Article

Hoehnea 49: e1092020, 1 tab., 6 fig., 2022 http://dx.doi.org/10.1590/2236-8906-109/2020

Leaf anatomy and photosynthetic parameters of *Vellozia squamata* Pohl (Velloziaceae) grown under different light intensities along *in vitro* cultivation[1]

Elson Rangel Calazans Júnior[2], Conceição Eneida dos Santos Silveira[2], Olegário Garcia de Freitas-Neto[2], Dario Martins Palhares de Melo[2,3], Luiz Alfredo Rodrigues Pereira[2] and Sueli Maria Gomes[2]

How to cite: Calazans Júnior, E.R., Silveira, C.E.S., Freitas-Neto, O.G., Melo, D.M.P., Pereira, L.A.R., Gomes, S.M. Leaf anatomy and photosynthetic parameters of *Vellozia squamata* Pohl (Velloziaceae) grown under different light intensities along *in vitro* cultivation. Hoehnea 49: e1092020. http://dx.doi.org/10.1590/2236-8906-109/2020

ABSTRACT – (Leaf anatomy and photosynthetic parameters of *Vellozia squamata* Pohl (Velloziaceae) grown under different light intensities along *in vitro* cultivation). The present work evaluates photosynthetic parameters and the leaf anatomy of plants of *Vellozia squamata* Pohl (Velloziaceae) in each phase of *in vitro* production, with wild plants serving as a control. The anatomical approach was done for the leaf middle portion. The photosynthetic curves were obtained with an infra-red gas analyzer. The *in vitro* plantlets showed the thinner and poorest developed leaves, the wild plants had the thickest and most developed leaves, and the plants in acclimatization showed intermediate features. The physiological pattern was similar, and the *in vitro* plantlets were not capable of net carbon absorption. The young seedlings at the garden showed a maximal net carbon assimilation rate inferior to the wild plants. In conclusion, the light intensity for the *in vitro* phase should be adjusted to produce seedlings.
Keywords: acclimatization, Cerrado, conservation, cultivation, shading

RESUMO – (Anatomia foliar e parâmetros fotossintéticos de *Vellozia squamata* Pohl (Velloziaceae) desenvolvendo-se sob diferentes intensidades luminosas ao longo do cultivo *in vitro*). O presente trabalho avalia parâmetros fotossintéticos e a anatomia foliar de *Vellozia squamata* Pohl (Velloziaceae) em cada etapa da produção *in vitro*, sendo as plantas silvestres o grupo-controle. A abordagem anatômica foi feita para a porção mediana foliar. As curvas fotossintéticas foram obtidas com analisador de gases por infravermelho. As plântulas *in vitro* apresentaram folhas mais delgadas e menos desenvolvidas, as silvestres apresentaram folhas mais espessas e desenvolvidas e aquelas em aclimatação exibiram características intermediárias. As plântulas *in vitro* não foram capazes de absorção líquida de carbono. As plântulas jovens de casa-de-vegetação exibiram taxa líquida de assimilação de carbono inferior às selvagens. Em conclusão, para o cultivo *in vitro* as intensidades luminosas para a produção de mudas devem ser melhor ajustadas.
Palavras-chave: aclimatação, Cerrado, conservação, cultivo, sombreamento

Introduction

Plants of the genus *Vellozia* (Velloziaceae) grow in the African and South American savannahs and are more frequent in open vegetations, such as rupestrian fields or Cerrado stricto sensu (Ayensu 1969, Coetzee 1974, Mello-Silva & Menezes 1999, Batista 2016). They are highly resistant to fire and drought (Owoseye & Sanford 1972) and show stems capable to absorb water (Oliveira *et al.* 2005). Often, they produce resins and several secondary metabolites of medicinal interest (Quintao *et al.* 2013), including the inhibition of the venom of snakes (Tribuiani *et al.* 2014). Some species have been used as ornamental (Souza 2005), and it can be attributed to their big showy

1. Part of Master of Science of Olegário Garcia de Freitas-Neto
2. Universidade de Brasília, Instituto de Ciências Biológicas, Campus Universitário Darcy Ribeiro, Bloco E s/n 1º andar, Asa Norte, 70919-900 Brasília, DF, Brazil
3. Corresponding author: dariompm@unb.br

flowers and peculiar architecture. Unfortunately, these plants are somehow endangered by the extractivism and expansion of crop cultures.

The plants of the genus *Vellozia* produce a large amount of small seeds that are transported by wind, they are resistant to high temperatures, and show high and quick germinability (Menezes 1977, Oliveira *et al.* 1991, Garcia & Diniz 2003, Palhares 2004, Garcia *et al.* 2007, Jacobi & Sarto 2007, Mota & Garcia 2013, Vieira *et al.* 2017). However, Alves (1994) observed that the growth is very slow in *Vellozia* species, ranges from about 0.5 to 2.0 cm per year, and almost no young plants were seen in the field, in spite of their high frequency in the Campo Rupestre. The plants of about 1.80 to 3.30 m were actually centenary plants, therefore, although they occupy the open fields, they are not pioneer plants but establish later in the ecosystem, *i.e.* they are climax plants (Alves 1994).

Vellozia squamata Pohl, sometimes named by its synonym, *Vellozia flavicans* Mart *ex* Schult (Smith & Ayensu 1974), is a common species from the Cerrado of the central region of Brazil (Flora do Brasil 2020). The obtention of seedlings from seeds of this species failed due to a high mortality of the young plants (Neto 2009), just as other *Vellozia* species (Alves 1994). Also, the production of seedlings by stem cuttings ended unsuccessfully (Neto 2009).

While Borges (2015) reported only a partial success in the micropropagation of *V. sincorana*, Neto (2009) managed to micropropagate *V. squamata*. Some individuals of the micropropagated *V. squamata* were planted in gardens and have been growing since then, for more than a decade, having reached about 20 cm (figure 5 e, C.E.S. Silveira, not published data). So, the *in vitro* propagation protocol of *Vellozia squamata* somehow overcame unknown factors that generate high loss of seedlings produced via seeds.

Overall, the transition between the *in vitro* phase to rooting phase has been a limiting point in the production of seedlings, as the seedlings, which had grown heterotrophically and under low light intensities, are then planted in a place where they must turn into plentifully autotrophic (Kaur 2015). In the protocol of Neto (2009), this transition has given to a loss of about 55% of the seedlings, but once passed this phase, the survival has been high, of about 95%, that is, once surviving and grown under greenhouse conditions, the seedlings could be cultivated outside. As for a comparison, in the protocol of micropropagation of the Cerrado species *Jacaranda ulei* Bureau & K. Schum., the survival in the transition between *in vitro* and rooting phase has been around 80% (Silveira *et al.* 2018).

To improve the *in vitro* protocol, it is important to know physiological aspects of the wild species and seedlings at each one of their phases (Kaur 2015). The leaf photosynthetic curves are one of the most important physiological features to be known, as these curves can point out the ability of a small plant in establishing an autotrophic metabolism (Leite *et al.* 2017).

There have been many reports on the anatomy of Velloziaceae species (Ayensu 1968, 1969, 1974; Menezes 1977, 1988; Coetzee 1974; Ayensu & Skvarla 1974; Menezes & Semir 1991; Amaral & Mello-Silva 2008; Mello-Silva 2010), including *V. squamata* (Ayensu 1974, Menezes 1977). However, none of which described the leaf anatomy of micropropagated plants either anatomical responses to shading for those species. In addition, there is no anatomical report of *Vellozia* species grown *in vitro* at all. Therefore, the present paper aims to verify the impact of the conditions of *in vitro* micropropagation in the photosynthetic responses and in the leaf anatomical structure of *V. squamata*.

