

Seed Germination and Early Seedling Growth of Bitter Kola (*Garcinia kola* Heckel): a Multipurpose Tree Species

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[Summary]

This study investigated seed germination and seedling growth of *Garcinia kola* under cold-water pretreatments, with sowing media, and under different light conditions. Seeds extracted from mature fruit of *G. kola* were subjected to different treatments: control, soaking seeds in water for 1 wk without changing the water, soaking seeds in water for 1 wk while changing the water daily, and soaking seeds in water for 1 wk while changing the water every other day respectively represented as T1, T2, T3, and T4. Seeds were sown in cured sawdust, and some were put in sealable polythene bags to aid sprouting. Seeds raised in cured sawdust were placed under 3 light conditions: direct light (DL), medium light (ML), and low light (LL). Germination with DL was observed under T1 at 89 d after sowing (DAS), under T2 at 99 DAS, under T3 at 94 DAS, and under T4 at 89 DAS, producing 65% germination in T1 and 60% in the other pretreatments. Under ML, germination began under T1 at 149 DAS, under T2 at 129 DAS, under T3 at 124 DAS and under T4 at 129 DAS, producing 15%, 55%, 45%, and 65% germination, respectively. Also, under LL, seeds germinated in T1 at 154 DAS, in T2 at 135 DAS, in T3 209 DAS, and in T4 at 172 DAS, respectively producing 15%, 20%, 20%, and 40% germination. In the polythene bags, seeds began sprouting across the pretreatments within 2~3 wk of setting up the experiment producing different germination percentages. Seedlings under DL could not be used for growth assessment due to mortality. Student's *t*-test was used to analyze seedling growth data for ML and LL, and it revealed a significant difference ($p \leq 0.05$) at specific assessment periods. ML produced higher mean seedling height, collar diameter, and leaf number compared to LL during the course of the experiment. Incubation in sealable polythene bags aided faster germination of *G. kola* seeds, although a suitable substrate was still required for proper seedling emergence. Also, moderate shade is needed in the early stage to raise *G. kola* seedlings.

Key words: pretreatment, sowing media, incubation, light condition, seedling growth variable.

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研究報告

多用途樹種-藤黃的種子萌發和幼苗早期生長

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摘 要

研究藤黃的種子經過冷水預處理、播種介質和不同光照條件下，種子發芽和幼苗之生長。成熟藤黃果實取出種子，進行不同試驗處理，包括對照(T1)，種子浸水1週而不換水(T2)，種子浸水1週且每天換水(T3)，種子浸水1週並間隔一天換水(T4)。種子播種在經過處理的木屑中，部分放入密封的聚乙烯袋中以幫助發芽。木屑中培育的種子被置於3種光照條件下培養，分別為直射光(DL)、中光(ML)以及低光(LL)。直射光條件下，T1處理種子播種後第89天開始發芽，T2需99天、T3需94天和T4需89天，而其發芽率T1為65%，而其他處理則為60%。中光條件下，T1處理種子播種後第149天開始發芽、T2需129天、T3需124天以及T4需129天，而其發芽率分別為15%、55%、45%和65%。低光條件下，T1處理種子播種後第154天開始發芽、T2需135天、T3需209天以及T4需172天，而其發芽率分別為15%、20%、20%和40%。在聚乙烯袋中，種子經過前處理後的2~3週內開始萌發，產生不同的發芽率。直射光下的幼苗，由於死亡率無法生長評估。t檢定比較中光度與低光度的幼苗生長，結果顯示兩者在試驗期間存在顯著性差異 ($p \leq 0.05$)。在實驗過程中，與低光度相比，中光度環境下，平均幼苗高度較高、苗木基徑較大以及葉片數量多。密封的聚乙烯袋中培育有助於藤黃種子更快發芽，而適合的育苗介質仍然是必需的，育苗初期也需要適度遮蔭。

關鍵詞：預處理、播種介質、孵育、光照條件、幼苗生長變化量。

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INTRODUCTION

Garcinia kola is one of the many tree species of humid rainforests and moist semi-deciduous forests found throughout West and Central Africa (Ajayi and Eyong 2016). It is an indigenous medicinal and economic tree which is highly branched, belongs to the family Clusiaceae, formerly the Guttiferae, and is widespread in evergreen forests. Its common name is bitter kola, which might have been derived from the bitter taste of its seeds. Many tribes in Nigeria have different names for bitter kola such as *orogbo* (Yoruba), *edun* (Benin), *okan* (Ijaw), *aku-ilu* (Ibo), *efiari* (Efik), *efiat* (Ibibio), and *cida-goro* (Hausa).

