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Karyomorphological Studies in Three Species of *Alocasia* (Schott.) G.Don.- An Ethno-medicinally and Economically Important Genus

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ABSTRACT

Karyomorphological studies in *Alocasia macrorrhiza* (L.) G.Don., *Alocasia fornicate* (Roxb.) Schott, *Alocasia longiloba* Miq. belonging to the family *Araceae* using root tip squash technique was carried out. It was observed that the chromosome number of the three species was found to be 2n=28 and chromosomes were smaller in size. The chromosomes in *A. longiloba* were found to be longer in length in comparison to *A. macrorrhiza, A. fornicata.* Present studies also revealed that the karyotype was asymmetric type. The present karyomorphological study had been undertaken as it was an established fact that karyomorphological analysis forms a prerequisite for the genetic improvement of any plant species. This study would be helpful in the protection, conservations of the species by the establishment of germplasm bank.

Key-words *Alocasia macrorrhiza,* Chromosomes, Karyomorphology, Karyotypes, Symmetric type

INTRODUCTION

The genus *Alocasia* (Schott) G. Don belong to the family Araceae consisting of about 2,500 species [1]. There are 79 species native to tropical and sub-tropical Asia to Eastern Australia and wildly cultivated elsewhere [2]. The species of this genus have been distributed in the Northeast and South India, Sri Lanka, and Bangladesh [3]. These are generally grown in marshy lands, but sometimes also in shady damp places in the forest and in village thickets. In this study, three species of *Alocasia* (Schott) G.Don, *A. macrorrhiza* (L.) G.Don*, A. fornicate* (Roxb.) Schott*, A. longiloba* Miq. were selected. These species are ethno-medicinally and economically important plants. According to IUCN Red list, *Alocasia atropurpurea* and *Alocasia sanderiana* are tagged as Critically Endangered [4]. Several medicinal applications of *Alocasia* have been reported for South-East Asia.

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Boiled stems of *A. macrorrhizos* are used as a laxative, chopped-up roots and leaves as a rubefacient, and juice from the petiole against a cough. The plants are applied for stimulating the skin, e.g. in cases of fever and to remove blotches. The rhizome is sometimes used as a poultice to treat furuncles. The pounded stems are applied as a paste to snakebites and scorpion stings. *A. fornicata* (Roxb.) Schott is important as food and ethno-medicine in Asia and Africa since time immemorial. In India, many people have been using rhizome paste to treat wounds, cure heel cracks and kill worms in domestic animals. A number of chemicals could be isolated from this species. The antioxidant properties of the edible parts of different plants have already been established [5]. There is growing industrial interest towards the production of herbal antioxidants that can be supplemented in medicine, cosmetics, and nutraceuticals in the modern world.

The tissues of *Alocasia* contain calcium oxalate crystals, which produce irritation of the skin and inflammations of the oral cavity and mucous membranes. Sapotoxin is also present and the toxic effects include gastroenteritis and paralysis of the nerve centers. Hydrocyanic acid is often present. The poisonous substances can be removed by repeated cooking, but the rhizomes and bases of petioles

of *A. macrorrhizos* which are sometimes used for food usually contain few poisonous substances. A lectin has been isolated from the rhizome of *A. macrorrhizos*, which showed potent mitogenic activity on human peripheral blood lymphocytes in the [3H]-thymidine uptake assay. It was a T-cell mitogen and did not induce any appreciable DNA synthesis in B-enriched lymphocytes. This species also contains a protein which inhibits both the enzymes trypsin and chymotrypsin. The seed extract showed antifungal activity. There is also a report on HIV-1 protease inhibitory activity [6].

Alocasia is a plant of great economic value. Experimentally antimicrobial, antifungal, antioxidant, hepato-protective, anti-diarrheal, anti-protozoal, anticancer properties have been found in many species of *Alocasia*. As such it needs conservation and detailed chromosome studies would aid in creating germplasm [7].

Hence, the present study has been undertaken:
To conduct karyomorphological study in three species of *Alocasia* (Schott) G. Don:

- *A. macrorrhiza* (L.) G.Don
- *A. fornicate* (Roxb.) Schott
- *A. longiloba* Miq.

MATERIALS AND METHODS

In the present investigation three species of *Alocasia* (Schott) G. Don was collected from different localities of Greater Guwahati, India. The saplings were potted inside the institute campus for collection of fresh roots for the experiment. The following investigation proceeded for 5 months i.e. from January to May 2017.