Materials and Methods

Plant material and treatments – The control group was five individuals of *Vellozia squamata* naturally found at Parque Ecológico Burle Max, Brasília, DF, Brazil, selected in a convenience sample due to easy access and apparently healthy vigor. For details of climate and soil, consult Bucci *et al.* (2006). Voucher specimens were deposited at the herbarium UB under the numbers UB0028356 and UB0028358. Seeds were harvested, put to germinate and the plantlets were sectioned and submitted to the protocol of micropropagation of Neto (2009). The hallmarks of this protocol for the present study area: a) plantlets in culture medium (Murashige-Skoog medium added with sucrose and phytoregulators kinetin, indol-butyric acid and gibberellin); b) seedlings under acclimatization in a greenhouse in pots containing soil; c) cultivation outside, in garden conditions. Thus, the following experimental treatments were: a) plantlets in culture medium growing under photosynthetically active radiation (PAR) of 32 µmol $m^{-2} s^{-1}$, photoperiod of 12/12 h and controlled temperature of 27 ± 2 °C; b) rooted seedlings in condition of a greenhouse, under partial shade, with maximal PAR of 412 µmol $m^{-2} s^{-1}$, as measured by a portable refractometer Apogee Instruments®; c) plants obtained by micropropagation and cultivated in an external garden, in treated and fertilized soil, exposed to the natural environmental conditions of Cerrado (annual cycle of drought and rains). The wild plants were the control.

Leaf anatomy – The leaf anatomy was carried out by transversal sections of the middle third of three well developed leaves in each of the groups. The fresh sections were fixed in FAA_{70} under vacuum, dehydrated in ethanolic series, included in resin Leica® and stained with toluidine blue or safranine plus Astra blue, according Johansen (1940) and Kraus & Arduin (1997). The slides were mounted with colourless glass varnish according to Paiva *et al.* (2006).

Light response curves – For each treatment, two leaves per plant from five plants (n = 10 measurements) were analyzed. From each leaf, the curve of light intensity/net carbon absorption (A, µmol CO_2 $m^{-2} s^{-1}$) was obtained with a portable infra-red gas analyzer (IRGA) model LI-6400XT,

manufactured by LI-COR Bioscience®. Since the leaves of the *in vitro* plantlets and the recently rooted seedlings were too small to completely fill the leaf chamber of the IRGA, data were adjusted according to the leaf area.

The IRGA was adjusted as to keep an air flow of 500 $\mu L.s^{-1}$ and carbon dioxide of reference of 400 ppm. The relative air humidity of the reference air was about the same in the treatments, as follows: control group: 45%; plantlets in vitro: 38%; seedlings in the greenhouse: 36%; garden condition: 44%. The air temperature at the time of the measurements were in a favorable physiologic condition, as follows: control group: 25.5 ± 0.5 °C; *in vitro* plantlets: 26.0 ± 0.1 °C; seedlings in the greenhouse: 30.4 ± 0.7 °C; garden condition: 31.3 ± 1.2 °C. The light intensities were previously selected, varying from zero to 1,600 $\mu mol\ m^{-2}\ s^{-1}$.

After each change in the light intensity, the IRGA was programmed to record the value after it had stabilized. In half of the measurements, the curve was carried out in ascendant way and in the other half, in descendant way of light intensity. For the plants of open conditions (garden and control group – ecological park), the measurements were taken between 09:00 and 10:00 AM during the rainy season; and besides the curve A/light, the gas exchange rates were also measured under the natural light intensity, of about 2,200 $\mu mol\ m^{-2}\ s^{-1}$.

Also, the transpiration rate was measured, and the water use efficiency (WUE) was considered as the ratio between the mean of the carbon absorption rate and the transpiration rate as seen in Field *et al.* (1983) and Medrano *et al.* (2015).

Chlorophyll content – The leaf chlorophyll content was estimated with a chlorophylometer Opti-Sciences CCM-200®, in two leaves per plant of five plants in each experimental treatment. According to the manufacturer, this device does not measure the chlorophyll content directly, since it analyzes the leaf transmittance of light, expressing the results in CCI – chlorophyll content index. Hence, it serves as for comparison among treatments.

Statistical analysis – The light response curves were adjusted to the model of Prado & Moraes (1997): $A_{meas} = A_{max}\ (1- e^{-k(PAR-Lcp)})$, in which A_{meas} means carbon assimilation rate under a given light intensity; A_{max} means the maximal carbon assimilation rate measured; k is a constant calculated for each curve; PAR means the given light intensity and Lcp means the light compensation point. Data were compared via Student´s T-test with the support of the open access graph software Libre Office.

Results

Physiological parameters and light response curves – The physiological parameters obtained are summarized in table 1 and figures 1 to 4 show the curves of light response of CO_2 absorbance. In the control group and in the plants growing in the garden, at a light intensity of 900 $\mu mol\ m^{-2}\ s^{-1}$, the leaves reached about 90% of the maximum carbon absorption rate. Since most of the leaves had peaked in the carbon absorption rate, this was considered as the light saturation point. For the light intensities of more than the double of this point, the carbon absorption rate was kept, hence there were no signs of photoinhibition (figures 1, 2, 4). In the open groups (wild plants and garden plants), the carbon absorption rates under the natural sun light (PAR of about 2,000 $\mu mol\ m^{-2}\ s^{-1}$) were the same as the maximal rates measured by the IRGA (maximal PAR of 1,600 $\mu mol\ m^{-2}\ s^{-1}$), so the data of A_{max} were grouped together.

There were clear differences in the photosynthetic parameters among the treatments. In the *in vitro* plants, some

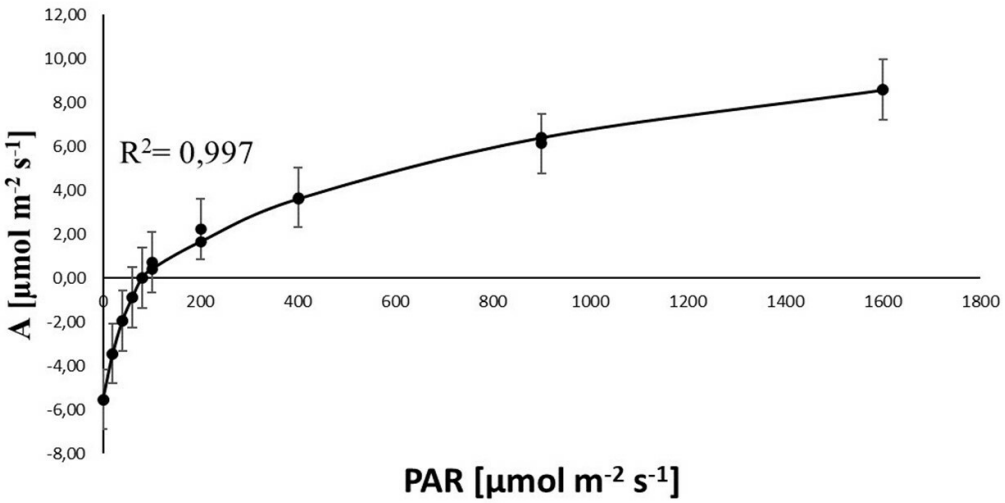

Figure 1. Light-response curve of A (net carbon assimilation rate) of leaves (n=10) of *Vellozia squamata* Pohl (Velloziaceae) in field conditions during the rainy season under different values of photosynthetically active radiation (PAR). Non-linear adjustment by the equation A_{meas}: $8.57\ (1-e^{-0.001658(PAR-80)})$

Table 1. Parameters of the experimental treatments with *Vellozia squamata* Pohl (Velloziaceae). A max: maximal CO_2 absorption rate, µmol CO_2.m^{-2}.s^{-1}; E: transpiration rate, mmol H_2O .m^{-2}.s^{-1}; LCP: light compensation point, µmol photon.m^{-2}.s^{-1}; LSP: light saturation point, µmol photon.m^{-2}.s^{-1}; L max: maximal PAR to which the plants are exposed, µmol photon.m^{-2}.s^{-1}; Chlorophyll: chlorophyll content, unities of chlorophyll (CCI); WUE: water use efficiency (µmol CO_2/mmol H_2O). Letters compare data in the same column. Distinct letters mean statistically significant difference (P<0.05) by Student´s T-test.

