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Nigeria's *Cola* Genetic Resources: The Need for Renewed Exploration

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Abstract: Understanding the genetic resource status of an economic crop such as *Cola* is a very relevant pre-requisite for its improvement and advancing its research attention. This will, no doubt, also improve the adoption of these grossly under-utilized species of West African origin. Sterility, self and cross incompatibilities, unpleasantly tall trees and low nut yield are among the problems faced by growers of this crop. As the crop takes up to seven years to produce its first fruit, while some trees may not bear fruit within the first fifteen years of field establishment or even throughout life, many farmers have adopted other crops as their source of livelihood. This poses a great threat to the livelihood of the rural populace whose main occupation revolves around kola trading. Hand-pollinated experiments showed a yield of 3,000-10,000 nuts per tree per year, as against 250 nuts per tree per year usually obtained in open-pollinated plants. In Nigeria, recent efforts in *Cola* breeding and improvement include plant introduction, progeny trials, hybrid trials, clonal trials, root stock-scion compatibility trials and variability studies resulting in further plant selections. The highest diversity of *Cola* spp. has been observed in Nigeria 's boundary with Cameroon. This review is intended to beam the searchlight on *Cola* breeding in Nigeria and the availability of genetic diversity that can be assembled and utilized for the improvement of this all-important tree crop.

Key words: *Cola* species, genetic diversity, incompatibility, nut yield, sterility

INTRODUCTION

Cola is a tropical African genus that belongs to Sterculiaceae. The genus comprises of about 140 species (Onomo *et al*., 2006). Fifty species of this genus have been described in West Africa (Adebola, 2003). Three new species were later identified (Cheek, 2002). Twenty eight of these species occur in the forest between the Cross River of extreme SE Nigeria and the Mungo River of western Cameroon (the eastern boundary of the *Flora of West and Tropical Africa*, FWTA). Of these, only a few are fruit bearing while majority are woody species of economic importance. The most commonly used species are *Cola nitida* [(Vent) Schott and Endlicher], *Cola acuminata* [(Pal de. Beuav) Schott and Endl] and *Cola anomala* (Schott and Endlicher). Known in Cameroon as non timber forest products, they are cultivated by small farmers in association with *Theobroma cacao* or coffee in the centre and south provinces (*C. acuminala*), west and north provinces (*C. anomala*) and south-west and littoral provinces (*C. nitida*).

The earliest record on kola was that of Johannus Leo Africanus who was the first to refer to kolanut in 1556 as reported by Russel (1955). The Portuguese Odoardo Lopez recorded the occurrence of kola trees in Congo in 1591; followed by the record of Andre Alvares, who saw kola trees in Gambia and Guinea in 1594 (Nzekwe, 1961).

Van Eijnatten (1969) stated that kola tree was recorded all along the west coast of Africa from Gambia to Angola. *C. nitida* was originally distributed along the west coast of Africa from Sierra Leone to Benin Republic (Opeke, 2005) with the highest frequency and variability in the forest areas of Cote d'Ivoire and Ghana. These areas are now adjudged the centre of origin of *C. nitida*. In the early twentieth century, the nuts used for trade and local consumption were obtained from spontaneously occurring trees, as the trees were seldom planted (Opeke, 2005). The areas stretching from Nigeria to Gabon was the original area of distribution of *C. acuminata* (Opeke, 2005). *C. acuminata* occurred spontaneously in mountainous areas of Angola, Zaire and Cameroon, while it has long been in cultivation on the islands of Sao Thome and Principe. Southern Nigeria (Ijare area of Ondo state) is currently regarded as the centre of origin of *C. acuminata* (Opeke, 2005).

The objectives of this study were to:

- Understand the genetic resource status of *Cola* in Nigeria
- Highlight the importance of *Cola* in Nigeria

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- Identify the limitations for *Cola* production in Nigeria
- Propose possible means of overcoming the observed limitations

IMPORTANCE OF THE SPECIES

C. nitida and *C. acuminata* are of major importance in Nigeria, as they are cultivated for their edible seeds (kolanuts). *C. nitida*, characterized by nuts of two cotyledons, is however of much greater commercial importance as the seeds are in higher demand locally and for export. The cotyledons of *C. acuminata* range from three to six, and this specie is of high social, religious and ceremonial values among some tribes in Nigeria and Ghana (Town, 1967). *C. verticillata* (or slimy kola) *C. ballayi*, *C. milleni*, *C. heterophylla* (often called 'monkey kola') and *C. anomala* can also be chewed, and are often used to adulterate the commercial produce in times of scarcity. Other uncultivated economic species include *C. gigantean*, *C. laurifolia*, *C. lepidota*, *C. lateritia*, and *C. polycarpa*. These are found growing throughout the rain forest and southern guinea savanna belt of Nigeria and extracts from their roots, leaves and bark are traditionally prepared into medicine for curing various ailments.

