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The aclimatization of plants of *Ananas comosus* MERR var. MD-2 is stimulated with irrigation with magnetically treated water

A aclimatização de plântulas de *Ananas comosus* MERR var. MD-2 é estimulada com a irrigação com água tratada magneticamente

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Abstract

Several researches confirm the existence of a high relationship between the magnetic field and many biological processes at cellular scale. There are several methodologies of micro-propagation, however, the obtained results in the acclimatization phase, are not yet desired. The objective of the present study was to characterize the quality of the stomata of *vitroplants* of pineapple during the acclimatization phase and to evaluate the influence of irrigation with magnetically treated water (MTW) six months after the acclimatization. The magnetizer employed had an induction level of 50-70 mT. When evaluating the results, it was observed that the stomata conserve its anatomy while increasing the stomata and stomatal opening area values, with $64x10^3$ stomata mm^{-2} in the treated pineapple *vitroplants* compared to the untreated ones, that presented $61.6x10^3$ stomata mm^{-2}; as well as, also presented 3.06 μm^2 of stomatal opening area, in relation to the untreated ones that presented 2 μm^2. These results show the ease of treatment and the potential of MTW technology as an effective management via in the acclimatization areas of plants produced "*in vitro*".

Keywords: stomatal density, stomatal opening area, pineapple, *in vitro* production, magnetic field, *ex vitro*

Resumo

Várias pesquisas confirmam a existência de uma relação estreita entre o campo magnético e muitos processos biológicos a escala celular. Existem diversas metodologias de micro-propagação, entretanto, os resultados obtidos na fase de aclimatização, ainda não são os desejados. O objetivo do nosso trabalho foi caracterizar a qualidade dos estomas de *vitroplantas* de abacaxi na fase de aclimatização e avaliar a influência da irrigação com água tratada magneticamente (ATM) após 6 meses de aclimatizadas. O magnetizador empregado tinha um nível de indução de 50-70 mT. Ao avaliar os resultados, observou-se que os estomas conservam sua anatomia ao mesmo tempo que incrementaram os valores de densidade estomática e de área da abertura estomática com $64x10^3$ estomas mm^{-2} nas *vitroplantas* de abacaxi tratadas comparadas com as não tratadas que apresentaram $61.6x10^3$ estomas mm^{-2}; assim como, também apresentaram 3.06 μm^2 de área de abertura estomática, em relação as não tratadas que apresentaram 2 μm^2. Estes resultados mostram a facilidade do tratamento e o potencial da tecnologia de ATM como tática eficaz de manejo nas áreas de aclimatização de plantas produzidas "*in vitro*".

Palavras-chave: densidade estomática, área de abertura estomática, abacaxi, produção *in vitro*, campo magnético*, ex vitro*

Introduction

Pineapple (*Ananas comosus* (L.) Merr.) originates from South America, being a very produced crop in many countries, in particular Gold "Extra Sweet" MD-2 cultivar, that due to its content of soluble solids, aroma and color has been preferred in major world markets. The variety MD-2, also recognized as Yellow or Gold, is a cultivar obtained from the hybrid of PRI 581184 x PRI 59443 (RODRÍGUEZ et al., 2016).

The widespread consumption of pineapple has forced the entire production chain to constantly seek new techniques that allow greater productivity in the field. The micro-propagation *in vitro*, which allows a large volume of plants to be obtained from small propagules (meristematic tissue or meristems) that after *ex vitro* adaptation, constitute a new source of production (GONZÁLEZ-OLMEDO et al., 2005; RODRÍGUEZ et al*.,* 2016).

The conditions of *in vitro* culture promote an environment with a high relative humidity, low light intensity, constant temperature, scarce gas exchange and a medium with abundance in organic compounds, especially sucrose. These conditions cause changes in plants morphology and physiology, which make them differ from those that grow under field conditions (AGRAMONTE, 1998).

One of the factors to be considered during the acclimatization phase is the relative humidity, which is very high during *in vitro* crop, and due to the lack of control of perspiration that the plants have in the environment, a gradual change of the high relative humidity during adaptation is good. If this process of gradual adaptation is not carried out the plants do not survive, so, the development of a good stomatal apparatus can help the *vitroplants* during the adaptation process (ORTEGA, RÓDEZ, 1986; PÉREZ, 1997).

