

# Germination of *Garcinia kola* (Heckel) seeds in response to different hormone treatments

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## Germination of *Garcinia kola* (Heckel) seeds in response to different hormone treatments.

**Abstract — Introduction.** Despite its socio-economic importance, cultivation of *Garcinia kola* Heckel is very limited due to poor seed germination. The literature gives contradictory information concerning this fact. Our study therefore aimed at (1) evaluating the variability in germination traits among seeds collected from different areas of Cameroon; and (2) testing the efficiency of some hormone treatments in improving the seed germination rate, which would promote cultivation of *G. kola* by rural farmers. **Materials and methods.** Six collections of seeds originating from six locations in Cameroon were subjected to pre-sowing treatments; soaking for 3 days at room temperature in cool distilled water (control), or in cool distilled water supplemented with either 10<sup>-4</sup> M gibberellic acid (GA<sub>3</sub>), 10<sup>-4</sup> M naphthalene acetic acid (NAA), 10<sup>-4</sup> M 2,4-dichlorophenoxyacetic acid (2,4-D), 10<sup>-4</sup> M benzylaminopurine (BAP) or 10<sup>-4</sup> M kinetin; they were then placed to germinate in laboratory conditions. Cumulative seed germination data were recorded for 30 weeks. **Results.** The pre-germination treatments had profound effects on the phenology of *G. kola* seed germination. Multiple shoots, multiple roots and callus formation were induced from seeds soaked in BAP, NAA and 2,4-D solutions, respectively. Analysis of variance showed a significant effect ( $p < 0.01$ ) of seed collection on the germination velocity. Although the rate of germination was higher and the complete dormancy period lower in seeds treated with NAA than in seeds with other treatments, none of these seed treatments significantly enhanced germination. **Conclusion.** Variations in phenology responses of *G. kola* seeds to hormone treatments indicate that the tissues of this plant may be responsive in *in vitro* culture. Variations in seed germination velocity among collections may explain the current controversy over *G. kola* seed germination, and could help in further selection and domestication processes of this species.

**Cameroon / *Garcinia kola* / plant propagation / seeds / dormancy breakers / germination / plant growth substances**

## Germination des graines de *Garcinia kola* (Heckel) en réponse à différents traitements hormonaux.

**Résumé — Introduction.** En dépit de son importance socio-économique, la culture de *Garcinia kola* Heckel est très limitée du fait de la faible germination de ses graines. La littérature fournit des informations contradictoires sur ce sujet. Notre étude a donc cherché (1) à évaluer la variabilité des caractéristiques de germination parmi des graines collectées dans différentes régions du Cameroun ; (2) à tester l'efficacité de quelques traitements hormonaux pour améliorer le taux de germination des graines, ce qui favoriserait la culture de *G. kola* par les agriculteurs. **Matériel et méthodes.** Six lots de graines provenant de six localisations différentes du Cameroun ont été soumis à des traitements avant semis ; immersion pendant 3 jours à la température ambiante dans de l'eau fraîche distillée seule (contrôle), ou dans de l'eau fraîche distillée additionnée de 10<sup>-4</sup> M d'acide gibbérellique (GA<sub>3</sub>), 10<sup>-4</sup> M d'acide naphthalène (ANA), 10<sup>-4</sup> M d'acide 2,4-dichlorophénoxyacétique (2,4-D), 10<sup>-4</sup> M de benzylaminopurine (BAP) ou de 10<sup>-4</sup> M de kinétine ; les graines ont alors été mises à germer dans les conditions ambiantes du laboratoire. Des données cumulatives de la germination des graines ont été enregistrées pendant les 30 semaines suivantes. **Résultats.** Les traitements de prégermination ont eu des effets prononcés sur la phénologie de la germination de graines de *G. kola*. Des tiges multiples, des racines multiples et la formation de calcs ont été induites à partir des graines immergées dans les solutions de BAP, ANA et 2,4-D, respectivement. L'analyse de la variance a montré un impact significatif ( $p < 0.01$ ) de l'origine des lots de graines sur la vitesse de germination. Bien que le taux de germination ait été plus haut et la période de dormance plus courte pour les graines traitées avec l'ANA que pour les graines soumises aux autres traitements, aucun traitement n'a augmenté la germination de manière significative. **Conclusion.** Les variations de la réponse phénologique des graines de *G. kola* aux traitements hormonaux indiquent que les tissus de cette plante peuvent répondre à la culture *in vitro*. Les variations de la vitesse de germination des graines en fonction de leurs lieux de collecte peuvent expliquer la polémique actuelle à propos de la germination des graines de *G. kola* ; Ces variations pourraient orienter les prochains travaux de sélection et de domestication de cette espèce.

