# Bacillus subtilis as a growth promoter of forest species seedlings

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**Aloisio Freitas Chagas Junior**
Doctor in Biotechnology, Federal University of Tocantins.
E-mail: chagasjraf@uft.edu.br

**Albert Lennon Lima Martins**
State University of Tocantins, Doctor in Plant Production.
E-mail: eng.Albertlennon@gmail.com

**Lillian França Borges Chagas**
Federal University of Tocantins, Doctor in Plant Production.
E-mail: lillianfbc@uft.edu.br

**Marcos Giongo**
Federal University of Tocantins, Doctor in Environment and Territory.
E-mail: giongo@uft.edu.br

**Damiana Beatriz da Silva**
Federal University of Tocantins
Doctor in Chemical and Biological Technologies
E-mail: damisb@gmail.com

**Kellen Angela O. de Sousa**
Federal University of Tocantins
Doctor student in Plant Production
E-mail: sousaka_@hotmail.com

**Adilon Martins Rocha**
Federal University of Tocantins
Master's student in Plant Production
E-mail: adilonrc@gmail.com

**Dallila Moreira de Oliveira Moura**
Federal University of Tocantins
Graduate in Agronomy
E-mail: dalilla.moreira@mail.uft.edu.br

**Leticia Bezerra de Almeida**
Federal University of Tocantins, Graduate in Agronomy
E-mail: leticia.almeida@mail.uft.edu.br

**Bruna Marques de Souza**
Federal University of Tocantins, Graduate in Agronomy
E-mail: bruna.marques@mail.uft.edu.br

**ABSTRACT**
Native forest species have been employed for several purposes, such as the recovery of degraded areas, reforestation, and afforestation. The growth-promoting microorganisms are a viable alternative to producing native forest seedlings. The Bacillus subtilis is an alternative since it stands out by promoting plant growth through the solubilization of phosphate, production of phytohormones, and the availability of nutrients. The present work aimed to evaluate the influence of Bacillus subtilis in the initial growth of fava-tamboril (Enterolobium maximum), amarelão (Apuleia leiocarpa), and paricá (Schizolobium amazonicum) seedlings. Inoculations were made with two strains of Bacillus subtilis (Bs08, Bs10) and the commercial product based on Bacillus subtilis PANTA©, using 1 mL of the solution of each strain, and a control sample with water only, using a graduated pipette. An entirely randomized design with three treatments and eight repetitions was used. The variables analysed were plant height (PH), stem diameter (SD), root volume (RV), root dry weight (RDW), above-ground biomass weight (AGB), and Dickson quality index (DQI). In general, inoculation with Bacillus subtilis, with both strains evaluated and the commercial inoculant (PANTA ©), provided gains in biomass and in the DQI of plants at 30 and 60 days after emergence. Thus, the inoculation of Bacillus subtilis showed promising results as a growth promoter for paricá, fava-tamboril, and amarelão.

**Keywords:** Microorganisms, enterolobium maximum, apuleia leiocarpa, schizolobium amazonicum.

# 1 INTRODUCTION

New technologies are constantly being used to improve the production of seedlings from forest species through management, as is the case of the use of tubes, which helped in several ways production of seedlings forms, facilitated transport, and saved substrate among others (LIMA FILHO, 2019).

To be successful in planting forest seedlings, it is necessary to have well-formed seedlings without diseases that can establish themselves as presenting their best performance. The rhizobacteria are presented as an alternative that benefits the seedlings in different ways, as growth promoters and fighting pests (CAIXETA, 2015; ZEILINGER et al., 2016; SARAVANAKUMAR et al., 2016).

The mechanism of plant growth promoted by microorganisms happens with biological nitrogen fixation, phosphate solubilization, production of phytohormones, nutrient availability, and other mechanisms that indirectly increase resistance to stresses (BULGARELLI et al., 2013; AHMAD et al., 2017; BRAGA et al., 2018; DIAZ et al., 2019).

With the use of rhizobacteria, the use of pesticides and mineral fertilizers can be reduced. Consequently, the negative impact on the environment is minimized (MORENO, 2019). Tanuja et al. (2015), in a study with several strains of *Bacillus,* proved their ability to establish colonies in roots and transport them to the other parts of the plants, such as stems and leaves. However, it is important to emphasize that this influence on plant growth may be altered according to biotic or abiotic factors (CAIXETA., 2015).

