Full Length Research Paper

Possibility of using the intermediate mature stage of *Garcinia kola* heckel seeds to shorten the germination time

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*Garcinia kola* is used as chew-stick for dental care in West Africa. The species is nearly commercially extinct in nature, and its propagation is hampered by seed germination problems. The current study investigated seed germination responses of *Garcinia kola* at different maturity stage. The experiments were conducted at the Central Laboratory of Biotechnology of the Centre National de Recherche Agronomique (CNRA) located in Adiopodoumé (North Abidjan - Côte d’Ivoire) while the fruit samples were collected from Yapo a protected forest area with very humid equatorial climate. The experimental design was factorial design with two factors. Seeds processed from green fruits of 6 weeks old did not germinate, whilst those of 10 weeks old fruits, at the intermediate stage, germinated at the rate of 46%. Seeds from orange-coloured mature fruits had a germination rate of 54 to 62%. The adventitious roots of intermediate maturity seeds protruded at the same time as mature ones after 9 weeks of sowing and normal germination, with first shoot growth occurring simultaneously after 12 weeks in the two (intermediate maturity and full maturity) stage of fruits. Desiccation tests after 4 weeks revealed similar trends of weight decrease and moisture content in the two kinds of seed. Seeds from partly intermediate mature fruits showed germinating characteristics comparable to mature fruits. These indicate that the seeds from this stage could be used to germinate *Garcinia kola* Heckel, for conservation, post harvesting and commercial purposes.

Key words: *Garcinia kola*, planting material, germination, seeds, fruits, Côte d’Ivoire.

INTRODUCTION

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| Kingdom: Plantae | Unranked: Eudicots |
| Unranked: Angiosperms | Unranked: Rosids |
| Order: Malpighiales | Family: Clusiaceae |

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Genus: *Garcinia*
Species: *G. kola*

*G. kola* Heckel belongs to the Guttiferae (Clusiaceae) family. There are more than 600 species in this genus distributed widely in tropical and subtropical regions and only 16 species are found in West Africa (Steentoft, 1988). *G. kola* occurs in the wet and moist semi-deciduous forest zones. *G. kola* is a spreading forest tree growing up to 90 feet high and having a girth of over 5 feet. Flowers appear from December to March or from May to August. Male and female flowers are separate and distinct. Fruits are smooth, reddish yellow and the pulp orange yellow with three or four seeds. The acid, red, aril-like fruit pulp is edible. The twigs can be used as tapers and the roots yield the favourite bitter chew sticks sold in small bundles in local markets (Adu-Tutu et al., 1979). The seeds are an important product in West Africa markets. When chewed they have a bitter, astringent taste rather resembling that of the raw coffee bean, and followed by a slight sweetness. They are chewed as an adjuvant rather than as substitute for the true cola. The juice has a stimulating effect. Moreover, they also enhance the flavour of local alcoholic beverages (Irvine, 1961).

Extracts of various parts of the plant are used for the treatment of laryngitis, mouth infections, cough, heart burn, liver disorder, chest colds hoarseness and others inflammatory diseases (Ainslie, 1937; Iwu, 1982). The seeds are also used in the treatment of bronchitis, throat troubles, post-partum haemorrhage, urinary tract infections and emesis (Irvine, 1961). Complex mixture of phenolic compounds including billavonoids, xanthones, benzophenones and related triterpenes has been reported as constituents of this genus (Iwu et al., 1990; Geiger and Quine, 1988; Waterman and Hussain, 1983). The antimicrobial activity of this plant is attributed to the benzophenones and flavonones (Hussain et al., 1982; Iwu et al., 1987). It is also known to have anti-viral, anti-inflammatory, anti-diabetic, bronchio-dilator and anti-heptotoxic attributes. Fruits extracts from *G. kola* have proven effective at stopping the deadly Ebola virus replication in laboratory tests (Wikipedia Contributors, 2006). The sap of *G. kola* is used for the treatment of parasitic skin diseases while the latex is orally ingested for the treatment of gonorrhoea. It is also useful in the eradication of guinea worm infestation (Ofakansi et al., 2008).