**Cameroon / *Garcinia kola* / multiplication des plantes / graine / substance levant la dormance / germination / substance de croissance végétale**

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## 1. Introduction

*Garcinia kola* (Heckel) of the Guttiferae family is a medium-sized tree growing up to 12 m high in 8 years in moist forest throughout West and Central Africa [1]. The seed has a bitter taste and hence the species is locally known as “bitter kola”. It is an economically significant and highly valued tree, used extensively in African traditional medicine. 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 other inflammatory diseases [2, 3]. Seeds are used in the treatment of bronchitis, throat troubles, postpartum haemorrhage, urinary tract infections and emesis [4]. *Garcinia kola* yields a complex mixture of phenolic compounds including biflavonoids, xanthenes, benzophenones and related triterpenes [5–7]. The antimicrobial activity of this plant is attributed to the benzophenones and flavanones [8, 9]. Thus, *G. kola* contributes substantially to the socio-economic uplift of the people of West and Central Africa [10].

In spite of great demand for *G. kola* seeds, its cultivation is not popular owing to the difficulty in germination [10] although *G. kola* is one of the useful indigenous trees prioritised by farmers in West and Central Africa [11].

The literature gives contradictory information concerning the germination of *G. kola* seeds. Authors describe *G. kola* seed as easy to germinate [12–14] as well as difficult to germinate [10, 11, 15]. Nigerian and Ghanaian collections reveal varying results, making it difficult to prescribe a standard procedure for enhancing germination.

In *G. kola*, although the seed coat is neither hard nor thick, a thin, leathery and water-permeable testa surrounds the endosperm. Thus, seed dormancy is suspected to be due to embryo dormancy. In many species, embryo dormancy is released by hormonal treatments using cytokinins, gibberellins or auxins [16]. Information on studies on treatments with hormones is lacking for *G. kola*.

Our study reports the response of *G. kola* seeds to pre-germination treatments using two auxins (naphthalene acetic acid and 2,4-dichlorophenoxyacetic acid), two cytotoki-

nins (benzylaminopurine and kinetin) and gibberellic acid. It involved six seed sources from different geographic areas of Cameroon.

## 2. Materials and methods

Six collections of seeds originating from six locations in Cameroon – Loum in the Littoral province, Yokadouma in the East province, Kumba in the South-west province, Bafia in the Centre province, Sangmelima in the South province and Mamfe in the South-west province – were used.

For each collection, mature seeds were harvested from a single tree in July 2006 and surface-sterilised using sodium hypochlorite (1%) for 10 min, followed by thorough rinsing with distilled water and air drying.

Seed viability was determined using 2,3,5-triphenyl-tetrazolium chloride (TZ). The TZ staining procedure is a standard test prescribed by the Association of Official Seed Analysts (AOSA) to determine the percentage of viable seeds in a lot; it was as follows: one hundred seeds from each collection were hydrated for 24 h at room temperature, cut longitudinally, placed in 0.1% TZ solution, and incubated at 30 °C for 5 h and examined for colour change [17].

Six treatments were studied for each collection resulting from the six locations surveyed. Treatments consisted of soaking seeds for 3 days at room temperature [(25 ± 1) °C] in distilled water (control), or in distilled water supplemented with 10<sup>-4</sup> M gibberellic acid (GA<sub>3</sub>), 10<sup>-4</sup> M naphthalene acetic acid (NAA), 10<sup>-4</sup> M 2,4-dichlorophenoxyacetic acid (2,4-D), 10<sup>-4</sup> M benzylaminopurine (BAP) or 10<sup>-4</sup> M kinetin. The hormone concentration (10<sup>-4</sup> M) and period of treatment (3 days) were determined in a preliminary experiment. Four replicates of one hundred seeds were used per treatment for each collection.

