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Germination of Garcinia kola (heckel) seeds in response to seed sectioning, chemical pretreatment and different temperatures

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Key words: Garcinia kola, dormancy, seed sectioning, chemical pre-treatment, germination temperature.

Abstract

Garcinia kola Heckel is a multi-purpose tree widely used in West Africa resulting in its over-exploitation, the species is extinction-threatened. On farm conservation through cultivation has been recommended. However, seeds of the species can take about 18 months to germinate. This study aims at generating information on how the combinations of the techniques of seed sectioning, chemical treatment and temperature can be used to enhance germination of the seed. Seed sections and whole seeds pretreated with dormancy breaking chemicals were germinated on a gel of 1% water agar at 20, 25, 30 and 35°C. Statistical design used in the investigation was a completely randomized design in a 5×7×4 factorial (germination materials × chemical treatments × temperature). Germination data showed significances ($p<0.001$) namely: germination materials, germination temperatures, germination materials x germination temperatures, germination materials x chemicals, germination temperatures x chemicals.

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Introduction

Garcinia is a tropical plant genus including several species in Africa, America and Asia. These species are commonly useful for many purposes. The seeds of G. kola have pharmacological uses in treating coughs, throat infections, bronchitis and hepatitis (Farombi *et al.*, 2005) The seeds which serve as a bitter stimulant also serve as snake repellent when they are placed round the compound (Nair, 1990). Other medicinal uses include: purgative, antiparasitic, antimicrobial. The seeds are used to prevent and relieve colic, cure head or chest colds.

This plant has shown bronchodilator effect (Orie and Ekon 1993), anti-inflammatory, antimicrobial, antibacterial and antiviral properties (Akoachere *et al.*, 2002). In laboratory tests, Garcinia kola was found to halt the deadly disease caused by Ebola virus in its tracks. The virus causes Ebola hemorrhagic fever - an often-fatal condition (Anonymous, 1999). Compounds from the plant have also proved effective against some strains of flu, a contagious respiratory disease also commonly known as influenza (Iwu, 1993). Its by-products are also useful: the wood makes excellent fuel wood; its dense rounded crown makes it an ideal tree for shade around homestead; the branches are used as chewing stick because of its bitter taste and antibacterial activities of its extracts (Taiwo *et al.*, 1999). The bark of the stem is used in the tanning and dyeing industry (Irvine, 1961).

Because of its high interest resulting in its over-exploitation, Garcinia kola is extinction-threatened in several West and Central African countries such as Ivory Coast (FAO (1996), Ghana (Wong, 1997), Congo and Cameroon (Tchatat, 1999). It is therefore useful to undertake on farm conservation by small holder farmers through agroforestry systems in order to decrease the pressure on wild population of the species. However, the major difficulty in Garcinia kola propagation as for several species of Garcinia genus is related to seeds germination. Due to dormancy in Garcinia, seeds can take as long as 18 months to germinate (Aduse-Poku *et al.*, 2003). Some studies to investigate seed germination of some species in the genus Garcinia has been done at the farmer's level and under laboratory conditions. These include Garcinia gummi-gutta (Geeta *et al*, 2006); Garcinia indica (Malik *et al.*, 2005); Garcinia kola (Agyili *et al.*, 2007; Kanmegne and Omokolo 2008).

The present work aims at generating information on how the combination of the techniques of seed sectioning, chemical treatment and temperature can be used to enhance the germination of G. kola. The information will be useful in raising large quantities of seedlings for several farming communities in Ghana who are being encouraged to plant the species on their cocoa farms as a shade crop and as a means of conserving the species.

Materials on methods

Fruit collection and processing

Fruit samples were collected in August 2016 at full maturity when they had turned yellow from trees growing on three cocoa farms located at New Edubiase in the Ashanti Region of Ghana. Fruits were packed in jute bags filled with moisten sawdust and quickly transported to the processing site. These measures were to prevent fruits from drying as Garcinia kola seed has been described as desiccation sensitive. Sorting of fruits was carried out by manual removal of damaged, infected and immature fruits. Matured cleaned fruits were placed in tap water in a large plastic bowl for 2 days after which seeds were extracted by depulping. A total of 1200 seeds of the desired quality were obtained after extraction. Seed samples for the initial moisture content determination were extracted from fruits without placing fruits in water.

