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Development of *Epidendrum denticulatum* (Orchidaceae) plants subjected to water deficit

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ABSTRACT - Water deficit is one of the main abiotic stresses that negatively affect plant growth. This work aimed to evaluate the survival and development of *Epidendrum denticulatum* Barb. Rodr. plants under water stress. Six-month-old acclimatized plants from asymbiotic germination were subjected to the following treatments: daily irrigation, biweekly irrigation, and no irrigation. Variables of plant development and pigment production were evaluated after 75 and 150 days of cultivation under the above conditions. At 75 days, the survival ranged between 95 and 100% under the different irrigation regimes; however, the number of leaves and new roots decreased in plants irrigated fortnightly and not irrigated. At 150 days, survival ranged between 35% and 95% in plants not irrigated and irrigated daily, respectively. The effects on development were more severe at this stage, inhibiting the emission of new roots entirely and reducing the content of photosynthetic pigments. Despite this inhibition, the high survival after 75 days of irrigation interruption indicated that *E. denticulatum* is resistant to water deficit, tolerating the drought, and can be used to enrich areas of native vegetation.

Keywords: irrigation, orchid, survival, water deficiency

RESUMO - Desenvolvimento de plantas de *Epidendrum denticulatum* (Orchidaceae) submetidas à deficiência hídrica. A deficiência hídrica é um dos principais estresses abióticos que atuam negativamente sobre o crescimento vegetal. Este trabalho teve como avaliar a sobrevivência e o desenvolvimento de plantas de *Epidendrum denticulatum* Barb. Rodr. submetidas a estresse hídrico. Plantas aclimatizadas desta espécie com seis meses de idade, oriundas da germinação assimbiótica, foram submetidas aos seguintes tratamentos: irrigação diária, irrigação quinzenal e sem irrigação. Estas plantas foram avaliadas após 75 e 150 dias de cultivo, por meio da coleta de dados de variáveis de desenvolvimento e de produção de pigmentos. Após 75 dias, a sobrevivência variou de 95 a 100% nos diferentes regimes de irrigação, entretanto, houve redução dos números de folhas e de raízes novas, nas plantas irrigadas quinzenalmente e não irrigadas. Aos 150 dias, a sobrevivência variou de 35% (não irrigadas) a 95% (irrigadas diariamente) e os efeitos sobre a inibição do desenvolvimento foram mais severos, por exemplo, inibindo completamente a emissão de raízes novas e reduzindo o teor de pigmentos fotossintéticos. Apesar dessa inibição, a elevada sobrevivência obtida após 75 dias de interrupção da irrigação permite concluir que *E. denticulatum* é resistente ao déficit hídrico, tolerando a seca, e pode ser utilizada no enriquecimento de áreas de vegetação nativa.

Palavras chave:- déficit de água, irrigação, orquídea, sobrevivência.

INTRODUCTION

Water deficit is one of the main abiotic stresses that negatively affect plant growth and development, causing stem and root reductions, in addition to inhibiting leaf expansion,which can trigger plant death (Silva *et al*. 2009, Dias-López *et al*. 2012).

Climate change has caused water deficits in different locations, as well as seasonal problems. According to Nobre *et al*. (2011), climate change is associated with increased temperatures and changes in the rainfall regime. These authors found that heavy rains increased from nine annual occurrences between 1933 and 1940 to 40 between 2000 and 2009. De Lima & Rueda (2018) also pointed out a reduction in drizzle occurrence (below 1 mm/day) in the last 20 years. Meteorological data showed an increase in annual precipitation but a decrease in the number of rainy days (IAG 2017), indicating higher rainfall concentration. Consequently, there was an increase in the annual number of days with low relative humidity (< = 30%) from 1958 to 2017 (IAG 2017).

