



Techniques for the Cultivation of ‘Mamacadela’ (*Brosimum Gaudichaudii* Tréc.Moraceae) for the Extraction of Furanocoumarins from the Roots

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Authors' contributions

This work was carried out in collaboration among all authors. Author CESS wrote the protocol and carried out the experiments. Author CA performed the chemical analysis. Author DP helped in writing the protocol and in the experiments. Author IA carried out part of the experiments. Author LA helped in writing the protocol and in handling the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Brosimum gaudichaudii is a common plant of Cerrado, which is of pharmaceutical interest owing to the high accumulation of furanocoumarins in the bark of the roots. The production chain is based on extractivism, which exerts a considerable ecological pressure. In this paper, a technique for its cultivation under garden conditions has been presented. Seeds were made to germinate, and the seedlings were cultivated in 1, 45 m deep PVC tubes filled with Cerrado soil. After nine years, the plants started producing flowers and fruits. The concentration of furanocoumarins was considerably high in the cultivated plants. Five plants were sampled for morphological description and chemical analysis. Root cuttings were obtained. After six months of observation,

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more than 70% of the cuttings had emitted shoots and leaves. The cultivation in PVC tubes is a suitable method for harvesting the roots, and it is easy to carry out vegetative propagation of this plant by root cuttings.

Keywords: *Brosimum gaudichaudii*; clay soil; Moraceae; root cutting; vitiligo.

1. INTRODUCTION

Mamacadela (*Brosimum gaudichaudii*) is the only species belonging to the genus *Brosimum* that is found commonly in Cerrado [1]. There is an enormous interest in cultivating this plant because of its alimentary and pharmaceutical properties [2,3]. The fruits are edible and have been used industrially in the production of ice creams and candies [4,5]. The pharmaceutical interest is based on the fact that the roots accumulate considerable amount of furanocoumarins, mainly bergapten and psoralen. Such compounds are used in PUVA-therapy for the control of several skin diseases, including vitiligo [6,7,8,9]. Medications are produced from the root extracts of *B. gaudichaudii* [8,9]. Moreover, popular herbalists have bundles of mamacadela meant for personal use as a phytotherapeutic agent, usually in the form of infusions and decoctions [10].

Presently, the productive chain of mamacadela relies on extractivism. Extractivism of fruits exerts less impact on the populations of this plant, however, the process involving the harvesting of roots puts a great pressure, as it kills the plant and poses risk of extinction [5,10].

The Cerrado is a biome with a wide geographical occurrence; hence *B. gaudichaudii* shows regional variations in seed germination: Faria et al. [11,12] observed that seeds from the Mato Grosso state display germinability higher than 80% after the removal of the seed coat. However, the seeds collected in Goias state germinate after simply being embedded in water, without needing any scarification [13,14]. Evidences exist that the seeds and seedlings largely vary in quality and vigor according to the precedence, reflecting the genetic variability of this wild plant [11].

Similarly, differences in the subterranean system have been observed. In samples obtained from Central Brazil, the plants possess deep taproots with few ramifications and re-sprouting occurs basically from the xylopodium [1,15,16]. Contrarily, in samples sourced from the southern frontiers of Cerrado in the sub-tropical region, the

plants had taproots that were densely ramified so that the lateral roots were gemiferous, that is, producing new shoots, which indicates a great potential for vegetative propagation via root cuttings [17].

Regarding cultivation, [14] caused seeds to germinate and placed the seedlings in PVC tubes of 5 cm diameter and 1 m length, filled them with soil from Cerrado and observed that after one year, the taproot had reached about 50 cm depth. Additionally, [18] documented the production of *B. gaudichaudii* saplings from root cuttings with a success rate of about 30% independently from a treatment with phyto regulators. Both Lima et al. [13] and Silva et al. [18] reported difficulties in obtaining saplings from shoot/branch cuttings.

The objective of the present study is to point to a mode for cultivating this species for providing raw material for extraction of furanocoumarins. Moreover, it aims to verify the efficiency of cultivation in PVC tubes for harvesting the roots and ascertain the production of saplings from root cuttings.

