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Role of water stress as a stimulus for *in vitro* multiplication and its effects on biochemical response in *Vellozia* species

Papel do estresse hídrico como estímulo para multiplicação in vitro e seus efeitos na resposta bioquímica em espécies de *Vellozia*

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ABSTRACT

Water stress impairs plant growth, resulting in the death of the plant in extreme cases. *In vitro* studies on stress-tolerant species can serve as the basis for improvement through genetic modifications aimed at minimizing damage and providing a controlled environment for performing biochemical and physiological assessments of plants under stress. The Velloziaceae family includes desiccation-tolerant and fire-resistant species; thus, these species can be used for analyzing protective mechanisms and reproductive responses to stress. We hypothesized that species with adaptability to survival under extremely dry conditions would respond to *in vitro* water stress through resprouting. This study evaluated the extent of water stress induced by the addition of sucrose, mannitol, or polyethylene glycol, the role of water stress as a trigger for *in vitro* multiplication, and its effect on biochemical responses in *Vellozia jolyi*, *Vellozia punctulata*, *Vellozia pyrantha*, and *Vellozia seubertiana*. Independent experiments were conducted by supplementing the following concentrations of sucrose, mannitol, and polyethylene glycol to the MS culture medium: sucrose (30 g L^{-1} [control], 75 g L^{-1}, and 120 g L^{-1}) and mannitol (0.0 g L^{-1} and 15.96 g L^{-1}) for *V. pyrantha*; sucrose (15 g L^{-1} [control], 45 g L^{-1}, and 60 g L^{-1}) and mannitol (0.0 g L^{-1} and 7.9 g L^{-1}) for the other species; and polyethylene glycol (50 g L^{-1}, 100 g L^{-1}, and 150 g L^{-1}). When sucrose and mannitol were added, shoots had grown in all species, showing significant differences between treatments only for *V. pyrantha*. Polyethylene glycol did not induce shoot growth but, instead, diminished plant survival. The highest concentration of polyethylene glycol increased proline levels in *V. pyrantha*. All four species were resistant to water stress, owing to their ability to survive and reproduce under high concentrations of osmoregulators. Our study provides evidence that proline acts as an osmoprotectant of *V. pyrantha*.

Index terms: *In vitro* propagation; osmoregulator; proline; stress-tolerant species.

RESUMO

O estresse hídrico prejudica o crescimento das plantas, causando a morte em casos extremos. Estudos *in vitro* com espécies tolerantes ao estresse podem ser a base para o melhoramento genético, visando minimizar esses danos, além de fornecer um ambiente controlado para avaliações bioquímicas e fisiológicas de plantas sob estresse. A família Velloziaceae possui espécies tolerantes à dessecação e resistentes ao fogo, tornando-se um material valioso para a análise de mecanismos de proteção e resposta reprodutiva ao estresse. O objetivo foi avaliar o estresse hídrico com sacarose, manitol e polietilenoglicol como gatilho para multiplicação *in vitro* e seu efeito na resposta bioquímica de *Vellozia jolyi*, *Vellozia punctulata Vellozia pyrantha* e *Vellozia seubertiana.* Experimentos independentes foram realizados com concentrações de sacarose (30 controle; 75; 120 g L^{-1}) + manitol (0.0; 15.96 g L^{-1}) para *V. pyrantha*; sacarose (15 controle, 45 e 60 g L^{-1}) + manitol (0.0; 7.9 g L^{-1}) nas demais espécies e polietilenoglicol (0,0, 50, 100 e 150 g L^{-1}) ambos em meio de cultura MS. No teste com sacarose + manitol, foram observadas brotações em todas as espécies com diferença estatística entre os tratamentos apenas para *V. pyrantha*. O polietilenoglicol não influenciou a indução de brotações e interferiu na porcentagem de sobrevivência de todas as espécies. A maior concentração de polietilenoglicol aumentou o teor de prolina de *V. pyrantha*. As quatro espécies são resistentes ao estresse hídrico, devido à sua capacidade de sobreviver e se reproduzir em altas concentrações de osmorreguladores. Há evidências que a prolina é um dos osmoprotetores de *V. pyrantha*.

Termos para indexação: Propagação *in vitro*; osmorreguladores; prolina; espécies tolerantes a estresse.

