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ORIGINAL RESEARCH ARTICLE

Heterochromatin Distribution Pattern of Six Wild *Corchorus* L. Species with Differential Staining from Bangladesh

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Abstract: In this study pattern of heterochromatin distribution at mitotic interphase and prophase stage of six wild *Corchorus* L. species, viz. *C. brevicornatus*, *C. septentrionalis*, *C. urticifolius*, *C. hirtus*, *C. siliquosus* and *C. pinnatipartitus* were investigated following orcein, CMA- and DAPI- staining. After orcein staining, interphase nuclei of *C. brevicornatus*, *C. septentrionalis* and *C. urticifolius* had "Diffuse Type" while *C. hirtus*, *C. siliquosus* and *C. pinnatipartitus* had "Simple Chromocenter Type". In case of prophase chromosomes, *C. septentrionalis* and *C. urticifolius* were detected as "Gradient Type", *C. brevicornatus* and *C. hirtus* as "Continuous Type" and *C. siliquosus* and *C. pinnatipartitus* as "Interstitial Type". Distinct amount of CMA- and DAPI- fluoresced bands were observed in the studied six wild *Corchorus* L. species.

Keywords: Chromosome banding, Interphase nuclei, Prophase chromosomes, Heterochromatins, *Corchorus*.

The species, *Corchorus* L. is considered ass one of the most important natural fiber crop that is widely distributed throughout the tropical and sub-tropical regions of Africa, America, Australia and Asia (1-3). For centuries jute (*Corchorus* L.) contributes towards in economy, agriculture and industry of Bangladesh and eastern part of India. As most of *Corchorus* species possess similar morphology, it is very difficult to identify them at vegetative stage.

Cytological studies are particularly useful for characterization of various species and germplasm to get a clear idea about the evolutionary trends. Different scientist were used Orcein, CMA- and DAPI- staining technique to characterize various species by detecting the nature and distribution pattern of heterochromatin at mitotic interphase and prophase stage (4-6). Therefore, in this investigation the sequential staining were conducted to detect the heterochromatin distribution pattern of six wild *Corchorus* L. species.

MATERIALS AND METHOD

Six wild species of *Corchorus* were investigated in this study. The detail of studied materials was tabulated in Table 1. Healthy roots from these plants were collected kept in pretreated chemical (0.002 M 8-hydroxyquinoline) for 1 h and 10 m at 6-10 ºC. Then the materials were kept in a fixative solution (45% acetic acid) for 12 m at 4 ºC. These were then hydrolyzed for 45 s at 65 °C (2 part 1N HCl and 1 part 45% acetic-acid). Orcein- staining was followed by Sultana and Alam (2016) (7).

Table 1. The detail of six wild *Corchorus* species collected from Bangladesh Jute Research Institute.

Species	Acc. No. (By BJRI)	Collection area
C. brevicornatus Vollesen	Acc. 3719	Kenya
C. septentrionalis Planch	Acc. 3122	USA
C. urticifolius Wight & Arn.	Acc. 3707	Kenya
C. hirtus L.	Acc. 1474	Australia
C. siliquosus L.	Acc. 1475	West Bengal, India
C. pinnatipartitus Wild	Acc. 4541	Thailand

In 1995, Alam and Kondo used a technique for used a technique for fluorescent (CMA- and DAPI-banding) which was used in this investigation with some moderation (8).

RESULTS

Interphase Nuclei

Classical cytological analysis with orcein-staining and fluorescent banding technique with CMA and DAPI were used to detect the staining property of six wild *Corchorus* species. For every staining, at least 50 interphase nuclei were observed for each species.

Orcein staining: The six wild *Corchorus* spp. were found to possess a prominent nucleolous in the interphase nuclei following orcein staining except *C. hirtus* (Figs. 1a-3a and 5a-6a). Heterochromatins were homogenously distributed in the interphase nuclei of *C. brevicornatus*, *C. septentrionalis* and *C. urticifolius* (Figs. 1a-3a). Meanwhile, *C. hirtus*, *C. siliquosus* and *C. pinnatipartitus* showed a number of small darkly stained heterochromatic blocks were scattered in the nucleus (Figs. 4a-6a).

