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Seed Germination and Growth of *Irvingia gabonensis* (Aubry-Lecomte ex O'Rorke) Baill. (Irvingiaceae) Seedlings in a Controlled Environment East of Taï National Park (Buyo, South-West of Ivory Coast)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Anthropogenic activities tend to destroy forest resources such as *Irvingia gabonensis*, a species much prized by the local populations of the Taï National Park (TNP). The enormous pressure on the natural stands of this species is leading to its rarefaction and even extinction. This study was to

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identify the best methods for germinating *Irvingia gabonensis* seeds, with a view to facilitating their integration into cropping systems on the outskirts of the park, with a view to conserving the species and promoting nutritional security. The methodological approach consisted of a series of five (5) treatments to lift dormancy and control seeds (T0) and measure germination and seedling growth parameters, all in a randomised Fisher experimental set-up. In order to control soil moisture, watering was carried out morning and evening, except in the event of rain. The seedlings were placed under a shade canopy to reduce their exposure to light. The results showed that seeds subjected to treatments T3 (soaking seeds in 33% dilute sulphuric acid for 96 h followed by rinsing with well water), T4 (soaking seeds in well water for 96 h) and T5 (manual scarification of seeds using a lime) had a better germination rate (83.33%, 80% and 66.66% respectively). The shortest germination times were recorded in T5 (5 days), T2 (heating at 45°C for 10 min followed by soaking the seeds in well water for 24 h) and T3 (8 days) and T1 (soaking the seeds in well water for 24 h followed by heating at 45°C for 10 min) with 11 days. T1 and T4 gave the shortest germination times (4 and 6 days respectively). In addition, the height growth of seedlings from T2, T3 and T4 was better. However, serious regressions were observed in the diameter growth of seedlings from T2 and T3 after 21 days. Treatments T3 and T4 are the most appropriate for growing *Irvingia gabonensis*. The results of this research could be applied to the conservation of *Irvingia gabonensis* by facilitating its integration into local cropping systems, thereby improving agricultural practices.

Keywords: Utility species; anthropogenic activity; rarefaction; domestication; natural reserve.

1. INTRODUCTION

Tropical forests, due to their biological richness, play a vital role in human life. Indeed, for many rural communities, the forest represents not only an economic, but also a social and cultural importance (Joiris 1998). Thus, many species of forest ecosystems are used in different areas of life. Unfortunately, these natural ecosystems, habitats of these plants, at current scenario particularly threatened by the rise of human activities. As anthropogenic pressures on these ecosystems are increasing, the availability of forest species remains dependent on resource exploitation techniques and the conditions of the natural environment (N'Da et al. 2008 and Dadjo 2011).

This problem of rapid disappearance of natural plant resources due to human activities is a worrying subject, as highlighted by various studies (Rice& Greenberg 2000, Oszwald 2000, Traore et al. 2011, Dimobe et al. 2012, Adingra et al. 2014]

In Côte d'Ivoire, human pressure is resulting in an alarming reduction in forest areas, mainly due to the excessive collection of useful plants (Kouamé 1998).

In this Ivorian context, the Taï National Park (PNT), rich in biodiversity, is facing increasing threats due to human activity, particularly the excessive collection of non-timber forest products (NTFPs) by local populations (Soro et al. 2021). The over-exploitation of species such as *Ricinodendron heudelotii, Beilschmiedia mannii* and *Irvingia gabonensis,* essential for food and the local economy, has led to their scarcity in the peripheral areas of the park. This situation is now pushing populations to turn to the park for the collection of products from these species, thus disrupting the biodiversity of the park. Despite several studies on the valorization and conservation of plant species essential for rural populations (Schreckenberg et al.2006, Kouamé et al. 2008, N'Driet al. 2012 and Ouattara2016) few efforts have been made in the country for the domestication of these species, a crucial step for their valorization (Djaha & Gnahoua 2014).

The objective of this study is to identify the best methods for germinating Irvingia gabonensis seeds in order to facilitate their integration into cultivation systems on the outskirts of the park, with a view to conserving the species and promoting nutritional security.

