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Effects of Pre-Germination Treatments on Seed Germination of *Pachypodium mikea* Lüthy, an Endemic Xerophytic Species from Southwestern Madagascar

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Abstract

Pachypodium mikea Lüthy (Apocynaceae) is a xerophytic species endemic to southwestern Madagascar whose germination ecology remains poorly documented. This study aimed to characterize its seeds and evaluate the effects of different pre-germination treatments under nursery conditions. A total of 400 seeds were subjected to four treatments: boiling water (100°C), warm water (45°C), room-temperature water (24°C), and an untreated control. Germination was monitored for 45 days using germination percentage (GP), mean germination time (MGT), and germination speed index (GSI).

Seeds were elongated and fusiform, with mean dimensions of 8.04 ± 0.71 mm in length, 2.83 ± 0.35 mm in width, and 1.29 ± 0.19 mm in thickness. Pre-germination treatments significantly affected germination performance ($\chi^2 = 25.28$; df = 3; $p < 0.001$). The boiling water treatment yielded the highest germination percentage (63%), the shortest mean germination time (9 days), and the highest germination speed index (9.12). Post-germination observations showed the early development of the caudex, succulent stem, and first spines. These results suggest the presence of partial seed coat dormancy that can be alleviated by thermal treatment. Boiling water immersion therefore represents a simple and effective technique for improving germination and supporting the conservation and propagation of *P. mikea*.

Keywords: *Pachypodium mikea*; seed germination; pre-germination treatment; seed morphometry; ex situ conservation; Madagascar

I. Introduction

I.1. General context

Madagascar is recognized as one of the world's major centers of plant endemism and harbors a remarkable diversity of ecosystems, among which the dry and xerophytic formations of the southwestern region occupy a prominent position. These environments are characterized by low rainfall, prolonged dry seasons, high temperatures, and persistent water deficits, conditions that have promoted the evolution of a highly specialized flora (Koechlin et al., 1997; Goodman & Benstead, 2003).

However, the dry ecosystems of southwestern Madagascar are increasingly subjected to intense anthropogenic pressures, including deforestation, bushfires, slash-and-burn agriculture, and the exploitation of forest resources. These disturbances contribute to the progressive degradation of natural habitats and threaten the conservation of numerous endemic species (Goodman & Benstead, 2003).

Among the emblematic species of these ecosystems is *Pachypodium mikea* Lüthy (Apocynaceae), a species strictly endemic to southwestern Madagascar. Officially described in 2005, this species is mainly associated with the dry forests and xerophytic formations of the Mikea region. Its restricted distribution range, combined with the continuing degradation of its habitat, highlights the importance of developing scientific knowledge to support its conservation and propagation (Lüthy, 2005; IUCN, 2024).

Seed germination constitutes a crucial stage in the plant life cycle and largely determines the success of both natural regeneration and nursery propagation. In many plant species, dormancy mechanisms may limit or delay germination. Appropriate pre-germination treatments can enhance seed imbibition and improve both germination percentage and germination rate (Baskin & Baskin, 2014).

In the context of conserving endemic species of southwestern Madagascar, improving propagation techniques for *Pachypodium mikea* is particularly important. Understanding and optimizing seed germination represent essential preliminary steps for the production of seedlings intended for *ex situ* conservation, ecological restoration, and environmental rehabilitation programs.

I.2. Presentation of species

I.2.1. Botanical Description

Pachypodium mikea was formally described by Lüthy in 2005 based on populations discovered in the Mikea Forest of southwestern Madagascar. Prior to its taxonomic recognition, this species was often confused with *Pachypodium geayi* due to their strong morphological similarity. However, detailed analyses of vegetative and reproductive characteristics confirmed its status as a distinct species (Lüthy, 2005).

The species is a small succulent tree, typically reaching 2.5 to 3.7 m in height, with a trunk diameter ranging from 24 to 45 cm. The trunk is cylindrical to fusiform, sparsely branched, and greatly thickened by water-storage tissues, enabling the plant to withstand the prolonged droughts characteristic of the arid regions of southwestern Madagascar (Lüthy, 2005).

The spines are arranged in groups of three on spine cushions distributed along the trunk surface. The two lateral spines are generally more developed than the central spine. This arrangement is one of the distinguishing features of the genus *Pachypodium* and provides protection to the succulent tissues (Lüthy, 2005; Rapanarivo & Leeuwenberg, 1999).

The leaves are clustered at the ends of the branches. They are narrowly lanceolate, dark green on the upper surface, and densely pubescent on the lower surface. The numerous and conspicuous secondary veins represent an important diagnostic characteristic of the species (Lüthy, 2005).

The terminal inflorescences bear several fragrant flowers with a white corolla and a yellow throat. Unlike *Pachypodium geayi*, the stamens remain enclosed within the floral tube, which is the main feature distinguishing the two species (Lüthy, 2005).

The fruits consist of two fusiform follicles that can reach approximately 9 cm in length. At maturity, they split longitudinally to release numerous seeds equipped with silky tufts that facilitate wind dispersal (Lüthy, 2005; Rapanarivo & Leeuwenberg, 1999).

According to Koechlin, Guillaumet, and Morat (1974), the vegetation formations of southwestern Madagascar include numerous species adapted to arid conditions. Among them, species of the genus *Pachypodium* exhibit distinctive morphological traits, particularly succulent tissues that enhance water storage capacity.

