 0, 1.3 and 10 mM of N in both forms ammonium and nitrate. They reported a significant effect of interaction on dry biomass; the higher plant growth was obtained when used 3 mM of N as ammonium. Castellanos-Morales *et al.* (2012) reported significant increases in leaf area, dry biomass and colonization percentage on strawberry plants inoculated with *Gl. intraradices* and irrigated with a solution 3 mM of nitrate compared with plants with the same concentration but without inoculation. Karagiannidis *et al.* (2007) recorded an increase in the shoot dry weight and leaf number on grapevine inoculated with *Gl. mosseae* and fertilized with calcium nitrate respect to other nitrogen sources. Azcón *et al.* (2001, 2008) cultivated lettuce plants under drought conditions, tested the effects of AM fungi on the percentage of N uptake from N fertilization under different levels of soil N. They mentioned that AM fungi enhanced plant N concentration, the activity of nitrate reductase and proline content in plant. At the medium level of N fertilization (6 mM N), AM fungi resulted in the higher N uptake from fertilization, relative to the lowest (3 mM N) and highest (9 mM N) N levels, which reduced N uptake from fertilization. In our studied *C. papaya* plants inoculated with Agua Dulce inoculum and supply with 1.5 mM of N as ammonium reached the higher values of most variables evaluated. When the N concentration was 4 mM, the effect concomitant between both N and mycorrhizal was ameliorated. High amounts of N fertilization can significantly decrease N uptake by mycorrhizal plants (Miransari, 2011). These results indicate that mycorrhizal plants may regulate plant N uptake with respect to the amounts of N supply.

Conclusion

In conclusion, the *C. papaya* cv 'Maradol' plant growth was determined by inoculum, N concentration and their interaction. The inoculum Agua Dulce enhanced significantly the root colonization and promoted the plant growth, compared to plant with mono-spore inoculums or non-inoculated. However these results depended on the N nitrogen concentration in the irrigation solution. The fact that the inoculum Agua Dulce was the best, might be due to the presence of different AM fungi species. The poor response of others inoculums would result from the specificity by host plant to fungi. The low N concentration results benefic to plant growth respect to high concentration, where the growth was reduced. This apparent response may be the result of the contribution to mycorrhizal in N uptake at low concentration. Finally the treatment where we obtained the best growth of *C. papaya* plants was with Agua Dulce (native) inoculum and 1.5 mM of N concentration.

Acknowledgements

This research was sponsored by the Fondo Mixto CONACYT-Gobierno del Estado de Michoacán under contract "Utilización de recursos microbianos para el control biológico de la pudrición del cogollo de agave tequilero en la DOT-Michoacán" (MICH-2010-03-148208).

References

Artursson, V., R.D. Finlay and J.K. Jansson, 2006. Interactions between arbuscular mycorrhizal fungi and bacteria and their potential for stimulating plant growth. *Environ. Microbiol*., 8: 1–10

Azcón, R., R. Rodríguez, E. Amora-Lazcano and E. Ambrosano, 2008. Uptake and metabolism of nitrate in mycorrhizal plants as affected by water availability and N concentration in soil. *Eur. J. Soil. Sci*., 59: 131–138

Azcón, R., J. Ruiz-Lozano and R. Rodriguez, 2001. Differential contribution of arbuscular mycorrhizal fungi to plant nitrate uptake (N-15) under increasing N supply to the soil. *Can. J. Bot*., 79: 1175–1180

Barnes, J., D.L., Balaguer, E. Manrique, S. Elvira and A. W. Davison, 1992. A reappraisal of the use of DMSO for the extraction and determination of chlorophylls a y b in lichens and higher plants. *Environ. Exp. Bot*., 32: 85–100

Baslam, M., R. Esteban, J.I. García-Plazaola and N. Goicochea, 2013. Effectiveness of arbuscular mycorrhizal fungi (AMF) for inducing the accumulation of major carotenoids, clorophylls and tocopherol in green and red leaf lettuces. *Appl. Microbiol. Cell Physiol*., 97: 3119–3128