Various parts of *G. kola* plants have been put to diverse uses. The fruits, seeds, and bark of the plant are extensively used in African traditional medicine to treat various diseases (Ekene and Erhirhie 2014). *Garcinia kola* seeds are chewed to cure cough, dysentery, chest colds, and liver disorders. Also, the pericarp is used as an anti-inflammatory agent and is also used in diarrhea treatment and chemotherapy. The stems and twigs are used to produce chewing sticks in many parts of Africa (Adesina et al. 1995). Seeds of bitter kola are used as food and snacks with a stimulant effect due to their high caffeine con-

tent, and the seed extracts are substituted for hop extract in brewing industries as well as in local alcoholic drinks. They are also used as a flavor enhancer in the beverage industry. The seeds are used as an antidote for food poisoning, snake bites, and overdoses as well as a snake repellent (Ofor et al. 2004). The extract from powdered seeds is made into various forms such as tablets, creams, and toothpaste. Many pharmacological effects are associated with the phenolic compounds found in *G. kola* seeds (Adaramoye et al. 2014).

Garcinia kola is an essential and ancient trade product that has high local market demands which have increased its level of exploitation by humans. *Garcinia kola* is listed in the International Union for Conservation of Nature (IUCN) *Red List of Threatened Species* as "vulnerable", and its population is globally decreasing due to severe fragmentation (Cheek 2004). Similarly, Ajayi (2016) reported that *G. kola* has reduced populations in the wild. Assogbadjo et al. (2017) reported that *G. kola* was classified as one of the priority species that needs evaluation and improvement. The high exploitation of this species is due to the high demand for its seeds and other plant parts used in traditional medicine, direct consumption, confectioneries, and the pharmaceutical industry (Yakubu et al. 2014). It is one of the non-timber forest products valued in national and international markets. Although *G. kola* is an economically important indigenous tree species from which many benefits are derived, its domestication has been hindered by difficulties in raising new seedlings from seed, in addition to a long gestation period before flowering and fruiting. The species is said to be slow-growing (Gyimah 2000). A significant problem that prevents the regeneration of some tropical tree species like bitter kola is that their seeds are recalcitrant. Low germination rates of bitter

kola are a problem for its natural regeneration and in several initiatives to promote seedling production. Its natural regeneration is poor and is grossly insufficient for its sustenance in the wild, rendering the species close to commercial extinction. Similarly, Ogunlade et al. (2013) opined that germination of bitter kola seeds can last for as long as 356 d, particularly with natural regeneration in the wild. It was also reported that regeneration conditions for the species are not well known (Ajayi and Echi 2016).

The dormancy in bitter kola seed is most likely to be endogenous (embryonic) and not exogenous because the seed coat has a thin, leathery, water-permeable testa. Although some authors (Nzegbule and Mbakwe 2001, Anegbah et al. 2006, Eyog-Matig et al. 2007, Yakubu et al. 2014) reported on the germination of this species, there is disparity in the results obtained which has thus necessitated further investigations of the silvicultural requirements of *G. kola*.

MATERIALS AND METHODS

Freshly harvested fruits (Fig. 1) of *Garcinia kola* were obtained from an area of its natural range in Ekiti-State, Nigeria. Ekiti State is located at latitude 7°23'N to 7°46'N and longitude 4°47'E to 5°45'E. The climate of Ekiti State is of the West-African monsoon type with rainy and dry seasons. The rainy season normally begins in March through October with occasional strong wind and thunderstorms, usually at the onset and the end. The dry season usually ranges from November to February, although with occasional variations. The annual rainfall ranges from 750 mm in the northern zone to 1500 mm in the southern zone. The diurnal temperature ranges from 21 to 31 °C with little variation throughout the year. The annual average rela-



Fig. 1. Mature fruit of *Garcinia kola*.