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INTRODUCTION

Water stress is a major abiotic factor that suppresses plant growth and reduces agricultural cultivation (Rai; Rai, 2020), and the severity of the changes depends on the stress duration (Bita; Gerats, 2013; Hopkins, 2008). Water stress usually occurs due to drought, extreme temperature variation, saline soil, and high light intensity (Farooqi et al., 2020). In some stress-tolerant plants, water stress advances sexual or asexual reproduction (Hopkins, 2008).

Water restriction and high light intensity are marked stress factors in the natural environment of tropical regions, Velloziaceae species are widely and abundantly distributed (Porembski; Bathlott, 2000). Although Velloziaceae species are native of Africa, they occur predominantly in the *campos rupestres* of neotropical regions (Alcantara et al., 2018). In this region, peculiar plants of the Velloziaceae family, such as *Vellozia pyrantha* A.A.Conc. and *Vellozia seubertiana* Goethart & Henrard, have gained tolerance to recurrent fires through the resprouting mechanism (Conceição et al., 2016, 2017), and some others, such as *Vellozia punctulata* Seub. and *Vellozia jolyi* L.B.Sm., have gained tolerance to desiccation, as they withstand extreme water deficit through the anabiosis mechanism (Conceição; Pirani; Meirelles, 2007), that is, their metabolic functions are temporarily interrupted during water stress, but then they resume their normal function (Dinakar; Djilianov; Bartels, 2012).

In ecosystems with extreme environmental conditions, drought can act as a filter in the selection of plants with vegetative reproduction, as the plant tends to regenerate new branches in a dry environment (Chomicki, 2021). Resprouting depends on bud protection, bud development and distribution, and viable bud bank size of the plant, and the new branches originate from the bulb, rhizome, tuber, dormant axillary buds, and plant fragments, which enabled the plant to gain tolerance to different types of disturbances (Clarke et al., 2013; Klimes˘ova´; Klimes, 2007). Stress-tolerant plants exert their response through mechanisms that cause minimal damage to the plant through the production of osmoregulators and protective substances such as glycine, sorbitol, and proline, one of the most widespread (Molinari et al., 2007; Taiz et al., 2017). Therefore, understanding and predicting plant responses are important owing to evolving climate changes, which tend to intensify abiotic stresses (Pausas et al., 2015).

Studies investigating physiological and biochemical mechanisms related to plant stress have performed plant tissue culture (Claeys et al., 2014; Mollo et al., 2019; Moyankova et al., 2014; Wu; Zeng; Zhang, 2017), which is a set of biotechnological techniques that enable carrying out experiments in a controlled environment and reducing the physical space and time of collection of results, when compared with field studies (George; Debergh, 2008). During micropropagation, the multiplication process promotes shoot production, which is influenced by several factors, such as the balance between plant regulators (Grattapaglia; Machado, 1998) and sucrose concentration, which is implicated in plant growth and osmoregulation of the culture medium (Cao et al., 2003; Grattapaglia; Machado, 1998; Vinterhalter; Vinterhalter; Calovic, 1997). Apart from sucrose, mannitol and polyethylene glycol (PEG) are commonly added to the culture medium to simulate water stress, which stimulates shoot regeneration and enables the selection of plants with characteristics of interest (Rai et al., 2011).

In vitro culture that uses stress as a stimulus to induce vegetative regeneration in Velloziaceae species is still incipient, although thermal stress has been successfully applied to induce *in vitro* multiplication in *V. pyrantha*, wherein plants were directly exposed to fire for a reduced time (Borges et al., 2020). As for water stress, sucrose supplementation to the culture medium can induce vegetative regeneration, as seen in *Vaccinium corymbosum* L. (Cao et al., 2003) and *Solanum tuberosum* L. (Vinterhalter; Vinterhalter; Calovic,1997). Thus, considering the evidence on the activation of lateral buds in dry environments (Chomicki, 2021; Zeppel et al., 2015) and the tendency of plants to resprout from dormant axillary buds under extreme conditions (Chomicki, 2021), we hypothesized that plant species with adaptability to survival under severely dry conditions would respond to *in vitro* water stress through the resprouting mechanism. In this study, we evaluated the role of water stress as a trigger for *in vitro* multiplication and its effects on the biochemical response in four *Vellozia* species.

