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Changes in anatomy and chlorophyll synthesis in orchids propagated *in vitro* in the presence of urea

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ABSTRACT. The environmental and nutritional conditions during plant growth can influence cell differentiation, resulting in anatomical and physiological adaptations. The objective of this study was to investigate the anatomical characteristics and chlorophyll content of *L. purpurata* during *in vitro* propagation when different concentrations of urea were applied. After 120 days of culture in a greenhouse, the growth characteristics, chlorophyll content, leaf anatomy and root features were assessed. Plants cultivated in a medium containing 75% urea exhibited modifications in their growth, increase in the number of leaves and roots, changes in the leaf and root dry matter content. There was an increase in the contents of "a" and "b" chlorophyll in plants treated with 50, 75 and 100% urea. An increase in the ratio of chlorophyll a to chlorophyll b ("a/b") was observed in plants treated with 25% urea compared with the controls. In plants treated with 75% urea, increases in the thicknesses of the mesophyll and the leaf blade were observed, and reductions in the number of layers of root cells, the velamen thickness, the exodermis and the vascular cylinder were observed. These results indicate that this species has a tolerance to urea and exhibits higher growth *in vitro*.

Keywords: *Laelia purpurata*, cultivation *in vitro*, sources of organic nitrogen.

Alterações anatômicas e síntese de clorofila em orquídea propagada *in vitro* na presença de uréia

RESUMO. O ambiente assim como as condições de cultivo e tipo de nutrição podem influenciar na diferenciação celular, resultando em adaptações anatômicas e fisiológicas. O objetivo foi estudar características anatômicas e o teor de clorofila de *L. purpurata* "carnea" propagadas *in vitro* quando aplicado diferentes concentrações de uréia. Após 120 dias em sala de crescimento, foram avaliadas características de crescimento, teor de clorofila, aspectos anatômicos foliares e radiculares. Ocorreram modificações nas características de crescimento das plantas cultivadas em meio com 75% de uréia, resultando em aumento do número de folhas, raízes, teor de massa seca foliar e radicular. Houve aumento no teor de clorofila "a" e "b" nos tratamentos com 50, 75 e 100% de uréia. Foi observado aumento da razão de clorofilas "a/b" no tratamento com 25% de uréia, quando comparado com o controle. Na concentração de 75% de uréia foi observado aumento da espessura do mesofilo, limbo foliar, redução do número de camada de células radiculares, espessura do velame, exoderme e cilindro vascular. Tais resultados indicam que a espécie possui tolerância à uréia, possibilitando maior taxa de crescimento em condições *in vitro*.

Palavras-chave: *Laelia purpurata*, cultivo *in vitro*, fontes de nitrogênio orgânico.

Introduction

The flower production sector has increased in Brazil during recent years and has reached significant importance in the international market. In Brazil, the *in vitro* production of flowers is essential to produce plants with higher quality and higher commercial value (MATA-ROSAS et al., 2011).

The genus *Laelia* comprises a group of 22 species of the family Orchidaceae located in Central America and South America. *Laelia* are beautiful plants, which favors investment in their production (ÁVILA-DIAZ et al., 2009). Due to the destruction of the *Laelia* habitat as a result of intense exploration activities, some species are at risk of extinction. Thus, biotechnological techniques such as *in vitro* culture provide essential tools (RODRIGUES et al., 2009; RODRIGUES et al., 2012) to aid in the preservation and production of these plants (SILVA JÚNIOR et al., 2012).

The epiphytes, including orchids, are found in a wide range of ecosystems. These plants withdraw

their nutrients from throughfall and stemflow water and/or from decomposed organic matter that reaches their roots. Some epiphytes have evolved anatomical adaptations such as water-storing tanks, succulence, and water-absorbing leaf scales to absorb water and trap litter more efficiently (KALIMUTHU et al., 2007). In contrast, terrestrial orchids withdraw their nutrients from the pedosphere, which provides a more stable source of water and nutrients that allows the plants to react more slowly to environmental changes in the nutrient status (ENDRES; MERCIER, 2001).

