ANATOMICAL AND PHYSIOLOGICAL MODIFICATIONS OF MICROPROPAGATED 'CAIPIRA' BANANA PLANTS UNDER NATURAL LIGHT

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ABSTRACT: Research about the use of natural light associated to changes in sucrose levels demonstrated potential in promoting *in vitro* hardiness of tropical climate species, as well as reducing production costs. However, little is known about physiological and structural changes that happen in the process. This study evaluated the physiological and anatomic performance, and *ex vitro* survival of micropropagated banana plants in response to cultivation conditions, in the stage of *in vitro* rooting. Shoots of the 'Caipira' cultivar were cultivated in MS medium, supplemented with 1 mg L^{-1} NAA and 6 g L^{-1} agar, in which the following treatments were applied: two sucrose concentrations (15 g L^{-1} or 30 g L^{-1}) and two cultivation conditions (Natural light – greenhouse and Artificial light – growth chamber). At the end of 45 days, the contents of chlorophyll *a*, *b* and *total*, the relative water content in the tissues, anatomic characteristics and the *ex vitro* survival were evaluated. Effects of growth environment and sucrose concentration were observed on micropropagated 'Caipira' banana anatomy, physiology and survival. *In vitro* rooting of the shoots under natural light in the medium containing 15 g L^{-1} or 30 g L^{-1} sucrose promoted major alteration in the increase of palisade and spongy parenchyma, as well as reducing leaf water loss and plant death. The results obtained in the present study confirm the potential of the use of natural light as a substitute for artificial light for micropropagation of tropical species.
Key words: *Musa* spp., solar light, carbohydrate, water loss, anatomy

MODIFICAÇÕES ANATÔMICAS E FISIOLÓGICAS DE BANANEIRAS 'CAIPIRA' MICROPROPAGADAS SOB LUZ NATURAL

RESUMO: Pesquisas acerca do uso da luz natural associado a alterações nos níveis de sacarose têm apresentado potencial para promover a rustificação *in vitro* de espécies de clima tropical bem como reduzir os custos de produção. Todavia, pouco se conhece sobre as modificações fisiológicas e estruturais ocasionadas. Avaliaram-se o comportamento fisiológico, anatômico e a sobrevivência *ex vitro* de bananeiras micropropagadas em resposta às condições de cultivo, na fase de enraizamento *in vitro*. Para isso, brotações axilares da cultivar Caipira foram cultivadas em meio MS, adicionado de ANA (1 mg L^{-1}) e agar (6 g L^{-1}), onde foram aplicados os seguintes tratamentos: duas concentrações de sacarose (15 g L^{-1}e 30 g L^{-1}) e dois ambientes de cultivo (Luz natural – casa de vegetação e Luz artificial – sala de crescimento). Ao final de 45 dias, foram avaliados os teores de clorofila *a*, *b* e *total*, conteúdo relativo de água nos tecidos, características anatômicas e a sobrevivência *ex vitro*. Houve efeito do ambiente de cultivo e concentrações de sacarose sobre a anatomia, fisiologia e sobrevivência de plantas de bananeira 'Caipira' micropropagada. O enraizamento *in vitro* das brotações sob luz natural e meio contendo 15 g L^{-1} ou 30 g L^{-1} de sacarose promove como principais alterações o aumento na espessura dos parênquimas paliçádico e esponjoso, bem como reduz a perda de água foliar e morte de plantas. Os resultados alcançados no presente estudo confirmam o potencial da luz natural em substituir a iluminação artificial para a micropropagação de espécies tropicais.
Palavras-chave: *Musa* spp, luz solar, carboidrato, perda de água, anatomia

INTRODUCTION

In vitro propagation has been extensively used as an alternative and efficient method for quick propagation of several plant species (Hazarika, 2006). In banana, the application of this technology has enabled the

fast diffusion and validation of new genotypes, consisting on the basis for the recovery of propagated material with high phytosanitary standard (Gübbük & Pekmezci, 2004).

