

ORIGINAL ARTICLE

Germination and sensitivity to desiccation of *Cola anomala* (K. Schum.) seeds

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Abstract – Introduction. As a step in the process of *Cola anomala* domestication, investigations were undertaken on germination requirements and desiccation tolerance of its seeds. **Materials and methods.** Three seed provenances (Bamenda, Bayangam and Dschang), three substrates (forest top soil, river sand and a mixture of forest top soil and river sand) and two photoperiods (12 h day⁻¹ and continuous darkness) were investigated for their effects on seed germination. To evaluate their desiccation tolerance, fresh seeds were dried at room temperature (24 ± 1 °C) for 16 days during which seed moisture content, seed germination percentage and electrical conductivity of seed leachate were monitored at two-day intervals. **Results and discussion.** Germination rates were significantly ($P < 0.05$) higher both on forest top soil alone (86.04 ± 4.8%), and on a mixture of forest top soil and river sand (83.56 ± 4.5%) than on river sand alone (69.96 ± 4.7%). Seeds from Bamenda had the highest germination (91.4 ± 4.7%) compared to those from Bayangam (77.36 ± 4.7%) or Dschang (70.8 ± 4.8%). As response to seed drying, the mean germination rate started to slightly decrease as moisture got lost, then considerably decreased when moisture content was below 50%. No germination was observed from 32.24% seed moisture. Electrical conductivity of seed leachate exhibited a strong correlation with loss of viability as well as with desiccation. **Conclusion.** There is a significant variation in germination responses between *C. anomala* seed batches. The best substrate for germination is forest top soil supplemented or not with river sand in a 1/1 (v/v) ratio. The seeds of *C. anomala* are particularly desiccation-sensitive which is a significant constraint for conservation storage.

Keywords: Cameroon / *Cola anomala* / seed germination / water stress / electrical conductivity / shelf live

Résumé – Germination et sensibilité à la dessiccation des graines de *Cola anomala* (K. Schum.). Introduction. Des études menées sur les conditions de germination et la tolérance à la dessiccation des graines de *Cola anomala* constituent une étape dans le processus de domestication de cette espèce. **Matériel et méthodes.** Trois provenances des graines (Bamenda, Bayangam et Dschang), trois substrats de germination (sol forestier superficiel, sable de rivière, et un mélange des deux) et deux photopériodes (12 h jour⁻¹ et obscurité continue) ont été étudiés pour leurs effets sur la germination des graines. Pour évaluer leur tolérance à la dessiccation, les graines fraîches ont été séchées à température ambiante (24 ± 1 °C) pendant 16 jours au cours desquels la teneur en eau, le taux de germination et la conductivité électrique du lixiviat des graines ont été suivis tous les 2 jours. **Résultats et discussion.** Le taux de germination des graines a été significativement plus élevé ($P < 0,05$) à la fois sur le substrat de sol forestier superficiel seul (86,04 ± 4,8 %), et sur le mélange avec sable de rivière (83,56 ± 4,5 %), que sur sable de rivière seul (69,96 ± 4,7 %). Les graines provenant de Bamenda ont mieux germé (91,4 ± 4,7 %) que celles de Bayangam (77,36 ± 4,7 %) ou de Dschang (70,8 ± 4,8 %). En réponse à la dessiccation des graines, le taux moyen de germination a commencé à légèrement diminuer avec la perte en eau, puis a chuté considérablement à partir d'un taux d'humidité des graines de 50 %. Aucune germination n'a été observée dès lors que le taux d'humidité des graines a atteint 32,24%. La conductivité électrique du lixiviat des graines de cola est en forte corrélation avec la perte de viabilité ainsi qu'avec la dessiccation des graines. **Conclusion.** Une variation significative de la germination des graines existe entre différents lots de semences de *Cola anomala*. Le sols forestier superficiel, complété ou non de sable de rivière, s'est montré le meilleur substrat de germination. Les graines de *C. anomala* se sont révélées particulièrement sensibles à la dessiccation, ce qui constitue une contrainte significative de stockage pour la conservation de l'espèce.

