THE INFLUENCE OF ARBUSCULAR MYCORRHIZAL COLONIZATION ON THE GROWTH PARAMETERS OF CAPE GOOSEBERRY (*Physalis peruviana* L.) PLANTS GROWN IN A SALINE SOIL

D. Miranda[1]*, G. Fischer[1], and C. Ulrichs[2]

[1]Universidad Nacional de Colombia, Faculty of Agronomy, Department of Agronomy, Bogota, Colombia. [2]Humboldt-Universität zu Berlin, Faculty of Agriculture and Horticulture, Division Urban Plant Ecophysiology, Berlin, Germany.
*Corresponding author: dmirandal@unal.edu.co

ABSTRACT

With the objective of determining whether arbuscular mycorrhizal (AM) colonization would alleviate salt stress on the growth of cape gooseberry plants, a saline soil (ECs of 5.65 dS m^{-1}, available phosphorous of 48.1 mg kg^{-1}) was inoculated with AM fungi (Mycoral®) (+AM) and compared to a non-inoculated saline soil (–AM). The open-field experiment was conducted over the course of 131 days on the Marengo farm of the Universidad Nacional de Colombia (near Bogotá, 4º42' N, 74º12' W, 2543 m a.s.l., 14ºC mean temperature, and 800 mm a^{-1} precipitation) where the plants were irrigated with water (ECs of 1.65 dS m^{-1}) from the salt-contaminated Bogota river. Mycorrhizal dependence, AM colonization, relative field mycorrhizal dependency ($RFMD^{100}$), dry matter (DM) accumulation and growth parameters (unit leaf rate [ULR], leaf area ratio [LAR] and specific leaf area [SLA]) were determined. The percentage of AM-colonization was 29.7% in +AM plants, but only 12.5% in –AM plants. The $RFMD^{100}$ index peaked at day 61 (42.5%) and decreased to 7.8% by day 89. Inoculation with AM fungi increased plant dry matter accumulation by 7%, especially stem DM, compared to –AM plants. Generally, growth rates were higher in the +AM plants; ULR increased more in the second half of the experiment in inoculated plants compared to non-inoculated. The mycorrhizal infection enhanced leaf area growth, which resulted in increased LAR and SLA, especially during the initial phases of the experiment.

Keywords: Mycorrhizae, mycorrhizal dependence, dry matter, growth indices, saline soil, *Physalis peruviana*

INTRODUCTION

A water supply with high salinity has detrimental effects on soil fertility and reduces plant growth and productivity (Al-Karaki, 2006). Salinity reduces water availability and nutrient uptake by plants (Al-Karaki 2000).

Arbuscular mycorrhizal (AM) fungi are ubiquitous in the terrestrial ecosystem, forming symbiotic associations with the roots of the majority of plant species (Smith and Read, 1997). AM fungi consist of an internal phase inside the root and an external phase, or extraradical mycelium phase, which can form an extensive network within the soil (Gosling *et al*., 2006).

Many studies have demonstrated that inoculation with AM fungi improves the growth of plants under salt stress conditions (Giri and Mukerji, 2004; Ghazi and Al-Karaki, 2006). The beneficial effects of arbuscular mycorrhizae in improving tolerance to environmental stress conditions such as water stress (e.g., Cruz *et al*., 2000), and high soil salinity (e.g., Ghazi and Al-Karaki, 2006) in some plant species have been widely reported. The improved growth of AM inoculated plants has been attributed to enhanced acquisition of mineral nutrients with low mobility, such as P, Zn, Cu and Fe (Al-Karaki, 2000), and to decreased uptake of Na^+ (Al-Karaki, 2006). Porras-Soriano *et al*. (2009) found increased plant growth and ability to acquire N, P and K from saline media in young olive trees inoculated with the *Glomus* species.

Just like several other solanaceous plants, the cape gooseberry (*Physalis peruviana* L.) originates from the Andean highlands of South America. The *P. peruviana* has become commercially promising during the last 15 years, especially for Colombian fresh-fruit exporters. In spite of several areas in Colombia where the cape gooseberry is cultivated having soils catalogued as being saline, e.g., on the Bogota Plateau, the crop's response to salt stress conditions using mycorrhizal inoculation during different stages of development has still not been evaluated.

The objective of this study was to determine the beneficial effects of a commercial inoculum with arbuscular mycorrizhae on dry weight and growth parameters of cape gooseberry plants grown in a saline soil in the area influenced by the salt-contaminated Bogota river.

MATERIALS AND METHODS

Plant material and site conditions

Seed propagated 15 cm high cape gooseberry plants (ecotype Colombia) were transplanted on August, 2006, in an open field of the Marengo experimental farm (near Bogotá, 4º42' N, 74º12' W, 2543 m a.s.l.) of the Universidad Nacional de Colombia, situated in an area influenced by the Bogota river. The site has mean values of 13.11ºC air temperature, 82.5% relative humidity, 20.6 m s^{-1} wind speed; and yearly: 582.29 mm rainfall, 1010.5 mm evaporation and 1134.43 hours of sunshine.

The corresponding clay loam soil had the following chemical properties: pH (H_2O) 5.59; EC 5.65 dS m^{-1} (at 32°C); organic C 4.08 %; N 0.35 %; available P 48.1 mg kg^{-1}; K 0.46, Ca 10.5, Mg 3.51 and Na 4.94 meq 100 g^{-1}, B 0.94, Zn 21.8, Cu 1.04, Fe 426 and Mn 1.49 mg kg^{-1}.

