

# Optimization of *Acacia auriculiformis* A. Cunn. ExBenth. seed germination by pretreatment analysis

## ABSTRACT

Agroforestry resources play a crucial role in conserving biodiversity, stabilizing soils and improving the livelihoods of rural populations. This study investigates the impact of different treatments on the germination of *Acacia auriculiformis*, an important tree species for soil restoration in Côte d'Ivoire. The work was carried out at University Jean Lorougnon GUÉDÉ in Daloa. The seeds used for this work were subjected to four treatments, including a control (T0); those soaked for 3 days in ordinary water (T1); a hot water treatment at 60°C (T3); a boiling water treatment at 100°C and a flame treatment (T4). A randomized block design consisting of four treatments in three blocks. The results showed that seed treatment in hot water at 100°C until cooling gave the best germination percentages and germination speed. The results also showed that soaking the seeds in water for three days produced seedlings with the shortest germination time. The study underlines the importance of pregermination for the selection of better-performing varieties and the implementation of effective reforestation programs. It also highlights the need for further research into the impacts of climate change on tree germination and growth. However, for a better policy of extension, production and improvement of this endangered forest species of socio-economic interest, mastery of the requirements of this species while maintaining optimal conditions for its germination in the nursery is more necessary.

**Keywords :** *Acacia auriculiformis*, Agroforestry, Treatment, Pregermination, Biodiversity

## INTRODUCTION

Agroforestry resources are essential for conserving biodiversity, stabilizing soils and improving the livelihoods of rural populations [1]. Among these resources, *Acacia auriculiformis* occupies a prominent place due to its multiple uses, such as wood and fodder production, and soil restoration [2]. However, this species is facing increasing threats from deforestation, degradation of its natural habitat, and the effects of climate change, compromising its natural regeneration [3]. Natural regeneration of *A.auriculiformis* is often limited by rigid integumentary dormancy, which reduces germination rates and, consequently, the species' survival in degraded ecosystems [4]. This complex dormancy requires specific interventions to be lifted effectively, making pregermination a crucial step in improving germination and seedling survival [5]. Furthermore, improving germination rates through pregermination is also fundamental to breeding programs, as it results in a more homogeneous and vigorous population, facilitating the selection of traits desired [6]. Pre-treatments such as mechanical scarification, hot water quenching and acid application have been shown to break seed dormancy and increase germination rates in various tropical species [7]. These methods are not only important for natural regeneration, but also play a key role in the genetic improvement of species. Rapid, uniform germination enables better assessment of phenotypic characteristics, thereby speeding up selection and crossing processes [8]. In the context of the restoration of degraded ecosystems, the application of well-studied and optimized pregermination techniques can greatly contribute to the preservation of threatened agroforestry species [9] such as *Acacia auriculiformis*. Also, genetic improvement, facilitated by better germination, can offer new varieties that are more resistant and better adapted to changing environmental conditions, thus enhancing the sustainability of agroforestry ecosystems [10].

In Côte d'Ivoire, scientific data on *A.auriculiformis* is fragmentary. This lack of information makes it impossible to implement sustainable management and conservation strategies for the species. In addition, seed dormancy with slow and difficult germination hampers reforestation efforts [11]. However, information on the application of the main, less costly methods used to lift seed dormancy in *A. auriculiformis* is rudimentary. This constitutes an obstacle to the popularization of the plant's cultivation to encourage the rural population to reforest. This study sets out to explore the effects of different pre-treatments on seed germination in *A.auriculiformis*, with the dual aim of determining the best pre-treatment that can improve germination percentage and demonstrating its importance within the agroforestry program in order to explore potential implications for genetic improvement of the species. The results obtained could provide valuable guidance for the conservation and sustainable management of genetic resources, while supporting efforts to preserve biodiversity in agroforestry ecosystems.