Figure 1: Adult specimen of Pachypodium mikea Lüthy (Moratahina R.,2025)

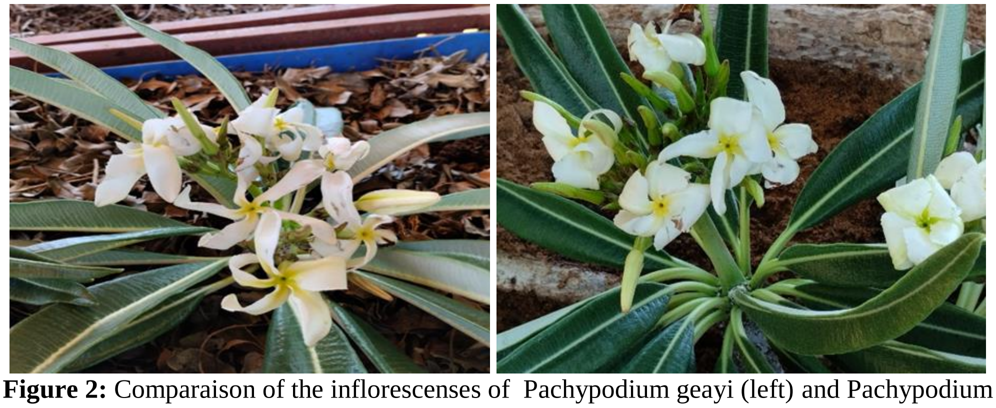

Figure 2: Comparaison of the inflorescenses of Pachypodium geayi (left) and Pachypodium mikea (right) (Moratahina R.,2025)

Figure 3: Trunk of Pachypodium mikea(Moratahina R.,2026)

I.2.2. Geographic Distribution

Pachypodium mikea is a species strictly endemic to Madagascar. Its distribution is restricted to the arid and semi-arid regions of southwestern Madagascar, particularly within the Mikea Forest complex located between Manombo, Andavadoaka, Lake Ihotry, and the surroundings of Morombe (Lüthy, 2005; IUCN, 2024).

This limited distribution range gives the species significant conservation value, as it is found nowhere else in the world. Known populations are generally fragmented and associated with the remaining patches of dry forests and xerophytic thickets within the Mikea region (Lüthy, 2005; Goodman & Benstead, 2003).

I.2.3. Habitat

The species primarily occurs in dry deciduous forests, xerophytic thickets, and spiny shrublands of southwestern Madagascar (Koechlin et al., 1974; Lüthy, 2005).

It grows preferentially on deep, well-drained sandy soils with low organic matter content, sometimes associated with calcareous substrates. These environments are characterized by low annual rainfall, high evapotranspiration rates, and a prolonged dry season that may exceed eight months (Koechlin et al., 1974; Goodman et al., 2021).

Like many species inhabiting the Mikea Forest, *P. mikea* is highly adapted to extreme water stress through its succulent trunk, which functions as a water reservoir during prolonged drought periods (Lüthy, 2005; Koechlin et al., 1974).

I.2.4. IUCN Consevation Status

According to the IUCN Red List, *Pachypodium mikea* is classified as **Endangered (EN)** due to its restricted distribution range and the continuing decline in the extent and quality of its natural habitats (IUCN, 2024).

The major threats affecting the species include:
- Deforestation of dry forests;
- Slash-and-burn agriculture (*hatsake*);
- Charcoal production;
- Bushfires;
- Forest resource exploitation;
- Increasing fragmentation of forest landscapes.

These anthropogenic pressures result in the progressive loss of suitable habitats and a reduction in the natural regeneration potential of wild populations (Goodman & Benstead, 2003; IUCN, 2024).

I.2.5. Ecological importance

Pachypodium mikea is a characteristic component of the xerophytic vegetation formations of southwestern Madagascar.

The flowers of *P. mikea*, like those of other species within the genus, are likely to attract insect pollinators through their nectar rewards and floral characteristics (Rapanarivo & Leeuwenberg, 1999; Lüthy, 2005). Consequently, the species contributes to ecological interactions within the xerophytic ecosystems of southwestern Madagascar.

Its seeds, equipped with silky tufts, facilitate wind dispersal (anemochory), a trait common among several species of the genus *Pachypodium* (Rapanarivo & Leeuwenberg, 1999; Lüthy, 2005). This dispersal strategy may enhance the colonization of favorable microsites for seedling establishment. Furthermore, *P. mikea* contributes to the vegetation structure of the xerophytic plant communities where it naturally occurs.

I.2.6. Socio-economic Importance

Like several other species of the genus *Pachypodium*, *P. mikea* possesses significant heritage, scientific, and horticultural value.

Its remarkable growth form, swollen trunk, and ornamental flowers make it highly attractive for botanical collections, xerophytic gardens, and international succulent plant conservation programs (Rapanarivo & Leeuwenberg, 1999; Lüthy, 2005).

The species also has ecotourism value due to its rarity and strict endemism to the Mikea Forest, one of the major biodiversity hotspots of southwestern Madagascar (Goodman & Benstead, 2003).

I.2.7. Importance for Ecological Restoration

Within the framework of ecological restoration programs in the arid ecosystems of southwestern Madagascar, Pachypodium mikea represents a particularly valuable species.

Its high tolerance to drought, ability to survive on nutrient-poor soils, and capacity for water storage allow it to withstand the harsh environmental conditions characteristic of degraded areas in the Toliara region (Koechlin et al., 1974; Lüthy, 2005).