Treatments	A max	E	LCP	LSP	Lmax	Chlorophyll	WUE
Cerrado	8.57±1.07 a	4.76± 0.90 a	80	900	1,925	24.6±3.97 a	1.8
Greenhouse	3.42±0.28 b	0.92±0.54 b	60	200	412	2.99±1.48 b	3.7
In vitro	n/a	3.44±0.03 c	n/a	n/a	40	1.37±0.44 c	n/a
Garden	4.97 ± 1.43 d	1.16 ± 0.06 b	60	900	1,925	8.70 ± 3.2 d	4.3

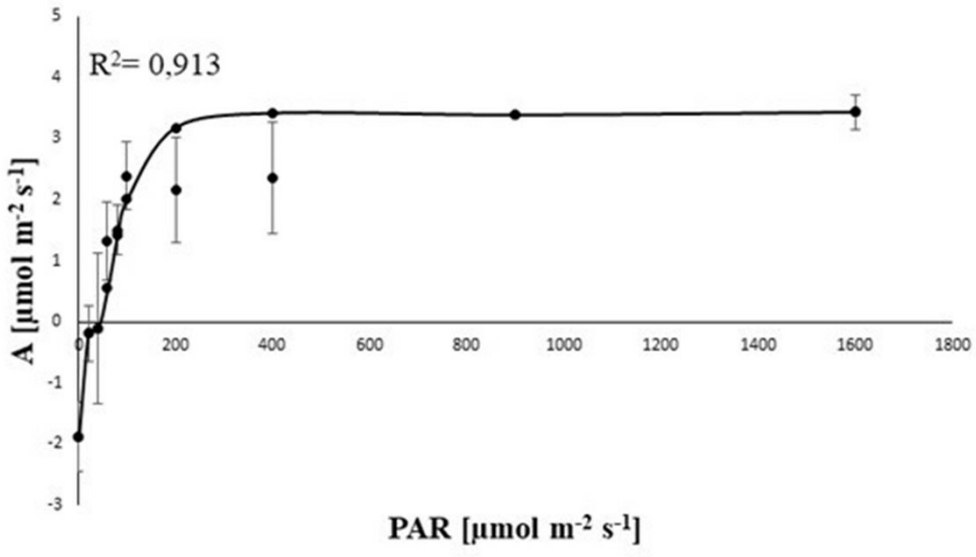

Figure 2. Light-response curve of A (net carbon assimilation rate) of leaves (n=10) of seedlings of *Vellozia squamata* Pohl (Velloziaceae) acclimatizing in greenhouse conditions under different values of photosynthetically active radiation (PAR). Non-linear adjustment by the equation A_{meas}: $3.42(1-e^{-0.0176833(PAR-60)})$

leaves were capable to absorb some carbon dioxide, mostly under light intensities between 100 and 200 µmol m^{-2} s^{-1}, but overall, the measurements showed only the respiration values, as the maximal values of A were of -0.35± 1.25 µmol CO_2 m^{-2} s^{-1}. As seen in figure 3, the leaves of the *in vitro* plantlets showed a random pattern of respiration up to light intensity of 100 µmol m^{-2} s^{-1}, above which they showed a reduction of the respiration rates up to the light intensity of 900 µmol m^{-2} s^{-1}. To light intensities higher than that, there was a slight deterioration in the respiration rate, suggesting photodamage. In this way, to the *in vitro* plants, there was no light compensation point.

The plants growing in the greenhouse were exposed to a maximum light intensity of about 412 µmol m^{-2} s^{-1}, that is, a little bit less than half of the light saturation point of the wild (control) plants (table 1). The maximum carbon absorption rate was inferior to the wild and the garden plants, but the shape of the light response curve followed a pattern as expected. To the greenhouse plants, although the light saturation point was of about 200 µmol.m^{-2}.s^{-1}, the carbon absorption rate was kept under higher light intensities, suggesting there was no photoinhibition (figure 2).

The leaf transpiration rates were kept constant throughout the measurements for the light response curves but varied among the experimental groups. The wild plants showed the higher transpiration rates, followed by the *in vitro* plants. The plants growing in the greenhouse and at the garden showed similar transpiration rates, but inferior to the wild plants. Thus, the instantaneous water use efficiency was higher in the young, garden growing plants than adult wild plants (table 1).

The chlorophyll content was slightly greater in the plants growing in the greenhouse than *in vitro*, but significantly inferior to those in the garden (table 1). This content was

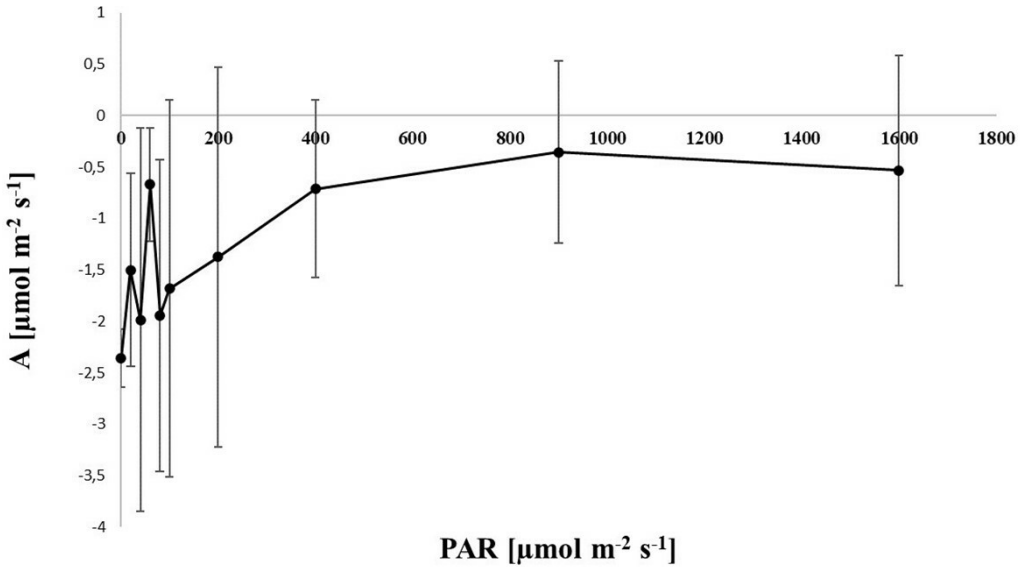

Figure 3. Light-response curve of A (net carbon assimilation rate) of leaves (n=10) of plantlets of *Vellozia squamata* Pohl (Velloziaceae) *in vitro* conditions under different values of photosynthetically active radiation (PAR). No adjustment of the curve was carried out.