2. MATERIALS AND METHODS

2.1 Plant Materials

The fruit of *Irvingia gabonensis* is a smooth drupe resembling a small mango, greenish becoming yellow when ripe (Bourobou& Arbonnier 2008) (Fig. 1). It contains a very fibrous yellow juicy pulp adhering to the stone. The latter is hard, subspherical, slightly flattened, 3 to 5 cm long. It contains a white, fleshy,

mucilaginous, discoid and very flattened almond. It is this edible almond that is sought after by local populations for food and marketing.

2.2 Choice of Study Site

The tests took place at the monitoring station of the ADK/V6 sector of the Taï National Park (PNT), in the Nawa region, more precisely in the Buyo sub-prefecture (Fig. 2). This monitoring station, bordered by the V villages, offered the best conditions for carrying out the germination and vegetative multiplication tests. Indeed, the monitoring station of this sector is located almost inside the Park. This geographical location offers the experimental site favorable edaphic and climatic conditions. Also, this sector benefited in 2014 from a plant production project (Project financed by GIZ and piloted by OIPR) of three species including Irvingia gabonensis. This made it easy to access plant material of different ages.

Fig. 1. Fruits of *Irvingia gabonensis*

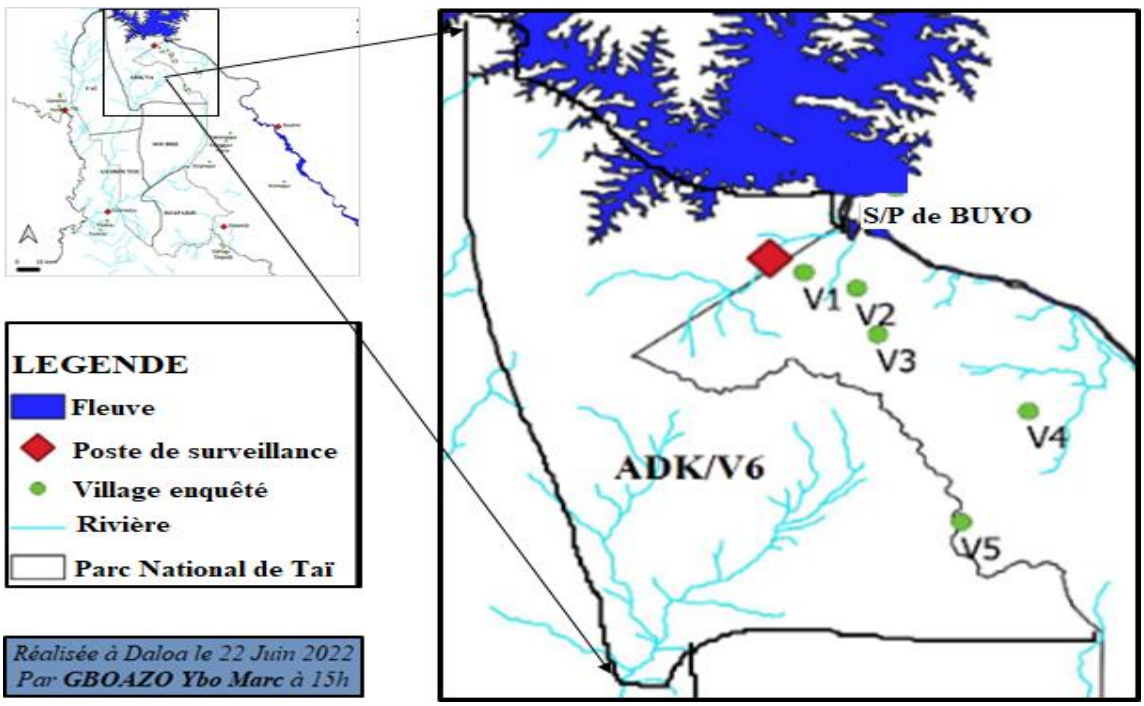

Fig. *2*. Location map of the study area

2.3 Data Collection

2.3.1 Installation of the shade

A 96 m² (8 mx 12 m) shade house was made with local materials (palm stalks, wooden poles, Chinese bamboo and raffia) and covered with palm leaves to reduce solar intensity on the seedlings (Fig. 3).