2. METHODS

Seeds of *Brosimum gaudichaudii* Tréc (Moraceae) were harvested from Cerrado areas of Central Brazil in 2007. The seeds were depulped, air dried at room temperature for 48 hours, and stored for about a month in capped bottles at ambient temperatures. The seeds were then made to germinate using a simple moist substrate at room temperature, with a germinability of more than 80% after 10-15 days. One week after the protrusion of the radicle, the visually stronger seedlings were planted in Cerrado soil using hollow-ended PVC tubes of 20 cm diameter and 1.45 m length. The tubes were placed in the garden (Fig. 1-A). Initially, 30 plants were cultivated in such PVC tubes. Further, in the spaces between the tubes, seedlings were planted directly in the Cerrado soil. The cultivation was done during the rainy season. In the following dry season, manual irrigation of the tubes was executed at an interval of two weeks. From the second year onwards, only a little random irrigation was done.

After nine years of cultivation, 25 plants reached maturity and started to produce flowers and fruits. Five tubes were randomly selected for morphological observations, biochemical dosages, and obtainment of 10-12 cm long root cuttings. The following parameters related to root sprouting were tested: a) orientation of the root cuttings – vertical or horizontal; b) partially buried or totally buried cuttings; c) fresh cuttings or those subjected to a quick burn of the extremities for 10 to 15 s; d) cuttings from the upper (xylopodium), middle, or apical regions of the roots. Also, cuttings of 10-12 cm from the main stem were taken.

The cuttings were then cultivated in small tubes containing soil from the Cerrado region and kept under moist conditions in a greenhouse with amended extreme variations in temperature, air humidity and solar exposure.

The chemical analyses of the following parameters were performed in triplicate with the medium portion of the roots for each sampled plant: humidity, ash, proteins, dietary fibers, lipids, pH, titratable acidity, vitamin C, bergapten and psoralen. Ash was determined by measuring the weight after muffle incineration at 550°C for 6-8 hours; humidity was calculated from the weight difference observed after drying in oven at 105°C for 24 hours. The protein content was estimated by the micro-Kjeldahl method. The content of dietary fiber was computed by the enzyme-gravimetric essay. The lipid content was assessed by the Goldfish method. The determination of vitamin C was carried out by using the potassium iodine method. Detailed protocols for these experiments can be found in [19,20]. The quantifications of bergapten and psoralen were done by liquid chromatography using a Shimadzu chromatograph according to the methods described by several authors [21,22,23,24,25,26].

3. RESULTS

None of the seedlings directly planted in the soil survived. Among the 30 plants initially cultivated in PVC tubes, 25 survived and reached maturity. The aerial parts of the plants showed a slow growth with periodical deciduousness. The leafless branches sometimes were either substituted by new branches produced from the xylopodium or kept growing and producing new leaves. It took nine years for the attainment of maturity (production of flowers and fruits).

Tables 1 and 2 display the data obtained from morphometric and chemical analyses.

In three out of the five sampled plants, the roots had grown only reaching the end of the PVC tube (about 1.45 m deep), while in other two plants, the roots had escaped from the tube and merged with the soil. The plants with the most developed shoot system also exhibited the most extensive roots.

The roots ramified at about 50 cm depth in bifurcations or multiple ramifications with a vertical growth (Fig. 1-B). The diameters of all the main roots were the same for all the root lengths, that is, showing almost no conicity. In transversal sections, about 40% of the root diameter consisted of wood and the other 60% was bark (Fig. 2-A).

No cuttings from the aerial portions sprouted, and after about 15 days, all of them were clearly dead and dry. Among the roots, none of the six cuttings that were planted horizontally sprouted. From the total of 63 cuttings planted vertically, two had died at the end of the observation period (six months). The partially buried cuttings sprouted earlier than the ones totally buried; however, as the percentage of sprouting was identical, the data were grouped together. Also, the treatment of burning the extremities of the cuttings did not lead to earlier and/or more frequently sprouting; therefore, the data were grouped together. This way, the first saplings producing shoots and leaves were observed after 60 days of cultivation. During the six months of observation, 45 out of the 61 cuttings had produced shoots and leaves; the others, however, remained visually showy, but none has sprouted after some more weeks of observation. The sprouted saplings were then transferred to bigger pots, and it was observed that 16 out of 45 had already produced roots of secondary order (Figs. 2- B and 2-C).

4. DISCUSSION

The cultivation method of *B. gaudichaudii* adopted in the present study proved to be suitable for root harvesting, the part that contains high concentrations of pharmaceutically important substances. The seedlings were capable of growing in the PVC tubes. Although the plants were exposed to natural environmental conditions, the regular irrigation in the first years seemed to be important for survival. One of the advantages of this cultivation method was the easy removal of the plants at the moment of

harvesting the roots. It takes only a few minutes extractivism, digging is necessary, which to recover the plants, while in the regular demands considerably greater energy and time.

