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Evaluation of the seed germination behavior of *Prunus africana* (Hook.f.) Kalkman (Rosaceae) under different pre-sowing treatments in Ethiopia

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Abstract: The seeds of *P. africana* pose challenges for propagation due to their harder seed coat and recalcitrant nature, as well as long flowering cycles. Therefore, the main objective of this study is to evaluate the seed germination capability of *P. africana* under different pre-sowing treatments. Mature seeds of *P. africana* were collected in April 2024 from the Walmara district of West Shewa (Oromia region). Seeds were treated with four types of treatments and sown in pots filled with a 3:2:1 ratio of forest topsoil, manure, and sand, respectively, in a completely randomized design. One-way ANOVA indicated that there exists a mean significant difference among treatment groups in both mean germination percentage (GP) and mean germination index (GI) at $p \leq 0.05$. Tukey's HSD pairwise comparisons for GP and GI at $p \leq 0.05$ indicated that the GP and GI for seeds soaked in cold normal water for 24 hours at room temperature (25°C) are highly significant compared to other pre-sowing treatments. Generally, this study implicates the potential pre-sowing treatment that enhances the seed germination of *P. africana*. The outputs of this study can be used for mass propagation, restoration, and conservation of *P. africana*.

Key words: Germination; *Prunus africana;* Seeds; Treatments.

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Prunus africana (Hook.f.) Kalkman (Rosaceae), commonly known as Pygeum or African cherry, is a tall evergreen tree found in mainland montane forests scattered across Central and Sub-Saharan Africa, and the Indian Ocean islands (Sacandé *et al*., 2004; CABI, 2022; Rubegeta *et al*., 2023). *P. africana* is a significant tree species found in Ethiopia (Tadesse *et al*., 2023). It is primarily located in moist and dry afromontane forests in Ethiopia (Seid and Mulatu, 2022; Chebet, 2022). It can reach heights of up to 40 meters and produces creamy-white flowers and purplish-red cherries that turn black when ripe (Viljoen *et al*., 2022). *P. africana* is identified by its deciduous leaves which have serrated margin. It displays specific adaptations such as evergreen foliage and smooth leaf margins, which may be a response to environmental factors (Were *et al*., 2001). The bark of *P. africana* has a long history of traditional use in treating various conditions, such as benign prostate hyperplasia (BPH), inflammation, stomach and chest pain, malaria, heart problems,

gonorrhea, urinary tract and kidney infections and cancer (Deresa *et al*., 2022; Mpouam *et al*., 2022; Rubegeta *et al*., 2023). The timber obtained from *P. africana* is used to make axe handles and other household needs, and is also suitable for timber production (Rubegeta *et al*., 2023).

The seeds of *P. africana* pose challenges for propagation due to their harder seed coat and recalcitrant nature as well long flowering cycles (Custódio *et al*., 2022). The seed viability of *P. africana* decreases when stored at room temperature after two-three months (Nzweundji *et al*., 2020; Custódio *et al*., 2022). However, innovative micro-propagation techniques using nodal segment explants have been developed to effectively address this issue (Nzweundji *et al*., 2020). Seed germination of *P. africana* can be influenced by various factors, such as pre-sowing techniques, endocarp removal, incubation temperature, and gibberellic acid concentration (Rubegeta *et al*., 2023). Moreover, previous research suggests that different methods, including acid or water immersion for specific durations, can significantly affect germination rates (Chukwu *et al*., 2020; Moreira *et al*., 2020). Collectively, these insights contribute to a better understanding of the properties and challenges associated with seeds of *P. africana*. Therefore, the main objective of this study is to evaluate the seed germination capability of *P. africana* under different pre-sowing treatments. It was, hence, hypothesized that application of pre-sowing treatments could enhance the seed germination of *P. africana*.

Materials and methods

The seeds were in April 2024 from Walmara district of West Shewa (Oromia region). Walmara district is located between 8.86°N to 9.26°N and 38.39°E to 38.72°E (Fig. 1). The annual mean temperature of Walmara district ranges between 11.57°C and 17.76°C, and the annual mean precipitation ranges from 772 mm to 1265 mm based on the Worldclim database (www.worldclim.com/version2) (Fick and Hijmans, 2017).