## 2. MATERIALS AND METHODS

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## 2.1 Experimental site

The study took place in one of the experimental nurseries of the University Jean Lorougnon GUEDE located in the town of Daloa between 6°53 north latitude and 6°27 west longitude. The town is located in the Haut Sassandra region in west-central Côte d'Ivoire. It is around 141 km from Yamoussokro, the political capital, and around 400 Km from Abidjan, the economic capital [12]. Daloa is bordered to the north by the town of Vavoua, to the south by Issia, to the west by Duekoue and to the east by Bouafle. The vegetation, which belongs to the mesophilic sector, is largely made up of dense forest, which has now disappeared to make way for various cash crops [13]. The soil is ferralitic and the climate is humid tropical, with one rainy season and two dry seasons [14]. Dry and wet seasons alternate with temperatures ranging from 24.65°C to 27.75°C on average.

## 2.2 Plant material

The plant material used consists of *Acacia auriculiformis* seeds. The seeds used for these tests are mainly from the experimental station of the Centre National de Recherche Agronomique de Katiola located in central northern Côte d'Ivoire between 8°10' north latitude and 5°4' west longitude, 55 km to the north.

## 2.3 Methods

### 2.3.1. Pre-germination treatment

Four pre-treatments were applied to the seeds, based on the methods of [15] and [16]:

- Control seeds (T0) of *Acacia auriculiformis* were directly sown without pre-treatment;
- Treatment 1 (T1) seeds were soaked for 3 days in a bucket of ordinary water. They were removed from the water on the third day (72 hours after soaking) and planted directly;
- Seeds from treatments 2 and 3 were respectively soaked in water heated to 60°C (T2) and 100°C (T3) until cooled before sowing;
- Seeds from treatment 4 were flamed (T4), the seeds were spread out on the ground and then a rapid passage (eight seconds) of a lighted straw beam (flame thrower) was made over the seeds. After this operation, seeds that had been severely affected by the fire were not used during sowing.

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In each of these environments, the seeds remained until they had cooled down completely. These pre-treatments are easy and inexpensive to carry out, and therefore directly applicable by farmers, unlike treatments with sulfuric acid or hydrogen peroxide, which require financial resources.

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The duration and season of collection  
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### 2.3.2. Sowing and maintenance

Sowing was carried out in 18 cm x 10 cm x 8 cm polyethylene bags containing Daloa red soil. After filling, the bags were placed under the shade. Maintenance consisted of watering twice a day (in the morning at 8:00 a.m. and in the evening at 5:00 p.m.). Regular weeding was also carried out. This enabled the plant to develop better. An insecticide treatment was applied when foliar attacks appeared to eliminate the insects.

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### 2.3.3. Experimental set-up

After sowing, the bags were laid out in a randomized block design consisting of four treatments in one block. Each treatment was repeated three times. Each experimental unit comprised 30 bags, each containing three seeds. The trial ran from April 13, 2022 to June 15, 2022.

### 2.3.4. Data collection

From April 13, 2022, we systematically counted seedlings that had emerged every day until May 13, 2022. Emergence corresponds to the appearance of a seedling with two cotyledonary leaves on the

soil surface. Reaching this stage was also taken into account when assessing germination date and emergence time.

### 2.3.5. Parameters measured

The following parameters were determined: waiting time (germination time), which is the time elapsed between sowing and the first germination; germination speed (staggering), which is the time elapsed between the first and last germination; germination rate, which is obtained from the following formula:  
Germination rate = (Number of germinated seeds/Total number of seeds sown per treatment) x100

### 2.3.6. Data analysis

Statistica version 7.1 software was used for statistical analysis of this work. An analysis of variances was used to distinguish the different treatments analyzed through these parameters. Where there was a significant difference, the Small Significant Difference (SSD) test was used to classify the means.

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## 3. Results and discussion

### 3.1 Results

Table 1 shows the results of a multivariate analysis of variance (MANOVA) that was carried out to determine whether the different treatments applied to *Acacia auriculiformis* seeds. The multiple analysis of variance showed that there was a significant difference between the different treatments carried out ( $P < 0.001$ ).

