http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 9 (3): 391-397, 2006
ISSN 1028-8880
© 2006 Asian Network for Scientific Information

Isoenzyme Variability of Three Cola (*Cola acuminata* (Pal. de Beauv, Schott and Endlicher), *Cola nitida* ((Vent) Schott and Endlicher) and *Cola anomala* (Schott and Endlicher)) Germplasm in Cameroon

Pierre Effa Onomo, Nicolas Niémenak and Denis Omokolo Ndoumou
Department of Biological Sciences, Laboratory of Biochemical and Plant Physiology,
High Teacher Training School, P.O. Box 47 Yaounde, Cameroon

Abstract: The aim of this study were to add information on the genetic structure of *Cola* sp. and to verify the possibility of classifying *Cola* sp. entries based on isoenzyme traits. Polyacrylamide gel electrophoresis was employed to study the isoenzyme variation of peroxydase, polyphenoloxydase and amylase in forty nine accessions of three Cameroonian *Cola* species (*Cola acuminata*, *Cola nitida* and *Cola anomala*). Band frequencies were calculated for each entry and for each isoenzyme system. The intrapopulation variation was estimated by Shannon-Weaver (H') diversity index. Based on the matrix of band frequencies and standardised data, the interpopulation variation was examined by cluster analysis. A total of 14 bands with frequency values ranging from 0 to 1 were observed including 12 polymorphic and 2 monomorphic. The average value of H' estimated for each entry range from 0.07 to 0.63 suggesting that *Cola* sp. entries showed a wide polymorphism for all the enzyme systems being tested. Cluster analysis revealed two distinct groups for both *Cola acuminata* and *Cola nitida* and six for *Cola anomala* in which entries revealed a greater similarity.

Key words: *Cola* sp., genetic diversity, accessions, isoenzyme variation, polymorphic, monomorphic

INTRODUCTION

Cola, a tropical African genus belong to Sterculiaceae family. This genus comprises about 140 species, but the most commonly used are *Cola acuminata* (Pal. de Beauv) Schott and Endl), *Cola nitida* ((Vent) Schott and Endlicher) and *Cola anomala* (Schott and Endlicher). Known in Cameroon as non timber forest products, they are cultivated by small farmers in association with *T. cacao* or coffee in the centre and south provinces (*C. acuminata*), west and north west provinces (*C. anomala*) and south west and littoral provinces (*C. nitida*). The spread of cola nuts has resulted from its reputation as a stimulant-increasing energy and strength dispelling drowsiness and staving off hunger (Morton, 1992). Cola has strong cultural significance in West Africa, partly due to the fact that it is a valuable commodity. Yet, researchers grant few interests to this plant and its systematic is again very little studied. However, a systematic based on the morphological traits of Cola species has been reported in literature (Nkongmeneck, 1982).

Isozymes are multiple molecular forms of enzymes, used extensively as molecular identification tags in strain or stock identification and taxonomic and evolutionary studies (McAndrew and Majumber, 1983; Richardson *et al*., 1986; Basaglia, 1988). Isozymes are generally expressed in a co-dominant fashion and rarely exhibit epistatatic interaction (Tanksley and Rick, 1980). Thus, isoenzyme profile of specific enzymes which display sufficient polymorphism could be used as reliable genetic markers for genetic identification when all other conditions remain constant (Sarangi and Mandal, 1996). Among molecular methods, the electrophoretic analysis of isoenzyme variation has proved to be particular useful in defining more precisely the size and structure of genetic diversity in the gene pools of different plants (Labdi *et al*., 1996; Polignano *et al*., 1998; Rodriguez *et al*., 2000). In *Cola* sp., the literature on both genetic diversity and intra and interspecific-relationships among collections is quite poor.

The aims of this study were to add information on the genetic structure of *Cola* sp. and to verify the possibility of classifying *Cola* sp. accessions based on isoenzyme traits.