Mots clés : Cameroun / *Cola anomala* / germination / stress hydrique / conductivité électrique / aptitude à la conservation

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1 Introduction

Cola is a tropical African genus that belongs to the family Malvaceae (sub-family Sterculiaceae) [1]. The genus comprises about 140 species, but the most commonly used are *Cola nitida* (Vent.) Schott & Endl, *Cola acuminata* (Pal. de Beauv) Schott & Endl and *Cola anomala* (K. Schum) [1, 2]. These three species are cultivated by subsistence farmers in association with cacao and/or coffee as shade plants for their edible seeds commonly called kola nuts [3]. Kola nut has for hundreds of years served as an important article of internal trade in Nigeria and other parts of Africa [4]. It has been an item of trade in West Africa and in the Trans-Saharan trade routes for many centuries [5]. The spread of kola nuts has resulted from its reputation as a stimulant, increasing energy and strength, dispelling drowsiness and staving off hunger [6]. These properties have been attributed to the large amounts of caffeine and smaller amounts of theobromine, kolatin and glucose it contains, all of which act as stimulants and may be mildly addictive [5, 7, 8]. It is also used to flavor drinks and in the manufacture of pharmaceuticals, and it is exported to Europe and North America for these purposes [9]. The most recent and remarkable advancement in kola by-product utilization is the use of kola pod husk in the replacement of up to 60 % of the maize used in the formulation of poultry feed [10]. Kola nut is one of the many non-timber forest products that are of socio-economic importance. Its commercialization in both the domestic and national markets raises the standard of living of those involved in its trading activities [5].

Given the socio-economic importance and the possibility of growing these trees in farms, *Cola* spp. (*C. anomala*, *C. acuminata*, and *C. nitida*) have been suggested as candidates for domestication by agroforestry. As an important step in the process of domestication of these species that constitute a vital resource for millions of people in sub-Saharan Africa, researches on seed germination physiology are needed. There have been some attempts to domesticate *C. nitida* and *C. acuminata* through seed handling, plant regeneration via seed and vegetative propagation [11–14]. However, for *C. anomala* there is little knowledge about the biology of this species. It is known that the species naturally regenerate through seeds [1, 2], but the characteristics of seed germination have not been documented to date. Moreover, classification of seed storage behavior has not been made for this last species. Indeed, among the different cola species producing edible nuts, *Cola anomala* has suffered neglect in the areas of research and development despite its socio-economic importance.

This paper reports an investigation on germination and sensitivity to desiccation of *C. anomala* seeds. The aim of the present study which represents a step in the process of *C. anomala* domestication was to determine: 1) the influence of three important factors (provenance, photoperiod and substrate) on seeds germination, and; 2) the desiccation sensitivity and storage behavior classification of seeds

2 Materials and methods

2.1 Seed material

The seeds of *Cola anomala* (K. Schum.) used in the present study originated from three localities situated in the western

highlands of Cameroon: Bayangam (5°18' N, 10°29' E, altitude 1,500 m), Bamenda (5°47' N, 10°10' E, altitude 1,300 m) and Dschang (5°27' N, 10°3' E, altitude 1,400 m). In each locality, mature and disease-free pods were harvested from a minimum of 10 randomly selected trees in March 2012. A minimum of 100 pods (containing 1 to 10 seeds each) was harvested from each site and immediately brought to the laboratory of applied botany, department of Plant biology, university of Dschang (see geographical situation above). In the laboratory, seeds were extracted from pods 24 h after harvest and processed by removing their white and soft testa to obtain red or pink colored fresh nuts, which were used for the determination of initial characteristics (*i.e.*, seed weight, moisture content, electrical conductivity and viability percentage) and for further experiments.

2.2 Viability test

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: Four replications of 25 seeds each from each locality were hydrated for 24 h at room temperature (24 ± 1 °C), then their embryos were excised incubated at 30 °C for 5 h in 0.1% TZ solution and examined for color change [15].

2.3 Moisture content determination

Moisture content determination was done by the oven dry method which consisted in weighing seed pieces before and after drying them in the oven at 103 °C for 17 h. Since *C. anomala* seeds are quite big seeds, instead of using whole seed for moisture content determination, only one of the four to six cotyledon pieces that make up each seed was used. Moisture content, which was expressed as a percentage of fresh weight, was calculated using the formula

$$MC\% = [(FW - DW)/(FW)] \times 100$$

where FW (fresh weight) is the weight of samples before drying and DW (dry weight) is the weight of samples after drying [16]. The value of the moisture content was the mean of six measurements at each time (six replications of one sample each).