Soil preparation, planting and AM inoculation

The soil was kept fallow for a year to reduce indigenous mycorrhizal fungi and decompose the root fragments of the previous crop to eliminate propagules. Since levels of mycorrhizal spores extracted from the native soil were extremely low (1–2 per kg), no attempt was made to fumigate the soil. Soil preparation consisted of plowing, raking two times and preparing elevated plant beds. Plants were transplanted in rows, 2.0 m apart, and the space between plants within a row was 2.5 m. A completely randomized block design was used, with three replications. The treatments included soil inoculated with AM (+AM) or non-inoculated soil (–AM). The total experiment area was 2625 m^2, thus each block consisted of 875 m^2. The total plant

number, including those on the edge, was 262. In each of the evaluations, three plants from each treatment and block (replication) were randomly selected (n=3).

Before planting, half of the experimental areas were inoculated with Mycoral® (AM fungi) applying 200 g of the product to the bottom of each plant site. Mycoral® (Fa. Mycoral Ltda., Cali, Colombia) is a commercial inoculum consisting of three mycorrhizal species, *Glomus sp., Acaulospora sp.* and *Entrophospora sp.,* composed of spores, mycelium, and root segments (K. Raddatz, personal communication, 2003).

After transplanting, irrigation with water (ECs of 1.65 dS m^{-1}) from the salt-contaminated Bogota river was applied uniformly to all plots with overhead sprinklers. All treatments received a recommended dose of 200 g of fertilizers 15:15:15 ($N:P_2O_5:K_2O$) per ha in the form of urea : single superphosphate : potassium sulphate, respectively, per plant at transplanting and the remaining amount was top-dressed in four equal splits and doses at 30, 60, 90, 120 and 150 days after transplanting. Additionally, a full dose of micronutrients (Agrimins® granulated) 4 g per plant was applied at these intervals.

Mycorrhizal colonization

Roots were assessed for AM colonization at 33, 61, 75 and 89 d of the experiment. Root samples of three plants from each treatment were rinsed free of soil, then cleaned with 10% KOH and stained with 0.05% Trypan blue in lactophenol as described by Phillips and Hayman (1970), and cut into 1 cm pieces. Colonization of AM in terms of percentage of root segments, containing mycorrhizal structures (hyphae, arbuscules and vesicles) was estimated according to Bierman and Linderman (1981), using a modified line intersect method (McGonigle *et al.*, 1990), in which the presence of AM structures was determined in the root samples with 100 line intersections, with three replications. Furthermore, a light microscope (Zeiss®, model Axioplan equipped with a Kodak Camera MC-100) was used at 40x magnification to find the degree of AM fungal infection in the root of each three replicate plants. The following equation was used to calculate the percentage of AM infection:

Percentage of AM infection = (Root length infected/Root length observed) x 100.

For plants that formed mycorrhizae, the relative field mycorrhizal dependency ($RFMD^{100}$) index was calculated per Plenchette *et al.* (1983), using five samplings:

$RFMD^{100}$ = (DW of +AM plants − DW of -AM plants) x 100 / DW of +AM plants

Growth parameters

At 33, 47, 61, 75, 89, 103, 117 and 131 d of the experiment, three plants were taken from each treatment, to determine the total expanded leaf area (measured by a LiCor Li-3000 Planimeter (LiCor Inc., Lincoln, Nebraska)) and dry weight (DW) of roots, leaves, stems, flowers and fruits (70ºC in a forced-air oven for 80 h). In the Tables, the DWs for the plants' organs are presented for the different growth phases (experimental time), the total accumulation over the course of the experiment, and the percent of distribution of the total DW for each organ. Taking into account that evaluations were carried out during a short period of vegetative and reproductive growth, the changes in growth obtained from the DW and leaf

area were fitted to an exponential model. In order to observe the behavior of plant growth in field conditions in response to AM inoculation, the growth parameters of unit leaf rate (ULR), leaf area ratio (LAR) and specific leaf area (SLA) were determined during the sampling days according to Hunt (1990) (Table 1).

The equation to calculate growth rates was: $W = ae^{bT}$, where W is the total DW, coefficient a is the value of W or $\log_e W$ and corresponds to initial DW when the growth rate $T = 0$ and coefficient b is the rate of increase of W or $\log_e W$ (Hunt, 1990).

Statistical Analysis

All data were statistically analyzed with analysis of variance (ANOVA) using the software SAS 9.1. Duncan's and Tuckey´s tests were performed at $p \leq 0.05$ to determine the differences among the treatments.

RESULTS AND DISCUSSION

Arbuscular mycorrhizal root colonization

Colonization by AM fungi induced root morphological changes in cape gooseberry plants. Early evaluation (four leaf pair stage) showed little presence of vesicles, hyphae or arbuscules; whereas later observations (five leaf pair stage) revealed infections characterized by a high presence of hyphae and fewer vesicles as observed on the segments of the evaluated roots. These salt-induced changes were attributed by Yano *et al.* (1996) and Mickelson and Kaeppler (2005) to the influence of the genotype used, symbiont inoculum ratio and water stress conditions.

