Australian Journal of Crop Science AJCS
AJCS 8(8):1160-1167 (2014) ISSN:1835-2707

Photosynthesis and leaf anatomy of *Anthurium* cv. Rubi plantlets cultured *in vitro* under different silicon (Si) concentrations

Gabrielen de Maria Gomes Dias[1*], Joyce Dória Rodrigues Soares[1], Moacir Pasqual[1], Renata Alves Lara Silva[1], Luiz Carlos de Almeida Rodrigues[2], Fabricio José Pereira[2] Evaristo Mauro de Castro[2]

[1]Federal University of Lavras (UFLA), Departamento of Agriculture, Plant Tissue Culture Laboratory, Postal Office 3037, 37200-000, Lavras- MG, Brazil
[2]Federal University of Lavras (UFLA), Departamento of Biology, Postal Office 3037, 37200-000, Lavras- MG, Brazil

***Corresponding author: gabriellen@gmail.com**

Abstract

The silicon can induce beneficial changes in plants, such as the further development of tissues and increased photosynthetic rate. Thus, studies on the anatomical changes resulting from *in vitro* culture are key to better understanding the development of micropropagated plants. Therefore, this study was undertaken to evaluate the morphological and physiological differences in plants with the use of silicon added to the medium for the *in vitro* culture of *Anthurium adreaenum* cv. Rubi. Nodal segments of seedlings were established *in vitro* and inoculated in Pierik medium supplemented with 30 g L^{-1} sucrose and solidified with 1.8 g L^{-1} Phytagel™. Different concentrations of sodium silicate (Na_2SiO_3) (0.0, 0.5, 1.0 and 2.0 mg L^{-1}) were added to the medium. The experimental design was completely randomized with 30 repetitions. The segments were maintained for 100 days in a growth chamber under controlled conditions and evaluated anatomically and scanning electron microscopy (ultrastructurally) and for their photosynthetic capacities. Medium containing 1.0 mg L^{-1} sodium silicate promoted the development of higher stomatal densities on the sheets. For the polar (31.38 µm) and equatorial (31.33 µm) diameter of the stomata of the abaxial leaf, the highest averages occurred in the treatment with 2.0 mg L^{-1}. Greater relative polar and equatorial diameters were estimated with a peak concentration of 1.2 mg L^{-1}. The increase in the sodium silicate concentration led to thinning of the abaxial and adaxial epidermis. The thickness of the central rib had a sharp decrease up to 1.3 mg L^{-1}. For the mesophyll, the control displayed a higher thickness, whereas the addition of sodium silicate to the culture medium promoted a decrease. Seedlings grown in sodium silicate displayed significant differences, with increased photosynthetic and transpiration rates, stomatal conductance and internal CO_2 concentrations. As for the ratio between the internal and external concentrations of CO_2, no significant differences were observed. The addition of sodium silicate resulted in increased epicuticular wax deposition and the formation of structures reserved for depositing calcium. Therefore, under *in vitro* conditions, the addition of sodium silicate to the culture medium affected the photosynthesis and leaf anatomy of *A. andraeanum* cv. Rubi, developing anatomical and physiological characteristics that contributed to the survival *ex vitro*.

Keywords: *Anthurium andraeanum* cv. Rubi; Ornamental; Silicate; Structural changes; Stomata.
Abbreviations: FAA_formaldehyde - glacial acetic acid - 70% ethanol; DE_equatorial diameter; DP_polar diameter; DE/DP_equatorial diameter ratio; Ep ada_adaxial epidermis; Msf_mesophyll; Ep aba_abaxial epidermis; Na_2SiO_3_ sodium silicate; CRD_ completely randomized design.

Introduction

In Brazil, the commercial production of *Anthurium* species previously originated from seedlings produced by sexual propagation. Plants grown in this manner produce flowers with a great variability of colors, shapes and sizes of sheaths, making it difficult to harvest flowers of the same type. The production of domestic cultivars resulting from research undertaken by the IAC (Agronomic Institute of Campinas), combined with *in vitro* propagation techniques, is reflected in the improvement of production quality, developing flowers within the desired standards for consumers (Dias et al., 2012). Currently, most varieties of *Anthurium* available for sale as potted plants in the international market, are produced by tissue culture (Maira et al., 2010). The use of this technique has been suggested as an alternative to increase the production of *Anthurium* (Jahan et al., 2009). During the last years, the micropropagation of *Anthurium* has evolved from a purely scientific technique to an applied tool for plant production. Despite the high quality reached for plant production, there are important points to be enhanced on the efficiency of micropropagation techniques. There is also a significant amount of genotypes in which this technique is not succeeded on inducing the plant regeneration, and in some materials, the *in vitro* development is too slow and inconsistent so that it can be used in large-scale propagation (Castro et al. 2012). Thus, tissue culture techniques have been employed for the rapid clonal propagation of new genotypes of *Anthurium* species. In this context, the production of large amounts of these plants has been enhanced by *in vitro* culture because spreading by traditional methods, such as cutting clump division, leads to only a few units obtained annually (Tombolato et al., 2004). Various changes in the leaf structures of plants grown *in vitro* have been reported, such as an increased leaf size, stomatal density, stomata reduction control, the amount of wax

