OPTIMIZATION OF THE TIME AND GERMINATION RATE OF SMALL COLA SEEDS (*Garcinia kola Heckel*, *Clusiaceae*)

Aabstract :

Despite its socio-economic importance, the cultivation of *Garcinia kola* is very limited due to the slow germination of its seeds. In order to facilitate the domestication of this plant species, the present study set itself the objective of improving the germination rate of its seeds in a short time. The methodology involved subjecting 150 G. kola seeds to six (06) treatments which are: the treatment with seed coat without soaking in tap water (absolute control), the 24 hour soaking treatment with seed coat, the 48 hour soaking treatment with seed coat, the treatment without seed coat or soaking (relative control), the 24 hour soaking treatment without seed coat and the 48 hour soaking treatment without seed coat. The seeds treated differently were sown the same day in the nursery in bags filled with soil. Evaluation ~~Monitoring~~ of seed germination, diameter and height of seedlings, length and number of leaves were measured every seven (07) days. The results obtained indicate that soaking G. kola seeds devoid of seed coat in water for 24 hours before sowing promotes a good germination rate of 36% within seven (07) days. After 11 days, 50% of the seeds in this treatment germinated. The longest germination time was observed in seeds sown with seed coat without soaking. Soaked seeds without seed coat produced the most vigorous plants with height growth of 0.34 cm/day and diameter increment of 0.15 mm/day. The variations in time and germination rate of G. kola seeds at different treatments indicate that the tissues of this plant species can respond to in vitro culture.

Keywords : Shortening germination time, Garcinia kola seeds, soaking, tap water

1. INTRODUCTION

*Garcinia kola*of the Guttiferae family (APG IV, 2016), commonly known as “little cola”, is a medium-sized intertropical dense humid evergreen forest tree that can reach 12 m in height (Isawumi *et al*.,1993). Extracts from different parts of this plant species are used for the treatment of oral infections, coughs, heart burns, liver disorders, infectious diseases, postpartum hemorrhage, urinary tract infections, vomiting and many more~~.~~ other diseases (Kanmegne and Omokolo, 2008; Dogara *et al*., 2022). The wood is also used as toothpick, energy and service wood, then the leaves as fodder for animal feed (Codjia *et al*, 2018). These numerous virtues make Garcina kola a highly sought-after species today. Despite this strong demand, especially in West and Central Africa (Anegbeh *et al*., 2006), the cultivation of G. kola is not very widespread due to the difficulty of germination of itsseeds, ranging from sixat twelve months (Adebisi, 2004). In addition, the few trees of the species in the rare forest relics

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are strongly threatened by the national and international development of the almond market (Kouamé *et al.*, 2016), combined with agriculture. The excessive picking of fruits for the marketing of seeds and the uncontrolled cutting of wood and other parts of the species for various uses are the main threats to its survival. All these factors could, obviously, lead to the extinction of G. kola. To avoid such a situation, it is wise to recommend a technology to improve the germination time and rate to facilitate its domestication. The work of Kanmegne and Omokolo (2008) on shortening the germination time of G. kola seeds focused on hormonal treatments of the seeds. This study showed that the best germination time for seeds treated with Naphthalene Acetic Acid (NAA) was nine weeks with germination rates ranging from 60 to 70%. As for the studies of Oboho and Ogana. (2011), they were spent soaking G. kola seeds with tap water for two to five days. His results indicated 10 weeks as the best germination time with seeds stripped of seed coat and soaked in water for two days. Although these technologies are proven, it is still appropriate to seek to further reduce the germination time of G. kola seeds with a technology that is easily transferable to farmers for its large-scale domestication. Thefore,~~It is with this in mind that the present study set itself~~ the general objective of ~~further~~ this study is designed to ~~improving~~ improve the time and germination rate of G. kola seeds. ~~Specifically, this involved determining the times and germination rates of different treatments and evaluating the stage of development of the seedlings.~~

