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Anatomy and micromorphometric analysis of leaf *Catasetum* x *apolloi* Benelli & Grade with addition of potassium silicate under different light sources

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Abstract

The aim of this study was to compare vitroplants *Catasetum* x *apolloi* grown under natural light and artificial light and different concentrations of potassium silicate, providing data on the anatomical differentiation that aids the acclimatization process of this species. Plants from *in vitro* seeding were used; 5 protocorms of approximately 0.5 cm were inoculated into vials with a capacity of 500 mL containing 100 mL of alternative culture medium plus potassium silicate (0.0, 0.5; 1.0 mL L^{-1}), pH adjusted to 5.5 ± 0.5 and gelated with 4GL^{-1} agar before the autoclaving process. Cultures were maintained under natural light (TNE) and artificial light (TAE) for 90 days, and micromorphometric analysis was performed for polar and equatorial diameter, density and stomatal index, blade thickness in the central rib, and secondary veins. Applications in K_2SiO_4 alternative medium provided the following: elongation of the hypodermis, thicker mesophyll, and more prominent midrib; elipptical guard cells; formation of epistomatal chamber; and lower stomatal density and stomatal with lower equatorial and polar diameters. The conditions that favored the acclimatization were lower light intensities and lower potassium silicate doses.

Keywords: leaf anatomy, light, orchids, plants vitro.

Anatomia e análise micromorfométrica foliar de *Catasetum* x *apolloi* Benelli & Grade com adição de silicato de potássio em diferentes fontes de luz

Resumo

O objetivo desse trabalho foi comparar vitroplantas de *Catasetum* x *apolloi* cultivadas sob luz natural e luz artificial e diferentes concentrações de silicato de potássio, fornecendo dados sobre diferenciação anatômica que auxiliem no processo de aclimatação dessa espécie. Utilizou-se plantas provenientes da semeadura *in vitro,* 5 protocormos de aproximadamente 0,5 cm foram inoculados em frascos com capacidade para 500 mL contendo 100 mL de meio de cultura alternativo, acrescido de silicato de potássio (0,0; 0,5; 1,0 mL L^{-1}), pH ajustado para 5,5 ±0,5 e gelificado com 4gL^{-1} de ágar antes do processo de autoclavagem. As culturas foram mantidas sob luz natural (TAA) e luz artificial (TAN) por 90 dias, e feitas análises micromorfométricas (diâmetro polar e equatorial, densidade e índice estomático, espessura do limbo na nervura central e nervuras secundárias). As aplicações de K_2SiO_4 em meio alternativo, propiciaram: alongamento da hipoderme; mesofilo mais espesso e nervura central mais proeminente; células guardas elípticas; formação de câmaras supraestomáticas; menor densidade estomática e estômatos com menores diâmetros equatorial e polar. As condições que podem favorecer a aclimatação são menores intensidades de luz e menores doses de silicato de potássio.

Palavras-chave: anatomia foliar, luminosidade, orquídeas, vitroplantas.

1. Introduction

The Orchidaceae is considered the largest botanical family among species in the scientific world; it has a range between 20,000 and 35,000 species in over 800 genera and thousands of hybrids (Souza and Lorenzi, 2005). This number increases every year, since there are new discoveries (Silva, 2010). Orchids are among the most appreciated ornamental plants for their beauty and exoticism, which add greater commercial value. They are constantly harvested and removed from their natural habitat, whether for private collections or for marketing.

In the Neotropics the family is broadly diversified, especially in the equatorial region, with a great diversity of

species in Colombia, Ecuador, Brazil, and Peru. Brazil has one of the highest diversity of orchids of the America and the world, with approximately 2,433 species and 236 genera, of which 1,620 are endemic to this country (Barros et al., 2015). The Midwest has the lowest representation, with 458 species and 112 genera.

Catasetum Rich. ex Kunth is a genus of 95 species and 7 natural hybrids found in Brazil, of which 28 species are found in Mato Grosso State. Of these, 21 species are found in Mato Grosso State – Amazon (Benelli, 2012).

In nature, the propagation of these species occurs through lateral seedlings, but this process is slow and the productivity is low and insignificant, given the amount of the produced seeds. Millions of seeds are sown in the soil and, when in contact with the mycorrhizae from adult plants of the same species, they associate and germinate (Ramos and Carneiro, 2007).

In comparison to germination under natural conditions for the spread of many species of orchids, *in vitro* seeding is an important alternative, especially for the endangered species, as it is a protocol for production of orchids in short periods of time (Moraes et al., 2009). Several culture media have been formulated based on nutritional requirements of each species, in order to obtain a greater number of germinated plants and their full development.

The mineral elements have been included in the culture media as inorganic salts such as potassium. According to some authors, potassium deficiency in the culture media leads to hyperhydricity and the decrease in phosphate absorption rate (Pasqual, 2001).

