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Effect of sucrose concentrations on *in vitro* growth and subsequent acclimatization of the native bromeliad *Vriesea inflata* (Wawra) Wawra

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Resumo

Efeito de concentrações de sacarose sobre o crescimento *in vitro* e a aclimatização da bromélia nativa *Vriesea inflata* (Wawra) Wawra. O uso de sacarose é essencial para o crescimento de plantas *in vitro*, devido à fotossíntese reduzida nessa condição. Contudo, espécies epífitas ornamentais, que apresentam crescimento lento, têm mostrado melhor desenvolvimento em meios nutritivos com concentrações reduzidas desse carboidrato. *Vriesea inflata* (Wawra) Wawra é uma bromélia epífita nativa da Mata Atlântica de alto valor ornamental. O objetivo deste estudo foi avaliar os efeitos de diversas concentrações de sacarose no meio nutritivo sobre o crescimento *in vitro* e a posterior aclimatização em condições *ex vitro*. Plantas foram cultivadas *in vitro* em meio basal na ausência de carboidratos; e em meios contendo 1,0; 1,5; 3,0; 4,5 e 6,0% (p/v) de sacarose por 60 dias. Posteriormente, as plantas foram aclimatizadas por 90 dias. Plantas cultivadas em concentrações acima de 3,0% de sacarose tiveram o menor desenvolvimento depois de cultivo *in vitro* e aclimatização. No entanto, aquelas cultivadas em concentrações menores apresentaram o maior crescimento radicular *in vitro*, e originaram plantas maiores depois da aclimatização. Em média, 1,7% de sacarose foi definida como adequada para cultivar *V. inflata in vitro*, para obter plantas mais vigorosas durante a aclimatização.

Palavras-chave: Bromeliaceae; *Ex vitro*; Fonte de carbono; Micropropagação; Ornamental

Abstract

Using sucrose is crucial for *in vitro* plant growth, due to decreased photosynthesis under this condition. However, ornamental epiphytic species, which have slow growth, have shown better development in nutrient media with low concentrations of this carbohydrate. *Vriesea inflata* (Wawra) Wawra is an epiphytic bromeliad native to the threatened Atlantic Rain Forest and it has a high ornamental value. This study aimed to evaluate the effects of various sucrose concentrations in the culture medium on *in vitro* growth and subsequent *ex vitro* acclimatization. Plants were cultured *in vitro* in basal media without carbohydrates; and in media containing 1.0; 1.5; 3.0; 4.5; and 6.0% (w/v) of sucrose for 60 days. After, plants were acclimatized for 90 days. Plants cultured in sucrose concentrations over 3.0% had the smallest development after *in vitro* culture and acclimatization.

Nevertheless, those cultured at lower concentrations had the highest *in vitro* root growth, and they generated larger plants after acclimatization. On average, 1.7% sucrose was defined as appropriate to cultivate *V. inflata in vitro*, in order to obtain more vigorous plants during acclimatization.

Key words: Bromeliaceae; Carbon source; *Ex vitro*; Micropropagation; Ornamental

Introduction

The bromeliad *Vriesea inflata* (Wawra) Wawra is native to the threatened Atlantic Rain Forest, and it has morphological traits of high ornamental value (PEDROSO et al., 2010) (Figures 1a; 1b). This species and many other bromeliads are extracted from their original habitat to supply the ornamental trade, something which can pose a significant threat to the Bromeliaceae biodiversity (NUNES, 2002; NEGRELLE et al., 2012). Many *in vitro* culture protocols have been developed for bromeliad species to effectively propagate them, aiming both at conservation and commercialization (SILVA et al., 2012; SANTA-ROSA et al., 2013; KURITA et al., 2014).

The development of plants *in vitro* depends to a large extent on the supply of carbon sources in the culture media, since morphogenesis and growth are high energy requiring processes. However, the required carbohydrate concentration varies according to species, something which demands the definition of specific protocols (YASEEN et al., 2013). Sucrose is one of the more frequently used carbon sources on *in vitro* cultivation, where concentrations from 2 to 4% usually lead to optimal growth, as reported by Hazarika (2003). Accordingly, bromeliads are mostly cultured *in vitro* with 3% sucrose in the medium (DAL VESCO et al., 2011; CARVALHO et al., 2013; MARTINS et al., 2013).

