



Cytogenetic studies of three cultivated hill cotton (*Gossypium arboreum* L.) varieties from Bangladesh

Afsana Hossain, Chandan Kumar Dash and Syeda Sharmeen Sultana^{1,*}

Cytogenetics Laboratory, Department of Botany, University of Dhaka, Dhaka-1000, Bangladesh; ¹Syeda Sharmeen Sultana, Cytogenetics Laboratory, Department of Botany, University of Dhaka, Bangladesh

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Abstract

Three hill cotton (*Gossypium arboreum* L.) varieties viz., HC-1, HC-2 and HC-3, released by Bangladesh Cotton Development Board were investigated through orcein, CMA- and DAPI-banding for cytogenetical characterization and to elucidate the karyotypic diversity among these varieties. All these three varieties were found to possess $2n = 26$ metacentric chromosomes with '1A' karyotype. Based on TF%, AsK% and Syi index, HC-3 was little advanced over HC-1 and HC-2. These three varieties showed differential Chromomycin A₃ (CMA)- and 4⁺-6 Diamidino-2-Phenyl Indole (DAPI)-banding patterns and a tendency of accumulation of repetitive sequences at the terminal regions was observed. Despite possessing same somatic chromosome number these three hill cotton varieties could be characterized by diversified karyotypic parameters through differential staining.

Key words: Cytogenetics, karyotype, CMA, DAPI, *Gossypium arboreum* L.

Cotton (*Gossypium* spp.) is one of the important cash crops and two species of *Gossypium* are grown in Bangladesh; these are *G. hirsutum* (Upland cotton) and *G. arboreum* (Hill cotton) (CDB 2017). Bangladesh Cotton Development Board (CDB) was able to release 15 upland cotton varieties of *G. hirsutum* namely- CB (1-15) with one hybrid variety- CB Hybrid 1 and three hill cotton varieties (HC-1, HC-2 and HC-3) of *G. arboreum* (CDB 2017). Although Niger and Alam (2007) had done cytogenetical analysis on two forms of *G. arboreum* (white and brown) but no such research was conducted for three hill cotton varieties of *G.*

arboreum released by CDB in Bangladesh. Therefore, the current study was done to characterize three *G. arboreum* varieties with orcein, Chromomycin A₃ (CMA) and 4⁺-6 Diamidino-2-Phenyl Indole (DAPI) staining.

Healthy roots of HC (1-3) were collected and pretreated with 8-hydroxyquinoline (0.002 mole/L) for 3 hours at 20-25°C followed by 15 minutes fixation in 45% acetic acid at 4°C. Roots were hydrolyzed in a mixture of 1N HCl and 45% acetic acid (2:1) at 65°C for 1 minute. The root tips were stained and squashed in 1% aceto-orcein for 2 hours (Niger and Alam 2007). Afterwards, these slides were observed under a compound microscope (Nikon eclipse 100). For CMA- and DAPI-banding, Alam and Kondo's (1995) method was followed. Various karyomorphological parameters and asymmetry indices calculated with the help of KaryoType software (Altinordu et al. 2016).

Three varieties of *Gossypium arboreum* i.e. HC-1, HC-2 and HC-3 were found to possess $2n = 26$ chromosomes (Fig. 1, Table 1), thus supported the previous findings of Wang et al. (1995), Dhamayanthi (2005) and Niger and Alam (2007). However, different chromosome number reports for this species were also available such as $2n = 25$ (Wang et al. 1995) and $2n = 52$ (Stephens 1942). According to the present and previous works, it can be said that $x = 13$ more likely the basic chromosome number for this species. Therefore, the origin of individuals with $2n = 25$ chromosome number might be a cause of aneuploidy.

*Corresponding author's e-mail: rumana_botany@yahoo.com

On the other hand, progenies with $2n = 52$ might be considered as tetraploid (Stephens 1942). The karyotype analysis showed slight variations in karyotype formulae and asymmetry indices among three hill cotton varieties (Table 1). These three varieties possessed 13 pairs of metacentric chromosomes and the karyotype formulae were $26m$ (Fig. 1, Table 1). Niger and Alam (2007) reported $10m + 16sm$ and $14m + 12sm$, in *G. arboreum* brown form and white form, respectively. Lower TF% and Syi index shows more asymmetric nature of karyotype whereas higher AsK% represents more asymmetry in the karyotype. According to the TF%, Syi index and AsK%, HC-3 had more asymmetric karyotype over HC-1 and HC-2. Therefore, in the evolutionary point of view, HC-3 was little advanced among these three varieties of *G. arboreum* and the predicted evolutionary sequence may be $HC-1 > HC-2 > HC-3$ (primitive to advance). The total chromosome length was highest in HC-1 ($96.14 \pm 2.17 \mu m$) while gradually decrease in HC-2 ($84.09 \pm 4.21 \mu m$) and in HC-3 ($78.06 \pm 3.59 \mu m$). Díaz-Castillo and Golic (2007) reported that evolutionary forces acting on heterochromatins are not bound by the same considerations that apply to euchromatins of the genome. Consequently, heterochromatic or repetitive sequences are subjected to rapid evolutionary change (Díaz-Castillo and Golic 2007). In this investigation, it has been observed that total chromosome length of these three varieties was reduced with the course of evolution.