Thus, matured seed were collected (Fig. 2). Then, the seeds were homogenized, and cleaned from inert and unwanted materials. As the seed storage behavior of *P. africana* is recalcitrant, the collected seeds were not exposed to sun to avoid moisture loss. The moisture content (MC) of seeds was checked using the moisture meter (KERN, DBS60-3 German-made moisture tester) and found to be 38.8%.

The experiment was conducted following the protocols established by the International Seed Testing Association (ISTA) for seed germination testing (ISTA, 1976 and 2018). Four pre-sowing treatments, detailed in Table 1, were applied based on established literature and ISTA guidelines (ISTA, 2005; Nzweundji *et al*., 2020; Chukwu *et al*., 2020; Moreira *et al*., 2020). Germination was assessed across four replications, each consisting of 36 seeds. The treatments, including a control, were prepared accordingly to ensure consistency and reliability in the results.

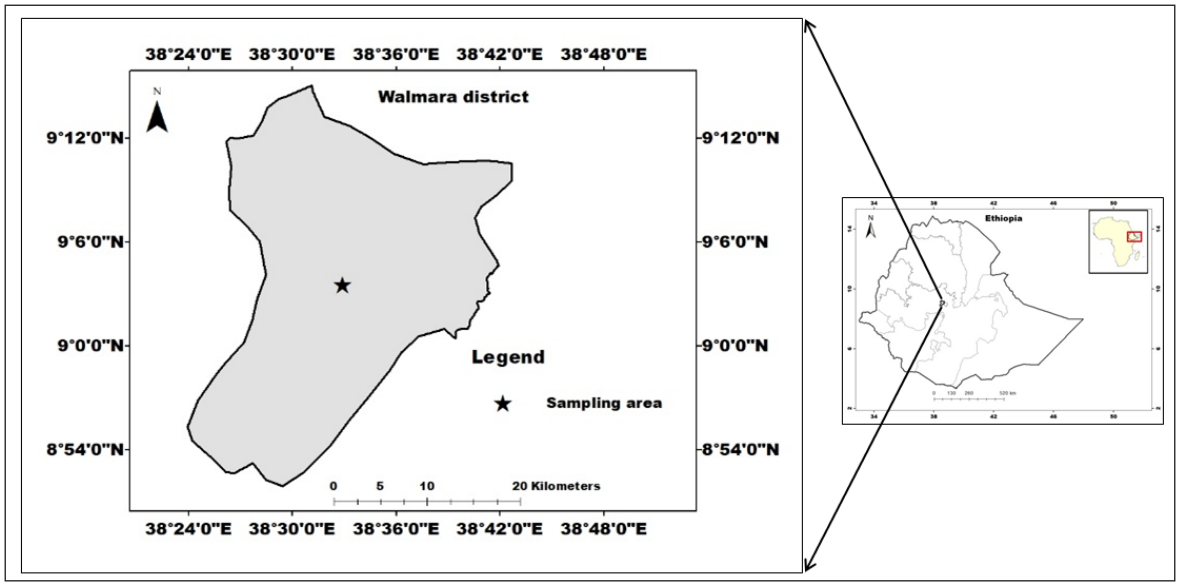

Fig. 1. Map of seed collection site.

Fig. 2. The tree (A) and branches (B) of P. africana bearing matured seeds.

Each experiment was arranged in a Completely Randomized Design (CRD) following the randomization tables of Gomez and Gomez (1984), with four replications. Germination trials were conducted using a substrate composed of a 3:2:1 ratio of topsoil, manure, and sand, respectively, filled into 20 cm diameter polyethylene plastic pots. Seeds were considered germinated once the radicle emerged, in accordance with the criteria outlined by Bewley and Black (1994) and Zietsman and Botha (1987). The germination tests were carried out from March 25, 2024, to April 22, 2024.