The fruits of *G. kola* in Cote d’Ivoire are sold in local markets or exported to Sahelian countries where the fruits are in high demand. The export of fruits permits the development of commercial activities that has increased the household incomes of many families. To face the increasing demand, drastic methods are used to collect the fruits. In most of the cases, trees are cut down instead of climbing up or using long pole to collect fruits. The harvesting of wood and roots has contributed to the scarcity of the population of *G. kola* in natural forests. *G. kola* is found in a spontaneous status in natural forest. To prevent its disappearance, it is preserved in forest reserves or domesticated. Even in the forest reserves, *G. kola* is still endangered as people enter the forest to collect fruits by cutting the trees.

The flowering that takes place in December to March leads to production of immature green fruits between May to June. The second development phase occur in August to September with the formation of intermediate mature fruit with partly orange colour and finally the last phase that lead to mature fruit with totally orange fruits occur in October to November. These three maturity stages are not strictly separated, so that the maturity stages overlap from one to another. Seed development is in accordance with fructification stages that are expressed by colours. In fact, when fruits are green, seeds are white, not well developed (smooth) and are difficult to be extracted from the pulp. At the partly orange stage, seed are extractible from the pulp with a well-developed seed coat and a consistent (hard) body. In the last stage where fruits are totally orange, seeds have the same consistency as in the previous stage with a more distinguishable seed coat (Agyili et al., 2007).

As aforementioned, the fruiting phenomenon takes place over many months. Conventionally, it is required to wait till the stage of ripe fruits to obtain seedlings or more time due to the fact that the degeneration of flesh surrounding seeds (pulp) before germination occurs, could take one to two months. Due to the long periods it takes for the seeds to germinate, it’s of paramount importance to evaluate ways to shorten time for the seeds to germinate.

Most of species of the genus *Garcinia* are sensitive to desiccation, exhibit low viability and short-lived, and are described as being recalcitrant (Morton, 1987; Chacko and Pillai, 1997; Geeta et al., 2006). Studies conducted on *G. kola* indicated that this species reduced its viability to about 10% at approximately 25 to 27% in moisture content categorizing it as recalcitrant in storage behaviour (Asomaning et al., 2011).

The available literature gives contradictory information concerning the germination of *G. kola* seeds. Some authors (Okafor, 1982; Kengue and Ndo, 2003; Mbolo, 2002) describe *G. kola* seed as easy to germinate, while others (Gyimah, 2000; Adebisi, 2004; Anegbeh et al., 2006) describe it as exhibiting a high degree of dormancy. According to observations made in Cote d’Ivoire, germination in *G. kola* could start at least three to four months in ideal conditions and last ten to eleven months after sowing (Agyili et al., 2007). Such duration seems obviously relatively long compared to most tree species or non-wood forest products. In such conditions, reducing germination duration seems quite crucial as *G. kola* tends to be domesticated according to products and off products of economic value related to the specie.

The overall objective of the study was to contribute in
the preservation of *G. kola* from extinction by domestication. Specifically, the research conducted aimed at shortening the germination duration process by exploring germination of seeds from different maturity stages during fruiting in order to quicken the process of producing planting material.

MATERIALS AND METHODS

Sites characteristics

Experiments were conducted at the Central Laboratory of Biotechnology of the Centre National de Recherche Agronomique (CNRA) located at Adiopodoumé PK 17 (North Abidjan - Côte d’Ivoire). This site is approximately 50 km from the fruit collection site (Yapo forest). Both sites are located in the southern region of Côte d’Ivoire. Yapo forest is located South-East of Côte d’Ivoire (5° 43'13 "N, 4° 9'0" W). It is a protected forest area with equatorial climate, very humid, characterized by long (from April to mid-July) and short (from September to November) rainy seasons with an average rainfall of 1400 mm separated by dry seasons. The maximum temperature is between 29 and 32°C.

Plant material

Fruits of *G. kola* were collected from four trees at three different stages of maturity namely immature green fruits of 6 weeks old, intermediate, partly orange-green fruits of 10 weeks old and from mature, fully orange fruits of 14 weeks old (Figure 1). Encrusted seeds were extracted from fruits by removing the fleshy mesocarp surrounding them (Figure 2). Fruits have a thick skin and a pulp that must be removed. Removing these parts before extracting seeds was found to be very difficult for all the three stages.