The amount of root colonization was significantly increased ($p \leq 0.05$) by the mycorrhizal colonization at all sampling dates showing remarkably reduced micorrhizal infection in the –AM treatment (Table 1). Whereas the mean infection percentage of –AM plants was 12.5%, those of +AM plants was 29.7%., i.e. 17.2% higher. Increased AM fungal sporulation and colonization under salt-stress conditions has also been reported by Giri and Mukerji (2004). Furthermore, these results confirmed that AM colonization of plant roots depends on the developmental stage of the plants (Hartwig *et al*., 2002).

The results of the present study clearly indicate that AM colonization occurs naturally in saline environments (Johnson-Green *et al*., 1995). We cannot ascertain which of the three AM fungi (*Glomus* sp., *Acaulospora* sp. or *Entrophospora* sp.) contained in Mycoral® (K. Raddatz, personal communication, 2003) responded with a higher or lower amount of infection. Some studies show that hosts exposed to a mix of AM fungi are preferentially colonized by certain strains, suggesting host-endophyte or ecological specificity (Zhu *et al*., 2000).

Edaphic factors have been shown to greatly affect AM function and association with host plants, and some species of AM tend to be more effective at colonizing in certain soils than others (Sylvia *et al*., 1998). In our study, the infection could be associated with the salinity in the soil (5.65 dS m^{-1}) and the presence of native mycorrhizae (Table 1). This suggests that in rich soils the proliferation of inefficient indigenous fungi over that of efficient fungi could reduce the effectiveness of the latter and affect the yield potential and that it may be advantageous to introduce selected fungi to these soils based on the soil

Table 1. Infection percentage and relative field mycorrhizal dependency ($RFMD^{100}$) index in cape gooseberry plants established in inoculated soil (+AM) and non-inoculated soil (–AM) at different days after transplantation, n=3.

Day	Infection percentage		($RFMD^{100}$) index %
	+AM	–AM	
33	20.3 **a**	8.5 **b**	0
47	34.0 **a**	12.0 **b**	18
61	41.0 **a**	19.3 **b**	42.5
75	37.6 **a**	16.4 **b**	25.3
89	15.2 **a**	6.3 **b**	7.8

Means followed by different letters, at the same sampling date, are significantly different according to Tuckey's test ($p \leq 0.05$).

fertility as well as mycorrhizal dependency of the crop (Daei *et al*., 2009; Joner, 2000).

In our soil, the pH value was 5.9, which is considered low, and that could have influenced the colonization by AM fungi. It was found that the response of arbuscular mycorrhizal to soil pH seems to be dependent primarily on the fungal species (Sylvia *et al.*, 1998.) Arbuscular mycorrhizae respond markedly to the presence of organic matter in their environment as well as to the fertilization used (Joner, 2000). The soil used in the experiment showed a high percentage of organic matter (7.2%) which could have negatively affected the rate of infection in the inoculated soil, in addition to the frequency of fertilization used for crop management. High-input soil management, involving mineral fertilization, can have a profound negative effect on mycorrhizal fungi, as reported by Galvez *et al.* (2001) who found that the abundance of spores of AM fungi was much lower in high input agriculture compared to that in low-input agriculture. Hartwig *et al*. (2002) suggested that the level of AM fungal colonization in *Lolium perenne* has been controlled by the relative availability of P or other micro-nutrients.

It is supposed that the phosphorous soil content of 48.1 mg kg^{-1}, to some extent, affected AM colonization. Olsson *et al.* (1997) confirmed that high P levels in the soil can reduce not only spore germination and hyphal growth from the germinated spores, but also early colonization of the roots and growth of the extra radical mycelium. By all manners, the authors suppose that the influence of the P content in the experimental soil cannot be too high since Sylvia and Schenk (1983) proposed 200 mg kg^{-1} as a concentration that limits the AM infection.

Relative field mycorrhizal dependency+

Mycorrhizal dependency is defined as the degree to which a plant relies upon mycorrhizal condition to produce its maximum growth or yield at a given level of soil fertility (Janos, 2007). In the present study, $RFMD^{100}$ increased with the experiment's duration and peaked at day 61 (Table 2). Increasing $RFMD^{100}$

indices were also registered by Giri and Mukerji (2004) who revealed that in saline soil, the micorrhizal dependency of *Sesbania aegyptiaca* and *S. grandiflora* increased with the plant's age. Day 61 of the evaluation is considered as the moment of the highest infective activity of the AM fungi.

Probably, the low $RFMD^{100}$ index could had been influenced by the relative high fertility of the experiment's soil field (48.1 mg kg^{-1} P, 4.1% organic C and 0.35% N). Menge *et al.* (1978) stated that mycorrhizal dependency is linked to a given level of soil fertility and it is well known that P is the element which has the closest relationship to AM development and efficiency.

The results of the present study were similar to those obtained by Plenchette *et al.* (1983) for the tomato ($RFMD^{100}$ of 41.9%), but much lower than those in the carrot (99.2%) under salinity conditions.

Dry weight of vegetative and generative growth

The present study demonstrated that AM inoculation significantly increased ($p \leq 0.05$) DW accumulation in vegetative and generative plant organs of cape gooseberry plants (Table 3 and 4). From day 61, in the case of vegetative plant DW and from day 89, for generative plant DW, AM inoculation promoted markedly higher DW accumulation as compared to –AM plants. Mycchorizal infection especially increased the accumulation of the stem (Table 2) and fruit (Table 3) dry mass of +AM plants. Independent of mycorrhizal inoculation, plants showed a very similar percentage DW distribution pattern, only +AM plants accumulated a slightly higher percentage of DW in the stem, whereas –AM plants did the same in the leaves.

