Journal of Global Biosciences
ISSN 2320-1355
Volume 5, Number 6, 2016, pp. 4258-4266
Website: www.mutagens.co.in
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Research Paper

GROSS MACRO AND MICRO- MORPHOLOGIC STUDIES ON FOUR SPECIES OF *Codiaeum* IN NORTH CENTRAL NIGERIA

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Abstract

Gross phenotyping of four species of *Codiaeum* was undertaken in North Central Nigeria. Specimens of four duly identified taxa of *Codiaeum* (*C.bractiferum*, *C.hirsutum, C.trichocalyx* and *C.variegatum*) across different locations in Makurdi, Lafia and Jos were collected. Propagation was done in the Biological Sciences garden of the University of Agriculture, Makurdi, Nigeria. Twenty (20) stems of each species were propagated, totaling sixty (80) accessions. A total of Fifteen (15) macroscopic characters were studied at maturity. Foliar epidermal features of all accessions were evaluated. Mean values of all quantitative characters were computed and analysed on the SPSS software. Degree of relationship amongst variable qualitative characters was established through Spearman's rank correlation. All characters were classified using the Ward's method to generate a dendrogram for phylogenetic study. The four species displayed a high level of variability (in all cases) and correlation (to some extent) in qualitative characters with respect to leaf shape, apex, margin, base, venation and colour. *C.trichocalyx* had the longest leaf (20.37cm) while *C.bractiferum* had the narrowest leaf sizes (0.84cm in breadth) but the tallest plant recorded (40.7cm) which is *C.bractiferum*. All the species had three types of stomata (Anomocytic, Tetracytic and Anisocytic) with the exception of *C.trichocalyx* lackingAnomocytic stomata. The highest stomatal index (55.5%) was recorded on the abaxial surface of *C.bractiferum*. Dendrogram revealed two partitions and combinations amongst the species: hirsutum-variagetumand bractiferum-trichocalyx. However, the genetic distance between former was shorter than that of the latter. This suggests that *C.hirsutum* and *C.variegatum* may exist at varietal level but not as different species based on the taxonomic evidence employed in this study. The high level of intra and interspecific variability amongst the species is beneficial to floriculture and breeders for improvement purposes. Desirable characteristics may be exploited to breed more alluring variants that would express combined features of interestfor aesthetic purposes based on the information obtained in this study.

Key words: Phenotyping, *Codiaeum*, Dendrogram, Variability, Improvement, Taxonomy.

INTRODUCTION

The genus *Codiaeum* (A. Juss) belongs to the family Euphorbiaceae and encompasses 17 species native to tropical forest from Indonesia and Philippine to New Guinea and Australia (Brown, 1995; Govaerts*et. al.,* 2000; Deng *et al*., 2010a). Brown (1995) as cited in Deng *et al*. (2010a) stated that the plant was first introduced to England in1804, but hybrids were developed mainly in Belgium and France in the 1800's. Many of the European hybrids were introduced into the United States in 1871. At least 70 hybrids commonly known as Florida hybrids were developed in South Florida during the 1920's and 1930's as adoring ornamental plants with colourful leaf pigmentation. There are only six known basic species of *Codiaeum* from which, all other cultivars arose as mutants or hybrids(Deng *et al*., 2010b). Generally, *Codiaeum* species are a group of beautiful variegated leafy perennial tropical ornamental herbs, shrubs or trees with glabrous branches and prominent leaf scars (Dutta, 2004). The most commonly cultivated species is the *C.variegatum* popularly known as croton, from where other species probably originate (Deng *et al*., 2010a). Crotons are evergreen trees and shrubs with glossy leathery leaves that have varied leaf shapes and vivid foliage colours displayed as shades, blends, combination or solid patches of red, pinks, orange, yellow lavender, black and green. Leaf sizes ranges from small (6cm long x 1cm wide) to large (35cm long x 13cm wide) and margins can be entire or trifurcate. The leaves are alternate, non-serrated but sometimes lobbed. The leaf shape varies from simple-ovate, linear-lanceolate, oblong, elliptic, lanceolate, spathulate, fiddle-shaped to broad and obovate.Sometimes the leaf blade is interrupted along the midrib and become divided into an upper and lower part. The leaf is probably green in its original natural state, but in cultivated forms, it is variously marked, streaked, bloched or banded with green, white, yellow, crimson, scarlet, brown or cream colourand the reds (orange, purple, pink, indigo, violet) when grown in appropriate light conditions (Deng *et al*., 2010a).