Vriesea inflata has been propagated *in vitro*, as previously reported by our research group (PEDROSO et al., 2010). Studies report that the species have slow growth, even when cultured *in vitro*. Therefore, it is important to optimize the *in vitro V. inflata* growth in order to obtain vigorous plants that can acclimatize in a more effective way. It has been reported that lower concentrations of carbon sources in the culture medium can improve acclimatization (JO et al., 2009), which have been associated with higher photosynthetic ability (LANGFORD; WAINWRIGHT, 1987; HUYLENBROECK; DEBERGH, 1996). Lower sucrose concentrations in the nutrient medium were applied to other epiphytic bromeliads and high survival rates after acclimatization were obtained, as described for *Vriesea gigantea* Gaudich. (BENCKE; DROSTE, 2008) and *Acanthostachys strobilacea* (Schult. f.) Link, Klotzsch & Otto (SANTOS et al., 2010).

This study aimed to evaluate the effects of various sucrose concentrations on *in vitro* and subsequent *ex vitro V. inflata* growth, in order to verify if lower concentrations improve the species' acclimatization.

Material and Methods

Plant material

Seeds of *Vriesea inflata* (Wawra) Wawra were collected from the "Alto da Serra de Paranapiacaba" Biological Reserve (Santo André, São Paulo, Brazil) (Figure 1a). They were surface-sterilized and aseptically transferred to 250 mL flasks containing 20 mL of modified Murashige and Skoog (1962) medium, according to Pedroso et al. (2010). The medium pH was adjusted to 5.8, followed by the addition of agar (5 $g.L^{-1}$) and autoclaving for 15 min at 121°C. After autoclaving the media, seeds were placed in the flasks and maintained in a growth room at 25 ± 2°C, with 12 h photoperiod and 30 $\mu mol.m^{-2}.s^{-1}$ irradiance (Osram® Fluorescent Lamps).

In vitro culture at different sucrose concentrations

One month after the seeds germinated (Figure 1c), 10 plants were transferred to each 250 mL flask containing 40 mL of basal media, without sucrose and supplemented with 1.0, 1.5, 3.0, 4.5 and 6.0% (w/v) sucrose (Synth®). Flasks were kept in growth room under

FIGURE 1: *Vriesea inflata* as seen in the natural environment, its ornamental use, and the stages of *in vitro* culture: **a)** adult plant located at the "Alto da Serra de Paranapiacaba" Biological Reserve; **b)** flowering plants in vases indicating ornamental value; **c)** 1-month-old *in vitro* seedlings (bar: 1 cm); **d)** general aspect of 4-month-old *in vitro* plants after acclimatization for 60 days (bar: 2 cm).

the same conditions described above. After 60 days of culture, 10 plants from each treatment were evaluated regarding the biometric features of survival rate, number of leaves and roots, length of the longest leaf and root, fresh and dry shoot mass.

Ex vitro acclimatization

After 60 days of culture under different sucrose concentrations, 10 additional seedlings from each treatment were transferred from the flasks to trays containing fine sterilized commercial medium-sized *Pinus* bark (100%) (Figure 1d). Plants from each treatment were fertilized biweekly with 10 mL of a solution with 50% macronutrient salts as proposed by Murashige and Skoog (1962). Trays containing plants were kept in growth room under the same conditions cited above for 90 days, then plants were evaluated regarding the features described in the previous section.

Statistical analysis

The experiments' design was completely randomized. For the *in vitro* culture and acclimatization experiment, the number of samples was 10 per treatment (n = 10). Shoot mass was determined in triplicates (n = 3). Data underwent analysis of variance, followed by regression analysis at $p < 0.05$ by using the software SISVAR.