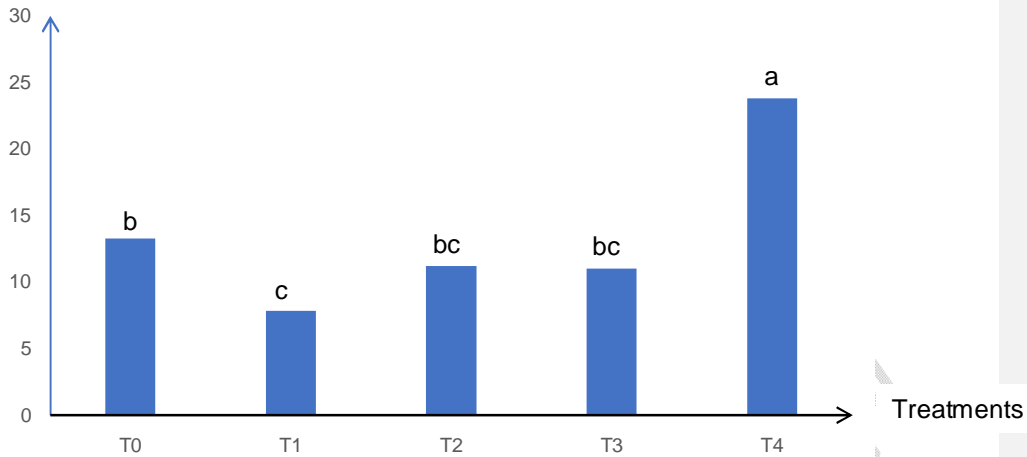
**Table 1. Multivariate analysis of treatment parameters**

	Test	Value	F	Error dl	P
Ord.Orig.	Wilk	0.000083	216930,3	90.00	< 0,001
Treatments	Wilk	0.000221	174,6	299.446	< 0,001

#### 3.1.1. Germination delay

Figure 1 shows the germination times of *Acacia Auriculiformis* seeds subjected to five different treatments. Germination times are affected by the treatments. Treatment T1 (seeds soaked for 3 days in water) has the shortest delay (7.79 days), followed by treatment T3 (seeds soaked in boiling water at 100°C until cooled (11 days). The control (T0) has a delay of 13.22 days, while treatments T2 (seeds soaked in hot water at 60°C) and T4 (partially flamed seeds) have delays of 11.15 and 23.83 days respectively. These results show that seeds soaked in water for 3 days have the best germination times (figure1).

Days

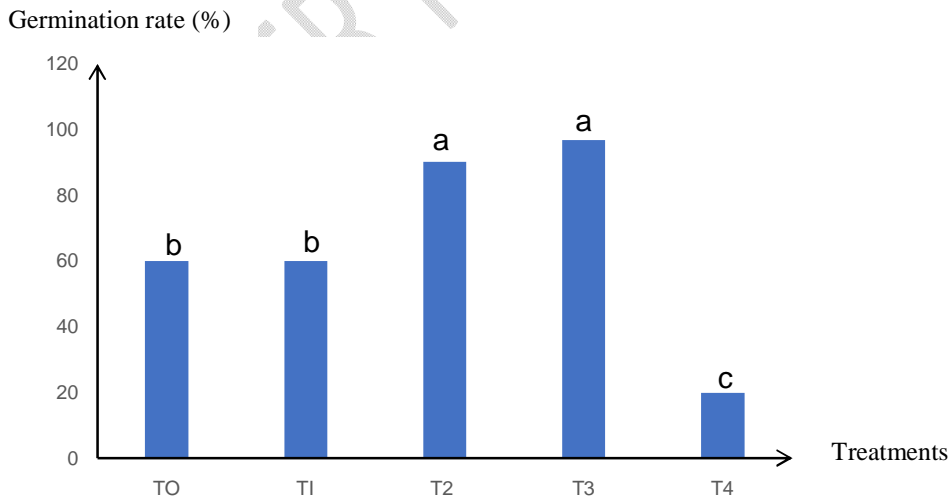


**Fig. 1. Histogram of evaluation of different pretreatments on average germination time**

(T0) Control seeds; (T1) seeds were soaked for 3 days in a bucket of ordinary water; (T2) Seeds were soaked in water heated to 60°C ;(T3) Seeds were soaked in water heated to 100°C ;(T4): Seeds were flamed;Histograms bearing the same alphabetical letters in each zone are not statistically different ( $p \leq 0.05$ ) (Newman and Keuls)