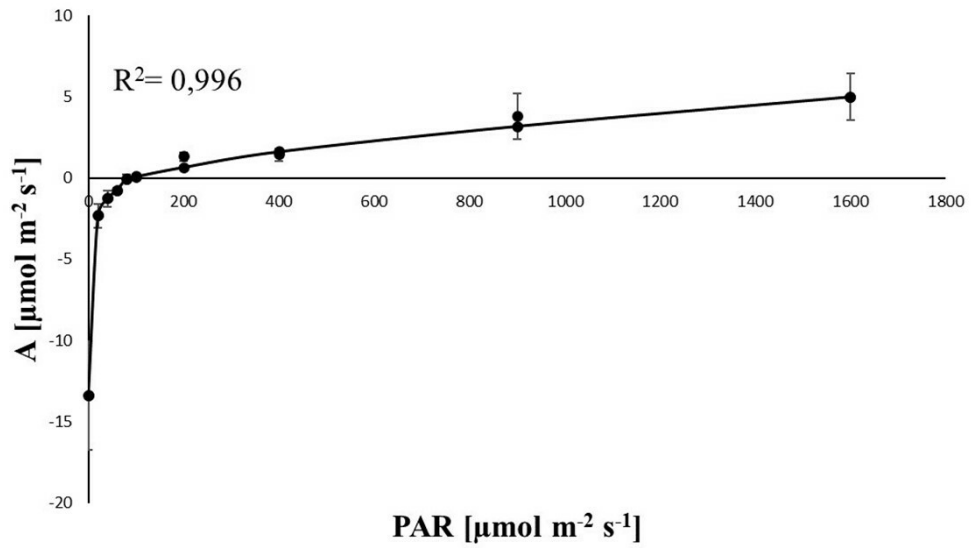

Figure 4. Light-response curve of A (net CO_2 assimilation rate) of leaves (n=10) of *Vellozia squamata* Pohl (Velloziaceae) seedlings obtained by *in vitro* propagation and growing in a garden during the rainy season under different values of photosynthetically active radiation (PAR). Non-linear adjustment by the equation A_{meas}: $4.97\ (1\text{-}e^{-0.00125(PAR-60)})$

greater in the garden plants than the greenhouse ones, but inferior to the wild plants, and the leaves presented a tonality of a lighter green, but not suggesting chlorosis, since the color intensity was uniform (figure 5). Leaf anatomy – The anatomical analysis showed the effects of environmental and growth conditions on the structure of *V. squamata* leaves (figure 6). In all leaves, the epidermis was single layered and without trichomes. In leaves of field grown plants (control group) and of garden condition (figures 6 a, f), the abaxial surface was smooth and the adaxial was corrugated. On the

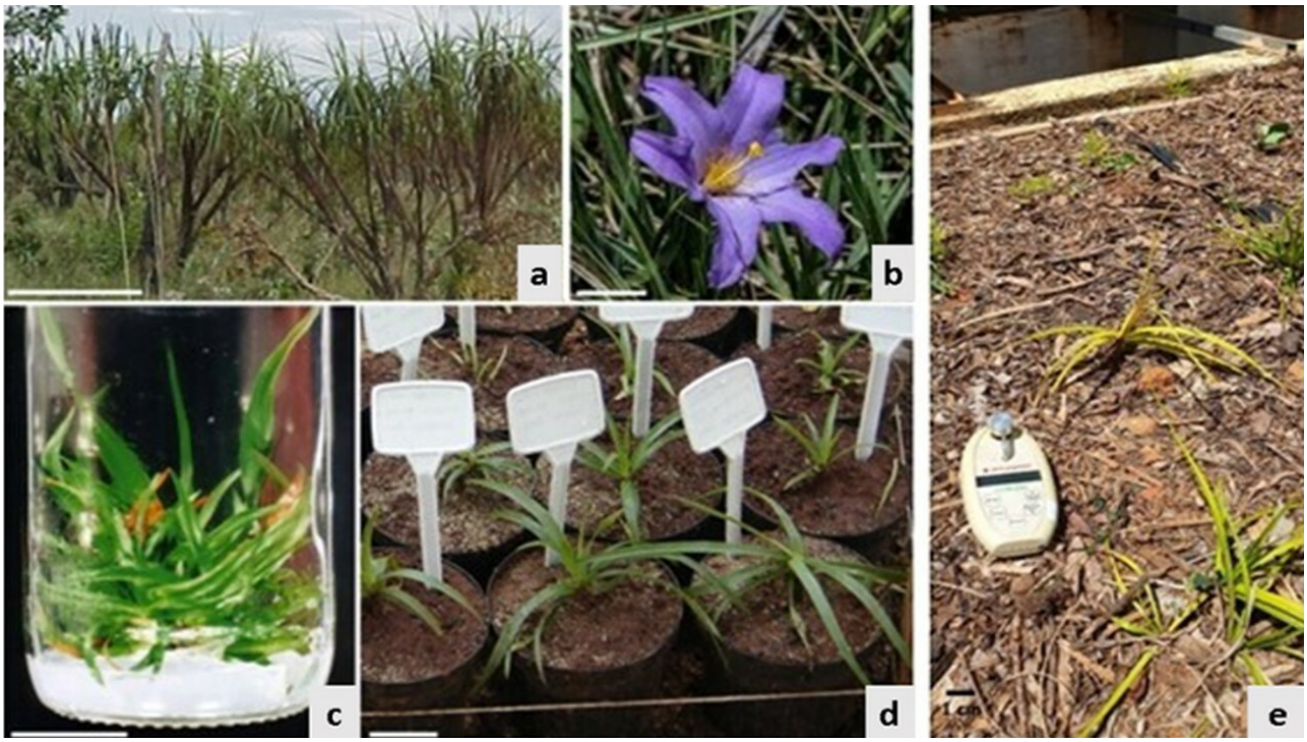

Figure 5. Plants of *Vellozia squamata* Pohl (Velloziaceae). a-b. In the field, showy lilac flower (b). c. *In vitro* condition. d. Greenhouse condition. e. Plants produced *in vitro* now growing at the garden. Bars: A = 1 m, B = 5 cm, C = 1 cm, D = 5 cm, E = 1 cm.

other hand, there was no visible epidermal ornamentation in leaves of *in vitro* grown plantlets nor of greenhouse acclimatizing plants (figures 6 d, e).

In the control group and in the garden group, the external periclinal cell wall and cuticle could not be distinguished from one another (figures 6 a, b, f), and these layers are called here the outer layer of epidermal cells. Hence, this layer in the plants under plentiful sunlight was considerably thicker (figures 6 a-c) than those in the other growth conditions (figures 6 d-e). Nonetheless, the epidermis presented an intermediate thickness in plants in the greenhouse condition (figure 6 e).

With respect to stomata disposition, *V. squamata* leaves were amphistomatic, and the stomata on the abaxial face were mainly found in crypts, which reached about a quarter of the leaf thickness (figure 6 a). These features were more noticeable in the control group and in the garden plants. It is noteworthy that *in vitro* plantlets did not develop stomatal crypts, and the stomata were rudimentary (figure 6 d). Additionally, plants in the greenhouse process had smaller and shallower crypts (figure 6 e) than those of field plants (control group) with fully developed stomata (figure 6 a).

