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Effect of Treatments on Seed Germination and Seedling Growth of *Irvingia gabonensis* in a Nursery in Soubre (South-West Côte d'Ivoire)

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Abstract

Anthropogenic pressures, climate change, and certain factors, including seed coat dormancy, hinder the natural regeneration of some tree species such as *Irvingia gabonensis*. This study, conducted in the city of Soubre, aimed to evaluate the germination potential of *Irvingia gabonensis* seeds and investigate the growth of seedlings from various treatments within an agroforestry perspective. The methodology involved subjecting seeds to fourteen different pre-treatments. These included seeds 1) treated with water at 100˚C; 2) soaked in tap water for varying periods (days); 3) treated with concentrated sulphuric acid at 96%; 4) soaked directly in GA3 at different concentrations; 5) scarified and soaked in GA3; and 6) untreated seeds, which served as controls. For the growth tests, the growth of seedlings from seeds treated with GA3 was compared with seedlings from control and scarified seed lots. The most satisfactory results were observed with scarified seeds soaked in gibberellin solution at 0.5 and 2 mg/L, yielding 46.66% and 56.66% germination, respectively. However, the best result was obtained with seeds soaked in GA3 at a concentration of 2 mg/L (50%). The findings showed that control seedlings exhibited similar growth to those derived from GA3-treated seeds.

Keywords

Treatments, Germination, Seeds, *Irvingia gabonensis*, Growth

1. Introduction

Forests represent a considerable reservoir of biological resources and play an essential role in meeting certain human needs. However, this reservoir is exposed to various natural and human phenomena. In tropical countries, particularly Côte d'Ivoire, the economy is closely linked to increasingly intensive forestry activities [1]. This situation presents several risks for the country. Initially, through its economic policy mainly based on agriculture, Côte d'Ivoire became the world's largest producer of cocoa. In fact, this industry propelled Côte d'Ivoire to become the leader in cocoa bean exports since 1977, representing 15% of the Gross Domestic Product (GDP) and over 40% of global production [2]. However, the economic success of Côte d'Ivoire, particularly in cocoa farming, masks a significant degradation of its forest area. According to [3] and [4], Côte d'Ivoire's humid forest area began to disappear in the 1960s due to cocoa cultivation. [5] and [4] estimate that about 80% of the Ivorian forest area in the South-West is now occupied by cocoa plantations. In addition, high climate variability, combined with human actions, has negatively impacted ecological balances by causing the deterioration of natural resources, soils, and agricultural ecosystems. Agricultural production systems have become vulnerable due to this situation. In response, development initiatives have been implemented, focusing on fast-growing plant species with socio-economic benefits, which are well-adapted to local climates and offer numerous advantages for rural development [6]. Agroforestry has also been identified as a sustainable solution to deforestation and shifting cultivation, aiming to mitigate the ecological and carbon impact of agriculture. According to [7] and [8], integrating trees into agricultural systems helps preserve and diversify agricultural production, promoting the fundamental principles of sustainable development. Furthermore, international research organizations, such as the International Cooperation Center of Agricultural Research for Development (CIRAD) and the International Center for Research in Agroforestry (ICRAF), have developed agroforestry systems incorporating fruit trees like *Irvingia gabonensis* to boost farmers' productivity while combating climate change [9]. This species, known as the "wild mango", is considered a forest species of high commercial value. Despite its socio-economic potential, little scientific information is available on its biology, phenology, reproduction, and germination potential. Additionally, *Irvingia gabonensis* is a forest species whose seeds exhibit extreme dormancy, which likely hampers its germination, hence the need for various treatments to promote and sustain its germination. This issue has prompted interest in this study, which aims to preserve the species *Irvingia gabonensis* in an agroforestry context. Thus, the general objective of this study is to assess the germination potential of *Irvingia gabonensis* seeds and the growth of its seedlings to produce vigorous plants for integration into agroforestry practices for sustainable conservation. More specifically, the study aims to 1) determine the effect of treatments on the germination of *Irvingia gabonensis* seeds and 2) evaluate the effect of treatments on the growth parameters of *Irvingia gabonensis*.

2. Materials and Methods

2.1. Study Area

Soubré, established on May 21, 1979, and inaugurated on October 6, 1982, is the largest city in the Nawa region of Côte d'Ivoire, covering an area of 4779 km^2. It is located between longitudes 6˚19' and 6˚57' West and latitudes 5˚26' and 6˚13' North, bordered by Issia to the north, Méagui and Sassandra to the south, Gagnoa and Gueyo to the east, and Buyo to the west (Figure 1). The climate is sub-equatorial,