MATERIAL AND METHODS

Cultivation conditions

In vitro studies were conducted at the Laboratory of Plant Tissue Culture, and the analysis of proline concentration was performed at the Germination Laboratory, both at the State University of Feira de Santana. The cultures were incubated in a growth chamber at a temperature of 25 ±3 °C and for a 14-h photoperiod with photosynthetic radiation of 60 μmol m^{-2} s^{-1}. The MS culture medium (Murashige; Skoog, 1962) was used with half of the salt concentration (MS/2) and one-third of the

salt concentration (MS/3). In all experiments, 0.7% agar was added to the culture media for solidification (except for the PEG test), and after pH adjustment to 5.7 ±1, the media were autoclaved at 121 °C for 15 min.

Seed collection and *in vitro* germination

Seeds were collected in *campos rupestres* at Bahia in Brazil; particularly, *V. pyrantha* seeds were collected in Serra do Candombá (12°33′S 041°28′W), at the Chapada Diamantina National Park, Palmeiras, and the seeds of *V. seubertiana* (12°59′S 041°20′W), *V. punctulata* (12°59′S 041°20′W), and *V. jolyi* (12°59′S 041°20′W) were collected at the Mucugê Municipal Park. The seeds were removed from the capsules and stored in paper bags in the refrigerator at a temperature range of 6-10 °C for 3 months; after refrigeration, the seeds were washed under running tap water with detergent to remove surface dirt, after which they were disinfected with 70% alcohol (1′) and 2.5% hypochlorite (10′) in a laminar flow hood, with additional immersion in Bendasol fungicide (10′) and in hypochlorite (15′ for a prolonged time) for *V. punctulata* and *V. seubertiana* seeds owing to the persistence of fungal and bacterial contamination observed in preliminary tests.

V. jolyi, *V. seubertiana*, and *V. pyrantha* seeds were taken in vials containing 50 mL of MS culture medium (Murashige; Skoog, 1962), with half of the salt concentration (MS/2) and, for *V. punctulata*, one-third of the salt concentration (MS/3). Subsequently, sucrose (30 g L^{-1} for *V. pyrantha* and 15 g L^{-1} for the other species) was added to the culture medium, based on the results obtained in the *in vitro* culture of the species.

Effects on water stress induced by sucrose and mannitol on *in vitro* multiplication

In vitro germinated plants were inoculated in MS/2 (*V. jolyi*, *V. seubertiana*, and *V. pyrantha*) and MS/3 (*V. punctulata*) culture media. For *V. pyrantha*, the medium was supplemented with different concentrations of sucrose (30 g L^{-1} [control], 75 g L^{-1}, and 120 g L^{-1}) and mannitol (0.0 g L^{-1} and 15.96 g L^{-1}). For the other species, sucrose (15 g L^{-1} [control], 45 g L^{-1}, and 60 g L^{-1}) and mannitol (0.0 g L^{-1} and 7.9 g L^{-1}) were added to the media. For *V. pyrantha* and *V. seubertiana*, activated carbon (1 g L^{-1}) was added to the medium based on results obtained in the *in vitro* culture of the species in preliminary tests.

The experimental design used was complete randomization, totaling five treatments, with six replicates and three plots each for *V. seubertiana* and *V. pyrantha* and six replicates and two plots each for *V. punctulata* and *V. jolyi*.

Effects of water stress-induced with polyethylene glycol (PEG_{6000}) on *in vitro* multiplication, relative leaf water content, and proline content

In vitro germinated plants were inoculated in MS/2 (*V. seubertiana*, *V. pyrantha*, and *V. jolyi*) and MS/3 (*V. punctulata*) culture media supplemented with 50, 100, and 150 g L^{-1} PEG (PEG_{6000}) for *V. seubertiana* and *V. pyrantha* and 50 and 150 g L^{-1} PEG for *V. jolyi* and *V. punctulata*, with PEG_{6000} of 0.0 g L^{-1} as the control for all species. Germitest® paper was used as support for plantlet cuttings in the liquid medium, supplemented with sucrose (30 g L^{-1} for *V. pyrantha* and 15 g L^{-1} for the other species). The experimental design used was complete randomization, wherein each treatment contained nine replicates with two plots each for *V. pyrantha* and *V. seubertiana* and 10 replicates with one plot each for *V. jolyi* and *V. punctulata*.