Silicon is another nutrient used in *in vitro* seeding protocols, providing benefits to plants by contributing to the structure of the cell wall of roots and leaves. However, this element has no defined metabolic role in plants and, according to Malavolta (2006), its action causes indirect effects, which, together, contribute to higher productivity.

The natural luminosity provides high stomatic density with functional stomata, favoring the plant adaptation to heterotrophic conditions (Araújo et al., 2009). Lee et al. (1988) and Dousseau et al. (2008) suggest that high light intensities provide an increase in the size of the mesophyll cells, greater leaf thickness, as well as more pronounced cell compaction in cultured leaves of *Liquidambar styraciflua L*. These authors also affirm that the low light intensities reduce the cell division, resulting in smaller leaf areas, producing thinner leaves. Several anatomic studies have shown that vegetative organs of vitroplants have poorly differentiated tissues and structures, when compared to plants grown in a greenhouse.

This study aimed to compare vitroplants of *Catasetum x apolloi* grown under natural light and artificial light and different concentrations of potassium silicate, providing data on anatomical plasticity that might assist in the acclimation process of this endemic species from the northern Mato Grosso.

2. Material and Methods

The work was conducted at the University of Mato Grosso State, Campus of Alta Floresta (UNEMAT), in the Laboratory of Cytogenetics and Plant Tissue Culture, and in the Laboratory of Plant Biology, between July 2012 and April 2014.

Alta Floresta is situated in the north of Mato Grosso State and has an invaluable wealth, which consists of an area with immense biodiversity, hydrous potential, and other natural resources in abundance, as well as complex vegetation mosaics (FEMA, 2002).

The municipality is situated in the swath of semidecidual seasonal forests of transition between the Amazon and Cerrado biomes. The regional relief varies from flat to mountainous. The climate is Awi type, according to the Köppen classification, i.e. rainy tropical, reaching a high pluviometric index in the summer, sometimes with averages exceeding 2750 mm, and a dry winter, with prevailing high temperatures. The average annual temperature is around 20 °C (IBGE, 2013).

This study used seeds of the *Catasetum x apolloi Benelli & Grade* species from the Orquidário Alta Florestense, in the Campus of Alta Floresta-MT (Figure 1). Seeds were taken from ripe fruits, stored at 4 °C for about 24 h, and soaked in distilled water and then sterilized. 2ml were inoculated in the alternative culture media consisting of 30 g L^{-1} of commercial sugar, 2 g L^{-1} of fertilizer B & G®, 200 ml L^{-1} of coconut water, and 2 g L^{-1} of activated carbon. They were de-jellied with 4 g L^{-1} of Agar (Rodrigues et al., 2012) and the pH of the culture media was adjusted to 5.5 ± 0.05.

Prior to the addition of the agar, they were distributed into 500 ml glass flasks; 100 ml of culture media were placed in each jar and they were autoclaved at 121 °C under pressure of 1 kg cm^2 for 20 minutes.

The treatments were maintained in light (2,000 lux) for 16 hours a day at a temperature of 27 ± 1 °C for 60 days. The treatments were observed weekly during this period to evaluate the development of protocorms into seedlings.

To carry out studies with seedlings of *C. x apolloi* after 60 days from the germination of seeds *in vitro*, five protocorms of approximately 0.5 cm were inoculated into flasks with the capacity of 500 ml containing 100 ml of alternative culture media. Potassium silicate (0; 0.5; 1.0 U ml^{-1}) was added, and pH was adjusted to 5.5 ± 0.5 and de-jellied with 4 g L^{-1} of agar before the autoclaving process at 121 °C and 1 kg cm^2 for 20 minutes. The cultures were kept under natural light and artificial light for 90 days.

The experimental design was completely randomized (DIC) in a 2 × 3 factorial scheme (2 environments and 3 culture medias), with four repetitions for each treatment. The treatments were: TAE (Treatment in Artificial Environment) at doses 0.0, with 0.5 and 1.0 ml L^{-1} of potassium silicate in alternative media; and TNE (Treatment in Natural Environment) at doses 0.0 with 0.5 and 1.0 ml L^{-1} of potassium silicate in alternative media.

In the artificial environment, in the growth room, the lighting was provided by fluorescent lamps, from special

Figure 1. *Catasetum x apolloi* in their natural habitat.

daylight type (OSRAM® 20W), with average irradiance of 602.27 lux (lumen/m^2), photoperiod of 16 hours and temperature of 22 ± 2 °C. In the natural environment (air-conditioned room with natural light), the following environmental parameters were maintained: maximum and minimum temperatures of 32.05 °C and 23.05 °C, and irradiance levels of 939.21 Lux (lumen/m^2).

At the end of 150 days of cultivation, 10 seedlings of 4 replications per treatment were taken randomly, sectioning 4 leaves of each seedling. The botanic material was fixed in FAA_{50} (formaldehyde, glacial acetic acid, and ethanol 50%, 5: 5: 90 v/v) for 48 hours and placed in 70% ethanol (Johansen, 1940).