Cytological investigation- For chromosome characterization in *A. macrorrhiza, A. fornicata and A. longiloba* detailed karyotypic studies were undertaken. Karyotypes were prepared from the somatic chromosomes. For cytological studies, root tips squash technique had done. Very young root tips were collected from the plant between 7:45 AM - 8:15 AM washed in double distilled water and pre-treated with saturated solution of Para dichlorobenzene at a suitable temperature for 3 hours at 4°C±2°C. Pretreated young root tips are fixed in a suitable fixative such as Carnoy's fluid-2 (1:3:6; Glacial acetic acid: Chloroform: Ethanol) for 24 to 28 hours at room temperature. After fixation the root tips were thoroughly washed with 70% ethanol and finally, they were stored used for cytological work. For the preparation of slides the root tips were first hydrolyzed with 1 N HCl and stained with aceto orecin and warmed over flame for 10 to 15 minutes and kept for 2–3 hours at room temperature. Single root tip was taken on a drop of 45% acetic acid on a slide. Only the dividing tip region was taken discarding the other tissue. Cover slip was placed over the tip and squashed by applying uniform pressure with the thumb through a piece of blotting paper, a gentle tapping followed by heat fixing and finally sealed with paraffin for further studies [8,9].

The temporary slides thus prepared were observed under compound microscope at a magnification of 1000*x* using oil immersion (10×100*x*, oil immersion). This procedure was standardized through trial and error method. Metaphase plates were selected for karyomorphological analysis of the chromosomes. Perfectly stained chromosomes were photographed using Trinocular microscope-N400-M, CMOS camera 5M with image analysis system. The drawings of the chromosomes were made with the help of camera lucida apparatus. Idiograms were then constructed on tracing paper.

Following parameters of the chromosomes were considered:

i) Length of the long arm
ii) Length of the short arm
iii) Total length of the chromosome
iv) Volume of the chromosome
v) Relative length
vi) Centromeric position

On the basis of the length, the chromosomes lengths were categorized under the following types-

Type A= 3.00 µm and above
Type B= 2.50 µm - 2.90 µm
Type C= 2.00 µm - 2.49 µm
Type D= 1.00 µm - 1.90 µm
Type E= 0.01 µm - 0.99 µm

The volume of an individual chromosome was calculated as chromosome volume-

$$(V) = \pi r^2 h$$

Where, r= Radius of the chromosome; h= Length of the whole chromosome.

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The total chromosome volume was then expressed by adding the volumes of all the chromosomes. On the basis of centromere position, the chromosomes were classified into metacentric, sub-metacentric, sub-telocentric, and telocentric following the nomenclature system of Levan *et al*. [10].