2. MATERIALS AND METHODS

1. Description of ~~S~~study site

The study was carried out at the Research Station of the National Agronomic Research Center (CNRA) of Bimbresso, a locality in the District of Abidjan, located precisely in the commune of Songon, between 5°18'35" and 5°18'55" north latitude and 4°9'15" and 4°9'30" west longitude (Figure 1). The climate of the commune of Songon is described by Assiri *et al.* (2015) of Subequatorial type with bimodal rainfall. The average annual temperature recorded between 2013 and 2023 in the Abidjan District is 28.8°C with a minimum of 22.5°C in September and a maximum of 32.3°C in February. The total annual rainfall is 1,545 mm. According to climatological data from SODEXAM (2023), the District of Abidjan remained subject to four seasons: a long dry season from December to February, followed by a long rainy season, from March to July, a short dry season in August and a short rainy season from September to November. Agriculture is the main activity in the commune of Songon. This

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agriculture is mainly dominated by food crops (corn, cassava, rainfed rice, pineapple and banana, tarot, market gardening, etc.) and perennial crops such as oil palm, rubber and cocoa (Yéo and Amani, 2016).

Figure 1 :Representative map of~~Location of the~~ study site (CNRA, 2024)

~~II. STUDY METHODS~~

Treatments and design

~~1. Collection of data~~

Treatments

The methodology of this study focused on the germination of Garcinia kola seeds by different treatments. The experiment was set up with 150 seeds (25 seeds per treatment), selected on the basis of their viability and vigor. These seeds were subjected to six (06) treatments which are:

- seeds with unsoaked seed coat (absolute control)
- seeds with seed coat soaked in tap water for 24 hours
- seeds with seed coat soaked in tap water for 48 hours
- seeds without seed coat not soaked in tap water (relative control)

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- seeds without seed coat soaked in tap water for 24 hours
- seeds without seed coat soaked in tap water for 48 hours

Design of the experiment

Experimental procedures

After subjecting the seeds to these different treatments, they were sown the same dayin a nursery on the same substratein bags filled with soil, one seed per bag, placed under a greenhouse (Figure 2). During the experiment, data collection consisted of monitoring seed germination by observing the appearance of rootlets and seedlings every seven days. The diameter and height of the seedlings were measured every week using calipers and a graduated ruler. The leaves of each seedling were counted, the length of each leaf was also measured every week.

Data collection

Figure 2: Garcinia kola nursery underOmbrière at the CNRA research station in Bimbresso

~~**2. Data determination**~~

2.~~1~~. Determination of ~~G~~germination delay (GD)

The germination delay is a time interval, a period of time during which a seed still has the capacity to germinate. ~~He~~It is the time between the first day of sowing and the appearance of the first rootlets. It is expressed in days and is formulated as follows:

with Pf, the germination period of the seed, Pi, the sowing period. It is obtained directly from the first day of emergence of the rootlet.DG

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2.2. Average germination time (GMT)

The average germination time represents the germination energy responsible for depleting the seed's reserves. It expresses the speed of germination of seeds of a species (Diallo*et al*., 2023). In other words, the average germination time is the time after which 50% of the seeds of a plant species germinate (Berka and Abdelkader, 2001). It is determined by the formula:

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With: nor the number of seeds germinated at time ti (i ranging from 1 to 70 days); N the number of seeds germinated at the end of the test.

2.3. Germination kinetics

Germination kinetics correspond to variations over time in the seed germination rate during the experiment. It represents the daily germination rate of seeds up to "n" days of the experiment (Hajlaoui *et al*., 2007). The germination rate, also called germination power, is the percentage of live seeds germinated under the most favorable conditions (Chedya, 2008). The germination kinetics were determined from thefollowing equation:

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With TqG, daily germination rate; n, number of seeds germinated daily and N, total number of seeds germinated.

2.4. Developmental stage (SD)

The stage of development of the seedlings was assessed by periodically measuring the height and diameter of all the seedlings every seven (07) days.The total number of leaves was counted. Leaf length from the base of the petiole to the acumen was also measured.