The acclimatization efficiency is transcendental in the commercial propagation, this process will determine the final quality of plants (PÉREZ, 1997). The use of effective acclimatization techniques aimed at gradually lowering humidity, more luminosity, leads to greater plant growth, as part of the micro-propagation process (AGRAMONTE, 1998; RODRÍGUEZ et al*.,* 2016).

In the process of producing propagules of pineapple *vitroplants*, excellent micro-propagation protocols have been developed, such as methodologies that employ temporal immersion systems (GONZÁLEZ-OLMEDO et al., 2005). These allow excellent results in terms of the number of *vitroplants* obtained from an initial propagule; however, the obtained results during acclimatization

are still inefficient (PÉREZ, 1997). In this context, the objective of the present work was to characterize the stomata of *vitroplants* of pineapple during the acclimatization phase, and to evaluate the influence of the irrigation with magnetically treated water (MTW) after six months of acclimatization.

Material and methods

The studies were carried out at the National Center for Applied Electromagnetism (CNEA) of the Universidad de Oriente (UO). MD-2 pineapple *vitroplants*, produced under *in vitro* conditions and soon acclimatized in a greenhouse were used, maintaining a relative humidity between 60-80% and internal temperature of 27-31° C. The lighting was controlled using a sombrite screen that allowed the passage of 30% of the luminosity. As substrate a mixture of soil and organic matter in a ratio of 3:1 (v.v), respectively was used.

The evaluations were carried out on *vitroplants* at seven days, two months and six months of acclimatization. The influence of magnetically treated water on the variable stomatal opening area (SOA) and stomata density (SD) was also evaluated for the 6-month pineapple *vitroplants*.

The treatment with magnetically treated water was carried out with a magnetizing of permanent magnets with a magnetic induction of 50 - 70 mT, calibrated in the laboratory of the CNEA with a Soviet Microwebermeter 192041, of relative error less than 5%, in an Equipment of Nuclear Magnetic Resonance and with a Tesla 410 Gaussmeter type of the company Lakeshore. The calibration with the three appliances demonstrated a high repeatability among the three methods used. The magnetizer was placed in the irrigation system only six months after planting the pineapple seedlings in the greenhouse.

From digital photographs taken through an inverted phase microscope, Model 37XB, the structure of the stomata was analyzed (Figure 1). The SD was evaluated by measuring the number of stomata in the field area observed and the SOA following the methodology of ORTEGA and RÓDEZ (1986). In each treatment 10 plants were used, the selected leaves were the second and third leaves pair. Afterwards, the choice of leaves was applied to epidermal impressions.

A completely randomized design with three replications was used. Statistical analysis was based on an analysis of variance of double classification to 95% of significance, using "STATGRAPHICS Plus" program (STATISTICAL GRAPHICS CROP, 2000).

Results and discussion

In vitro plant acclimatization remains a bottleneck faced by most *in vitro* plant propagation systems (PÉREZ, 1997). In this acclimatization phase the main objective is to obtain the maximum survival percentage of plants, and for this, the improvement in the quality and the plants adaptation improvement is necessary. The magnetic treatment in the irrigation water, although it is used in the plant development stimulation (MÉNDEZ et al., 2005; BOIX et al., 2008; AGUILERA, MARTÍN, 2016; BOIX et al., 2018), numerous beneficial effects have been reported, however, during the acclimatization phase *in vitro* cultures has been little used (DUBOIS et al., 2004; AGUILERA et al., 2018).

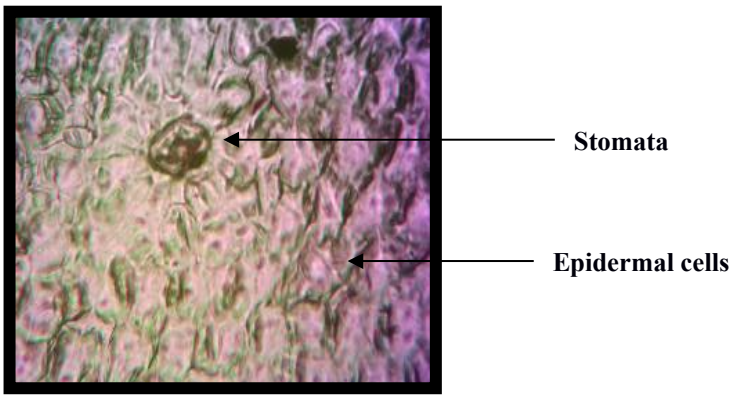

Figure 1. Microphotography of the stomata of *in vitro* plants of *Ananas comosus* during the acclimatization phase. (Increase 25x25).

