CHARACTERIZATION OF SOME COTTON GERMPLASMS THROUGH MOLECULAR CYTOGENETICS

Ph.D. THESIS

BY

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MARCH 2014

CYTOGENETICS LABORATORY
DEPARTMENT OF BOTANY
UNIVERSITY OF DHAKA
DHAKA 1000, BANGLADESH

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CHARACTERIZATION OF SOME COTTON GERMPLASMS THROUGH MOLECULAR CYTOGENETICS

A DISSERTATION
SUBMITTED TO THE UNIVERSITY OF DHAKA
IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
IN
BOTANY

BY
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MARCH 2014

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Dedicated
To
My Beloved Parents,
Husband
and beautiful daughter
Riyana

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Certificate

*This is to certify that the thesis entitled "**Characterization of some cotton germplasms through molecular cytogenetics**" submitted by Syeda Sharmeen Sultana has been carried out under my supervision in this laboratory. This is further to certify that it is an original work and suitable for submission for the award of doctor of philosophy in Botany.*

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– The Author

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List of abbreviations

BARI	:	Bangladesh Agricultural Research Institute
CDB	:	Cotton Development Board
CB	:	Cotton Board
CTAB	:	Cetyl trimethyl ammonium bromide
PVP	:	Polyvinyl pyrrolidone
EDTA	:	Ethylene diamine tetra acetic acid
PCR	:	Polymerase Chain Reaction
RAPD	:	Random Amplified Polymorphic DNA
SSR	:	Simple Sequence Repeat
AFLP	:	Amplified Fragment Length Polymorphism
RFLP	:	Restriction Fragment Length polymorphism
ISSR		Inter-simple sequence repeat
Kb	:	Kilo base
bp	:	Base pair
UPGMA		Unweighted Pair Group Method of Arithmetic Means
UV	:	Ultra violet
TE	:	Tris-EDTA
ddH_2O	:	Double distilled water
rpm	:	Revolution per minute
TAE	:	Tris-acetate-EDTA
FISH		Fluorescent *in situ* hybridization
MT	:	Metric Ton
t/ha	:	Ton/hectare
m	:	Metacentric
sm	:	Sub-metacentric
ac	:	Acrocentric
t	:	Telocentric
CI	:	Centromeric Index
RL	:	Relative length
RTs	:	Root tips
CMA	:	Chromomycin A_3
DAPI	:	4′-6-diamidino-2-phenylindole

List of abbreviations

GC	:	Guanine-Cytocine
AT	:	Adenine-Cytocine
m	:	Minute
s	:	Second
h	:	Hour
1N	:	1 Normal
MW	:	Molecular weight
g	:	Gram
mg	:	Milligram
ng		Nanogram
µ	:	Micron
µm	:	Micrometer
µM	:	Micro molar
µl	:	Micro liter
µg	:	Microgram
α	:	Alpha
β	:	Beta
γ	:	Gama
φ	:	Phi (small letter)
θ	:	Theta
Φ	:	Phi (capital letter)
λ	:	Lambda
δ	:	Delta
Ω	:	Omega
%	:	Percentage
˚C	:	Degree centigrade
et al.	:	et alii (and others)
i.e.	:	id est (that is)
viz.	:	Videlicet, Namely
No.	:	Number
Fig(s).	:	Figure(s)
Vol.	:	Volume
W/V	:	Weight/Volume

ABSTRACT

Abstract

Eleven germplasms of *Gossypium hirsutum* L. (cotton) released by Bangladesh Cotton Development Board *viz.* CB-1 (Cotton Board-1), CB-2, CB-3, CB-4, CB-5, CB-6, CB-7, CB-8, CB-9, CB-10 and CB-11 were investigated cytogenetically and at the molecular level using RAPD- and SSR-markers for authentic characterization. The 11 cotton germplasms represented a broad spectrum of variation for several phenotypic and agronomic traits. These germplasms were found to possess a prominant nucleolus in the interphase nuclei and prophase chromosomes after orcein staining. The interphase nuclei and prophase chromosomes of these germplasms showed different types of orcein-staining pattern. Although the germplasms 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 46m + 6sm were found in CB-1 and CB-4 while it was 48m + 4sm in CB-8 and CB-10. The rest germplasms have 52 metacentric chromosomes. A wide range of CMA-positive bands (5-20) was found in the metaphase chromosomes of 11 germplasms. Different number of satellites such as 4 (CB-1), 6 (CB-2), 6 (CB-3), 2 (CB-4), 3 (CB-6), 2 (CB-7), 6 (CB-8) and 6 (CB-10) were found after CMA-staining. Entirely DAPI-fluoresced chromosomes were frequent in these germplasms. The number, location and distributions of GC- and AT-rich repeats are specific for each germplasm. Fluorescent banding revealed the occurrence of genomic alteration within these germplasms. DNA from the 11 cotton germplasms was studied with 10 oligonucleotide primers and 5 microsatellite primer pairs for RAPD and SSR assay, respectively. The ten RAPD primers generated 335 distinct bands with 100% polymorphisms indicated highly diversed nature. In

addition to polymorphism, 29 unique RAPD sequences were identified in 11 cotton germplasms. The five SSR primer pairs generated 69 distinct bands of which 39 were considered as polymorphic (56.52% polymorphisms). Moreover, four unique SSR sequences were identified in CB-1, CB-5 and CB-9. The combined RAPD and SSR dendrogram made CB-11 distinct from the rest and placed alone in a separate cluster that correlated with its phenotypic, agronomic and cytogenetical features. Therefore, each germplasm could be characterized authentically by cytogenetical and molecular analysis.

1. INTRODUCTION

1. Introduction

Cotton is a kind of bast fibre obtained from the epidermis of the seeds. Cotton is often called the king of fibers. It is the 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).

The common name "cotton" comes from the Arabic "quotn" and generally refers to species that produces spinnable fibres on their second coat (Lee 1984). The oldest known words for cotton are "karpasara-i", in the language Sanskrit and "karapas" used in early Bible manuscripts (Smith 1995). The genus *Gossypium* was named by Linneaus in the middle of the 18th century.

Commercial cotton fibres are mainly obtained from different *Gossypium* species. The genus *Gossypium* L. belongs to the family Malvaceae. There is a controversy about the number of species of this genus. According to Sauer (1993), *Gossypium* comprises of 39 species. On the other hand, 50 different species of this genus were reported by Chaudhary *et al.* (2010).

The origin, distribution and domestication of cotton has long antiquity. One of the most remarkable stories in the annals of crop domestication is the origin of cultivated cotton. Perhaps the most striking aspect of this history is that it is global in scope, involving ancient human cultures in both the Old- and New Worlds and a convergent or parallel plant domestication process from divergent and geographically isolated wild ancestors. Indeed, cotton is unique among crop plants in that four separate species were independently

domesticated for the specialized single-celled trichomes (Brubaker *et al.* 1999, Brubaker and Wendel 1994, Percy and Wendel 1990, Wendel 1989, Wendel *et al.* 1992, Wendel *et al.* 1999).

This parallel domestication process involved four species, two from Americas, *viz. Gossypium hirsutum* and *G. barbadense* and two from Africa–Asia, namely *G. arboreum* and *G. herbaceum*. In each of these four cases, aboriginal people discovered several thousand years ago that the unique properties of cotton fibers made them useful for ropes, textiles and other applications. As a consequence, cotton cultivation became increasingly widespread, such that over the millennia cotton became firmly established as the world's most important fiber crop and an important source of seed oil and protein meal. Due to increasing its commercial utilization this genus distributed in 70 countries of the world (Farzaneh *et al.* 2010), although it is believed that they are native to the tropics and warm temperate regions (Sauer 1993).

There are 50 known *Gossypium* species of which 45 species are diploid (2n = 2x = 26) and five allotetraploids (2n = 4x = 52) (Brubaker *et al.* 1999a). Out of 50 *Gossypium* species, only four are cultivated such as *Gossypium herbaceum* L., *G. arboreum* L., *G. barbadense* L. and *G. hirsutum* L. The species *Gossypium hirsutum* L. was named due to its hairiness (hirsute) around seeds. It is commonly known as "upland cotton", American cotton or Mexican cotton. *Gossypium barbadense* L. was named after its assumed habitat of Barbados. It is commonly known as Egyptian cotton or Indian cotton. On the basis of origin, cotton has been grouped into three groups *viz.* (i) Asian or Old-World diploid cotton, (ii) American or New-World diploid

cotton, and (iii) American or new-world polyploidy cotton (Zaman 1998). *Gossypium arboreum* and *G. herbaceum* belong to the old world diploid group with 'A' genome, where as the new world tetraploid cultivated species are *Gossypium hirsutum* and *G. barbadense* with 'AD' genome (Chaudhary *et al.* 2010). The new world polyploidy cotton is an allopolyploid. Although there is a contradiction about the putative parents of new world cotton, most of the scientists believed that it is a segmental allopolyploid (Beasly 1942).

Gossypium species exhibit extraordinary morphological variation, ranging from herbaceous perennials to small trees with a diverse array of reproductive and vegetative characteristics. A parallel level of cytogenetic and genomic diversity has arisen during the global radiation of the genus, leading to the evolution of eight groups of diploid species. The evolutionary history of the genus included multiple episodes of trans-oceanic dispersal, invasion of new ecological niches and a surprisingly high frequency of natural interspecific hybridization among lineages that are presently both geographically isolated and intersterile. Allopolyploid cottons appear 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. Subsequent to formation, allopolyploids radiated into three modern lineages, including those containing the commercially important species *G. hirsutum* and *G. barbadense*. Genome doubling has led to an array of molecular genetic interactions, including inter-locus concerted evolution, differential rates of genomic evolution, inter-genomic genetic transfer and probable alterations in gene expression (Wendel and Cronn 2003, Brubaker *et al.* 1999, Brubaker and Wendel 1994,

Percy and Wendel 1990, Wendel 1989, Wendel *et al.* 1992, Wendel *et al.* 1999).

Among the cultivated species, *G. hirsutum* has the highest yield potential and is the largest globally cultivated species with about 90% contribution to the world cotton market. The next in importance is *G. barbadense* which is a donor for good quality fiber. The two cultivated diploid species, *G. arboreum* and *G. herbaceum* have wide adaptability and high degree of resistance to biotic and abiotic stresses (Patel and Mehta 1990, Katagiri et al. 2004, Lee 1984).

Besides fibre importance, *Gossypium hirsutum* L. and related species have pigment glands located throughout the plant and these glands contain a polyphenolic compound called "gossypol" (Adams *et al.* 1960). Gossypol and other related compounds are an integral part of cotton's self-defense mechanism and protect the plants from pests and possibly some diseases (Bell and Stipanovic 1977, Hedin *et al*. 1992, Jenkins and Wilson 1996). This compound also has been reported to have antitumor activity (Blackstaffe *et al.* 1997) and possess contraceptive properties (Matlin 1994). Unfortunately, gossypol also has a detrimental effect on humans as well as other monogastric animals. Gossypol is known to have anti-nutritional effects on animals fed cottonseed products (Blom *et al*. 2001, Eisele 1986) and its presence in cotton seed has limited its use in feeding rations (Berardi and Goldblatt 1980).

Bangladesh has a glorious history in textile production. The finest cotton fabric- "Moslin" once produced in medieval Bengal was famous through out

the world. However, the production and trading of Moslin gradually declined during the British rule ultimately resulting to closure of the industry by early nineteenth century. During Pakistan era, there had been limited effort to introduce cotton in this part (former East Pakistan) with little support for research and development of the crop and as a consequence cotton remained confined to laboratory until early seventies of the last centuries. Production of cotton domestically has become a strong challenge soon after the liberation of the country in 1971 when the supply of raw cotton was suspended from Pakistan (CDB 2014).

At present, cotton is one of the important cash crops in Bangladesh. It is the main raw materials of textile industry. Annual requirement of raw cotton for textile industry of Bangladesh is estimated around 2.5 million bales. Local production is only about 0.1 million bales. The highest domestic cotton production was 93,000 bales in 1997-98 against the total requirement of 1,20,000 bales for the year 2001-2002 (CDB 2014). Cotton Development Board (CDB) was targeted 50000 ha cultivation and expected production of 94000 bales lint in the year of 2007-2008. This amount of domestic production of the total could provide only negligible proportion of the total requirement of the country's textile industry. The textile industry therefore, predominantly depends on imported cotton (CDB 2014).

The four most domesticated cotton species in Bangladesh are *Gossypium herbaceum* (African-West Asian cotton), *G. arboreum* (Pakistan-Indian cotton), *G. barbadense* (South-American cotton) and *G. hirsutum* (Mexican cotton). Being an important cash crop, breeders of different national agencies are attracted to this crop. As a consequence, Bangladesh Cotton

Development Board (CDB) has been conducting research since 1991 and was able to release so far eleven germplasms (Upland Cotton) of *G. hirsutum* namely- CB-1, CB-2, CB-3, CB-4, CB-5, CB-6, CB-7, CB-8, CB-9, CB-10 and CB-11.

Main germplasms of American cotton grown in Bangladesh are CB-1 (Deltapine-90), CB-3 (Deltapine-50), CB-5 (a cross between Deltapine and indigenous variety), CB-9 (Developed from SI4/91/646)) and CB-10 (developed from BC-0397). Among the 11 germplasms, CB-5 and CB-9 has some tolerance to Jassids. In addition, CB-9 is the most commonly grown germplasms covering 60% of the total area under the cultivation of American cotton.

Breeders have evolved these germplasms through selection based on morphological and physiological features (yield, fiber quality, resistance against certain pests and diseases etc.)(Iqbal *et al.* 1997). Although great progress has been made in the field of improvement of cotton with conventional breeding methodology, it is time consuming and commercialization of new cotton germplasms often takes 6 to 10 years (Farzaneh *et al.* 2010, Zhang *et al.* 2000).

Successful breeding program depends on the complete knowledge and understanding of the genetic diversity within and among genetic resources of the available germplasms. This will enable plant breeders to choose parental sources that generate diverse populations for selection (Esmail *et al.* 2008). The extent of genetic diversity in species is important in determining the selection to be performed and for the ability to adapt to variable

environmental conditions. At the onset of a breeding program, information concerning genetic relationships can be used to improve the breeding population by complementing phenotypic features (Surgun *et al.* 2011). Though various breeding procedures like hybridization, mutation and polyploidy breeding etc. can be used for enlarging the available variability, it is always essential to quantify and qualify the genetic variability.

Moreover, a number of germplasms have been collected all over Bangladesh and stored in the gene bank of Bangladesh Agricultural Research Institute (BARI). These varieties with many lines are being characterized on the basis of their morphological features. This kind of characterization sometimes creates problem since phenotypic features are not always reliable. There are many lines evolved from these varieties but the screening of these lines difficult due to the hybrid possibilities among them. For this purpose, an authentic characterization of each germplasms is needed to choose the most compatible parents, germplasm conservation and patenting as well.

Only a few earlier workers tried to characterize cotton with classical karyotype analysis (Beasly 1942, Kammacher 1959, Mehetre *et al.* 1980, Nei and Li 1985, Wang *et al.* 1996, Mehetre and Thombre 1877, Davie 1933, Mehetre and Thombre 1980, Marina *et al.* 2011, Farzaneh *et al.* 2010). 2n= 52 chromosomes for *G. hirsutum* L. was reported earlier by different scientists (Beasly 1942, Kammacher 1959, Mehetre *et al*. 1980, Nei and Li 1985, Wang *et al.* 1996). In addition, an attempt was taken to determine the aneuploid nature of certain cotton germplasms. Different chromosome numbers were also reported for *G. hirsutum, s*uch as 2n = 26 (Mehetre and Thombre 1877, Davie 1933), 2n = 39 (Mehetre and Thombre 1980), 2n = 51

(Marina *et al.* 2011) and 2n = 56 (Farzaneh *et al.* 2010). In comparison to mitosis, more information was available for meiotic chromosome analysis (Mehetre and Thombre 1877, Mehetre and Thombre 1980, Marina *et al.* 2011). Few works on cotton karyotype as well as meiotic analysis of some gamma irradiated cotton germplasms and their hybrids were undertaken (Shedai and Koobaz 2003, Shedai *et al.* 2004, Shedai and Dezfulian 2008, Shedai *et al.* 2007). Most of the earlier cytological research on cotton were confined to 2n chromosome count. There was no available report on detail karyotype analysis of cotton germplasms.

Karyotype is a stable character and specific for each specimen. However, conventional karyotype analysis is alone unable to express critically the differences among different germplasms of a species since the germplasms of a species possess similar 2n chromosomes numbers and even other karyotype parameters (Khantun and Alam 2010, Kahtun et al. 2011). Moreover, the consideration of chromosome length, arm ratio, position and number of secondary constrictions are not always sufficient to differentiate individual chromosome. Minute deletion, inversion, tandem duplication etc. could not be possible to detect by conventional karyotype analysis. In such a case, a combination of modern cytogenetical and molecular techniques is necessary for comparative study among different germplasms of a species. In addition, other karyomorphological parameters *viz*. staining property of interphase nuclei and prophase chromosomes should be considered to get more data about each germplasms.

Staining with DNA-base specific banding with fluorochromes such as chromomycin A_3 (CMA) and 4´-6 diamidino-2-phenylindole (DAPI) is

relatively recent method for karyotype study. Schweizer (1976) for the first time initiated this technique. CMA binds with GC (Guanine-Cytosine)-rich repetitive sequences of the genome and gives characteristics yellow color bands. On the other hand, DAPI binds to AT (Adenine-Thymine)-rich repeats giving characteristic blue color (Schweizer 1976, Alam and Kondo 1995, Kondo and Hizume 1982, Jessy *et al.* 2005, Akhter and Alam 2005, Islam and Alam 2011, Sultana *et al.* 2011). Thus it seems that fluorescent banding is quite satisfactory for detail and critical chromosome analysis such as identification of individual chromosome, determination of amount and site of AT- and GC-rich base pairs in chromosomes etc. Nigar and Alam (2007) made a comparative fluorescent banding with 3 *Gossypium* species. They were able to differentiate the 3 species with the help of CMA- and DAPI-banding pattern.

Study of staining properties of interphase nuclei and prophase chromosomes are another karyomorphological parameters. Tanaka (1971) classified the different types of 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 and Kondo 1995, Fawzia and Alam 2011, Alam *et al.* 2011, Shahla and Alam 2011). The outcome of this study showed that various taxa including varieties of many plant species could be distinguished by their staining properties of interpahse nuclei and prophase chromosomes.

Molecular markers have become important tools in studding genetic diversity (Bered *et al.* 2005). The utility of molecular markers are generally determined by the technology that is used to reveal DNA based

polymorphism. Recent studies have shown that inter-simple sequence repeats (ISSRs), amplified fragment length polymorphism (AFLPs), simple sequence repeats (SSRs), random amplified polymorphic DNA (RAPDs) could able to detect a certain degree of polymorphism in different plant species (Ferguson *et al.* 2004a, He and Prakash 1997, He *et al.* 2003, Hopkins *et al.* 1999, Subrammanian *et al.* 2000, Raina *et al.* 2001).

DNA fingerprinting by RAPD is one of the molecular methods for characterizing germplasms. The term DNA fingerprinting/profiling describes the combined use of several single locus detection systems. This method has been using as versatile tool for investigating various genomic aspects of organism. It includes characterization of genetic variability, genome fingerprinting, genome mapping, gene localization, analysis of genome evolution, population genetics, taxonomy etc. The advantages of RAPD analysis over other methods are its low sample DNA requirement and the high frequency of polymorphic bands detected (Williams *et al.* 1990).

Simple sequence repeats (SSRs) are a class of molecular microsatellites markers based on tandem repeats of short (2-6 bp) DNA sequences (Litt and Lutty 1989). These repeat sequences are often highly polymorphic, even among closely related cultivars, due to slippage mutations during DNA replication causing variation in the number of repeating units. Different alleles of a given locus can readily be detected using primers designed from the conserved DNA sequences flanking the SSR and the polymerase chain reaction (PCR). SSR markers are generally reported to detect higher levels of polymorphism than RFLPs, RAPDs and AFLPs (Powel *et al*. 1996b, Milbourne *et al.* 1997, Russel *et al.* 1997) and have been widely adopted for genetic

analysis in plants (Panaud *et al.* 1996, Powell *et al.* 1996a). Thus it is believed that SSR markers will provide the molecular genetic differentiation to facilitate routine diversity analysis and molecular breeding applications.

RAPD and SSR molecular markers have been used for revealing genetic diversity among some germplasms and genotypes in cotton (Esmail *et al.* 2008, Maleia *et al.* 2010, Saravanan *et al.* 2006, Hussain *et al.* 2007, Menzel and Brown 1978, Kumar *et al.* 2003, Vafaie-Tabar *et al.* 2003, Mehetre *et al.* 2004). Moreover, genetic analysis using DNA markers have been used to reveal genetic bases of both qualitative and quantitative traits in different crop plants including cotton (Saha *et al.* 1998). These DNA markers have advantage over morphological traits as they are polymorph with no pleiotropic or epistatic effects and are not affected by environmental conditions, thereby used to exploit the available cotton gene pools and enhance germplasm resources (Cantrell *et al.* 1999).

Although some conventional karyotype and PCR based marker analysis of cotton have been undertaken, these were scattered not exactly used for characterization. There was no report on fluorescent banding for comparative karyotype analysis of cotton germplasms. No attempt has been taken earlier to combine cytogenetical and molecular data for characterizing cotton germpalsms. Moreover, no cytogenetical and molecular analyses have yet been initiated for the cotton germplasms available in Bangladesh. As a result, our germplasms have not yet been characterized. Genomic characterization is necessary for successful breeding and patenting each germplasms to prevent illegal trade.

Therefore, in the present study, a combination of cytogenetical and molecular analysis was carried out for the first time to characterize 11 cotton germplasms released by CDB with the following aims:

i. to compare the staining property of the interphase nuclei and prophase chromosomes after staining with orcein and CMA and DAPI.

ii. to make the conventional orcein-stained karyotype of each germplasms.

iii. to compare the fluorescent banding pattern after staining with CMA- and DAPI- fluorochromes.

iv. to compare RAPD and SSR patterns.

v. to characterize each germplasms with cytogenetical and molecular markers.

vi. to elucidate the phylogenetic relationship among 11 cotton germplasms on the basis of cytogenetical and molecular data.

2. MATERIALS AND METHODS

2. Materials and Methods

2.1. Materials

2.1.1. Plant materials

The following 11 germplasms of *Gossypium hirsutum* L. were collected from Cotton Development Board (CDB), Bangladesh and maintained in the Botanic garden, Department of Botany, University of Dhaka.

Eleven germplasms of *Gossypium hirsutum* L. :

i. CB-1 (Fig. 1)

ii. CB-2 (Fig. 2)

iii. CB-3 (Fig. 3)

iv. CB-4 (Fig. 4)

v. CB-5 (Fig. 5)

vi. CB-6 (Fig. 6)

vii. CB-7 (Fig. 7)

viii. CB-8 (Fig. 8)

ix. CB-9 (Fig. 9)

x. CB-10 (Fig. 10)

xi. CB-11 (Fig. 11)

2.1.2. Morphological description of *Gossypium hirsutum*

Gossypium hirsutum L. is annual herb or perennial shrub up to 3 m tall, usually much branched, with nearly all parts irregularly dotted with black

oil glands. Leaves spirally arranged, stipules ovate to lanceolate, often falcate, 5–20 mm × 2–5 mm, petiole 2–10 cm long, blade orbicular in outline, 3–15 cm in diameter, palmately 3-lobed, rarely palmatifid, lower ones sometimes 5-lobed, upper ones occasionally ovate and entire, base cordate, lobes broadly ovate to triangular, apex acute to acuminate, sinuses acute to rounded, margin entire, densely pubescent to glabrous, palmately 5–7-veined, with nectaries on the central basal veins beneath. Flowers solitary, usually on sympodial branches, pedicel 1–4 cm long, not articulated, with 3 nectaries below the insertion of the epicalyx segments, epicalyx segments (bracteoles) 3, free, closely enveloping the flower and fruit, ovate to triangular, 2–6.5 cm × 1.5–4 cm, cordate at base, margin with 3–19 acuminate teeth, persistent, calyx campanulate to cupular, 5–7 mm long and 6 mm in diameter, truncate or with 5 rounded (rarely acuminate) segments, outside with 3 inconspicuous nectaries, ruptured after flowering, corolla usually pale yellow to white, rarely with a purplish centre, petals 5, imbricate, obovate, 2–5.5 cm long, stamens numerous, forming a column 1–2 cm long, filaments 3–4 mm long, anthers 1-celled; pistil with 3–5-celled ovary and one short style with clavate, 3–5-sulcate stigma. Fruit ('boll') an ovoid or globose capsule 2–5 cm × 1–1.5 cm, rostrate at the apex, coarsely pitted, glabrous, opening loculicidally, 3–5-celled with several seeds per cell. Seeds ovoid, 3.5–10 mm long, acute at the hilum, black or brown with a dense covering of white or rusty, long, woolly hairs (lint or floss) and with a fine, short tomentum (fuzz) everywhere or only at the hilum. Seedling with epigeal germination (Burkill 1997, Kerkhoven and Mutsaers 2003). Besides the above general morphological description, each germplasm has specific morphological features and agronomic performance which were listed in Table 1.

Table 1. Major morphological features and agronomic performance of 11 *Gossypium hirsutum* L. germplasms (According to Cotton Developent Board, Bangladesh, 2014):

Germplasm	CB-1	CB-2	CB-3	CB-4	CB-5	CB-6	CB-7	CB-8	CB-9	CB-10	CB-11
NSB Registration No.	05(06) 06/2005	05(06) 07/2005	05(06) 08/2005	05(06) 09/2005	05(06) 10/2005	05(06) 11/2005	05(06) 12/2005	05(06) 13/2005	05(06) 14/2005	05(06) 15/2005	-
Days to first flower	57	53	52	54	51	52	50	68	52	45	47
Days to first boll split	127	130	117	136	122	117	117	124	119	114	112
Number of boll per plant	56	33	43	42	47	35	34	35	44	42	43
Single boll weight (gm)	4.8	5.4	6.4	6.0	5.1	5.6	5.6	4.0	5.8	5.5	5.7
Plant height at harvest (cm)	151	121	130	134	133	129	121	105	106	126	106
Seed cotton yield (ton/ha)	1.75-2.00	1.50-1.80	1.75-2.00	1.32-1.50	1.75-2.00	1.50-2.00	1.50-2.00	1.50-2.50	2.00-2.50	1.75-2.00	2.10-3.50
Fibre length (2.5%) inch	1.12	1.18	1.14	1.17	1.13	1.17	1.16	1.08	1.10	1.11	1.14
Suitable area for cultivation	Jessore & Kushtia	Not under cultivation	Rangpur, Rajshahi, Thakurgaon, Bogra, Dhaka, Mymensingh	Not under cultivation	Jessore & Kushtia	Not under cultivation	Not under cultivation	Not under cultivation	Jessore, Kushtia,Jhenaidah, Chuadanga,Rangpur, Rajshahi,Thakurgaon, Bogra,Dhaka, Mymensingh	Rangpur, Rajshahi, Thakurgaon ,Bogra	Not under cultivation
Jassid resistancy	-	-	-	-	Jassid resistant	-	-	-	Jassid resistant	-	-

2.2. METHODS -Cytogenetical studies

2.2.1. Preparation of reagents

2.2.1.1. Pre-fixative 8-hydroxyquinoline (0.002 M)

8-hydroxyquinoline (0.29 gm) crystals were thoroughly dissolved in a liter of distilled water by constant stirring and kept at below 20°C for future use.

2.2.1.2. Fixative (45% acetic acid)

Glacial acetic acid (45 ml) was mixed with 55 ml of distilled water.

2.2.1.3. Preservative (70% alcohol)

Absolute alcohol (70 ml) was mixed with 30 ml of distilled water.

2.2.1.4. Hydrolyzing agent (1N HCl)

HCl (36.5 ml) were thoroughly dissolved in a liter of distilled water by constant stirring and kept at room temperature for future use.

2.2.1.5. Hydrolyzing solution

Two parts of 1N HCl was mixed with one part of 45% acetic acid.

2.2.2. Stains

2.2.2.1. Aceto-orcein (1%)

One g of orcein dye (Sigma) was added to a flask containing 45 ml (100%) acetic acid. These were heated (not boiling) for about 20 h and messed up to 100 ml by adding distilled water. It was then filtered quickly and stored at room temperature for future use.

2.2.2.2. Chromomycin A_3 (CMA) (0.1 mg/ml)

One mg of chromomycin A_3 (CMA) (Sigma) was thoroughly dissolved in 10 ml of McIlvaine's buffer supplemented with Mg^{+2} by stirring and kept at -20 °C for future use.

2.2.2.3. 4′-6 Diamidino-2-Phenylindole (DAPI) (0.01 mg/ml)

One mg 4′-6-Diamidino-2-Phenylindole (DAPI) (Sigma) was thoroughly dissolved in 100ml McIlvaine's buffer (without $Mg^{+2)}$ by stirring and kept at –20 °C for future use.

2.2.3. Buffer

2.2.3.1. McIlvaine's buffer (pH 7.0)

About 59 (58.99) g of $Na_2HPO_4.12H_2O$ (FW 358.14) and 3.71 g citric acid were measured and mixed in one litre distilled water. It was then autoclaved for 10 m and kept at 4 °C for future use.

2.2.3.2. McIlvaine's buffer with Mg^{+2} (pH 7.0)

About 59 (58.99) g of $Na_2HPO_4.12H_2O$ (FW 358.14) and 3.71 g of citric acid were mixed in one litre of distilled water. After autoclaving for 10 m, 2.46 g of $MgSO_4$ was added to it and kept at 4 °C for future use.

2.2.4. Antibiotics

2.2.4.1. Distamycin A (0.1 mg/ml)

Two mg of distamycin A (Sigma) thoroughly dissolved in 20 ml of McIlvaine's buffer by stirring and kept at -20 °C for future use.

2.2.4.2. Actinomycin D (0.25 mg/ml)

Two mg of Actinomycin D (Sigma) thoroughly dissolved in 8 ml of McIlvaine's buffer by stirring and kept at -20 °C for future use.

2.2.5. Photography

An automatic digital canon camera (8 mega pixels Canon power shoot PC1564 model), Magnification-8.9x, Mode-Auto and Canon PC1251 Magnification-6x, Mode- Portrate) was used.

2.2.6. Procedure for studying mitotic chromosomes

2.2.6.1. Collection of root-tips (RTs)

Roots were collected from the Botanic garden of the Department of Botany, University of Dhaka. The young healthy roots were cut 0.5 cm away from the tip by a clean blade.

2.2.6.2. Pre-treatment

The collected RTs were soaked on a filter paper to remove surface water and pretreated with 8-hydroxyquinoline (0.002 M) for 3 h at room temperature (28 -30˚C).

2.2.6.3. Fixation

Root tips were fixed in 45% acetic acid for 15 m at 4 ˚C.

2.2.6.4. Preparation of slide for orcein-staining

The pretreated RTs were hydrolyzed for 20-40 s (depending on thickness of root) at 60 ˚C in a mixture of 1N HCl and 45% acetic-acid (2:1). Then the hydrolyzed RTs were soaked on a filter paper and taken on a clean slide. The meristematic region was cut with a fine blade. A drop of 1% aceto-orcein was added to the material and kept in an acetic acid chamber for 12 h. A clean cover glass was placed on the material. At first the materials were tapped gently by a tooth pick and then squashed by placing thumbs. During tapping and squashing care was taken so that the cover glass should not be moved because a minute displacement of it

could damage the entire preparation. The slides were observed under Olympus microscope.

2.2.7. Preparation of slide for fluorescent staining

2.2.7.1. Preparation of air-dried slides

After hydrolyzing and dissecting, the materials were tapped and squashed with 45% acetic-acid and kept in -80 $^{\circ}$C freeze for 3 m. The cover glass was removed quickly and dried in air for at least 24 h before study.

2.2.7.2. CMA-staining

Method proposed by Alam and Kondo (1995) was followed with slight modifications. After 24 h of air drying, the slide was first pre-incubated in McIlvaine's buffer (pH 7.0) for 30 m. At once one drop of 0.1 mg/ml distamycin-A was added to the materials of slides and a cover glass placed on it. The slide was kept in a humid chamber for 10 m. Then the slide was washed with distilled water in such a way that the cover glass removed. The slide was rinsed mildly in McIlvaine's buffer supplemented with $MgSO_4$ for 15 m. Then one drop of chromomycin A_3 (0.1 mg/ml) was added to the materials of slide and a clean cover glass placed on it. The slides were kept in a humid chamber for 12 m. The slide was washed with distilled water in such a way that the cover glasses removed. The slide was treated again for 10 m in McIlvaine's buffer with Mg^{+2} and 10 m in McIlvaine's buffer without Mg^{+2}. Slide was mounted in 50% glycerol and kept at 4 $^{\circ}$C for over night before observation. These were observed under Nikon (Eclipse 50i) fluorescent microscope with blue violet (BV) filter cassette.

2.2.7.3 DAPI-staining

For DAPI-staining, method proposed by Alam and Kondo (1995) was followed after slight modifications. After 48 h of air drying, the slide was first pre-incubated in McIlvaine's buffer (pH 7.0) for 25 m. The slide was treated in 0.25 mg/ml actinomycin-D for 10 m in a humid chamber. After antibiotic treatment, the slide was washed with distilled water in such a way that the cover glass removed. The slide was immersed again in McIlvaine's buffer (pH 7.0) for 15 m followed by treating in DAPI solution (0.1mg/ml) for 12 m. After rinsing in McIlvaine's buffer (pH 7.0) for 10 m, the slide was mounted with 50% glycerol and kept at 4 $^{\circ}$C. These were observed under a Nikon (Eclipse 50i) fluorescent microscope with ultra violet (UV) filter cassette.

2.2.8. Determination of centromeric type, relative length and centromeric index

2.2.8.1. Centromeric type

Procedure proposed by Levan *et al.* (1964) for determining centromeric types of chromosomes was followed with slight modification. In this study, the l/s arm ratio (l = length of the longer arm and s = length of the shorter arm) was considered in the following manner:

	Chromosome type (ct)	l/s ratio
(a)	Metacentric (m)	1.00- 1.50
(b)	Sub-metacentric (sm)	1.51- 3.00
(c)	Acrocentric (ac)	3.01- 7.00
(d)	Telocentric (t)	above 7.00

2.2.8.2. Relative length (RL) of chromosomes

The measurement of relative length was done by dividing the length of a particular chromosome with the total length of the diploid complements. It is represented as follows:

$$RL = \frac{\text{length of a particular chromosome}}{\text{total length of the diploid complements}}$$

2.2.8.3. Centromeric index (CI)

It was measured by the ratio of short arm to total length of that chromosome, expressed as per cent. It can be shown as below:

$$CI = \frac{\text{length of short arm}}{\text{total length of that chromosome}} \times 100$$

2.2.9. Classification of interphase nuclei and prophase chromosomes in orcein-staining

The classification proposed by Tanaka (1971) was followed:

2.2.9.1. Interphase nuclei

(1) Diffuse type

(2) Simple chromocenter type

(3) Complex chromocenter type

(4) Rod prochromosome type

(5) Round prochromosome type

2.2.9.2. Prophase chromosomes

(1) Continuous type

(2) Interstitial type

(3) Gradient type

(4) Proximal type

(5) Tenuous type

2.2.10. Classification of fluorescent bands

According to the different fluorescent banding patterns observed in this study, bandings were classified as follows:

α= Band in centromeric region

γ= Band in whole chromosome

φ= Band in whole short arm

λ= Band in whole long arm

θ= Band in terminal region

β= Band in satellite

Ω= Band in above centromere

ϕ= Two dot in two terminal region of same chromosome

δ= No band

2.2.11. Idiogram

To get an accurate measurement of lengths, chromosomes from at least three metaphase plates were measured for each germplasm. Their average arm length was used to prepare the idiogram. The chromosomes

were arranged gradually from bigger to smaller in length. The short arm placed on the upper side of the axis and long arm on the lower side.