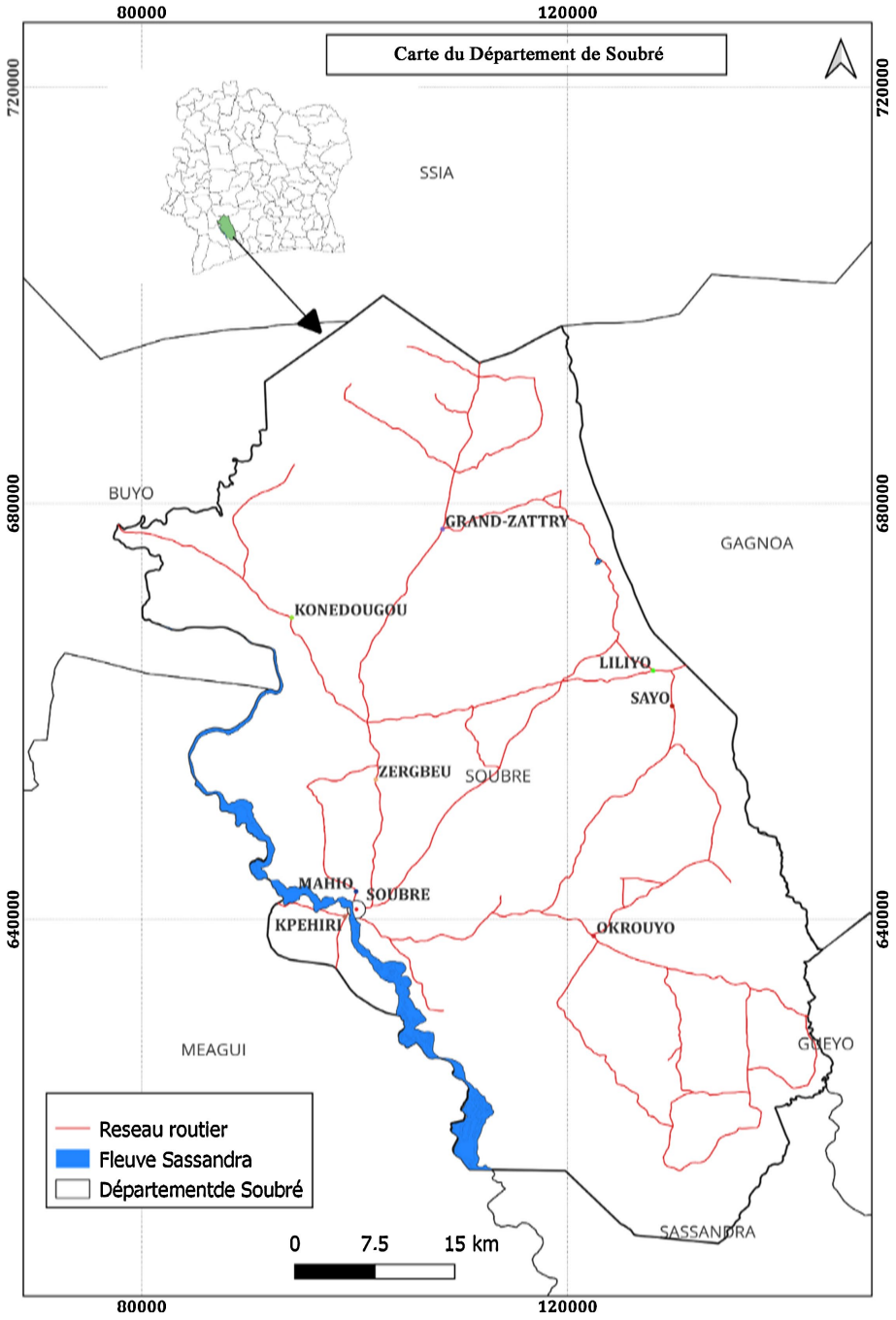

Figure 1. Soubré study area.

characterized by two rainy seasons and two dry seasons, with annual rainfall ranging from 1600 mm to 1800 mm and temperatures between 26˚C and 32˚C. The vegetation consists of secondary forests, gallery forests, swampy formations, fallow lands, and previously cultivated areas, hosting specific species such as *Uapaca heudelotii* and *Ceiba pentandra*. Soubré has an estimated population of around 587,441 inhabitants, including indigenous groups (Beté, Bakwé), non-native populations (Baoulé, Malinké, Wê), and foreign residents, primarily from Burkina Faso. Socio-economic activities are diverse, ranging from agriculture to non-agricultural sectors, with uneven distribution based on the origin of the inhabitants.

2.2. Seed Collection and Treatment

Sampling was carried out in Adzopé, in the Mé region. It involved the collection of 600 fresh fruits from different mature *Irvingia gabonensis* trees. The fruits (**Figure 2**) were stored in bags and transported by car the following day to the Regional Directorate of Water and Forests in Soubré. Once collected, the fruits were placed in a shaded, humid area, and if necessary, they were watered regularly for two weeks to promote pulp decomposition and facilitate depulping. After the fruits were depulped, the seeds were dried for three days. They were then soaked in water in a basin to conduct a purity test, which allowed for distinguishing between healthy, viable seeds and those with rotted albumen. These seeds subsequently underwent various treatments before sowing. The purpose of these treatments was to determine the most effective method for promoting successful germination of *Irvingia gabonensis* seeds.

Figure 2. Overview of *Irvingia gabonensis* seeds pulped and soaked in GA3.

2.3. Experimental Design and Seed Treatments

The experiment was set up using a complete randomized block design. Fourteen treatments were applied to the seeds, which were divided into 14 groups of 10 seeds each, with three replications per treatment. A total of 420 seeds (14 × 3 × 10) were used in the trial. Seeded bags were arranged by treatment and uniformly watered twice daily: once between 6:00 and 8:00 am, and again between 4:00 and

6:00 pm. Observations were made on the seeds over a 45-day period, during which the following data were collected: the start and end dates of germination for each group and test, and the number of germinated seedlings per group and per test recorded daily until germination was complete. At the end of the study, germination rates for each test were calculated. After 45 days of the experiment, 30 seedlings of the same age were selected from the control, scarified, gibberellin treatments (0.5 mg/L and 2 mg/L), and the scarification + gibberellin treatments (0.5 mg/L and 2 mg/L) for the growth test. This selection aimed to evaluate the appropriate gibberellin concentration (minimum 0.5 mg/L and maximum 2 mg/L) for optimal growth and effective regulation of *Irvingia gabonensis* juveniles. Gibberellin is a powerful hormone involved in plant growth, and its effect was compared to the normal growth of the control plants. To assess this, measurements were taken every two days over a 30-day period. The experiment was set up in a single block shaded by trees, with regular watering each morning and evening, except on rainy days.