In vitro plant culture allows for control over nutritional factors and is therefore an important strategy for large-scale production (RODRIGUES et al., 2012). One of the most important constituents of plant nutrition is nitrogen. The assimilation of this element is essential for the vital processes that control growth and development, and it has marked effects on the biomass and productivity of crops.

Changes in the final balance of the salt concentration in the nutritional medium, particularly nitrogen-containing media, can cause modifications in the rate of growth and development of plants and affect the morphology and cell totipotency (ARAÚJO et al., 2009; VILLA et al., 2009). Nitrogen (N) availability often limits plant performance in natural ecosystems, causing selective pressure to optimize the use of N resources. Because N is a limiting resource, plants do not only require efficient N uptake mechanisms, but they also possess enzymatic pathways for N remobilization (ELSER et al., 2006).

The epiphytic habit presents some physiological restrictions. Plants of this group can only access N derived from the atmosphere (via wet and/or dry deposition or through N_2 fixation), the canopy (organic forms derived from leaching or decomposition of trapped canopy litter and also from input by animals) and symbiotic relationships with microorganisms (INSELSBACHER et al., 2007).

The growth and development phases are extremely important for the successful acclimatization of micro-plants. Generally, growth regulators stimulate rooting and elongation of the shoots, but other factors can also be used as new sources of nitrogen in the basal medium, including organic sources (RAMOS et al., 2009). Culture media usually possess ammonium nitrate and potassium nitrate as sources of inorganic nitrogen, but organic nitrogen can also be used by the plant. Urea may be used in the medium as an additional source of nitrogen and has been tested by some authors (FRÁGUAS et al., 2003).

Furthermore, modifications in the mesophyll thickness and the epidermis, changes in the stomata density and the differentiation of the endoderm, cortex and vascular cylinder may occur. Environmental conditions and nutrient availability can also influence the "a" and "b" chlorophyll content, altering the a/b ratio and influencing the photosynthetic efficiency (ZHANG et al., 2006, 2008).

Nitrogen is essential for the synthesis of chlorophyll, which is required for photosynthesis. Therefore, low levels of nitrogen and chlorophyll will lead to impairments in energy utilization and a reduced ability to perform essential functions such as the absorption of nutrients and the production of carbohydrates for growth and development. The chlorophyll content of the leaves may also correlate with the N content in the plant. This relationship is primarily attributable to the fact that 50-70% of the total N in the leaves is incorporated into enzymes that are associated with chloroplasts (TAIZ; ZEIGER, 2009).

Nitrogen is essential for the synthesis of chlorophyll and for amino acid metabolism, which are essential for the growth and development of plants. The epiphytes (including orchids), have restrictions on the physiological absorption of nutrients (including nitrogen). Thus, the objectives of this work was to investigate the growth characteristics of *Laelia purpurata* exposed to different concentrations of ammonium nitrate (NH_4NO_3) or urea (CH_4N_2O). We evaluated the anatomical changes in the root tissue and analyzed the chlorophyll content of leaves to evaluate the nitrogen requirements in the orchid species *Laelia purpurata* "carnea".

Material and methods

The experiment was performed in the Tissue Culture Laboratory in the Agriculture Department of the Federal University of Lavras (UFLA) in Lavras, Minas Gerais State.

Laelia purpurata "carnea" plants that were 1 cm in length after *in vitro* germination were inoculated in MS culture medium (MURASHIGE; SKOOG, 1962) that was supplemented with 2.68 μmol of naphtalenacetic acid (ANA) + 13.72 μmol of gibberellic acid (GA_3) + 2 g L^{-1} of activated carbon + 100 g L^{-1} of banana nanica pulp solidified with 6 g L^{-1} of agar. The pH was adjusted to 5.8, and the medium was autoclaved at 121°C and 0.1 atm for 20 minutes.

The inoculation of the plants was performed in a laminar flow chamber in bottles of 200 mL

containing 30 mL of MS medium that was modified according to the treatment. The bottles were sealed with propylene covers and transferred to a greenhouse where they were kept at 27±1°C, irradiance of 32 μmol m^{-2} s^{-1} and photoperiod of 16 hours.