2.2.12. Magnification

Photographs were taken by using 8 mega pixels canon power shoot A720 model with the magnification of 8.9X at Auto mode. For measuring the magnification, at first the magnification was calculated by multiplying the magnification of objective (100X), tube length (1.25X) and camera lens (7X). From print the final magnification was calculated.

2. 3. MOLECULAR STUDY (RAPD and SSR marker analysis)

2.3.1. Collection of leaf sample

To extract genomic DNA, young and actively growing fresh leaves were collected from each of the 11 germplasms of cotton. These leaves were cut apart with sterilized scissors and washed well initially in distilled water and then ethanol. These were dried on fresh tissue paper to remove spore of microorganisms and other sources of foreign DNA.

2.3.2. Preparation of different stock solutions and working solutions used for DNA isolation

For conducting the isolation procedures, the following stock solutions and working solutions were prepared:

2.3.2.1. 1M stock solution of Tris-HCl pH 8.0 (100 ml)

12.14 g of Trizma base (MW=121.14) was dissolved in 75 ml of distilled water. The pH of this solution was adjusted to 8.0 by adding about 5 ml of concentrated HCl in a fume hood. The volume of the solution was adjusted

to a total of 100 ml with de-ionized distilled water. Then it was sterilized by autoclaving and stored at 4 °C.

2.3.2.2. Stock solution of EDTA (0.5 M) pH 8.0 (100 ml)

18.61 g of EDTA (EDTA. $2H_2O$, MW = 372.24) was added to 75 ml of distilled water and stirred thoroughly with a magnetic stirrer. Approximately 2 g of NaOH pellets was added to adjust the final pH to 8.0. It may mention that EDTA alone will not dissolve, unless NaOH is added. The final volume of the solution was adjusted to 100 ml by adding sterile de-ionized distilled water. The solution was sterilized by autoclaving and stored at 4 °C.

2.3.2.3. Stock solution of NaCl (5 M) (100 ml)

29.22 g of sodium chloride (NaCl, MW = 58.44) was dissolved slowly (not at once) in 75 ml of distilled water. The total volume of the solution was adjusted to 100 ml with distilled water. The solution was then heated by oven for 15 s and stirred thoroughly on a magnetic stirrer to dissolve NaCl. It was then sterilized by autoclaving and stored at 4 °C.

2.3.2.4. β-Mercaptoethanol

β-Mercaptoethanol (Merck, Germany) was obtained as a 14.4 M solution from company and it was stored in a dark bottle at room temperature.

2.3.2.5. Ribonuclease-A stock solution

10 mg RNase-A (Merck, Germany) was dissolved in 1 ml of deionized distilled water and stored in -20 °C.

2.3.2.6. Tris-HCl saturated phenol

The crystal phenol was melted in a water bath at 65 °C for 30 m. Melted phenol (100 ml) was added to same volume of Tris-HCl (pH 8.0). It was mixed initially for at least 10 m with a magnetic stirrer and then kept in

rest for 5 m. At this stage, two distinct phases were visible, colorless upper phase and colorful lower phase. With the help of a dropper, the upper phase was removed as much as possible. The same procedure was repeated until the pH of the lower phase rose up to 7.8. Repetition for several times was needed. In this experiment, six times repetitions were done which required about 3.5 h for obtaining the pH 7.75. After saturation, the phenol became the half of the initial volume.

2.3.2.7. Phenol: Chloroform: Isoamyl alcohol (25: 24: 1) (100 ml)

50 ml of Phenol, 48 ml of Chloroform and 2 ml of Isoamyl alcohol were added and mixed properly by vortex mixture under a fume hood. The solution was then stored at 4 $^{\circ}$C. The solution was shaken well before each use.

2.3.2.8. 70% Ethanol (100 ml)

30 ml double distilled water (ddH$_2$O) was added in 70 ml absolute ethanol.

2.3.2.9. Stock Solution of TE (Tris-HCl EDTA) buffer pH 8.0 (100 ml)

1 ml of 1 M Tris-HCl was added to 0.2 ml (200 μl) of 0.5 M EDTA. The final volume was adjusted to 100 ml with sterile de-ionized distilled water. The solution was sterilized by autoclaving and stored at 4 $^{\circ}$C.

2.3.2.10. Sodium acetate (3 M) pH 5.2 (100 ml)

40.824 g of sodium acetate was mixed with 70 ml of ddH$_2$O, adjusted the final volume to 100 ml with ddH$_2$O and sterilized by autoclaving.

2.3.2.11. Extraction buffer (Homogenization buffer 100 ml)

To prepare extraction buffer the following components with proper concentrations were used. For the economic use of chemicals, different volumes of solutions were prepared as in the tabular form given below:

Table 2. Volume of solutions to prepare extraction buffer

Chemicals	Molecular weight	Stock con.	Working con.	Working volume	
				100 ml	1000 ml
CTAB	---	---	2%	2 g	20 g
NaCl	58.44	5 M	1.4 M	28 ml	280 ml
EDTA (pH 8)	372.24	0.5 M	20 mM	4 ml	40 ml
Trisbase (pH 8)	121.1	1.0 M	100 mM	10 ml	100 ml
β-Mercaptoethanol	---	14.4 M	100 mM	700 µl	7 ml

Steps of extraction buffer preparation (100 ml)

- 10 ml of 1 M Tris HCl (pH 8.0) was taken in a 250 ml conical flask
- 28 ml 5 M NaCl was added to it
- 4 ml of 0.5 M EDTA (pH 8.0) was taken in the conical flask
- The mixture was then autoclaved
- After autoclaving, 2 g CTAB was added and stirred very carefully.
- 700 µl β-mercaptoethanol was added prior use and mixed by glass rod under fume hood
- pH of all solutions were adjusted to 5 with HCl and make up to 100 ml by adding sterile de-ionized distilled water

2.3.3. Protocol used for genomic DNA isolation

DNA was isolated using the mini preparation CTAB method (Doyle and Doyle 1987) with minor modification. The protocol was as follows:

2.3.3.1. Required equipments

1. Plant tissues (leaf tissues in this experiment)
2. Autoclave machine
3. Digital balance
4. Beaker
5. Centrifuge machine with 10,000-14,000 rpm
6. Conical flasks
7. Eppendorf tubes
8. Gloves
9. Ice machine
10. Water bath capable of maintaining 65 °C
11. Micropipettes and nuclease free micropipette tips
12. Mortar and pestle
13. pH meter
14. Refrigerator
15. Water de-ionizer
16. Water distillation plant
17. Fume hood

2.3.3.2. Required Chemicals

1. Liquid nitrogen
2. 100% ethanol
3. Tris base
4. EDTA (Ethylenediaminetetraacetic acid)
5. NaCl (Sodium chloride)
6. CTAB (Cetyl Trimethyl Ammonium Bromide)
7. Sodium acetate
8. PVP (Polyvinyl pyrrolidone)
9. β-Mercaptoethanol
10. Phenol `
11. Chloroform
12. Isoamyl alchohol
13. Isopropanol
14. 70% ethanol

2.3.3.3. Procedure

1. Freshly harvested leaf tissue of 200 mg was grinded to fine powder in liquid nitrogen and taken in 2 ml centrifuge tube. 800 µL extraction buffers was added into 2 ml centrifuge tubes and vortexed for 5-10 s to mix the contents well. The tubes were put into 65 ˚C of pre-heated water bath and invert every 5-10 m to allow mixing properly. The samples were then cooled down to room temperature.
2. The samples were centrifuged at 13,000 rpm for 10 m at room temperature to remove non soluble debris. The supernatant was transferred to fresh tubes.
3 The supernatant was mixed with equal volume of Phenol: Chloroform: Isoamyl alcohol solution (25:24:1) by inverting the tubes and centrifuged at 13,000 rpm for 10 m. This process was repeated 2-3 times.
4. The supernatant was taken into another fresh tubes and 0.1 vol. of 3 M sodium acetate (pH 5.2) was added followed by 0.6 vol. of 100% chilled Isopropanol. The solution was shaken slowly. In this step, DNA became visible as whitish fibre in the solution.
5. The solution was centrifuged for 10 m at 13,000 rpm at room temperature. The upper layer of the solution was discarded carefully by using adjustable micropipette.
6. The pellet was washed with 70% ice-cold ethanol. This washing step was repeated at least 2-3 times. Residual CTAB was removed by this step. The liquid was poured out and the tubes put on a paper towel for about 1 h at inverted position. Pellets should neither contain residual ethanol, nor allow for too dry. In both the cases, re-dissolving may be difficult.
7. The dried DNA was dissolved in 100 µl of TE buffer and treated with RNase A for 30 m at 37 ˚C and store at -20 ˚C.

2.3.4. Qualification and quantification of isolated DNA

Before PCR amplification it is important to know the concentration of genomic DNA because different DNA extraction methods produced DNA of widely different purity. It is necessary to optimize the amount of DNA for reproducibility and strong signal in PCR assay. Excessive genomic DNA may result smears lack of clearly defined bands on gel. On the other hand, too little DNA will give non-reproducible patterns (Williams *et al*. 1993). Measurement of isolated DNA concentration can be done by comparing DNA with the standard DNA on agarose gel electrophoresis or by estimating the absorbance of DNA by spectrophotometer at 260 nm wave lengths. Both the methods were carried out in this experiment.

2.3.4.1. Measurement of DNA concentration and quality by agarose gel electrophoresis

The following equipments and chemicals were used to conduct agarose gel electrophoresis:

1. A horizontal electrophoresis chamber and power supply
2. Gel casting tray and combs
3. Gel documentation system (BioSciTec, Gelscan 6.0 Professional, Germany)
4. Gloves
5. Pipette and tips
6. DNA ladder (1 kb for RAPD and 123 bp for SSR)
7. Electrophoresis buffer (TAE)
8. 6X sample loading buffer
9. Agarose
10. DNA stain (ethidium bromide)

2.3.4.1.1. Preparation of stock solutions used for gel electrophoresis

For conducting the gel electrophoresis, the following stock solutions and other solutions were prepared:

2.3.4.1.1.1. TAE buffer (50X, pH 8.3, 1 litre)

242 g Trizma base (MW=121.14) was dissolved into 900 ml of sterile de-ionized distilled water. Then 57 ml glacial acetic acid was added to the solution. Finally, 100 ml 0.5 EDTA (pH 8.0) was added to it. These were mixed well. The pH of the solution was adjusted by mixing concentrated HCl to pH 8.3. The final volume of the solution was adjusted to 1000 ml.

2.3.4.1.1.2. Loading dye (10X)

For making 10 ml of 10X loading dye, 40 mg of Bromophenol blue (i.e., the final concentration was 0.4%), 40 mg of Xylene cyanol FF (i.e., the final concentration was 0.4%) and 5 ml of 98% glycerol (i.e., the final concentration was 50%) were added to 4 ml of sterile de-ionized distilled water and mixed properly. The final volume was adjusted to 10 ml with sterile de-ionized distilled water and stored at 4 $^{\circ}$C.

2.3.4.1.1.3. Ethidium bromide solution

For preparing 1ml solution, ethidium bromide (10 mg) was added to 1 ml of sterile de-ionized distilled water. It was then mixed by hand shaking. The solution was then transferred to a dark bottle and stored at room temperature. Stock solution of 10 mg/ml can be purchased directly from companies.

2.3.4.1.2. Preparation of 1% Agarose Gel

For agarose gel electrophoresis, 100 ml of 1% agarose gel was prepared (100 ml was required for 15 ×15 cm tray with 0.5 cm thickness).

The following steps were followed during casting the gel-

1.0 g agarose powder was measured and put in a 250 ml conical flask. 100 ml of 1X TAE buffer was added to the flask. The agarose was melted in a microwave oven for several short intervals until the solution became clear. The solution did not allow boiling for long period. When the agarose solution was cooled to about 50 °C (the flask was cooled enough to hold comfortably with bare hand), 10 μl (10mg/ml) of ethidium bromide was added and mixed well by gentle shaking.

2.3.4.1.3. Comb set-up

The ends of the gel casting tray was sealed or fitted with casting dams and the combs placed in the gel casting tray. The melted agarose solution was poured into the casting tray and let it to cool until solid. The comb was removed carefully from the solidified gel. The casting dams or gates were removed from the edges of the gel tray carefully so that the gel did not slide off the tray.

2.3.4.1.4. Preparation of DNA sample for electrophoresis

At first 2 μl 6X loading dye was placed on a piece of parafilm by a micropipette. 10 μl DNA sample (after thawing from frozen stock) was added to it and mixed 2-4 times with the adjustable micropipette.

2.3.4.1.5. Electrophoresis

The gel was placed in the electrophoresis chamber in such a way that the sample wells remained near to the cathode (negative end generally marked as black). DNA sample would migrate towards the anode (positive end generally marked as red) during electrophoresis.

The gel was covered by sufficient amount of 1X TAE buffer. The volume of the electrophoresis buffer should not above the maximum buffer mark on electrophoresis system. The prepared samples were loaded slowly and

allowed to sink to the bottom of the wells. 10 μl of 1 kb DNA ladder (marker) was also loaded at least in one well (generally the first one). Electrophoresis was carried out at 50 volts and 100 mA for 1.5 hours. The separation process was monitored by the migration of the dye on the gel. When the first dye (bromophenol blue) had reached about three-fourths of the gel length, the electrophoresis was completed and stopped.

2.3.4.1.6. Documentation of the DNA sample

After electrophoresis, the gel was taken out carefully from the electrophoresis chamber and placed in gel documentation system (BioSciTec, Gelscan 6.0 Professional, German) for observing the DNA bands. The DNA was observed as band and photographed using gel documentation system. The electrophoregram of DNA samples of 11 cotton germplasms are shown in figure 177.

2.3.5. Quantification and qualification of DNA by spectrophotometer

For more confirmation, DNA was also quantified through spectrophotometer. Spectrophotometer is commonly used in laboratories for the measurement of DNA concentration and purity. The DNA concentration was obtained by multiplying the absorbance at 260 nm wave length by a constant. The DNA purity was measured by dividing the absorbance at 260 nm wave length with the absorbance at 280 nm wave length.

Good quality DNA should give the ratio (A260/A280) ranging from 1.8–2.0. The A260/A280 ratio higher than 2.0 and lower than 1.8 generally indicates RNA and protein contamination, respectively during extraction process. Following Sambrook *et al.* (1989), the DNA concentration was calculated (1 O. D.) at 260 nm corresponds to 50 μl/ml of double standard DNA.

2.3.5.1. Set-up the spectrophotometer

To estimate genomic DNA concentration, absorbance readings of the DNA samples were recorded at 260 nm using a spectrophotometer (Analylikjena, Specord 50, Germany).

At first, the spectrophotometer UV-lamp was turned on. After warming up the wave length was set at 260 nm. After washing, the cuvette (the 'zero' or 'blank' cuvette) was filled with 2 ml sterile distilled water and placed on cuvette chamber. The absorbance reading was adjusted to zero for standardization.

2.3.5.2. Preparation of the DNA samples for spectrophotometry

The test samples were prepared by taking 2 μl of each DNA sample in the cuvette containing 1,998 μl sterile distilled water. The samples were mixed well by using an adjustable micropipette. The reading was taken by viewing the monitor of spectrophotometer at 260 nm and 280 nm wave length. After recording the absorbance readings, the cuvette was rinsed out with sterile distilled water, tamped out on a paper and wiped. The absorbance reading for each sample was recorded in the same way. The ratios of spectrophotometric absorption readings at 260 nm of different samples for quality determination of DNA are shown in the Table 2.

2.3.5.3. Calculation for the concentration of DNA

Before PCR, DNA concentrations were determined according to the following formula:

$$\text{DNA concentration} = \text{Dilution factor} \times \text{Conversion factor}$$

$$= A260 \times \frac{\text{Volume of distilled water } (\mu l)}{\text{Amount of DNA sample } (\mu l)} \times 50$$

$$= (ng/\mu l)$$

$$= (\mu g/ml) [\text{since } 1\ \mu g = 10^{-3} ng \text{ i.e., } \mu g/ml = ng/\mu l]$$

A260 = spectrophotometric absorbance reading at 260 nm of DNA sample.

Dilution factor = the ratio of distilled water (µl) to amount of DNA sample (µl).

Conversion factor 50 = the 50 µg/ml of DNA contained in a solution which gives the spectrophotometric absorbance reading at 260 nm equal to 1.

Note: The calculated DNA concentration expressed in µg/ml gave a fraction. To avoid fraction it was converted into ng/µl (1µg = 10^{-3} ng) and therefore, multiplied with 1000.

Table 3. Spectrophotometric absorbance readings at 260 nm wave length and concentration of DNA of 11 germplasms of *Gossypium hirsutum* L.

Germplasms	Absorbance reading at 260 nm	Concentration of DNA (µg/µl)
CB-1	0.094	4700
CB-2	0.098	4900
CB-3	0.082	4100
CB-4	0.067	3350
CB-5	0.058	2900
CB-6	0.083	4150
CB-7	0.044	2200
CB-8	0.078	3900
CB-9	0.094	4700
CB-10	0.098	4900
CB-11	0.082	4100

2.3.6. Amplification of DNA by polymerase chain reaction (PCR) using RAPD and SSR primers

To perform the amplification of target DNA, RAPD and SSR primer sequences were mixed with genomic DNA in the presence of a thermostable DNA polymerase with suitable buffer and subjected to temperature cycling conditions typical for PCR.

2.3.6.1. Preparation of working solution (25 ng/µl) of DNA samples for PCR

Original stock solution concentration of each DNA sample was adjusted to a unique concentration (25 ng/µl) using the following formula:

$S_1 \times V_1 = S_2 \times V_2$

$V_1 = S_2 \times V_2 / S_1$

Where,

S_1= stock DNA concentration (ng/µl)

V_1= volume require (µl)

S_2= working DNA concentration (ng/µl)

V_2= working volume of DNA solution (µl)

Original stock DNA (2 µl) was taken in a 2 ml eppendorf tube and required amount of TE buffer calculated from the above formula added to it. Calculated required volume of TE buffer for each sample was shown in Table 3.

Table 4. Preparation of working DNA solution (25 ng/µl) for PCR reaction

Germplasms	Working Solution (25 ng/µl) for PCR	
	TE buffer/ de-ionized water (µl) required	**DNA (µl) required**
CB-1	374	2
CB-2	390	2
CB-3	326	2
CB-4	266	2
CB-5	230	2
CB-6	330	2
CB-7	174	2
CB-8	310	2
CB-9	374	2
CB-10	390	2
CB-11	326	2

2.3.6.2. Primer test

Twenty five (25) decamer primers were tested for RAPD amplification of which ten primers exhibited good quality banding patterns and sufficient variability. These ten primers were selected for further analysis. The details of the ten primers were given in Table 4.

Five (5) SSR primers were used to amplify simple sequence repeats of genomic DNA from 11 germplasms of *G. hirsutum*. Primers were evaluated on the basis of intensity or resolution of bands, repeatability of markers and consistency within individual and potential to differentiate germplasms (polymorphism). The details of the four primers were given in Table 5.

Table 5. Ten arbitrary RAPD primers used in the present study

Primer code	Sequence (5′—3′)	Annealing Temp. (°C)	G+C content (%)
OPA-1	5′-CAG GCC CTT C-3′	36.40	70
OPA-3	5′-AGT CAG CCA C-3′	34.30	60
OPA-4	5′-AAT CGG GCT G-3′	35.10	60
OPA-6	5′-GGT CCC TGA C-3′	35.20	70
OPA-7	5′-GAA ACG GGT G-3′	33.20	60
OPA-10	5′-GTG ATC GCA G-3′	33.10	60
Primer-1	5′-GAA ACG GGT G-3′	34.50	70
Primer-2	5′-GTT GCG ATC C-3′	32.20	60
Primer-18	5′-GTT TCG CTC C-3′	33.70	60
Primer-23	5′-GTC AGG GCA A-3′	34.70	60

Table 6. Five SSR primers were used in the present study

Primer code	Sequence (5′—3′)	Annealing Temp. (°C)	G+C content (%)
BA00175679	forward-5′-GCTTCTTCCATTTTATTCAAG-3′	57.1	33.3
BA00175680	reverse-5′-CAGCGGCAACCAAAAAG-3′	63.1	52.9
BA00175681	forward-5′-ACCCTTAAATCATAAGAGAAC-3′	53.2	33.3
BA00175682	reverse-5′-CCGTAAGTTAAGGTACAAGG-3′	55.5	45.0
BA00175683	forward-5′-GGAGTTAAAGCTAATGCCTG-3′	61.4	50.0
BA00175684	reverse-5′-CGGGTCATTGGTTGTTTTTG-3′	64.9	45.0
BA00175685	forward-5′-GCCTAGGTGGAGTTCGTG-3′	60.9	61.1
BA00175686	reverse-5′-CTGAACCTGCTCCTGAATC-3′	60.0	52.6
BA00175687	forward-5′-CATCGGAAAACTCTGAAC-3′	55.5	44.4
BA00175688	reverse-5′-GTAGCAGTACAGATGAAAGAG-3′	53.2	42.8

2.3.6.3. Preparation of primers

The supplied primers were diluted to 100 μM. To make 100 μM of each primer, the following mathematical deduction was followed:

We know,

n = cv (where n= number of mole)

c= molarity *i.e.* concentration in molarity

v= volume.

Generally, 100 μM concentration of primer has to prepare as main stock solution.

In this case, c=100 μM since 53.4 nM of primer was present in the vial used in the investigation, supplied from the company, n= 53.4 nM.

v= required volume of TE buffer has to add in the supplied vial to make 100 μM main stocks, using the formula-

$$v = \frac{n}{c}$$

$$v = \frac{53.4 \text{ nmol}}{100 \text{ μmol}}$$

$$v = \frac{53.4 \times 10^{-9} \text{ mol}}{100 \times 10^{-6} \text{ mol}}$$

$$v = \frac{53.4 \times 10^{-5} \text{ mol}}{\text{mol}}$$

$$v = \frac{53.4 \times 10^{-5} \text{ mol}}{\text{mol} L^{-1}}$$

$$v = \frac{53.4 \times 10^{-5}}{L^{-1}}$$

$$v = 53.4 \times 10^{-5} L$$

$$v = 53.4 \times 10^{-5} \times 100 \text{ ml}$$

$$v = 53.4 \times 10^{-2} \text{ ml}$$

$$v = 53.4 \times 10^{-2} \times 1000 \ \mu l$$

$$v = 534 \ \mu l$$

Therefore, 534 µl of TE buffer was added to the vial to make 100 µM main stocks. Using the above deduction method, all primer pairs were diluted to 100µM main stock. All primers were diluted to 10 times *i.e.* 10 µM to make working solution for use.

2.3.6.4. Preparation of PCR reaction mixture/ PCR Cocktail

The following components were used to prepare PCR cocktail (Table 7). The total volume of PCR cocktail was 25 µl per sample.

Table 7. Component of PCR cocktail (12 reactions) for RAPD primers

Sl. No.	Reagents	Amount per sample	Total
1	Sterile de-ionized distilled water	18.8 µl	225.6 µl
2	*Taq* Buffer A 10X (Tris with 15 mM $MgCl_2$)	2.5 µl	30.0 µl
3	Primer (10 µM)	1.0 µl	12.0 µl
4	dNTPs (10 mM each)	0.5 µl	6.0 µl
5	*Taq* DNA Polymerase (5U/µl)	0.2 µl	2.4 µl
6	Template DNA (25 ng/µl)	2.0 µl	---
	Total	**25.0 µl**	

Table 8. Component of PCR cocktail (12 reactions) for SSR markers

Sl. No.	Reagents	Amount per sample	Total
1	Sterile de-ionized distilled water	17.9 µl	214.8 µl
2	*Taq* buffer A 10X (Tris with 15 mM $MgCl_2$)	2.5 µl	30.0 µl
3	Primer forward (10 µM)	1.0 µl	12.0 µl
4	Primer reverse (10 µM)	1.0 µl	12.0 µl
5	dNTPs (10 mM each)	0.5 µl	6.0 µl
6	*Taq* DNA polymerase (5U/µl)	0.2 µl	2.4 µl
7	Template DNA (25 ng/µl)	2.0 µl	---
	Total	**25.0 µl**	

During the experiment, PCR buffer, dNTPs, primers and DNA sample solution were thawed from frozen stocks, mixed well by vortexing and kept on ice. Template DNA (25 ng/µl) were pipetted (2.0 µl) first into PCR tubes (0.5ml) compatible with the thermocycler. For each DNA sample being tested, a pre-mix was prepared in the following order- buffer, dNTPs, DNA template and sterile distilled water. *Taq* DNA polymerase enzyme was added to the pre-mix. The pre-mix was then mixed well and aliquot into the tubes containing primers. The tubes were then sealed and placed in a thermocycler and the cycling started immediately.

2.3.6.5. PCR amplification

PCR amplification was done in an oil-free thermal cycler (Biometra UNOII, Germany). The optimum amplification cycle was as follows:

Table 9. Arbitrary primer-PCR profile

Denaturation/ Annealing/ Extension		Temperature	Time
	Initial denaturation	94 °C	5 m
45 cycles	Denaturation at	94 °C	1 m
	Annealing at	34-36 °C	30 s
	Extension at	72 °C	3 m
	Final extention at	72 °C	5 m

Table 10. SSR marker-PCR profile

Denaturation/ Annealing/ Extension		Temperature	Time
	Initial denaturation	95 °C	3 m
35 cycles	Denaturation at	95 °C	30 s
	Annealing at	53-60 °C	30 s
	Extension at	72 °C	1 m
	Final extention at	72 °C	2 m

After completion of cycling programme, the reactions were held at 4 °C.

2.3.6.6. Electrophoresis of the amplified products (RAPD and SSR) and documentation

The amplified products were resolved by electrophoresis on 1% agarose gel for RAPD and 2% agarose gel for SSR. The gel was prepared using 1.0g agarose for RAPD and 2.0 g agarose for SSR with 10 µl (10 mg/ml) ethidium bromide and 100 ml 1X TAE buffer at 50 volts and 100 mA for 1.5 h. 1 kb and 123 bp DNA ladder was electrophoresed alongside the RAPD and SSR product as marker, successively. DNA bands were observed on UV-transilluminator and photographed by a gel documentation system for both the markers.

2.3.7. RAPD and SSR marker data analysis

The PCR products were analyzed after gel electrophoresis. The photographs were critically discussed on the basis of presence (1) or absence (0), size of bands and overall polymorphism of bands. The scores obtained using all primers in the RAPD and SSR markers analysis were then pooled for constructing a single data matrix. This was used for estimating polymorphic loci, Nei's (1972) gene diversity, genetic distance (D) and constructing a UPGMA (Unweighted Pair Group Method of Arithmetic Means) dendrogram among the germplasm using computer program "POPGENE 32" (Version 1.32).

3. RESULTS

3. Results

3.1. Appropriate season for obtaining maximum number of dividing cells from the root-tips

Although a few dividing cells was observed throughout the year, maximum number of dividing cells were found in the root tip during March to August (about 60%). The number of dividing cells were very poor in extreme high or low temperature.

3.2. Appropriate time for obtaining maximum number of dividing cells from the root tips

Root tips of eleven germplasms of *Gossypium hirsutum* L. were collected from 8.30 a.m. with 30 minutes interval *viz.* 9.00, 9.30, 10.00, 10.30, 11.00 and 11.30 a.m. It was found that 10.30 a.m. to 11.30 a.m. was the optimum time for obtaining maximum number of dividing cells (about 60 %).

3.3. Appropriate chemical for pretreatment

Different chemicals like 8-hydroxyquinoline, colchicines and paradichloro benzene (PDB) were tried for pretreatment to find out proper contraction and intact morphology of chromosomes. It was found that only 8-hydroxyquinoline solution (0.002 M) for 3 hours gave the best result.

3.4. Karyotypes and Idiograms

On the basis of overall length and centromeric position, somatic chromosomes of these eleven germplasms could be assembled in 26 pairs

(Figs. 112-122, Tables 12-22). From the data, idiograms were made supplementing the karyotypes (Figs. 145-155).

3.5. Interphase nuclei

For every staining, at least 50 interphase nuclei were observed in each germplasm.

3.5.1. Orcein staining

The eleven germplasms of *Gossypium hirsutum* L. were found to possess a prominant nucleolus in the interphase nuclei after orcein-staining. Some darkly stained small heterochromatic regions were observed in the nucleus of CB-1, CB-3, CB-5, CB-6, CB-8, CB-9 and CB-10 (Figs. 13, 15, 17, 18, 20, 21, 22). On the other hand, CB-2, CB-4 and CB-7 were found to possess some darkly stained large heterochromatic regions in the nucleus (Figs. 14, 16, 19). In the later germplasms, few heterochromatin were fused together forming bigger heteropycnotic regions. In contrast, some less stained heterochromatic regions were present in interphase nuclei of CB-11 (Fig. 23).

3. 5.2. CMA-staining

Several CMA-positive bands were found at different location of interphase nuclei in these eleven *Gossypium hirsutum* L. germplasms of which highest number (15-18) observed in CB-2, CB-6 and CB-10 (Figs. 25, 29, 33). In contrast, 6-10 CMA-positive bands were present in the rest germplasms (Figs. 24, 26, 27, 28, 30, 31, 32, 34).

3.5.3. DAPI staining

A number of smaller and brightly stained regions was scattered around the interphase nuclei of these germplasms (Figs. 35-45).

3.6. Prophase chromosomes

For every staining, at least 50 prophase stages were observed in each germplasm.

3.6.1. Orcein staining

The prophase chromosmoes 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. 47, 48, 49, 50, 54). In CB-1, CB-7, CB-8, CB-10 and CB-11 most of the prophase chromosomes were stained homogenously along the entire length (Figs. 46, 52, 53, 55, 56). The prophase chromosomes of CB-6 stained only at the interstitial regions (Fig. 51). A nucleolus was found in almost every prophase plate in these germplasms (Figs. 46-56).

3.6.2. CMA-staining

Several CMA-positive bands were found at different location of prophase chromosomes in these germplasms (Figs. 57-67).

3.6.3. DAPI staining

A number of DAPI-positive band was found at different location of prophase chromosomes in these eleven cotton (*Gossypium hirsutum* L.) germplasms (Figs. 68-78).

3.7. Metaphase Chromosomes

For every staining, at least 50 metaphase cells were observed in each germplasm.

3.7.1. Orcein-staining

Total length, l/s ratio, centromeric index, relative length and centromeric type of each chromosome of eleven germplasms of *Gossypium hirsutum* L. were tabulated (Tables 12-23).

The eleven germplasms of cotton were found to possess 2n=52 metacentric chromosomes except CB-1, CB-2 and CB-5. These three germplasms possessed few sub-metacentric chromosomes (Figs. 79, 80, 83, Tables 12, 13, 16). No sharp difference in respect of chromosomal length was observed. The conventional karyotype features of each germplasm were described below.

In CB-1, the total length of the chromosome complements was 86.27 µm. This length was the biggest among the eleven germplasms. The relative length of chromosome and individual chromosomal length were ranging from 0.01 to 0.03 and 1.02 to 2.56 µm, respectively (Table 12). The centromeric formula of this germplasm is 10 sm + 42 m (Table 12). A pair of satellites (0.30 µm) was found on both the members of chromosome pair XII (Figs. 79, 112, 145, arrow).

The total length of diploid complements in CB-2 was 78.05 µm (Table 13). The relative length of individual chromosome ranged from 0.01 to 0.03 whereas individual chromosomal length was ranging from 1.12 to 2.40 µm (Table 13). The centromeric formula of this germplasm is 2sm + 50m

(Figs. 80, 113, 146, Table 13). No satellite or secondary constriction was found in this germplasms after orcein-staining.

The total length of 2n chromosome complements of CB-3 was 69.82 μm (Table 14). The relative length of the individual chromosomes was ranging from 0.01 to 0.03 and individual chromosome length from 0.76 to 1.98 μm (Table 14). The centromeric formula of this germplasm was determined as 52m (Figs. 81, 114, 147, Table 14). After orcein-staining no satellite or secondary constriction was found in this germplasms.

In CB-4, total length of 2n chromosome complements was 81.22 μm. The range of relative length of chromosome and the range of individual chromosomal length were ranging from 0.01 to 0.03 and 0.77 to 2.62 μm, respectively (Table 15). The centromeric formula of this germplasm was 52m (Figs. 82, 115, 148, Table 15). No satellite or secondary constriction was found in this germplasms.

In CB-5, the total length of 2n chromosome complements was 79.32 μm (Table 16). The relative length of individual chromosome was ranging from 0.01 to 0.03 while range of individual chromosome length was 1.28 to 1.85 μm (Table 16). The centromeric formula of this germplasm was 6 sm + 46m (Figs. 83, 116, 149, Table 16). No satellite or secondary constriction was found in this germplasms.

The total length of diploid complements in CB-6 was 82.72 μm (Table 17). The relative length of individual chromosome ranged from 0.01 to 0.03 whereas individual chromosomal length ranging from 0.80 to 2.30 μm (Table 17). The centromeric formula of this germplasm is 52m (Figs. 84,

117, 150, Table 17). No satellite or secondary constriction was found in this germplasms after orcein-staining.

The total length of 2n chromosome complements of CB-7 was 61.82 µm (Table 18). The relative length of the individual chromosome was ranging from 0.01 to 0.03 and the range of individual chromosome length was 0.80 to 1.73 µm (Table 18). The centromeric formula of this germplasm was determined as 52m (Figs. 85, 118, 151, Table 18). After orcein-staining no satellite or secondary constriction was found in this germplasms.

In CB-8, total length of 2n chromosome complements was 66.05 µm. The relative length of chromosome and range of individual chromosomal length were 0.01 to 0.03 and 0.83 to 1.98 µm, respectively (Table 19). The centromeric formula of this germplasm was 52m (Figs. 86, 119, 152, Table 19). No satellite or secondary constriction was found in this germplasms.

In CB-9, the total length of 2n chromosome complements was 55.58 µm (Table 20). This length was the lowest among the eleven germplasms. The relative length of individual chromosome was ranging from 0.01 to 0.03 while range of individual chromosome length was 0.82 to 1.82 µm (Table 20). The centromeric formula of this germplasm was 52m (Figs. 87, 120, 153, Table 20). No satellite or secondary constriction was found.

The total length of diploid complements in CB-10 was 77.58 µm (Table 21). The relative length of individual chromosome ranged from 0.01 to 0.03 with individual chromosomal length from 0.90 to 2.37 µm (Table 21).

The centromeric formula of this germplasm is 52m (Figs. 88, 121, 154, Table 21). No satellite or secondary constriction was found.

The total length of 2n chromosome complements of CB-11 was 83.62 μm (Table 22). The relative length of the individual chromosomes was ranging from 0.01 to 0.03 and the range of individual chromosome length was 0.96 to 2.31 μm (Table 22). The centromeric formula of this germplasm was determined as 52m (Figs. 89, 122, 155, Table 22). After orcein-staining no satellite or secondary constriction was found in this germplasms.

3.7.2. CMA-staining

In CB-1, CMA-positive bright bands of different size and at different location were observed in 20 chromosomes (Figs. 90, 123, 156, Table 24). Four CMA-positive satellites were found on short arm in both the members of chromosome pair XII and XIX (Figs. 123, 156). The long arms of the members of pair I fluoresced while members of pair IV and V were entirely fluoresced with CMA fluorochrome. On the other hand, the upper arm of both members of pair VIII and IX were fluoresced with CMA. Centromeric CMA-positive bands were observed in both members of pair X and XI (Figs. 123, 156). Heteromorphicity in respect of banding pattern was found in pair VII where a member was entirely fluoresced with CMA while its homologue member had upper terminal CMA-positive bands. The total length of CMA banded region was 14.37 μm which occupied about 16.66 % of the total chromatin length. The CMA banded karyotype formula of this germplasm was determined as $4\alpha+4\beta+5\gamma+4\varphi+2\lambda+1\theta+32\delta$ (Table 24).

