Effects of Coconut (*Cocos Nucifera* L.) Drupe Water and Cotyledon Wounding On Seed Germination and Seedling Performance in Kola Nuts (*Cola Spp*.)

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Abstract
Naturally occurring hormones in plant organs and wounding have been demonstrated to induce sprouting in dormant or intact plant organs. However, practical application of phytohormones found in coconut water or induced by wounding of plant organs has not been widely reported to break dormancy in kola nut or to stimulate subsequent growth in the seedlings. This study therefore was undertaken with the objective of evaluating the role of coconut water and wounding in germination of dormant seeds and growth performance of germinated seedlings in kola nuts. The study was a screened-house experiment conducted at the Faculty of Agriculture and Forestry, Cross River University of Technology, Obubra, Nigeria. Treatments were two varieties of cola nuts: C. nitida (V_1) and C. acuminata (V_2), which were either wounded (W_1) or not wounded (W_2) and soaked for 24 hours in coconut water (C_1) or in water (C_2). This gave treatment combinations of 8: (V_1 C_1 W_1, V_2 C_1 W_1, V_1 C_2 W_1, V_2 C_2 W_1, V_1 C_1 W_2, V_2 C_1 W_2, V_1 C_2 W_2 and V_2 C_2 W_2). The 2 x 2 x 2 factorial combinations were replicated three times in a randomized complete block design. The results obtained showed that both wounding (C_1) and coconut water (W_1) significantly enhanced the germination of kola nut. The C_1 x W_1 treatments gave the highest emergence rate in both the species of cola nut seeds (54% in C. nitida and 93% in C. acuminata) compared to when neither of the treatments was applied. Similarly, wounding enhanced the response of kola nut to coconut water treatment, in all the treatment combinations. However, while coconut water alone significantly influenced the vegetative parameters studied, wounding alone had no significant effect on these parameters in the two species of kola nut.

Keywords: *phytohormones, biostimulant, wounding, germination, kola nuts, coconut water.*

Introduction

Kola nuts (*Cola nitida and C. acuminata*) are tropical tree crops in the Family Sterculaceae. They are widely cultivated in the High Rainforest agro-ecology of West Africa (Akinbode, 1982; Asogwa *et al*., 2012). The two species are cultivated mainly for their seeds as sources of caffeine and theobromine alkaloids taken as stimulants, anti-fatigue and for flavouring cola drink (Beatie, 1970, Raven *et al*., 2005). The seeds feature prominently in traditional marriages, burial ceremonies and in everyday entertainment of guests (Isawumi, 2003). Kola nuts are said to be the third most important stimulant after cocoa and coffee (Lovejoy, 1980).

World production of kola nuts was put at 300,000 metric tonnes in 2011, with Nigeria accounting for over 70 percent of that figure (Asogwa *et al*., 2012). This production level is short of demand, either locally or for international trade (Oluwalana *et al*., 2016). The slow rate of expansion has partly been attributed to prolonged seed dormancy, irregular seed germination and poor seedlings performance (Oladokun, 1982). Induction of seed germination by early breaking of seed dormancy and production of vigorous seedlings has been advocated to encourage expansion in production by local farmers.

Hormonal termination of dormancy and cell division initiation has long been demonstrated in many dormant plants and plant parts. Arteca (1996) demonstrated the application of cytokinins exogenously to potato tubers to terminate tuber dormancy. Similarly, Kanmegne and Ndoumou(2008) reported the use auxins, cytokinins and GAs to terminate dormancy in *Garcinia cola*. However, they observed that while auxins and cytokinins terminated the dormancy, GA was ineffective in this species. This indicated that the response to exogenously applied hormones vary among plant species. Synthesized hormones are, however, beyond the reach of the poor and subsistence farmers in the tropics. These farmers have no technological know-how or resources to acquire the hormones, even if they were available. Naturally available hormones in plant organs may be more available to, and within reach of, the farmers.