2.4 Electrical conductivity of seed leachate

For the conductivity test, a square of seed piece (cotyledon) was cut, weighed and soaked in distilled water in a 100 mL beaker (*i.e.*, 3 g of seed piece in 50 mL distilled water). The beaker was covered and left in the laboratory at 24 ± 1 °C for 24 h. Two beakers with 50 mL distilled water, but no seed piece, were treated similarly as blanks to determine the base conductivity of the water. After 24 h, the seed

piece was strained from the water and conductivity of the water was measured using a HACH Model of Conductivity/TDS Meter. The mean conductivity of the blanks was subtracted from each sample reading. Conductivities were expressed in microSiemens (μS). Six replications were carried out at each time.

2.5 Germination assay

Seeds from each provenance were sown in black plastic perforated polythene bags (20 cm high and 10 cm diameter) filled with substrate which was either forest top soil, river sand or a mixture of forest top soil and river sand in 1:1 (v/v) ratio. As light regime (photoperiod) effect was one of the topics of investigation, sowing was done in such a way that 3/4 of the volume of seed was buried in the substrate while the remaining 1/4 was visible above. The seeded polythene bags were then placed in the nursery ($22 \pm 3^\circ\text{C}$) at two different photoperiods: natural photoperiod (*i.e.* 12 h day⁻¹ and continuous darkness). Darkness was provided by placing the seeded polythene bags in wooden boxes (1 m \times 1 m \times 0.5 m) whose internal walls were lined with black and thick polythene paper.

To investigate on *Cola anomala* seed germination, a total of 1,350 seeds were sown in three blocks of a split-split plot experimental design. Each main plot contained three substrates (Forest top soil, River sand and, 1:1 (v/v) mixture of Forest top soil and River sand), whereas three different provenances (Bamenda, Bayangam and Dschang) were tested at the subplot level. At the sub-subplot level, two photoperiods (12 h day⁻¹ and continuous darkness) were investigated. At each level, treatments were assigned at random to experimental units so as to have 3 substrates \times 3 provenances \times 2 photoperiods \times 3 replications \times 25 seeds.

Manually, water was applied daily to the seeded polythene bags so that the medium (substrate) was kept moist without getting waterlogged. Nuts with radicle protrusions and/or with the emergence of the plumule were recorded as having germinated. Germination was recorded daily, and the experiment was finished after 20 days, when no further germination was observed over a period of two consecutive weeks.

2.6 Desiccation tolerance test

Mature seeds harvested from a single tree in Bamenda were used for the desiccation tolerance test. Fresh seeds were extracted from mature pods 24 h after harvest and processed as described above. They were then spread in a single layer on the laboratory bench top and left to dry under shade at laboratory temperature ($24 \pm 1^\circ\text{C}$). Laboratory relative humidity was $55 \pm 5\%$. Seed samples were withdrawn every 2 days for moisture content measurement, conductivity test and germination test.

Moisture content measurements and conductivity tests were done as described above. For the germination test, three replications of 25 seeds each were performed at 2-day intervals in the conditions of a nursery: at $22 \pm 3^\circ\text{C}$, under natural photoperiod (*i.e.* 12 h day⁻¹ light) and in black polythene bags

filled with forest top soil as substrate. The seeded polythene bags were then regularly watered and germination percentage was computed for 20 days as described above. The non-germinated seeds were then assessed for their viability through TZ test.

2.7 Data analysis

Data analyses were performed using SPSS 12.0 software package. The dependent variable was the mean germination percentage, whose data were transformed into arcsine square root values before statistical analysis. Analyses of variance (ANOVA) were performed to detect the level of significance of the main effects of seed provenance, germination substrate, and photoperiod as well as the significance of their interaction effects. Means that exhibited significant differences ($P < 0.05$) were further compared using Duncan's multiple comparison test or t-test as appropriate.

The 2-tailed Pearson correlation coefficient was used to establish correlations between the different seed variables investigated (moisture content, germination percentage, and electrical conductivity of leachate). The critical water content of seeds was recorded at the point in which the germination significantly decreased whilst the lethal water content was recorded at the point where there was total failure of germination.

