Karyotype Analysis in Three Morphological Forms of *Colocasia fallax* Schott.

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**Summary** The 3 forms of *Colocasia fallax* Schott. complex, namely green petiole form, light purple petiole form and deep purple petiole form, available in Bangladesh were studied cytogenetically to confirm their taxonomic status after staining with orcein and CMA. The green petiole form and the deep purple petiole form were found to possess $2n=28$ chromosomes whereas $2n=30$ chromosomes were observed in the light purple petiole form. The centromeric formula was determined as $20 \text{m} + 8 \text{sm}$ in the green petiole form, $26 \text{m} + 2 \text{sm} + 2 \text{ac}$ in the light purple petiole form and $24 \text{m} + 4 \text{sm}$ in the deep purple petiole form. Acrocentric chromosomes were found only in the light purple petiole form. 4 satellites were found in the green petiole form after orcein staining while only 1 satellite observed in CMA staining of the same form. These satellites showed stain specific nature in this form. 8 and 11 CMA positive bands were found in the green petiole form and the deep purple petiole form, respectively. Only 2 centromeric CMA positive bands were found in the light purple petiole form. Different CMA banding pattern and percentage of GC-rich repeats were found in these 3 forms. CMA banding was able to identify some chromosomes in the green petiole form and the deep purple petiole form. The diploid chromosome numbers and overall karyotypic features indicated that the light purple petiole form possessed quite different genomes than the other 2 forms and thus may be placed in a different taxonomic rank. Except a minute difference in the karyotypic features, the green petiole form and the deep purple petiole form possessed almost entirely similar genomes and therefore could be considered as different varieties of *Colocasia fallax*.

**Key words** Fluorescent banding, Karyotype, *Colocasia*, Araceae.

Araceae is a big family consisting of 110 genera and 1400–1500 species (Lawrence 1966). The members of this family are distributed globally but are most frequent in tropical regions (Shaw 1966). Due to its economic, ethno botanic and horticultural importance, taxonomists in Bangladesh were attracted to this family. As a consequence, a wild relative of *Colocasia* was recently reported by Ara (2000). This specimen was identified as *Colocasia fallax* Schott. Ara (2000) reported 3 morphological forms of *Colocasia fallax* from different regions of Bangladesh, namely: i) green petiole form, ii) light purple petiole form and iii) deep purple petiole form. In spite of few sharp morphological differences among these different forms of species, other common characteristics allowed the taxonomists to regard these forms as a complex of *Colocasia fallax*. Therefore, confusion exists regarding the taxonomic status of these various morphological forms of *Colocasia fallax*. It is already proved that where morphological differences are not enough, cytological parameters help to distinguish different specimen. However, when different taxa possess the same chromosome number and almost similar karyotypic features, it is hard to distinguish between such taxa by conventional karyotype analysis. Minute alteration regarding the distribution pattern of GC-rich repeats in the karyotypes cannot be detected through orcein-stained karyotype analysis. Moreover, deletion of heterochromatic regions may change the karyotype of a variety without affecting the morphology (Sumner 1990). In such a situation, some modern cytogenetical methods may be

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used (Alam et al. 1998, Alam and Zarin 1998). Staining with fluorochromes such as Chromomycin A\(^3\) is one of such methods. CMA binds with GC-rich repetitive sequences of the genome and gives characteristic yellow colour bands. With the help of the above mentioned techniques it was possible to characterize karyotypically the 3 forms of *Colocasia esculenta* (Alam and Deen 2002) and 2 forms of *Xanthosoma violaceum* (Deen and Alam 2002). They confidently suggested placing the dark purple petiole form of *Colocasia esculenta* and the light purple form of *Xanthosoma violaceum* in different taxonomic ranks. However, no such work has yet been done with *Colocasia fallax*. The karyotype analysis with both conventional and fluorescent staining might be helpful to distinguish the 3 forms of *Colocasia fallax*. Therefore, the aim of this study was to: i) compare the orcein and CMA-stained karyotypes of 3 morphological forms, ii) find out marker chromosomes (if any) and iii) ascertain the taxonomic status of these 3 forms.

**Materials and methods**

The 3 morphological forms of *Colocasia fallax* used in this study are: i) green petiole form collected from Madhobkundo, Moulovibazar district, the petiole colour of which is green; ii) light purple petiole form collected from Madhobkundo, Moulovibazar district, the petiole colour is light purple; and iii) deep purple petiole form collected from Shuvlong, Rangamati district, the petiole and peduncle colours of which are deep purple. These specimens were maintained in the Botanical Garden, Department of Botany, University of Dhaka, Bangladesh.

