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KARYOTYPE ANALYSIS WITH ORCEIN AND CMA IN TWO SPECIES OF *ALOCASIA* (SCHOTT) G. DON (ARACEAE)

SYEDA SHARMEEN SULTANA, HOSNE ARA[1] AND SHEIKH SHAMIMUL ALAM*

Department of Botany, University of Dhaka, Dhaka-1000, Bangladesh

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Abstract

Alocasia fallax Schott and *A. odora* (Roxb.) Koch (Araceae) were investigated cytogenetically to confirm their taxonomic status. There is no report of 2n chromosome number for *A. fallax* in the available literature and internet information. Therefore the 2n chromosome number (2n = 28) found in this study is probably the first report for *A. fallax*. *Alocasia odora* showed exactly double 2n chromosome number (2n = 56) from A. *fallax.* In addition to chromosome number, the other karyotypic features of *A. odora* were exactly double for that of *A. fallax*. The centromeric formulae of *A. fallax* was 24 m + 4 sm whereas it is just double in *A. odora*. Total length of 2n chromosome complement of *A. odora* (62.58 µm) was almost double to *A. fallax*. The range of chromosomal length of the two species was almost same. Moreover, *A. odora* plant is much taller than *A. fallax.* All of these data suggests that *A. odora* might be an autotetraploid of *A. fallax* which in course of evolution had undergone some changes in GC-rich repeats.

Introduction

The genus *Alocasia* (Schott) G. Don (Araceae) is a medium-sized perennial herbs to rarely arborescent and gigantic. *Alocasia* has wide distribution in India (Darjeeling, Sikkim, Himalaya, Khasia Hills, Assam and Meghalaya of eastern India) and Bhutan. In Bangladesh, there are 11 *Alocasia* spp. (Siddiqui *et al.* 2007) widely found in the shady areas of hill slopes and foot hills in rain forest. *Alocasia fallax* and *A. odora* are two of them. *Alocasia fallax* has been reported from Netrokona and Mymensingh whereas *A. odora* from Bogra, Faridpur, Mymensingh and Sylhet districts in Bangladesh (Uddin *et al*. 2001). The rhizomes of *Alocasia odora* possess medicinal value in curing stomach ache, abdominal pain and cholera. The same is crushed into a paste and applied externally on the human body to cure abacessess and insect or snake bites (Heng 1979).

The Bangladesh National Herbarium (BNH) has been collecting different *Alocasia* species from all over the country and maintained in the garden of BNH. The different *Alocasia* species are mainly collected and identified on the basis of plant morphology. Therefore, a problem regarding authentic identification is existing since specimen may show different morphology in different environment due to phenotypic plasticity.

Generally karyotype analysis plays important role in determining the taxonomic status. When the different taxa showed same chromosome number and similar karyotypic features, then it is hard to distinguish between them by conventional cytological analysis. Fluorescent chromosome banding is one of the modern cytogenetic methods commonly used for critical karyotype analysis. Recently Deen and Alam 2002 and Alam and Deen 2002 were able to distinguish different forms of *Colocasia esculenta* and *Xanthosoma violaceum* by differential fluorescent banding.

The present work was undertaken to confirm the taxonomic status of *A. fallax* and *A. odora* by extensive karyotype analysis after staining with orcein and CMA.

Materials and Methods

Alocasia fallax and *A. odora* were collected from different districts of Bangladesh and maintained in the garden of Bangladesh National Herbarium (BNH).

*Corresponding author: <ssalam81@yahoo.com>. [1]Bangladesh National Herbarium, Chiriakhana Road, Mirpur-1, Dhaka-1216, Bangladesh.

Alocasia fallax is a herb with short, suberect rhizome under 10 cm long. Leaves are moderately round shaped and slightly wrinkled inside. It shows 3 - 4 straight inflorescence. *Alocasia odora* is a large evergreen perennial herb, above-ground stem stout, 0.3 - 1.0 m high, 5 - 15 cm thick, unbranched, base with stolons. Leaves are round in shape. It have 3 curved inflorescence.

Healthy and young root tips were collected and pretreated with 0.002 M 8-hydroxyquinoline for 3 hrs at room temperature (28 - 30º C) followed by 15 min fixation in 45% acetic acid at 4º C. These were then hydrolysed in a mixture of 1 N HCl and 45% acetic acid (2 : 1) at 60º C for 30 sec. The shoot apices were stained and squashed in 2% aceto orcein. For fluorescent banding, Alam and Kondo's (1995) method was followed with slight modification. After hydrolysing and dissecting, the materials were squashed with 45% acetic acid. The cover glasses were removed quickly on dry ice and allowed to air dry for at least 48 hrs before study. The air-dried slides were first pre-incubated in McIlvaine's buffer (pH 7.0) for 30 min followed by Distamycin A (0.1 mg/ml) treatment for 10 min. The slides were rinsed mildly in McIlvaine's buffer supplemented with $MgSO_4$ (5 mM) for 15 min. One drop of CMA (0.1 mg/ml) was added to the materials for 15 min and rinsed with McIlvaine's buffer with $MgSO_4$ for 10 min. Slides were mounted in 50% glycerol and kept at 4º C for overnight before observation. These were observed under Hund fluorescent microscope with blue violet (BV) filter cassette.

Results and Discussion

Alocasia fallax and *A. odora* were found to possess 2n = 28 and 2n = 56 chromosomes, respectively (Figs 1, 3). Peterson (1989) reported 2n = 28 chromosomes for *A. odora*. However, in this study this species showed 2n = 56 chromosomes which is a clear disagreement with that of Peterson (1989). On the other hand, there is no report of 2n chromosome number for *A. fallax* in the available literature and internet information (Encyclopedia of Flora and fauna of Bangladesh, Vol. 11, 2007). Therefore the 2n chromosome number (2n = 28) found in this study is probably the first report for *A. fallax*.