Considering its threatened conservation status, nursery propagation and a thorough understanding of its germination mechanisms are essential for strengthening ex situ conservation strategies. The use of this species in restoration projects could simultaneously contribute to the conservation of Madagascar's endemic biodiversity and the recovery of the vegetation structure of severely degraded xerophytic ecosystems (Baskin & Baskin, 2014; IUCN, 2024).

I.3. Justification de l'étude Justification of the Study

Pachypodium mikea Lüthy is of considerable scientific and conservation importance due to its strict endemism and restricted distribution in southwestern Madagascar (Lüthy, 2005; IUCN, 2024). Despite its botanical significance, knowledge regarding seed morphology, germination behavior, and seedling development remains limited. Acquiring data on these aspects is essential for improving our understanding of its reproductive biology (Baskin & Baskin, 2014).

Furthermore, the ongoing degradation of natural habitats represents one of the major threats to Madagascar's endemic plant species (IUCN, 2024). In this context, mastering sexual propagation constitutes an important tool for supporting *ex situ* conservation programs, ecological restoration initiatives, and biodiversity conservation efforts within the xerophytic ecosystems of southwestern Madagascar (Guerrant et al., 2004; Baskin & Baskin, 2014).

The identification of effective pre-germination treatments is also of practical importance for the production of seedlings intended for conservation actions, specialized nurseries, and restoration programs targeting Madagascar's dry forests and xerophytic thickets. The findings of this study will contribute to the development of propagation protocols adapted to *Pachypodium mikea* and may serve as a reference for the propagation of other endemic plant species from Madagascar's arid environments.

I.4. Research Problem

Germination is a critical stage in the plant life cycle, determining both regeneration capacity and nursery propagation success (Baskin & Baskin, 2014). In many species, seed characteristics influence germination rate and speed, often requiring specific pre-germination treatments to optimize seedling emergence (Hartmann et al., 2018).

For *Pachypodium mikea*, information concerning germination biology remains poorly documented. In particular, the effects of different pre-germination treatments on the germination performance of this species are still insufficiently understood. This situation limits the development of reliable protocols for improving seedling production in nurseries.

Therefore, it is necessary to evaluate the effectiveness of different pre-germination treatments in order to determine their influence on germination percentage, germination speed, and seedling emergence in *Pachypodium mikea*.

Research Question:

What is the effect of different pre-germination treatments on the germination of Pachypodium mikea Lüthy seeds?

I.5. General Objective

To evaluate the effect of different pre-germination treatments on the germination of *Pachypodium mikea* seeds in order to contribute to its propagation and conservation.

I.6. Specific objectives

- To describe the morphological and morphometric characteristics of Pachypodium mikea seeds;
- To evaluate the influence of different pre-germination treatments on seed germination dynamics;
- To compare the germination performance of treatments using germination percentage, mean germination time, and germination speed index;
- To describe the main stages of post-germination seedling development.

I.7. Hypotheses

General Hypothesis

Pre-germination treatments significantly influence the germination of *Pachypodium mikea* seeds.

Specific Hypotheses

- *Pachypodium mikea* seeds exhibit partial seed-coat dormancy that can be reduced by thermal treatments;
- Immersion of seeds in boiling water significantly improves germination percentage compared with other treatments;
- Thermal treatments accelerate seedling emergence and increase germination speed;
- The main morphological characteristics of the species are established during the early stages of post-germinative development.

II. Materials and Methods

II.1. Seed collection Site

The present study focused exclusively on *Pachypodium mikea*, a xerophytic species investigated using seeds collected from natural populations located in the Anjaha sector within the Ranobe PK32 Protected Area. Formerly designated as the PK32 Ranobe New Protected Area (NAP) at the time of its establishment under Madagascar's Protected Areas System, this protected area is situated in the Atsimo-Andrefana Region, mainly within the Toliara II District (Randrianarison, 2011).

The most distinctive feature of the vegetation in the Anjaha area is the xerophytic thicket, a characteristic plant formation of southwestern Madagascar. Its physiognomy is strongly marked by the presence of species belonging to the endemic family Didiereaceae, numerous Euphorbiaceae, several baobab species (*Adansonia* spp.), and a diverse assemblage of succulent plants adapted to semi-arid climatic conditions (Koechlin et al., 1974; Goodman, 2008).

The Ranobe PK32 Protected Area belongs to the Southwestern Dry Forest and Spiny Thicket Ecoregion, recognized as one of Madagascar's most important biodiversity conservation areas. This ecoregion is characterized by vegetation formations adapted to water-limited conditions and dominated by succulent, spiny, or reduced-leaf plant species (Cornet & Guillaumet, 1976; WWF, 2012).