Inoculation with AM fungi has been shown to increase biomass production in many plant species (Al-Karaki, 2000). A variety of mechanisms have been proposed to determine how mycorrhizae ameliorate the effects of salinity stress on plants (Marschner, 2002). A positive effect of AM fungi on dry matter growth may be attributed to the increased nutrient acquisition of plants under salinity conditions (Al-Karaki, 2000), predominantly phosphate and micronutrients (Bresinski *et al*., 2008), but also nitrogen and potassium (Porras-Soriano *et al*., 2009). Like root hairs, mycorrhizae increase the roots' absorptive surface (Lambers *et al*., 1998). Other authors have observed that AM can alleviate the salt stress on plants by inhibiting the high uptake of Na^+ and Cl^- and their transfer to the shoot (Al-Karaki, 2006; Giri and Mukerji, 2004).

Due to the uptake of carbohydrates from the plant, the AM fungi enhance the sink capacity of the root system and thus, in turn, increase the photosynthetic performance of the plant leading to improved plant growth (Bresinski *et al*., 2008). The enhanced mycorrhizal tolerance to salt stress was attributed by Feng *et al.* (2002) to a higher accumulation of soluble sugars in plant roots.

Also, mycorrhizal fungal infection can increase water uptake through higher photosynthate demand and thus enhanced transpiration rates (Marschner, 2002). Ruiz-Lozano *et al*. (1996) found that colonization with AM fungi compensates for water deficit stress by increasing the absorption power of roots. This fact could be important in the case of the experimental site where only 529 mm $year^{-1}$ rain fell.

Porras-Sorriano *et al*. (2009) found a 468% increase in root DW growth of young olive trees under saline conditions in AM fungi plants. In the present study, the +AM treatment did not generate a higher root DW at the different sampling

dates, but the accumulated DW during the evaluated period was higher in inoculated plants, compared to non-AM plants. Similar results were obtained by Kaya *et al*. (2009) in pepper plants which showed no improvement in root dry matter for +AM-plants in control, 50 mM or 100 mM NaCl salt conditions. Marschner (2002) described that the higher shoot growth, compared to the root growth, is a typical plant response to higher nutrient supply as the AM fungus competes with the roots for photosynthates. Probably, the elevated root respiration in +AM plants (Marschner, 2002) inhibited a higher root dry matter accumulation than in –AM plants.

The positive effect of +AM on DW production was visible up to day 103, after that, leaf DW was not influenced by AM inoculation, probably, at this phase, –AM plants were able to acclimate to the stress (Buchanan *et al*., 2000), thus the growth parameters URL, LAR and SLA (Figures 1 to 3) showed only slight differences. Also, the organic matter content and mineral fertilization of the experiment field could have inhibited a higher response by the +AM plants (Joner, 2000).

The low effect of AM-inoculation on the reproductive parameters, with the +AM plants accumulated only slightly more DW in flowers and fruits (Table 3), may have several causes. Supposedly, added AM fungi only slightly influenced leaf dry matter growth for a better photoassimilate production and, on the other hand, flowering and fruiting are affected differently than the vegetative growth processes and, sometimes, stress conditions can alter reproductive responses (Bresinski *et al*., 2008).

Growth parameters

The general effect of salinity is the reduction of growth, resulting in smaller and fewer leaves and shorter plant height, thus in the cape gooseberry mycorrhizal inoculation led to a greater biomass accumulation ($p \leq 0.05$), as found Daei *et al.* (2009) in wheat.

Unit leaf rate

The unit leaf rate (ULR), also called the net assimilation rate (NAR), is the dry matter accumulation rate of the whole plant per unit of leaf area (and is a measure of the average efficiency of leaves of plants or crop stand (Hunt 1990; Lambers *et al*., 1998). ULR values obtained for –AM plants decreased from 0.00085 down to 0.00054 g cm^{-2} day^{-1} by day 75, which coincides with the time flowering began. Starting with day 89 after transplanting, ULR values increased reaching 0.0077 g cm^{-2} day^{-1} at the end of evaluation period. Mycchorizal inoculated plants showed lower ULR due to the higher leaf area, with increasing values, from 0.00046 at 30 d up to 0.0013 g cm^{-2} day^{-1} at 131 d (Figure 1).

The observed early ULR reduction in –AM plants could be related with the increased leaf area production of the plant at the early stages of development, but with lower leaf efficiency of DW accumulation over time, due to the effect of salinity (Erdei and Taleisnik, 1993).
The mycorrhized plants produced more assimilates than the –AM plants from 117 d on (Table 3), at which point, the assimilates were also attracted by the induced reproductive organs (flowers and fruits) (Miranda *et al*., 2010).

The increasing URL values from the middle of the experiment on could indicate an adaptation of the plants to salinity conditions (Taleisnik *et al*., 1997) and this effect was much higher in +AM plants toward the experiment's end where the promoting effect of mycorrhizal inoculation were evident.

