Ciência Rural, Santa Maria, v.50:3, e20180941, 2020
http://dx.doi.org/10.1590/0103-8478cr20180941
ISSNe 1678-4596
CROP PRODUCTION

Vernonia condensata Baker: an alternative for large-scale seedling production

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ABSTRACT: *The increasing use of Vernonia condensata Baker highlights the importance of developing strategies to reduce the impact of exploitation on nature reserves. The aim of this study was to establish a micropropagation protocol to produce homogenous plants with high phytosanitary quality. Apical, nodal, and internodal segments of plants grown in the field were used for in vitro growth. The segments were disinfected in sodium hypochlorite solution (1.0 and 2.0%) for 15 and 30 minutes and then transferred to Petri dishes containing MS culture medium for 30 days. A completely randomized factorial experiment (3 x 2 x 2) with five replicates was designed. After this period, a completely randomized in vitro multiplication experiment was carried out with six treatments (BAP - 0.0; 0.5; 1.0; 1.5; 2.0; 2.5 mg L^{-1}) and six replicates. The shoots obtained in the best treatment were transferred to flasks with rooting medium (MS, MS/2 or MS/4). The experiment was completely randomized with 12 replicates. Microplants were acclimatized in polyethylene terephthalate (PET) bottles filled with autoclaved topsoil. Our results showed that 40.0% of the nodal segments (immersed in 1.0% sodium hypochlorite for 30 minutes) were adequately disinfected and survived. In the in vitro multiplication experiment, the 0.5 mg L^{-1} concentration of BAP yielded the highest number of shoots and the best vegetative growth. With regard to the assessed characteristics, MS/4 was the best rooting medium, with 100% survival during acclimatization. This study showed that V. condensata in vitro culture might produce 32,000 seedlings in 7 months.*
Key words: *cytokinin, in vitro culture, environmental sustainability.*

Uma alternativa para a produção de mudas de *Vernonia condensata* Baker em larga escala

RESUMO: *A crescente utilização de Vernonia condensata Baker evidencia a importância de desenvolver estratégias que reduzam o extrativismo nos ambientes naturais. O estudo objetivou estabelecer protocolo de micropropagação produzindo plantas homogêneas e de alta qualidade fitossanitária. Foram utilizados, na etapa de estabelecimento in vitro, segmentos apicais, intermodais e nodais de plantas cultivadas no campo. Desinfestados em solução de hipoclorito de sódio (1,0 e 2,0%) no tempo de 15 e 30 minutos e em seguida introduzidos em placa de Petri, com meio de cultura MS, durante 30 dias. Foi utilizado o delineamento inteiramente casualizado no esquema fatorial (3 x 2 x 2), com cinco repetições. Após este período, foi montado o experimento de multiplicação in vitro, em delineamento inteiramente casualizado com seis tratamentos (BAP - 0,0; 0,5; 1,0; 1,5; 2,0; 2,5 mg L^{-1}) e seis repetições. As brotações obtidas no melhor tratamento foram transferidas para frascos com meio de cultura de enraizamento (MS, MS/2 e MS/4). Esse experimento foi conduzido no delineamento inteiramente casualizado, com 12 repetições. A aclimatização foi realizada introduzindo as microplantas em garrafas do tipo PET contendo terra vegetal autoclavada. Os resultados mostraram que 40,0% dos segmentos nodais (imersos em 1,0% de hipoclorito de sódio, durante 30 minutos) foram desinfestados e sobreviveram. No experimento de multiplicação in vitro obteve-se a melhor resposta no número de brotos e no desenvolvimento vegetativo, na concentração de 0,5 mg L^{-1} de BAP. O meio de cultura de enraizamento que possibilitou a melhor resposta, nas características avaliadas, foi o MS/4. Durante a aclimatização obteve-se 100% de plantas sobreviventes, oriundas do meio MS/4. A realização desse trabalho permite estimar a obtenção de 32.000 mudas de V. condensata, após sete meses de cultivo in vitro.*
Palavras-chave: *citocinina, cultivo in vitro, sustentabilidade ambiental.*

INTRODUCTION

The use of plants with therapeutic properties has been increasingly recognized by society due to the search for healthier practices and the challenges encountered in treating some diseases, such as microbial resistance (BUGNO et al., 2005).

Phytotherapy has adapted to the reality and the need of many municipalities for basic health care. The reasons for the expansion of this type of alternative medicine included consumers' preference for natural treatments, scientific support of the pharmacological properties of medicinal plants, and low cost (MELO et al., 2007).