Nineteen chromosomes had CMA-positive bands in CB-2 (Figs. 91, 124, 157, Table 24). Among these bands, six CMA-positive satellites were found on upper arms of both the members of chromosome pair XII, XIX and XXVI (Figs. 124, 157). A member of pair I had CMA-positive banded region in whole long arm whereas no band was found in its homologue. Whole chromosomal portion of both members of pair IV, V and VI were fluoresced with CMA fluorochrome. Both members of pair VIII and IX were found to possess CMA-positive bands in short arm (Figs. 124, 157). Heteromorphicity in respect of banding pattern was found in pair VII where a member was entirely fluoresced with CMA and its homologue member had upper terminal CMA-positive band. The total length of CMA-banded region was 13.44 μm which covered about 17.22 % of total chromatin length. The CMA- banded karyotype formula of this germplasm was $6\beta+5\theta+7\gamma+1\lambda+33\delta$ (Table 24).

In CB-3, nine CMA-positive bright bands were observed of which six were at the satellited portions of both the members of chromosome pair XII, XIX and XXVI (Figs. 92, 125, 158, Table 24). Almost the short arms of both members of pair VIII fluoresced entirely with CMA (Figs. 92, 125). An upper terminal CMA-band was observed in a member of pair VII whereas no such band present in its homologue member (Figs. 92, 125). The total length of CMA banded region was 2.56 μm which occupied about 3.67 % of the total chromatin length. The CMA banded karyotype formula of this germplasm was $6\beta+2\varphi+1\theta+43\delta$ (Table 24).

Thirteen chromosomes of CB-4 showed CMA-positive bands (Figs. 93, 126, 159, Table 24). The two satellited portions of pair XII were CMA-positive (Figs. 93, 126). The long arm in a member of pair I fluoresced with CMA. Whole chromosomal portion of both members of pair IV, V and

VI were fluoresced with CMA. Both members of pair VIII were found to possess CMA-positive bands on upper terminal region. Centromeric CMA-positive bands were observed in both members of pair X (Figs. 93, 126). The total length of CMA-banded region was 10.88 μm which covered about 13.39 % of total chromatin length. The CMA- banded karyotype formula of this germplasm was $2\alpha+2\beta+6\gamma+2\varphi+1\lambda+39\delta$ (Table 24).

The germplasm CB-5 is totally different from the rest ten in respect of CMA-banding pattern since having only 2 dot like terminal bands on the short arm of both the members of chromosome pair II (Figs. 94, 127, 160, Table 24). No satellite was found after CMA-staining. The total length of CMA banded region was only 0.32 μm which occupied about 0.40 % of the total chromatin length. The CMA banded karyotype formula of this germplasm was $2\theta+50\delta$ (Table 24).

Five CMA-positive bands were found in CB-6 (Figs. 95, 128, 161, Tables 24). Two CMA-positive satellites were found on short arm of both the members of chromosome pair XII. In contrast, only one CMA-positive satellite was observed in a member of pair XIX. There was no such band in the other member of this pair (Figs. 95, 128). Both members of pair VIII were found to possess CMA-positive bands in terminal region of short arms (Figs. 95, 128). The total length of CMA-banded region was 0.80 μm and that covered 0.97% of total chromatin length. The CMA- banded karyotype formula of this germplasm was $3\beta+2\theta+47\delta$ (Table 24).

Eighteen CMA-positive bright bands were observed in CB-7 (Fig. 96, 129, 162, Table 24). A pair of CMA-positive satellites was found in pair XII (Figs. 96, 129). The long arm of both the members of pair I fluoresced with CMA. Both members of pair VIII, IX, X, XIX and XXVI were found to

possess CMA-positive bands on the terminal region of short arms (Figs. 96, 129). Heteromorphicity in respect of banding pattern was found in two different chromosome pairs. In pair VII, a member was entirely fluoresced with CMA and its homologue member had an upper terminal CMA-positive bands (Figs. 96, 129). On the other hand, a centromeric CMA-positive band was found in a member of pair XI whereas its homologue had a thick upper band (Figs. 96, 129). The total length of CMA banded region was 6.08 µm which occupied about 9.84 % of the total chromatin length. The CMA banded karyotype formula of this germplasm was $1\alpha+2\beta+12\theta+1\gamma+2\lambda+34\delta$ (Table 24).

Highest number of CMA-positive bands (22) were found in CB-8 (Figs. 97, 130, 163, Table 24). Six CMA-positive satellites were found on short arm of both the members of chromosome pair XII, XIX and XXVI (Figs. 97, 130). Both the members of pair I and X had CMA-positive banded region in whole long arm. The entire chromosomal portion of both members of pair IV, V, VI, VII and VIII were fluoresced with CMA fluorochrome (Figs. 97, 130). In pair IX, one member fluoresced entirely and the other one fluoresced along only the long arm (Figs. 97, 130). The total length of CMA-banded region was 20.96 µm that covered about 31.73% of total chromatin length. The CMA- banded karyotype formula of this germplasm was $6\beta+5\lambda+11\gamma+30\delta$ (Table 24).

In CB-9, 8 CMA-positive bright bands were observed at different location of eight chromosomes (Figs. 98, 131, 164, Table 24). Although no satellite was found, a pair of dot like upper terminal CMA-positive bands observed in each member of chromosome pair XII, XIX and a member of pair XXVI (Figs. 98, 131). These pairs showed distinct satellite in other germplasms (Figs. 98, 131, Table 24). Both the members of pair VIII were found to

possess a thick CMA-positive band that covered almost the short arm. A centromeric CMA-positive band was observed in only a member of pair X, no such band present on its homologue member (Figs. 98, 131). The total length of CMA banded region was 2.40 µm which occupied about 4.32 % of the total chromatin length. The CMA banded karyotype formula of this germplasm was $1\alpha+2\varphi+5\theta+44\delta$ (Table 24).

Eleven CMA-positive bands were found in CB-10 of which six at the satellited regions on short arm of both the members of chromosome pair XII, XIX and XXVI (Figs. 99, 132, 165, Table 24). The short arm of each member of pair VIII and IX was entirely fluoresced with CMA (Figs. 99, 132). Heteromorphicity in respect of banding pattern was found in pair VII where a member was fluoresced along the length with CMA and its homologue member had no CMA-positive band at all. The total length of CMA-banded region was 4.96 µm which covered about 6.40 % of total chromatin length. The CMA- banded karyotype formula of this germplasm was $6\beta+4\varphi+1\gamma+41\delta$ (Table 24).

In CB-11, five CMA-positive bright bands were observed (Figs. 100, 133, 166, Table 24). Satellites were not distinguishable after CMA-staining. However, upper terminal prominent bands were present in each member of pair XII and XIX. These chromosomes had CMA-positive satellites in other germplasms (Figs. 100, 133, Table 24). Heteromorphicity in respect of banding pattern was found in pair XI where a member had a centromeric CMA-positive bands but no such band present in its homologue member. The total length of CMA banded region was 2.24 µm which occupied about 2.68 % of the total chromatin length. The CMA banded karyotype formula of this germplasm was $4\theta+1\alpha+47\delta$ (Table 24).

3.7.3. DAPI-staining

Ten DAPI-positive bright bands were observed in CB-1 (Figs. 101, 134, 167, Table 25). Both the members of pair I and II were found to possess DAPI-positive regions along in short arms (Figs. 101, 134). Centromeric bands were present in both members of pair IX. Chromosomes of pair XI and XXIII were entirely fluoresced with DAPI fluorochrome (Figs. 101, 134). No satellite or secondary constriction was found after DAPI-staining. The total length of DAPI banded region was 6.65 µm which occupied about 7.60 % of the total chromatin length. The CMA banded karyotype formula of this germplasm was $2\alpha+4\gamma+4\varphi+42\delta$ (Table 24).

Twelve DAPI-positive bands were found in CB-2 (Figs. 102, 135, 168, Table 25). Both the members of pair I were found to possess DAPI-positive bands that covered whole short arms (Figs. 102, 135). Centromeric bands were present in both members of pair IX. Both the members of pair XI, XXIII and XXIV were entirely fluoresced with DAPI fluorochrome (Figs. 102, 135). Heteromorphicity in respect of banding pattern was found in pair VIII. In this case, a centromeric DAPI-positive band was observed in a member whereas an upper terminal band present in its homologue (Figs. 102, 135). No satellite or secondary constriction was found after DAPI-staining. The total length of DAPI-banded region was 6.08 µm which covered about 7.79 % of total chromatin length. The DAPI- banded karyotype formula of this germplasm was $1\theta+3\alpha+6\gamma+2\varphi+40\delta$ (Table 25).

In CB-3, six chromosomes showed DAPI-positive bright bands (Figs. 103, 136, 169, Table 25). The whole short arm of both the members of pair I fluoresced with DAPI (Figs. 103, 136). Members of pair XI and XXIII were

entirely fluoresced (Figs. 103, 136). No satellite or secondary constriction was found after DAPI-staining. The total length of DAPI banded region was 4.80 µm which occupied about 6.87 % of the total chromatin length. The DAPI banded karyotype formula of this germplasm was $2\varphi+4\gamma+46\delta$ (Table 25).

CB-4 is unique among the 11 germplasms studied because of having 16 entirely DAPI-fluoresced chromosomes (Figs. 104, 137, 170, Table 25). The other germplasms did not have such big number of entirely DAPI-fluoresced chromosomes. Both members of pair VIII showed different banding pattern. Here a centromeric band was found in a member and an upper terminal band present in another member (Figs. 104, 137, Table 25). No satellite or secondary constriction was found after DAPI-staining. The total length of DAPI-banded region (15.20 µm) and % of DAPI-positive banded region (18.71 %) of this germplasm were the highest among 11 germplasms. The DAPI- banded karyotype formula of this germplasm was $16\gamma+1\alpha+1\theta+34\delta$ (Table 25).

In CB-5, six DAPI-positive bright bands were observed (Figs. 105, 138, 171, Table 25). A centromeric band was present in both members of pair IX. The members of pair XI were entirely fluoresced with DAPI fluorochrome (Figs. 105, 138). Heteromorphicity in respect of banding pattern was found in pair VIII where a member had centromeric and its homologue member showed an upper terminal DAPI-positive band (Figs. 105, 138). No satellite or secondary constriction was found after DAPI-staining. The total length of DAPI banded region was 3.36 µm which occupied about 4.24 % of the total chromatin length. The DAPI banded karyotype formula of this germplasm was $3\alpha+1\theta+2\gamma+46\delta$ (Table 25).

Sixteen chromosomes showed DAPI-positive regions in different location in CB-6 (Figs. 106, 139, 172, Table 25). Both the members of pair XI, XII, XIII, XIV, XV, XVI and XXIII were entirely fluoresced with DAPI fluorochrome (Figs. 106, 139). Heteromorphicity in respect of banding pattern was found in pair VIII. In this pair, a member had centromeric DAPI-positive band whereas an upper terminal DAPI-positive band was found in its homologue member (Figs. 106, 139). No satellite or secondary constriction was found after DAPI-staining. The total length of DAPI-banded region was 11.84 μm which covered about 14.31 % of total chromatin length. The DAPI- banded karyotype formula of this germplasm was $1\alpha+1\theta+14\gamma+36\delta$ (Table 25).

In CB-7, six DAPI-positive bright bands were observed (Figs. 107, 140, 173, Table 25). Both the members of pair XI and XXIII were entirely fluoresced with DAPI fluorochrome (Figs. 107, 140). Heteromorphicity in respect of banding pattern was found in pair VIII where a centromeric DAPI-positive band was found in one member and an upper terminal band present on its another homologue member (Figs. 107, 140). No satellite or secondary constriction was found after DAPI-staining. The total length of DAPI banded region was 4.48 μm which occupied about 7.25 % of the total chromatin length. The DAPI banded karyotype formula of this germplasm was $1\alpha+4\gamma+1\varphi+46\delta$ (Table 25).

Ten DAPI-positive bands were found in CB-8 (Figs. 108, 141, 174, Table 25). The whole short arm of both the members of pair I were fluoresced with DAPI (Figs. 108, 141). Members of pair XI, XII and XXIII were entirely fluoresced (Figs. 108, 141). The two members of pair VIII showed DAPI-bands in different locations, one band present at the centromeric region of a chromosome whereas another band was on the upper terminal

region of other member. Satellite or secondary constriction could not be found after DAPI-staining. The total length of DAPI-banded region was 7.52 µm which covered about 11.38 % of total chromatin length. The DAPI- banded karyotype formula of this germplasm was $1\alpha+6\gamma+1\theta+2\varphi+42\delta$ (Table 25).

In CB-9, fourteen chromosomes showed DAPI-positive bright bands (Figs. 109, 142, 175, Table 25). The members of pair I were found to possess DAPI-positive regions in whole short arm (Figs. 109, 142). DAPI-positive bands in whole long arm were present in both members of pair II. The members of pair XI and XXIII were entirely fluoresced with DAPI (Figs. 109, 142). In pair VIII, an upper terminal and a centromeric band were present in different chromosomes. In contrast, one member of pair XII was fluoresced entirely whereas an upper terminal band present in its homologue (Figs. 109, 142). A pair of satellite was found in pair VIII and XII. The total length of DAPI banded region was 7.28 µm which occupied about 13.10 % of the total chromatin length. The DAPI banded karyotype formula of this germplasm was $1\alpha+2\theta+5\gamma+2\lambda+2\varphi+2\beta+38\delta$ (Table 25).

Thirteen DAPI-positive bands were found in CB-10 (Fig. 110, 143, 176, Table 25). Both the members of pair I were found to possess DAPI-positive fluoresced regions in whole short arms (Figs. 110, 143). A centromeric band was present in both members of pair VIII. The members of pair XI, XII, XIII and XIV were entirely fluoresced with DAPI (Figs. 110, 143). On the other hand, a member of pair XV fluoresced entirely but no DAPI-band was found in its another member (Figs. 110, 143). No satellite or secondary constriction was found after DAPI-staining. The total length of DAPI-banded region was 9.76 µm which covered about 12.58% of total

chromatin length. The DAPI- banded karyotype formula of this germplasm was $2\alpha+9\gamma+2\varphi+39\delta$ (Table 25).

Twelve DAPI-positive bright bands were observed in CB-11 (Figs. 111, 144, 177, Table 25). Short arms of pair I and II were entirely fluoresced. A pair of dot like terminal DAPI-bands was present in each member of pair III and IV. Both the members of pair XI were entirely fluoresced with DAPI (Figs. 111, 144). A member of pair XIII showed a band in both the terminal regions. This chromosome is unique since this feature (two terminal bands in a chromosome) absent in other germplasms (Figs. 111, 144). A pair of satellites was found one in each member of chromosome pair XII (Figs. 111, 144). The total length of DAPI banded region was 5.44 µm which occupied about 6.51 % of the total chromatin length. The DAPI banded karyotype formula of this germplasm was $4\theta+2\gamma+2\beta+4\varphi+1\phi+39\delta$ (Table 25).

3.8. RAPD analysis

Ten oligonucleotide primers viz. i) OPA-1 (5′-CAG GCC CTT C-3′), ii) OPA-3 (5′-AGT CAG CCA C-3′), iii) OPA-4 (5′-AAT CGG GCT G-3′), iv) OPA-6 (5′-GGT CCC TGA C-3′), v) OPA-7 (5′-GAA ACG GGT G-3′), vi) OPA-10 (5′-GTG ATC GCA G-3′), vii) Primer-1 (5′-GAA ACG GGT G-3′), viii) Primer-2 (5′-GTT GCG ATC C-3′), ix) Primer-18 (5′-GTT TCG CTC C-3′) and x) Primer-23 (5′-GTC AGG GCA A-3′) were used for RAPD analysis of 11 cotton germplasms. Each primer showed different banding patterns. The RAPD analysis of 11 germplasms of cotton was described below:

3.8.1. OPA-1 (5′-CAG GCC CTT C-3′)

Similar RAPD-banding pattern (5 bands – 1700, 850, 600, 350 and 250 bp) were found in CB-1, CB-2, CB-3 and CB-5 with primer OPA-1 of which 850 bp was brighter and thicker (Fig. 179). Four bands were found in CB-4 where 2000 bp was unique since absent in other germplasms. Among the six bands of CB-9, three were unique (1400, 400 and 300 bp). CB-11 was found to possess only one bright band (650 bp). No band was found in CB-6, CB-7, CB-8 and CB-10 (Fig. 179, Table 26).

3.8.2. OPA-3 (5′-AGT CAG CCA C-3′)

With this primer, CB-1 and CB-2 shared similar DNA fragments (7000, 2700, 1400, 900 and 800 bp) of which 2 bands (1400 and 800 bp) were brighter. Including a unique band (1600 bp), CB-3 had three light bands (7000 and 2700 bp). The germplasms CB-4, CB-5, CB-6, CB-7 and CB-8 were found to possess two bands (1400 and 800 bp) in each. Similar banding pattern (4 bands – 7000, 2700, 1400, 800 bp) was found in CB-9 and CB-10. Six bands were present in CB-11 in which 3 were unique (1500, 750, 600 bp). Dispite of different banding patterns, fragment size 1400 and 800 bp were common in all germplasms except CB-3 and CB-11 (Fig. 180, Table 27).

3.8.3. OPA-4 (5′-AAT CGG GCT G-3′)

A number of different sized bands were found in each germplasms except CB-8 where no band was observed. The germplasms CB-1, CB-2, CB-3, CB-4, CB-5 and CB-6 showed almost similar banding pattern with a little difference, such as 500 and 350 bp bands were absent in only CB-1. A band of 650 bp was found extra in CB-5 and CB-6. On the other hand,

only two bands (650 and 300 bp) were found in CB-7. In CB-9 and CB-11, six bands were common (1450, 1000, 850, 650, 350 and 300 bp). Moreover, CB-11 had three unique bands (4000, 3000 and 1700 bp) which totally absent in other 10 germplasms. In CB-10, the 4 bands were common with those of CB-9 (Fig. 181, Table 28).

3.8.4. OPA-6 (5′-GGT CCC TGA C-3′)

With primer OPA-6, no band was found in CB-1, CB-2, CB-3, CB-4, CB-6 and CB-7. Only one bright band (750 bp) was found in CB-5 and CB-8. The germplasm CB-9 was found to possess 3 bands (2000, 1000 and 650 bp) of which 2000 bp was unique. Two bands (750 and 500 bp) were found in CB-10 and four (1300, 1000, 750 and 500 bp) present in CB-11 in which 1 band was unique (1300 bp) (Fig. 182, Table 29).

3.8.5. OPA-7 (5′-GAA ACG GGT G-3′)

The germplasms CB-1, CB-3, CB-4, CB-7, CB-8, CB-9 and CB-11 did not show any band with this primer. Only one band (750 bp) was found in CB-2 and CB-10. This band in CB-2 was thicker and bigger than CB-10. The germplasm CB-5 and CB-6 showed 3 similar bands (1700, 1300 and 750 bp). Besides these bands, a unique band (500 bp) was present in CB-6 (Fig. 183, Table 30).

3.8.6. OPA-10 (5′-GTG ATC GCA G-3′)

No band was found with this primer in CB-11. The germplasms CB-1, CB-2, CB-3, CB-4, CB-8 and CB-9 showed similar banding pattern (5 bands – 2400, 1500, 1000, 650 and 500 bp). On the other hand, four light bands were present in CB-5 and CB-7 (2400, 1500, 1000 and 650 bp). The

germplasm CB-6 was found to possess 3 bright bands (1000, 650 and 500 bp). Three bands of different size (1400, 650 and 500 bp) were found in CB-10 of which fragment size 1400 bp was unique.

Besides the above bands, few common bands were found in these germplasms such as: i) a band of 650 bp was common in each germplasms, ii) except CB-5 and CB-7, each germplasms had a band of 500 bp. Two bands (2400 and 1500 bp) were common in all germplasms except CB-6 and CB-10 and iii) the band size 1000 was absent in only CB-10, however, this band in CB-6, CB-8 and CB-9 were germplasm specific since it was very thick, bright and big (Fig. 184, Table 31).

3.8.7. Primer-1 (5′-GAA ACG GGT G-3′)

A very bright and thick band (850 bp) was present in all germplasms except CB-11. A fragment of 2100 and 1800 bp were common in CB-1, CB-2, CB-3, CB-5, CB-6 and CB-11. In addition, a band of 1400 bp was present in CB-1, CB-2, CB-3, CB-5, CB-6 and CB-10. A unique band (1600 bp) was found in CB-1 whereas two such bands (2750, 1000 bp) observed in CB-11 (Fig. 185, Table 32).

3.8.8. Primer-2 (5′-GTT GCG ATC C-3′)

In CB-5, CB-6 and CB-7, no band was found. The germplasms CB-1 and CB-10 showed almost similar banding patterns except a band of 2500 bp in CB-10. This band was absent in other germplasms, thus considered as unique band (Fig. 186). Three bands (600, 350 and 250 bp) were found in CB-2, CB-3 and CB-4. Only one band of 300 bp was found in CB-8. The germplasm CB-9 had two bands (450 and 300 bp) of which 450 bp was unique since absent in other germplasms. Only a band of 750 bp was

found in CB-1, CB-10 and CB-11 of which the band in CB-11 was thicker and bigger than the other two germplasms and thus could easily be identified (Fig. 186, Table 33).

3.8.9. Primer-18 (5'-GTT TCG CTC C-3')

All germplasms showed different RAPD banding pattern. The highest numbers of bands (7 bands) were found in CB-10 and CB-11. Band size 1750 bp and 1600 bp were unique for CB-10 and CB-11, respectively. These two bands were very thick, big and bright, could easily be differentiated from other bands. These two germplasms had two bands of 4000 bp and 3000 bp which were absent in other germplasms. On the other hand, six bands were present in CB-9, five in CB-4 and CB-5, four in CB-3 and three in the rest germplasms. A fragment of 600 and 500 bp were unique for CB-9. Fragment size of 3500, 2000 and 650 bp were common in CB-1, CB-2, CB-3, CB-4, CB-5, CB-6, CB-7, CB-8 and CB-10 (Fig. 187, Table 34). In addition, a band of 1500 bp was present in only CB-4, CB-5, CB-8 and CB-9.

3.8.10. Primer-23 (5'-GTC AGG GCA A-3')

No band was found in CB-2. The germplasm CB-1 had maximum of six bands (2000, 1750, 1400, 1000, 800 and 650 bp) of which four bands (2000, 1000, 800 and 650 bp) were unique since absent in the rest germplasms (Fig. 188). Three bands of 1850, 1400 and 1250 bp were common in CB-3, CB-4, CB-5, CB-6, CB-7, CB-8, CB-9, CB-10 and CB-11. However, a fragment size of 1850 and 1400 bp were absent in CB-7 and CB-11, respectively. In addition to the above bands, CB-5, CB-6, CB-8,

CB-9 and CB-10 had one more band of 850 bp. Moreover, CB-9 had one unique band of 600 bp (Fig. 188, Table 35).

3.9. SSR analysis

Five primer pairs *viz*. i) forward-BA00175679 - reverse-BA00175680, ii) forward-BA00175681 - reverse-BA00175682, iii) forward-BA00175683 and reverse-BA00175684, iv) forward-BA00175685 - reverse-BA00175686 and iv) forward-BA00175687 - reverse-BA00175688 were used for SSR analysis of 11 cotton germplasms. Each primer pair showed different banding patterns. The pair-wise SSR analysis of 11 germplasms of cotton was described below:

3.9.1. Primer pair forward-5'-GCTTCTTCCATTTTATTCAAG-3'- reverse-5'-CAGCGGCAACCAAAAAG-3'

Only one bright unique band (180 bp) was found in CB-7 with this primer sequence. Rest 10 germplasms did not show any band with this sequence (Fig. 189, Table 36).

3.9.2. Primer pair forward-5'-ACCCTTAAATCATAAGAGAAC-3'- reverse-5'-CCGTAAGTTAAGGTACAAGG-3'

All germplasms were found to possess one common band of 50 bp. A band of 123 bp was found in CB-3 and CB-9. In addition to the above bands, a unique band (738 bp) was found in CB-9 (Fig. 190, Table 37).

3.9.3. Primer pair forward-5′-GGAGTTAAAGCTAATGCCTG-3′-reverse-5′-CGGGTCATTGGTTGTTTTTG-3′

No band was found in CB-4. A fragment of 50 bp was common in all germplasms except CB-4. The germplasms CB-1, CB-2, CB-5, CB-6, CB-8, CB-9 and CB-10 shared a fragment of 2000 bp. In addition, a band of 123 bp was present only in CB-5 and CB-10. A unique band of 1968 bp was found in CB-9 (Fig. 191, Table 38).

3.9.4. Primer pair forward-5′-GCCTAGGTGGAGTTCGTG-3′-reverse-5′-CTGAACCTGCTCCTGAATC-3′

No band was found in CB-2, CB-7, CB-9 and CB-11. The rest germplasms shared a fragment of 123 bp (Fig. 192, Table 39).

3.9.5. Primer pair forward-5′-CATCGGAAAACTCTGAAC-3′-reverse-5′-GTAGCAGTACAGATGAAAGAG-3′

The germplasm CB-1 had 3 bands (246, 123 and 50 bp) of which 123 bp was unique since absent in other germplasms. The germplasms CB-5, CB-6, CB-7, CB-8 and CB-9 shared 3 common bands (246, 180 and 50 bp). In addition, a unique band of 492 bp was present only in CB-5. All the germplasms have a common band of 50 bp. (Fig. 193, Table 40).

3.10. Genetic distances

The values of pair-wise Nei's (1972) genetic distances analyzed by using computer software "popgene32" among 11 germplasms of cotton were computed from combined data for the fifteen primers (Ten RAPD primers and five SSR primer pairs), ranging from 0.0991 to 0.8348 (Table 43).

The highest genetic distance (0.8348) was found between CB-11 and two germplasms i.e. CB-5 and CB-9. The lowest (0.0991) genetic distance was observed between CB-7 and CB-8.

3. 11. Cluster analysis (Tree Diagram)

A combined cluster analysis on the basis of DNA fingerprinting by RAPD and SSR was carried out. Dendrogram based on Nei's (1972) genetic distance using Unweighted Pair Group Method of Arithmetic Means (UPGMA) segregates 11 germplasms of cotton into four major clusters *viz.* cluster-1 (C_1), cluster-2 (C_2), cluster-3 (C_3)and cluster-4 (C_4). The germplasms CB-1, CB-2, CB-3, CB-4, CB-5, CB-6, CB-7 and CB-8 formed cluster-1 (C_1). Cluster C_1 was further divided into 6 sub-clusters SC_1, SC_2, SC_3, SC_4, SC_5 and SC_6. The germplasms CB-9, CB-10 and CB-11 formed C_2, C_3 and C_4, respectively. Therefore, CB-9, CB-10 and CB-11 were separated from rest of the germplasms (Fig. 194).

Table 11. Types of interphase nuclei and prophase chromosomes of eleven germplasms of *Gossypium hirsutum* L. after staining with orcein

Cotton germplasms	Orcein-stained interphase nuclei	Orcein-stained prophase chromosomes
CB-1	Simple chromocenter type	Continuous type
CB-2	Complex chromocenter type	Gradient type
CB-3	Simple chromocenter type	Gradient type
CB-4	Complex chromocenter type	Gradient type
CB-5	Simple chromocenter type	Gradient type
CB-6	Simple chromocenter type	Continuous type
CB-7	Complex chromocenter type	Continuous type
CB-8	Simple chromocenter type	Continuous type
CB-9	Simple chromocenter type	Gradient type
CB-10	Simple chromocenter type	Continuous type
CB-11	Simple chromocenter type	Continuous type

Table 12. Length, arm ratio, centromeric index, relative length and centromeric type of mitotic metaphase chromosomes of CB-1 germplasm of *Gossypium hirsutum* L.

Chromosome pair	Long arm (l) µm	Short arm (s) µm	Total length (T) µm	Arm ratio (l/s)	Relative length (RL)	Centromeric index (CI)	Centromeric type (CT)
I	1.60	0.96	2.56	1.67	0.03	0.38	sm
	1.50	0.83	2.34	1.81	0.03	0.36	sm
II	1.38	0.96	2.34	1.43	0.03	0.41	m
	1.38	0.96	2.34	1.43	0.03	0.41	m
III	1.50	0.83	2.34	1.81	0.03	0.36	sm
	1.44	0.86	2.30	1.67	0.03	0.38	sm
IV	1.34	0.86	2.21	1.56	0.03	0.39	sm
	1.34	0.86	2.21	1.56	0.03	0.39	sm
V	1.12	0.96	2.08	1.17	0.02	0.46	m
	1.12	0.96	2.08	1.17	0.02	0.46	m
VI	1.12	0.80	1.92	1.40	0.02	0.42	m
	1.12	0.80	1.92	1.40	0.02	0.42	m
VII	1.12	0.70	1.82	1.59	0.02	0.39	sm
	1.12	0.70	1.82	1.59	0.02	0.39	sm
VIII	0.86	0.86	1.73	1.00	0.02	0.50	m
	0.86	0.86	1.73	1.00	0.02	0.50	m
IX	1.02	0.64	1.66	1.60	0.02	0.38	sm
	1.02	0.64	1.66	1.60	0.02	0.38	sm
X	0.90	0.70	1.60	1.27	0.02	0.44	m
	0.90	0.70	1.60	1.27	0.02	0.44	m
XI	0.80	0.80	1.60	1.00	0.02	0.50	m
	0.80	0.80	1.60	1.00	0.02	0.50	m
XII	0.80	0.80	1.60	1.00	0.02	0.50	m
	0.80	0.80	1.60	1.00	0.02	0.50	m
XIII	0.80	0.80	1.60	1.00	0.02	0.50	m
	0.80	0.80	1.60	1.00	0.02	0.50	m

Table Contd.

Table Contd.

Chromosome pair	Long arm (l) μm	Short arm (s) μm	Total length (T) μm	Arm ratio (l/s)	Relative length (RL)	Centromeric index (CI)	Centromeric type (CT)
XIV	0.80	0.80	1.60	1.00	0.02	0.50	m
	0.80	0.80	1.60	1.00	0.02	0.50	m
XV	0.80	0.80	1.60	1.00	0.02	0.50	m
	0.80	0.80	1.60	1.00	0.02	0.50	m
XVI	0.86	0.74	1.60	1.17	0.02	0.46	m
	0.86	0.74	1.60	1.17	0.02	0.46	m
XVII	0.96	0.64	1.60	1.50	0.02	0.40	m
	0.86	0.74	1.60	1.17	0.02	0.46	m
XVIII	0.86	0.70	1.57	1.23	0.02	0.45	m
	0.86	0.70	1.57	1.23	0.02	0.45	m
XIX	0.70	0.70	1.41	1.00	0.02	0.50	m
	0.70	0.70	1.41	1.00	0.02	0.50	m
XX	0.70	0.70	1.41	1.00	0.02	0.50	m
	0.70	0.70	1.41	1.00	0.02	0.50	m
XXI	0.70	0.70	1.41	1.00	0.02	0.50	m
	0.70	0.70	1.41	1.00	0.02	0.50	m
XXII	0.64	0.64	1.28	1.00	0.01	0.50	m
	0.64	0.64	1.28	1.00	0.01	0.50	m
XXIII	0.64	0.64	1.28	1.00	0.01	0.50	m
	0.64	0.64	1.28	1.00	0.01	0.50	m
XXIV	0.64	0.64	1.28	1.00	0.01	0.50	m
	0.64	0.64	1.28	1.00	0.01	0.50	m
XXV	0.58	0.58	1.15	1.00	0.01	0.50	m
	0.58	0.58	1.15	1.00	0.01	0.50	m
XXVI	0.51	0.51	1.02	1.00	0.01	0.50	m
	0.51	0.51	1.02	1.00	0.01	0.50	m
		GT=	**86.27**				

m = metacentricc, sm = sub-metacentric chromosome.

Table 13. Length, arm ratio, centromeric index, relative length and centromeric type of mitotic metaphase chromosomes of CB-2 germplasm of *Gossypium hirsutum* L.

Chromosome pair	Long arm (l) µm	Short arm (s) µm	Total length (T) µm	Arm ratio (l/s)	Relative length (RL)	Centromeric index (CI)	Centromeric type (CT)
I	1.44	0.96	2.40	1.50	0.02	40.00	sm
	1.44	0.64	2.08	2.25	0.03	30.77	sm
II	1.12	0.80	1.92	1.40	0.02	41.67	m
	1.12	0.80	1.92	1.40	0.02	41.67	m
III	1.02	0.90	1.92	1.14	0.01	46.67	m
	1.02	0.90	1.92	1.14	0.01	46.67	m
IV	0.96	0.80	1.76	1.20	0.02	45.45	m
	0.96	0.80	1.76	1.20	0.02	45.45	m
V	0.96	0.77	1.73	1.25	0.02	44.44	m
	0.96	0.70	1.66	1.36	0.02	42.31	m
VI	0.96	0.70	1.66	1.36	0.02	42.31	m
	0.90	0.77	1.66	1.17	0.01	46.15	m
VII	0.90	0.70	1.60	1.27	0.02	44.00	m
	0.90	0.70	1.60	1.27	0.02	44.00	m
VIII	0.80	0.80	1.60	1.00	0.01	50.00	m
	0.80	0.80	1.60	1.00	0.01	50.00	m
IX	0.80	0.80	1.60	1.00	0.01	50.00	m
	0.80	0.80	1.60	1.00	0.01	50.00	m
X	0.80	0.80	1.60	1.00	0.01	50.00	m
	0.80	0.80	1.60	1.00	0.01	50.00	m
XI	0.83	0.77	1.60	1.08	0.01	48.00	m
	0.83	0.77	1.60	1.08	0.01	48.00	m
XII	0.83	0.77	1.60	1.08	0.01	48.00	m
	0.83	0.77	1.60	1.08	0.01	48.00	m
XIII	0.80	0.77	1.57	1.04	0.01	48.98	m
	0.80	0.70	1.50	1.14	0.01	46.81	m

Table Contd.

Table Contd.

Chromosome pair	Long arm (l) µm	Short arm (s) µm	Total length (T) µm	Arm ratio (l/s)	Relative length (RL)	Centromeric index (CI)	Centromeric type (CT)
XIV	0.80	0.70	1.50	1.14	0.01	46.81	m
	0.83	0.64	1.47	1.30	0.02	43.48	m
XV	0.83	0.64	1.47	1.30	0.02	43.48	m
	0.77	0.67	1.44	1.14	0.01	46.67	m
XVI	0.74	0.70	1.44	1.05	0.01	48.89	m
	0.70	0.70	1.41	1.00	0.01	50.00	m
XVII	0.70	0.70	1.41	1.00	0.01	50.00	m
	0.70	0.64	1.34	1.10	0.01	47.62	m
XVIII	0.70	0.64	1.34	1.10	0.01	47.62	m
	0.64	0.64	1.28	1.00	0.01	50.00	m
XIX	0.64	0.64	1.28	1.00	0.01	50.00	m
	0.64	0.64	1.28	1.00	0.01	50.00	m
XX	0.64	0.64	1.28	1.00	0.01	50.00	m
	0.64	0.64	1.28	1.00	0.01	50.00	m
XXI	0.64	0.64	1.28	1.00	0.01	50.00	m
	0.64	0.64	1.28	1.00	0.01	50.00	m
XXII	0.64	0.64	1.28	1.00	0.01	50.00	m
	0.64	0.64	1.28	1.00	0.01	50.00	m
XXIII	0.64	0.58	1.22	1.11	0.01	47.37	m
	0.64	0.58	1.22	1.11	0.01	47.37	m
XXIV	0.58	0.58	1.15	1.00	0.01	50.00	m
	0.58	0.58	1.15	1.00	0.01	50.00	m
XXV	0.58	0.58	1.15	1.00	0.01	50.00	m
	0.58	0.58	1.15	1.00	0.01	50.00	m
XXVI	0.64	0.48	1.12	1.33	0.02	42.86	m
	0.64	0.48	1.12	1.33	0.02	42.86	m
		GT=	**78.05**				

m = metacentricc, sm = sub-metacentric chromosome.

Table 14. Length, arm ratio, centromeric index, relative length and centromeric type of mitotic metaphase chromosomes of CB-3 germplasm of *Gossypium hirsutum* L.