Fig. 2. Depulped and washed seeds of *Garcinia kola*.

tive humidity is about 90% at 07:00 and 65% at 16:00.

Harvested *G. kola* fruits were allowed to soften for about 5 d to facilitate seed extraction. The fruits were split open by hand and de-pulped, and seeds were extracted and rinsed in water (Fig. 2). The seeds were composited with those of comparatively similar sizes selected and subjected to 4 treatments. The 4 treatments were represented as T1, T2, T3, and T4 respectively indicating the control, seeds soaked in water for 1 wk without changing the water, seeds soaked in water for 1 wk while changing the water daily, and seeds soaked in water for 1 wk while changing the water every other day. Seeds were soaked in water as a practical method in this study, because it is easy and safe to adopt unlike some other pretreatment methods, particularly those that use chemicals, which may require special handling and thereby pose risks to rural people.

Sowing pretreated *G. kola* seeds in cured sawdust under various light conditions

In total, 240 seeds were used for this experiment, with 60 seeds drawn for each pretreatment and placed under 3 light conditions. Various light intensities were used to ascertain the light requirement in the early stage of bitter kola seedlings. Tropical tree species differ in their light requirements particularly in the early stage. The light conditions were direct light (DL; 100%), medium light (ML; 75%), and low light (LL; 50%). ML was achieved by making a box with a single layer of 1-mm netting (mosquito net), while LL was achieved with 2 layers of netting. Seeds were sown in seed trays filled with cured sawdust at 2 cm in depth, and these were watered once daily in the morning. Germination was taken to have occurred when the plumule emerged above the soil surface.

Incubation of *G. kola* seeds in sealable polythene bags

In total, 120 *G. kola* seeds with 30 seeds

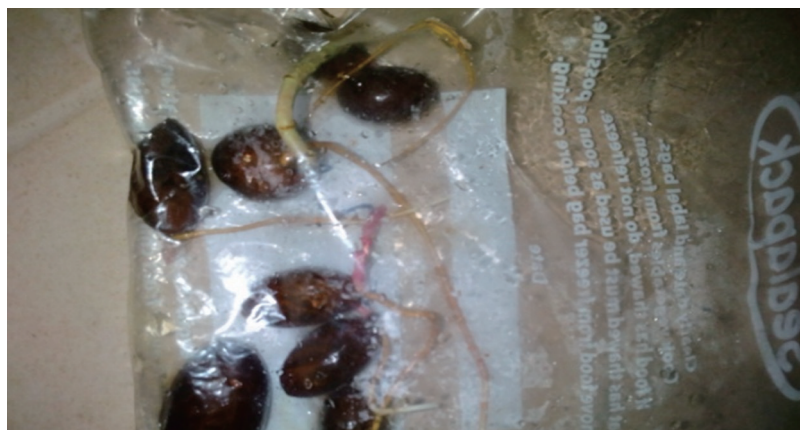


Fig. 3. Sprouted seeds of *Garcinia kola* placed in a sealable polythene bag.

drawn for each pretreatment were placed in sealable polythene bags in 3 replicates to initiate early germination (Fig. 3). These were moistened to prevent the seeds from drying out and placed on a laboratory desk at room temperature. The light intensity in the laboratory was comparable to that of ML (75%). Moistening of the seeds was done when deemed necessary. A seed was taken to have germinated when the radicle (at times both the radicle and plumule) protruded from the seed.

Seedling growth assessment

Uniformly growing seedlings from cured sawdust were transplanted into polythene pots filled with standard nursery soil for growth assessment under various light conditions. However, only seedlings under ML and LL produced data for growth assessment. The height, collar diameter, and number of leaves of seedlings were assessed every 2 wk for 12 wk. Germinated seeds from the sealable polythene bags were also sown in polythene pots containing nursery soil for proper seedling emergence.

It should be noted that although the germination experiments began during the rainy

season, they were not completed until the dry season had begun. The excessive heat of the dry season led to mortality of the germinated seeds under the DL condition. Similarly, seedlings obtained by sowing germinated seeds obtained through incubation and placed under DL suffered mortality due to the same reason aforementioned.