deposited and the mesophyll thickness by increasing the proportion of intercellular spaces (Khan et al., 2003; Hazarika, 2006). According to Santana et al. (2008), the intensities of these anatomical changes are quite variable depending on the characteristics of each species, and its quantification may help to improve the growing conditions for each plant group. Leaf anatomy may contribute to our understanding of the physiological conditions and structural changes of micropropagated plants, providing information applicable to the plants in adapting to the new environment *ex vitro* (Marin, 2003). It has been shown that silicon (Si) is related to increased chlorophyll content and may lead to improvement on plant metabolism. This element also promoted increased tolerance to environmental stress like heat, cold, and drought, reducing the imbalance of nutrients and toxic metals in the plant, strengthening the cell walls of plants and increasing the resistance to pathogens and pests (Epstein, 2001; Ahmed et al., 2013). The addition of silicon to the culture medium for plants can be beneficial because of the increased production of hemicellulose and lignin, providing greater resistance to the cell wall. Such modifications increase the survival rate of plants during acclimatization (Asmar et al., 2011). The direct effects of silicon are accompanied by various indirect effects, including increases in the photosynthetic capacity, reduced rates of transpiratory, plant growth and increased cellular mechanical resistance (Asmar et al., 2013). Given the lack of comparative studies on different species of *Anthurium*, work in this area is of utmost importance, in order to distinguish these species and contributing to future research. For a more efficient micropropagation technique of *Anthurium*, the addition of Si to the culture medium may lead to more vigorous plantlets. Si may contribute to the higher plant quality, because its accumulation in the leaves allows protection to plants, increased photosynthetic capacity, reduction of water loss and also promotes faster growth, these desirable characteristics in the acclimatization of micropropagated plants process. Thus, this study aimed to measure the possible changes in the leaf tissues of *Anthurium andraeanum* cv. Rubi to identify anatomical and ultrastructural changes of photosynthetic seedlings grown *in vitro* in the presence of sodium silicate at different concentrations.

Results and discussion

Anatomical Analysis and Scanning electron microscopy

The leaves have stomata flanked on each side by two subsidiary cells parallel to larger stomatal axis (Fig 1A and B). Stomata showing two subsidiary cells displayed parallel to the larger stomatal axis are often classified as type brachyparacytic type (Castro et al., 2009). So, *A. andraeanum* stomata may be classified as paracytic type. Stomatal types in Araceae are very diverse and some species may show anomocytic, actinocytic, paracytic, and cyclocytic stomata (Wang and Zhao, 2002). Therefore, the brachypracytic stomata described by *A. andraeanum* may be an important characteristic for correct identification and was also reported by Mantovani et al. (2010) for some *Anthurium* species. Stomata guard cells showed a large number of chloroplasts and the usual bean-like shape (Fig. 1A). Despite the chloroplasts in stomatal guard cells is a very common anatomical characteristic (Castro et al., 2009) it was not highlighted by previous works in *Anthurium* anatomy (Wang and Zhao, 2002; Mantovani et al., 2010). Anticlinal cell walls are deeply sinuous in epidermal cells (Fig. 1A-D). Araceae leaves may show straight to very sinuous anticlinal cell walls in epidermal cells but a given species often show just one type (Keating, 2003). In nine Brazilian *Anthurium* species, Matovani et al. (2010) described from straight to undulated anticlinal cell walls. Therefore, the very sinuous anticlinal cell walls from *A. andraeanum* may be an important anatomical trait. The stomata are distributed only on the abaxial surface of leaves, classifying the se organs as hypostomatous (Fig 1). Hypostomatous leaves were also described in another *Anthurium* species by Mantovani et al. (2010) and Saito and Lima (2009). A more significant deposition of epicuticular wax on the adaxial epidermal surface of *A. andraeanum* subjected to treatment with sodium silicate (Fig 2B, C, D) was observed compared to the control (Fig 2A). Wax deposition has also been observed during the cultivation of strawberries using sodium silicate by Braga et al. (2009). Second, Mohammadian et al. (2007) suggest that waxes may decrease the temperature of the leaves, reducing transpiration, which is closely linked to photosynthesis. Increased cuticle deposition is a common response to water deficiency and is important to water-limited conditions (Kosma et al., 2009). Therefore, reduced transpiration and tolerance to water deficit are both important to *A. andraeanum* adaptation to *ex vitro* conditions and desirable characteristics to its micropropagation. Stomata with larger polar and equatorial diameters occurred in the treatment with 2.0 mg L^{-1} sodium silicate. However, larger polar and equatorial ratio were estimated with a peak concentration of 1.2 mg L^{-1} sodium silicate. As for the stomatal density, the medium containing 1.0 mg L^{-1} sodium silicate showed the highest value for this trait compared to other treatments; however, there was actually a decrease with a concentration of 2.0 mg L^{-1} (Fig 3). Khan et al. (2002) reported that the polar and equatorial diameter ratio (DP/DE) is associated with guard cells and that it constitutes an important characteristic feature of the stomata, which is due to the fact that an elliptical shape (higher DP/DE) is characteristic of more functional stomata, whereas a rounded shape (smaller DP/DE) is associated with stomata that do not have appropriate function. However, the type and condition of cultivation may modify these results. Elliptical stomata may lead to a higher CO_2 uptake, leading to more photosynthetic potential whereas, this stomatal shape shows reduced transpiration rate (Castro et al., 2009). Thus, in this work, the stomata may be more functional in the presence of sodium silicate and this is important to plant tolerance to water deficiency conditions as on the *ex vitro* environment. The mesophyll of *A. andraeanum* is homogeneous, showing no differentiation between palisade and spongy parenchyma, which was observed in all treatments in this study (Fig 4). Mantovani et al. (2010) when working with nine *Anthurium* species reported dorsiventral leaves to all species. Keating et al. (2003) also described dorsiventral leaves to several Araceae species. However, as *A. andraeanum* showed homogenous mesophyll structure, it may be an important characteristic of this species differing to other genera and species among Araceae. For the mesophyll, the control showed increased thickness (Fig 4A and 5), indicating that the addition of sodium silicate to the culture medium promoted a decrease in the thickness (Fig 4B, C, D). Plant cell expansion is related to the cell turgor pressure and cell wall strength (Dupy et al., 2010). Plant cells can accumulate silicon on cell walls (Currie and Perry, 2007) increasing its strength. Therefore, the lower thickness of the leaf mesophyll of the silicon treated plants may be related to a higher deposition of this element in the cell walls of mesophyll cells, reducing the coefficient of extensibility and producing shorter