The karyotypes of three hill cotton varieties were compared after CMA-staining. Each variety showed distinct CMA-banding pattern (Fig. 1, Table 2). In addition to four centromeric band (pair IX and XI), two terminal (pair X) and two intercalary band (pair I) were also observed in HC-1 whereas all the five banded chromosomes (pair IV, VI and one member of pair XIII) of HC-2 possess only terminal band. However, HC-3 showed three terminal (pair III and one member

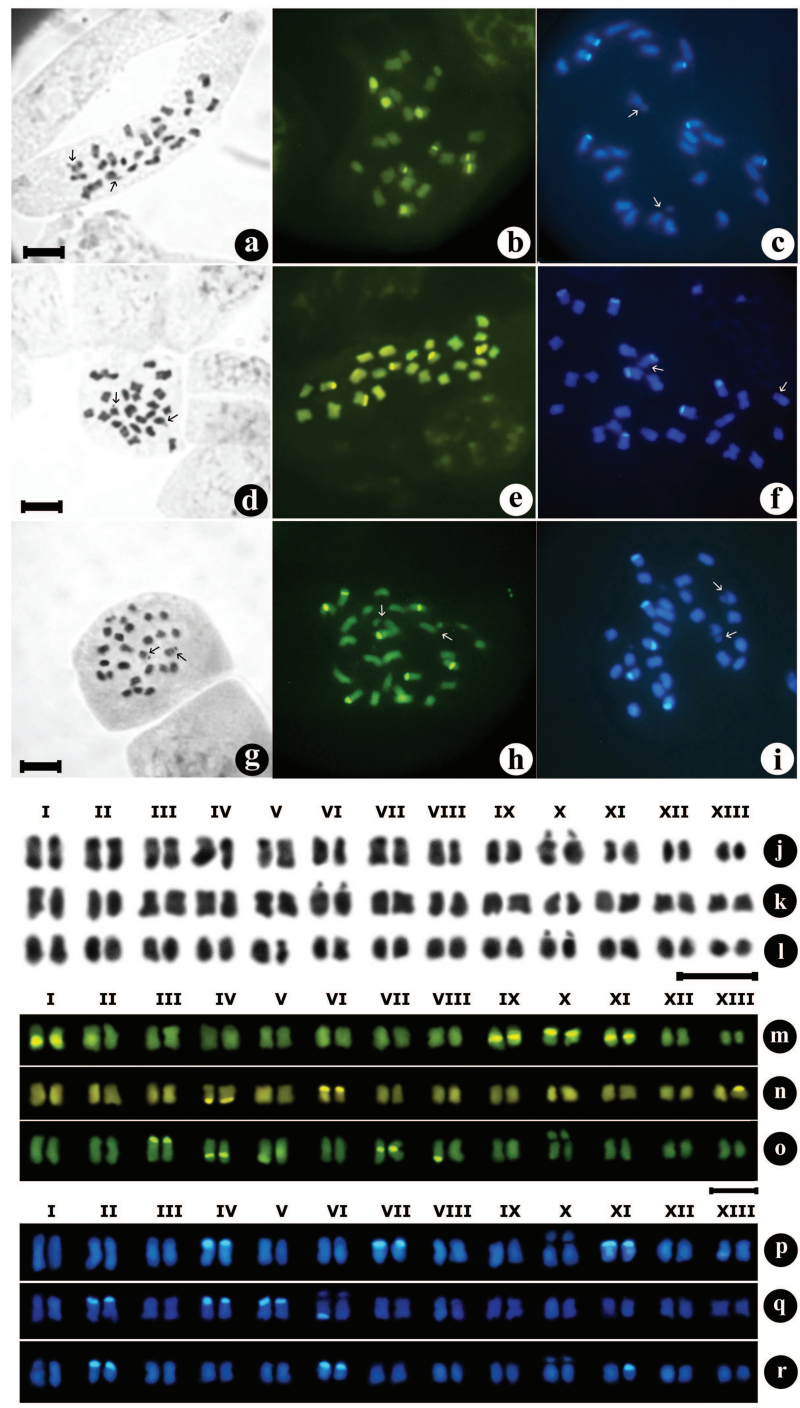


Fig. 1. Metaphase chromosomes and karyotypes of three hill cotton varieties with Orcein-, CMA- and DAPI-staining. a-c. Orcein-, CMA- and DAPI-stained metaphase of HC-1; d-f. Orcein-, CMA- and DAPI-stained metaphase of HC-2; g-i. Orcein-, CMA- and DAPI-stained metaphase of HC-3; j-l. Orcein-karyotypes of HC-1, HC-2 and HC-3; m-o. CMA-karyotypes of HC-1, HC-2 and HC-3; p-r. DAPI-karyotypes of HC-1, HC-2 and HC-3. Bars = $10 \mu m$

Table 1. Comparative karyotype analysis of three varieties of cotton (*Gossypium arboreum* L.)