When evaluating the results, it was observed that for all plants, irrespective of acclimatization time, the stomata conserve their anatomy with their stomatal pore, occlusive cells and typical epidermal cells (Figure 1). The pineapple plants have modifications of the xeromorphic type, adaptability that allows to tolerate drought stress. The stomata in these plants appear mainly in the abaxial part of the leaf, in epidermal chambers, which coincides with the results shown above. The precise structure of the stomatal apparatus can vary considerably from one species to another; and a precise feature of guiding cells is that they are thickened and may be up to 5 mm thick, in contrast to a typical epidermal cell that normally are between 1 to 2 μm. The stomata of the acclimatized seedlings, maintains this characteristic (Figure 1).

After checking that the leaves structures of the *vitroplants* were able to perform their functions, the behavior of the SOA of *vitroplants* was evaluated over time (Figure 2). At the Figure 2 it is observed that with 7 days the adapted plants showed their maximum value for this characteristic and over time this variable was decreasing. When comparing the introduction of the magnetized water treatment at 6 months, it is observed that the treated plants presented 3.06 μm^2 of stomatal opening area, in relation to 2 μm^2 of those not treated, although the differences are not significant between the two treatments.

The magnetic fields promote morphological and physiological changes in many plant species of greater agronomic interest (DUBOIS et al., 2004; AGUILERA et al., 2018), so that the increase in SEA can be related with this phenomenon. The stomatal opening and closing, among other factors, is related to the accumulation of osmotically active solutes in the guiding cells that causes the accumulation of water, an increase in the turgidity pressure finally promotes the stomata opening, favored by the magnetic treatment, as is observed in Figure 2.

SD is one of the parameters to consider when performing a morpho-anatomical study of the leaves, allow knowing the relation between the number of stomata by epidermal cells and area respectively. In Figure 2, the mean values of SD evaluations in acclimated plants at seven days, two and six months are shown. The obtained results in the application of magnetically treated water are also shown in Figure 2. For this variable it was obtained a linear increase in the values of the initial (seven days after the beginning of acclimatization with $16.1x10^3$ stomata mm^{-2}) and end evaluation (six months after the start of acclimatization with $61.6x10^3$ stomata mm^{-2}), and when the magnetic treatment of irrigation water was added, these values were increased ($64.0x10^3$ stomata mm^{-2}), even though statistical differences were not obtained when comparing the obtained values in both

treatments at 6 months. These results were similar to those obtained by DUBOIS et al. (2004), when acclimatizing *vitroplants* of coffee (*Coffea arabica* L.). The results of DUBOIS et al. (2004) and those obtained in this work show that, because there is a greater number of stomata per surface, they plays a more active role in gas exchange regulation of the plants, allowing an improvement in the development and as a result a higher survival rate during the acclimatization phase.