2.4. Statistical Analysis

Data collection in this study was based on germination delay, duration, and rate. Growth measurements were taken every two days using a graduated ruler, and the number of leaves per seedling for each treatment was counted over a 30-day period. Analysis of variance (ANOVA) was used to compare the means of germination parameters (rate, delay, and duration) and simultaneously assess the growth data across different treatments. ANOVA helps compare intra- and intergroup variances, which is a parametric analysis that assumes the measured variable follows a normal distribution. The aim of this analysis was to determine if the mean values across the different germination tests were significantly different. Before performing ANOVA, three preliminary tests were conducted to verify data normality, distribution, and homogeneity of variances. First, the Shapiro-Wilk normality test was performed to check whether the data followed a normal distribution. If the data were normally distributed, the Levene's test was used to assess the homogeneity of variances. Lastly, Tukey's test was applied to compare means pairwise and identify significant differences between them whenever the calculated probability was significant. According to Martin (2008), biological data often do not follow a normal distribution. For the collected data, ANOVA tests enabled the comparison of the mean germination and growth parameters. Additionally, the Kruskal-Wallis test was used to rank the means at a significance level of 5%. All statistical analyses were performed using RStudio software.

3. Results

3.1. Germination Delay Based on Treatments

The Kruskall Wallis test showed a highly significant difference between the germination delays of the different treatments ($P < 0.05$) (Table 1).

Table 1. Comparison of average germination delays for *Irvingia gabonensis* seeds.

Treatments	Germination Delay (Days)
Control	8.66 ± 4.33[a]
Scarified	11.66 ± 0.33[a]
GA3 0.5 mg/L	16.66 ± 0.66[b]
GA3 1 mg/L	19.66 ± 3.17[b]
GA3 2 mg/L	17 ± 2.88[b]
Scarified + GA3 0.5 mg/L	11 ± 1[a]
Scarified + GA3 1 mg/L	12 ± 1.15[a]
Scarified + GA3 2 mg/L	10.66 ± 0.66[a]
H_2SO_4 3 min	--
H_2SO_4 6 min	--
H_2SO_4 12 min	--
Water 3 days	16.33 ± 8.56[b]
Water 7 days	7.33 ± 7.33[a]
Boiling Water 100˚C	18.66 ± 9.40[b]
Mean	**10.95**
P-Value	**0.04**

3.1.1. Germination Delay of Seeds Soaked in Sulfuric Acid (H_2SO_4)

At the end of the 45-day experiment, it is important to note that the seeds soaked in 96% concentrated sulfuric acid did not germinate at all.

3.1.2. Germination Delay of Seeds Soaked in Water

Soaking *Irvingia gabonensis* seeds in water for 3 days and in boiling water extended the germination delay, with average germination times of 16.33 and 18.66 days, respectively. However, shorter germination delays were observed in the control seeds and seeds soaked in water for 7 days, with average delays of 8.66 and 7.33 days, respectively (**Figure 3**).

3.1.3. Germination Delay of Seeds Soaked in Gibberellin

GA3 does not shorten the germination delay. Compared to the control group, which has a germination delay of 8 days, the seeds soaked in GA3 have a delay that varies between 16 and 19 days (**Figure 4**).

3.1.4. Germination Delay of Seeds under the Combined Effect of Scarification and Gibberellin

The analysis showed that the combined effect of scarification shortened the germination delay. However, GA_3 at 0.5 mg/L reduced the germination delay to a level very close to the control group (11 days). A slightly longer delay compared to scarification alone was observed at 1 mg/L of GA_3, with a delay of 12 days. At a

concentration of 2 mg/L, the germination delay was very close to both the control and the scarification-only treatment, with delays of 10.66 days and 11 days, respectively (Figure 5).

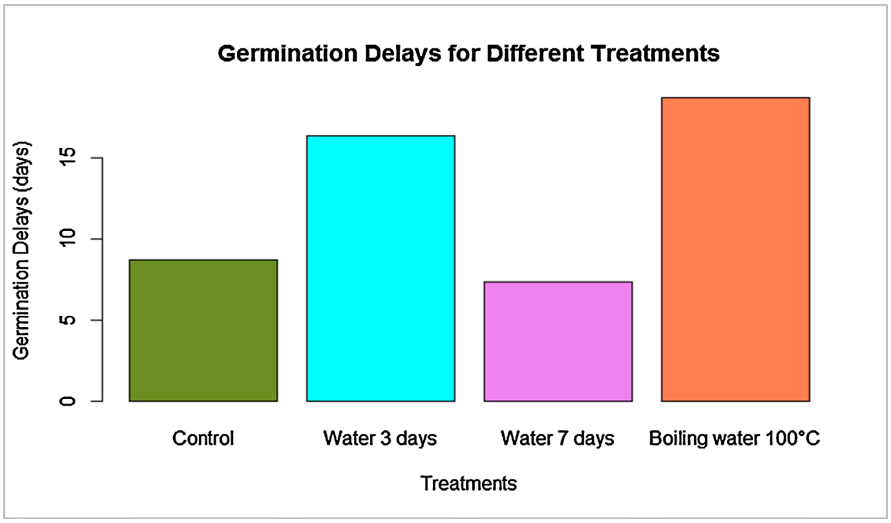

Figure 3. Germination delays of *Irvingia gabonensis* seeds with different water treatments

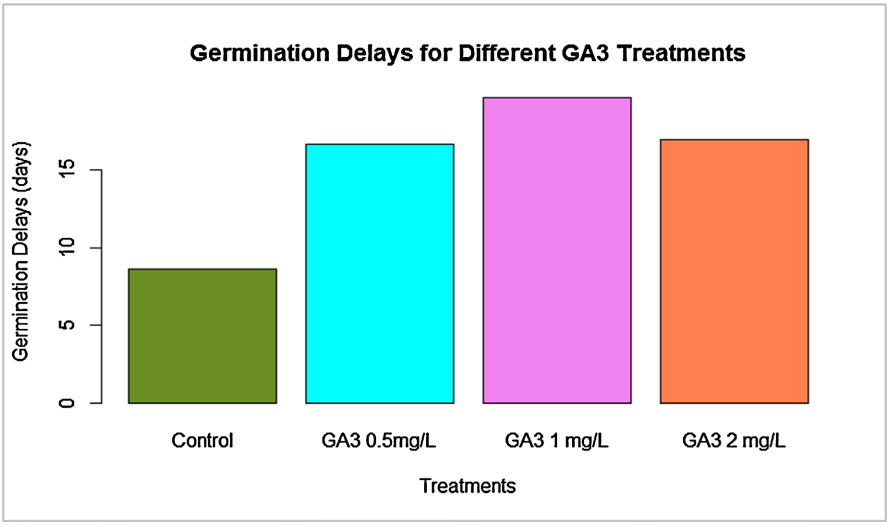

Figure 4. Germination delays of *Irvingia gabonensis* seeds soaked in Gibberellin