Seed moisture content determination

Twenty (20) seeds drawn from the sample specially extracted without placing fruits in water were used for this experiment. Moisture content of seeds was determined gravimetrically. Five whole seeds in four replications were used. Each seed was cut into 2 halves using a sharp knife to facilitate effective drying in the oven. Cut seed samples were dried in the laboratory oven at 103°C for 17 hours (ISTA, 1999) and cooled after

drying for 45 minutes in a desiccator over silica gel. Dried samples were weighed again and moisture content expressed as a percentage of fresh weight.

Preparation of seed and seed sections for germination

Five hundred seeds were drawn from the bulk sample and seeds cut into the following germination materials (sections/fragments) using a sharp kitchen knife.

- Distal End Cut, DEC (90% of the seed)
- Proximal End Cut, PEC (90% of the seed)
- Proximal Section, PS (50% of the seed)
- Half Proximal Section, HPS (25% of the seed)
- Whole Seed, WS (100% of seed)

By a careful examination of the seed, it is observed that one end of the seed is thicker than the other end. The proximal end of the seed is the thicker end whilst the smaller end is the distal end (Agyili *et al.*, 2007).

Treatments applied to the seeds and seed sections

Each of the above seed/ seed sections were treated by immersion in the following chemicals dissolved in distilled water for 24 hours at 25°C prior to the germination testing. In the control treatment no chemicals were applied to seeds and seed sections.

- Citric acid ($C_6H_8O_7$) {1g/l}
- Citric acid ($C_6H_8O_7$) {2g/l}
- Gibberellic acid (GA_3) {500mg/l}
- Gibberellic acid (GA_3) {1000mg/l}
- Potassium nitrate (KNO_3) {1g/l}
- Potassium nitrate (KNO_3) {1g/l}
- Control (No treatment with chemicals)

Germination trials

Seeds and seed sections removed from the various chemical solutions were placed on a gel of 1% water agar in plastic sandwich boxes with dimensions 17.3cm x 11.3cm x 6cm without rinsing. 25 seeds or seed sections were sown per each chemical treatment in a sandwich box with each treatment being replicated four times. The germination trials were carried out in incubators with constant temperature regimes of 20°C, 25°C, 30°C and 35°C, each with a [12h day / 12h night] photoperiod at the Seed Testing Laboratory of the National Tree Seed Centre located at the CSIR-Forestry Research Institute of Ghana. The statistical design used in the investigation was a completely randomized design in a 5×7×4 factorial (germination materials × chemical treatments × temperature). Germination count was done at 1-week interval from the 4th week after seeds and seed sections have been set for germination till the 11th week. Germination patterns of seeds and seed sections were closely observed. Seeds or seed sections were considered to have germinated when the root or the shoot had emerged from the distal or proximal end of the germinating material. Germination percentage of seeds or seed sections was calculated as follows:

$$\text{Germ percentage} = \frac{\text{Total number of seeds or seed sections which germinated}}{\text{Total number of seeds or seed sections in all replicates}} \times 100$$

Data Analysis

Germination data collected was subjected to a two way analysis of variance (ANOVA) using the Genstat Discovery Edition statistical package to determine significance. Mean separation was done after ANOVA, using standard error of the difference.

Results

Seed moisture content

Seed moisture content measured from samples extracted without soaking fruits in water was 56.8%.

Germination pattern of seed and seed sections

Germination pattern of seed and seed sections of G. kola are summarized in Table 1 and depicted by Fig.s 1a to 1e. All germinating materials which successfully germinated gave rise to complete seedlings. In situations where there was the emergence of seed roots from the distal ends, namely, Whole Seed (WS) and Proximal End Cut (DEC), such roots degenerated soon after the emergence of adventitious roots from the base of the shoots. Proximal Section (PS), Half Proximal Section (HPS) and Distal End Cut off pieces never produced seed roots but rather only adventitious roots from the bases of the shoots at the proximal ends of the seed pieces.

Table 1. Germination pattern observed in seed and seed sections of Garcinia kola.