Corresponding Author: Pierre Effa Onomo, Department of Biological Sciences, Laboratory of Biochemical and Plant Physiology, High Teacher Training School, P.O. Box 47 Yaounde, Cameroon Tel: +237 740 59 49

MATERIALS AND METHODS

Plant material: This study was conducted during the period from February to May 2005 in the Laboratory of Biochemical and Plant Physiology, Department of Biological Sciences, High Teacher Training School of the University of Yaounde I in Cameroon. Sixteen accessions of *Cola acuminata* (Pal. de Beauv) Schott and Endl), twelve of *Cola nitida* ((Vent) Schott and Endlicher) and twenty one of *Cola anomala* (Schott and Endlicher) were collected from different geographic sites in Cameroon. The principal characteristics of all accessions studied are summarised in Table 1. In each population, leaf material was harvested from 20 randomly collected plants and stored at -20°C until later uses.

Isoenzyme analysis: Leaf tissues (2 g) were crushed in 5 mL of 80% acetone in a motar. Acetonic extract was moved by filtration using Whatman N°3 filter paper. The residue was homogenized in 15 mL pre-chilled extraction buffer (0.1 M Tris-HCl, containing 0.1% (w/v) cystein-HCl, 0.1% (w/v) ascorbic acid, 17% (w/v) sucrose, pH 7.4). The homogenates were spun at 6000 g for 30 min at 4°C. The supernatants were collected and kept in -20°C until future uses.

Vertical polyacrylamide gel electrophoresis (PAGE) was performed at 4°C. The separation was performed on 6% stacking and 12% separating gel containing 0.375 M Tris-HCl, pH 8.8. The tank buffer containing 0.02 M Tris and 0.19 M glycine, pH 8.3. Samples of 50 μg were loaded for each cultivar. The electrophoresis was carried out at 220V for about 5 h at 4°C. Gels were removed, washed and stained for peroxidase (EC 1.11.1.7), polyphenoloxidase (EC 1.10.3.1) and amylase (EC 3.2.1.2).

Analysis of the data: Band frequencies were calculated for each accession and for each isozyme system. The intra-population variation was estimated by the Shannon-weaver (H') diversity index.

$$H' = -\sum_{i=1}^{n} Pi \log_2 Pi$$

Where Pi is the frequency of the ith band and n is the number of bands observed for each enzyme. This index applied by different authors (Hutchenson, 1970; Polignano *et al.*, 1998) provides a quantitative estimate of intra-population diversity. Based on the matrix of band frequencies and standardized data, the inter-population variation was examined by cluster analysis, and the Unweighted Pair-group Mean Arithmetic (UPGMA) method using SPSS program version 10.1 for windows was utilized to calculate the square of Euclidian distance.

RESULTS

Peroxidase (POX): Four patterns and one zone of activity (a cathodal POX-1) were detected. In *Cola acuminata*, POX-1 was polymorphic with two bands (A and B). This zone contained three bands (A, C and D) in *Cola nitida* and three (A, B and D) in *Cola anomala*. Band A in the latter specy was monomorphic and the three others polymorphics (Table 2).

Polyphenoloxidase (PPO): Six bands and two zones of activity were detected; a cathodal PPO-1 and an intermediate zone (PPO-2). PPO-1 comprised one band (band B) in *Cola acuminata,* two (band A and C) in *Cola nitida* and three (band A, B and C) in *Cola anomala.* All these bands were polymorphics.