Despite of its importance, micropropagation under heterotrophic conditions is responsible for the induction of plant's physiological and structural modifications, considered as different from that obtained in *ex vitro* plants (Yokota et al., 2007), such as low regulation mechanism of water loss, mainly due to inefficient stomata functionality and the formation of epicuticular wax (Lamhanedi et al., 2003), and reduced development of photosynthetic tissues (Amâncio et al., 1999).

Some strategies have been adopted, such as reduction of carbohydrate level and relative humidity inside the flasks (Mohammed & Vidaver, 1990), variations on irradiancy levels (Navarro et al., 1994), use of natural light (Kodym & Zapata-Arias, 1999; Talavera et al., 2005) and the increase in gas exchange (Valero-Aracama et al., 2007). Light is considered to be one of the most important due to the decisive influence on plant development. Light enables the photostimulation of the biosynthesis of compounds necessary for growth (Larcher, 2000) or promotes structural modifications needed for better plant adaptation to the external environment (Whatley & Whatley, 1982). Nevertheless, studies include only few species, mainly using the natural light as an alternative to the illumination with fluorescent bulbs, but without evaluating the effects on plant morphology and physiology. Thus, the objective in this study was to evaluate the physiological and anatomic performance, and *ex vitro* survival of micropropagated banana plants in response to the cultivation conditions, in the stage of *in vitro* rooting.

MATERIAL AND METHODS

This study was carried out in Lavras (21º14'S, 45º17'W and 918 m asl), MG, Brazil, from March to July 2006. The plant material consisted of *Musa* cv. Caipira (AAA) shoots (2-3 cm and two expanded leaves), obtained from the *in vitro* establishment and multiplication of shoot tips of elite plants.

MS medium (Murashige & Skoog, 1962), containing 5 mg L^{-1} BA (6-Benzylaminopurine) was used to obtain the explants, and subculturing was carried out at 35 days interval. Standard growth conditions were 25 ± 2°C and 16 hours irradiance at 35 μmol m^{-2} s^{-1}. Subsequently, the explants were transferred to culture medium composed of salts and vitamins used in the MS medium, containing 1 mg L^{-1} NAA (α-naphthalene acetic acid), in which the treatments for *in vitro* rooting/elongation were applied, during 45 days. The treatments were analyzed in a 2 × 2 factorial design, and were composed of sucrose concentrations (15 g L^{-1} or 30 g L^{-1}) and culturing environments (greenhouse – natural light, and growth chamber – artificial light). All the tested media were solidified with 6 g L^{-1} agar, and the pH adjusted to 5.8 ± 0.1 before the addition of the solidifying agent. Cultivation was carried out in 250-mL flasks containing 40 mL of medium and closed with clear plastic film. At the end of 45 days, the following parameters were evaluated: survival percentage, chlorophyll contents, relative water content and anatomical characteristics.

The artificial light environment consisted of a growth chamber, with lighting provided by two cool white fluorescent bulbs (Osram 20 W each) (16 h irradiance at 35 μmol m^{-2} s^{-1}) at 25 ± 2°C, while the natural light environment corresponded to a greenhouse, covered by a clear polyethylene film (150 microns) and 70% shading, having the following environmental parameters: maximum, minimum and average temperature of 26°C/32°C; 16°C/16°C and 20°C/23°C, and maximum, minimum and average levels of irradiance of 432.17 μmol m^{-2} s^{-1}/918.57 μmol m^{-2} s^{-1}; 51.20 μmol m^{-2} s^{-1}/49.04 μmol m^{-2} s^{-1} and 227.15 μmol m^{-2} s^{-1}/ 457.38 μmol m^{-2} s^{-1}, corresponding to cloudy and clear days typical of the experimentation period. The data of diurnal solar irradiance, incident at the top of the flasks, were obtained by irradiance sensors (LI-200SA, Li-cor, Lincoln, Nebrasca, USA), connected to a recording system (LI 1400; Li-cor, Neb.), with readings occurring at 30-minute intervals for 11 hours (from 7:00 AM to 6:00 PM). At the artificial environment, irradiance was measured only during 6 h, because the environmental conditions were rigorously controlled. Data corresponding to the weekly temperature means were obtained with a thermohygrograph.