In addition to chromosome number, the other karyotypic features of *A. odora* were exactly double for that of *A. fallax*. Such as: (i) The centromeric formulae of *A. fallax* was 24 m + 4 sm whereas it is just double in *A. odora* (Figs 5, 6, Table 1). (ii) Total length of 2n chromosome complement of *A. odora* (62.58 µm) was almost double to *A. fallax* (Table 1). (iii) The range of chromosomal length of the two spp. was almost same (Table 1).

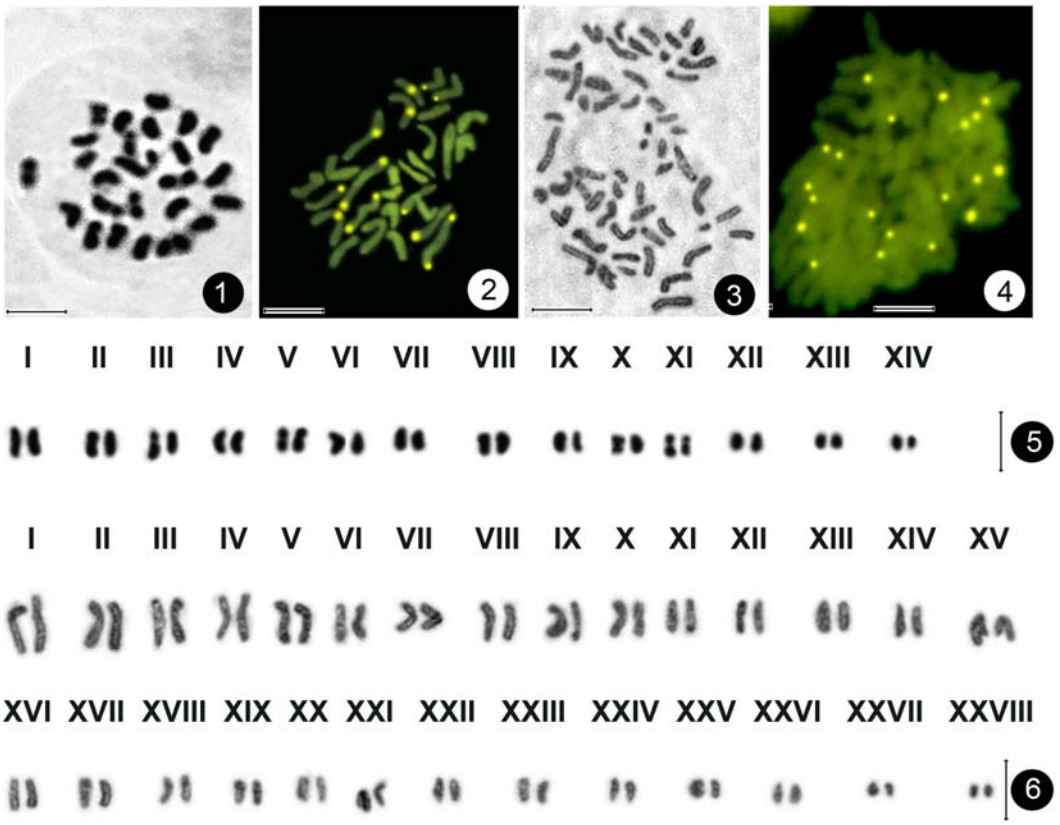
Table 1. Comparative orcein and CMA-karyotype analysis in two species of *Alocasia*.

Species	2n	Total length of 2n chromosome complement (µm)	Range of chromosomal length (µm)	Orcein karyotype formulae	No. of CMA positive bands	% of GC-rich repeats
A. fallax	28	29.30	0.42 - 1.35	24 m + 4 sm	15	21.50
A. odora	56	62.58	0.42 - 1.42	48 m + 8 sm	20	13.42

m = metacentric chromosome, sm = sub-metacentric chromosome.

However, these two *Alocasia* spp. possessed different CMA-banding pattern. Fifteen comparatively large CMA-positve bands were found in *A. fallax* whereas smaller 20 CMA bands present in *A. odora* (Figs 2, 4). The percentage of GC-rich repeats in *A. fallax* and *A. odora* were 17.59 and 21.50%, respectively. The CMA-banded regions of chromosomes are the GC-rich repeats (Schweizer 1976). It indicates that *A. odora* possessed little more GC-repeats.

The *A. odora* has exactly double 2n chromosome number, total length of diploid complement and centromeric formulae to those of *A. fallax* (Figs 5-6). The range of chromosomal length is almost same in these two species. Although the GC-rich repeats are not double in *A. odora*, the



Figs 1-6. Mitotic metaphase chromosomes of two species of *Alocasia*. 1. Orcein-stained mitotic metaphase of *Alocasia fallax*. 2. CMA-stained mitotic metaphase of *Alocasia fallax*. 3. Orcein-stained mitotic metaphase of *Alocasia odora*. 4. CMA-stained mitotic pro-metaphase of *Alocasia odora*. 5. Karyotype prepared from orcein-stained mitotic metaphase of *Alocasia fallax*. 6. Karyotype prepared from orcein-stained mitotic metaphase of *Alocasia odora*. Bars = 10 µm.

percentage is more than *A. fallax*. Moreover, *A. odora* is much taller than *A. fallax*. All of these data suggests that *A. odora* might be an autotetraploid of *A. fallax* which in course of evolution had undergone some changes in GC-rich repeats.

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