Table 2. Dry weight of vegetative plant organs, accumulated dry weight and distribution of dry weight of cape gooseberry plants, grown in soil inoculated with AM fungi (+AM) and soil without the inoculum (–AM) as measured on various days after transplantation, n=3.

Day	+AM				-AM			
	Root (g)	**Stem (g)**	**Leaf (g)**	**Total veg. DW (g)**	**Root (g)**	**Stem (g)**	**Leaf (g)**	**Total veg. DW (g)**
33	0.7 **a**	1.7 **a**	4.0 **a**	6.4 **a**	0.7 **a**	1.9 **a**	3.8 **a**	6.4 **a**
47	1.5 **a**	2.9 **a**	5.9 **a**	10.3 **a**	1.7 **a**	2.2 **a**	4.5 **a**	8.7 **a**
61	5.4 **a**	7.2 **a**	14.3 **a**	26.9 **a**	4.0 **a**	5.4 **b**	9.8 **b**	19.2 **b**
75	7.5 **a**	15.1 **a**	23.4 **a**	46.0 **a**	7.4 **a**	10.7 **b**	18.7 **b**	36.8 **b**
89	13.6 **a**	36.2 **a**	45.4 **a**	95.2 **a**	11.4 **a**	35.2 **a**	42.0 **a**	88.6 **b**
103	22.5 **a**	53.4 **a**	65.0 **a**	140.9 **a**	19.5 **a**	53.8 **a**	60.0 **b**	133.3 **b**
117	29.2 **a**	86.6 **a**	82.8 **a**	198.6 **a**	24.4 **a**	88.8 **a**	86.8 **a**	200.0 **a**
131	39.6 **a**	152.9 **a**	136.9 **a**	329.4 **a**	38.2 **a**	133.8 **b**	134.6 **a**	306.6 **b**
Accumulated DW	120.0 **a**	356.0 **a**	397.5 **a**	873.5 **a**	107.3 **b**	331.8 **b**	360.2 **b**	799.3 **b**
DW distribution (%)	10.6	40.8	36.6	88.0	11.0	38.6	38.9	88.5

Means of dry weight followed by different letters, at the same sampling date, are significantly different according to Tuckey's test ($p \leq 0.05$).

Table 3. Dry weight of generative plant organs, total dry weight, accumulated dry weight and distribution of dry weight weight of cape gooseberry plants, grown in soil inoculated with AM fungi (+AM) and soil without the inoculum (–AM) as measured on various days after transplantation, n=3.

Day	+AM				-AM			
	Flowers (g)	**Fruits (g)**	**Total gen. DW (g)**	**Total plant DW (g)**	**Flowers (g)**	**Fruits (g)**	**Total gen. DW (g)**	**Total plant DW (g)**
33	-	-	-	6.4 **a**	-	-	-	6.4 **a**
47	0.1 **a**	-	0.1 **a**	10.4 **a**	0.1 **a**	-	0.1 **a**	8.8 **a**
61	0.2 **a**	-	0.2 **a**	27.1 **a**	0.2 **a**	-	0.2 **a**	19.4 **b**
75	0.5 **a**	0.1 **a**	0.6 **a**	46.6 **a**	0.4 **a**	-	0.4 **a**	37.2 **b**
89	1.5 **a**	0.9 **a**	2.4 **a**	97.6 **a**	1.2 **a**	0.7 **a**	1.9 **b**	90.5 **b**
103	2.1 **a**	7.1 **a**	9.2 **a**	150.1 **a**	2.3 **a**	7.0 **a**	9.3 **a**	142.6 **b**
117	3.6 **a**	24.2 **a**	27.8 **a**	226.4 **a**	3.0 **a**	20.1 **a**	23.1 **b**	223.1 **a**
131	5.4 **a**	39.5 **a**	44.9 **a**	374.3 **a**	4.6 **a**	35.3 **a**	39.9 **b**	346.5 **b**
Accumulated DW	13.4 **a**	71.8 **a**	85.2 **a**	938.9 **a**	11.8 **b**	63.1 b	74.9 **b**	874.3 **b**
DW distribution (%)	1.4	10.6	12.0	100	1.3	10.2	11.5	100

Means of dry weight followed by different letters, at the same sampling date, are significantly different according to Tuckey's test ($p \leq 0.05$).

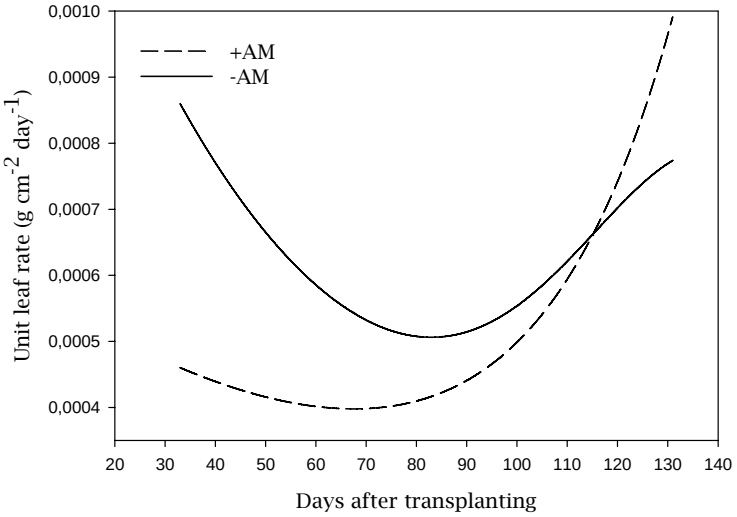

Figure 1. Unit leaf rate (ULR) of cape gooseberry plants grown in an inoculated soil with AM fungi (+AM) and soil without the inoculum (–AM).