Results and Discussion

Vriesea inflata plants cultured *in vitro* without sucrose had low survival rates (20%), perhaps because most plants could not photosynthesize in an effective way under this condition to generate energy for other metabolic processes (VON ARNOLD, 2008). However, varying sucrose concentration from 1.0 to 6.0% enabled total survival of *V. inflata* plants (100%). Those treatments did not significantly influence the development of new leaves (Figure 2a). On the other hand, the number of roots, leaf and root length increased linearly with the reduction of sucrose in the medium, therefore, plants at 1.0% sucrose showed the highest mean values (Figure 2a; 2b). Similar responses were noticed for banana and apple plants, where both had higher development when cultured at lower sucrose concentrations (< 3.0%) (YASEEN et al., 2009; WAMAN et al., 2014). Fresh and dry shoot mass showed a quadratic response to the varying concentrations, where plants kept at 1.0% sucrose also had the highest values (Figure 2c). The alteration in *V. inflata* biometric features must derive from the influence sugars such as sucrose have on growth and development, because they have high nutritional value and control over many genes related to morphogenetic and metabolic processes (KOCH, 1996; SOTIROPOULOS et al., 2006).

All plants cultured *in vitro* at 1.0-6.0% sucrose survived after *ex vitro* acclimatization (100%). The varying sucrose concentrations had significant effects on all biometric characters of acclimatized plants, in which they showed a quadratic response (Figure 3). The maximum average of leaf and root growth were 1.6 and 1.5% sucrose, respectively, whereas plants from higher concentrations had smaller values (Figure 3a; 3b). The results are in accordance with those observed before acclimatization (Figure 2). According to Rolland et al. (2006), carbon sources are essential for the production of growth hormones (auxins), which induce root development. However, the carbohydrate concentration required to produce auxins depends on the species. In fact, high sugar concentrations can lead to reduced osmotic potential in the media, resulting in lower availability of water for the plants, which in turn hampers growth (CALVETE et al., 2002; JO et al., 2009).

FIGURE 2: Regression analysis of biometric features of *Vriesea inflata* plants cultivated *in vitro* for 60 days under different sucrose concentrations in the nutrient media: **a)** number of leaves and roots; **b)** leaf and root length; **c)** fresh and dry shoot mass (*: 5% significant ($p \leq 0.05$)).