3 Results and discussion

3.1 Influences on seed germination

Values of different parameters characterizing seeds from different provenances (*table I*) showed that except for viability percentage which was higher in Bamenda batch ($98.6 \pm 2.3\%$) than in Bayangam ($84.0 \pm 4.0\%$) and Dschang ($81.3 \pm 2.3\%$) batches, there was no significant difference between the three provenances for the other investigated parameters (weight, moisture content and conductivity of leachate). *Cola anomala* seed germination was spread out over the period from day-4 to day-20 after sowing. From the 20th day after sowing, there was no further germination over a period of two consecutive weeks. The rest of the non-germinated seeds were assessed for their viability and the result of the TZ test revealed that none of them were viable.

Germination percentages were significantly ($P < 0.05$) higher both on forest top soil ($86.04 \pm 4.8\%$) and on [forest top soil + river sand] mixture ($83.56 \pm 4.5\%$) than on river sand alone ($69.96 \pm 4.7\%$) (*table II*). Seeds from Bamenda showed a higher germination percentage ($91.4 \pm 4.7\%$) than those from either Bayangam ($77.36 \pm 4.7\%$) or Dschang ($70.8 \pm 4.8\%$). There was no significant difference between the germination percentages for seeds from Bayangam and Dschang. The highest germination percentage ($98.22 \pm 8.2\%$) was recorded with seeds from Bamenda sown on forest top soil, while the lowest ($65.2 \pm 8.4\%$) was recorded with seeds from Dschang sown on river sand. Analysis of variance (*table III*) showed that the germination percentage was significantly ($P = 0.03$) influenced

Table I. Initial characteristics of *Cola anomala* seeds from different provenances in Cameroon. Each value is the mean \pm standard error of n measurements ($n = 25$ for weight, $n = 6$ for moisture content and conductivity) and four replications ($n = 25$ seeds each) for viability.

Provenances	Weight ^y (g)	Moisture content ^y (% Fresh weight)	Viability ^y (%)	Conductivity ^y (μ S)
Bamenda	36.4 \pm 6.7 ^a	67.57 \pm 0.89 ^a	98.66 \pm 2.30 ^a	30.43 \pm 1.33 ^a
Bayangam	38.2 \pm 4.8 ^a	66.53 \pm 1.23 ^a	84.00 \pm 4.00 ^b	31.12 \pm 1.40 ^a
Dchang	34.8 \pm 5.2 ^a	67.08 \pm 1.03 ^a	81.33 \pm 2.30 ^b	30.90 \pm 1.20 ^a

^y Within the same column, values followed by the same letter are not significantly different at the 5% level according to Duncan multiple comparison test.

Table II. Germination percentages of *Cola anomala* seeds from different provenances in Cameroon 20 days after sowing on different germination substrates. Each value is the mean \pm standard error of tree replications ($n = 25$ seeds).

Provenances	Substrates			Provenance main effect
	Top soil	Top soil + River sand	River sand	
Bamenda	98.2 \pm 8.2 ^a	98.0 \pm 8.0 ^a	78.0 \pm 8.2 ^a	91.4 \pm 4.7 ^a
Bayangam	82.4 \pm 8.2 ^b	83.0 \pm 8.2 ^b	66.7 \pm 8.0 ^b	77.4 \pm 4.7 ^b
Dschang	77.5 \pm 8.4 ^b	69.7 \pm 8.2 ^b	65.2 \pm 8.4 ^b	70.8 \pm 4.8 ^b
Substrate main effect	86.0 \pm 4.8 ^{ns}	83.6 \pm 4.5 ^{ns}	70.0 \pm 4.7 [*]	

Within the same column, values followed by the same letter are not significantly different at the 5% level according to Duncan multiple comparison test. ^{ns} No significant difference; ^{*} Significant difference at 5% level.

Table III. ANOVA of the effects of substrate, photoperiod, seeds provenance and their interactions on mean germination percentage of *Cola anomala* seeds in a Substrate \times Provenance \times Photoperiod split-split plot experiment at 20 days after sowing. DF: degree of freedom; S: substrate; Ph: photoperiod; Pr: provenance.

