Effect of storage temperature and dormancy breaking pre-treatments on germination and early seedling growth of *Garcinia kola* (Heckel): a threatened medicinal fruit tree in Benin

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Abstract
Germination and early growth of *Garcinia kola*- Heckel were conducted to determine the suitable temperature of storage to maintain germinability and growth of the species. The viable seeds of the species were divided into two groups, one stored at room temperature and the other at 4°C in a refrigerator for 2 months before pre-treatments. Seeds of each group were soaked in distilled water for 48 or 72 hours, with and without their seed coat. The treated seeds were kept in a growth chamber for germination assessment. The results indicated that independently of pre-treatments, 66.67–96.67% of *G. kola* seeds stored at room temperature germinated after 5 months while 0–23.33% of *G. kola* seeds stored at 4°C germinated. Seeds stored at room temperature, soaked without coat in distilled water for 72 hours gave the highest germination percentage of 96.67%. Seedlings from seeds stored at room temperature, soaked without coat in distilled water for 72 hours had the highest radicle and plumule length as well as the highest leaf number. Only radicle length and plumule length were highly correlated. The present study demonstrates that storage at room temperature for a short period does not alter *G. kola* germination.

1. Introduction
Storage of seeds as *ex situ* germplasm is an essential step for the long-term conservation of plant genetic resources. The operational cost of storage facility per unit seed stored increases considerably as the requirements of temperature and relative humidity become more stringent. Therefore, prior to storing seeds, a decision must be made on the type of storage needed. This can be related to the time period for which storage is expected and the storage characteristics of the species (Paroda and Arora 1991). Simple techniques have been adopted to maintain seed viability in both domesticated and wild sources (Onyekwelu and Fayose 2007; Pradhan and Badola 2008). Inappropriate storage medium often results in low seed germination, seed deterioration, and loss of viability, which are natural phenomena during storage (Müller et al. 2011). Several factors, namely, temperature, nature of the seeds, seed moisture content, relative humidity, and so forth, influence the seed longevity during storage (Roberts 1972; Onyekwelu and Fayose 2007; Pradhan and Badola 2008). Proper storage conditions, however, may effectively retain substantial viability in seeds over a considerable storage period (Pradhan and Badola 2012). Such approaches are especially crucial in case of endangered species, where judicial use of seeds as valuable genetic material through standardizing proper storage mechanism is a precondition to strengthen species conservation programme (Pradhan and Badola 2012).

*Garcinia kola* Heckel, popularly known as ‘bitter kola tree’ belongs to the family Clusiaceae and is distributed throughout West and Central Africa. (Adebisi 2004). The tree is one of the many non-timber forest products that have a high socio-economic importance (Kangneme and Omokolo 2007). The fruit, seeds, nuts, and bark of the plant are extensively used in African traditional medicine for the treatment of various diseases (Ekene and Erhirhie 2014). Because of its importance the species has been overexploited which, together with an ongoing habitat loss and degradation caused by humans, have led to its inclusion in the list of IUCN extinct in the wild species in Benin (Neuenschwander et al. 2011). Considering its importance and to prevent genetic erosion, appropriate strategies should be developed to promote its sustainable use. Recently, *G. kola* has also been classified as one of the top ten priority species that need valuation and improvement in Benin (Assogbadjo et al. 2017). One important approach for the conservation of plant species is cultivation (Sanchez et al. 2010), as in restoration programmes. Unfortunately, farmers are reluctant to plant the species because it is characterized...
by dormant seeds with non-uniform and low germination rate. Previous studies used freshly collected seeds in Nigeria (Nzegbule and Mbakwe 2001; Kangneme and Omokolo 2008), Ivory Coast (Kouakou et al. 2016) and Ghana (Agyili et al. 2007). Dormancy is one of the greatest obstacles for the germplasm conservation of forest species, which frequently produce dormant seeds (Gonçalves et al. 2011). Pre-treatments methods used were scarification, mechanical coat removal, hormone pre-treatments and water pre-treatments to improve G. kola germination. However, these methods gave mixed results. It has been shown that seed germinability in the species varies widely from one provenance to another (Kangneme and Omokolo 2008). To the best of our knowledge, no germination test has been done on G. kola seeds collected from Benin. Furthermore, no G. kola germination work has ever tested the effect of storage temperature on germinability of G. kola seeds. The aims of the present study are to (i) investigate the effects of storage temperature and different dormancy breaking pre-treatments on G. kola seed germination, and (ii) evaluate the effect of these treatments on early seedling growth. It was hypothesized that storage temperatures would maintain germinability and the pre-treatments methods applied in this study should improve G. kola seed germination under controlled conditions.