The leaf mesophyll from control group plants was dorsiventral and composed of 21-25 parenchyma layers, which cells are elongated on the adaxial side and gradually diminishes in height towards the abaxial side, getting a rounded shape (figure 6 a, f). Both *in vitro* and greenhouse plants had a dorsiventral mesophyll (figures 6 d-e). Nevertheless, in these plants, the mesophyll presented a less dense cell arrangement than the mature leaves from the control (figures 6 a, f). Additionally, each leaf face of greenhouse, garden and control plants, the mesophyll possessed subepidermal fibre fascicles, which was more abundant in leaves from the control and garden plants (figures 6 a, f). Conversely, these fibre fascicles were not observed in leaves from the *in vitro* grown plants (figure 6 d).

The paralellodromous leaf veins from the control plants, garden and in greenhouse had a conspicuous vascular bundle sheath (figures 6 a, e-f). In addition, the bundle sheaths had extensions on the adaxial side with large colourless palisade cells termed as aquiferous (figures 6 a, e-f). This parenchyma connected the bundle sheath to subepidermal fibre fascicles on the adaxial epidermis (figures 6 a, e-f). On the abaxial epidermal face, the sheath was in direct contact with the epidermis (figures 6 a, e-f). Leaves from *in vitro* plants have bundle sheaths with no extensions (figure 6 d).

The vascular bundles were heavily fibrous with two strands of phloem placed laterally to the xylem (figures 6 a, e-f). However, this feature was less pronounced in leaves from *in vitro* plants (figure 6 d). The vascular fibres had thick lignified cell walls (figures 6 a, e-f). The vascular fibres were present in a greater amount in the control plants, in an intermediate amount in the greenhouse plants and in a small amount in the *in vitro* plants (figures 6 a, e-f).

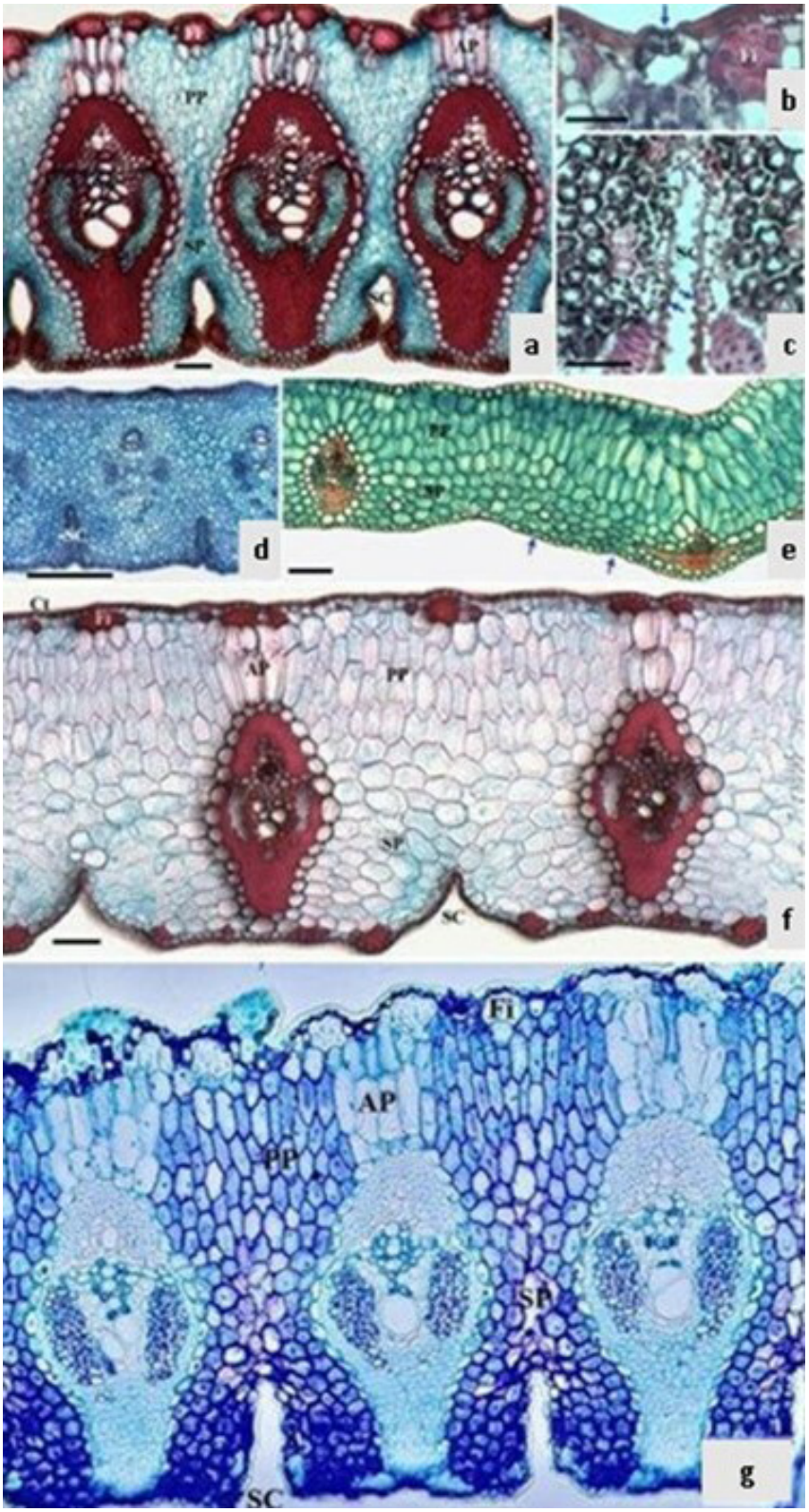

Figure 6. Leaf anatomy of *Vellozia squamata* Pohl (Velloziaceae). a-d. Plants from the field (b-c: details of a): adult (a-c) and young (d) leaves, showing the early formation of the stomatal crypts and later differentiation of the cuticle, subepidermal fibres, palisade parenchyma, and the vascular bundle sheath. e. Plants from micropropagation, not acclimatized: absence of stomatal crypts and subepidermal fibres. f. Plants from acclimatization similar to the adult leaf of the field, but with less deep crypts. g. Plants growing at garden condition. Arrows: stomata. AP: aqueous parenchyma, Ct: cuticle, Fi: fibres, PP: palisade parenchyma (elongated cells), SC: stomatal crypt, SP: spongy parenchyma (isodiametric cells). Bars a, d-g = 50 µm, b-c = 25 µm.

Discussion

The species of *Vellozia* grown in open fields show a strong photodissipating apparatus, which is important to protect the leaves from the stressful conditions of Cerrado – the dry season together with a high solar irradiance. Tocotrienols, which are strong antioxidant compounds of vitamin E complex basically found in seeds, have been identified in leaves of *V. gigantea* (Morales *et al.* 2014, 2015). Thus, at the same time those plants dissipate excess of light, they need high light intensities for total development of the photosynthetic apparatus and leaf structure.

The light intensity effect on photosynthetic aspects of *Vellozia* leaves has been more frequently evaluated by the electron transport rate. In several species of *Vellozia,* this rate is maximal under photosynthetically active radiation (PAR) intensities of about 2,100 $\mu mol.m^{-2}.s^{-1}$, which is coincident to the values commonly found in Cerrado, *i.e.* ca. 1,800 to 2,200 $\mu mol.m^{-2}.s^{-1}$ at midday (Lüttge *et al.* 2007). As for a comparison, the maximal electron transport rate of *Smilax goyazana,* a common shrub of Cerrado, occurs at about half of the maximal PAR of the environment (Palhares *et al.* 2011).

