Original article

Effect of pre-sowing and incubation treatment on germination of *Garcinia kola* (Heckel) seeds

Emmanuel Nzegbule*, Roy Mbakwe

College of Natural Resources and Environmental Management, Federal University of Agriculture, Umudike, PMB 7267 Umuahia, Nigeria

nzegbule@yahoo.com

Effect of pre-sowing and incubation treatment on germination of *Garcinia kola* (Heckel) seeds.

Abstract — **Introduction**. *Garcinia kola*, a multi-purpose fruit tree, produces fruit, seeds, roots and stem which are extensively used in Nigeria, Ghana and other West African countries for dental care. Cultivation of this fruit tree is limited because of poor seed germination. Our study therefore aimed at improving seed germination properties which will promote cultivation of *G. kola* by rural farmers. **Materials and methods**. Seeds taken from a single tree of *G. kola* were treated with cold water (22 °C), hot water (60 °C) and gibberelic acid (0.5 $g \times L^{-1}$) for various lengths of time. They were then put to incubate in sterilized river sand or a thick transparent polyethylene bag to evaluate the capacities of seed to be germinated under such conditions. In addition, the seed moisture content at harvest was also reduced to various levels before incubation in polyethylene bags to determine the critical moisture rate necessary for seeds to germinate. **Results**. The seeds incubated in the river sand without any treatment before sowing had an average time of germination of 71.2 d and a total percentage of germination of 28% whereas those incubated in polyethylene bags had an average time of germination of 25.8 d and a total percentage of germination of 62%. The cold water treatment, irrespective of the incubation method, did not significantly affect either the mean germination time or the total germination percentage. However, it significantly reduced the spreading out of the seed germination to 5.4 d. The seed pretreatment with gibberelic acid prolonged at the same time the average time of germination and the spreading out of the germination. The seed water content at harvest (50.4%) allowed the best rate of seed germination and the shortest spreading out of the germination period. **Conclusion**. The pretreatment of freshly harvested seeds with cold water followed by an incubation in a thick transparent polyethylene bag proved most effective in enhancing the germination of *G. kola* seeds. This procedure has to be recommended to promote the cultivation of this species.

Nigeria / *Garcinia kola* / plant propagation / seed / germination / germinability / seed treatment

Effet des traitements avant semis et des techniques d'incubation sur la germination de graines de *Garcinia kola* (Heckel).

Résumé — **Introduction**. *Garcinia kola*, arbre fruitier polyvalent, produit des fruits, des graines, des racines et une tige, très utilisés au Nigeria, au Ghana et dans d'autres pays d'Afrique de l'Ouest pour les soins dentaires. La culture de l'arbre est pourtant peu courante du fait de la difficulté de germination des graines. L'étude présentée a donc tenté d'améliorer les propriétés de germination de cette graine afin d'encourager la culture de l'arbre par les cultivateurs ruraux. **Matériel et méthodes**. Des graines prélevées sur un même arbre de *G. kola* ont été traitées avec de l'eau froide, de l'eau chaude (60 °C) et de l'acide gibbérélique (0,5 $g \times L^{-1}$) pendant des temps différents. Elles ont été ensuite mises à incuber dans du sable de rivière stérilisé ou dans un sac en polyéthylène transparent épais pour évaluer les capacités de la graine à germer dans de telles conditions. Par ailleurs, la teneur en eau de la graine à la récolte a été ramenée à différents taux avant incubation en sacs en polyéthylène afin de déterminer le taux critique d'humidité nécessaire à la graine pour germer. **Résultats**. Les graines incubées dans le sable de rivière sans aucun traitement avant semis ont eu un temps moyen de germination de 71,2 j et un pourcentage total de germination de 28 %, alors que celles incubées en sacs de polyéthylène ont eu un temps moyen de germination de 25,8 j et un pourcentage total de germination de 62 %. Le traitement à l'eau froide, indépendamment de la méthode d'incubation, n'a pas affecté de manière significative le temps moyen de germination et le taux de germination. Cependant, il a réduit de manière significative l'étalement de la germination des graines à 5,4 j. Le prétraitement des graines avec de l'acide gibbérélique a augmenté à la fois le temps moyen et l'étalement de la germination. Le taux d'humidité à la récolte (50,4 %) a permis le meilleur taux de germination des graines et l'étalement le plus court de la période de germination. **Conclusion**. Le prétraitement à l'eau froide de graines de *G. kola* fraîchement récoltées, suivi d'une incubation en sacs de polyéthylène transparent épais, s'est montré le plus efficace pour améliorer la germination des graines de cet arbre. Ce procédé doit être recommandé pour favoriser la culture de cette espèce.

