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Characterization of *Colocasia esculenta* var. *esculenta* by cytogenetical analysis from Bangladesh

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Abstract

The study was undertaken to characterize *Colocasia esculenta* var. *esculenta* by cytogenetical analysis after staining with orcein. Complex chromocentric type of interphase nuclei and Continuous type of prophase chromosomes was observed in this study. *Colocasia esculenta* var. *esculenta* was found to possesses 2n=28 chromosomes. Persistent nucleoli was observed in this plant material suggested the late transcription of rDNA to rRNA and late transportation of rRNA from the nucleus to the cytoplasm. This material possessed metacentric and submetacentric chromosome representing heterogeneous karyotype. Individual chromosome length ranged from 0.72±0.05-2.34±0.05µm. Distance between small and large chromosome was about 1.62µm indicating the gradual decrease of chromosome length. The Total form percentage was 41.56%. Karyotype symmetry and asymmetry index was 71.11% and 58.44%, respectively. Moderately asymmetric karyotype was found in this material. Therefore, the interphase nuclei, prophase chromosome and metaphase chromosome of *Colocasia esculenta* var. *esculenta* can be characterized cytogenetically with the above features.

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Introduction

Colocasia esculenta var. *esculenta* belongs to Araceae which is highly heterogenous family and consists of about 1400-1500 species under 150 genera (Lawrance, 1966). They are commonly known as taro, eddo, dasheen and kalo and one of the most ancient cultivated crops *(Denham, 2011)*. It is a starchy root crop in tropical to temperate regions of Africa, the Mediterranean, Asia and the Pacific (Matthews, 2006). Many cultivars adapted to saline conditions, low water availability and/or seasonal flooded soils have been created and are also widely cultivated throughout tropical and subtropical regions (Onwueme, 1999; Acevedo-Rodríguez and Strong, 2005). In Bangladesh, different cultivated and wild taros are distributed in all over the country (Kreike *et al.,* 2004).

The different members of this widely cultivated crops have some economical and ethnobotanical importance. The tubers and roots are acrid stimulant at fresh stage and also an effective drug in case of snake bites when applied both externally and internally (Ghani, 1998; Rao and Henry, 1996). The young leaves and petioles are used as vegetables. This is used to cure piles also. The tubers are used as poultice. They also meet the iron deficiency. The rhizomes of *Colocasia* posses medicinal value in curing stomach ache, abdominal pain and cholera. The same is crushed into a paste and applied externally on the physical body to cure abscesses and bug bites (Heng, 1979). It is a very popular vegetable known as kochu or mukhi in Bangladesh. The stolons or stems are usually cooked with small prawns which are very favoured by Bangladeshis. It is used to cooked with the head of the ilish fish into a curry, but some dishes are cooked with dried fish. Green leaves and stem are also eaten as a favourite dish and usually ground to a paste or finely chopped to make shake. Food and Agriculture Organization (FAO) reported that taro production has doubled over the past decade (FAOSTAT, 2000) and is now the 5th most-consumed vegetable worldwide.

Colocasia esculenta is an extremely variable species and has many different varieties probably because its chromosomes are prone to unpredictable behaviour during cell division. Because of this natural variablity, many different varieties, cultivars have also arisen. Different regions produce many different types and even within one type there can be a marked variablity over the course of a season or two. Barrau (1957) recognized two species namely *C. esculenta* and *C. antiquorum*. The two prominent chromosome numbers in *Colocasia esculenta* are 2n=28 and 2n=42: the diploids with 2n=28 chromosomes being mainly *Colocasia esculenta* var. *esculenta* genotypes characterised by a large central tuber surrounded by smaller ones. In addition, different chromosome number such as 2n=36 (Huang *et al*., 1989), 2n=38 (Tanimoto and Matsumoto, 1986), 2n=42 (Zhang and Yang, 1984; Coates and Gaffey, 1988; Subramanian and Munian, 1988; Zhang, 1998), and 2n=84 (Sreekumari and Mathew, 1991) chromosomes number was also reported.

The Bangladesh National Herbarium (BNH) has been collecting different taro including *Colocasia esculenta* var. *esculenta* from all over the country and they maintained it in the garden of BNH. They are mainly collected and identified on the basis of plant morphology. Therefore, authentic identification problem is existing since specimen may show different morphology in different environment due to phenotypic plasticity.