Experimental procedure

Germination tests were conducted in the laboratory and seeds were sown in sand or in sawdust at 1 cm depth with 2 replicates of 25 seeds each. A factorial experimental design comprising two factors was implemented. Factors were the maturity stage of the seeds (with three levels: immature, intermediate and mature) and the substrate (sawdust and sand). For desiccation tests, two (2) replicates of 65 seeds each were used for the three stages of maturity. Their weight was followed up for 30 days. Moisture content of intermediate and mature seeds was assessed using 50 seeds according to ISTA (2007). In each phase of maturity, 50 seeds divided into 5 replicates of 10 seeds each were analysed within a period of 4 weeks.

Data collection

Initial characteristics (weight, length, width, water content and moisture content) of seeds were determined before the germination tests. Seeds from different maturity stages were extracted from fruits and full seeds with testa were weighed using Sartorius electronic balance. Length and width of seeds were determined using “Pied à coulisse” instrument. Water content was determined according to ISTA (2007).

For germination tests, data on the germination rate, the presence or absence of adventitious roots as well as shoots and the time taken for them to appear were recorded. Germination process was

Figure 1. Different stages of maturity in *Garcinia kola* fruits: (A) green fruits (immature seeds); (B) partly orange (intermediate mature) fruits, and (C) fully orange (mature) fruits.

Figure 2. Cross section of ripe fruit of *Garcinia kola* showing encrusted seeds patterns in mesocarp and the extracted seeds.
Seeds weight increased with the maturity. Seeds from green fruits showed a relative low weight compared to those from intermediate fruits. The later fruits were found to have lower weight than the mature fruits. It was observed that water and moisture content varied with the stages of maturation. Mature seeds had a lower water and moisture content than that of intermediate fruits. These seeds recorded lower values compared to immature seed. The moisture content of intermediate mature fruits was observed to be close to that one of mature fruits but very different compared to immature fruits (Table 1). As shown, seeds weight increased according to maturation process. However, an opposite observation was made with water and moisture content. In this case, mature seeds expressed lower water and moisture content than that of intermediate ones that indicated lower values of these two characteristics compared to non-mature seed. Physiological phenomena occurring during seed maturation could explain such situation whereby maturity is related to water lost and seed tissues firmness acquired (Fiona and Roger, 2003). When considering the length, intermediate fruits registered a higher value than both mature and green fruits. Moisture content decrease could be related also to tissue maturity acquirement.

Mean weight (of about 5.9 g) and moisture content (37.2) of mature seeds from Côte d’Ivoire during the current study were found to be inferior to that from Ghana (Agyili et al., 2007; Asomaning et al., 2011). This result showed the diversity of seed characteristics that could be linked to the distribution area of the Garcinia kola species that extend from Sierra Leone to Congo (Vivien and Faure, 1985; Eyog-Matig et al., 2007).

### RESULTS AND DISCUSSION

#### Seeds characteristics

Seeds from green fruits did not germinate, on sawdust and sand substrates. Germination occurred earlier (9 weeks) on sawdust compared to 14 week on sand substrate in mature seeds. Seeds sown in sawdust germinated faster than those sown in sand (Table 2). The germination occurred from fully orange fruits after 5 weeks while they appeared 8 weeks after sowing in the partly orange ones. Mature seeds recorded a relatively high number of germinated seedlings compared to seeds from partly orange fruits despite the same evolution of seed germination curves (Figure 3).

