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FIRST KARYOTYPE REPORT ON *Colocasia oresbia*: A COMPARATIVE CYTOGENETIC STUDY BETWEEN TWO VARIETIES

PRIMER REPORTE DEL CARIOTIPO DE *Colocasia oresbia*: UN ESTUDIO CITOGENÉTICO COMPARATIVO ENTRE DOS VARIEDADES

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ABSTRACT

Karyotypes of two *Colocasia oresbia* botanical varieties from Bangladesh were analyzed and compared with orcein, chromomycin A_3 (CMA) and 4´-6 diamidino-2-phenylindole (DAPI). Both varieties had 2n= 2x = 26 chromosomes (karyotypic formula: 20m+ 6sm) and a pair of satellites each. Total chromosome length was 144.18 ± 2.45 µm in *C. oresbia* var. *oresbia* and 133.02 ± 2.75 µm in *C. oresbia* var. *stolonifera*. The karyotype of *Colocasia oresbia* var. *oresbia* is 2A whereas that of *C. oresbia* var. *stolonifera* is 1A. Six CMA and four DAPI bands were observed in *C. oresbia* var. *oresbia* and eight CMA and six DAPI bands in *C. oresbia* var. *stolonifera*. However, in these two morphologically distinct *C. oresbia* varieties of two different ecological zones, the same somatic chromosome number, diversification in various karyotypic parameters and CMA/DAPI-banding patterns were observed. In addition to taxonomic characters, the studied karyotype features will contribute to the characterization of these two *C. oresbia* varieties and to establish a base for future research.

Key words: chromosome banding; CMA; DAPI; Karyotype.

RESUMEN

Se analizaron y compararon los cariotipos de dos variedades botánicas de *Colocasia oresbia* de Bangladesh con orceína, chromomicina A_3 (CMA) y 4-6 diamidino-2-phenilindol (DAPI). Ambas variedades presentaron 2n= 2x = 26 cromosomas (fórmula cariotípica: 20m+ 6sm) y un par de satélites cada una. La longitud total de cromosomas fue 144,18 ± 2,45 µm en *C. oresbia* var. *oresbia* y 133.02 ± 2.75 µm en *C. oresbia* var. *stolonifera*. El cariotipo de *Colocasia oresbia* var. *oresbia* es 2^a, y 1^a el de *C. oresbia* var. *stolonifera*. Se observaron seis bandas CMA y cuatro DAPI en *C. oresbia* var. *oresbia* y ocho bandas CMA y seis DAPI en *C. oresbia* var. *stolonifera*. Sin embargo, en estas dos variedades morfológicamente distintivas de *C. oresbia* de dos zonas ecológicas diferentes se observó el mismo número cromosómico somático, diversificación en varios parámetros cariotípicos y en patrones de bandeo CMA/DAPI. En adición a los caracteres taxonómicos, las características de los cariotipos estudiados contribuirán a la caracterización de estas dos variedades de *C. oresbia* y a establecer una base para futuras investigaciones.

Palabras clave: bandeo cromosómico; CMA; DAPI; cariotipo

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INTRODUCTION

The genus *Colocasia* Schott belonging to the Araceae family, comprises about 20 species over the world (Li and Boyce, 2010). A total of nine of these species has been reported for Bangladesh so far, such as *C. affinis* Schott, *C. esculenta* (L.) Schott, *C. fallax* Schott, *C. gigantea* (Blume) Hook. f., *C. heterochroma* H. Li *et* Z.X. & Wei, *C. lihengiae* C.L. Long *et* K.M. Liu, *C. mannii* Hook. f., *C. oresbia* A. Hay and *C. virosa* Kunth. (Ara and Hassan, 2019). This genus is popular because it is edible and has medicinal, ornamental and cultural importance. Ara and Hassan (2019) reported and differentiated two varieties of *C. oresbia* from Bangladesh *viz. C. oresbia* A. Hay var. *stolonifera* H. Ara & M.A. Hassan, var. nov. and *C. oresbia* A. Hay var. *oresbia* based on several prominent morphological features. In fact, most species of this genus are morphologically distinct although the morphological features of a few of them are very confusing. In those cases, karyo-morphological information can open a new direction for evaluating the relationship among them. The nature and degree of karyotype differences obtained from conventional and fluorescent banding techniques could be useful to discuss plant phylogeny. In addition, the cytogenetical information will be useful for development of successful breeding programs in this crop. So far *C. oresbia* is unexplored cytogenetically. Therefore, in the present study, a combination of morphological and cytogenetical analyses with orcein, chromomycin A_3 (CMA) and 4´-6 diamidino-2-phenylindole (DAPI) were carried out for the first time to present karyotype data from two varieties of *C. oresbia viz. Colocasia oresbia* var. *stolonifera* and *Colocasia oresbia* var. *oresbia* to determine chromosomal relationships among them.