2. Materials and methods

2.1. Plant materials and experimental design

Fully mature (orange) fruits of G. kola were collected in the Guineo-Congolian zone (6°25′–7°30′ N) of Benin, which corresponds to the distribution area of the species. Within this agro-ecological zone, G. kola fruits were collected from different trees in Atlantique, Oueme and Plateau. Seeds were removed from the collected fruits and washed. The extracted seeds were mixed into a single batch and dried under shade for 2 days to avoid fungal contamination during storage. Flotation and manual selection were used to remove empty and damaged seeds and to select seeds of uniform size. Two storage temperatures were tested (D’Antonio and McHale 1988): (i) in a cloth bag to offer oxygen for moist storage at room temperature (25 ± 5°C) for 2 months, water is sprayed weekly to keep the seeds moist and (ii) in polyethylene bag in a refrigerator at 4°C for 2 months. After the storage period, four dormancy-breaking pre-treatments were applied and a control were used for both seeds stored at room temperature and seeds stored in the refrigerator (Table 1). Dormancy-breaking treatments consisted on mechanically removing the seed coat and soaking in distilled water for different time periods, soaking the seed in distilled water for different time periods without removing the coat of the seeds. During the soaking period, water was decanted and renewed after 24 hours until the duration of the soaking is reached in order to remove and wash off possible inhibitors. The experimental design was a complete randomized with 3 replicates of 30 seeds.

2.2. Surface sterilization and germination conditions

To minimize the effect of environmental factors on germination, germination test was undertaken under controlled environment in a growth chamber. Pre-treated seeds were first surface-sterilized with liquid soap containing 2 drops of tween-20 for 15 minutes, then soaked in 0.5% Ridomil® (fungicide) containing 2 drops of tween-20 for 30 minutes and finally in 15% bleach (JIK®) containing 2 drops of tween-20 for 20 minutes. Thereafter, the seeds were rinsed thrice with autoclaved distilled water and placed to germinate in the growth chamber in sterilized plastic dishes lined within two sheets of sterile paper and containing sufficient amount of autoclaved distilled water. The culture conditions were kept at 28 ± 2°C for the temperature, a relative humidity of 80% and 12 hours photoperiod. Autoclaved distilled water was added to each dish every 2 days to prevent desiccation.

2.3. Data collection and analysis

Seeds were checked daily for 150 days for germination and early growth. The criterion of germination is the emergence of 1 mm of either the radicle or the plumule. Germination was recorded daily, from the day of sowing through to the end of the experiments, while seedling growth (number of radicle, longer of radicle, longer of plumule, number of leaves) was measured at the end of the experiment.

To estimate treatments’ germination ability, we calculated:

\[ PG = \frac{msp}{N} \] (1)

| Storage temperature (°C) | Dormancy-breaking treatments | Treatment combination definition |
|--------------------------|-----------------------------|---------------------------------|
| Room temperature (25°C)  | Seeds with coat removed and soaked in distilled water for 48 hours | RT1                             |
|                          | Seeds with coat removed and soaked in distilled water for 72 hours | RT2                             |
|                          | Seed intact soaked in distilled water for 48 hours | RT3                             |
|                          | Seed intact soaked in distilled water for 72 hours | RT4                             |
|                          | Untreated seeds | RT0                             |
| Refrigerator (4°C)       | Seeds with coat removed and soaked in distilled water for 48 hours | FT1                             |
|                          | Seeds with coat removed and soaked in distilled water for 72 hours | FT2                             |
|                          | Seed intact soaked in distilled water for 48 hours | FT3                             |
|                          | Seed intact soaked in distilled water for 72 hours | FT4                             |
|                          | Untreated seeds | FT0                             |

Table 1. Treatments applied for Garcinia kola germination.
with nsp being the number of seeds that germinated (ns) in the treatment p and N, the total number of seeds sown in the seed lot (30 for all lots).

- The germination speed was assessed by calculating (a) the time to first germination (TFG); (b) the time necessary to reach 20% germination (t20); (c) the time necessary to reach 50% germination (t50).