Bettio (2015) evaluated different doses of *Bacillus subilis* in forest species and found significant results regarding plant growth. However, considering that there are many other tree species of economic interest, there are still few studies on the use of these microorganisms in seedlings of forest species.

The present study aims to analyse the efficiency of *Bacillus subtilis* in promoting the growth of paricá (*Schizolobium amazonicum*), fava-tamboril (*Enterolobium maximum*), and amarelão (*Apuleia leiocarpa*) seedlings.

# 2 METHODOLOGIES

## 2.1 PLACE OF EXPERIMENT

The experiment was conducted in the seedling nursery production at the experimental station of the Federal University of Tocantins - Gurupi Campus, located in the southern region of the state of Tocantins (11º48'29" S, 48º56'39" W, 280 m altitude). The predominant climate is Aw type, considered an equatorial and dry winter, according to Köppen - Geiger (PEEL et al., 2007) and the regional climate is type B1WA 'a' humid with moderate water deficiency. The annual precipitation is 1600 mm, with a dry winter and rainy summer.

## 2.2 IDENTIFICATION OF BACILLUS SUBTILIS ISOLATES

The *Bacillus subtilis* UFT-Bs08 and UFT-Bs10 isolates were identified by morphological, biochemical, and structural tests following the methodology of Bergey's Manual of Determinative Bacteriology (SLEPECKY; HEMPHILL, 2006; RABINOVITCH; OLIVEIRA, 2015). The isolates UFT-Bs08 and UFT-Bs10 were acquired from soils of the cerrado of Tocantins and are maintained in the Microbiology Laboratory of the Federal University of Tocantins, Gurupi Campus (PPGPV/UFT) being periodically reported in Luria - Bertani (LB) medium. The *B. subtilis* strain UFMT-Pant001 (PANTA©) was identified as described by Machado et al (2010), also cultivated in LB medium and kept in the Microbiology Laboratory of the Medical School, Federal University of Mato Grosso collection.

## 2.3 OBTAINING SEEDS AND SOWING

The seeds of Paricá (*Schizolobium amazonicum*), fava-tamboril (*Enterolobium maximum*), and amarelão (*Apuleia leiocarpa*) were collected by the company EletroNorte and were donated for the experiment. Then these seeds were submitted to the mechanical scarification method, performed on the opposite side of the hilum with a 220-water sandpaper, this procedure was performed until the tegument had a hole so that there was exposure of the endosperm, facilitating the entry of water in the seed and, consequently, germination.

After scarification, the paricá seeds were placed in water at room temperature for 12 hours. The amarelão and fava-tamboril seeds, soon after scarification, were sown, and the paricá seed was sown after 12 hours in water. Tubes with a volume of 55 $cm^3$ were used. The substrate for seedling production was composed of a commercial substrate called Plantmax (pine bark, vermiculite of fine and superfine granulometry and humus) and sand in a proportion of 1:1. Forty-eight seeds of each species were used, planting two seeds in each tube. Seven days after emergence, the plants were thinned, leaving only one plant per tube.

## 2.4 TREATMENTS AND INOCULATION

The treatments used were inoculation of the two *Bacillus subtilis* isolates, UFT- Bs08 and UFT-Bs10, the commercial product based on *Bacillus subtilis* UFMT-Pant001 (PANTA), and a sample without inoculation. The isolates of *B. subtilis* UFT-Bs08 and UFT-Bs10 were cultivated on LB medium for three days, and the concentration of colonies in colony forming units (CFU) was determined by the serial dilution method, presenting an average concentration of 1 x $10^9$ CFU per mL of suspension, using 1 mL of suspension per tube. For the treatment with PANTA, also at a concentration of 1 x $10^9$ CFU per mL, a dose of 200 mL per kg of seed was used, according to the manufacturer's recommendations. For the control treatment, without inoculation, only water was used

in the volume of 1 mL per tube. The inoculation was performed at planting time, and the substrate was perforated by placing the seeds, then 1 mL of inoculant, and soon afterward, the seeds were covered with the substrate.

The seeds were planted 2 cm deep and maintained at 60% field capacity with daily irrigation. Each experiment was conducted separately in an entirely randomized design, composed of four treatments and eight repetitions.