In addition, 500 nursery bags measuring 15 cm x 5 cm were filled with soil from the forest undergrowth and placed in the shade to receive the seeds.

2.3.2 Acquiring and sorting fruits

The fruiting and harvesting periods of *Irvingia gabonensis* were identified thanks to the work of Soro et al. (2021) coupled with information collected from agents of the Ivorian Office of Parks and Reserves (OIPR). Thus, approximately 800 fresh fruits were collected in the Park, at the foot of the mother trees (seed trees) in December 2022. In order to keep only good quality fruits (well formed), careful sorting was carried out (Fig. 4).

Fig. 3. Shade for germination tests

Fig. 4. Sorting *Irvingia gabonensis* fruits

2.3.3 Seed extraction and pre-treatment

The fruits have been stripped of their fleshy mesocarp covering the hard endocarp or stone containing the seed. It is this stone which represents the seed of the species (Fig. 5).

With reference to the work of Afizou et al. (2020) five pre-treatments (T1, T2, T3, T4, T5) and a control (T0) were adopted, at a rate of 60 seeds per treatment.

Treatment T0 consisted of untreated seeds, serving as a control. Treatment T1 involved immersing the seeds in well water for 24 hours, away from sunlight, before heating them in the laboratory in an autoclave at 45°C for 10 minutes. In treatment T2, the seeds were first heated, then soaked as in treatment T1. The pre-treated seeds were then placed in a moist clod of soil and transported to the experimental site. Treatment T3 involved immersing the seeds in sulphuric acid (H_2SO_4) diluted to 33% for 96 hours (4 days), followed by rinsing with well water. Treatment T4 consisted of soaking the seeds in well water for 96 hours. Finally, treatment T5 involved manually scarifying the seeds with a new lime, sanding off the part close to the embryo A(domou et al. 2007).

2.3.4 Experimental device

In order to standardize the level of the tests, an experimental design in completely randomized blocks with three repetitions per treatment was adopted (Fig. 6), in accordance with Gnamkoulamba et al. 2018 and Diouf et al. 2019. Indeed, this design makes it possible to reduce the effect of variations between blocks and to better evaluate the effect of treatments. Thus, three blocks were set up under the shade to accommodate the seeds (Fig. 8). Each block was subdivided into six sub-blocks (from T0 to T5) of 20 sachets each, for a total of 360 sachets.

2.3.5 Sowing and measuring seedling germination and growth

One seed was sown per sachet followed by systematic watering of the entire device, once a day (in the morning) until germination. When the leaves appeared, watering was done twice a day (in the morning and in the evening) except on rainy days to ensure good growth of the seedlings.

In this work, a seed was considered to have germinated when its seedling appeared above the surface of the substrate contained in the sachet (Afizou et al. 2020). The germination parameters measured were: time to first germination, time to last germination and number of seeds germinated.

Growth parameters measured on seedlings were: seedling height (from collar to terminal bud) using a graduated ruler (Fig. 8), diameter at the collar using a caliper (Fig. 9). Data collection took place weekly for four weeks.

Fig. 5. Seeds of *Irvingia gabonensis* tripped of the fleshy part

Fig. 6. View of the experimental device under the shade

Fig. 7. Measuring the hauthor of a seedling of *Irvingia gabonensis* using a graduated ruler

Fig. 8. Measuring the Diameter at the collar of an *Irvingia gabonensis* seedling using a vernier caliper

2.4 Data Analysis

Excel spreadsheet was used for data entry. R software version 4.3.2 was used to perform statistical analyses, including analysis of variance (ANOVA) to compare treatments.

2.4.1 Calculating the germination rate

For each treatment, the germination rate (Tg) was determined according to the following formula proposed by Heller et al. (1990)

$$Tg = \frac{n_i}{N} \times 100 \text{ with}$$

•ni: number of seeds germinated on date i
•N: total number of seeds sown.