MATERIALS AND METHODS

Two varieties of *Colocasia oresbia viz. C. oresbia* A. Hay var. *stolonifera* H. Ara & M.A. Hassan, var. nov. and *C. oresbia* A. Hay var. *oresbia* were studied. *Colocasia oresbia* var. *oresbia* was collected from Chittagong, Cox's Bazar, Khagrachari, Moulvibazar, Rangamati, Kaptai, Rajbari area, Shubalong and Dhaka (flat regions) of Bangladesh whereas *C. oresbia* var. *stolonifera* was found and collected only from Rangamati district (hilly regions), Bangladesh. For cytogenetic investigation, healthy roots of ten individuals of each variety were collected and pretreated with 2 mM 8-hydroxyquinoline for 3 h at room temperature followed by 15 min fixation in 45% acetic acid at 4 °C, then hydrolyzed in a mixture of 1 N HCl and 45% acetic acid (2:1 v/v) at 60 °C for 3 min. The root tips were stained and squashed in 1% aceto-orcein. For CMA- and DAPI-banding, Alam and Kondo's (1995) method was used with slight modifications. Slides were observed under a Nikon (Eclipse 50i) fluorescent microscope with a blue violet (BV) filter cassette for CMA and an ultraviolet (UV) one for DAPI-banding. CMA binds with GC (Guanine-Cytosine)-rich repetitive sequences of the genome expressing yellow fluorescence, and DAPI binds to AT (Adenine-Thymine)-rich repeats giving a characteristic blue color (Schweizer, 1976).

For every staining, at least 50 cells were observed in each variety. The idiograms were made on the basis of chromosome size in decreasing order. Levan *et al.* (1964) was followed for determining centromeric type of chromosomes. Karyotype asymmetry index (AI) was also calculated to determine the degree of karyotype heterogeneity (Paszko, 2006).

RESULTS AND DISCUSSION

Morphological investigation

The two studied varieties of *Colocasia oresbia* show some prominent morphological dissimilarities. *Colocasia oresbia* var. *stolonifera* has stolons, which are absent in *C. oresbia* var. *oresbia*. They also show differences in inflorescence formation: the inflorescence of *C. oresbia* var. *stolonifera* is normally formed in group of up to 3 but in *C. oresbia* var. *oresbia* inflorescence occurred in group of up to 8 (never less than 4).

Somatic chromosome number and karyotype analysis

This present study provides detailed chromosomal information of *C. oresbia* for the first time. The two varieties are found to possess 2n=26 chromosomes (Figure 1A, B; Table 1). Somatic chromosome numbers 2n=28 and 42 have been reported for most of the studied species of this genus. Besides, some infrequent records such as 2n=26 in *C. gigantea* and *C. esculenta*, 2n=38 in *C. antiquorum*, and 2n=56 (tetraploid) in *C. esculenta* have also been reported (Wang *et al.*, 2017). Previous literature has stated the basic chromosome number of *Colocasia* is x=14 since most of the species belonging to this genus have 2n=28 chromosomes (Yang *et al.*, 2003). Other researchers have suggested that chromosomal variation regarding ploidy levels and aneuploidy occurred frequently in this genus (Fedorov, 1974; Kumar and Subramanian, 1979; Cao and Long, 2004; Huang *et al.*, 2012). Moreover, the presence of euploid and aneuploid cytotypes in different species represents inconstancy in the basic chromosome number. The reported basic chromosome numbers are x=13, 14, 19, present in 2x, 3x, and 4x cytotypes (Wang *et al.*, 2017). Previous studies concerning genus *Colocasia* showed that x=14 should be considered as ancestral basic chromosome number (Yang *et al.*, 2003; Wang *et al.*, 2017). In two varieties of *C. oresbia* of the present study, the basic chromosome