Two leaves per replicate were used for the anatomical analysis, in which transverse sections were made in the region of the middle third through the inclusion of samples in synthetic resin. The blocks were transversely cut in a rotary microtome with automatic advance, with the use of steel disposable knives. The cuts, with thickness of 8 mm, were stained with toluidine blue. These were then fixed between slides and coverslipping with Permont metacrilanato Leica® type. The analyzed variables for the transversal sections were the thickness of the blade in the midrib region and secondary vascular bundles, which were measured with the Anati Quant 2® UFV program (Aguiar et al., 2007).

For the epidermal analyses, the Franklin protocol (1947) (Kraus and Arduin, 1997) modified was used; leaf portions were placed in Ependorf tubes with hydrogen peroxide (30 v) and glacial acetic acid in the ratio of 1: 1 and kept in the oven at 50 °C for about 20 hours. After this period, the samples were washed in distilled water and 50% ethanol. The two epidermal surfaces were separated, stained with basic fuchsin (Roeser, 1962), and assembled in glycerine jelly.

The stomatic index (SI) was performed using the formula of Cutter (1986), in which (SI) = [NS/(EC + NS)] ×100sd, NS is the number of stomata, and EC is the number of epidermal cells. The middle third region of one leaf of each repetition was evaluated, and 10 fields were qualified. For studies related to the characterization of the stomata (average number per mm^2, polar and equatorial diameter), the slides were photomicrographed with a 20x objective lens through the image capturer, coupled to the light microscope Leica® DMLB type, and the analyses were made through the program Anati Quant 2® UFV (Aguiar et al., 2007).

Data were subjected to analysis of variance (ANOVA) and the averages were compared by the Tukey test at 5% of probability (Ferreira, 2003).

3. Results and Discussion

In the front view, the stomata in the contact region between the ostiole gave a circular aspect in both environments tested with alternative media without the addition of potassium silicate (Figure 2C, D). It has been observed that vitroplants leaves have less stomata, which are circular rather than elliptical (Desjardins, 1995), corroborating the data obtained in this work.

In plants grown *in vitro*, when potassium silicate is added at a dosage 0.5 mL L^{-1} and 1.0 mL L^{-1} in a natural environment, the cuticle gets thicker, demonstrating that the silicate has promoted an increase in thickness, an interesting characteristic for the acclimatization of plants in an *ex vitro* environment because these variables together increase the water storage in the plant. Silva et al. (2006) studied the leaf anatomy of 13 species of orchids and

