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KARYOMORPHOLOGY OF ELEVEN VARIETIES OF *GOSSYPIUM HIRSUTUM* L.

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Abstract

The present study deals with the karyological behavior at different mitotic stages in 11 varieties of *Gossypium hirsutum* L. On the basis of orcein staining pattern, the interphase nuclei of 11 cotton varieties were grouped into two categories i.e. "Simple Chromocenter Type" and "Complex Chromocenter Type". "Gradient Type" of prophase chromosomes were observed in five varieties where chromosomes stained darker in one end and gradually faint to the other end. Six varieties were found to possess "Continuous Type" of prophase chromosomes with homogenously stained along the entire length. Several CMA and DAPI positive bands were found in the interphase nuclei and prophase chromosomes of these eleven varieties of cotton. Although the varieties were found to possess 2n = 52 chromosomes, differed in respect of other karyotypic features such as total length of 2n chromosome complements, number of satellites, range of relative length, centromeric index, etc. The centromeric formula of 42m + 10sm were found in CB-1, 50m + 2sm in CB-2 while it was 46m + 6sm in CB-5. Rest of the varieties have 52 m chromosomes. After orcein staining, a nucleolus was found in almost every interphase, prophase and sometimes even in metaphase stages in each variety indicating its persisting nature.

Introduction

Cotton, often called the king of fibers obtained from the epidermis of the seeds. It is most dominating textile crop and the world's second most important oilseed crop after soybean (Kantartzi 2010, Farzaneh *et al.* 2010). Cotton is also known as "White Gold" because it provides 70% of the raw material needed for the textile industry in the world (Saravanan *et al.* 2006).

In Bangladesh, cotton is considered as one of the most important cash crops cultivated in various regions of the country. Due to continuous demand, the breeders of Bangladesh have been trying to develop better variety with suitable agronomic traits. As a consequence, breeders of Bangladesh Cotton Development Board (CDB) have released 11 varieties with desirable traits based on morphological features. But genetic information of these varieties are not available.

Successful breeding program depends on the complete genetic knowledge and understanding of the genetic diversity within and among genetic resources of the available varieties. This will enable plant breeders to choose parental sources that generate diverse populations for selection (Esmail *et al.* 2008). Karyomorphological study provides basic genetic knowledge of an organism. This includes the nature of interphase nuclei, prophase and metaphase chromosomes i.e. different stages of mitotic cell division. The total karyoporphological behavior enables to characterize even varieties of a species (Khatun and Alam 2010, Kahtun *et al.* 2011).

A few workers tried to characterize cotton with classical karyotype analysis but their research were confined mainly to 2n chromosome count (Beasly 1942, Nei and Li 1985, Wang *et al.* 1996, Davie 1933, Mehetre and Thombre 1980, Marina *et al.* 2011, Farzaneh *et al.* 2010).

Tanaka (1971) classified interphase nuclei and prophase chromosomes on the basis of orcien staining property. Later different workers tried to characterize interphase nuclei and prophase chromosomes by differential staining with orcein, CMA and DAPI (Alam *et al.* 1993, Begum and

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Alam 2004). The sequential staining with different stain provides more data that help to characterize each specimen. In addition, this type of study may reveal various taxa including varieties of many plant species for distinguishing them by their staining properties of interpahse nuclei and prophase chromosomes along with a complete karyomorphological study and that has been done for *Gossypium hirsutum* L. in the present study.

Materials and Methods

Eleven varieties of *Gossypium hirsutum* L. *viz.* CB-1, CB-2, CB-3, CB-4, CB-5, CB-6, CB-7, CB-8, CB-9, CB-10 and CB-11 released by Cotton Development Board (CDB), Bangladesh were investigated in this study. Seeds were initially collected from the gene bank of BARI and sown in the Botanic garden, Department of Botany, University of Dhaka. Healthy root tips were collected from the mature plants and used for this study.