Nigeria / *Garcinia kola* / multiplication des plantes / semence / germination / faculté germinative / traitement des semences

* *Correspondence and reprints*

Received 21 July 2000
Accepted 29 May 2001

Fruits, 2001, vol. 56, p. 437–442

***Resumen Español**, p. 442*

1. Introduction

Garcinia kola (Guttiferae) is a tree species occurring in the humid rainforest of West Africa [1]. It prevails as a multipurpose tree crop in the home gardens of southern Nigeria. The tree grows to a height of about 14 m and produces reddish yellow, orange-shaped fruit. Each fruit contains 2 to 4 yellow seeds and a sour-tasting pulp. The flowering of the plant occurs between December and January while the fruit matures between June and August. *Garcinia* sp. is highly valued because of its medicinal uses [2–4]. The pericarp is locally used in diarrhea treatment and as an anti-inflammatory agent. Some reports have shown the chemical composition of the fruit, seeds and stem of the plant, suggesting its usefulness in chemotherapy [5, 6]. In Ghana and Nigeria, the most popular use of the stem and root is in producing chewing sticks sold in bundles at local markets. The good dental health of the population in these countries is attributed to the very popular use of these chewing sticks [7]. It has been suggested that the combination of light elements (F, Na, Mg, Al, Cl) and Ca in high concentrations is responsible for the preventive and protective abilities of the chewing sticks for human teeth [6, 8].

Although *G. kola* seeds are used as food, there are strong prospects for using a seed extract of *G. kola* as a substitute for hop extracts in the brewing industries because of chemical similarities in these products [9]. The anti-microbial properties of the seed extract are valuable in controlling *Candida vini* and *Lactobacillus delbruckii* that cause beer spoilage [7]. The increasing demand for *G. kola* seeds, bole and roots for various uses has encouraged people to plant the tree in their homesteads. However, there is a shortage of planting stock as the seeds are difficult to germinate. Consequently, wildlings are used for planting. Unfortunately, as with the majority of indigenous species in Nigeria, *G. kola* has never been investigated as far as seed matters are concerned. Considering the importance of the species and the problem posed by the delayed seed germination, our study aimed at identifying a simple and effective method for improving the germination of *G. kola*.

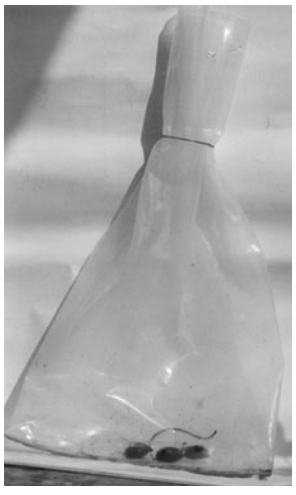

Figure 1.
Thick transparent polyethylene bag used for incubating the seeds of *Garcinia kola*.

2. Materials and methods

Ripe fruits of *G. kola* were harvested from a single parent tree in Umuahia, Nigeria (5° 38' N, 7° 20' E). Seeds were extracted soon after harvest using a knife. A total of 5000 seeds was extracted and washed with water to remove all adhering pulp-materials. The mean fresh weight of the seeds was determined before they were air-dried for 36 h. The seeds were divided into five samples of 200 seeds and exposed to five pre-treatments which included soaking in 0.5 g gibberellic acid (GA_3) $\times$ L^{-1} for 24 h; soaking in cold water (22 °C) for 48 h; soaking in warm water (60 °C) for 8 h and two controls without soaking the seeds : one using river sand and one using a polyethylene bag.

After the treatment, the samples were further divided into sub-samples of 100 seeds each and either sown in germination trays filled with a sterilized river sand or incubated in a thick transparent polyethylene bag of 0.35 mm in thickness (*figure 1*). Both the germination trays and the polyethylene bags were kept in a room with an average temperature of 25 °C and moistened daily with distilled water.

The experiment was conducted as a split-plot design with five replications. The incubation methods formed the main plots and the pre-treatments represented the sub-plots.

Daily observation was made of signs of a protruding epicotyl. Seeds with an epicotyl of up to 10 mm were scored as having germinated. Data collected were the time length between the germination of the first and the last seed (i.e., germination spread), the mean germination time (Mgt), and the total germination percentage. The mean germination time was obtained from the formula: $Mgt = (t \times n) / n$ [10], where t was the number of days starting from the day "0", and n, the number of seeds completing the germination on day t.