To verify the percentage of relative leaf water content (RLWC%) and the proline concentration, control (0.0) and stress (150 g L^{-1} PEG_{6000}) treatments were repeated. The treatments included 15 replicates with five plots for *V. seubertiana*, 15 replicates with two plots for *V. pyrantha*, and 17 replicates with three plots for *V. punctulata*.

RLWC was calculated in six leaves (three leaves per plant) from each treatment, according to the formula RLWC (%) = (LFM − LDM/LTM − LDM × 100) as described by Weatherley (1950), where LFM is the fresh mass of the leaf, LTM is the turgid mass of the leaf, and LDM is the dry mass of the leaf. Proline quantification was performed according to the method described by Bates (1973). Briefly, 100 mg of fresh mass of leaves was used to prepare the extract with 3% sulfosalicylic acid. The samples for analyses were collected from the control and 150 g L^{-1} PEG treatment groups after 10 days of *in vitro* culture.

Analysis of variables

At 45 days after the multiplication experiments were conducted, the following parameters were evaluated: survival percentage (S%), percentage of plants responsive to shoot formation (%PR), number of shoots (NS), longest shoot length (LSL), number of leaves (NL), and dry matter (DM) of the shoots.

Statistical analyses

The mean values obtained with the plot values were compared using the Scott-Knott test at a 5% probability level, and the variables NS, LSL, NL, and DM were transformed with the square root of y + 0.5 − sqrt (y + 0.5). Linear regression analysis was performed to S% with mean

values obtained with different concentrations of PEG. We used the SISVAR program, version 5.3 (Ferreira, 2011). Data normality was evaluated with the Shapiro-Wilk test, using R software, version 4.0.5 (R Core Team, 2020).

RESULTS AND DISCUSSION

Effects of water stress induced with sucrose and mannitol on *in vitro* multiplication

All four species showed high survival rates in all treatments analyzed, but the mean values obtained with sucrose 60 g L^{-1} + mannitol 7.9 g L^{-1} for *V. seubertiana* (83.33%) were significantly lower than those in the other treatments. For the other species, no difference in the survival percentage was observed between the treatments (Figure 1). Considering that successive exposure to stress events leads to the activation of protective mechanisms that may culminate in resistance or tolerance of plant species (Bruce et al., 2007), the high survival rate of *Vellozia* species subjected to water stress may be attributed to the *campos rupestres* environment where they live, characterized by the presence of a shallow substrate susceptible to recurrent dry periods (Conceição; Pirani; Meirelles, 2007; Fernandes, 2016).

When the species were treated with a high concentration of osmoregulators, a stress signal was generated, such as change in the color of leaves with a violet shade among *V. pyrantha*, *V. punctulata*, and *V. jolyi* (Figure 1).

In experiments involving water stress, *V. punctulata* and *V. jolyi* produced shoots in the control and other treatment groups, while *V. seubertiana* produced shoots only in three of the stress treatment groups. For the three species, no significant difference was observed between the treatments for any of the variables analyzed (Table 1). The highest number of activated axillary buds was observed in *V. punctulata* and then in *V. jolyi* (Table 1).

Shoot formation due to water stress in the control and other treatment groups in *V. punctulata* and *V. jolyi* suggested good vegetative reproduction ability, even in an environment with reduced water availability, which may be attributed to the tolerance to desiccation. This response was also observed in another desiccation-tolerant plant, namely, *Haberlea rhodopensis*, whose habitat is similar to that of the species assessed in our study (Djilianov et al., 2005). The smaller emission of shoots and of visual signs of stress in *V. seubertiana* than in *V. punctulata* and *V. jolyi* suggests the tolerance of the plant species to water stress (Figure 1), indicating the need for a higher sucrose concentration for the plant to respond to stress, as detected in the preliminary tests with *V. pyrantha*.

The stress treatments influenced ($P \leq 0.05$) NS, % PR, LSL, and NL of *V. pyrantha* shoots. The highest mean values for NS, %PR, and NL were obtained in treatments with 75 and 120 g L^{-1} sucrose and with 120 g L^{-1} sucrose + 15.6 g L^{-1} mannitol (Table 1). The highest values of LSL for this species were obtained using 75 or 120 g L^{-1} sucrose (Table 1).