Leaf area ratio (LAR)

Mycorrhizal inoculated plants increased LAR from (80 to 93 cm^2 g^{-1}) whereas –AM plants had a peak of LAR at day 75, 12% lower than in +AM plants, indicating a bigger leaf size for inoculated plants (Figure 2).

It is known that the LAR is the amount of leaf area per unit total plant mass (Lambers *et al*., 1998) and has recently been classified as the most important determination of growth (Bresinsky *et al*., 2008). The decreasing LAR values after the curves' reflection point indicate that salinity mainly affected leaf elongation and, hence, the development of photosynthetic leaf area (Curtis and Läuchli, 1986). The proportionally greater reduction of leaf area growth rate compared to plant DW accumulation can indicate that leaf thickness (Bosabalidis and Kofidis, 2002) and water content (Flowers *et al*., 1991) increased with the experiment's duration as a response to salinity stress. Plants may mitigate an increase in ion concentration with an increase in water content and therefore succulence. It appears that this behavior is similar in the whole plant (LAR refers to DM of the whole plant) and in the leaf (SLA refers to leaf DM), when comparing the plots of the curves after 89 d (Figure 2 and 3).

Specific leaf area (SLA)

SLA is a measure of density or of relative thinness of leaves, because it deals with the leaves' areas in relation to their dry mass (Hunt, 1990). The SLA increased from the beginning of the evaluation, the highest points were between days 80 and 89 in the mycorrhized plants (182 cm^{-2} g^{-1}), significantly higher ($p \leq 0.05$) than those of the –AM plants (175 cm^{-2} g^{-1}) (Figure 3). After the reflection point, the SLA decreased abruptly to values of 100 and 96 cm^{-2} g^{-1} at day 131 for +AM and –AM plants, respectively. The higher SLA

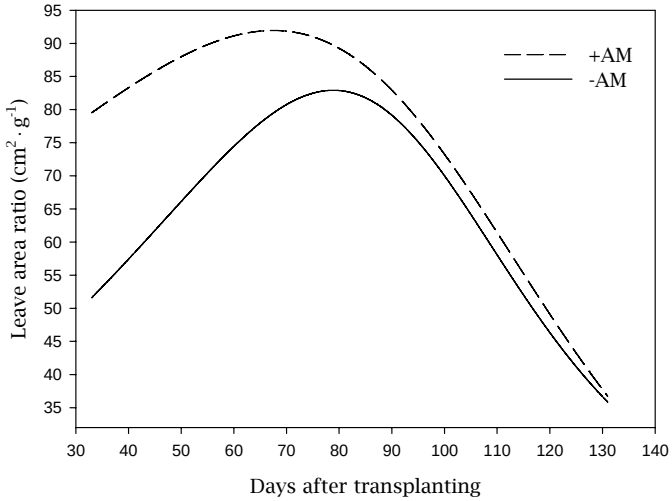

Figure 2. Leaf area ratio (LAR) of cape gooseberry plants grown in an inoculated soil with AM fungi (+AM) and soil without the inoculum (-AM).

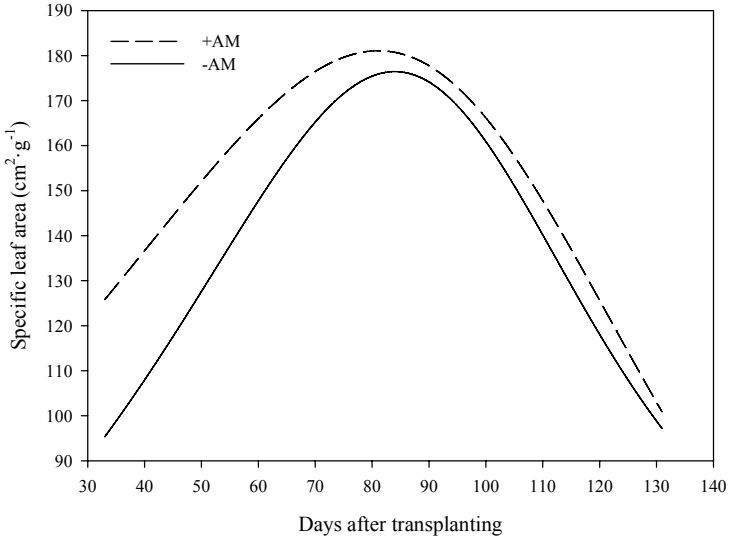

Figure 3. Specific leaf area (SLA) of cape gooseberry plants grown in an inoculated soil with AM fungi (+AM) and soil without the inoculum (–AM).

of +AM plants are in agreement with Amerian and Steward (2001) who found enhanced SLA in well watered and moderately drought stressed mycorrhized maize plants compared to those that were non–infected. These authors attributed the enhanced SLA rates to the beneficial effect of the *Glomus*-infection on the leaf water relations (higher leaf water potential) and CO_2 assimilation rates. Since SLA affects the amount of photosynthetically active radiation captured per unit of mass (Tholen *et al*., 2004); the higher SLA signifies that mycorrhized plants capture more light per unit mass than leaves with a low SLA (-AM plants).