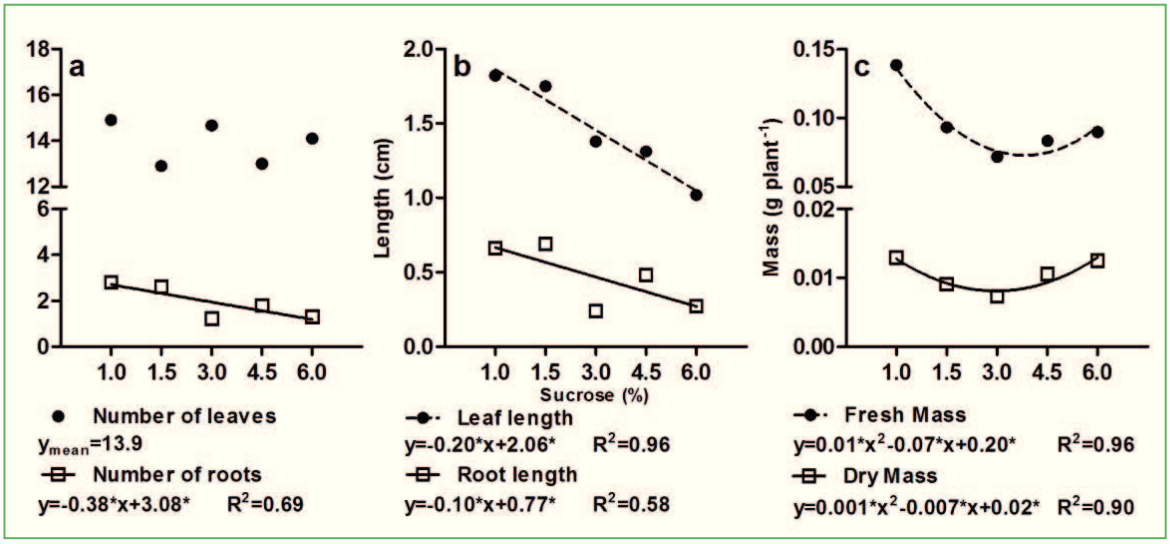
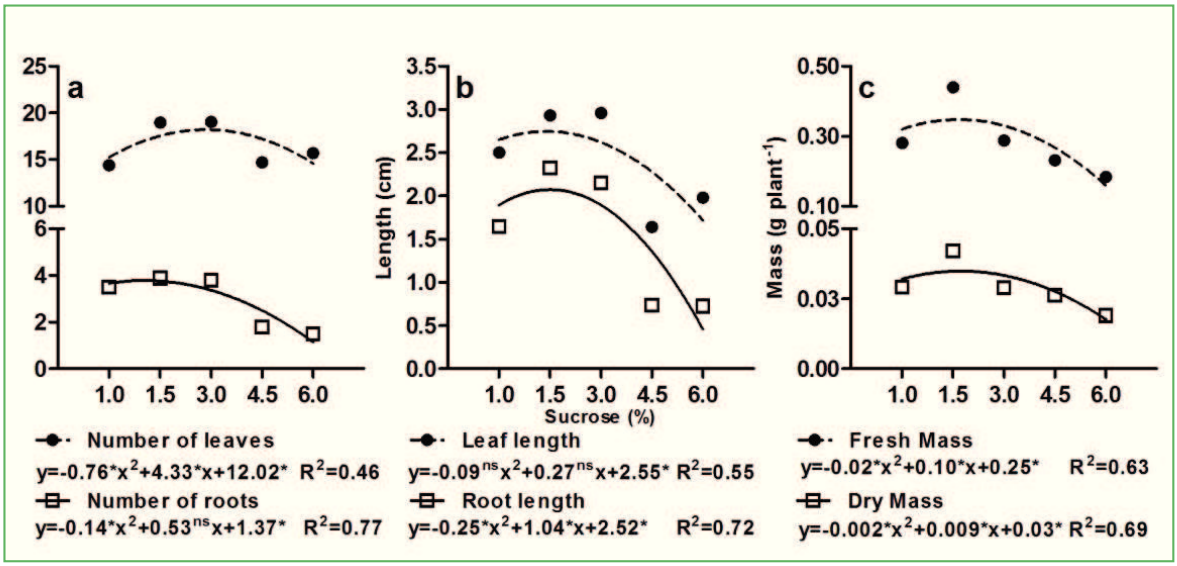


FIGURE 3: Regression analysis of biometric characters of *Vriesea inflata* plants acclimatized for 90 days after cultured *in vitro* for 60 days under different sucrose concentrations in the nutrient media: **a)** number of leaves and roots; **b)** leaf and root length; **c)** fresh and dry shoot mass (ns: not significant ($p > 0.05$), *: 5% significant ($p \leq 0.05$)).



Fresh and dry mass of acclimatized plants were maximum at 1.9 and 1.7% sucrose, respectively (Figure 3c), similar to leaf and root growth. In average, the maximum growth after acclimatization was observed in plants cultured *in vitro* at concentrations circa 1.7%, something which suggests that such sucrose levels generate *in vitro* plants more adapted to grow under *ex vitro* conditions for longer periods. These results might be due to the significant root development observed in plants cultured *in vitro* under 3.0% sucrose (Figure 2a; 2b). Likewise, Galdiano Junior et al. (2013) noticed that cultivating the orchid *Cattleya loddigesii* Lindley under 2.0% sucrose effectively induced *in vitro* root growth, which resulted in higher survival after acclimatization in comparison with plants obtained from 3.0-4.0% sucrose. Nievola and Mercier (1996) have shown that not only leaves but also roots of the bromeliad *Vriesea fosteriana* L.B. Sm., which can be found in epiphytic conditions, are important for nitrogen assimilation. Considering that *V. inflata* is also an epiphytic bromeliad, the best developed root system of plants obtained from < 3.0% sucrose concentrations may have led to a more effective nutrient assimilation, producing vigorous plants after acclimatization.

According to regression analysis, cultivating *V. inflata* plants *in vitro* at 1.7% sucrose for 60 days promotes significant root growth, which leads to more vigorous plants after 90 days of *ex vitro* acclimatization when compared to higher sucrose concentrations.

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