### 3.1.2. Germination rate

Figure 2 shows the variations in germination rate observed on *Acacia auriculiformis* seed treatments. These results show that pre-treatments have variable impacts on germination rate. Treatment T3, representing seeds soaked in water heated to 100°C, showed the highest germination rate (96.67%). These were followed by seeds soaked in water heated to 60°C (T2), seeds soaked in water for three days (T1), control seeds (T0) and partially flamed seeds (T4) with 90%, 63.33%, 52.94% and 20% respectively. These results show that the method of soaking seeds in water heated to 100°C gives the best germination rate in *A. auriculiformis*(fig. 2).

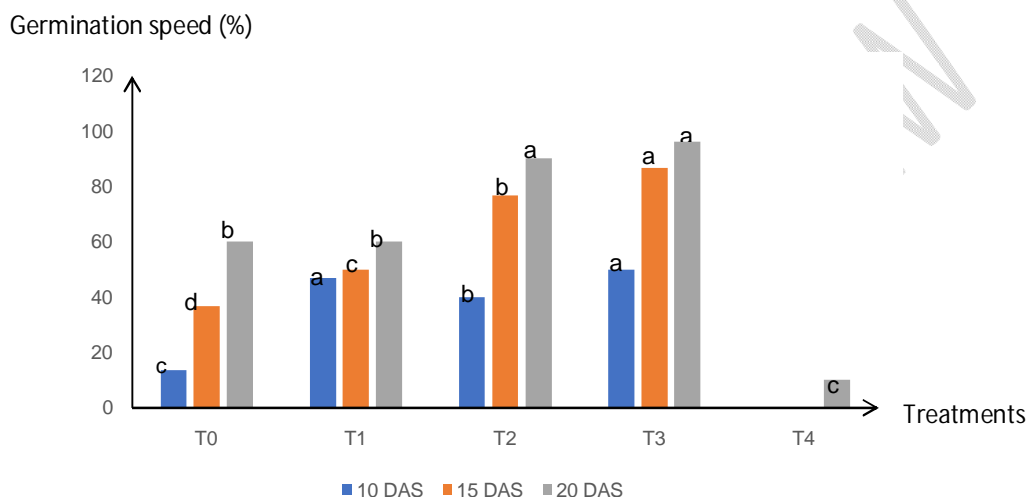


**Fig. 2. Histogram of evaluation of different pretreatments on germination rate**

(T0) Control seeds; (T1) seeds were soaked for 3 days in a bucket of ordinary water; (T2) Seeds were soaked in water heated to 60°C ;(T3) Seeds were soaked in water heated to 100°C;(T4) Seeds were flamed;Histograms bearing the same alphabetical letters in each zone are not statistically different ( $p \leq 0.05$ ) (Newman and Keuls)

### 3.1.3 Germination speed

The results for seed emergence speed in the different treatments are shown in figure 3. Treatments T3 and T2 show germination rates of 50% and 40% respectively from 10 days after sowing. It's not until 20 days after sowing that we see more than 50% emergence speed in treatments T0 and T1. However, treatment T4 showed the lowest emergence speed, suggesting less efficient germination. The results show that emergence speed is also affected by treatment.