Table 1. Leaf gas exchanges of *Anthurium andraeanum* cv. Rubi plantlets cultured in the presence of different concentrations of sodium silicate.

Sodium silicate (mg L^{-1})	A	C_i	E	g_s	C_i/C_a
Control	1.37^{a}	263.59^{b}	0.35^{b}	0.02^{b}	1.29^{a}
0,5	0.86^{b}	266.01^{a}	0.41^{a}	0.03^{a}	1.29^{a}
1,0	1.47^{a}	99.06^{d}	0.10^{c}	0.01^{b}	1.04^{a}
2,0	0.84^{b}	202.09^{c}	0.24^{c}	0.01^{b}	1.56^{a}

A= net photosynthesis (µmol CO_2 m^{-2} s^{-1}), Ci = internal carbon (µmol CO_2), E = transpiration rate (mmol H_2O m^{-2} s^{-1}), gs = stomatal conductance (mmol H_2O m^{-2} s^{-1}), and Ci/Ca ratio = internal and atmospheric carbon ratio. Means followed by the same letter in columns do not differ by the Scott - Knott test at 5% probability.

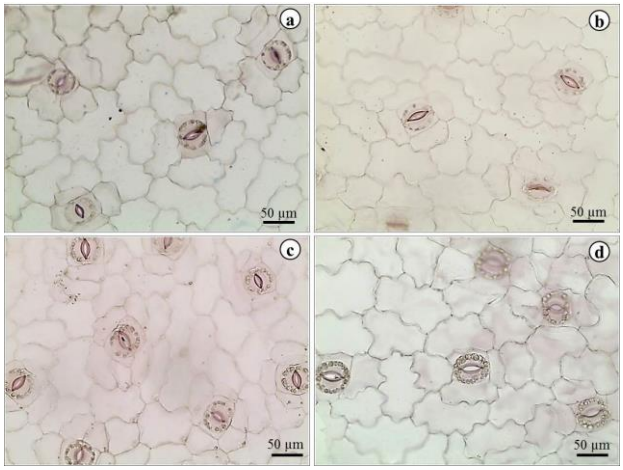

Fig 1. Paradermic sections displaying stomata on the abaxial surface of *Anthurium andraeanum* cv. Rubi leaves grown *in vitro* in culture medium containing various concentrations of sodium silicate. a) Control, b) 0.5 mg L^{-1}, c) 1.0 mg L^{-1}, d) 2.0 mg L^{-1}.

cells. Alves et al. (2001) working with hybrid *Trandescantia*, reported lower thickness of the mesophyll on silicon-exposed plants, possibly due to a reduction in intercellular spaces. However, this reduction of leaf mesophyll has not been observed in any work performed with silicon-exposed plant grown *in vitro* (Braga et al., 2009; Asmar et al., 2011; Soares et al., 2012; Asmar et al., 2013). As noted in Figure 5, increased concentration of sodium silicate lead to reduced thickness of both abaxial and adaxial epidermis. The thickness of the midrib also displayed a marked decrease with increasing concentrations of sodium silicate. This decrease may be due to the addition of sodium silicate to the culture medium, which is in agreement with Nwugo and Huerta (2008), who stated that the accumulation of silicon makes the leaf more rigid and straighter with a higher capacity for the interception of light, thereby increasing the efficiency of photosynthesis. In addition, as discussed for the leaf mesophyll, shorter cells may be produced due to stronger cell walls, reducing cell expansion (Currie and Perry, 2007; Dupy et al., 2010). The midrib of *A. andraeanum* shows one-seriated epidermis on both adaxial and abaxial sides of leaves. The adaxial side of the midrib is concave and the abaxial side shows a convex structure. There is only one collateral vascular bundle showing phloem to the abaxial side and xylem to adaxial side. The vascular bundle is surrounded by ground parenchyma and some idioblasts containing crystals may be found (Fig 6). Brazilian *Anthurium* species have variable structure of the midrib, some species may show palisade parenchyma on the midrib and collenchyma on both adaxial and abaxial sides (Mantovani et al., 2010). The midrib in the leaves of Araceae plants is variable in structure and may be symmetrically shaped with a rounded, flattened, concave, or convex surface or both adaxial and abaxial sides (Keating, 2003). The general structure of *A. andraeanum* midrib have some differences compared to previous described Brazilian species and this may be important to correct identification of this species. *Anthurium andraeanum* shows a more prominent midrib on the abaxial surface in the control treatment (Fig 6A). With the addition of sodium silicate to the culture medium, a noticeable change in the midrib size and thickness was evident, making the midrib more symmetrical on both surfaces (Fig 6B, C, D). The silicon-treated plants also reduced the curve angle in the adaxial side, producing more flattened adaxial surfaces in these treatments (Fig 6B, C, D). Therefore, beside the midrib of *A. andraeanum* is slightly different from some Brazilian *Anthurium* species, the environment may change its structure and silicon promotes flattened and thinner midribs in this species. The presence of sodium silicate during the *in vitro* cultivation of the species studied in this work provided structural benefits that led to the plant developing without affecting its ornamental structure or its commercial value. Calcium oxalate crystals were found in leaves from plants grown under all treatments, crystals were represented by druses (star-shaped crystals) and raphides (needle-shaped crystals) (Fig 7). Those crystals were present in the cells from mesophyll and epidermis. This feature has been reported by Mantovani et al. (2010) and Mantovani and Pereira (2005). The occurrence of different types of calcium oxalate crystals has been described for members of the *Araceae* (Keating, 2003). Keating (2002) has demonstrated that two or more types of crystals, can occur simultaneously in the same organ of plants in family *Araceae*, the author also emphasize that druses occur in *Anthurium* species. Because the presence of crystals in plants is listed as a defense mechanism against herbivores (Lucas et al., 2000; Xiang and Chen, 2004) and the crystals regulate the level of calcium in tissues (Volk et al., 2002), calcium oxalate crystals are recognized for their ecological importance. These structures also assist in the distribution of light to the chloroplast with the dissipation of excess light during periods of high radiation intensity (Franceschi, 2001). Additionally, some studies have demonstrated that excess calcium can be stored in the form of

