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Original Paper

Magnetically treated irrigation water improved the adaptation of *Spathoglottis plicata* produced in vitro

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Abstract

In the search for other methods that contributed to the success of the micropropagation of plant species, the man has been able to establish relationships with phenomena that consider other sciences such as physics, and within this, the use of magnetism has been used as a promising tool in Plant Biotechnology. The objective of this study was to evaluate the effect of magnetically treated water (MTW) during the adaptation process of the orchid *Spathoglottis plicata*. To perform the magnetic treatment, a permanent magnets magnetizer with induction of 0.12 T in the irrigation water was used. The survival percentage, plant height, number of leaves, number of roots, leaf area and photosynthetic pigments content were evaluated. The magnetic treatment presented mean values higher than the control in all evaluated traits. With MTW the plants showed an improvement in the survival and the physiological traits associated to the greater photosynthetic pigments content. Magnetically treated water is efficient to improve and anticipate the adaptation of plants to *ex vitro* conditions.

Key-words: Adaptation, Orchids, *ex vitro*, Magnetism, Physiology

Introduction

Orchids are plants that for the beauty of their flowers are among the favorites in the ornamental plants market (Sorace et al., 2009). Among them is the species *Spathoglottis plicata*, with perennial and bulbous herbaceous habit. Unlike most orchids, this species prefers environments with high humidity and well-irrigated soils. The beauty of its flowers and pleated leaves are striking and make the plant a good commercial and ornamental option even when devoid of its flowers.

Its traditional reproduction is carried out in an asexual way, where a part of it (bulbs) is used to give rise to a new plant. The few reserves of orchid seeds and the dependence of association with mycorrhizae make their sexual reproduction inefficient in nature, and when obtained by man (hybrids), they need an *in vitro* culture process to achieve a higher percentage of plants (Butcher and Ingran, 1976; Dixon, 1985). With the use of micropropagation this limitation on the germination of orchids is supplied. In micropropagation of orchids, the most important step is acclimatization, which is considered the most critical stage (Andrew et al., 1990; Pérez Ponce, 1998) as a consequence of the defective physiological functioning of the photosynthetic and transpiration apparatus of *in vitro* plants when transferred to *ex vitro* conditions, as well as susceptibility to pests and diseases.

The beneficial action of the magnetic treatment in the acclimatization of several species has promoted an increase in the survival percentage and accelerated the plant development in this stage (Rodríguez, 1998; Botta, 2000; Dubois et al., 2001,

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2004). Magnetically treated water has been used in the physiological recovery and in the hardening of i*n vitro* plants, however, there is a variable response to certain magnetic inductions that must be adequate to allow the maximum development of *in vitro* plants in the acclimatization stage (Alemán et al., 2014a, b; Maffei, 2014). The use of water treatment has been more extensive in horticultural crops (Putti et al., 2015; Aguilera and Martin, 2016; Haq et al., 2016; Surendran et al., 2016; Yusuf et al., 2016; Yusuf and Ogunlela, 2017). In view of the above, the objective of this study was to determine the effect of magnetically treated water under the acclimatization of *in vitro* orchids of the species *Spathoglottis plicata*.

Material and Methods

The trial was carried out at Laboratory of Plant Biotechnology belonging to the National Center for Applied Electromagnetism (CNEA) of the University of Oriente (UO). We used orchid in vitro plants of the species *Spathoglottis plicata* at 16 weeks of *in vitro* culture. The establishment under natural conditions was performed in an adaptation area (greenhouse), where they stayed for an initial period of 7 days. After this period, the seedlings were extracted from the culture flasks and washed in water with abundance to eliminate all excess agar in the roots, rinsed with distilled water and submerged in a 1% iodine solution for their total disinfection. To standardize the samples, we selected the plants that had the following morpho-anatomical characteristics: ≥ 2 cm length, ≥ 3 leaves and ≥ 2 roots.

The selected *in vitro* plants were planted in a tray containing 35 plants per treatment. Irrigation was performed three times a day during the first two weeks maintaining high relative humidity, similar to in vitro conditions, and after this initial period, the irrigation was performed twice a day by using aerial sprinklers. The greenhouse was maintained with relative humidity around 55-60%, temperature 27 ± 2 °C, and 30% natural brightness. The substrate employed had a mixture of soil, organic matter and sand in a ratio 4:3:2, with the following properties: electrical conductivity of 4.24 $\mu S\ m^{-1}$, pH 7.3 organic matter > 4 %, P_2O_5 57.25 mg L^{-1}, K_2O 960 mg L^{-1} and MgO 725.76 mg L^{-1}.

We evaluated two treatments with three replicates containing 5 plants, one with magnetically treated water (MTW) and other no magnetically treated water (NMTW) as control. In the magnetic treatment, water was treated with a permanent magnets and homogeneous field magnetizer, designed and built in the CNEA, with 20 cm in length and a magnetic induction of 0.12 T. The verification of the magnetic treatment was carried out using the crystal optic method, based on the decrease in size experienced by the salt crystals during the heating of the magnetically treated water regarding the crystals of the untreated water (Rodríguez et al., 1999).