Chromosome pair	Long arm (l) µm	Short arm (s) µm	Total length (T) µm	Arm ratio (l/s)	Relative length (RL)	Centromeric index (CI)	Centromeric type (CT)
I	1.02	0.96	1.98	1.07	0.03	48.39	m
	1.02	0.89	1.92	1.14	0.03	46.67	m
II	1.02	0.76	1.79	1.33	0.03	42.86	m
	0.96	0.83	1.79	1.15	0.03	46.43	m
III	0.96	0.83	1.79	1.15	0.03	46.43	m
	0.89	0.83	1.72	1.08	0.02	48.15	m
IV	0.89	0.76	1.66	1.17	0.02	46.15	m
	0.89	0.76	1.66	1.17	0.02	46.15	m
V	0.89	0.76	1.66	1.17	0.02	46.15	m
	0.89	0.70	1.60	1.27	0.02	44.00	m
VI	0.83	0.76	1.60	1.08	0.02	48.00	m
	0.96	0.64	1.60	1.50	0.02	40.00	m
VII	0.96	0.64	1.60	1.50	0.02	40.00	m
	0.76	0.76	1.53	1.00	0.02	50.00	m
VIII	0.76	0.76	1.53	1.00	0.02	50.00	m
	0.83	0.70	1.53	1.18	0.02	45.83	m
IX	0.76	0.70	1.47	1.09	0.02	47.83	m
	0.76	0.70	1.47	1.09	0.02	47.83	m
X	0.83	0.64	1.47	1.30	0.02	43.48	m
	0.76	0.70	1.47	1.09	0.02	47.83	m
XI	0.70	0.70	1.40	1.00	0.02	50.00	m
	0.83	0.57	1.40	1.44	0.02	40.91	m
XII	0.70	0.70	1.40	1.00	0.02	50.00	m
	0.76	0.57	1.34	1.33	0.02	42.86	m
XIII	0.70	0.57	1.28	1.22	0.02	45.00	m
	0.70	0.57	1.28	1.22	0.02	45.00	m

Table Contd.

Table Contd.

Chromosome pair	Long arm (l) µm	Short arm (s) µm	Total length (T) µm	Arm ratio (l/s)	Relative length (RL)	Centromeric index (CI)	Centromeric type (CT)
XIV	0.64	0.64	1.28	1.00	0.02	50.00	m
	0.64	0.57	1.21	1.11	0.02	47.37	m
XV	0.64	0.57	1.21	1.11	0.02	47.37	m
	0.64	0.57	1.21	1.11	0.02	47.37	m
XVI	0.57	0.57	1.15	1.00	0.02	50.00	m
	0.57	0.57	1.15	1.00	0.02	50.00	m
XVII	0.57	0.57	1.15	1.00	0.02	50.00	m
	0.57	0.57	1.15	1.00	0.02	50.00	m
XVIII	0.57	0.57	1.15	1.00	0.02	50.00	m
	0.57	0.57	1.15	1.00	0.02	50.00	m
XIX	0.57	0.57	1.15	1.00	0.02	50.00	m
	0.57	0.57	1.15	1.00	0.02	50.00	m
XX	0.57	0.57	1.15	1.00	0.02	50.00	m
	0.57	0.57	1.15	1.00	0.02	50.00	m
XXI	0.57	0.57	1.15	1.00	0.02	50.00	m
	0.57	0.51	1.08	1.13	0.02	47.06	m
XXII	0.57	0.51	1.08	1.13	0.02	47.06	m
	0.57	0.51	1.08	1.13	0.02	47.06	m
XXIII	0.51	0.51	1.02	1.00	0.01	50.00	m
	0.51	0.51	1.02	1.00	0.01	50.00	m
XXIV	0.51	0.51	1.02	1.00	0.01	50.00	m
	0.51	0.51	1.02	1.00	0.01	50.00	m
XXV	0.51	0.51	1.02	1.00	0.01	50.00	m
	0.51	0.51	1.02	1.00	0.01	50.00	m
XXVI	0.51	0.51	1.02	1.00	0.01	50.00	m
	0.38	0.38	0.76	1.00	0.01	50.00	m
			69.82				

m = metacentricc, sm = sub-metacentric chromosome.

Table 15. Length, arm ratio, centromeric index, relative length and centromeric type of mitotic metaphase chromosomes of CB-4 germplasm of *Gossypium hirsutum* L.

Chromosome pair	Long arm (l) µm	Short arm (s) µm	Total length (T) µm	Arm ratio (l/s)	Relative length (RL)	Centromeric index (CI)	Centromeric type (CT)
I	1.34	1.28	2.62	1.05	0.03	48.78	m
	1.54	1.02	2.56	1.50	0.03	40.00	m
II	1.54	1.02	2.56	1.50	0.03	40.00	m
	1.15	1.15	2.30	1.00	0.03	50.00	m
III	1.09	1.09	2.18	1.00	0.03	50.00	m
	1.22	0.90	2.11	1.36	0.03	42.42	m
IV	1.09	0.96	2.05	1.13	0.03	46.88	m
	1.09	0.96	2.05	1.13	0.03	46.88	m
V	1.02	1.02	2.05	1.00	0.03	50.00	m
	1.09	0.90	1.98	1.21	0.02	45.16	m
VI	1.02	0.96	1.98	1.07	0.02	48.39	m
	0.96	0.96	1.92	1.00	0.02	50.00	m
VII	1.02	0.90	1.92	1.14	0.02	46.67	m
	0.96	0.96	1.92	1.00	0.02	50.00	m
VIII	0.96	0.90	1.86	1.07	0.02	48.28	m
	0.96	0.90	1.86	1.07	0.02	48.28	m
IX	0.90	0.83	1.73	1.08	0.02	48.15	m
	0.90	0.83	1.73	1.08	0.02	48.15	m
X	0.90	0.83	1.73	1.08	0.02	48.15	m
	0.90	0.83	1.73	1.08	0.02	48.15	m
XI	0.83	0.83	1.66	1.00	0.02	50.00	m
	0.96	0.64	1.60	1.50	0.02	40.00	m
XII	0.83	0.77	1.60	1.08	0.02	48.00	m
	0.90	0.64	1.54	1.40	0.02	41.67	m
XIII	0.77	0.77	1.54	1.00	0.02	50.00	m
	0.77	0.77	1.54	1.00	0.02	50.00	m

Table Contd.

Table Contd.

Chromosome pair	Long arm (l) μm	Short arm (s) μm	Total length (T) μm	Arm ratio (l/s)	Relative length (RL)	Centromeric index (CI)	Centromeric type (CT)
XIV	0.77	0.70	1.47	1.09	0.02	47.83	m
	0.70	0.70	1.41	1.00	0.02	50.00	m
XV	0.70	0.70	1.41	1.00	0.02	50.00	m
	0.70	0.70	1.41	1.00	0.02	50.00	m
XVI	0.70	0.64	1.34	1.10	0.02	47.62	m
	0.70	0.58	1.28	1.22	0.02	45.00	m
XVII	0.70	0.58	1.28	1.22	0.02	45.00	m
	0.64	0.64	1.28	1.00	0.02	50.00	m
XVIII	0.64	0.64	1.28	1.00	0.02	50.00	m
	0.64	0.64	1.28	1.00	0.02	50.00	m
XIX	0.64	0.58	1.22	1.11	0.01	47.37	m
	0.64	0.58	1.22	1.11	0.01	47.37	m
XX	0.64	0.58	1.22	1.11	0.01	47.37	m
	0.64	0.58	1.22	1.11	0.01	47.37	m
XXI	0.58	0.58	1.15	1.00	0.01	50.00	m
	0.58	0.58	1.15	1.00	0.01	50.00	m
XXII	0.58	0.58	1.15	1.00	0.01	50.00	m
	0.58	0.58	1.15	1.00	0.01	50.00	m
XXIII	0.58	0.58	1.15	1.00	0.01	50.00	m
	0.58	0.58	1.15	1.00	0.01	50.00	m
XXIV	0.58	0.51	1.09	1.13	0.01	47.06	m
	0.51	0.51	1.02	1.00	0.01	50.00	m
XXV	0.51	0.51	1.02	1.00	0.01	50.00	m
	0.51	0.45	0.96	1.14	0.01	46.67	m
XXVI	0.45	0.38	0.83	1.17	0.01	46.15	m
	0.38	0.38	0.77	1.00	0.01	50.00	m
		GT=	**81.22**				

m = metacentricc, sm = sub-metacentric chromosome.

Table 16. Length, arm ratio, centromeric index, relative length and centromeric type of mitotic metaphase chromosomes of CB-5 germplasm of *Gossypium hirsutum* L.

Chromosome pair	Long arm (l) µm	Short arm (s) µm	Total length (T) µm	Arm ratio (l/s)	Relative length (RL)	Centromeric index (CI)	Centromeric type (CT)
I	1.02	0.83	1.85	1.23	0.02	44.83	m
	1.02	0.76	1.79	1.33	0.02	42.86	m
II	0.96	0.83	1.79	1.15	0.02	46.43	m
	0.89	0.83	1.72	1.08	0.02	48.15	m
	0.96	0.70	1.66	1.36	0.02	42.31	m
III	0.96	0.70	1.66	1.36	0.02	42.31	m
	0.96	0.70	1.66	1.36	0.02	42.31	m
IV	0.89	0.76	1.66	1.17	0.02	46.15	m
	0.89	0.76	1.66	1.17	0.02	46.15	m
V	0.96	0.70	1.66	1.36	0.02	42.31	m
VI	0.89	0.70	1.60	1.27	0.02	44.00	m
	0.89	0.70	1.60	1.27	0.02	44.00	m
VII	0.89	0.70	1.60	1.27	0.02	44.00	m
	0.83	0.76	1.60	1.08	0.02	48.00	m
VIII	0.83	0.76	1.60	1.08	0.02	48.00	m
	0.83	0.76	1.60	1.08	0.02	48.00	m
IX	0.89	0.70	1.60	1.27	0.02	44.00	m
	0.83	0.73	1.56	1.13	0.02	46.94	m
X	0.83	0.73	1.56	1.13	0.02	46.94	m
	0.89	0.64	1.53	1.40	0.02	41.67	m
XI	0.89	0.64	1.53	1.40	0.02	41.67	m
	0.83	0.70	1.53	1.18	0.02	45.83	m
XII	0.80	0.73	1.53	1.09	0.02	47.92	m
	0.80	0.70	1.50	1.14	0.02	46.81	m
XIII	0.80	0.70	1.50	1.14	0.02	46.81	m
	0.83	0.67	1.50	1.24	0.02	44.68	m

Table Contd.

Table Contd.

Chromosome pair	Long arm (l) µm	Short arm (s) µm	Total length (T) µm	Arm ratio (l/s)	Relative length (RL)	Centromeric index (CI)	Centromeric type (CT)
XIV	0.89	0.57	1.47	1.56	0.02	39.13	sm
	0.89	0.57	1.47	1.56	0.02	39.13	sm
XV	0.89	0.57	1.47	1.56	0.02	39.13	sm
	0.89	0.57	1.47	1.56	0.02	39.13	sm
XVI	0.83	0.64	1.47	1.30	0.02	43.48	m
	0.83	0.64	1.47	1.30	0.02	43.48	m
XVII	0.76	0.70	1.47	1.09	0.02	47.83	m
	0.83	0.64	1.47	1.30	0.02	43.48	m
XVIII	0.83	0.64	1.47	1.30	0.02	43.48	m
	0.83	0.64	1.47	1.30	0.02	43.48	m
XIX	0.89	0.57	1.47	1.56	0.02	39.13	sm
	0.89	0.57	1.47	1.56	0.02	39.13	sm
XX	0.83	0.64	1.47	1.30	0.02	43.48	m
	0.76	0.70	1.47	1.09	0.02	47.83	m
XXI	0.83	0.64	1.47	1.30	0.02	43.48	m
	0.76	0.64	1.40	1.20	0.02	45.45	m
XXII	0.83	0.57	1.40	1.44	0.02	40.91	m
	0.83	0.57	1.40	1.44	0.02	40.91	m
XXIII	0.83	0.57	1.40	1.44	0.02	40.91	m
	0.83	0.57	1.40	1.44	0.02	40.91	m
XXIV	0.76	0.64	1.40	1.20	0.02	45.45	m
	0.83	0.57	1.40	1.44	0.02	40.91	m
XXV	0.70	0.64	1.34	1.10	0.02	47.62	m
	0.76	0.57	1.34	1.33	0.02	42.86	m
XXVI	0.70	0.57	1.28	1.22	0.02	45.00	m
	0.70	0.57	1.28	1.22	0.02	45.00	m
		GT:	**79.32**				

m = metacentricc, sm = sub-metacentric chromosome.

Table 17. Length, arm ratio, centromeric index, relative length and centromeric type of mitotic metaphase chromosomes of CB-6 germplasm of *Gossypium hirsutum* L.

Chromosome pair	Long arm (l) µm	Short arm (s) µm	Total length (T) µm	Arm ratio (l/s)	Relative length (RL)	Centromeric index (CI)	Centromeric type (CT)
I	1.18	1.12	2.30	1.06	0.03	48.61	m
	1.28	1.08	2.36	1.18	0.03	45.95	m
II	1.21	1.02	2.24	1.19	0.03	45.71	m
	1.12	1.02	2.14	1.09	0.03	47.76	m
III	1.12	0.96	2.08	1.17	0.03	46.15	m
	1.12	0.96	2.08	1.17	0.03	46.15	m
IV	1.15	0.92	2.08	1.24	0.03	44.62	m
	1.15	0.92	2.08	1.24	0.03	44.62	m
V	0.76	1.28	2.04	0.6	0.02	62.50	m
	1.12	0.89	2.01	1.25	0.02	44.44	m
VI	1.05	0.92	1.98	1.14	0.02	46.77	m
	1.05	0.92	1.98	1.14	0.02	46.77	m
VII	0.96	0.96	1.92	1.00	0.02	50.00	m
	1.08	0.80	1.88	1.36	0.02	42.37	m
VIII	0.96	0.89	1.85	1.07	0.02	48.28	m
	1.05	0.80	1.85	1.32	0.02	43.10	m
IX	0.99	0.83	1.82	1.19	0.02	45.61	m
	0.99	0.83	1.82	1.20	0.02	45.61	m
X	1.02	0.76	1.79	1.33	0.02	42.86	m
	0.96	0.83	1.79	1.15	0.02	46.43	m
XI	0.96	0.80	1.76	1.20	0.02	45.45	m
	0.96	0.76	1.72	1.25	0.02	44.44	m
XII	0.89	0.80	1.69	1.12	0.02	47.17	m
	0.89	0.80	1.69	1.12	0.02	47.17	m
XIII	0.96	0.70	1.66	1.36	0.02	42.31	m
	0.83	0.80	1.63	1.04	0.02	49.02	m

Table Contd.

Table Contd.

Chromosome pair	Long arm (l) µm	Short arm (s) µm	Total length (T) µm	Arm ratio (l/s)	Relative length (RL)	Centromeric index (CI)	Centromeric type (CT)
XIV	0.80	0.76	1.56	1.04	0.02	48.98	m
	0.80	0.76	1.56	1.04	0.02	48.98	m
XV	0.80	0.73	1.53	1.09	0.02	47.92	m
	0.76	0.73	1.50	1.04	0.02	48.94	m
XVI	0.76	0.73	1.50	1.04	0.02	48.94	m
	0.80	0.64	1.44	1.25	0.02	44.44	m
XVII	0.73	0.70	1.44	1.05	0.02	48.89	m
	0.80	0.64	1.44	1.25	0.02	44.44	m
XVIII	0.73	0.70	1.44	1.05	0.02	48.89	m
	0.80	0.64	1.44	1.25	0.02	44.44	m
XIX	0.73	0.60	1.34	1.21	0.02	45.24	m
	0.51	0.80	1.31	0.64	0.02	60.98	m
XX	0.51	0.80	1.31	0.64	0.02	60.98	m
	0.64	0.64	1.28	1.00	0.02	50.00	m
XXI	0.64	0.64	1.28	1.00	0.02	50.00	m
	0.64	0.64	1.28	1.00	0.02	50.00	m
XXII	0.64	0.64	1.28	1.00	0.02	50.00	m
	0.64	0.64	1.28	1.00	0.02	50.00	m
XXIII	0.64	0.64	1.28	1.00	0.02	50.00	m
	0.64	0.64	1.28	1.00	0.02	50.00	m
XIV	0.64	0.57	1.21	1.11	0.01	47.37	m
	0.48	0.73	1.21	0.65	0.01	60.53	m
XV	0.64	0.48	1.12	1.33	0.01	42.86	m
	0.64	0.48	1.12	1.33	0.01	42.86	m
XVI	0.48	0.48	0.96	1.00	0.01	50.00	m
	0.48	0.32	0.80	1.50	0.01	40.00	m
		GT:	**82.72**				

m = metacentricc, sm = sub-metacentric chromosome.

Table 18. Length, arm ratio, centromeric index, relative length and centromeric type of mitotic metaphase chromosomes of CB-7 germplasm of *Gossypium hirsutum* L.

Chromosome pair	Long arm (l) µm	Short arm (s) µm	Total length (T) µm	Arm ratio (l/s)	Relative length (RL)	Centromeric index (CI)	Centromeric type (CT)
I	0.93	0.80	1.73	1.16	0.03	53.70	m
	0.90	0.74	1.63	1.22	0.03	54.90	m
II	0.83	0.77	1.60	1.08	0.03	52.00	m
	0.90	0.67	1.57	1.33	0.03	57.14	m
III	0.83	0.70	1.54	1.18	0.02	54.17	m
	0.80	0.70	1.50	1.14	0.02	53.19	m
IV	0.80	0.70	1.50	1.14	0.02	53.19	m
	0.83	0.64	1.47	1.30	0.02	56.52	m
V	0.77	0.70	1.47	1.09	0.02	52.17	m
	0.74	0.70	1.44	1.05	0.02	51.11	m
VI	0.77	0.64	1.41	1.20	0.02	54.55	m
	0.70	0.70	1.41	1.00	0.02	50.00	m
VII	0.70	0.67	1.38	1.05	0.02	51.16	m
	0.70	0.67	1.38	1.05	0.02	51.16	m
VIII	0.70	0.67	1.38	1.05	0.02	51.16	m
	0.70	0.64	1.34	1.10	0.02	52.38	m
IX	0.70	0.64	1.34	1.10	0.02	52.38	m
	0.67	0.64	1.31	1.05	0.02	51.22	m
X	0.64	0.64	1.28	1.00	0.02	50.00	m
	0.64	0.64	1.28	1.00	0.02	50.00	m
XI	0.64	0.64	1.28	1.00	0.02	50.00	m
	0.64	0.64	1.28	1.00	0.02	50.00	m
XII	0.64	0.58	1.22	1.11	0.02	52.63	m
	0.64	0.58	1.22	1.11	0.02	52.63	m
XIII	0.64	0.58	1.22	1.11	0.02	52.63	m
	0.64	0.58	1.22	1.11	0.02	52.63	m

Table Contd.

Table Contd.

Chromosome pair	Long arm (l) µm	Short arm (s) µm	Total length (T) µm	Arm ratio (l/s)	Relative length (RL)	Centromeric index (CI)	Centromeric type (CT)
XIV	0.58	0.58	1.15	1.00	0.02	50.00	m
	0.58	0.58	1.15	1.00	0.02	50.00	m
XV	0.58	0.58	1.15	1.00	0.02	50.00	m
	0.58	0.58	1.15	1.00	0.02	50.00	m
XVI	0.58	0.58	1.15	1.00	0.02	50.00	m
	0.58	0.51	1.09	1.13	0.02	52.94	m
XVII	0.61	0.48	1.09	1.27	0.02	55.88	m
	0.54	0.51	1.06	1.06	0.02	51.52	m
XVIII	0.54	0.48	1.02	1.13	0.02	53.13	m
	0.54	0.48	1.02	1.13	0.02	53.13	m
XIX	0.51	0.48	0.99	1.07	0.02	51.61	m
	0.54	0.45	0.99	1.21	0.02	54.84	m
XX	0.51	0.45	0.96	1.14	0.02	53.33	m
	0.51	0.45	0.96	1.14	0.02	53.33	m
XXI	0.51	0.45	0.96	1.14	0.02	53.33	m
	0.51	0.45	0.96	1.14	0.02	53.33	m
XXII	0.51	0.45	0.96	1.14	0.02	53.33	m
	0.51	0.42	0.93	1.23	0.02	55.17	m
XXIII	0.48	0.42	0.90	1.15	0.01	53.57	m
	0.48	0.38	0.86	1.25	0.01	55.56	m
XIV	0.45	0.38	0.83	1.17	0.01	53.85	m
	0.45	0.38	0.83	1.17	0.01	53.85	m
XV	0.42	0.42	0.83	1.00	0.01	50.00	m
	0.42	0.42	0.83	1.00	0.01	50.00	m
XVI	0.42	0.38	0.80	1.08	0.01	52.00	m
	0.42	0.38	0.80	1.08	0.01	52.00	m
		GT:	**61.82**				

m = metacentricc chromosomes.

Table 19. Length, arm ratio, centromeric index, relative length and centromeric type of mitotic metaphase chromosomes of CB-8 germplasm of *Gossypium hirsutum* L.

Chromosome pair	Long arm (l) µm	Short arm (s) µm	Total length (T) µm	Arm ratio (l/s)	Relative length (RL)	Centromeric index (CI)	Centromeric type (CT)
I	1.12	0.86	1.98	1.30	0.03	56.45	m
	0.99	0.77	1.76	1.29	0.03	56.36	m
II	0.96	0.70	1.66	1.36	0.03	57.69	m
	0.90	0.70	1.60	1.27	0.02	56.00	m
III	0.90	0.70	1.60	1.27	0.02	56.00	m
	0.96	0.64	1.60	1.50	0.02	60.00	m
IV	0.86	0.64	1.50	1.35	0.02	57.45	m
	0.77	0.64	1.41	1.20	0.02	54.55	m
V	0.77	0.64	1.41	1.20	0.02	54.55	m
	0.83	0.58	1.41	1.44	0.02	59.09	m
VI	0.77	0.64	1.41	1.20	0.02	54.55	m
	0.70	0.67	1.38	1.05	0.02	51.16	m
VII	0.70	0.64	1.34	1.10	0.02	52.38	m
	0.70	0.64	1.34	1.10	0.02	52.38	m
VIII	0.74	0.61	1.34	1.21	0.02	54.76	m
	0.74	0.61	1.34	1.21	0.02	54.76	m
IX	0.67	0.64	1.31	1.05	0.02	51.22	m
	0.64	0.64	1.28	1.00	0.02	50.00	m
X	0.64	0.64	1.28	1.00	0.02	50.00	m
	0.70	0.58	1.28	1.22	0.02	55.00	m
XI	0.70	0.58	1.28	1.22	0.02	55.00	m
	0.64	0.64	1.28	1.00	0.02	50.00	m
XII	0.70	0.58	1.28	1.22	0.02	55.00	m
	0.67	0.61	1.28	1.11	0.02	52.50	m
XIII	0.70	0.58	1.28	1.22	0.02	55.00	m
	0.67	0.58	1.25	1.17	0.02	53.85	m

Table Contd.

Table Contd.

Chromosome pair	Long arm (l) µm	Short arm (s) µm	Total length (T) µm	Arm ratio (l/s)	Relative length (RL)	Centromeric index (CI)	Centromeric type (CT)
XIV	0.67	0.58	1.25	1.17	0.02	53.85	m
	0.67	0.58	1.25	1.17	0.02	53.85	m
XV	0.67	0.58	1.25	1.17	0.02	53.85	m
	0.64	0.58	1.22	1.11	0.02	52.63	m
XVI	0.64	0.58	1.22	1.11	0.02	52.63	m
	0.64	0.58	1.22	1.11	0.02	52.63	m
XVII	0.67	0.54	1.22	1.24	0.02	55.26	m
	0.67	0.54	1.22	1.24	0.02	55.26	m
XVIII	0.64	0.58	1.22	1.11	0.02	52.63	m
	0.64	0.58	1.22	1.11	0.02	52.63	m
XIX	0.64	0.58	1.22	1.11	0.02	52.63	m
	0.67	0.54	1.22	1.24	0.02	55.26	m
XX	0.64	0.51	1.15	1.25	0.02	55.56	m
	0.64	0.51	1.15	1.25	0.02	55.56	m
XXI	0.64	0.51	1.15	1.25	0.02	55.56	m
	0.64	0.51	1.15	1.25	0.02	55.56	m
XXII	0.64	0.51	1.15	1.25	0.02	55.56	m
	0.64	0.51	1.15	1.25	0.02	55.56	m
XXIII	0.58	0.48	1.06	1.20	0.02	54.55	m
	0.58	0.48	1.06	1.20	0.02	54.55	m
XIV	0.58	0.42	0.99	1.38	0.02	58.06	m
	0.58	0.42	0.99	1.38	0.02	58.06	m
XV	0.51	0.38	0.90	1.33	0.01	57.14	m
	0.51	0.38	0.90	1.33	0.01	57.14	m
XVI	0.45	0.38	0.83	1.17	0.01	53.85	m
	0.45	0.38	0.83	1.17	0.01	53.85	m
		GT:	**66.05**				

m = metacentricc chromosome.

Table 20. Length, arm ratio, centromeric index, relative length and centromeric type of mitotic metaphase chromosomes of CB-9 germplasm of *Gossypium hirsutum* L.

Chromosome pair	Long arm (l) µm	Short arm (s) µm	Total length (T) µm	Arm ratio (l/s)	Relative length (RL)	Centromeric index (CI)	Centromeric type (CT)
I	0.96	0.86	1.82	1.11	0.03	52.60	m
	0.92	0.83	1.76	1.12	0.03	52.70	m
II	0.86	0.80	1.66	1.08	0.03	51.90	m
	0.76	0.64	1.40	1.20	0.03	54.50	m
III	0.76	0.64	1.40	1.20	0.03	54.50	m
	0.76	0.64	1.40	1.20	0.03	54.50	m
IV	0.76	0.64	1.40	1.20	0.03	54.50	m
	0.76	0.64	1.40	1.20	0.03	54.50	m
V	0.76	0.64	1.40	1.20	0.03	54.50	m
	0.76	0.64	1.40	1.20	0.03	54.50	m
VI	0.76	0.57	1.34	1.33	0.02	57.10	m
	0.76	0.57	1.34	1.33	0.02	57.10	m
VII	0.64	0.64	1.28	1.00	0.02	50.00	m
	0.64	0.64	1.28	1.00	0.02	50.00	m
VIII	0.64	0.64	1.28	1.00	0.02	50.00	m
	0.64	0.60	1.24	1.05	0.02	51.30	m
IX	0.64	0.64	1.28	1.00	0.02	50.00	m
	0.64	0.64	1.28	1.00	0.02	50.00	m
X	0.60	0.54	1.15	1.12	0.02	52.80	m
	0.60	0.54	1.15	1.12	0.02	52.80	m
XI	0.60	0.51	1.12	1.19	0.02	54.30	m
	0.51	0.48	0.99	1.07	0.02	51.60	m
XII	0.51	0.48	0.99	1.07	0.02	51.60	m
	0.51	0.48	0.99	1.07	0.02	51.60	m
XIII	0.51	0.48	0.99	1.07	0.02	51.60	m
	0.51	0.48	0.99	1.07	0.02	51.60	m

Table Contd.

Table Contd.

Chromosome pair	Long arm (l) µm	Short arm (s) µm	Total length (T) µm	Arm ratio (l/s)	Relative length (RL)	Centromeric index (CI)	Centromeric type (CT)
XIV	0.51	0.48	0.99	1.07	0.02	51.60	m
	0.51	0.48	0.99	1.07	0.02	51.60	m
XV	0.51	0.48	0.99	1.07	0.02	51.60	m
	0.51	0.48	0.99	1.07	0.02	51.60	m
XVI	0.51	0.48	0.99	1.07	0.02	51.60	m
	0.51	0.48	0.99	1.07	0.02	51.60	m
XVII	0.51	0.48	0.99	1.07	0.02	51.60	m
	0.51	0.48	0.99	1.07	0.02	51.60	m
XVIII	0.51	0.48	0.99	1.07	0.02	51.60	m
	0.51	0.48	0.99	1.07	0.02	51.60	m
XIX	0.48	0.48	0.96	1.00	0.02	50.00	m
	0.48	0.48	0.96	1.00	0.02	50.00	m
XX	0.48	0.48	0.96	1.00	0.02	50.00	m
	0.48	0.48	0.96	1.00	0.02	50.00	m
XXI	0.48	0.48	0.96	1.00	0.02	50.00	m
	0.48	0.48	0.96	1.00	0.02	50.00	m
XXII	0.48	0.48	0.96	1.00	0.02	50.00	m
	0.48	0.48	0.96	1.00	0.02	50.00	m
XXIII	0.48	0.48	0.96	1.00	0.02	50.00	m
	0.44	0.41	0.85	1.08	0.02	51.90	m
XXIV	0.44	0.41	0.85	1.08	0.02	51.90	m
	0.44	0.41	0.85	1.08	0.02	51.90	m
XXV	0.44	0.41	0.85	1.08	0.02	51.90	m
	0.44	0.41	0.85	1.08	0.02	51.90	m
XXVI	0.41	0.41	0.82	1.00	0.01	50.00	m
	0.41	0.41	0.82	1.00	0.01	50.00	m
		GT:	**55.58**				

m = metacentric chromosome.

Table 21. Length, arm ratio, centromeric index, relative length and centromeric type of mitotic metaphase chromosomes of CB-10 germplasm of *Gossypium hirsutum* L.

Chromosome pair	Long arm (l) µm	Short arm (s) µm	Total length (T) µm	Arm ratio (l/s)	Relative length (RL)	Centromeric index (CI)	Centromeric type (CT)
I	1.34	1.03	2.37	1.30	0.03	43.40	m
	1.34	1.03	2.37	1.30	0.03	43.40	m
II	1.18	1.03	2.21	1.20	0.03	46.60	m
	1.18	1.03	2.21	1.20	0.03	46.60	m
III	1.09	0.96	2.05	1.10	0.03	46.80	m
	1.09	0.96	2.05	1.10	0.03	46.80	m
IV	1.03	0.90	1.93	1.20	0.03	46.60	m
	0.96	0.90	1.86	1.10	0.02	48.30	m
V	0.90	0.77	1.67	1.20	0.02	46.10	m
	0.90	0.77	1.67	1.20	0.02	46.10	m
VI	0.90	0.77	1.67	1.20	0.02	46.10	m
	0.90	0.77	1.67	1.20	0.02	46.10	m
VII	0.86	0.71	1.57	1.20	0.02	45.20	m
	0.80	0.74	1.54	1.10	0.02	48.00	m
VIII	0.80	0.74	1.54	1.10	0.02	48.00	m
	0.80	0.74	1.54	1.10	0.02	48.00	m
IX	0.80	0.74	1.54	1.10	0.02	48.00	m
	0.80	0.74	1.54	1.10	0.02	48.00	m
X	0.80	0.74	1.54	1.10	0.02	48.00	m
	0.77	0.74	1.51	1.04	0.02	49.00	m
XI	0.77	0.74	1.51	1.04	0.02	49.00	m
	0.77	0.74	1.51	1.04	0.02	49.00	m
XII	0.77	0.74	1.51	1.04	0.02	49.00	m
	0.77	0.74	1.51	1.04	0.02	49.00	m
XIII	0.71	0.71	1.42	1.00	0.02	50.00	m
	0.71	0.71	1.42	1.00	0.02	50.00	m

Table Contd.

Table Contd.

Chromosome pair	Long arm (l) µm	Short arm (s) µm	Total length (T) µm	Arm ratio (l/s)	Relative length (RL)	Centromeric index (CI)	Centromeric type (CT)
XIV	0.74	0.64	1.38	1.16	0.02	46.30	m
	0.74	0.64	1.38	1.16	0.02	46.30	m
XV	0.74	0.64	1.38	1.16	0.02	46.30	m
	0.74	0.64	1.38	1.16	0.02	46.30	m
XVI	0.74	0.64	1.38	1.16	0.02	46.30	m
	0.74	0.64	1.38	1.16	0.02	46.30	m
XVII	0.74	0.64	1.38	1.16	0.02	46.30	m
	0.74	0.64	1.38	1.16	0.02	46.30	m
XVIII	0.74	0.64	1.38	1.16	0.02	46.30	m
	0.71	0.61	1.32	1.16	0.02	46.20	m
XIX	0.71	0.61	1.32	1.16	0.02	46.20	m
	0.71	0.61	1.32	1.16	0.02	46.20	m
XX	0.71	0.61	1.32	1.16	0.02	46.20	m
	0.71	0.61	1.32	1.16	0.02	46.20	m
XXI	0.71	0.61	1.32	1.16	0.02	46.20	m
	0.71	0.61	1.32	1.16	0.02	46.20	m
XXII	0.64	0.58	1.22	1.10	0.02	47.50	m
	0.64	0.58	1.22	1.10	0.02	47.50	m
XXIII	0.58	0.58	1.16	1.00	0.02	50.00	m
	0.58	0.58	1.16	1.00	0.02	50.00	m
XXIV	0.58	0.58	1.16	1.00	0.02	50.00	m
	0.58	0.51	1.09	1.14	0.01	46.70	m
XXV	0.58	0.51	1.09	1.14	0.01	46.70	m
	0.58	0.51	1.09	1.14	0.01	46.70	m
XXVI	0.45	0.45	0.90	1.00	0.01	50.00	m
	0.45	0.45	0.90	1.00	0.01	50.00	m
		GT:	**77.58**				

m = metacentricc chromosome.

Table 22. Length, arm ratio, centromeric index, relative length and centromeric type of mitotic metaphase chromosomes of CB-11 germplasm of *Gossypium hirsutum* L.

Chromosome pair	Long arm (l) µm	Short arm (s) µm	Total length (T) µm	Arm ratio (l/s)	Relative length (RL)	Centromeric index (CI)	Centromeric type (CT)
I	1.19	1.12	2.31	1.06	0.03	48.50	m
	1.19	1.12	2.31	1.06	0.03	48.50	m
II	1.19	1.06	2.25	1.12	0.03	47.10	m
	1.19	1.06	2.25	1.12	0.03	47.10	m
III	1.12	1.09	2.21	1.03	0.03	49.30	m
	1.09	1.06	2.15	1.03	0.03	49.30	m
IV	0.99	0.93	1.92	1.06	0.02	48.50	m
	0.99	0.93	1.92	1.06	0.02	48.50	m
V	0.99	0.93	1.92	1.06	0.02	48.50	m
	0.99	0.93	1.92	1.06	0.02	48.50	m
VI	0.99	0.93	1.92	1.06	0.02	48.50	m
	0.99	0.93	1.92	1.06	0.02	48.50	m
VII	0.99	0.93	1.92	1.06	0.02	48.50	m
	0.93	0.87	1.80	1.07	0.02	48.30	m
VIII	0.96	0.83	1.79	1.17	0.02	46.30	m
	0.96	0.83	1.79	1.17	0.02	46.30	m
IX	1.02	0.77	1.79	1.32	0.02	43.00	m
	1.02	0.77	1.79	1.32	0.02	43.00	m
X	0.87	0.80	1.67	1.08	0.02	47.90	m
	0.87	0.80	1.67	1.08	0.02	47.90	m
XI	0.80	0.80	1.60	1.00	0.02	50.00	m
	0.80	0.80	1.60	1.00	0.02	50.00	m
XII	0.80	0.77	1.57	1.04	0.02	49.00	m
	0.80	0.77	1.57	1.04	0.02	49.00	m
XIII	0.77	0.77	1.54	1.00	0.02	50.00	m
	0.77	0.77	1.54	1.00	0.02	50.00	m

Table Contd.

Table Contd.