3.2. Germination Duration Based on Treatments

The Kruskall-Wallis test reveals that there is a significant difference between the different germination duration ($P < 0.05$) (Table 2).

3.2.1. Germination Duration of Seeds Soaked in Water

Water treatments, particularly prolonged soaking or soaking at high temperatures, demonstrated a strong ability to reduce the duration of germination. However, soaking in water for 3 days resulted in a slightly shorter germination duration, similar to the control group (4.33 and 5.66 days, respectively). Prolonged soaking in water for 7 days significantly reduced the duration of germination,

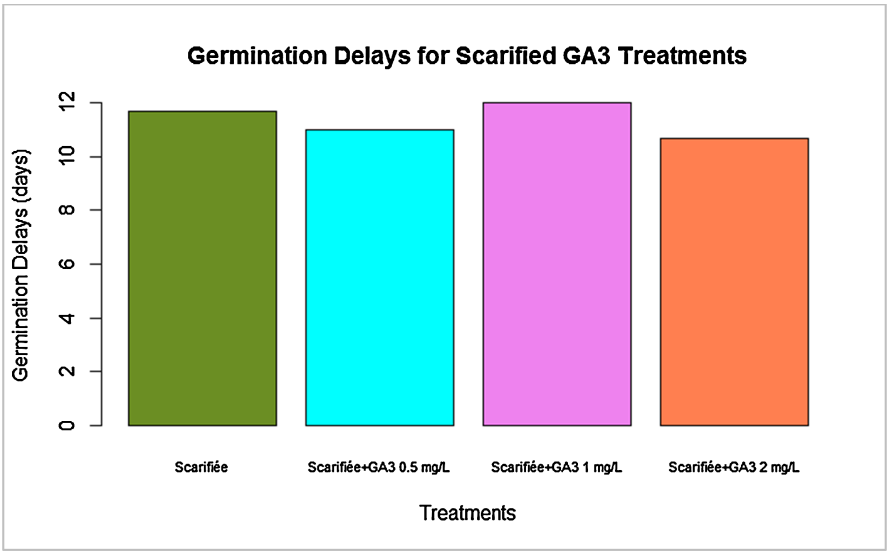

Figure 5. Germination delays of *Irvingia gabonensis* seeds with different scarified Gibberellin treatments.

Table 2. Comparison of average germination delays for *Irvingia gabonensis* seeds.

Treatments	Germination Duration (Days)
Control	5.66 ± 3.48^{ab}
Scarified	5 ± 4^{ab}
GA3 0.5 mg/L	9.66 ± 2.90^{b}
GA3 1 mg/L	1 ± 0^{a}
GA3 2 mg/L	9 ± 2.08^{b}
Scarified + GA3 0.5 mg/L	10.33 ± 1.45^{c}
Scarified + GA3 1 mg/L	9.33 ± 2.84^{b}
Scarified + GA3 2 mg/L	10 ± 5.03c
H_2SO_4 3 min	--
H_2SO_4 6 min	--
H_2SO_4 12 min	--
Water 3 days	4.33 ± 3.84^{ab}
Water 7 days	0.33 ± 0.33^{a}
Boiling Water 100˚C	0.66 ± 0.33^{a}
Mean	**4.78**
P-Value	**0.006**

leading to nearly immediate germination with low variability, indicating a very strong treatment effect (0.33 days). The treatment with boiling water also exhibited a very short duration of germination, similar to that of the 7-day soaking treatment (0.66 days) (Figure 6).

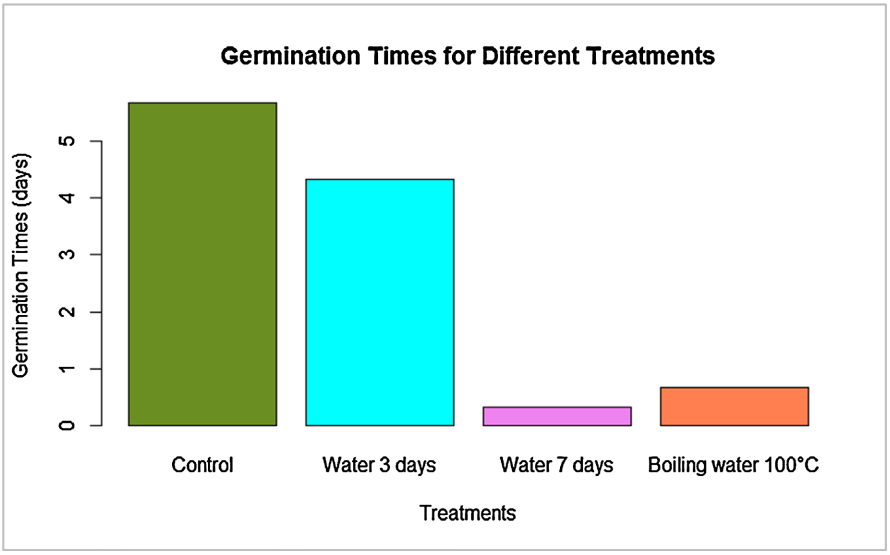

Figure 6. Germination duration of *Irvingia gabonensis* seeds with different water treatments.

