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DIFFERENTIAL CHROMOSOME BANDING AND ISOZYME ASSAY IN *CORCHORUS AESTUANS* L.

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Abstract

Karyotype of *Corchorus aestuans* L. was investigated with differential staining, *viz*. orcein, CMA and DAPI. In addition, activities of three isozyme system namely, esterase, acid phosphatase and peroxidase were studied to obtain specific molecular markers. This species was found to possess 2n = 14 metacentric chromosomes. In this species, three terminal CMA-bands appeared of which two in chromosome pairs I and the rest in a member of chromosome pair VI. Two thick DAPI-positive terminal bands were found in pair II and III. These two chromosomes showed deep CMA-negative bands at the terminal regions where DAPI-positive bands were found. Moreover, one member of pair VII fluoresced entirely with DAPI whereas an interstitial DAPI band occurred in its homologue. Five esterase, one peroxidase and two acid phosphatase bands were observed in this species. These isozyme bands were very stable and species specific. Therefore, differential fluorescent chromosome banding and isozyme assay were found to produce characteristic cytogenetical and molecular markers in *C. aestuans.*

Introduction

Jute is the most important natural bast fibre grown in the tropical region of the world. Jute fibre is traditionally used in making sacks, hessian, cotton bags, wool packs, carpet, carpet backing, yarn and twine. It is also used in making some special and fancy things such as handicraft, garments and paper pulp. According to Jennifer (1990), the largest number of *Corchorus* species found to grow in Africa and hence it is considered to be the centre of origin of the genus (Kundu 1951). The genus *Corchorus* belonging to Tiliaceae possesses about 170 spp. according to *Index Kewensis.* However, there is a difference of opinion regarding the number of valid species such as 40 (Kundu *et al.* 1959), 100 (Willis 1973) and 50 - 60 (Jennifer 1990).

The cultivated species of the genus *Corchorus*, namely *C. capsularis* L. and *C. olitorius* L. are susceptible to a number of diseases including stem rot caused by *Macrophomina phaseolina* and root rot caused by *Rhizoctonia solani* (Ahmed and Ahmed 1969). Such diseases cause a severe loss to yield every year in Bangladesh. The cultivated species possess very little genetic variability (Sobhan 1992). The wild *Corchorus* spp. on the other hand are the good sources of genetic variability. It has been reported that one of the wild species of jute, *C. aestuans* is resistant to stem rot.

The jute germplasm is mainly collected and identified on the basis of plant morphology. The different accessions are given based on the locality. Due to the phenotypic plasticity specimens belonging to the same taxon may show different morphology. It creates confusion in the identification of different germplasms. Karyotype is one of the most reliable parameters for identifying a taxon since it is generally a stable feature of a species. The karyotype analysis of *Corchorus* spp. by conventional techniques showed a number of problems. These were: (i) most of

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the *Corchorus* spp. contain the same somatic chromosome number (2n=14), (ii) no sharp chromosome difference exists among the karyotypes of the different species as all the chromosomes are metacentric and equal sized, and (iii) the satellites, a useful marker, are not always detectable. In such a situation, some recently developed cytogenetical techniques may be useful to study the karyomorphology of *Corchorus* spp. Among these techniques, DNA base-specific chromosome banding with different fluorochromes may be most suitable (Schweizer 1976). In this technique, usually two fluorochromes, *viz.* chromomycin A_3 (CMA) and 4- 6' diamidino-2-phenyl indole (DAPI) have been using widely. CMA binds with GC-(guanine-cytosine) rich and DAPI with AT-(adenine-thymine) rich repeats of the genomes, respectively. Thus it would be easier to identify even an individual chromosome with the help of differential fluorescent banding (Schweizer 1976, Alam and Kondo 1995, Akter and Alam 2005, Sultana and Alam 2007). To assess the genetic diversity at the molecular level, isozymes techniques with polyacrylamide gel electrophoresis are being used as an useful tool (Sammour 1994, Ladizinsky 1979, Arüs and Orton 1983, Scandalios 1974, Jacobs 1975, Alam *et al.* 1998, Alam and Zarin 1998, Alam *et al.* 1999). The electrical properties of enzymes can be employed to obtain mobility values for characterization of this species. The isozymes serve as unique molecular genetic marker to effectively differentiate the species.

Under these circumstances, it a needed to characterize wild *Corchorus* sp. authentically for successful breeding programme and germplasm conservation. In this study, an attempt was undertaken to characterize a wild *Corchorus* sp. namely *C. aestuans* with the help of cytogenetical and molecular markers. The aim was to: (i) prepare the conventional, CMA and DAPI banded karyotype of the *C. aestuans*, (ii) identify the CMA- and DAPI-banded species-specific marker chromosomes and (iii) explore of genetic marker by using esterase, peroxidase and acid phosphatase.