### 2.5 EVALUATIONS AND STATISTICAL ANALYSIS

Evaluations were performed 30 and 60 days after seed emergence. The height of the plant was determined with a millimetre ruler from the root collar to the last leaf insertion. The diameter of the root collar was determined with the aid of a pachymeter. Subsequently, the seedlings were sectioned to separate the root system from the aerial part. Then, the roots were washed in running water. The roots and aerial parts of the plants were placed in paper bags, identified, and taken to a forced circulation drying oven at 65 ºC for 72 hours.

After the drying period, the aboveground biomass (AGB) and the root dry weight (RDW) were determined using a precision balance (0.0001 g). The Dickson Quality Index (DQI) was also evaluated, where the relation between total dry weight (TDW) is calculated by the sum of the relation between height (H) and the stem diameter (SD) and the relation between aboveground biomass (AGB) and root dry weight (RDW): DQI = {TDW(g)} /{H(cm) / SD (mm) + AGB (g) / RDW(g)}.

The data were submitted to the ANAVA (Analysis of Variance) test and the means to the Tukey test, with a 5% probability error level, using the software SISVAR (FERREIRA,2019).

## 3 RESULTS AND DISCUSSION

The inoculation of *B. subtilis* isolates exerted a significant influence on quality biomass parameters in the two evaluation periods at 30 and 60 days after emergence (DAE).

For paricá, at 30 DAE, for plant height (PH), the treatments with UFT-Bs10 and the one with PANTA were superior ($p \leq 0.05$), followed by the treatment with UFT-Bs08 also superior when compared to the control sample without inoculation (Table 1, Figure 1). For root volume (RV), the treatment with UFT-Bs10 was superior ($p \leq 0.05$), followed by the other treatments with inoculation, when compared to the sample. For aboveground biomass (AGB), root dry weight (RDW), and total dry weight (TDW), the treatment with the product PANTA was superior ($p \leq 0.05$), followed by the other treatments also superior (Table 1). For stem diameter (SD) and Dickson quality index (DQI), there were no significant differences between treatments at 30 DAE (Table 1).

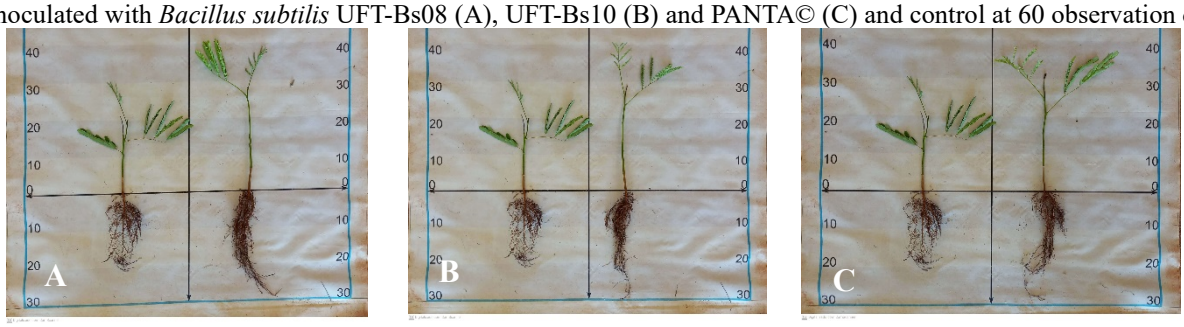
Table 1. Height (H), stem diameter (SD), root volume (RV), aboveground biomass (AGB), root dry weight (RDW), total dry weight (TDW), and Dickison's quality index (DQI) of Paricá (*Schizolobium amazonicum*) seedlings cultivated in substrate inoculated with *Bacillus subtilis*. [1]

