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Jacaranda ulei Bureau and K. Schum. (Bignoniaceae): *in vitro* seedling developmental study as contribution towards the domestication of this medicinal Brazilian savannah species.

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ABSTRACT

Jacaranda ulei is a Cerrado species used in traditional medicine and has been indicated for high cholesterol and inflammatory processes. This study has focused on *in vitro* seed germination, and initial seedling growth of *J. ulei*. Seed decontamination was effective (>95%), and germinability was above 90% for all collected seeds, except those from mother-plant-3 (75%). The majority of the seedlings were morphologically normal, with epigeal phanerocotylar germination, with leaf and cordate cotyledons, and the collet region was obliquely to the stem. The number of leaflets increased in the eophylls with the development. Around the 120th day of culture, seedlings continued to develop and the primary roots were well developed, with few lateral roots and no thickening at the collet region. This study showed that *J. ulei* may be germinated *in vitro*, which is an important step for domestication and will help to establish a propagation protocol for this species.

Keywords: Decontamination, seedling, young plant, germinability, morphology.

1. Introduction

Jacaranda ulei Bureau & K. Schum. is a Cerrado subshrub, popularly known as Carobinha and Carobinha-do-campo[1]. Leaves and subterranean system of *J. ulei* are used in traditional medicine for musculoskeletal pain relief, diarrhea control, and, more importantly, as a main ingredient in homeopathic remedies in which it is indicated for skin disorders and urinary tract diseases [2]. It has also been found that this plant is used for cholesterol reduction, as based on field interviews with the root-harvesting communities[3]. Furthermore, this plant has been indicated for veterinary treatment of skin complications, musculoskeletal, digestive and endocrinal system disorders, infections, parasite diseases, and inflammation[1].

The medicinal properties of the *Jacaranda* genus are due to the high phenolic, flavonoid, terpene, chinone, and complex polysaccharide contents, which have shown antitumor, anti-malarial, and anti-leishmaniasis activities in *in vitro* assays [4, 5, 6, 7, 8, 9, 10, 11, 12, 13]. These compounds may accumulate in several plant organs, including the leaves, flowers, and root system. For instance, phenolic compounds and terpenoids, more specifically monoterpene cineole, pentacyclic triterpenes, and steroids, many of which may have medicinal uses and were detected in the flowers of *J. oxyphylla*[9]. Furthermore, leaf extracts of *J. puberula* show anti-oxidant activity, contributing to prevent diseases associated with excess free radicals, including cancer, heart disease, atherosclerosis, and diabetes [12].

Plant tissue culture techniques have been used to propagate several medicinal species. For instance, *Poliomintha glabrescens* was micropropagated to provide a rational use of this species[14]. This plant, also known as Mexican oregano, has high contents of luteolin, a flavone that has been for several medicinal purposes such as antimicrobial, anti-inflammatory, antioxidant, and cancer prevention and treatment[15, 16]. Moreover, such studies are essential to ensure the domestication and sustainable use of Cerrado plant species,

Particularly those that are difficult to propagate vegetatively due to a low rate of explant rooting[17, 18]. Additionally, tissue culture protocols allow the genetic manipulation of such plants for utilization, for example, as bioreactors to produce compounds of pharmaceutical interest.

In non-domesticated species the use of mature plant material for *in vitro* propagation such as leaf disks, sprouts, and roots have not been usually very successful. In wild species, it is very difficult to establish aseptic culture using mature tissue samples because the explant source is highly contaminated. In such cases, the use of seeds as source of young and more reactive tissues is an alternative to overcome this hindrance. Seeds, as explant source, have not been used only for non-domesticated plants, but as starting explants in species with recognized commercial value such as in *Eugenia myrtifolia* Sims[19].

Thus, the aim of this work was to evaluate the seed sterilization efficiency, *in vitro* germination, early development, and morphology of *Jacaranda ulei* seedlings and plants, with the purpose of propagating this species by seed and the production of more suitable juvenile propagules for micropropagation.