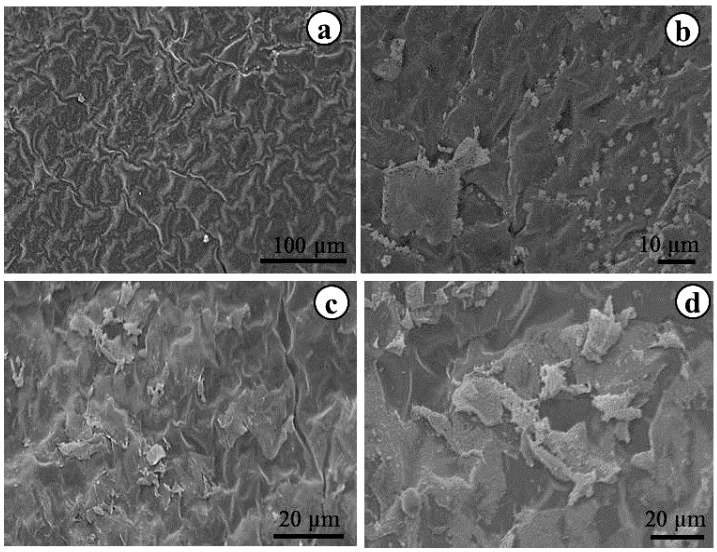

Fig 2. Electron micrographs showing the wax deposition on the adaxial surface of leaves of *Anthurium andraeanum* cv. Rubi grown *in vitro* in culture medium containing following concentrations of sodium silicate: a) Control, b) 0.5 mg L^{-1}, c) 1.0 mg L^{-1}, d) 2.0 mg L^{-1}.

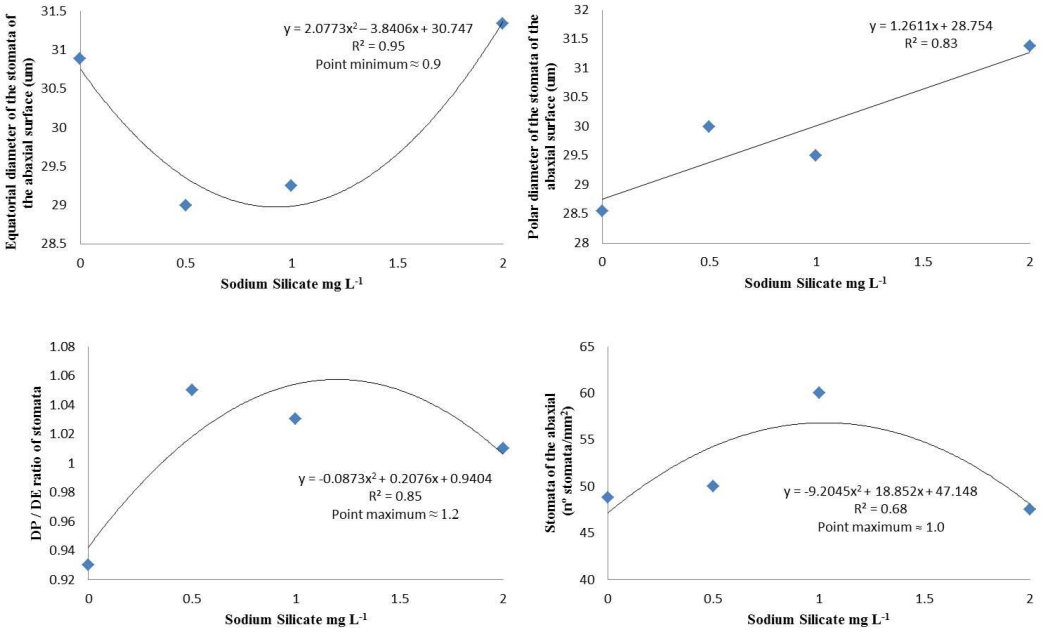

Fig 3. Leaf stomatal characteristics of *Anthurium andraeanum* cv. Rubi plantlets after 100 days of culture with different concentrations of sodium silicate.

calcium oxalate and that this calcium can be remobilized under certain conditions (Volk et al., 2002).