Investigation into the effect of the moisture level on the *G. kola* seed germination was conducted by oven-drying seeds of *G. kola* at 35 °C till moisture levels (%/weight)

Table I.
The effect of seed pre-treatment (soaking in cold water, warm water or gibberellic acid) and incubation method (in river sand or a polyethylene bag) on the mean germination time (Mgt), germination spread and cumulative germination of *Garcinia kola*.

Treatments	Mgt (d)	Germination spread (d)	Cumulative germination (%)
River sand	71.2 b	32.6 c	28.2 a
Polyethylene bag	25.8 a	12.2 b	62.4 bc
Cold water then river sand	69 b	27.2 c	31.6 a
Cold water then polyethylene bag	18.2 a	5.4 a	69.8 c
GA_3 then river sand	96.6 c	44.8 d	26.2 a
GA_3 then polyethylene bag	64.4 b	18.2 b	54.2 b
Warm water then river sand	0	0	0
Warm water then polyethylene bag	0	0	0

Figures followed by the same letter(s) along the columns are not significantly different from each other according to the Duncan test (5%).

of 50.8%, 40.6%, 35.1%, 30.6%, 20.2% and 10.3% were achieved. A sample of 50 seeds at each moisture level was kept in thick polyethylene bags and moistened. The germination percentage was determined after 6 weeks. All the germination data were analyzed using the analysis of variance procedure [11]. A Duncan test was applied for means comparison.

3. Results

The mean fresh weight of the seeds was 5.9 g and the moisture content when freshly harvested was 50.8% of the weight. About 10 d from the inception of incubation, signs of germination in the *G. kola* seed started with protrusion of a primary root at the posterior end of each seed. The mean germination time in the river sand and in the thick polyethylene bags was 71.2 d and 25.8 d, respectively (*table I*). The shortest mean germination time of 18.2 d was achieved when seeds treated with cold water were incubated in a polyethylene bag. The mean germination time increased to 96 d when seeds were incubated in river sand after GA_3 treatment.

There was no germination of seeds soaked in warm water. The mean germination of seeds incubated in river sand and the polyethylene bags were 28% and 62.4%, respectively. The immersion of the seeds in cold water or GA_3 did not influence the germination in either method of incubation (*table I*).

The spread of germination decreased from 32.6 d to 5.4 d with cold water pretreatment (*table I*). The widest seed germination spread was 44.8 d caused by seed pre-treated with gibberellic acid. Untreated seeds incubated in polyethylene bags had a significantly shorter germination spread than those incubated in river sand.

Freshly harvested seeds with a moisture percentage of 50.8% produced the maximum germination (68%) and no seed germinated at 20.2% moisture level (*table II*). At 30.6% seed moisture content, the germination was significantly reduced by 74.7% compared to that of freshly harvested seeds.

4. Discussion

Garcinia kola has a relatively prolonged period of seed germination due to an apparent existence of dormancy, and this, as in other indigenous trees, creates a problem of restricted use [12, 13]. The chemical

Table II.
The effect of the seed moisture level on the germination percentage of *Garcinia kola* seeds.

Moisture content (%)	Total germination (%) ± S.E.
50.8	68.1 a ± 7.2
40.6	56.2 a ± 8.5
35.1	22.6 b ± 4.4
30.6	12.8 b ± 5.0
20.2	0
10.3	0

Figures followed by the same letter along the columns are not significantly different from each other according to the Duncan test (5%).
S.E.: standard error.

examination of some other seeds has proved the existence of substances associated with germination inhibition [14, 15]. Such substances include the phenol group (C = C, O-H) which has been found in *G. kola* seeds [6, 8]. It appears that chemical inhibitors could be playing a significant role in the poor germination of the *G. kola* seeds.

Seed incubation in thick transparent polyethylene bags remarkably enhanced both the mean germination time and the seed germination percentage of *G. kola*. The mean germination time of *G. kola* was reduced by more than a half and the total germination percentage doubled when the polyethylene bag was used instead of the conventional river sand. It has been reported that tropical seeds need peculiar requirements for germination: stable humidity, adequate aeration and at times light [16], for instance. The transparent polyethylene bag perhaps promoted the seed germination of *G. kola* through the moderation of these major factors.