Chromosome pair	Long arm (l) µm	Short arm (s) µm	Total length (T) µm	Arm ratio (l/s)	Relative length (RL)	Centromeric index (CI)	Centromeric type (CT)
XIV	0.77	0.77	1.54	1.00	0.02	50.00	m
	0.74	0.74	1.48	1.00	0.02	50.00	m
XV	0.74	0.74	1.48	1.00	0.02	50.00	m
	0.74	0.74	1.48	1.00	0.02	50.00	m
XVI	0.74	0.74	1.48	1.00	0.02	50.00	m
	0.77	0.71	1.48	1.08	0.02	47.90	m
XVII	0.77	0.71	1.48	1.08	0.02	47.90	m
	0.77	0.71	1.48	1.08	0.02	47.90	m
XVIII	0.71	0.71	1.42	1.00	0.02	50.00	m
	0.71	0.71	1.42	1.00	0.02	50.00	m
XIX	0.71	0.71	1.42	1.00	0.02	50.00	m
	0.71	0.71	1.42	1.00	0.02	50.00	m
XX	0.71	0.67	1.38	1.06	0.02	48.50	m
	0.71	0.67	1.38	1.06	0.02	48.50	m
XXI	0.67	0.67	1.34	1.00	0.02	50.00	m
	0.67	0.67	1.34	1.00	0.02	50.00	m
XXII	0.67	0.64	1.31	1.05	0.02	48.80	m
	0.67	0.64	1.31	1.05	0.02	48.80	m
XXIII	0.64	0.64	1.28	1.00	0.02	50.00	m
	0.64	0.64	1.28	1.00	0.02	50.00	m
XXIV	0.64	0.64	1.28	1.00	0.02	50.00	m
	0.64	0.64	1.28	1.00	0.02	50.00	m
XXV	0.58	0.54	1.12	1.07	0.01	48.20	m
	0.58	0.54	1.12	1.07	0.01	48.20	m
XXVI	0.51	0.51	1.02	1.00	0.01	50.00	m
	0.48	0.48	0.96	1.00	0.01	50.00	m
		GT:	**83.62**				

m = metacentric chromosome.

Table 23. Comparative orcein stained karyotype analysis in eleven germplasms of *Gossypium hirsutum* L.

Germplasms	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.

Table 24. Comparative CMA-banding analysis of eleven germplasms of *Gossypium hirsutum* L.

Cotton germplasms	2n	No of satellites	No. of CMA-bands	Total length of CMA-positive banded region (μm)	% of CMA-positive banded region	CMA- banded karyotypic formulae
CB-1	52	4	20	14.37	16.66	4α+4β+5γ+4φ+2λ+1θ+32δ
CB-2	52	6	19	13.44	17.22	6β+5θ+7γ+1λ+33δ
CB-3	52	6	9	2.56	3.67	6β+2φ+1θ+43δ
CB-4	52	2	13	10.88	13.39	2α+2β+6γ+2φ+1λ+39δ
CB-5	52	-	2	0.32	0.40	2θ+50δ
CB-6	52	3	5	0.80	0.97	3β+2θ+47δ
CB-7	52	2	18	6.08	9.84	1α+2β+12θ+1γ+2λ+34δ
CB-8	52	6	22	20.96	31.73	6β+5λ+11γ+30δ
CB-9	52	-	8	2.40	4.32	1α+2φ+5θ+44δ
CB-10	52	6	11	4.96	6.40	6β+4φ+1γ+41δ
CB-11	52	-	5	2.24	2.68	4θ+1α+47δ

Classification of CMA positive bands:

α= Band in centromeric region
γ= Band in whole chromosome
φ= Band in whole short arm
λ= Band in whole long arm
θ= Band in terminal region
β= Band in satellite
Ω= Band in above centromere
δ= No band

Table 25. Comparative DAPI-banding analysis of eleven germplasms of *Gossypium hirsutum* L.

Cotton germplasms	2n	No of satellites	No. of DAPI-bands	Total length of DAPI-positive banded region (μm)	% of DAPI-positive banded region	DAPI-banded karyotypic formulae
CB-1	52	-	10	6.65	7.60	2α+4γ+4φ+42δ
CB-2	52	-	12	6.08	7.79	1θ+3α+6γ+2φ+40δ
CB-3	52	-	6	4.80	6.87	2φ+4γ+46δ
CB-4	52	-	18	15.20	18.71	16γ+1α+1θ+34δ
CB-5	52	-	6	3.36	4.24	3α+1θ+2γ+46δ
CB-6	52	-	16	11.84	14.31	1α+1θ+14γ+36δ
CB-7	52	-	6	4.48	7.25	1α+4γ+1φ+46δ
CB-8	52	-	10	7.52	11.38	1α+6γ+1θ+2φ+42δ
CB-9	52	-	14	7.28	13.10	1α+2θ+5γ+2λ+2φ+2β+38δ
CB-10	52	-	13	9.76	12.58	2α+9γ+2φ+39δ
CB-11	52	2	12	5.44	6.51	4θ+2γ+2β+4φ+1ϕ+39δ

Classification of DAPI positive bands:

α= Band in centromeric region
γ= Band in whole chromosome
φ= Band in whole short arm
λ= Band in whole long arm
θ= Band in terminal region
β= Band in satellite
ϕ= Two dot in two terminal region of same chromosome
δ= No band

Table 26. RAPD analysis with primer OPA-1 (5′-CAG GCC CTT C-3′) of 11 germplasms of *Gossypium hirsutum* L.

Germplasms	Total no. of bands with molecular weight (bp)	No. of light band with molecular weight (bp)	No. of bright band with molecular weight (bp)	No. of unique polymorphic band with molecular weight (bp)	No. of common band with molecular weight (bp)
CB-1	5 (1700, 850, 600, 350, 250)	4 (1700, 600, 350, 250)	1 (850)	—	
CB-2	5 (1700, 850, 600, 350, 250)	4 (1700, 600, 350, 250)	1 (850)	—	
CB-3	5 (1700, 850, 600, 350, 250)	4 (1700, 600, 350, 250)	1 (850)	—	
CB-4	4 (2000, 850, 600, 250)	3 (2000, 600, 250)	1 (850)	1 (2000)	
CB-5	5 (1700, 850, 600, 350, 250)	4 (1700, 600, 350, 250)	1 (850)	—	
CB-6	—	—	—	—	—
CB-7	—	—	—	—	
CB-8	—	—	—	—	
CB-9	6 (1700, 1400, 850, 650, 400, 300)	5 (1700, 1400, 650, 400, 300)	1 (850)	3 (1400, 400, 300)	
CB-10	—	—	—	—	
CB-11	1 (650)	—	1 (650)	—	

Table 27. RAPD analysis with primer OPA-3 (5′-AGT CAG CCA C-3′) of 11 germplasms of *Gossypium hirsutum* L.

Germplasms	Total no. of bands with molecular weight (bp)	No. of light band with molecular weight (bp)	No. of bright band with molecular weight (bp)	No. of unique polymorphic band with molecular weight (bp)	No. of common band with molecular weight (bp)
CB-1	5 (7000, 2700, 1400, 900, 800)	3 (7000, 2700, 900)	2 (1400, 800)	—	
CB-2	5 (7000, 2700, 1400, 900, 800)	3 (7000, 2700, 900)	2 (1400, 800)	—	
CB-3	3 (7000, 2700, 1600)	3 (7000, 2700, 1600)	—	1 (1600)	
CB-4	2 (1400, 800)	—	2 (1400, 800)	—	
CB-5	2 (1400, 800)	—	2 (1400, 800)	—	
CB-6	2 (1400, 800)	—	2 (1400, 800)	—	—
CB-7	2 (1400, 800)	—	2 (1400, 800)	—	
CB-8	2 (1400, 800)	2 (1400, 800)	—	—	
CB-9	4 (7000, 2700, 1400, 800)	2 (7000, 2700)	2 (1400, 800)	—	
CB-10	4 (7000, 2700, 1400, 800)	2 (7000, 2700)	2 (1400, 800)	—	
CB-11	6 (7000, 2700, 1500, 900, 750, 600)	2 (7000, 2700)	4 (1500, 900, 750, 600)	3 (1500, 750, 600)	

Table 28. RAPD analysis with primer OPA-4 (5′-AAT CGG GCT G-3′) of 11 germplasms of *Gossypium hirsutum* L.

Germplasms	Total no. of bands with molecular weight (bp)	No. of light band with molecular weight (bp)	No. of bright band with molecular weight (bp)	No. of unique polymorphic band with molecular weight (bp)	No. of common band with molecular weight (bp)
CB-1	5 (1600, 1450, 1250, 1000, 750)	4 (1600, 1450, 1250, 750)	1 (1000)	—	—
CB-2	7 (1600, 1450, 1250, 1000, 750, 500, 350)	6 (1600, 1450, 1250, 750, 500, 350)	1 (1000)	—	
CB-3	7 (1600, 1450, 1250, 1000, 750, 500, 350)	6 (1600, 1450, 1250, 750, 500, 350)	1 (1000)	—	
CB-4	7 (1600, 1450, 1250, 1000, 750, 500, 350)	5 (1600, 1450, 1250, 750, 350)	2 (1000, 500)	—	
CB-5	8 (1600, 1450, 1250, 1000, 750, 650, 500, 350)	7 (1600, 1450, 1250, 750, 650, 500, 350)	1 (1000)	—	
CB-6	7 (1600, 1450, 1250, 1000, 650, 500, 350)	6 (1600, 1450, 1250, 650, 450, 350)	1 (1000)	—	
CB-7	2 (650, 300)	1 (650)	1 (300)	—	
CB-8	—	—	—	—	
CB-9	8 (1450, 1250, 1000, 850, 650, 500, 350, 300)	2 (1450, 1250)	6 (1000, 850, 650, 500, 350, 300)	—	
CB-10	4 (650, 500, 350, 300)	4 (650, 500, 350, 300)	—	—	
CB-11	10 (4000, 3000, 1700, 1450, 1000, 850, 750, 650, 350, 300)	8 (4000, 3000, 1700, 1400, 850, 750, 350, 300)	2 (1000, 650)	3 (4000, 3000, 1700)	

Table 29. RAPD analysis with primer OPA-6 (5′-GGT CCC TGA C-3′) 11 germplasms of *Gossypium hirsutum* L.

Germplasms	Total no. of bands with molecular weight (bp)	No. of light band with molecular weight (bp)	No. of bright band with molecular weight (bp)	No. of unique polymorphic band with molecular weight (bp)	No. of common band with molecular weight (bp)
CB-1	—	—	—	—	
CB-2	—	—	—	—	
CB-3	—	—	—	—	
CB-4	—	—	—	—	
CB-5	1 (750)	—	1 (750)	—	
CB-6	—	—	—	—	—
CB-7	—	—	—	—	
CB-8	1 (750)	—	1 (750)	—	
CB-9	3 (2000, 1000, 650)	2 (2000, 1000)	1 (650)	1 (2000)	
CB-10	2 (750, 500)	1 (500)	1 (750)	—	
CB-11	4 (1300, 1000, 750, 500)	4 (1300, 1000, 750, 500)	—	1 (1300)	

Table 30. RAPD analysis with primer OPA-7 (5′-GAA ACG GGT G-3′) of 11 germplasms of *Gossypium hirsutum* L.

Germplasms	Total no. of bands with molecular weight (bp)	No. of light band with molecular weight (bp)	No. of bright band with molecular weight (bp)	No. of unique polymorphic band with molecular weight (bp)	No. of common band with molecular weight (bp)
CB-1	—	—	—	—	
CB-2	1 (750)	—	1 (750)	—	
CB-3	—	—	—	—	
CB-4	—	—	—	—	
CB-5	3 (1700, 1300, 750)	2 (1700, 1300)	1 (750)	—	
CB-6	4 (1700, 1300, 750, 500)	2 (1300, 500)	2 (1700, 750)	1 (500)	—
CB-7	—	—	—	—	
CB-8	—	—	—	—	
CB-9	—	—	—	—	
CB-10	1 (750)	1 (750)	—	—	
CB-11	—	—	—	—	

Table 31. RAPD analysis with primer OPA-10 (5′-GTG ATC GCA G-3′) of 11 germplasms of *Gossypium hirsutum* L.

Germplasms	Total no. of bands with molecular weight (bp)	No. of light band with molecular weight (bp)	No. of bright band with molecular weight (bp)	No. of unique polymorphic band with molecular weight (bp)	No. of common band with molecular weight (bp)
CB-1	5 (2400, 1500, 1000, 650, 500)	5 (2400, 1500, 1000, 650, 500)	—	—	
CB-2	5 (2400, 1500, 1000, 650, 500)	5 (2400, 1500, 1000, 650, 500)	—	—	
CB-3	5 (2400, 1500, 1000, 650, 500)	5 (2400, 1500, 1000, 650, 500)	—	—	
CB-4	5 (2400, 1500, 1000, 650, 500)	4 (2400, 1500, 650, 500)	1 (1000)	—	
CB-5	4 (2400, 1500, 1000, 650)	4 (2400, 1500, 1000, 650)	—	—	
CB-6	3 (1000, 650, 500)	—	3 (1000, 650, 500)	—	—
CB-7	4 (2400, 1500, 1000, 650)	4 (2400, 1500, 1000, 650)	—	—	
CB-8	5 (2400, 1500, 1000, 650, 500)	4 (2400, 1500, 650, 500)	1 (1000)	—	
CB-9	5 (2400, 1500, 1000, 650, 500)	4 (2400, 1500, 650, 500)	1 (1000)	—	
CB-10	3 (1400, 650, 500)	2 (650, 500)	1 (1400)	1 (1400)	
CB-11	—	—	—	—	

Table 32. RAPD analysis with primer-1 (5′-GAA ACG GGT G-3′) of 11 germplasms of *Gossypium hirsutum* L.

Germplasms	Total no. of bands with molecular weight (bp)	No. of light band with molecular weight (bp)	No. of bright band with molecular weight (bp)	No. of unique polymorphic band with molecular weight (bp)	No. of common band with molecular weight (bp)
CB-1	4 (2100, 1800, 1400, 850)	3 (2100, 1800, 1400)	1 (850)	—	—
CB-2	4 (2100, 1800, 1400, 850)	3 (2100, 1800, 1400)	1 (850)	—	
CB-3	4 (2100, 1800, 1400, 850)	3 (2100, 1800, 1400)	1 (850)	—	
CB-4	1 (850)	—	1 (850)	—	
CB-5	4 (2100, 1800, 1400, 850)	3 (2100, 1800, 1400)	1 (850)	—	
CB-6	4 (2100, 1800, 1400, 850)	3 (2100, 1800, 1400)	1 (850)	—	
CB-7	1 (850)	—	1 (850)	—	
CB-8	1 (850)	—	1 (850)	—	
CB-9	1 (850)	—	1 (850)	—	
CB-10	3 (1800, 1400, 850)	2 (1800, 1400)	1 (850)	—	
CB-11	4 (2750, 2100, 1800, 1000)	3 (2100, 1800)	1 (1000)	2 (2750, 1000)	

Table 33. RAPD analysis with primer-2 (5′-GTT GCG ATC C-3′) of 11 germplasms of *Gossypium hirsutum* L.

Germplasms	Total no. of bands with molecular weight (bp)	No. of light band with molecular weight (bp)	No. of bright band with molecular weight (bp)	No. of unique polymorphic band with molecular weight (bp)	No. of common band with molecular weight (bp)
CB-1	5 (900, 750, 600, 300, 200)	—	5 (900, 750, 600, 300, 200)	—	
CB-2	3 (600, 350, 250)	2 (600, 350)	1 (250)	—	
CB-3	3 (600, 350, 250)	3 (600, 350, 250)	—	—	
CB-4	3 (600, 350, 250)	3 (600, 350, 250)	—	—	
CB-5	—	—	—	—	
CB-6	—	—	—	—	—
CB-7	—	—	—	—	
CB-8	1 (300)	1 (300)	—	—	
CB-9	2 (400, 300)	1 (400)	1 (300)	1 (400)	
CB-10	6 (2500, 900, 750, 600, 350, 250)	—	6 (2500, 900, 750, 600, 350, 250)	2 (2500, 350)	
CB-11	1 (750)	1 (750)	—	—	

Table 34. RAPD analysis with primer-18 (5′-GTT TCG CTC C-3′) of 11 germplasms of *Gossypium hirsutum* L.

Germplasms	Total no. of bands with molecular weight (bp)	No. of light band with molecular weight (bp)	No. of bright band with molecular weight (bp)	No. of unique polymorphic band with molecular weight (bp)	No. of common band with molecular weight (bp)
CB-1	3 (3500, 2000, 650)	1 (650)	2 (3500, 2000)	—	
CB-2	3 (3500, 2000, 650)	1 (650)	2 (3500, 2000)	—	
CB-3	4 (3500, 2000, 1100, 650)	2 (1100, 650)	2 (3500, 2000)	—	
CB-4	5 (3500, 2000, 1500, 1100, 650)	3 (1500, 1100, 650)	2 (3500, 2000)	—	
CB-5	5 (3500, 2000, 1500, 1100, 650)	3 (1500, 1100, 650)	2 (3500, 2000)	—	
CB-6	3 (3500, 2000, 650)	3 (3500, 2000, 650)	—	—	—
CB-7	3 (3500, 2000, 650)	3 (3500, 2000, 650)	—	—	
CB-8	3 (3500, 2000, 650)	3 (3500, 2000, 650)	—	—	
CB-9	6 (3500, 2000, 1500, 1100, 600, 500)	2 (1500, 1100)	4 (3500, 2000, 600, 500)	2 (600, 500)	
CB-10	7 (5000, 3000, 1750, 1100, 650, 550, 400)	4 (5000, 1750, 1100, 650)	3 (3000, 550, 400)	3 (5000, 3000, 1750)	
CB-11	7 (4000, 3000, 2000, 1600, 1000, 550, 400)	6 (4000, 3000, 2000, 1000, 550, 400)	1 (1600)	3 (4000, 1600, 1000)	

Table 35. RAPD analysis with primer-23 (5′-GTC AGG GCA A-3′) of 11 germplasms of *Gossypium hirsutum* L.

Germplasms	Total no. of bands with molecular weight (bp)	No. of light band with molecular weight (bp)	No. of bright band with molecular weight (bp)	No. of unique polymorphic band with molecular weight (bp)	No. of common band with molecular weight (bp)
CB-1	6 (2000, 1750, 1400, 1000, 800, 650)	—	6 (2000, 1750, 1400, 1000, 800, 650)	4 (2000, 1000, 800, 650)	
CB-2	—	—	—	—	
CB-3	3 (1850, 1400, 1250)	—	3 (1850, 1400, 1250)	—	
CB-4	3 (1850, 1400, 1250)	—	3 (1850, 1400, 1250)	—	
CB-5	4 (1850, 1400, 1250, 850)	—	4 (1850, 1400, 1250, 850)	—	
CB-6	4 (1850, 1400, 1250, 850)	—	4 (1850, 1400, 1250, 850)	—	—
CB-7	2 (1400, 1250)	—	2 (1400, 1250)	—	
CB-8	4 (1850, 1400, 1250, 850)	—	4 (1850, 1400, 1250, 850)	—	
CB-9	5 (1850, 1400, 1250, 850, 600)	—	5 (1850, 1400, 1250, 850, 600)	1 (600)	
CB-10	4 (1850, 1400, 1250, 850)	—	4 (1850, 1400, 1250, 850)		
CB-11	2 (1750, 1150)	—	2 (1750, 1150)	1 (1150)	

Table 36. SSR analysis with primer pair Forward-5′-GCTTCTTCCATTTTATTCAAG-3′-Reverse-5′-CAGCGGCAACCAAAAAG-3′ of 11 germplasms of *Gossypium hirsutum* L.

Germplasms	Total no. of bands with molecular weight (bp)	No. of light band with molecular weight (bp)	No. of bright band with molecular weight (bp)	No. of unique polymorphic band with molecular weight (bp)	No. of common band with molecular weight (bp)
CB-1	—	—	—	—	
CB-2	—	—	—	—	
CB-3	—	—	—	—	
CB-4	—	—	—	—	
CB-5	—	—	—	—	
CB-6	—	—	—	—	—
CB-7	1 (180)	—	1 (180)	1 (180)	
CB-8	—	—	—	—	
CB-9	—	—	—	—	
CB-10	—	—	—	—	
CB-11	—	—	—	—	

Table 37. SSR analysis with primer pair Forward-5′-ACCCTTAAATCATAAGAGAAC-3′-Reverse-5′-CCGTAAGTTAAGGTACAAGG-3′ of 11 germplasms of *Gossypium hirsutum* L.

Germplasms	Total no. of bands with molecular weight (bp)	No. of light band with molecular weight (bp)	No. of bright band with molecular weight (bp)	No. of unique polymorphic band with molecular weight (bp)	No. of common band with molecular weight (bp)
CB-1	1 (50)	1 (50)	—	—	
CB-2	1 (50)	1 (50)	—	—	
CB-3	2 (123, 50)	1 (50)	1 (123)	—	
CB-4	1 (50)	1 (50)	—	—	
CB-5	1 (50)	—	1 (50)	—	
CB-6	1 (50)	1 (50)	—	—	1 (50)
CB-7	1 (50)	—	1 (50)	—	
CB-8	1 (50)	—	1 (50)	—	
CB-9	3 (738, 123, 50)	3 (738, 123, 50)	—	1 (738)	
CB-10	1 (50)	1 (50)	—	—	
CB-11	1 (50)	1 (50)	—	—	

Table 38. SSR analysis with primer pair Forward-5′-GGAGTTAAAGCTAATGCCTG -3′-Reverse-5′-CGGGTCATTGGTTGTTTTG-3′ of 11 germplasms of *Gossypium hirsutum* L.

Germplasms	Total no. of bands with molecular weight (bp)	No. of light band with molecular weight (bp)	No. of bright band with molecular weight (bp)	No. of unique polymorphic band with molecular weight (bp)	No. of common band with molecular weight (bp)
CB-1	2 (2000, 50)	—	2 (2000, 50)	—	
CB-2	2 (2000, 50)	—	2 (2000, 50)	—	
CB-3	1 (50)	—	1 (50)	—	
CB-4	—	—	—	—	
CB-5	3 (2000, 123, 50)	—	3 (2000, 123, 50)	—	
CB-6	2 (2000, 50)	—	2 (2000, 50)	—	—
CB-7	1 (50)	1 (50)	—	—	
CB-8	2 (2000, 50)	—	2 (2000, 50)	—	
CB-9	3 (2000, 1968, 50)	—	3 (2000, 1968, 50)	1 (1968)	
CB-10	3 (2000, 123, 50)	—	3 (2000, 123, 50)	—	
CB-11	1 (50)	—	1 (50)	—	

Table 39. SSR analysis with primer pair Forward-5'-GCCTAGGTGGAGTTCGTG-3'-Reverse-5'-CTGAACCTGCTCCTGAATC-3' of 11 germplasms of *Gossypium hirsutum* L.

Germplasms	Total no. of bands with molecular weight (bp)	No. of light band with molecular weight (bp)	No. of bright band with molecular weight (bp)	No. of unique polymorphic band with molecular weight (bp)	No. of common band with molecular weight (bp)
CB-1	1 (123)	—	1 (123)	—	
CB-2	—	—	—	—	
CB-3	1 (123)	—	1 (123)	—	
CB-4	1 (123)	—	1 (123)	—	
CB-5	1 (123)	—	1 (123)	—	
CB-6	1 (123)	—	1 (123)	—	—
CB-7	—	—	—	—	
CB-8	1 (123)	—	1 (123)	—	
CB-9	—	—	—	—	
CB-10	1 (123)	—	1 (123)	—	
CB-11	—	—	—	—	

Table 40. SSR analysis with primer pair Forward-5′-CATCGGAAAACTCTGAAC-3′-Reverse-5′-GTAGCAGTACAGATGAAAGAG-3′ of 11 germplasms of *Gossypium hirsutum* L.

Germplasms	Total no. of bands with molecular weight (bp)	No. of light band with molecular weight (bp)	No. of bright band with molecular weight (bp)	No. of unique polymorphic band with molecular weight (bp)	No. of common band with molecular weight (bp)
CB-1	3 (246, 123, 50)	—	3 (246, 123, 50)	—	1 (50)
CB-2	1 (50)	—	1 (50)	—	
CB-3	2 (246, 50)	—	2 (246, 50)	—	
CB-4	2 (246, 50)	—	2 (246, 50)	—	
CB-5	4 (492, 246, 180, 50)	—	4 (492, 246, 180, 50)	1 (492)	
CB-6	3 (246, 180, 50)	—	3 (246, 180, 50)	—	
CB-7	3 (246, 180, 50)	—	3 (246, 180, 50)	—	
CB-8	3 (246, 180, 50)	—	3 (246, 180, 50)	—	
CB-9	3 (246, 180, 50)	—	3 (246, 180, 50)	—	
CB-10	1 (50)	—	1 (50)	—	
CB-11	2 (246, 50)	—	2 (246, 50)	—	

Table 41. Compilation of RAPD analysis in 11 germplasms of *Gossypium hirsutum* L.

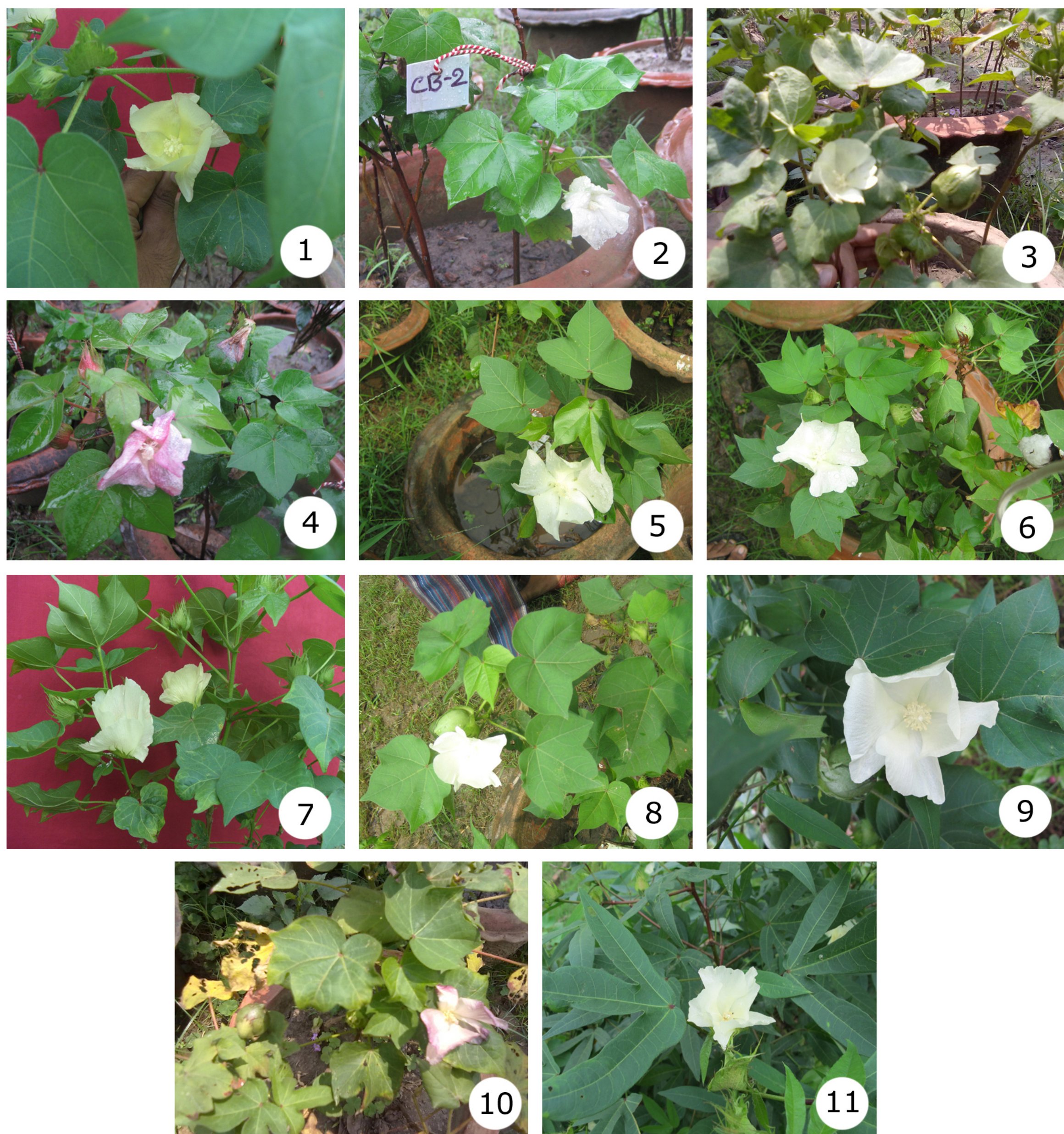
Primer codes	Sequences (5′—3′)	Total bands	Size ranges (bp)	Number of Polymorphic bands	Number and size (bp) of germplasms specific unique bands	Polymorphisms (%)
OPA-1	5′-CAG GCC CTT C-3′	31	250-2000	31	CB-4 (2000) CB-9 (1400, 400,300)	100
OPA-3	5′-AGT CAG CCA C-3′	37	600-7000	37	CB-3 (1600) CB-11 (1500, 750, 600)	100
OPA-4	5′-AAT CGG GCT G-3′	65	300-4000	65	CB-11 (4000, 3000, 1700)	100
OPA-6	5′-GGT CCC TGA C-3′	11	500-2000	11	CB-9 (2000) CB-11 (1300)	100
OPA-7	5′-GAA ACG GGT G-3′	09	500-1700	09	CB-6 (500)	100
OPA-10	5′-GTG ATC GCA G-3′	44	500-2400	44	CB-10 (1400)	100
Primer-1	5′-GAA ACG GGT G-3′	31	850-2750	31	CB-1 (1600) CB-11 (2750, 1000)	100
Primer-2	5′-GTT GCG ATC C-3′	21	200-2500	21	CB-9 (450) CB-10 (2500)	100
Primer-18	5′-GTT TCG CTC C-3′	49	400-5000	49	CB-9 (600,500) CB-10 (1750) CB-11 (1600)	100
Primer-23	5′-GTC AGG GCA A-3′	37	600-2000	37	CB-1 (2000, 1000, 800, 650) CB-9 (600)	100
Grand Total:		**335**	**200-7000**	**335**	**29**	100

Table 42. Compilation of SSR analysis in 11 germplasms of *Gossypium hirsutum* L.

Primer codes	Sequences (5′—3′)	Total bands	Size ranges (bp)	Number and size (bp) of germplasms specific unique bands	Polymo-rphic bands	Polymorph-isms (%)
BA00175679 BA00175680	forward-5′-GCTTCTTCCATTTTATTCAAG-3′ reverse-5′-CAGCGGCAACCAAAAAG-3′	01	180-180	-	01	100.00
BA00175681 BA00175682	forward-5′-ACCCTTAAATCATAAGAGAAC-3′ reverse-5′-CCGTAAGTTAAGGTACAAGG-3′	14	50-738	CB-9 (738)	03	21.42
BA00175683 BA00175684	forward-5′-GGAGTTAAAGCTAATGCCTG-3′ reverse-5′-CGGGTCATTGGTTGTTTTTG-3′	20	50-2000	CB-9 (1968)	20	100.00
BA00175685 BA00175686	pair forward-5′-GCCTAGGTGGAGTTCGTG-3′ reverse-5′-CTGAACCTGCTCCTGAATC-3′	07	123-123	-	-	00.00
BA00175677 BA00175678	forward-5′-CATCGGAAAACTCTGAAC-3′ reverse-5′-GTAGCAGTACAGATGAAAGAG-3′	27	50-492	CB-1 (123) CB-5 (492)	16	59.26
Grand total		**69**	**50-2000**	**4**	**39**	**56.52**

Table 43. Summary of Nei's (1972) genetic distances of 11 germplasms of *Gossypium hirsutum* L.

Germpl-asms	CB-1	CB-2	CB-3	CB-4	CB-5	CB-6	CB-7	CB-8	CB-9	CB-10	CB-11
CB-1	****										
CB-2	0.1975	****									
CB-3	0.2446	0.1417	****								
CB-4	0.3196	0.2091	0.1417	****							
CB-5	0.3594	0.2690	0.2446	0.2446	****						
CB-6	0.3868	0.3196	0.3196	0.2940	0.1861	****					
CB-7	0.4008	0.3327	0.3594	0.2567	0.3460	0.2446	****				
CB-8	0.3460	0.3594	0.3327	0.2326	0.3196	0.1975	0.0991	****			
CB-9	0.5691	0.4587	0.4008	0.3730	0.4439	0.4439	0.3730	0.3460	****		
CB-10	0.5859	0.4439	0.4439	0.4439	0.4587	0.3730	0.4149	0.3868	0.5859	****	
CB-11	0.7514	0.6931	0.7316	0.8133	0.8348	0.6745	0.5526	0.6931	0.8348	0.6204	****



Figs. 1-11. Morphology of eleven germplasms of *Gossypium hirsutum* L. 1. CB-1, 2. CB-2, 3. CB-3, 4. CB-4, 5. CB-5, 6. CB-6, 7. CB-7, 8. CB-8, 9. CB-9, 10. CB-10 and 11. CB-11.

