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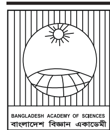

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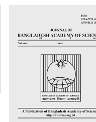

Research Article

Orcein and DAPI-stained karyotype analysis of *Alocasia macrorrhizos* (L.) G. Don

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ABSTRACT

Karyotype analyses are required for the identification, characterization, and genetic improvement of any organism. *Alocasia macrorrhizos* (L.) G. Don. was investigated cytogenetically to determine the karyotypic features. Complex chromocenter type, of interphase nuclei, and gradient type of prophase chromosomes were found in this study. *Alocasia macrorrhizos* was found to possesses 2n=28 chromosomes. The total length of the 2n chromosome complement was recorded as 98.83±1.39 µm. The range of chromosomal length was 2.50±0.10-4.70±0.10 µm. A gradual decrease in chromosomal length was observed. The total form (TF%) value was found to be 43.58%, Karyotype symmetry index (Syi %) was 77.00 % and karyotype asymmetry index (AsK %) was 56.66%. The centromeric formula was 18m+4sm+2ac, representing asymmetric karyotype. In DAPI banding, the 1.48% positive banded region indicates the lower amount of AT rich repeats in this material. Therefore, *Alocasia macrorrhizos* could be authentically characterized through karyotype analysis.

Introduction

Alocasia macrorrhizos (L.) G. Don is a fast growing herbaceous flowering plant belonging to the arum family Araceae. It is native to Malesia (including Peninsular Malaysia, the Philippines, and parts of Indonesia), Queensland, and the Solomon Islands. It is currently cosmo-politan and naturalized in many tropical and subtropical regions in North, Central and South America, the West Indies, tropical Africa and the Indo-Pacific Islands (Wagner et al., 1999). It is listed as invasive in Cuba, New Zealand, and a several numbers of islands within the Pacific, including Hawaii, French Polynesia, Fiji, New Caledonia, and Palau (Sykes, 1970; Smith, 1979; Wagner et al., 1999; González-Torres et al., 2012) and it is considered a weed in Vietnam (Koo et al., 2000). It is cultivated in India, Sri Lanka, Bangladesh, and also in Myanmar, Thailand, Peninsular Malaysia (Flach and Rumawas, 1996; Singh et al., 2017), and in tropical America, in some parts of Africa (Lebot, 2008). In Bangladesh, there are eleven Alocasia spp. (Siddiqui et al., 2007) found widely all over the country, and *Alocasia macrorrhizos* is one of them.

Different members of Araceae, such as *Colocasia esculanta* has three, *Xanthosoma violaceum* has two, Typhonium trilobatum has three morphological forms. Authentic identification of these taxa is difficult for Taxonomist (Ara, 2000). A latter extensive cytological investigation had been carried out in these taxa. The cytological data indicated a sharp difference among the different forms of

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Colocasia esculanta, *Xanthosoma violaceum* (Deen and Alam, 2002; Alam and Deen, 2002).

The common name of *Alocasia macrorrhizos* is Giant Taro and Elephant Ear Taro. It has been intentionally introduced in many tropical and subtropical regions to be used as an ornamental food crop and animal feed (Manner, 2011). The rhizomes of *Alocasia* possess medicinal value in curing stomach aches, abdominal pain, and cholera. The same is crushed into a paste and applied externally on the human body to cure abscesses and insect or snake bites (Heng, 1979).

However, it is well known that karyotypic feature plays an important role in determining the taxonomic status. But conventional karyotype analysis is alone unable to express critically the differences among different germplasm of a species since the germplasm of a species possess similar 2n chromosomes numbers and even other karyotypic features (Khatun and Alam, 2010; Khatun et al., 2011; Sultana and Alam, 2016a; Sultana et al., 2018). The somatic chromosome number 2n=14 (Subramanian and Munian, 1988), 2n=28 (Ramachandran, 1978; Chaudhuri and Sharma, 1979; Ankei, 1987; Ishida, 2001; Das, 2018; Senavongse et al., 2020) and 2n=42 (Bhattacharya, 1974.) in *A. macrorrhizos* were reported by a different scientist.