2. Material and Methods

J. ulei fruits were collected in February and March 2009 from five previously selected specimens growing in the Cerrado, Brasilia, DF (15º58'50.6"S and 47º56'58.8"W). An exsiccate was deposited in the Herbarium of the University of Brasilia (UB) under the registration number 96488. The dehiscent fruits were collected still closed at the end of the ripening period, a time when the exocarp color had changed from green to beige, and packed in paper bags.

After manual processing, the selected seeds were sterilized in 70% alcohol for 1 min and then in commercial bleach (2-2.5% active chlorine) for 15 min; the seeds were then rinsed 3 times in sterilized distilled water. After sterilization, the collected seeds from five wild specimens (mother-plants) were inoculated in test tubes (25x150 mm) containing autoclaved agar-water (8 $g.L^{-1}$) medium. The culture conditions were 27 ºC (±2 °C), 80% relative humidity, a 16 h photoperiod, and light intensity of 41 $\mu mol.m^{-2}.s^{-1}$. The experiment was completely randomized, with a sample size of 80 seeds per mother plant, and each experimental parcel was composed of 10 tubes, each containing one seed. The sterilization and germination efficiencies were observed on the 15th and 30th day of culture, respectively. Germination was characterized by the emergence of the radicle, and normal seedling development was considered plants with well-formed roots, shoots, and cotyledons. The ontogeny of the *in vitro* seedlings and young plants was described up to the 120th day of culture.

To determine the proper conservation period for the seeds, two sets of seeds from each mother plant (n = 48 each) were stored in paper bags at ambient temperature for six and 12 months; these seeds were then inoculated in test tubes containing agar-water and cultured under the same growth conditions described above.

The data were subjected to an analysis of variance (ANOVA) using the software SISVAR version 5.1, and the means were compared by Scott-Knott test at 5% significance[20].

3. Results

The sterilization rate was over 95% on the 15th day of culture for seeds from all the field-collected plants tested (Table 1). The sterilization treatment did not affect the *in vitro* germination process of *J. ulei* seeds and was between 90 and 100% on approximately the 30th day of cultivation for the seeds collected from all but a single mother-plant (number 3), which showed 75% germinability. The results showed that the difference in germinability among the mother-plants was only significant between plant 3 and the other plants (Table 1). It is noteworthy that all the plants used as a seed source were able to produce seedlings with normal morphology, which was above 90% on the 60th day after inoculation (Table 1).

Table 1: Germinability (%) on the 30th day of culture and normal plants (%) on the 60th day of culture for all field-collected *Jacaranda ulei* Bureau and K. Schum. seeds.

Plant	Germinated seeds (%)	Normal plants (%)
1	98.8 (80) **a**	92.4 (79) **a**
2	100.0 (80) **a**	93.7 (80) **a**
3	75.0 (80) **b**	91.7 (60) **a**
4	90.0 (80) **a**	90.3 (72) **a**
5	96.2 (80) **a**	92.2 (77) **a**

The values followed by the same letter in the same column do not differ from each other by the Scott-Knott ($p<0.05$) test. (n) = number of seeds.

The germinability rate decreased with seed storage, showing 47.9% and 29.2% for seeds stored for 6 to 12 months, respectively (data not shown).

The number of eophylls, leaves, and leaflets was observed after 60 days of culture. The first two pairs of eophylls had three leaflets. From the third pair and thereafter, and in some cases from the second pair, it was observed that the eophylls had five pairs of leaflets (Figure 1).