Table 1. Morphometric data of five plants cultivated in garden conditions after nine years of growth

Parameters	Measured values		
	Minimum	Maximal	Average
Length of the aerial part (cm)	63.0	100.5	85.7
Circumference of the aerial part close to soil (cm)	3.4	6.4	5.1
Length of the roots (cm)	109.0	155.0	128.3
Circumference of the upper portion of the taproot (cm)	5.0	7.5	5.9
Circumference of the lower portion of the taproot (cm)	3.8	5.7	4.7
Total mass of the subterranean system (g)	140.8	230.3	184.6

Table 2. Chemical composition of the roots of *Brosimum gaudichaudii* cultivated in garden conditions, after nine years of growth

Parameter	Mean ± Standard deviation
Humidity(g of H ₂ O.100 ⁻¹ g of fresh weight)	69.45±3.22
Proteins(g.100 ⁻¹ g of fresh weight)	1.51±0.09
Lipids (g. 100 ⁻¹ g of fresh weight)	0.53±0.02
Ash (g.100 ⁻¹ g of fresh weight)	0.79±0.03
Fibers (g.100 ⁻¹ g of fresh weight)	5.05±0.87
pH	5.95± 0.05
Totally titratable acidity	5.11± 0.10
Vitamin C (mg.100 ⁻¹ g of fresh weight)	13.53± 0.10
Furanocumarins (mg.g ⁻¹ of fresh weight)	Bergapten : 2.67 ± 0.10 Psoralen: 2,89 ± 0,10



**Fig. 1. A. Conditions of cultivation in a garden, seed propagation. Scale bar = 25 cm
B. General overview of an adult plant being removed from the cultivation tube, showing ramification of the taproot at about 50 cm depth. Scale bar = 25 cm**

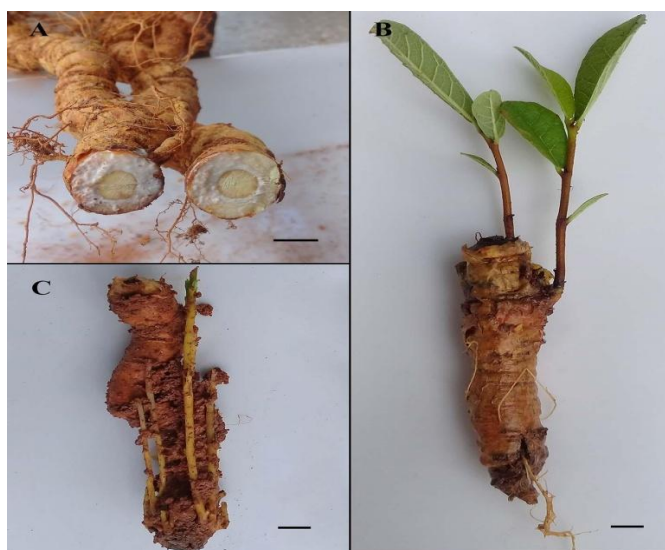


Fig. 2. A. Transversely sectioned root showing the proportionally thick bark exuding latex. Scale bar = 1cm. (B). Sapling produced in a small tube displaying aerial shoots and roots of the secondary order. Scale bar = 1 cm, (C). Sapling grown in a small tube exhibiting the production of aerial branches, but not roots of the secondary order. Scale bar = 1 cm

Morphologically, the roots of the cultivated plants differed from the wild ones; while the wild plants had a single, straight, deep taproot that ramified at a depth of about 1.5 – 2.0 m [1], in the cultivated plants, the ramification of the taproot started at a depth of 50 cm itself. Palhares and Silveira [14] observed that after the first year of cultivation, the seedlings of *B. gaudichaudii* produced roots that were 50 cm deep. Hence, the cultivation under horticultural conditions seemed to have induced the ramification of the roots at 50 cm length. If in the first year the root grew 50 cm, then it took around eight years for an additional growth of only 1 m, resulting in the average length of 1,30 m observed in Table 2. Such an observation is in accordance with the edaphic and ecophysiological studies of Jackson et al. [27], who exposed that from 50 cm and below, water is reasonably available for the Cerrado plants during the dry season. Therefore, perhaps the roots of *B. gaudichaudii* are genetically programmed for a quick growth in the first year, so that they could obtain water from the soil; after this initially fast growth, the root system would respond to other stimuli, such as the competition for water and nutrients with the surrounding vegetation and that is why the roots of the wild plants would ramify only after a depth of 1 m.