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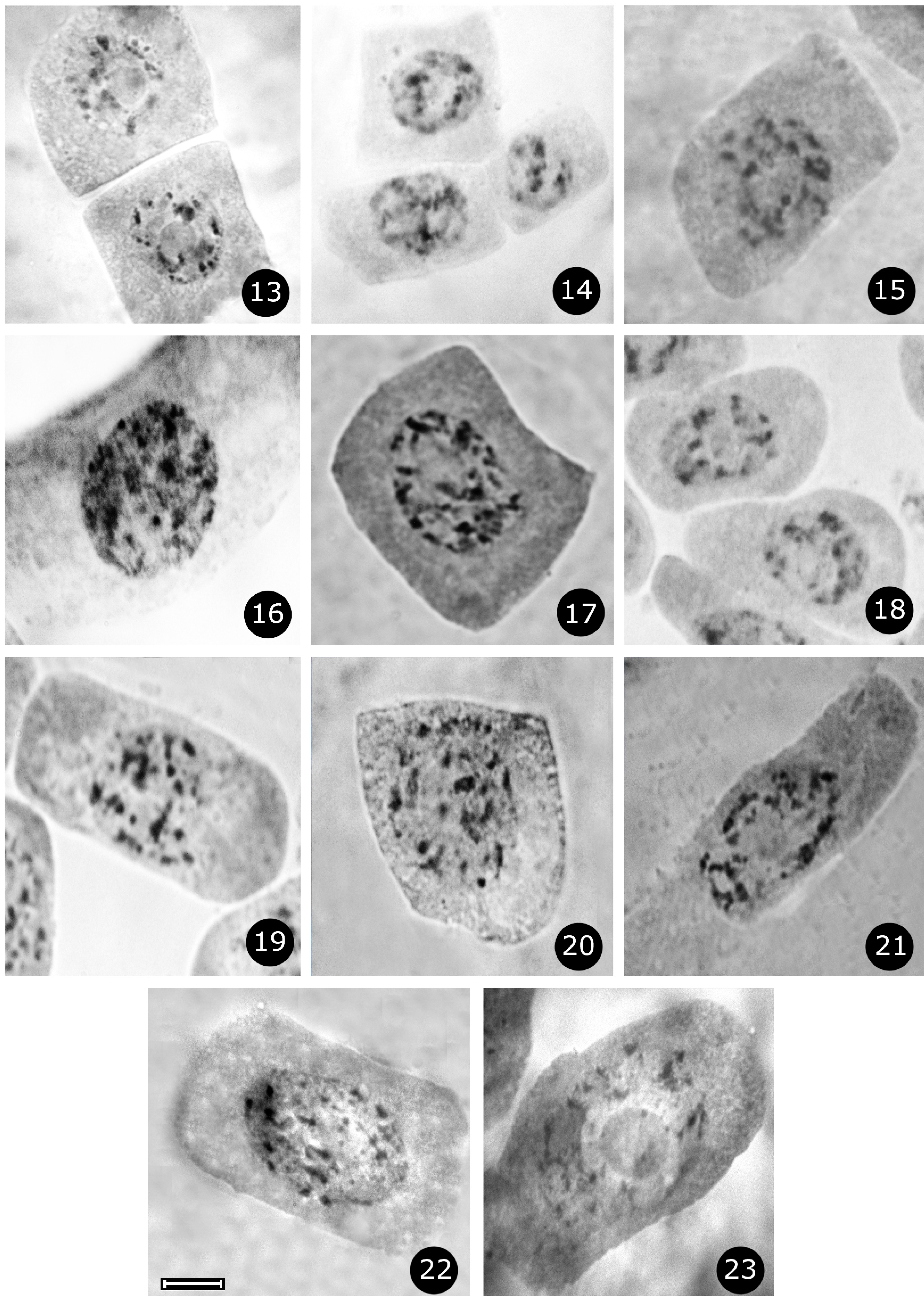
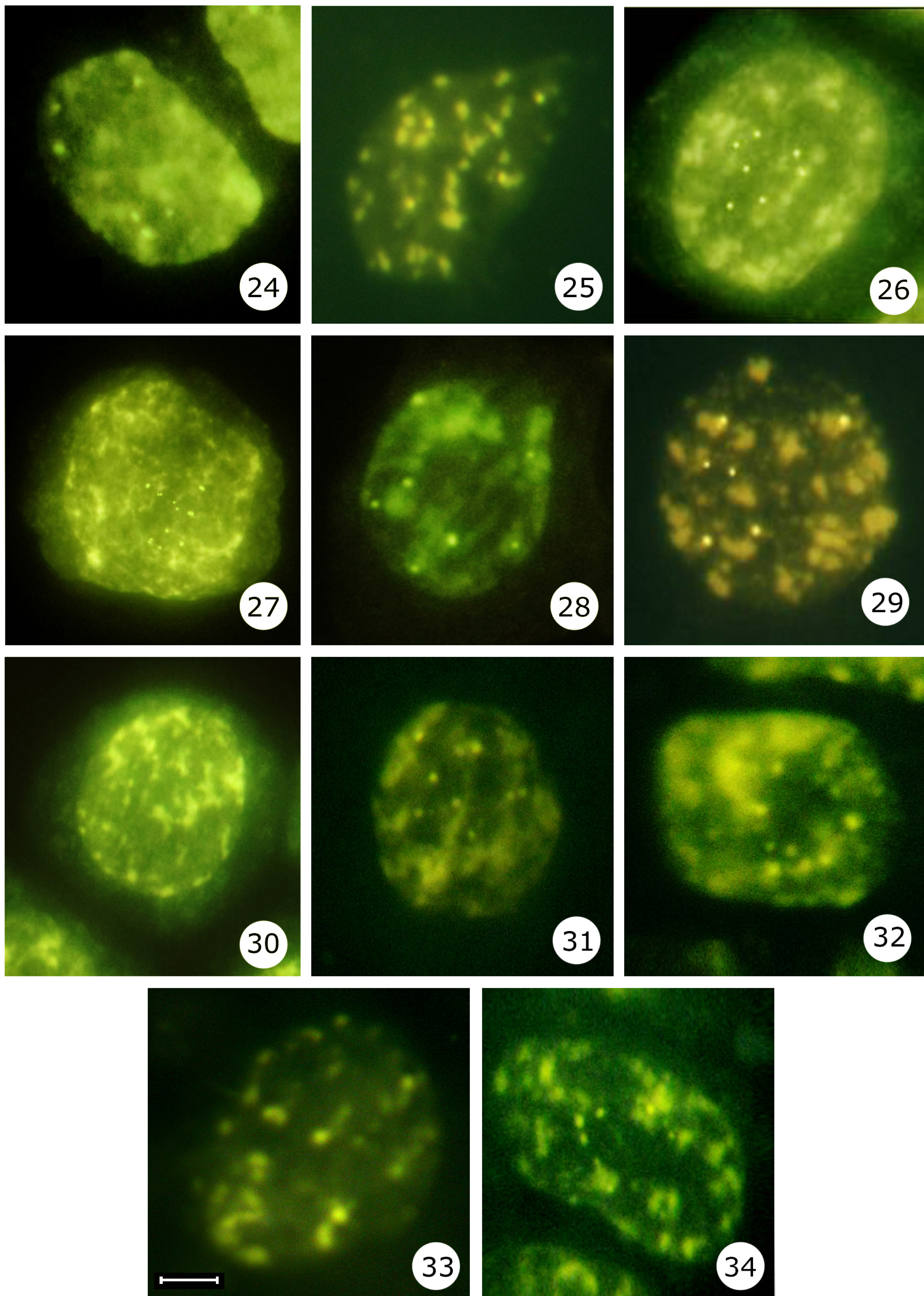
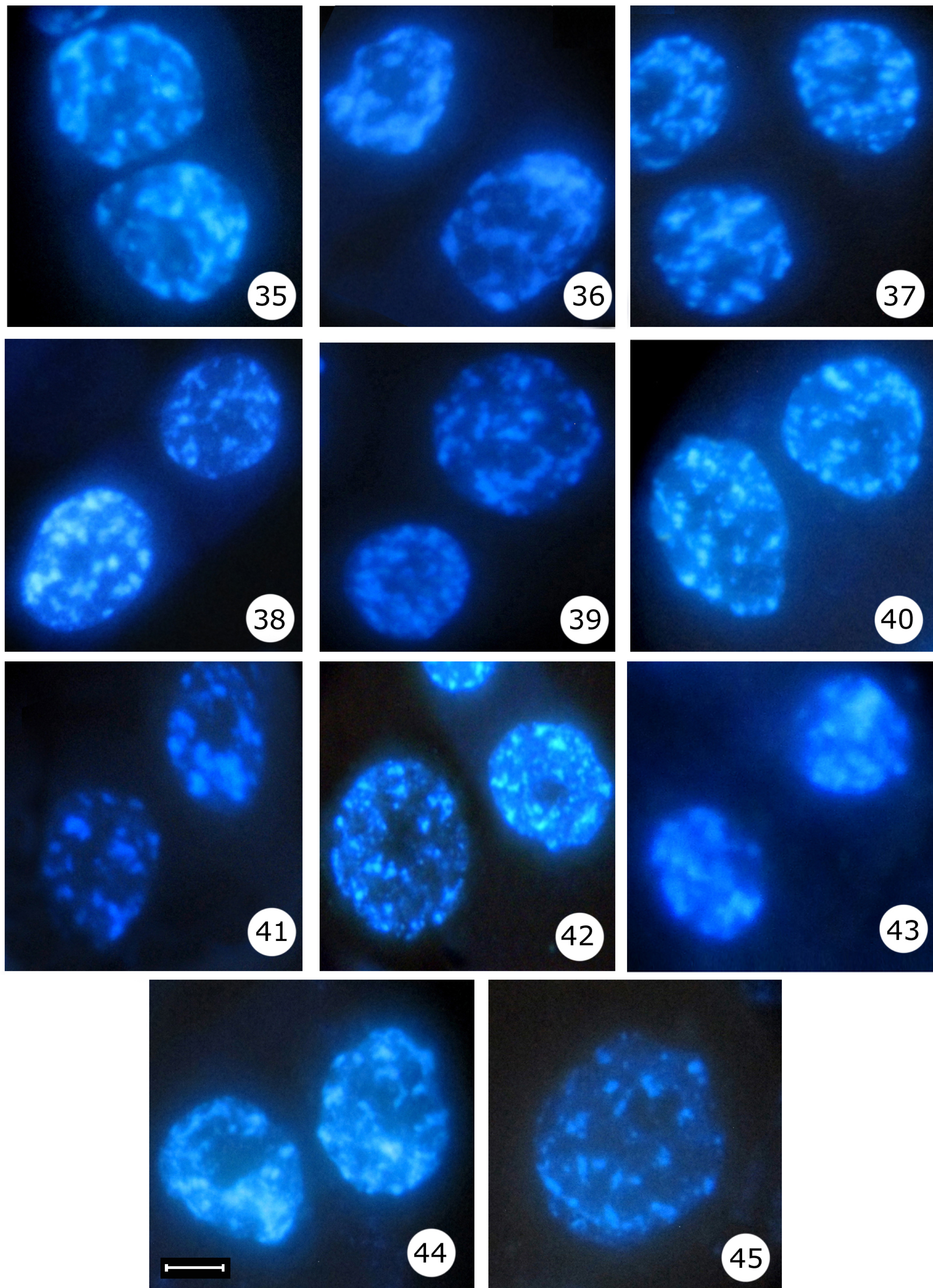
Fig. 12. Development of bloom in *Gossypium hirsutum* L. germplasms. i. A white flower emerges on day-1, ii. Gradually darkens and takes pink colour during days-2,3 and 4, iii. The bloom eventually dries up and falls off.



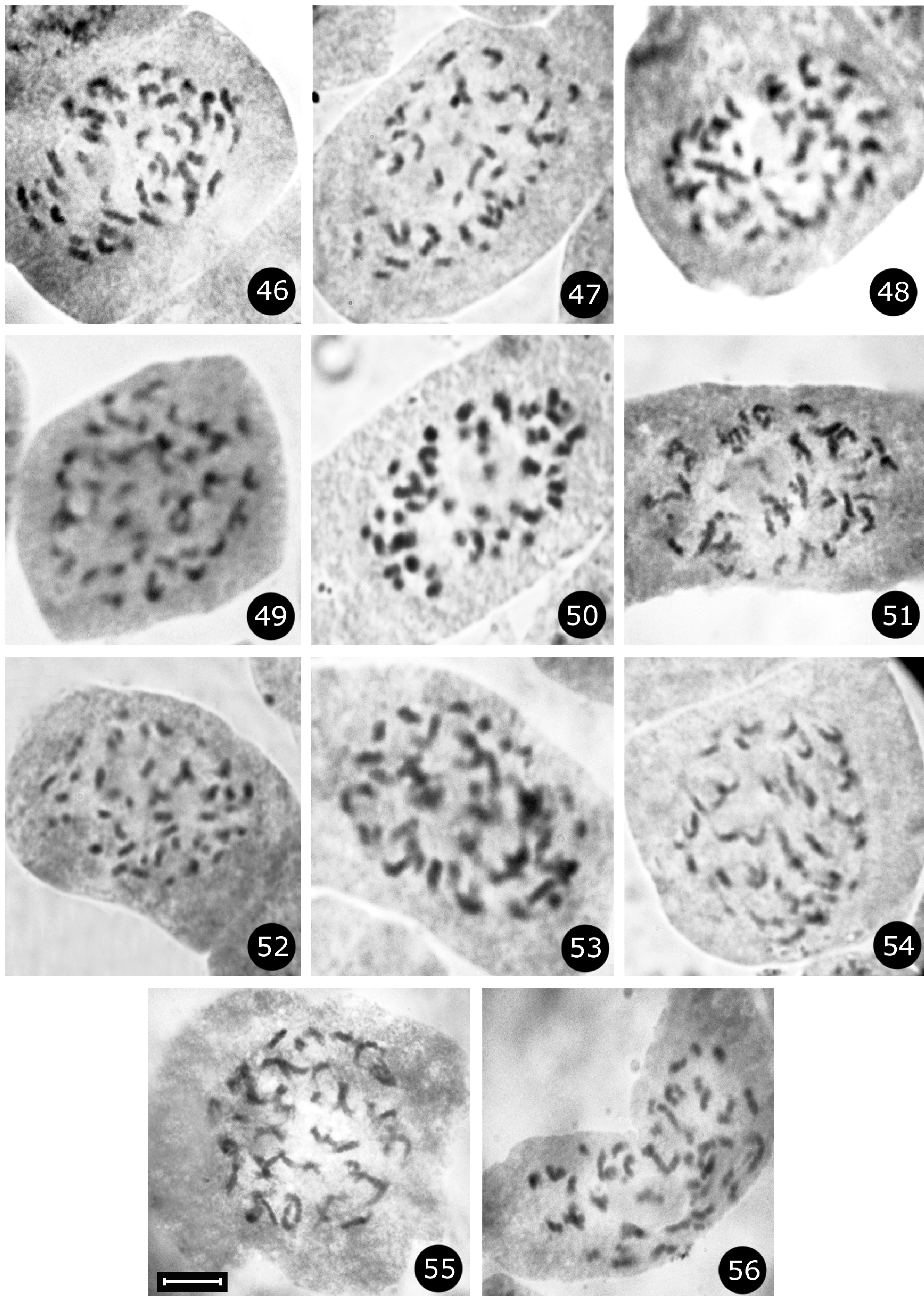
Figs. 13-23. Orcein-stained mitotic interphase nuclei of eleven germplasms of *Gossypium hirsutum* L. 13. CB-1, 14. CB-2, 15. CB-3, 16. CB-4, 17. CB-5, 18. CB-6, 19. CB-7, 20. CB-8, 21. CB-9, 22. CB-10 and 23. CB-11. Bar = 5 µm.



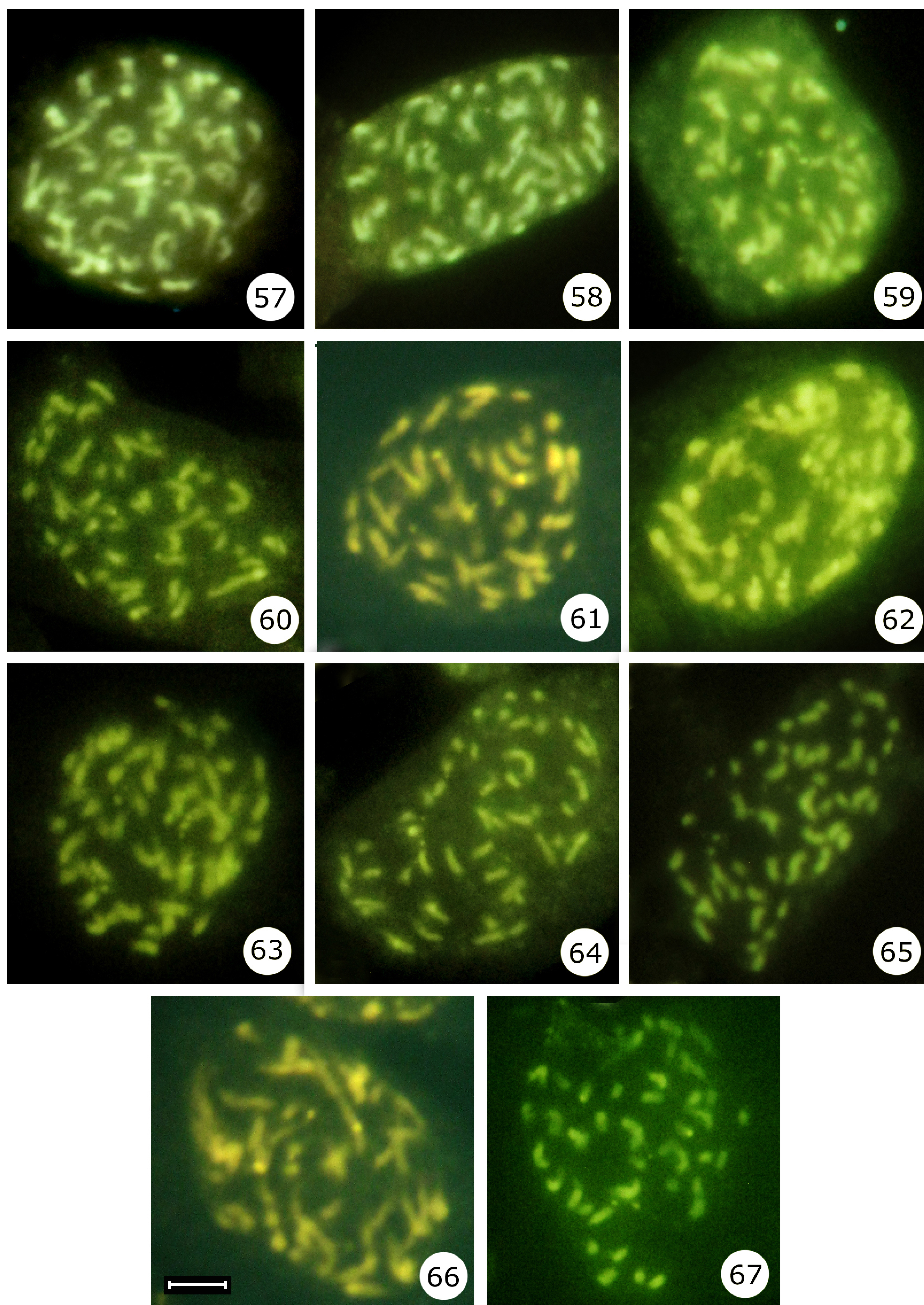
Figs. 24-34. CMA-stained mitotic interphase nuclei of eleven germplasms of *Gossypium hirsutum* L. 24. CB-1, 25. CB-2, 26. CB-3, 27. CB-4, 28. CB-5, 29. CB-6, 30. CB-7, 31. CB-8, 32. CB-9, 33. CB-10 and 34. CB-11. Bar = 5 µm.



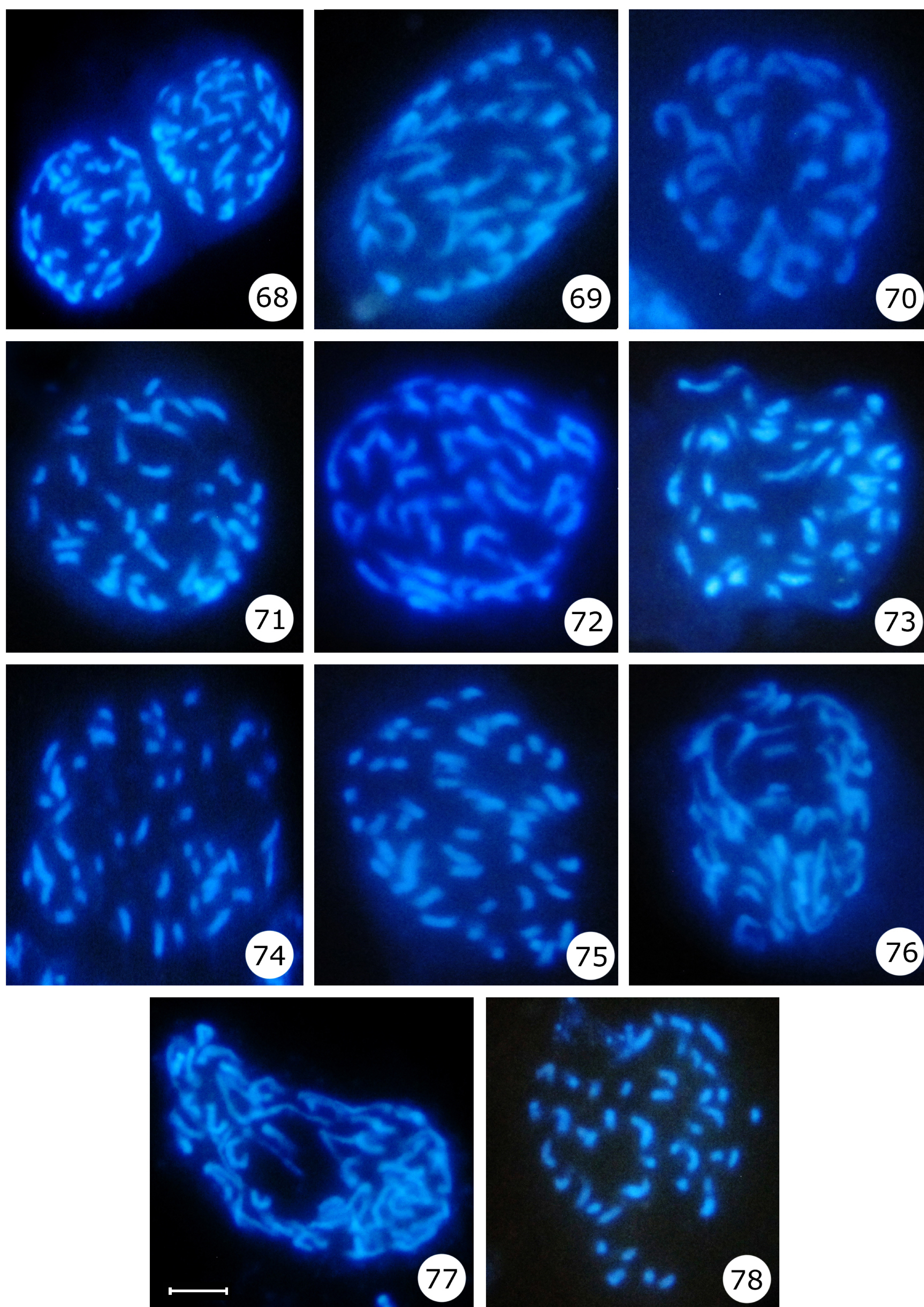
Figs. 35-45. DAPI-stained mitotic interphase nuclei of eleven germplasms of *Gossypium hirsutum* L. 35. CB-1, 36. CB-2, 37. CB-3, 38. CB-4, 39. CB-5, 40. CB-6, 41. CB-7, 42. CB-8, 43. CB-9, 44. CB-10 and 45. CB-11. Bar = 5 µm.



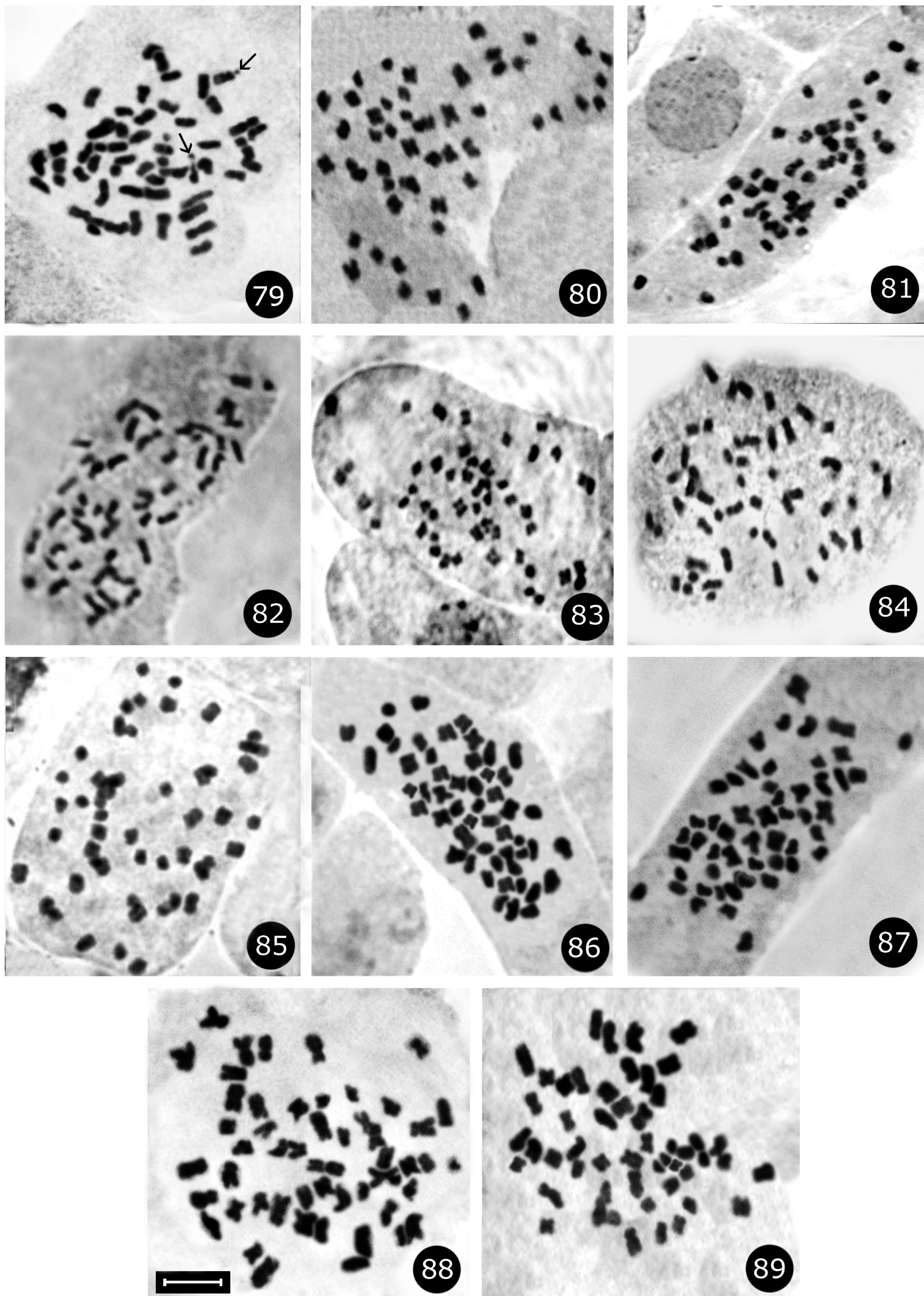
Figs. 46-56. Orcein-stained mitotic prophase chromosomes of eleven germplasms of *Gossypium hirsutum* L. 46. CB-1, 47. CB-2, 48. CB-3, 49. CB-4, 50. CB-5, 51. CB-6, 52. CB-7, 53. CB-8, 54. CB-9, 55. CB-10 and 56. CB-11. Bar = 5 µm.



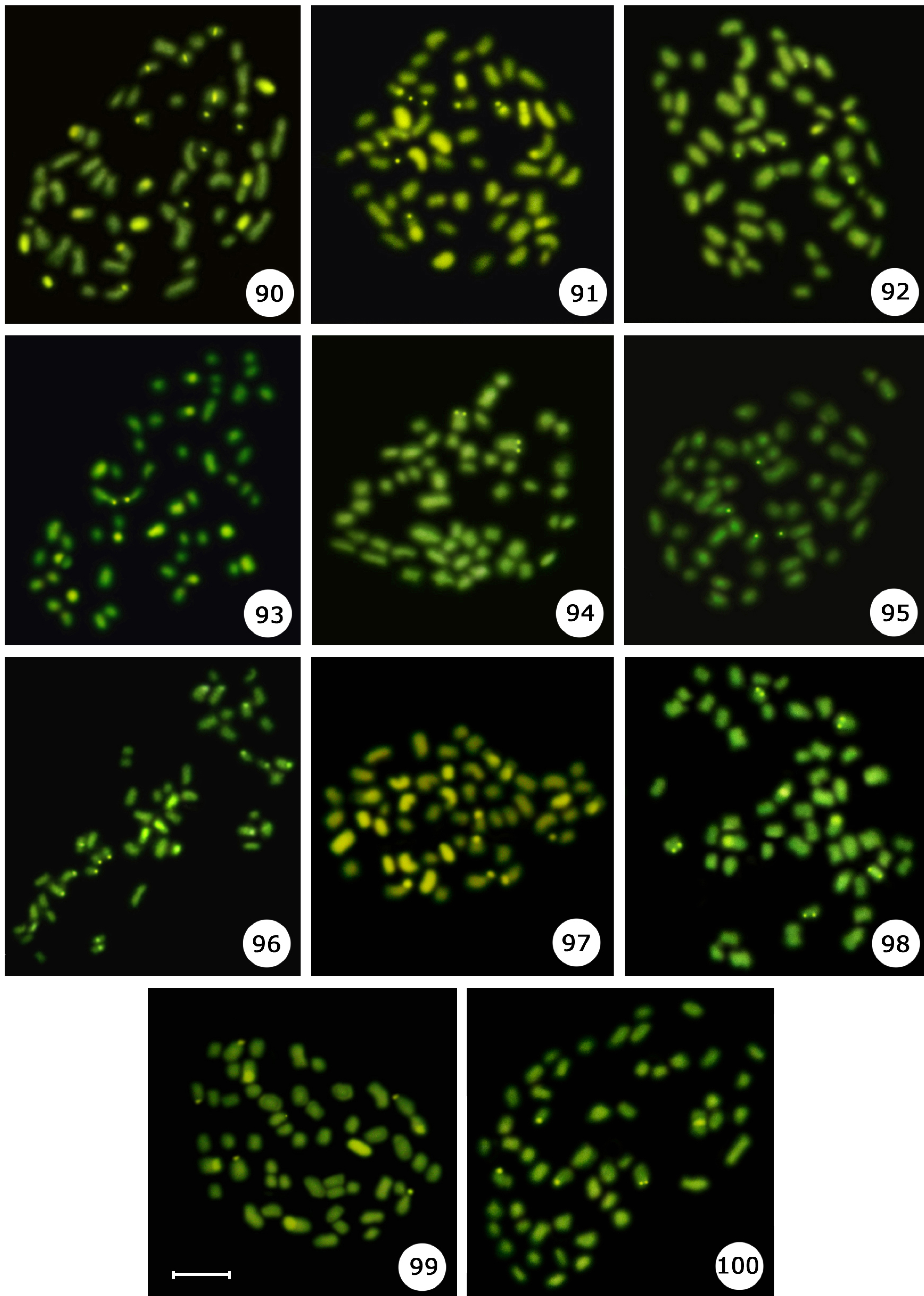
Figs. 57-67. CMA-stained mitotic prophase chromosomes of eleven germplasms of *Gossypium hirsutum* L. 57. CB-1, 58. CB-2, 59. CB-3, 60. CB-4, 61. CB-5, 62. CB-6, 63. CB-7, 64. CB-8, 65. CB-9, 66. CB-10 and 67. CB-11. Bar = 5 µm.



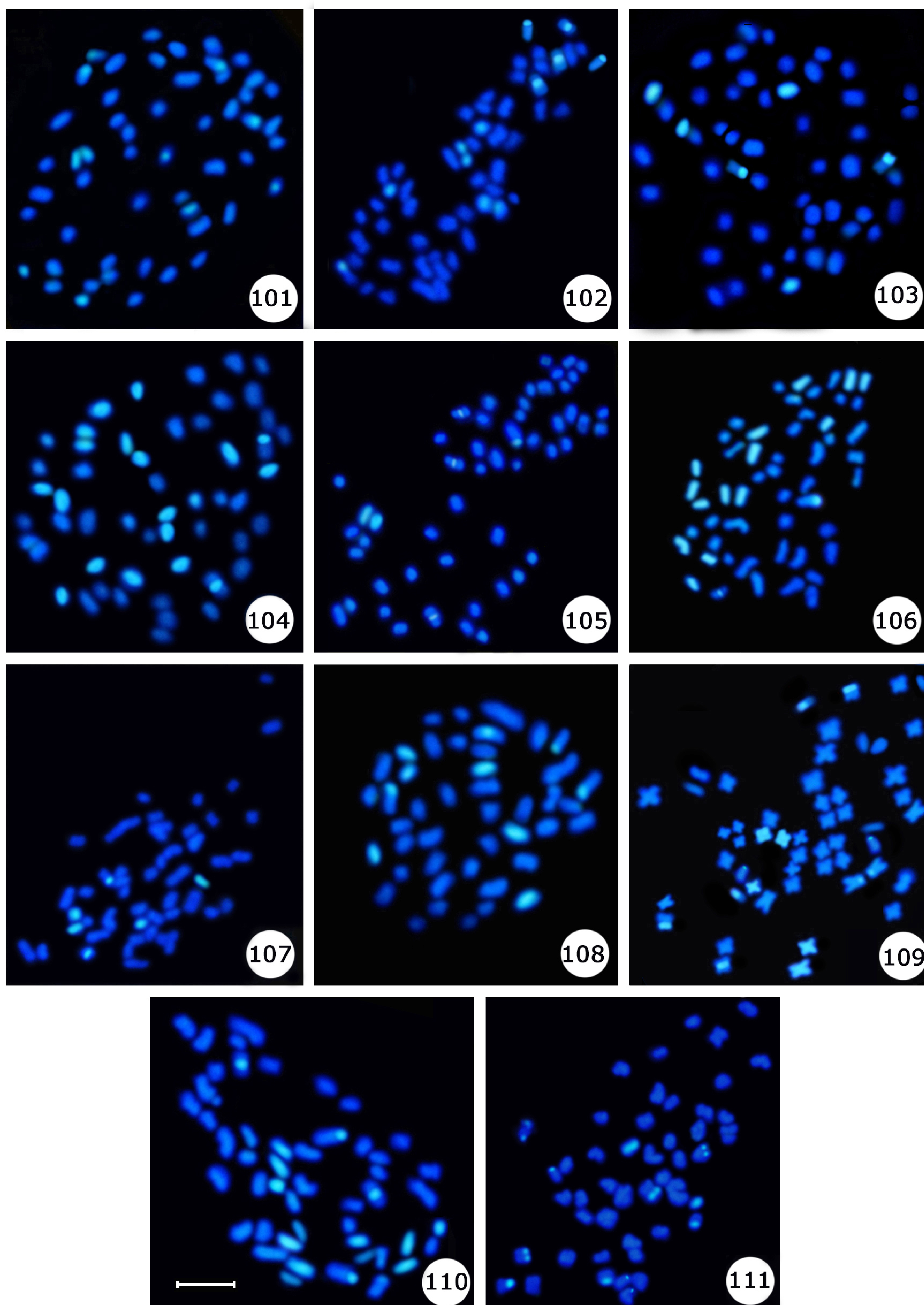
Figs. 68-78. DAPI-stained mitotic prophase chromosomes of eleven germplasms of *Gossypium hirsutum* L. 68. CB-1, 69. CB-2, 70. CB-3, 71. CB-4, 72. CB-5, 73. CB-6, 74. CB-7, 75. CB-8, 76. CB-9, 77. CB-10 and 78. CB-11. Bar = 5 µm.



Figs. 79-89. Orcein-stained mitotic metaphase chromosomes of eleven germplasms of *Gossypium hirsutum* L. 79. CB-1, 80. CB-2, 81. CB-3, 82. CB-4, 83. CB-5, 84. CB-6, 85. CB-7, 86. CB-8, 87. CB-9, 88. CB-10 and 89. CB-11. Bar = 5 µm.

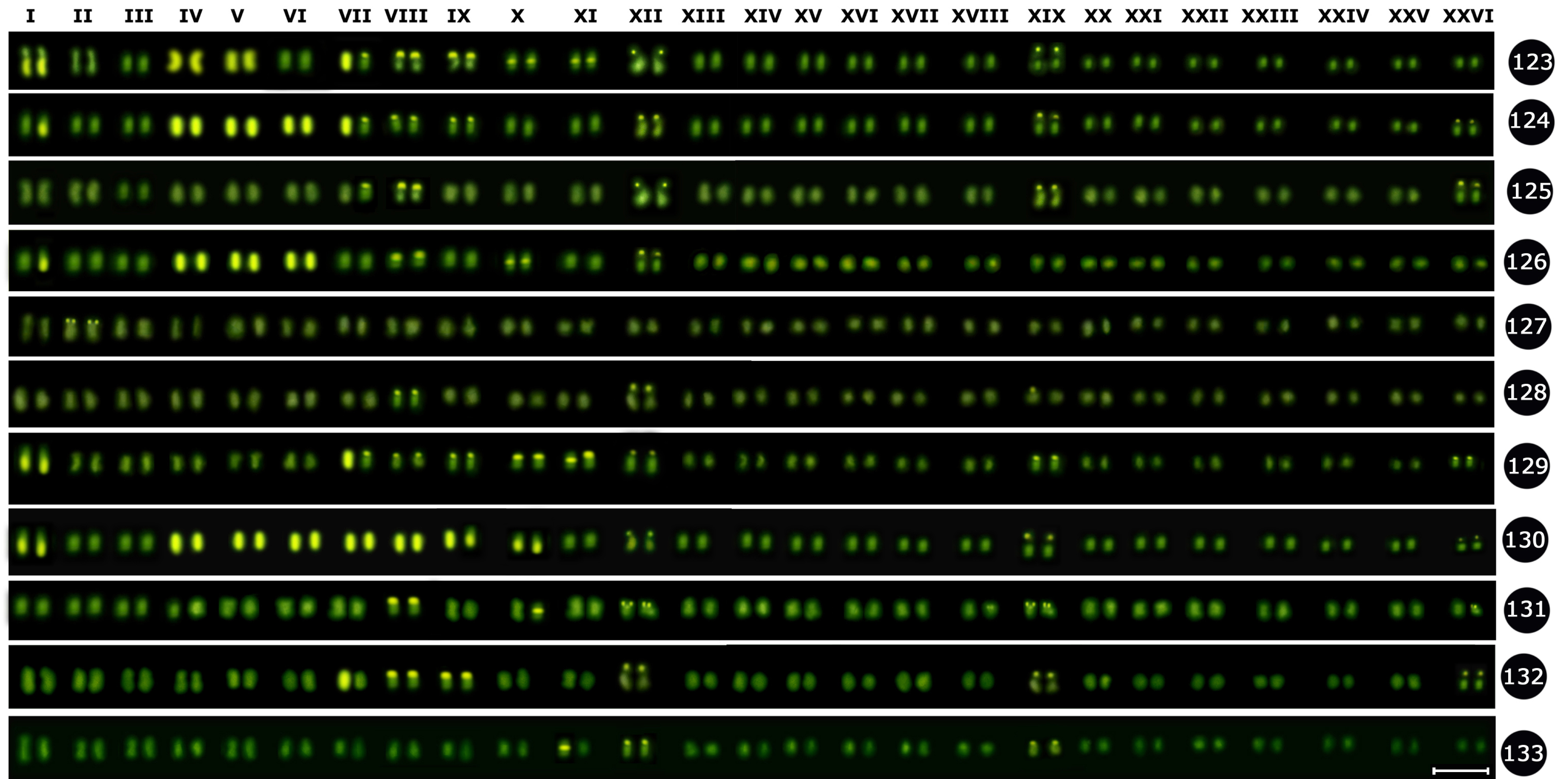


Figs. 90-100. CMA-stained mitotic metaphase chromosomes of eleven germplasms of *Gossypium hirsutum* L. 90. CB-1, 91. CB-2, 92. CB-3, 93. CB-4, 94. CB-5, 95. CB-6, 96. CB-7, 97. CB-8, 98. CB-9, 99. CB-10 and 100. CB-11. Bar = 5 µm.