The bulk of kola nuts produced in Nigeria is consumed within the country. A gift of kola among many cultural divides in Nigeria, especially white kola, means peace, friendship and sympathy. Offering kola is an important show of hospitality. Thus on such occasions the person being visited would prefer to have white or pale pink nuts. The pink nuts are chewed to dispel sleep, thirst, hunger and fatigue, and therefore serve as a substitute to coffee drinking. Kola nut contains active ingredients such as caffeine (2-3%) and smaller amounts of theobromin and kolatin a phenolic substance. The nuts also contain 10% protein, 1.35% fats and 45% starch (Van Eijnatten, 1969). The crop is therefore useful in pharmaceutical industries and in the production of kola type beverages, dyes, wines and confectionery (Ogutuga, 1975). Kola nuts are used to produce cardiac stimulants, laxatives, sedatives and sodas (Egbe and Oladokun, 1987). Consumption of Cola type beverage is not correlated with any of the heart disease risk factors (Stanton *et al*., 1978). According to Mshana *et al*. (2000), the bark of *C. gigantea* is used to treat yaws while that of *C. lateritia* is very efficient in the treatment of wound. The fruit of *C. nitida* is also reported to be used for the treatment of excessive appetite and fracture. The cotyledon is used to treat herpes and the bark is also good in the treatment of dystocia. *Cola* therefore will continue to play a significant role as an important economic crop for internal and regional trade and an increasingly important role in foreign trade. Estimated figures of trade in kolanuts are 2006 is presented in Table 1.

Table 1: Trade in kolanuts in 2006

Country	Producer price(U.S.$ per tonne)	Export (Tonnes)	Export value(in $ 1000)	Import (Tonnes)	Import value (in $1000)
Burkina Faso	0	0	0	7848	5046
Cameroon	0	87	200	1	3
Cote d'Ivoire	896.19	0	0	0	0
Ghana	1123.01	0	0	0	0
Maldives	0	0	0	715	1394
Nigeria	546.50	1	0	360	115
Sierra Leone	0	500	400	30	8

FAO (2009)

The foregoing strongly portrays the genus as very importance to West Africa. Yet not much is known in terms of attempts to unravel its genetic diversity with the aim of improving upon the crop through breeding and other improvement procedures. Despite the fact that *Cola* has strong cultural significance in West Africa, partly due to the fact that it is a valuable commodity, researchers grant limited interest to the genus. *C. nitida* has been favoured by most of the research works on kola in Nigeria because of economic tilt in its favour (Adebola, 2003).

Information on *C. Acuminata* and the other important species are hitherto very scarce, while it is almost non-existent in the uncultivated wild species. Considering the enormous production potential that exists, production is still very low in Nigeria, which is the world's largest producer of kolanuts (Adebona, 1992). The average kolanut production per tree of *C. nitida* is 250 nuts. This is in contrast to annual of 3,000 to 10,000 kolanuts per tree recorded in experimental plantings (Morakinyo and Olorode, 1984). The low yield has been attributed to several factors, some of which are sterility, incompatibility, inefficient pollination, fruit loss due to attack by pests and diseases, untimely harvesting as a result of cryptic green colour of the pod as well as unusually tall height of the trees.

As the germplasm of wild species exhibit large variability with respect to attributes of agronomic importance, these species offer opportunities for the exploitation of useful genes from them for the improvement of cultivated species. There is therefore the need to have an insight into the status of its breeding in Nigeria, and an outlook into the future of this genus so as to harness its economic importance to the fullest.

