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Anthurium andraeanum Lindl. cv. Eidibel *in vitro* rooting and acclimatization with arbuscular mycorrhizal fungi

ABSTRACT

The aim of the present study was to evaluate *A. andraeanum in vitro* rooting and acclimatization with arbuscular mycorrhizal fungi (AMF). In order to induce rooting, the consistence and ionic strenght of MS medium modified (5,71 μM de NAA) were tested: solid MS; liquid MS; solid half-strenght MS and liquid half-strength MS. After 60 days, plants were acclimatized in association with the AMF *Gigaspora albida*, *Glomus etunicatum*, *Acaulospora longula,* multiple inoculum or without AMF (control treatment). The liquid nutritive medium containing the complete concentration of MS salts induced better plant development, specially with regard to root number. Mycorrhization reduced the acclimatization impact, favoring water and phosphorus absorption, as well as plant growth, specially those associated with multiple inoculum.

Key words: *in vitro* culture, AMF, *Gigaspora albida*, *Glomus etunicatum*, *Acaulospora longula*

Enraizamento in vitro de *Anthurium andraeanum* Lindl. cv. Eidibel e aclimatização com fungos micorrízicos arbusculares

RESUMO

Realizou-se este trabalho com o propósito de se avaliar o enraizamento *in vitro* de *A. andraeanum* e a aclimatização das plantas com fungos micorrízicos arbusculares (FMA). Testaram-se, para o enraizamento, a consistência e a força iônica do meio MS modificado (5,71 mM de ANA): MS sólido; MS líquido; MS sólido com metade da força iônica e MS líquido com metade da força iônica. Após 60 dias, as plantas foram aclimatizadas em associação com os FMAs *Gigaspora albida*, *Glomus etunicatum*, *Acaulospora longula,* inóculo múltiplo e sem FMA (tratamento controle). O meio nutritivo líquido contendo a concentração completa de sais MS proporcionou maior desenvolvimento das plantas, sobretudo no que se refere ao número de raízes. A micorrização reduziu o impacto da aclimatização favorecendo a absorção de água e fósforo, e o crescimento das plantas, sobretudo naquelas associadas ao inóculo múltiplo.

Palavras-chave: cultivo *in vitro*, FMA, *Gigaspora albida*, *Glomus etunicatum*, *Acaulospora longula*

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INTRODUCTION

Floriculture moves a considerable volume of money in the productive chain, commercializing about 60 billion dollars per year (SEBRAE, 2002). Among the tropical cultivated flowers, *Anthurium* stands out for its beauty and durability.

Anthurium propagation can be done either sexually, or asexually by the separation of buds that emerge from the stock plant. More recently, *Anthurium* micropropagation from axillary buds (Kunisaki, 1980), lamina explants (Martin et al., 2003) and micro-cuttings (Vargas et al., 2004) has been successfully employed. Micropropagation is a promising method of true-to-type plant propagation with a high quality in a small area of the laboratory and a shorter period of time than that required for seeds production or vegetative propagation (Kane, 2000).

The acclimatization process is a critical point in plant micropropagation. In order to minimize losses during the acclimatization process, the arbuscular mycorrhizal fungi (AMF) inoculation shows out as a possible alternative. These obligate symbiotic microorganisms may have improved the growth rate of micropropagated hortensia, *Hydrangea* sp. (Varma & Schuepp, 1994) and peach, *Prunus* sp., (Estaun et al., 1999). The AMF favors the absorption of mineral nutrients that have little movement in the soil, including phosphorus, copper and zinc (Liu et al., 2000). Furthermore, the symbiosis favors the development of more robust plants due to the increased water absorption, hormone production, adverse environmental conditions tolerance, and pathogens resistance (Siqueira et al., 2002).

The aim of the present study was to assess the root induction and to evaluate the effect of arbuscular mycorrhizal fungi on *Anthurium andraeanum* acclimatization process.

MATERIAL AND METHODS

Shoots of *Anthurium andraeanum* Lindl., cv. Eidibel (IAC) obtained from nodal segments cultivated on MS medium (Murashige & Skoog, 1962) with 4.44 mM 6-benzylaminopurine (BAP) and 2.89 mM gibberelic acid (GA_3), either liquid or solid, were transferred to MS medium containing 5.71 mM of a-naphthaleneacetic acid (NAA) for root induction. Four treatments were established: T_1 = solid full strength MS; T_2 = liquid full strength MS; T_3 = solid half-strength MS and T_4 = liquid half-strength MS. The plants were placed in test tubes containing 10 mL of the nutritive medium. In the treatments with liquid nutritive medium, a paper filter was used as support to avoid total immersion of the shoots. Each treatment had 20 replicates. The plants were kept in a growth room at 25 ± 2 °C temperature, 50 mol m^{-2} s^{-1} light intensity under 16 hours photoperiod, for 60 days. After this period, the number of roots and leaves, the length of root and aerial parts were evaluated.