Chlorophyll contents determination was carried out randomly in five plants per treatment. Fifty milligram samples were collected from the first completely expanded leaf, at the center of the leaf blade, and were immediately wrapped in aluminum foil and placed in an ice box. Extraction and quantification of chlorophyll a, b and total was carried out as described by Arnon (1949), reading the extracts in a spectrophotometer at 663 nm and 645 nm, respectively, for chlorophyll a and b. Chlorophyll contents, on a fresh mass basis, in mg g^{-1}, was calculated according to Castro et al. (2005).

The relative water content (RWC) was determined using whole plants. Plants were removed from the growth flasks, subjected to a quick root rinse and gently blotted dry. Subsequently, they were placed on a bench top, over aluminum foil, and maintained at room temperature (about 63% relative moisture). Every ten minutes, for 240 minutes, six plants per treatment were weighed to determine the fresh mass. Sub-

sequently, the dry mass of the leaves was ALSO obtained (50°C) and the RWC for each time was estimated as: RWC (%) = $[(FW_t\text{-}DW) / (FW_S\text{-}DW)] \times 100$, where FW_t is the fresh matter at time t, FW_s is the initial fresh matter (time zero) and DW is the dry matter (Romano & Martins-Loução, 2003).

Anatomic evaluations were carried out on the central part of the second expanded leaf, obtained from different plants per treatment. Plant material was previously fixed in 70 FAA (formaldehyde, acetic acid and ethanol) (Johansen, 1940), during 72 h and conserved in 70% ethanol (v/v). The transversal and paradermal sections were obtained in a microtome and manually, by using a stainless-steel blade. Subsequently, they were subjected to clarification with sodium hypochlorite (50% v/v), rinsed three times in distilled water, stained with astra-safranine blue (transversal) and safranine 1% (paradermal), and mounted in water-glycerol for microscope observation (Kraus & Arduin, 1997). Transversal sections were observed in a Kena-vision 2100 light microscope, where the width of the epidermis and hypodermis (upper and lower faces), and the spongy and palisade parenchyma, after the fourth lateral leaf bundle sheath, were measured. Additionally, the foliar limb thickness was measured. For stomata characterization, only stomata density (number of stomata mm^{-2}) was obtained using an Olympus CBB light microscope (Labouriau et al., 1961).

The acclimatization was carried out in a greenhouse with constant misting. Plants were removed from the flasks, and had their roots washed in tap water and pruned, and were transferred to 0.3 L root plug containing a mixture of soil and a commercial substrate: carbonized rice husks (1:1:1 v/v), amended with 50 g L^{-1} of humus and 20 g L^{-1} of single superphosphate.

A completely randomized experimental design was used for the quantification of the chlorophyll content and for the anatomical analysis, with five and six replications of tissues measurements and stomata counting. The assay of the RWC was carried out as subdivided plots in time in a completely randomized design, with six replications per treatment. The data obtained were initially submitted to individual analyses of variance (for each cultivation environment), following a test of homogeneity of variances and the combined analyses of the environments, using the software Sisvar 4.3 (Ferreira, 2000) and test F ($p \leq 0.05$). Plant survival was determined by visual observation.