After 120 days of cultivation, the following characteristics were evaluated: number of leaves (NF), number of roots (NR), leaf dry matter, and root dry matter. The samples were dried in a forced-air circulation chamber at 70°C until they reached a steady mass.

To measure the chlorophyll content in the leaves, samples containing 1 g of leaf material were extracted with 30 mL 80% acetone. The extracts were filtered, diluted to 50 mL and evaluated in a spectrophotometer at wavelengths λ= 645 and 663 nm. Chlorophyll contents "a" and "b" and the total chlorophyll were calculated based on the following equations: chlorophyll "a" ($\mu g\ mL^{-1}$) = $12.7A_{663} - 2.69A_{645}$; chlorophyll "b" ($\mu g\ mL^{-1}$) = $22.9A_{645} - 4.68A_{663}$; total chlorophyll ($\mu g\ mL^{-1}$) = $20.2A_{645} + 8.02A_{663}$, according to the method of (KOIKE et al., 2001; ZHANG et al., 2006, 2008). The treatments were established by providing nitrogen substitution in the proportions presented in Table 1.

Table 1. Different nitrogen sources and concentrations for the *in vitro* growth of *L. purpurata* "carnea".

Source of N (%)	Ammonium nitrate (NH_4NO_3)	Urea (CH_4N_2O)
100% NH_4NO_3 (control)	577.7 mg L^{-1}	0 mg L^{-1}
75% NH_4NO_3 + 25% CH_4N_2O	433.2 mg L^{-1}	309.51 mg L^{-1}
50% NH_4NO_3 + 50% CH_4N_2O	288.85 mg L^{-1}	619.02 mg L^{-1}
25% NH_4NO_3 + 75% CH_4N_2O	144.4 mg L^{-1}	928.53 mg L^{-1}
100% CH_4N_2O	0 mg L^{-1}	1228.04 mg L^{-1}

The samples of the leaves and roots were fixed in 70% F.A.A. (formaldehyde, glacial acetic acid and ethanol) for 72 hours and then stored in 70% ethanol until the analysis. After performing transverse cuts of the leaves and roots, the samples were flushed with solution [safrablau (safranin (1%) and astra blue (1%) at a proportion of 7:3] and mounted on semi-permanent plates.

The blades were observed under an Olympus BX60 optical microscope coupled with a Canon A630 digital camera. For micromorphometry, the images were analyzed using Image tool-UTHSCSA software. The epidermis thickness of the undersurface (Eab), the adaxial (Ead), mesophyll thickness (MF) and the leaf blade (LF) were measured.

The experimental design was completely randomized with five treatments and ten repetitions, each composed of a bottle with five plants. The data were submitted to variance analysis, and the means were compared by the Tukey test at 5% probability using the Genes statistical software (CRUZ, 1997).

Results and discussion

To our knowledge, this study is the first to associate anatomical changes and synthesis of chlorophyll *L. purpurata* plants treated with different sources of N, including urea. *In vitro* culture conditions were chosen to prevent any conversion of the N forms supplied during the course of the experiments. Our results demonstrate that under the tested conditions, urea is directly taken up by root cells prior to hydrolysis.

The addition of urea at a concentration of 75% resulted in modifications in the evaluated characteristics. There was an increase of 121% in the number of leaves, 62% in the number of roots, 12% in the leaf dry matter and 41% in the root dry matter compared with the control treatment (Table 2). These results suggest that *L. purpurata* "carnea" plants are able to assimilate 75% urea as an inorganic source of nitrogen.

From these data, we can infer that *L. purpurata* have urea transporters in the membranes of their cells (roots), as mentioned by (WITTE, 2011), who reported that plants possess dedicated urea transporters and can efficiently hydrolyze urea as the sole source of nitrogen source without toxicity.

Table 2. Vegetative growth and development of *L. purpurata* "carnea" orchids propagated *in vitro* with different concentrations of urea. The number of leaves (NL), number of roots (NR), leaf dry matter (LDM) and root dry matter (RDM) were measured.