**Fig. 3. Histogram of evaluation of different pretreatments on germination speed**

(T0) Control seeds; (T1) seeds were soaked for 3 days in a bucket of ordinary water; (T2) Seeds were soaked in water heated to 60°C ;(T3) Seeds were soaked in water heated to 100°C ;(T4) Seeds were flamed;(DAS) Day after sowing;Histograms bearing the same alphabetical letters in each zone are not statistically different ( $p \leq 0.05$ ) (Newman and Keuls)

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### 3.2. Discussion

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Rapid and uniform seed germination is a crucial step in ensuring efficient and sustainable agricultural production. Pre-treatment methods are proving to be promising ways of overcoming obstacles such as integumentary dormancy. With regard to germination time, the best results were observed with seeds soaked in ordinary water for three days. According to [17], the optimum soaking time influences seed coat hardness and, consequently, seed germination time. Treatment T4, in which the seeds were exposed to an intense heat source, showed the longest germination time. This result is probably explained by the altered germinative properties of some seeds after prolonged exposure to fire, which was also observed by [18] with *Pterocarpus erinace* seeds. For growers, the choice of the right treatment is essential to ensure rapid and homogeneous germination, enabling better synchronization of crop cycles. In fact, T1 and T3 treatments with reduced germination times enable farmers to maximize production efficiency. Research such as that by [19,20] and [21], confirms the effectiveness of boiling water in softening the seed coat and reducing its impermeability, thus facilitating rapid germination. As a plant improvement tool, these pre-treatments not only speed up germination, but also make it easier to select the hardiest plants, an essential aspect of varietal breeding programs. The best germination rates were obtained with treatments T3 and T2. A high germination rate is a key indicator of seed performance and has a direct impact on crop productivity. Our results corroborate those of [7], who observed a significantly higher germination rate in *Acacia senegal* after pretreatment

with boiling water and high temperature. These treatments soften the seed coat, facilitating water absorption and, consequently, the germination process. Work by [22] on *Prosopis africana* also demonstrated that using boiling water as a pre-treatment resulted in a germination rate of 85%. For small-scale growers, this translates into reduced seed losses and increased crop yields, with immediate economic benefits. By optimizing seed use, farmers can increase productivity without having to spend resources on expensive inputs or invest in more advanced technologies. This improved germination rate also enables growers to manage their resources more efficiently, particularly in regions where access to quality seed is limited. By adopting practices such as boiling water pre-treatment, farmers benefit from an accessible, inexpensive and effective solution to improve their production.

Germination speed is a key factor in crop cycle management. Seeds that germinate quickly enable growers to better synchronize their crops with the seasons, optimizing the use of land and inputs. [7] have shown that pre-treatment with boiling water not only increases germination rates, but also the speed at which seeds germinate, which is particularly important for good plant development.

The effect of heat on the seed coat, which promotes softening and facilitates embryo emergence, results in more vigorous seedlings capable of rapid establishment. This is beneficial for growers, especially those in regions where cultivation periods are limited by climatic factors. By reducing seed dormancy time, farmers can carry out more cropping cycles in the same year, increasing the productivity of their farms. From an agronomic point of view, increasing germination speed through these techniques also contributes to better variety selection.

The best-performing plants can be identified more quickly, enabling the genetic quality of crops to be improved over the long term.

Tegmental dormancy, observed in many species, represents a major challenge for farmers seeking to obtain rapidly germinating seeds. [23] and [24] have observed that this type of dormancy can be lifted by thermal pre-treatment methods, such as soaking seeds in boiling water. This technique lifts the impermeability of the seed coat, facilitating water absorption and the activation of germination. Controlling this dormancy is essential, as it ensures more homogeneous and predictable germination, which is a crucial advantage for farms, especially those operating with limited resources. By adopting appropriate pre-treatment techniques, farmers can increase their chances of success and thus maximize their yields while reducing the risks associated with non-viable seeds [25]. From the grower's point of view, seed pre-treatment, particularly with boiling water, is of undeniable interest in terms of cost-effectiveness and ease of application. These methods do not require sophisticated equipment and can be carried out with limited means, making them an ideal solution for small-scale farmers and peasants in rural regions [22]. By reducing dormancy and increasing germination rate and speed, growers can improve their yields without the need to acquire hybrid seeds or expensive chemicals. This accessibility is a considerable advantage in contexts where financial resources are limited, but demand for productive and sustainable agriculture is high. From an agronomic point of view, the adoption of these techniques not only improves crop productivity, but also preserves the diversity of local varieties, often better adapted to specific ecological conditions. This strengthens the resilience of local farming systems and contributes to their long-term sustainability.