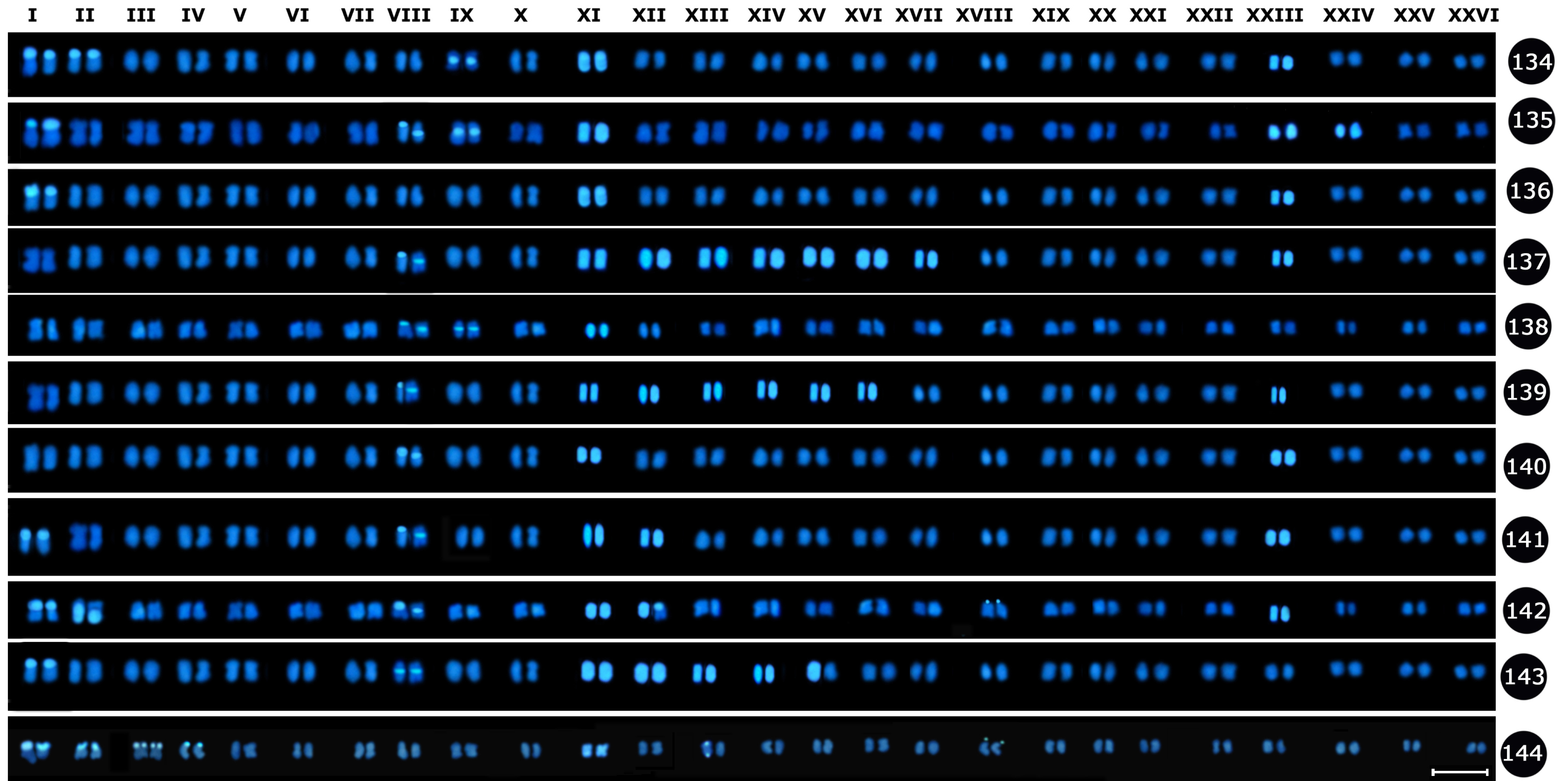


Figs. 101-111. DAPI-stained mitotic metaphase chromosomes of eleven germplasms of *Gossypium hirsutum* L. 101. CB-1, 102. CB-2, 103. CB-3, 104. CB-4, 105. CB-5, 106. CB-6, 107. CB-7, 108. CB-8, 109. CB-9, 110. CB-10 and 111. CB-11. Bar = 5 μm.

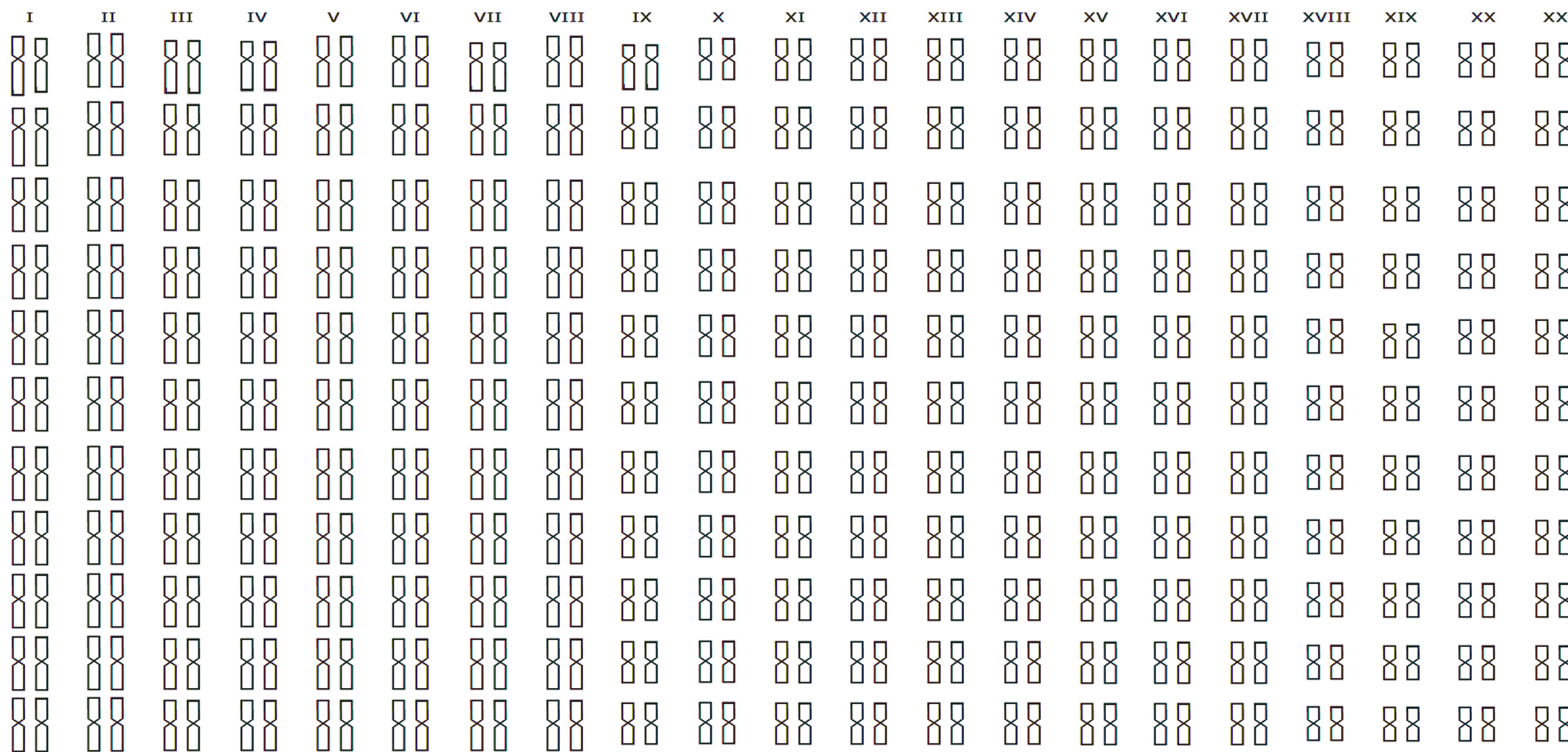
Figs. 112-122. Karyotypes prepared from orcein-stained mitotic metaphase chromosomes of eleven germplasms of *Gossypium hirsutum* L. 112. CB-1, 113. CB-2, 114. CB-3, 115. CB-4, 116. CB-5, 117. CB-6, 118. CB-7, 119. CB-8, 120. CB-9, 121. CB-10 and 122. CB-11. Bar = 5 μm.



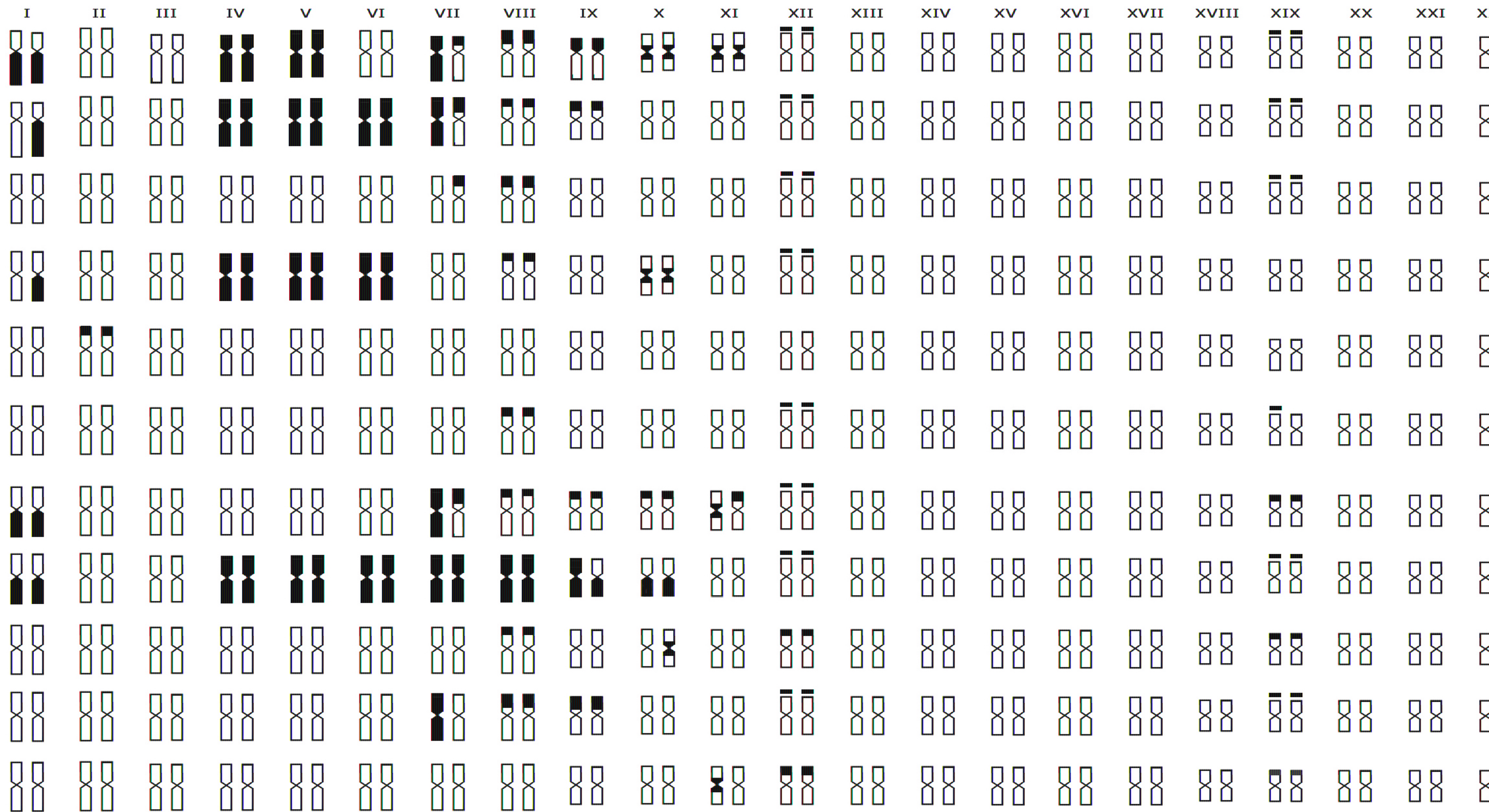
Figs. 123-133. Karyotypes prepared from CMA-stained mitotic metaphase chromosomes of eleven germplasms of *Gossypium hirsutum* L. 123. CB-1, 124. CB-2, 125. CB-3, 126. CB-4, 127. CB-5, 128. CB-6, 129. CB-7, 130. CB-8, 131. CB-9, 132. CB-10 and 133. CB-11. Bar = 5 µm.



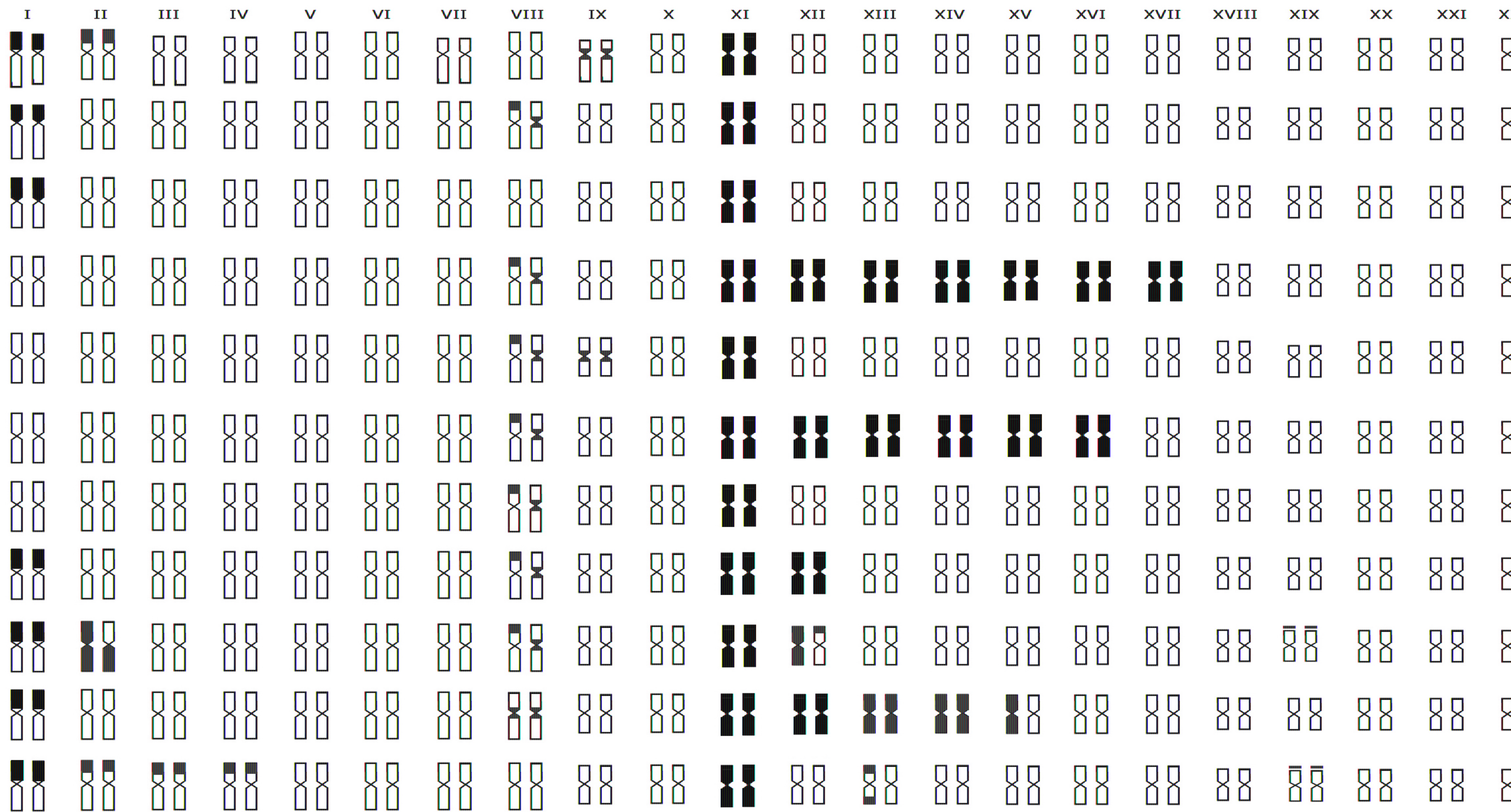
Figs. 134-144. Karyotypes prepared from DAPI-stained mitotic metaphase chromosomes of eleven germplasms of *Gossypium hirsutum* L. 134. CB-1, 135. CB-2, 136. CB-3, 137. CB-4, 138. CB-5, 139. CB-6, 140. CB-7, 141. CB-8, 142. CB-9, 143. CB-10 and 144. CB-11. Bar = 5 µm.



Figs. 145-155. Idiograms prepared from orcein-stained mitotic metaphase chr
germplasms of *Gossypium hirsutum* L. 145. CB-1, 146. CB-2, 147. CB-3, 1
150. CB-6, 151. CB-7, 152. CB-8, 153. CB-9, 154. CB-10 and 155. CB-11. Ba



Figs. 156-166. Idiograms prepared from CMA-stained mitotic metaphase chr
germplasms of *Gossypium hirsutum* L. 156. CB-1, 157. CB-2, 158. CB-3, 1
161. CB-6, 162. CB-7, 163. CB-8, 164. CB-9, 165. CB-10 and 166. CB-11. Ba



Figs. 167-177. Idiograms prepared from DAPI-stained mitotic metaphase chr germplasms of *Gossypium hirsutum* L. 167. CB-1, 168. CB-2, 169. CB-3, 172. CB-6, 173. CB-7, 174. CB-8, 175. CB-9, 176. CB-10 and 177. CB-11. Ba

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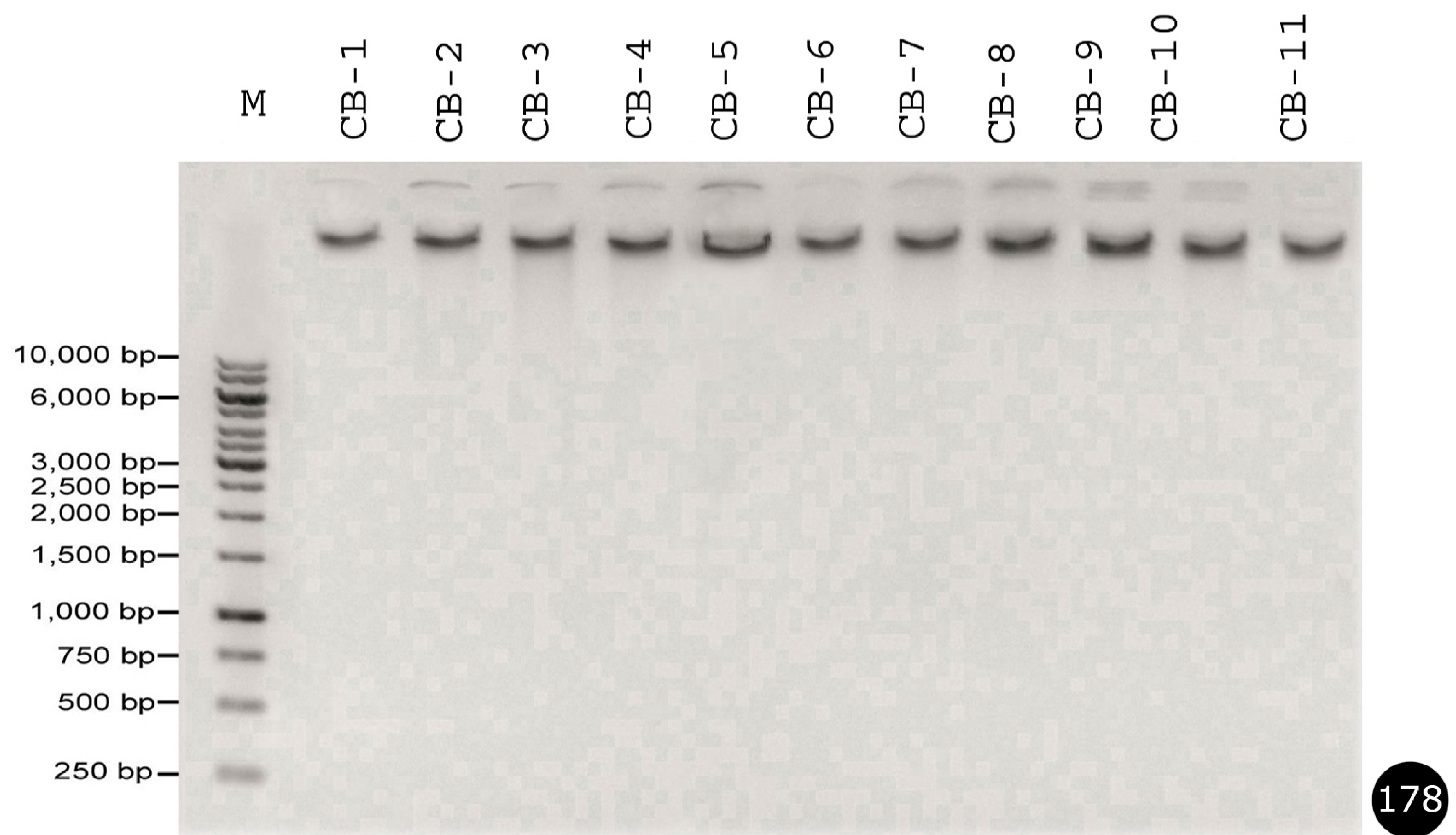

Fig. 178. Electrophoregram of Ethidium Bromide stained genomic DNA samples of 11 germplasms of *Gossypium hirsutum* L. M=1 Kb DNA ladder.

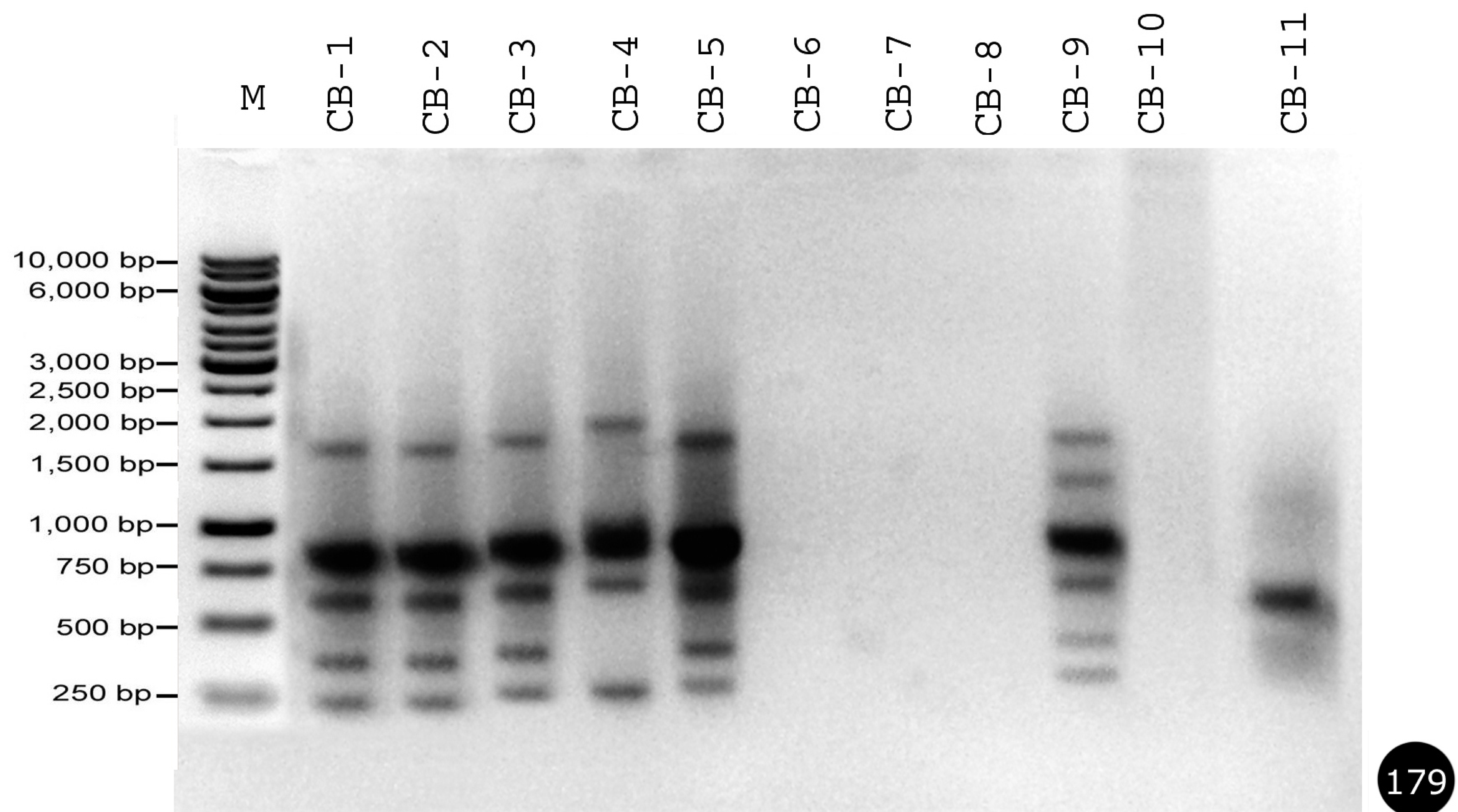

Fig. 179. RAPD analysis with primer OPA-1 (5′-CAG GCC CTT C-3′) of eleven germplasms of *Gossypium hirsutum* L. M=1 Kb DNA ladder.

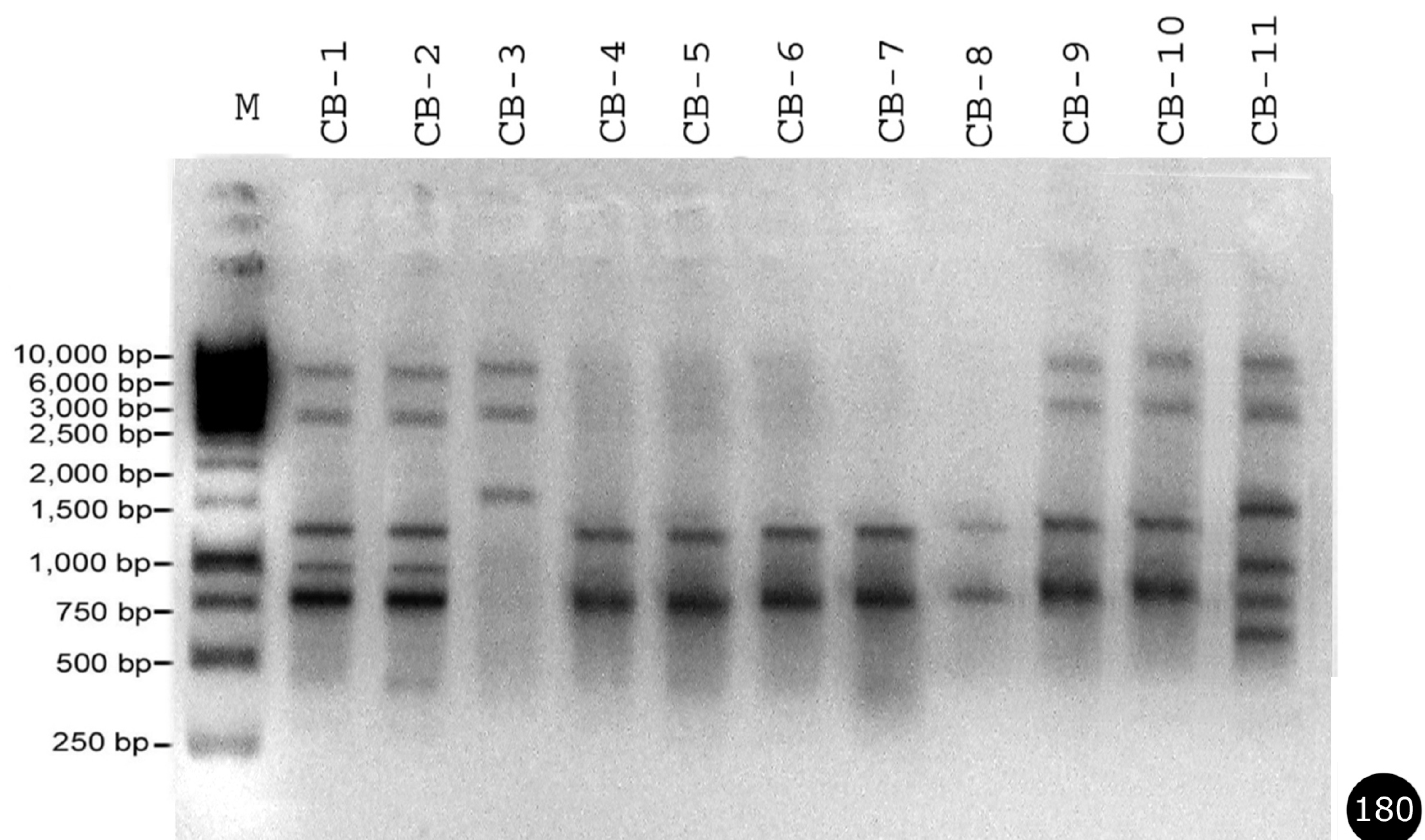

Fig. 180. RAPD analysis with primer OPA-3 (5′-AGT CAG CCA C-3′) of eleven germplasms of *Gossypium hirsutum* L. M=1 Kb DNA ladder.

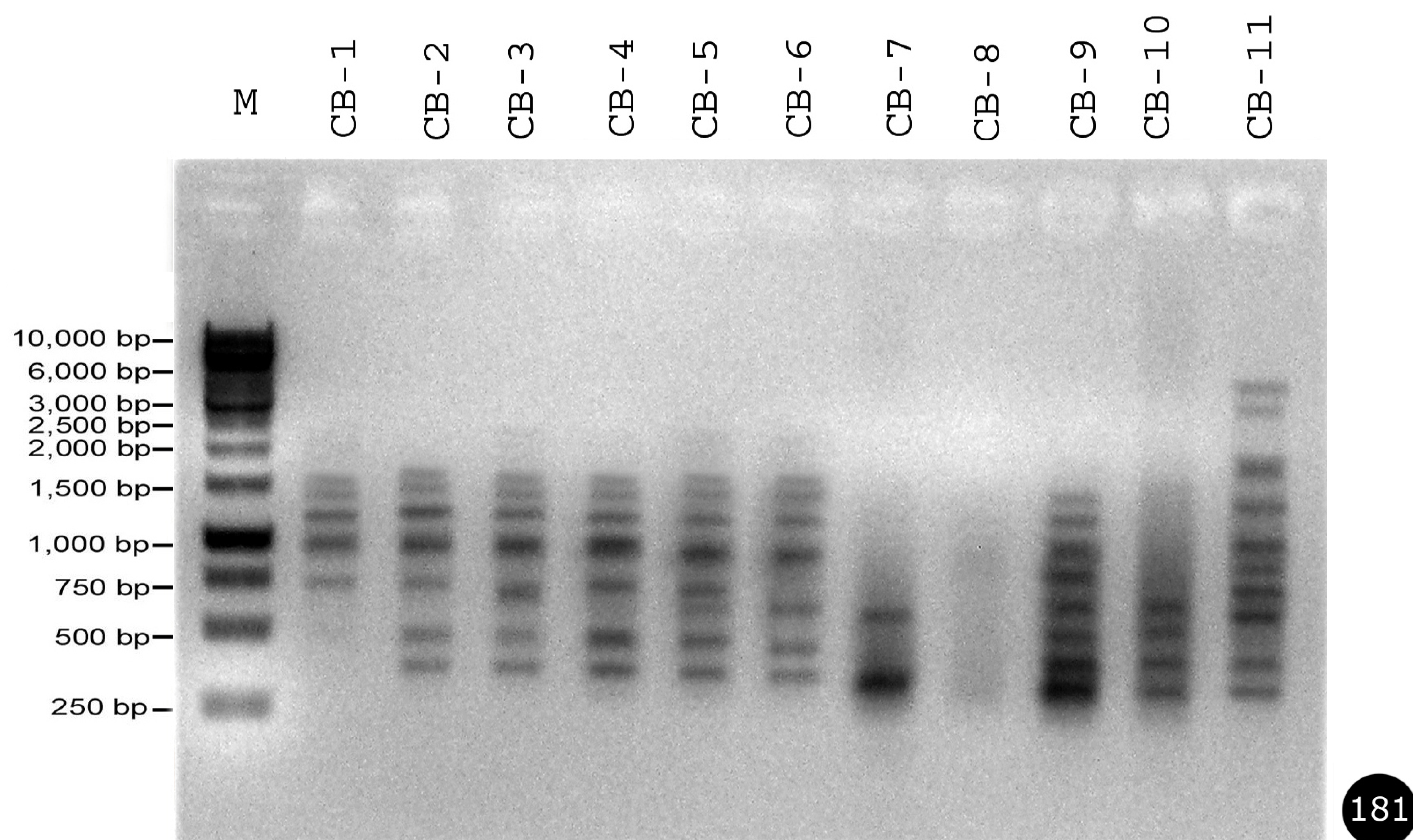

Fig. 181. RAPD analysis with primer OPA-4 (5′-AAT CGG GCT G-3′) of eleven germplasms of *Gossypium hirsutum* L. M=1 Kb DNA ladder.