3.2.2. Germination Duration of Seeds Soaked in Gibberellin (GA3)

The treatments with gibberellin (GA3), especially at lower concentrations, tended to extend the germination duration, with the exception of GA3 at 1 mg/L, which dramatically accelerated it. Specifically, treatment with GA3 at 0.5 mg/L increased the germination duration compared to the control group (5.66 days) and scarified seeds (5 days), showing moderate variability. In contrast, GA3 at 1 mg/L significantly reduced the germination duration to 1 day, with no variability, indicating a very strong effect of this concentration in accelerating germination. Finally, a higher concentration of GA3 (2 mg/L) also extended the germination duration, similar to the 0.5 mg/L treatment, but with lower variability (9 days) **Figure 7**.

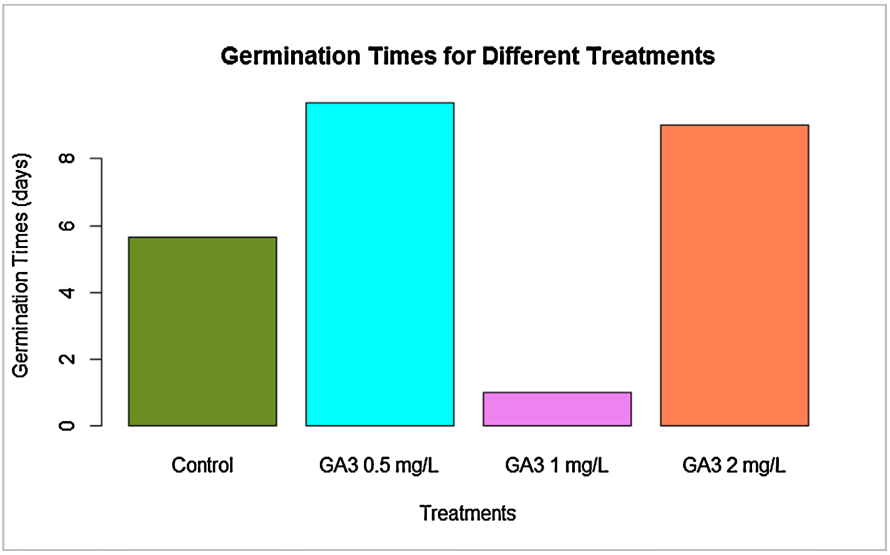

Figure 7. Germination duration of *Irvingia gabonensis* seeds with different Gibberellin treatments.

3.2.3. Germination Duration of Seeds under the Combined Effect of Scarification + Gibberellin (GA3)

Scarification alone did not significantly impact the germination duration. However, when combined with GA3, it tended to extend the germination duration. The combination of scarification and GA3 at concentrations of 0.5 mg/L, 1 mg/L, and 2 mg/L increased the germination duration, reaching approximately 10.33 days, 9.33 days, and 10 days, respectively. This suggests that the combined treatment delayed germination compared to scarification alone (Figure 8).

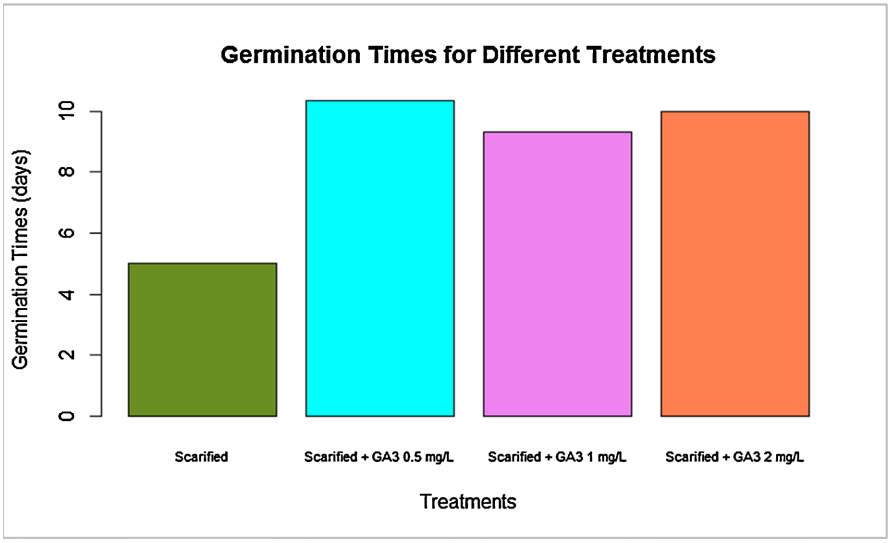

Figure 8. Germination duration of *Irvingia gabonensis* seeds with different Scarified + Gibberellin treatments

3.3. Variation of Germination Rates According to Treatments.

The Kruskall-Wallis test revealed a highly significant difference in germination rates $P < 0.05$ (Table 3).

Table 3. Comparison of average germination rates of *Irvingia gabonensis* seeds.

Treatments	Germination Rate (%)
Control	20 ± 11.54^{b}
Scarified	16.66 ± 3.33^{b}
GA3 0.5 mg/L	26.66 ± 3.33^{b}
GA3 1 mg/L	10 ± 0^{ab}
GA3 2 mg/L	50 ± 5.77c
Scarified + GA3 0.5 mg/L	46.66 ± 3.33^{c}
Scarified + GA3 1 mg/L	33.33 ± 3.33^{bc}
Scarified + GA3 2 mg/L	56.66 ± 16.66^{c}
H_2SO_4 3 min	--
H_2SO_4 6 min	--

Continued

H_2SO_4 12 min	--
Water 3 days	13.33 ± 8.8^{ab}
Water 7 days	3.33 ± 3.33^{a}
Boiling Water 100˚C	6.66 ± 3.33^{ab}
Mean	**20.73%**
P-Value	**0.001**

3.3.1. Variation of Germination Rates of Seeds Soaked in Water

The control seeds, meaning those that underwent no treatment, showed a relatively low germination rate, around 20%. Furthermore, water treatments, particularly prolonged soaking for 3 days, 7 days, and exposure to boiling water (100˚C), generally reduced germination rates, with respective rates of 13.33%, 3.33%, and 6.66%. This suggests that these conditions may be stressful or damaging to the seeds, negatively affecting their ability to germinate (Figure 9).