BREEDING EFFORTS SO FAR

According to Badaru (1989) and Okoloko and Jacob (1971) reviewed kola breeding in Nigeria since the inception of research efforts in this area and identified two periods of kola research activities, viz, the pre-CRIN era in which some work was initiated by botanists of the earstwhile/defunct Department of Agriculture, and the CRIN era. (CRIN, i.e. Cocoa Research Institute of Nigeria is the only body saddled with research into kola in

Nigeria). The pre-CRIN research activities consisted mainly of the introduction of planting materials, establishment of early experimental orchards and later on selection of private holdings. The subsequent CRIN era continued the selection study and extended it to clonal and progeny testing of selected trees. Previous scientists have encountered significant problems, amongst which are

- Narrow genetic base of the kola research materials
- Incompatibility of selected clones
- Sterility (of interspecific hybrid)
- Non-uniform and poor germination of kolanuts
- Storage pests and diseases

Okololoko and Jacob (1971) reported the selection of 57 kola trees from 6 different kola agro-ecologies. The number later increased to 74 with the addition of 17 trees from an additional ecology. Factors considered included nut yield, nut colour, and consistency of yield among others. These 74 selections formed the breeding population for further improvement programme in CRIN. The production of materials for progeny trials by controlled pollination of selected trees led to the discovery of self and cross incompatibilities (Jacob, 1971, 1973a, b; Okoloko and Jacob, 1971). A practical approach of solving the problem of incompatibility in *C. nitida* is the use of compatible clones of progenies and efforts in this direction have led to the identification of cross-compatible clones (Morakinyo *et al*., 1981). Ojo *et al*. (1982) identified some *C. nitida* clones in relation to general and specific combining abilities (gca and sca).Consistent observation of the field showed that the proportion of functional female to male flowers (flower sex ratio) on a tree depends on the state of maturity of the plant. This ratio is also believed to be determined by a combination of weather and soil conditions.

As many other kola groves have been surveyed 17 additional selections were made (Quarcoo, 1973) Table 2 and 3. Also CRIN kola breeders participated in a Kola Pilot Project sponsored by the Government of the Netherlands, supervised by the International Labour Organization (1971-1975) with the objective of introducing selected planting materials and improved production methods for the cultivation of kola (Furste and Odusolu, 1974). The exercise resulted in three sets of selections:

- Clonal Selection I (C.S.I): 28 trees
- Clonal Selection II (C.S.II): 73 trees
- Clonal Selection III (C.S.III): 530 trees

All these trees yielded between 532 and 2,893 nuts/ tree/ year. Selections observed to be self compatible include AA 31, AA 42, AB 6 and AD 44.

Table 2: Yield characteristics of C. nitida selections from Okuku (South-West Nigeria) (Quarcoo, 1973)

Code number	Avereage yield of nuts	Ave. nut wt.(g)	Proportion of productive years	Nut colour
EK 43	1008	14	6/6	White
EK 91	750	14	6/6	White
EK 92	901	16	6/6	White
EK 103	830	22	6/6	White
EK 133	761	14	6/6	White
EK 141	804	16	5/6	White
Average for the selections	842	16	5.83/6	

Progress in Tree Crop Research (2nd Edn., 1989), CRIN, Ibadan, Nigeria

Table 3: Yield characteristics of *C. nitida* selections from alata (South-West, Nigeria) (Quarcoo, 1973)

Code number	Average yield of nuts	Ave. nut wt. (g)	Proportion of productive years	Nut colour
AL 2	1801	13	3/3	Red
AL 4	690	39	3/3	Red
AL 9	1076	16	3/3	Red
AL 14	3058	15	3/3	Red
AL 15	2273	12	3/3	Red
AL 16	2820	11	3/3	Red
AL 19	1432	19	3/3	Red
AL 24	5602	13	3/3	Red
AL 25	1648	14	3/3	Red
AL 26	1633	15	3/3	Red
AL 31	1492	16	3/3	Red
Average for the selections	2138	16.6	3/3	

Progress in Tree Crop Research (2nd Edn., 1989), CRIN, Ibadan, Nigeria

Cross compatible selections are AA 42, AA 62, AA 86, AA 213, AA 231, AC 71, L 47 and L 54. They are also early bearing. The L clones are homozygous for white nuts. The AL and KD selections produce pink nuts which are highly marketable and are characterized by high nut weight.