Table 1: Passport of the accessions of *Cola sp.* studied

Species	No.	Name	Origin	Longitude	Latitude
Cola acuminata	01	zoa	Zoaetele	11°53'	3°15'2"
	02	nkot	Nkoteng	12°1'2"	4°30'
	03	ombes	Ombessa	11°15'3"	4°36'
	04	okola	Okola	11°23'3"	4°1'1"
	05	bafia	Bafia	10°14'	4°45'
	06	mkne	Makenene	10°48'	4°53'
	07	elfmo	Elig-Mfomo	11°22'	4°11'
	08	mbsna	Mbangassina	11°24'	4°33'3"
	09	esse	Esse	11°53'	4°5'2"
	10	bokit	Bokito	11°6'4"	4°34'2"
	11	akga	Akonolinga	12°16'	3°46'
	12	mbyo	Mbalmayo	11°30'	3°31'
	13	sangma	Sangmelima	11°48'	2°38'
	14	mbang	Mbang	NA	NA
	15	ngoulma	Ngoulmakong	NA	NA
	16	eseka	eseka	10°46'2"	3°38"
Cola nitida	01	esse	Esse	11°53'	4°5'2"
	02	eseka	Eseka	10°46'2"	3°38'
	03	akga	Akonolinga	12°16'	3°46'
	04	elfmo	Elig-Mfomo	11°22'	4°11'
	05	loum	Loum	9°45'	4°57'
	06	ayos	Ayos	12°31'3"	3°54'
	07	edea	Edea	10°8'2"	3°48'
	08	muy	Muyuka	NA	NA
	09	mbyo	Mbalmayo	11°30'	3°31'
	10	yde	Yaounde	11°32'	3°52'
	11	limb	Limbe	9°14'	4°1'
	12	buea	Buea	9°14'	4°9'
Cola anomala	01	bafang	Bafang	10°11'	5°29'
	02	bangwa	Bangoua	10°29'	5°12'
	03	bazou	Bazou	10°28'	5°3'3"
	04	bafou	Bafou	10°7'	5°28'
	05	mbui	Kumbo	10°42'	6°11'3"
	06	batcham	Batcham	10°3'2"	5°34'4"
	07	baham	Baham	10°22'	5°20'
	08	santa	Santa	10°10'	5°48'
	09	ntonga	Ntonga	10°42'2"	4°58'
	10	balven	Baleveng	10°9'	5°30'
	11	balgu	Balengou	10°28'	5°7'
	12	bamda	Bamenda	10°10'	5°47'
	13	bana	Bana	10°16'4"	5°9'
	14	bayg	Bayamgam	10°29'	5°18'
	15	batfam	Batoufam	10°29'8"	5°16'
	16	mboud	Mbouda	10°15'	5°37'3"
	17	fban	Foumban	10°53'	5°44'
	18	fbot	Foumbot	10°38'	5°30'
	19	kout	Koutaba	10°48'	5°41'2"
	20	dsch	Dschang	10°3'	5°27'
	21	mamf	Mamfe	9°19'	5°45'2"

Table 2: Band frequencies for 3 enzyme systems in 16 *Cola acuminata* accession (A), 12 *Cola nitida* accessions (B) and 21 *Cola anomala* accessions (C)

(A)

		Accessions																
Enzyme	Band	Sangma	Okola	Eseka	Zoa	Mbyo	Mknne	Ngoulma	Bok	Esse	Elmo	Akga	Ombes	Nkot	Mbangas	Mbang	Bafia	Mean
POX	A	1	1	0.9	1	1	1	0.8	1	0.75	0.9	0.9	0.85	0.8	0.9	1	0.85	0.91
	B	0.85	0.78	0.75	0.85	0.9	0.75	0.9	0.75	0.9	1	0.85	0.6	0.8	0.9	0.75	0.75	0.81
	C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PPO	A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	B	0	0	0	0	0	0	0	0	0.4	0.7	0	0.7	0.4	0	0.7	0	0.18
	C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	D	0.4	0.5	0.3	0.4	0.7	0.5	0.4	0	0.7	0.9	0.4	0.7	0.4	0.4	0.9	0.4	0.5
	E	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
AMY	A	0.2	0	0	0.2	0.2	0.4	0.2	0.2	0	0.2	0		0.2 0	0.2	0	0.2	0.13
	B	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	C	0	0.2	0.2	0	0.2	0.2	0	0.2	0.2	0	0.2	0.2	0.2	0	0.2	0.2	0.13
	D	0	0.2	0	0	0	0.2	0	0	0	0	0.2	0	0	0	0	0	0.03

(B)

