Acclimatization of *Tapeinochilos ananassae* plantlets in association with arbuscular mycorrhizal fungi

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Abstract – The objective of this work was to assess the potential of three isolates of arbuscular mycorrhizal fungi to promote growth of micropropagated plantlets of *Tapeinochilos ananassae* during acclimatization. The experiment was carried out in greenhouse, in a completely randomized block design, with four inoculation treatments: non-inoculated control and plants inoculated with *Glomus etunicatum*, *Acaulospora longula* or *Gigaspora albida*, with ten replicates. After 90 days, the following parameters were evaluated: survival rate, height, leaf and tiller number, leaf area, fresh and dry biomass, contents of macro- and micronutrients in the root and shoot, glomerospore number, and mycorrhizal colonization. The survival percentage was 100%, except for plants inoculated with *G. albida* (80%). The isolate *G. etunicatum* is more suitable for plant development, since it improves survival, growth, dry matter production, nutritional status, and vigor of *T. ananassae* micropropagated plants.

Index terms: Glomeromycota, growth promotion, micropropagation, mineral nutrition, tropical flowers.

Aclimatização de plântulas de *Tapeinochilos ananassae* em associação com fungos micorrízicos arbusculares

Resumo – O objetivo deste trabalho foi avaliar o potencial de três isolados de fungos micorrízicos arbusculares na promoção do crescimento de plântulas micropropagadas de *Tapeinochilos ananassae* durante a fase de aclimatização. O experimento foi realizado em casa de vegetação, tendo-se utilizado o delineamento inteiramente casualizado, com quatro tratamentos de inoculação: controle não inoculado e plantas inoculadas com *Glomus etunicatum*, *Acaulospora longula* ou *Gigaspora albida*, com dez repetições. Após 90 dias, foram avaliados os seguintes parâmetros: percentual de sobrevivência, altura, número de folhas e de perfilhos, área foliar, biomassa fresca e seca, conteúdo de macro e micronutrientes nas partes aérea e radicular, número de glomerosporos e colonização micorrízica. O percentual de sobrevivência foi de 100%, exceto para as plantas inoculadas com *G. albida* (80%). O isolado *G. etunicatum* é o mais adequado para o desenvolvimento das plantas, pois aumenta a sobrevivência, o crescimento, a produção de matéria seca, o conteúdo nutricional e o vigor de plantas micropropagadas de *T. ananassae*.

Termos para indexação: Glomeromycota, promoção do crescimento, micropropagação, nutrição mineral, flores tropicais.

Introduction

The great diversity of climates and soils in Brazil allow the cultivation of numerous species of ornamental plants and flowers, with potential to compete in the international market (Cançado Júnior et al., 2005). Among the tropical ornamental plants, the genus *Tapeinochilos* comprises 16 species, of which 80% are endemic to the island of New Guinea, and is characterized by the formation of inflorescences composed of bright red bracts of great beauty and post-harvest durability (Specht & Stevenson, 2006). *Tapeinochilos ananassae* (Hassk.) K. Shum. is still poorly known in Brazil, but has excellent prospects for acceptance by cultivators and consumers.

Commercial production of ornamental plants has evolved greatly to become an extremely competitive activity, but it requires technology, advanced knowledge, and efficient marketing (Pasqual et al., 2008). Consequently, vegetative, in vitro propagation (micropropagation) has been widely applied to produce, in a short time and at any time of the year, a large scale of high quality plantlets, ensuring varietal authenticity (Rout et al., 2006).

Efficiency of micropropagation involves an acclimatization step, which represents the transition

from the heterotrophic to the autotrophic phase, when plantlets must increase photosynthetic rate and absorption of water and minerals (Grattapaglia & Machado, 1998). Micropropagation techniques produce plantlets without pathogens, but eliminate arbuscular mycorrhizal fungi (AMF), which could bring great benefits to the hosts, whether in the production of seedlings in nurseries or in the acclimatization of micropropagated plants (Kapoor et al., 2008). Mycorrhization expands the absorption zone around the root, increasing the contact surface with the soil and favoring an increased uptake of minerals, such as phosphorus, zinc, copper, nitrogen, and potassium, resulting in increased plant tolerance to environmental stresses (Smith & Read, 2008).