Decreasing SLA after the curves peaked are in agreement with the LAR, which confirms the close relation between these two growth indices under salt stress conditions found by Miranda *et al*. (2010).

Supposedly, the marked decrease in the leaf area per unit DW was due to an accumulation of non-structural carbohydrates in the leaf and subsequent remobilization to reserve organs (Hartwig *et al*., 2002) in +AM plants or an increased concentration of minerals such as Na and Cl, in –AM plants (Perez *et al*., 2004).

CONCLUSIONS

The present study showed that the inoculation of AM fungi can alleviate and compensate for the growth limitations of cape gooseberry plants, imposed by saline conditions, thus increasing the production of dry matter of +AM plants.

The mycorrhizal fungal inoculum showed marked effects on leaf area growth, with increased LAR and SLA, relative to the non-inoculated plants, especially during the initial phases of the experiment.

The increased URL (NAR) values from the middle of the experiment on reflect a possible adaptation of plants to salinity conditions and this effect was more evident in +AM plants.

REFERENCES

Al-Karaki, G.N. 2000. Growth and mineral acquisition by mycorrhizal tomato grown under salt stress. Mycorrhiza. 10**,** 51–54.

Al-Karaki, G.N. 2006. Nursery inoculation of tomato with arbuscular mycorrhizal fungi and subsequent performance under irrigation with saline water. Scientia Hort. 109, 1–7.

Amerian, M.R., Stewart, W.S. 2001. Effect of two species of arbuscular mycorrhizal fungi on growth, assimilation and leaf water relations in maize (*Zea mays*). Aspects Appl. Biol. 63, 1–6.

Bierman, B., Linderman, R. 1981. Quantifying vesicular–arbuscular mycorrhizae: proposed method towards standardization. New Phytol. 87, 63–67.

Bosabalidis, A.M., Kofidis, G. 2002. Comparative effect of drought stress on leaf anatomy of two olive cultivars. Plant Sci. 163, 375–379.

Bresinsky, A., Körner, C., Kadereit, J.W. Neuhaus, G., Sonnewald, U. 2008. Strasburger - Lehrbuch der Botanik. 36. Auflage. Spektrum Verlag, Heidelberg, 1175 p.

Buchanan, B.B., Gruissem, W., Jones, R.L. 2000. Biochemistry and molecular biology of plants. American Society for Plant Physiologists, Rockville, MD, 1367 p.

Cruz, A.F., Ishii, T., Kadoya, K. 2000. Effect of arbuscular mycorrhizal fungi on tree growth, leaf water potential, and levels of 1-aminocyclopropane-1-carboxylic acid and ethelyne in the roots of papaya under water-stress conditions. Mycorrhiza. 10, 121–123.

Curtis, P.S., Läuchli, A. 1986. The role of area development and photosynthetic capacity in determining growth of kenat und moderate salt stress. Aust. J. Plant Physiol. 18, 553–565.

Daei, G., Ardekani, M.R., Rejali, F., Teimuri, S., Miransari, M. 2009. Alleviation of salinity stress on wheat yield, yield components, and nutrient uptake using arbuscular mycorrhizal fungi under field conditions. J. Plant Physiol. 166, 617-625.

Erdei, L., Taleisnik, E. 1993. Changes in water relation parameters under osmotic and salt stresses in maize and sorghum. Physiologia Plant. 89(2), 381–387.

Feng, G, Zhang, F.S., Li, X.L., Tian, C.Y., Tang, C., Rengel, Z. 2002. Improved tolerance of maize plants to salt stress by arbuscular mycorrhiza is related to higher accumulation of soluble sugars in roots. Mycorrhiza. 12, 185–90.

Flowers, T.J., Flowers, S.A., Hijibagheri, M.A., Yeo, A.R. 1991. Ion accumulation in the cell walls of rice plants growing under saline conditions: evidence for the Oertli hypothesis. Plant Cell Environ. 14, 319–325.

Galvez, L., Douds Jr., D.D., Drinkwater, L.E., Wagoner, P. 2001. Effect of tillage and farming system upon VAM fungus populations and mycorrhizas and nutrient uptake of maize. Plant Soil. 118, 299–308.

Giri, B., Mukerji, K.G. 2004. Mycorrhizal inoculant alleviates salt stress in *Sesbania aegyptica* and *Sesbania grandiflora* under field condition: evidence for reduced sodium and improved magnesium uptake. Mycorrhiza. 14, 307–312.

Gosling, P., Hodge, A., Goodlass, G., Bending, G.D. 2006. Arbuscular mycorrhizal fungi and organic farming. Agric. Ecosyst. Environ. 113(1–4), 17–35

Hartwig, U.A., Wittmann, P., Braun, R., Hartwig-Räz, B., Jansa, J., Mozafar, A., Lüscher, A., Leuchtmann, A., Frossard, E., Nösberger, J. 2002. Arbuscular mycorrhiza infection enhances the growth response of *Lolium perenne* to elevated atmospheric pCO_2**.** J. Exp. Bot. 53(371), 1207–1213.

Hunt, R. 1990. Basic growth analysis. Unwin Hyman, London, 112 p.

Janos, D.P. 2007. Plant responsiveness to mycorrhizas differs from dependence upon mycorrhizas. Mycorrhiza. 17, 75-91.