Treatments that did not reach 20 or 50% of germination were not included in the analysis. The generalized linear model with binomial structure was used to test the effects of the different treatments on germination. Effects of the treatments on germination speed were compared using the generalized linear model with a Poisson error structure. Early seedling growth parameters were analyzed using the generalized linear model with linear structure or Poisson structure on data recorded at the end of the experiment. Correlation tests were used to analyze relationships among growth parameters in the best-performing treatment. Statistical analyses were performed in IBM SPSS software version 23.

3. Results

3.1. Effect of treatment combinations on germination

From the second week after sowing, germination started with the emergence of a tiny radicle, then was followed by the emergence of plumule. It clearly appeared that there was a significant difference in the storage temperature effect on germination \( (p < 0.001) \). Seed stored at room temperature in general performed better than seeds stored at 4°C. When considering the combination of the storage temperature and the dormancy-breaking treatments, seeds stored at room temperature whose coat has been removed and soaked in distilled water for 72 hours (RT2) was the best treatment \( (p < 0.001) \) followed by seeds stored at room temperature whose coat has been removed and soaked in distilled water for 48 hours (RT1). Seeds of RT2 reached 96.67% germination before 2 months (Figure 1). The highest germination observed in seeds stored at 4°C was 23.33% for seeds whose coat was removed and soaked in distilled water for 72 hours (FT6). Untreated seeds stored at 4°C (FT0) did not germinated.

3.2. Effect of treatment combinations on germination speed

The germination speed was assessed using the time to first germination (TFG), the time to 20% germination \( (t20) \) and the time to 50% germination \( (t50) \). The trends in these parameters indicated that seed stored at room temperature in general performed better than seeds stored at 4°C \( (p < 0.001) \). The results of the GLM analysis with a Poisson error structure also indicated that the combination of storage temperature and dormancy-breaking treatments applied significantly

![Figure 1. Effect of different treatment combinations on germination percentage of G. kola seeds; RT1 seeds stored at room temperature, coat removed + soaked in distilled water for 48 hours; RT2 seeds stored at room temperature, coat removed + soaked in distilled water for 72 hours; RT3 seeds stored at room temperature, soaked intact in distilled water for 48 hours; RT4 seeds stored at room temperature, soaked intact in distilled water for 72 hours; RT0 seeds stored at room temperature, untreated; FT1 Seed stored at 4°C, coat removed + soaked in distilled water for 48 hours; FT2 seed stored at 4°C, coat removed + soaked in distilled water for 72 hours; FT3 seed stored at 4°C, soaked intact in distilled water for 48 hours; FT4 Seed stored at 4°C, soaked intact in distilled water for 72 hours; FT0 seeds stored at 4°C, untreated.](image-url)
affected the time to first germination \((p < 0.001)\), with quicker germination in seeds stored at room temperature with coat removed and soaked in distilled water for 72 hours (RT2; 18.23 days on average, with the earliest occurring at 12 days after sowing) and RT1 (24.39 days on average, with the earliest occurring at 16 days). The highest TFG (102.86 days on average) was observed in the seeds stored at 4°C whose seeds was removed and soaked in distilled water for 72 hours (FT2; Figure 2(a)). As for the threshold set at 20% germination, there was a very significant difference among treatments \((p < 0.01)\). Indeed, 20% of seeds germinated in the RT2, RT1 and RT4 seeds before 16.33 days and 19.33 days and 38 days after sowing, respectively. In contrast, even after two months (60 days after sowing), 20% germination was not yet achieved in the other seed lots (Figure 2(b)). No seed stored at 4°C germinated up to 50%. Among the seed lots stored at room temperature, RT2 showed the best performance with 50% of the seeds germinated before 18.67 days followed by RT1 seeds with the time to 50% germination being 21.67 days (Figure 2(c)).

### 3.3. Effect of treatments on early seedling growth

#### 3.3.1. Number of radicles

The number of radicles per seedling ranged from 0 to 2 during the experiment. There was no significant difference among treatments \((p > 0.05)\) for the number of radicles (Table 2). The highest mean number of radicles at the end of the experiment was recorded in RT1 (1.11) and RT2 (1.07).

#### 3.3.2. Radicle length

Highly significant difference was noted between seeds stored at 4°C and treatments with seeds stored at room temperature \((p < 0.001)\). When considering the combination of storage temperature and dormancy-breaking treatments, results revealed that the longest mean radicle (4.44 cm) was noted in RT2 (Table 2) followed by RT1 (3.06 cm). The shortest radicle was observed in FT1 and FT3 (0.8 cm).