Received 11.14.18 Approved 12.17.19 Returned by the author 02.12.20
CR-2018-0941.R2

The National List of Medicinal Plants of Interest to the Public Health System (Relação Nacional de Plantas Medicinais de Interesse ao Sistema Único de Saúde – RENISUS) currently contains 71 species, including *Vernonia condensata* Baker (BRASIL, 2010). This plant is commonly known in Brazil as *alumã* (VICENTE et al., 2009) and *boldo-da-bahia* (AFONSO et al., 2015), among other names. It belongs to the family *Asteraceae* and has been used since colonial times to treat gastrointestinal problems and because of its analgesic properties (BOORHEM, 1999).

There is little information related to the market, consumption, and use of medicinal plants in Brazil, aspects that reflect the current scenario of production of poor quality plant material in the country (VEIGA-JUNIOR, 2008). Therefore, the use of biotechnological tools, such as tissue culture, in plants with medicinal properties will contribute to the generation of competitive and adapted germplasm, as well as to the development of species that supply biologically active compounds of interest to the pharmaceutical industry. In this sense, tissue culture tools can facilitate the commercial propagation of plants and the *in vitro* industrial production of secondary compounds (MORAIS et al., 2012; CARVALHO et al., 2013).

The increased demand for products derived from medicinal plants justifies the need for the application of biotechnological tools for large-scale seedling production. Thus, the use of micropropagation techniques to define protocols for *in vitro* propagation of medicinal species of interest to the Brazilian Public Health System (SUS) and to the pharmaceutical industry is of great relevance (SALES, 2015). However, several factors may influence the establishment of micropropagation methods. The genotype, type of explant, and culture medium are the main factors that control the development of plants grown *in vitro* (COSTA et al., 2006).

Given the above information, the aim of this study was to establish an efficient micropropagation protocol for *V. condensata* plants to provide plants for future pharmacological studies, for commercial production, and/or for the establishment of a collection or germplasm bank of this species.

MATERIALS AND METHODS

The study was conducted at the Laboratory of Biotechnology Applied to Health of Maria Milza School (Faculdade Maria Milza – FAMAM). Plants grown in the field, collected in the Recôncavo da Bahia region, were used as sources of explants. Explants consisted of a) nodal segments, b) internodal segments, and c) apical segments, all approximately 0.5 cm long.

Disinfection and in vitro establishment

An experiment was conducted combining explant types with sodium hypochlorite concentrations (1.0 and 2.0%) and immersion times (15 and 30 minutes). The explants were washed three times in autoclaved distilled water after the disinfection treatment. They were then transferred to Petri dishes (90 x 15 mm) containing 20 mL of MS culture medium (MURASHIGE & SKOOG, 1962) supplemented with 30 g L^{-1} of sucrose and solidified with 8 g L^{-1} of agar, and the pH was adjusted to 5.8 before autoclaving (121 ºC for 20 minutes).

The Petri dishes were kept in a growth room with a controlled temperature set at 25 ºC ± 2 ºC, 16-hour photoperiod, and light intensity of 40 µM m^{-2} s^{-1}, for 30 days. A completely randomized 3 x 2 x 2 factorial experiment (three explant types, two sodium hypochlorite concentrations, and two immersion times) with five replicates was conducted, with each replicate consisting of a Petri dish containing four segments. After 30 days of culture, the percentage of contaminated and surviving explants was evaluated.

In vitro multiplication

The surviving explants (shoots of nodal segments) were placed in 268-mL flasks containing 20 mL of MS culture medium supplemented with 1.0 mg L^{-1} of BAP (6-benzylaminopurine), according to VICENTE et al. (2009), under the previously described growth room conditions, to increase the number of shoots over another 30 days.

For the induction of *in vitro* multiplication, an experiment was set up using the previously obtained shoots, which were cut into segments of ± 1.0 cm in length containing one node and one leaf. These segments were inserted vertically into 268-mL flasks containing 20 mL of MS nutrient medium plus BAP (0.0; 0.5; 1.0; 1.5; 2.0 and 2.5 mg L^{-1}) and 30 g L^{-1} sucrose, solidified with 8 g L^{-1} agar and pH adjusted to 5.8, to induce maximum shoot proliferation.

A completely randomized experiment with six treatments and six replicates was conducted, with each replicate consisting of a vial containing five segments of ± 1.0 cm. The flasks were stored in a growth room under the same conditions as described above. After 30 days of cultivation, the following characteristics were evaluated: a) number of responsive explants, b) number of shoots per responsive explant, c) number of leaves per shoot, and d) shoot height in cm.