The efficiency of sucrose in shoot induction in *V. pyrantha* may be attributed to its double function in the culture medium, as an osmotic agent and a carbon source. Water stress caused by sucrose supplementation possibly activated the physiological pathways related to the production of plant hormones that promote multiplication, similar to the result obtained for *Solanum tuberosum*; the activation of the genes was associated with the production of cytokinin, auxin, and gibberellin in the resprouting phase of the plant (Bisognin et al., 2018; Gong et al., 2021; Suttle, 2007). Sucrose-induced shoot growth has also been observed in other species, such as *Vaccinium corymbosum* L. (Duke variety), at a concentration of up to 44 mM (15.06 g L^{-1}) (Cao et al., 2003) and *Solanum tuberosum* L. (potato) at sucrose concentrations of 50 g L^{-1}, 80 g L^{-1}, and 100 g L^{-1} in the culture medium (Vinterhalter; Vinterhalter; Calovic, 1997).

The positive effect of sucrose on the *in vitro* growth of *Vellozia*, confirmed based on the greater length and number of leaves of the shoots (Table 1; Figure 1B and C), can be attributed to the primordial role of sucrose in *in vitro* culture, which serves as the basis for carbon skeleton construction (George; Debergh, 2008). Mannitol supplementation to the culture medium induced a higher level of water stress than sucrose supplementation, which is marked by the decrease in shoot growth and number of leaves (Figure 1D and 1E). This reduction may be attributed to the lower water potential in the medium, which caused a decrease in the absorption of carbohydrates and water apart from the consequent reduction in cell expansion, thereby triggering a decrease in cellular and metabolic activity (Taiz et al., 2017); this result justified the evaluation of growth as a relevant characteristic for measuring the stress level (Claeys et al., 2014).

The peculiar response of the species assessed, in terms of survival maintenance and shoot production, demonstrates cellular protection against the deleterious effects of water stress, such as antioxidant production through the biosynthesis of flavonoids such as anthocyanin (He et al., 2010; Taiz et al., 2017), a substance possibly produced by *V. pyrantha*, *V. seubertiana*, and *V. jolyi*, given the violet shades observed in their leaves. A similar response was obtained when *Vitis vinifera* L. suspension cells

were used, wherein higher concentrations of anthocyanin produced in the culture medium with osmotic potential were reduced by sucrose and mannitol supplementation (Do; Cormier, 1990). These substances can combat reactive oxygen species, which, despite playing an important role in activating defense against water stress, are deleterious to the stability of the membranes, DNA, and other cellular structures when their levels increase (Taiz et al., 2017).

Effects on water stress with polyethylene glycol (PEG_{6000}) on *in vitro* multiplication, relative leaf water content, and proline content

The high concentration of PEG_{6000} in the culture medium caused chlorophyll degradation, indicated by the brown color of the leaves. PEG did not induce shoot generation in any of the species assessed. The addition of osmoregulators to the culture medium altered the survival percentage of all *Vellozia* species, with linear variation descending as a function of increasing concentrations and lower rates for *V. seubertiana* (39%), *V. pyrantha* (6%), *V. punctulata* (60%), and *V. jolyi* (10%) when adding 150 g L^{-1} PEG in the culture medium (Figure 2). The reduction in the survival rate of *Vellozia* species in the presence of a high PEG concentration indicates extreme water stress. The plants *V. jolyi* and *V. pyrantha* showed a genotype-dependent response and had the highest susceptibility to water stress at 150 g L^{-1} PEG, with survival rates of only 10% and 5.55%, respectively, when compared