Later, seeds from each lot were washed with distilled water and placed to germinate at room temperature [(25 ± 1) °C] with a [12 h day / 12 h night] photoperiod in 20-cm sterile glass petri dishes lined with two sheets of Whatman No. 1 filter paper. This

photoperiod is the natural photoperiod in central Africa where the *G. kola* plant grows. Seeds were watered when necessary and considered to be germinated once the root or the shoot had emerged.

The complete dormancy period (number of weeks from sowing to start of germination) was noted, percent germination was recorded at 2-week intervals for 30 weeks, and the germination index (GI) was calculated as follows:  $GI = [(germination \% / viability \% ) \times 100]$ . Rate of germination was estimated by using a modified Timson's index of germination velocity: germination velocity =  $[(\Sigma GI) / t]$ , where (GI) is the germination index at 2-week intervals and  $t$  is the total germination period (in weeks) [18, 19]. The maximum value possible for our data using this index was 50, *i.e.*,  $[1500 / 30]$ . The higher the value, the more rapid the germination.

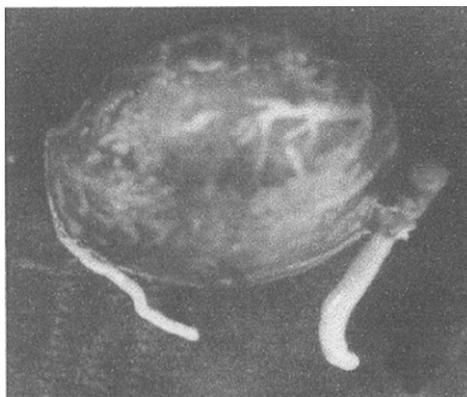
Germination percentages were transformed into arcsine values and dormancy periods into square root values before a statistical analysis was performed using the statistical software package GraphPad Prism Version 3.02 for Windows. A one-way ANOVA was used to determine variability among collections and treatment means, and Bonferroni's test was used ( $P < 0.05$ ) to determine significant differences between means of germination velocity and complete dormancy period.

### 3. Results

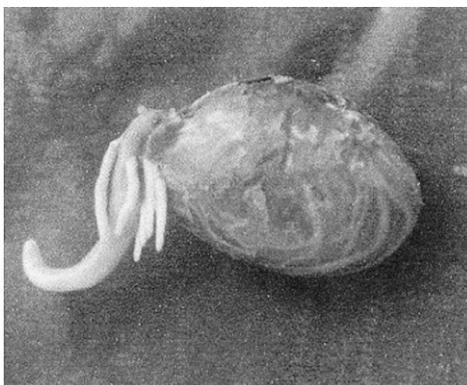
#### 3.1. Phenology of germination

The pre-germination treatments studied had profound effects on the phenology of *G. kola* seed germination. Germination of seeds in control and  $GA_3$  pre-germination treatments showed simultaneous protrusion of a radicle and a shoot apex at the two opposite ends of the ovoid-shaped seed, followed by the induction of a second root at the bottom of the shoot (*figure 1*).

Sixty percent of seeds which germinated under NAA treatments showed a single, thick and ramified root appearing first at one end of the seed (*figure 2*), followed two to four weeks later by the protrusion of the



**Figure 1.** Protrusion of a radicle and a shoot apex at the two opposite ends of a *Garcinia kola* seed and formation of a second root at the bottom of the shoot from seed soaked in distilled water supplemented or not with  $10^{-4}$  M  $GA_3$ .