| Treatments | Height (cm) | SD (cm) | RV (mL) | AGB (g) | RDW(g) | TDW(g) | DQI |
|---|---|---|---|---|---|---|---|
| Evaluation at 30 days | | | | | | | |
| UFT-Bs08 | 24,12b | 0,50a | 3,50c | 2,47b | 1,20ab | 3,67b | 0,07a |
| UFT-Bs10 | 26,12a | 0,51a | 5,50a | 2,48b | 1,10b | 3,59b | 0,06a |
| PANTA© | 26,25 a | 0,47a | 5,00b | 2,71a | 1,30a | 4,01a | 0,06a |
| Control | 20.00 c | 0,41a | 2,50d | 2,01c | 0,90c | 2,91c | 0,05a |
| COV (%)[2] | 5,61% | 18,34% | 6,67% | 4,56% | 7,52% | 4,15% | 20,11% |
| Evaluation at 60 days | | | | | | | |
| UFT-Bs08 | 27,87b | 1,11a | 7,12a | 3,50a | 2,22a | 5,72a | 0,21a |
| UFT-Bs10 | 29,25b | 1,10a | 6,37b | 3,51a | 2,10a | 5,62a | 0,19ba |
| PANTA© | 30,87a | 1,05b | 6,25b | 3,50a | 2,21a | 5,72a | 0,18cb |
| Control | 22,12c | 0,91c | 4,50c | 2,90b | 1,62b | 4,52b | 0,17c |
| COV (%)[2] | 3,77% | 2,55% | 8,95% | 8,28% | 9,82% | 5,77% | 8,23% |

Source: Author.

[1]Mediums followed by the same lowercase letter in the column do not differ by Tukey test at 5% probability ($p \leq 0.05$).
[2]C.O.V. = Coefficient of variation.

Figure 1. Comparison of aerial part growth and roots of paricá (*Schizolobium amazonicum*) seedlings cultivated in substrate inoculated with *Bacillus subtilis* UFT-Bs08 (A), UFT-Bs10 (B) and PANTA© (C) and control at 60 observation days.




Source: Author.

After 60 DAE, for PH, the treatment with Bs08 was superior ($p \leq 0.05$), followed by the other treatments, also superior to the control (Table 1). For SD, the treatments with UFT-Bs08 and UFT-Bs10 were superior ($p \leq 0.05$), followed by the treatment with UFT-Bs08, also superior to the control. For the RV, the treatment with UFT-Bs08 was superior ($p \leq 0.05$), followed by the other treatments with inoculation, which were also superior to the control. For the AGB, RDW, and TDW, the treatments with inoculations of *B. subtilis* were superior ($p \leq 0.05$) concerning the control. For DQI, the treatments with UFT-Bs08 and UFT-Bs10 were superior ($p \leq 0.05$) (Table 1).

For fava-tamboril seedlings, inoculation with UFT-Bs10, UFT-Bs08, and PANTA, at 30 DAE, was superior ($p \leq 0.05$) for the characteristics evaluated concerning the control (Table 2) (Source: Author Figure 2).

At 60 DAE, the treatments with inoculation were superior ($p \leq 0.05$) compared to the control. For AGB the treatments with UFT-Bs10 and PANTA were superior ($p \leq 0.05$). For DQI the treatment with UFT-Bs08 was superior ($p \leq 0.05$), followed by the treatments with UFT-Bs10 and PANTA, also superior to the control (Table 2).

Despite the scarcity of literature addressing the effects of bio-inoculation on fava-tamboril, it was observed that it is a plant that responds well to the action of biological growth promoters.

Table 2. Height (H), stem diameter (SD), root volume (RV), aboveground biomass (AGB), root dry weight (RDW), total dry weight (TDW), Dickison's quality index (DQI) of fava-tamboril seedlings (*Enterolobium maximum)* grown in substrate inoculated with *Bacillus subtilis*. [1]

| Treatments | Height (cm) | SD (cm) | RV (mL) | AGB(g) | RDW (g) | TDW (g) | DQI |
|---|---|---|---|---|---|---|---|
| Evaluation at 30 days | | | | | | | |
| UFT-Bs08 | 15,12a | 0,30[a] | 3,50b | 1,64a | 0,82a | 2,46a | 0,047a |
| UFT-Bs10 | 15,87a | 0,30[a] | 4,25ba | 1,60a | 0,74a | 2,34a | 0,042ba |
| PANTA© | 15,50a | 0,30[a] | 4,37a | 1,44a | 0,76a | 2,20a | 0,041ba |
| Control | 11,37b | 0,27b | 2,50c | 1,09b | 0,52b | 1,61b | 0,037b |
| COV (%)[2] | 5,48% | 0,00% | 15,83% | 13,02% | 18,43% | 10,50% | 13,72% |
| Evaluation at 60 days | | | | | | | |
| UFT Bs08 | 25,00b | 0,58b | 3,87b | 2,68b | 2,87a | 5,55a | 0,126a |
| UFT Bs10 | 31,62a | 0,60[a] | 5,37a | 2,94a | 2,81a | 5,75a | 0,108b |
| PANTA© | 30,75a | 0,60[a] | 5,12a | 2,88a | 2,54a | 5,43a | 0,103b |
| Control | 24,50b | 0,50c | 2,75c | 2,59b | 1,39b | 3,98b | 0,078c |
| COV (%)[2] | 3,66% | 0,80% | 15,99% | 5,25% | 12,90% | 5,23% | 6,20% |