The leaves from plants grown *in vitro* conditions show typical effects of shading (Barboza *et al.* 2006, Chaari-Rkhis *et al.* 2015, Ziv & Chen 2008), with poorly developed anatomical structures, including stomata. Moreover, some ultrastructural features of shaded leaves are the limited cytoplasmic content and flattened chloroplast with an irregularly arranged internal membrane system and a higher proportion of intercellular spaces (Wetzstein & Sommer 1982, Chirinéa *et al.* 2012). In the present work, some of these features include qualitative alterations, as the absence of aquiferous cells, subepidermal sclerenchyma fascicles, and stomatal crypts.

It is noteworthy that the depth of the crypts reflected the growth condition. The stomatal crypts in the leaves are deeper in the control (field) and garden than greenhouse plants, and they were totally absent in plants grown *in vitro*. They are already present in the young leaves of field plants (figure 6 d). It is surprising that the presence of leaf crypts is not a genetical imposition for *V. squamata*.

Furthermore, leaves from *in vitro* plants of *V. squamata* presented several structural differences in comparison with plants in acclimatization: they had no subepidermal fibres, subtler dorsiventral mesophyll, undifferentiated bundle sheath extension, fewer vascular system fibres, as well as a lack of aquiferous parenchyma. The aquiferous tissue remains in the leaves from pineapple (*Ananas comosus* L.) grown *in vitro* (Barboza *et al.* 2006), so qualitative alterations like those observed in *V. squamata* are no ordinary thing, mainly the absence of stomatal crypts and subepidermal fibres.

The above differences can be directly associated with the low light levels and perhaps to the high humidity of the *in vitro* condition. The anatomical results indicated that *V. squamata* grown *in vitro* requires a longer and careful conducted acclimatization process.

The anatomical immaturity is linked to the immaturity of the photosynthetic apparatus. In the present case, the *in vitro* plantlets have inability of net carbon absorption, together with a high leaf transpiration rate, indicating dysfunctional stomata. A given light intensity may be enough for the plain development of the leaves of a given species, but insufficient for others. Thus, comparing with other Cerrado species cultivated *in vitro*, *Lippia rotundifolia* develops leaves capable of liquid carbon absorption under PAR of 78 $\mu mol\ m^{-2}\ s^{-1}$ (Hsie *et al.* 2019), while *Hanconia speciosa* and *Hyptis marrubioides* showed carbon absorption under PAR of 50 $\mu mol\ m^{-2}\ s^{-1}$ (Costa *et al.* 2014).

Regarding crop cultures, in the production of *in vitro* seedlings of strawberry (Grout & Millan 1985) and grape (Amancio *et al.* 1999), the growth under a PAR of 90 $\mu mol\ m^{-2}\ s^{-1}$ generates photosynthetically capable leaves with light saturation point around 450 $\mu mol\ m^{-2}\ s^{-1}$, while in *V. squamata*, the growth under maximal PAR of 412 $\mu mol\ m^{-2}\ s^{-1}$ still showed shade effects. In the Cerrado species *Pouteria gardneriana,* there were clear differences in the structural development of leaves growing under PAR varying from 75 to 150 $\mu mol\ m^{-2}\ s^{-1}$, but the authors had not shown data of wild plants as a control (Leite *et al.* 2017).

According to the data herein presented, even under low PAR intensities there is chlorophyll formation. However, the photosynthetic ability depends on the development of other elements that require higher light intensities. According to Leite *et al.* (2017), in *Pouteria gardneriana* the chlorophyll fluorescence did not vary substantially among the plantlets grown under the different PAR intensities, but the anatomical structure was more developed under the higher ones. In grape, the leaves grown in very low PAR intensities (as of 20 $\mu mol\ m^{-2}\ s^{-1}$) release oxygen, indicating that the photosynthetic apparatus is capable of carrying out photolysis of water, but they are not capable of carrying out net absorption of carbon dioxide (Amancio *et al.* 1999).

In the present experiment, the chlorophyll content was not directly measured, but the comparison carried out showed that the plants in the greenhouse, partially shaded, had about the double of chlorophyll (2.99 CCI) as the plantlets *in vitro* (1.37 CCI) but a little bit less than half of the open garden plants (8.70 CCI). However, all the experimental groups showed much less chlorophyll compared to the wild plants (24.6 CCI). Certainly, the improvement of PAR intensity from *in vitro* to garden condition could explain those differences seen, but it was not clear why the young plants at the garden showed a chlorophyll content of about one third of the wild plants. It is noteworthy that the anatomical structure of the leaves of the plants grown in the garden was overall similar to the plants in the fields, except for a smaller thickness. So, the growth under plentiful sunlight resulted in anatomically

mature leaves, but photosynthetically they showed a pattern of relative immaturity.

Moreover, the maximum carbon absorption rate of the plants at the garden was about 45% higher than the plants in the greenhouse but about 70% smaller than the wild plants. Spite the statistically significant differences among the experimental groups, some measurements of the maximum carbon absorption rate of the plants at the garden were close to the plants in the greenhouse. It is difficult to find in the field young plants of *Vellozia squamata* that could serve as for a comparison. It is not clear if the photosynthetic performance of the younger plants is something inherent to their youthfulness or if it is sort of a side effect of the protocol of micropropagation. Indeed, Durkovic *et al.* (2016) described that the way of obtaining seedlings can interfere in the leaf development, generating leaves with different photosynthetic parameters.

Regarding the leaf transpiration rates, the leaves of the plants of either the garden or inside the greenhouse showed differences compared to the wild plants, suggesting that the youthfulness of the plant is a factor that interferes with the physiological parameter of the leaves. Compared to other Cerrado species (Palhares *et al.* 2010), the adult wild plants of *V. squamata* showed a low water use efficiency. In this way, the leaves of the younger plants (of garden and greenhouse) presented a lower carbon assimilation rate but a better water use efficiency. Regarding the plantlets of *in vitro* condition, the higher transpiration rate is common to *in vitro* plantlets overall. The epidermis and stomata are poorly developed, both structurally and functionally, hence the control of the transpiration is weak, and this is one of the stressing factors during transition from *in vitro* to greenhouse growth (Van Huylenbroeck *et al.* 1998, Amancio *et al.* 1999, Kaur 2015).

According Van Huylenbroeck *et al.* (1998) and Kaur (2015), the *in vitro* propagated plants can be divided into two functional categories regarding the transition from *in vitro* to greenhouse phase: plants with photosynthetically incapable, in a which the leaves serve only as reservoir organ for the sprouting, and those photosynthetically active, whose leaves have at least partial development. In this last group, it is often seen that during the first days of transplantation there is a typical stress acute response, such as great rates of transpiration and respiration and eventually a reduction in the chlorophyll content (photobleaching; Van Huylenbroeck *et al.* 1998, Kaur 2015).

After this initial period, not only the leaves become photosynthetically active, but also they often show anatomical changes, such as cellular growth with reduction of stomatal density and differentiation of hairs (Van Huylenbroeck *et al.* 1998, Kaur 2015). Anyway, in both functional groups the *in vitro* shaded leaves cannot tolerate the environmental light intensities, but the newer leaves formed under those intermediary light intensities tend to present a light compensation point and a light saturating point like the wild plants, as well as a photo-dissipating apparatus better developed (Van Huylenbroeck *et al.* 1998, Kaur 2015).