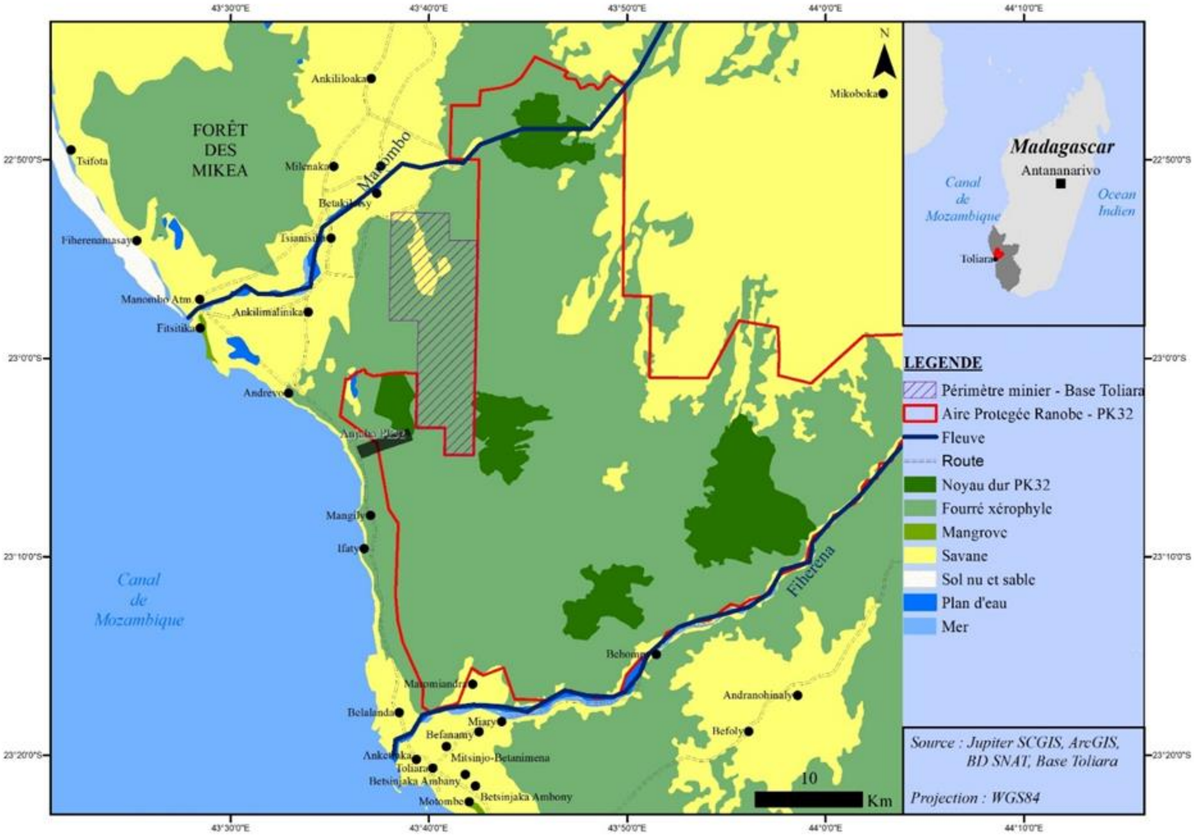

Figure 4: Geographic location of the Ranobe PK32 Protected Area and the Anjaha Site (Moratahina R., 2024)

II.2. Seed Collection and Preparation

Seeds of *Pachypodium mikea* were collected on 26 December 2025 from healthy, vigorous adult individuals exhibiting good phytosanitary conditions and abundant fruit production. Fruits were harvested directly from seed trees at physiological maturity, following recommendations for forest seed collection (Willan, 1985; Schmidt, 2000; ENSCONET, 2009).

After collection, the fruits were placed in labeled bags and transported to the experimental nursery. They were subjected to a pre-drying phase in a dry and well-ventilated environment to facilitate seed extraction. Seeds were then manually extracted, cleaned, and visually sorted. Malformed, damaged, or deteriorated seeds were discarded, and only healthy seeds were retained for morphometric analyses and germination experiments (FAO, 1994; Schmidt, 2000).

The seeds were temporarily stored in clean, labeled containers in a dry, ventilated room protected from direct sunlight. Sowing was carried out on 8 January 2026, corresponding to 13 days after collection.

II.3. Site expérimental

The experiments were conducted at the **Vara Mada experimental nursery**, located in Ambolimailaka, within the rural commune of Manombo, approximately **43 km north of Toliara** (**23°02′43.52″ S; 43°34′14.56″ E**). All activities related to seed preparation, pre-germination treatments, sowing, and germination monitoring were carried out at this site.

Figure 5: Vara Mada Nursery (Moratahina R., 2025)

The selection of this experimental site was justified by the availability of its infrastructure, its proximity to the Anjaha PK32 seed collection area, and its direct involvement in ecological restoration programs using native species. Conducting the experiments under these conditions provides knowledge that can be directly applied to **ex situ conservation**, the production of indigenous forest seedlings, and future restoration and rehabilitation initiatives targeting degraded ecosystems in southwestern Madagascar.

II.4. Morphological and Morphometric Characterization of Seeds

A morphological characterization of *Pachypodium mikea* seeds was carried out to describe their main external features. Observations focused on seed shape, color, texture, seed coat appearance, and any distinctive structures. The seeds were observed and photographed in order to document their morphological characteristics.

For the morphometric characterization, a random sample of **30 seeds** was used. Measurements were performed using a digital caliper with an accuracy of **± 0.01 mm**. Three orthogonal dimensions were recorded for each seed: length (L), width (W), and thickness (T), following the methods described by Mohsenin (1986) and Schmidt (2000).

The **geometric mean diameter (Dg)** was calculated using the following equation

$$\boxed{Dg = (L \times W \times T)^{1/3}}$$

where:

- **Dg** = geometric mean diameter (mm);
- **L** = seed length (mm);
- **W** = seed width (mm);
- **T** = seed thickness (mm).

The **sphericity (Φ)** was determined as:

$$\boxed{\Phi = \frac{Dg}{L}}$$

where:

- **Φ** = sphericity;
- **Dg** = geometric mean diameter (mm);
- **L** = seed length (mm).