CMA- staining: Two bright and prominent CMA-bands were observed in the interphase nuclei of *C. septentrionalis* and *C. hirtus* (Figs. 2b and 4b). In contrast, few small dot like CMA-bands were scatterdly distributed throughout the nucleus of *C. brevicornatus*, *C. urticifolius* and *C. siliquosus* (Figs. 1b, 3b and 5b). On the other hand, *C. pinnatipartitus* was found to possess numerous dots like CMA- bands (Fig. 6b).

DAPI- staining: The interphase nuclei of *C. brevicornatus* possessed few faintly stained DAPI-fluoresced region (Fig. 1c). Two bright DAPI-bands were found in *C. septentrionalis* (Fig. 2c) whereas 12-15 DAPI-bands were present in *C. urticifolius*, *C. hirtus* and *C. siliquosus* (Figs. 3c-5c). On the other hand, *C. pinnatipartitus* was found to possess numerous dot like DAPI- bands (Fig. 6c). In *C. brevicornatus*, *C. septentrionalis* and *C. pinnatipartitus* possessed non-staining area in the nuclei (Figs 1c. 2c and 6c).

Prophase Chromosomes

To find out the staining property of prophase chromosomes of six wild *Corchorus* species, fluorescent banding technique with CMA and DAPI were used. For every staining method, at least 50 prophase chromosomes were observed for each species.

Orcein staining: In prophase stage of six wild *Corchorus* species nucleolus was easily detectable (Figs. 1d-6d, arrow). In *C. brevicornatus* and *C. hirtus* most of the prophase chromosomes were stained homogeneously along the entire length (Figs. 1d and 4d). The prophase chromosomes of *C. siliquosus* and *C. pinnatipartitus* stained only at the interitial regions (Figs. 5d and 6d). On the other hand, the prophase chromosomes of *C. septentrionalis* and *C. urticifolius* were darker in one end and gradually faint to other end (Figs. 2d and 3d).

CMA- staining: In prophase chromosomes, two prominent and bright CMA-positive bands were found in *C. septentrionalis* and *C. urticifolius* (Figs. 2e and 3e). In addition, few bright bands were observed in that two species (Figs. 2e and 3e). On the other hand, several CMA-positive bands were found at different location of the prophase chromosomes in *C. pinnatipartitus* (Fig. 6e). In *C. brevicornatus*, *C. hirtus* and *C. siliquosus* few CMA- bands were present at different regions of the prophase chromosome (Figs. 1e, 4e and 5e).