The experiment was set up in completely randomized design. Data were subjected to variance analysis and the averages were compared using Tukey test at 5% level of probability.

Plants that presented three leaves and roots of approximately 2.0 cm were selected for acclimatization in 200 mL plastic pots containing sand and vermiculite (1:1) as substrate mixture. The substrate was sterilized in an autoclave at 121 °C for 1 hour on two consecutive days. The pH of the substrate was 5.8, after sterilization. The inocula consisted of soil inoculum with spores of *Gigaspora albida* Schenck & Smith, *Glomus etunicatum* Becker & Gerdemann and *Acaulospora longula* Spain & Schenck, growing in association with *Panicum milliaceum* L. Spores were extracted from soil by wet sieving and sucrose centrifugation (Jenkins, 1964). In *Anthurium* plants acclimatization, five inoculation treatments were established: M_0 = control – non-inoculated, M_1 = AMF mixture (*G. albida, G. etunicatum* and *A. longula*), M_2 = *G. albida,* M_3 = *G. etunicatum,* M_4 = *A. longula*. A total of 300 spores per plant were used. In the treatment with multiple inoculum, 100 spores from each species were applied. The substrate received the complete Hoagland nutritive solution, except for phosphorus, which was maintained in the substrate at a concentration of 0.31 mg L^{-1}.

The experimental design was completely randomized with eight replicates. The analysis of variance was based on the ANOVA procedure, using the software program STATISTICA for Windows. The averages were compared using Tukey test within a probability of 5%.

After 180 days of acclimatization, measurements on number of leaves, shoot and root fresh and dry matter, water content, P concentration in the aerial part and root colonization were taken. Shoots were separated from the roots and allowed to dry. The water content was calculated through the formula [FM-DM/FM] x 100, where: FM = total fresh matter and DM = total dry matter. After obtaining dry matter the roots were moisted and clarified with 10% KOH and 10% H_2O_2, stained with 0,05% Trypan blue, cut into 1,0-cm segments (100 segments per sample) and observed with a light microscope for evaluation of AMF colonization. The shoots dry matter was subjected to nitro-perchloric digestion and phosphorus content was determined using the molybdate-vanadate colorimetric method (Sarruge & Haag, 1979).

RESULTS AND DISCUSSION

The treatment with liquid full strength MS medium inducted the greatest number of roots (Table 1). Similar results were

Table 1. Number of leaves and roots in *A. andraeanum* plants cultivated in different nutritive media MS medium supplemented with 5.71 mM of NAA

Tabela 1. *Número de folhas e raízes em A. andreanum em diferentes meios nutritivos MS suplementado com 5,71 mM de NAA*

Treatments	Number of leaves	Number of roots
Solid medium	3.9 ab	4.5 b
Liquid medium	4.1 ab	6.5 a
Solid half strength medium	2.4 b	2.6 c
Liquid half strength medium	4.7 a	5.2 b

Means followed by the same letter in each column do not differ by Tukey test at 0.05 confidence level.

obtained with *Liquidambar styraciflua*, which presented a more complete root system in a liquid medium in comparison to a solid medium (Lee et al., 1986). The greater efficiency of the liquid medium maybe a result of its greater homogeneity, once nutrient gradients are established by tissue growth in solid medium, which does not occur in liquid medium (Caldas et al., 1998). Regarding the aerial part height (2.1 ± 0.3 cm) and roots length (1.2 ± 0.2 cm), there was no effect of the culture medium consistency or ionic concentration (Table 1).

During acclimatization, *Anthurium* plants associated with AMF generally exhibited a better performance than control plants. Multiple inoculum with three AMF species (*G. etunicatum*, *G. albida* and *A. longula*) promoted better plant development for all analyzed parameters (Table 2). Regarding the isolated effect of the AMF species, the best results related to growth parameters were observed in *G. albida* inoculated plants, whereas plants inoculated with *A. longula* presented growth parameters values similar to non-inoculated plants (Table 2).