The elongation index (EI) was calculated as:

$$\boxed{Ia = \frac{L}{W}}$$

où:

- **EI** = Elongation index;
- **L** = seed length (mm);
- **W** = seed witdh (mm).

Thus:

- When EI ≈ 1, the seed has a nearly spherical shape;
- When EI > 1, the seed exhibits an elongated shape;
- the higher the EI value, the more elongated the seed.

This index provides information on seed shape variation and complements sphericity in the morphometric characterization of seeds.

These parameters were used to characterize the overall size and shape of *Pachypodium mikea* seeds (Mohsenin, 1986).

II.4. Pre-germination Treatments and Germination Trial

Four treatments were compared: (i) immersion in hot water at 100°C, (ii) soaking in warm water at 45°C, (iii) soaking in water at ambient temperature (24°C), and (iv) a control treatment without pre-treatment. These treatments were designed to evaluate the effect of water temperature on seed germination response.

The effect of different pre-germination treatments on the germination of *Pachypodium mikea* seeds was assessed using four treatments:

- **T0 - Control:** direct sowing without pre-treatment;
- **T1 - Boiling water:** immediate immersion of seeds in boiling water (100°C), followed by a 24-hour soaking period during gradual cooling;
- **T2 - Warm water:** immersion of seeds in water at 45°C, followed by a 24-hour soaking period during gradual cooling;
- **T3 - Ambient water:** soaking of seeds in water at 24 ± 2°C for 24 hours before sowing.

For each treatment, 100 seeds of *Pachypodium mikea* were used. Following treatment application, seeds were immediately sown on germination beds established within the experimental nursery. This experimental design enabled the evaluation of the influence of water temperature on the germination behavior of the species (Schmidt, 2000; Baskin & Baskin, 2014).

II.5. Germination Monitoring and Parameters Assessed

Germination was monitored for 45 days after sowing. The evolution of cumulative germination was used to evaluate germination dynamics under the different treatments.

The **final germination percentage (GP)** was calculated using the following formula:

$$\boxed{GP = \frac{N_g}{N_s} \times 100}$$

where:

- **GP** = final germination percentage (%);
- **Ng** = total number of germinated seeds;
- **Ns** = total number of seeds sown.

The **Mean Germination Time (MGT)** was calculated according to:

$$\boxed{MGT = \frac{\sum(n_i \times t_i)}{\sum n_i}}$$

where:
- **MGT** = mean germination time (days);
- **ni** = number of seeds germinated at time *ti* ;
- **ti** = number of days elapsed since sowing.

Lower MGT values indicate faster germination.
The **Germination Vigor Index (GVI)** was calculated using the formula proposed by Maguire (1962):

$$\boxed{GVI = \sum \frac{n_i}{t_i}}$$

where:
- **GVI** = germination vigor index;
- **ni** = number of seeds germinated during observation *i*;
- **ti** = number of days elapsed since sowing.

High GVI values indicate rapid and uniform germination, whereas low values reflect slower or more dispersed germination over time (Maguire, 1962).

II.6. Data Processing and Analysis

Morphometric data obtained from 30 seeds were analyzed using descriptive statistics, including the mean, standard deviation, minimum and maximum values, and coefficient of variation. These parameters were used to characterize the morphometric variability of *Pachypodium mikea* seeds (Mohsenin, 1986; Schmidt, 2000).

For the germination experiment, four pre-germination treatments were evaluated: the untreated control (T0), boiling water treatment (T1), warm water treatment at 45°C (T2), and ambient-temperature water treatment (T3). Each treatment consisted of a single experimental unit comprising **100 seeds**, resulting in a total of **400 seeds**. No independent replicates were included.

For each treatment, the total number of germinated seeds, final germination percentage (GP), mean germination time (MGT), and germination vigor index (GVI) were calculated to assess germination performance (Maguire, 1962; Baskin & Baskin, 2014).

Due to the absence of independent replicates, statistical comparisons were restricted to analyses of final germination frequencies. The proportions of germinated and non-germinated seeds among treatments were compared using a **Chi-square (χ^2) test of independence** (Zar, 2010; Sokal & Rohlf, 2012). Observed frequencies were compared with expected frequencies to determine whether an association existed between pre-germination treatments and seed germination. **Pearson residuals** and **adjusted residuals** were examined to identify treatments contributing significantly to the observed differences (Agresti, 2019).

III. Results

III. 1. Morphological Characteristics of the Seeds

Figure 6 presents the general appearance of *Pachypodium mikea* seeds. The observed seeds exhibit an elongated and fusiform morphology, characterized by a narrow body with gradually tapering pointed ends. Their overall shape is distinctly slender, with a pronounced dominance of the longitudinal axis.

Seed coat color ranges from light beige to pale brown, occasionally displaying slightly darker grayish shades. The outer surface appears dry and dull, with fine longitudinal striations visible to the naked eye, giving the seeds a slightly fibrous appearance. The outline is generally regular, although some seeds exhibit slight curvature or subtle asymmetry.

Overall, the seeds show a high degree of uniformity in both shape and coloration within the studied seed lot.

These characteristics confer on *Pachypodium mikea* seeds a distinctive elongated and fusiform morphology. Such a morphology may be associated with the ecological constraints of the xeric environments of southwestern Madagascar.