Germination material (Seed/Seed Section	Germination Pattern Observed
Whole Seed (WS)	First there is the emergence of a primary root from the distal end of the seed. This is followed by the emergence of a shoot from the proximal end. An adventitious root later arises from the base of shoot at the proximal end followed by the degeneration of the primary root. The whole seed successfully gives rise to a complete seedling.
Distal End Cut (DEC)	Emergence of shoot from the proximal end. An adventitious root later arises from the base of shoot at the proximal end. No emergence of roots from the cut distal end is observed. Seed piece successfully gives rise to a complete seedling.
Proximal End Cut (PEC)	There is the growth of a primary root from the intact distal end of the seed piece. This is followed by emergence of a shoot from the cut proximal end. An adventitious root later arises from the base of shoot at the proximal end followed by the degeneration of the primary root. Seed piece successfully gives rise to complete seedling.
Proximal Section (PS)	Emergence of shoot from the proximal end. Adventitious root later develops from the base of the shoot. There is no emergence of a root at the cut end. Seed piece successfully gives rise to a complete seedling.
Half Proximal Section (HPS)	Emergence of shoot from the proximal end. Adventitious root later develops from the base of the shoot. There is no emergence of a root at the cut end. Seed fragment successfully gives rise to a complete seedling.

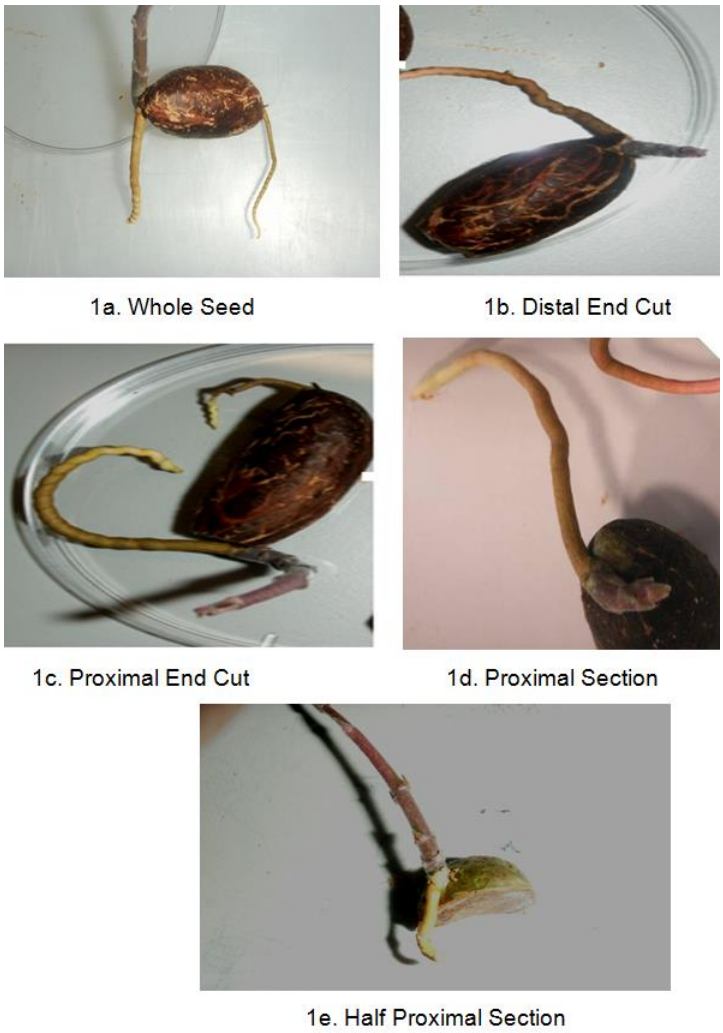

Fig. 1a to 1e. Pictures of germination pattern observed in seed and seed sections of G. Kola.

Effect of germination material on germination of G. kola

Results on the effect of germination material on germination of G. kola is presented in Fig. 2. Germination material had a significant effect on percent germination of G. kola seed ($P < 0.001$). Half Proximal Sections (HPS) of the seed gave a significantly higher germination percentage than all the other treatments. Proximal Section (PS) and the Proximal End Cut (PEC) also gave germination

percentages higher than the others. The lowest germination was recorded by the Whole Seed (WS).