Coconut water, obtained from drupe of *Cocos nucifera* has been shown to exhibit a wide range of active growth regulatory activities in plants (Yong *et al*., 2009). Kende and Zeevant (1997) and Yong (2009) reported that the growth regulatory agent in coconut water mainly and most importantly cytokinins. Subsequently, Arditi (2008) showed that the active growth regulators in coconut water also included amino acids, nitrogenous compounds, organic acids, enzymes, sugars and lipids. He demonstrated that these extracts play various functional roles in plant growth and development. Plantlets treated with coconut water have been reported to be more active, bigger and more robust than those in the control (Yong *et al*., 2009). Coconut water, therefore, not only initiates cell division and expansion but also supply nutrients that aid plant growth and development.

Cocos nucifera, from which coconut water is obtained, belongs to the Family Arecaceae. It is cultivated in over 90 countries in the tropics (FAOSTAT, 2011). It is easily and cheaply accessible to the peasant, kola nut, farmers in the tropics. Many of these farmers may have a tree or two within their homestead gardens.

Another bioactivity that has been reported to initiate cell division in instant plant organs or whole plants is wounding. Burton (1989) reported long ago that wounding increased sprout time in potato. Similarly, Mader *et al*, (2003) observed that mitosis was initiated within an hour after cutting dormant potato tubers. Subsequently, they extracted cytokinin as the growth stimulator in wounded chickpea.

Practical application of cytokinins found in coconut water or induced by wounding of plant organs has not been widely reported to break dormancy in kola nut or to stimulate subsequent growth in the seedlings. This study therefore was undertaken with the objective of evaluating the role of coconut water and wounding in germination of dormant seeds and growth performance of germinated seedlings in kola nuts.

Materials and Methods

This study was a screened-house experiment conducted at the Faculty of Agriculture and Forestry, Cross River University of Technology, Obubra ($06^0 06^{'}$ N, $08^0 18^{'}$ E), Nigeria.

Treatments were two varieties of cola nuts: *C. nitida* (V_1) and *C. acuminata* (V_2),

which were either wounded (W_1) or not wounded (W_2) and soaked for 24 hours in coconut water (C_1) or in water (C_2). This gave treatment combinations of 8: (V_1 C_1 W_1, V_2 C_1 W_1, V_1 C_2 W_1, V_2 C_2 W_1, V_1 C_1 W_2, V_2 C_1 W_2, V_1 C_2 W_2 and V_2 C_2 W_2). The 2 x 2 x 2 factorial combinations were replicated three times in a randomized complete block design.

A total of 240 freshly harvested and dormant cola nut seeds (120 each for *C. nitida* and *C. acuminata*) were purchased each from the same source at Bebuatsuan village, Ipong, in Obudu, South Eastern Nigeria. The fresh exodermises of seeds of each species were removed, the seeds washed in water and stored to dry for a day at room temperature. Out of the 120 seeds for each species, sixty (60) seeds were wounded and sixty (60) were left unwound. From each species, thirty (30) of the wounded seeds were treated with coconut water and the remaining 30 were treated with ordinary stream water. Similarly, thirty (30) of the non-wounded seeds were treated with coconut water and the other thirty (30) were treated with stream water. Wounding was by cutting off about one-third of the seed leaf (cotyledon) from the tail (basal) end of the seed.

Fresh coconut water was obtained from stored, fully matured coconut fruits that showed activities of sprouting within the batch. All seeds were soaked, as per treatment for 24 hours before planting into polypots.

Large polypots measuring 12 cm x 12 cm were filled with 10 kg of thoroughly heated smooth river sand. Each pot was watered and placed in a screened house. After 24 hours, the pre-treated seeds were sown, one to a pot as per treatment. The pots were arranged as RCBD with three replications. All the treatments were watered every 7 days. At the end of every two weeks, each polypot was inspected for seed emergence. Emerged seedlings were measured with a metre rule from the base of epicotyls to the tallest plant part for plant height. An electronic vernier caliper was used for stem girth at 5 cm above the epicotyls. The number of leaves per plant was visually counted at each inspection.

The experiment was terminated at 120 days after planting (DAP). Analysis of variance (ANOVA) was performed on all data collected, using windows SPSS (version 20). Means were separated using Duncan Multiple Range Test (DMRT) at 5% level of probability.