The PEFI, Conservation Unit considered in the present study, is one of the few forest fragments of the Atlantic Forest plateau (Pivello & Peccinini 2002) and can be

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regarded as a corridor between the humid coastal forests and the drier inland forests (Strufaldi-De-Vuono, 1985). Despite being isolated by urbanization, it is the third-largest remnant of Atlantic Forest in the city of São Paulo with 345 ha. (Costa & Gomes 2020). Although it is considered a secondary forest, it has a good conservation status due to the large number of epiphytes found in floristic studies carried out between the 1980s and 2000s, with more than 145 species identified, mainly composed of Orchidaceae, Bromeliaceae, Araceae, Cactaceae, and Piperaceae (Barros *et al*. 2002).

Over the decades, the PEFI vegetation has changed its structure, reducing the heterogeneous and tall canopy forest and the sparse homogeneous canopy forest, with a consequent increase in the heterogeneous and low canopy forest, of the homogeneous canopy forest dense and degraded forest (Peccinini & Pivello 2002). Among the leading causes of degradation, we can mention the atmospheric pollution of a conservation unit located in a large metropolis like São Paulo, the existence of a neighboring steelwork, which operated until 1991 (Struffaldi-De-Vuono & Marzolla 1984), and a large proportion of fires in 1993 (Pivelo & Peccini 2002). However, the rainforest shows clear signs of recovery, with an increase in tree numbers (Pivelo & Peccini 2002).

The changes above, combined with a rainfall regime with more days of drought, possibly modified the PEFI orchid composition, given that in various incursions carried out in 2018 and 2019, we could not identify the same richness observed by Barros *et al*. (1983). Specifically, for *Epidendrum denticulatum* Barb. Rodr., a significant reduction in the number of specimens possibly occurred, as only one specimen was found during these incursions.

Thus, to evaluate the ability of *E. denticulatum* plants to survive a future relocation in the PEFI, in the current scenario of less constant rainfall, we assessed the survival and development of young plants of this species subjected to water deficit.

MATERIAL AND METHODS

Epidendrum denticulatum Barb. Rodr. is a terrestrial or rupicolous orchid found in the Atlantic Forest and the Cerrado. It has a non-thickened cylindrical stem in a pseudobulb, covered by leaf sheaths and distichous, usually numerous, thick, and leathery leaves, distributed