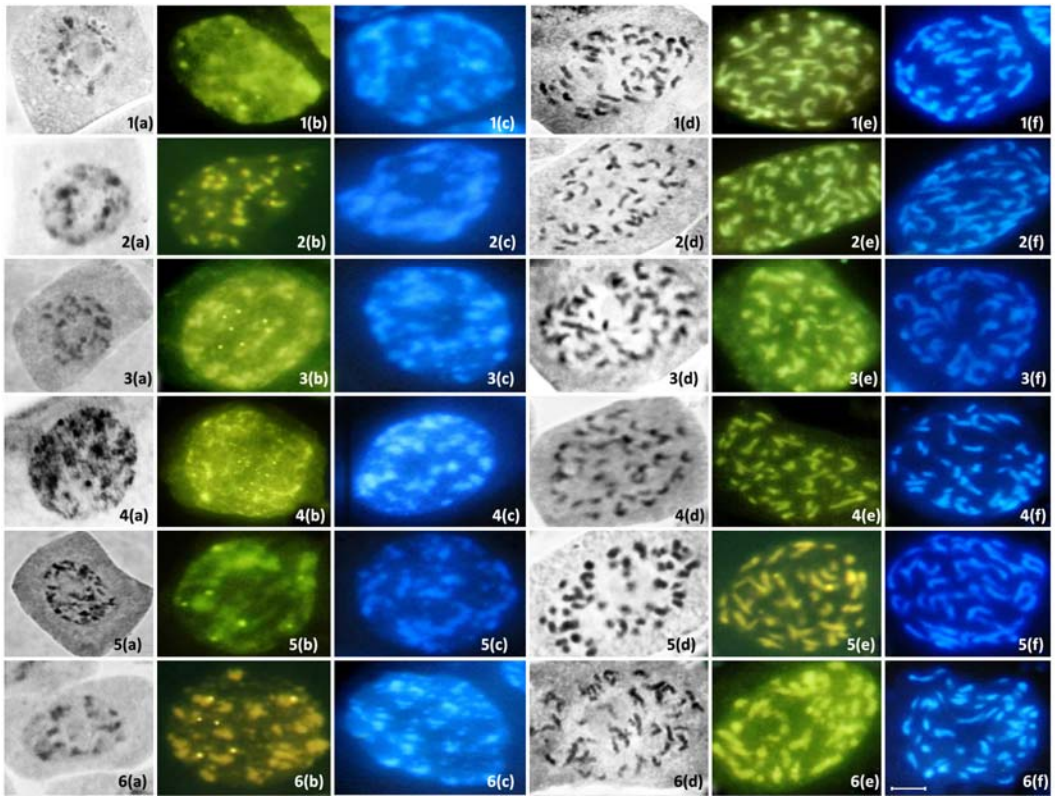
Healthy roots were pretreated with 0.002 M 8-hydroxy quinoline for 3.5 hrs at 18°C followed by 15 m fixation in 45% acetic acid at 4°C. These were then hydrolyzed in a mixture of 1N HCl and 45% acetic acid (2 : 1) at 60°C for 20 sec. The root tips were stained and squashed in 1% aceto-orcein. For CMA- and DAPI 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 24 hrs before study. The air-dried slides were first pre-incubated in McIlvaine's buffer (pH 7.0) for 30 m 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. One drop of CMA (0.1 mg/ml) was added to the materials for 15 min in a humid chamber and then rinsed with McIlvaine's buffer with $MgSO_4$ for 10 min. Slides were mounted in 50% glycerol and kept at 4ºC for overnight before observation. These were observed under Nikon (Eclipse 50i) fluorescent microscope with blue violet (BV) filter cassette. For DAPI-staining, after 24 hrs of air drying, the slides were first pre-incubated in Mcllvaine's buffer (pH 7.0) for 27 m and treated in actinomycin D (0.25 mg/ml) for 10 min in a humid chamber. The slides were immersed in DAPI solution (0.01 mg/ml) for 20 min and mounted with 50% glycerol. These were observed under a Nikon (Eclipse 50i) fluorescent microscope with ultra violet (UV) filter cassette.

Results and Discussion

The staining properties for interphase nuclei and prophase chromosomes sometimes provide karyomorphological features that help to characterize different varieties. Tanaka (1971) was the pioneer of proposing these criteria for karyomorphological features. 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 in each case. Later different workers applied these criteria in characterizing different plant materials (Alam *et al*. 1993, Begum and Alam 2004).

