Note

ACCLIMATIZATION OF MICROPROPAGATED *Heliconia bihai* (HELICONIACEAE) PLANTS

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ABSTRACT: To evaluate the behavior of micropropagated heliconia seedlings during the acclimatization process using different substrates and shade levels, seedlings of *H. bihai* (Lobster Claw I) were planted in plastic trays using the substrates: washed sand, vermiculite (medium texture), and PlantMax® Horticultura, and shade cloths with percentages of 0% (full sun), 30%, 40%, 50%, 60%, 70%, and 80% of shade in relation to full sun. Best results were obtained for washed sand and PlantMax substrates at shade conditions starting from 50%, especially at 70% and 80%. Pre-existing roots from *in vitro* rootings were not functional, not presenting secondary roots, and showing root tissue necrosis. Acclimatized seedlings developed new roots. This finding suggests that this plant can be acclimatized without going through a laboratory rooting stage.
Key words: Heliconia, *ex vitro* culture, rooting

ACLIMATIZAÇÃO DE MUDAS MICROPROPAGADAS DE *Heliconia bihai* (HELICONIACEAE)

RESUMO: A cultura da helicônia é a que apresenta maior crescimento entre o cultivo de flores tropicais para a exportação. Avaliou-se o comportamento de mudas de helicônias micropropagadas no processo de aclimatização, em diferentes substratos e níveis de sombreamento. Foram plantadas mudas de *H. bihai* (Lobster Claw I) em bandejas plásticas contendo os diferentes substratos: areia lavada, vermiculita (textura média) e PlantMax® Horticultura. Na avaliação de níveis de sombreamento foi utilizado o telado (sombrite), com ordem de sombreamento 0% (pleno sol), 30%, 40%, 50%, 60%, 70% e 80%. O desempenho foi superior nos substratos areia lavada e PlantMax® Horticultura e nas condições de sombreamento a partir de 50 %, em especial 70% e 80%. As raízes pré-existentes, oriundas do enraizamento *in vitro*, mostraram-se não funcionais, sem a emissão de raízes secundárias e apresentando necrose do tecido radicular. As mudas aclimatizadas desenvolveram novas raízes, o que sugere que esta cultura pode ser aclimatizada sem que as plântulas passem pela fase de enraizamento em laboratório.
Palavras-chave: Helicônia, cultura *ex vitro*, enraizamento

INTRODUCTION

The tropical flower agribusiness has become prominent as an important source of hard currency for some countries, such as Colombia, Costa Rica, Honduras, Jamaica, the United States (Hawaii and Florida), Puerto Rico, Suriname, and Venezuela (Castro & Graziano, 1997). In Brazil, the states of Rio de Janeiro, São Paulo, Santa Catarina, Pernambuco, in addition to Amazonas, Ceará, and Alagoas have carried out commercial plantings, especially of heliconias (Castro, 1995).

One of the obstacles found during attempts to multiply heliconias *in vitro* is a consequence of the fact that the presence of an endophytic bacterium of the species *Pseudomonas solanacearum* frequently occurs in organs that serve as explants (Atehortua, 1997; Dias & Rodrigues, 2001). After this initial impediment was overcome (Dias, 2002), with the development of a micropropagation protocol for *H. bihai* using the shoot apex, the acclimatization of the produced material became a limiting factor in obtaining micropropagated seedlings.

Acclimatization is an important step in micropropagation. During *in vitro* growth, plants develop under controlled conditions, including enclosed environments, without gaseous exchanges, with high moisture in the air, low light intensity, and the use of sugars from the medium as a source of carbon and energy (Preece & Sutter, 1991; Sciutti & Morini, 1993; Pospísilová et al.,

1999). Thus, the transplanting of *in vitro*-grown plantlets and the complete establishment in the greenhouse can be complex for some species (Van Huylenbroeck & Debergh, 1996; Ross-Karsten et al., 1998).

The selection of a suitable substrate can be decisive for acclimatization. The low effectiveness of vermiculite as a substrate in the acclimatization of apple tree rootstocks and gloxinia plantlets demonstrates the importance of this parameter in this process (Hoffmann et al., 2001; Silva et al., 2003).

In view of these difficulties and of the absence of data regarding acclimatization of the genus *Heliconia*, the present work had the objective of evaluating the behavior of such plantlets on different substrates and under different shade levels in the acclimatization process.

MATERIAL AND METHODS

The experiment was carried out in a plant nursery, in Natal, RN, Brazil (5°46'S, 35°12'W), from March to August, 2002. The material consisted of micropropagated *H. bihai* plantlets (Lobster Claw I), obtained from shoot apices, which were at the twelfth *in vitro* subculture. Part of this material was rooted in a solid culture medium containing one half of the MS (Murashige & Skoog, 1962) salts concentration and 6.0 g L^{-1} Agar with Morel vitamin (Morel & Wetmore, 1951), 30.0 g L^{-1} sucrose, 0.1 mg L^{-1} indolebutyric acid (IBA), and the pH adjusted to 5.8.