Characteristics of gas exchange

Plantlet grown with different concentrations of sodium silicate showed significant differences in their photosynthetic and transpiration rates, stomatal conductance and internal CO_2 concentrations. However, the ratio between internal and atmospheric concentrations of CO_2 showed no significant differences. The internal CO_2 concentration, transpiration rate and stomatal conductance were increased by the concentration of 0.5 mg L^{-1} sodium silicate, followed by a reduction in the higher silicon concentrations. However, the net photosynthesis data showed variations, were lower means were observed in the 0.5 and 2.0 mg L^{-1} sodium silicate but the 1.0 mg L^{-1} concentration showed data very similar to the control group (Table 1). Photosynthesis corresponds to the basic input energy to plants and is essential for growth, being directly connected to the leaf morphology (Castro et al., 2009). Photosynthesis can vary by the environment of the plant, and the two main environmental limitations to the photosynthetic rate are the availability of CO_2 and radiation (Zhou and Han, 2005). However, *in vitro* grown plants often show little photosynthetic capacity due to the heterotrophic medium providing external carbon sources such as sucrose (Capellades et al., 1991). The poor photosynthesis showed by *in vitro* plants may also be due to lack of functionality of stomata and photosynthetic tissues (Hazarika, 2006). Therefore, modifications in the net photosynthesis of *A. andraeanum* plants have showed variable results because of

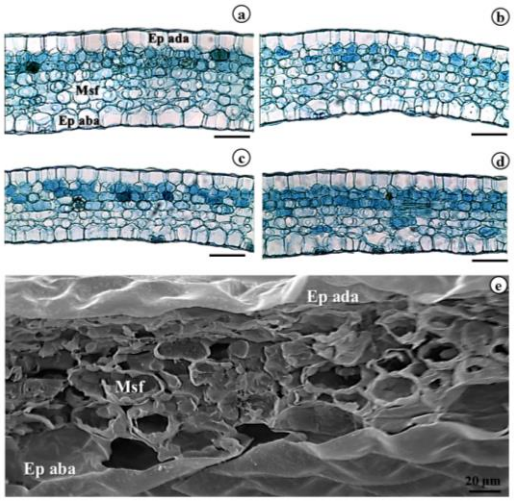

Fig 4. Photomicrographs (a, b, c and e) of transversal sections of *Anthurium andraeanum* cv. Rubi leaves exposed to the following concentrations of sodium silicate. a) Control, b) 0.5 mg L^{-1}, c) 1.0 mg L^{-1}, d) 2.0 mg L^{-1}. Electron micrograph (e) of a transversal section of the leaf from treatment of 0.5 mg L^{-1} of sodium silicate. Ep ada = adaxial epidermis; Msf= mesophyll; Ep aba= abaxial epidermis. Bars = 100 µm (a, b, c and d).

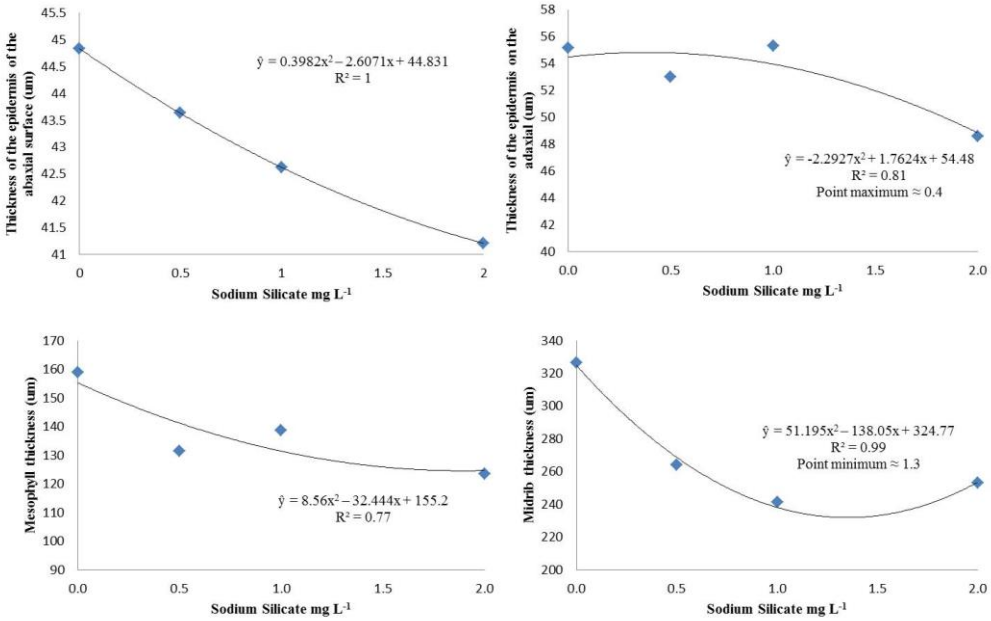

Fig 5. Leaf anatomical characteristics of *Anthurium andraeanum* cv. Rubi plantlets after 100 days of culture with different concentrations of sodium silicate.

the poor developed tissues as well as the heterotrophic culture medium. One of the most important environmental condition that *in vitro* plants face when are further transported to *ex vitro* environment is the lower water availability (Hazarika, 2006). In that condition, must be able to control transpiration and reduce water loss. The reduction of transpiration and stomatal conductance showed by silicon-treated plants may be an important enhancement promoted by higher silicon concentrations because it may lead to more tolerant plants to *ex vitro* environment. Additionally, the changes occurring in the internal structure of the leaves are the determining factors in the acclimatization ability of species (Hanba et al., 2002).These modifications promoted reduced water loss maintaining plant water status; this may be related to the higher epicuticular wax deposition observed in silicon treated plants.