Figure 6: General morphology of Pachypodium mikea (Moratahina R., 2026)

III.2. Morphometric Characteristics of the seeds

The morphometric characterization of *Pachypodium mikea* seeds was conducted on a random sample of thirty (30) seeds collected from the Anjaha PK32 seed lot.

Table 1: Morphometric characteristics of Pachypodium mikea (n = 30)

Parameter	Minimum	Maximum	Means ± SD	CV (%)
Length (mm)	6,19	9,18	8,04 ± 0,71	8,82
Width (mm)	2,12	3,59	2,83 ± 0,35	12,43
Thickness (mm)	0,93	1,67	1,29 ± 0,19	15,00
Geometric Mean Diameter (Dg) (mm)	2,52	3,58	3,07 ± 0,25	7,98
Sphericity (Φ)	0,33	0,44	0,38 ± 0,03	8,08
Elongation Index (L/l)	2,20	3,88	2,88 ± 0,41	14,36

Source: Moratahina R., 2026

Morphometric analysis revealed a mean length of 8.04 ± 0.71 mm, a mean width of 2.83 ± 0.35 mm, and a mean thickness of 1.29 ± 0.19 mm. The observed values demonstrate a clear predominance of length over the other two dimensions, with width representing on average only about one-third of the seed length, while thickness remains particularly low. This dimensional pattern confirms the narrow and elongated appearance observed during the morphological characterization.

The recorded minimum and maximum values indicate that this trend is maintained across all examined seeds. Even the shortest seeds remain substantially longer than wide, reflecting a homogeneous morphology dominated by the longitudinal axis. This feature is also evident in the photographs, where the seeds display a fusiform silhouette with gradually tapered extremities.

The geometric mean diameter of 3.07 ± 0.25 mm indicates a relatively small overall seed size. Despite their small dimensions, the seeds exhibit well-defined proportions that contribute to their distinctly elongated shape.

The mean sphericity value of 0.38 ± 0.03 represents one of the most remarkable findings. Such a low value indicates that the seeds differ greatly from a spherical shape and instead possess a strongly elongated geometry. This low sphericity directly confirms the previously described fusiform morphology and reflects the marked difference between the longitudinal and transverse dimensions.

The elongation index averaged 2.88 ± 0.41, with values reaching 3.88 in some seeds. These results indicate that seed length is, on average, nearly three times greater than seed width. This pronounced dominance of the longitudinal axis explains the narrowly elliptical appearance observed during morphological characterization and constitutes the main distinguishing trait of Pachypodium mikea seeds.

Overall, all morphometric parameters strongly support the morphological observations. Pachypodium mikea seeds are characterized by a fusiform to narrowly elliptical shape, reduced transverse dimensions, low thickness, and marked longitudinal elongation. Furthermore, the relatively low coefficients of variation recorded for most parameters indicate that these traits are fairly consistent within the studied seed lot.

III.2. Germination of Seeds

III.2.1. Germination Dynamics

Figure 7 shows the cumulative germination rate of *Pachypodium mikea* seeds subjected to different pre-germination treatments over the 45-day monitoring period.

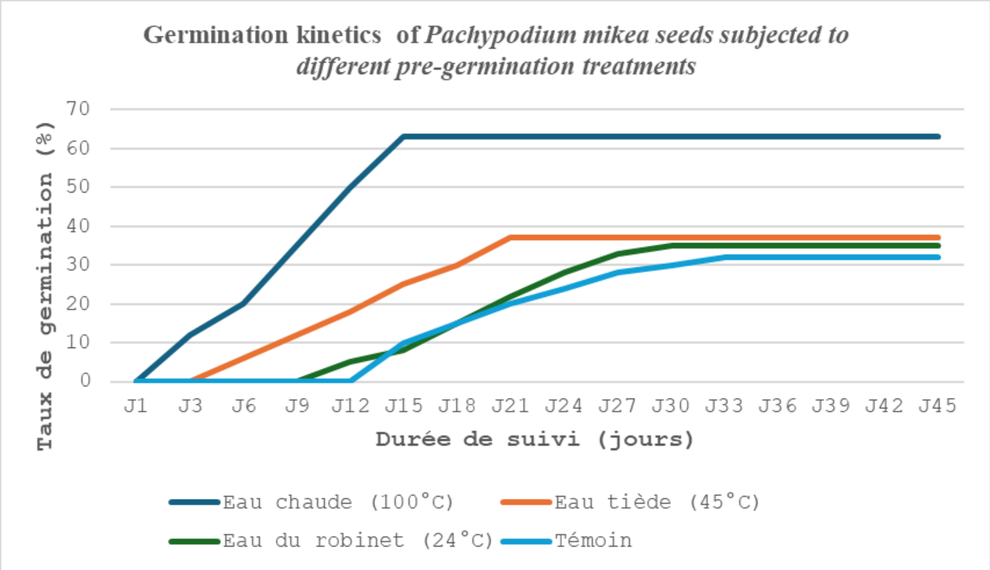

Figure 7: Germination kinetics of Pachypodium mikea seeds subjected to different pre-germination treatments

The first germination events were observed as early as the third day after sowing in seeds treated with boiling water (100°C). This treatment also exhibited the most rapid germination progression, reaching a final plateau of 63% by the fifteenth day. The warm-water (45°C) and ambient-water (24°C) treatments showed slower germination responses, with final germination percentages of 37% and 35%, respectively. The latest and poorest germination performance was observed in the control treatment, which reached only 32% germination.