RESULTS AND DISCUSSION

Chlorophyll contents

Differences for chlorophyll *a* and *total*, regarding the cultivation environment were found only for the treatment containing 30 g L^{-1} sucrose. At this concentration, high contents of both types of chlorophyll were obtained in conditions of artificial light, with 1.24 mg g^{-1} and 1.67 mg g^{-1} respectively. However, no differences were observed among the concentrations of carbohydrates in both environments ($p \leq 0.05$) (Table 1). This result could be due to the lower irradiance observed in the growth chamber (artificial) environment, because the processes of synthesis and degradation (photo-oxidation) of chlorophylls are directly correlated with irradiance, with pronounced reduction on chlorophyll content (per mass unit and, or area) under high levels (Lee et al., 2000; Atroch et al., 2001; Kitajima & Hogan, 2003; Deccetti, 2004).

No effects were observed for chlorophyll *b* and the *a/b* ratio for all treatments, isolated or in association. This is consistent with the findings of Deccetti (2004) with plants of *Amona glabra* L., at the *in vitro* rooting stage, in which lower influence of the increase in irradiance (artificial) levels on *a* and *b* chlorophylls was observed. Nevertheless, Lee et al. (2000) observed a reduction in chlorophyll concentrations and in the ratio of chlorophyll *a/b* by the exposure to a high PFD (Photon Flux Density) in two tree species native to Asia.

Relative water content (RWC)

A significant interaction of the three factors (Environment × Sucrose × Time) evaluated was obtained for the RWC, where greater water loss (lower RWC) with exposition time occurred in plants grown in the artificial environment, and this loss was greater at 30 g L^{-1} sucrose (Figure 1). Lower RWC in plants

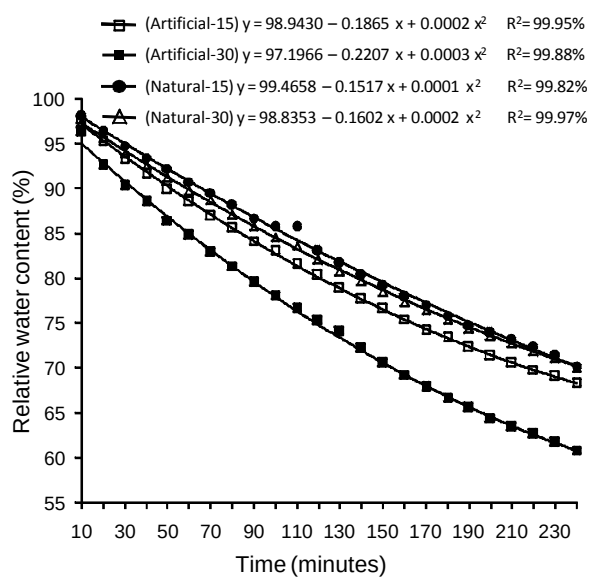

Figure 1 - Relative water contents of in micropropagated 'Caipira' (AAA) banana plants as a function of the cultivation environment and sucrose concentration.

Table 1 - Chlorophyll *a* and *total* contents of micropropagated 'Caipira' (AAA) banana plants as a function of the cultivation environment and sucrose concentration.

Sucrose	Chlorophyll *a*		Mean	Chlorophyll *total*		Mean
	Natural	Artificial		Natural	Artificial	
g L^{-1}	---------- mg g^{-1} of fresh mass ----------					
15	1.05 aA	1.12 aA	1.08 a	1.42 aA	1.50 aA	1.46 a
30	0.96 aB	1.24 aA	1.10 a	1.34 aB	1.67 aA	1.50 a
Mean	1.01 B	1.18 A		1.38 B	1.59 A	
CV(%)	8.48			8.54		

Means followed by distinct letters, small caption in the column and capital in the row, are different (F test at 5%).

grown under artificial lighting and medium containing 30 g L^{-1} sucrose is observed only when the average of each growth environment and sucrose concentration is evaluated, with 82.6% and 78.1% for the natural and artificial conditions, and 81.8% and 78.9% for 15 and 30 g L^{-1}. Similar results have been observed reported by Decetti (2004) in leaf disks of *Amona glabra* L., who observed reduced water loss in leaf disks from plants grown under high *in vitro* irradiance (300 μmol m^{-2} s^{-1}).