RESULTS

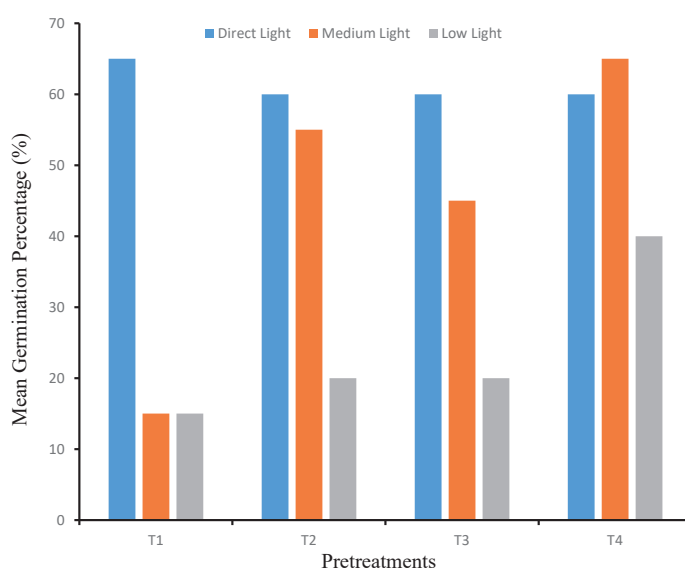
Germination of pretreated seeds sown in cured sawdust under various light conditions

Germination percentages of *G. kola* seeds subjected to different pretreatments and sown under different light conditions are shown in Fig. 4. Under DL, germination commenced on the 89th day after sowing (DAS) for T1 and T4, the 94th DAS for T3, and the 99th DAS for T2 (Table 1). Likewise, germination was completed on the 119th DAS in T2, T3, and T4 with a mean germination percentage of 60% for the 3 treatments. However for T1, germination ended on the 125th DAS with a mean percentage of 65%. In ML, germination began on the 124th DAS in T3 and the 129th DAS in both T2 and T4, while in T1, it began on the 149th DAS. Germina-

Table 1. Mean germination of *Garcinia kola* seeds subjected to different pretreatments and light conditions

Light conditions	Onset of germination (DAS)				End of germination (DAS)			
	T1	T2	T3	T4	T1	T2	T3	T4
Direct light	89	99	94	89	125	119	119	119
Medium light	149	129	124	129	229	247	172	229
Low light	154	135	209	172	229	172	219	247

DAS, days after sowing; T1, control; T2, soaking seeds in water for 1 wk without changing the water; T3, soaking seeds in water for 1 wk while changing the water daily; T4, soaking seeds in water for 1 wk while changing the water every other day.

**Fig. 4. Mean germination percentages of *Garcinia kola* seeds subjected to different pretreatments and light conditions.**

tion was completed on the 172th DAS for T3, and on the 229th DAS for both T1 and T4, while it ended on the 247th DAS for T2. Mean germination percentages of 15%, 45%, 55%, and 65% were respectively obtained for T1, T2, T3, and T4. Also, in LL, germination was first observed on the 135th DAS in T2, the 154th DAS in T1, the 172nd DAS in T4, and the 209th DAS in T3. It was completed on the 172nd, 229th, 247th, and 219th DAS in T2, T1, T4, and T3, respectively. The pretreatments produced germination percentages under the 3 light conditions (Fig. 1).

Germination of *G. kola* seeds incubated in sealable polythene bags

Germination of *G. kola* seeds in sealable polythene bags in T1 began on the 13th DAS and was completed on the 91st DAS with a germination percentage of 73.3%. That of T2 began on the 21st DAS and was completed on the 84th DAS with a germination percentage of 53.3%. Also, sprouting commenced in T3 on the 18th DAS and ended on the 87th DAS with a germination percentage of 46.7%. In T4, sprouting began on the 17th DAS and was completed on the 79th DAS with a mean

germination percentage of 46.7% (Table 2). However, germinated seeds of *G. kola* subjected to incubation could not continue into the seedling growth assessment phase because harsh environmental factors negatively led to mortality as soon as the seedlings emerged.