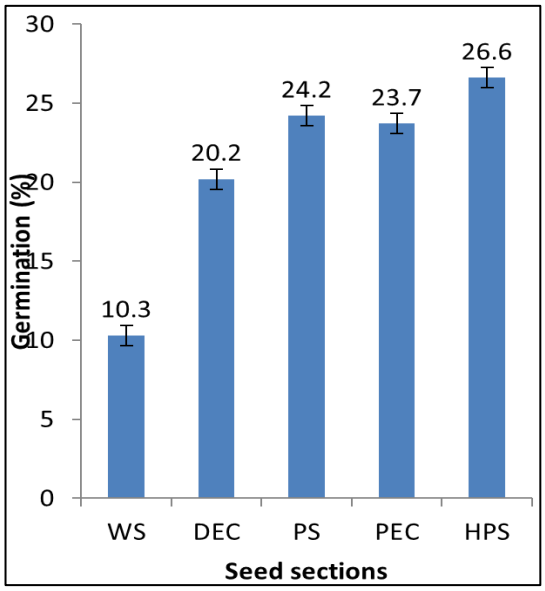

Fig. 2. Effect of germination material on germination of G. kola . Bars represent two standard error of the difference (2 SED).

Effect of temperature on the germination of Garcinia kola

Temperature had significant effect (p< 0.001) on germination of G. kola as shown in Fig. 3. Germination temperatures of 25°C and 30°C gave significantly higher germination percentages than germination at 35°C. The lowest germination percentage of the seed was recorded at 20°C.

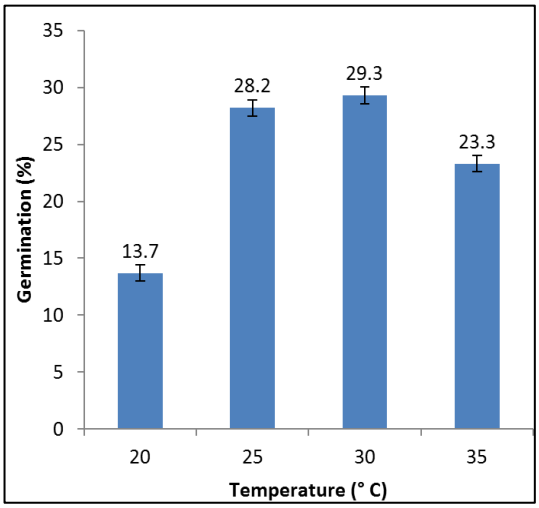

Fig. 3. Effect of temperature on the germination of Garcinia kola. Bars represent two standard error of the difference (2 SED).

Effect of chemical treatment on the germination of G. kola

Chemical treatment of seed and seed sections resulted in significant differences ($p < 0.001$) in germination of G. kola as shown in Fig. 4. Seeds and seed sections treated with GA_3 (1000mg/l) gave the highest germination percentage. This was followed by those treated with GA_3 (500mg/l) and KNO_3 (2g/l) and then those treated with Citric acid (1g/l), Citric acid (2g/l) and KNO_3 (1g/l). The control treatment where seeds and seed sections received no chemical treatment recorded the lowest germination percentage.

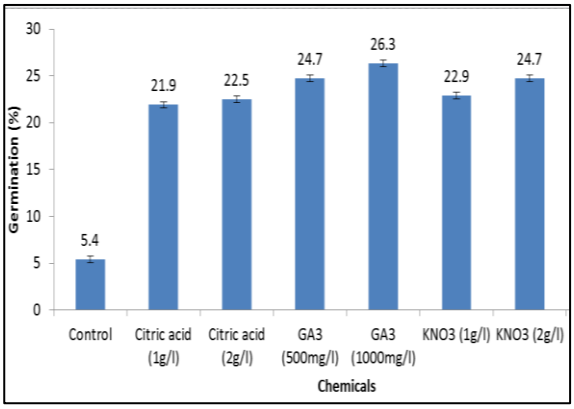

Fig. 4. Effect of chemical treatment on the germination of G. kola. Bars represent two standard error of the difference (2 SED).

Germination temperature and chemical treatment effect on germination of G. kola

The interactions between germination temperature and chemical treatment resulted in significant differences ($p < 0.001$) in germination of the seeds of G. kola. Seed and seed sections treated with chemicals and provided with germination temperatures of 25 and 30°C gave significantly higher germination percentages than those which received chemical treatment but placed at 35°C. Chemically treated seeds and seed sections placed at 20°C recorded lower germination percentages compared to those placed at higher temperatures.

In the control experiment where no chemical treatment was used, germination materials placed at all temperatures recorded the lowest germination percentages as indicated in Fig. 5.