In this study, the nature of orcein staining of interphase nuclei of 11 cotton varieties was grouped into two categories i.e. "Simple Chromocenter Type" and "Complex Chromocenter Type". "Simple Chromocenter Type" of interphase nuclei was observed in CB-1, CB-3, CB-5, CB-6, CB-8, CB-9, CB-10 and CB-11 with some darkly stained small heterochromatic regions in the nucleus (Figs 1a, 3a, 5a, 6a, 8a, 9a, 10a, 11a and Table 1). On the other hand, CB-2, CB-4 and CB-7 were found to possess "Complex Chromocenter Type" of interphase nuclei where some darkly stained large heterochromatic regions were found in the nuclei (Figs 2a, 4a, 7a and Table 1). In these varieties, a few heterochromatic regions were aggregated together forming bigger

heteropycnotic regions in the interphase nuclei. This result indicated the presence of different kind of heterochromatins in these 11 cotton varieties. In the former group (Simple Chromocenter Type), the heterochromatins were scattered and stained less while the other group (Complex Chromocenter Type) had aggregated heterochromatins.



Figs 1 - 6. Mitotic interphase nuclei and prophase chromosomes of 6 *Gossypium hirsutum* L. varieties. 1(a). Orcein-stanied interphase nuclei of CB-1, 1(b). CMA-stanied interphase nuclei of CB-1, 1(c). DAPI-stanied interphase nuclei of CB-1, 1(d). Orcein-stanied prophase chromosomes of CB-1, 1(e). CMA-stanied prophase chromosomes of CB-1, 1(f). DAPI-stanied prophase chromosomes of CB-1, 2(a). Orcein-stanied interphase nuclei of CB-2, 2(b). CMA-stanied interphase nuclei of CB-2, 2(c). DAPI-stanied interphase nuclei of CB-2, 2(d). Orcein-stanied prophase chromosomes of CB-2, 2(e). CMA-stanied prophase chromosomes of CB-2, 2(f). DAPI-stanied prophase chromosomes of CB-2, 3(a). Orcein-stanied interphase nuclei of CB-3, 3(b). CMA-stanied interphase nuclei of CB-3, 3(c). DAPI-stanied interphase nuclei of CB-3, 3(d). Orcein-stanied prophase chromosomes of CB-3, 3(e). CMA-stanied prophase chromosomes of CB-3, 3(f). DAPI-stanied prophase chromosomes of CB-3, 4(a). Orcein-stanied interphase nuclei of CB-4, 4(b). CMA-stanied interphase nuclei of CB-4, 4(c). DAPI-stanied interphase nuclei of CB-4, 4(d). Orcein-stanied prophase chromosomes of CB-4, 4(e). CMA-stanied prophase chromosomes of CB-4, 4(f). DAPI-stanied prophase chromosomes of CB-4, 5(a). Orcein-stanied interphase nuclei of CB-5, 5(b). CMA-stanied interphase nuclei of CB-5, 5(c). DAPI-stanied interphase nuclei of CB-5, 5(d). Orcein-stanied prophase chromosomes of CB-5, 5(e). CMA-stanied prophase chromosomes of CB-5, 5(f). DAPI-stanied prophase chromosomes of CB-5, 6(a). Orcein-stanied interphase nuclei of CB-6, 6(b). CMA-stanied interphase nuclei of CB-6, 6(c). DAPI-stanied interphase nuclei of CB-6, 6(d). Orcein-stanied prophase chromosomes of CB-6, 6(e). CMA-stanied prophase chromosomes of CB-6, 6(f). DAPI-stanied prophase chromosomes of CB-6, Bar = 5 µm.

The prophase chromosomes of CB-2, CB-3, CB-4, CB-5 and CB-9 were darker in one end and gradually faint to the other end (Figs 2d, 3d, 4d, 5d, 9d and Table 1). According to Tanaka

(1971), this type of prophase chromosomes were regarded as “Gradient Type”. The varieties CB-1, CB-6, CB-7, CB-8, CB-10 and CB-11 were found to possess “Continuous Type” of prophase chromosomes (Tanaka 1971) where most of the chromosomes stained homogenously along the entire length (Figs 1d, 6d, 7d, 8d, 10d, 11d and Table 1).

Table 1. Types of interphase nuclei and prophase chromosomes of 11 varieties of *Gossypium hirsutum* after staining with orcein.