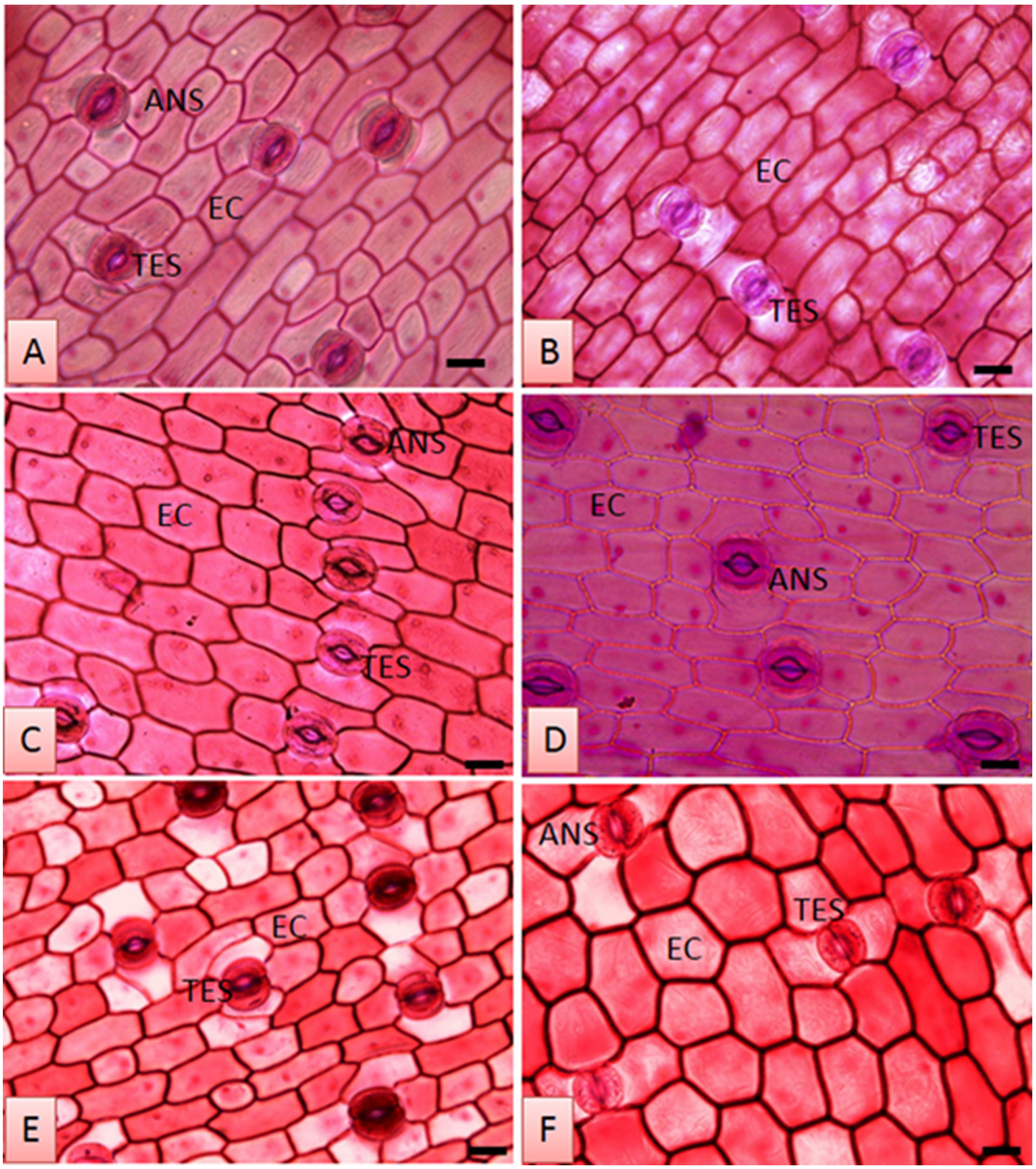

Figure 2. (A-F) Photomicrograph of paradermic sections from the epidermis of *Catasetum x apolloi*. 1(A, B) adult plant; (C) TNE (natural environment treatment with alternative means / natural light); (D) TAE (treatment with artificial light in alternative means); (E) TNE with potassium silicate 0.5 mL L^{-1}; (F) TAE with potassium silicate 0.5 mL L^{-1}. (EC = epidermal cells; SC = subsidiary cells; TES = tetracytic stomata; ANS = anomocytic stomata). Bar = 50 µm (A, B); 40 µm (C, E, F); 60 µm (D).

reported that in species with reduction of the aerial part, the adaxial epidermal cells presented a thin cuticle, usually devoid of striations. Despite presenting a reduction of the aerial part in a period of the year, the species studied in this work does not present a thin cuticle, but a thick cuticle in treatments with application of potassium silicate, in both environments.

The cuticle is the first protective barrier between the surface of the aerial part of the plant and the environment; it is also the main obstacle to the movement of water, including the flow of perspiration (Evert, 2006). The most thickened cuticle has a lipid nature, avoiding excessive loss of water through transpiration, being fundamental in the tolerance mechanism to hydric deficiency (Castro et al., 2009). Especially when they are transplanted from *in vitro* to *ex vitro* environment, where the environmental conditions of acclimation are quite different, because plants pass from the heterotrophic condition to the autotrophic condition, the relative humidity is changed as well as the temperature and the luminosity.

In treatment in a natural environment (TNE 0.5), the epidermis presents cells with straight thick walls, in rectangular, polygonal, and hexagonal shapes (Figure 2E). Zhou (1995) confirms that the presence of silicate provides to the plants greater resistance to water loss when compared to the control treatment, providing better arrangement of epidermal cells without loss by dehydration of the tissues, which would harm the acclimatization process.

There was elongation of the adaxial cells in plants grown *in vitro*, adding potassium silicate with 0.5 mL L^{-1} to the alternative media in an artificial environment (TAE) (Figure 3C-D). This characteristic, associated with the thickening of the epidermis, can contribute to the maintenance of water in plants (Kurzweil et al., 1995). In the leaf epidermis, silicon combines with the cellulose (Silva et al., 2006).

Transversally, in the anatomical sections, epistomatal chamber were observed in the treatment with alternative media with the addition of 0.5m L^{-1} of potassium silicate in a natural environment (Figure 4C). These chambers that keep a small moist air compartment that reduces transpiration are adaptive characteristics very common in epiphytic orchids, which face high temperatures and limited water availability (Rasmussen, 1987).

In all treatments, in an environment with natural light, the stomata have guard cells with external periclinal walls that present a rather sharp thickening, forming a crest around the external atrium, and underdeveloped substomatic chambers (Figures 3A, E, 4A, C, D). According to Fahn and Cutler (1992), substomatic chambers establish a long and shallow diffusion gradient between the chlorophyllic parenchyma and the environment, as well as high resistance to water loss. Thus, even when the stomata are open, the low stomatal density and high cuticular and substomatal resistance allow only low transpiration rates. These characteristics are essential for water saving in cultivated plants and also an excellent functional apparatus for plants in the acclimatization process, in which hydric stress is higher.

There was no interaction between the factor luminosity for density and the stomatic index (SI) (Table 1). For doses of 0.0 and 1.0 mL L^{-1} of potassium silicate in alternative media,there was no statistical difference in the stomatic index. Treatment with addition of 0.5 mL L^{-1} of potassium silicate to alternative means presented higher SI, surpassing the other treatments with 7.33% of stomata per mm^2.

