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Effects of Seed Treatment and Bio-inoculants on *Baikiaea plurijuga* DC. and *Pterocarpus angolensis* Harms Seed Germination

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ABSTRACT

Background: This study examined the effect of pre-sowing seed treatments and microbial inoculation on the germination of *Pterocarpus angolensis* and *Baikiaea plurijuga* at the Botswana University of Agriculture and Natural Resources.

Methods: This tree nursery study employed a 2 × 4 × 2 factorial, randomised experiment to test four pre-sowing treatments: mechanical scarification, soaking in rainwater and warm water (40°C) and untreated seeds- alongside two levels of microbial inoculation: with and without *B. ripae*.

Result: Mechanical scarification slightly improved germination, leading to earlier and more uniform germination in both species, with *P. angolensis* exhibiting a mean germination time of 10.5 days and a germination velocity coefficient of 9.57. *P. angolensis* germination decreased from 80.83% to 66.7%, indicating an incompatibility with *B. ripae*. Conversely, *B. plurijuga* germination increased from 70.84% to 91.67%. The findings demonstrate that scarification enhances germination in both species and can be used to improve native seedling protocols for the regeneration of dryland forests.

Key words: *Baikiaea plurijuga*, *Bradyrhizobium ripae*, Native tree species, Pre-sowing treatments, *Pterocarpus angolensis*, Seed germination.

INTRODUCTION

Forests and woodlands provide essential ecosystem services, including climate change mitigation (Vargas, 2022), habitat for numerous plant and animal species (Pan *et al*., 2011; Watson *et al*., 2018) and the supply of timber, fuelwood and non-timber forest products (Sunderlin *et al*., 2007). They aid in carbon sequestration (Pramova *et al*., 2012), enhance landscape aesthetics (Mundher *et al*., 2022; O'Brien *et al*., 2024) and improve urban environments by filtering pollutants, reducing temperatures and promoting mental well-being (Tyrväinen *et al*., 2005; McDonald *et al*., 2007). *Pterocarpus angolensis* (African teak or mukwa) and *Baikiaea plurijuga* DC (Zambezi teak) are key deciduous hardwood species native to Miombo woodlands (lipumbu *et al*., 2024). *P. angolensis* offers durable, attractive timber used in veneering, flooring, carpentry, furniture and construction (Palgrave, 2002). Its bark and roots are also employed in traditional medicine to treat various ailments (Samie *et al*., 2009; Sadiki and Tshikhwawe, 2018). Additionally, this species contributes to nitrogen fixation and provides pollen and nectar for bees (Orwa *et al*., 2009). *B. plurijuga* supplies durable timber suitable for construction, furniture and tools, with its bark used in medicine and tanning (Palgrave, 2002).

B. Plurijuga, *P. angolensis* Harms and other native woody species are threatened by overexploitation, forest fires, pests, diseases, drought, herbivory and climate change. Their natural recruitment has declined due to droughts, seed shortages, litter loss, fire incidents and herbivory (McLaren and McDonald, 2003; Vieira and Scariot, 2006; Gavinet *et al.,* 2018; Zhang *et al.,* 2017). Furthermore, the germination of native woody species seeds in their natural habitats is hindered by tough seed coats, unfavourable environmental conditions and human activities; however, factors such as temperature fluctuations, abrasion and animal digestion (Ooi *et al.,* 2014; Traveset *et al.,* 2001; Renison *et al.,* 2010), along

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with heat from the soil (Mbalo and Witkowski, 1997; Lamont and Pausas, 2023), contribute to breaking seed dormancy.

Various methods, including mechanical, acid and water treatments, are employed to break seed dormancy and enhance germination (Tiwari *et al*., 2023; Mojeremane *et al.,* 2017, 2024; Taghizadeh and Sajadi, 2023; Mohamed *et al*., 2024; Alzandi *et al.,* 2025). The advantages of seed treatments include higher germination rates, uniform seedling emergence and protection of seeds or seedlings from early-season diseases and insect pests, which promotes crop emergence and growth (Govindaraj *et al*., 2017). Besides pre-sowing treatments, bio-inoculants are increasingly recognised as sustainable and environmentally friendly methods to improve seed germination, root growth and seedling development (Khan *et al.,* 2011, 2014; Ahemad and Kibret, 2014; Mridha *et al.,* 2016). They are especially crucial in semi-arid and savanna areas characterised by water shortages and poor soil quality (Maitra *et al.,* 2021). Despite growing interest, their effects on the germination of native trees remain largely unexplored. This study examined the impacts of seed pre-treatments and *Bradyrhizobium ripae* WR4T inoculation on germination in a nursery setting.