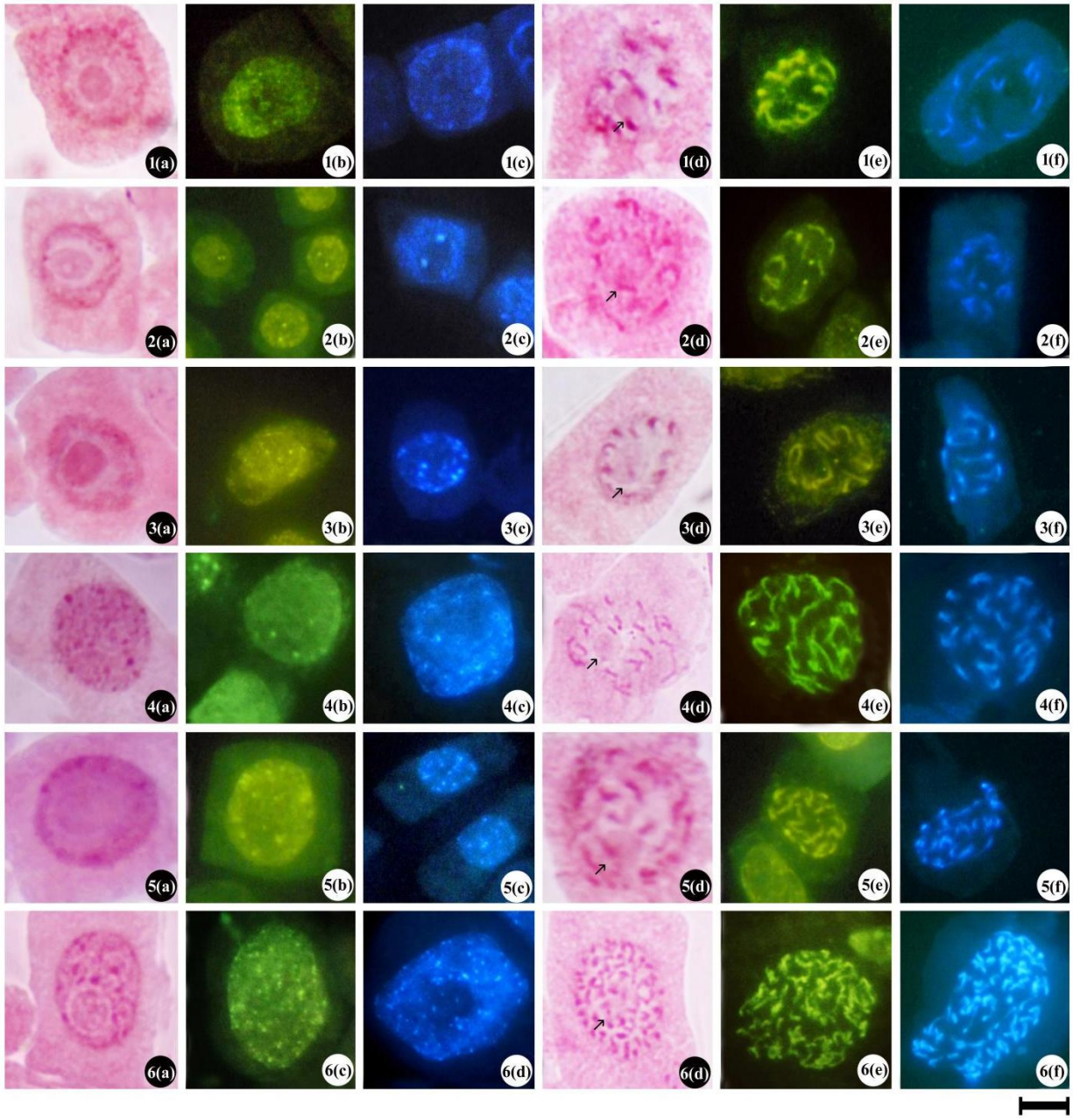
DAPI- staining: No prominent DAPI-positive band was observed in the prophase chromosomes in *C. brevicornatus* and *C. hirtus* (Figs. 1f and 4f). A number of DAPI-positive bands were found to be scattered among the prophase chromosomes of *C. septentrionalis*, *C. urticifolius*, *C. hirtus* and *C. siliquosus* (Figs. 2f-5f). In contrast, numerous DAPI fluoresced bands were scatterdly distributed in the prophase chromosomes of *C. pinnatipartitus* (Fig. 6f). A non staining region was observed in *C. brevicornatus* and *C. septentrionalis* (Figs. 1f and 2f).

DISCUSSION

The nature of interphase nuclei and prophase chromosomes considerd as a karyomorphological features to characterize different germplasm. These criteria first propsed by Tanka in 1971 (6). Tanaka observed that the nature and distribution of heterochromatins varies in different species after orcein staining. He classified interphase nuclei and prophase chromosome into five categories according to the staining property. Further, it was applied to characterize different plant materials by many researchers (7-13).