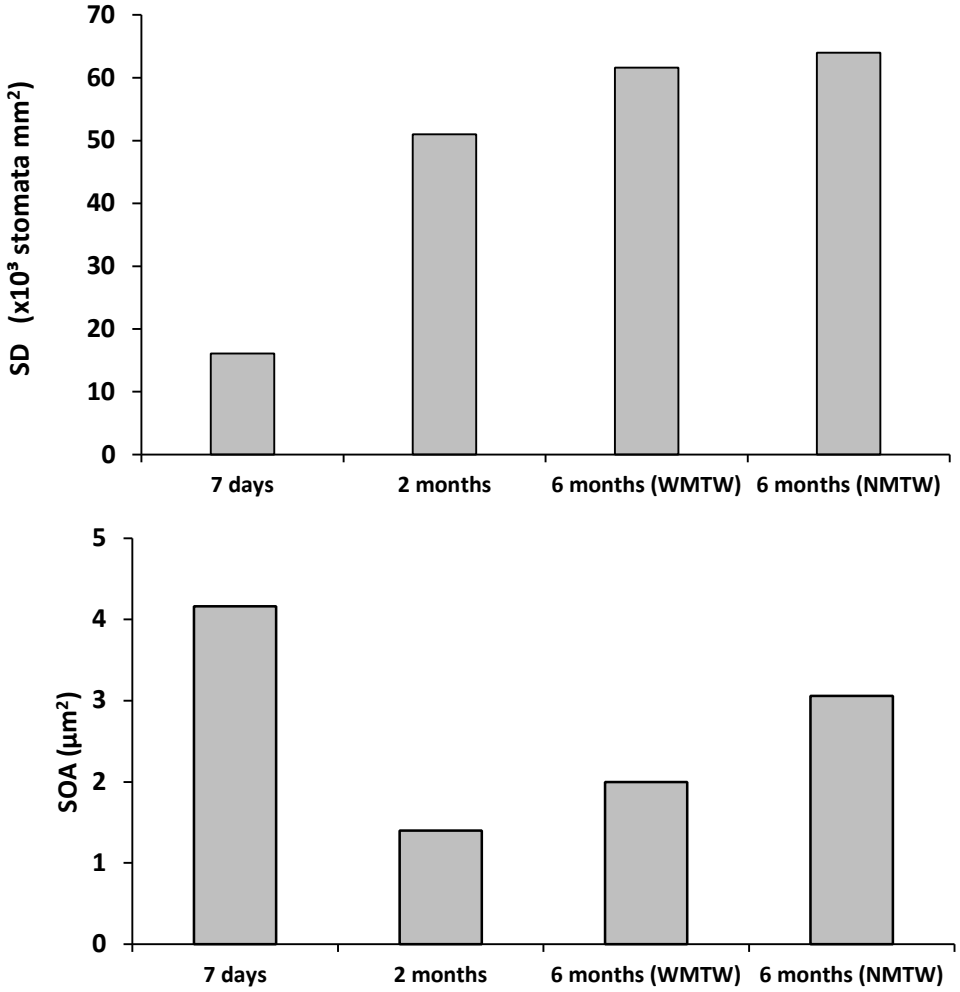

Figure 2. Averages values obtained when evaluating *vitroplants* of MD-2 variety pineapple for the stomata density (SD) and stomatal opening area (SOA) characteristics, measured over three different dates (seven days, two and six months). In the X-axis, WMTW and NMTW represent the treatment with magnetically treated water and the absence thereof, respectively.

The MTW has been used in the physiological recovery and in the hardening of *vitroplants*, however, there is a variable response to certain magnetic inductions that needs to be adequate to allow the maximum development of the *vitroplants* in the acclimatization stage (ALEMÁN et al., 2014a, b; MAFFEI, 2014). AGUILERA et al. (2018) when acclimatizing *vitroplants* of *Spathoglottis plicata* found that the magnetic treatment of irrigation water allowed an increase of 11.43% of the survival rate of orchid *vitroplants*. When measuring the plant height and the number

of leaves during the evaluated period of 126 DAT, AGUILERA et al. (2018) observed that only the plant height showed significant differences at 5% of significance by the F test, with increments that were evident from the 84 DAT in favor of the MTW treatment, although without statistical significance, and in the last two epochs (105 and 126 DAT), these differences were more notable and verified by the statistical test, evidencing the positive effect that this treatment has during the acclimatization phase of *vitroplants* with respect to *ex vitro* conditions, a greater height of the plant, a greater leaf area and number of roots, as well as a higher content of a + b chlorophyll and carotenes was also obtained.

On the other hand, RAPÔSO et al. (2014), using irrigation water with treatment of 0.12 mT, stimulated the growth of *Adenathera pavoniva*. These researchers explained that the movement of water through the lipid bilayer through the pores forming the aquaporins could be increased. Anything that could allow an increase in water content, accompanied by turgor and cell stretching could have as a consequence the stimulation in the stomatal opening process and transpiration, as physiological events of great importance for plants metabolism and survival. Thus allowing a better efficiency of these processes (photosynthesis, perspiration, respiration, nutrition) and thereby improves its ability to adapt to the new *ex vitro* conditions.

Conclusions

The *in vitro* multiplication process did not affect the stomata of the pineapple *vitroplants*, variety MD-2, showing, after the period of normal multiplication, of the anatomy with its guard and epidermal cells. The stomatal opening area and the stomata density throughout the evaluated period showed increases which were more notable when the magnetic treatment was added to the irrigation water applied at six months after the beginning of the acclimatization phase, but without significant statistical differences. The incorporation of magnetic treatment in the irrigation water in systems of adaptation of *vitroplants* is an important tool that could verify the increase of the rate of survival and development of adapted plants in these conditions, representing a valuable management strategy.

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