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Original Paper

Baccharis vulneraria (Asteraceae) cuttings in different substrates

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Abstract

The objective of the study was to identify the most suitable substrate and type of cutting to obtain seedlings from *Baccharis vulneraria* Baker cuttings. Apical and stem cuttings were collected from stock plants grown in a greenhouse. After collection, the cuttings were grown in the following substrates: commercial peat-based substrate (CPS); Vermiculite (VM); carbonized rice husk (CRH); Coconut fiber (CF); VM and CRH (1:1) (VM + CRH); VM and CF (1:1) (VM + CF); and CRH and CF (1:1) (CRH + CF) mixtures. The experiment used a completely randomized factorial design and, after 60 days, the percentage of survival and rooting (% SR), aerial part length (APL) and roots length (RSL), number of shoots (SN) and new leaves (LN), root volume (RSV), aerial part dry mass (APDM), and roots dry mass (RSDM) were evaluated. An interaction was found between the type of substrate and cutting for APL, RSL, LN, RSV, and RSM. On the other hand, %SR, SN and APDM differed only by cutting type. Apical cuttings showed higher APL, while internode cuttings showed higher SN, LN, RSV, and APDM. CPS promoted higher RSL in both types of cuttings, being a suitable option for the propagation of *Baccharis vulneraria*, particularly in internode cuttings.

Key words: *Asteraceae*, bioprospection, medicinal plants, propagation.

Resumo

O estudo objetivou identificar o substrato e o tipo de estaca adequados para a obtenção de mudas por estaquia de *Baccharis vulneraria* Baker. Foram coletadas estacas apicais e caulinares de plantas matrizes mantidas em casa de vegetação e cultivadas nos substratos: substrato comercial à base de turfa (CPS); Vermiculita (VM); casca de arroz carbonizada (CRH); Fibra de coco (CF); VM e CRH (1:1) (VM + CRH); VM e CF (1:1) (VM + CF); CRH e CF (1:1) (CRH + CF). O delineamento foi inteiramente casualizado em esquema fatorial e, após 60 dias foi avaliado: percentual de sobrevivência e enraizamento (% SR), comprimento da parte aérea (APL) de raízes (RSL), número de brotos (SN) e de folhas novas (LN), volume de raiz (RSV), massa seca da parte aérea (APDM) e de raízes (RSDM). Houve interação entre o tipo de substrato e estaca para APL, RSL, LN, RSV e RSM. Já %SR, SN e APDM diferiram somente para o tipo de estaca. Estacas apicais apresentaram maior APL enquanto as de entrenó demonstraram maior SN, LN, RSV e APDM. CPS promoveu maior CSR nos dois tipos de estacas, sendo uma opção adequada para propagação de *Baccharis vulneraria*, principalmente em estacas de entrenó.

Palavras-chave: *Asteraceae*; bioprospecção; plantas medicinais; propagação.

Introduction

The genus *Baccharis* is represented by more than 500 species, most of which are distributed in Brazil, Argentina, Chile, Colombia, and Mexico (Verdi *et al.* 2005). Species in this genus are relevant in folk medicine, with several uses for diseases control and treatment, as well as economic and environmental aspects. Studies on the species

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of this genus have shown considerable progress due to their extensive use in folk medicine in Brazil and other South American countries (Campos *et al.* 2016). Among its biological activities, the allelopathic, antimicrobial, cytotoxic, and anti-inflammatory effects stand out (Verdi *et al.* 2005).

Baccharis vulneraria Baker (Asteraceae) is a medicinal plant, popularly known as *Erva-santa*, found in southern and southeastern Brazil, in the Atlantic Forest and Pampa biomes. It also occurs in Paraguay, Uruguay, and Argentina (Heiden *et al*. 2012). It is used in folk medicine as an aid in wound healing (Stuker 2007). Although it has a kind of popular therapeutic value, there are no reports in the literature regarding its propagation methods.

Works on propagation of medicinal plants are important to ensure the standardization of seedlings, as well as quality and large-scale production. Furthermore, knowledge of appropriate techniques for their use is also necessary (Lusa & Biasi 2010). Standardization in seedling production and growing conditions can prevent variations in essential oil production, as well as mitigate the interference in metabolic compound production caused by abiotic factors in the sites where the plants grow, including soil nutrient availability (Morais 2009).