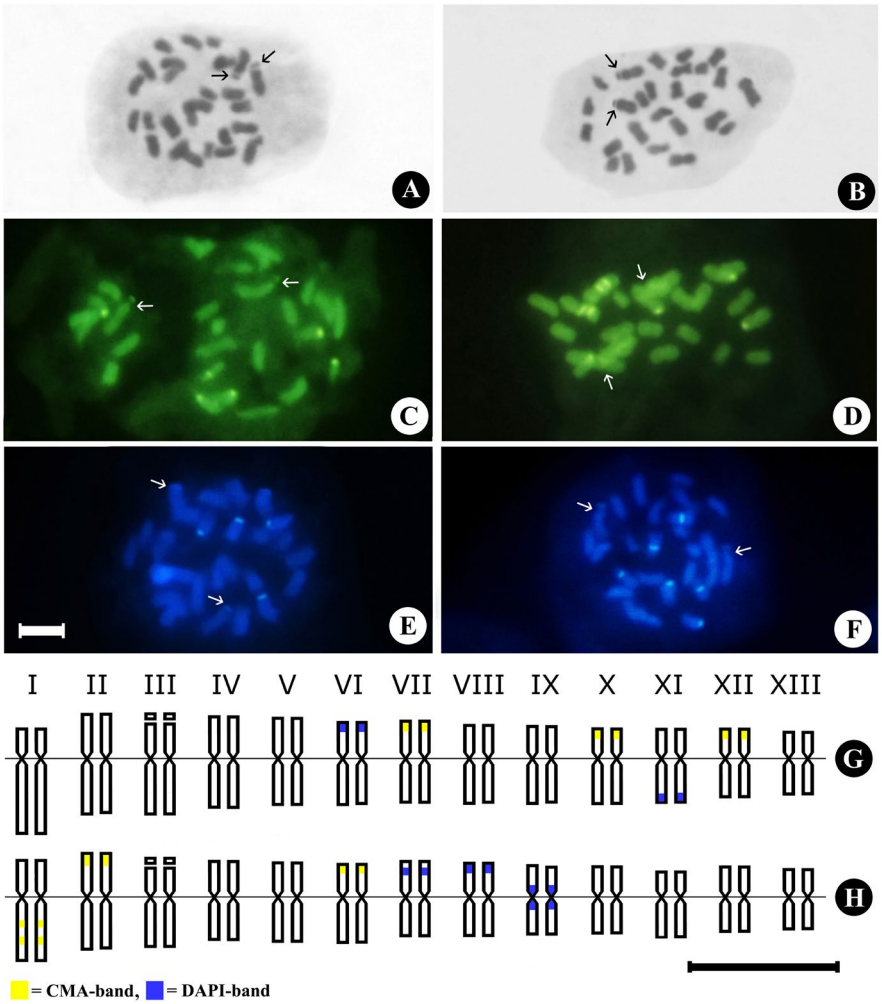

Figure 1. Metaphase chromosomes and idiograms of two *Colocasia oresbia* varieties. A. Orcein-stained mitotic metaphase of *C. oresbia* var. *oresbia*, B. Orcein-stained mitotic metaphase of C. *oresbia* var. *stolonifera*, C. CMA-stained mitotic metaphase of C. *oresbia* var. *oresbia*, D. CMA-stained mitotic metaphase of C. *oresbia* var. *stolonifera*, E. DAPI-stained mitotic metaphase of C. *oresbia* var. *oresbia*, F. DAPI-stained mitotic metaphase of C. *oresbia* var. *stolonifera*, G. Idiogram of C. *oresbia* var. *oresbia*, H. Idiogram of C. *oresbia* var. *stolonifera*. Arrows indicate satellites. Bars=10 µm.

number is x=13. Other previously reported basic chromosome numbers of x=13 and x=19 indicate that these two basic numbers probably originated from x=14 by secondary modifications (Leong-Škorničková *et al.*, 2007).

Both varieties of *C. oresbia* display relatively homogeneous karyotype arrangement with metacentric and submetacentric chromosomes with a KF of 20m + 6sm, and have one pair of satellites in chromosome pair III (Figure 1G, H). However, these two varieties show differences in other karyotype parameters. *Colocasia oresbia* var. *oresbia* and *C. oresbia* var. *stolonifera* has TCL of 144.18 ± 2.45 µm and 133.02 ± 2.75 µm, respectively (Table 1). The ACL is lower in *C. oresbia* var. *stolonifera* (5.12 µm) than *C. oresbia* var. *oresbia* (5.55 µm). The RCL is 4.23-7.02 µm in *C. oresbia* var. *oresbia* and 4.05-6.75 µm in *C. oresbia* var. *stolonifera*. The RL is 2.93-4.87% in *C. oresbia* var. *oresbia* whereas 3.04-5.07% in *C. oresbia* var. *stolonifera*.