For stomatic density (SD), doses of 0.0 and 0.5 mL L^{-1} of potassium silicate were more effective; however, with an increase of the doses there was a decrease in SD (Table 1). Higher stomata densities are a form of adaptation to drought, as they allow the plants to regulate the transport of water and transpiration more efficiently (Luković et al., 2009); this can promote the stomatic opening in a short period of time, favoring a proper CO_2 capture and reducing the period in which these stomata remain open, reducing, consequently, the transpiration (Ribeiro et al., 2012). This factor is essential in that it allows a better adaptation of plants cultivated *in vitro* to conditions of low water availability, when transplanted to an *ex vitro* environment.

The highest stomatic indexes were observed in treatment with addition of 0.5 mL^{-1} of potassium silicate, with 7.33%. For stomatal density, the same dosage afforded 45.98 mm^2, not statistically differentiating it from the treatment with alternative means without the addition of the potassium silicate. On the other hand, Pompelli et al. (2010) assert that the stomatic index is an intrinsic characteristic of the plant, remaining constant. This information does not apply to treatments performed in this study because there were changes in the stomatic index when potassium silicate was added to the alternative means. There are no mentioned bibliographic data for the relation of the potassium silicate and the increase in SI and SD, but these data were observed in this study.

There was no statistical difference in the environment with artificial luminosity or for potassium silicate doses applied to the alternative media for polar diameter (Table 2). For natural luminosity, the application of potassium silicate had higher averages for the two concentrations of 0.5 L^{-1} mL and 1.0 mL L^{-1}, with no difference between the doses. However, without the application of silicate,

Table 1. Index (SI) and stomatic density (SD) of *Catasetum x apolloi* cultured *in vitro* with different luminosities and potassium silicate concentrations.

Doses K_2SiO_4 (mL L^{-1})	Variables	
	Index stomatic%	Stomatic density (mm^2)
0	6.37 b	46.49 a
0.5	7.33 a	45.98 a
1	5.88 b	38.43 b
VC (%)	17.75	33.87

SI = stomatic index; SD = stomatic density; VC = variation coefficient. Medias followed by the same letter in the column do not differ by the ANOVA test P <0.05 through the Sisvar program (Ferreira, 2003).

Table 2. Equatorial diameter (EDI), Polar diameter (PD), leaf blade (FL) vitroplants in different environments, natural light / artificial light and potassium silicate concentrations to the alternative means.

Doses K_2SiO_4	LF (µm)		DP (mm²)		DIE (mm²)	
	Natural	Artificial	Natural	Artificial	Natural	Artificial
0	183.38 B a	199.54 A a	18.84 B b	22.66 A a	36.57 A b	36.15 A a
0.5	181.76 B a	207.21 A a	22.98 A a	22.11 A a	44.50 A a	37.76 B a
1	188.02 A a	173.95 A b	21.81 A a	21.46 A a	36.25 A b	37.92 A a
VC%	11.97		10.57		9.36	

VC = variation coefficient. Medias followed by the same letter in the column do not differ by the ANOVA test P <0.05 through the Sisvar program (Ferreira, 2003).