MATERIALS AND METHODS

Study area

This experiment was conducted at the forestry nursery of Botswana University of Agriculture and Natural Resources (BUAN) located in Sebele Content Farm (latitude 23°34′S, longitude 25°57′E, elevation 994 m), approximately 10 km north of Gaborone near the A1 highway (Rasebeka *et al.,* 2014). The soil composition is a well-drained sandy loam. The semi-arid climate of Sebele is characterised by hot summers and mild winters, receiving between 250 and 600 mm of rainfall each year, mainly from November to March. The study was carried out from March 2024 to March 2025.

Seed and potting soil collection

Fruits were harvested from two experimental trees collected from Kazuma, Kasane and Chobe Forest Reserves in the Chobe District of Northern Botswana. A GPS device was used to record the locations of the mother trees. Pods were gathered using a long-hooked stick and a looper, subsequently packed into paper bags and transported to BUAN for extraction and storage. For *B. plurijuga*, the pods were manually crushed at the BUAN Herbarium to remove the seeds, whereas for *P. angolensis*, secateurs were used. Any damaged or immature seeds were discarded, while the healthy seeds were stored at 5°C until they were required for experiments. Soil samples were collected from beneath the two study species at Pandamatenga Farms in the Chobe District. An electric fence protects the farm from elephants and other animals. The soil was taken to a depth of 20 cm using a spade. It was, then transported to BUAN in 25-kg plastic bags and subsequently filled into 2-litre black polyethene bags.

Experimental design and layout

This study employed a 2 × 4 × 2 randomised factorial design, incorporating four pre-sowing treatments: (i) mechanical scarification, (ii) soaking in rainwater, (iii) exposure to warm water at 40°C and (iv)seeds that were not treated. The second factor pertained to inoculation with *Bradyrhizobium ripae* WR4T, which was either applied or omitted. Each treatment was replicated three times, with 10 seeds per species in each replication. A single seed was sown in a 2-litre black polythene bag containing 2 kg of soil. The bags were placed on raised metal benches under a 40% shade net in the nursery.

Seed germination treatments

Each presowing treatment used 60 seeds, for a total of 240 per species. The 240 seeds were divided into two batches of 120 seeds each, one for presowing and the other for inoculation. Each treatment had three replicates, with 10 seeds in each. A nail cutter was used to scarify the seeds of both species by nipping the convex end opposite the embryo, avoiding damage. For a warm-water (40°C) soaking treatment, a water bath was filled with tap water and kept at 40°C. After reaching equilibrium, the water bath was turned off and the seeds were soaked for 24 hours. Rainwater was collected in a bucket overnight and transferred to a 250 ml beaker containing seeds. Seeds were soaked for 24 hours. Untreated seeds served as a control. The inoculation experiment employed seed batches that were mechanically scarified, pretreated in a water bath at 40°C and soaked in rainwater. *B. ripae* from the University of Bremen, Germany, was used as an inoculant. Approximately 5 g of inoculum was mixed with 25 mL of distilled water to create a slurry in a sterile 250 mL glass beaker. Seeds were, then coated with bacterial slurry in peat, following the method described by Somasegaran and Hoben (1994) and immediately sown in black polyethene bags. To prevent cross-contamination, inoculated seeds were sown after the non-inoculated ones.

Germination parameters

Seed germination was monitored daily from the day after sowing until measurements ceased after 12 months. The collected germination data were then used to calculate the specified parameters.

Seed germination percentage (GP)

The GP was determined using the method described by Scott *et al*. (1984).

$$GP = \frac{\text{Number of germinated seeds}}{\text{Total number of seed sown}} \times 100 \qquad ...(1)$$

Mean germination time (MGT)

Mean germination time was calculated using the method outlined by Ellis and Roberts (1981).

$$MGT = \frac{\Sigma\,(n \times t)}{\Sigma n} \qquad ...(2)$$

Where,

n= The number of seeds germinated on *t.*

t= Days from the start of the experiment to when those seeds germinated.

Σ (t*n)= The product of the number of seeds that have germinated and the corresponding number of days.

Σn= The total number of seeds that germinate successfully.

Germination index (GI)

The germination index was evaluated following Esechie (1994).

$$\text{Germination index} = \Sigma\,(G_t/T_t) \quad ...(3)$$

Where,

Gt= The quantity of seeds that have germinated by T_t.

T_t= The number of days from sowing to germination.