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2.5. Seedling development rate (VD)

The determination of the speed of seedling development focused on the parameters diameter and height. The speed of seedling development is the ratio between heights or diameters and treatment time. It is calculated by the following formula:

with Pf = final diameter or height parameter; Pi = initial diameter or height parameter; tf= final time and ti = initial time.

3. ~~Statistical~~ Data analysis

3.1. Influence of different parameters on the vegetative vigor of seedlings

The influence of the different parameters on the vegetative vigor of the seedlings was highlighted by the correlation test. This test makes it possible to predict a variable Y based on another variable X (Tomassone et al., 1992). In this study, Y is the dependent variable and X is the independent variable. This means that the time to germination (DG), the number of leaves and the length of leaves can predict the vigor of the seedlings resulting from the germination of G. kola seeds of the different treatments, but it is not certain that the reciprocal is true. This analysis was carried out with XLSTAT software version 16.0.

2.4.2. Analysis of variance

An analysis of variance and comparison of means was applied to the different parameters calculated, to observe or not possible significant differences, with an error of 5% ($p < 0.05$). This analysis was carried out with XLSTAT software version 16.0.

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~~3H.~~ RESULTS

3.1. Germination delay

Figure 3: A- Histogram of germination times of Garcinia kola seeds treated differently; B- Garcinia kola seed stripped and soaked, germinated 7 days after sowing.**PST:**seeds sown with seed coat without soaking; PT24H: seeds sown with seed coat but soaked in water for 24 hours; PT48H: seeds sown with seed coat but soaked in water for 48 hours; SPST: seeds sown without seed coat and without soaking; SPT24H: seeds sown without seed coat with soaking in water for 24 hours; SPT48H: seeds sown without seed coat with soaking in water for 48 hours.

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Determination of the germination time indicates that seeds stripped of their seed coat and soaked in tap water for 24 and 48 hours germinate from seven (07) days after sowing (Figure 3 A and B). The longest germination times were observed in seeds sown with seed coat. Germination times for these seeds range from 42 days for soaked seeds to 48 days for those sown directly with seed coat without soaking.

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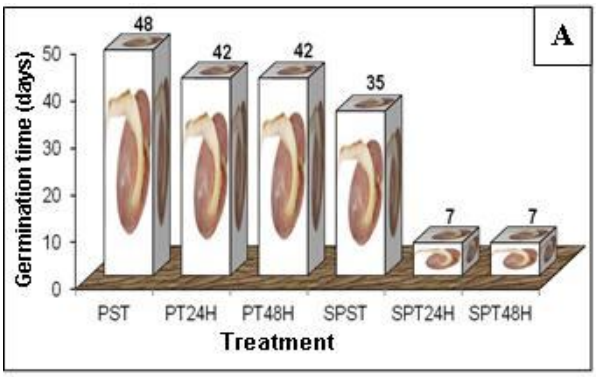

~~3.~~ 3.2. Average germination time (GMT)

The average germination time represents the germination energy responsible for depleting the seed's reserves. The average time, after which 50% of the seeds germinate, was better (11 days) for seeds devoid of seed coat and soaked in tap water for 24 hours (SPT24H). Seeds devoid of seed coat and soaked for 48 hours (SPT48H) follow with an average time of 24 days (Table I). This time was quite long for the seeds whose kernel had not been removed from its seed coat. However, among these seeds with long germination time, those that were soaked for 48 hours (PT48H) showed a shorter average germination time (48 days).

Table I: Average seed germination time under the influence of different treatments.