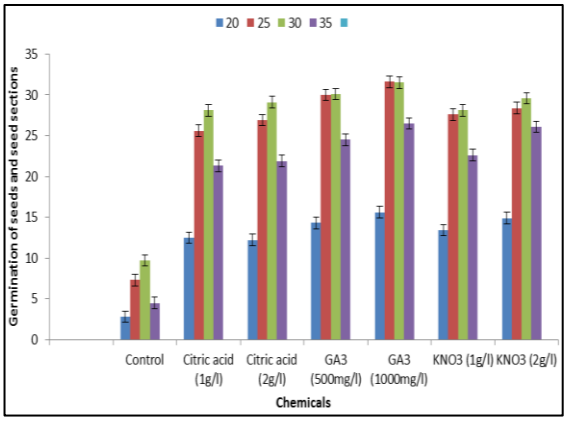

Fig. 5. Germination temperature and chemical treatment effect on germination of G. kola. Bars represent two standard error of the difference (2 SED).

Germination material and chemical treatment effect on germination of G. kola

The interaction between chemicals and germination materials resulted in significant differences ($p< 0.001$) in germination percentages of germination materials. In situations where GA_3 (500mg/l), GA_3 (1000mg/l), KNO_3 (2g/l), Citric acid (2g/l) and the Control were applied, Half Proximal Section (HPS) recorded significantly higher germination than the others.

The germination percentages of Proximal Section (PS) came next to that of Half Proximal Section (HPS) when the chemicals KNO_3 (2g/l), GA_3 (500mg/l) and GA_3 (1000mg/l) were applied to them prior to germination. Whole Seed (WS) gave the lowest germination percentages in all chemical treatments in addition to the control as indicated in Fig. 6.

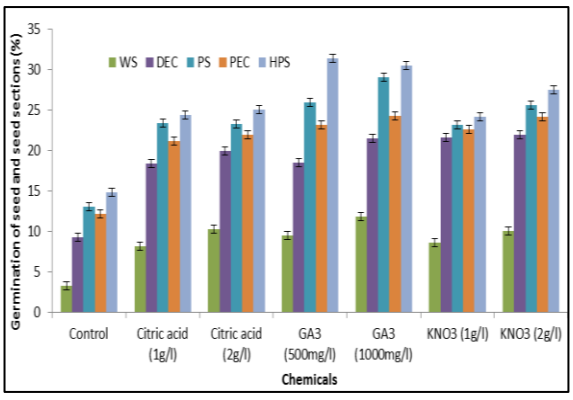

Fig. 6. Germination material and chemical treatment effect on germination of G. kola. Bars represent two standard error of the difference (2 SED).

Effect of germination material and time on the germination of G. kola

The interaction between the kind of germination material and time resulted in significant differences ($p<0.001$) in germination percentages of G. kola as indicated in Fig. 7. There was no significance differences between germination performances of Distal End Cut (DEC), Proximal End Cut (PEC), Half Proximal Section (HPS) and Proximal Section (PS) between weeks 4 and 5. However, between weeks 5 and 6, Proximal End Cut (PEC), Half Proximal Section (HPS) and Proximal Section (PS) significantly performed better than Distal End Cut (DEC). Half Proximal Section (HPS) and Proximal Section (PS) also performed significantly better than Proximal End Cut (PEC) between weeks 6 and 7. Half Proximal Section (HPS) performed better than all the other germination materials between weeks 8 and 11. From week 4 up to week 11, Whole Seed (WS) performed significantly poorer than the other germination materials.

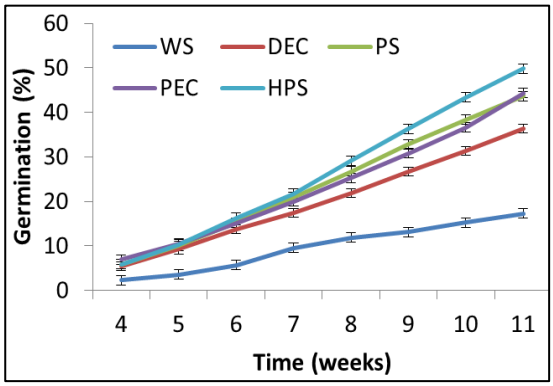

Fig. 7. Effect of germination material and time on the germination of G. kola. Bars represent two standard error of the difference (2 SED).