In vitro rooting and acclimatization

The shoots obtained in the treatment with the best induction of multiplication were used in the rooting step. In this step, the shoots were placed in flasks containing the rooting medium, which consisted of MS, MS/2 (50% of the mineral salts in the MS medium), and MS/4 (25% of the mineral salts in the MS medium). All media were supplemented with 30 g L^{-1} of sucrose and solidified with 8 g L^{-1} of agar, and the pH was adjusted to 5.8.

The flasks were kept in a growth room under the same conditions as in the previous steps. The experimental design was completely randomized with three treatments and twelve replicates, each consisting of a flask containing one shoot. After 30 days, the following characteristics were evaluated: a) percentage of rooted shoots (microplants), b) number of roots per shoot, and c) length of the longest root in cm.

After the rooting step, the microplants were washed in running water to remove the excess culture medium for evaluation of the aforementioned characteristics. They were then used in the acclimatization step, which consisted of placing the plants in polyethylene terephthalate (PET) soda bottles (2,000 mL) containing substrate (autoclaved topsoil), according to the method described by VICENTE et al. (2009). They were kept in an area covered with a shade cloth (70% light reduction), and the bottle tops were progressively removed for 10 minutes on the first day, 20 minutes on the second day, and so on, gradually increasing until complete removal of the bottle tops. The seedlings were irrigated daily with the aid of a spray bottle. The experimental design of the rooting step was maintained, and after 30 days, the percentage of surviving plants was evaluated. Statistical analysis was performed using SAS (Statistical Analysis System; SAS Institute, 2004).

RESULTS AND DISCUSSION

Explant disinfection and establishment

Results obtained in this experiment showed that, among the explants used, only the nodal segment showed survival. According to BRONDANI et al. (2013), the type of explant, asepsis method, and physiological conditions of the mother plant are factors that influence *in vitro* establishment. Results obtained herein reinforce the assertion of these authors because different types of explants from the same plant presented different responses to the asepsis treatments. This result might be attributed to the different amounts of microorganisms present in these plant parts, as well as to the possibility that the disinfection process did not effectively target some microorganisms. According to EVERT (2013), apical segments, for example, have leaf primordia that cover the meristems. Therefore, these primordia can protect microorganisms, not allowing the effective action of the decontaminating agent. The internodal segments are more exposed due to the absence of these structures; however, they may contain a greater amount of microorganisms on their surfaces, thus requiring a more rigorous disinfection processes.

According to table 1, there were significant differences among the tested treatments for the nodal segment explants. The combination of the concentration of 2.0% sodium hypochlorite with the immersion time of 30 minutes demonstrated the lowest percentage of contamination (30%). However, the non contaminated explants showed necrosis, marked darkening (oxidation), and did not survive (Figure 1A). It is possible that immersion of the explants at the 2.0% sodium hypochlorite concentration for 30 minutes was an important cause of the death of 70% of the microorganisms present but also presented a phytotoxic effect, leading to cell death by oxidation (Table 1).

VICENTE et al. (2009) reported that oxidation, observed in 2 to 14%, of their plants, affected the morphogenetic response in the disinfection of *V. condensata* explants. Both the concentration and immersion time in sodium hypochlorite can, in addition to eliminating microorganisms, cause cell death due to excess chlorine or a long exposure to the solution (SILVA et al., 2015). These authors disinfected seeds of the medicinal plant *Byrsonima intermedia* A. Juss. using a solution containing 1.0% active chlorine and varied the immersion time to up to 10 minutes to avoid phytotoxic effects. SOUZA et al. (2011) reported that the oxidation caused by sodium hypochlorite may be due to changes in cell membranes as well as in the oxygen supply to the explants. Therefore, it is possible that the supply of oxygen, released by sodium hypochlorite, contributed to enzymatic reactions involving phenolic compounds, which led to the death of the explants by oxidation.

With regard to the contaminated explants, it is important to mention that only fungal contamination was observed. In addition, the contaminated explants did not survive; the surviving explants were those that were not contaminated (Table 1). Additionally, according to table 1, the 1.0% sodium hypochlorite concentration combined with the 30 minutes immersion time was less effective for disinfection compared with the 2.0% concentration combined with the same immersion time, but it ensured the survival of the explants (Figure 1B). This result indicated that the immersion time of 30

Table 1 - Percentage of contamination and survival of nodal segments (explants) of *V. condensata* as a function of the sodium hypochlorite concentration and immersion time.

Immersion time (minutes)	----------Sodium hypochlorite concentration (%) ----------	
	1.0	2.0
---------- Percentage of contamination----------		
15	90aA	80aA
30	60bA	30bB
----------Percentage of survival----------		
15	10bA	20aA
30	40aA	0bB

Means followed by the same lowercase letter in the column and uppercase letter in the row do not differ significantly by the F-test ($P<0.05$).

minutes was not the main factor causing phytotoxicity to the explants, because when combined with the lowest concentration (1.0%), it ensured the survival of 40% of the explants (Table 1). It is possible that subdividing the sodium hypochlorite concentrations between 1.0 and 2.0% combined with different immersion times between 15 and 30 minutes may increase the efficiency of this process in future experiments. In addition to subdivision of the concentration and immersion time, other disinfection agents should also be tested.