Result

Table 1 and 2 present the result of cumulative emergence of kola nut species at 8 and 12 weeks after planting (WAP) in response to the treatments. Treatments had significant effects on germination of cola nut in both the species. Cumulative emergences obtained in *C. acuminata* were significantly higher than those obtained in *C. nitida* at each level of the treatments in both the sampling periods.. Wounding x coconut water gave the highest emergence rate in both the species of cola nut seeds (54% more emergence in *C. nitida* and 93% emergence in *C. acuminate* at 12 WAP) compared to when neither of the treatments was applied. In both the species, the unwounded seeds not treated with coconut water gave the lowest emergence rate.

Table 1: Cumulative emergence of kola nut seedlings at 8 weeks after planting as affected by cocoa nut fruit water and seed wounding

Germination (%)						
	Cola nitida			*Cola acuminata*		
Coconut Fruit water	Seeds wounded	Seeds not wounded	Difference	Seeds wounded	Seeds not wounded	Difference
With	31.26	22.20	9.06*	54.15	34.45	49.70*
Without	21.49	0.00	21.49*	32.30	0.00	32.30*
Difference	09.77*	22.20*		21.85*	34.45*	

*Significant at 5% probability level (DMRT).

In all of the vegetative parameters of number of leaves/plant (Table 3), plant height (Table 4) and stem girth (Table 5), *C. acuminata* was significantly superior to *C. nitida* at all treatment levels measured. Similarly, for each of the above parameters studied, wounded seeds treated with coconut water gave the highest value and these were significantly higher than those obtained with any other treatment combinations. Similarly, unwounded seeds not treated with coconut water gave the lowest value for each of the vegetative parameters. For example, with no coconut water or wounding, plant height was 8.12 cm in *C. nitida* and 13.30 cm in *C. acuminata*. Addition of coconut water to wounded *C. intida* increased plant height by 23.08 cm, and that of *C. acuminata* by 27.95 cm, and these increases were significant.

Table 2: Cumulative emergence of kola nut seedlings at 12 weeks after planting as affected by cocoa nut fruit water and seed wounding

Germination (%)						
	Cola nitida			*Cola acuminata*		
Coconut Fruit water	Seeds wounded	Seeds not wounded	Difference	Seeds wounded	Seeds not wounded	Difference
With	73.15	49.81	23.34*	98.23	67.95	30.28*
Without	46.33	04.11	42.22*	61.64	09.36	52.28*
Difference	26.82*	45.70*		36.59*	58.59*	

*Significant at 5% probability level (DMRT).

Table 3: Number of seedling leaves/plant of kola nut at 12 weeks after planting as affected by cocoa nut fruit water and seed wounding

Number of seedling leaves						
	Cola nitida			*Cola acuminata*		
Coconut Fruit water	Seeds wounded	Seeds not wounded	Difference	Seeds wounded	Seeds not wounded	Difference
With	3.20	2.06	1.14*	4.46	2.98	1.48*
Without	1.03	0.33	0.7*	1.66	0.92	2.58*
Difference	2.17*	1.73*		2.80*	2.06*	

*Significant at 5% probability level (DMRT).

Table 4: Plant height of kola nut seedlings at 12 weeks after planting as affected by cocoa nut fruit water and seed wounding

Plant height (cm)						
	Cola nitida			*Cola acuminata*		
Coconut Fruit water	Seeds wounded	Seeds not wounded	Difference	Seeds wounded	Seeds not wounded	Difference
With	31.28	14.41*	16.87 **	41.25	20.03	21.22**
Without	10.18	10.29*	6.06*	14.02	6.30	7.72*
Difference	23.10*	4.29*		27.23*	13.73*	

*Significant at 5% probability level (DMRT).

Table 5: Stem girth of kola nut seedlings at 12 weeks after planting as affected by cocoa nut fruit water and seed wounding

Stem girth (cm)						
	Cola nitida			*Cola acuminata*		
Coconut Fruit water	Seeds wounded	Seeds not wounded	Difference	Seeds wounded	Seeds not wounded	Difference
With	5.20	3.23	1.97*	10.01	6.86	3.15*
Without	2.27	1.33	0.94*	3.66	2.23	1.43*
Difference	2.93*	1.90*		4.35	4.63*	

*Significant at 5% probability level (DMRT).