In this study, the interphase nuclei of diploid *Corchorus* species. viz. *C. brevicornatus*, *C. septentrionalis* and *C. urticifolius* stained homogenously after orcein staining which was classified as “Diffuse Type” by Tanaka 1971 (6) (Figs. 1a-3a, Table 2). On the other hand, the polyploid *Corchorus* species viz. *C. hirtus*, *C. siliquosus* and *C. pinnatipartitus* had small heterochromatic blocks distributed scatterdly in the interphase nuclei after orcein staining. According to Tanaka 1971, it could be considered as “Simple chromocenter type” (Figs. 4a-6a, Table 2) (6).

Uniformly stained prophase chromosomes were found in *C. brevicornatus* and *C. hirtus* after orcein staining (Figs. 1d and 4d, Table 2). According to Tanaka 1971, this type of prophase chromosomes could be regarded as “Continuous type”.



Figs 1 - 6. Differential staining of six wild *Corchorus* L. species. 1(a-c). Intephase nuclei *C. brevicornatus* (a). Orcein-stanied, (b). CMA-stanied, (c). DAPI-stanied, 1(d-f). Prophase chromosomes of *C. brevicornatus* (d). Orcein-stanied, (e). CMA-stanied, (f). DAPI-stanied, 2(a-c). Interphase nuclei of *C. septentrionalis* (a). Orcein-stanied, (b). CMA-stanied, (c). DAPI-stanied, 2(d-f). Prophase chromosomes of *C. septentrionalis* (d). Orcein-stanied, (e). CMA-stanied, (f). DAPI-stanied, 3(a-c). Interphase nuclei of *C. urticifolius* (a). Orcein-stanied, (b). CMA-stanied, (c). DAPI-stanied, 3(d-f). Prophase chromosomes of *C. urticifolius* (d). Orcein-stanied, (e). CMA-stanied, (f). DAPI-stanied, 4(a-c). Interphase nuclei of *C. hirtus* (a). Orcein-stanied, (b). CMA-stanied, (c). DAPI-stanied, 4(d-f). Prophase chromosome of *C. hirtus* (d). Orcein-stanied, (e). CMA-stanied, (f). DAPI-stanied, 5(a-c). Interphase nuclei of *C. siliquosus* (a). Orcein-stanied, (b). CMA-stanied, (c). DAPI-stanied, 5(d-f). Prophase chromosomes of *C. siliquosus* (d). Orcein-stanied, (e). CMA-stanied, (f). DAPI-stanied, 6(a-c). Interphase nuclei of *C. pinnatipartitus* (a). Orcein-stanied, (b). CMA-stanied, (c). DAPI-stanied, 6(d-f). Prophase chromosomes of *C. pinnatipartitus* (d). Orcein-stanied, (e). CMA-stanied, (f). DAPI-stanied, arrow indicates nucleolus. Bar = 5 µm.

Table 2. Types of interphase nuclei and prophase chromosomes of six wild *Corchorus* L. species after staining with orcein.

Species	Type of interphase nuclei	Type of prophase chromosomes
C. brevicornatus	Diffuse	Continuous
C. septentrionalis	Diffuse	Gradient
C. urticifolius	Diffuse	Gradient
C. hirtus	Simple Chromocenter	Continuous
C. siliquosus	Simple Chromocenter	Interstitial
C. pinnatipartitus	Simple Chromocenter	Interstitial

Chromosomes were observed in *C. siliquosus* and *C. pinnatipartitus* as the prophase chromosomes stained at the interstitial region with orcein (Figs. 5d and 6d, Table 2). However, the prophase chromosomes of *C. septentrionalis* and *C. urticifoilus* possessed "Gradient type" because the chromosomes were darkly stained at one end and gradually become faint towards another end (Figs. 2d and 3d, Table 2).