Overall, most germination occurred during the first thirty days following sowing. Thermal treatments enhanced both the onset and magnitude of germination, with boiling water proving to be the most effective treatment.

III.2.2. Germination Parameters

Table 2: Germination parameters of Pachypodium mikea seeds subjected to different pre-germination treatments

Traitement	GP (%)	MGT (jours)	GVI
Boiling water (100 °C)	63	9	9,12
Warm water (45 °C)	37	14	3,24
Ambiant water (24 °C)	35	20	1,84
Control	32	21	1,62

Source: Moratahina R., 2026.

Germination parameters varied according to treatment. The boiling-water treatment (100°C) produced the best overall performance, with the highest germination percentage (**63%**), the lowest mean germination time (**9 days**), and the highest germination vigor index (**9.12**). In contrast, the control treatment showed the poorest performance, with a germination percentage of **32%**, a mean germination time of **21 days**, and a germination vigor index of **1.62**.

These results indicate that boiling-water treatment simultaneously improves germination percentage, seedling emergence rate, and overall germination vigor.

II.2.3. Effet of Pre-germination Treatments on Final Germination

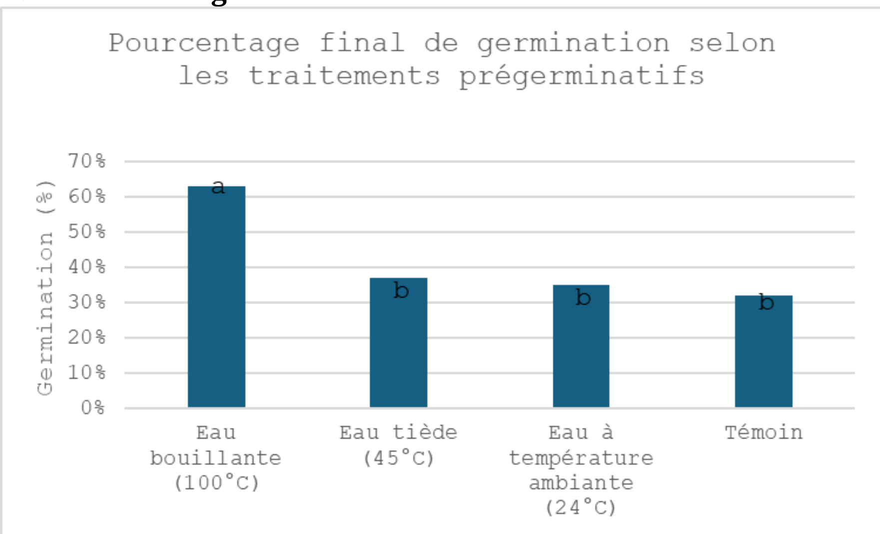

Figure 8: Final Germination Percentage under Different Pre-germination Treatments

Final germination percentages differed significantly among the pre-germination treatments (**$\chi^2 = 25.28$; df = 3; $p < 0.001$**). The boiling-water treatment (100°C) achieved the highest final germination percentage (**63%**), followed by warm water (**37%**), ambient water (**35%**), and the control (**32%**).

Analysis of adjusted residuals revealed that the boiling-water treatment contributed most strongly to the observed differences, with a significantly greater number of germinated seeds than expected under the null hypothesis of no treatment effect. Conversely, the control treatment exhibited a significantly lower germination proportion than expected.

These findings confirm the effectiveness of boiling-water treatment in promoting germination of *Pachypodium mikea* and suggest the presence of partial seed-coat dormancy that can be alleviated through the action of heat on the seed coat.

III.2.3 Post-germinative Development

The first seedlings of *Pachypodium mikea* displayed a fleshy and slightly swollen hypocotyl axis during the earliest stages of development, foreshadowing the formation of the characteristic caudex of the species.

Young seedlings rapidly developed simple, narrow, lanceolate leaves arranged in an apical rosette. As growth progressed, the basal swelling became increasingly pronounced, while the stem gradually acquired a succulent structure.

The appearance of the first spines near the apical meristem marked an important stage in juvenile development. Despite their young age, the seedlings already exhibited several morphological traits characteristic of the genus *Pachypodium*, including a succulent axis, foliage concentrated at the apex, and developing spines.

This developmental phase highlights the early establishment of the principal morphological features of the species, particularly caudex formation, stem succulence, and progressive spine development.

Figure 9: Young individual of Pachypodium mikea during post-germinative development (Moratahina R., 2026)

IV. Discussion

IV.1. Seed Morphology and Morphometry

Pachypodium mikea seeds exhibited an elongated and fusiform morphology, characterized by low sphericity (**0.38 ± 0.03**) and a relatively high elongation index (**2.88 ± 0.41**). These characteristics indicate a strong dominance of the longitudinal axis and confirm the morphological observations made during seed description. The low coefficients of variation recorded for most parameters further suggest a relatively homogeneous seed lot.

To our knowledge, no previous study has provided a detailed morphometric description of *Pachypodium mikea* seeds. Therefore, the present results represent an original contribution to the biological knowledge of this endemic species from southwestern Madagascar. The reported values may serve as a reference for future studies focusing on the ecology, germination, conservation, and propagation of the species.