Source of N (%)	NF	NR	LDM (g)	RDM (g)
100% NH_4NO_3 (control)	2.46 c	3.71 b	0.165 b	0.253 b
75% NH_4NO_3 + 25% CH_4N_2O	4.27 b	4.65 b	0.174 b	0.267 b
50% NH_4NO_3 + 50% CH_4N_2O	4.99 a	4.31 b	0.151 b	0.298 b
25% NH_4NO_3 + 75% CH_4N_2O	5.45 a	6.03 a	0.185 a	0.357 a
100% CH_4N_2O	3.33 b	6.78 a	0.161 b	0.260 b

The means that are followed by the same letter in the column do not differ from each other according to the Tukey test at 5% of probability.

Similar results were observed in *Arabidopsis* grown with 0.5 mM NH_4NO_3 and 0.5 mM CH_4N_2O; the dry weights of leaves and roots were higher than those of the control plants grown with 1 mM NH_4NO_3.

Hyssopus officinalis cultured in the presence of N-phenyl-N'-benzothiazol-6-yl-urea (PBU), a new derivative of urea that shows cytokine-like activity,

exhibited significantly better performance in terms of the percentage of shoots that rooted and the mean number of roots per rooted shoot during *in vitro* propagation (ROLLI et al., 2011).

When 100% urea was applied, there was a reduction in the leaf and root dry matter when compared to the treatment with 75% urea (Table 2). However, this reduction did not hinder the ex vitro development because all of the plants were acclimatized and the survival was 100%.

Although the administration of urea as a sole source of nitrogen may cause disturbances in the morphophysiology and metabolism of some groups of plants, a better understanding of plant urea metabolism, including the mechanisms of uptake, storage, internal transport, hydrolysis and assimilation of urea nitrogen, will be required to assess and possibly improve the direct usage of urea by plants (without prior soil conversion) in agricultural settings that employ urea fertilization (WANG et al., 2008; WITTE, 2011).

There was an increase of 54% in the chlorophyll "a" content of plants treated with 100% urea compared with the control treatment, which was not statistically different from the results of treatment with 75% of urea. An increase of 209% in chlorophyll "b" content was observed in the plants treated with 75% urea compared with the control treatment, which was not statistically different from plants treated with 50 and 100% urea.

The total chlorophyll content increased by 89% in the plants treated with 100% urea compared with the controls, which was not statistically different from the plants treated with 75% urea. However, we detected an 18% increase in the chlorophyll "a/b" ratio in plants treated with 25% urea compared with the controls. We also noted a reduction of 58% in this production in the plants treated with 50% urea, which was not statistically different from the plants treated with 75 and 100% urea (Table 3).

Depending on the availability of N, the plants show signs of your own, such as increased chlorophyll synthesis, for which glutamate is an important precursor. Glutamate is an ammonium-containing compound, which, in addition to being a precursor for other amino acids, is also the precursor for δ-aminolevulinic acid (ALA), which is considered a tetrapyrrolic universal precursor. Thus, N deficiency leads to decreased synthesis of glutamate, reduced ALA porphobilinogen synthesis and, consequently, a decrease in the biosynthesis of chlorophyll, which leads to the development of chlorosis in plants (CHU et al., 2007).

Table 3. Chlorophyll "a" and "b" contents, total chlorophyll and the chlorophyll "a/b" ratio in the leaves of *L. purpurata* "carnea" propagated *in vitro* with different concentrations of ammonium nitrate and urea in the MS culture medium.

	Chlorophyll $\mu g\ mL^{-1}$			
Source of N	"a"	"b"	Total	"a/b"
100% NH_4NO_3 (control)	9.54 c	3.17 b	12.71 c	3.01 b
75% NH_4NO_3 + 25% CH_4N_2O	10.62 c	2.96 c	13.58 c	3.58 a
50% NH_4NO_3 50% + CH_4N_2O	11.43 b	9.21 a	20.64 b	1.24 c
25% NH_4NO_3 + 75% CH_4N_2O	13.87 a	9.80 a	23.67 a	1.41 c
100% CH_4N_2O	14.77 a	8.32 a	24.09 a	1.58 c

The means followed by the same letter in the column do not differ from each other according to the Tukey test with 5% probability.