Cotton varieties	Type of chromocentric interphase nuclei	Type ofprophase chromosomes
CB-1	Simple	Continuous
CB-2	Complex	Gradient
CB-3	Simple	"
CB-4	Complex	"
CB-5	Simple	"
CB-6	"	Continuous
CB-7	Complex	"
CB-8	Simple	"
CB-9	"	Gradient
CB-10	"	Continuous
CB-11	"	"

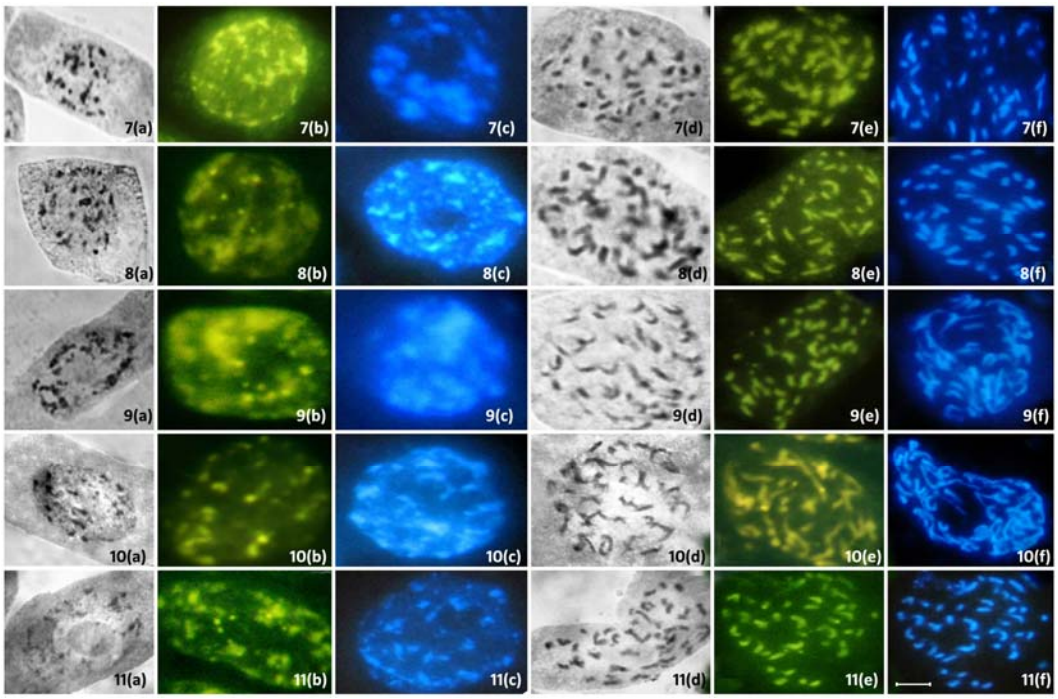
Generally the localized heterochromatin (as observed in the interphase nuclei) is not homogeneously distributed in the prophase chromosomes, rather occupy different locations. The present findings did not support the usual regulation regarding the distribution of heterochromatin in prophase chromosomes. The comparative staining property of interphase nuclei and prophase chromosomes indicated the presence of facultative heterochromatin which firmly aggregated in the interphase nuclei and then somehow had been either homogenously or gradually distributed in the prophase chromosomes. Presence of facultative heterochromatin might be one of the reasons for this disagreement. Whatever the reason is, the 11 varieties could be characterized on the basis of these characters.

A nucleolus was found in almost every interphase, prophase and metaphase stages in all these cotton varieties (Figs 1 - 22). Usually the nucleolus disappears at late prophase of mitosis. There are considerable evidence that for plant nucleoli persist 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 and round shaped entities. They varied in size from small, hardly detectable structures to large conspicuous ones. These suggested the late transcription of rDNA to rRNA and late transportation of rRNA from the nucleus to the cytoplasm. Persistent nucleolus was observed in a few species such as *Spartocera fusca* (Cattani and Papeschi 2004), *Zea mays* (Zirkle 1928), telophase stage in *Oryza sativa* (Ramanujam 1938), *Ceiba pentandra* (Tijo 1948) and 45 species of the family Gramineae (Walter and Emery 1957). No such report was found in any species of *Gossypium*. Therefore, the persistent nature of nucleolus is a salient feature of *G. hirsutum*.