2.4.2 Seed latency time evaluation

Lag time (LT) is the number of days between sowing and first germination Heller et al. (1990). It was determined using the formula below:

$$T_L = [d_1 - d_0] \text{ with}$$
•d0: sowing date
•d1: date of first germination

2.4.3 Evaluation of the extent of germination

The germination extent (Eg) is the time between the first and last germination (Afizou et al. 2020). It was determined using the following formula:

$$Eg = t_1 - t_0 \text{ with}$$

•t0: number of days between sowing and first germination
•t1: number of days between sowing and last germination

3. RESULTS

3.1 Effect of Treatments on Germination

Treatments applied to the kernels significantly influenced seed germinationof Irvingia gabonensis (p=0.000013) (Table 1). Indeed, the Newman-Keuls test indicates that the treatments T3, T4 and T5 induced the best germination rates, Approximately 83.33%; 80% and 66.66% according to standard deviation. On the other hand, the lowest germination rates were observed in the control T0 (26.66%) and in the pretreatments T1 (38.33%) and T2 (21.66%).

Table 1. Effect of treatments on germination ratefrom *Irvingia gabonensis* based on standard deviation

Treatment	Germination rate (%)
T0 (witness)	26.66 ± 7.63a
T1 (H2O soaking + heating)	38.33 ± 7.63a
T2 (heating + H2O soaking)	21, 66 ± 7.63a
T3 (H2SO4 soaking)	83.33 ± 16.07b
T4 (H2O soak)	80 ± 10.40b
T5 (scarification)	66.66 ± 7.63b
F	21,6139
p	0.000013

Table 2. Effects of treatments on latency and extent of germination based on standard deviation

Treatment	Latency time (days)	Extent of germination (days)
T0 (witness)	32 ± 4.04c	8 ± 1.15ad
T1 (H2O soaking + heating)	11 ± 2.00b	4 ± 1.00c
T2 (heating + H2O soaking)	8 ± 1.15ab	11 ± 1.15ab
T3 (H2SO4 soaking)	8 ± 1.00ab	12 ± 2.51b
T4 (H2O soak)	37 ± 2.51d	6 ± 0.57cd
T5 (scarification)	5 ± 0.57a	11 ± 2.08ab
F	114,1295	13,3864
p	0.000000	0.000147

Table 3. Effects of treatments onthe diameter and height of the seedlings from *Irvingia gabonensis* based on standard deviation

Treatment	Average height (cm)	Average diameter (mm)
T0 (witness)	15 ± 2.85a	6.07 ± 1.30c
T1 (H2O soaking + heating)	16.33 ± 1.25a	5.66 ± 0.90bc
T2 (heating + H2O soaking)	16.50 ± 2.65a	5 ± 0.81abc
T3 (H2SO4 soaking)	16.53 ± 2.70a	4.13 ± 0.35ab
T4 (H2O soak)	15.50 ± 1.01a	3.40 ± 0.20a
T5 (scarification)	14 ± 0.62a	5.20 ± 0.62abc
F	0.7253	6,4051
p	0.617486	0.004037

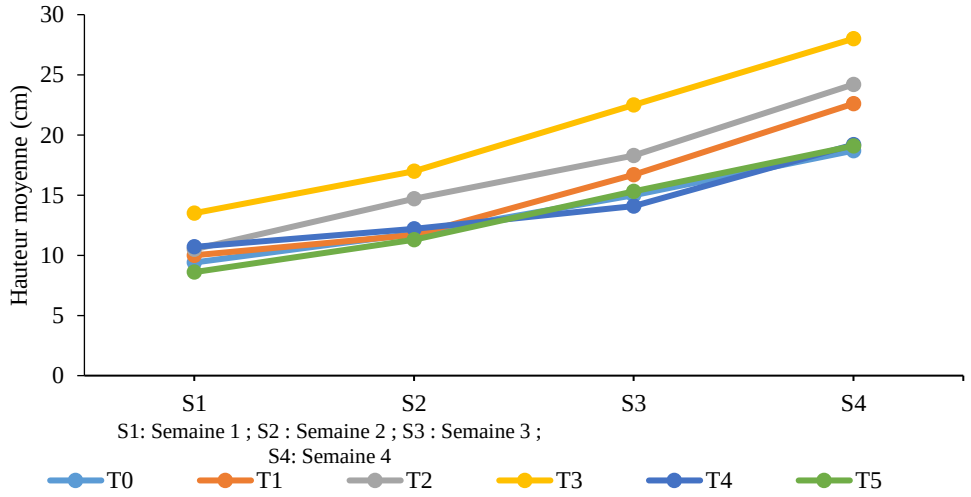

Fig. 9. Height growth dynamics of seedlings of *Irvingia gabonensis* over four weeks