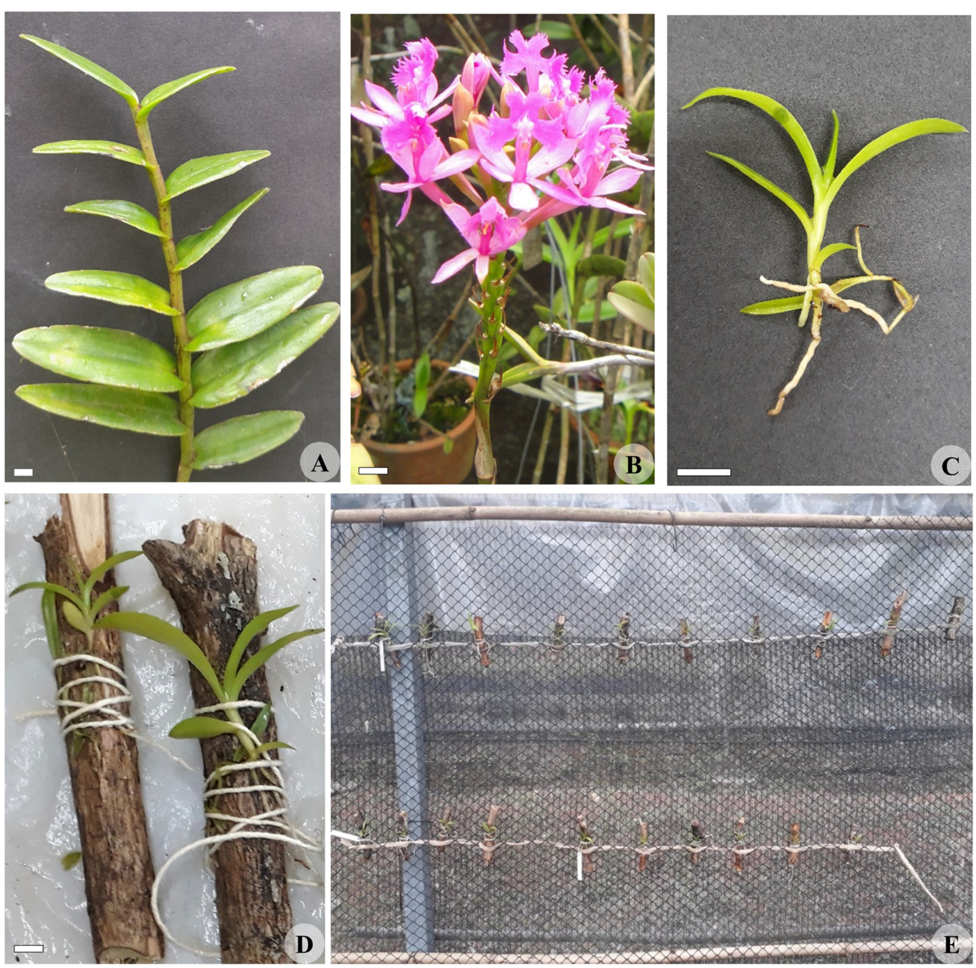

Figure 1. Plant model and method used. **A.** Vegetative aspect of *Epidendrum denticulatum*; **B.** General aspect of the inflorescence; **C.** Orchid phenotype used in the experiment; **D.** Model adopted to simulate the phorophyte in nature; **E.** .Arrangement of plants on the phorophytes, placed on a nursery net curtain. Scale bar A = 2 cm, B-D = 1 cm.

equidistantly along the stem (Fig. 1A). The inflorescence is the same length or longer than the stem and has pink to lilac flowers generally arranged in corymbs (Fig. 1B) (Pinheiro & Barros 2007, Stancik *et al*. 2009). Another important characteristic of *E. denticulatum* is the possibility that photosynthesis is CAM because this metabolism was demonstrated in *E. secundum* (Pinheiro & Barros 2007, Moreira *et al*. 2009).

Our experiments used six-month-old acclimatized plants of *E. denticulatum* obtained from asymbiotic germination of seeds from "Frederico Carlos Hoehne" orchid collection, Environmental Research Institute (IPA), São Paulo-SP, Brazil. The experiment was carried in greenhouses of the IPA Experimental Field from October 2020 to April 2021. These greenhouses were covered with a shade screen to reduce the light incidence by 50%.

The plants initially had, on average, 6 cm of stem length, 6 cm of root length, five leaves, five roots, and approximately 0.8 g of fresh mass (Fig. 1C). Each plant was tied to a plant branch of 20 cm long and 7 cm in circumference (Fig. 1D) to simulate the phorophyte on which the plant is found in nature. These branches were placed on a 13 mm-mesh plastic curtain hung in a greenhouse (Fig. 1E) and were subjected to three treatments: daily irrigation, biweekly irrigation, and no irrigation.

At 75 and 150 days of cultivation, survival, stem length, the number of leaves per plant, and new roots per plant were evaluated. The reading of leaf photosynthetic pigments was also collected with SPAD equipment (Minolta SPAD-502). In addition to the parameters mentioned above, we determined the average fresh and dry masses and the water content of plants at 150 days.

The concentration of photosynthetic pigments was measured by organic extraction and spectrophotometric analysis. These data and the data output from the Minolta SPAD-502 foliar chlorophyll meter were correlated according to Markwell *et al*. (1995).

The experimental design was completely randomized, with five plants in each treatment and four replications (totaling 20 plants). The experiment was carried out for 150 days when the results stabilized. Data were subjected to the analysis of variance, and means were subsequently compared using Tukey´s test ($p \geq 0.05$) with SISVAR® software (Ferreira 2011).