		Accessions												
Enzyme	Band	Esse	Loum	Akga	Edea	Muy	Buea	Elmo	Limbe	Eseka	Mbyo	Ayos	Yde	Mean
POX	A	1	0.9	0.85	0.95	0.8	0.9	0.85	0.75	0.8	0.8	0.9	0.9	0.86
	B	0	0	0	0	0	0	0	0	0	0	0	0	0
	C	0.8	1	1	0.75	1	0.9	1	1	0.9	0.8	0.75	0.8	0.89
	D	0	0.3	0	0	0.3	0	0	0.3		0 0	0	0	0.07
PPO	A	0.4	0.4	1	0.9	0.4	0.4	0.4	0.4	0	1	1	0.4	0.54
	B	0	0	0	0	0	0	0	0	0	0	0	0	0
	C	0.4	0.5	0.9	0.9	0	0.4	0.3	0.5	0	0.9	1	0.4	0.54
	D	1	1	1	1	1	1	1	1	1	1	1	1	1
	E	0	0.4	0.9	0	0.4	0.3	0.5	0	0.6	1	1	0.4	0.45
	F	0	0	0	0	0.4	0	0	0	0	0	0	0	0.03
AMY	A	0	0	0	0	0.2	0.2	0	0.2	0	0	0	0	0.05
	B	1	1	1	1	1	1	1	1	1	1	1	1	1
	C	0.2	0.4	0.2	0.4	0	0	0	0.2	0	0.4	0	0.2	0.16
	D	0	0.2	0	0	0.4	0	0	0.2	0	0	0	0	0.06

(C)

		Accessions															
Enzyme	Band	Sta	Mfe	Bafang	Ktba	Btfam	Fbot	Bmda	Fban	Btcham	Balven	Mbui	Dsch	Mbda	Balgu	Bazu	
POX	A	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
	B	0.3	0.6	0.6	0.3	0.6	0.3	0.3	0.3	0.6	0.6	0.6	0.6	0.6	0.6	0.6	
	C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	D	0	0	0.3	0	1	0	1	0	1	1	1	0.75	0.3	0.3	0	
PPO	A	0.4	0.7	0.4	0	0.9	0.4	0.4	1	0.4	0.9	0.4	0	0	1	1	
	B	0	0	0	0.9	0	0	0	0	0	0	0	0	0	0	0	
	C	0.4	0.7	0.4	0	0.4	0.4	0.4	0.4	0.4	0.9	0	0	0.4	1	1	
	D	0	0.7	0.4	0.7	0	0.4	0	0.4	0.4	1	0.4	0.7	0.7	0.4	1	
	E	0.4	1	0	0.7	0.4	0.4	0.4	1	0.4	1	0.4	0.7	0	0	0.9	
	F	0	0	0	0	0	0	0	0	0	0	0		0 0	0	0	
AMY	A	0.4	0.2	0.4	0	0.2	0.4	0.2	0.2	0.3	0.2	0.2	0.2	0.2	0.2	0.4	
	B	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
	C	0	0	0.4	0	0.2	0	0	0	0.2	0.3	0	0.4	0.2	0.3	0.2	
	D	0.2	0.2	0	0.2	0	0	0.2	0	0.2	0.2	0.4	0.2	0	0	0	

		Baygam	Bana	Bafu	Baham	Bangwa	Ntonga	Mean
POX	A	1	1	1	1	1	1	1
	B	0.8	1	0.3	0.3	0.3	0.3	0.5
	C	0	0	0	0	0	0	0
	D	0	0.3	0.3	0	0.3	0.3	0.34
PPO	A	0	0	0	0	0	0	0.37
	B	0	1	1	0	0.9	0	0.18
	C	0.4	0	0	0	0	0	0.34
	D	0.9	0.9	0.9	0.7	0.9	0.4	0.45
	E	0.9	0.9	0.9	1	1	0.4	0.6
	F	0	0	0	0	0	0	0
AMY	A	0	0.2	0.3	0.2	0.3	0.4	0.24
	B	1	1	1	1	1	1	1
	C	0	0	0	0	0.3	0.4	0.13
	D	0.2	0.2	0.2	0	0	0	0.11

Table 3: Shannon-weaver (H') diversity indices for 3 enzyme systems observed in 16 *Cola acuminata* accessions (A), 12 *Cola nitida* accessions (B) and 21 *Cola anomala* accessions (C)

(A)