IV.2. Effect of Pre-germination Treatments on Germination

The results demonstrated that the boiling-water treatment (**100°C**) significantly improved the germination of *Pachypodium mikea* seeds. This treatment produced the highest final germination percentage (**63%**), the lowest mean germination time (**9 days**), and the highest germination vigor index (**9.12**). The Chi-square test confirmed a significant effect of pre-germination treatments on seed germination (**$\chi^2 = 25.28$; df = 3; $p < 0.001$**).

The effectiveness of the boiling-water treatment suggests the presence of **partial physical or seed-coat dormancy** in *Pachypodium mikea* seeds. According to Bewley et al. (2013), physical dormancy is generally associated with low seed-coat permeability, which restricts water uptake required for the initiation of germination. Heat exposure may therefore have altered the seed-coat structure, promoting imbibition and activating the physiological processes necessary for embryo growth resumption.

The warm-water (**45°C**) and ambient-water (**24°C**) treatments also allowed germination to occur, but with substantially lower performance than the boiling-water treatment. These findings suggest that lower temperatures were probably insufficient to produce significant modifications of the seed coat. According to Baskin and Baskin (2014), the intensity of thermal treatment is often a critical factor in breaking physical dormancy in species adapted to harsh environmental conditions.

These findings are particularly important for the conservation of *Pachypodium mikea*, an endemic species of southwestern Madagascar. Propagation within the genus *Pachypodium* relies primarily on seed production, while vegetative propagation through cuttings is generally limited. Consequently, seed germination represents an essential pathway for producing individuals intended for **ex situ conservation programs** and the restoration of natural populations.

The analysis of adjusted residuals showed that the boiling-water treatment contributed most strongly to the observed differences among treatments. This finding confirms that thermal treatment constitutes a simple and effective method for improving germination percentage, accelerating seedling emergence, and enhancing overall germination performance in *Pachypodium mikea*.

IV.3. Post-germinative Development.

Observations made during post-germinative development revealed the early appearance of a basal swelling, indicating the progressive formation of the characteristic caudex, together with the development of a succulent stem and the first spines. These features emerged during the earliest growth stages and correspond to the principal morphological traits described for species of the genus *Pachypodium* (Rapanarivo & Leeuwenberg, 1999; Lüthy, 2005).

The early formation of the caudex observed in young seedlings suggests the rapid establishment of specialized storage structures characteristic of the species. In *Pachypodium*, the development of succulent tissues constitutes a key adaptation to the drought conditions prevailing in the arid and semi-arid regions of Madagascar and southern Africa (Rapanarivo & Leeuwenberg, 1999; Koechlin et al., 1974). This adaptation enables individuals to store water and better withstand prolonged periods of water deficit.

The gradual appearance of spines during juvenile development is also consistent with available morphological descriptions of *Pachypodium mikea* and other species of the genus (Lüthy, 2005; Rapanarivo & Leeuwenberg, 1999). These structures represent one of the defining characteristics of the genus and contribute to the protection of succulent tissues.

Overall, the results indicate that the principal morphological traits of *Pachypodium mikea* are established at very early developmental stages. These observations enhance current knowledge of the species' biology and provide useful information for propagation, **ex situ conservation**, and ecological restoration programs.

IV.4. Study Limitations

The present study generated novel information on the seed morphology, germination, and post-germinative development of *Pachypodium mikea*. Nevertheless, several limitations should be considered when interpreting the results.

The germination experiment was conducted using a single seed lot collected from one location, and treatments were not replicated independently. Consequently, the findings should be regarded as a preliminary contribution to the understanding of the germination biology of the species. Future studies incorporating multiple seed lots, different collection periods, and experimental replicates would help confirm and strengthen the robustness of the observed results.

In addition, only thermal treatments were evaluated in this study. The effectiveness of other dormancy-breaking methods, such as mechanical scarification, chemical scarification, and the use of plant growth regulators, also deserves investigation in order to further optimize germination protocols for *Pachypodium mikea*.

Conclusion

This study provided the first detailed description of the seed morphology, germination behavior, and post-germinative development of *Pachypodium mikea*, an endemic species of southwestern Madagascar. The seeds are characterized by an elongated and fusiform shape, low sphericity, and a high elongation index. These morphometric data constitute a valuable contribution to the biological knowledge of the species and may serve as a reference for future ecological, conservation, and propagation studies.

Among the pre-germination treatments evaluated, immersion in boiling water (100 °C) proved to be the most effective, resulting in the highest germination percentage (63%), the shortest mean germination time (9 days), and the highest germination vigor index. The significant effect of the treatments on germination was confirmed by the Chi-square test. In addition, observations of post-germinative development revealed the early establishment of key morphological traits, including caudex formation, stem succulence, and spine development.

Overall, the results demonstrate that boiling-water treatment represents a simple, effective, and practical technique for enhancing the germination of Pachypodium mikea. This method could be applied in nursery propagation, ex situ conservation programs, and ecological restoration initiatives involving the species. Future research should investigate the effects of different soaking durations, alternative dormancy-breaking methods such as mechanical and chemical scarification or gibberellic acid treatments, and seed storage. Further studies involving replicated experiments, multiple seed lots, and post-transplant seedling performance would also contribute to optimizing propagation protocols and strengthening conservation strategies for *Pachypodium mikea*.

Future research should investigate the effects of different soaking durations, alternative dormancy-breaking methods such as mechanical and chemical scarification or gibberellic acid treatments, and seed storage conditions. Further studies involving replicated experiments, multiple seed lots, and post-transplant seedling performance would also contribute to optimizing propagation protocols and strengthening conservation strategies for *Pachypodium mikea*.

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