Usually, the equivalently distributed heterochromatin of interphase nuclei further located homogeneously in prophase chromosomes and localized heterochromatin of interphase nuclei firmly aggregated at the different region of prophase chromosomes. In this study, *C. brevicornatus* followed the above general rule having "Diffuse Type" interphase nuclei and "Continuous type" of prophase chromosome (Figs. 1a and 1d, Table 2). In addition, *C. siliquosus* and *C. pinnatipartitus* also followed the rule having "Simple chromocenter type" of interphase nuclei and "Gradient type" of prophase chromosomes (Figs. 5a, 5d, 6a and 6d, Table 2). However, homogeously distributed heterochromatin of interphase nuclei aggregated in different position of prophase chromosomes in *C. septentrionalis* and *C. urticifolius* (Figs. 2a, 2d, 3a and 3d, Table 2). In contrast, heterochromatins are condensed to form block whether in interphase nuclei these are homogeneously distributed along the prophase chromosomal length of *C. hirtus* (Figs. 4a and 4d, Table 2). This disagreement might be due to presence of facultative heterochromatin in *C. septentrionalis*, *C. urticifolius* and *C. hirtus*. Whatever the reason is, six wild *Corchorus* species could be characterized on the basis of these characters of interphase nuclei and prophase chromosomes.

Distinct amount of CMA- and DAPI- fluoresced bands were observed in the studied six wild *Corchorus* L. species which also could be used as a parameter to characterize them.

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CONFLICT OF INTEREST

This article has no conflict of interest.

REFERENCES

1. Kundu BC. Origin of Jute. Indian J. Genet. Plant Breed. 1951;11:95-99.
2. Kubitzki K, Bayer C. Flowering plants, dicotyledons: Malvales, Capparales, and non-betalain Caryophyllales. Berlin Heidelberg: Springer Verlag. 2003. pp 252.
3. Benor S. Phylogeny of the genus *Corchorus* (Malvaceae s.l.) and diversity analyses in selected species: evidence from morphology, flow cytometry and molecular data, (Ph.D. Dissertation) Kassel University, Kassel, Germany; 2011.
4. Khatun M, Alam SS. Conformation of species status of *Corchorus trilocularis* by differential chromosome banding and isozyme assay. Cytologia. 2010;75(1):83-88. **[doi]**
5. Khatun M, Sultana SS, Ara H, Islam MN, Alam SS. Differential chromosome banding and isozyme assay of three *Corchorus* spp. Cytologia. 2011;76(1):27-32. **[doi]**
6. Tanaka R. Type of resting nuclei in Orchidaceae. Bot. Mag. Tokyo. 1971;84:118-122.
7. Sultana SS, Alam SS. Karyomorphology of eleven varieties of *Gossypium hirsutum*. Bangladesh J. Bot. 2016;45(1):151-159.
8. Alam SS, Kondo K. Differential staining with orcein, Giemsa, CMA and DAPI for comparative chromosome study of 12 species of Australian *Drosera* (Droseraceae). American J. Bot. 1995; 82(10):1278-1286. **[doi]**
9. Alam SS, Kondo K, Tanaka R. A chromosome study of eight orchid species in Bangladesh. La Kromsomo II. 1993; 11-71-72:2456-2464.
10. Begum R, Alam SS. Karyomorphological study in two orchid species. Dhaka Univ. J. Biol. Sci. 2004; 13(1):99-101.
11. Vosa CG. Acetic-orcein, a new stain for the detection of constitutive heterochromatin in plant and animal chromosomes. Expt. Cell Res. 1973;79 (2):463-465.
12. Hossain MU, Islam M, Afroz M, Sultana SS, Alam SS. Karyotype and RAPD analysis of male and female *Coccinia grandis* L. from Bangladesh. Cytologia. 2016;81(3):349-355. **[doi]**
13. Bonna IJ, Alam SS, Sultana SS. Cytogenetical characterization of *Acalypha indica* L. in Bangladesh. Dhaka Univ. J. Biol. Sci. 2018;27(2):183-189. **[doi]**

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AUTHOR(S) CONTRIBUTION

Mahin Afroz performed this experiment and prepared the manuscript. Dr. Syeda Sharmeen Sultana planed, participated in data analysis and finally edited the manuscript.

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