The primary (false) root appears first (one week) followed by the root and the shoot (ninth week) (Figure 4) which occurred 9 weeks on sawdust and 14 weeks on sand (Table 2). The mature seeds recorded the highest percentage (56%) of roots appearance, while the intermediate mature seeds recorded a lower percentage of 36%. Concerning the shoot appearance, a rate of 28% was recorded for the intermediate mature seeds at the 12th week, while at the 13th week mature seeds registered

#### Seeds germination tests

<table>
<thead>
<tr>
<th>Duration (Week)</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
<th>16</th>
<th>17</th>
<th>18</th>
<th>19</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substrate</td>
<td>Sawdust</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Sand</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>7</td>
<td>15</td>
<td>3</td>
<td>5</td>
<td>1</td>
</tr>
</tbody>
</table>

### Data analysis

Data (weight, length, width and moisture content) were statistically analysed for significance by analysis of variance with mean separation by Newman-Keuls test and means were separated using a threshold at 5% level of significance with Statistical software (release 7.5).

### Table 1. Weight, length, width and moisture content of Garcinia kola seeds at different stages of maturity.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Weight (g)</th>
<th>Length (cm)</th>
<th>Width (cm)</th>
<th>Moisture content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immature</td>
<td>2.72 ± 0.69b</td>
<td>3.45 ± 0.34a</td>
<td>2.02 ± 0.21a</td>
<td>65.10 ± 40.37a</td>
</tr>
<tr>
<td>Intermediate</td>
<td>3.88 ± 1.09b</td>
<td>4.02 ± 0.49a</td>
<td>2.26 ± 0.22a</td>
<td>40.37 ± 2.60b</td>
</tr>
<tr>
<td>Mature</td>
<td>5.88 ± 0.85a</td>
<td>3.28 ± 0.23a</td>
<td>2.52 ± 0.13a</td>
<td>37.18 ± 2.19b</td>
</tr>
</tbody>
</table>

Values represent means ± SE. Means within a column followed by different letters are significantly different at P = 5%.

### Table 2. The effect of sowing substrate on shoots appearance in Garcinia kola mature seeds.

<table>
<thead>
<tr>
<th>Duration (Week)</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
<th>16</th>
<th>17</th>
<th>18</th>
<th>19</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substrate</td>
<td>Sawdust</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Sand</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>7</td>
<td>15</td>
<td>3</td>
<td>5</td>
<td>1</td>
</tr>
</tbody>
</table>

followed up when seeds were sown. Seeds were removed from substrates to evaluate germination stage on a weekly basis. The time of root or shoot emergency and the number of seeds germinated were recorded. Desiccation tests were conducted with seeds from fruits at the three stages of maturity. Desiccation was carried out in a desiccators whose upper compartment contained seeds while the lower compartment was filled with silicagel. The weight of these desiccated seeds was measured daily till the weight became stable.

### Data analysis

Desiccation tests were conducted with seeds from fruits that extend from Sierra Leone to Congo (Vivien and Faure, 1985; Eyog-Matig et al., 2007). This result was made with water and moisture content. However, an opposite observation was made with water and moisture content.
Figure 3. Germination trend of *Garcinia kola* seeds (from intermediate mature: partly orange and mature: fully orange fruits) on sawdust. Intermediate stage: mature seeds from partly orange fruits. Mature stage: mature seeds from fully orange fruits.

Figure 4. Germination process steps from mature seeds of *Garcinia kola* on sawdust: One week (A), 9\textsuperscript{th} week (B) and 13\textsuperscript{th} week (C).

the highest (40\%) shoot appearance percentage (Table 3). The highest (40\%) number of roots was observed at 13\textsuperscript{th} week in mature seeds and the intermediate mature seeds recorded a lower (24\%) number of roots at the
Table 3. Percentage of shoot and root appearance according to stage of maturity of seeds from *Garcinia kola* observed over 16 weeks.

<table>
<thead>
<tr>
<th>Organ</th>
<th>Maturity</th>
<th>6</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>16</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roots (%)</td>
<td>Intermediate</td>
<td>12</td>
<td>08</td>
<td>36</td>
<td>08</td>
<td>00</td>
<td>00</td>
<td>04</td>
<td>00</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>Mature</td>
<td>16</td>
<td>08</td>
<td>56</td>
<td>04</td>
<td>00</td>
<td>00</td>
<td>04</td>
<td>04</td>
<td>96</td>
</tr>
<tr>
<td>Shoot (%)</td>
<td>Intermediate</td>
<td>00</td>
<td>04</td>
<td>04</td>
<td>00</td>
<td>28</td>
<td>24</td>
<td>08</td>
<td>00</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>Mature</td>
<td>04</td>
<td>04</td>
<td>24</td>
<td>12</td>
<td>00</td>
<td>40</td>
<td>08</td>
<td>04</td>
<td>96</td>
</tr>
</tbody>
</table>