The percentage of living plants in the first six weeks of the trial was evaluated, quantifying the number of plants at 21 and 42 days after transplanting (DAT). Morpho-anatomical traits were evaluated every three weeks: leaf number, plant height and number of roots; this latter was evaluated at the beginning and at the end of the trial. At the end of the evaluated period (18 weeks), the leaf area was evaluated using the gravimetric method and the content of photosynthetic pigments (chlorophylls (a + b) and carotene) was assessed by the spectrophotometric method (Ortega and Ródez, 1986).

Data normality was previously tested by the Kolmogorov-Smirnov test and then the data were submitted to analysis of variance (ANOVA). When necessary, square root of x transformation was applied to the data of the number of leaves and number of roots; and logarithm of x transformation for plant height and leaf area. Statistical analysis was based on a simple variance analysis at 95% significance using of the STATGRAPHICS Plus software version 3.0.

Results and discussion

In vitro plants survival was evaluated in Table 1. It was verified that the magnetically treated water (MTW) allowed an increase of 11.43% of the survival percentage of the orchid in vitro plants. Similar results regarding the survival response were obtained in *Dioscorea alata* L. *in vitro* plants with 95 % compared to 85% in control (Dubois et al., 2001) and in coffee tree (*Coffea arrabica* L.) with 75% compared to 70% in control, respectively, by applying MTW in irrigation (Botta, 2000). When studying this same response *in vitro* plants of *Oncidium luridum*, Rodríguez (1998) observed that the magnetic treatment stimulated the survival percentage with values of 63% compared to 59% in the control. These results corroborate those obtained in this study.

Table 1. Evaluation of the survival of orchid in vitro plants of the species *Sphatoglottis plicata*, irrigated with magnetically treated water (MTW) and no magnetically treated water (NMTW).

Treatment	Number of plants		Survival
	21 DAT	42 DAT	(%)
MTW	30	27	77.14
NMTW	31	31	88.57

DAT: days after transplantation (n = 35).

The development of *in vitro* plants adapted to *ex vitro* conditions needs to be efficient and with high rates of plant development that guarantee a good adaptation to the new culture environment. When measuring the plant height of and number of leaves over the evaluated period (126 DAT), we observed that only the plant height showed significant differences at 5% significance level by the F test (Table 2). In this trait, increases in plant height were evident from 84 DAT in favor of MTW, although without statistical significance. In the last two times (105 and 126 DAT), these differences were more notable and verified by the statistical test. Increased plant height (evaluated as the length of the largest leaf) could be correlated with the leaf area that could result in a greater interception of light to increase the photosynthetic rates, and indirectly the plant response to the acclimatization stress. This same trait was evaluated by Botta (2000) when applying a treatment with MTW with an induction of 0.4 T in adapted vitro plants of *Dioscorea alata*, obtaining a higher height of the treated seedlings in relation to the control, and therefore, verifying the same behavior obtained in our study. The number of leaves under the conditions tested in our trial was not influenced by the magnetic treatment. These MTW stimuli for overall plant growth have been widely reported in horticultural crops. Tomato (*Lycopersicon esculentum*) irrigated with MTW presents better performance with greater stem thickness under low fertility soil and irrigation deficit than NMTW, attenuating the adverse effect of the water stress that the crop could suffer with the irrigation deficit (Aguilera and Martin, 2016; Yusuf et al., 2016). The root of the turnip (*Brassica rapa* L.) and shoot height, dry and fresh weights were increased as a result of irrigation with MTW (Haq et al., 2016).

Table 2. Evaluation of the plant height and number of leaves of orchid *in vitro* plants of the species *Sphatoglottis plicata,* irrigated with magnetically treated water (MTW) and no magnetically treated water (NMTW).

Treatment	Plant height (cm)					
	21 DAT	42 DAT	63 DAT	84 DAT	105 DAT	126 DAT
NMTW	3.70 ^{NS}	3.47 ^{NS}	3.44 ^{NS}	3.66 ^{NS}	4.72*	6.38*
MTW	3.39	3.33	3.39	4.54	5.91	7.79
	Number of leaves (unit)					
NMTW	3.23 ^{NS}	3.73 ^{NS}	4.89 ^{NS}	5.81 ^{NS}	6.44 ^{NS}	7.25 ^{NS}
MTW	2.54	3.35	4.39	5.34	6.00	6.83

DAT: days after transplantation. ^{NS} not significant and * significant by F test at 5% probability level.

In Figure 1 four traits assessed at 126 DAT are shown. The leaf area was stimulated by the use of MTW with 12 cm^2 compared to 8 cm^2 manifested by the control (Figure 1a). This trait is directly related to the number of leaves, then we can state that, although the treatment with MTW manifested a smaller number of leaves, they had a larger leaf area, which also had positive effect on the survival percentage. The larger leaf area results in an increased ability of the plant to perform photosynthesis, and hence an increased ability to withstand the adverse conditions if we consider the changes in the environment to which in vitro plants have to survive when they are acclimatized *ex vitro* (Pérez Ponce, 1998).