Seedling growth assessment

Seedlings that emerged under ML and LL were used for growth assessments. As mentioned earlier, seedlings that emerged under DL were killed by excessive heat.

Seedling height

Student's *t*-test showed a significant difference ($p \leq 0.05$) throughout the assessment period between ML and LL, from 2 to 12 wk. Moreover, when mean values for height were examined, ML had the highest mean value of

6.42 cm obtained at 10 and 12 wk, followed by 6.10 cm at 8 wk under ML. Low light produced the smallest value of 2.72 cm obtained at 2 wk (Table 3).

Collar diameter

Student's *t*-test did not reveal significant ($p > 0.05$) differences in seedling collar diameter between ML and LL from 2 to 12 wk across the assessment period. However, when mean values were considered, ML had the highest mean collar diameter value of 0.35 cm obtained at 12 wk, closely followed by 0.34 cm under ML at 10 wk. LL produced the smallest mean collar diameter value of 0.25 cm at 2 wk (Table 4).

Number of leaves

Student's *t*-test indicated significant differences ($p \leq 0.05$) in the mean number of

Table 2. Mean germination percentages of *Garcinia kola* seeds placed in sealable polythene bags

Treatment	Start of germination (DAS)	End of germination (DAS)	Germination (%)
T1	13	91	73.3
T2	21	84	53.3
T3	18	87	46.7
T4	17	79	46.7

DAS, days after sowing; T1, control; T2, soaking seeds in water for 1 wk without changing the water; T3, soaking seeds in water for 1 wk while changing the water daily; T4, soaking seeds in water for 1 wk while changing the water every other day.

Table 3. Student's *t*-test results for seedling height under medium light (ML) and low light (LL) across the assessment period

Assessment period (wk)	Mean \pm mean standard error		<i>p</i> value
	ML (cm)	LL (cm)	
2	5.30 \pm 0.40	2.72 \pm 0.76	0.03*
4	5.45 \pm 0.39	3.10 \pm 0.80	0.04*
6	5.90 \pm 0.47	3.42 \pm 0.62	0.02*
8	6.10 \pm 0.39	3.52 \pm 0.64	0.02*
10	6.42 \pm 0.41	3.62 \pm 0.70	0.01*
12	6.42 \pm 0.41	3.62 \pm 0.70	0.01*

* $p \leq 0.05$.

Table 4. Student's t-test results for collar diameter under medium light (ML) and low light (LL) during the assessment period

Assessment period (wk)	Mean \pm mean standard error		<i>p</i> value
	ML (cm)	LL (cm)	
2	0.28 \pm 0.01	0.25 \pm 0.04	0.52
4	0.30 \pm 0.01	0.27 \pm 0.03	0.36
6	0.33 \pm 0.01	0.29 \pm 0.03	0.34
8	0.33 \pm 0.01	0.30 \pm 0.04	0.46
10	0.34 \pm 0.01	0.30 \pm 0.04	0.35
12	0.35 \pm 0.01	0.31 \pm 0.04	0.35

Table 5. Student's t-test results for the number of leaves under medium light (ML) and low light (LL) across the assessment period

Assessment period (wk)	Mean \pm mean standard error		<i>p</i> value
	ML (cm)	LL (cm)	
2	2.50 \pm 0.28	2.00 \pm 0.00	0.13
4	2.50 \pm 0.28	2.00 \pm 0.00	0.13
6	2.50 \pm 0.28	2.00 \pm 0.00	0.13
8	3.00 \pm 0.70	2.00 \pm 0.00	0.20
10	4.50 \pm 0.28	2.00 \pm 0.00	0.00*
12	4.50 \pm 0.28	2.50 \pm 0.05	0.01*

* $p \leq 0.05$.

leaves at 10 and 12 wk of the assessment period between ML and LL. In contrast, there was no significant difference ($p > 0.05$) from 2 to 8 wk. Nevertheless, consideration of mean values showed that ML and LL had the highest values of 4.50 at 10 and 12 wk, and this was followed by 3.00 under ML at 8 wk, while LL had the smallest value of 2.00 from 2 to 10 wk (Table 5).