RESULTS AND DISCUSSION

Table 1 shows that plant survival did not differ significantly between the irrigation treatments at 75 days, ranging between 95% (daily irrigation) and 100% (fortnightly irrigation and no irrigation). At 150 days, the survival decreased in plants not irrigated (35%) and irrigated every two weeks (50%), compared to plants irrigated daily (95%). Non-irrigated plants had a significant decrease in survival associated with the duration of irrigation interruption, between 75 (100%) and 150 days (35%). *Epidendrum denticulatum* proved to be very resistant to drought for 75 days, and even a relatively high percentage survived after six months without irrigation. Therefore, the relocation of this orchid as a management strategy in forest restoration seems very promising. Survival of other relocated orchids was 92% for *Sauroglossum nitidum* (Vell.) Schltr. (Suzuki *et al*. 2021), 83% for *Cattleya intermedia* Graham ex Hook (Dorneles & Trevelin 2011), and between 70 and 80% for *Vanda coerulea* Griff. ex Lindl. (Seeni & Latha 2000), without being subjected to a severe water deficit. However, our results showed inhibition development in *E. denticulatum* associated with the time exposed to reduced irrigation conditions. As shown in Table 1, the stem length varied significantly between 75 and 150 days of cultivation in plants irrigated fortnightly (5.8 and 4.0, respectively) and not irrigated (5.2 and 4.1, respectively). During the initial 75 days, the stem length did not vary significantly between the irrigation regimes,

Table 1. Plants of *Epidendrum denticulatum* subjected to daily irrigation (DI), biweekly irrigation (BI), and no irrigation (NI). Average survival, stem length, number of leaves per plant, and new roots per plant, at 75 and 150 days. Fresh and dry mass values and water content after 150 days of cultivation.

	75 days			150 days		
	DI	BI	NI	DI	BI	NI
Survival (%)	95 aA	100 aA	100 aA	95 aA	50 bA	35 bB
Stem lenght (cm)	5,4 aA	5,8 aA	5,2 aA	4,6 aA	4,0 aB	4,1 aB
Leaves*plant^{-1}	4,5 aA	2,9 bA	3,0 bA	4,2 aA	2,0 bA	1,9 bB
Roots*plant^{-1}	2,7 aA	1,4 abA	0,9 bA	1,5 aB	0 bB	0 bB
Fresh Mass (mg)	–	–	–	697,8 a	192,3 b	161,1 b
Dry Mass (mg)	–	–	–	89,5 a	49,2 a	51,7 a
Water Content (%)				87,6 a	73,4 ab	67,9 b

Note: Means followed by the same letter do not differ significantly by Tukey's test ($p > 0.05$). Lowercase letters compare irrigation treatments within an assessment date, and uppercase letters compare assessment dates within an irrigation treatment.

ranging between 5.2 in treatments without irrigation and 5.8 in fortnightly irrigation.

At 75 days of cultivation, plants irrigated every two weeks and without irrigation had a significantly reduced number of leaves (2.9 and 3.0, respectively) than plants irrigated daily (4.5) (Tab. 1). This inhibitory effect was even more pronounced at 150 days of cultivation. The leaf number in plants without irrigation also differed significantly between 75 and 150 days of cultivation (Tab. 1). Noya *et al*. (2014) also verified a decrease in the leaf number in *Stenachaenium megapotamicum* (Spreng.) Baker plants subjected to irrigation shifts of three days compared to plants irrigated daily. Although leaves contribute to water storage in orchids (Pridgeon & Stern 1982), they are also the main organ responsible for water loss. Therefore, it is necessary for orchid leaves to regulate water balance and stomatal control during drought as a tolerance strategy to drying conditions (Rosa-Manzano *et al*. 2014). According to Tay *et al*. (2015), one of these strategies is to reduce the leaf area and the abscission of the leaves, reducing water loss considerably.

The number of new roots showed the same behavior as the number of leaves. At 75 days, the number of new roots decreased significantly in non-irrigated plants, compared to plants irrigated daily (0.9 and 2.7, respectively). At 150 days, the number of roots in the irrigated plants was significantly lower than in the 75-day plants (Tab. 1). After 150 days of cultivation, the other treatments no longer presented new roots. Water deficit proved to inhibit root development in *Medicago sativa* Lindl. (Zeid & Shedeed 2006), *Helianthus annuus* Lindl. (Manivannan *et al*. 2007), and *Oryza sativa* Lindl. (Pandey & Shukla 2015). However, Manivannan *et al*. (2007) observed that root development in *Helianthus annuus* initially increased under water deficit but decreased at a later stage due to severe water stress. In our study, even for the shortest evaluation time (75 days), the plants would already be in this last stage of root development inhibition.