The greatest RWC observed under natural light could have been favored by variations in relative humidity, irradiance and temperature inside the flasks, which would have favored a better response of stomata opening and closing mechanism after plant exposure to the external cultivation conditions, thus favoring the hardening of the plants. This hypothesis is supported by the observations of Silva (2006) with pineapple, who also conducted an experiment in the municipality of Lavras and under the same cultivation, irradiance and temperature conditions. According to Silva (2006), the relative humidity and temperature inside the flasks, under natural light environment, varied from 58% to 95% and 10°C to 40°C; however, in the artificial environment they ranged from 96% to 98% and 23°C to 27°C. Hazarika (2003) stated that plants developed *in vitro* under conditions of low relative humidity showed lower transpiration and less *ex vitro* translocation problems, and the persistent leaves are quite similar to the ones developed naturally.

Sciutti & Morini (1995) stated that the high relative humidity within the cultivation flasks was the main factor leading to modifications in tissue structure and functioning. According to those authors, the increase in irradiance levels and or reduction in relative humidity during *in vitro* cultivation have resulted in anatomical modifications similar to those observed during acclimatization in greenhouse, with the increase in deposition of epicuticular wax, reduction on stomata size and density, decreasing the loss of turgor and improving stomata functionality (Capellades et al., 1990; Sciutii & Morini, 1995; Decceti, 2004).

Moreover, Sciutti & Morini (1995) stated that *Prunus cerasifera* plants rooted under 100% relative humidity lost more water ($p \leq 0.05$) than plants rooted under relative humidity from 70% to 80%. In addition, plants grown under relative humidity from 70% to 80% presented a visible thicker waxy layer on the leaves, the stomata were closed, not raised and without collapse, even after 30 minutes of exposure in growth chamber. In contrast, under 100% humidity the stomata were more numerous, raised on the leaf surface, with larger apertures and the epidermal cells collapsed after 30 minutes of exposure. In this aspect, Capellades et al. (1990) reported that it is likely that the collapse of the epidermal cell could cause loss of integrity of the leaf surface, leading to more desiccation.

These results are consistent with those of Khan et al. (1999), where greater water loss was observed in leaves of *in vitro Quercus robur* plants, with 90% and 80% loss in plants obtained in the multiplication and rooting stages, within 30 to 90 min. This was attributed to a high density of stomata and the heterogeneous functionality of the stomata developed in this environment. Similar results about greater water loss in *in vitro* leaf tissues was observed by Romano & Martins-Loução (2003), which occurred in leaves developed during the *in vitro* rooting, compared to the ones formed in the acclimatization stage, losing approximately 53% and 14%, respectively, in the first 30 minutes when exposed to 50% of relative humidity at 21 ± 2°C. Additionally, the leaves formed *in vitro* showed opened stomata and collapse of guard-cells, which was not observed in acclimatized plants. High *in vitro* humidity, resulting from the low gas exchange in the flasks, lead the plants to open their stomata to keep the equilibrium with the atmosphere inside the flask, inducing an abnormal development of stomata, thus remaining opened even when they are subjected to a drastic change in the environment (Shackel et al., 1990; Sutter et al., 1992).

Anatomical features

Differences related to sucrose concentration were observed only on the width of the upper epidermis and on the foliar limb, with greater width in the medium amended with 15 g L^{-1} ($p \leq 0.05$). For the cultivation environments, the plants grown under natural light showed significant thickening in both the palisade and spongy parenchyma, in contrast to the upper and lower epidermis, and lower hypodermis, which had greater thickening in the artificial environment ($p \leq 0.05$) (Table 2). Sandoval et al. (1994) found that, despite *in vitro* 'Grande Naine' banana plants had epidermis and hypodermis with relatively thick cells, they showed thin walls, which could explain the greatest thickenings observed in the present study. As to the parenchyma, the increase in the width of these tissues due to increased irradiance was also observed by Hanba et al. (2002) in *in vitro Acer* species.