Enzyme	Sangma	Okola	Eseka	Zoa	Mbyo	Mknne	Ngoulma	Bok	Esse	Elmo	Akga	Ombes	Nkot	Mbangas	Mbang	Bafia	Mean
POX	0.09	0.13	0.14	0.09	0.06	0.14	0.18	0.14	0.21	0.06	0.16	0.23	0.24	0.13	0.14	0.24	0.14
PPO	0.25	0.25	0.25	0.25	0.17	0.25	0.25	0.06	0.42	0.23	0.25	0.34	0.50	0.25	0.23	0.25	0.26
AMY	0.22	0.44	0.22	0.22	0.44	0.70	0.22	0.44	0.22	0.22	0.44	0.44	0.22	0.22	0.22	0.44	0.33
Mean	0.18	0.27	0.20	0.18	0.22	0.36	0.21	0.21	0.28	0.17	0.28	0.33	0.32	0.20	0.19	0.31	0.24

(B)

Enzyme	Esse	Loum	Akga	Edea	Muy	Buea	Elmo	Limbe	Eseka	Mbyo	Ayos	Yde	Mean
POX	0.12	0.06	0.09	0.18	0.12	0.13	0.09	0.14	0.18	0.24	0.21	0.18	0.14
PPO	0.50	0.49	0.13	0.13	1	0.75	0.73	0.50	0.21	0.06	0	0.76	0.43
AMY	0.22	0.47	0.22	0.25	0.47	0.22	0	0.66	0	0.25	0	0.22	0.24
Mean	0.28	0.34	0.14	0.18	0.53	0.36	0.27	0.43	0.13	0.18	0.07	0.38	0.27

(C)

Enzyme	Sta	Mfe	Bafang	Ktba	Btfam	Fbot	Bmda	Fban	Btcham	Balven	Mbui	Dsch	Mbda	Balgu	Bazu
POX	0.25	0.21	0.46	0.25	0.21	0.25	0.25	0.25	0.21	0.21	0.46	0.46	0.21	0.21	0.46
PPO	0.76	0.51	0.76	0.41	1	1	0.76	0.50	1	0.13	0.76	0.34	0.42	0.25	0.06
AMY	0.47	0.44	0.50	0.22	0.44	0.25	0.44	0.22	0.69	0.69	0.47	0.70	0.44	0.47	0.47
Mean	0.49	0.38	0.57	0.29	0.55	0.50	0.48	0.32	0.63	0.34	0.56	0.50	0.35	0.31	0.33

	Baygam	Bana	Bafu	Baham	Bangwa	Ntonga	Mean
POX	0.12	0.25	0.50	0.25	0.50	0.50	0.30
PPO	0.38	0.13	0.13	0.17	0.13	0.50	0.48
AMY	0.22	0.44	0.25	0.22	0.50	0.50	0.41
Mean	0.24	0.27	0.29	0.21	0.37	0.50	0.39

The intermediate PPO-2 zone was formed by two bands (band D polymorphic and band E monomorphic) in *Cola acuminata*, three in *Cola nitida* (band D monomorphic, bands E and F polymorphics) and two in *Cola anomala* (bands D and E, all polymorphics). Band F was found only in Muyuka *Cola nitida* accession (Table 2).

Amylase (AMY): This enzyme system exhibited two zones of activity (AMY-1 and AMY-2) corresponding to four phenotypes. In all Cola species, AMY-1 exhibited both homozygous and heterozygous phenotypes while some electrophoretic variation was found for AMY-2.

In general, a total of fourteen bands with frequency values ranging from 0 to 1 were observed (Table 2), nine in *Cola acuminata*, twelve in *Cola nitida* and *Cola anomala*, respectively.

Average frequencies below 5% indicate a rare allelic presence; instead, if the frequency of the most frequent allele to a fixed locus is less than 95%, the population is considered polymorphic (Brown and Weir, 1983).

Values lower than 5% were estimated for the following bands:

POX-D in Esse, Akonolinga, Edea, Buea, Elig-mfomo, Eseka, Mbalmayo, Ayos and Yaounde accessions for *Cola nitida*; Santa, Mamfe, Koutaba, Foumbot, Mbouda, Mbalengou, Bayangam and Baham accessions for *Cola anomala*.

PPO-A in all *Cola acuminata* accessions, Eseka for *Cola nitida*, Koutaba, Dschang, Mbouda, Bayangam, Bana, Bafou, Baham, Bangoua and Ntonga accessions for *Cola anomala*.

PPO-B in all accessions except Koutaba, Bana, Bafou and Bangoua for *Cola anomala*.