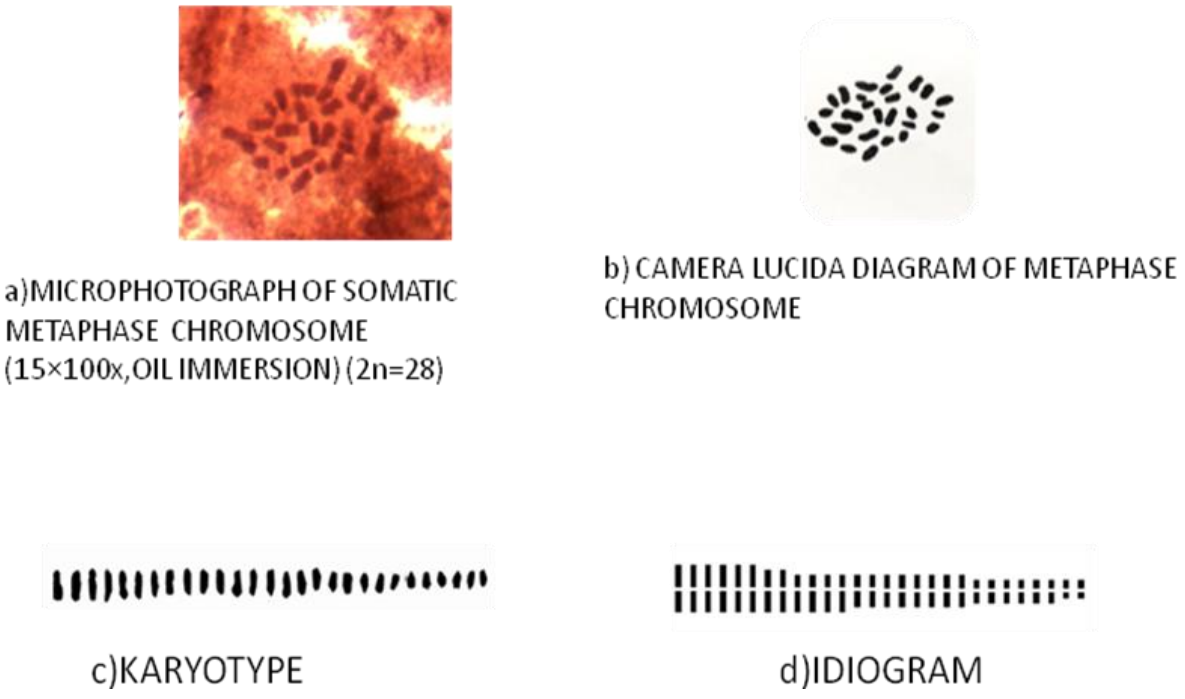

Fig. 1 (a-d): Karyotype and Ideogram of somatic chromosomes of *A. macrorrhiza* (L) G.Don

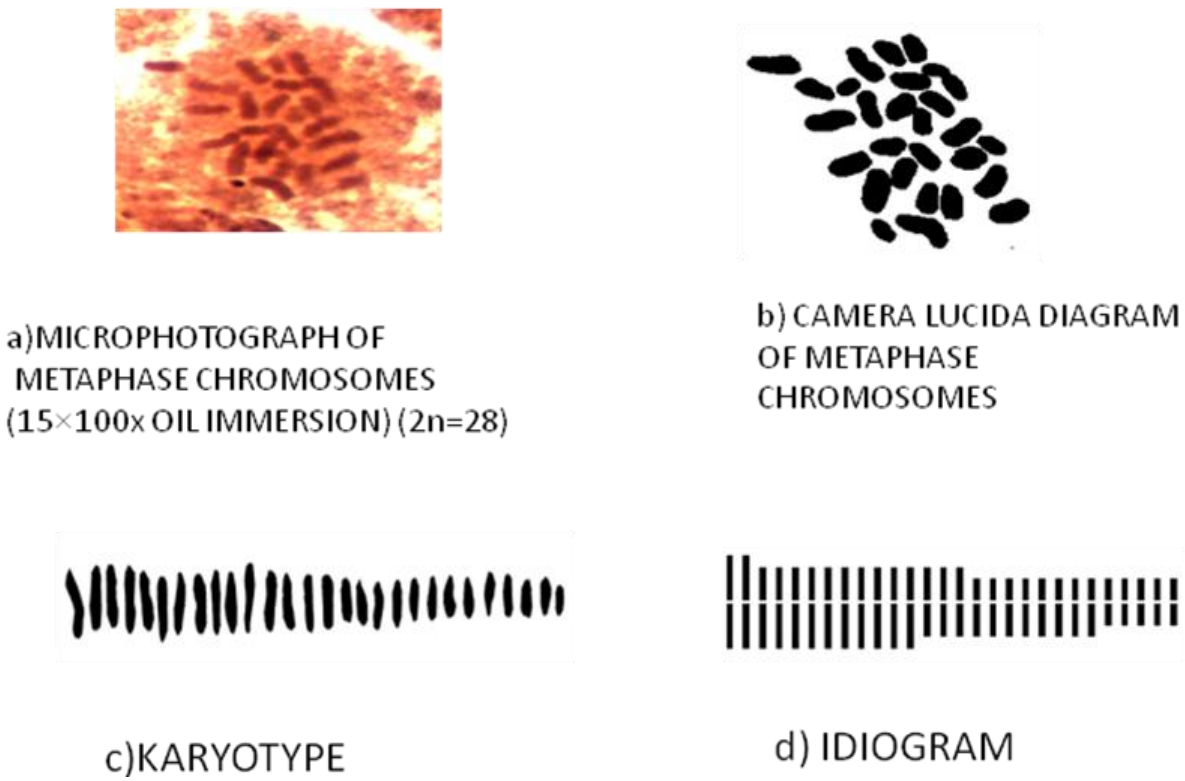

Fig 2 (a-d): Karyotype and Ideogram of somatic chromosome of *Alocasia fornicate* (Roxb.) Schott.