DISCUSSION

Germination of *G. kola* seeds subjected to pretreatments and sown in cured sawdust

The germination of *G. kola* seeds sown in cured sawdust was very slow during this

experiment. Also, the germination rate did not improve by pretreatments used compared to the control. This might have been due to the fact that the outer seed coat contains chemical depositions, which prevented water from penetrating the seeds to effect the germination process (Nzegbule and Mbakwe 2001). The main aim of seed pretreatment is to enhance rapid and even germination (Schmidt 2000). However, the effect of different pretreatments in this study did not achieve that aim, for it took about 3 mo before germination and seedling emergence were observed. This even contradicted the time to germination of *G. kola* seeds reported by Oboho and Nwaihu (2016) of 72 d.

Furthermore, observations in this study contradict a report by Yakubu et al. (2014) that physiological dormancy present in *G. kola* can be reduced by soaking its seeds in cold water. This contradiction could be because the testa of *G. kola* seeds used in this study was not removed before soaking in water, whereas Yakubu et al. (2014) removed the testa before the seeds were soaked in water. Eyog-Matig et al. (2007) earlier reported that the germination rate of *G. kola* decreases with a lowering of the seed's moisture content; therefore, dormancy-breaking through seed coat removal and soaking of the seeds in cold water was suggested. Incidentally, soaking of some tropical tree seeds in water improved germination in certain species. Baatuuwie et al. (2019) observed that soaking *Detarium microcarpum* seeds in cold water for 48 h produced the highest germination percentage. Likewise, El-Juhany et al. (2009) reported that *Terminalia catappa* had increased germination percentages and rates after soaking seeds in cold water. However, Danthu et al. (1995) reported that soaking seeds of *Adansonia digitata* in cold water did not aid germination. The response of tropical tree seeds to dousing in water could thus be species-specific.

Seeds incubated in sealable polythene bags

Placing seeds in transparent sealable polythene bags led to the early germination of *G. kola* seeds with the protrusion of both a plumule and radicle in less than 2 wk. The reason could be attributed to the fact that the seeds were not in any substrate unlike the use of an organic substrate such as cured sawdust. Munjuga et al. (2008) recommended treating seeds of *Allanblackia* species in a polybag (black or transparent) as one of the methods for raising *Allanblackia* sp. This is also in agreement with the view of Nzegebule and

Mbakwe (2001) that treating freshly collected *G. kola* seeds with cold water and incubating them in a polythene bag favored a relatively high germination percentage and germination spread. Although *G. kola* seeds placed in sealable nylon germinated early, the germinated seeds had to subsequently be placed in nursery soil for seedling emergence to occur.

Germination and seedling growth under various light conditions

The different light conditions showed variations in germination percentages, with DL having the highest percentage, but the seedlings that emerged died due to scorching heat. Furthermore, light conditions had a significant effect on seedling height and the number of leaves but not on the collar diameter. ML seemed to produce the best height for seedling growth. This result aligns with that of Bolanle-Ojo et al. (2014), who reported that the development of *Kigelia africana* seedlings under an ML condition performed well. Nevertheless, Olayode and Olatunji (2014) and Olayode (2016) observed that LL conditions favored the growth of *Parkia biglobosa* and *Terminalia superba* in the early stage. Moreover, the effects of light on seedling growth variables in this study were similar to those reported by Bolanle-Ojo et al. (2015), that light conditions had a significant impact on the height of seedlings but no significant effect on the collar diameter. The poor performance of *G. kola* seedlings under DL affirmed that *G. kola* may require some level of shading in the early stage. This agrees with findings of Bolanle-Ojo et al. (2019) that *G. kola* requires dense shade with a small amount of light for its early growth. Different tropical tree species vary in their light requirements particularly in the early stage of development. This also influences the stratum occupied by tree species in a mature forest

stand either as an emergent or an understory species.

CONCLUSION

Soaking *G. kola* seeds with intact testa in cold water is not recommended, for it did not appear to aid germination. Incubation of freshly extracted seeds in a sealable polythene bag proved effective in enhancing the germination of *G. kola* despite the fact that the seed coat was not removed. Medium light condition favored *G. kola* seedling growth, and this implies that *G. kola* species requires moderate shade for its early development.

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