The stability of the chlorophyll content in plants treated with 75% urea may be partially related to what is called the photosynthetic maturation point, from which the chlorophyll levels will not change even when the N content increases inside the cells (COSTA et al., 2001). An "a/b" ratio of 3:1 is considered acceptable (HE; TEO, 2007; LIN; HSU, 2004); this ratio close to that found with the application of nitrate (Table 3).

However, with the application of 50% urea, the "a/b" ratio reached 1.24:1. Chlorophyll "a" receives energy from the light-harvesting complex of the photosystems, which is responsible for photochemical dissipation (HE; TEO, 2007; LIN; HSU, 2004).

Shade-grown orchids exposed to different irradiance levels exhibit varying sensitivities to sunlight. Leaves exposed to intermediate sunlight and maximal photosynthetic photon flux density are more sensitive than leaves grown under full sunlight or maximal shade (HE; TEO, 2007).

In one study, a reduction of the "a/b" ratio was reported in shaded plants (ZHANG et al., 2006, 2008) due to an increase in the production of accessory pigment "b", which is important for optimizing the efficiency of photosystem II under conditions of shade or stress (KOIKE et al., 2001).

The photosynthetic rate, chlorophyll fluorescence, leaf nitrogen content and chlorophyll content were studied in the leaves of *Cypripedium flavum* at different ages. The photosynthetic capacity changed significantly with leaf age. The net photosynthesis and chlorophyll content peaked at a leaf age of 60 days and decreased at 30, 90 and 120 days (ZHANG et al., 2008).

In regard to the anatomical characteristics, the leaves in the transverse section showed a uniseriated epidermis on both sides, homogeneous collenchyma with isodiametric cells and a thin cell wall. The leaf epidermis thickness was similar on both sides, and there were no significant differences due to the different concentrations of urea used in the MS medium. Nevertheless, there was an increase of 25%

in the mesophyll thickness in plants treated with 75% urea compared with the control treatment. The leaf blade thickness followed a pattern similar to that of the leaf mesophyll, with an increase of 18% in plants treated with 75% urea when compared with the control treatment (Table 4).

Table 4. Leaf micromorphometry of *Laelia purpurata* "carnea" propagated *in vitro* with different concentrations of urea. EAd = epidermis of adaxial side, EAb = epidermis of undersurface, MT = mesophyll thickness, LBT = leaf blade thickness.

Source of N (%)	EAd (μm)	EAb (μm)	MT (μm)	LBT (μm)
100% NH_4NO_3 (control)	22.76 a	21.98 a	87.78 b	132.52 b
75% NH_4NO_3 + 25% CH_4N_2O	23.87 a	19.03 a	86.98 b	129.88 b
50% NH_4NO_3 + 50% CH_4N_2O	24.59 a	22.65 a	85.87 b	133.11 b
25% NH_4NO_3 + 75% CH_4N_2O	24.21 a	23.66 a	110.01 a	156.88 a
100% CH_4N_2O	22.76 a	21.76 a	60.09 c	108.61 c

The means followed by the same letter in the column do not differ from each other according to the Tukey test with 5% of probability.

Thus, the type of nutrition available influenced the quantitative variations and differentiation of leaf tissues in orchids. However, the mesophyll thickness and the leaves of *L. purpurata* are directly related to the mesophyll thickness, may affect the photosynthetic rate. When size increases mesophyll thickness, the leaves of this species have a higher photosynthetic efficiency when urea is applied at a concentration of 75%. However, higher concentrations of urea can cause toxicity, resulting in a reduction in the thickness.

The increase of the leaf thickness blade is directly related to the increase of the mesophyll thickness, whereas the epidermis had no significant modifications. Thus, a urea concentration of 75% is favorable for the cultivation of *L. purpurata*, and it can contribute to an increase in the efficiency of the photosynthetic process.