When evolutionary positions are taken into consideration in relation to the karyotypic nature, symmetric karyotypes are usually regarded as primitive and asymmetrical as advanced, since karyotype asymmetry can be considered to be the dynamic force behind speciation (Stebbins, 1971). Furthermore, a higher AI value represents more asymmetric karyotypes (Paszko, 2006). The studied asymmetry index of karyotype reveals that the karyotype of *C. oresbia* var. *oresbia* is more asymmetric than the karyotype of *C. oresbia* var. *stolonifera*. Thus, *C. oresbia* var. *oresbia* is more advanced from an evolutionary point of view. Chromosome number and size along with karyotypic

Table 1. Comparative cytogenetical analysis of two *Colocasia oresbia* varieties.

Features	C. *oresbia* var. *oresbia*	C. *oresbia* var. *stolonifera*
Orcein staining		
2n	26	26
No. of satellites	2	2
KF	20m+6sm	20m+6sm
TCL (µm)	144.18±2.45	133.02±2.75
RCL (µm)	4.23–7.02	4.05–6.75
RL (%)	2.93–4.87	3.04–5.07
ACL (µm)	5.55	5.12
AI	1.65	1.20
Karyotype category	2A	1A
CMA		
No. of bands	6	8
Total banded region (µm)	7.65±0.53	9.03±0.74
Banded region (%)	5.31	6.79
DAPI		
No. of bands	4	6
Total banded region (µm)	4.50±0.78	7.20±0.68
Banded region (%)	3.12	5.41

2n=Somatic chromosome number; KF=Karyotypic formula; TCL=Total chromosome length; RCL=Range of chromosomal length; RL=Relative length of chromosome; ACL=Average chromosome length; AI=Asymmetry index of karyotype.

features are subjected to evolutionary change (Lavia *et al.*, 2009). Chromosome evolution can take place either by increasing or decreasing chromosomal length (Brandham and Doherty, 1998; Martel *et al.*, 2004). In this case, the total length of the chromosome complements increase in the course of evolution, since both varieties have similar 2n numbers and karyotype formula. *Colocasia oresbia* var. *oresbia* and *C. oresbia* var. *stolonifera* have 2A and 1A karyotypes, respectively, which also correlate with the asymmetric index (Table 1).

Fluorescent banding

Each variety exhibited distinct CMA-banding pattern (Figure 1C, H; Table 1). Six and eight CMA-bands were found in *C. oresbia* var. *oresbia* and *C. oresbia* var. *stolonifera*, respectively, with 5.31% GC-rich repeats in *C. oresbia* var. *oresbia* and 6.79 % in *C. oresbia* var. *stolonifera*. Six chromosomes (pairs VII, X and XII) of *C. oresbia* var. *oresbia* and four chromosomes (pairs II and VI) of *C. oresbia* var. *stolonifera* exhibited terminal CMA-bands. In addition, two chromosomes (pair I) of *C. oresbia* var. *stolonifera* had a peculiar CMA-banding pattern. In this variety, two chromosomes possess a pair of interstitial bands that may be used as chromosome markers. Four and six DAPI-bands were observed in *C. oresbia* var. *oresbia* and *C. oresbia* var. *stolonifera*, respectively. The DAPI-banded regions are 3.12% and 5.41% of the total chromosome complements in *C. oresbia* var. *oresbia* and *C. oresbia* var. *stolonifera*, respectively. Four terminal DAPI-bands (pairs VI and XI) in *C. oresbia* var. *oresbia* and two terminal DAPI-bands (pair VIII) in *C. oresbia* var. *stolonifera* were found. In addition, two centromeric (pair IX) and two intercalary DAPI-bands (pair VII) were also observed in *C. oresbia* var. *stolonifera* (Figure 1G, H). The mentioned findings suggest that each variety has a characteristic CMA and DAPI banded pattern with different number, location, total banded regions and percentage of GC- and AT-rich segments. Most of the bands are present at the terminal regions of the short arms of the respective chromosomes (Figure 1C, H). The presence of terminal bands indicated the tendency of accumulating GC- and AT-rich repetitive sequences at the chromosomal ends. Even though both varieties have the same chromosome number, diversification in karyotypic features and reshuffling of GC- and AT-rich

banded regions were observed. The variation in karyotype indices and fluorescence banding patterns may be the result of inversions, deletions or unequal translocations, among other chromosomal aberrations. The diversity in karyotypes of these two varieties may have arisen due to the exposure to different environmental conditions.

This research is the first cytogenetical report for *C. oresbia*. The findings of the present study would be useful for future breeding programs and a contribution to the systematics of the species.

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