Effect	DF	F-value	P-value
S	2	3.39	0.030
Ph	1	3.29	0.070
Pr	2	5.94	0.003
S \times Ph	2	0.64	0.520
S \times Pr	4	0.32	0.850
Ph \times Pr	2	0.69	0.500
S \times Ph \times Pr	4	0.10	0.980

by substrate and highly significantly ($P = 0.003$) influenced by the provenance, meanwhile the photoperiod had no significant influence on the germination percentage.

Seed provenances have displayed significant differences in germination percentages of *C. anomala* seeds. Some authors [17] showed a relatively low correlation between seed provenances and their germination parameters. In some plant species nevertheless, seeds vary in their degree of germinability between and within populations and between and within individuals [18–20]. Some of this variation has been suggested to be of genetic origin [19], but much of it is known to be phenotypic, caused by the local conditions under which the seed matured. Indeed, germination characteristics are not only affected by the current environmental conditions but also by conditions experienced by the mother plants in the previous generation [18, 21]. The differences in germination data as reported in the present work are nevertheless not sufficient to conclude a provenance-related variation in *C. anomala* seed germination. These differences may be attributed to differential

germination responses for the various samples of populations and individuals, which is very common in seed batches.

Substrates are not limiting factors in seed germination per se in the nursery, but rather the growth of the seedling after germination. Nevertheless, a substrate needs to have adequate aeration and moisture for germinating seeds [22]. The results of the present study showed that forest top soil and mixture of forest top soil and river sand showed a higher germination percentage compared to river sand alone. As seeds were only partially buried, there was no problem of seed aeration. The differences among different substrates for their germination percentage as reported here may be attributed to the differences in moisture retention. It is well known that both forest top soil and the mixture of forest top soil and river sand have higher moisture retention capacity than river sand alone. Germination of *C. anomala* seeds may have been enhanced by the constant moisture condition, which is not possible in sand. Similar results have been reported for seeds of many others species [23, 24], although ISTA [25] has suggested sand as a suitable medium for seed germination in some species.

3.2 Criteria of seed viability

The desiccation tolerance test showed that fresh seeds of *C. anomala* had an initial moisture content of 67.57 \pm 0.89%. As seeds were dried, their moisture content gradually decreased and reached the value of 32.24 \pm 1.08% after 16 days of drying (figure 1). At the same time, germination percentage declined gradually with decreasing moisture content, while the electrical conductivity of leachate increased (figure 2). When the seeds moisture content decreased from 67.57 \pm 0.89 to 50.28 \pm 2.53% (corresponding to the period from 0 to 10 days drying), the germination percentage was maintained between 90 and 55%; meanwhile the electrical conductivity of leachate

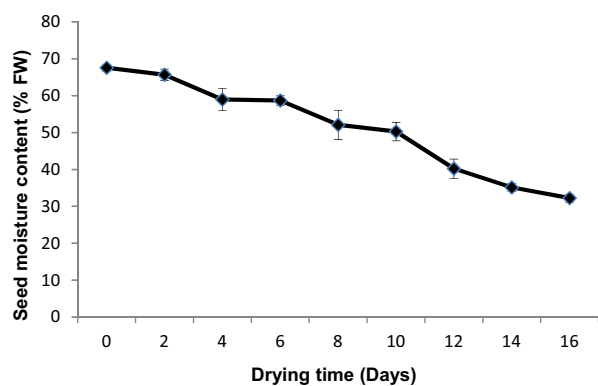


Figure 1. Variation in moisture content of *Cola anomala* seeds during drying. Each value is the mean and standard error of six measurements (FW: fresh weight).