Treatments	Total number of seeds germinated after 70 days	Time after which 50% of seeds have germinated (days)	Numerical value of 50% of germinated seeds after 70 days	GMT (days)
PST	13	70	13	**70 ± 4.2 d**
PT24H	15	70	15	**70 ± 3.9 d**
PT48H	17	63	13	**48 ± 3.2c**
SPST	19	63	15	**50 ± 4.3c**
SPT24H	25	21	13	**11 ± 3.6 a**
SPT48H	25	42	14	**24 ± 4.1 b**
p-value				**0.0001*** **

Significance threshold for tukey tests: ***< 0.001; a, b, c, d: treatments assigned to the same letters are not statistically different; **VMG:** germination speed; PST: seeds sown with seed coat without soaking; PT24H: seeds sown with seed coat but soaked in water for 24 hours; PT48H: seeds sown with seed coat but soaked in water for 48 hours; SPST: seeds sown without seed coat and without soaking; SPT24H: seeds sown without seed coat with soaking in water for 24 hours; SPT48H: seeds sown without seed coat with soaking in water for 48 hours.

3.3.~~2.~~ Germination kinetics

The study of germination kinetics indicates variations in germination rate of seeds from different treatments. The highest germination rate in seeds sown with seed coat without soaking (PST) was observed on the 63rd day after sowing (Figure 4). The maximum germination rate of seeds soaked with seed coat (PT24H and PT48H) is located on the 42nd

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day. As for seeds devoid of integument and soaked for 24 hours (SPT24H), they recorded a significant germination rate of 36%, only in seven (07) days after sowing.

The cumulative germination rate curves display three (03) phases in the SPT24H and SPT48H treatments: progressive phase, exponential acceleration phase followed by the plateau phase, corresponding to the germination of all the seeds. The phase of exponential acceleration of germination of seeds sown without seed coat and soaked for 24 hours (SPT24H), starts from the 21st to the 42nd day after sowing (Figure 5). Those soaked for 48 hours with the integuments (SPT48H) start from the 46th to the 56th day. After the phase of exponential acceleration of the seeds of these two treatments, comes the plateau phase on the 42nd and 56th day respectively. This plateau phase corresponds to 100% germination of seeds from these treatments. The seeds covered with seed coat and especially not soaked did not reach maximum germination after 70 days of experimentation.

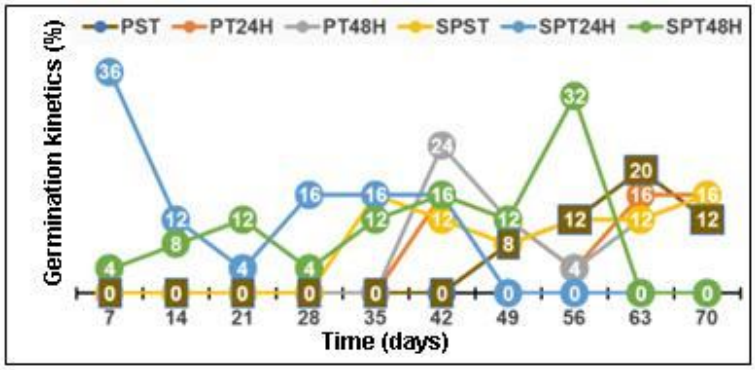

Figure 4: Germination kinetics curves of G. kola seeds treated differently before sowing.

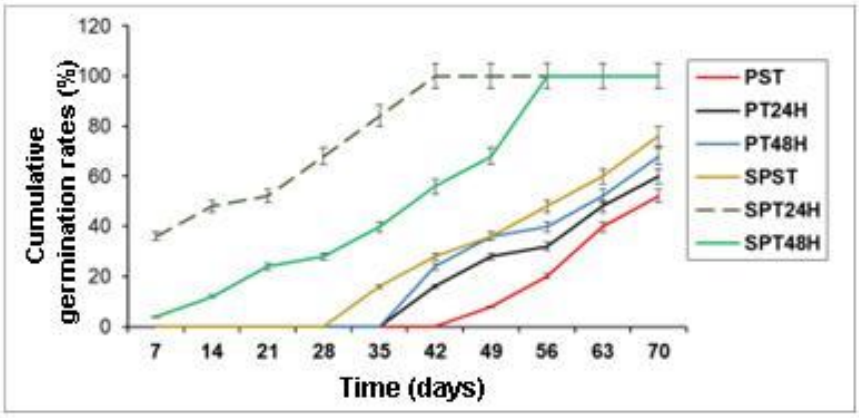

Figure 5: Cumulative germination rates according to the different treatments.**PST:**seeds sown with seed coat without soaking; PT24H: seeds sown with seed coat but soaked in water for 24 hours; PT48H: seeds sown with seed coat but soaked in water for 48 hours; SPST: seeds sown without seed coat and without soaking; SPT24H: seeds sown without seed coat with soaking in water for 24 hours; SPT48H: seeds sown without seed coat with soaking in water for 48 hours.