Work carried out on gerbera (*Gerbera* sp.) demonstrated an increase in both fresh and dry matter of the aerial part, as well as an increase on aerial part and roots length in micropropagated plants that received multiple inoculum with the mycorrhizal fungi *Glomus clarum, G. etunicatum* and *Gigaspora margarita* (Sato et al. 1999). AMF inoculation also proved to be an efficient and viable system in the acclimatization phase of pears (*Pyrus communis),* peaches (*Prunus persica* x *P. amygdalis)* and bananas (*Musa* spp) (Rapparini et al., 1994; Lins et al., 2003).

Plants associated with AMF presented a water content ranging from 85.0 to 86.9% (Table 2), in contrast to non-inoculated plants water content which did not surpass 80%. Considering that plants, whether inoculated or not, were under the same environmental conditions, and that balance between water absorbed and water lost through transpiration defines water content in vegetal tissue (Calbo & Moraes, 2000), the role of AMF in promoting greater water absorbtion in micropropagated *Anthurium* plants is evident.

Table 2. Number of leaves (NL), foliar fresh matter (FFM), foliar dry matter (FDM), root fresh matter (RFM), root dry matter (RDM) and water (%) in *A. andraeanum* plants acclimatized with AMF*

Tabela 2. *Número de folhas (NL), matéria fresca foliar (FFM), matéria seca foliar (FDM), matéria fresca da raiz (RFM), matéria seca da raiz (RDM) e água (%) em A. andraeanum aclimatizadas com AMF**

Treatments	NL	FFM	FDM	RFM	RDM	Water
			(g)			(%)
Control	3.4 c	0.12 e	0.02 d	0.28 e	0.05 d	80.0
G. etunicatum	7.6 b	0.83 c	0.12 b	0.78 c	0.09 c	86.9
G. albida	7.8 b	0.90 b	0.13 b	0.92 b	0.13 b	86.6
A. longula	3.0 c	0.42 d	0.07 c	0.38 d	0.05 d	85.0
Mixture of AMF	13.6 a	1.07 a	0.15 a	1.67 a	0.21 a	86.8

* Means followed by the same letter in each column do not differ by Tukey test at 0.05 confidence level.

Another mycorrhizal association beneficial effect on plant growth is directly related to phosphorus absorption. Plants associated with inoculum mixture presented highest values for the growth variables, as well as highest phosphorus content (0.092 g plant^{-1}). On the other hand, a low P concentration in plants associated with inoculum mixture indicates a dilution effect as a result of the greatest plant growth (Tabela 3). These plants presented 0.26% of P, an adequate phosphorus concentration (Raven et al., 2004). The increase in P absorption was also demonstrated in a number of mycorrhized fruit trees, such as the yellow passion fruit (Cavalcante et al., 2002), guava (Samarão & Martins, 1999) and citrus (Graham et al., 1997).

Table 3. Phosphorus content and concentration in *Anthurium andreanum* plants mycorrhized with distinct inoculates*

Tabela 3. *Conteúdo e concentração de fósforo em Anthurium andreanum inoculadas com diferentes micorrizas**

P	AFM Treatments				
	AMF Mixture	*G. albida*	*G.etunicatum*	*A. longula*	Control
P content (g.plant^{-1})	0.092 a	0.060 b	0.046 c	0.023 d	0.023 d
P concentration (%)	0.26 c	0.23 d	0.22 d	0.32 b	0.38 a

* Means followed by the same letter in each column do not differ by Tukey test at 0.05 confidence level.

The greatest colonization was observed on *G. etunicatum* (68.28%), followed by AMF mixture (41.40%), *G. albida* (37.08%) and *A. longula* (26.60%). The difference on root colonization rate between the AMF studied isolates may be related to the degree of fungus-plant compatibility, which is genetically controlled by the symbionts (Silveira, 1992). Although the highest mycorrhizal root colonization occurred on *G. etunicatum*, the best growth and absorption of P was observed on plants cultivated with AMF mixture. Heliconia (*Heliconia* sp.) presented higher root colonization in treatments with *G. margarita* (55.95%), however, the AMF did not promote the growth of this plant as much as gerbera (*Gerbera* sp.), in which the root colonization with *G. margarita* did not surpass 31.29% (Sato et al., 1999). The authors emphasize that the greatest colonization rates do not always result in highest benefits for the plant.

Conclusions

The growth of micropropagated *Anthurium andraeanum* plants is improved by mycorrhization with multiple inoculum (*Gigaspora albida* Schenck & Smith, *Glomus etunicatum* Becker & Gerdemann and *Acaulospora longula* Spain & Schenck).

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