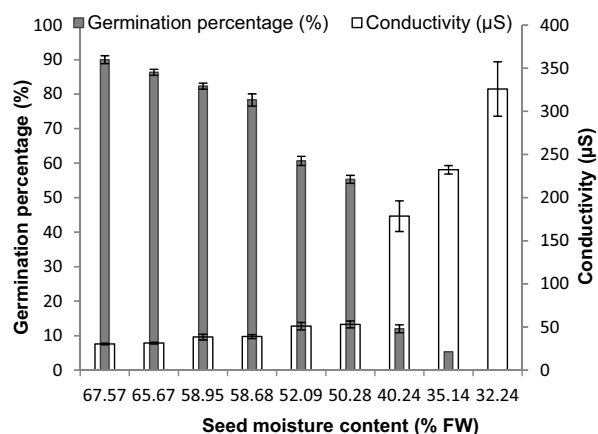


Figure 2. Germination percentage and leachate conductivity of *Cola anomala* seeds at different moisture contents. Each value of germination percentage is the mean and standard error of tree replications ($n = 25$ seeds), while each value of conductivity is the mean and standard error of six measurements.

was maintained between 30.43 and 53.13 μS . The germination percentage then drastically dropped to $12.00 \pm 1.15\%$ when moisture content dropped below $50.28 \pm 2.53\%$ at 12 days drying. At the same time the electrical conductivity of leachate abruptly increased to $178.5 \pm 17.7 \mu\text{S}$. The value of 50.28% was then recorded to be the critical moisture content. After 16 days of drying, the moisture content had reduced to $32.24 \pm 1.08\%$ and germination was 0%. The value of 32.24% was consequently recorded to be the lethal moisture content.

In relation to the desiccation sensitivity test, the moisture content value (67.57%) recorded for fully matured fresh seeds of *Cola anomala* is very high. According to MSBP [26], such moisture content indicates that seeds are potentially immature or are in the post abscission phase. This is however applicable to orthodox seeds which go through the process of maturation drying. Recalcitrant seeds on the contrary do not undergo maturation drying and even after shedding from the mother plant they have high moisture content ranging from 30 to 70%, leading to their wetness [27, 28].

The loss in mean germination percentage as a result of seed dehydration indicated that *C. anomala* seeds can be cate-

Table IV. Two-tail Pearson's correlation coefficients between different *Cola anomala* seed parameters: moisture content, conductivity and germination rate.

	Moisture	Conductivity	Germination
Moisture	1	-0.915**	0.983**
Conductivity		1	-0.937**
Germination			1

** Correlation significant at $P \leq 0.01$.

gorized as desiccationsensitive. This was not surprising, since *C. anomala* seeds exhibit most of the characteristics of recalcitrance such as being fleshy, large in size and produced from plants growing in the humid forest environment [29]. The critical moisture content value of 50.28% recorded in the present study is higher than those reported for many other recalcitrant tree seeds such as *Garcinia kola* [30, 31], *Corypha umbraculifera* [32] as well as *Acer* spp. [33], although similar values were obtained for *Euterpe edulis* seeds [34]. This is an indication that the critical moisture content of recalcitrant seeds varies greatly among species.

Seed leachate conductivity was highly and negatively correlated both to the germination percentage ($r = -0.93$) and to the moisture content ($r = -0.91$), meanwhile the germination percentage and the moisture content were positively correlated ($r = 0.98$) (table IV). Electrical conductivity of seed leachate exhibited a strong correlation with loss of viability as well as with desiccation. It has been suggested that there is a linear relationship between seed desiccation and membrane damage, while electrical conductivity and membrane damage are also directly correlated events [35]. Indeed, if dehydration stress disrupts membrane integrity in desiccationsensitive seeds, then increases in the amount of solutes leaked may be detectable in response to dehydration, and these increases should be associated with loss of viability [36, 37]. The results presented here are complementary to these findings. These results clearly indicate that cellular membranes of desiccation sensitive *Cola anomala* seeds may have been damaged as seeds were dried further, and for that matter increases in the levels of solute leakages were observed. Excessive dehydration of the seeds beyond the critical moisture content may have severely disrupted the integrity of the cellular membranes of seed tissues, resulting in the uncontrollable rate of solute losses from seeds.

4 Conclusion

The *Cola anomala* seeds are clearly desiccation sensitive, with 50.28 and 32.24% as values of the critical and the lethal moisture contents, respectively. Loss in seed viability during dehydration was found to be associated with increased electrolyte leakage following the disruption of cellular membranes integrity. For propagating *C. anomala* from seeds, it is recommended that fresh seeds from mature pods be sown without being previously dried, and that the seeding be done in substrate composed of either forest top soil or a mixture of forest top soil and river sand in a 1:1 (v/v) ratio.

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