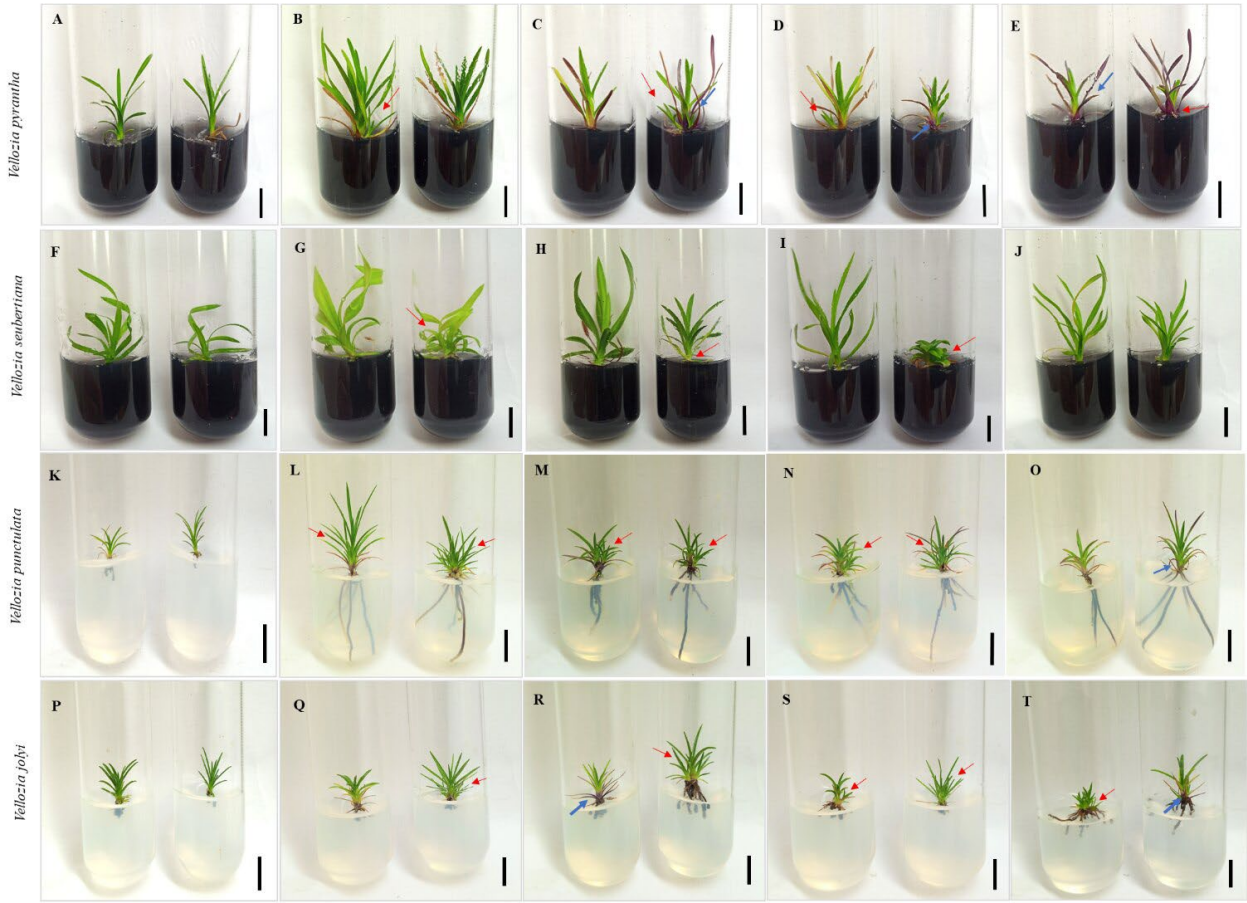

Figure 1: Four species of the genus *Vellozia* subjected to different concentrations of sucrose and mannitol: 30 g L^{-1} sucrose (control) (A), 75 g L^{-1} sucrose (B), 120 g L^{-1} sucrose (C), 75 g L^{-1} sucrose + 15.6 g L^{-1} mannitol (D) and 120 g L^{-1} sucrose + 15.6 g L^{-1} mannitol (E); 15 g L^{-1} sucrose (control) (F, K, and P), 45 g L^{-1} sucrose (G, L, and Q), 60 g L^{-1} sucrose (H, M, and R), 45 g L^{-1} sucrose + 7.9 g L^{-1} mannitol (I, N, and S), and 60 g L^{-1} sucrose + 7.9 g L^{-1} mannitol (J, O, and T) at 45 days after *in vitro* culture. The red arrow indicates the shoot, and blue arrow indicates leaves in violet shade. Scale bar = 1 cm

with the value of approximately 50% survival at high PEG concentrations in *V. punctulata* and *V. seubertiana*. Surprisingly, the plants had the ability to protect and maintain the cell structure after 45 days *in vitro*, a relatively long period of water restriction. In addition, the diversity of plant responses to extreme abiotic stresses is attributed to the dependence among genetic load, water stress duration, and water stress intensity (Zeppel et al., 2015).