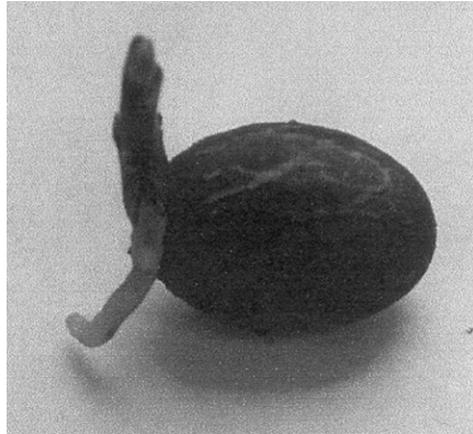
**Figure 2.** Single, thick and ramified root formation from *Garcinia kola* seed soaked in  $10^{-4}$  M NAA.



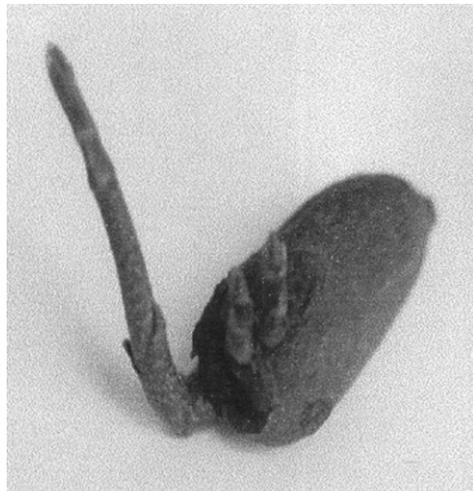
**Figure 3.** Multiple root formation from *Garcinia kola* seed soaked in  $10^{-4}$  M NAA.

shoot at the other end. Multiple root (2 to 5) formation was observed in the other 40% of germinated seeds (*figure 3*).

**Figure 4.** Appearance of a shoot and a root at the same end of the *Garcinia kola* ovoid-shaped seed soaked in  $10^{-4}$  M kinetin.



**Figure 5.** Multiple shoot formation from *Garcinia kola* seed soaked in  $10^{-4}$  M BAP.



**Figure 6.** Development of an undifferentiated tissue from the endosperm, at one end of *Garcinia kola* seed soaked in  $10^{-4}$  M 2,4-D.



For seeds treated with BAP or kinetin, the shoot appeared at one end of the seed, followed 2 weeks later by the appearance of the root at the bottom of the shoot (figure 4). In the case of BAP treatment, 50 percent of germinated seeds developed a single shoot while the other 50% developed one to three supplementary shoots (figure 5).

Seeds treated with 2,4-D swelled and 70% of them developed an undifferentiated tissue from the endosperm (figure 6). Shoot or root protrusion was highly delayed and very poor in terms of percentage with that treatment.

### 3.2. Rate of germination

Analysis of variance indicated significant individual influences of seed collections ( $P < 0.01$ ) and treatments ( $P < 0.05$ ) on the germination rate. Seed collections could be separated into two main groups according to their germination rate: group A, comprising the Loum, Yokadouma, and Sangmelima collections, whose seed germination rates were significantly lower than those of group B, comprising the Bafia and Mamfe collections, which performed best. The Kumba collection belonged to both groups since its index of germination velocity was not different either to those of group A or to those of group B (figure 7).

When considering the six collections taken all together, although the germination rate was higher with seeds treated with NAA, none of the treatments studied significantly enhanced germination compared with the control. Compared with other treatments, 2,4-D treatment significantly slowed germination (figure 8).

### 3.3. Complete dormancy periods

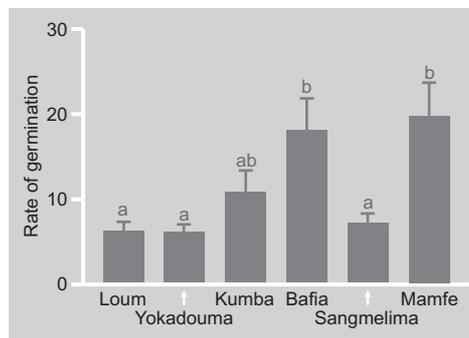
The complete dormancy period of seeds of different sources varied between (10 and 14) weeks and the difference was not significant. Although the complete dormancy period was shortest with seeds treated with NAA, none of the treatments studied significantly reduced this parameter. Moreover, 2,4-D treatment significantly delayed germination compared with other treatments (figure 9).

