Research Article
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Herbaceous and woody cuttings of *Cissus verticillata* (princess vine)

Abstract

Cissus verticillata (L.) Nicolson & C.E. Jarvis, commonly known as princess vine, has been traditionally used to treat several diseases, particularly diabetes. This study evaluated different strategies for the ex-vitro vegetative propagation of *C. verticillata*. Stem cuttings approximately 5 cm long, each containing one axillary bud, were subjected to combinations of three factors: cutting type (herbaceous with one-half leaf or leafless woody), tube size (19 × 5.5 cm or 13 × 3.1 cm), and substrate (oxisol or organic substrate), totaling eight treatments. The cuttings were maintained in a greenhouse at 22 ± 2 °C and irrigated every two days. After 45 days, survival, rooting, shoot formation, callus formation, cutting establishment, root number, and root length were evaluated. The experiment followed a completely randomized 2 × 2 × 2 factorial design with ten replicates per treatment. Continuous variables were analyzed by analysis of variance or generalized linear models, whereas binary variables were analyzed using generalized linear models with a binomial distribution ($p \leq 0.05$). *Cissus verticillata* exhibited high vegetative propagation capacity, with rooting rates ranging from 60 to 100%. Significant interactions between cutting type and tube size were observed for shoot formation and cutting establishment, indicating superior performance of herbaceous cuttings in larger containers and greater tolerance of woody cuttings to smaller ones. Callus formation was favored by the organic substrate, while the greatest root lengths were obtained in 19 × 5.5 cm tubes. The highest number of shoots was recorded in herbaceous cuttings grown in larger tubes filled with oxisol. These findings demonstrate the high potential of *C. verticillata* for ex vitro vegetative propagation and support the use of herbaceous cuttings in 19 × 5.5 cm tubes to maximize early seedling development and cutting establishment.

Keywords: medicinal plant, vegetative propagation, stem cuttings, substrate, nursery tube

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Introduction

Plant propagation is essential for the multiplication of species of agronomic, medicinal, and ecological importance, since the indiscriminate collection of plants from natural populations may lead to environmental degradation and even species extinction. Vegetative propagation by stem cuttings enables rapid plant multiplication, particularly for species with low seed germination capacity, allowing the production of large numbers of seedlings within a short period and at relatively low cost.[1]

The genus *Cissus* belongs to the family Vitaceae, which comprises approximately 20 genera and 900 species.[2] *Cissus verticillata* (L.) Nicolson & C.E. Jarvis[3] (syn. *Cissus sicyoides* L.) is commonly known as vegetable insulin, princess vine, blister bush, wild grape, *bejuco-caro*, *cipó-puca*, *uva-brava*, and several other regional names.[4-7] It is a shrubby climbing vine with simple opposite leaves, membranous and ovate to oblong, measuring approximately 4-7 cm in length and 2.5-4.5 cm in width, with occasionally serrated margins and an acute apex.[7] The inflorescences are corymbiform and bear small white flowers with a pale green calyx (approximately 2 mm long), four free petals of similar size, and an androecium composed of four rounded stamens. The gynoecium has a glabrous globose ovary that develops into black berries at maturity, containing seeds approximately 6 mm long.[5]

Interest in this species has increased considerably because of its remarkable medicinal properties and its importance in ethnopharmacology.[8] The main secondary metabolites reported for *C. verticillata* include polysaccharides, simple phenolic compounds, flavonoids, tannins, and alkaloids.[9] Polysaccharides are associated with hypoglycemic activity, flavonoids contribute to reductions in triglyceride and transaminase levels, whereas alkaloids and tannins have been associated with antinociceptive activity.[9-12] Both *in vitro* and *in vivo* preclinical studies have demonstrated the antidiabetic activity of *C. verticillata*.[10,13,14] Furthermore, treatment with 50 µg mL^{-1} of an aqueous extract significantly attenuated H_2O_2-induced cellular damage (including reactive oxygen species production, DNA fragmentation, and cell death) in HT22 cells.[15] In addition, hydroalcoholic leaf extracts have shown low toxicity in animal models.[16] It should be noted that domestication may alter the production of plant defense-related secondary metabolites, either increasing or decreasing their accumulation.[17] However, no studies have yet compared the phytochemical profiles of wild and cultivated populations of *C. verticillata*.