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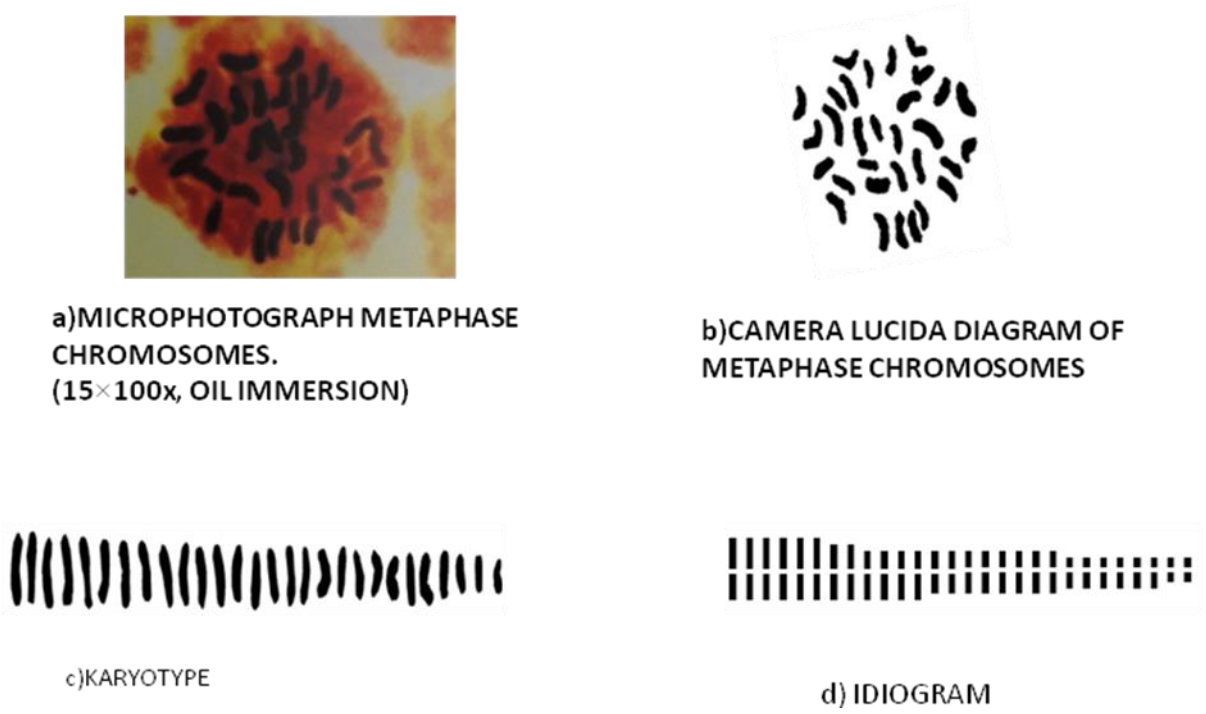

Fig 3 (a-d): Karyotype and Idiogram of somatic chromosomes of *A. longiloba* Miq.

RESULTS

Cytological and karyotypic investigated in *A. macrorrhiza* (L) G.Don, *A. fornicata* (Roxb.) Schott. and *A. longiloba* showed that all three species consist of 2n=28 was the chromosome (Table 1). On the basis of length of chromosomes, it was observed that in *A. macrorrhiza* 10 chromosomes belong to type A and 18 chromosomes belong to type B and the chromosomes formula can be written as: A_{10}+ B_{18}+ C_0+D_0+ E_0= 2n= 28. Depending on the position of their centromere, the karyotypic formula can be written as M_{17}+Sm_{11}= 28. In *A. fornicate* (Roxb.) Schott. 15 chromosomes belong to type A and 13 chromosomes belong to type B. So the Chromosome formula can be written as: A_{15}+B_{13}+C_0+D_0+E_0= 2n= 28, and the karyotypic formula can be written as: M10+ Sm18= 28. However, *A. longiloba* Miq. Total 20 chromosomes belong to type A and 8 chromosomes belong to type B, therefore their chromosomes formula can be written as: A_{20}+B_8+ C_0+ D_0+E_0= 2n= 28 and the karyotypic formula can be written as: M_8+ Sm_{20}= 28. The microphotograph, camera lucida diagram, karyotype and idiogram of the chromosomes are represented in Fig. 1 A, B, C, and D respectively.