Identification problems were also found in different members of Araceae. Taxonomist faced problem to identify them authentically (Ara, 2000). Latter extensive cytological investigation had been carried out for proper identification of these taxa. The cytological data indicated sharp difference among the various sort of *Colocasia esculanta*, *Xanthosoma violaceum, Typhonium trilobatum* etc. (Alam and Deen, 2002; Deen and Alam, 2002; Warasy and Alam 2009). For authentic identification of a specimen, genomic information is very essential. Karyotype analysis is one among the traditional methods that provide a preliminary idea about the genome of a specimen. Generally karyotype analysis plays important role in determining the taxonomic status. Staining properties of interphase nuclei and prophase chromosomes is another approach for distinguishing different individual.

This is usually done by differential staining (Fawzia and Alam, 2011; Bonna *et al.*, 2018). Tanaka (1971) classified the different types of interphase nuclei and prophase chromosomes on the basis of orcien staining property. The outcome of this study showed that various taxa including sort of many plant species might be distinguished by their staining properties.

Therefore, an extensive research should be undertaken to characterize *Colocasia esculenta* var. *esculenta* through cytogenetical analysis.

The aim of the present study was-
i. to compare the staining property of the interphase nuclei and prophase chromosomes after staining with orcein.
ii. to find out the chromosome number of *Colocasia esculenta* var. *esculenta*.
iii. to prepare and analysis the convantional karyotype from mitotic metaphase plate.

Material and methods

Material

For Cytogenetical analysis, *Colocasia esculenta* var. *esculenta* was used in this study as material.

Study area

The plant materials was collected from Bangladesh National Herbarium (BNH) and maintained in the botanic garden of the Department of Botany, Jahangirnagar University, Bangladesh.

Cytogenetical methods

Healthy roots were collected from the Botanical garden, Department of Botany, Jahangirnagar University. The optimum time of root collection for obtaining maximum number of dividing cells was 9.30-10.00 am. The collected root were pretreated with 0.002 M 8-hydroxyquinoline for 1.30 h at room temperature (28-30° C) followed by 15 min fixation in 45% acetic acid at 4°C. The pretreated roots were then hydrolyzed in a mixture of 1 N HCl and 45% acetic acid (2:1) at 60° C for 7-8 sec. Then the hydrolyzed root were soaked on a filter paper and taken in a clean slide. The meristematic region was cut with fine blade. A drop of 1% aceto-orcein was added to the material. A clean cover glass was placed on the material. The materials were tapped gently by a tooth pick and squashed by placing thumbs. Finally the slides were observed under microscope (Olympus-DP72, Japan) with a digital camera. Final measurement was calculated by dividing the magnification of the objective 100x.

Results and discussion

The conducting investigation disclosed the karyotypic features of *Colocasia esculenta* var. *esculenta*. The nature of staining property of interphase nuclei and prophase chromosomes were accomplished to get additional information towards characterization of this germplasm.

Orcein-staining properties of interphase nuclei and prophase chromosomes

The staining properties of interphase nuclei and prophase chromosomes usually provide karyomorphological features that help to characterize different germplasm. Tanaka (1971) was the pioneer of proposing these criteria for karyomorphological investigation. He found that the nature of staining of heterochromatins present in the interphase nuclei and prophase chromosomes were different in different species. On the basis of the staining property he classified interphase nuclei and prophase chromosomes in five different categories. Later different investigators applied these criteria in characterizing different plant materials of diverge nature (Alam *et al.,* 1993; Begum and Alam, 2004; Hossain *et al.,* 2016; Sultana and Alam, 2016; Bonna *et al.*, 2018).

In the present study, few darkly stained large heterochromatic regions were found in interphase nuclei of *Colocasia esculenta* var. *esculenta*. A clear nuclear boundary was visible. A big and prominent nucleolus was found in the interphase nuclei (Fig 1). The presence of prominent nucleoli indicated the active transcription of rDNA for the synthesis of rRNA. According to Tanaka (1971) it may be regarded as complex chromocentric type.

The prophase chromosomes stained uniformly from one end to another with orcein. A prominent nucleolus was also found in this stage (Fig 2). According to Tanaka (1971) it may be regarded as continuous type.