Among all the factors that might interfere with the active principles of plants, nutrition is one of the most critical. The deficiency or excess of one or more minerals in the soil is directly related to the variation in the production of active substances (Silva 2015). Therefore, seedling production for essential oil extraction is extremely important, as it allows the control of various factors, such as temperature, irrigation, substrate type and nutrients (Azambuja 2012).

Studies have shown that different substrates can have significant impacts on germination, plant growth, flowering, and fruit quality (Ahmad *et al.* 2012, El-Sayed *et al.* 2016; Leroy *et al.* 2017). This is largely because of the difference in factors such as nutrient composition and form, pH, electrical conductivity, and availability of organic compounds (Caballero *et al.* 2007; Ahmad *et al.* 2012). Moreover, plant propagation from cuttings can also be affected by the length and position of the cutting on the branch, resulting in different survival, rooting, and growth rates (Zigene & Kassahun 2016; Verdi *et al.* 2020).

In addition to controlling environmental factors, the use of a protected environment, such as greenhouses, can provide different benefits for seedling production. These include reducing physiological stresses on plants, promoting better growth, increased production, improvement of the quality of essential oil production, and enabling greater control of pests and diseases (Silva 2015). Given the potential impacts of substrates and cutting types on seedling production, this study aimed to identify the most suitable substrate and cutting type for obtaining seedlings from *B. vulneraria* cuttings.

Material and Methods

Specimens were identified by the botanist Elisete M. de Freitas with the use of dichotomous keys and herbarium validation. The scientific name followed the *Flora e Funga do Brasil* classification. One fertile sample was deposited in the HVAT Herbarium at the Science Museum of Univates, under the registration number HVAT 5846. *Baccharis vulneraria* propagation tests were carried out in automated agricultural greenhouse (*Van Der Hoeven*®), with mean temperature of 27°C and 82% relative air humidity. Stock plants (60) were obtained from the germination of propagules collected in situ from the population in the municipality of Sério, Rio Grande do Sul, Brazil (29°24'10.5''S and 52°12'54.8''W). The climate in this region is classified as Cfa-type (subtropical climate with hot summers) according to the Kppen and Geiger classification system, with an average temperature of 17.6 °C and annual rainfall of 2041 mm (Climate 2021). The plants were grown in 2.5-L pots filled with commercial peat-based substrate and kept in a greenhouse with a sprinkler irrigation system. After eight months of cultivation, the apical and stem cuttings (internodes) with 5 to 7 cm in length and containing two buds were collected and the leaves were removed. From the preparation of the cuttings to planting, these were kept immersed in water to prevent dehydration. For planting, the cuttings were placed in polypropylene trays containing 96 cells, each cell with a capacity of 100 cm^3, filled with different substrates and seven treatments: (1) Commercial peat-based substrate (CPS); (2) Vermiculite (VM); (3) Carbonized rice husk (CRH); (4) Coconut fiber (CF); (5) Vermiculite and carbonized Rice Husk (1:1) (VM + CRH); (6) Vermiculite and Coconut Fiber (1:1) (VM + CF); (7) carbonized rice husk and Coconut fiber (1:1) (CRH + CF).

The experiment was designed using a completely randomized factorial scheme [two types of cuttings (apical and Internode) x seven substrates], with five repetitions of ten cuttings each, totaling 50 cuttings per treatment. Intermittent nebulization was controlled by a timer, with cycles of 15 seconds every 20 minutes, and relative humidity was maintained above 90%. The temperatures recorded during the experiment were obtained using an Akso AK174 thermo-hygrometer placed inside the greenhouse throughout the experiment, with average, maximum and minimum temperatures of 28 °C, 33 °C and 18 °C, respectively, over the experimental period. After 60 days of the experiment, the following parameters were measured: percentage of survival and rooting (% SR), aerial part length (APL), root system length (RSL), number of shoots (SN), new leaves (NL), root system volume (RSV), aerial part dry mass (APDM) and root dry mass (RSDM).

A milimeter ruler was used to measure the length of the aerial parts and roots. Root volume was measured using the water displacement method in a beaker with a known volume of water. The root system was immersed, and the displaced volume was equivalent to the volume of the root system (mL). The dry mass of the aerial parts and roots was determined by weighing the material on an analytical balance (0.0001 g) after drying in an oven at 65°C until reaching constant weight.

Data were submitted to analysis of variance (ANOVA) followed by Fisher's Least Significant Difference (LSD) test with a 5% significance level to compare the means, using Costat 6.45 and SigmaPlot 11.0 software. The percentage of survival, root system volume, and shoot dry mass data did not meet the ANOVA assumptions and were transformed using *Asen* (√x)/100, √x and √x, respectively.