Yield in Cola production is generally poor. The progeny takes 6 to 7 years to produce its first fruit and many trees remain unproductive for up to 15 years and sometimes throughout life. These problems are attributed to factors earlier outlined. Many kola farmers have therefore adopted other crops as their source of livelihood. This poses a great threat to the livelihood of many rural women whose main occupation revolves around kola nut trading. There is at present, a grave danger with respect to the future of this crop. If the current generation of kola farmers retires it is not certain that others will take over from them. Extreme variability in nut production is observed across West Africa (Adebola, 2003) and this could be attributed to the fact the centre of diversity of *C. nitida* is in the West African kola belt. Bodard (1962) reported that *Cola spp* was evolving towards a dioecious condition. High level of male fertility was observed in *C. nitida* (Adebola, 2003) and inter-specific variability was reported among various plants of the genus *Cola*, giving a strong bearing on the taxonomy of the groups

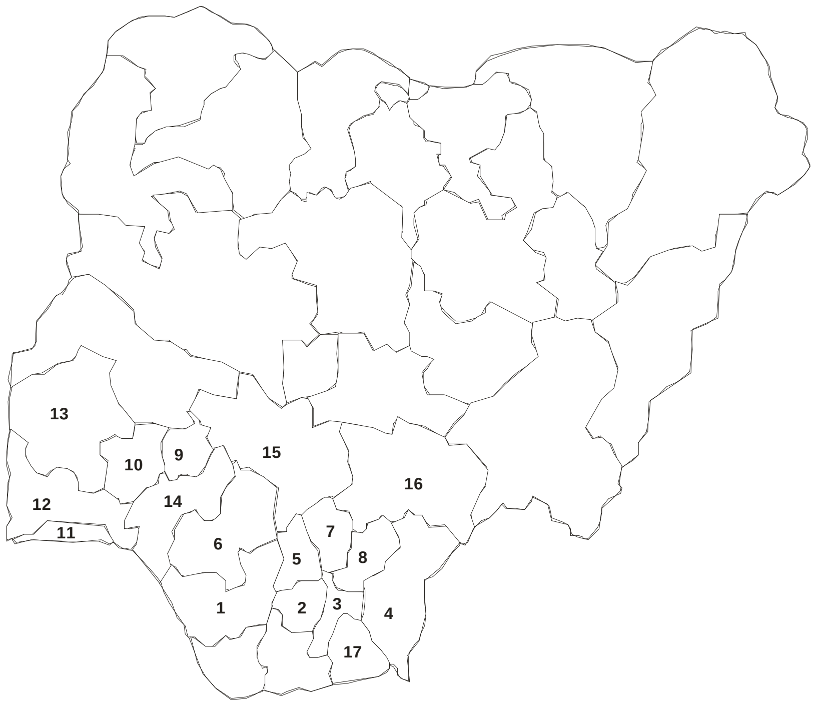

Fig. 1: Map showing the distribution of kola production in Nigeria,1: Delta; 2: Imo; 3: Abia; 4: Cross River; 5: Anambra; 6: Edo; 7: Enugu; 8: Ebonyi; 9: Ekiti; 10: Osun; 11: Lagos; 12: Ogun; 13: Oyo; 14: Ondo; 15: Kogi; 16: Benue; 17: Akwa Ibom

(Adebola, 2003). Sie *et al*. (2005), using enzymatic markers reveal a common origin for *C. nitida* trees from Cote d'Ivoire, Guinea and Nigeria but relatively few collections were used in the study. Onomo *et al*. (2006), on the Isoenzyme variability of *C. acuminata, C. nitida* and *C. anomala* reported polymorphism for all the enzyme systems tested. Yet some authors demonstrated that Isoenzyme analysis did not differentiate clones of taro (*Colocasia esculenta* L. Schott) germplasm (Monzano *et al*., 2001), and date palm (*Phoenix dactilifera* L.) (Bendiab, 1992).

FUTURE OUTLOOK

Beyond yield, the scope of kola improvement programme has broadened to include such important factors as pest and disease incidence, nutrient and climatic factors and other factors that directly or indirectly contribute to yield and quality of the crop. Since white and pale pink nuts usually fetch a higher price to the farmer than the darker pink or red nuts, the colour of the seed produced by the kola tree is a matter of concern. Yet the behavior of the tree in this respect could be unpredictable, as a single follicle may contain seed of different colours, and on occasion a seed has been found in which one cotyledon was red, the other pink or white (Russel, 1955).