Usually, germplasm with "Complex chromocenter type" of interphase nuclei showed "Gradient type" of prophase chromosomes. In this investigation, the studied material did not followed the usual regulation of heterochromatin. Presence of facultative heterochromatin might be one of the reasons for this type of observation. Whatever the reason is, this germplasm could be characterized on the basis of these characters of interphase nuclei and prophase chromosomes.

Somatic chromosome Number

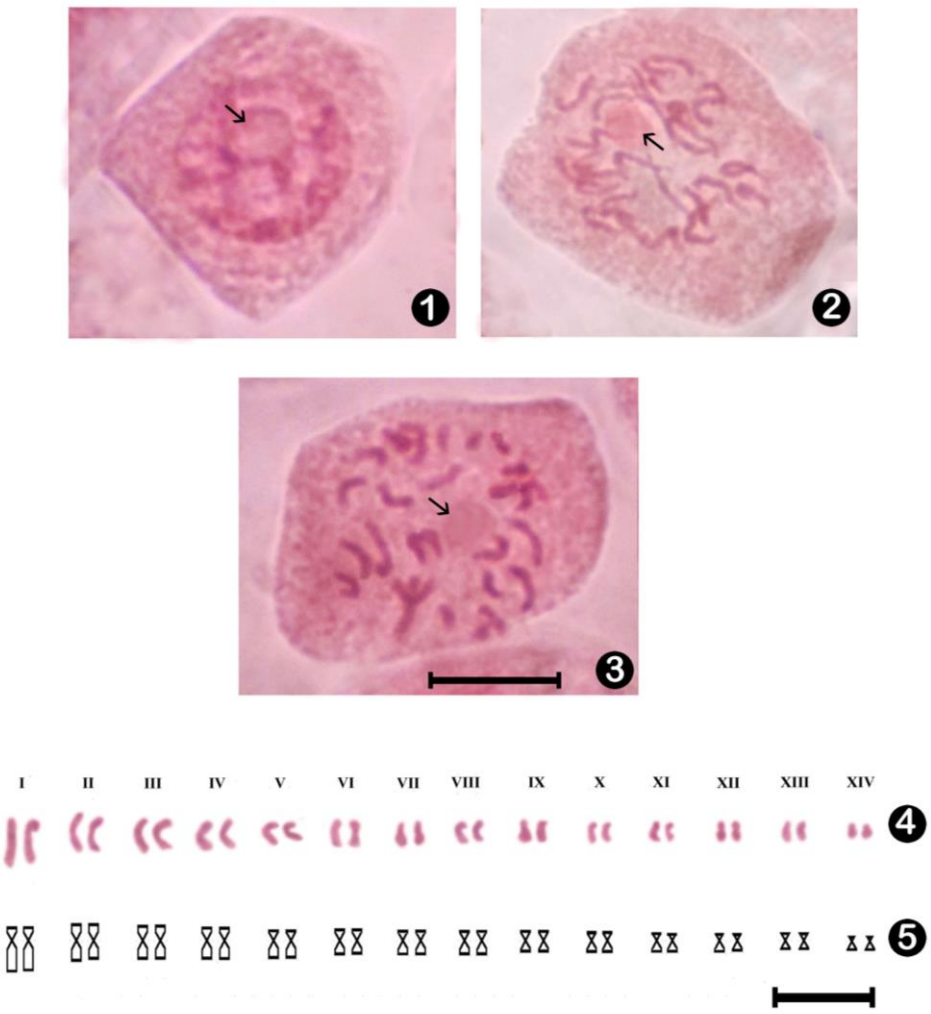
Colocasia esculenta var. *esculenta* was found to possess 2n=28 chromosomes (Figs. 3,4,5 Table 1,2). Same chromosome number was reported earlier by different scientist (Zhang and Yang, 1984; Coates and Gaffey, 1988; Sreekumari and Mathe, 1989; Kuruvilla *et al.,* 1989; Ivancic and Lebot, 1999).

In addition, different chromosome number such as 2n=36 (Huang *et al.*, 1989), 2n=38 (Tanimoto and Matsumoto, 1986), 2n=42 (Zhang and Yang, 1984; Coates and Gaffey, 1988; Subramanian and Munian, 1988; Zhang, 1998), and 2n=84 (Sreekumari and Mathew, 1991) chromosomes number was also reported. Several scientist considered the basic chromosome number as x=14 for this species, then specimen with 2n=28 could be regarded as deploid, 2n=42 could be the triploid and 2n=84 is considered as hexaploid, respectively. So the present findings correlate with 2n=28 of the earlier reports and agree with the basic number of x=14. However, 2n=36 and 38 report previously did not agree with the basic number of x=14. In this case, they might be evolved from nearer ploidy level by chromosomal aberration.