Karyotypic parameter	Hill cotton-1	Hill cotton-2	Hill cotton-3
Somatic Chromosome number	2n = 2x = 26	2n = 2x = 26	2n = 2x = 26
Centromeric formula	26m	26m	26m
No. of satellites	2	2	2
Range of chromosomal length (µm)	2.69–4.46	2.51–4.12	2.46–3.75
Total chromosome length (µm)	96.14 ± 2.17	84.09 ± 4.21	78.06 ± 3.59
Average chromosome length (µm)	3.63	3.23	3.00
Total Form (TF) value (%)	46.37	45.94	45.67
Karyotype asymmetry index (AsK) %	53.63	54.06	54.33
Karyotype symmetry index (Syi) %	86.46	84.98	84.05
Karyotype asymmetry degree according to Stebbin's classification (Stebbin 1971)	1A	1A	1A

m=metacentric chromosome, sm=sub-metacentric chromosome

Table 2. Comparative fluorescent banding analysis of three varieties of cotton (*Gossypium arboreum* L.)

Banding parameter	Hill cotton-1	Hill cotton-2	Hill cotton-3
Banding with Chromomycin A ₃ (CMA)			
No. of CMA band	8	5	7
Total length of CMA- banded segment (µm)	7.9	4.05	6.12
% of CMA- banded segment	8.22	4.82	7.84
No. of satellites	-	-	2
Banding with 4'-6 diamidino-2-phenylindole (DAPI)			
No. of DAPI band	6	7	5
Total length of DAPI- banded segment (µm)	5.35	5.78	4.74
% of DAPI- banded segment	5.56	6.87	6.07
No. of satellites	2	2	2

of pair VIII), two intercalary (pair IV) and two centromeric (pair VII) CMA-bands. Heteromorphism in respect of banding pattern was observed in chromosome pair XIII in HC-2 and pair VIII in HC-3. In those pair one chromosome showed terminal CMA-bands whereas their homologue did not possess any band (Fig. 1, Table 2). The above findings suggested the probable deletion of the banded region from the respective chromosomes. Three hill cotton varieties differed in respect of DAPI-banding pattern. Six, seven and five DAPI-bands were present in HC-1, HC-2 and HC-3, respectively. Moreover, the percentage of AT-rich repeats was another parameter for distinguishing each karyotype (Table 2). The AT-rich regions occupied 5.56% area in HC-1, 6.87% area in HC-2 and 6.07% area in HC-3. All the DAPI-bands were present at the terminal regions of short arm of

respective chromosomes in three hill cotton varieties (Fig. 1, Table 2). The presence of terminal DAPI-bands indicated the tendency of accumulating AT-rich repetitive sequences at the chromosomal ends. Chromosome pair VI in HC-2 and pair XI in HC-3 showed heteromorphic banding pattern. One chromosome in those pair possesses terminal DAPI-bands whereas other chromosome of same pair did not have any band (Fig. 1, Table 2). These results might be due to the occurrence of deletion of the banded region from the respective chromosomes. After orcein- and DAPI-staining, a pair of secondary constriction was found on the short arms of chromosome pair X in HC-1 and HC-3 and pair VI in HC-2. Only in HC-3, a pair of satellite was observed on the short arm of chromosome pair X after CMA-staining. However, other two varieties did not show

any satellite with CMA. This finding suggested that the satellite portions of HC-1 and HC-2 might have stain specific nature (AT-rich) thus visible with orcein- and DAPI-stain and hardly detectable with CMA fluorochrome. Despite similar 2n chromosome number, diversification and reshuffling of CMA- and DAPI-banded regions were observed in these three varieties of hill cotton. The variation in different karyotypic indexes of these three varieties of hill cotton might be caused by dysploidy, inversion, deletion, unequal translocation, intraspecific hybridization, etc. Each variety showed characteristic CMA and DAPI banded pattern with different number, location and percentage of GC- and AT-rich segments. This report is a preliminary cytogenetical investigation in three *Gossypium arboreum* varieties and more investigations such as Single-nucleotide polymorphism (SNP) is needed. This result will help in future breeding program and to conserve each germplasm with proper characterization.

Authors' contribution

Conceptualization of research (AH, CKD, SSS); Designing of the experiments (AH, SSS); Contribution of experimental materials (AH, CKD); Execution of field/lab experiments and data collection (AH, CKD); Analysis of data and interpretation (CKD, SSS); Preparation of manuscript (CKD, AH, SSS).

Declaration

The authors declare no conflict of interest.

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