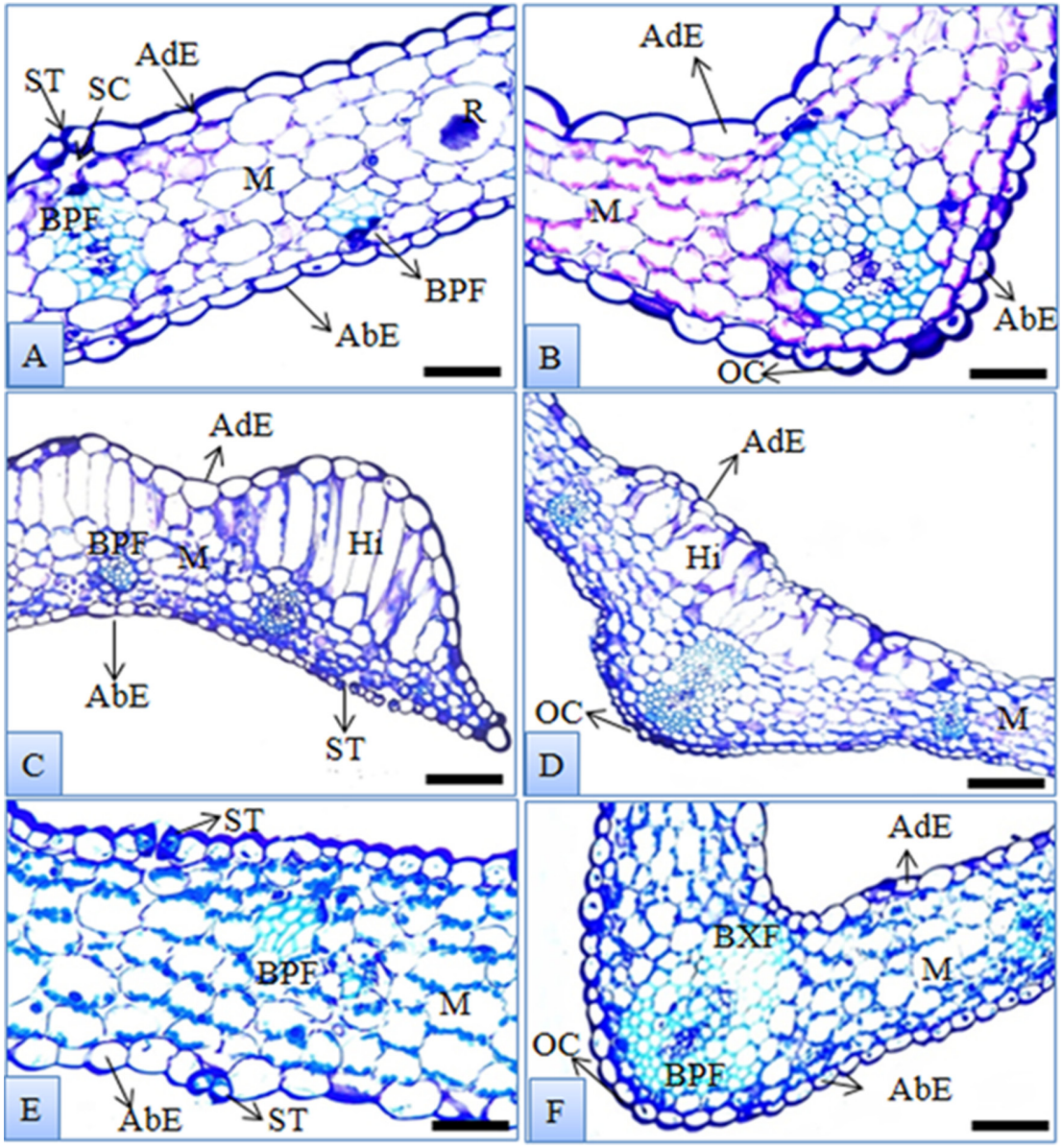

Figure 3. (A-F) Photomicrographs of transverse cuts from vitroplants of *Catasetum x apolloi*: mesophyll and midrib TNE (treatment with natural luminosity). (A, B) Control; (C, D) Addition of potassium silicate 0.5 mL L^{-1}; (E, F) Addition of potassium silicate 1mL L^{-1}. (ST = stomata; SC = substomatal chamber; M = mesophyll; AdE = adaxial epidermis; AbE = abaxial epidermis; X = xylem; P = phloem; BPF = bundle of phloematic fibers; BXF = bundle of xylem fibers; Hi = hipodermis; R = raphides; OC = ornamented cuticle). Bar = 80 µm (A-F).