PPO-C in all *Cola acuminata* accessions, Eseka for *Cola nitida* and 8 of 21 *Cola anomala* accessions.

PPO-D in Bokito (*Cola acuminata*), Santa and Batoufam (*Cola anomala*).

PPO-E in Esse, Edea and Limbe for *Cola nitida*, Bafang, Mbouda and Balengou for *Cola anomala*.

PPO-F in all accessions except Muyuka *Cola nitida* accession.

AMY-A in 5, 9 and 2 accessions for *Cola acuminata*, *Cola nitida* and *Cola anomala*, respectively.

AMY-C in 5 for *Cola acuminata* and *Cola nitida*, 11 for *Cola anomala*

AMY-D in 13, 9 and 10 accessions for *Cola acuminata*, *Cola nitida* and *Cola anomala*, respectively.

The Shannon-weaver diversity index H' was calculated to compare the diversity expressed by the systems and the accessions (Table 3). For a single enzyme system, a low H' value indicates unbalanced frequency and lower diversity levels. On the contrary, higher H' values indicate a greater balance among frequency classes and greater diversity levels.

The average value of H' estimated for each accession, ranged from 0.18 (Sangmelima) to 0.36 (Makenene) for *Cola acuminata*, 0.18 (Edea and Mbalmayo) to 0.53 (Muyuka) for *Cola nitida* and 0.21 (Baham) to 0.63 (Batcham) for *Cola anomala*.

In *Cola acuminata*, lower H' values were estimated for peroxidase in all accessions excepted in Esse, Ombessa, Nkoteng and Bafia. Intermediate H' values were estimated for polyphenoloxidase system excepted for Bokito (H' = 0.06) and Mbalmayo (H' = 0.17). Amylase system also displayed intermediate H' values, but this value remained high in Makenene (H' = 0.70). In *Cola nitida*, lower H' values for peroxidase were observed in 10 of the 12 accessions, meanwhile, higher values for this Cola specy were estimated for polyphenoloxidase excepted in Ayos, Mbalmayo, Akonolinga, Edea and Eseka. For this enzyme system, Muyuka was monomorphic. Higher values of H' for amylase was only noticed in Limbe. In *Cola anomala*, Bafou, Bangoua and Ntonga accessions presented a greater diversity for peroxidase. Bafang, Bangoua, Ntonga, Batcham, Baleveng and Dschang accessions presented high values for amylase system. On the contrary, PPO system was monomorphic in Batoufam, Foumbot and Batcham accessions.

For peroxidase system, while *Cola acuminata* and *Cola nitida* showed a common mean value of H' (0.14), this value in *Cola anomala* was 0.3 suggesting that peroxidase indicates a greater diversity level in the latter specy than the two other Cola species. *Cola anomala* also displayed a greater level of diversity for PPO (H' = 0.48) followed by *Cola nitida* (H' = 0.43) and *Cola anomala* (H'=0.26). If for amylase system, *Cola anomala* gathered the highest diversity level (H' = 0.41), *Cola nitida* gathered the lowest (H' = 0.24).

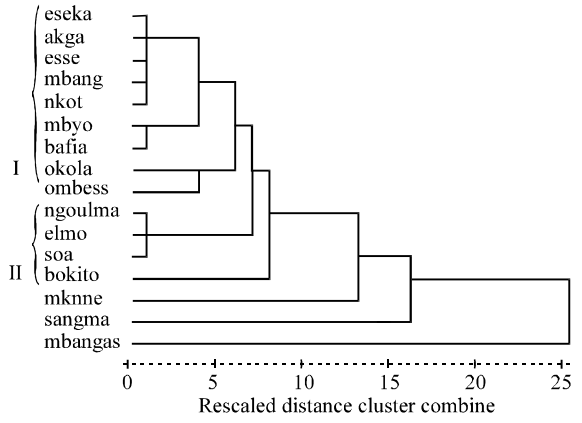

Fig. 1: Dendrogram of 16 accessions of *Cola acuminata* based on a distance matrix of band frequencies of 7 polymorphic isozymes