Greater thickening due to increase irradiance levels was also reported by Deccetti (2004). According to that author, *Annona glabra* plants rooted *in vitro* at 300 μmol m^{-2} s^{-1} had increase in the width of the palisade and spongy parenchyma, besides the pronounced differentiation of these tissues, responses that could be determinant to the photosynthetic process and *ex vitro* survival. The occurrence of more elongated palisade cells consists of classic pattern of response to plant adaptation to greater light intensity, thus highlighting the adaptable plasticity to the new environment (Lee et al., 2000). Moreover, structural modifications related to the development of the photosynthetic tissues could have an important role in increasing photosynthetic activity, survival and acclimatization of plants (Amâncio et al., 1999; Serret & Trillas, 2000.

As to the upper hypodermis, greater thickening ($p \leq 0.05$) was observed in plants grown under artificial light environment, in both sucrose concentrations (Table 3). Greater width of the lower and upper hypodermis of 'Prata-Anã' banana plants rooted in a growth chamber (artificial light) was also observed by Rocha (2005). Regarding the characteristics of the stomata, the leaves of banana plants have stomata distributed on both faces of the epidermis, however, with greater number on the lower face (Table 3), condition that classifies this species as amphistomatic, which agreed with Sandoval et al. (1994) and Rocha (2005).

Greater number of stomata per mm^{-2} was observed in the lower hypodermis in the natural environment, only with 30 g L^{-1} sucrose ($p \leq 0.05$). In contrast, no effect for the factors studied was observed

Table 2 - Tissue width and stomata density in micropropagated 'Caipira' (AAA) banana plants as a function of cultivation environment and sucrose concentration.

Sucrose	Epidermis *upper*	Palisade parenchyma	Spongy parenchyma	hypodermis lower	Epidermis *lower*	Foliar limb	Stomate density *upper*
g L^{-1}	---------- μm ----------						
15	20.5 a	58.1 a	76.7 a	47.7 a	13.7 a	278.6 a	32.2 a
30	16.7 b	60.4 a	72.6 a	49.1 a	13.3 a	266.5 b	34.6 a
Environment							
Natural	17.8 b	67.3 a	82.4 a	46.6 b	11.9 b	272.7 a	35.6 a
Artificial	19.4 a	51.1 b	67.0 b	50.2 a	15.1 a	272.4 a	31.3 a
CV (%)	12.47	13.11	15.50	11.24	17.73	6.45	27.40

Means followed by distinct letters in the vertical, within each variable, are different (F test at 5%).

Table 3 - Width of the upper hypodermis and stomata density of the lower hypodermis 'Caipira' (AAA) micropropagated banana plants as a function of the cultivation environment and sucrose concentration.

Sucrose	Hypodermis *upper*		Mean	Stomata density *lower*		Mean
	Natural	Artificial		Natural	Artificial	
g L^{-1}	---------- μm ----------					
15	44.1 Ba	79.7 Aa	61.9 a	158.1 Aa	150.7 Aa	154.4 a
30	49.5 Ba	59.7 Ab	54.6 b	167.2 Aa	131.1 Bb	149.2 a
Mean	46.8 B	69.7 A		162.6 A	140.9 B	
CV(%)	11.97			12.43		

Means followed by distinct letters, small caption in the vertical and capital in the horizontal, within each variable, are different (F test at 5%).

on the upper surface (Table 3). Similar results were obtained by Rocha (2005) for the 'Prata-Anã' cultivar that showed greater stomata density on the lower surface in artificial environment with 15 g L^{-1} and natural with 15 g L^{-1} or 30 g L^{-1} sucrose. Similar effect of irradiance was also observed by Deccetti (2004) in *Annona glabra* plants subjected to *in vitro* rooting. The increase in stomata density can provide the plant with increased gas conductance, thus avoiding that photosynthesis to be limited under different environmental conditions (Kundu & Tigerstedt, 1998). Nevertheless, the occurrence or not of differences in the number of stomata depends on the species and the cultivation condition.