Moreover, the consideration of chromosome length, arm ratio, position, and number of secondary constrictions aren't always sufficient to differentiate individual chromosomes. Minute deletion, inversion, tandem duplication, etc., could not be detected by conventional karyotype analysis. In such a case, combining of modern cytogenetical and molecular techniques is necessary for comparative study among different germplasm of a species.

In addition, other karyomorphological parameters *viz.* Characteristic features of the staining property of interphase nuclei and prophase chromosomes should be considered to get more data about each germplasm. Tanaka (1971) classified the different types of interphase nuclei and prophase chromosomes based on orcein staining property. Later different scientists tried to characterize interphase nuclei and prophase chromosomes by differential staining with orcein, CMA, and DAPI (Alam and Kondo, 1995; Fawzia and Alam, 2011; Alam et al., 2011; Shahla and Alam, 2011; Sultana and Alam, 2016b). The outcome of these studies showed that various taxa, including varieties of many plant species, could be distinguished by their staining properties of interphase nuclei and prophase chromosomes.

Staining with DNA-base specific banding with fluorochromes such as DAPI (4´-6 diamidino-2-phenylindole) is a relatively recent method for karyotype study. Schweizer (1976), for the first time, initiated this fluorescence technique for cytogenetical study. DAPI binds to AT (Adenine-Thymine)-rich repeats giving characteristic blue color (Schweizer, 1976; Kondo and Hizume, 1982; Alam and Kondo, 1995; Jessy et al., 2005; Akhter and Alam, 2005; Islam and Alam, 2011; Manzum et al., 2014; Bonna et al., 2017; Dash et al., 2017). Thus it seems that the fluorescent banding technique is quite satisfactory for critical and details chromosomal study such as identification of individual chromosomes, determination of amount and site of AT-rich base pairs in chromosomes, etc.

In this investigation, an attempt was made for karyotypic analysis of *Alocasia macrorrhizos* found in Bangladesh, and after that, staining with orcein and DAPI is a continuation of above works.

Materials and Methods

In the present investigation, *Alocasia macrorrhizos* was used as experimental material. This plant species was transplanted in the botanic garden of the Department of Botany, Jahangirnagar University, Savar, Dhaka, Bangladesh, to collect fresh roots for the experiment. The following investigation proceeded for 6 Months.

Healthy young root tips were collected and pretreated with 0.002 M 8-hydroxyquinoline for 1.5 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 12 sec. Then the hydrolyzed roots were soaked on a filter paper and taken in a clean slide. The meristematic region was cut with a fine blade. A drop of 1% aceto-orcein was added to the material. A clean cover glass was placed on the material. Then the materials were tapped gently by a toothpick and then squashed by placing thumbs. The slides were observed under Nikon (Eclipse 100) microscope. For fluorescent banding, Alam and Kondo's (1995) method was followed with slight modification. After hydrolyzing and dissecting, the materials were squashed with 45% acetic acid. The cover glasses were removed quickly on dry ice and allowed for air drying for at least 48 hrs before the study. After 48 h of air drying, the slide was first pre-incubated in McIlvaine's buffer (pH 7.0) for 25 m. Next the slide was treated in 0.25 mg/mL actinomycin D for 10 m in a humid chamber. After antibiotic treatment, the slide was washed with distilled water so that the cover glass was removed. Next, the slide was immersed again in McIlvaine's buffer (pH 7.0) for 15 m followed by treating in DAPI solution (0.1 mg/mL) for 10 m. After rinsing in McIlvaine's buffer (pH 7.0) for 10 m, the slide was mounted with 50% glycerol and kept at 4 °C. These were observed under a Nikon (Eclipse 50i) fluorescent microscope with an ultraviolet (UV) filter cassette.

Results and Discussion

Orcein-staining

Interphase nuclei and prophase chromosomes

The staining properties of interphase nuclei and prophase chromosomes provide karyomorphological features that help to characterize different germplasm. In this study, darkly stained large heterochromatic regions were found at the peripheral region of nucleus in the orcein staining of interphase nuclei. A distinct nuclear boundary was observed here (Fig. 1). The presence of prominent nucleoli indicated the active transcription of rDNA for the synthesis of rRNA. The prophase chromosomes of orcein staining were darkly stained at one end and gradually become faint towards another end (Fig. 2).