#### 4. Discussion

Multiple roots, multiple shoots and callus formation could be induced in *G. kola* seeds by treatment with NAA, BAP and 2,4-D, respectively. At least some of these roots and shoots may be adventitious instead of originating from the embryo, which is known to generate a single shoot and radicle. The efficiency of NAA, BAP and 2,4-D in the induction of roots, shoots and callus formation, respectively, from tissues cultured *in vitro*, has been reported previously for a large range of plants [20]. This developmental pattern is being reported for the first time from germinating seeds. These results are an indication of the regeneration potential of the tissues of this plant, which propagates strictly by sexual means [21]. It would therefore be interesting to examine the conditions needed for the regeneration and multiplication of this plant through tissue culture, which could be a useful alternative for mass production of elite plants and the development of new varieties. Our ongoing research work is partly focused on this attempt.

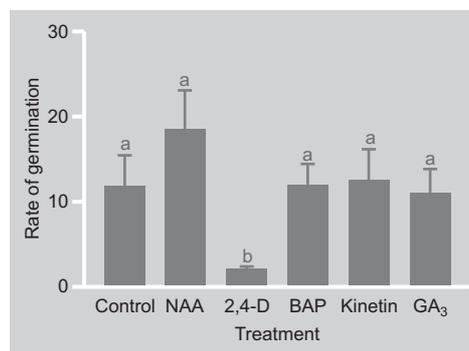
Information on intraspecific variability in *G. kola* is lacking. Our results showed significant differences in seed germination rate among the six studied collections, indicating that seed germination traits may be variable in the species. Similar variations in germination traits have been found in some other tropical species such as *Dalbergia sissoo* and *Acacia nilotica* [22]. These variations may explain the current controversy over *G. kola* seed germination. These results contribute to the knowledge on intraspecific variation in *G. kola* and could help in further selection and domestication processes of this species, which has suffered neglect in the areas of research and development, despite its socio-economic importance [10]. Exploiting natural variation and using seeds which perform the best germination rate could be promising tools for domestication and development of the cultivation of the species.

The cause of embryo dormancy in seeds is widely reported to be due to the high level of abscisic acid (ABA), a growth substance of an inhibiting nature which interrupts gene expression or evokes enzyme inhibition [23–26]. In many species, reversal of



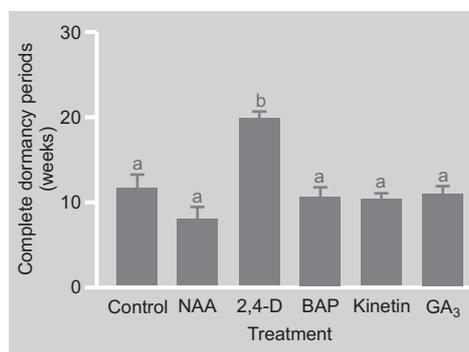
**Figure 7.**

Germination rate of different collections of *Garcinia kola* seeds. Bars represent means  $\pm$  standard error (SEM) of germination rates with six different pre-germination treatments. Bars with the same letter are not significantly different at  $p < 0.05$  (Bonferroni's test).



**Figure 8.**

Germination rate of *Garcinia kola* seeds with different pre-germination treatments. Bars represent means  $\pm$  SEM of germination rates of six of seeds resulting from six collections. Bars with the same letter are not significantly different at  $p < 0.05$  (Bonferroni's test).



**Figure 9.**

Complete dormancy period of *Garcinia kola* seeds with different pre-germination treatments. Bars represent means  $\pm$  SEM of complete dormancy periods of seeds resulting from six collections. Bars with the same letter are not significantly different at  $p < 0.05$  (Bonferroni's test).

