Scientific Research Publishing

Open Journal of Genetics, 2025, 15(3), 73-82
https://www.scirp.org/journal/ojgen
ISSN Online: 2162-4461
ISSN Print: 2162-4453

In Vitro Pollen Germination Testing for Controlled Pollination Success in Shea Tree (*Vitellaria paradoxa* Subsp. *paradoxa*)

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How to cite this paper: Diallo, R., Diarrassouba, N., Yao, S.D.M., Attikora, A.J.P., Agongnon, H.J., Daramcoum, W.A.M.P., Koffi, E.-B.Z. and Diarrassouba, N. (2025) *In Vitro* Pollen Germination Testing for Controlled Pollination Success in Shea Tree (*Vitellaria paradoxa* Subsp. *paradoxa*). *Open Journal of Genetics*, **15**, 73-82.
https://doi.org/10.4236/ojgen.2025.153007

Received: June 6, 2025
Accepted: August 22, 2025
Published: August 25, 2025

Abstract

In order to better plan controlled pollination conducted by the shea tree improvement program of Côte d'Ivoire hosted at Peleforo Gon Coulibaly University (UPGC, Korhogo), a study was carried out to optimize the *in vitro* germination tests of shea tree pollen. Two successive experiments were conducted at the UPGC laboratory of the African Centre for Shea Research and Applications (CRAK). The first experiment aimed to determine the most suitable culture medium composition for optimal *in vitro* germination of shea pollen. Four media coded as M1 (10 g sucrose, 1.5 g agar, 100 ml distilled water), M2 (10 g sucrose, 0.6 g agar, 100 ml distilled water), M3 (20 g sucrose, 0.6 g agar, 100 ml distilled water), and M4 (9 g sucrose, 0 g agar, 100 ml distilled water) commonly used for forest tree species were tested with four incubation durations (5, 10, 30, and 48 hours) at 30˚C. The second experiment aimed to identify the optimal temperature for pollen germination. Five temperature conditions were tested: 25˚C, 30˚C, 35˚C, 40˚C, and ambient temperature (29˚C - 37˚C). The results showed culture media M1 (10 g sucrose, 1.5 g agar) and M2 (10 g sucrose, 0.6 g agar) gave the highest *in vitro* pollen germination rate (75.73%) after 30 hours of incubation. Heat significantly influenced the *in vitro* germination capacity of shea pollen. Based on these findings, the Shea Tree Improvement Program of Côte d'Ivoire can now assess pollen viability

using a gel-based medium with 10 g sucrose and either 0.6 g or 1.5 g agar, incubated for 30 hours at a temperature ranging from 25˚C to ambient conditions (29˚C - 37˚C).

Keywords

Shea Tree, Pollen, *In Vitro* Germination, Culture Medium, Temperature

1. Introduction

The shea tree (*Vitellaria paradoxa* C.F. Gaertn.) is a perennial species endemic to the Sudanian savannahs of sub-Saharan Africa. It is an agroforestry species with a very slow growth rate. In Côte d'Ivoire, its natural distribution ranges from the North to the Center, between 7˚30' and 10˚15' North latitude [1] [2].

Shea is one of the main sources of plant-based fat and is highly valued by local communities for its nutritional, financial, and environmental contributions. It is also a multimillion-dollar industry on the global market, making the species a strategically important resource for Africa [3]. The pulp of the fruit is edible, and the kernel is rich in fat. Shea butter is one of Africa's oldest edible oils and is widely used in the food industry (especially in oil and chocolate production), cosmetics (skin hydration and UV protection), and pharmaceuticals [4]. The economic exploitation of shea has become a dynamic industry largely due to the initiative and resilience of rural African women [5].

Despite its importance, the shea tree faces several challenges, including intentional destruction for charcoal production [6] and irregular fruiting from year to year [7].

Several studies have been conducted to improve the productivity of shea genetic resources in Africa. These have contributed to the conservation and improvement of shea across its native range. However, the creation of new varieties aligned with farmers' and breeders' ideal plant types is now essential for the establishment of shea plantations. To support this goal, information on flowering and fruiting phenophases has been gathered to guide breeders on optimal periods for pollen collection and application to receptive female flowers, resulting in the optimization of manual pollination techniques in Côte d'Ivoire [8].