Regarding the phenology of *B. gaudichaudii*, [28] observed that under natural condition, this

species showed deciduousness, at least partly. Thus, this appears to be a genetically controlled aspect that is unrelated to the environmental conditions.

One of the most important fields of research interest in the domestication of *B. gaudichaudii* is its relevance to the pharmaceutical industry. According Miranda [9] and Cunha et al. [29], an oral dose of furanocoumarins for PUVA-therapy contains about 4 mg of the ingredient. Since the roots of *B. gaudichaudii* contained about 5,58 mg of furanocoumarins per gram of fresh weight and the roots weighed about 184 mg in average, the content in the cultivated plants was considerably high, indicating that the cultivation of this plant can be economically viable in the conditions presented here. Martins et al. [30] reported that the concentration of furanocoumarins in the roots of *B. gaudichaudii* is 0.18% (w/w), which is still considerably high. Nonetheless, these discrepancies can be related to sampling methods, harvesting conditions, and measurement techniques. Indeed, in the wild plants, the diameter of the bark was less than 20%, [1,16] while in the cultivated plants, the bark was thicker, amounting to 60% of the radicular diameter. According to Jesus et al. [5] and Pozetti [6] the bark of the root contains the highest concentration of furanocoumarins.

It is necessary to optimize the production of valuable substances, for example, by identifying

more productive cultivars and/or by improving the cultivation practices. In the experiments performed as a part of this study, it was necessary to wait for the plants to reach maturity, but it is possible that the plants could be harvested earlier within a few years of cultivation, aspects which need further research.

In the field conditions, xylopodium re-sprouts after the fire, which in the Cerrado is often quick and superficial. So, it was hypothesized that the stimulation by fire would induce the subterranean system to re-sprout. Hence, a quick burn was done for a part of the root cuttings. However, the obtained results did not confirm this hypothesis.

The results derived from our work are more promising than the ones of Silva et al. [18], who reported a sprouting in about 30% of the cuttings. However, the authors did not specify the time of observation. The matrices of the present paper came from relatively young plants cultivated in garden conditions; thus, it is necessary to verify if root cuttings from wild plants will present similar results.

In the growth of saplings from cuttings, there is a preoccupation if indeed the cutting emits roots [18]. In fact, in most of the plant species, the cuttings are obtained from aerial parts. The meristems of the aerial parts are partly differentiated so that they are programmed to produce new shoots and leaves. Hence, the simple fact of a branch cutting producing branches of secondary orders does not guarantee that the meristems of the buried portions would have produced roots. However, when a root cutting emits shoots and leaves, it is probable that it will be capable of generating roots of secondary order, as the root meristems are already programmed to do so. In the present sample, not all saplings had emitted roots of lower orders, but this is in accordance with the observation of wild plants, as the taproot produces basically a few radicles along its length and roots of the secondary order are less frequent [1]. Anyhow, it is now necessary to wait longer for documenting the growth of the saplings obtained herein.

Although the xylopodium is an organ anatomically different from the root, the radicular system of *B. gaudichaudii* also proved to be capable of sprouting and producing new saplings. Starch is present in the parenchyma of the root wood, which is not seen in the stem wood [15,16]. Therefore, it is probable that the reservoirs in the roots have a direct influence on

the ability to survive for several weeks. Small cuttings of 3-4 cm from the xylopodium sprouted in less than 50% of the cases (data not shown), indicating that the length of 10-12 cm is ideal for root cuttings.

Pereira et al. [31] evaluated the growth of *B. gaudichaudii* seedlings in a substrate less compact than the clay soil of the Cerrado, by mixing the soil with an equal amount of river sand, concluding that a less dense substrate may facilitate the root growth of *B. gaudichaudii*, while Carvalho et al. [32] showed that the quality of the substrate influences in the cultivation of medicinal plants.

5. CONCLUSION

As a conclusion, this work has provided a simple and promising method for the cultivation of macadela (*Brosimum gaudichaudii*) from the seeds or by vegetative propagation, thereby alleviating the extractivist pressure on the Cerrado and allowing a more stable and planned supply of raw material for the industry. So, instead of simply harvesting wild plants, one can cultivate them in tubes, and seedlings can be obtained either by seeds or by root cuttings.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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