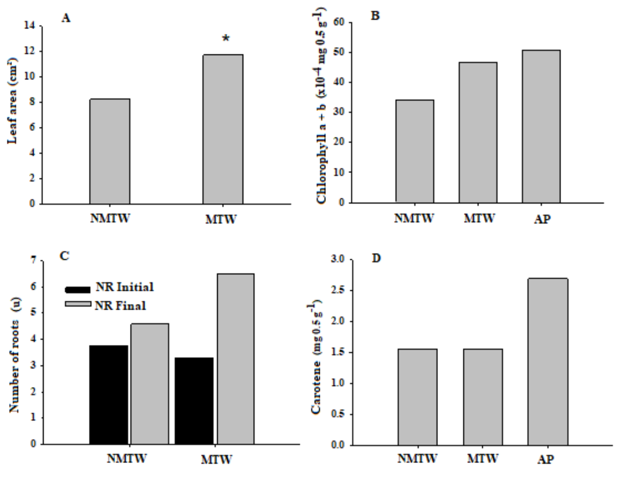

Figure 1. Mean values for leaf area (A), chlorophyll (B), number of roots (C) and carotene (D) obtained by evaluating of orchid in vitro plants of the species Sphatoglottis plicata, irrigated with magnetically treated water (MTW) and no magnetically treated water (NMTW). AP represents one adult plant sample and * significant differences by F test at 5% probability level.

The number of roots (Figure 1c) did not show statistically differences between the two treatments, however the MTW, which initially (7 DAT) showed values lower than the control, surpassed the control at the end of the trial (126 DAT), evidencing the beneficial effect of MTW in our experiment. The fresh and dry root weight, and the root length of lettuce (*Lactuca sativa* L.) was influenced by the type of water and the irrigation, being the treatments irrigated with MTW the ones promoted the highest growth rates, with significant differences (Putti et al., 2015).

By measuring the content of photosynthetic pigments (chlorophylls (a + b) and carotenes) in the leaves of the adapted in vitro plants, there were no statistical differences between the treatments tested (Figure 1). For these two traits, an adult plant sample was added in the study and compared with the content measured in the two treatments. The chlorophyll a + b content (Figure 1b) was higher (51 x 10^{-4} mg 0.5 g^{-1}) in the adult plant as expected, however, the treatment with MTW maintained values above the control, with 47 x10^{-4} mg 0.5 g^{-1} and 34 x10^{-4} mg 0.5 g^{-1}, respectively. Carotenes content (Figure 1d) was higher (3 mg 0.5 g^{-1}) in the adult plant repeating the chlorophyll a + b result, however, the values of both treatments were very close, with 2 mg 0.5 g^{-1} and 1 mg 0.5 g^{-1} in NMTW and MTW, respectively. Chlorophyll content was increased in response to MTW irrigation, also presenting 29%, 11% and 15% higher protein content, alpha-amylase and protease activities, respectively, in relation to the control in turnip plants (Haq et al., 2016). The results showed that irrigation with MTW has potential to improve turnip germination, seedling growth and enzymatic activities, and this study is also extensible to other plants and crops for improved germination and growth.

The favorable results obtained in the development of *Spatholglottis plicata in vitro* plants during the acclimatization process using MTW in irrigation corroborates the results obtained by several authors (Rodríguez,1998; Botta, 2000; Dubois et al., 2001). The use of MTW in crop irrigation promotes variation in physical, chemical and biological fluids properties (Rodríguez et al., 1999; Maffei, 2014; Surendran et al., 2016), which generates among other issues an increased permeability of biological membranes. This change contributes to a better nutrient uptake (Rodríguez, 1998; Aguilera and Martin, 2016; Surendran et al., 2016), and hence the plant develops and increases the efficiency of the metabolic processes occurring in it.

In the acclimatization stage, Alemán et al. (2014) found an increased cuticular wax content around the stomata and development of xylem vessel thickness. These changes may indicate that the metabolism of the treated plants ranges probably due to an increase in the conductance and size of the ion channels in their cell membranes and other pores induced by the action of this magnetic field. As a result, there is an increase in nutrient uptake, cell metabolism and plant growth as observed in our study.

The treatment with MTW in the orchid species evaluated promoted a higher plant height, a greater number of roots, a higher photosynthetic pigments content, and a larger leaf area with a lower number of leaves, hence promoting a greater survival and adaptation of these plants to the new cultivation conditions. Overall, these traits allow a better capacity and efficiency of the plants to carry out the processes of photosynthesis, transpiration, breathing, nutrition and thereby further improve their ability to adapt to the new *ex vitro* conditions.

Conclusion

The use of magnetically treated water favored the survival percentage in more than 85% of the in vitro orchids under acclimatization stage. This percentage is satisfactory when compared to commercial propagation.

Irrigation with magnetically treated water had a stimulatory effect on the development of the acclimated plants for all traits analyzed associated to the higher photosynthetic pigments content.

Irrigation with magnetically treated water has been established as an excellent technique in improving the *in vitro* micropropagation process.

Conflict of Interest: All authors declare no conflict of interest.

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