Beyond its pharmacological potential, *C. verticillata* can also be used as an ornamental plant and in the restoration of degraded forest and coastal (*restinga*) ecosystems in urban environments, provided that adequate management practices are adopted because of its vigorous growth habit.[5] Nevertheless, despite its therapeutic and commercial potential, information regarding efficient propagation methods for this species remains scarce, representing a major obstacle to large-scale cultivation. Abreu et al.[7] evaluated the propagation of *Cissus sicyoides* using 10 and 20 cm stem cuttings treated with 0, 80, or 160 mg L^{-1} indole-3-butyric acid (IBA), with or without sucrose plus boric acid for two hours. They concluded that propagation using 10 cm cuttings was most effective with 160 mg L^{-1} IBA, whereas 20 cm cuttings performed best with 80 mg L^{-1} IBA, with no significant differences in root number or root length. Subsequently, Cruz-Silva, Marcon, and Nobrega[18] investigated cuttings without plant growth regulators, testing lengths of 5, 10, and 15 cm combined with three leaf conditions (two intact leaves, two half leaves, or no leaves). Their results indicated that 5 cm cuttings bearing intact or half leaves, as well as 10 cm cuttings with intact leaves, were the most suitable for propagation. Therefore, establishing an efficient propagation protocol for *C. verticillata* is an important step toward ensuring sustainable cultivation and enabling the commercial exploitation of its medicinal properties.

The present study aimed to evaluate the vegetative propagation of *C verticillata* using different cutting types, container sizes, and substrates in order to identify the most suitable conditions for seedling production, thereby contributing to the expansion of its cultivation and promoting its sustainable commercial and medicinal use.

Materials and methods

Plant material of *Cissus verticillata* (L.) Nicolson & C.E. Jarvis was obtained from the VPA Plant Nursery, located in Porto Amazonas, Paraná State, Brazil.

The experiment was conducted in a greenhouse, and the evaluations were carried out in the laboratory in Ponta Grossa, Paraná, Brazil (25°05′23″ S, 50°06′23″ W; 912 m above sea level). Stem cuttings approximately 5 cm long were prepared with a basal bevel cut. Three experimental factors were evaluated: cutting type (herbaceous with one-half leaf or leafless woody), container size (large nursery tubes, 19 × 5.5 cm, or small nursery tubes, 13 × 3.1 cm), and substrate (oxisol or organic substrate), resulting in eight treatment combinations. The cuttings were maintained in a greenhouse at 22 ± 2 °C. Plastic bags were placed around the nursery tubes to maintain high humidity, and irrigation was performed every two days by applying 10 mL of water per cutting. After 45 days, the following variables were evaluated: rooting percentage (cuttings bearing roots at least 1 mm long), number of roots per cutting, mean length of the three longest roots (cm), percentage of cuttings showing basal callus formation without roots, percentage of sprouted cuttings without roots, cutting establishment percentage, and survival percentage.

The experiment followed a completely randomized design arranged in a 2 × 2 × 2 factorial scheme, comprising eight treatments with ten replicates per treatment and one cutting per experimental unit. Statistical analyses were performed using the R statistical software[19] through the RStudio interface version 2024.09.1+394 "Cranberry Hibiscus".[20] Root number and mean root length were analyzed by analysis of variance (ANOVA) in a 2 × 2 × 2 factorial design. Whenever significant effects were detected, treatment means were compared using Tukey's test at the 5% significance level.

Shoot number was analyzed using a generalized linear model (GLM) with a quasi-Poisson distribution and log link function because of the discrete nature of the data and the presence of overdispersion. The binary variables (survival, rooting, shoot formation percentage, callus formation, and cutting establishment) were analyzed using generalized linear models (GLMs) with a binomial distribution and logit link function. The significance of model effects was assessed by analysis of deviance (Chi-square test) at the 5% significance level. Whenever appropriate, estimated marginal means were calculated and multiple comparisons were performed using Tukey's test.