In vitro multiplication

The results for the responsive explants and number of shoots are shown in table 2 and illustrated in figure 2, as it was not possible to fit equations with biological meaning and a high R^2 (coefficient of determination) to show the variation observed in these characteristics as a function of the BAP concentrations. Figure 2A shows small shoots established *in vitro*, which were transferred to flasks to ensure sufficient development for use as a source of explants (Figure 2B and 2C). Figure 2D shows explants from elongated shoots (nodal segments measuring ± 1.0 cm in length) containing one leaf, which were vertically introduced into the culture medium, representing an innovation of the method used by VICENTE et al. (2009) in the micropropagation of *V. condensata* Baker. Table 2 shows that the addition of BAP was essential for

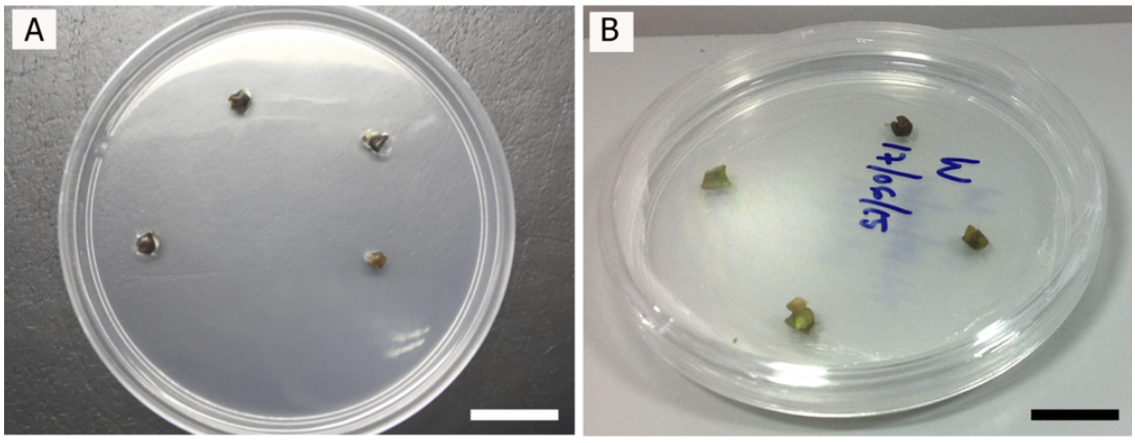

Figure 1 - Disinfection of nodal segments (explants) of *V. condensata*. A) Explants that were disinfected but which did not survive at a concentration of 2.0% sodium hypochlorite combined with 30 minutes of immersion time. B) Surviving explants at a concentration of 1.0% sodium hypochlorite combined with 30 minutes of immersion time. Bar = 50 µm.

Table 2 - Percentage of responsive explants and number of shoots per responsive explant as a function of the BAP concentration.

BAP concentrations (mg L^{-1})	Responsive explants (%)	Number of responsive shoots/explant
0.0	46.6 b	1.0 b
0.5	100.0 a	4.0 a
1.0	96.6 a	3.0 a
1.5	83.3 a	2.8 a
2.0	100.0 a	3.1 a
2.5	100.0 a	4.3 a

Means followed by the same letter in the column do not differ significantly by Tukey's test ($P<0.05$).

obtaining responsive explants, as the absence of this growth regulator resulted in only 46.6% responsive explants, differing significantly from all the other concentrations of BAP. The effect of BAP on this characteristic has already been widely demonstrated in the micropropagation of several plants of economic importance and has been prominent in the *in vitro* culture of medicinal species, as demonstrated by BEZERRA et al. (2014) for *Mimosa caesalpiniifolia* BENTH and by HOULLOU et al. (2015) for *Azadirachta indica* A. Juss, among others.

The addition of BAP was also essential for the induction of adventitious buds, increasing the number of shoots compared with the treatment without this growth regulator (Table 2). It was also observed that the 0.5 mg L^{-1} BAP concentration resulted in, on average, 4.0 shoots/explant (Table 2 and Figure 2E); although, it did not differ from the other treatments, except for the absence of BAP. However, this result might be associated with the residual effect of the cytokinin present in the culture medium (1.0 mg L^{-1} BAP) after the *in vitro* establishment step due to the need to increase the number of shoots for use in the *in vitro* multiplication experiment. In the low-cost micropropagation of banana cv. Maçã, BERNARDI et al. (2004) increased the BAP concentration in the nutrient medium after the *in vitro* establishment step, also with the aim of increasing the number of shoots.