***Ex vitro* survival**

Thirty days after transplanting, 100% survival was recorded in plants from the natural environment, in both sucrose concentrations. Conversely, in plants from the artificial environment, losses of 28.0% and 6.3% were observed with 15 g L^{-1} and 30 g L^{-1} of carbohydrate, respectively (Table 4).

Possibly, the exposure of the shoots to greater irradiance and temperature amplitudes promoted the hardening of the plants still *in vitro*, since the greenhouse conditions were more similar to that in which the plants were acclimatized, thus favoring lower stress in the transplanting and the *ex vitro* reestablishment.

Actually, the modifications observed to the RWC and anatomic parameters under natural light confirm the better structural and physiological adaptation of the plants and justify the greater survival rates. Moreover, these results are consistent with those obtained by Kodym & Zapata-Arias (1999) in *Musa* cv. Grande Naine (AAA), and Talavera et al. (2005) in *Cocos nucifera* plants grown under natural light. Moreover, according to Kodym & Zapata-Arias (1999), plants grown under natural light were successfully hardened, with 100% survival after two months, and presented normal development.

Table 4 - Survival of 'Caipira' (AAA) micropropagated banana plants as a function of the cultivation environment and sucrose concentrations, 30 days after the *ex vitro* transplanting.

Environment	Sucrose (g L^{-1})		Mean
	15	30	
	---------- % ----------		
Natural	100.0	100.0	100.0
Artificial	72.0	93.7	82.9
Mean	86.0	96.9	

Data obtained by the rate between the number of dead plants and or not developed and the total number of plants transferred to the *ex vitro* conditions, not being statistically analyzed.

The effect of sucrose on the success plant hardening is reported by Folliot & Marchal (1992), Skrebsky et al. (2004) and Pacheco et al. (2006) for *Musa* spp., *Pfaffia glomerata* Spreng. Pedersen and *Arachis retusa*, using between 15 g L^{-1} and 60 g L^{-1}. In contrast, negative effects from the partial or total removal of sucrose on the *ex vitro* development of micropropagated plants were obtained by Skrebsky et al. (2004), Fuentes et al. (2005) and Pacheco et al. (2006). The low survival and the slow development of *Cocos nucifera* L. cultivated under the absence or low sucrose concentration is a result of the deficiency in the formation of carbon backbone and the allocation of the stocks from the leaves formed *in vitro* to sustain the *ex vitro* reestablishment (Fuentes et al., 2005).

With a profound knowledge of the physiological and structural alterations induced *in vitro*, it is possible to establish strategies for the most efficient manner of transferring micropropagated plants to *ex vitro* conditions and, consequently, to maximize survival rates (Smith et al., 1997; Apóstolo et al., 2005). Thus, the results obtained in this study provide an explanation for the high survival rate observed in plants grown in natural light and contributes for the use of this kind of lighting for *in vitro* establishment. Similarly, the association of survival rate with morphological and anatomical features is important to demonstrate or not the hardening of plants subjected to modifications in the *in vitro* environment, thus reducing the losses after the exposition to the *ex vitro* environment (Capellades et al., 1990).

CONCLUSIONS

In vitro rooting of 'Caipira' banana shoots, under natural lighting, with 15 g L^{-1} or 30 g L^{-1} sucrose promoted the thickening of the palisade and spongy parechyma, reduced leaf water loss, and increased *ex vitro* plant survival.

The study of structural and physiological characteristics provided a better understanding of the changes in banana plants subjected to environmental alterations in *in vitro* cultivation.

Substituting fluorescent lights for natural lighting, during *in vitro* rooting confirms the potential of this lighting source in banana micropropagation protocols.

ACKNOWLEDGMENTS

To Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the financial support and the fellowship for the first author.

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Received October 22, 2007
Accepted October 06, 2008