It is possible that the increase in the thickness of the mesophyll of *L. purpurata* grown in the presence of 75% urea is related to crassulacean acid metabolism (CAM). The conditions provided in this study (low radiation, low temperature and water vapor saturation) may have stimulated the plants to increase their photosynthetic area (leaf area) by increasing the thickness of the tissue and the metabolism-related conversion of photons into chemical energy by activating photosystems II and I. Under the conditions described above, the source of inorganic nitrogen (urea) may have triggered plant growth, making the plants that were treated with urea more robust compared to the controls.

Crassulacean acid metabolism is a physiological feature that increases the use of water and nutrients, such as crassulacean in leaves containing highly thickened and cutinized cell walls, and it is the main mechanism of survival of epiphytes (KERTEN; KUNIOSHI, 2009; SILVA et al., 2006).

The roots of *L. purpurata* are of the polyarc type and have a compound endodermis with a thick layer of cells forming an "O"-shaped passage that is lined with metaxylem poles (Figure 1A). We observed reductions in the size of the medulla of 20, 56, 69 and 81% in plants treated with 25, 50, 75 and 100% urea, respectively (Figure 1B-E).

Changes in the morphology of the endodermal cells were observed *in vitro*, including an increase in the size and a change in the shape from longitudinal to isodiametric (Figure 1). Modifications in the endodermis and in the medulla can be caused by different factors, such as hydric and osmotic potential. The endodermis is an anatomical barrier that can help in the control of water flow, as well as prevent the entry of contaminants and microorganisms present in the substrate (LIN; HSU, 2004).

Moreover, Villa et al. 2009 observed that concentrations of urea over 20% can cause a decrease in the emission of new roots. These results demonstrate that *L. purpurata* has mechanisms that allow it to develop in environments with a high concentration of urea (Table 2).

The presence of urea resulted in concentration-dependent changes in the velamen of the roots of *L. purpurata* as illustrated in Figure 2. We observed a reduction in the velamen thickness and changes in the shape of the cells from an isodiametric (Figure 2A) to a tabular morphology (Figure 2B).

There was also a reduction in the number of cell layers when the plants were cultivated in medium containing greater than 50% urea. The velamen is a specialized feature that is associated with water capitation and assimilation of atmospheric gases, as well as protection of the root system (SILVA et al., 2010). The authors (SEGECIN; SCATENA, 2004) can be attributed to the roots of this epiphyte. In addition to storing water and minerals, the velamen provides mechanical protection and also prevents excessive sweating, functions that allow the survival of the plants in extreme environments, such as severe heat, water shortage, constant wind and friction with the substrate.