Several CMA-positive bands were found at different location of interphase nuclei and prophase chromosomes in these 11 *Gossypium hirsutum* L. varieties, where highest number (15 - 18) were observed in CB-2, CB-6 and CB-10 (Figs 2b,e, 6b,e, 10b,e). In contrast, 6 - 10 CMA-positive bands were present in the rest varieties (Figs 1b,e, 3b,e, 4b,e, 5b,e, 7b,e, 8b,e, 9b,e, 11b,e). On the other hand, a number of smaller and brightly DAPI stained regions were scattered

around the interphase nuclei of these varieties. Several DAPI-positive bands were found at different location of prophase chromosomes in these 11 cotton varieties (Figs 1c,f, 2c,f, 3c,f, 4c,f, 5c,f, 6c,f, 7c,f, 8c,f, 9c,f, 10c,f, 10c,f).

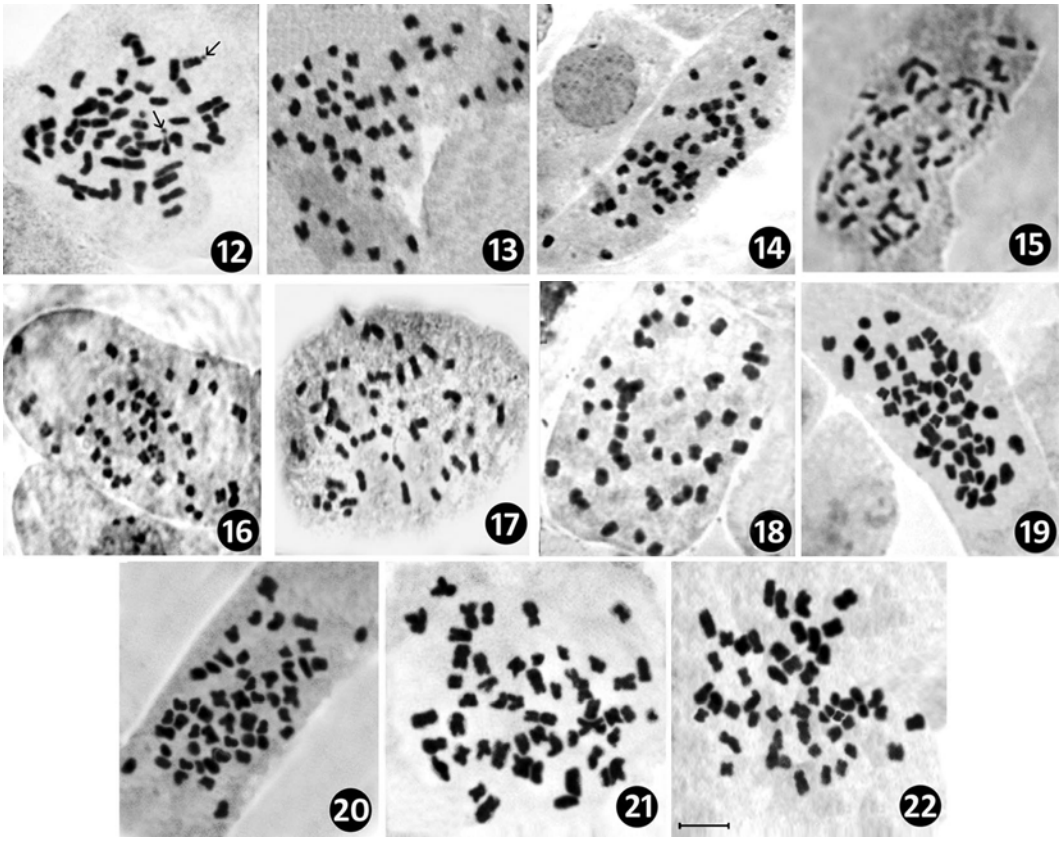
CMA bands are bigger than those of DAPI suggesting that these varieties have more GC-repeats than AT-repeats (Schweizer 1976). Moreover, number of DAPI bands in the interphase nuclei were more than in the prophase chromosomes. This finding revealed that the AT-rich repeats aggregated in the prophase chromosomes during the contraction of chromatin as cell cycle proceeded.