~~4.~~ 3.4 Seedling development stage

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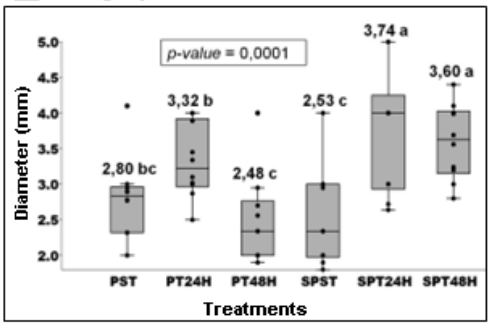
The evaluation of the diametric growth of G. kola seedlings indicates significant differences (p-value = 0.0001) between the six treatments. Seedlings from seeds devoid of seed coat and soaked for 24 and 48 hours (SPT24H and SPT48H) recorded the highest average diameters with values of 3.74 mm and 3.60 mm, respectively (Figure 6). Seedlings from seeds soaked for 24 hours with seed coat (PT24H) follow with an average diameter of 3.32 mm. Those of seeds soaked for 48 hours with the integuments (PT48H) contain the smallest diameters (2.48 mm).

In terms of height growth, seedlings from seeds soaked for 24 hours without seed coat (SPT24H) gave an average height of 9.55 cm (Figure 6). Those from seeds of the PT24H treatment recorded the lowest average height which is 4.81 cm.

According to these results, seeds devoid of seed coat and soaked in tap water promote good diametric and height development of G. kola seedlings in bag nurseries with large leaves (Figures 7 A and B).

Figure 6: A- Measurement of the diameter of a young 63-day-old Garcinia kola plant from the experiment; B- Measurement of the length of a leaf of a young 63-day-old Garcinia kola plant from the experiment



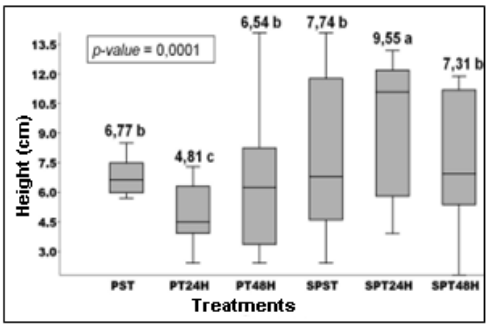


Figure 7: Box plots of mean diameters and mean heights of Garcinia kola seedlings from different treatments.**PST:**seeds sown with seed coat without soaking; PT24H: seeds sown with seed coat but soaked in water for 24 hours; PT48H: seeds sown with seed coat but soaked in water for 48 hours; SPST: seeds sown without seed coat and without soaking; SPT24H: seeds sown without seed coat with soaking in water for 24 hours; SPT48H: seeds sown without seed coat with soaking in water for 48 hours.

3.5~~5.~~ Speed of seedling development

The study of the speed of seedling development indicates significant differences between the treatments with p-values, ranging from 0.021 (growth speed) to 0.036 (diametric growth speed). The growth rates in height of the seedlings of the SPT24H and SPT48H treatments are 0.34 and 0.35 cm/day, respectively (Table II). The seedlings of these same treatments still contain the highest diameter growth rates with values of 0.15 mm/day for the seedlings of the SPT24H treatment and 0.17 mm/day for those of the SPT48H treatment (Table II). .