Four parameters, namely the mean germination percentage (*GP*), mean daily germination percentage (*GD*), mean germination time (*GT*), and mean germination index (*GI*) were calculated as follows: Where; n_i = number of seeds germinated in the i^{th} time, and N= total number of seeds used.

$$GP = \frac{\sum_{i=1}^{k} ni}{N} x\ 100 \quad \ldots 1$$

$$GI = \frac{\text{No. of germinating seeds}}{\text{days of first count}} + \cdots + \frac{\text{No. of germinating seeds}}{\text{days of final count}} \quad \ldots 2$$

where *GI* is a measure of the percentage and speed of germination (AOSA, 1983). The higher values for this measure indicate a greater rate of germination (Benech-Arnold *et al*., 1991). Furthermore, a one-way ANOVA and Tukey's Honestly Significant Difference (HSD) (Steel *et al*., 1997) were computed to check the level of significance difference among the treatments groups at $p \leq 0.05$.

Table 1. Pre-sowing treatments employed study the seed germination

No	Seed treatment types	No	Seed treatment types
1	Control	3	Soaked in 10 mM GA for 24 hours at room temperature (25°C) (*T2*)
2	Soaked in cold normal water for 24 hours at room temperature (25°C) (*T1*)	4	Soaked in 57°C hot water for 10 minutes, and cooled for 24 hours (*T3*)

Fig. 3. Illustration of and fully grown seedlings of P. africana.

Results and Discussion

The image below (Fig. 3) shows fully grown seedlings of *P. africana* after two months of nursery management. The seedlings reached an average shoot height of 10 cm after eight weeks' period.

Seed batches soaked in cold normal water for 24 hours at room temperature (25°C) i.e. T_1 showed maximum cumulative germination (144), followed by those sdoaked in GA for 24 hras(T_2; 102). The shortest germination time was also observed for seeds batches soaked in cold normal water for 24 hours at room temperature (25°C) (Fig. 4). Soaking in hot water treatment totally reduced the germination.

The one-way ANOVA indicated that there exists a mean significant difference among treatments groups in both GP and GI at $p \leq 0.05$ (Table 2). Tukey's HSD pairwise comparisons for GP and GI at $p \leq 0.05$ indicated that the GP and GI for seeds soaked in cold normal water for 24 hours at room temperature (25°C) are highly significant compared to other pre-sowing treatments (Table 3).

In this study, soaking the seeds of *P. africana* in cold water for 24 hours can indeed improve both the germination percentage (GP) and the germination index (GI), which are important indicators of seed viability and vigor. The process of soaking softens the seed coat, helping break dormancy and enhancing water absorption, which in turn boosts germination rates. *P. africana* is often slow to germinate due to its hard seed coat, which acts as a barrier to water and oxygen, essential for seed germination

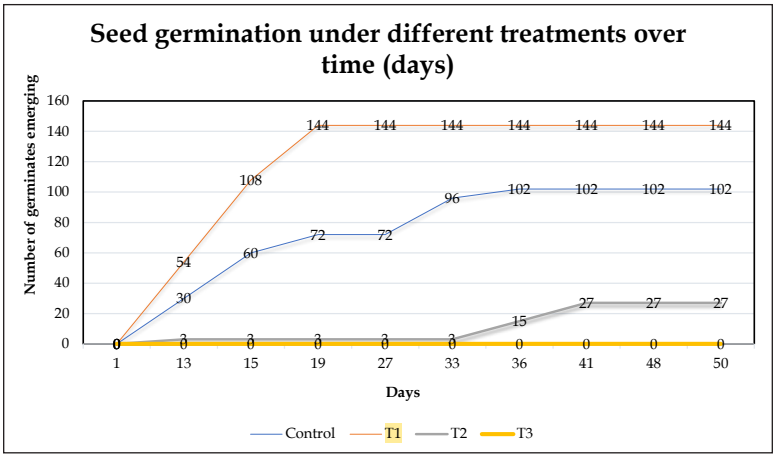

Fig. 4. Cumulative germination of P. africana under different treatments over days: T1 = Soaked in cold normal water for 24 hours, T2 = Soaked in 10 mm GA for 24 hours, and T3 = Soaked in 57°C hot water for 10 minutes, and cooled for 24 hours.