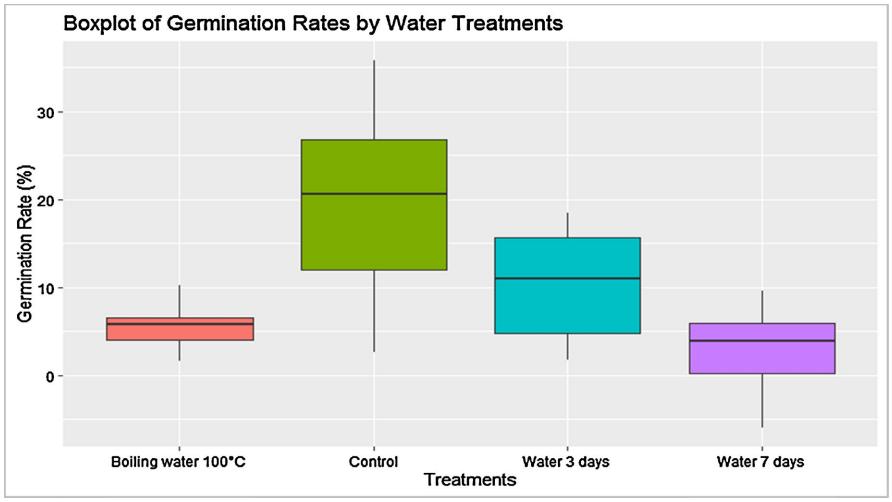

Figure 9. Germination rates of *Irvingia gabonensis* seeds with different water treatments.

3.3.2. Variation of Germination Rates of Seeds Soaked in Gibberellin

The analysis of results showed that, compared to the control group, direct soaking in gibberellin significantly increased germination rates, particularly at concentrations of 0.5 mg/l and 2 mg/l, yielding germination rates of 26.66% and 50%, respectively. However, gibberellin at 1 mg/l reduced the germination rate, with a rate of only 10% (Figure 10).

3.3.3. Variation in Seed Germination under the Combined Effect of Scarification + Gibberellin

During the experiment, the seeds were scarified in the same way. A slit is made along the line of dehiscence of the seed from the base opposite the apex to prevent damage to the embryo. Therefore, the results obtained by this method of seed treatment were not analyzed according to the location of the scar on the

seed, but rather on the effect of the opening of the shell on the germination rate and time.

The combined effect of scarification and gibberellin significantly increases the germination rate, in contrast to scarified seeds without gibberellin, which show a slightly lower germination rate than the scarified control (16%). The combination of scarification with GA3 at concentrations of 0.5 mg/L, 1 mg/L, and 2 mg/L increases the germination rate to 46.66%, 33.33%, and 56.66%, respectively. The highest germination rate was obtained with scarified seeds soaked in a 2 mg/L GA3 solution, demonstrating the effectiveness of this method compared to other treatments (Figure 11).

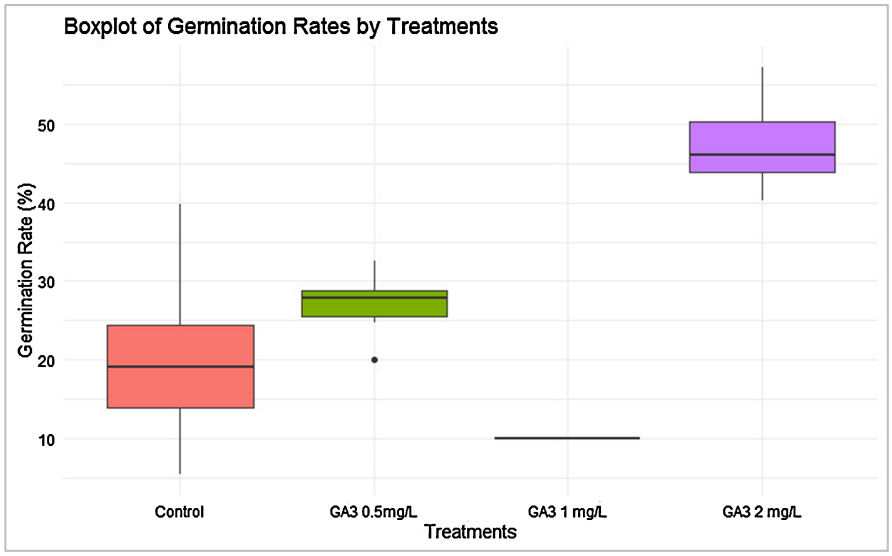

Figure 10. Germination rates of *Irvingia gabonensis* seeds with different Gibberellin treatments.