Effect of temperature and time on the germination of G. kola

The interaction between temperature and time gave significant differences ($p< 0.001$) in germination percentages of G. kola as indicated in Fig. 8. The interaction between the two factors of temperature and time consistently gave significantly higher germination percentages values from week 4 to week 11 for germination materials set at temperatures 25°C and 30°C. Germination at 35°C was also consistently higher than germination at 20°C from week 4 to week 11.

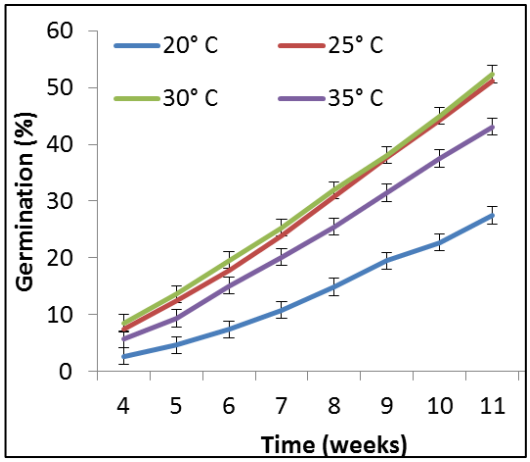

Fig. 8. Effect of temperature and time on the germination of G. kola. (Bars represent two standard error of the difference (2 SED).

Discussions

The initial seed moisture content of 57.8% indicates that seeds matured at very high moisture content. Asomaning (2009), reported that initial seed moisture content of the species was 58.0% during a desiccation work on the seed which has been described as desiccation sensitive. High moisture content at maturity is a characteristic of desiccation sensitive seeds as such seeds do no undergo the process of maturity drying (Chin, 1998).

The germination patterns of seeds and seed sections of Garcinia kola observed in this study is similar to the patterns observed in the species by (Asomaning *et al.*, 2011). whose work however, did not include chemical treatment of the germination materials. This pattern of germination was described as "garcinia-type" of seed germination by de Vogel, (1980). In this pattern of germination, a primary root first arises from the distal end of a whole seed after which a shoot emerges from the proximal end. Prior to leaf differentiation, a robust adventitious root arises from the base of the shoot and takes over as the main root of the developing seedling in place of the primary root which degenerates. Seed sections or seed pieces of species in the Genus, Garcina had the ability to develop into complete seedlings as shown in Table 1. This has also been proven in other Garcinia species including, Garcinia gummi-guta (Geeta *et al.*, 2006) where every section of the seed including the middle sections produced complete seedlings and in G. indica (Malik *et al.*, 2005). The Middle Section (MS) of the seed was not included in this present work because earlier report (Asomaning, 2009), indicated that there was neither the production of roots nor shoots from this fragment. Ofori *et al.,* (2015), also reported of this pattern of germination in Allanblackia parviflora (a species belonging to the family Clusiaceae in which the genus Garcinia is found). This pattern of germination observed among the species of the genus is probably attributed to root-shoot polarity exhibited by the seed (Geeta *et al.*, 2006). Kanmegne and Omokolo (2008), reported that multiple roots, multiple shoots and callus formation could be induced in G. kola seeds by treatment with NAA, BAP and 2, 4-D, respectively. In the present study, treating seeds and seed sections with chemicals did not result in the production of multiple adventitious roots, multiple shoots nor the formation of callus. Sectioning the seed into the germination materials namely: Distal End Cut (DEC), Proximal End Cut (PEC), Proximal Section (PS) and Half Proximal Section(HPS) resulted in improved germination compared to using Whole Seed,(WS).

Seed sectioning can be viewed as a method of mechanical scarification or nicking of the seed which is reported to have dormancy thereby enhancing germination. The observation that the species germinated better between 25 and 35°C is in agreement with the germination temperatures of most tropical species. Smith *et al.* (2002), stated that germination temperatures of 25 to 35°C or even higher is typical of many tropical species. This is probably explained by the fact that in the natural environment dispersed seeds are likely to experience soil temperatures of 36°C or more (Smith, 2002).

A constant temperature of 20°C is not favorable for the germination of most tropical species as noted in this study. However tropical species can germinate well at alternative temperature regimes with 20°C or even lower as one of the alternating temperatures. Asomaning (2009), observed this with Terminalia superba (15/35, 15/40, 40/15 and 20/35°C; Terminalia

ivorensis (15/35, 20/35°C and Khaya anthotheca (20/25, 20/30, 25/15, 30/10, 25/20, 30/15°C) in a series of thermogradient plate experiments.