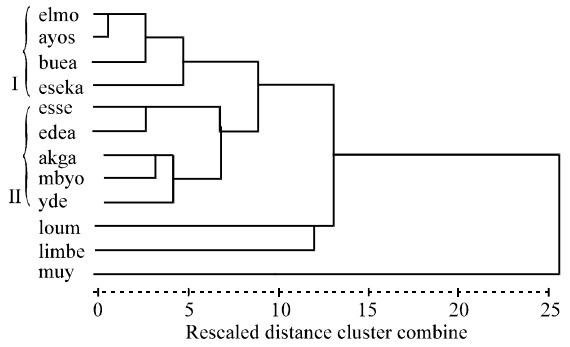

Fig. 2: Dendrogram of 12 accessions of *Cola nitida* based on a distance matrix of band frequencies of 10 polymorphic isozymes

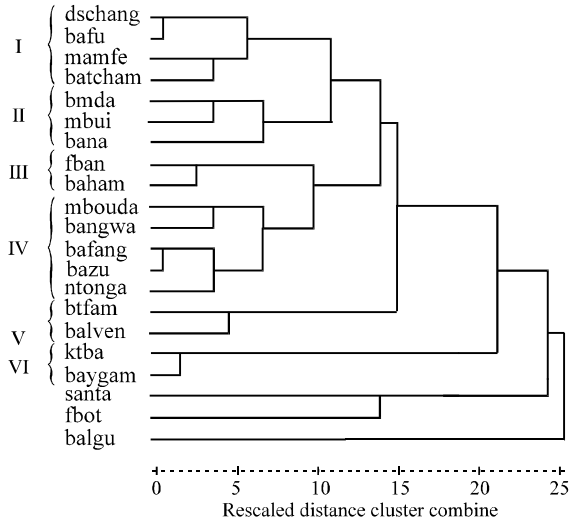

Fig. 3: Dendrogram of 21 accessions of *Cola anomala* based on a distance matrix of band frequencies of 10 polymorphic isozymes

Figure 1, 2 and 3 are dendrograms from cluster analysis of 16 accessions of *Cola acuminata*, 12 of *Cola nitida* and 21 of *Cola anomala,* respectively based on a distance matrix of band frequencies of 12 polymorphic isozymes.

Figure 1 presented 2 clusters. Nine of the 16 accessions are included in cluster one while cluster two is represented by three accessions. Makenene, Sangmelima and Mbangassina accessions are very far from the other clusters. In Fig. 2, *Cola nitida* accessions are grouped in two mains clusters where the first comprises 4 and the second 5. Loum, Limbe and Muyuka do not belong to any cluster. Figure 3 is characterized by 6 clusters and Santa, Foumbot and Balengou are very distant from these clusters.

DISCUSSION

For all Cola species studied, peroxidase displayed the lower average H' value followed by amylase and polyphenoloxidase. However, *Cola* sp. accessions showed polymorphism for all the enzyme systems being tested. Similar results have been reported in *Lathyrus sativus* (Alba *et al*., 2001) in 44 cultivars of Indian banana and plantains (Bhat *et al*., 1992), in rice (Maheswaran and Rangasamy, 1992) and potato (Giovanni *et al*., 1993). Yet, some authors demonstrated that peroxidase analysis did not differentiate clones of taro (*Colocasia esculenta* L. Schott) germplasm (Manzano *et al*., 2001). In the same way, peroxidase, alcohol deshydrogenase and superoxide dismutase did not expressed variability in date palm (*Phoenix dactilifera* L.) (Bendiab, 1992).

Clusters analysis revealed 2 distinct groups for *Cola acuminata* and *Cola nitida* and 6 for *Cola anomala* in which accessions revealed a greater similarity. In the other words, accessions belonging to a cluster showed similar patterns for the enzyme system compared to those located in different clusters. This similarity among cultivars might be due to their origin from a common stock following an identical evolutionary pathway or domestication under similar natural selection pressure that has helped develop homology and expression of similar traits for survival under identical agro-niches. It might also be due to up-regulation of similar kinds of domain in the genomes of different cultivars governing the expression of isomorphs of an enzyme.

The intra-population variation observed suggested the opportunity to extend survey to wide segments of the collection. More detailed studies including more differentiated materials could be useful to investigate the associations among morpho-agronomic and isozyme traits. Future research should be directed to more accurate investigations on both biochemical characters and dominant markers to better understand the genetic diversity of cultivars. This could be important for hybrid breeding and strategy formulation for conserving valuable indigenous germplasm.