Although plant-fungus association is not specific, the natural occurrence of mycorrhizal associations in representatives of the family Costaceae has been documented (Santos et al., 2000). Specific favorable combinations between AMF and ornamental plant genotypes have been observed in anthurium (*Anthurium andraeanum* Lindl.) (Stancato & Silveira, 2006), chrysanthemum (*Chrysanthemum morifolium* Ramat.) (Sohn et al., 2003), and gerbera daisy (*Gerbera* sp.) (Sato et al., 1999). However, lack of plant growth promotion has also been reported in associations of AMF with heliconia (*Heliconia* sp.) (Sato et al., 1999), red ginger [*Alpinia purpurata* (Vieill.) K. Shum.], and beehive ginger (*Zingiber spectabile* Griff.) (Silva et al., 2006). Therefore, the selection of mycorrhizal inocula that are effective in plantlet acclimatization and development is desirable for the improvement of propagation technology.

The objective of this work was to assess the potential of three AMF species to promote growth of micropropagated plantlets of *T. ananassae* during acclimatization.

Materials and Methods

The experiment was carried out in greenhouse, under controlled environmental conditions (27±2ºC; 75% relative humidity; light intensity of 250–560 µmol m^{-2} s^{-1}). The substrate used was soil (Oxisol) and expanded vermiculite (2:1 v v^{-1}), previously sterilized in autoclave for two periods of 1 hour at 120°C. The substrate had the following characteristics: 6.41 g kg^{-1} of organic matter, pH 5.6, electrical conductivity of 0.54 dS m^{-1}, cation-exchange capacity of 5.69 $cmol_c$ dm^{-3}, 93 g kg^{-1} of P, 0.44 g kg^{-1} of K, 1.5 g kg^{-1} of Ca, 1.3 g kg^{-1} of Mg, 0.84 mg kg^{-1} of Cu, 68.9 mg kg^{-1} of Fe, 34.8 mg kg^{-1} of Mn, 2.10 mg kg^{-1} of Zn, and 0.14 mg kg^{-1} of Na. The plantlets were irrigated with about 50 mL of distilled water, daily, without supplement of nutrient solution.

Micropropagated plantlets of *T. ananassae*, provided by the Laboratório de Biotecnologia of Embrapa Semiárido, Petrolina, PE, Brazil, were multiplied in MS medium (Murashige & Skoog, 1962).

Isolates of the AMF *Glomus etunicatum* Becker & Gerd., *Acaulospora longula* Spain & Schenck, and *Gigaspora albida* Schenck & Smith were propagated in greenhouse in pots containing previously disinfected sand:soil (1:1 v v^{-1}), with sorghum [*Sorghum bicolor* (L.) Moench.] as host. The inoculum produced was evaluated for the number of glomerospores by wet sieving and decanting (Gerdemann & Nicolson, 1963), followed by centrifugation in water and sucrose (40% w v^{-1}) (Jenkins, 1964). Counting was done in channeled plates, using a stereomicroscope (40×). After counting the glomerospores, soil inoculum was prepared to contain approximately 200 glomerospores, in addition to fragments of mycorrhizal-colonized root and mycelium.

The following treatments were established: non-inoculated control, and plants inoculated with *G. etunicatum*, *A. longula*, and *G. albida*. A completely randomized block design with ten replicates was used.

The micropropagated plantlets of *T. ananassae* were removed from growth flasks and washed in running water until the culture medium was completely removed. Specimens were selected in order to achieve uniformity of size and number of leaves and roots. At the moment of transplanting to 2-kg bags containing substrate (soil and vermiculite, 2:1 v v^{-1}), plantlets were inoculated or not, according to the treatment. In the control treatment, 2 mL of filtrate were added, which derived from the screening (45 µm) of all inoculum tested, in order to standardize microbiota.