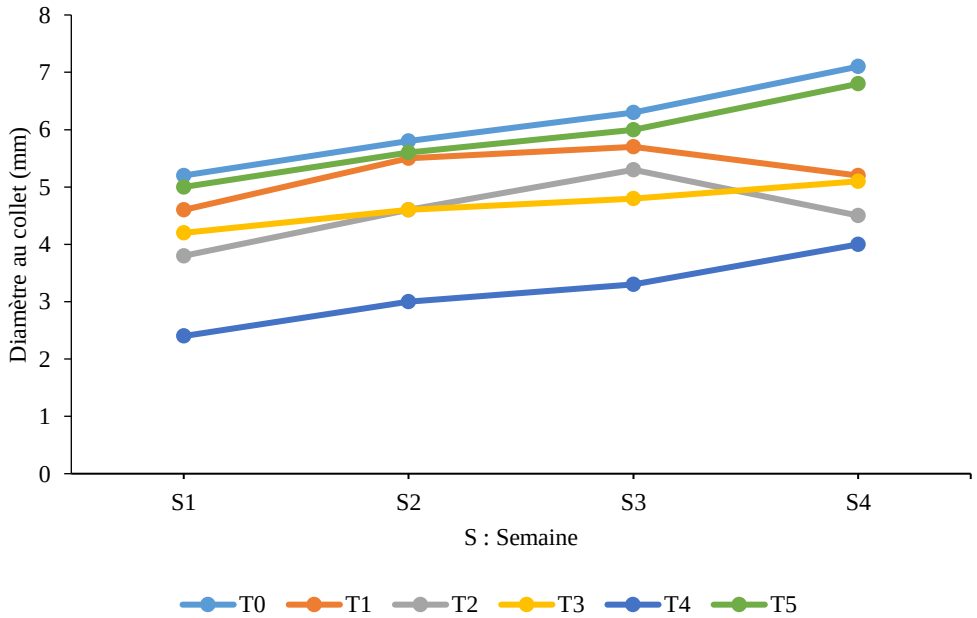

Fig. 10. Diameter growth dynamics of seedlings of *Irvingia gabonensis over four weeks*

3.2 Effect of Treatments on Germination Kinetics

The applied pretreatments significantly influenced the latency time and extent of germination. Indeed, the Treatments T2, T3 and T5 made it possible to shorten the germination time (Table 2), whilecontrol seeds (T0) and those from treatment T4 took much longer to germinate (respectively 32 and 37 days after sowing). However, the shortest germination times were observed in treatments T0, T1 and T4.

3.3 Effect of Treatments on Seedling Growth

Treatments applied to Irvingia gabonensis cores had no significant effect on seedling height (p=0.617486) aged 28 days (Table 3). However, the growth dynamics during the first four weeks favors better height growth of seedlings from treatments T1, T2 and T3 compared to the others (Fig. 9). On the other hand, the diameter was significantly influenced by the treatments (p =0.004037) (Table 3). Thus, the best diametric performances were observed with treatments T4 and T5 (Fig. 10). This influence was also marked by a regression of the diameter after 21 days in seedlings from T1 and T2.