#### 3.3.3. Plumule length

The longest mean plumule was noted in RT2 (5.03 cm) followed by RT1 (3.97 cm). The generalized linear model indicated that the dormancy-breaking treatments very significantly affected plumule length \((p < 0.001)\). Highly significant difference was also observed between treatments of seeds stored with stored at 4°C and seeds stored at room temperature (Table 2).

#### 3.3.4. Number of leaves

The generalized linear model on the number of leaves produced per seedling revealed that there were highly significant differences in the effect of treatments and temperature of storage \((p < 0.001)\). The highest number of leaves was produced by RT2. No seeds stored at 4°C produced leaves (Table 2).

#### 3.3.5 Correlation among growth parameters

Correlations among number of radicles, radicle length, plumule length and number of leaves produced by G. kola seedlings were studied in the best performing treatment (Table 3). It was observed a high and significant correlation \((r = 0.91, p < 0.001)\) between the length of the plumule and the length of radicle. The number of leaves was fairly and significantly correlated with the length of plumule \((r = 0.41, p < 0.05)\) and length of radicle \((r = 0.36, p < 0.05)\).
Table 2. Effect of seed storage temperature and dormancy-breaking treatments on early seedling growth for seeds of G. kola.

| Treatments | Number of radicle | Radicle length | Plumule length | Number of leaves |
|------------|------------------|----------------|----------------|-----------------|
| RT1        | 1.11 ± 0.04a     | 3.06 ± 0.16b   | 3.97 ± 0.16a   | 0.43 ± 0.16ab   |
| RT2        | 1.07 ± 0.03a     | 4.44 ± 0.16a   | 5.03 ± 0.16b   | 1.00 ± 0.24a    |
| RT3        | 1.00 ± 0.04a     | 2.51 ± 0.18bcd | 3.4 ± 0.18bc   | 0.18 ± 0.12ab   |
| RT4        | 1.00 ± 0.04a     | 2.88 ± 0.18bc  | 3.9 ± 0.18b    | 0.09 ± 0.08b    |
| RT0        | 1.00 ± 0.05a     | 1.89 ± 0.23de  | 2.63 ± 0.23cd  | 0.43 ± 0.23ab   |
| FT1        | 1.00 ± 0.13a     | 0.8 ± 0.66de   | 1.05 ± 0.06de  | 0.00 ± 0.00ab   |
| FT2        | 1.00 ± 0.07a     | 0.94 ± 0.32e   | 1.16 ± 0.32e   | 0.00 ± 0.00ab   |
| FT3        | 1.00 ± 0.19a     | 0.8 ± 0.85bcde | 1 ± 0.85cde    | 0.00 ± 0.00ab   |
| FT4        | 1.00 ± 0.13a     | 0.85 ± 0.60dde | 1.1 ± 0.60dde  | 0.00 ± 0.00ab   |
| FT0        | 0                | 0              | 0              | 0               |

Means followed by the same letter within a column are not statistically different; RT1 seeds stored at room temperature, coat removed + soaked in distilled water for 48 hours; RT2 seeds stored at room temperature, coat removed + soaked in distilled water for 72 hours; RT3 seeds stored at room temperature, soaked intact in distilled water for 48 hours; RT4 seeds stored at room temperature, soaked intact in distilled water for 72 hours; RT0 seeds stored at room temperature, untreated; FT1 seed stored at 4°C, coat removed + soaked in distilled water for 48 hours; FT2 seed stored at 4°C, coat removed + soaked in distilled water for 72 hours; FT3 seed stored at 4°C, soaked intact in distilled water for 48 hours; FT4 seed stored at 4°C, soaked intact in distilled water for 72 hours; FT0 seeds stored at 4°C, untreated.

Table 3. Pearson correlations among growth parameters for seedlings of Garcinia kola.

| Treatments | Number of radicle | Radicle length | Plumule length | Number of leaves |
|------------|------------------|----------------|----------------|-----------------|
| RT2        |                  |                |                |                 |
| Number of radicle | 0.30             | –              |                |                 |
| Radicle length   | 0.22             | 0.91***        | –              |                 |
| Plumule length   | 0.19             | 0.36*          | 0.40*          | –               |

***Correlation significant at p < 0.001 level; *correlation significant at p < 0.05 level; RT2 seeds stored at room temperature, coat removed + soaked in distilled water for 72 hours.