Results

After 45 days under greenhouse conditions, the rooting percentages of *Cissus verticillata* cuttings ranged from 60% to 100% (Table 1). Leafless woody and herbaceous cuttings grown in 19 × 5.5 cm tubes filled with organic substrate both achieved 100% rooting. In the same tube size filled with oxisol, woody and herbaceous cuttings exhibited 90% rooting. In the smaller tubes (13 × 3.1 cm), leafless woody cuttings grown in oxisol also reached 90% rooting, whereas herbaceous cuttings showed rooting percentages of 70% and 60% in oxisol and organic substrate, respectively. Despite these numerical differences, generalized linear models with binomial distribution detected no significant effects of cutting type, substrate, container size, or their interactions on survival and rooting percentage.

Table 1 Survival, rooting, shoot formation, callus formation, and establishment percentages of *Cissus verticillata* cuttings as affected by cutting type, tube size, and substrate, 45 days after planting.

Type of cutting	Tube size	Substrate	Survival (%)	Rooting (%)	Shoot formation (%)	Callus formation (%)	Establishment (%)
Woody	Larger	Oxisol	90	90	70	0	60
Woody	Larger	Organic Substrate	100	100	60	50	50
Woody	Smaller	Oxisol	90	90	90	0	90
Woody	Smaller	Organic Substrate	100	100	90	0	90
Herbaceous	Larger	Oxisol	90	90	90	0	90
Herbaceous	Larger	Organic Substrate	100	100	100	0	100
Herbaceous	Smaller	Oxisol	70	70	60	0	60
Herbaceous	Smaller	Organic Substrate	60	60	50	20	50

Note: A significant interaction between cutting type and container size was observed for the variables sprouting ($\chi^2 = 12.66$; $p = 0.0004$) and rooting ($\chi^2 = 14.32$; $p = 0.0002$). Regarding callogenesis, significant effects were observed for the substrate ($\chi^2 = 10.72$; $p = 0.0011$) and the interaction between cutting type and container size ($\chi^2 = 9.96$; $p = 0.0016$), based on generalized linear models with a binomial distribution.

The highest mean number of roots was observed in herbaceous cuttings grown in 19 × 5.5 cm tubes filled with oxisol (3.8 roots per cutting), followed by thos observed in leafless woody cuttings grown in 13 × 3.1 cm tubes containing oxisol (3.5 roots per cutting), although these means did not differ significantly (Table 2).

Table 2 Type of tube, cutting, and substrate for the rooting of *Cissus verticillata* cuttings.

Type of cutting	Tube size	Substrate	Number of roots	Mean root length
Herbaceous	Larger	Oxisol	3,8 ± 2,1 ns	11,91 ± 6,16a
Herbaceous	Larger	Organic Substrate	3,1 ± 1,73	14,77 ± 4,42 a
Herbaceous	Smaller	Oxisol	3,3 ± 3,16	5,8 ± 5,83 b
Herbaceous	Smaller	Organic Substrate	2 ± 1,89	5,53 ± 4,95 b
Woody	Larger	Oxisol	2,9 ± 1,6	14,02 ± 5,68 a
Woody	Larger	Organic Substrate	2,2 ± 0,79	12,1 ± 3,99 a
Woody	Smaller	Oxisol	3,5 ± 1,35	10,28 ± 5,18 b
Woody	Smaller	Organic Substrate	2,7 ± 1,25	9,16 ± 3,35 b

Note: ns = not significant by the F-test. Means followed by different letters differ according to Tukey's test ($p \leq 0.05$). Letters refer to the main effect of the tube size factor, as there was no significant interaction between the factors.

Regarding root length, the greatest mean values were observed for leafless woody cuttings grown in 19 × 5.5 cm tubes filled with oxisol and for herbaceous cuttings grown in 19 × 5.5 cm tubes filled with organic substrate, with no significant differences between these two treatments (Table 2). Herbaceous cuttings grown in 13 × 3.1 cm tubes, regardless of substrate, showed the lowest mean root lengths (5.80 and 5.33 cm), whereas the remaining treatments did not differ significantly from one another.

Table 3 Type of tube, cutting, and substrate on the shoot formation of *Cissus verticillata* cuttings.