4. DISCUSSION

The results obtained provide a positive overview of the effectiveness of the pre-treatments applied to *Irvingia gabonensis* seeds. Seeds soaked in sulphuric acid for 96 h followed by rinsing with water (T3), those soaked in well water for 96 h (T4) and those scarified (T5) gave the best germination results, with over 65% of seeds germinating. The performance observed with scarified seeds is corroborated by the work of Afizou et al. (2020). who showed that seed scarification was a better germination method for hard-shelled seeds. Soaking the seeds in water and sulphuric acid would have been sufficient to stimulate germination. In fact, the hard shell of the seeds would have been softened by the long soaking through the activation of enzymes, which increased their permeability, essential for water absorption. Sulphuric acid, with its corrosive power, would have led to a degradation of the hull, thus facilitating germination. This observation is supported by the work of Bakar et al. (2019), who point out that chemical pre-treatments can improve germination by altering the structure of the shell, thus allowing better penetration of water and nutrients. The control seeds (T0), those soaked in water for 24 h followed by heating (T1) and those heated then soaked in water for 24 h (T2) did not germinate sufficiently (less than 40% germination rate). The germination rate of the control seeds was close to those obtained by Kouamé et al. (2016), Soro et al. (2021) and Koné (2022) with untreated seeds of the same species, i.e. less than 50%. These results could be explained by the variability of the agro-ecological experimental conditions. In addition, the control seeds (T0) and those from T4 required a long germination time (36 and 39 days respectively), confirming that untreated *Irvingia gabonensis* seeds germinate with difficulty under natural conditions. In contrast, the shortest germination time (5 days) for scarified seeds (T5) corroborates that of Kouamé et al. (2016), who also reported a short germination time for stripped seeds (9 days) in contrast to unstripped seeds (around 1 month). In addition, seeds from a treatment that took longer to germinate tended to have a shorter germination time, while those that germinated more quickly tended to have a more extensive germination over time. This relationship is illustrated by treatments T0, T1 and T4 whose seeds, having taken longer to germinate, had a shorter germination time, while treatments T2 and T3 showed the opposite effect. These observations, also described by Koné (2022) could be explained by the influence of internal or external factors on both germination time and duration. Internally, seed viability, water content and nutrient content are key determinants of the ability of seeds to germinate. Recent studies have shown that moisture and nutrient management are essential for maximising seed germination potential, particularly for tropical species such as *Irvingia gabonensis* Bhandari et al. (2022). Externally, environmental conditions play a major role. Temperature, humidity, light and the availability of nutrients in the soil must be conducive to germination. Inhibitors present in the environment or interactions with soil micro-organisms can also delay or promote germination.

Seedlings from treatments T1, T2 and T3 showed greater growth in height than those from the other treatments, although this difference was not statistically significant. However, diameter growth was better in seedlings subjected to treatments T4 and T5. These results indicate that the different treatments have varying effects on seedling growth, depending on the parameter studied. Height growth seems to

be stimulated more by treatments T1, T2 and T3, while diameter growth is optimised by the treatments.

A crucial point to note is the observation of a regression in diameter growth for seedlings in treatments T1 and T2 after 21 days, despite faster initial height growth. This trend could be the result of limiting factors affecting the overall development of seedlings in these treatments. Constraints such as soil compaction would have led to root expansion, thus limiting the uptake of essential nutrients (Adegbite et al. 2012). In addition, an imbalance in nutrients such as nitrogen, phosphorus or potassium could have contributed to this reduction in diameter growth, a phenomenon observed when seedlings are subjected to sub-optimal conditions in terms of soil fertility (Lacerda & Silva 2023). Recent research has concluded that root system development is directly influenced by soil quality and nutrient availability. Poor soil structure or inadequate pH could inhibit root growth, thus affecting the absorption of water and nutrients required for balanced growth (Monténégro et al. 2022 and Guerrero& Delgado 2022).

5. CONCLUSION

The present study identified the best methods for germinating *Irvingia gabonensis* seeds in order to facilitate their integration into cropping systems on the periphery of the park. The results indicate that *Irvingia gabonensis* seeds need to be pre-treated to improve seed germination and growth. Soaking the seeds in sulphuric acid diluted to 33% for 96 h followed by rinsing with well water (T3) and soaking the seeds in well water for 96 h (T4) are the best techniques applicable to *Irvingia gabonensis* seeds . These practices accelerated seed germination while limiting losses in terms of germination rate. These results offer promising prospects for improving propagation practices for this valuable species. The practices observed are in line with agro-ecology, favouring the sustainability and good production of the species. However, a better understanding of the mechanisms underlying the performance of these techniques and an assessment of the long-term impact of the various pre-treatments on the health of the seedlings could have both ecological and socio-economic benefits.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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