Similarly seeds not wounded but treated with coconut water had significant increases in plant height in both the species. However, wounding alone had no significant effect on plant height in the two species of kola nut. But wounding significantly enhanced the response of kola nut to coconut water treatment in each of the two species. This response trend was similar in all the treatment combinations.

Discussion

Wounding and the application of coconut water to cola nut had similar effects in both the species, though the responses varied between the species. In this study, the response of *C. nitida* was observed to be inferior to that of *C. acuminata* in all the treatment combinations. In the two species, wounding and coconut water had positive effects on cola nut seed emergence.

The significant increase in plant height, stem girth and number of leaves/plant in cola nut as observed in this study indicated that coconut water had positively affected not only the germination of the seeds but also their growth and development. Arditi (2008) and Yong *et al*. (2009) showed that the active growth regulators in coconut water also included amino acids, nitrogenous compounds, organic acids, enzymes, sugars and lipids. These might have played the various functional roles in cola nut seedling growth and development as observed in this work. Similar to our work, Yong (2009) reported that addition of coconut water to plant organs produced plantlets that were more active, bigger and more robust than those in the control.

However, wounding alone had no significant effect on the developing seedling of cola nuts. This might be due to the fact that wounding stimulated production of cytokinins only for cell division, and not growth stimulating substances. Buston (1989) demonstrated long ago

that wounding increased sprout time in potato, but not seedling development. Similarly, Mader *et al*. (2003) observed that mitosis was initiated within an hour after cutting dormant potato tubers, but no report has been given that wounding of seeds has any growth stimulating effect on seedling growth.

Conclusion

Coconut water and wounding may be utilized in combination or singularly to induce germination in dormant seeds of kola nuts and for effective seedlings development.

References

Akinbode, A. (1982). *Kolanut production and trade in Nigeria*. Nigerian Institute of Social and Economic Research (NISER), Ibadan.

Arditi, J. (2008). *Micropropagation of Orchids.* Second edition. Blackwell Publishing, Oxford.

Arteca, R. N. (1996). *Plant growth substances: principles and practices*. Chapman and Hall, New York.

Asogwa, E. U., Otuenye, A. H., Oluyole, K. A., Ndubuaka, T. C. and Owagboe, E. O. (2012). Kola production, processing and marketing in the South Eastern States of Nigeria. *American- Eurasia Journal of Agriculture and Environmental Science* 4:463-468.

Beatie, G. B. (1970). Soft drink flavor, their history and characteristic: cola or "kola" flavors. *Flavoring* 6:390-394.

FAOSTAT (2011). Production. Crops. Coconut. Htpp://faostat.fao.org.

Isawumi, A. M. (2003). The common edible fruits of Nigeria: 11. *The Nigeria Field* 58:1-2

Kanmegne, G. and Ndoumou, D. (2008). Germination of *Garcinia kola* (Heckel) seeds in response to different hormones. *Fruits* 63(3):156-161.

Kende, H. and Zeevaart, J. (1997). The five 'Classical' plant hormones. *Plant Cell* 9: 1197-1210.

Mader, J. C., Emery, R. J. N. and Turnbull, C. G. N. (2003). Spatial and temporal changes in multiple hormone groups during lateral bud release shortly following apex decapitation of chickpea (*Cicer arietinum*) seedlings. *Physiologia Plantanum* 119:295-308.

Oladokun, M. A. O. (1982). *Morpho-physiological aspect of germination and seedling growth in kola*. PhD Thesis, University of Ibadan, Nigeria.

Oluwalana, E. O. A., Okojie, L. A., Ashaolu, F. O. and Olaniran, V. O. (2016). A analysis of yields of kolanuts production and marketing in Shagamu, Ogun State, Nigeria. *Journal of Humanities, Social Science and Arts* 11(1):88-103.

Raven, P. H., Event, F. R. and Eichnom, S. E. (1999). *Biology of Plants*. 6th edition. W. H. Freeman and Company, New York.

Yong, J. W. H., Ge, L., Ng, Y. F. and Tan, S. N. (2009). The chemical composition and biological properties of coconut (*Cocos nucifera* L.) water. *Molecules* 14:5144-5164.