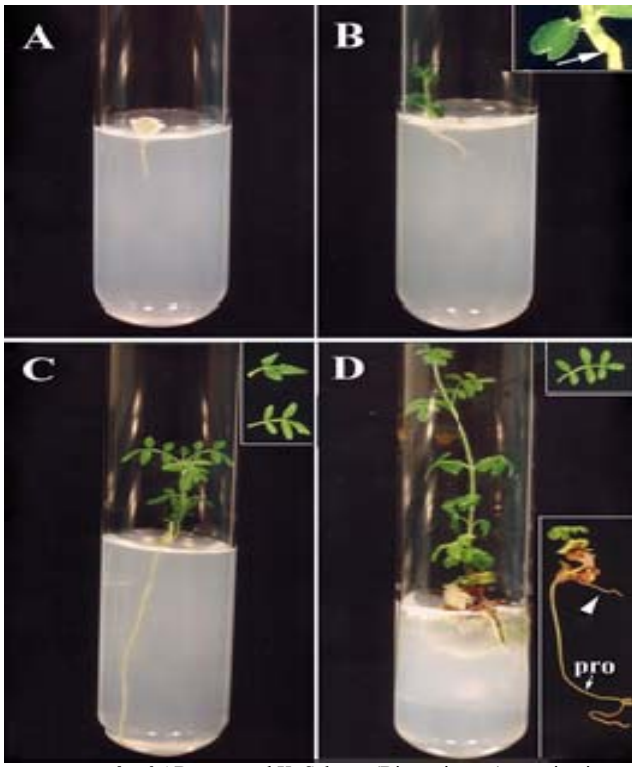

Fig 1: *Jacaranda ulei* Bureau and K. Schum. (Bignoniaceae) germination and seedling/young plant development in agar-water medium (8%). A. Germination observed on the 12th day of culture. B. Plants at approximately the 20th day of culture. The shoot had foliaceous cotyledons, cordate shape, and hypocotyl/collet region (arrow). Notice that the collet was oblique to the hypocotyl (inset). C. Culture day 60. First eophylls composed of 3 to 5 leaflets (inset). D. Plant appearance at 120 days. Note that the upper internodes have elongated and that the eophylls have also expanded and possess 7 leaflets (upper inset). Root system (lower inset). Note the primary root (pr) with a lateral root (arrow).

Additionally, the plants had four to six pairs of leaves at this age (Figure 1). Up to 120 days of age, occurred an increase in the number of leaflets, and the plants exhibited leaves with five to seven leaflets (Figure 1). Consistently, the number of leaves also increased, as observed in Figure 1d.

The vast majority of the seedlings were normal (Figure 2a), with no significant differences related to the origin of the seedling (Table 1). Figure 2 depicts representative specimens of normal and abnormal seedlings, which was seen in less than 10% of seedlings from all mother-plants (Table 1). Besides, the most common abnormalities observed were mainly related to poor root formation (Figures 2b-c), which usually manifested in the apical region of the primary root with signs of necrosis (Figure 2c).

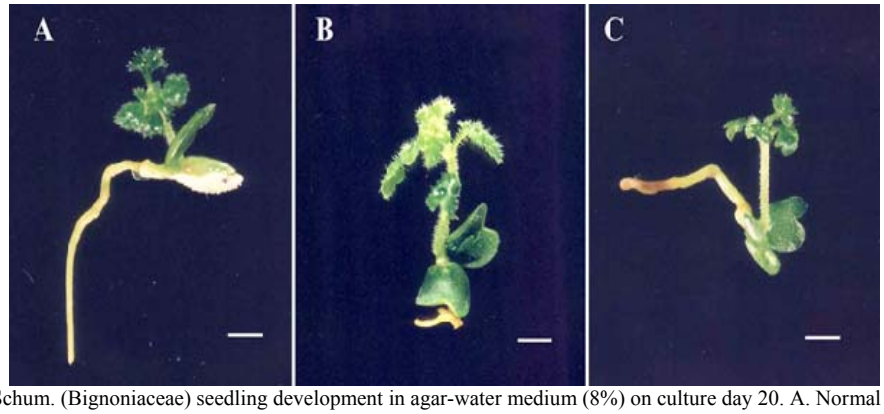

Fig 2: *Jacaranda ulei* Bureau and K. Schum. (Bignoniaceae) seedling development in agar-water medium (8%) on culture day 20. A. Normal seedling with well-developed primary root. A-C. Abnormal seedlings showing impaired primary root, with necrosis signs on the apical region. Note that the shoot of all plants was relatively well developed. Scale bar = 0.5 cm

It is noteworthy to mention that the collet was oblique to hypocotyl as well as to the primary root (Figure 1 b). In addition, up to 120 days of cultivation no root thickening was observed in *J. ulei* seedlings.

4. Discussion

For woody plants, different types of propagules have successfully been used to initiate *in vitro* culture; however, success depends heavily on the phytosanitary conditions of the mother-plants. In general, plants from the field have high rates of infestation, which jeopardizes the continuation of micropropagation. To overcome this limitation, *in vitro* seed germination has been widely used, particularly for savannah species [21, 22, 23] because seeds are more easily sterilized.