Table 1. Length (inμm), arm ratio, centromeric index, relative length and centromeric type of metaphase chromosomes of *Collocasia esculenta* var. *esculenta*.

Pair	Long arm (l)μm ($\bar{x}$ ± SD)	Short arm (s)μm ($\bar{x}$ ± SD)	Total length (T)μm ($\bar{x}$ ± SD)	Arm ratio (l/s)	Relative length (RL)	Centro-meric Index (CI)	Centro-meric type (CT)
I	1.62± 0.03	0.72± 0.02	2.34± 0.05	2.25	0.06	30.77	sm
	1.53± 0.01	0.72± 0.02	2.25± 0.03	2.13	0.06	32.00	sm
II	1.17± 0.03	0.90± 0.01	2.07± 0.04	1.30	0.05	43.48	m
	0.99± 0.02	0.90± 0.01	1.89± 0.03	1.10	0.05	47.62	m
III	0.99± 0.01	0.81± 0.01	1.80± 0.01	1.22	0.05	45.00	m
	0.99± 0.01	0.81± 0.00	1.80± 0.01	1.22	0.05	45.00	m
IV	0.99± 0.03	0.72± 0.01	1.71± 0.04	1.38	0.05	42.11	m
	0.99± 0.03	0.72± 0.02	1.71± 0.04	1.38	0.05	42.11	m
V	0.90± 0.02	0.54± 0.02	1.44± 0.04	1.67	0.04	37.50	sm
	0.90± 0.03	0.54± 0.02	1.44± 0.04	1.67	0.04	37.50	sm
VI	0.72± 0.02	0.63± 0.03	1.35± 0.05	1.14	0.04	46.67	m
	0.72± 0.04	0.63± 0.02	1.35± 0.05	1.14	0.04	46.67	m
VII	0.72±0.01	0.54± 0.01	1.26± 0.02	1.33	0.03	42.86	m
	0.72± 0.02	0.54± 0.01	1.26± 0.03	1.33	0.03	42.86	m
VIII	0.72± 0.02	0.54± 0.01	1.26± 0.03	1.33	0.03	42.86	m
	0.72± 0.02	0.54± 0.01	1.26± 0.03	1.33	0.03	42.86	m
IX	0.63± 0.03	0.54± 0.02	1.17± 0.05	1.17	0.03	46.15	m
	0.63± 0.03	0.54± 0.02	1.17± 0.05	1.17	0.03	46.15	m
X	0.63± 0.03	0.45± 0.01	1.08± 0.04	1.40	0.03	41.67	m
	0.63± 0.03	0.45± 0.02	1.08± 0.04	1.40	0.03	41.67	m
XI	0.63± 0.01	0.45± 0.03	1.04± 0.03	1.54	0.03	41.67	sm
	0.63± 0.02	0.36± 0.01	0.99± 0.03	1.75	0.03	36.36	sm
XII	0.63± 0.02	0.36± 0.01	0.99± 0.03	1.75	0.03	36.36	sm
	0.55± 0.01	0.36± 0.02	0.91± 0.03	1.53	0.02	39.56	sm
XIII	0.45± 0.02	0.45± 0.01	0.90± 0.03	1.00	0.02	50.00	m
	0.45± 0.03	0.45± 0.01	0.90± 0.04	1.00	0.02	50.00	m
XIV	0.45± 0.04	0.27± 0.01	0.72± 0.05	1.67	0.02	37.50	sm
	0.45± 0.04	0.27± 0.01	0.72± 0.05	1.67	0.02	37.50	sm
			GT=37.83± 0.91				

m = metacentric, sm = sub-metacentric chromosome.



Figs. 1-5. Orcein-stained mitotic interphase, prophase, metaphase, karyotype and Idiogram of *Colocasia esculenta* var. *esculenta*. 1. Interphase nuclei, 2. Prophase chromosome, 3. Mitotic metaphase chromosome, 4. Karyotype prepared from mitotic metaphase chromosomes, 5. Idiogram. In figure, arrow (→) indicates nucleolus. Bar=5µ.