#### 4. Conclusion

This study highlights the importance of seed pre-treatment, particularly for *Acacia auriculiformis*, to optimize germination and improve crop yields. Of the various methods tested, soaking the seeds for three days (T1) proved the most effective in terms of speed of germination. Next, immersion in boiling water until cooled (T3) offered the highest germination rate, reaching 97.67%. These two pre-treatment techniques offer several advantages for small-scale growers. They are simple, accessible and inexpensive, while offering significant results in terms of improving germination rates and lifting seed dormancy. By optimizing germination, they enable farmers to maximize the use of their seeds and increase their crop yields, which is crucial in a context of food security and farm profitability.

This study opens up several avenues for future research. It would be interesting to combine the two most effective methods, namely prolonged soaking and boiling water immersion, to see if this combination could offer better results. In addition, the addition of scarification techniques, adapted to the delicate size of *A.auriculiformis* seeds, could lead to even more uniform and rapid germination. These are promising prospects for large-scale use of this species in agroforestry systems.

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#### REFERENCES

1. FAO. The State of the World's Forests 2022. Forest solutions for a green recovery and inclusive, resilient and sustainable economies. Rome, FAO. 2022  
<https://doi.org/10.4060/cb9360en>
2. Fonton HN, Clausiaux JJ, Agbahungba G. Performance of *Acacia auriculiformis* (Cunn. A.) in the agroforestry system in Benin. *Annals of Agronomic Sciences of Benin*. 2002; (3): 65-79.
3. Van Noordwijk, Ekadinata MA, Leimona B, Catacutan D, Martini E, Tata H L, Ôborn I, Hairiah K, Wangpakapattanawong P, Mulia R, Dewi Sonya, Rahayu S & Zulkarnain T. (2020). Agroforestry Options for Degraded Landscapes in Southeast Asia. In: Dagar, JC, Gupta, SR, Teketay, D. (eds) *Agroforestry for Degraded Landscapes*. Springer, Singapore. 2020; 307-347. [https://doi.org/10.1007/978-981-15-4136-0\\_11](https://doi.org/10.1007/978-981-15-4136-0_11).
4. Ouattara N, Kouassi BAT, Soro D, Soro D. Effect of Pretreatments on Seed Dormancy of *Acacia auriculaeformis* A. Cunn ex. Benth (Fabaceae). *European Scientific Journal*. 2019; 15(18):202-215.
5. Gbadamosi IT and Kalejaye AO. Comparison of the antioxidant activity, phytochemical and nutritional contents of two antihypertensive ethnomedicinal plants. *Ife Journal of Science*. 2017; 19 (1):147 DOI:10.4314/ijfs.v19i1.15.
6. Kumar M, Sarvade S, Kumar R and Kumar A. Pre-Sowing Treatments on Seeds of Forest Tree Species to Overcome the Germination Problems. *Asian Journal of Environment & Ecology*. 2024; 23(5):1-18.
7. Hamawa Y, Baye-Niwah C, Kepwa FBFS, Mapongmetsem P M. Effect of Pretreatments on the Germination of *Acacia senegal* (L.) Seeds Willd. (Mimosaceae) in the Sahelian Zone of Cameroon. *European Scientific Journal*. 2020; 16(3):263-274.
8. Pedrini S, Lewandowski W, Stevens JC, Dixon KW. Optimization of seed production techniques for improving germination and seeding of native grasses for ecological restoration. *Plant Biology*. 2019; 21:415–424. <https://doi.org/10.1007/s11692-019-0911-1>.
9. Wanogo S H, Abdou MO, Adamou MM, Garba A, Souman B, Mahamane KI and Kona HM. Ecological impacts of degraded land restoration activities in three village terroirs of the Maradi region (Niger). *Int. J. Biol. Chem. Sci.* 2024; 18(2): 389-404.
10. Oyebamiji NA, Suleiman RO and Jegede, OC. Seeds germination pre-sowing techniques and growth performance of some selected savanna agroforestry tree species. *Journal of Research in Forestry, Wildlife & Environment*. 2021; 13(3): 42 - 49.
11. Rakoto FNJ, Vahatra AT R, Herizo R, Martial DA, Rondro HB, Faly R, Jean BR, Tsoushima ER, Vonjison R, and Heriniaina R. "Chapter 43. Pre-germination scarification treatment of seeds for ecological restoration of degraded areas". In *Biodiversity of intertropical ecosystems*, edited by Jean-Pierre Profizi, Stéphanie Ardila-Chauvet, Claire Billot, Pierre Couteron, Maïté Delmas, Thi My Hanh Diep, Philippe Grandcolas, et al. Marseille: IRD Éditions, 2022. <https://doi.org/10.4000/books.irdeditions.42144>
12. Diomandé M, Kouamé KB & Koko AC. Comparison of the chemical properties of peanut and cashew nut oil and cake on the Daloa market, Côte d'Ivoire. *Engineering and Applied Sciences*. 2017; 4(11): 28-32.
13. Sangare A, Koffi E, Akamou F & Fall C A. State of plant genetic resources for food and agriculture. Second national report. Ministry of Agriculture, Republic of Côte d'Ivoire. 2009; 64p
14. N'guessan CA, Abo K, Fondio L, Chiroleu F, Lebeau A, Poussier S, Wicker E. & Koné D. So Near and Yet So Far: The Specific Case of *Ralstonia solanacearum* Populations from Côte d'Ivoire in Africa. *Bacteriology*. 2012; 102: 733-740
15. Houehoun, HAR, Hermane, TA, Brice, S., André, MT. Artificial regeneration approaches of *Daniellia oliveri* (Rolfe) Hutchison and Dalziel. *International Journal of Biological and Chemical Sciences*. 2009; 3 (1): 7-19.
16. Lachiheb K, Neffati M, Zid E. Germination abilities of some spontaneous halophytic grasses from southern Tunisia. Zaragoza: CIHEAM, Cahiers Options Méditerranéennes. 2004; 62: 89-93.