The proline levels increased in *V. pyrantha* in the culture medium containing 150 g L^{-1} PEG; however, the level did not differ from that of the control to *V. seubertiana* (Table 2). The increase in proline levels in *V. pyrantha* promotes tolerance against water stress, considering its role in the protection and maintenance of the cell structure with osmoregulatory activity (Rai; Rai, 2020). Proline can also be advantageous as an alternative source of carbon and nitrogen for plant homeostasis after stress (Taiz et al., 2017).

Each plant group has one or two compatible osmolytes (Taiz et al., 2017), and probably, Velloziaceae plants accumulate another type of osmoprotective substance to minimize the damage, as a reduction in the RLWC of two species (*V. pyrantha* and *V. seubertiana*) was observed when they were treated with 150 g L^{-1} PEG (Table 3), but the proline concentration did not differ from that of the control for *V. seubertiana*.

Table 1: Number of shoots (NS) and percentage of responsive plants (%PR) of the four species of the genus *Vellozia* subjected to treatment with different concentrations of sucrose combined with mannitol at 45 days of *in vitro* culture.

Sucrose (g L^{-1})	Mannitol (g L^{-1})	NS	% PR	LSL (mm)	NL
			Vellozia punctulata		
15 (control)	------	0.33 a	25.00 a	0.13 a	0.75 a
45	------	1.41 a	50.00 a	0.50 a	3.25 a
60	------	1.00 a	41.66 a	0.49 a	2.58 a
45	7.9	0.91 a	50.00 a	0.38 a	2.91 a
60	7.9	0.66 a	41.66 a	0.15 a	1.08 a
			Vellozia jolyi		
15 (control)	------	0.16 a	8.33 a	0.09 a	0.50 a
45	------	1.50 a	41.66 a	0.31 a	2.16 a
60	------	0.50 a	25.00 a	0.26 a	1.33 a
45	7.9	0.75 a	25.00 a	0.21 a	1.00 a
60	7.9	0.83 a	33.00 a	0.21 a	2.25 a
			Vellozia seubertiana		
15 (control)	------	0.00 a	0.00 a	0.00 a	0.00 a
45	------	0.16 a	11.11 a	0.24 a	0.94 a
60	------	0.22 a	16.66 a	0.06 a	0.61 a
45	7.9	0.05 a	5.55 a	0.01 a	0.16 a
60	7.9	0.00 a	0.00 a	0.00 a	0.00 a
			Vellozia pyrantha		
30 (control)	------	0.00 b	0.00 b	0.00 b	0.00 b
75	------	0.44 a	27.77 a	2.14 a	0.77 a
120	------	1.16 a	41.66 a	2.13 a	1.08 a
75	15.6	0.11 b	11.11 b	0.39 b	0.16 b
120	15.6	0.72 a	27.77 a	0.61 b	0.55 a

Mean values with the same letters do not differ from each other, as assessed using the Scott-Knott test ($P \leq 0.05$).