At the end of the experiment, the fresh mass was higher in plants irrigated daily than in plants irrigated fortnightly and not irrigated, with values more than three times higher (Tab. 1, $p < 0.05$). However, the dry mass did not differ significantly between irrigation regimes (daily irrigation: 89.5 mg and biweekly irrigation: 49.2 mg; $p \geq 0.05$).

After 150 days, the plant water content reflected the stress caused by the decrease in irrigation since plants irrigated daily had a significantly higher content (approximately 88%) than non-irrigated plants (~68%). Similarly, water deficit caused a decrease in the fresh mass and water content in *Vanilla planifolia* Jacks. ex Andrews, which reached its critical point of dehydration 48 to 94 days after irrigation interruption (Gantiva *et al*. 2020). Likewise, the orchid *Lophiaris oerstedii* (Rchb.f.) reduced its relative water content by 40% during the dry season in the natural environment (Rosa-Manzano *et al*. 2014).

The chlorophyll *a* content was significantly higher in plants irrigated daily (289 $\mu g.gDW^{-1}$) than not irrigated (~179 $\mu g.gDW^{-1}$). As shown in Table 2, chlorophyll *b* showed a similar pattern, in which the content was significantly higher in plants irrigated (~182 $\mu g.gDW^{-1}$) than not irrigated (~105 $\mu g.gDW^{-1}$). The total content of chlorophylls *a* + *b* varied significantly between irrigation regimes. Plants irrigated daily had the highest content (~362 $\mu g.gDW^{-1}$), followed by plants irrigated every two weeks (229 $\mu g.gDW^{-1}$) and not irrigated (188 $\mu g.gDW^{-1}$). The carotenoid content was similar in the plants irrigated daily and biweekly (107.6 and 108.3, respectively).

Regarding photosynthetic pigments, the chlorophyll *a* content was higher than that of chlorophyll *b*, which was higher than the carotenoid content in the three irrigation treatments.

The effect of water deficit on photosynthetic pigments can vary between species. Some studies demonstrated a decreased chlorophyll content in plants subjected to abiotic stresses due to pigment photo-oxidation and chlorophyll degradation (Asharaf & Harris 2013). Similarly, Manevanann *et al*. (2007) observed a significantly reduced content of chlorophyll *a*, *b*, total chlorophyll, and carotenoids in *Helianthus annuus* under water stress treatments. In contrast, the total chlorophyll in *Xerosicyos danguyi* H. Humb. plants did not change significantly after exposure to severe drought (Bastide *et al*. 1993). De França *et al*. (2017) found that the content of chlorophyll *a* and

Table 2. Mean values of chlorophyll *a*, chlorophyll *b*, chlorophyll *a* + chlorophyll *b,* and carotenoids in *Epidendrum denticulatum*, subjected to daily irrigation (DI), biweekly irrigation (BI), and no irrigation (NI) after 150 days.

	Pigments	DI	BI	NI
Photosynthetic pigments ($\mu g.gMS^{-1}$)	Chlorophyll *a*	289,2 a	235,3 ab	178,7 b
	Chlorophyll *b*	181,6 a	137,2 ab	104,8 b
	Chlorophyll *a* + Chlorophyll *b*	361,7 a	229,4 b	188,4 c
	Carotenoids	107,6 a	108,3 a	80,8 b

Note: Means followed by the same letter do not differ significantly by Tukey's test ($p > 0.05$). Lowercase letters compare irrigation treatments within a given pigment.

carotenoids in *Calophyllum brasiliense* Cambess did not change under water stress.