[1] Averages followed by the same lowercase letter in the column do not differ by Tukey test at 5% probability ($p \leq 0.05$).
[2]C.O.V = Coefficient of variation

Figure 2. Comparison of growth of aerial part and roots of fava-tamboril (*Enterolobium maximum)* seedlings cultivated in substrate inoculated with *Bacillus subtilis* UFT-Bs08 (A), UFT-BS10 (B), and PANTA© (C) and control at 60 days of observation.


Source: Author

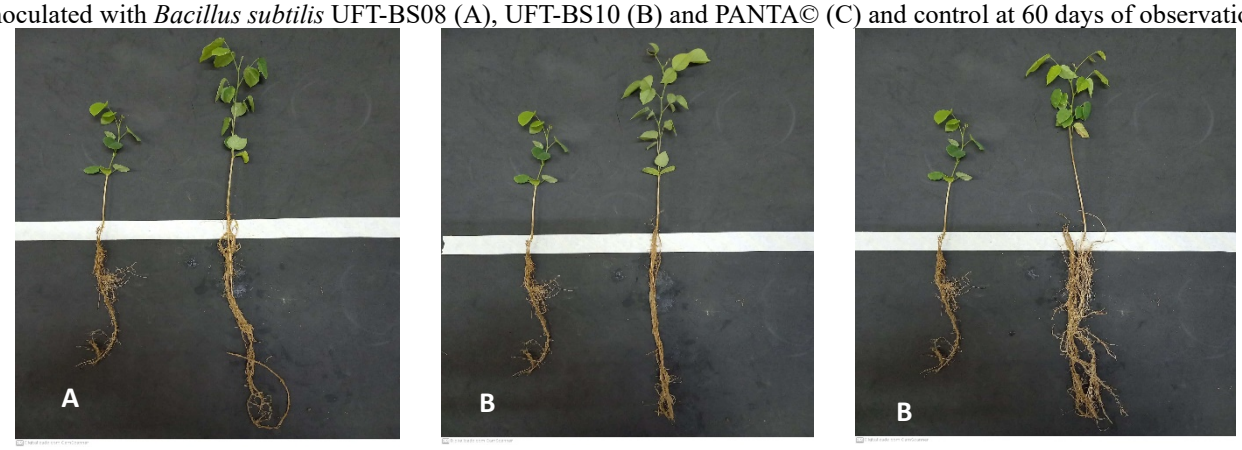
For the seedlings of *amarelão*, it was observed that there were significant differences for H, SD, RV, and TDW (Table 3) (Figure 3). At 60 DAE, it was observed that inoculation with the different treatments was superior ($p \leq 0.05$) compared to the control (Table 3).

Table 3. Height (H), stem diameter (SD), root volume (RV), above-ground biomass weight (AGB), root dry weight (RoWM), total dry mass (TDS), and Dickison's quality index (DQI) of amarelão (*Apuleia leiocarpa*) seedlings grown in substrate inoculated with *Bacillus subtilis*. [1]