Coefficient of velocity of germination (CVG)

The velocity coefficient of germination was determined as described by Maguire (1962).

$$\text{Coefficient of velocity of germination} = [\Sigma\,(N \times T)/\Sigma N\,) \times 100 \quad ...(4)$$

Where,

N= The number of seeds germinated on t.

T= Days from the start of the experiment to when those seeds germinated.

Σ(T*N)= The product of the number of seeds germinated each day.

ΣN= The total quantity of seeds that germinated.

Data analysis

Data analysis was conducted using Statistix Software (Version 10) using descriptive statistics and three-way ANOVA. The germination percentage data were transformed to meet normality assumptions before performing the ANOVA. Tukey's HSD was employed to test mean differences at p<0.05.

RESULTS AND DISCUSSION

Table 1 presents the effects of seed pre-treatments and inoculation on the germination of *P. angolensis* seeds. No significant differences were observed among pre-sowing treatments (P>0.05), with germination rates ranging from 71.7% to 75%. Mechanical scarification and warm-water treatment resulted in a slight increase in germination, though this was not statistically significant. Germination time ranged from 10.5 to 22.81 days, with mechanical scarification being the fastest at 10.5 days. Soaking in warm water and untreated seeds reduced germination time compared to rainwater, but these differences were not significant. The germination index varied slightly, from 12 to 13 across treatments. Mechanical scarification had the highest germination velocity coefficient of 9.57; although this was not statistically significant, it indicated a faster germination rate. Other treatments showed lower velocities with no significant differences. Germination parameters were similar overall, but mechanical scarification was most effective, as evidenced by shorter germination time and a higher velocity coefficient.

The results showed that inoculating *P. angolensis* seeds with *B. ripae* significantly (P<0.05) decreased the germination percentage (Table 1). There were no significant differences in mean germination time or the coefficient of velocity of germination between inoculated and non-inoculated seeds (Table 1). A slight, non-significant reduction in germination time was observed for inoculated seeds (Table 1). Although non-inoculated seeds exhibited a higher germination index and velocity, these differences were not statistically significant. Inoculation may negatively affect germination, possibly due to microbial competition. Seed pre-treatment and inoculation effects appear to be independent, with no significant interaction.

The results presented in Table 2 showed no significant differences between pre-treatments. The results revealed

Table 1: Effect of presowing treatments and inoculation on the germination parameters of *P. angolensis*.

Presowing treatment	GP	GMT	GI	CVG
Soaking in rainwater	73.33	22.81	0.12	4.68
Control	71.67	17.34	0.12	6.07
Mechanical scarification	75.00	10.50	0.13	9.57
Soaking in warm water	75.00	19.50	0.13	5.27
Significance	ns	ns	ns	ns
Inoculum status				
Inoculated	66.67	17.32	0.11	6.37
Non-inoculated	80.83	17.75	0.14	6.43
Significance	*	ns	ns	ns
Interaction				
There is no interaction effect across all treatments				
Significance	ns	ns	ns	ns

*Significant at p>0.01 and ^{ns} non-significant at p>0.05. Means separated using Tukey's Honestly Significant Difference (HSD) Test at *p*≤0.05. Means within columns followed by the same letters are not significantly different (GP is germination percentage, GMT is germination mean time, GI is germination index and CVG is Coefficient of velocity of germination).

that germination rates varied from 73.33% to 88.33%, with the highest value recorded in the mechanically scarified seeds (88.33%), followed by untreated seeds (85.00%), rainwater-treated seeds (78.33%) and warm water-soaked seeds (73.33%). The results showed no significant differences between the mean germination time (ranged from 26.37 to 28.15 days). The results displayed slightly shorter mean germination times due to mechanical scarification of seeds, with a germination index of 0.18 and a coefficient of velocity of germination of 3.82, indicating marginally faster germination, with no significant differences.

B. plurijuga seeds inoculated with *B. ripae* showed significantly ($P<0.05$) higher germination rates and a higher germination index than non-inoculated seeds (Table 2). Microbial inoculation appears to enhance germination by improving seed health and suppressing pathogens. No interaction was observed between pre-treatment and inoculation in *B. plurijuga.*