Healthy roots were collected and pretreated with 0.002 M 8-hydroxyquinoline for 1.10 h at room temperature (25°C) followed by 15 min fixation in 45% acetic acid at 4°C. These were then hydrolysed in a mixture of 1 N HCl and 45% acetic acid (2 : 1) at 60°C for 6 sec. The root tips were stained and squashed in 1% aceto orcein. For CMA banding, Alam and Kondo’s (1995) method was used 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 h 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. 1 drop of CMA (0.1 mg/ml) was added to the materials for 15 min and rinsed with McIlvaine’s buffer with Mg\(^{2+}\) for 10 min. Slides were mounted in 50% glycerol and kept at 4°C for overnight before observation. These were observed under a Nikon (UFX-IIA) fluorescent microscope with Blue Violet (BV) filter cassette.

**Results and discussion**

3 morphological forms of *Colocasia fallax* were cytogenetically investigated to confirm their taxonomic status. The karyotypes of these species were compared after staining with orcein and CMA.

**Orcein-stained Karyotype**

2\(n\)=28 chromosomes were found in both the green petiole form and the deep purple petiole form whereas 2\(n\)=30 chromosomes were found in the light purple petiole form (Figs. 1a–c). However, 2\(n\)=28 and 2\(n\)=42 chromosomes were reported for *Colocasia fallax* by both Larsen (1963) and Rattenburg (1957). The different chromosome numbers indicated the existence of triploid series in *Colocasia fallax* along with the predominant disomic population. The range of chromosomal length was 2.82–5.04 \(\mu\)m in the green petiole form, 2.22–5.24 \(\mu\)m in the deep purple petiole form and 1.00–3.43 \(\mu\)m in the light purple petiole form (Table 1). The range of chromosomal length of these 3 forms did not show a sharp gradual decrease. The karyotype formula of 20 m+8 sm was
Table 1. Comparative orcein and CMA karyotype analysis in 3 morphological forms of *Colocasia fallax*

<table>
<thead>
<tr>
<th>Morphological forms of <em>Colocasia fallax</em></th>
<th>2n</th>
<th>Total length of 2n chromosome complement (µm)</th>
<th>Karyotype formulae</th>
<th>Range of chromosomal length (µm)</th>
<th>No. of CMA positive bands</th>
<th>% of GC-rich repeats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Green petiole form</td>
<td>28</td>
<td>106.03</td>
<td>20 m + 8 sm</td>
<td>2.82–5.04</td>
<td>8</td>
<td>13.58</td>
</tr>
<tr>
<td>Deep purple petiole form</td>
<td>28</td>
<td>109.9</td>
<td>24 m + 4 sm</td>
<td>2.22–5.24</td>
<td>11</td>
<td>27.30</td>
</tr>
<tr>
<td>Light purple petiole form</td>
<td>30</td>
<td>60.74</td>
<td>26 m + 2 sm + 2 ac</td>
<td>1.00–3.43</td>
<td>2</td>
<td>4.91</td>
</tr>
</tbody>
</table>

m=metacentric chromosome, sm=submetacentric chromosome, ac=acrocentric chromosome.

Fig. 1. Mitotic chromosomes of 3 morphological forms in *Colocasia fallax*. a. Orcein-stained mitotic metaphase of green petiole form. b. Orcein-stained mitotic metaphase of deep purple petiole form. c. Orcein-stained mitotic metaphase of light purple petiole form. d. CMA-stained mitotic metaphase of green petiole form. e. CMA-stained mitotic metaphase of deep purple petiole form. f. CMA-stained mitotic metaphase of light purple petiole form. g. CMA-banded karyotype of green petiole form. h. CMA-banded karyotype of deep purple petiole form. i. CMA-banded karyotype of light purple petiole form. Small bar=10 µm for Figs. 1a–f, Big bar=10 µm for Figs. 1g–i.
found in green petiole form, 24 m+4 sm in deep purple petiole form and 26 m+2 sm+2 ac in light purple petiole form (Table 1). The karyotype formula revealed the presence of maximum metacentric chromosomes in their karyotypes and hence less heterogenous. On the basis of the range of chromosomal length and centromeric type these 3 forms may be considered as symmetric type. Stebbins (1971) stated that plant with symmetric karyotype was primitive. Therefore these 3 forms of *Colocasia fallax* possessed a primitive karyotype. Alam and Deen (2002) reported symmetrical karyotypes in the 3 forms of *Colocasia esculenta*. The previous and present results suggested that members of *Colocasia* possessed primitive karyotypes.