Tanaka (1971) found that the nature of staining properties of heterochromatin presents in the interphase nuclei and prophase chromosomes were different in different species. He was the pioneer of proposing these criteria for karyomorphological features. Tanaka (1971) classified interphase nuclei and prophase chromosomes in five different categories in each case on the basis of the staining property. In the present study, complex chromocenter types of interphase nuclei and gradient type of prophase chromosomes were found. Constitutive heterochromatic nature may be indicated by this observation. Usually, germplasm with complex chromocenter type of interphase nuclei showed "Gradient type" of prophase chromosomes. In this study, the selected germplasm followed the general rule of heterochromatin.

Chromosome number

In the present investigation, *Alocasia macrorrhizos* was found to possess 2n=28 chromosome (Figs. 3 & 6; Tables 1 & 2). The same chromosome number was reported earlier by several scientists (Ramachandran, 1978; Chaudhuri and Sharma, 1979; Ankei, 1987; Ishida, 2001; Das, 2018; Senavongse et al., 2020). In addition, different chromosome numbers such as 2n=42 (Bhattacharya, 1974) and 2n=14 (Subramanian and Munian, 1988) chromosome count were also reported. Several scientists considered the basic chromosome number as x=7 for this species, then specimen with 2n=14 could be regarded as diploid, 2n=28 as tetraploid, and 2n=42 as hexaploid. However, 2n=28 observed in this study agreed with the basic number of x=7, and thus, the present experimental plant species may be considered as tetraploid due to 2n=4x=28 chromosome complement.

Karyotype analysis

In the present study, the total length of 2n chromosome complement was recorded as 98.83±1.39 µm. The range of chromosomal length was 2.50±0.10–4.70±0.10 µm. The difference between large and small chromosomes is 2.20 µm; thus, a gradual decrease in chromosomal length was observed. The range of relative length of an individual chromosome was 0.03 to 0.05. The total form (TF%) value was found to be 43.58%. and the karyotype symmetry index (Syi%) was 77.00%. On the other hand, karyotype asymmetry index (AsK%) was 56.66% (Table 2). The value of AsK% increases with the increasing asymmetry. The centromeric formula was 18m+4sm+2ac (Table 2). There was no heteromorphic in respect of centromeric position found in this material. This species possessed metacentric, sub-metacentric and acrocentric chromosomes representing asymmetric karyotype. According to Stabbins (1971), heterogenous or asymmetric karyotype indicates the advanced character, and thus, this material possessed the advanced karyotype.

DAPI-staining

Fluorescent banding gives a decisive analysis of karyotype, even chromosome having similar morphology and other conventional karyotypic features. The fluorescent banding technique with DAPI fluorochromes helps to provide information regarding AT-rich repeats in the genome. In addition, different types of chromosomal aberration, such as deletion, duplication, inversion, etc., could also be detected by this method. In this study, this DAPI fluorochrome was used for critical analysis of karyotype.

In the present study, a big and prominent nucleolus and a distinct nuclear boundary were observed in interphase nuclei. It was easy to differentiate the nucleus from the cytoplasm. The interphase nuclei were fluoresced brightly. A non-staining region was found in the nuclei of the specimen (Fig. 4). No band was found in the prophase chromosome of this specimen. The chromosomes were uniformly stained along the length (Fig. 5). In the metaphase stage, DAPI positive bands were found in only 2 chromosomes on their short-arm among 28 chromosomes (Figs. 6, 8, 10). Both the members of chromosome pair IX showed DAPI positive band in their short-arm. The total length of the DAPI-positive banded region was 1.46±0.06 µm. The DAPI positive banded region was 1.48%. DAPI-banded karyotypic formulae was $2\alpha+26\delta$ (Table 3). This result indicates the lower amount of AT rich repeats in these materials.

Therefore, karyomorphologically *Alocasia macrorrhizos* could be characterized in authentic way with the help of orcein and DAPI staining.