In addition to its aesthetic value, crotons are also well known for their medicinal values. The leaf extracts are reported to have many medicinal properties including purgative, sedative, antifungal antiamoebic and anticancerous activities (Deshmukh and Borle, 1975; Kupchane*et. al.,* 1976). This is because the plant contains valuable secondary metabolites such as alkaloids, terpenes and flavonoids (Puebla *et. al.,* 2003; Maciel*et. al.,* 1998; Martins *et. al.,* 2002). Other medicinal uses of crotons as well as other species of *Codaieum*have been reported (Robert *et al.,* 1988; Burkill, 1994; Moundpa*et. al.,* 2005; Gertrudes, 2006; Deng *et al*., 2010a). Seed propagation results in plants with different phenotypes (Sharma and Bal, 1958; Mulabagal and Tsay, 2004). In commercial production, once a hybrid selection is made, it is propagated asexually through stem cuttings (Chen and Stamps, 2006; Nasib*et. al.,* 2008; Deng *et al*., 2010). The plant is very high in demand and constitutes a huge source of income to floriculturists due to its foliar diversity. Micro-propagation is a relatively new technology and application of innovative method have served to overcome barriers to progress in the multiplication of elite species and further improvements are anticipated (Shibata *et. al.,* 1996; Orlikowska*et. al.,* 2000; Deng *et al*., 2010a).

The croton society (2010) as cited in Deng *et al*. (2010a) categorized croton cultivars into nine (9) types based on their leaf morphology which are: broad leaf, oak leaf, semi oak, spiral leaf, narrow leaf, very narrow leaf, small leaf, interrupted leaf and re-curved leaf. However, information regarding thephylogenetic relationship of cultivated croton is incomplete. Cytological studies of cultivar produced in India (Sharma and Bal, 1958),

the Philippines (Pancho and Hilario, 1963), Nigeria (Ogunwenmo*et. al.,* 2007), and Florida (Deng *et. al.,* 2010b) showed that crotons are predominantly polyploid with 2n=60-120. Endo-reduplication, irregular chromosome segregation, sindlemultipolarity, andunusual nucleus shape were observed in crotons and proposed to be the mechanisms underlying the wide range of chromosome variation culminating in diverse phenotypes (Deng *et al*., 2010a; 2010b). As a result, establishment of genetic relationships among cultivarsis difficult and is hardly undertaken thereby placing enormous challenge on plant taxonomists (Deng *et. al.,* 2010a; 2010b). This has hampered efforts on croton germplasm conservation and new cultivar development. This perennial problem may however be solved using appropriate combination of taxonomic evidences as applied in other plants with such taxonomic complications in Nigeria (Aguoru *et al*., 2014a; Aguoru *et al*., 2015a; 2015b; 2015c; 2015d). The present study intended to carry out detailed phenotyping of four species of *Codiaeum* found across the North Central zone of Nigeria. The overall aim was to establish the phylogenetic relationship amongst the species and delimitate them accordingly on the basis of foliar macro and micro phenotyping.