During the 90 days of acclimatization, height and number of leaves and tillers were assessed. At the end of the experiment, survival rates, leaf area, fresh and dry biomass of shoot and root, root colonization, number of glomerospores, and mineral contents in shoots and roots were determined.

To determine dry biomass, *T. ananassae* leaf and root were placed in oven (65°C), until constant weight. After weighing, shoot samples were ground in a Wiley mill,

in which 0.5-g portions of sample were mineralized by nitric perchloric acid digestion for subsequent determination of Ca, Mg, Fe, Zn, Cu, and Mn levels by atomic absorption spectrophotometry. Phosphorus was determined by colorimetry; and K by flame emission photometry. All tests were performed according to Silva (1999), and the values obtained were multiplied by dry biomass to determine mineral content.

Roots (1 g) from each treatment were washed in tap water, followed by clearing in 10% KOH and 10% H_2O_2, acidification in 1% HCl, and staining with trypan blue (0.05%) (Phillips & Hayman, 1970). Then, AMF colonization was estimated by the gridline-intersect method (Giovannetti & Mosse, 1980). The number of glomerospores was determined by counting (Gerdemann & Nicolson, 1963; Jenkins, 1964) in 50 g of soil of each treatment, and leaf area was estimated using the metering device Li 3100 (LI-Cor Inc., Lincoln, NE, USA). In order to determine the increment resulting from the treatments, the following formula was used: $I\ (\%) = [(Tr - T)T^{-1}] \times 100$, in which: I (%) is the increment of the variable; Tr is the average value for the inoculated treatment; T is the average value for the non-inoculated treatment.

Data were subjected to analysis of variance and variables that showed significant difference were compared by the Tukey test, at 5% probability, using Sanest software (Zonta & Machado, 2007).

Results and Discussion

Plants colonized by *G. etunicatum* and *A. longula* showed a considerable increase in leaf area (Table 1): 175.67 and 95.12%, respectively. In addition, inoculation with *G. etunicatum* had the best results for all biomass variables of *T. ananassae*, which that exceeded 100% (Table 1). The treatment with *A. longula* provided significantly higher biomass than control. Inoculation benefits with *G. etunicatum* and *Acaulospora* sp. in acclimatization have also been reported for micropropagated gerbera (Sato et al., 1999) and anthurium plants (Stancato & Silveira, 2006), and for chrysanthemum plants inoculated with different species of *Glomus* (Sohn et al., 2003).

Despite the high mycorrhizal colonization achieved with *G. albida*, inoculation with this AMF did not result in growth benefits or increase survival of *T. ananassae* plantlets (Tables 1 and 2). The values obtained were significantly similar to the control. According to Piotrowski et al. (2004), the lack of growth increment in root biomass may be related to excessive carbon sink for the formation of hyphae of some species of AMF with high colonization. This may be the case for members of the family Gigasporaceae, which were characterized by Hart & Reader (2002) as producing larger amounts of extra-radicular mycelium, in comparison to other representatives of families of AMF. Moreover, the number of recovered glomerospores in the other treatments was significantly higher than that obtained with *G. albida*. A possible explanation is that the substrate used had a high phosphorus concentration (93 g kg^{-1}), which may have directly influenced the functionality of the symbiosis (Siqueira et al., 2004), germination, and subsequent colonization (Lovelock & Ewel, 2005).

After 45 days of evaluation, plantlets inoculated with *G. etunicatum* differed from the other treatments regarding height and number of leaves (Table 2), but not number of tillers, which differed significantly from the non-mycorrhizal plantlets after 75 days. More advanced stages of the plant-fungus relationship may allow the visualization of greater benefits for the host (Van der Heijden & Kuyper, 2001). However, until the last day of evaluation (90 days), only inoculation with *G. etunicatum* and *A. longula* resulted in a significant increase in plant height, when compared to the control.

The content of macro- and micronutrients (Tables 3 and 4) in shoots and roots of plantlets inoculated

Table 1. Leaf area, mycorrhizal colonization, number of glomerospores, and fresh and dry biomass (g) of *Tapeinochilos ananassae* shoots and roots, in plants with or without mycorrhizal associations, after 90 days in greenhouse[(1)].