**CMA-banding**

The 3 forms of *Colocasia fallax* showed different types of CMA banding pattern. 6 prominent CMA-positive bands and 2 entirely fluoresced chromosomes were found in the green petiole form (Figs. 1d, g). 4 entirely and 7 almost entirely fluoresced chromosomes were found in the deep purple petiole form (Figs. 1e, h). Only 2 centromeric CMA positive bands were observed in the light purple petiole form (Figs. 1f, i). Therefore, the 3 forms possessed distinct CMA positive karyotypes (Table 1).

**Satellite**

In the green petiole form, 2 pairs of satellite were observed when stained with orcein (Fig. 1a). After CMA staining only 1 satellite was found in a member of pair IV (Figs. 1d, g). The satellite that was observed in both orcein and CMA did not show stain specificity. Since the satellites portion was stained brightly with CMA, indicated GC-rich nature. Schweizer (1976) reported that the rDNA satellites were generally GC-rich and thus CMA-positive. Therefore, the CMA-positive satellite found in this study was probably rDNA repeats. However, other 3 satellites those found in the orcein staining could not be detected in CMA stained chromosomes. This revealed their stain specific nature. Alam and Kondo (1995) found stain specific satellites in *Drosera* species. Khatun (2008) reported stain specific satellites in different jute species. Therefore, like *Drosera* and jute, *Colocasia fallax* also possessed stain specific satellites.

**Heteromorphicity**

After CMA-staining 4 heteromorphic pairs were found in the green petiole form, such as: i) 1 member of pair V was fluoresced entirely while no fluorescence was found in its homologue (Fig. 1g), ii) 1 member of pair XIII fluoresced entirely and the other member had a CMA-positive band on the long arm (Fig. 1g) and iii) 1 member of pair IX and XII had a centromeric CMA-positive bands; however, no band was found in the respective homologue (Fig. 1g). In the deep purple petiole form, 1 member of pair XII was fluoresced almost entirely where as no fluorescent band was found in its homologue. The probable reasons for this type of heteromorphicity were due to tandem duplication of the GC-rich repeats or small deletion of the banded area. Khatun (2008) reported the tandem duplication and deletion as probable results of heteromorphicity in jute.

**Marker chromosomes**

In the green petiole form, the 2 entirely CMA-positive fluoresced chromosomes and 1 CMA-positive satellited chromosome were so unique that could they be used as marker for this form (Figs. 1d, g, arrows). All the fluoresced chromosomes (4 entirely and 7 almost entirely fluoresced chromosomes) of the deep purple petiole form were stable in respect of the location and the intensity of fluorescence (Figs. 1e, h). Therefore, these chromosomes could also be used as marker for this form.
Karyotype comparison

The light purple petiole form differed sharply from other 2 forms, such as:

i. The light purple petiole form possessed 2n=30 chromosomes whereas the other 2 forms had 2n=28 chromosomes (Fig. 1a–c).

ii. The light purple petiole form had 1 pair acrocentric chromosome whereas no acrocentric chromosome was found in the other 2 forms (Table 1).

iii. The light purple form possessed 7 pairs of very small chromosomes (1.2–1.8 µm). This type of small chromosome was not found in the other 2 forms (Fig. 1i).

iv. The total length of chromosomes was about 60 µm in the light purple petiole form; it was above 100 µm in the other 2 forms (Table 1).

v. Only 2 CMA-positive bands were found in the light purple petiole form. On the other hand, 8 and 11 CMA-positive bands were observed in the green petiole form and deep purple petiole form respectively (Figs. 1d–i, Table 1).

vi. The total GC-rich area in the light purple petiole form was 4.91% and other 2 forms had 13.58% and 27.30% (Table 1).

Minute differences between green and dark purple forms

On the basis of satellites, CMA-banding patterns and the percentage of GC-rich regions, the green petiole form and the deep purple petiole form showed minute differences (Table 1). However, these 2 forms are more or less similar in respect of morphology and other karyotypic features.

Taxonomic status of three forms

The foregoing discussion clearly indicated that the karyotype of the light purple petiole form is differed sharply from the other 2 forms. These karyotypic data together with morphological difference suggested for placing this specimen in separate taxonomic rank. The karyotypic features of the green petiole form and the deep purple petiole form revealed the presence of almost similar genomes (Table 1), although they differed minutely only in certain characters. Therefore, these 2 forms could be considered as different variety of *Colocasia fallax*.

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References


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