MATERIALS AND METHODS

Stems were cut from four duly identified species of *Codiaeum* collected from different locations in Makurdi (Benue State), Lafia (Nasarawa State) and Jos (Plateau State).All in North Central Nigeria. Stem propagations were done in soil-filled polythene bags in the Biological Sciences garden of the University of Agriculture, Makurdi, Nigeria. Twenty (20) stems of each species were propagated, totaling sixty (80) accessions. Plants were nursed and allowed to grow to full maturity. Gross macro morphological observations and measurements were carried out. A total of Fifteen (15) macroscopic characters were studied.Abaxial and adaxial foliar epidermal features of all accessions were examined on the compound light microscope after slide preparations (Aguoru *et al*., 2014a, 2014b). Stomatal types were observed and recorded. Stomatal densities and number of epidermal cells were evaluated and employed in the calculation of stomatal indices following the method of Aguoru *et al*. (2014a). Photomicrographs were captured using digital camera with electronic microchip slot and transferred to a computer system. Mean values of all quantitative characters were computed and analysed on the SPSS software. Degree of relationship amongst variable qualitative characters was established through Spearman's rank correlation. All characters were classified using the Ward's method to generate a dendrogram for phylogenetic study. Data were described and presented in tables and graphs.

RESULTS AND DISCUSSION

The four species displayed a high level of interspecific variability in qualitative characters with respect to leaf shape, apex, margin, base, venation and colour (Table 1). Leaf shapes were linear, pinnatifid, lanceolate and ovate amongst the species (Plate 1). Leaf apices were rounded, cuspidate, acyte and acute. Leaf bases were rounded, cuneate, cordate and aricalate. Leaf venation was inconspicuous in *C.bractiferum* and *C.hirsutum*but palmately netted in *C.trichocalyx* and *C.variegatum*. Leaves were multi-coloured which ranged from light or dark green with yellow and pink spots. Mean quantitative characters revealed that *C.trichocalyx* had the longest leaf (20.37cm) with the widest breadth at same time (6.21cm). *C.bractiferum* had the narrowest leaf sizes (0.84cm in breadth) but the tallest plant recorded (40.7cm).Speaman's correlation showed a high positive correlation of +1 between leaf shape (LS) and apex (LA) as well

as between leaf shape (LS) and base (LB). All leaf characters were negatively correlated with leaf colour (Table 2).All the species had three types of stomata (Anomocytic, Tetracytic and Anisocytic) with the exception of *C.trichocalyx* which lacked Anomocytic stomata.*C.bractiferum* had the highest stomatal index (55.5%) found on the abaxial surface (Table 3) while *C.trichocalyx* recorded the lowest stomatal index found the adaxial surface with 7.8% (Figure 1).Dendrogram(Figure 2) has revealed two partitions among the four species: *C.hirsutum-C. variagetum*and *C.bractiferum-C. trichocalyx* combinations However, the genetic distance between hirsutum-variagatum (<5) is shorter than that of bractiferum-trichocalyx suggesting that *C.hirsutum* and *C.variegatum* may exist at varietal level but not as different species. From a far distance, *C.trichocalyx* arose from *C. bractiferum* as the mother stock. Photomicrograph of the latter species displayed unique epidermal features close to the guard cells arranged in beadlike or gland-like fashion (Plate 2c).

Results are consistent with earlier reports on *Codiaeum* species being genetically diverse (Brown, 1995; Govaerts*et. al.,* 2000; Deng *et al*., 2010a; 2010b). The plant has been reported to exhibit a high level of plasticity, polyploidy and hybridization (Ogunwenmo*et. al.,* 2007; Deng *et. al.,* 2010a; 2010b). This may suggest a form of sympatric speciation frequently occurring in the genus *Codiaeum*with constant genetic diversification. Chennaveeraiah and Wagley(1985) explained the mechanism of chromosome mosaicism in cultivars of garden crotons (*Codiaeumvariegatum*Blume). As far back as 1963, Pancho and Hilario reported incidence of intraspecific variability among members of *C.variegatum*and proposed that they may likely give rise to new species in the future.