The carbon assimilation rates verified in *Vellozia squamata* are relatively low, compared to other Cerrado species (Palhares *et al.* 2010), and according to Nogueira *et al.* (2004) are more typical of secondary than pioneer plants. That is, in spite of the large production of seeds with high germinability, the establishment of *Vellozia squamata* is related to a complex ecological interaction, highlighting the importance of the conservation of this species in the environment, since even the seedlings herein obtained are of a very slow growth.

As a conclusion, for improving the protocols, it is useful to compare the responses of the wild and the *in vitro* plants. The data herein presented may help to adjust the PAR intensity to obtain more mature seedlings with perhaps a higher survival rate at the transition between *in vitro* and greenhouse conditions. Therefore, the development of technology for producing seedlings of Cerrado species is a complex task, but of great importance for their conservation and domestication.

Acknowledgements

To the Programa de Pós-Graduação em Botânica, Universidade de Brasília, Instituto de Ciências Biológicas, Brasília, DF, Brazil. This research was sponsored by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Fundação de Empreendimentos Científicos e Tecnológicos, and Conselho Nacional de Desenvolvimento Científico e Tecnológico. Janet Nimoh Sallis Mensah reviewed the English grammar and spelling.

Author Contributions

Elson Calazans: Contribution to data collection; Contribution to data analysis and interpretation; Contribution to critical revision; Contribution to manuscript preparation.

Conceição Eneida: Contribution in the concept and design of the study; Contribution to manuscript preparation; Contribution to critical revision, adding intellectual content.

Olegário Garcia: Contribution to data collection.

Dario Palhares: Contribution in the concept and design of the study; Contribution to data analysis and interpretation; Contribution to manuscript preparation; Contribution to critical revision, adding intellectual content.

Luiz Alfredo: Contribution in the concept and design of the study; Contribution to manuscript preparation; Contribution to critical revision, adding intellectual content.

Sueli Gomes: Contribution in the concept and design of the study; Contribution to manuscript preparation; Contribution to critical revision, adding intellectual content.

Conflicts of interest

There is no conflict of interest.

Literature cited

Alves, R. 1994. Morphological age determination and longevity in some *Vellozia* populations in Brazil. Folia Geobotanica et Phytotaxonomica 29: 55-59.

Amancio, S., Rebordão, J. & Chaves, M. 1999. Improvement of acclimatization of micropropagated grapevine: photosynthetic competence and carbon allocation. Plant Cell Tissue and Organ Culture 58: 31-37.

Amaral, M. & Mello-Silva, R. 2008. Ontogenesis of stomata in Velloziaceae: paracytic versus tetracytic. Acta Botanica Brasilica 31(3): 529-536.

Ayensu, E.S. 1968. The anatomy of *Barbaceniopsis* a new genus recently described in the Velloziaceae. American Journal of Botany 55(4): 399-405.

Ayensu, E.S. 1969. Leaf anatomy and systematics of Old World Velloziaceae. Kew Bulletin 23(2): 315-335.

Ayensu, E.S. 1974. Leaf anatomy and systematics of New World Velloziaceae. Smithsonian Contributions to Botany 15:1-125.

Ayensu, E.S. & Skvarla, J.J. 1974. Fine Structure of Velloziaceae pollen. Bulletin of the Torrey Botanical Club 101(5): 250-206.

Barboza, S.B., Graciano-Ribeiro, D., Teixeira, J.B., Pontes, T.A. & Souza, L.A. 2006. Anatomia foliar de plantas micropropagadas de abacaxi. Pesquisa Agropecuária Brasileira 41(2): 185-194.

Batista, D. 2016. *Vellozia ramosissima*: estrutura populacional, anatomia foliar e avaliação nutricional em áreas de complexos rupestres, sob diferentes substratos, Serra do Espinhaço, MG. Dissertação de Mestrado, Universidade Federal do Vale do Jequitinhonha e Mucuri, Diamantina.

Borges, B. 2015. Regeneração in vitro de *Vellozia sincorana* Ayensu & Smith. M.Sci Dissertation, University of Feira de Santana.

Bucci, S.J., Scholz, F.G., Goldstein, G., Meinzer, F.C., Franco, A.C., Campanello, P.,Villalobos-Vega, R., Bustamante, M. & Miralles-Wilhelm, F. 2006. Nutrient availability constraints the hydraulic architecture and water relation in savannah trees. Plant Cell and Environment 29(12): 2153-2167.

Chaari-Rkhis, A., Maalej, M., Chelli-Chaabouni, A., Fki, L. & Drira N. 2015. Photosynthesis parameters during acclimattization of *in vitro*-grown olive plantlets. Photosynthetica 53(4): 613-616.

Chirinéa, C.F., Pasqual, M., Araujo, A.G., Pereira, A.R. & Castro, E.M. 2012. Acclimatization and leaf anatomy of micropropagated fig plantlets. Revista Brasileira de Fruticultura 34(4): 1180-1188.

Coetzee, H. 1974. Anatomy of the leaves of the Velloziaceae in South Africa and South West Africa and a key based on leaf anatomy. Dinteria 10: 19-33.

Costa, A.C., Rosa, M., Megguer, C.A., Silva, F.G., Pereira, F.D. & Otoni, W. 2014. A reliable methodology for assessing the in vitro photosynthetic competence of two Brazilian savanna species: *Hyptis marrubioides* and *Hancornia speciosa.* Plant, Cell, Tissue and Organ Culture 117: 433-454.

Durkovic, J., Canova, I., Javorikova, L., Kardosova, M., Lagana, R., Priwitzer, T., Longauer, R. & Krajnakova, J. 2016. The effects of propagation techniques on leaf vascular anatomy, modulus of elasticity and photosynthetic traits in micropropagated and grafted plants of the Dutch elm hybrid "Dodoens". Journal of the American Society for Horticultural Science 141(4): 351-362.

Field, C., Merino, J. & Mooney, H. 1983. Compromises between water-use efficiency and nitrogen-use efficiency in five species of California evergreens. Oecologia 60: 384-389.

Flora do Brasil. 2020. Jardim Botânico do Rio de Janeiro. Available at http://floradobrasil.jbrj.gov.br (access 26-VI-2021).

Garcia, Q. & Diniz, I. 2003. Germinative behavior of three species of *Vellozia* from Serra do Cipo, MG. Acta Botanica Brasilica 17(4): 487-494.

Garcia, Q., Jacobi, C. & Ribeiro, B. 2007. Germination response of two species of *Vellozia* (Velloziaceae) from the campos rupestres of Minas Gerais, Brazil. Acta Botanica Brasilica 21(2): 451-456.

Grout, B. & Millam, S. 1985. Photosynthetic development of micropropagated strawberry plantlets following transplanting. Annals of Botany 55: 129-131.

Hsie, B.S., Bueno, A.I., Bertolucci, S.K., Carvalho, A.A., Cunha, S.H., Martins, E.R. & Pinto, J.E. 2019. Study of the influence of wavelengths and intensities of LEDs on the growth, photosynthetic pigment, and volatile compounds production of *Lippia rotundifolia* Cham *in vitro.* Journal of Photochemistry and Photobiology B. 198: 111577.

Jacobi, C. & Sarto, M. 2007. Pollination of two species of *Vellozia* (Velloziaceae) from high altitude quartzitic grasslands, Brazil. Acta Botanica Brasilica 21(2): 325-333.