Results and Discussion

Analysis of variance (ANOVA) showed a significant interaction between type of cutting and substrate for the variables APL, RSL, NL, RSV, and RDM. However, no interaction was observed for %SR, SN, and APDM. These variables differed only for the type of cutting, and no significant differences were observed for the type of substrate (Tab. 1).

The average percentage of survival was 88.7%, regardless of the type of substrate; however, internode cuttings had the highest percentage of survival and rooting (96%) when compared to apical cuttings (81.4%) (Tab. 2). These results suggest that *B. vulneraria* has a high survival rate, particularly for internode cuttings. All the cuttings that survived had developed roots, while the cuttings that did not root were dead at evaluation. Winhelmann *et al.* (2018) also found that the type of substrate did not affect the percentage of survival of cuttings of *Angelonia integerrima* Sprengel, which had an average rooting of 79%. In the rooting of semi-hardwood *Sanchezia oblonga* cuttings, Weiss *et al.* (2018) found that the type of substrate also showed no interference and observed 88.7% of average rooting, in addition to a high survival rate (average of 83.33%).

Table 1 – Analysis of variance (ANOVA) of *Baccharis vulneraria* cutting considering different types of cutting and substrates.

Analyzed variables	P-value substrate	Cutting type p-value	Interaction p value	CV (%)	Overall mean
% SR	0.67 ns	< 0.001	0.83 ns	18.01	88.7
APL (cm)	< 0.001	0.95 ns	0.03	18.01	4.9
RSL (cm)	< 0.001	< 0.001	0.0108	14	11.6
LN	< 0.001	< 0.001	0.0105	15	8.7
SN	0.34 ns	< 0.001	0.78 ns	7,5	1.5
RSV (mL)	< 0.001	< 0.001	< 0.001	11.9	0.9
APDM (g)	0.071 ns	< 0.001	0.9072 ns	13.8	0.11
RSDM (g)	< 0.001	< 0.001	< 0.001	23.4	0.05

%SR = survival and rooting percentage; APL = aerial part length; RSL = root system length; LN = number of new leaves; SN = shoot number; RSV = root system volume; APDM = aerial part dry mass; RSDM = root system dry mass; CV = Coefficient of variation.

Table 2 – Influence of substrate and cutting type on survival and rooting percentage (% SR), number of shoots (SN) and aerial part dry mass (APDM) (g) of *Baccharis vulneraria*.

Substrate	**% SR**	**NS**	**APDM (g)**
CPS	86 ns	1.55 ns	0.14 ns
VM	91	1.47	0.11
CRH	92	1.52	0.12
CF	83	1.53	0.1
VM+CRH	94	1.48	0.11
VM+CF	90	1.47	0.09
CRH+CF	85	1.44	0.09
Mean	88.7%	1.5	0.11
Cutting type	% S	NB	MS PA (g)
Apical	81.4 b	1.0 b	0.05 b
Internode	96.0 a	2.0 a	0.17 a
Overall mean	88.7	1.5	0.11

% SR= percentage of survival and rooting, SN = number of shoots, APDM = aerial part dry mass, CPS = commercial peat-based substrate, VM= vermiculite, CRH= carbonized rice husk, CF=coconut fiber, VM+CRH= vermiculite + carbonized rice husk, VM+FC= vermiculite + coconut fiber, CRH+CF= carbonized rice husk + coconut fiber.

In the study conducted by Bona *et al.* (2005), *Baccharis articulata* (Lam.) Pers. and *Baccharis stenocephala* Baker showed a higher rooting rate in apical cuttings, which was not significantly different from the median ones, but higher than the basal cuttings. On the other hand, the internode cuttings in the present work had the highest percentage of survival and rooting (96%); however, the apical cuttings did not perform as well (81.4%) in these variables. These findings suggest that for *Baccharis*, both internode and apical cuttings can be used for propagation.

The cuttings had an average of 1.5 shoots and 0.11 g of dry matter in the aerial part (APDM), regardless of the type of substrate, but the internode cuttings had higher values for both variables (2.0 and 0.17 g, respectively) when compared to the apical cuttings (1 and 0.05 g, respectively).