A big and prominent nucleolus was observed in metaphase stage. Usually the nucleolus disappears at late prophase of mitosis. There is considerable evidence that it is not unusual for plant nucleoli to continue in mitotic metaphase or later. Persistent nucleolar materials were of frequent occurrence at prometaphase, metaphase, anaphase and even sometimes in telophase. In the majority of cases, nucleoli appeared as clear entities, usually almost round in shape. They varied in size from small, hardly detectable structures to large conspicuous ones. These observations suggested the late transcription of rDNA to rRNA and late transportation of rRNA from the nucleus to the cytoplasm. Persistent nucleolus was observed in few species such as *Spartocera fusca* (Cattani and Papeschi, 2004), *Zea mays* (Zirkle, 1928), *Ceiba pentandra* (Tijo, 1948), 11 species of *Gossypium* (Sultana and Alam, 2016) and 45 species of the family Gramineae (Walter and Emery, 1957). However, no available report on persistent nucleolus was found in the studied species. So the persistent nature of nucleolus is considered as a salient feature of *Colocasia esculenta* var. *esculenta*.

Chromosomal length

In the present study, the total length of 2n chromosome complement was recorded as 37.83± 0.91µm (Table 1,2). The range of chromosomal length was 0.72±0.05 - 2.34±0.05µm. Distance between small and large chromosomes was about 1.62µm indicating the gradual decrease of chromosome length (Table 1, 2). According to Stebbins (1971) the

asymmetric karyotype showed gradual decrease of chromosome length in their karyotype representing advance nature of germplasm. Average chromosomal length was about 1.48µm. The range of relative length was 0.02-0.06. Difference between relative lengths was 0.04 (Table 2). These features have been limitedly considered as cytological parameters in this species. Therefore, this is very important approaches by which this species could be characterized.

Table 2. Orcein stained karyotype analysis of *Collocasia esculenta* var. *esculenta*.

Cytologgenetical Parameters	*Collocasia esculenta* var. *esculenta*.
2n	28
Total length of chromosome complement (µm)	37.83± 0.91
Range of individual chromosome length (µm)	0.72± 0.05-2.34± 0.05
Average chromosomal length (µm)	1.48
Range of relative length	0.02-0.06
Difference of relative length (DRL)	0.04
Centromeric index range (CI)	31.77-50.00
Centromeric formula	18m+10 sm
Total form percentage (TF%)	41.56
Karyotype symmetry index (Syi%)	71.11
Karyotype Asymmetry index (AsK%)	58.44

m = metacentric, sm = sub-metacentric chromosome.

Centromeric feature

Centromeric index range of *Colocasia esculenta* var. *esculenta* was 31.77-50.00. In case of centromeric formula, studied material was found to possess 18 metacentric and 10 sub-metacentric chromosomes. Combination of meta and sub-metacentric chromosomes were also found in previous reports (Sreekumari and Mathew, 1991; Parvin *et al.,* 2008; Rattanavalee *et al.,* 2018). Beside meta and submeta, acrocentric chromosome were reported earlier by different scientist (Sreekumari and Mathew, 1991; Parvin *et al.,* 2008). All metacentric chromosome also reported (Parvin *et al.,* 2008). The result of present study representing moderately asymmetric karyotype. Stebbins (1971) mentioned that the asymmetric karyotype indicate advance nature and from that point of view the studied plant is comparatively advanced nature from the different members of this species.

Karyotype symmetry and asymmetry index

In this study, the Total form percentage (TF%) was found 41.56% (Table 3). Karyotype symmetry index (Syi%) was 71.11% (Table 3). Karyotype symmetry index values decreased with increasing asymmetry. On the other hand, karyotype asymmetry index (AsK%) was 58.44**%** (Table 3). The value of karyotype asymmetry index increases with the increasing asymmetry. Thus the above findings indicating the moderately asymmetric nature of *Colocasia esculenta* var. *esculenta*.

These karyotypic features were not available in previous reports. So, these features could be helpful to identification of this species.

Therefore, the interphase nuclei, prophase chromosome and metaphase chromosome of *Colocasia esculenta* var. *esculenta* can be characterized cytogenetically with the above features.

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