Type of cutting	Tube size	Substrate	Shoot number
Herbaceous	Larger	Oxisol	9 ± 4,97 c
Herbaceous	Larger	Organic Substrate	5,1 ± 1,91 bc
Herbaceous	Smaller	Oxisol	2,5 ± 2,55 ab
Herbaceous	Smaller	Organic Substrate	1,2 ± 1,62 a
Woody	Larger	Oxisol	4,8 ± 3,36 bc
Woody	Larger	Organic Substrate	1,4 ± 1,35 a
Woody	Smaller	Oxisol	3,4 ± 1,43 ab
Woody	Smaller	Organic Substrate	2,2 ± 1,32 ab

Note: Means followed by the same letter do not differ from each other based on multiple comparisons of adjusted means obtained via a generalized linear model with a Quasi-Poisson distribution and a log link function ($p \leq 0.05$).

Shoot formation was significantly affected by the interaction between cutting type and container size, both for the percentage of cuttings producing shoots ($\chi^2 = 12.66$; $p = 0.0004$) and for the number of shoots produced (Tables 1 and 3). All cuttings that produced shoots also developed roots, regardless of cutting type (leafless woody or herbaceous with half a leaf). The highest values were recorded for herbaceous cuttings grown in large containers, which produced an average of 9.0 shoots per cutting in oxisol and 5.1 shoots per cutting in organic substrate, while also exhibiting high shoot formation percentages (90% in oxisol and 100% in organic substrate). In contrast, herbaceous cuttings grown in smaller containers showed reductions in both shooting percentage (60% in oxisol and 50% in organic substrate) and number of shoots (2.5 and 1.2 shoots per cutting, respectively).

Callus formation was significantly influenced by substrate type ($\chi^2 = 10.72$; $p = 0.0011$) and by the interaction between cutting type and container size ($\chi^2 = 9.96$; $p = 0.0016$). Callogenesis occurred mainly in treatments containing organic substrate, particularly in leafless woody cuttings grown in large containers, in which approximately 50% of the cuttings formed callus tissue. In contrast, most of the remaining treatments exhibited little or no callus formation.

Establishment percentage was also significantly influenced by the interaction between cutting type and container size ($\chi^2 = 14.32$; $p = 0.0002$). The highest establishment rates were observed in herbaceous cuttings grown in large containers, reaching up to 100%. In contrast, herbaceous cuttings cultivated in smaller containers exhibited reduced establishment. Leafless woody cuttings, however, maintained high establishment rates regardless of container size, demonstrating greater tolerance to the limitations imposed by the reduced container volume.

Discussion

The present study demonstrates the high capacity of *Cissus verticillata* for vegetative propagation under the evaluated conditions.

Particularly remarkable was the performance of leafless woody cuttings, which achieved rooting percentages ranging from 90 to 100%. These findings contrast with those reported by Cruz-Silva, Marcon, and Nobrega,[18] who observed no rooting in leafless cuttings and only 2.5% shoot formation in 10- and 15-cm-long cuttings without leaves. This discrepancy may be explained by differences in the physiological condition of the propagules, since the leafless cuttings used by those authors were obtained from green shoots, whereas woody cuttings were used in the present study.

According to Foladori-Invernizzi, Maggioni, and Zuffellato-Ribas,[21] lignification may be advantageous by reducing tissue dehydration. Although herbaceous cuttings generally present fewer anatomical barriers to adventitious root formation, excessively tender tissues with low biosynthetic activity may impair rooting. The same authors also suggest that leaves supply carbohydrates and endogenous auxins that promote adventitious root formation. Therefore, for leafless cuttings, woody or semi-hardwood cuttings are considered the most suitable propagules, whereas young herbaceous cuttings require the presence of at least half a leaf to ensure successful rooting.

Compared with the study of Cruz-Silva, Marcon, and Nobrega,[18] the number of roots per cutting was generally lower. However, it should be considered that cuttings in the present study were evaluated only 45 days after planting, which provided less time for root development. Furthermore, it is noteworthy that leafless woody cuttings produced an average of 2.7 to 3.5 roots per cutting, whereas Cruz-Silva, Marcon, and Nobrega[18] reported no root formation in leafless cuttings.