Burleigh, S.H., T. Cavagnaro and I. Jakobsen, 2002. Functional diversity of arbuscular mycorrhizas extends to the expression of plant genes involved in P nutrition. *J. Exp. Bot.*, 53: 1593–1601

Castellanos-Morales, V., J. Villegas, S. Wendelin, H. Vierheilig, R. Eder and R. Cárdenas-Navarro, 2010. Root colonization by the arbuscular mycorrhizal fungus *Glomus intraradices* alters the quality of strawberry fruits (*Fragaria x ananassa* Duch.) at different nitrogen levels. *J. Sci. Food Agric*., 90: 1774–1782

Castellanos-Morales, V., J. Villegas-Moreno, H. Vierheilig and R. Cárdenas-Navarro, 2012. Nitrogen availability drives the effects of *Glomus intraradices* on the growth of strawberry (*Fragaria x ananassa*Duch.) plants. *J. Sci. Food Agric*., 92: 2260–2264

Chapin, I.I.I., F.S. Mooney, H.A. Chapin and P. Matson, 2004. *Principles of Terrestrial Ecosystem Ecology*. Springer, USA

Constantino, M., R. Gómez, J.D. Álvarez, J.M. Pat and E.G. Espín, 2011. Efecto de la inoculación de *Azotobacterchroococcum* y *Glomus intraradices* en el crecimiento y nutrición de plántula de papaya en fase de vivero. *Agron. Costarricense*, 35: 15–31

Constantino, M., R. Gómez-Álvarez, J.D. Álvarez-Solís, J.M. Pat-Fernández and E.G. Espín, 2010. Efecto de la biofertilización y los bioreguladores en la germinación y el crecimiento de *Carica papaya* L. *Rev. Colomb. Biotecnol*., 2: 103–115

Demoling, F., D. Figueroa and E. Bäth, 2007. Comparison of factors limiting bacterial growth in different soils. *Soil Biol. Biochem*., 39: 2485–2495

Díaz, F.A. and C.I. Garza, 2006. Colonización micorrízica arbuscular y crecimiento de genotipos de pasto buffel (*Cenchrus ciliaris* L.). *Rev. Fitotec. Mex*., 29: 203–206

Díaz, F.A., C.M. Alvarado, C.F. Ortiz and C.O. Grageda, 2013. Nutrición de la planta y calidad de fruto de pimiento asociado con micorriza arbuscular en vivero. *Rev. Mex. Ciencias. Agríc*., 4: 315–321

Frey, B. and H. Schüepp, 1993. Acquisition of nitrogen by external hyphae of arbuscular mycorrhizal fungi associated with *Zea mays* L. *New Phytol*., 124: 221–230

Gianinazzi, S., A. Trouvelot and P.V. Gianinazzi, 1990. Role and use of mycorrhizas in horticulture crop production. *XXII. International Horticulture Congress Florence*, pp: 25–30

Govindarajulu, M., P.E. Pfeffer, H.R. Jin, J. Abubaker, D.D. Douds, J.W. Allen, H. Bucking, P.J. Lammers and Y. Shachar-Hill, 2005. Nitrogen transfer in the arbuscular mycorrhizal symbiosis. *Nature*, 435: 819–823

Grace, E.J., O. Cotsaftis, M. Tester, F.A. Smith and S.E. Smith, 2009. Arbuscular mycorrhizal inhibition of growth in barley cannot be attributed to extent of colonization, fungal phosphorus uptake or effects on expression of plant phosphate transporter genes. *New Phytol*., 181: 938–949

Habte, M., 1999. Soil acidity as a constraint to the application of arbuscular mycorrhizal technology. *In: Mycorrhiza*, 2th edition, pp: 557–569. Varma, A. and B. Hock (eds.). Springer, Berlin

Hawkins, H.J., A. Johansen and E. George, 2000. Uptake and transport of organic and inorganic nitrogen by arbuscular mycorrhizal fungi. *Plant Soil*, 226: 275–285

Hodge, A., 2003. Plant nitrogen capture from organic matter as affected by spatial dispersion, interspecific competition and mycorrhizal colonization. *New Phytol*., 157: 303–314