Furthermore, material from young seedlings is more responsive to *in vitro* conditions[24]. Moreover, *in vitro* germinability of the seeds is sometimes higher than under conditions of field breeding[25], thereby obtaining explants to induce aseptic multi-sprouting in the next step. The sterilization protocol used for *J. ulei* (70% alcohol for 1 min and commercial bleach for 15 min) was also effective for many other Cerrado plants, such as *Jacaranda mimosaefolia*[26] and *Alibertia edulis*[22].

The *J. ulei* seeds were not dormant and generally showed a high germinability rate. However, the lower germinability rate observed for the seeds of mother-plant-3 indicated that seed germination may vary between plants of the same population, emphasizing the importance of selecting the most productive plants. This procedure will certainly facilitate the domestication of this plant species with the purpose of using it in a sustainable way. As mentioned above, *J. ulei* has been attracting attention as a medicinal plant; nonetheless, before implementing this plant as a source of pharmaceutically active molecules, it must be domesticated to prevent predatory extractivism of the species. Accordingly, micropropagation techniques may accelerate the domestication of *J. ulei*.

The seeds used in these experiments were freshly collected (used no longer than three months after collection), which is very important because it has been found that the seed germinability rate significantly decreased in seeds stored for six months. This pattern has been observed in other species of the *Jacaranda* genus[1, 27] and, other genera of the Bignoniaceae family, such as in *Tabebuia*[28].

Morphological studies of seedlings and young plants indicate that the connection between the hypocotyl and principal root is, in general, a vertically continuous structure, which appears genetically determined[29]. Conversely, an oblique connection between the primary root and xylopodium has been observed in several Cerrado species, such as *Brosimum gaudichaudii* Tréc. [30] and *Byrsonima basiloba* Juss.[31], and these species have been shown to have a xylopodium. Similar to *Brosimum gaudichaudii* and *Byrsonima basiloba,* the *J. ulei* seedlings exhibited a lateral connection between the hypocotyl and collet. Likewise, field observations show that *J. ulei* possesses xylopodium (Silveira and Miranda, personal communication). Therefore, it appears that an oblique hypocotyl-collet connection is an important morphological step to developing a xylopodium. Nonetheless, the ontogeny of xylopodia requires further investigation, both *in vitro* and in nature. The xylopodium is a structure that is important for plant vegetative propagation, and it commonly accumulates medicinal substances. In *Brosimum gaudichaudii*, the xylopodium is the source of compounds traditionally used in the treatment of vitiligo[30].

A low percentage of abnormal seedlings was observed during *in vitro J. ulei* seedling development. The occurrence of abnormal seedlings has been reported for many species of Bignoniaceae, such as *Tabebuia impetiginosa* (Mart.). Standl, which showed approximately 20% abnormal plants *in vitro*[32]. In the Cerrado, these abnormalities are often associated with malformation of the root system, which generally involves the degeneration or necrosis of the apical region of the primary root[31, 33], similar to our observations for the abnormal *J. ulei* plants.

Studies on seedling and young plant ontogeny within the scope of micropropagation will certainly help to determine the most appropriate time to transfer the plants to the field. Moreover, comparing the morphology of micropropagated plants with naturally grown plants is an important quality parameter for the

success of *in vitro* native plant propagation and, consequently, for the conservation of the species. To the best of our knowledge, there are no available studies on the ontogeny of *J. ulei* seedlings or the morphology the young plants. Therefore, the results presented here may contribute to the characterization of the different developmental stages, allowing field recognition. Indeed, it is a relatively common fact that young individuals may be very different from the adults. For instance, adult *J. ulei* plants have bipinnate leaves, whereas the leaves of the young plants are pinnate. Accordingly, a lack of such knowledge may deceive the collector in the field.

Therefore, describing plant ontogeny is crucial for conservation efforts because it will allow the recognition and protection of young individuals in nature, which is important for a rational and sustainable use of the plant, which includes medicinal and pharmaceutical applications.

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