Johnson-Green, P.C., Kenkel, N.C., Booth, T. 1995. Distribution and phenology of arbuscular-mycorrhizae along a salinity gradient at an inland salt pan. Can. J. Bot. 73, 1318–1327.

Joner, E.J. 2000. The effect of long-term fertilization with organic and inorganic fertilizers on mycorrhiza-mediated phosphorus uptake in subterranean clover. Biol. Fertil. Soils 32, 435–440.

Kaya, C., Ashraf, M., Sonmez, O., Aydemir, S. 2009. The influence of arbuscular mycorrhizal colonisation on key growth parameters and fruit yield of pepper plants grown at high salinity. Scientia Hort. 212, 1–6.

Lambers, H., Chapin, F.S., Pons, T.L. 1998. Plant physiological ecology. Springer, New York, NY, 540 p.

McGonigle, T.P., Miller, M.H., Evans, D.G., Fairchild, D.L., Swan, G.A. 1990. A new method which gives an objective measure of colonisation of roots by vesicular–arbuscular mycorrhizal fungi. New Phytol. 115, 495–501.

Marschner, H. 2002. Mineral nutrition of higher plants. Academic Press, Amsterdam, The Netherlands, 889 p.

Menge, J.A., Johnson, E.L.V. Platt, R.G. 1978. Mycorrhizal dependeny of several citrus cultivars under three nutrient regimes. New Phytol. 81, 553–559.

Mickelson, S.M., Kaeppler, S.M. 2005. Evaluation of six mycorrhizal isolates for their ability to promote growth of maize genotypes under phosphorus deficiency. Maydica. 50, 137–146.

Miranda, D., Fischer, G., Ulrichs, Ch. 2010. Growth of cape gooseberry (*Physalis peruviana* L.) plants affected by salinity. J. Appl Bot. Food Qual. 83(2), 175-181.

Olsson, P.A., Bååth, E., Jakobsen, I. 1997. Phosphorus effects on the mycelium and storage structures of an arbuscular mycorrhizal fungus as studied in the soil and roots by analysis of fatty acid signatures. Appl. Environ. Microbiol. 63, 3531–3538.

Pérez, J.A., Garía, E., Enriquez, J.F., Quero, A.R., Pérez, J., Hernández, A. 2004. Análisis de crecimiento, área foliar específica y concentración de nitrógeno en hojas de pasto 'Mulato' (*Brachiaria* híbrido, cv.). Téc. Pecu. Méx. 42(3), 447-458.

Phillips, J.M., Hayman, D.S. 1970. Improved procedures for cleaning roots and staining parasitic and vesicular arbuscular mycorrizal fungi for rapid assessment of infection. Trans. Br. Mycol. Soc. 55, 158-161.

Plenchette, C., Fortin, J.A., Furlan, V. 1983. Growth response of several plant species to mycorrhizae in a soil of moderate P-fertility. I. Mycorrhizal dependency under field conditions. Plant Soil. 70, 199–209.

Porras-Soriano, A., Soriano-Martín, M.L., Porras-Piedra, A., Azcón, R. 2009. Arbuscular mycorrhizal fungi increased growth, nutrient uptake and tolerance to salinity in olive trees under nursery conditions. J. Plant Physiol. 166, 1350–1359.

Ruíz-Lozano, J.M., Azcón, R., Gómez, M. 1996. Alleviation ofsalt stress by arbuscular-mycorrhizal *Glomus* species in *Lactuca sativa* plants. Physiol. Plant. 98, 767–72.

Smith, S.E., Read, D.J. 1997. Mycorrhizal symbiosis. 2nd edition. Academic Press, London, 605 p.

Sylvia, D.M., Fuhrmann, J.J., Hartel, P.T., Zuberer, E. 1998. Principles and applications of soil microbiology. Prentice Hall, London. 550 p.

Sylvia, D.M., Schenck, N.C. 1983. Application of superphosphate to mycorrhizal plants stimulates sporulation of phosphorus-tolerant vesicular-arbuscular mycorrhizal fungi. New Phytol. 95, 655-661.

Taleisnik, E., Peyrano, G., Arias, C. 1997. Response of *Chloris gayana* cultivars to salinity. 1. Germination and early vegetative growth. Tropical Grasslands. 31, 231-240.

Tholen, D., Voesenek, L.A.C.J., Poorter, H. 2004. Ethylene insensivity does not increase leaf area or relative growth rate in Arabidobsis, *Nicotiana tabacum*, and *Petunia x hybrida*. Plant Physiol. 134, 1803–1812.

Yano-Melo, A.M., Saggin, O.J. Jr., Maia, L.C. 2003. Tolerance of mycorrhized banana (*Musa* sp. cv. Pacovan) plantlets to saline stress. Agric. Ecosyst. Environ. 95, 343–348.

Zhu, J., Kaeppler, S.M., Lynch, J.P. 2005. Topsoil foraging and phosphorus acquisition efficiency in maize (*Zea mays*). Funct. Plant Biol. 32, 749–762.

Zhu, Y.G., Laidlaw, A.S., Christie, P., Hammond, M.E.R. 2000. The specificity of arbuscular mycorrhizal fungi in perennial ryegrass-white clover pasture. Agric. Ecosyst. Environ 77, 211–218.