However, evaluating the germination potential of pollen grains prior to manual pollination would be highly beneficial for plant breeding programs. The present study aims to estimate the quantity and quality of shea pollen during manual hybridization by developing an *in vitro* pollen germination test to support the planning of controlled pollination programs in Côte d'Ivoire.

2. Materials and Methods

2.1. Study Area

The study was conducted in the laboratory of the African Centre for Shea Research

and Applications (CRAK) at Peleforo Gon Coulibaly University in Korhogo. The climate in this area is classified as dry tropical of the Sudanian-Sahelian type [9], characterized by two main seasons: a wet season (June to September) and a dry season (October to May). Average annual rainfall is about 1200 mm, with mean temperatures around 30˚C and limited variation throughout the year [10].

2.2. Preparation of Culture Media

Two experiments were carried out successively. The first aimed to determine the ideal culture medium composition for optimal *in vitro* pollen germination at 30˚C. Four culture media and four incubation durations were tested. Pollen grains were cultured on M1 (10 g sucrose, 1.5 g agar, 100 ml distilled water), M2 (10 g sucrose, 0.6 g agar, 100 ml distilled water), M3 (20 g sucrose, 0.6 g agar, 100 ml distilled water), M4 (9 g sucrose, 0 g agar, 100 ml distilled water. These media are commonly used for evaluating the *in vitro* germination potential of woody tree species such as *Picea pungens* (M1), *Populus deltoides* and *Populus nigra* (M2), *Populus maximowiczii* (M3), and *Pinus pinaster* and *Pinus pinea* (M4) [11] [12]. Once the optimal medium was identified, the second experiment tested the influence of five temperature conditions: 25˚C, 30˚C, 35˚C, 40˚C, and ambient temperature (29˚C - 37˚C).

Media were prepared by mixing agar with distilled water in an Erlenmeyer flask. After the agar was hydrated, sucrose was added. The mixture was heated to 100˚C for 2 minutes until fully dissolved. Then, 10 ml of the solution was poured into 8-cm diameter glass Petri dishes and allowed to cool. Once solidified, pollen from eight shea tree stamens was sprinkled over each medium to assess *in vitro* germination at different durations and temperatures.

2.3. Experimental Setup for the *in Vitro* Germination Test of Shea Pollen

The germination tests were conducted using two replicates per treatment (**Figure 1**). In the first experiment, 8 Petri dishes were arranged in pairs—two dishes for each of the four media, incubated for each of the four time durations. The second experiment used 10 Petri dishes to assess the effect of different temperatures using the best-performing culture medium.

Each Petri dish was divided into four equal sections. One random field of view was observed per section, and germinated and non-germinated pollen grains were counted. This setup yielded four data points per Petri dish and was used for each *in vitro* germination trial.

2.4. Collection and Statistical Analysis of Data on Pollen Germination

Pollen grains were counted as germinated or non-germinated across the four media (M1, M2, M3, M4) at 5 h, 10 h, 30 h, and 48 h of incubation in the first experiment. In the second experiment, germination was assessed on the best-performing medium at five temperature levels. A pollen grain was considered viable if the pollen tube was at least half the diameter of the grain [13] (**Figure 2**). Pollen via-

bility was calculated using the following formula:

Germination rate (%) = (Number of germinated pollen grains/Total number of pollen grains (germinated and non-germinated)) × 100.