Table II: Development speed of Garcinia kola seedlings from seeds treated differently

Treatments	Seedling growth rate (cm/day)	Diametric growth rate of seedlings (mm/day)
PST	0.29 ± 0.09 ab	0.11 ± 0.03b
PT24H	0.26 ± 0.09 ab	0.15 ± 0.03 a
PT48H	0.28 ± 0.09 ab	0.13 ± 0.03b
SPST	0.24 ± 0.09b	0.10 ± 0.03b
SPT24H	0.34 ± 0.09a	0.15 ± 0.03 a
SPT48H	0.35 ± 0.09a	0.17 ± 0.03 a
p-value	0.021*	0.036*

Significance threshold for tukey tests: *< 0.05;a, b: treatments assigned the same letters are not statistically different;**PST:**seeds sown with seed coat without soaking; PT24H: seeds sown with seed coat but soaked in water for 24 hours; PT48H: seeds sown with seed coat but soaked in water for 48 hours; SPST: seeds sown without seed coat and without soaking; SPT24H: seeds sown without seed coat with soaking in water for 24 hours; SPT48H: seeds sown without seed coat with soaking in water for 48 hours.

~~8.~~ 3.6 Influence of different parameters on the vegetative vigor of seedlings

The evaluation of the influence of different parameters on the vegetative vigor of seedlings indicates two trends. The first trend is characterized by the influence of the germination time and the number of leaves on the vegetative vigor of the seedlings with coefficients of determination R^2 varying between 0.59 and 0.97 (Figure 8 A and B). The germination time negatively influences the vigor of the seedlings. In other words, when the seed germination time is long, the G. kola seedlings produced from these seeds are less

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vigorous. As for the number of leaves, it positively influences the vigor of the seedlings. Indeed, when the number of leaves is greater, the seedlings develop vigorously. Concerning the length of the leaves, it has no influence on the vigor of the seedlings (Figure 8 C). The coefficient of determination is 3.23.10-6.

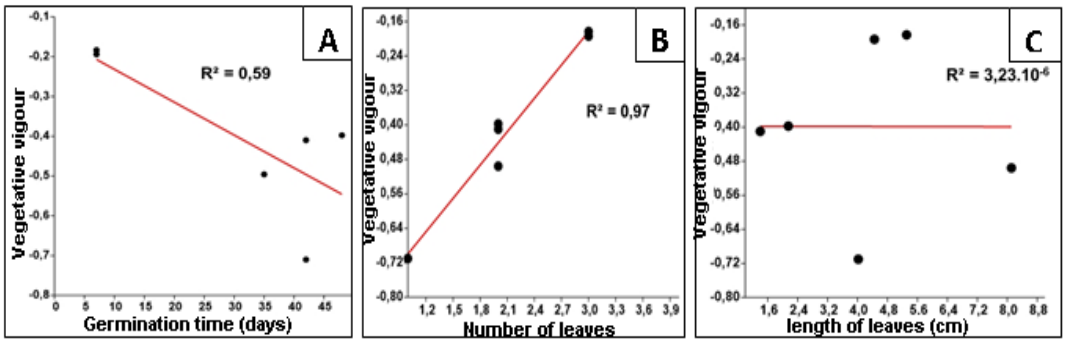

Figure 8: Influence of different parameters on the vegetative vigor of seedlings.

4. DISCUSSION

The shortest germination time for Garcinia kola seeds achieved in this experiment is seven (07) days with the seeds devoid of seed coat and soaked in tap water for 24 hours. This short germination time achieved with these seeds could be due to the high humidity level favoring the infiltration of water inside the seed which subsequently swells hence oxygenation and therefore quickly triggers the process germination after sowing (Ndiaye et al., 2022). This delay is better than that observed by Kanmegne and Omokolo (2008). Indeed, these authors in their work showed that the best germination time for G. kola seeds treated with Naphthalene Acetic Acid (NAA) was nine (09) weeks. This indicates that soaking G. kola seeds in water potentially influences germination time. Liksowe et al. (2008) working on Terminalia sericea seeds showed that soaking in water for 24 hours improves germination. Odebunmi (2009) has already discovered in his work that water is an important factor favoring the germination of seeds of tropical trees. The germination time obtained with seeds devoid of seed coat and soaked in water for 24 hours in this study is much better than that obtained by Oboho and Ogana (2011). Indeed, the work of these authors indicated ten (10) weeks as the best time for germination of G. kola seeds stripped of their seed coat and soaked in water for two (02) days. The difference in germination time observed between these two experiments with water could