Treatment	Survival	Colonization	Leaf area	Number of merospores	Fresh		Dry	
	---------- (%) ----------		(cm^2)	(50 g^{-1} substrate)	Shoot	Root	Shoot	Root
Control	100	0.03d	186.48c	0.01c	9.68c	7.37bc	0.97bc	1.25bc
Glomus etunicatum	100	93.74b	514.07a	23.90a	26.78a	21.26a	2.96a	3.69a
Acaulospora longula	100	81.45c	363.87b	16.38b	18.55b	11.12b	1.45b	1.92b
Gigaspora albida	80	99.12a	189.53c	1.00c	9.41c	5.62c	0.81c	0.83c
CV (%)		10.9	21.2	19.9	21.6	25.8	23.4	27.1

[(1)]Means followed by equal letters do not differ by Tukey test, at 5% probability.

Table 2. Height and number of leaves and tillers of *Tapeinochilos ananassae* with or without mycorrhizal associations with *Glomus etunicatum*, *Acaulospora longula* or *Gigaspora albida*, after 15, 30, 45, 60, 75, and 90 days in greenhouse[(1)].

Treatment	Time (days)					
	15	30	45	60	75	90
			Height (cm)			
Control	3.56a	3.75ab	3.62b	5.18b	5.31b	6.06c
Glomus etunicatum	3.62a	4.43a	5.87a	9.62a	12.31a	15.68a
Acaulospora longula	3.25a	3.31b	3.81b	4.81b	6.06b	9.18b
Gigaspora albida	3.43a	3.86ab	4.43b	4.86b	5.00b	5.78c
CV (%)	17.4	17.7	18.0	18.1	16.2	16.6
			Number of leaves			
Control	4.37a	6.17ab	7.25b	9.94b	12.50b	15.20b
Glomus etunicatum	4.62a	6.77a	11.75a	18.00a	22.37a	27.20a
Acaulospora longula	5.12a	5.68ab	5.00b	9.76b	14.62b	19.71ab
Gigaspora albida	4.50a	4.64b	8.87b	7.98b	10.62b	15.11b
CV (%)	27.1	11.7	26.2	14.1	24.4	12.8
			Number of tillers			
Control	1.00a	1.11a	1.37b	2.03a	2.25b	2.82b
Glomus etunicatum	1.10a	1.22a	2.37a	3.28a	3.62a	4.57a
Acaulospora longula	1.20a	1.22a	1.50ab	2.06a	2.50ab	3.86bc
Gigaspora albida	1.10a	1.11a	1.50ab	2.04a	2.00b	3.44ab
CV (%)	27.9	11.3	38.3	17.9	32.0	13.9

[(1)]Means followed by equal letters, in the columns, do not differ by Tukey test, at 5% probability.

Table 3. Macronutrient content (g per plant) of *Tapeinochilos ananassae* with or without mycorrhizal associations with *Glomus etunicatum*, *Acaulospora longula* or *Gigaspora albida*, 90 days after transplantation[(1)].

Treatment	N	P	K	Ca	Mg	S
			Shoots			
Control	18.77b	0.77d	30.24c	6.29c	3.43c	1.58c
Glomus etunicatum	36.81a	4.41a	81.11a	18.61a	18.18a	6.55a
Acaulospora longula	31.94a	2.62b	63.57b	11.34b	9.31b	3.91b
Gigaspora albida	19.76b	1.63c	30.82c	7.54c	5.37c	1.83c
CV (%)	20.5	13.3	19.6	19.0	17.8	17.4
			Roots			
Control	6.40c	0.20b	14.49c	3.05b	28.11bc	1.36c
Glomus etunicatum	15.79a	1.18a	71.54a	11.00a	85.00a	5.39a
Acaulospora longula	9.93bc	1.00a	30.48b	4.41b	34.50b	2.85b
Gigaspora albida	11.71ab	0.79b	20.35bc	3.93b	21.23c	1.52c
CV (%)	25.2	31.0	19.0	23.9	18.7	27.8

[(1)]Means followed by equal letters do not differ by Tukey test, at 5% probability.