Old discovery also exists that some cultivars of *Codiaeum*arose as a result of frequent mutation (Chennaveeraiah and Wagley, 1985). Thus, it seems that genetic hybridization, mutation and diversification are the driving forces behind the enormous diversity of foliar features (like leaf shapes, bases, pigmentations, apices and venations amongst other qualitative characters) in existence today.*C.trichocalyx* which lacked Anomocytic stomata may be described as a mutant of *C.bractiferum* from all indications. This report has confirmed that foliar epidermal characters are indispensable in the determination of phylogenetic relationship amongst the *Codiaeum* species studied. Hence, anatomical and gross phenotypic characterization may be employed to solve taxonomic problems existing in the genus. They may also be combined with molecular tools and markers study to arrive at a more robust analysis and conclusion (Deng *et al*., 2010a; Aguoru *et al*., 2015a). Stomatal indices are considered low among all the species of *Codiaeum*investigated (the highest being 55.5% in *C.bractiferum*) compared to other ornamental plants such as *Plumeria*(Aguoru *et al*., 2015d) with higher stomatal indices (63.3%). The lower stomatal indices of the leathery leaf with thick cuticle thus suggest a good water conservation mechanism of the genus. This may account for the high rate of adaptability, acclimatization and survival of the plant in harsh environment as characteristic of exotic plants. The high level of variability coupled with rapid hybridization amongst the species is an added benefit to floriculturists and breeders. This is because improvement exercise is anchored on the existence of genetic diversity among plants (IBPGR, 2015). These properties may be exploited to breed more alluring variants that would express unique characteristics of different cultivars for aesthetic purposes based on the information given in this report.

Table 1: Morphological characters

Morphological Characters	*Codiaeumbractiferum*	*Codiaeum trichocalyx*	*Codiaeum Hirsutum*	*Codiaeumvariegatum*
Leaf type	Simple	Simple	Simple	Simple
Leaf shape	Linear	Pinnatifid	Lanceolate	Ovate
Leaf apex	Rounded	Cuspidate	Acyte	Acute
Leaf margin	Entire	Pinnatifid	Pinnatifid	Pinnatifid
Leaf arrangement	Alternate	Alternate	Alternate	Alternate
Leaf base	Rounded	Cuneate	Cordate	Aricalate
Leaf venation	NV	Palmately netted	NV	Palmately netted
Leaf pubescence	Absent	Absent	Absent	Absent
Leaf colour	LgYs	DgYs	DgYPs	DgYs
Leaf length	10.92cm	20.37cm	12.25cm	11.35cm
Leaf breadth	0.84cm	6.21cm	4.14cm	13.32cm
Petiole length	1.83cm	2.55cm	1.94cm	2.12cm
Plant height	40.7cm	27.5cm	21.40cm	30.32cm
Stem colour	GB	GB	GB	GB
Root colour	Brownish	Brownish	Brownish	Brownish

Legend: LgYs=Light green with yellow spots; DgYs= Dark green with yellow spotsDgYPs=Dark gree with yellow-pink spots; NV=Not visible; GB= Greenish brown

Table 2: Spearman's rank correlation of variable qualitative characters

			LS	LA	LM	LB	LV	LC
Spearman's rank	LS	Correlation Coeff	1.000	1.000	.775	1.000	.447	-.211
	LA	Correlation Coeff	1.000	1.000	.775	1.000	.447	-.211
	LM	Correlation Coeff	.775	.775	1.000	.775	.577	-.272
	LB	Correlation Coeff	1.000	1.000	.775	1.000	.447	-.211
	LV	Correlation Coeff	.447	.447	.577	.447	1.000	-.943
	LC	Correlation Coeff	-.211	-.211	-.272	-.211	-.943	1.000

LS=Leaf shape, LA=Leaf apex, LM=Leaf margin, LB=Leaf base, LV=Leaf venation, LC=Leaf colour

Table 3: Stomatal Types and Indices of the four species

Species	Abaxial Stomatal Types	Abaxial Stomatal Indices (%)	Adaxial Stomatal Types	Adaxial Stomatal Indices (%)
Codiaeumvariegatum	Anomocytic Tetracytic Anisocytic	31.19	Anomocytic Tetracytic Anisocytic	38.39
Codiaeumhirsutum	Anomocytic Tetracytic Anisocytic	32.57	Anomocytic Tetracytic Anisocytic	35.78
Codiaeumbractiferum	Anomocytic Tetracytic Anisocytic	55.51	Anomocytic Tetracytic	12.22
Codiaeumtrichocalyx	Tetracytic Anisocytic	37.61	Tetracytic	7.80