ABA inhibition of germination has been attempted by pre-treating seeds with cytokinins or gibberellins [16]. Some species are known for which auxins also seem to play a prominent role in the germination process [27, 28]. None of the growth promoters used in this work significantly increased the rate of *G. kola* seed germination, nor reduced the complete dormancy period. The results from this study are in agreement with those of Nzegebulu and Mbakwe [29], who also reported that GA<sub>3</sub> treatment was ineffective at enhancing *G. kola* seed germination. This indicates that responses of seeds to exogenously applied hormones may vary

with plant species. It seems, therefore, more appropriate not to make a generalisation including all plants about the role of growth promoters in germination and breaking dormancy of seeds.

In relation to the variable responses given by angiosperm seeds to various growth regulator applications, Khan [30] has proposed a model whereby gibberellins, cytokinins and ABA play primary, permissive and preventive roles, respectively, in germination. On the basis of this model, the primary agents are gibberellins, and cytokinins act in the presence of ABA to remove its blockage; gibberellin-mediated germinative processes cannot occur in the presence of ABA unless there are sufficient cytokinins present to overcome its inhibitory effects. This model has been accepted by most authors [24, 31]. There is a need, therefore, to test in further research work Khan's hypothesis in *G. kola* seeds, by studying the effects of associations of cytokinin and gibberellin treatments on seed germination. It would also be interesting to extend these studies to other chemical treatments, such as thiourea and nitrite treatments, which have shown beneficial effects in enhancing seed germination in other species [32, 33].

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### Germinación de las semillas de *Garcinia kola* (Heckel) en respuesta a diferentes tratamientos hormonales.

**Resumen — Introducción.** A pesar de su importancia socioeconómica, el cultivo de *Garcinia kola* Heckel es muy limitado a causa de la floja germinación de sus semillas. La literatura proporciona informaciones contradictorias sobre este tema. Por ello nuestro estudio pretendió (1) evaluar la variabilidad de las características de germinación entre las semillas colectadas en diferentes regiones de Camerún ; (2) testear la eficacia de ciertos tratamientos hormonales para mejorar el índice de germinación de las semillas, lo que favorecería el cultivo de *G. kola* por los agricultores. **Material y métodos.** Seis lotes de semillas procedentes de seis localizaciones diferentes del Camerún se sometieron a un tratamiento antes de la siembra ; inmersión durante tres días a temperatura ambiente en agua fresca destilada sola (control), o en agua fresca destilada con adiciones de  $10^{-4}$  M de ácido giberélico ( $GA_3$ ),  $10^{-4}$  M de ácido naftalenacético (ANA),  $10^{-4}$  M de ácido 2,4- diclorofenoxiacético (2,4-D),  $10^{-4}$  M de bencilaminopurina (BAP) o de  $10^{-4}$  M de quinolina; seguidamente las semillas se colocaron para germinar bajo las condiciones ambiente del laboratorio. Se registraron los datos cumulativos de la germinación de las semillas durante las 30 semanas siguientes. **Resultados.** Los tratamientos de pregerminación tuvieron efectos pronunciados sobre la fenología de la germinación de las semillas de *G. kola*. A partir de las semillas sumergidas en las soluciones de BAP, ANA y 2,4-D, respectivamente, se indujeron múltiples tallos, múltiples raíces, así como la formación de cal. El análisis de la variación mostró un impacto significativo ( $p < 0.01$ ) del origen de los lotes de las semillas sobre la velocidad de germinación. A pesar de que el índice de germinación fuese más alto y el periodo de latencia más corto para las semillas tratadas con el ANA que para aquellas semillas con otros tratamientos, ninguno de los tratamientos aumentó la germinación de modo significativo. **Conclusión.** Las variaciones de la respuesta fenológica de las semillas de *G. kola* a los tratamientos hormonales indican que los tejidos de esta planta pueden responder al cultivo *in vitro*. Las variaciones de la velocidad de germinación de las semillas en función de los respectivos lugares de cosecha pueden explicar la polémica actual sobre la germinación de las semillas de *G. kola*; dichas variaciones podrían orientar los próximos estudios de selección y de domesticación de esta especie.

**Camerún / *Garcinia kola* / propagación de plantas / semilla / interruptores de latencia / germinación / sustancias de crecimiento vegetal**