Figure 1 is a map of Nigeria showing seventeen (17) states in Southern Nigeria where *Cola spp* are grown. The forest of the Cross River-Mungo River is located in the Eastern region of Southern Nigeria, specifically Cross River state (labeled '4' on the map). Southern Nigeria is also bounded by the Gulf of Guinea. Southern Nigeria, therefore, is a good area for further germplasm collection activities, with the aim of broadening the genetic base of available germplasm at experimental stations.

The extreme floristic richness of the Gulf of Guinea area has been underscored by Cheek (2002), as about half of the 7,349 species in FWTA exist on this same small area (Cheek, 2002). The forest of the Cross River-Mungo River has been the subject of a series of collecting expeditions that have yielded thousands of new specimens, as well as botanical inventories of protected areas for the purpose of conservation management (Cable and Cheek, 1998; Cheek *et al*., 2002). This therefore makes a case for further exploration of this area for useful genetic resources for further improvement of this all-important genus that should be the pride of Nigeria, and indeed the entire West African region, as three new species (*C. cecidiifolia*, *C. metallic*a and *C. suboppositifolia*) of the genus were recently discovered in this Cross River-Mungo River forest zone (Cheek, 2002).

Given the efforts of previous scientists to improve the species in the genus, there is the need to be-up to-date with recent trends in scientific investigations, so as to arrive at precise and overwhelming results. The role of biotechnology becomes germane for significant progress to be achieved in this, as morphological characterization seems to be exhaustive. Given the summations of Sie *et al.* (2005), Onomo *et al.* (2006), Monzano *et al.* (2001) and Bendiab (1992), it is sufficient to consider the use of further molecular markers to investigate individuals in this genus across the distribution area in Nigeria. In recent years different marker systems such as Restriction Fragment Length Polymorphism (RFLPs), Random Amplified Polymorphic DNAs (RAPDs), Sequence Tagged Sites (STS), Amplified Fragment Length Polymorphism (AFLPs) and Simple Sequence Repeats (SSRs) or microsatellites among others have been developed and applied to a range of crops. Investigations have revealed that AFLPs and SSRs are the most popular markers in various crops due to their high effectiveness at detecting polymorphism. The advantages of microsatellites (or SSRs markers) are summarized as follows:

- The method is relatively simple and can be automated
- Most of the markers are monolocus and show Mendelian inheritance
- SSRs markers are high informative
- A high number of public SSR primer pairs are available
- Effective cost per genotype and primer (similar to that for RAPDs)

SSRs markers have been developed for *Theobroma cacao* (Alves *et al.*, 2007). Cross-specie amplification of microsatellite loci represents an appealing alternative, with transferability rates between congeners typically above 50%, decreasing proportionally to phylogenetic divergence (Varshney *et al*., 2005), with reported successful amplification in population genetics studies (Brondani *et al*., 2005; Varshney *et al*., 2005; Zucchi *et al*., 2003). Microsatellite primers developed for *Theobroma cacao* were tested in *T. grandiflorum* (Alves *et al*., 2007) and *Cola nitida* (Lanaud *et al*., 1999) with success. As the germplasm of wild species exhibit high level of variability with respect to attributes of agronomic importance, these species offer opportunities for the exploitation of useful genes from them for the improvement of cultivated species. With molecular characterization, the extent of genetic variability among the tested genotypes is expected be revealed. Genetic information on desirable and economic traits on the genotypes will become available. Appropriate selection/hybridization will be done. Inherent problems such as sterility and incompatibility as well as the problems of height (as most kola trees are tall), disease and pest susceptibiity will be solved. Yield will eventually be improved, thereby encouraging better farmer interest in the crop. Hence hope of livelihood can be restored to the many rural women whose survival is threatened by a drift away from this crop. The future of the genus will also be guaranteed, as this is not assured with the current trend in kola farming in Nigeria.

In conclusion, the findings of this study bring forth an understanding of the current status of *Cola* genetic resources in Nigeria. Availability of *Cola* genetic diversity at various locations, in Southern Nigeria, especially the Cross-River area of Nigeria has been highlighted. An update is therefore presented for further exploration of the genetic diversity present across the country, as this is a pre-cursor to further breeding and improvement of this tree crop.

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