Figs 7 - 11. Mitotic interphase nuclei and prophase chromosomes of five *Gossypium hirsutum* L. varieties. 7(a). Orcein-stanied interphase nuclei of CB-7, 7(b). CMA-stanied interphase nuclei of CB-7, 7(c). DAPI-stanied interphase nuclei of CB-7, 7(d). Orcein-stanied prophase chromosomes of CB-7, 7(e). CMA-stanied prophase chromosomes of CB-7, 7(f). DAPI-stanied prophase chromosomes of CB-7, 8(a). Orcein-stanied interphase nuclei of CB-8, 8(b). CMA-stanied interphase nuclei of CB-8, 8(c). DAPI-stanied interphase nuclei of CB-8, 8(d). Orcein-stanied prophase chromosomes of CB-8, 8(e). CMA-stanied prophase chromosomes of CB-8, 8(f). DAPI-stanied prophase chromosomes of CB-8, 9(a). Orcein-stanied interphase nuclei of CB-9, 9(b). CMA-stanied interphase nuclei of CB-9, 9(c). DAPI-stanied interphase nuclei of CB-9, 9(d). Orcein-stanied prophase chromosomes of CB-9, 9(e). CMA-stanied prophase chromosomes of CB-9, 9(f). DAPI-stanied prophase chromosomes of CB-9, 10(a). Orcein-stanied interphase nuclei of CB-10, 10(b). CMA-stanied interphase nuclei of CB-10, 10(c). DAPI-stanied interphase nuclei of CB-10, 10(d). Orcein-stanied prophase chromosomes of CB-10, 10(e). CMA-stanied prophase chromosomes of CB-10, 10(f). DAPI-stanied prophase chromosomes of CB-10, 11(a). Orcein-stanied interphase nuclei of CB-11, 11(b). CMA-stanied interphase nuclei of CB-11, 11(c). DAPI-stanied interphase nuclei of CB-11, 11(d). Orcein-stanied prophase chromosomes of CB-11, 11(e). CMA-stanied prophase chromosomes of CB-11, 11(f). DAPI-stanied prophase chromosomes of CB-11, Bar = 5 µm.

In the available literatures and internet sources, reports on the nature of mitotic interphase and prophase chromosomes of *Gossypium hirsutum* L. after differential staining with orcein, CMA and DAPI were not found. Therefore, characterization of 11 varieties of *Gossypium hirsutum* L. by the above parameters was the pioneer attempt.

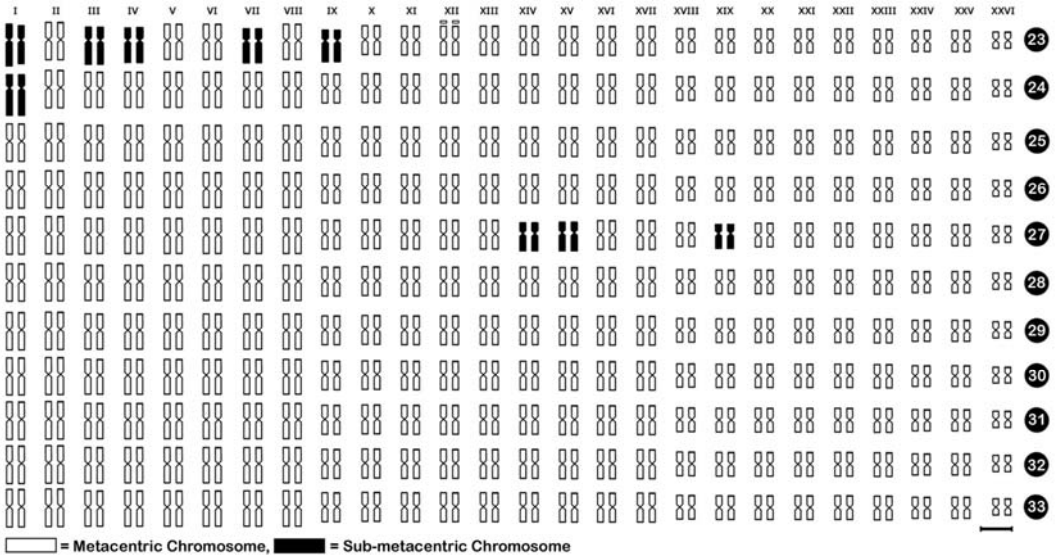
In this study, 11 varieties of *Gossypium hirsutum* L. were found to possess 2n = 52 chromosomes (Figs 12-22). Similar chromosome number for *G. hirsutum* L. was reported earlier by different workers (Beasly 1942, Nei and Li 1985, Wang *et al.* 1996). Besides, different chromosome numbers were also reported either for *G. hirsutum*, such as 2n = 26 (Davie 1933), 2n = 39 (Mehetre and Thombre 1980), 2n = 51 (Marina *et al.* 2011) and 2n = 56 (Farzaneh *et al.* 2010). 2n = 26 and 2n = 39 might be a rare case of diploidy and triploidy, respectively. On the other hand, the monosomic condition (2n =51) of *G. hirsutum* was frequently observed by Marina *et al.* (2011). They found 92 primary monosomic stocks of *G. hirsutum*. Those monosomic conditions were not found for a particular chromosome of *G. hirsutum* rather for different chromosomes. The reasons for obtaining 2n = 56 chromosomes were not explained by the authors. This type of chromosomal aberration was very rare and would not be classified in usual way. In this study, no numerical annomalies was observed in the 11 varieties of *G. hirsutum.* Therefore, the 11 cotton varieties released by CDB have strict 2n chromosome number.