The residual effect of BAP in the preculture medium was observed by JESUS et al. (2002), who reported an increase in the studied coffee plant variables when the explants were precultured in medium with BAP and subcultured in medium supplemented with this cytokinin. In *Hancornia speciosa* Gomes stem explants, SOARES et al. (2011) reported a greater number of shoots at the end of the preculture due to the presence of BAP in the culture medium, and the values remained constant in three subcultures. According to LENCINA et al. (2018), supplementation of the nutrient medium with BAP (2 mg L^{-1}) and subsequent subculture in culture medium without this cytokinin favors shoot induction and growth in *Apuleia leiocarpa* microstumps.

The BAP concentration used in the present study before the *in vitro* multiplication step was determined based on the results observed by VINCENT et al. (2009), who selected a BAP concentration of 1.0 mg L^{-1} from among the evaluated ones (0.0, 1.0, 2.0, 3.0, 4.0, and 5.0 mg L^{-1}) in a micropropagation study of the same species.

MARTINS et al. (2011) emphasized that lower growth regulator concentrations are more commonly used *in vitro* propagation methods to reduce the occurrence of somaclonal variation and to reduce cost.

VICENTE et al. (2009) worked with *V. condensata* Baker and reported the highest number of shoots at the concentration of 1.0 mg L^{-1} of BAP, observing a phytotoxic effect at BAP concentrations greater than 2.0 mg L^{-1}. For this reason, concentrations lower than those evaluated in that study were tested in the present study. However, a phytotoxic effect was observed at the highest concentrations used due to the reduction in the number of shoots and the production of hyperhydric shoots. Although, the concentration of 2.5 mg L^{-1} of BAP resulted in 4.3 shoots per explant, the vegetative growth of these shoots was negatively affected (Figure 2F). As the BAP concentration increased, there was lower shoot vegetative growth (Figure 3).

With the increase in BAP concentration, there was a decrease in the number of leaves (Figure 3A) and in shoot height (Figure 3B). The phytotoxic effect of the highest BAP concentrations was evidenced at the concentration of 2.5 mg L^{-1} of BAP, where many shoots showed hyperhydricity, a phenomenon known as vitrification. In addition, some studies have emphasized that shoots obtained from nutrient medium with lower concentrations of BAP exhibit greater vegetative

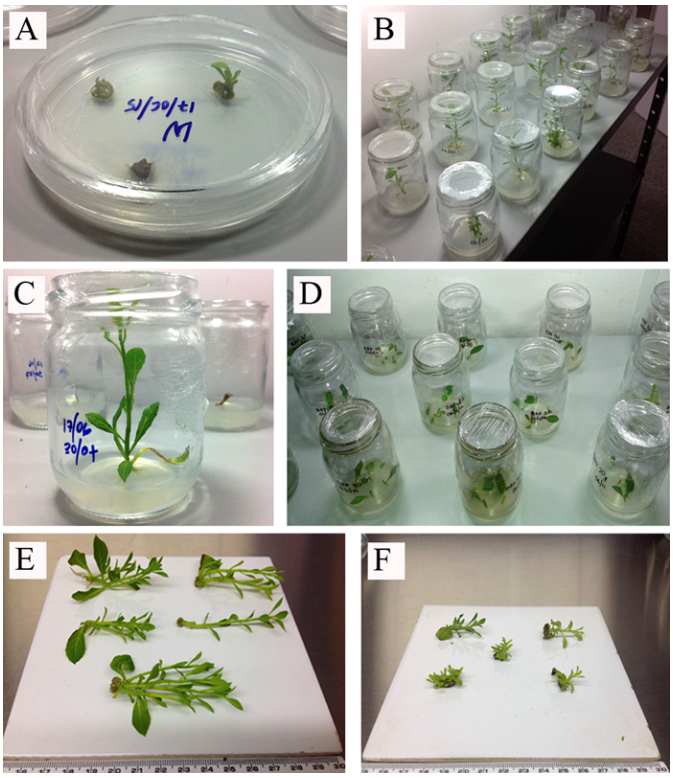

Figure 2 - *V. condensata in vitro* multiplication step. A) shoots established in Petri dishes after disinfection, containing MS culture medium. B) Elongated shoots in flasks containing MS culture medium supplemented with 1.0 mg L-1 of BAP. C) Shoot used as a source of explants for assembling the in vitro multiplication experiment. D) Explants used in the in vitro multiplication experiment: nodal segments ± 1.0 cm in length containing one leaf and introduced vertically. E) Multiple shoots from the treatment with 0.5 mg L^{-1} of BAP. F) Multiple shoots showing reduced size or hyperhydricity from the treatment with 2.5 mg L-1 of BAP.