For the number of shoots (SN), apical cuttings produced an average of one shoot, while internode cuttings produced two, with no statistical differences observed among the substrates. Weiss *et al.* (2018) found that the type of substrate significantly influenced the number of shoots per cutting, with organic substrate promoting an average of 2.36 shoots per cutting and organic substrate-vermiculite mixture producing an average of 1.95. Moreover, Winhelmann *et al.* (2018) reported the influence of the substrate on shoot formation in *Angelonia integerrima* Spreng cuttings. In that study, the average number of shoots formed in the commercial peat-based substrate was approximately 2.4 times higher than in the rice husk substrate. The lower number of shoots in apical cuttings observed in the present study may be attributed to the high concentration of endogenous auxin, a phytohormone that regulates apical dominance and inhibits the growth of axillary buds (Weiss *et al.* 2018).

No significant interaction was observed for any variable evaluated in this study on the vegetative propagation of *Mentha arvensis* L. using different types of cuttings and substrates. However, the substrate was found to be significant, while the type of cutting was not (Amaro *et al.* 2013). Such results differ from those obtained in the present work, in which both the type of substrate and type of cutting had significant differences for most of the variables analyzed. This highlights that results may be different according to the species, which explains the importance of conducting experiments in this area.

On the other hand, when comparing the cutting types, in all treatments, the mean MSPA was higher in internode cuttings and differed from all treatments using apical cuttings.

The commercial peat-based substrate (CPD) promoted the highest increase in the length of the aerial part (APL) in apical cuttings (6.82 cm) and was statistically different from the others, except for VM+CRH treatment (5.9 cm). For the internode cuttings, no difference was found among the substrates, with an average length of 4.91 cm (Tab. 3). In general, cuttings of the apex in CPS treatment showed higher APL. Comparing the APL between the two types of cuttings showed a statistically significant difference between the STC and VM substrates, as the STC substrate promoted the highest RSL in apical cuttings compared to internode cuttings. In the VM treatment, the length was longer for internode cuttings compared to apical cuttings.

Regarding the root system, the SCT substrate promoted the longest root length (RSL) in both types of cuttings (apical: 16.87 cm; internode: 18.45 cm), which was statistically different from all other substrates assessed. For root system length (RSL), internode cuttings differed statistically from apical ones only in CF, VM+CRH, CRH+CF substrates, presenting the longest root length, with means values of 9.91; 13.64 and 9.87 cm, respectively.

The number of leaves was significantly higher in internode cuttings, with an average of 12.75 per cutting, compared to apical cuttings which had, on average, 4.63 new leaves per cutting (Tab. 4). Among the treatments, the substrates VM+FC, CPS, CAC, and FC promoted the highest NL (13.98; 13.93; 13.71 and 12.93, respectively), which were not statistically different from each other (Tab. 4), but differed from all others. For apical cuttings, the CPS substrate promoted higher NL, which was statistically different from all other substrates. Paulus *et al.* (2011), when evaluating the best organic substrates for the production of *Mentha gracilis* R. Br. and *Mentha* x *villosa* Huds seedlings for field cultivation, found that the commercial substrate Plantmax® provided the highest average number of leaves in cuttings in all evaluation periods, which were seven, 14 and 21 days after experiment establishment.

Internode cuttings also showed higher RSV, with an average of 1.14 mL compared to apical cuttings (0.73 mL) (Tab. 4). The CPS substrate promoted the highest SR volume, statistically different from all other substrates in both types of cuttings. When considering the types of cuttings, only CPS substrate did not show any statistical difference. However, the other substrates showed a greater volume in internode cuttings and differed from all treatments with apical cuttings. The volume of the root system, according to Metivier

Table 3 – Aerial part length (APL) and root system length (RSL) of *Baccharis vulneraria* cuttings.

Substrate	APL (cm)		RSL (cm)	
	Cuttings			
	Apical	Internode	Apical	Internode
SCT	6.82 aA	5.46 aB	18.45 aA	16.87 aA
VM	4.13 dB	5.28 aA	11.87 bA	13.65 bA
CAC	5.32 bcA	4.91 aA	11.38 bA	12.04 bA
FC	3.83 dA	4.6 aA	6.6 cB	9.91 cA
VM + CAC	5.9 abA	5.21 aA	10.83 bB	13.64 bA
VM + FC	4.72 cdA	4.54 aA	10.24 bA	11.4 cA
CAC + FC	3.74 dA	4.39 aA	6.2 cB	9.87 cA
Mean	4.92	4.91	10.80	12.48