Materials and Methods

The seeds of *C. aestuans* L. were initially collected from BJRI and were sown in the Botanic garden, Department of Botany, University of Dhaka. The seeds were harvested from the mature plants and used for this study.

Healthy roots were collected and pretreated with 0.002 M 8-hydroxyquinoline for one hr at room temperature followed by 15 m 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 7 s. The root tips were stained and squashed in 1% aceto orcein. For fluorescent 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 hr 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 and then 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 Mg^{2+} for 10 min. Slides were mounted in 50% glycerol and kept at 4^0C overnight before observation. These were observed under Hund fluorescent microscope with blue violet (BV) filter cassette. For DAPI-staining, after 48 hr of air drying, the slide was first pre-incubated in Mcllvaine's buffer (pH 7.0) for 20 minutes in a humid chamber and treated in actinomycin D (0.25 mg/ml) for 10 min. The slides were immersed in DAPI solution (0.01 mg/ml) for 20 min and mounted with 50% glycerol. These were observed under Hund fluorescent microscope with ultra violet (UV) filter cassette.

For the extraction of proteins, the germinating seeds of this species grinded with a mortar-pistol. The mortar-pistol was placed in ice in such a way that most of mortar's body remained inside the ice blocks. Then the seeds were homogenized well with adding water. The paste was taken in the Eppendorf tubes and centrifuged at 10,000 rpm for 10 min. The supernatant was used for isozyme experiment. A mixture of 50 ml was prepared by the following proportion 49 ml of Na_2CO_3 (1%), 0.5 ml of $CuSO_4$ (1%) and 0.5 ml of Na-K tartrate (2%). The quantities of proteins in various extracts were estimated as per Lowery *et al.* (1951). Seven test tubes were taken which marked as A, B, C, D, E, F and 1. These tubes contained materials in the following proportion: A- 1 ml of distilled water, B- 200 µl bovine serum albumin (BSA) + 800 µl distilled water, C- 400µl BSA + 600 µl distilled water, D- 600 µl BSA + 400 µl distilled water, E- 800 µl BSA + 200 µl distilled water, F-1 ml BSA and 1 (sample)- 20 µl extract + 980 µl distilled water. Five ml of above mixture and 0.5 ml of 50% FCPR were added to each test tube and kept for 30 min at room temperature. Then spectrophotometer reading was taken for standard and sample by a spectro-photometer (UV-120-02). Three isoenzyme systems namely, esterase, acid phosphatase and peroxidase were used in the present investigation. These experiments were carried out following the method of Arüs and Orton (1983). Before loading, the samples were diluted in the ratio of 2 : 1 with the sample buffer. Gel was run at 60V until the tracking dye reached the separating gel and then at 200V for 4 hr. Sample containing 200 µg of proteins was applied during the running of gel. After running, the gel was stained by different methods for three different types of enzyme localization. The banded gels were photographed quickly with Fuji colour film.