Water deficit causes several biochemical changes in plants, such as reducing stem growth, nutrient uptake by the roots, and transpiration rates and promoting stomatal closure and cessation of CO_2 uptake (Zayed & Zeid 1997, Cui *et al*. 2004, Anjum *et al*. 2017). Stancato *et al*. (2001) observed strong photoinhibition and reduced photosynthetic capacity in the CAM epiphytic hybrid *Cattleya forbesii* Lindl. × *Laelia tenebrosa* Rolfe. after 42 days of water deficit. In a variety of the Aranda orchid, Fu & Hew (1982) observed a considerable reduction in CO_2 assimilation under severe water stress. Likewise, but *in vitro*, Barbero *et al*. (2011) found that as they reduced the water potential of the culture medium, there was a delay in *Acianthera* development. Water deficit caused a decline in photosynthesis in *Coelogyne rochussenii* de Vriese, *Coelogyne mayeriana* Rchb. f., *Bulbophyllum vaginatum* (Lindl.) Rchb.f, *Bulbophyllum membranaceum* Teijsm. and Binn., *Dendrobium leonis* Rchb. f., and *Phalaenopsis cornu-cervi* (Breda) Blume and Rchb.f. (Tay *et al*. 2019). These changes possibly occurred in *E. denticulatum* and were reflected in almost all growth parameters and pigments evaluated in the present study, leading to plant development inhibition (Tables 1 and 2).

At 75 days, the plants irrigated daily had a reddish color of greater or lesser intensity (Figs. 2A and 2B), which was maintained at 150 days (Figs. 2C and D). The plants irrigated fortnightly showed the first signs of leaf wilting at 75 days (Figs. 2E and F). This wilting was more pronounced at 150 days (Figs. 2 G and H) when we observed more dead leaves than before (Figs. 2 F and H). The effect of water stress was even more severe in non-irrigated plants at 75 days (Figs. I and J) and 150 days (Figs. 2K and L), causing the senescence and death of most plants. Figs. 2M, N, and O show the appearance of the leaves in greater detail at 150 days of cultivation under the different irrigation regimes. Fig. 2M shows the leaves subjected to daily irrigation. Fig. 2N shows the leaves irrigated biweekly with leaf wilting. In Fig. 2O, leaves in non-irrigated plants present wilting and other characteristics under severe water stress, such as folding and wrinkling. This last feature was recurrent

Figure 2. General aspect of *Epidendrum denticulatum* plants subjected to different irrigation regimes at 75 and 150 days of greenhouse cultivation. **A-B.** General appearance of plants subjected to daily irrigation at 75 days; **C-D.** General appearance of plants subjected to daily irrigation at 150 days; **E-F.** General appearance of plants subjected to biweekly irrigation at 75 days; **G-H.** General appearance of plants subjected to biweekly irrigation at 150 days; **I-J.** General appearance of non-irrigated plants at 75 days; **K-L.** General appearance of non-irrigated plants at 150 days; **M.** Leaves of plants irrigated daily at 150 days; **N.** Leaves of plants irrigated biweekly at 150 days; **O.** Leaves of non-irrigated plants at 150 days of cultivation.

in plants subjected to water deficit and was observed, for example, in *Oryza sativa* L. (Pandey & Shukla 2015).

As previously mentioned, water deficit can result in reduced leaf area, leaf abscission, and even death of individual plant parts (Tay *et al*. 2015). The pseudobulb protects the orchids against water stress due to its ability to retain water (Ng & Hew 2000), delaying the decrease in leaf water content and water potential (He *et al*. 2013). Since *E. denticulatum* does not have a pseudobulb, the hypodermis of leaves acts as a water reservoir (Oliveira & Sajo 1999), storing up to 80% of the total leaf volume (Pridgeon 1986). The root canopy in this orchid could have contributed to the plants' tolerance to reduced irrigation at 75d, maintaining a high survival percentage (Table 1). According to Zotz & Winkler (2013), the dry canopy can absorb moisture in seconds, minimizing, at least partially, the effects of reduced irrigation in *E. denticulatum*.

Plants with CAM photosynthesis are usually tolerant to severe water stress, prioritizing survival over growth, as observed in *E. denticulatum*. Therefore, we conclude that *E. denticulatum* is resistant to water deficit, tolerating moderate and severe drought for more than 75 days, and can be relocated to restore or enrich forest areas.

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