In *Cissus sicyoides* (currently recognized as a synonym of *Cissus verticillata*), vegetative propagation is an effective method for producing plants genetically identical to the mother plant. According to Abreu,[7] cutting length (10 or 20 cm) and the application of 80 or 160 mg L^{-1} indole-3-butyric acid (IBA), combined with sucrose and boric acid under greenhouse conditions, influence the rooting and subsequent cutting development. The authors reported that 10-cm cuttings treated with 160 mg L^{-1} IBA achieved the greatest root dry mass and concluded that propagation using 10 cm cuttings is effective when this concentration of IBA is applied. In contrast, the present study obtained rooting percentages ranging from 60 to 100% using 5 cm cuttings without the application of any plant growth regulator.

Vegetative propagation has also been investigated in *Piper umbellatum*, another medicinal species subject to extractive harvesting because of its anti-inflammatory, analgesic, and antimicrobial properties, similarly to *C. verticillata*. Gomes and Krinski[22] evaluated cuttings of different lengths (10, 15, and 20 cm) grown in either Plantmax® or fine-grade vermiculite and concluded that 20-cm cuttings cultivated in Plantmax® produced higher-quality seedlings, with establishment rates ranging from 37.5 to 60%.

Santos et al.[23] demonstrated that container size influenced root length in herbaceous cuttings of *Passiflora cincinnata*. Cuttings grown in 50-cm^3 tubes developed shorter roots than those cultivated in 288 and 573 cm^3 containers, although no significant differences were observed between the two larger container sizes. Likewise, although the mean number of roots did not differ significantly among treatments in the present study, root length was consistently greater in the larger containers, regardless of cutting type or substrate. These findings indicate that, although similar numbers of roots were produced, larger containers promoted greater root length after root initiation.

Herbaceous cuttings grown in smaller containers showed a lower percentage of shoot formation, particularly when cultivated in organic substrate. These findings suggest that herbaceous cuttings are more sensitive to the physical constraints imposed by smaller containers, a response consistent with the effects of root confinement described by Poorter et al.[24] Root confinement reduces water and nutrient availability while increasing substrate temperature fluctuations, factors that limit early plant growth and make herbaceous cuttings more dependent on favorable conditions during the establishment phase. During the rooting phase, these limitations may become even more critical for herbaceous cuttings because their tissues exhibit high metabolic activity and greater resource demand to simultaneously support root and shoot formation. In addition, leaves and young tissues constitute important sources of endogenous auxins, particularly indole-3-acetic acid (IAA), which is transported to the basal region of the cutting and plays a key role in the induction of adventitious root formation.[25]

Shoot formation in leafless woody cuttings (60–90%) contrasts with the findings of Cruz-Silva, Marcon, and Nobrega,[18] who reported only 2.5% shoot formation in leafless cuttings measuring 10 and 15 cm, even in the absence of rooting. Moreover, leafless woody cuttings maintained a high capacity for shoot formation even when grown in smaller containers. Because of their higher degree of lignification and lower meristematic activity, woody cuttings generally have a lower capacity for the synthesis and transport of plant hormones, particularly auxins, which may impair rooting.[26] On the other hand, woody cuttings may contain larger carbohydrate reserves that, under favorable environmental conditions, compensate for the lower hormonal activity and promote root development. Oliveira et al.[27] demonstrated that woody cuttings are capable of resynthesizing carbohydrates during rooting, especially under adequate temperature and humidity conditions. Therefore, the contrasting responses observed in the present study may be attributed to the physiological condition of the plant material, which may have synchronized shoot formation and root initiation. Overall, these findings support the hypothesis that container volume plays an important role in the initial development of cuttings, although the magnitude of this effect depends on the type of cutting used.

Callus formation was favored by the use of organic substrate. The higher frequency of callus formation in organic substrate may be related to the physical and nutritional characteristics of the substrate, which promote intense cellular activity in wounded tissues.[28] Callus formation was not associated with the highest establishment percentages observed in the experiment, indicating that callogenesis was not a prerequisite for rooting or successful establishment of the cuttings. According to Hartmann et al.,[28] although adventitious root formation and callus development may occur simultaneously, these processes are physiologically independent and do not necessarily exhibit a direct correlation.