REFERENCES

Alba, E., G.B. Polignano, D. De Carlo and A. Mincione, 2001. Electrophoretic phenotypes of different enzymes in some entries of *Lathyrus sativus* L. *Lathyrus*. Lathyrism Newslett., 2: 15-20.

Basaglia, F., 1988. The isozymes of the lactate dehydrogenase, malate dehydrogenase and glucose phosphate in *Halian ictalunds*. Comp. Biochem. Physiol., 89: 731-736.

Bendiab, K., 1992. Contribution in the study of enzymatic and proteic variability of datte palm (*Phœnix dactilifera* L.). Contribution in the genetic improvement of the plant. Superior study diploma, Cadi Ayyad University, Marrakech. Morocco, pp: 190.

Bhat, K.V., S.R. Bhat and K.P.S. Chandel, 1992. Survey of isozyme polymorphism for clonal identification in *Musa* L. Esterase, acid phosphatase and catalase. J. Hortic. Sci., 67: 737-742.

Brown, A.H.D. and R.S. Weir, 1983. Measuring Genetic Variability In Plant Populations. In: Tankley S.D., (Eds, T.J. Orton And Elsevier) (Eds.) Amsterdam, Nederland. pp: 219-239.

Giovanni, T., R. Alicchio, and L. Cinilo, 1993. Genetic analysis of isozyme and restriction fragment patterns in the genus *Salanum*, J. Genet. Breed., 47: 237-244.

Hutchenson, K., 1970. A test for comparing diversities based on the Shannon formulae. J. Theor. Biol., 29: 251-254.

Labdi, M., L.D. Robertson., K.B. Singh and A. Charrier, 1996. Genetic diversity and phylogenetic relationship among the annual Cicer species as revealed by isozyme polymorphism. Euphytica, 88: 181-188.

Maheswaran, M. and S.R. Sree Rangasamy, 1992. Changes in peroxidase activity during shoot formation in *Orysa sativa* L., J. Genet. Breed., 46: 15-20.

Manzano, A.R., A.A.R. Nodals., M.I.R. Gutierrez., F.Z. Mayor and C.I. Alfonso, 2001. Morphological and isozyme variability of taro (*Colocasia esculenta* L. Schott) germplasm in Cuba, Plant Genet. Resourc. Newslett., 126: 31-40.

McAndrew, B.J. and K.C. Majumber, 1983. Tilapia stock identification using electrophoretic markers, Aquaculture., 30: 249-261.

Morton, J.F., 1992. Widespread Tannin Intake Via Stimulants And Masticories, Especially Guaranana, Kola Nuts, Betel Vine And Accessories. In: Plant Polyphenols, Plenum Press, New York. pp: 739-765.

Nkongmeneck, B.A., 1982. Contribution to the study of Cameroon Cola genus. Ph.D Thesis University of Yaounde, Cameroon. pp: 51.

Polignano, G.B., G. Quintano., V. Bisignano., P. Uggenti., C. Della Gatta and E. Alba, 1998. Enzyme polymorphism in faba bean (*Vicia faba* L. minor) accessions. Genetic interpretation and value for classification. Euphitica., 102: 169-179.

Richardson, B.J., P.R. Baverstock M. Adams, 1986. Allozyme Electrophoresis-a Handbook for Animal Systematic and Population Studies. Academic Press, New York, pp: 15-218.

Rodriguez, M.M., O.M. Parades and V.L. Becerra, 2000. Isozyme diversity of Chilean lentil germplasm (*Lens culinaris* Medik). pp: 135 In: Proceedings of International Food legume Research Conference III. Adelaide, Australia, September 1997. pp: 22-26.

Sarangi, N. and A.B. Mandal, 1996. Isozyme polymorphism in diploid and heat shock-induced tetraploid Indian Major Carp, *Labeo rohita* (Hamilton). Curr. Sci., 71: 227-236.

Tanksley, S.D. and C.M. Rick, 1980. Isozymic gene linkage map of the tomato: Applications in genetics and breeding. Theor. Applied Genet., 57: 161-170.