Figs 12 - 22. Orcein-stained mitotic metaphase chromosomes of 11 varieties of *Gossypium hirsutum*. 12. CB-1, 13. CB-2, 14. CB-3, 15. CB-4, 16. CB-5, 17. CB-6, 18. CB-7, 19. CB-8, 20. CB-9, 21. CB-10, 22. CB-11, Bar = 5 µm.

Out of 11 cotton varieties, 8 were found to possess all metacentric chromosomes (52 m) (Figs 23-33 and Table 2). Only three varieties (CB-1, CB-2, CB-5) had few submetacentric chromosomes (sm) *viz.* 10 sm in CB-1, 2 sm in CB-2 and 6 sm in CB-5 (Figs 23, 24, 27 and Table

2). Nie and Li (1985) observed 16 submetacentric and 2 subtelocentric chromosomes in *G. hirsutum.* The submetacentric chromosomes might have originated from metacentric chromosomes by some chromosomal aberrations *viz.* terminal deletion, pericentric inversion, non reciprocal or unequal translocation between fragments of chromosomes within *G. hirsutum*.



Figs 23-33. Idiogram prepared from orcein-stained mitotic metaphase chromosomes of 11 varieties of *Gossypium hirsutum* L. 23. CB-1, 24. CB-2, 25. CB-3, 26. CB-4, 27. CB-5, 28. CB-6, 29. CB-7, 30. CB-8, 31. CB-9, 32. CB-10, 33. CB-11, Bar = 2 µm.

Table 2. Comparative karyotype analysis in eleven varieties of *Gossypium hirsutum* L.

Varieties	2n	No. of satellite	Total length of 2n chromosome complement (µm)	Range of chromosomal length (µm)	Centromeric formulae
CB-1	52	2	86.27	1.02 - 2.56	10sm + 42m
CB-2	52	-	78.05	1.12 - 2.40	2sm + 50m
CB-3	52	-	69.82	0.76 - 1.98	52m
CB-4	52	-	81.22	0.77 - 2.62	52m
CB-5	52	-	79.32	1.28 - 1.85	6sm + 46m
CB-6	52	-	82.72	0.80 - 2.30	52m
CB-7	52	-	61.82	0.80 - 1.73	52m
CB-8	52	-	66.05	0.83 - 1.98	52m
CB-9	52	-	55.58	0.82 - 1.82	52m
CB-10	52	-	77.58	0.90 - 2.37	52m
CB-11	52	-	83.62	0.96 - 2.31	52m

m = Metacentric chromosome, sm = Sub-metacentric chromosome.

In these 11 cotton varieties, the range of chromosomal length was almost negligible i.e. distance between the smallest and largest chromosomes was about 1 µm (Table 2). As a result, no gradual decrease of chromosomal length was observed in their karyotypes (Table 2). These

features indicated that *G. hirsutum* has a strict symmetric karyotype. Stebbins (1971) mentioned that the symmetric karyotypes were primitive character. The origin of *G. hirsutum* was in the pre-historic era. Allopolyploid cottons appeared to have arisen within the last million years, as a consequence of trans-oceanic dispersal of an A-genome taxon to the New World followed by hybridization with an indigenous D-genome diploid (Wendel and Cronn 2003). The symmetric karyotype proved its primitive nature. The species might alter its genomic constituents without changing the apparent karyotype.

The full strength karyomorphological study at different mitotic stages provided much genomic information about 11 cotton varieties released by CDB. This will be helpful for improved breeding programme of upland cotton.

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