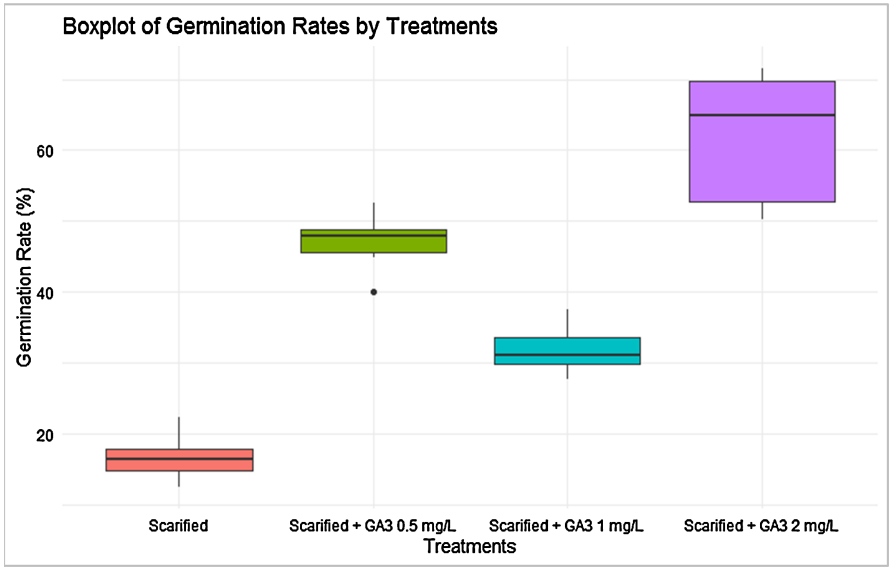

Figure 11. Germination rates of *Irvingia gabonensis* seeds with different Scarificaton + Gibberellin treatments.

3.4. Seedling Growth Based on Treatments

3.4.1. Height Growth of Seedlings by Treatment

Seedlings from seeds treated with scarification and gibberellin (T7) at 2 mg/L for 24 hours showed the fastest growth, reaching an average height of 21.95 cm after 30 days. By comparison, seedlings from untreated control seeds (T0) reached an average height of 20.83 cm. Seedlings from seeds treated with scarification and gibberellin at 0.5 mg/L (T5) reached an average height of 17.35 cm. Additionally, seedlings from seeds directly soaked in gibberellin at 0.5 mg/L (T2) and 2 mg/L (T4) reached heights of 17.93 cm and 19.53 cm, respectively. Finally, control seedlings from scarified seeds (T1) exhibited the slowest growth, with an average height of 14.60 cm (Figure 12, Figure 13).

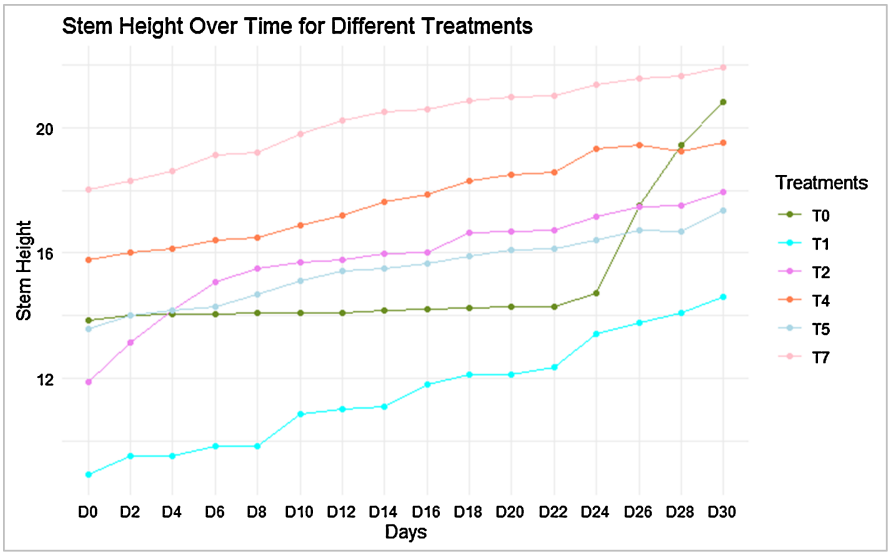

Figure 12. Evolution of *Irvingia gabonensis* stem height with different treatments for 30 days.

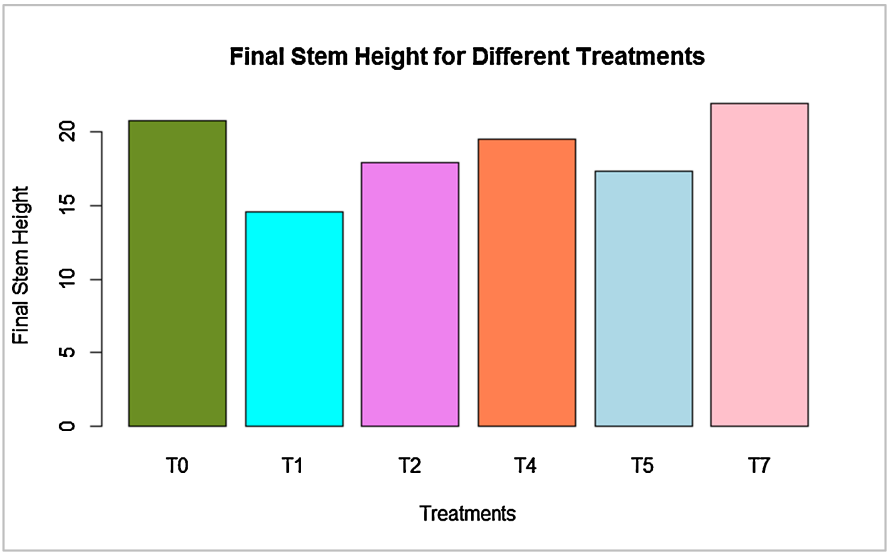

Figure 13. Final *Irvingia gabonensis* stem height with different treatments after 30 days.

3.4.2. Number of Leaves Based on Treatments

Observations on leaf development showed similar results across all treatments. Notably, seedlings from seeds treated with scarification and GA3 at 0.5 mg/L (T5) for 24 hours exhibited the fastest leaf production, with an average of 9 leaves. In comparison, seedlings from seeds treated with scarification and GA3 at 2 mg/L (T7) developed an average of 6 leaves. Additionally, seedlings from seeds directly soaked in GA3 at 0.5 mg/L (T2) and 2 mg/L (T4) produced an average of 7 leaves. Lastly, seedlings from control seeds, whether scarified (T1) or untreated (T0), each developed around 6 leaves (**Figure 14**).