The gibberellic acid prolonged the mean germination time by 25 d; however, the total germination percentage at the end of the trial was similar to those of untreated seeds. A similar suppression of sprouting was reported on *Dioscorea* sp. when it was treated with gibberellic acid [17, 18]. The effect of soaking of the seed in cold water was insignificant in both improving the total germination percentage and the mean germination time. However, incubation of seeds treated with cold water in polyethylene bags increased the germination percentages by 8%. It was observed that the seed imbibition rate increased when seeds were soaked in water before incubation and this similarly contributed to the enhanced germination of *G. kola* [19].

Both the pre-treatment and incubation methods used influenced the spread of the seed germination of *G. kola*. Some authors have reported that a very short germination spread protects seedlings from pathogen infestation as well as encouraging the uniformity of seedling stands [13, 20]. The contribution of the cold water treatment and the incubation with the polyethylene bag to the achievement of a shorter germination spread, as obtained in this trial, appears to relate to a consistent moisture availability needed for embryo activation and, perhaps, helped in reducing the effects of germination-inhibiting substances. A low moisture content seems to affect irreversibly the seed germinability of *G. kola*. Freshly collected seeds with the highest moisture percentage had the maximum germination percentage and a decline followed as the moisture level decreased. Storage of *G. kola* before sowing would affect germinability, particularly if the moisture content was not being conserved [21]. A local method of using layers of red clay for storing seeds of *G. kola* [22] from the harvest time (June) to a time of higher market price (January) may not be favoring seed germinability.

The pre-treatment of freshly harvested seeds with cold water followed by an incubation in a thick transparent polyethylene bag proved most effective in enhancing the germination of *G. kola* seeds. This procedure, which is economical and fast, has to be recommended to promote the cultivation of this species.

References

[1] Keay R.W.J., Onochie C.I.A., Stanfield D.F., Nigerian trees, vol. 2, Dep. For. Res., Ibadan, Nigeria, 1964.

[2] Irvine F.R., Woody plants of Ghana, Oxf. Univ. Press, Oxford, UK, 1961.

[3] Nattaya C., Kazuya T., Yasushi O., Shigeo N., Tomihisa O., Mangostanol, a prenyl xanthone from *Garcinia mangostana,* Phytochemistry 43 (5) (1996) 1099–1102.

[4] Hiroyuki M., Emi T., Mitsuaki K., Yoshiyasu F., Three xanthones from *Garcinia subelliptica*, Phytochemistry 41 (2) (1996) 629–633.

[5] Eka O.U., Studies in the feasibility of replacing hop with other bittering substances in brewing, Niger. J. Microbiol. 20 (1984) 84–89.

[6] Achinewhu S.C., Ogbonna C.C., Hart A.D., Chemical composition of indigenous wild herbs, spices, fruits, nuts and leafy vegetables used as food, Plant Foods Hum. Nutr. 48 (1995) 341–348.

[7] Leakey R.R.B., Farmers top – Priority fruit trees, Agroforest. Today 2 (3–4) (1995) 11–15.

[8] Olabanji S.O., Makanju O.V. Haque A.M.I., Buoso M.C., Ceccato D., Cherbini R., Moschini G., PIGE-PIXE analysis of chewing sticks of pharmacological importance, Nucl. Instrum. Meth. B 113 (1996) 368–372.

[9] Ogu E.O., Agu R.C., A comparison of some chemical properties of *Garcinia kola* and hops for assessment of *Garcinia* brewing value, Bioresource Technol. 54 (1995) 1–4.

[10] Bewley J.D., Black M., Seeds physiology of development and germination, Plenum Press, New York, USA, 1986.

[11] Steele R.G.D., Torrie J.H., Principles and procedures of statistics, 2nd edition, McGraw Hill, New York, USA, 1982.

[12] Schaefer C., Storage of tree seed in Kenya – Recommendations and problems, Proc. 1st Natl. Tree Seed Workshop, Kenya For. Seed Cent., Muguga, Kenya, 1992.

[13] Piotto B., The effects of scarification on the germination of the seeds of *Trachycarpus fortunei* (Hookes) Wendl, in: Some L.M., de Kam M. (Eds), Tree seed problems, with special reference to Africa, Proc. IUFRO Symp., Ouagadougou, Burkina Faso, 1992, pp. 154–160.

[14] Brookman-Amissah J., Coumarin like substances in the fruit of *Terminalia ivorensis* A. Chev. inhibit its germination, in: Proc. 2nd Int. Symp. physiology of seed germination, Gov. For. Exp. Stn, Tokyo, Japan, 1976.