Table 1. Length (in µm), arm ratio, relative length, centromeric index and centromeric type of metaphase chromosomes of *Alocasia macrorrhizos*.

Chrom-osome pairs	Long arm (l) ($\bar{x}\pm$SD)	Short arm (s) ($\bar{x}\pm$SD)	Total length (TL) ($\bar{x}\pm$SD)	Arm ratio (l/s)	Relative length (RL)	Centromeric index (CI)	Centromeric type (CT)
I	2.70±0.10	2.20±0.26	4.70±0.10	1.23	0.05	46.81	m
	2.70±0.10	1.90±0.10	4.60±0.20	1.42	0.05	41.30	m
II	2.20±0.20	2.03±0.06	4.23±0.21	1.08	0.04	48.03	m
	2.10±0.00	2.00±0.00	4.10±0.00	1.05	0.04	48.78	m
III	2.10±0.10	1.93±0.06	4.03±0.06	1.09	0.04	47.93	m
	2.07±0.06	1.90±0.00	3.97±0.06	1.09	0.04	47.90	m
IV	2.03±0.06	1.80±0.10	3.80±0.00	1.13	0.04	47.37	m
	1.90±0.00	1.90±0.00	3.80±0.00	1.00	0.04	50.00	m
V	2.00±0.00	1.80±0.00	3.80±0.00	1.11	0.04	47.37	m
	2.00±0.00	1.80±0.00	3.80±0.00	1.11	0.04	47.37	m
VI	1.93±0.06	1.80±0.00	3.73±0.06	1.07	0.04	48.21	m
	1.90±0.00	1.80±0.00	3.70±0.00	1.06	0.04	48.65	m
VII	1.90±0.00	1.80±0.00	3.70±0.00	1.06	0.04	48.65	m
	1.90±0.00	1.80±0.00	3.70±0.00	1.06	0.04	48.65	m
VIII	1.93±0.06	1.60±0.00	3.53±0.06	1.21	0.04	45.28	m
	1.90±0.00	1.60±0.00	3.50±0.00	1.19	0.04	45.71	m
IX	2.70±0.00	0.73±0.06	3.43±0.06	3.68	0.03	21.36	ac
	2.60±0.00	0.73±0.06	3.33±0.06	3.55	0.03	22.00	ac
X	2.10±0.00	1.20±0.00	3.30±0.00	1.75	0.03	36.36	sm
	2.10±0.10	1.10±0.00	3.20±0.10	1.91	0.03	34.38	sm
XI	1.60±0.00	1.53±0.06	3.13±0.06	1.04	0.03	48.94	m
	1.60±0.00	1.50±0.10	3.10±0.10	1.07	0.03	48.39	m
XII	2.00±0.00	0.93±0.06	2.93±0.06	2.14	0.03	31.82	sm
	2.00±0.00	0.90±0.00	2.90±0.00	2.22	0.03	31.03	sm
XIII	1.60±0.00	1.30±0.00	2.90±0.00	1.23	0.03	44.83	m
	1.47±0.06	1.40±0.00	2.87±0.06	1.05	0.03	48.84	m
XIV	1.50±0.00	1.03±0.06	2.53±0.06	1.45	0.03	40.79	m
	1.47±0.06	1.03±0.06	2.50±0.10	1.42	0.03	41.33	m
	Total complement length		98.83±1.39				

m = Metacentric, sm = Sub-metace, ac = Acrocentric

Table 2. Orcein-stained karyotype analysis of *Alocasia macrorrhizos*.