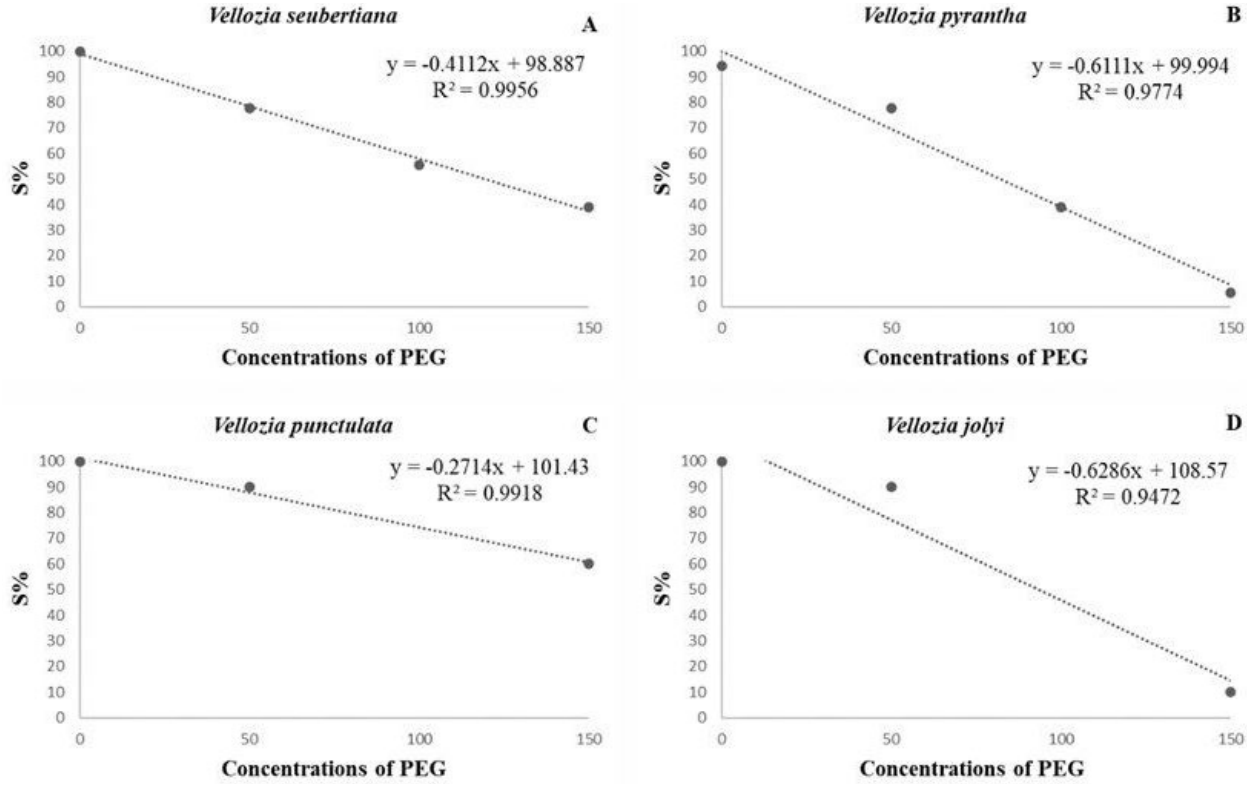

Figure 2: Survival percentage of plants (S%) of the genus *Vellozia* (four species) subjected to different concentrations of polyethylene glycol (PEG_{6000}) at 45 days of *in vitro* culture.

Table 2: Proline content in two species of the genus *Vellozia* after 10 days of *in vitro* culture under a high concentration of polyethylene glycol (PEG_{6000}).

PEG_{6000} (g L^{-1})	*Vellozia pyrantha*	*Vellozia seubertiana*
Control	9.46 b	10.25 a
150	16.11 a	11.56 a

Mean values with the same letters do not differ from each other, as assessed using the Scott-Knott test ($P \leq 0.05$).

The RLWC% in *V. punctulata* showed no difference between the PEG concentration (150 g L^{-1}) and the control (Table 3).

The stability of RLWC in *V. punctulata* may be attributed to its tolerance to desiccation because a similar response was observed in another desiccation-tolerant Velloziaceae species, *Barbacenia purpurea* Hook, which showed maintenance of leaf water potential even after 8 days of water stress, and the potential decreased only after 16 days of stress in plants evaluated in a greenhouse in winter (Suguiyama et al., 2016).

Table 3: Relative leaf water content (RLWC) of three species of the genus *Vellozia* after 10 days of exposure to a high concentration of polyethylene glycol (PEG).

PEG (g L^{-1})	*Vellozia pyrantha*	*Vellozia seubertiana*	*Vellozia punctulata*
Control	65.16 a	76.51 a	77.27 a
150	53.18 b	52.03 b	72.02 a

Mean values with the same letters do not differ from each other, as assessed using the Scott-Knott test ($P \leq 0.05$).

CONCLUSIONS

To the best of our knowledge, this study is the first to report *in vitro* vegetative reproduction in *Vellozia* species under induced water stress and to demonstrate alterations in endogenous hormone levels sufficient for clonal reproduction. Sucrose was efficient to induce shoots in *V. pyrantha* and caused a smaller visible stress level in *V. seubertiana*. The species *V. punctulata* and *V. jolyi* have the potential for easy *in vitro* multiplication. Furthermore,

we provide evidence that proline acts as a biochemical osmoprotectant in *V. pyrantha*.

AUTHORS CONTRIBUTION

Conceptual idea: Borges, B.P.S.; Methodology design: Borges, B.P.S.; Lima-Brito, A.; Data collection: Borges, B.P.S.; Data analysis and interpretation: Borges, B.P.S; Lima-Brito, A.; Conceição, A.A and Writing and editing: Borges, B.P.S; Lima-Brito, A.; Conceição, A.A.

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