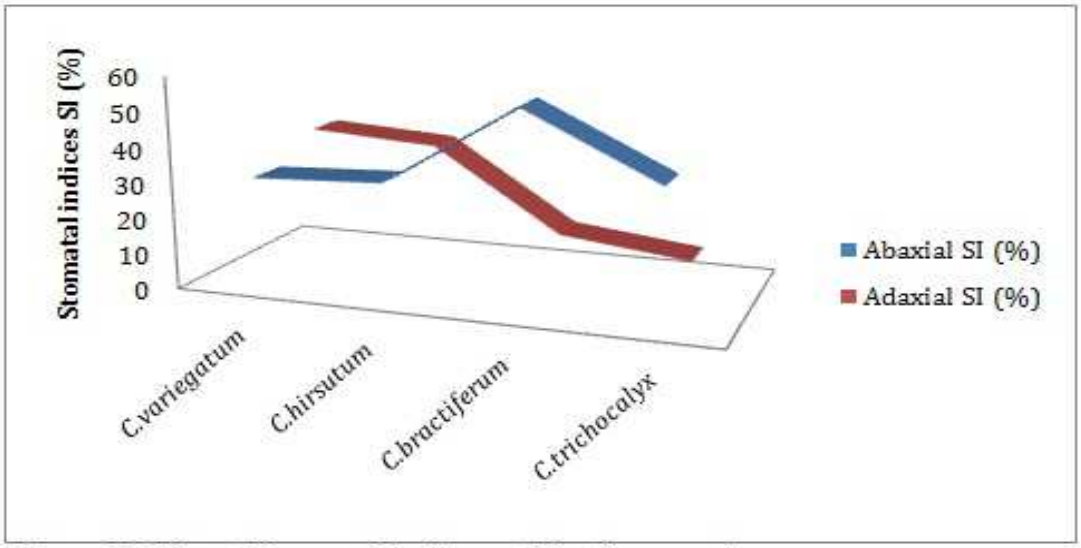


Figure 1: Plot of Stomatal indices of the four species

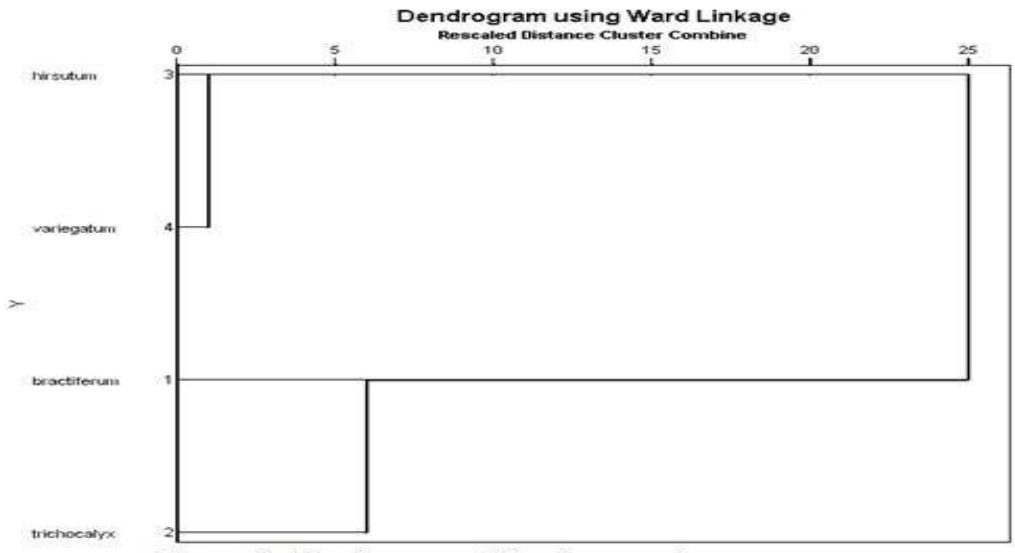


Figure 2: Dendrogram of the four species

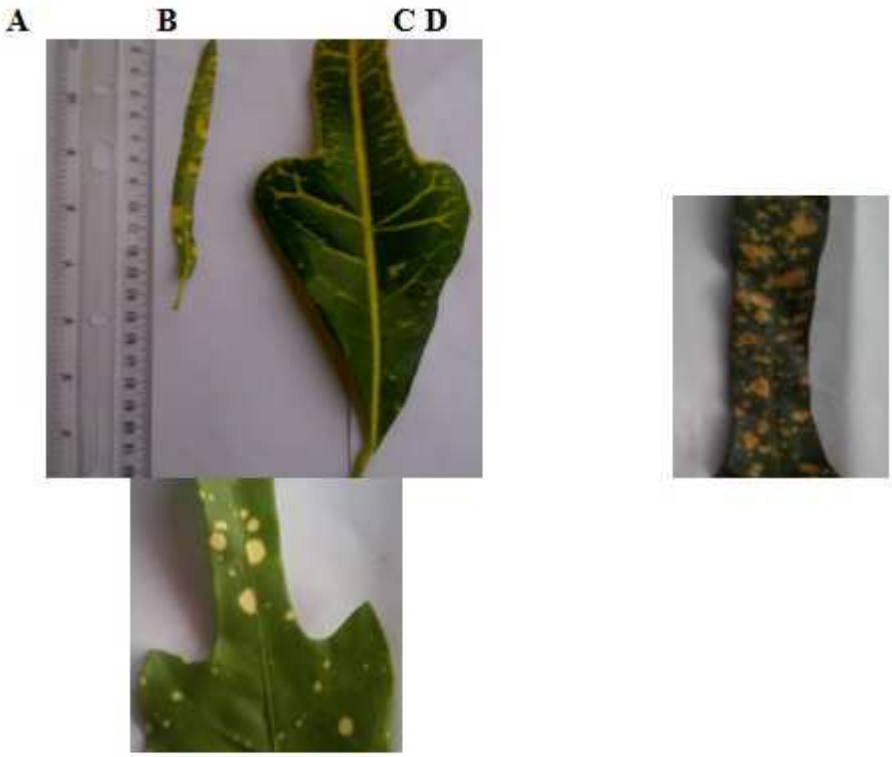
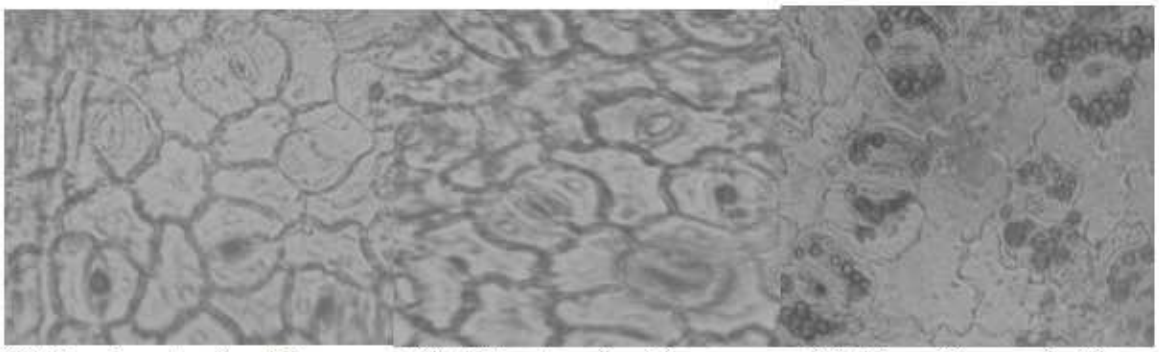


Plate A-D: Leaf morphologies of the four species
A= *Codieaumbractiferum* leaf
B= *Codieaumtrichocalyx*leaf
C= *Codieaumhirsutum*leaf
D= *Codieaumvariegetum*leaf



(a) *C.variegatum*abaxial (b) *C.hirsutum*abaxial (c) *C.bractiferum*adaxial
Plate 2a-c: Photomicrographs of selected epidermal surfaces

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