[15] Lompo M., Étude de La dormance et identification des inhibiteurs de germination ou de croissance au niveau des semences de *Terminalia avicenniodes* Guill. et Perr., *Terminalia macroptera* Guill. et Perr. et *Terminalia mantaly* Perr., mém. ITDR, Univ. Ouagadougou, Burkina Faso,1990.

[16] Come D., Rôle des facteurs du milieu dans la germination et la survie des semences, in: Some L.M., de Kam M. (Eds), Tree seed problems, with special reference to Africa, Proc. IUFRO Symp., Ouagadougou, Burkina Faso, 1992, pp. 154–160.

[17] Wickham L.D., Extension of dormancy in cushcush yams (*Dioscorea trifida*) by treatment with gibberellic acid, Trop. Sci. 28 (1988) 75–77.

[18] Nnodu E.C., Alozie S.O., Using gibberellic acid to control sprouting of yam tubers, Trop. Agric. (Trinidad) 69 (1992) 329–332.

[19] Ellis R.H., Hong T.D., Roberts E.H., Tao K.L., Low moisture-content limits to relation between seed longevity and moisture, Ann. Bot. 65 (1990) 493–504.

[20] Senaratne R., Seresinhe P.S.J.W., Effect of seed treatment on the germination of lpil-lpil (*Leucaena leucocephala* (L.) Benth) seeds, Beitr. Zurtropi. Landwirtsch. Vet. (Germany) 22 (1) (1984) 69–72.

[21] Wang B.S.P, Scheer G.C, Coleman S.J., Effects of moisture content and storage temperature on germination of white spruce seeds, in: Some L.M., de Kam M. (Eds), Tree seed problems, with special reference to Africa, Proc. IUFRO Symp., Ouagadougou, Burkina Faso, 1992, pp. 234–238.

[22] Miranda I.D., Ekarika C.J., Chemical evaluation of the nutritive value and changes in ascorbic acid contend during storage of the fruit of “bitter kola” (*Garcinia kola*), Food Chem. 54 (1995) 67–72.

Efecto de tratamientos antes de la siembra y de técnicas de incubación en la germinación de semillas de *Garcinia kola* (Heckel).

Resumen — **Introducción**. *Garcinia kola*, árbol frutal polivalente, produce frutos, semillas, raíces y un tallo, muy utilizados en Nigeria, Ghana y otros países de África occidental para los cuidados dentales. Sin embargo, el cultivo del árbol es raro debido a la dificultad de germinación de las semillas. El objetivo de este estudio era intentar mejorar las propiedades de germinación de esta semilla para favorecer el cultivo del árbol entre los campesinos. **Material y métodos**. Las semillas, tomadas de un mismo árbol de *G. kola*, fueron tratadas con agua fría, agua caliente (60 °C) y ácido giberélico (0,5 g $\times$ L^{-1}) durante tiempos diferentes. Posteriormente, se pusieron a incubar en arena de río esterilizada o en una bolsa de polietileno transparente espesa para evaluar las capacidades de germinación de la semilla en dichas condiciones. Por otra parte, el contenido de agua de la semilla durante su cosecha se llevó a diferentes tasas, antes de la incubación en bolsas de polietileno, para determinar la tasa crítica de humedad que necesita la semilla para germinar. **Resultados**. Las semillas incubadas en arena de río, sin ningún tratamiento antes de la siembra, tuvieron un tiempo promedio de germinación de 71,2 d y un porcentaje total de germinación del 28%, mientras que aquellas que se incubaron en bolsas de polietileno tuvieron un tiempo promedio de germinación de 25,8 d con un porcentaje total de germinación del 62%. El tratamiento con agua fría, independientemente del método de incubación, no afectó de manera significativa al tiempo promedio de germinación y a la tasa de germinación. No obstante, redujo de manera significativa el escalonamiento de la germinación de las semillas a 5,4 d. El pretratamiento de semillas con ácido giberélico aumentó, a la vez, el tiempo promedio y el escalonamiento de la germinación. La tasa de humedad en cosecha (50,4%) permitió la mejor tasa de germinación de semillas y el escalonamiento más breve del período de germinación. **Conclusión**. El pretratamiento con agua fría de semillas de *G. kola* recién cosechadas, seguido de una incubación en bolsas de polietileno transparente espeso resultó ser el más eficaz para mejorar la germinación de las semillas de este árbol. Este procedimiento debe aconsejarse para favorecer el cultivo de esa especie.

Nigeria / *Garcinia kola* / propagación de plantas / semillas / germinación / poder germinativo / tratamiento de semillas