The assay was comprised of 2,520 plantlets with an average size of 7.0 cm, which were planted in plastic trays with 24 cells each, containing different substrates: washed sand (autoclaved), Eucatex vermiculite (medium texture), and Eucatex PlantMax® Horticultura. Shade levels were evaluated with the use of shade cloth (sombrite), consisting of the union of 1.5 m wide screen pieces, with a randomic shading order: 0% (full sun), 40%, 50%, 80%, 70%, 30%, and 60%, arranged in the north-south direction to prevent the interference among treatments. The trays containing different types of substrates were arranged side by side for each shade level. The seven different shade levels with three substrates resulted in 21 treatments, each consisting of a plastic tray with 24 cells.

Irrigation was made through automatically-controlled fogging nozzles, installed every 1.0 m, 1.0 m above the trays, providing a relative humidity of 80%, common to all treatments. The experimental design was organized as randomized blocks in a 3 × 7 factorial scheme (substrate × shading), and each treatment was repeated five times. Each replicate lasted 28 days, after which the live acclimatized plants were counted. The results thus obtained were evaluated through analysis of variance in a factorial scheme, and the substrate means were compared by the Tukey test. The degrees of freedom corresponding to the shading factor were partitioned by polynomial regression (α = 5%).

RESULTS AND DISCUSSION

The survival percentage of plants was affected by substrate and by shading level. The substrates used for acclimatization revealed that vermiculite has a low yield as compared to washed sand and PlantMax® Horticultura, at all shading levels. Inexpressive survival, ranging from 5.83% (30% shade) to 32.50% (80% shade), indicate that the use of vermiculite should not be recommended for the acclimatization of this crop. In vermiculite, the dead heliconia seedlings did not present new roots, and their leaves were severely affected, showing rapid wilting of the leaf blade with later necrosis. The acclimatized seedlings that survived had at least one leaf in good condition, which contributed toward the emission of roots; preexisting roots, originated during the *in vitro* rooting, were not functional, without the emission of secondary roots, and presented root tissue necrosis. Vermiculite showed a low capacity of aggregation with the roots, which negatively affected plant development, in agreement with Hoffmann et al. (2001), who worked with apple tree rootstocks, and Silva et al. (2003), with gloxinia plantlets.

The substrates washed sand and PlantMax® Horticultura had similar yields, with expressive survival rates (72.50% and 71.66%) beginning at 50% shade, reaching up to 95% and 93.33% respectively, at 80% shade. Survival was inexpressive below 50% shade, with rates lower than 27.50%. The abnormalities caused by *in vitro* culturing, such as cuticle alterations, epicuticular wax, and lack of functionality of the stomatal apparatus, observed by Marin et al. (1988); Preece & Sutter (1991); Desjardins (1995); and Pospísilová et al. (1999; 2000) could be the reason for the difficult acclimatization at shade levels below 50%.

Shade levels of 70 and 80% had a survival rate stability for the substrates washed sand (94.16% and 95.0%) and vermiculite (31.66% and 32.5%), and a slight decrease for the PlantMax® Horticultura (95.0% and 93.3%), which indicates that the maximum shading limit for this heliconia species is 80%.

The acclimatized seedlings that survived presented emission of new roots and the same non-functional behavior, and root tissue necrosis of *in vitro*-induced roots. The non-functionality of the roots produced during the *in vitro* process suggests that this crop may become acclimatized without plantlets going through a rooting stage in the laboratory, which would eliminate one step in the micropropagation process. Plantmax® Horticultura provided good aggregation to roots and water retention, whereas sand had an excellent drainage with satisfactory aggregation to the roots.

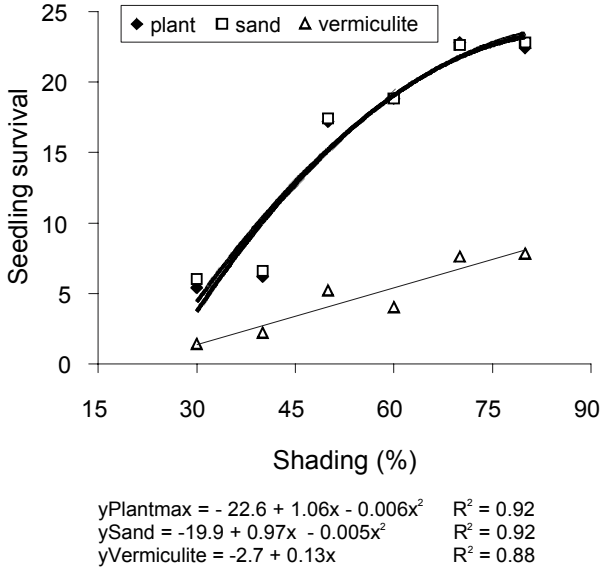

Figure 1 - Effect of shade levels and substrates on the survival of heliconia seedlings during acclimatization, 28 days of *ex vitro* culture.
Treatments followed by distinct letters are different by Tukey test. ($P < 0.05$).

Plant survival rate was zero for all substrates evaluated in the 0% shade treatment (full sun). Figure 1 presents the middle point distributions, their respective equations, and coefficients of determination (R^2) for the interval between 30 and 80% shade. For the substrates washed sand and Plantmax® Horticultura, plant survival increase was quadratic, with a linear increase for vermiculite. Increased levels of shading promoted a quadratic increase in the percentage of surviving plants during the acclimatization stage for Plantmax® Horticultura and Sand, and a linear increase for vermiculite.

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