4. Discussion

Seed viability and germinability vary greatly by species and storage conditions (Siddique and Wright 2003). Our present investigation, testing seeds for the appropriate storage conditions (at 4°C and room temperature) confirms that the storage temperature significantly affects the seed germination capacity of G. kola. A greater percentage of germination was registered at room temperature, independently of the pre-sowing treatments. The greatest percentage of germination was at room temperature (25°C) while minimal percentage of germination was reported at 4°C. A drastic reduction of germination from 96.67% to 0% was observed when comparing seeds stored at room temperature and seeds stored at 4°C. In one hand, the reduction of germination of seeds store at 4°C may be due to the effect that cold exercises in the seeds caused the decrease in the activity of enzymes such as α- and β-amylase which are involved in the main breakdown of carbohydrates used during respiration, specifically starch; this activity is critical to provide the embryo with energy and a carbon skeleton. On the other, storage at room temperature probably favoured the increase of those enzymes, which positively affected germination (da Mata et al. 2016). Previous studies reported that seed of Garcinia subelliptica seeds are intolerant to low temperature (Yang et al. 2010). This confirms that Garcinia species are better stored at relatively high temperature. Percent seed germination reported for fresh seeds collected from different locations if they are provided with the same dormancy-breaking treatments is similar to G. kola collected from Benin and stored at room temperature. Indeed, our present study revealed that seeds stored at room temperature and soaked in distilled water for 48 or 72 hours resulted in 93.33–96.67% germination which roughly is similar to what was reported for fresh seeds from Nigeria (Yakubu et al. 2014) and Ivory Coast (Kouakou et al. 2016). This is an indication that the initial germination potential may remain similar after a short-term storage if the right condition is maintained.

Germination in G. kola is known to be spread over a long period, but this can be shortened. In our study, regardless the pre-treatment methods, seeds stored at room temperature started germination before seed stored at 4°C. The first germination was recorded 12 days after sowing. The earliest germinations were recorded in RT2 and RT1, which are seeds without coat stored at room temperature, soaked without coat in water for 72 and 48 hours respectively. This effect was also observed in fresh G. kola from Nigeria (Yakubu et al. 2014). In addition, the time to first germination obtained, is comparable to the one reported by the same authors. Another parameter used to measure germination speed is the time to reach the threshold germination rate, which is 20% (t20). Our results revealed that, only seed stored at 4°C soaked for 72 hours without coat reached 20% germination while more than 20% of all the seeds stored at room temperature regardless the pre-treatments germinated. In 38 days, just over 1 month, 20% germination can be achieved with G. kola seeds stored at room temperature and soaked without coat (RT1, RT2). As for the untreated seeds stored at room temperature, 20% can be achieved after 96 days. For both seeds stored at room temperature and at 4°C, the lowest germination and germination speed in seeds soaked with their coat
compared to seeds soaked without coat for the same period of time revealed that removing the seed coat followed by soaking in water for 48 or 72 hours is necessary to break the physical and physiological dormancy that have been observed in *G. kola*.

According to N’danikou et al. (2014), the vigour of the seedling will be affected by the time taken to germinate. In fact, the seed embryo is weakened over time if it takes longer than normal to germinate. Also, soil and other environmental conditions became poorer over time. However in this study, experiments were undertaken under controlled conditions to maintain the environmental conditions uniform throughout the study. Independently of the seed dormancy-breaking treatments, seeds stored at room temperature were the most effective. These seeds soaked in water for 72 hours (RT2) significantly affected early seedling growth parameters. At 150 days’ age, the length of radicle and plumule of seedlings on average were 4.44 cm and 5.03 cm, respectively; whereas they produced one leave on average. This corroborates the common opinion that *G. kola* is a slow-growing species (Agyili et al. 2007). The results of the correlations among growth parameters at 5 months in the best performing treatment (RT2) indicated that plumule length and radicle length are strongly and positively correlated. Positive correlations among growth parameters were also observed in V. *doniana* seedlings (N’danikou et al. 2014).

### 5. Conclusion

The storage of *G. kola* seeds stored at room temperature (25°C) has proved not to affect seed viability and germinability contrary to seeds stored at 4°C in the refrigerator for the same period. Moreover, removing the seed coat and soaking the seeds stored at room temperature in water for 72 hours was the most effective dormancy-breaking treatment in enhancing the germination of *G. kola* seeds. This method, which is economical and fast, has to be recommended to promote the cultivation of this species. Future study should focus on testing varying storage period that will allow conservation as well as maintain seed viability.

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