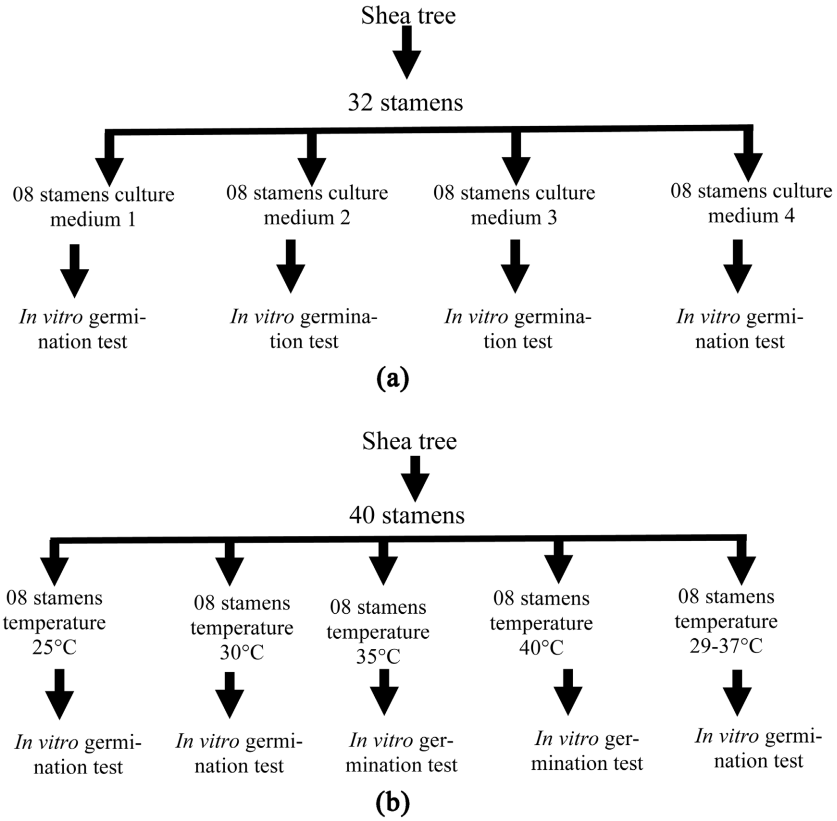

Figure 1. An *in vitro* pollen germination test was set up for the shea tree. (a) Set up for evaluating culture media; (b) Set up for evaluating incubation temperature.

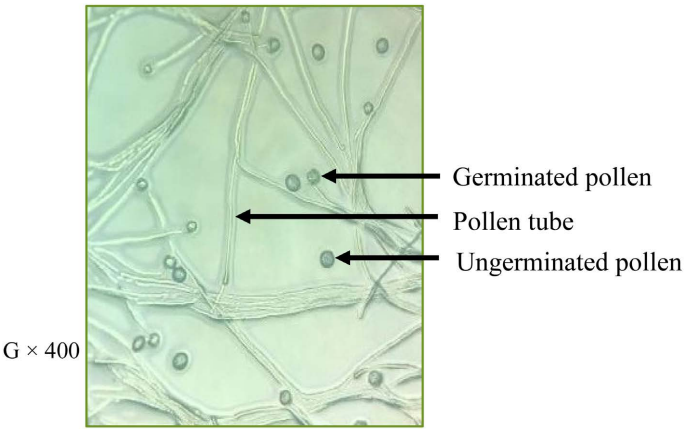

Figure 2. Germination status of shea pollen grains on culture medium M1 (10 g sucrose, 1.5 g agar, 100 ml distilled water) at different incubation durations.

Data transformation was performed when the data did not follow a normal distribution. A two-factor analysis of variance (ANOVA) was then conducted to evaluate the impact of the *in vitro* culture medium type (four modalities) and incuba-

tion duration (four modalities) on the *in vitro* germination rate of shea pollen, as well as their interactions. A one-factor ANOVA was performed to assess the effect of incubation temperature on the *in vitro* germination rate of shea pollen. An error threshold of 5% was chosen. When the ANOVA test was significant ($p < 0.05$), a Student-Newman-Keuls (SNK) post hoc test was performed to classify the statistical units studied. The analysis was performed using SPSS version 26 (IBM, USA) and R version 4.4.0 (Posit Software, USA).

3. Results

3.1. Effect of Culture Medium on *in Vitro* Germination of Shea Pollen

A significant difference was observed in the germination rates of pollen grains among the different culture media ($F = 10.63$; $p < 0.001$). Media M1 and M2 showed the highest germination rates at 58.56% and 56.43%, respectively. The lowest *in vitro* germination rates were recorded on media M3 and M4, with 43.58% and 33.56%, respectively (Figure 3; Figure 4).