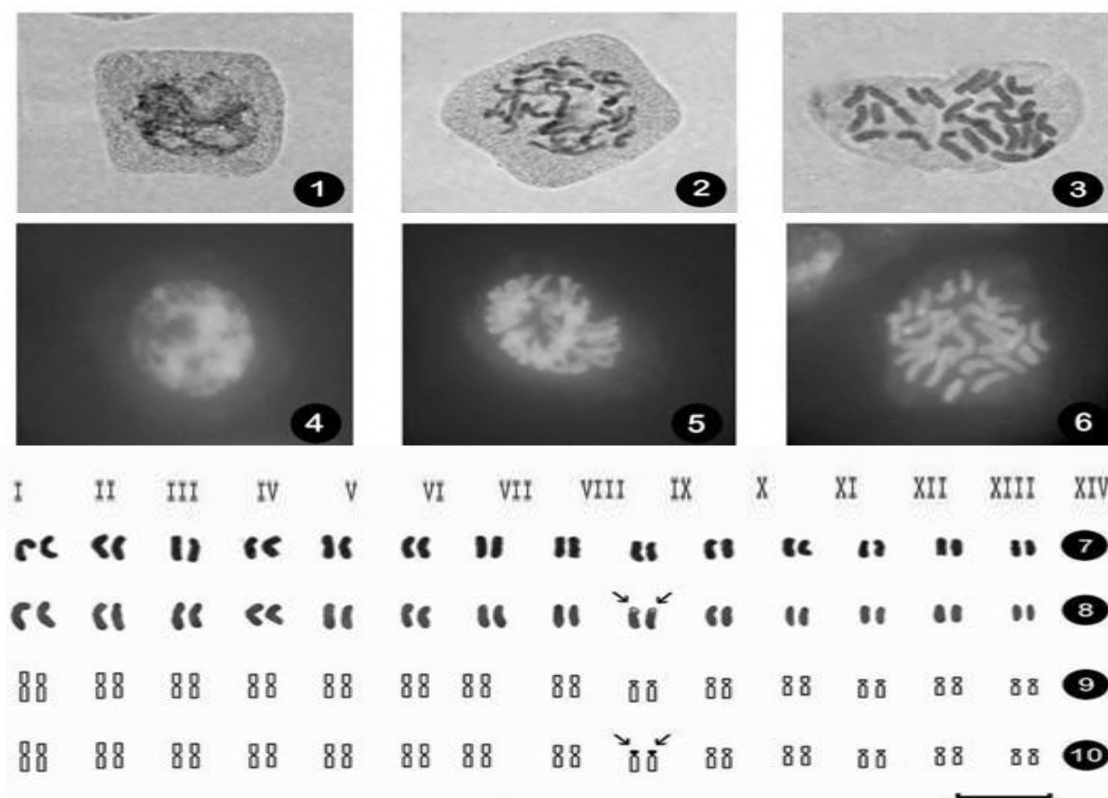
2n	RCL (μm) ($\bar{x} \pm$ SD)	TCL (μm)	RRL	TF %	Syi %	AsK %	CF	Nature of chromosomes
28	2.50±0.10-4.70±0.10	98.83±1.39	0.03-0.05	43.58	77.00	56.66	18m+4sm+2ac	Asymmetric

RCL = Range of chromosomal length, TCL= Total chromosomal length, RRL = Range of relative length, TF = Total form, Syi = Karyotype symmetry index, AsK = Karyotype asymmetry index, CF = Centromeric formula, m = Metacentric, sm = Sub-metace, ac = Acrocentric.

Table 3. DAPI-stained karyotype analysis of *Alocasia macrorrhizos*.

Species	2n	No. of DAP-bands	Total length of DAP-positive banded region (μm) ($\bar{x} \pm$ SD)	% of DAPI-positive banded region (μm)	DAPI- banded karyotypic formulae
Alocasia macrorrhizos	28	2	1.46 ± 0.06	1.48	2α + 26δ

Classification of CMA-positive bands : α=Band in whole short arm, δ=No band



Figs. 1-10. Orcein and DAPI-stained mitotic interphase, prophase, metaphase and karyotype of *Allocasia macrorhizos*.

1. Orcein-stained interphase nuclei, 2. Orcein-stained prophase chromosome, 3. Orcein-stained metaphase chromosome, 4. DAPI-stained interphase nuclei, 5. DAPI-stained prophase chromosome, 6. DAPI-stained metaphase chromosome, 7. Orcein-stained karyotype prepared from mitotic metaphase chromosomes, 8. DAPI-stained karyotype prepared from mitotic metaphase chromosomes, 9. Orcein-stained idiogram, 10. DAPI-stained idiogram, Bar=10μ. Arrow (→) indicates the DAPI band.

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