Materials and Methods

Sources of explants

Nodal segments of *Anthurium andraeanum* seedlings established *in vitro* were inoculated in Pierik medium (Pierik, 1976) supplemented with 30 g L^{-1} sucrose and solidified with 1.8 g L^{-1} Phytagel™. Sodium silicate (Na_2SiO_3) was added to the culture medium at concentrations of 0.0, 0.5, 1.0 or 2.0 mg L^{-1}. The pH of the culture medium was adjusted to 5.8, and the medium was then autoclaved at 121°C and 1.2 atm for 20 minutes. Subsequently, the yolk contained in the nodal

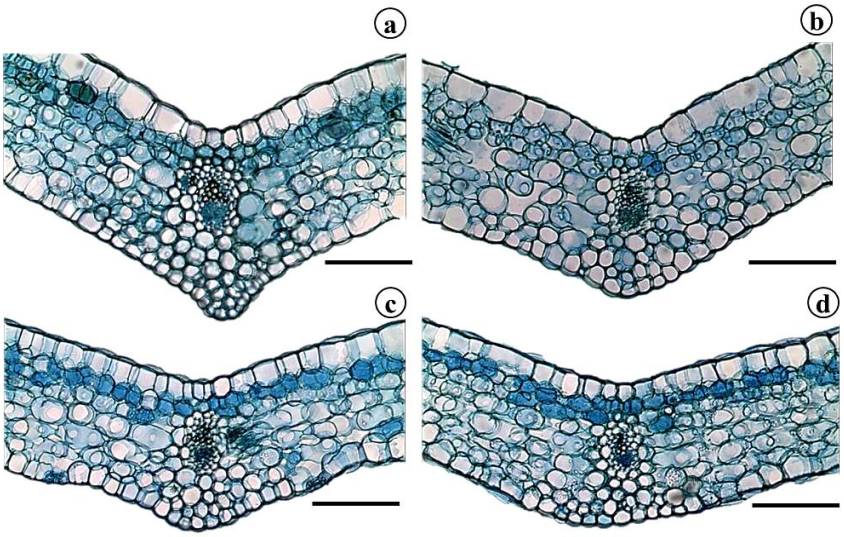

Fig 6. Photomicrographs of transversal sections of *Anthurium andraeanum* cv. Rubi leaves showing the midrib at following sodium silicate concentrations: a) control, b) 0.5 mg L^{-1} c) 1.0 mg L^{-1} d) 2.0 mg L^{-1}. Bars= 100 µm.

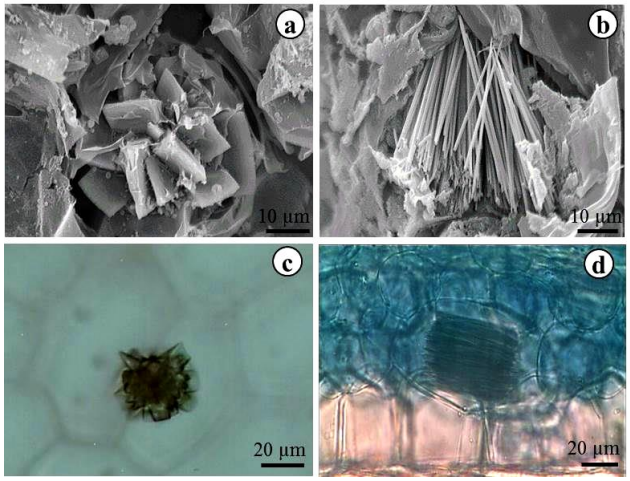

Fig 7. Electron micrographs (a and b) and photomicrographs (c and d) showing calcium oxalate crystals in *Anthurium andraeanum* cv. Rubi *in vitro*. Druses are shown in a and c figures in the treatment supplemented with 2.0 mg L^{-1} sodium silicate. Raphides are shown in b and d figures in the treatment supplemented with 1.0 mg L^{-1} sodium silicate.

segments was inoculated into 400 ml flasks containing 50 ml of culture medium with the respective treatments in a laminar flow hood.

Culture conditions

The vials were maintained in a growth chamber with a photoperiod of 16 hours at 25 ± 2°C and a radiation rate of 52.5 W m^{-2}. After 100 days, we evaluated the parameters described below:

Anatomical features

The middle third of the second fully expanded leaves collected from 4 different plants per treatment were fixed in advance with F.A.A (formaldehyde - glacial acetic acid - 70% ethanol at a ratio of 0.5: 0.5: 9 v^{-1}) (Johansen, 1940) for 72 hours and subsequently stored in 70% ethanol (v / v). The cross sections were obtained using a microtome table-type LPC and sectioned paradermic freehand using a steel blade. The sections were subjected to clarification with sodium hypochlorite (1%-1.25% active chlorine) and triple-rinsed in distilled water for 10 minutes. A safra-blue staining solution (0.1% astra blue and safranin 1% in 7:3 v^{-1}) was used for cross sections, whereas 1% aqueous safranin was used for the paradermic sections (Kraus and Arduin, 1997). Subsequently, the sections were mounted on slides semipermanently. Slides were observed and photographed under an optical microscope (model Olympus BX 60, Olympus, Tokyo, Japan) attached to a Canon A630 digital camera (Canon Inc., Tokyo, Japan). The images were analyzed using the image analysis software UTHSCSA - ImageTool, and five fields per repetition for each variable were evaluated. We evaluated the following characteristics: the epidermal thickness of the abaxial surface, the thickness of the adaxial epidermis and the mesophyll thickness. To characterize the stomata, the stomatal density (number of stomata per mm^2) and polar and equatorial diameters of the stomata were analyzed.