*Means followed by a distinct lowercase letter in the columns and distinct uppercase letter in the row are different from each other by the LSD test at 5% error probability. Lowercase letter in the columns = substrate. Uppercase letters in the row = cuttings. CPS = commercial peat-based substrate, VM= vermiculite, CRH= carbonized rice husk, CF=coconut fiber, VM+CRH= vermiculite + carbonized rice husk, VM+CF= vermiculite + coconut fiber, CRH+CF= carbonized rice husk + coconut fiber, APDM = aerial part dry mass

Table 4 – Number of new leaves (LN), root system volume (RSV), and aerial part dry matter (APDM) of *Baccharis vulneraria* internode and apical cuttings.

Substrate	LN		RSV (mL)		APDM (g)	
	Cuttings					
	Apical	Internode	Apical	Internode	Apical	Internode
CPS	7.55 aB	13.93 aA	1.94 aA	1.72 aA	0.079 aA	0.08 aA
VM	4.72 bB	11.42 bA	0.81 bB	1.11 bA	0.027 bB	0.068 aA
CAC	4.55 bB	13.71 aA	0.52 bB	1.08 bA	0.029 bB	0.075 aA
FC	3.65 bB	12.93 abA	0.44 cB	0.95 bA	0.02 bB	0.071 aA
VM + CAC	4.46 bB	11.47 bA	0.61 bcB	1.11 bA	0.27 bB	0.066 aA
VM + FC	3.81 bB	13.98 aA	0.46 cB	1.01 bA	0.24 bB	0.072 aA
CAC + FC	3.66 bB	11.84 bA	0.33 cB	0.98 bA	0.016 bB	0.072 aA
Mean	4.63	12.75	0.73	1.14	0.10	0.07

*Means followed by a distinct lowercase letter in the columns and distinct uppercase letter in the row are different from each other by the LSD test at 5% error probability. Lowercase letter in the columns = substrate. Uppercase letters in the row = cuttings. CPS = commercial peat-based substrate, VM= vermiculite, CRH= carbonized rice husk, CF=coconut fiber, VM+CRH= vermiculite + carbonized rice husk, VM+CF= vermiculite + coconut fiber, CRH+CF= carbonized rice husk + coconut fiber, RSV= root volume, APDM = aerial part dry mass.

(1985) and Marschner (1986), is associated with a longer period of leaf retention in the plant, which delays the effect of leaf senescence as cytokinins are synthesized by the root meristems.

The success of the commercial peat-based substrate (CPS) for the growth of the aerial part and the root of cuttings, and for the considerable development in the number of new leaves and the volume of the root system, may be related to its composition. CPS is formed by the combination of different materials, which provide better chemical and physical characteristics than the materials used in isolation (Kampf 2000).

The RSDM variable showed an average of 0.10 g for apical cuttings and 0.07 g for internode cuttings. Moreover, only the apical cuttings showed a statistical difference between the substrates (Tab. 4). The commercial peat-based substrate (STC) differed statistically from the others. In the remaining substrates, the internode cuttings were statistically better compared to the apical ones. Similarly, Silva *et al.* (2017), when evaluating the influence of different substrates and indolebutyric acid (IBA) on cuttings of *Eplingiella fruticosa* (Salzm. *ex* Benth.) Harley & JFBPastore, found that the commercial substrate Biomax® used in the test promoted the highest accumulation of total dry mass in apical cuttings. These results corroborate those obtained in the present work, where the dry mass differed only in the STC substrate and not by the cutting type.

These results indicate that this substrate has satisfactory performance in both types of cuttings for root formation, confirming that the use of a substrate with adequate chemical and physical properties, much like the one used in the present work and in the study by Silva *et al.* (2017), is beneficial for seedling production using cuttings.

The results observed by several authors (Klein 2015; Orlando da Ros *et al*. 2015; Silva *et al.* 2019) show the need for studies to establish the most suitable substrates for the production of quality seedlings. This is because, depending on the medicinal species, some substrates can produce different results.

Conclusions

The internode cuttings had the best results for most of the evaluated variables, except for the length of the aerial part, in which the apical cuttings stood out. The commercial peat-based substrate provided the greatest increase in root system length, number of leaves, root system volume in both types of cuttings, as well as the greatest shoot length and root system dry mass in apical cuttings. The results obtained showed that CPS is a suitable option for the cultivation of *Baccharis vulneraria*, especially in internode cuttings.

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