These findings suggest that the response of *C. verticillata* cuttings is strongly influenced by the interaction between the physiological characteristics of the propagule and the growing conditions. In particular, container volume appears to play a more critical role for herbaceous cuttings than for leafless woody cuttings, emphasizing the importance of considering both propagule type and cultivation conditions when developing propagation protocols for this species.

Conclusion

Vegetative propagation of *Cissus verticillata* (L.) Nicolson & C.E. Jarvis by stem cuttings proved to be highly effective under the conditions evaluated in this study, resulting in high survival, rooting, and establishment rates even in the absence of plant growth regulators. These findings demonstrate the species' excellent potential for clonal propagation and highlight stem cuttings as a promising strategy for cultivation and conservation programs.

Although no significant differences were detected in survival or rooting percentages, container size influenced cutting development, particularly in combination with the type of propagative material. Herbaceous cuttings exhibited superior performance when grown in larger tubes (19 × 5.5 cm), showing higher shoot formation percentages (90% in oxisol and 100% in organic substrate), a greater number of shoots (an average of nine shoots per cutting in oxisol and 5.1 in organic substrate), and higher establishment rates, reaching 90% in oxisol and 100% in organic substrate. In contrast, leafless woody cuttings showed more consistent performance across container sizes, indicating greater tolerance to the restrictions imposed by the smaller rooting volume.

Root length was favored by the larger containers regardless of cutting type or substrate, indicating that greater substrate volume provides more suitable conditions for root growth after root initiation. Callus formation was promoted by the use of organic substrate, particularly in leafless woody cuttings grown in larger containers; however, callogenesis was not directly associated with rooting or successful establishment.

Overall, the use of herbaceous cuttings grown in larger tubes (19 × 5.5 cm) is recommended to maximize the efficiency of vegetative propagation in *C. verticillata*. Nevertheless, leafless woody cuttings also exhibited high propagation potential and can be successfully used in smaller containers. Stem cutting therefore represents a simple, inexpensive, and efficient propagation technique for Cissus verticillata, contributing to its sustainable cultivation, reducing harvesting pressure on natural populations, and providing standardized plant material of known origin for commercial and medicinal applications.

According to the meta-analysis conducted by Poorter et al.,[24] plant biomass increases by approximately 43% for every doubling of pot volume, with no significant differences between herbaceous and woody species. According to the authors, this response results from a reduction in photosynthetic rate caused by a combination of factors, including lower nutrient availability, reduced water-holding capacity, greater substrate temperature fluctuations, and root confinement, all of which delay plant growth and consequently reduce leaf expansion. The findings of the present study suggest that this effect may occur even during the rooting phase of cuttings, particularly in herbaceous material. Poorter et al.[24] also emphasized that younger plants are less affected by growth restriction and that the ratio of total plant biomass to root-zone volume is more important than pot volume itself, recommending values below 2 g L^{-1}. Regarding woody cuttings, Thomas, Schreiber, and Kamelchuk,[29] while evaluating the effects of container type on the growth and survival of dormant cuttings from three hybrid poplar cultivars used as windbreak trees in Canada, found that trays with deeper and larger cavities, as well as lower cavity density, produced seedlings with greater stem diameter, higher root-to-shoot ratios, and better cost-effectiveness than other tray designs.

The results of the present study suggest that herbaceous cuttings are more dependent on favorable conditions to maintain water balance and support initial growth, whereas leafless woody cuttings can compensate for environmental constraints through larger internal carbohydrate reserves stored in lignified tissues. The high establishment rates observed in several treatments, reaching up to 100%, demonstrate the excellent potential of *Cissus verticillata* for vegetative propagation by stem cuttings. Furthermore, the results indicate that selecting an appropriate container is particularly important when herbaceous cuttings are used, whereas leafless woody cuttings exhibit more stable performance under different cultivation conditions.

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Conflict of interest

All authors declare that there are no conflicts of interest.

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