Scanning electron microscopy

Samples from the middle third of four leaves were fixed using the method of Karnovsky (Karnovsky, 1965), post-fixed in osmium tetroxide (OsO_4), dehydrated in increasing acetone solutions (30%, 50%, 70%, 90% and 100%) and then subjected to critical point drying using liquid CO_2 as a

transition (Robards, 1978). Later, they were coated with gold (20 nm) and analyzed by scanning electron microscopy (LEO – EVO) following the protocol of Alves (2004). We analyzed the stomata and wax deposition in the leaf epidermis.

Characteristics of gas exchange

The photosynthetic and transpiration rates of the plants were evaluated using an infrared gas analyzer [(IRGA) model LI - 6400 (Li-COR Biosciences, Lincoln, USA)]. To evaluate these variables, fully expanded leaves on four plants per treatment were selected and evaluated at 10:00 am. Flux density photosynthetic photons were fixed in an appliance chamber to 100 µmol m^{-2} s^{-1}.

Experimental design and statistical analysis

A completely randomized design (CRD) with four treatments, four replications and 5 fields for anatomical analyzes the cross sections and paradermic sections was utilized. For gas exchange analysis four treatments and four replications was performed. Data was submitted to one-way ANOVA and means compared to Scott-Knott test at 5% probability or regression analysis depending on data adjustment. The data were analyzed using the statistical program SISVAR (Ferreira, 2011) along with analysis of variance and data regression.

Conclusions

The addition of sodium silicate to appropriate medium led to the development of leaf tissues, and also the increase in the number of functional stomata of *Anthurium andraeanum* cv. Rubi. The addition of up to 1.0 mg L^{-1} sodium silicate promotes increased rate of photosynthesis rate of seedlings *Anthurium andraeanum* cv. Rubi.

Acknowledgements

We thank the Coordination of Improvement of Higher Education Personnel (CAPES) for funding, the Foundation for Research Support of the State of Minas Gerais (FAPEMIG) and National Council for Scientific and Technological Development (CNPq) for granting the scholarship.

References

Ahmed M, Kamran A, Asif M, Qadeer U, Ahmed ZI, Goyal A (2013) Silicon priming: a potential source to impart abiotic stress tolerance in wheat: A review. Aust J Crop Sci. 7:484-491.

Alves ES, Giusti PM, Domingos M (2001) Anatomic studies on *randescantia* hibrid clone 4430 leaves: changes caused by urban air pollution. Rev Bras Bot. 24: 561-566.

Alves E (2004) Introdução à microscopia eletrônica. Lavras: UFLA/FAEPE: 88

Asmar AS, Pasqual M, Rodrigues FA, Araujo AG, Pio LAS, Silva SO (2011) Fontes de silício no desenvolvimento de plântulas de bananeira 'Maçã' micropropagadas. Cienc Rural. 41:1127-1131.

Asmar SA, Castro EM, Pasqual M, Pereira FJ, Soares JDR (2013) Changes in leaf anatomy and photosynthesis of micropropagated banana plantlets under different silicon sources. Sci Hortic. 161: 328–332.

Braga FT, Nunes CF, Favero AC, Pasqual M, Carvalho JG, Castro EM (2009) Anatomical characteristics of the strawberry seedlings micropropagated using different sources of silicon. Pesqui Agropecu Bras. 44: 128-132.

Capellades M, Lemeur R, Debergh P (1991) Effects of sucrose on starch accumulation and rate of photosynthesis in rosa cultured *in vitro*. Plant Cell Tiss Organ. 25: 21-26.

Castro EM de, Pereira FJ, Paiva R (2009) Histologia vegetal: estrutura e função de órgãos vegetativos. Lavras: UFLA: 234.

Castro ACR de, Terao D, Carvalho ACPP de, Lages V (2012) Antúrio. Brasília, DF: Embrapa, 163 p.Currie HA, Perry CC (2007) Silica in Plants: Biological, Biochemical and Chemical Studies. Ann Bot. 100:1383–1389.

Dias GM, Tombolato AFC, Leme JM, Mosca JL (2012) Pós-colheita. In: Castro ACR, Terao D, Carvalho ACPP, Loges V. Antúrio. Brasília, DF: EMBRAPA: 163.

Dupy L, MacKenzie J, Haseloff J (2010) Coordination of plant cell division and expansion in a simple morphogenetic system. Proc Natl Acad Sci USA. 107: 2711–2716.

Epstein E (2001) Silicon in plants: facts vs concepts. In: Datnoff LE, Snyder GH, Korndörfer GH. Silicon in agriculture. Netherlands: E. Science.

Ferreira DF (2011) SISVAR: A computer statistical analysis system. Ciência Agrotecnologia, Lavras, 35: 1039-1042

Franceschi V (2001) Calcium oxalate in plants. Trends Plant Sci. 6: 361-427.

Hanba YT, Kogami H, Terashima L (2002) The effects of growth irradiance on leaf anatomy and photosynthesis in *Acer* species differing in light demand. Plant Cell Environ. 25: 1021-1030.

Hazarika BN (2006) Morpho-physiological disorders in *in vitro* culture of plants. Sci Hortic. 108: 105-120.