Hodge, A., C.D. Campbell and A.H. Fitter, 2001. An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material. *Nature*, 413: 297–299

Johansen, A., R.D. Finlay and P.A. Olsson, 1996. Nitrogen metabolism of external hyphae of the arbuscular mycorrhizal fungus *Glomusintraradices*. *New Phytol*., 133: 705–712

Johnson, C.R., W.M. Jarrel and J.A. Menge, 1984. Influence of ammonium: nitrate ratio and solution pH on mycorrhizal infection, growth and nutrient composition of *Chrysanthemum morifolium* var. circus. *Plant Soil*, 77: 151–157

Karagiannidis, J., N. Nikolaou, I. Ipsilantis and E. Zioziou, 2007. Effects of different N fertilizer on the activity of *Glomus mosseae* and on grape nutrition and berry composition. *Mycorrhiza*, 18: 43–50

Khade, S.W., M.J. Bukhari, V. Jaiswal, U.C. Gaonkar and B.F. Rodrigues, 2002. Arbuscular mycorrhizal status of medicinal plants: a field survey of AM fungal association in shrubs and trees. *J. Eco. Tax. Bot*., 26: 571–578

Khade, S.W. and B.F. Rodrigues, 2008a. Ecology of arbuscular mycorrhizal fungi associated with *Carica papaya* L. in agro-based ecosystem of Goa, India. *Trop. Subtrop. Agroecosyst*., 8: 265–278

Khade, S.W. and B.F. Rodrigues, 2008b. Spatial variations in arbuscular mycorrhizal (AM) fungi associated with *Carica papaya* L. in a tropical agro-based ecosystem. *Biol. Agric. Hortic*., 26: 149–174

Khade, S.W. and B.F. Rodrigues, 2009. Arbuscular mycorrhizal fungi associated with varieties of *Carica papaya* L. in tropical agrobased ecosystem of Goa, India. *Trop. Subtrop. Agroecosyst*., 10: 369–381

Leigh, J., A. Hodge and A.H. Fitter, 2009. Arbuscular mycorrhizal fungi can transfer substantial amounts of nitrogen to their host plant from organic material. *New Phytol*., 181: 199–207

Li, Y., Y.N. Zou and Q. Wu, 2013. Effects of *Diversispora spurca* inoculation on growth, root system architecture and clorophyll contents of four citrus genotypes. *Int. J. Agric. Biol*., 15: 342–346

López-Moctezuma, H., R. Ferrera-Cerrato, J. Farias-Larios, S. Aguilar-Espinosa, M.R. Bello and J.G. López-Aguirre, 2005. Arbuscular mycorrhizae, *Bacillus* and substrate enriched with vermicompost on the development of papaya plants. *Terra Latinoamericana*, 23: 523–531

Marschner, H., 1998. *Mineral Nutrition of Higher Plants*, 2nd edition. Academic Press, London

Marzluf, G.A., 1996. Regulation of nitrogen metabolism in mycelial fungi. *In: The Mycota III: Biochemistry and Molecular Biology*, pp: 357–368. Brambl, R. and G.A. Marzluf (eds.). Springer, Berlin, Germany

McFarland, J.W., R.W. Ruess, K. Kielland, K. Pregitzer, R. Hendrick and M. Allen, 2010. Cross-ecosystem comparisons of in situ plant uptake of amino acid-N and NH_4^+. *Ecosystem*, 13: 177–193

Mcgonigle, T., M. Miller, D. Evans, G. Fairchild and J. Swan, 1990. A new method wich gives and objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. *New Phytol*., 115: 495–501

Miransari, M., 2011. Arbuscular mycorrhizal fungi and nitrogen uptake. *Arch. Microbiol*., 193: 77–81

Ngwene, B., G. Elke and G. Eckhard, 2013. Influence of different mineral nitrogen sources (NO_3^--N vs. NH_4^+-N) on arbuscular mycorrhizal development and N transfer in a *Glomus intraradices*-cowpea symbiosis. *Mycorrhiza*, 23: 107–117