Poor germination, often caused by seed coat hardness in woody species, is common in arid regions and hampers nursery propagation (Mojeremane *et al.,* 2021). This study investigated the impact of seed pre-treatments and inoculation on *P. angolensis* and B. plurijuga, two ecologically and economically significant species in Botswana. In nature, seed coats are typically broken by fire (Walters *et al.,* 2004). To counter dormancy caused by seed coat hardness and promote faster, more uniform germination, several methods have been suggested (Fredrick *et al.,* 2017; Cañizares *et al*., 2025; Motbaynor *et al.,* 2025). The study found that seed responses to pre-treatment vary by species and method. All treatments increased germination, with mechanically scarified seeds showing higher rates; however, this difference was not statistically significant. Results for *P. angolensis* align with studies reporting higher germination in scarified seeds (Chisha-Kasumu *et al*., 2007; Botsheleng *et al.,* 2014; Tselakgosi, 2021; Latiwa *et al.,* 2023). Similarly, Botsheleng *et al.* (2014) observed higher but insignificant germination in scarified *B. plurijuga* seeds. The findings suggest that seed treatment may not be essential for *B. plurijuga* and *P. angolensis*; however, the germination rate (speed) is crucial, as slower germination can lead to uneven seedling growth (Chisha-Kasumu *et al.,* 2007). Pre-treatments did not significantly influence mean germination time, germination index, or coefficient of velocity of germination. Scarified seeds showed marginally faster germination, indicating that this process could be expedited.

The benefits of mechanical scarification have been observed in various woody species in Botswana (Koobonye *et al.,* 2018; Botumile *et al.,* 2020; Mojeremane *et al.,* 2021; 2024) and in other regions (Fredrick *et al.,* 2017; Salazar and Ramírez, 2018; Alzandi *et al.,* 2025). However, this method has its limitations. It is physically demanding and labour-intensive, especially when only a small number of seedlings are needed (Todd-Bockarie *et al.,* 1993) and it is not suitable for large-scale seed treatment (Danthu *et al.,* 1992).

Soil inoculants can affect seed germination rates depending on the type of inoculant and the plant species (Balshor *et al.,* 2017; Mawarda *et al.,* 2020). This study shows the different responses of *B. plurijuga* and *P. angolensis* seeds to *B. ripae*. The effects of inoculants on woody seed germination vary depending on the strain used (Lindström and Mousavi, 2020; Mendoza-Suárez *et al.,* 2021). In this case, inoculated *P. angolensis* seeds had lower germination rates than non-inoculated seeds, although germination metrics remained unchanged. Since *P. angolensis* naturally forms an effective symbiosis, introducing a non-native strain might have disrupted essential early plant-microbe communication (Bünger *et al.,* 2021). Incompatibility between host and inoculant strains can reduce seed germination and seedling vigour in legumes, especially if the non-native strain fails to form a symbiosis (Allito *et al.,* 2021; Etesami, 2025). Inoculating

Table 2: Effect of different seed pre-treatments and inoculum on germination of *Baikiaea plurijuga* seeds.

Presowing treatment	GP	GMT	GI	CVG
Soaking in rainwater	78.33	28.12	0.16	3.57
Control	85.00	28.15	0.17	3.58
Mechanical scarification	88.33	26.37	0.18	3.82
Soaking in warm water	73.33	26.91	0.15	3.74
Significance	ns	ns	ns	ns
Inoculum status				
Inoculated	91.67	26.99	0.18	3.73
Non-inoculated	70.83	27.78	0.14	3.62
Significance	*	ns	*	ns
Interaction				
There is no interaction effect across all treatments				
Significance	ns	ns	ns	ns

*Significant at $p>0.01$ and ^{ns}non-significant at $p>0.05$. Means separated using Tukey's honestly significant difference (HSD) Test at $p\leq0.05$. Means within columns followed by the same letters are not significantly different (GP is germination percentage, GMT is germination mean time, GI is germination index and CVG is Coefficient of velocity of germination).

B. plurijuga significantly improved germination and the germination index, indicating higher rates and more vigorous, uniform sprouting. These results agree with other studies, which demonstrate that inoculants, either alone or in combination, can enhance seed germination in woody plants (Khan *et al.,* 2011, 2014; Singh *et al.,* 2011; Sreedhar and Mohan, 2014; Mridha *et al.,* 2016).

CONCLUSION

Seeds of *P. angolensis* and *B. plurijuga* can germinate without pre-treatment, but mechanical scarification proved to be the most effective method for both species. It produces the highest, though statistically insignificant, germination rates, with faster germination and better uniformity. This approach is suitable for small seed quantities due to its labour intensity. Inoculation reduced germination in *P. angolensis* but enhanced it for *B. plurijuga*. These findings highlight the importance of tailoring propagation strategies to meet the unique biological needs of each species.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for the experiments were approved by the Committee of Experimental Animal and handling techniques were approved by the University of Animal Care Committee.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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