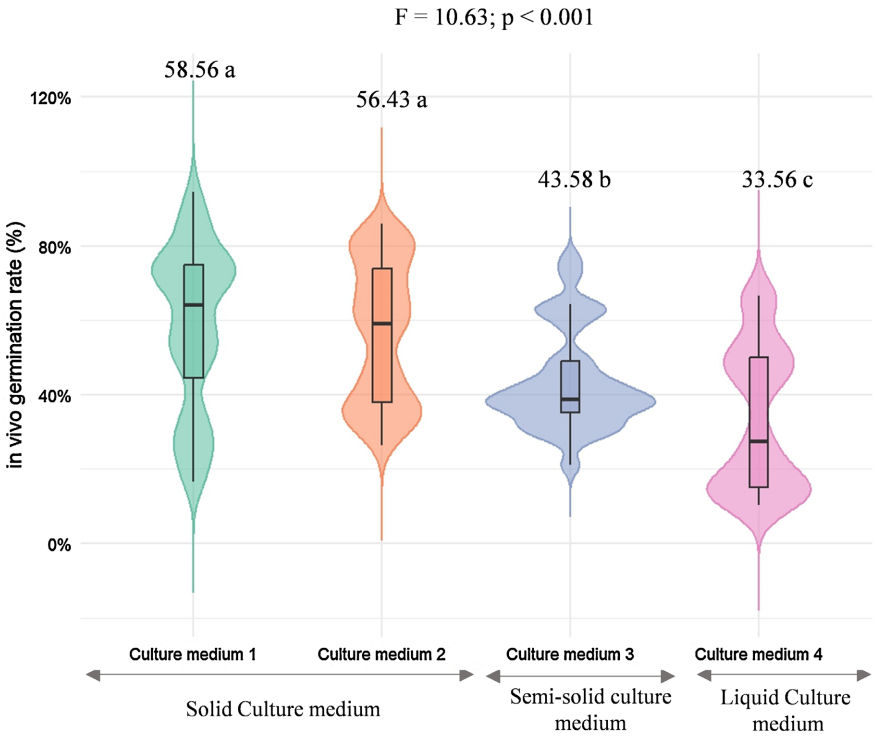

Figure 3. *In vitro* germination rate of shea pollen grains by type of culture medium.

3.2. Effect of Incubation Duration on *in Vitro* Germination of Shea Pollen

Figure 5 reveals a significant variation in the *in vitro* germination rates of shea pollen according to incubation time ($F = 10.49$; $p < 0.001$). Incubation durations of 30 and 48 hours yielded the highest germination rates (60.18% and 56.70%, respectively), while the shortest durations (5 and 10 hours) produced the lowest germination rates (40.19% and 38.37%).

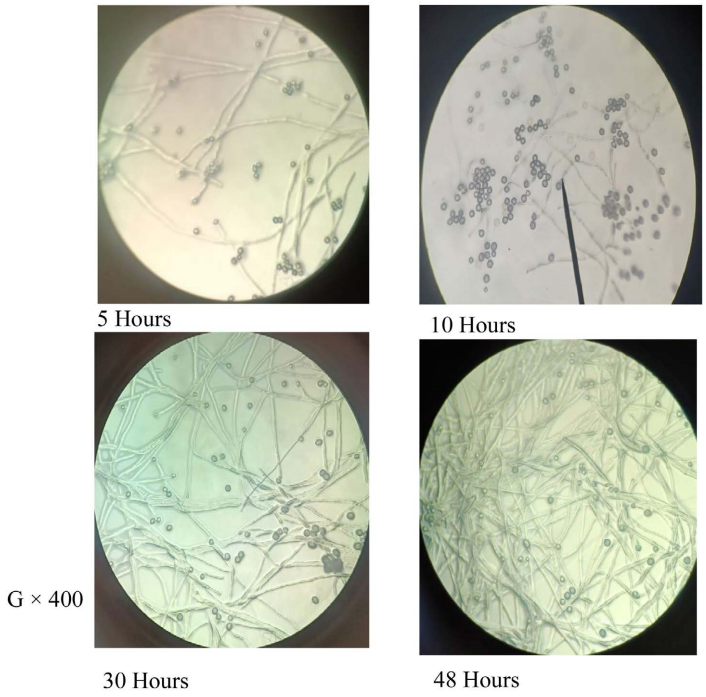

Figure 4. *In vitro* germination pattern of shea pollen on culture medium M1 (10 g sucrose, 1.5 g agar, 100 ml distilled water) at different incubation durations.