Table 4. Micronutrient content (mg per plant) of *Tapeinochilos ananassae* with or without mycorrhizal associations with *Glomus etunicatum*, *Acaulospora longula* or *Gigaspora albida*, 90 days after transplantation[(1)].

Treatment	B	Cu	Fe	Mn	Zn	Na
			Shoots			
Control	46.28c	11.82b	238.47c	1191.19c	60.43c	652.27c
Glomus etunicatum	108.05a	41.42a	1032.83a	5009.90a	252.06a	1841.59a
Acaulospora longula	75.70b	34.46a	554.48b	1740.40b	153.48b	1321.25b
Gigaspora albida	66.92bc	13.92b	321.18bc	436.29d	77.10c	1074.20bc
CV (%)	21.0	17.1	27.4	15.5	18.5	25.8
			Roots			
Control	32.77c	37.34bc	31027.50c	536.07bc	81.85c	709.05c
Glomus etunicatum	134.83a	116.27a	96232.33a	1949.04a	365.12a	3957.13a
Acaulospora longula	67.63b	50.84b	57454.66b	675.03b	159.97b	1542.38b
Gigaspora albida	50.01bc	27.33c	27244.16c	372.21c	109.34bc	1108.68bc
CV (%)	28.4	23.4	23.0	17.2	18.4	22.7

[(1)]Means followed by equal letters do not differ by Tukey test, at 5% probability.

with *G. etunicatum* and *A. longula*, in general, was significantly greater than that of the control and *G. albida*. Similarly, Sohn et al. (2003) observed increased concentrations of P, K, Mg, Ca, Fe, Mn and Cu in leaves and K, Ca, Fe, Mn, Cu and Zn in roots of chrysanthemum seedlings associated with *Glomus* sp. Using *G. clarum*, Leal et al. (2005) observed differential accumulation of N, P, and K in micropropagated banana plantlets. These results may be related to the compatibility between host and environment (Cavagnaro et al., 2005) or to the preferential association of certain combinations of plant genotype x species of AMF (Sanders, 2004), since not all isolates equally promoted the content of mineral nutrients (Table 3). Although mycorrhization with *G. etunicatum* has favored mineral accumulation in *T. ananassae*, this same strain reduced the concentration of some macronutrients, in spite of favoring the development of micropropagated banana plantlets (Yano-Melo et al., 1999).

Mycorrhization by *A. longula* provided aerial shoots of *T. ananassae* with greater accumulation of all macro- and micronutrients, in comparison to the control. In the roots, the nutrient content in mycorrhizal plantlets showed significant difference only when compared to the control, regarding the macronutrients P, K, and S (Table 3) and the micronutrients B, Fe, Zn, and Na (Table 4). The use *G. albida* inoculum did not increase the content of macro- and micronutrients, except for P in the shoot and N in the root portion, in comparison to the control (Table 3). Freitas et al. (2006) also observed that, in the absence of phosphate fertilizer in mint (*Mentha arvensis* L.), the use of *G. margarita* inoculum was responsible for the increased content of N, P, and K. However, inoculations with *G. margarita* also caused low levels of Ca and Mg in rootstocks of avocado (*Persea* sp.) (Silveira et al., 2002). Therefore, the nutritional benefits induced by AMF depend on the relative availability of elements in the environment (Siqueira et al., 2002). In addition, the evaluation of different isolates under the same conditions can assist in selection of more efficient AMF (Avio et al., 2006).

Conclusions

1. The isolate *Glomus etunicatum* has higher potential for application in *Tapeinochilos ananassae* acclimatization, with improved survival, plant growth, vigor and contents of mineral nutrients, besides promoting high fungal colonization and sporulation.

2. The inoculum of *Acaulospora longula* increase leaf area, biomass measures, and the concentration of macro- and microminerals in shoots and roots.

3. Inoculation with *Gigaspora albida* does not improve the contents of mineral nutrients and may even hamper the development of *T. ananassae*.

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