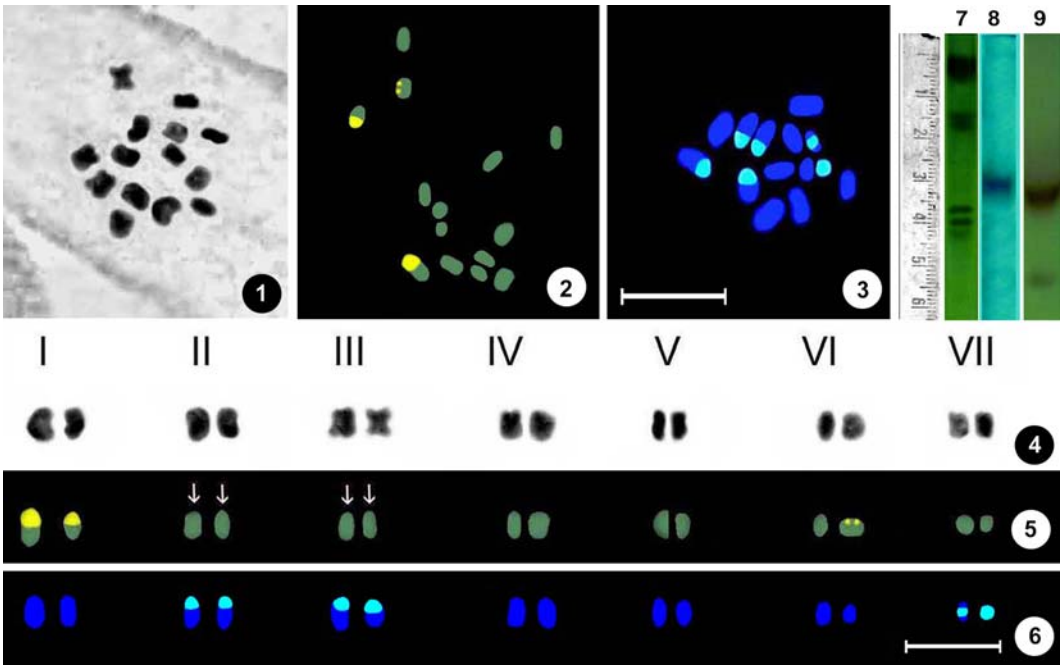
Results and Discussion

This species was found to possess 2n = 14 metacentric chromosomes (Fig. 1). Therefore, the centromeric formula of *C. aestuans* is 14 m. The total length of 2n chromosome complement was 40.68 µm which is bigger in comparison with other *Corchorus* species (Khatun and Alam 2010, Khatun *et al.* 2011). Individual chromosome length ranged from 2.13 to 4.27 µm. Remarkable gradual decrease in chromosomal length was not found in this species (Fig. 4). The relative length of each chromosome ranged from 0.052 to 0.105. No satellite or secondary constriction was found. Two satellites were reported by Alam and Rahman (2000) in *C. olitorius*, *C. capsularis* and *C. trilocularis*. Therefore, *C. aestuans* is different from these three spp. in respect of these features.

The CMA-bands appeared at the terminal region of three chromosomes of which two were observed in pair I (Figs 2, 5). A pair of minute CMA-bands found in only a member of chromosome pair VI whereas no band present in its homologue (Figs 2, 5). The probable reason for absence of CMA band might be a deletion of banded region. This feature was so unique that this chromosome could be used as a marker. The CMA-banded regions are the GC-rich repeats in the chromosomes (Schweizer 1976). Total length of CMA-positive banded region was 3.73 µm which covered 9.17% of the total chromatin length. Khatun *et al.* (2011) reported 10, 7 and 4 CMA positive bands in *C. tridens*, *C. fascicularis* and *C. pseudo-olitorius*, respectively. On the other hand *C. trlocularis* and *C. pseudo-olitorius* were found to possess 7 and 5 CMA bands, respectively (Khatun and Alam 2010). Therefore, the CMA banding pattern of *C. aestuans* is different from the other jute spp.

Two thick terminal bands were found in pairs II and III in *C. aestuans*. These two chromosomes showed deep CMA-negative band. Since these negative bands appeared at the terminal regions of the respective chromosomes, these chromosomes looked smaller when compared with DAPI-staining ones (Figs 3, 5, 6). DAPI-banded regions indicate the presence of AT- rich repeats in the genome (Schweizer 1976). Reversible banding pattern (the DAPI-positive

and CMA- negative features) of these regions revealed that those regions were fully composed of AT-rich repeats (Schweizer 1976). These two chromosomes could easily be identified. No reversible banding pattern was found in other jute spp. (Khatun *et al.* 2011, Khatun and Alam 2010). Moreover, one member of pair VII fluoresced entirely whereas an interstitial band occured in its homologue (Figs 3, 6). The reason for entirely fluorescence was due to tandem duplication of the AT-rich repeats. Several authors reported this type of duplication in different species (Begum *et al.* 2009). Total length of DAPI- positive banded region was 7.73 µm which covered 19.00% of the total chromatin length. Khatun *et al.* (2011) reported 4 DAPI positive bands in *C. tridens*, *C. fascicularis* and *C. pseudo-olitorius*. Therefore, the DAPI banding pattern of *C. aestuans* is different from the other jute spp.



Figs 1-6. Mitotic metaphase chromosomes of *Corchorus aestuans*. 1. Orcein-staining. 2. CMA-staining. 3. DAPI-staining. 4. Orcein-karyotype. 5. CMA- karyotype. 6. DAPI- karyotype. 7. Esterase activities 8. Peroxidase activities. 9. Acid phosphatase activities. Bars = 10 µm

Activities of three isozyme systems *viz.* esterase, peroxidase and acid phosphatase were investigated to characterize *Corchorus aestuans* since electrophoretic and isozyme techniques have proved to be powerful and useful tool (Sammour 1994, Ladizinsky 1979, Lowery *et al.* 1951, Arüs and Orton 1983, Jacobs 1975, Alam *et al.* 1998, Alam *et al.* 1999 and Alam and Zarin 1998).

In total five bands were observed in esterase system (Fig. 7). Whereas only one light thick band was found at the middle portion of the profile in peroxidase (Fig. 8). In Acid phosphatase, one thick dark band and one thin light light band were found at the middle and the lower portion of the profile respectively (Fig. 9). Although one peroxidase and an esterase band common in other wild jute species (Khatun *et al.* 2011, Khatun and Alam 2010), the other bands were unique and species specific. The unique bands could be used as genetic markers of *C. aestuans.*

Corchorus aestuans showed characterstic CMA and DAPI bands. Moreover, this species has different specific isozyme markers. Both these features could be used for authentic identification of this species.

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