17. Neffati M. Morpho-biological characterization of some North African plant species: implications for pastoral improvement. PhD thesis: University of Ghent (Belgium). 1994.
18. Bamba N, Ouattara ND, Konan D, Bakayoko A & Tra Bi FH. Effects of five pretreatments on the germination of veneer (*Pterocarpus erinaceus* Poir., Fabaceae) in the Haut Bandama Reserve (Ivory Coast). *European Scientific Journal*. 2018;14(30):438-453.
19. Aduradola AM & Badru. Aspects of germination in seeds of *Azelia Africana* Sm. And *Terminalia ivorensis* A. Chev. *Annals of Agronomic Sciences of Benin*. 2004; 6(2): 175-184.
20. Rolston MP. Water impermeable seed dormancy. *Bot. Rev.* 1978; 44:365-396. 16 21.
- Tran & Cavanagh (1984) Tran VN & Cavanagh AK. Structural aspects of dormancy: In: D.R. Murray (ed.) *Seed Physiology*. V.II. Academic Press, Melbourne. 1984; 1-44.
22. Ahoton, LE, Adjakpa, JB, M'po, IM, Akpo, EL. Effect of seed pretreatments on the germination of *Prosopis africana* (Guill., Perrot. Et Rich.) Taub., (Cesalpiniaceae). *Tropicultura*. 2009; 27 (4): 233-238.
23. FAO. Forest seed handling guide. FAO Forest Study 20/2.1992; <http://www.fao.org/docrep/006/AD232F/AD232F00.HTM>.1992; page consulted on March 24, 2024.
24. Laurie MV. Treeplanting practices in African savannas. Food and Agriculture Organization of the United Nations, Rome. 1974; 42-43.
25. N'dri AAN, Vroh-Bi I, Kouamé PL & Zoro Bi IA. Genetic and biochemical bases of seed germination capacity: implications for seed systems and food production. *Sciences & Nature*. 2011;8(1):119-137.

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