Table: 1: Details of karyotype analysis in three species of Genus *Alocasia* (Schott) G. Don

Taxa	Chromosome No. (2n)	Range of Chromosomes					Types of chromosomes	Karyotype formula
		Length (µm)	Relative length	radius	volume	Arm ration		
***A. macrorrhizos* (L.) G.Don**	28	1.5 to 3.5	2.7–4.9	0.50	1.5–3.0	1–1.5	A_{10}+ B_{18}	M_{17}+Sm_{11}= 28
***A. fornicate* (Roxb.) Schott.**	28	1.5 to 3.5	2.4–4.8	0.50	1.5–3.2	1–1.5	A_{15}+ B_{13}	M_{10}+Sm_{18}= 28
***A. longiloba* Miq.**	28	1.5 to 3.5	1.9–4.9	0.50	1.5–4	1–1.6	A_{20}+ B_8	M_8+Sm_{20}= 28

DISCUSSION

It is well known that karyotype analysis often plays an important role in determining the taxonomic status of a taxon where the taxonomic parameters are insufficient, because the karyotype indicates a very stable character that was specific for each specimen. However, a problem arises when different taxa possess the same chromosome number and similar karyotype features. In this situation, it was hard to distinguish between different taxa by conventional karyotype analysis. Even the consideration of chromosome length, arm ratio, position, and number of secondary constrictions are not always sufficient to differentiate individual chromosomes. Minute alterations regarding the distribution pattern of GC- and AT-rich repeats in the karyotypes cannot be detected through conventional karyotype analysis. Moreover, the deletion of heterochromatic regions may change the karyotype of a specimen without affecting the morphology [11].

Different scientists have studied karyotype and RAPD analysis of three morphological forms of *A. fornicata* (Roxb.) Schott. The three forms of *A. fornicata* (Roxb.) Schott were found to possess 2n=28 chromosomes. The similar diploid chromosome numbers were reported by Ramachandran [12] and Petersen [13].

Singh *et al*. [14] in their article, aim to evaluate the biological activities, pharmacological applications and clinical studies of *A. macrorrhiza* in an attempt to provide a direction for further research. They found that apart from household decorative purposes, this plant has some pharmacological activities like antifungal, weak hemagglutinating activity, antidiuretic, laxative, antitubercular and reduces the activity of human immunodeficiency virus reductase and also has antioxidant properties. This plant contains flavonoids, Oxalic acid, cyanogenic glycosides, alocasin, cholesterol, amino acids, gallic acid, mallic acid, ascorbic acid, succinic acid, glucose, fructose, sucrose and betalectins.

In this present study, the karyotypes of the three species of *A. macrorrhiza* (L.) G.Don, *A. fornicata* (Roxb.) Schott., *A. longiloba* Miq. were considered by following the root tip squash method. All the three species were found to posses 2n= 28 chromosomes. A centromeric formula of M_{17}+ Sm_{11}= 28 was found in *A. macrorrhiza* (L,) G.Don, M_{10} +Sm_{18}= 28 in *A. fornicata* (Roxb.) Schott. and M_8 +M_{20}=28 in *A. longiloba* Miq. *A. macrorrhiza* (L,) G.Don, showed more symmetric karyotype consisting of maximum metacentric chromosomes. The other two forms had relatively fewer metacentric chromosomes and more sub-metacentric chromosomes. This result shows, the karyotypic relatedness between the species, which could be taken as a positive output to do further works on the commonly available species of this genus, so as to conserve the other threatened species of this same genus. The following study also revealed the symmetric karyomorphology between species which shown the primitiveness in this genus [15,16].

CONCLUSIONS

There was growing industrial interest towards the production of herbal antioxidants and aim to evaluate the biological activities, pharmacological applications and clinical studies of *A. macrorrhiza* (L.) G.Don, in an attempt to provide a direction for further research. It was an established fact that karyomorphological analysis forms a prerequisite for the genetic improvement of any plant species. Therefore the detailed cytological investigation has been undertaken in these species of *Alocasia* (Schott.) G.Don. Realizing their importance and utility, ex-situ conservation measures may be initiated for protection, preservation, and regeneration of such medicinally and economically important plants.

CONTRIBUTION OF AUTHORS

All authors equally contributed in this article.

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