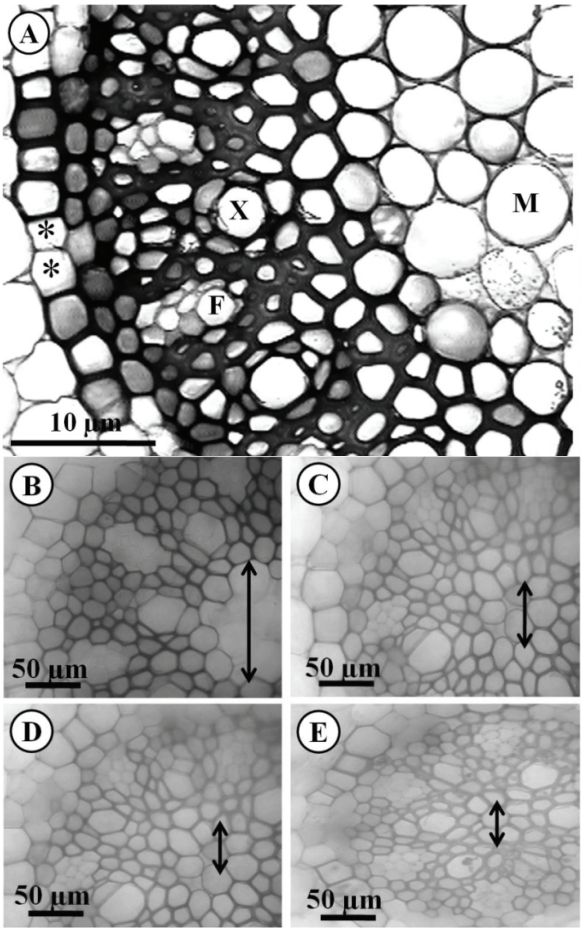

Figure 1. Transverse sections of roots of *L. purpurata* "carnea" cultivated with different concentrations of ammonium nitrate and urea. A) MS 100% ammonium nitrate; B) MS + 25% urea; C) MS + 50% urea; D) MS + 75% urea; E) MS + 100% urea. F, phloem; X, xylem; M, medulla; (*) cells passage; (arrows) medulla.

As previously reported (SANDFORD; ADANLAWO, 1973), environmental conditions, such as the availability of water and the temperature can affect the anatomical structure of the roots of Orchidaceae. The size and thickness of the cells of the canopy are particularly affected by environmental factors. The authors conclude that in dry environments, the canopy contains numerous layers of thick cells, whereas in humid environments, the canopy presents fewer layers of cells, and the exodermis shows higher numbers of idioblasts in plants treated with at least 50% urea (Figure 2B). The exodermis is an important apoplastic barrier for water flow and prevents the entry of pathogens (LIN; HSU, 2004).

The cells *in vitro* have periclinal and anticlinal thickening the walls of the exodermis, providing greater resistance to drying conditions ex vitro (acclimatization) and reducing the transpiration rate of the root. Environmental pressures can influence in the characteristics of the roots, causing a reduction of the diameter of the vascular cylinder, which is associated with a reduction in the hydraulic conductivity (SILVA et al., 2010). The differentiation of the exodermis of *L. purpurata* may be related to the reduction of the functions of the velamen under *in vitro* conditions (Figure 2B).

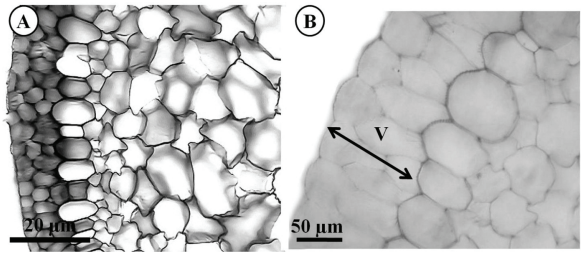

Figure 2. Transverse sections of roots of *L. purpurata* "carnea" cultivated with ammonium nitrate and urea. A) MS 100% ammonium nitrate; B) MS + 25% ammonium nitrate and 75% urea. V, velamen.

The ability of a plant to dynamically acclimate to different light environments is generally genetically determined. The photosynthetic performance of the leaves is related to irradiance, making it possible to improve the photosynthetic production of the plant by increasing the exposure to light. This can also induce an increase in starch production in the leaves of the orchid (LIN; HSU, 2004).

In plants that were selected based on their resistance to hydric stress, changes in the diameter of the vascular cylinder of corn roots were reported (PEREIRA et al., 2008). The same authors also verified an increase in the number of metaxylem vessels associated with a higher efficiency of the vascular system of these plants. However, a reduction in the vascular cylinder due to an increase in the concentration of urea was observed in this study (Figure 1).

In vitro cultivation led to modifications in the root structure of *L. purpurata*, leaving them less able to reduce the hydraulic conductivity and reducing the protection of the roots against phytopathogens. Such factors do not restrict the production of this species, but these results indicate the necessity of an acclimatization period for the better development of these structures.

Conclusion

The plants of *L. purpurata* are able to metabolize urea at concentrations up to 75% during propagation *in vitro*; toxic effects were observed when the concentration of urea was 100%. Urea caused changes in the mesophyll of the plants and in the

chlorophyll content, favoring the development of photosynthetic tissues. Changes were also observed in the morphological characteristics of the roots, which were less functional in plants cultured in the presence of urea, demonstrating the necessity of the acclimatization phase for these plants.

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