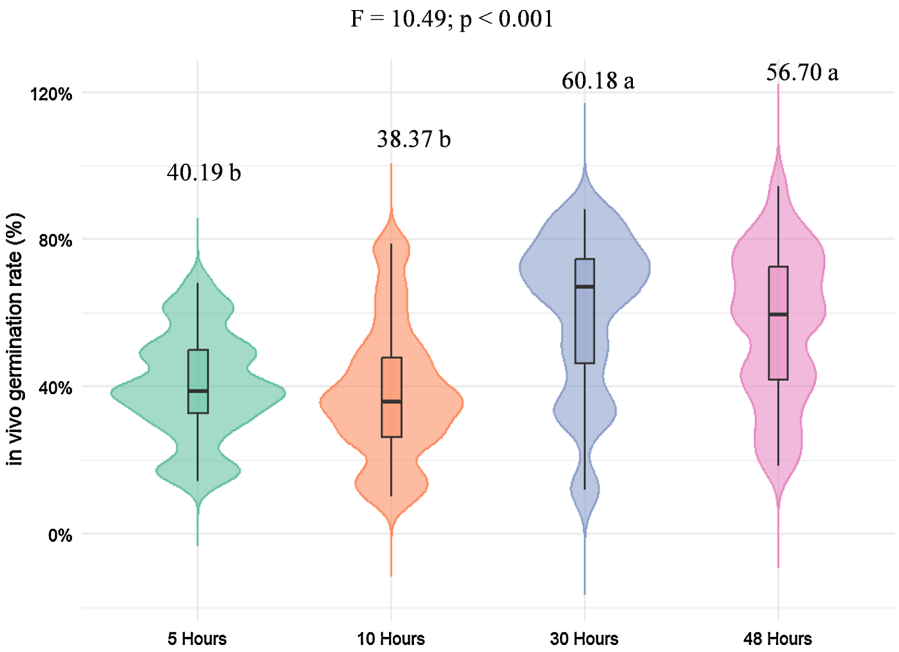

Figure 5. *In vitro* germination rate of shea pollen at different incubation durations.

3.3. Combined Effect of Culture Medium and Incubation Duration on *in Vitro* Germination of Shea Pollen

The results of descriptive analysis and comparison tests are presented in Table 1. A significant difference in pollen germination rates was observed across media for each incubation time.

Table 1. Effect of culture medium and incubation duration on *in vitro* germination of shea pollen.

Incubation duration for culture media	Solid culture medium		Liquid culture medium		F	p
	Culture medium 1	Culture medium 2	Culture medium 3	Culture medium 4		
	In vitro germination rate (%)					
5 Hours	40.01 ± 22.76[a]	37.23 ± 7.43[a]	37.28 ± 4.40[a]	18.95 ± 14.69[b]	4.44	0.012
10 Hours	43.98 ± 14.93[a]	42.36 ± 10.54[a]	40.53 ± 7.00[a]	24.01 ± 17.67[b]	3.362	0.035
30 Hours	75.73 ± 8.10[a]	75.24 ± 8.46[a]	44.17 ± 16.04[b]	42.37 ± 24.46[b]	11.599	<0.001
48 Hours	73.98 ± 11.94[a]	70.86 ± 9.32[a]	39.06 ± 11.26[b]	40.90 ± 17.27[b]	11.599	<0.001

F: ANOVA test statistic; p: ANOVA p-value; a and b: Statistical grouping indicators; Mean ± Standard Deviation.

At 5 hours, media M1, M2, and M3 recorded the highest germination rates: 40.01%, 37.23%, and 37.28%, respectively. The lowest was M4 at 18.95%.

At 10 hours, media M1, M2, and M3 again performed best: 43.98%, 42.36%, and 40.53%, respectively. M4 recorded 24.01%.

At 30 hours, M1 and M2 showed the highest germination rates: 75.73% and 75.24%, respectively. M3 and M4 followed with 44.17% and 42.37%.

At 48 hours, M1 and M2 had the best results with 73.98% and 70.86%, while M3 and M4 yielded 39.06% and 40.90%, respectively.

3.4. Effect of Incubation Temperature on *in Vitro* Pollen Germination Rate

As shown in Figure 6, temperature significantly affected the *in vitro* germination rate of shea pollen (F = 2.97; p = 0.035). Incubation temperatures of 25˚C and 30˚C produced the highest germination rates (75.19% and 76.27%). The lowest rate was at 40˚C (61.97%). Intermediate germination rates were observed at 35˚C (70.28%) and the ambient temperature range of 29˚C - 37˚C (68.01%).