All the chemical treatment applied had some significant effect on the germination of G. kola even though not to very effective levels these chemicals are reported to have had on other species. However, Kanmegne and Omokolo (2008), reported that none of the growth promoters they used in their work including GA_3 significantly increased the rate of G. kola seed germination. Nzegbule and Mbakwe (2001), also reported that GA_3 treatment was ineffective at enhancing G. kola seed germination. The differences in these reports probably may have come from the types of germination media used as well as the concentrations of these chemicals applied in the various studies.

Seeds and seed sections treated with chemicals and provided with germination temperatures of 25, 30 and 35°C gave significantly higher germination percentages than those which received chemical treatment but were placed at temperature 20°C. The most practicable reason for the observation is that these higher temperatures are more conducive for germinating G. kola which is a typical tropical species. For most tropical tree species, temperatures of 25-30°C are suitable for optimum germination (Daws *et al*., 2004).

The observation that higher germination occurred in Half Proximal Sections (HPS) and Proximal Sections (PS) after treating with chemicals compared with the other seed sections could be due to the following reason. Half Proximal Sections (HPS) and Proximal Sections (PS) offered greater surface area for penetration of water into the impenetrable seed coat as described by Anegbeh *et al*., 2006). This might have enhanced the ability of HPS and PS germination materials to imbibe water necessary for hydrolysing substances stored in the dormant embryos to improve germination. The Proximal End Cut (PEC) pieces also performed well coming next to the Half Proximal Sections (HPS) and Proximal Sections (PS). In preparing the PEC pieces, the proximal end of the seed, which is larger than the distal end was severed exposing a relatively larger surface area for imbibition of water to enhance germination.

In general, germination of all the seed and seed sections studied was erratic under the various chemical treatments and germination temperatures regimes. It took 8 weeks after first germination for HPS, PS, and PEC pieces of the seed to gain cumulative germination of 49.8, 43.6 and 43.3% respectively whilst Whole Seed (WS) recorded a cumulative germination of 17.3% during the same period. According to Watson and Dallwitz (2009), the embryo of G. kola seed is rudimentary at the time the seed is matured. It is not well differentiated into cotyledons and embryonic axis. Most of the seed is a mass of undifferentiated tissue at maturity (Agyili *et al.,* 2007). Asomaning *et al*., (2011), reported that an attempt to locate the embryo of fresh seeds of G. kola through staining by the tetrazolium testing (TZ) was not possible because no staining of the seed parts was achieved. Choudhary (1975), indicated that a rudimentary embryo is one of the factors responsible for poor and erratic germination of seeds. Anegbeh *et al.* (2006), recorded a cumulative germination rate of 100% in a period of 28 weeks for seeds of G. kola which were nicked. Eyog-Matig *et al*., (2007) recorded 40% germination from coated G. kola seeds placed in banana trunk after 20 weeks. The highest cumulative germination of 49.8% recorded for HPS over a period of 11 weeks in the current study may have been more if the experiment had continued for more weeks. The relatively longer period required for the species to gain higher germination percentages could be that the rudimentary embryo takes time to mature as seeds remain on the germination media awaiting germination. Weekly cumulative germination of germination materials recorded at temperatures 25 to 35°C and the final germination percentage 8 weeks after first germination were higher compared to germination recorded at 20°C on 1% water-agar. This is not unexpected as temperatures of 25 to 35°C have been recognized as the optimum temperature range for the germination of most tropical species.

Conclusion

Chemical pretreatment did not affect the germination patterns of seeds and seed sections of Garcinia kola. Sectioning the seed into Proximal End Cut (PEC), Proximal Section (PS) and Half Proximal Section (HPS) resulted in improved germination compared to using Whole Seed (WS). Higher germination occurred in Half Proximal Sections (HPS) and Proximal Sections (PS) after treating with chemicals compared with the other seed sections. In general, seeds and seed sections treated with chemicals and provided with germination temperatures of 25, 30 and 35°C gave significantly higher germination percentages compared to those which received chemical treatment but were placed at temperature 20°C. Germination of Half Proximal Sections (HPS) is mentioned for extra perfection as it has the potential for generating two seedlings from a single seed.

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