Ngwene, B., E. George, W. Claussen and E. Neumann, 2010. Phosphorus uptake by cowpea plants from sparingly available or soluble sources as affected by N-form and arbuscular-mycorrhiza-fungal inoculation. *J. Plant. Nut. Soil Sci*., 173: 353–359

Pérez-Tienda, J., A. Valderas, G. Camañes, N. García-Agustín and N. Ferrol, 2012. Kinetics of NH_4^+ uptakes by the arbuscular mycorrhizal fungus *Rhizofagus irregularis*. *Mycorrhiza*, 22: 485–491

Quiñones-Aguilar, E.E., E. Hernández-Acosta, G. Rincón-Enríquez and R. Ferrera-Cerrato, 2012. Interaction of arbuscular mycorrhizal fungi and phosphorus fertilization in papaya.*Terra Latinoamericana*, 30: 165–176

Raven, J.A. and F.A. Smith, 1976. Nitrogen assimilation and transport in vascular land plants in relation to intracellular pH regulation. *New Phytol*., 76: 415–431

Rohyadi, A., F.A. Smith, R.S. Murray and S.E. Smith, 2004.Effects of pH on mycorrhizal colonization and nutrient uptake in cowpea under conditions that minimize confounding effects of elevated available aluminum. *Plant Soil*, 260: 283–290

Salgado-Barreiro, C.S., A. Bravo-Patiño, E.T. Wang and R. Cárdenas-Navarro, 2012. Effect of the inoculation with *Glomus intraradices* and nitrogen fertilization on growth of strawberry plants. *Sci. Agrop*., 2: 171–179

Sharda, W.K., B.F. Rodrigues and P.K. Sharma, 2010. Arbuscular mycorrhizal status and root phosphatase activities in vegetative *Carica papaya* L. varieties. *Acta Physiol. Plant*., 32: 565–574

Smith, S.E. and F.A. Smith, 2012. Fresh perspectives on the roles of arbuscular mycorrhizal fungi in plant nutrition and growth. *Mycologia*, 104: 1–13

Tobar, R., R. Azcón and J.M. Barea, 1994. Improved nitrogen uptake and transport from 15N-labeled nitrate by external hyphae of arbuscular mycorrhiza under water-stressed conditions. *New Phytol*., 126: 119–122

Toussaint, J.P., M. St-Arnaud and C. Charest, 2004. Nitrogen transfer and assimilation between the arbuscular mycorrhizal fungus *Glomus intraradices* Schenck & Smith and Ri T-DNA roots of Daucuscarota L. in an in vitro compartmented system. *Can. J. Microbiol*., 50: 251–260

Trindale, A.V., J.O. Siquiera and P.J. de Almeida, 2001. Dependencia micorrízica de variedades comerciais de mamoeiro. *Pes. Agrop. Bras*., 36: 1485–1494

Valentine, A.J. and A. Kleinert, 2006. Respiratory costs of P uptake in arbuscular mycorrhizal roots supplied with NH_4^+ and NO_3^- nutrition. *Symbiosis*, 41: 119–125

Vázquez-Hernández, M.V., L. Arévalo-Galarza, D. Jaen-Contreras, J.L. Escamilla-García, A. Mora-Aguilera, E. Hernández-Castro, J. Cibrián-Tovar and D. Téliz-Ortiz, 2011. Effect of *Glomus mosseae* and *Entrophospora colombiana* on plant growth, production and fruit quality of ‘Maradol’ papaya (*Carica papaya* L.). *Sci. Hortic*., 128: 255–260

Vierheilig, H., A.P. Coughlan, U. Wyss and Y. Pich´e, 1998. Ink and vinegar, a simple staining technique for arbuscular-mycorrhizal fungi. *Appl. Environ. Microbiol.*, 12: 5004–5007

Williams, L. and A. Miller, 2001.Transporters responsible for the uptake and partitioning of nitrogenous solutes. *Annu. Rev. Plant Physiol*., 52: 659–688

(Received 27 December 2013; Accepted 18 June 2014)