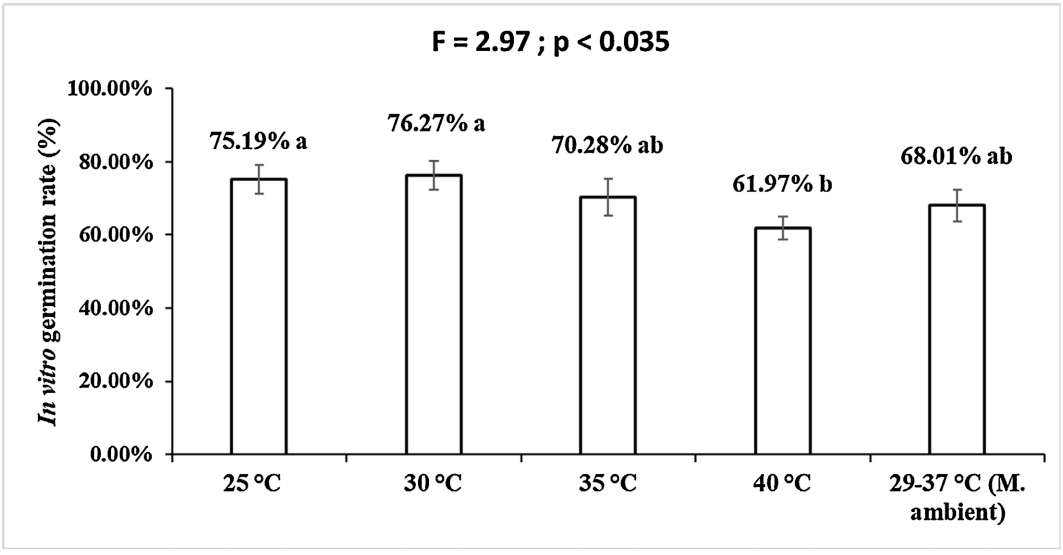

Figure 6. *In vitro* germination rate of shea pollen at different incubation temperatures of the culture medium.

4. Discussion

The ability of pollen to germinate under favorable conditions reflects its viability. Establishing an *in vitro* germination test for pollen allows for predicting pollen viability prior to controlled pollination, thereby improving fruit set success in the field. This study aimed to optimize such a test for shea tree pollen in Côte d'Ivoire.

Findings show that culture media M1 and M2, with 30 hours of incubation, are the most effective for *in vitro* germination of shea pollen. These media (10 g sucrose with either 1.5 g or 0.6 g agar) provided the highest germination rates. Solid media likely mimic the cell matrix of the stigma and style more closely than liquid media, thus enhancing germination. These results were reported by [14] in their study on an optimised protocol for the *in vitro* germination of yam pollen. According to Silva *et al.* (2020) [15], agar has several functions in the composition of the *in vitro* pollen germination medium. It facilitates the solidification of the medium, establishes its osmosis balance, ensures a constant humidity level, and promotes nutrient uptake. Furthermore, it facilitates the formation of the pollen tube.

The low germination rate observed on M3 (20 g sucrose, 0.6 g agar) at 30 hours is likely due to the high sucrose concentration (>0.1 g/ml), which may inhibit germination by causing plasmolysis through osmotic imbalance. According to Colas and Mercier (2000) [12], an osmotic imbalance in the *in vitro* culture medium may cause cytoplasm leakage at the end of the pollen tube or result in the pollen tube's absence.

Pollen germination improved with increased incubation time, with the best results occurring between 30 and 48 hours, indicating this as the optimal incubation duration for shea pollen germination *in vitro*. This information is key for planning germination testing protocols.

Temperatures between 25˚C and the ambient range (29˚C - 37˚C) were favorable for shea pollen germination, supporting optimal enzymatic and metabolic activity. Kellal Siham and Hadjer (2021) [16] found that temperature influences the viability of pollen grains. And Youmbi *et al.* (2011) [17] found 30˚C to be optimal for shea pollen germination in Cameroon.

Conversely, the reduced germination rate at 40˚C is likely due to enzyme denaturation. Latruffe (2017) [18] demonstrated that temperatures above a certain threshold cause thermal denaturation of enzymes, leading to the loss of their functional three-dimensional structure.

5. Conclusion

This study was conducted to optimize *in vitro* pollen germination testing for shea trees, aiming to better plan controlled pollination. Results indicate that optimal germination occurs on solid media containing 10 g sucrose and either 0.6 g or 1.5 g agar, incubated for 30 hours at 25˚C or within the ambient temperature range of 29˚C - 37˚C.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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