| Treatments | Height (cm) | SD (cm) | RV (mL) | AGB(g) | RDW (g) | TDW (g) | DQI |
|---|---|---|---|---|---|---|---|
| Evaluation at 30 days | | | | | | | |
| UFT-Bs08 | 7,62a | 0,15a | 4,00b | 0,86a | 0,43a | 1,30a | 0,02ba |
| UFT-Bs10 | 6,12b | 0,15a | 4,50a | 0,80a | 0,41a | 1,21ba | 0,02ba |
| PANTA© | 8,25a | 0,14b | 4,00b | 0,84a | 0,44a | 1,29a | 0,02b |
| Control | 4,50c | 0,13c | 2,00c | 0,72a | 0,38a | 1,10b | 0,03a |
| COV (%)[2] | 7,13% | 4,96% | 1,95% | 14,09% | 22,70% | 10,78% | 19,75% |
| Evaluation at 60 days | | | | | | | |
| UFT-Bs08 | 15,00a | 0,30[a] | 8,37b | 2,44a | 1,11a | 3,55a | 0,068a |
| UFT-Bs10 | 15,50a | 0,30[a] | 8,75ba | 2,26a | 1,12a | 3,38a | 0,063ba |
| PANTA© | 15,87a | 0,30[a] | 9,12a | 2,41a | 1,10a | 3,52a | 0,063ba |
| Control | 10,25b | 0,21b | 5,37c | 1,51b | 0,80b | 2,31b | 0,048b |
| COV (%)[2] | 7,83% | 16,70% | 6,81% | 7,57% | 7,22% | 5,63% | 12,91% |

[1]Averages followed by the same lowercase letter in the column do not differ by Tukey test at 5% probability ($p \leq 0.05$).
[2]C.O.V. = Coefficient of variation

Figure 3: Comparison of growth of aerial part and roots of amarelão (*Apuleia leiocarpa*) seedlings cultivated in substrate inoculated with *Bacillus subtilis* UFT-BS08 (A), UFT-BS10 (B) and PANTA© (C) and control at 60 days of observation.




Source: Author

In general, the inoculation with *B. subtilis* with both strains evaluated and the commercial inoculant (PANTA ©) demonstrated efficiency as a growth promoter for seedlings of the species evaluated in this work. Although the literature for native tree species is scarce, the use of *B. subtilis* as a growth promoter and as a biocontrol pathogens agent is described and proven for tree species of commercial interest as *Pinus ellioti* (MACIEL et al., 2014), *Eucalyptus urograndis* (MOREIRA; ARAÚJO, 2013; LUCIANO et al., 2023) and *Jacaranda mimosifolia* (MISSIO, 2016).

The present study indicates contrasting results with those found by Lima et al. (2020), who observed that biological inoculation with *Bacillus subtilis* does not influence the growth of paricá seedlings. However, the results observed corroborate the growth-promoting effect of *Bacillus subtilis* described in the literature (JEONG et al., 2010; WANG et al., 2010).

One of the effects observed for the three plant species evaluated was the increase in the root volume and above-ground biomass weight of plants grown in the substrate with inoculum of *B. subtilis*, whether in the form of inoculant from strains grown in the laboratory or the form of a commercial

product. This increase in root system weight was also observed in seedlings of commercial species such as eucalyptus (MAFIA et al., 2005) and pine (SANTOS et al., 2018).

The results found may also be related to the fact that the inoculation of *B. subtilis* promotes greater growth in plants due to the induction of phyto hormones, which favours root growth and an increase in the number of root hairs and, consequently, increasing root mass (CUSTÓDIO et al., 2013; ANGUIANO CABELLO et al., 2019).

Regarding commercial inoculants, formulated products represent a practical alternative increasing in the field since they can be marketed and stored more easily. The efficiency of commercial inoculant products of *B. subtillis* at a physiological level has already been demonstrated for other commercial crops (QIAO et al., 2017; JAMILY et al., 2019; MORETTI et al., 2020).

The production of forest seedlings has increased due to the immense use in commercial planting, which has also been used for the recovery of degraded areas. With the increase of this demand, new technologies are needed to accelerate growth and reduce the cost of inputs during cultivation. Chemical stimulants only contain temporary control, which makes repeated applications necessary during crop growth. Microorganism-based bio stimulants can establish, colonize, and reproduce in the soil (SUASSUNA et al., 2019). Thus, the use of microorganisms that promote plant growth, such as *B. subtilis,* provides significant results in increasing biomass of forest seedlings such as the species paricá (*Schizolobium amazonicum),* fava-tamboril (*Enterolobium maximum*) and amarelão (*Apuleia leiocarpa*), demonstrating to be an efficient technology for the formation of forest seedlings.

## 4 CONCLUSIONS

The inoculation of *Bacillus subtilis* showed a promising result as a growth promoter for Paricá (*Schizolobium amazonicum),* Fava-tamboril (*Enterolobium maximum*), and amarelão (*Apuleia leiocarpa*).

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