Figure 5. Root growth length between seeds from intermediate mature (partly orange) and mature (fully orange) fruits in *G. kola*. Blue colour: Adventitious root length from intermediate mature seeds from partly orange fruits. Pink colour: Adventitious root length from mature seeds from fully orange fruits. Numbers indicate seeds showing shoots.

It was observed that the mature seeds gave a lower root length from 6th to 11th week. However, this trend was reversed after the 12th week (Figure 5). The mature seeds had the highest percentage (96%) germination rate compared to intermediate mature seeds (68%) (Table 3).

Roots and shoot formation appeared at the same period in both mature and intermediate mature seeds. However the mature seeds recorded an highest germination rate than the intermediate mature seeds. It could be assumed that intermediate mature seeds reached the potential maturity needed for germination and they could, therefore, be harvested early, in addition to full mature seeds for propagation, postharvest storage, conservation and marketing purposes (Ofor et al., 2010). It takes two months for the intermediate mature seeds to become mature and thus the use of intermediate mature seeds could be quite advantageous on an economic point of view. Water is required for germination as shown with intermediate maturity seeds that germinated at the same time as mature seeds. But the reduced number of plantlets in intermediate maturity seeds in comparison to mature seeds could be explained by incomplete physiological maturity of these seeds. Nevertheless, the germination characteristics in respect to the two physiological maturity stages could favour the use of intermediate maturity seeds.

The fact that there was high shoot development in mature seeds at the 13th week, in comparison to intermediate mature seed, could be explained by the maturity stage (Fiona and Roger, 2003). Related to the substrate, sawdust used alone or in combination with forest soils appeared as a good substrate for seed germination. This result is in agreement with that of
Desiccation tests

During these tests, it was observed that the weight of seeds decreased and was negatively correlated to the stage of maturity. The weight of immature seeds was drastically reduced when compared to that of intermediate and mature stage fruits (Figure 6).

The similarity of weight decrease in mature and intermediate mature seeds suggests that the two maturation stages led to a comparable physiological maturity. Earlier work on germination tests and desiccation tests were carried out in mature seeds (Agyili et al., 2007; Ofor et al., 2010), while this study, permitted a comparative analysis of germination and desiccation conditions in seeds from partly orange fruits and seeds from fully orange fruits. Basically, desiccation tests are conducted to confirm whether the seeds are orthodox or recalcitrant. In general orthodox seeds are less sensitive to desiccation in comparison with recalcitrant ones that do not germinate after removal of water. G. kola seeds belongs to the genus Garcinia that is described as having recalcitrant species (Morton, 1987; Chacko and Pillai, 1997). Thus, during the current study, the intermediate mature and mature seed did not germinate after desiccation. These results are contrary to those of Asomaning et al. (2011), who found that the seeds were able to germinate up to 30% with low moisture content. This could be probably due to the difference in the experimental conditions. The G. kola seeds require higher moisture to germinate compared to orthodox seeds where germination occur at 5% moisture content after desiccation.

Conclusion

The exploration of the three stages (Green, partly orange and totally orange) of maturity of G. kola fruits in accordance with seed maturity (immature, intermediate maturity and full maturity) showed that intermediate mature seeds had a comparable behaviour with mature seeds in terms of germination. We conclude that, partly orange fruits contain intermediate mature seeds whose physiological potential is high. These seeds could be harvested to for postharvest storage, conservation and germination for marketing purposes and propagation of planting materials. Such intermediate mature seeds could allow gain of time to make available sufficient quantity in the process of plant material production. The mature seeds of G. kola seeds are known to have numerous medicinal values and we recommend that there is need to evaluate the composition of intermediate mature seeds using chemical, organic, alkaloid, antioxidant and biochemical tests.

Conflict of Interests

The authors have not declared any conflict of interests.
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