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be due to the soaking time. Indeed, when the seeds of a plant species stay in water for a long time, the oxygenation of the embryo becomes limited, and this causes its respiration to stop (Sridhar et al., 2013). According to these authors, the seeds, in such conditions, will see their germination delayed or even stopped. Nzegbule and Mbakwe (2001) showed that the phenolic substances contained in the seeds of G. kola are the factors that inhibit its germination. Soaking for 24 hours would be enough to remove these inhibiting chemicals. The water in this short soaking time hydrolyzes the nutrients stored in the embryos and stimulates seed germination.

The germination time of 35 days was long for seeds lacking seed coat without soaking. This means that only integumentary dormancy has been removed, while the inhibitory chemical parameters remain. The germination time was even longer for seeds with seed coat. This long germination time could be explained by the combination of integumentary and physiological dormancy induced by the chemical substances contained in the seed (Oboho and Ogana, 2011). According to these authors, theThe seminal envelope which surrounds the seed constitutes a real obstacle to the passage of water or oxygen, hence the prolonged dormancy action of these seeds.

The differences in height growth and diameter increase between seedlings are linked to the treatments of their seeds. Indeed, the seedlings from seeds devoid of integument and soaked in water for 24 hours were the most vigorous, and showed better growth in height and diameter increase. This could be due to the action of the soaking water which would have stimulated the chemical substances contained in the seed, responsible for the development of the seedlings after germination. The work of Harris et al. (2000) also showed that seed soaking improves germination and increases plant vigor by approximately 30%. The study of the correlation indicates a negative influence of the germination time on the vigor of the seedlings. In other words, the longer it takes for G. kola seeds to germinate, the less vigorous the seedlings resulting from these seeds are. The negative influence of germination time on seedling vigor could be due to their root system. Indeed, the short time for seed germination would have favored good root development and an increase in the number of secondary roots as shown by the work of Hassane (2023). According to Paul et al. (2019) and Barrajón-Catalán et al. (2020), such root development improves the vigor of plants by supplying them with sufficient nutrients essential for their growth. Plants with less developed roots have reduced aerial apparatus. As for the number of leaves, it positively influences the vigor of the seedlings. This could be explained by the important photosynthetic function of the leaves, allowing the plant to have sufficient nutrients for its development. Ikoungou (2003), by

studying the germination of Terminalia superba seeds, observed a strong positive correlation between the diametric development of the plants and the number of leaves of around 94.31%. His work showed that increasing the number of Terminalia superba leaves considerably promotes plant vigor.

5. CONCLUSION

The study of the kinetics and germination rate showed that Garcinia kola seeds stripped of their integuments and soaked in tap water for 24 hours before sowing promotes a good germination rate within seven days after sowing with a high germination rate of around 36%. The average time after which 50% of seeds devoid of seed coat and soaked in water for 24 hours germinate is 11 days. The exponential acceleration phase of germination of these seeds is between the 21st and 42nd day after sowing. The longest germination times were observed in seeds not soaked and sown with seed coat. These seeds did not reach their maximum germination after 70 days of the experiment.

Soaking seeds stripped of their seed coat in tap water has a positive effect on the optimal development in height and diameter of G. kola seedlings in bag nurseries. The development speed of these seedlings is 0.34 cm/day for height growth and 0.15 mm/day for diameter growth. This development is positively influenced by the seed germination time. The shorter the seed germination time, the more vigorous the plants. The longer the germination time, the less vigorous the plants.

Conflect of interest

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