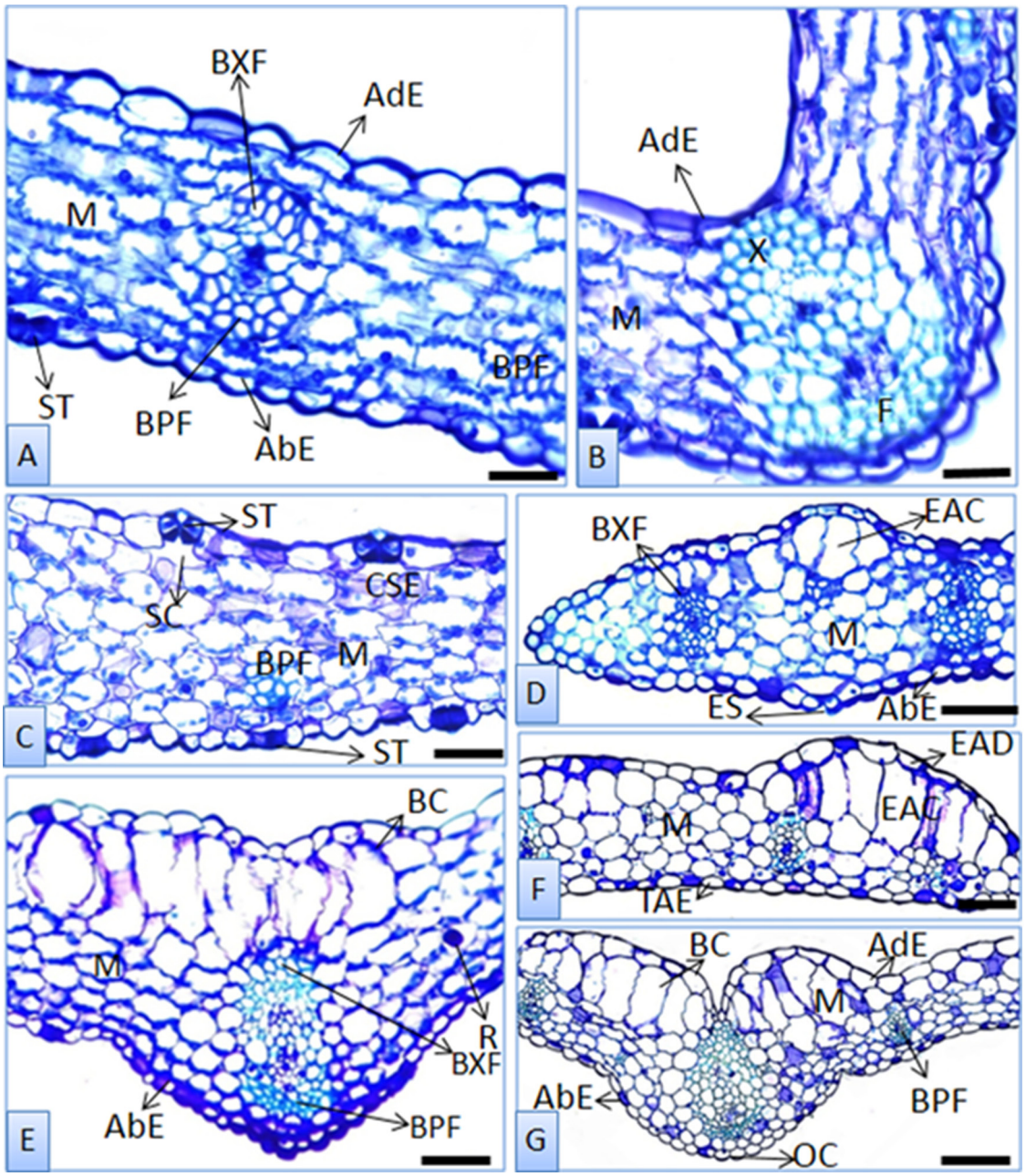

Figure 4. (A-G) Photomicrographs of transverse cuts from vitroplants of *Catasetum x apolloi*: mesophyll and midrib TNE (treatment with natural luminosity). (A, B) Control; (C, D) Addition of potassium silicate 0.5 mL L^{-1}; (E, F) Addition of potassium silicate 1mL L^{-1}. (SC = substomatal chamber; ST = stomata; M = mesophyll; AdE = adaxial epidermis; TAE = thicker abaxial epidermis; X = xylem; P = phloem; BPF = bundle of phloematic fibers; BXF = bundle of xylem fibers; R = raphides; EAC = elongated adaxial cells; OC = ornamented cuticle). Bar = 80 μm (A-F).

there was smaller polar diameter in the growth room with artificial luminosity.

When the equatorial diameter was analyzed, it was verified that there was an interaction between the luminosity factors and the potassium silicate doses in the alternative means (Table 2). It was verified that the smaller stomatic diameters, polar and equatorial, are formed with the doses 0.0 and 0.5 ml L^{1} of silicate in the culture media in an artificial environment. The decrease in the size of the stomata is essential because its importance consists of a larger functionality of the gas exchange, presenting a decrease in the pore size and also a smaller water loss through transpiration.

The shape of the guard cells together and also the relation between polar and equatorial diameters of the stomata are indicative of its functionality (Rocha et al.,

2007). Some authors agree that the elliptical shape is characteristic of functional stomata, while the rounded shape is associated with stomata that present abnormal operation (Khan et al., 2002). Rocha et al. (2007) found in his research with micropropagation of dwarf silver banana that the higher the relation between polar diameter and equatorial diameter, the more ellipsoid the stomata, which may result in increased functionality.

It was observed that plants of the control treatment presented circular stomata, differing from the other treatments with application of the potassium silicate (Figure 2A-F). In the treatments with the application of potassium silicate, the stomata became more elliptical. These characteristics are essential to optimize the survival and the photosynthetic capacity during the acclimatization phases.

Table 2 shows that the increase of the blade was affected not only by the applied doses of Si, but also by the luminous incidence in both environments. This verifies that the artificial light associated with silicate doses 0.0 and 0.5 U ml^{-1} provided the same significance for the increase of the blade, different from the environment with natural light, where the dose of potassium silicate was the double 1 mL L^{-1} to present similar thickness.

Different from these results, Lee et al. (1988) and Dousseau et al. (2008) attributed an increase in the size of the mesophyll cells to high light intensities, and consequently a greater leaf thickness, as well as a more pronounced cell compression in cultivated leaves of *Liquidambar*. The authors also stated that low luminosity intensity decreases cell division, resulting in a reduced leaf area, producing thinner leaves. For the studied plant, the results were reversed, with lower luminosity causing an increases to thickness in the blade (Table 2).

The differences in the results can also be interpreted by the fact that the growth of plants, organs, tissues and cells *in vitro* depends on the formulation of the culture media, because it is necessary to develop optimized culture media for each species and the perfect interaction of the essential components such as carbon sources and mineral nutrients (Pasqual, 2001).