development (VICENTE et al., 2009; ASMAR et al., 2011; ZURAIDA et al., 2016). According to BARRUETO CID & TEIXEIRA (2010), high cytokinin levels are among the factors that cause vitrification. BARBOSA et al. (2013) cultured strawberry *in vitro* and reported that the increase in the BAP concentration increased the odds of occurrence of hyperhydricity in the plants; concentrations greater than 2.0 mg L^{-1} of BAP induced greater formation of hyperhydric and oxidized shoots due to increased antioxidant activity and lipid peroxidation, in addition to cellular modifications (deformation of the stomata and epidermal cells).

To avoid obtaining hyperhydric shoots in the *in vitro* culture of *V. condensata* Baker, BAP doses below 2.0 mg L^{-1} should be used. VICENTE et al. (2009) reported vitrified shoots at BAP concentrations above 2.0 mg L^{-1} (starting at 3.0 mg L^{-1}) for *V. condensata* Baker, corroborating the data found in the present study. Even at the lowest BAP concentrations, those authors observed the beneficial effect of adding 1.0 mg L^{-1} GA_3 (gibberellic acid) to induce shoot elongation. Gibberellins are important in cell elongation and are synthesized mainly in young tissues of shoots, leaves, fruits, and seeds (TAIZ & ZEIGER, 2004). Therefore, it is possible that the vertical placement of the explants (young stem branch) and the presence of a young leaf favored the endogenous synthesis of gibberellins in the present study because the shoots elongated without the need for exogenous addition of GA_3, which is

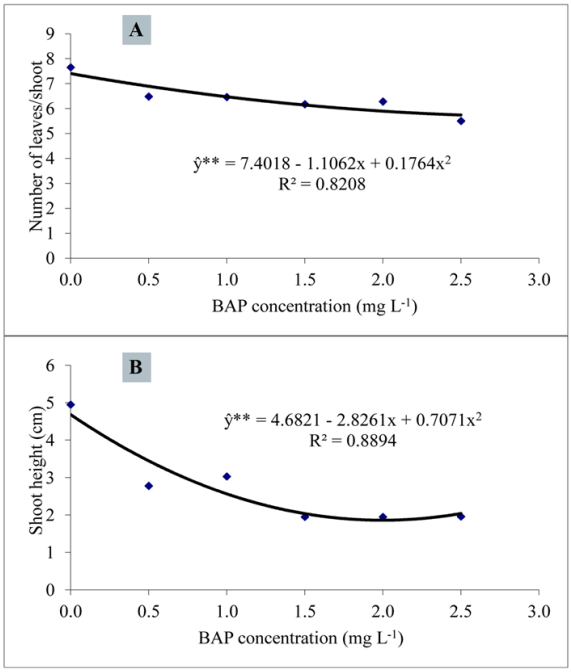

Figure 3 - Number of leaves and shoot height as a function of the BAP concentration. A) Number of leaves per shoot. B) Shoot height.

an economic advantage for the *V. condensata* Baker micropropagation protocol.

The use of 0.5 mg L^{-1} of BAP in the induction of shoots *in vitro* in *V. condensata* Baker, even with a preculture of 30 days in culture medium with 1 mg L^{-1} of BAP after the establishment step, was found to be the best condition, not only due to the reduced cost but also the absence of hyperhydric shoots and satisfactory *in vitro* elongation. Furthermore, it is estimated that 32,000 shoots can be produced with three subcultures in the *in vitro* multiplication step, as shown in figure 4.

In vitro rooting and acclimatization

The *in vitro* rooting and acclimatization data are shown in figure 5. Reducing the amount of salts in the MS medium resulted in 100% rooting of the shoots, demonstrating a significant difference from the MS medium, which showed only 58% rooting (Figure 5A). Regarding the number of roots and length of the longest root, reduction of the salts to 25% (MS/4) showed the best response, with a mean of 16 roots and 6.15 cm, respectively, demonstrating a significant difference from the other treatments, as shown in figures 5B and 5C. The performance of the culture media used in the rooting of the shoots is also illustrated in figure 6A. A lower rooting performance was observed in plants grown in MS and MS/2 culture media compared with MS/4 (Figure 6A).