Johansen, D.A. 1940. *Plant microtechnique*. Mc Graw Hill, New York, 140 p.

Kaur, R. 2015. Photoautotrophic micropropagation, an emerging new vista in micropropagation. Agricultural Reviews 36(3): 198-207.

Kraus, J.E. & Arduin, M. 1997. Manual básico de métodos em morfologia vegetal. – Editora da Universidade Rural, Seropédica: Editora da Universidade Rural.

Leite, M., Silva, F.G., Assis, E.S., Neto, A.R., Mendes, G.C. & Rosa, M. 2017. Morphoanatomy and physiology of *Pouteria gardneriana* Radlk plantlets grown *in vitro* at varied photosynthetic photon flux densities. Acta Scientiarum 39(2): 217-224.

Lüttge, U., Duarte, H., Scarano, F., Mattos, E., Cavalin, P., Franco, A. & Fernandes G. 2007. Physiological ecology of photosynthesis of five sympatric species of Velloziaceae in the rupestrian fields of Serra do Cipo, Minas Gerais, Brazil. Flora 202: 637-646.

Medrano, H., Tomás, M., Martorell, S., Flexas, J., Hernández, E., Rosselló, J., Pou, A., Escalona, J.M., Bota, J. 2015. From leaf to whole-plant water use efficiency (WUE) in complex canopies: Limitations of leaf WUE as a selection target. The Crop Journal 3(3): 220-228.

Mello-Silva, R. & Menezes, N. 1999. Two new Brazilian Velloziaceae, *Vellozia auriculata* and *Vellozia gigantea* and a key to the related dracenoid species of *Vellozia*. Novon 9(4): 536-541.

Mello-Silva, R. 2010. Circumscribing *Vellozia hirsuta* and *V. tubiflora* (Velloziaceae). Hoehnea 37(3): 617-646.

Menezes, N.L. 1977. Aspectos morfológicos e anatômicos do desenvolvimento de plântulas de Velloziaceae. Boletim de Botânica da Universidade de São Paulo 5:65-77.

Menezes, N.L. 1988. Evolution of the anther in the family Velloziaceae. Boletim de Botânica da Universidade de São Paulo 10: 33-41.

Menezes, N.L. & Semir, J. 1991. *Burlemarxia*, a new genus of Velloziaceae. Taxon 40(3): 413-426.

Morales, M., Garcia, Q., Siqueira-Silva, A., Silva, M. & Munné-Bosch S. 2014. Tocotrienols in *Vellozia gigantea* leaves: occurrence and modulation by seasonal and plant size effects. Planta 240: 437-446.

Morales, M., Garcia, Q. & Munné-Bosch, S. 2015. Ecophysiological response to seasonal variations in water availability in the arborescent, endemic plant *Vellozia gigantea*. Tree Physiology 35: 253-265

Mota, L. & Garcia, Q. 2013. Germination patterns and ecological characteristics of *Vellozia* seeds from high-altitude sites in south-eastern Brazil. Seed Science Research 23: 67-74.

Neto, O. 2009. Micropropagação e anatomia foliar de canela-de-ema (*Vellozia flavicans* Mart. *ex* Schult f. – Velloziaceae) em diferentes condições ambientais. Masters dissertation, University of Brasília, Brasília.

Nogueira, A., Martinez, C., Ferreira, L. & Prado, C. 2004. Photosynthesis and water use efficiency in twenty tropical tree species of differing succession status in a Brazilian reforestation. Photosynthetica 42(3): 351-35.

Oliveira, P., Gibbs, P. & Proença, C. 1991. Pollination and breeding system of *Vellozia squamata* (Liliales: Velloziaceae): a species of the Brazilian Cerrados. Botanica Acta 104(5): 392-398.

Oliveira, R., Dawson, T. & Burgess, S. 2005. Evidence for direct water absorption by the shoot of the desiccation-tolerant plant *Vellozia flavicans* in the savannas of Central Brazil. Journal of Tropical Ecology 21: 585-588.

Owoseye, J. & Sanford, W. 1972. An ecological study of *Vellozia schnitzleinia*, a drought-enduring plant of Northern Nigeria. Journal of Ecology 60(3): 807-817.

Paiva, J., Fank-de-Carvalho, S., Magalhães, M. & Graciano-Ribeiro, D. 2006. Verniz vitral incolor 500: a mounting medium alternative and economically viable**.** Acta Botanica Brasilica 20(2): 257-264.

Palhares, D. 2004. Practical class proposal based on the germination of *Vellozia flavicans* Mart. *ex* Schult (Velloziaceae). Lecta-USF 22(1/2): 7-9.

Palhares, D., Franco, A. & Zaidan, L. 2010. Respostas fotossintéticas de plantas do Cerrado nas estações seca e chuvosa. Revista Brasileira de Biociências 8(2): 213-220.

Palhares, D., Franco, A. & Zaidan, L. 2011. Photosynthetic responses of *Smilax goyazana* (Smilacaceae). Phyton 51(1): 115-132.

Prado, C.H.B. & Moraes, J.A. 1997. Photosynthetic capacity and specific leaf mass in twenty woody species of Cerrado vegetation under field conditions. Photosynthetica 33(1): 103-112.

Quintao, F., Tavares, R., Vieira-Filho, S., Souza, G. & Santos, H. 2013. Hydroalcoholic extracts of *Vellozia squamata*: study of its nanoemulsions for pharmaceutical or cosmetic applications. Brazilian Journal of Pharmacognosy 23(1): 101-107.

Silveira, C., Fukuda, D., Palhares, D., Cury, N. & Pereira, L. 2018. A micropropagation protocol for the domestication of *Jacaranda ulei* (Bignoniaceae). Phyton 58(2): 165-174.

Smith, L.B. & Ayensu, E.S. 1974. Classification of Old World Velloziaceae. Kew Bulletin 29(1): 181-205.

Souza, V.C. 2005. Systematic botany: an illustrated guide for the identification of angiosperm families of the Brazilian flora, based on APG II. Instituto Plantarum, Nova Odessa.

Tribuiani, N., Silva, A., Ferraz, M., Silva, M., Bentes, A., Graziano, T., Santos, M., Cogo, J., Varanda, E. & Groppo, F. 2014. *Vellozia flavicans* Mart. *ex* Schult. Hydroalcoholic extract inhibits the neuromuscular blockade induced by *Bothrops jararacussu* venom. BMC complementary and alternative medicine 14: 48.

Van Huylenbroeck, J.M., Piqueras, A. & Debergh, P.C. 1998. Photosynthesis and carbon metabolism in leaves formed prior and during *ex* vitro acclimatization of micropropagated plants. Plant Science 134: 21-30.

Vieira, B., Bicalho, E., Munné-Bosch, S. & Garcia, Q. 2017. Abscisic acid regulates seed germination of *Vellozia* species in response to temperature. Plant Biology 19: 211-216.

Wetzstein, H.Y. & Sommer, H.E. 1982. Leaf anatomy of tissue-cultured *Liquidambar stryraciflua* (Hamamelidaceae) during acclimatization. American Journal of Botany 69(10):1579-1586.

Ziv, M. & Chen, J. 2008. The anatomy and morphology of tissue cultured plants. Pages 465-478 *In*: George EF, MA Hall, G-JD Klerk (eds.). Plant propagation by tissue culture. Vol. 1. Springer, Dordrecht.

Associate Editor: Ana Paula Souza Caetano
Received: 04/05/2020
Accepted: 18/01/2022

ISSN da publicação *online* 2236-8906