According to Sæbø et al. (1995) and Schuerger et al. (1997), the quality and quantity of the light can interfere in thickness, in the differentiation of the mesophyll and the vascular system, in the cell division, and in the development of the leaf stomata, providing a high physiological and anatomical plasticity in plants. Larger mesophyll tissues in the leaves give the plant cultivated *in vitro* a better chance of survival during transfer to the *ex vitro* environment, which is an important factor for the success of tissue culture (Barboza et al., 2006; Silva et al., 2008).

At concentrations of 0.0 and 0.5 U ml^{-1} of potassium silicate, in an artificial environment with concentrations of 0.5 and 1 mL L^{-1} of potassium silicate in a natural environment, an elongation and expansion of the hypodermal cells occur (Figure 4D-G). This characteristic is considered an extremely important factor for the seedlings that will be transferred to the acclimatization since the presence of hypodermis with periclinal cell elongation provides greater concentration or water reserve, fundamental processes to eliminate hydric stress in *ex vitro* plantlets.

The leaf anatomy can be influenced by the luminosity levels during growth due to its plasticity. The ability to change the structure of the leaves in response to different levels of light is a common attribute of species with broad potential of acclimation (Bjorkman, 1981). The adaptation of the plant to the natural light environment depends on the adjustment of its photosynthetic apparatus, so that the environmental luminosity is used more efficiently. The responses to these adjustments will be reflected in the general development of the plant. Thus, the growth efficiency is related to the adaptability of plantlets to the luminous intensity conditions of the environment. In this experiment, when we observed Figures 3C, D in the artificial environment and Figures 4D-G in the natural environment, this verified that cell elongation occurred.

The increase in the thickness of the leaf, mainly by the elongation or addition of palisade cells, is related to the reduction in resistance of the mesophyll to the carbon dioxide (Nobel, 1977), and correlated with an increase of potentially limiting factors for the photosynthesis, such as the Rubisco, chargers electrons, or stomatal conductance (Bjorkman, 1981). Chazdon and Kaufmann (1993) studied two species of Piper and observed that the photosynthetic capacity was correlated with the thickness of the mesophyll. In this context, mesophyll with larger dimensions are fundamental to the photosynthetic process and essential in the process of transplanting from the *in vitro* environment to the *ex vitro* environment, where the plant undergoes stresss due mainly to the differentiation of the substrate in which it was submitted, with dosages of sugar in half, going from autotrophic condition to heterotrophic.

The plantlets presented extravascular fibers in the midrib (Figure 3B, F, 4B, E), which perform different functions such as support, protection against water loss, and attenuation of the luminous intensity (Eames and Mac Daniels, 1925).

Several changes in the leaf structure of plants kept *in vitro* have been reported, such as the increase in size in the density of the stomata and the reduction in the stomatal control and in the thickness of the mesophyll, with a high proportion of intercellular spaces (Hazarika, 2006). In this study, broad intercellular spaces were not observed (Figure 3A-F, 4A-G), and in plantlets with the addition of potassium silicate there was an increment in the cuticle and in the cell wall, providing greater thickening when compared to the vitroplants of the control treatment.

Silva et al. (2006) also affirm that both the water and mineral nutrients associated with abiotic factors influence the structural characteristics of the plant. In this context, in this study, the inclusion of potassium silicate proves to be efficient for plants grown *in vitro*, and this is an indication that they would have greater survival in the *ex vitro* environment.

4. Conclusion

The best results seen with the application of K_2SiO_4 in alternative means, in TAE environments (artificial luminosity) and TNE (natural luminosity), were stretching of the hypodermis (doses 0.5 and 1 mL L^{-1}); thicker mesophyll (0.0 and 0.5 mL L^{-1} TAE) and more prominent midrib (0.0 mL L^{-1} TAE); shape of the elliptical guard cells (0.5 mL L^{-1} and L^{-1} 1mL TAE); formation of the epistomatal chamber (0.5 mL L^{-1}); lower stomatal density (1 mL L^{-1}); and stomata with smaller polar and equatorial diameters (0.0 and 0.5 mL L^{-1}).

The characteristics mentioned above promote lower hydric stress of plants grown *in vitro*, implying that this set of characteristics also provides a greater chance of *ex vitro* survival. Furthermore, the conditions that favored the acclimatization were lower light intensities and lower potassium silicate doses.

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Erratum

In the article "Anatomy and micromorphometric analysis of leaf *Catasetum* x *apolloi* Benelli & Grill with addition of potassium silicate under different light sources", DOI http://dx.doi.org/10.1590/1519-6984.12015, published in *Brazilian Journal of Biology*, vol. 77, ahead of print, in the title of the article:

Where it reads:

Anatomy and micromorphometric analysis of leaf *Catasetum* x *apolloi* Benelli & Grill with addition of potassium silicate under different light sources

It should be read:

Anatomy and micromorphometric analysis of leaf *Catasetum* x *apolloi* Benelli & Grade with addition of potassium silicate under different light sources