Some studies have shown that the use of only basic MS medium is sufficient for the *in vitro* rooting of medicinal plants (OLIVEIRA et al., 2011; MONFORT et al. 2012). In addition, other studies have demonstrated the need to use plant growth regulators (especially auxin) or activated charcoal (NAVROSKI et al., 2014; RESENDE et al., 2015; HOULLOU et al., 2015). Reducing the amount of salts in the nutrient medium has been a strategy that is frequently used to induce *in vitro* rooting (DINIZ, 2014). For example, the use of MS/4 culture medium has provided promising responses for obtaining *in vitro* adventitious roots in some species, as observed in studies conducted with *Azadirachta indica*, *Lychnophora pinaster*, and *Pluchea sagittalis* (CHATURVEDI et al., 2004; SOUZA et al., 2004; ROSSATO et al., 2015). According to WAREING & PHILLIPS (1982), rooting is favored in more diluted culture media, which may be explained by the cumulative synthesis of endogenous auxins due to the low availability of salts in the culture medium, increasing the metabolic activity of the tissue and, consequently, root induction.

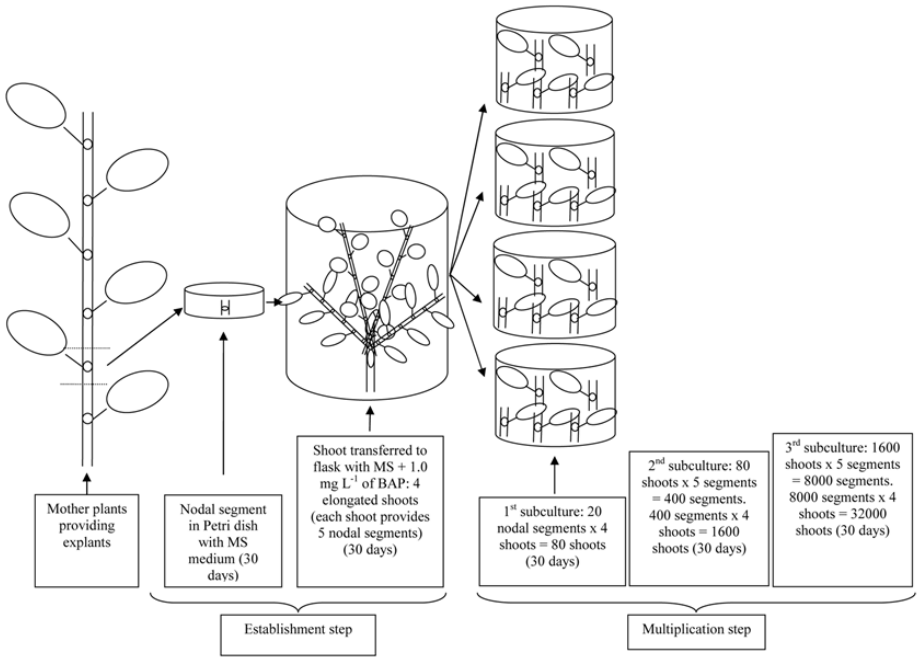

Figure 4 - Diagram illustrating the estimation of the *in vitro* production of 32,000 *V. condensata* shoots from a nodal segment cultured in Petri dishes and flasks containing MS culture and MS culture medium supplemented with 1.0 mg L^{-1} of BAP, respectively, in the establishment step and grown in flasks containing MS medium supplemented with 0.5 mg L-1 of BAP in the multiplication step.