Jahan MT, Islam MR, Khan R, Mamun ANK, Ahmed G, Hakim L (2009) *In vitro* clonal propagation of anthurium (*Anthurium andreanum* L.) using callus culture. Plant Tissue Cult Biotec. 19: 61-69.

Johansen DA (1940) Plant microtechnique. New York: McGraw Hill: 523

Karnovsky M J (1965) A formaldehyde-glutaraldehyde fixative of high osmolality for use in eletron microscopy. J Cell Biol. 27: 137-138.

Keating RC (2002) Anatomy of the monocotyledons: IX Araceae and Acoraceae. Oxford University Press. New York.

Keating RC (2003) Leaf anatomic characters and their value in understanding morphoclines in the Araceae. Bot Rev. 68: 510-523.

Khan PSSV, Kozai T, Nguyen QT, Kubota C, Dhawan V (2002) Growth and net photosynthetic rates of *Eucalyptus tereticornis* Smith under photomixotrophic and various photoautotrophic micropropagation conditions. Plant Cell Tiss Org Cult. 71: 141-146.

Khan PSSV, Kozai T, Nguyen QT, Kubota C, Dhawan V (2003) Growth and water relations of Paulownia fortunei under photomixotrophic and photoautotrophic conditions. Biol. Plant. 46: 161-166

Kosma DK, Bourdenx B, Bernard A, Parsons EP, Lu S, Joubès J, Jenks MA (2009) The Impact of Water Deficiency on Leaf Cuticle Lipids of Arabidopsis. Plant Physiol. 151: 1918-1929.

Kraus JE, Arduin M (1997) Manual básico de métodos em morfologia vegetal. UFRRJ: 198.

Lucas PW, Turner IM, Dominy NJ, Yamashita N (2000) Mechanical defenses to herbivory. Ann Bot. 86: 913-920.

Maira O, Alexander M, Vargas TE (2010) Micropropagation and organogenesis of *Anthurium andraeanum* Lind cv. Rubrun. In: Jain SM, Ochatt SJ. Protocols for *in vitro* propagation of ornamental plants. New York: Human: 3-14 (Methods in Molecular Biology, 589).

Mantovani A, Pereira TE (2005) Comparative anatomy of leaf and spathe of nine species of *Anthurium* (section urospadix; subsection flavescentiviridia) (araceae) and their diagnostic potential for taxonomy. Rodriguesia. 56: 145-160..

Mantovani A, Filartiga LDP, Coelho MAN (2010) Anatomia comparada da folha e espata de espécies de *Anthurium* (Araceae) ocorrentes na Mata Atlântica. Rev Bras Bot. 33: 185-200.

Marin JA (2003) High survival rates during acclimatization of micropropagated fruit tree rootstocks by increasing exposures to low relative humidity. Acta Hortic. 616: 139-142.

Mohammadian MA, Watling JR, Hill RS (2007) The impact of epicuticular wax on gas-exchange and photoinhibition in Leucadendron lanigerum (Proteaceae). Acta Oecologica. 31: 93-101.

Nwugo CC, Huerta AJ (2008) Effects of silicon nutrition on cadmium-uptake, growth andpho to synthesis of rice (*Oryza sativa* L.) seedlings exposed to long-term low level cadmium. Plant Soil. 311: 73-86.

Pierik RLM (1976) *Anthurium andraeanum* Lindl. Plantles produced from callus tissues cultivated *in vitro*. Physiologia Plantarum. 37: 80-82.

Robards AW (1978) An introduction to techniques for scanning electron microscopy of plant cells. In: Hall J L. Electron Microscopy and Cytochemistry of Plant Cells. Elsevier: 343-444.

Santana JRF, Oliveira LM, Paiva R, Resende RKS, Castro EM, Pereira FD (2008) Anatomia foliar de seis espécies de anonáceas cultivadas *in vitro* e em casa de vegetação. Cienc Rural. 38: 2362-2365.

Saito SEM, Lima VFGAP (2009) Estudo anatômico e variação na concentração de idioblastos com ráfides em folhas de Araceae, mantidas sob diferentes condições de luminosidade. Rev Saude- UNG. 3: 1-9.

Soares JDR, Pasqual M, Araujo AG, Castro EM, Pereira FJ, Braga FT (2012) Leaf anatomy of orchids micropropagated with different silicon concentrations. Acta Sci Agron. 34: 413-421.

Tombolato AFC, Furlani PR, Castro CEF, Mathes LAF, Tagliacozzo GMD, Saes LA, Rivas EB, Coutinho LN, Bergamann EC, Leme JM (2004) Antúrio: *Anthurium andraeanum* Lindl. Cultivo Comercial de Plantas Ornamentais. Instituto Agronômico de Campinas: IAC. 61-94.

Volk G, Lynch-holm V, Kostman T, Franceschi VR (2002) The role of druse and raphide calcium oxalate crystals in tissue calcium regulation in *Pistia stratiotes* leaves. Plant Biol. 41: 34-45.

Wang W, Zhao N (2002) Epidermal Characters of Leaves in Araceae. Plant Sci J. 20: 343-349.

Xiang H, Chen J (2004) Interspecific variation of plant traits associated with resistance to herbivory among four species of *Ficus* (Moraceae). Ann Bot. 94: 377-384.

Zhou YM, Han SJ (2005) Photosynthetic response and stomatal behaviour of *Pinus koraiensis* during the fourth year of exposure to elevated CO_2 concentration. Photosynthetica. 43: 445-449.