Table 2. One-way ANOVA of the mean significance difference among treatment groups for mean germination percentage (GP), mean daily germination percentage (GD), mean germination time (GT) and mean germination index (GI) of P. africana

	Df	Sum Square	Mean Square	F-value	Pr(>F)
		Mean Germination Percentage			
Treatments	3	36328	12109	30.2	1.27e-07 ***
Residuals	20	8021	401		
		Mean Germination Index			
Treatments	3	0.25958	0.08653	44.7	4.71e-09 ***
Residuals	20	0.03872	0.00194		

Significance codes: Highly significant '***' if $p \leq 0.001$, Very significant '**' if $p \leq 0.01$, Significant '*' if $p \leq 0.05$.

Table 3. Tukey's HSD pair-wise comparisons test ($p \leq 0.05$) for GP and GI of seed treatments (Note that any confidence intervals that do not contain 0 provide evidence of a difference in the groups)

Mean Germination Percentage		
	Mean	SD
Control	71[a]	29.2
T1	100[b*]	0.0
T2	25[c]	27.4
T3	0[d]	0.0
Mean Germination Index		
Control	0.16[a]	0.1
T1	0.26[b*]	0.0
T2	0.042[c]	0.1
T3	0[d]	0.0

(Custódio *et al*., 2022). Pre-treatment methods like soaking can help overcome this natural dormancy and improve the chances of successful seedling establishment (Chukwu *et al*., 2020; Moreira *et al*., 2020; Rubegeta *et al*., 2023). This technique is especially useful in reforestation or conservation projects involving this species. By soaking the seeds in cold water, we are mimicking a natural process that seeds might undergo in the wild, such as exposure to rain or prolonged moisture during a wet season. The water softens the seed coat, allowing increased permeability, which enhances the seed's ability to absorb water and oxygen. This process can trigger the onset of germination. Sacandé *et al*. (2004) indicated the importance of maintaining sufficient moisture content on the success of seed germination of *P. africana.*

Enhancing seed germination of *P. africana* involves understanding various biological factors, e.g., frugivores such as birds **(**Yagihashi *et al*., 1999**),** and environmental factors, *e.g.*, dispersal that enhances germination success (Farwig *et al*., 2006). Previous research indicates that both seed treatment and environmental conditions play crucial roles in improving germination rates. The imbibition (water absorption) process is critical for seed germination. For germination to begin, seeds must absorb enough water to rehydrate their tissues, activate enzymes, and kick-start metabolic processes. Soaking the seeds for prolonged hours, e.g., 24 hours, accelerates imbibition, ensuring the seeds take in enough water to initiate enzymatic activities. This results in a higher germination percentage and germination index. Thus, the current findings are consistent with previous studies on *P. africana* that have reported increased germination rates after soaking in cold water for extended hours (Chukwu *et al*., 2020; Moreira *et al*., 2020). However, Nzweundji *et al*. (2020) and Rubegeta *et al*. (2023) suggested that application of GA could enhance the seed germination of *P. africana*. Seeds that undergo cold water treatment are more likely to germinate uniformly, with higher overall success, because the barriers to water and gas exchange are reduced. This enhances the germination index, which reflects not only the percentage of seeds that germinate but also how quickly they do so.

Conclusion

The findings of the current study align well with existing dormancy and germination theories that emphasize the role of water uptake, seed coat permeability, and pre-treatment in improving the germination performance of *P. africana*. Pre-treatment methods, such as cold water soaking, are commonly used to break dormancy in seeds with physical barriers caused by seed coats in *P. africana* and many other tree species. These

findings support the principle that stratification or scarification treatments (mechanical and chemical, or thermal) can enhance germination by disrupting dormancy mechanisms. Cold water soaking functions similarly to stratification by simulating natural conditions that soften the seed coat or increase moisture availability in the environment. The improved germination percentage and germination index observed after cold water soaking illustrate these principles, demonstrating that this pre-treatment method effectively breaks dormancy and enhances seedling emergence. The findings of the present study can be used by restoration, conservation, and management initiatives of *P africana* in Ethiopia and beyond.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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