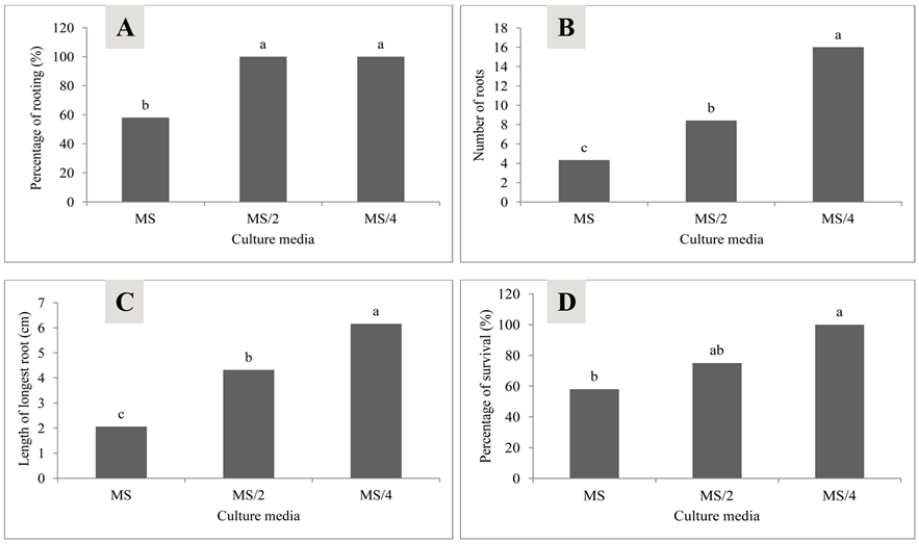

Figure 5 - *In vitro* rooting and acclimatization of *V. condensata* microplants as a function of the culture media. A) Percentage of rooting. B) Number of roots. C) Length of the longest root. D) Percentage of survival during acclimatization. MS = 100% of the mineral salts in the MS culture medium; MS/2 = 50% of the mineral salts in the MS medium; MS/4 = 25% of the mineral salts in the MS medium. Bars followed by the same letter do not differ significantly by Tukey's test (P<0.05).

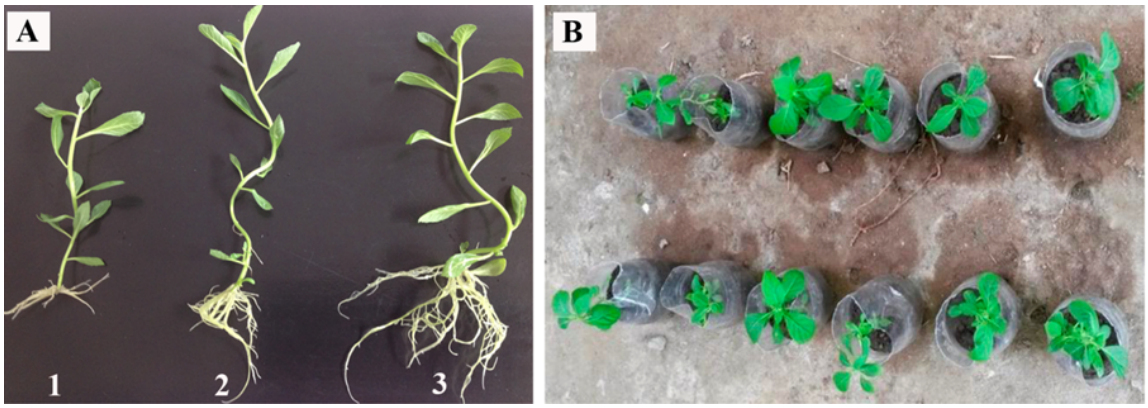

Figure 6 - *In vitro* rooting and acclimatization in the micropropagation of *V. condensata*. A) Root development: 1) microplant from MS culture medium (100% salts), 2) microplant from MS/2 culture medium (50% of the MS salts), and 3) microplant from MS/4 culture medium (25% of the MS salts). B) Plants acclimatized in PET soda bottles containing autoclaved topsoil, with 100% survival and originating from MS/4 medium.

The results of the acclimatization step are shown in figure 5D. Microplants from MS/4 medium showed 100% survival, which did not differ significantly from those from the MS/2 culture medium, with 75% survival. In turn, microplants from MS medium showed only 58% survival. These results can be explained by the higher percentage, number, and length of roots provided by the MS/4 culture medium (Figure 6A), which might have been essential for ensuring the survival of all evaluated microplants (Figure 6B). Plants from standard MS medium presented only 58% rooting at the rooting step (Figure 5A) and this same percentage of survival in the acclimatization step (Figure 5D), whereas plants from MS/2 and MS/4 media presented 75 and 100% acclimatized plants, respectively (Figure 5D). Based on these results, the rooting step is crucial for successful acclimatization of plants from *in vitro* culture, as well as for their transfer to field conditions, as observed in another study conducted with *V. condensata* Baker (VICENTE et al., 2009). Thus, the nonsurvival of shoots from MS medium might be explained by the absence of roots. Similar results were observed by RESENDE et al. (2015) in the micropropagation of *Lippia rotundifolia* and by HOULLOU et al. (2015) in the micropropagation of *Azadirachta indica* (neem).

CONCLUSION

The concentration of 1.0% sodium hypochlorite combined with 30 minutes of immersion ensures the survival and development of explants.

A concentration of 0.5 mg L^{-1} of BAP allows the proliferation of shoots with the best vegetative development conditions. The best conditions for rooting are provided in MS/4 culture medium. The developed acclimatization system enables 100% survival of microplants originating from MS/4 culture medium. It is possible to estimate a production of 32,000 *V. condensata* Baker seedlings with three subcultures.

ACKNOWLEDGMENTS

The authors thank Faculdade Maria Milza for providing the physical infrastructure and financial support essential for this study and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for partial finance support, Brasil - Finance code 001.

DECLARATION OF CONFLICT OF INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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