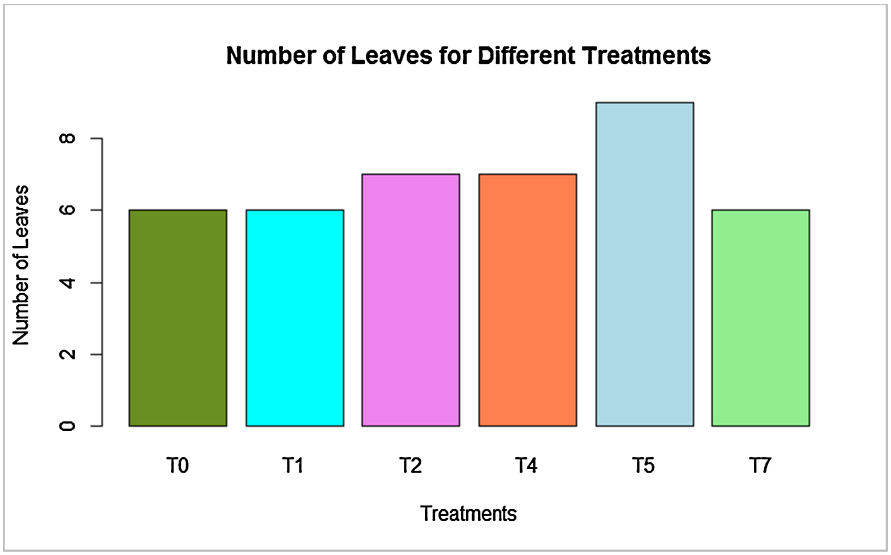

Figure 14. Number of *leaves of Irvingia* gabonensis with different treatment 30 days after seedling germination.

4. Discussion

The current study confirms that the seeds of Irvingia gabonensis exhibit diverse responses to physical, hormonal, and chemical treatments during germination. Pre-treatments not only shorten the germination period but also increase the germination rate. The results from the sulfuric acid treatment showed that *Irvingia gabonensis* seeds did not germinate during the experiment. The lack of germination could be due to the harmful effects of concentrated H_2SO_4 (96%), which may have a lethal effect on the embryo due to the hyperthermic conditions caused by the high concentration. According to this author, the 96% concentration of H_2SO_4 could be too strong and therefore harmful to the seeds [10] [11].

For the control seeds, a natural germination rate of 20% was recorded. This low rate may reflect the naturally limited germination capacity of *Irvingia gabonensis* seeds [12]. According to these authors, prolonged storage of seeds decreases germination potential, as observed in seeds stored for a month. These results corroborate those of [12], who observed a low natural germination rate for *Irvingia gabonensis*. Furthermore, *Irvingia gabonensis* seeds contain fats, which may reduce germination potential over time. Indeed, as noted by [13], seeds of forest species

containing fats lose their germination viability when dried and stored for extended periods. The germination trial also showed that untreated *Irvingia gabonensis* seeds (controls) had a relatively short germination period of 8.66 days. This period significantly differs from that reported by [13] for the same species, which ranged from 1 to 3 months. The germination rate for seeds treated with boiling water was relatively low (6.66%). This low rate could be due to the embryo's sensitivity to heat. Prolonged exposure to hot water at 100˚C may lead to a loss of germination viability. These findings are consistent with those of [14], who found that soaking *Brachychiton populneus* seeds in boiling water for 48 to 72 hours asphyxiated the embryos, resulting in low germination rates. Conversely, some authors, including [15]-[17], argue that boiling water treatment can effectively speed up germination by softening the seed coat and reducing water impermeability. In this study, using water at 100˚C reduced both the time to first germination and the overall germination period but also lowered the germination percentage. Seeds soaked in tap water for 3 and 7 days had germination rates of 13.33% and 3.33%, respectively. Longer incubation in water reduces germination rates, possibly due to pericarpic dormancy limiting embryo softening, or the extended soaking period could harm the seeds. Similar results were obtained by [18] with *Simmondsia chinensis*, [19] *Cetonia siliqua*, and *Cassia corymbosa*, as well as with *Prosopis juliflora* seeds [20]. The germination rate for scarified seeds without additional treatment was 16.66%. This result might be due to potential parasitic attacks on the exposed embryo, water saturation of the cotyledons, or protein exposure leading to rot [21]. Although scarification can increase the germination rate, these factors may reduce the number of germinated seeds, as seen in this study. When seeds were scarified and soaked in GA3 for 24 hours at concentrations of 0.5, 1, and 2 mg/L, the germination rates were 46.66%, 33.33%, and 56.66%, respectively. These high germination rates suggest that GA3 may accelerate germination in scarified seeds. GA3 may act as an embryo stimulant, promoting softening of the embryo and cotyledons. These findings align with those of [22] who reported germination rates of 46.60%, 57.60%, and 75.5% for Myrica rubra seeds soaked in GA3 at 1.3, 2.6, and 5.2 mM, respectively, compared to 31.3% for seeds soaked in water. According to these authors, GA3 plays a critical role in regulating seed germination.

5. Conclusions

This study revealed that GA3 significantly increases the germination rate of Irvingia gabonensis seeds, raising the natural germination rate from 20% to 33% and even up to 56%. However, its effect does not shorten the germination time. This germination time is rather shortened by soaking in water for 7 days. Furthermore, scarification also has a shortening effect on the germination time. Additionally, the study found that GA3 stimulates the growth of young plants.

For the production of vigorous Irvingia gabonensis plants from seeds, one can soak the seeds in water for seven days or scarify them to shorten the germination time, then treat them with gibberellin for 24 hours at concentrations of 0.5, or 2

mg/L to increase the germination rate and ensure rapid growth of young plants.

Conflicts of Interest

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

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