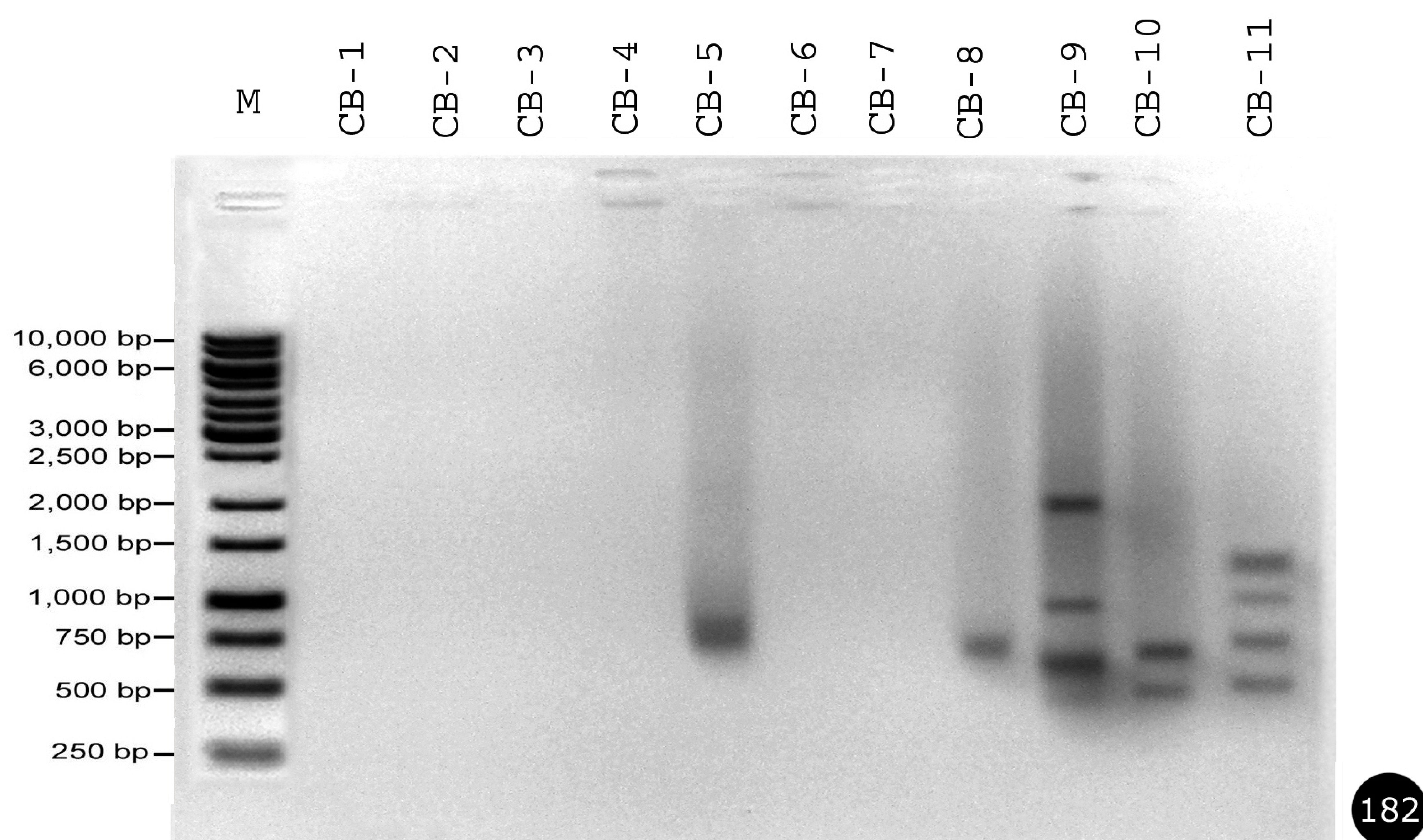

Fig. 182. RAPD analysis with primer OPA-6 (5′-GGT CCC TGA C-3′) of eleven germplasms of *Gossypium hirsutum* L. M=1 Kb DNA ladder.

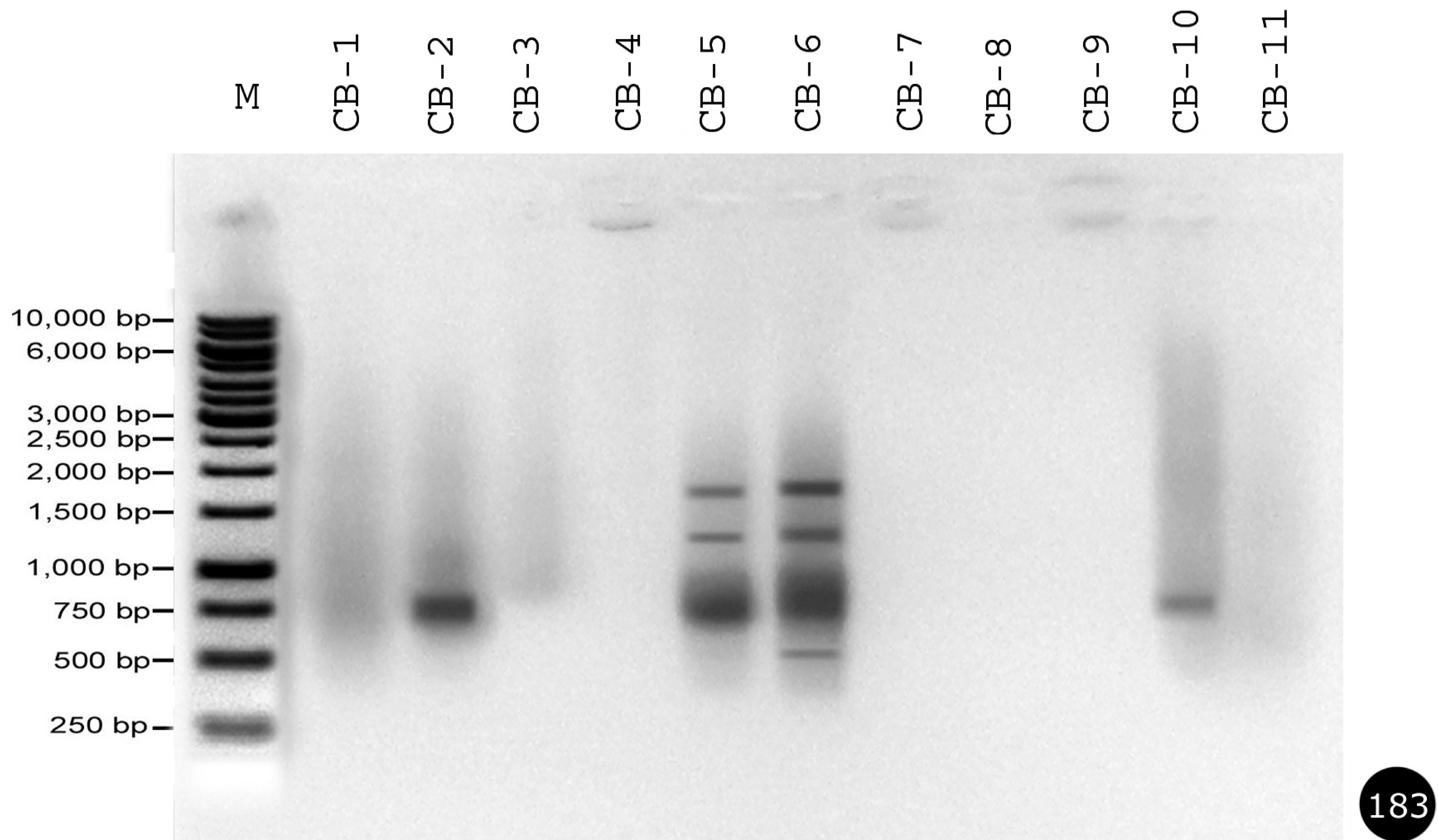

Fig. 183. RAPD analysis with primer OPA-7 (5′-GAA ACG GGT G-3′) of eleven germplasms of *Gossypium hirsutum* L. M=1 Kb DNA ladder.

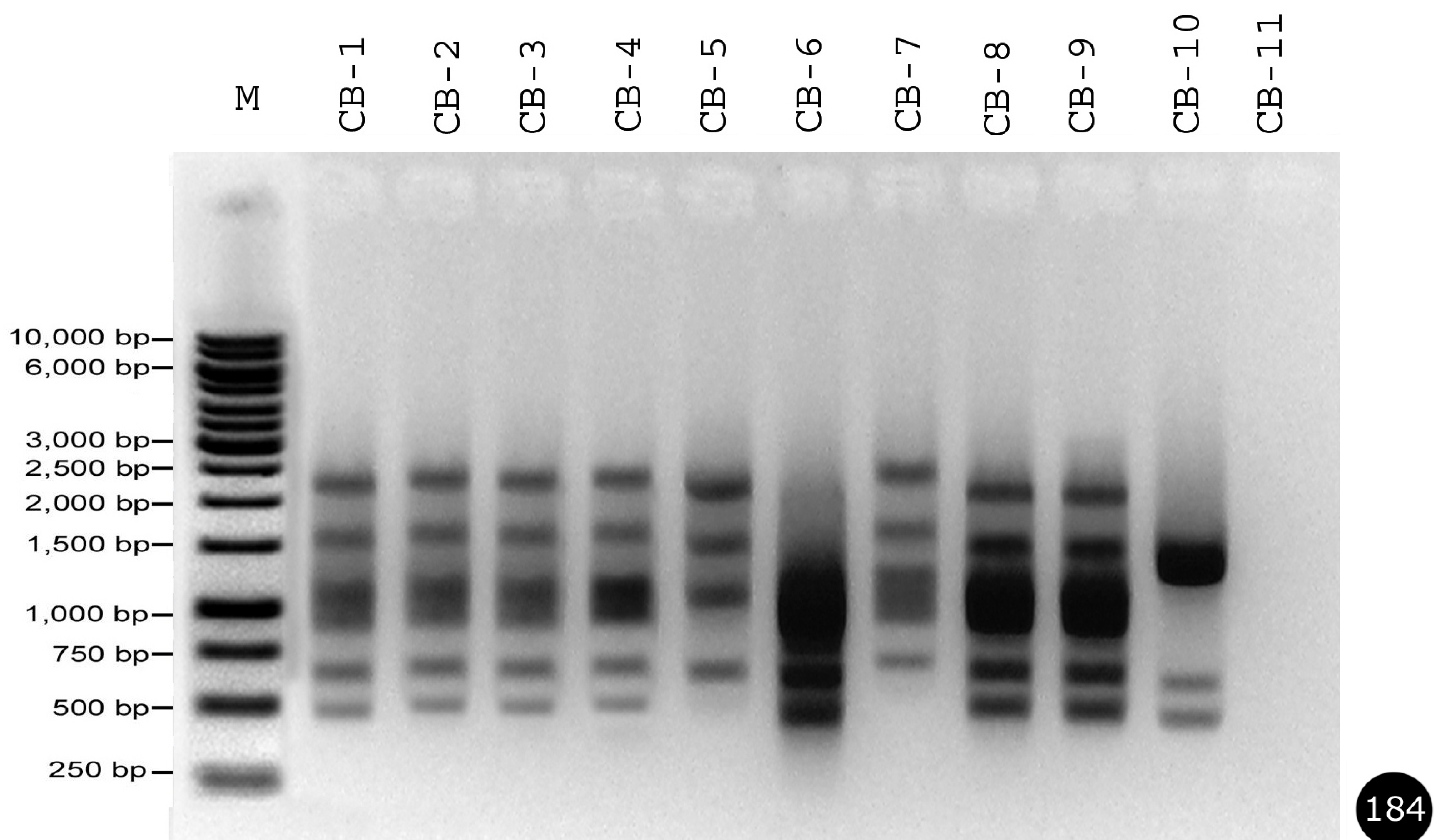

Fig. 184. RAPD analysis with primer OPA-10 (5′-GTG ATC GCA G-3′) of eleven germplasms of *Gossypium hirsutum* L. M=1 Kb DNA ladder.

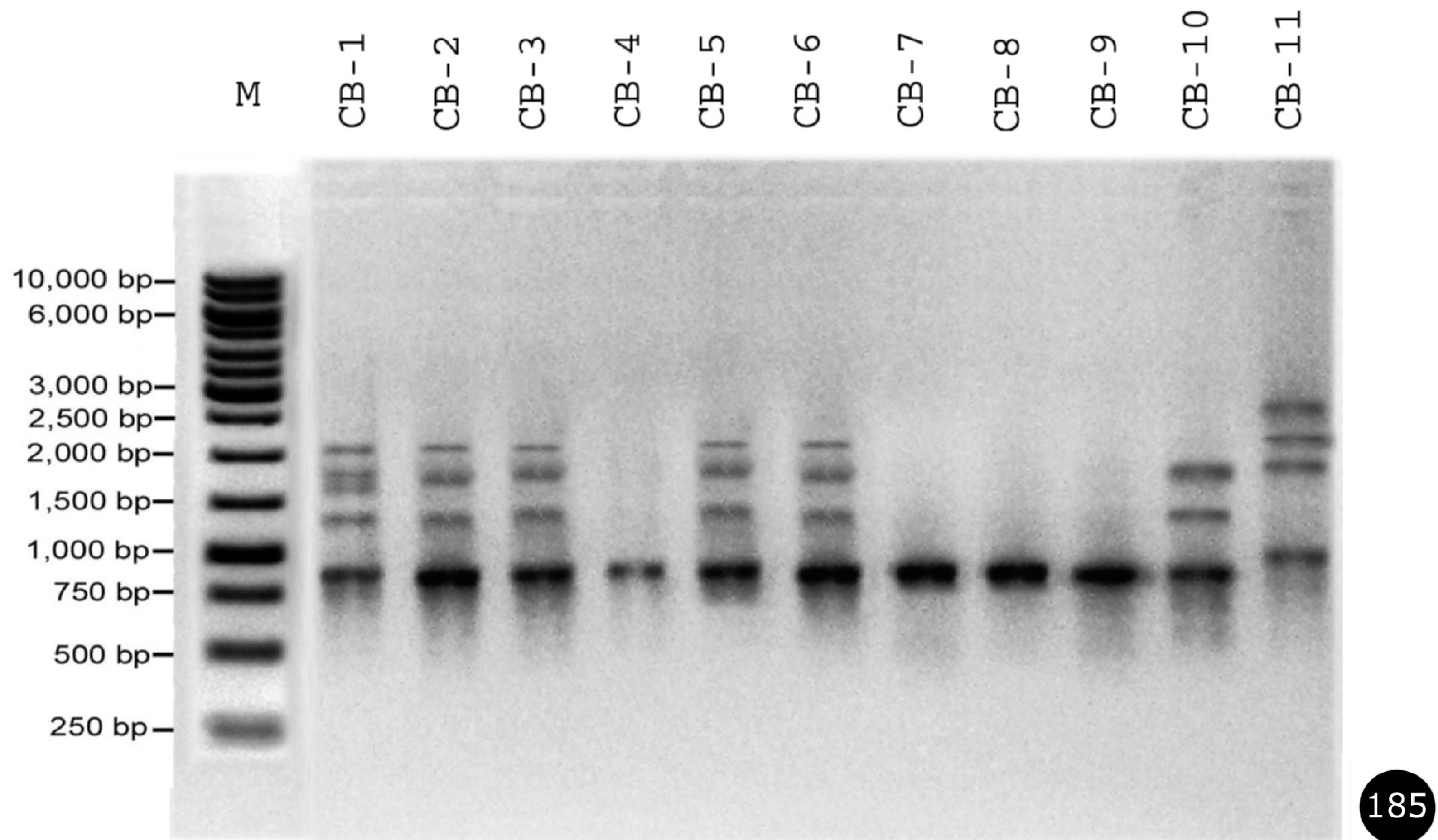

Fig. 185. RAPD analysis with primer-1 (5′-GAA ACG GGT G-3′) of eleven germplasms of *Gossypium hirsutum* L. M=1 Kb DNA ladder.

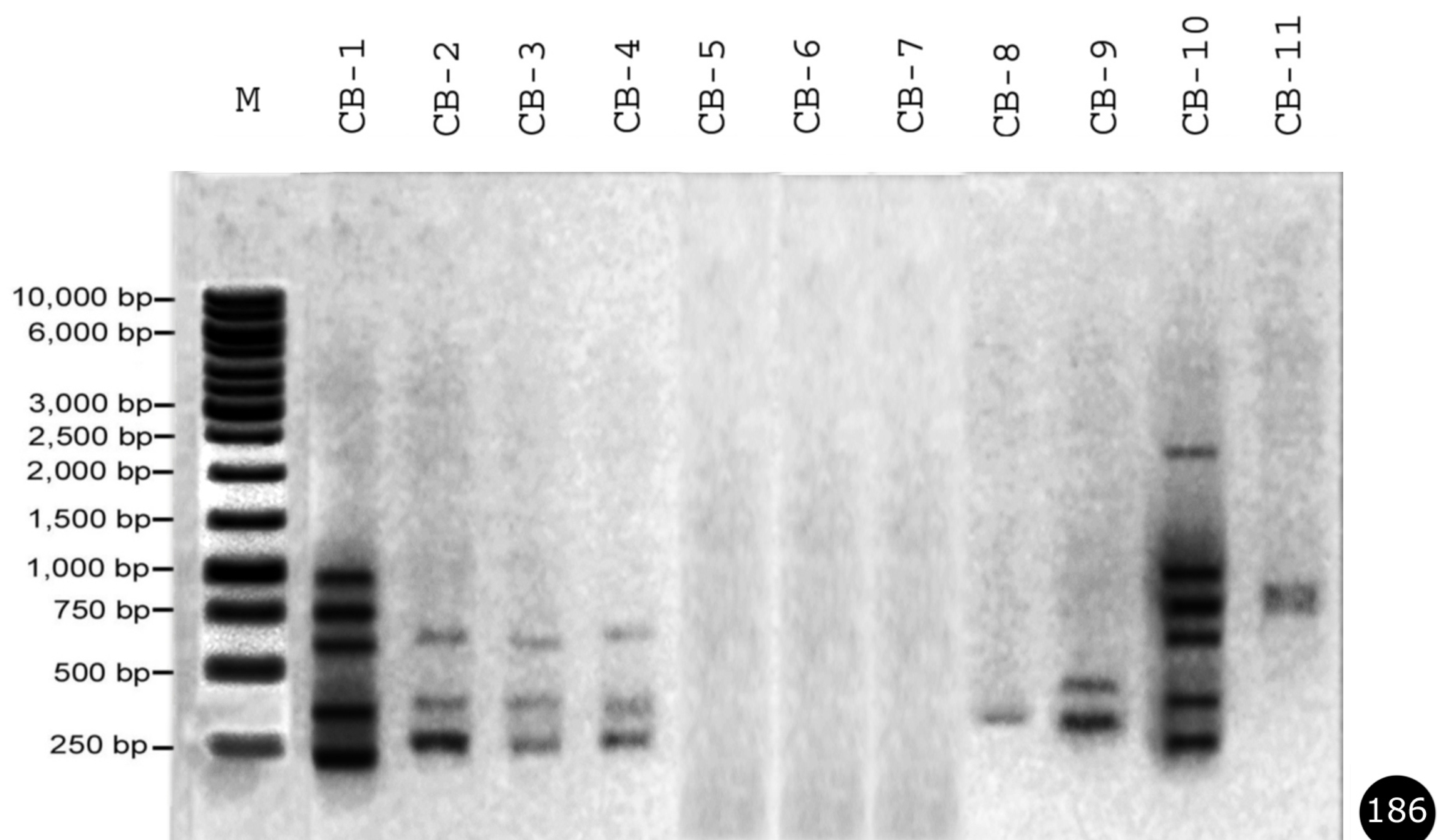

Fig. 186. RAPD analysis with primer-2 (5′-GTT GCG ATC C-3′) of eleven germplasms of *Gossypium hirsutum* L. M=1 Kb DNA ladder.

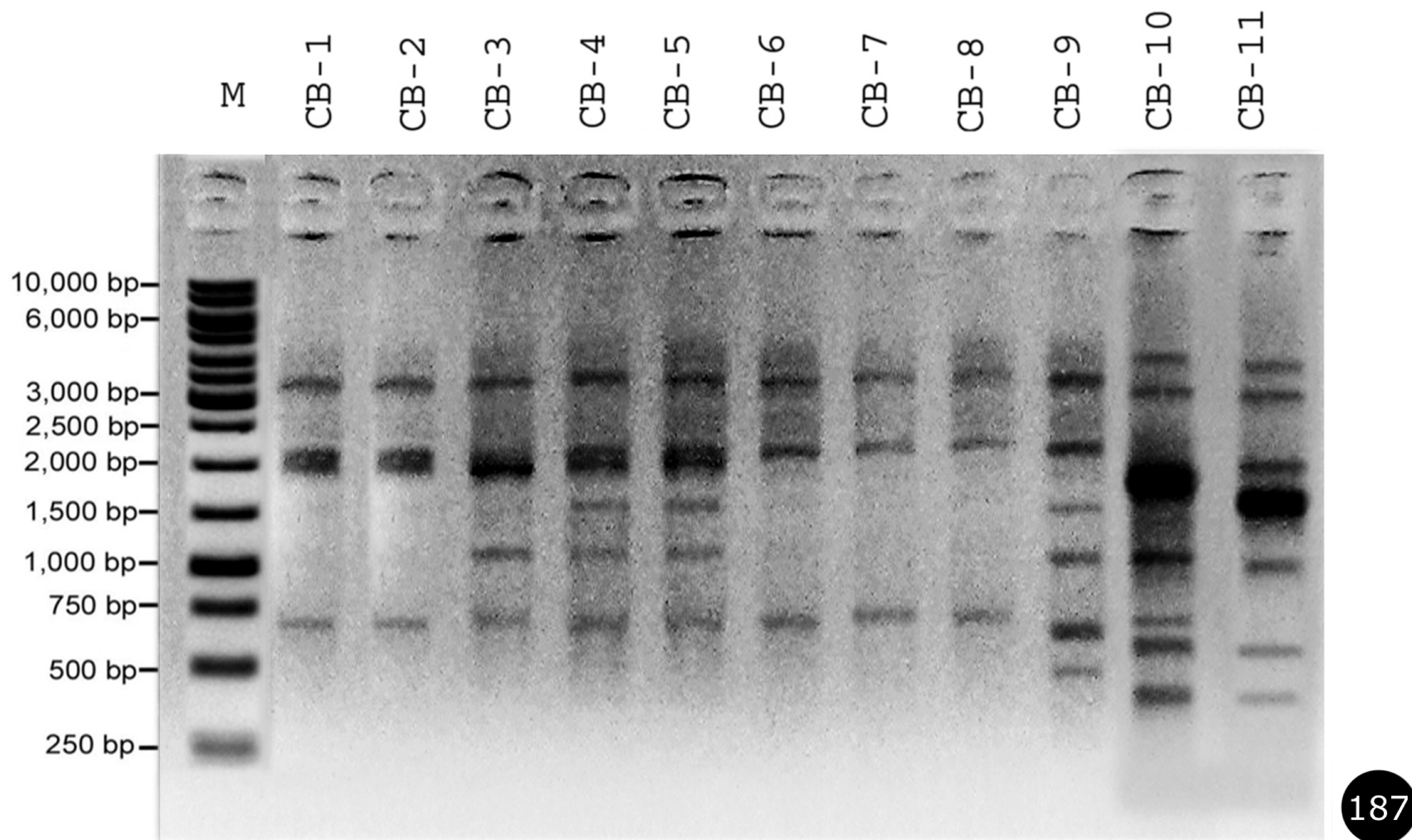

Fig. 187. RAPD analysis with primer-18 (5′-GTT TCG CTC C-3′) of eleven germplasms of *Gossypium hirsutum* L. M=1 Kb DNA ladder.

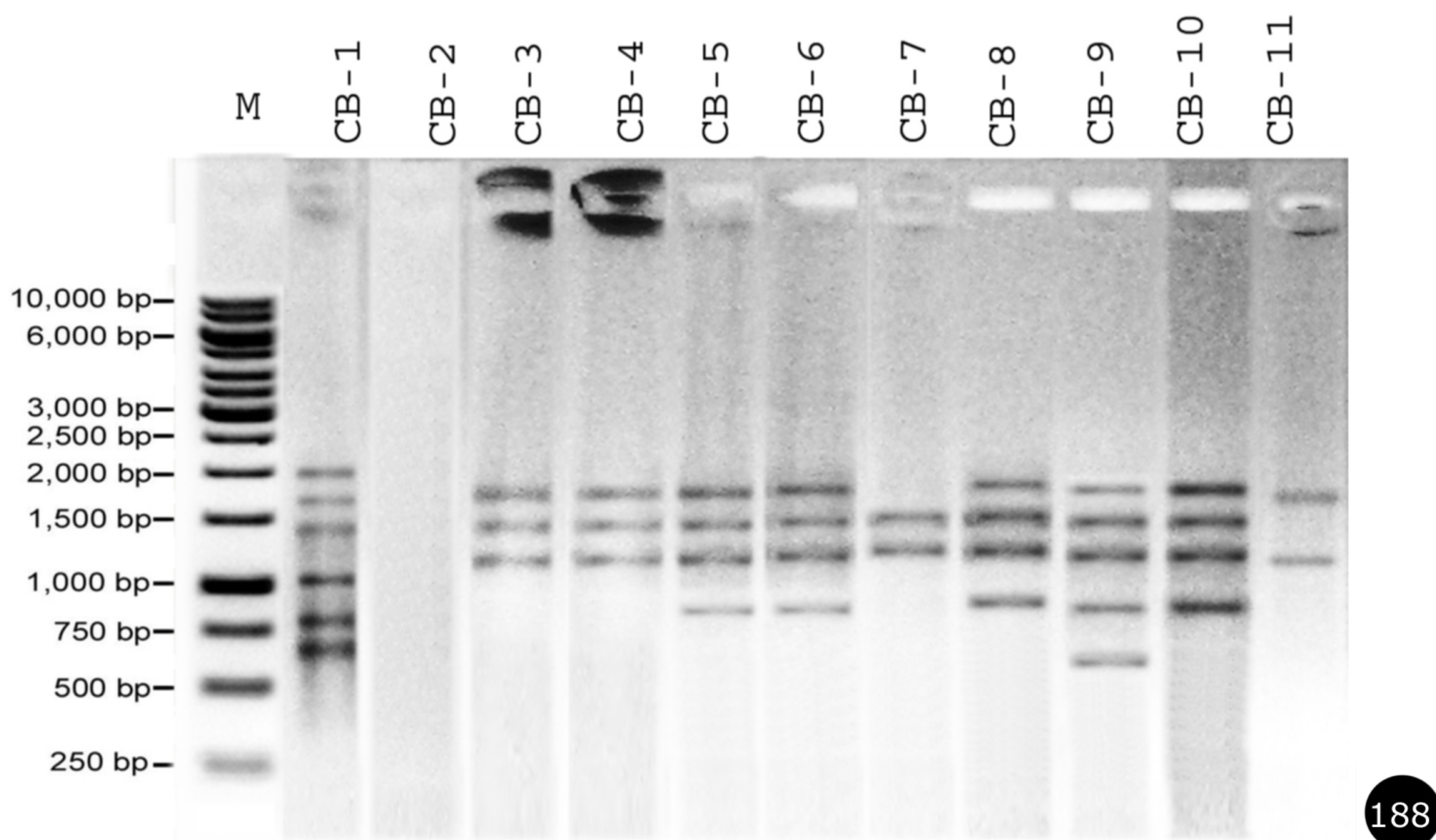

Fig. 188. RAPD analysis with primer-23 (5′-GTC AGG GCA A-3′) of eleven germplasms of *Gossypium hirsutum* L. M=1 Kb DNA ladder.

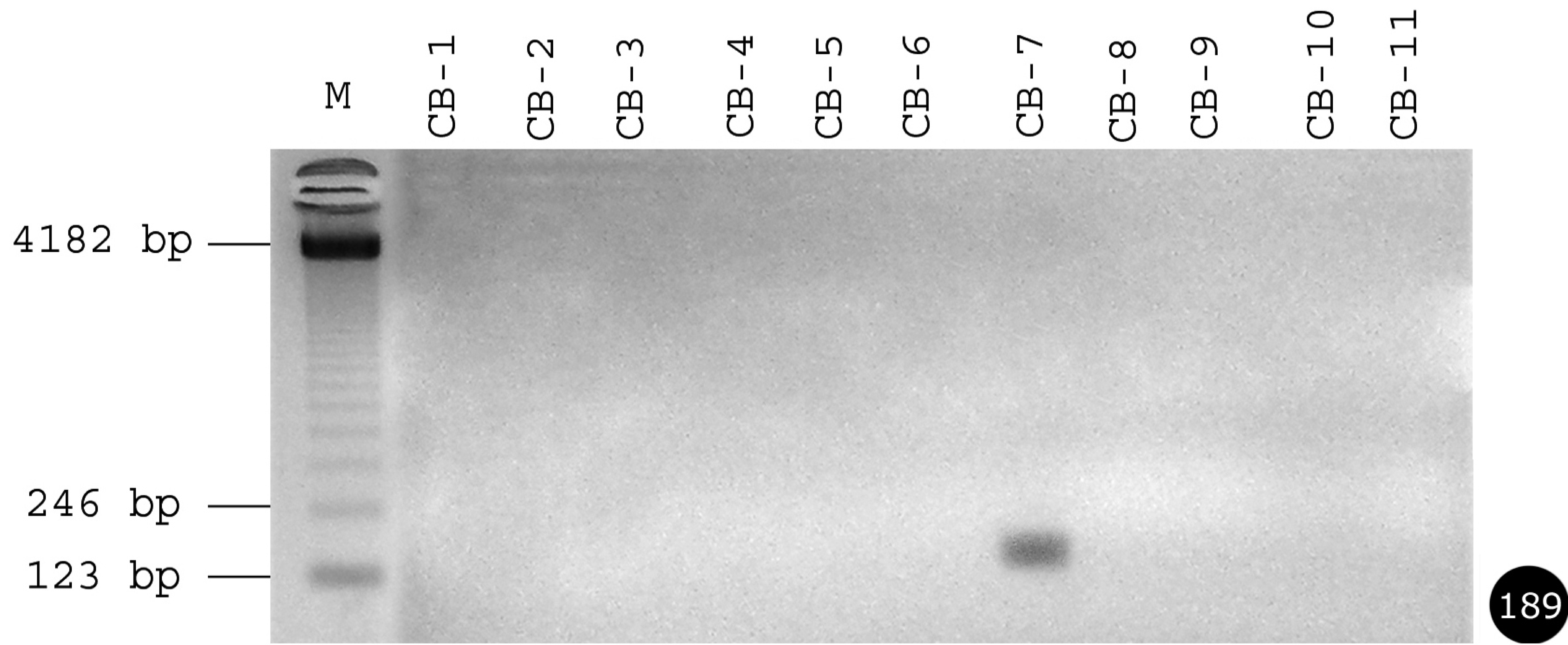

Fig. 189. SSR analysis with primer pair Forward-5′-GCTTCTTCCATTTTATTCAAG-3′-Reverse-5′-CAGCGGCAACCAAAAAG-3′ of eleven germplasms of *Gossypium hirsutum* L. M = 123 bp DNA ladder.

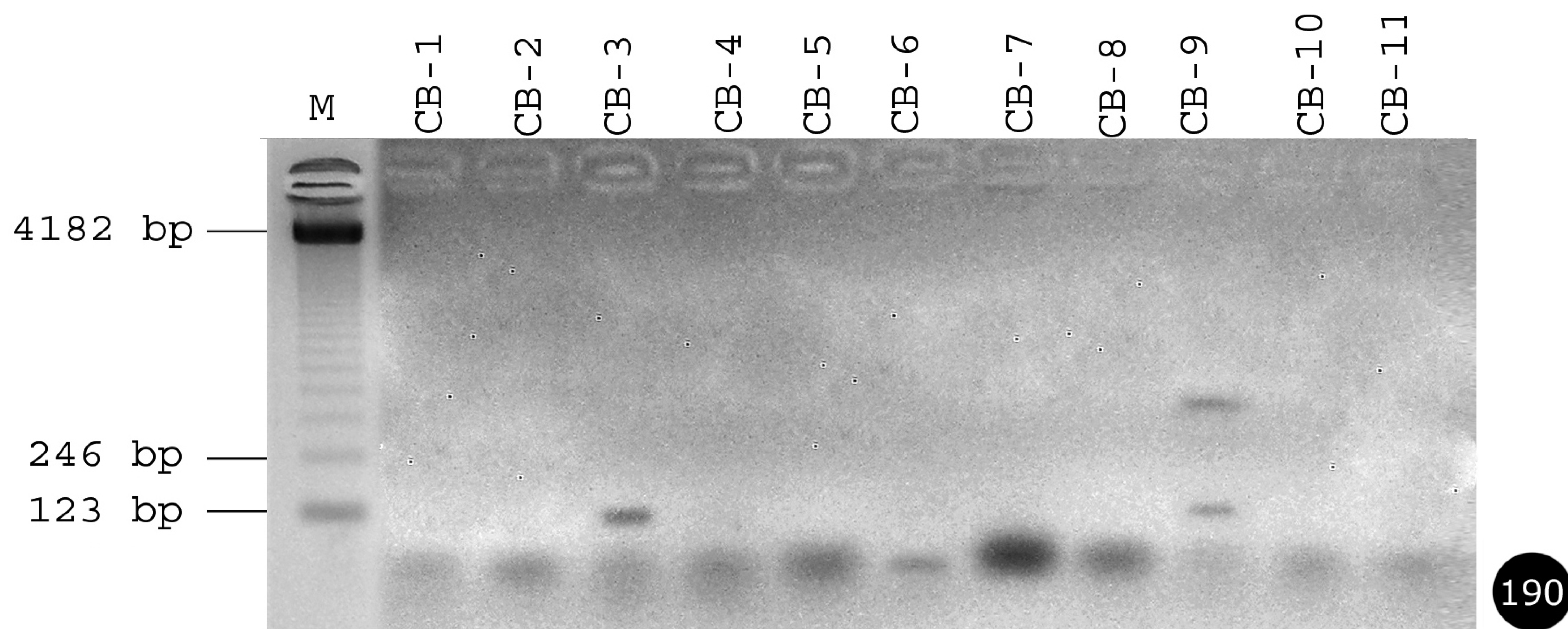

Fig. 190. SSR analysis with primer pair Forward-5′-ACCCTTAAATCATAAGAGAAC-3′-Reverse-5′-CGTAAGTTAAGGTACAAGG-3′ of eleven germplasms of *Gossypium hirsutum* L. = 123 bp DNA ladder.

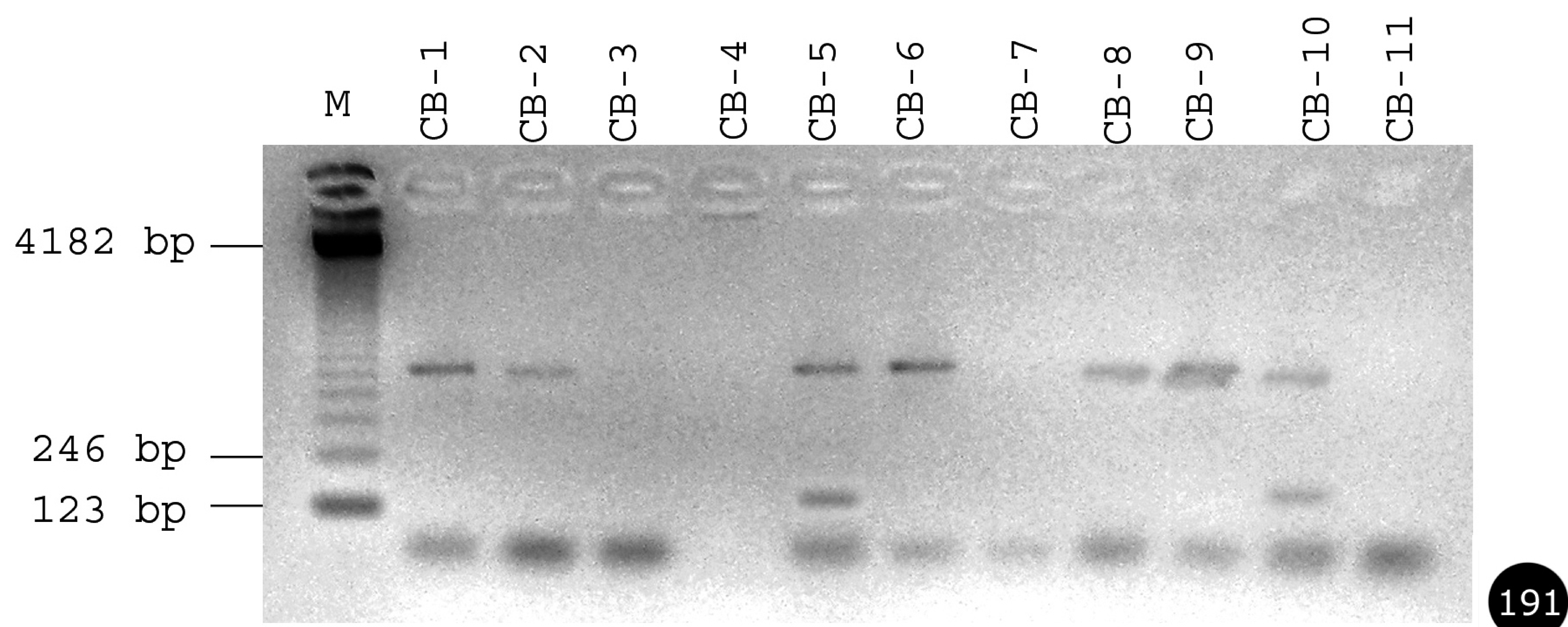

Fig. 191. SSR analysis with primer pair Forward-5′-GGAGTTAAAGCTAATGCCTG-3′-Reverse-5′-CGGGTCATTGGTTGTTTTTG-3′ of eleven germplasms of *Gossypium hirsutum* L. M = 123 bp DNA ladder.

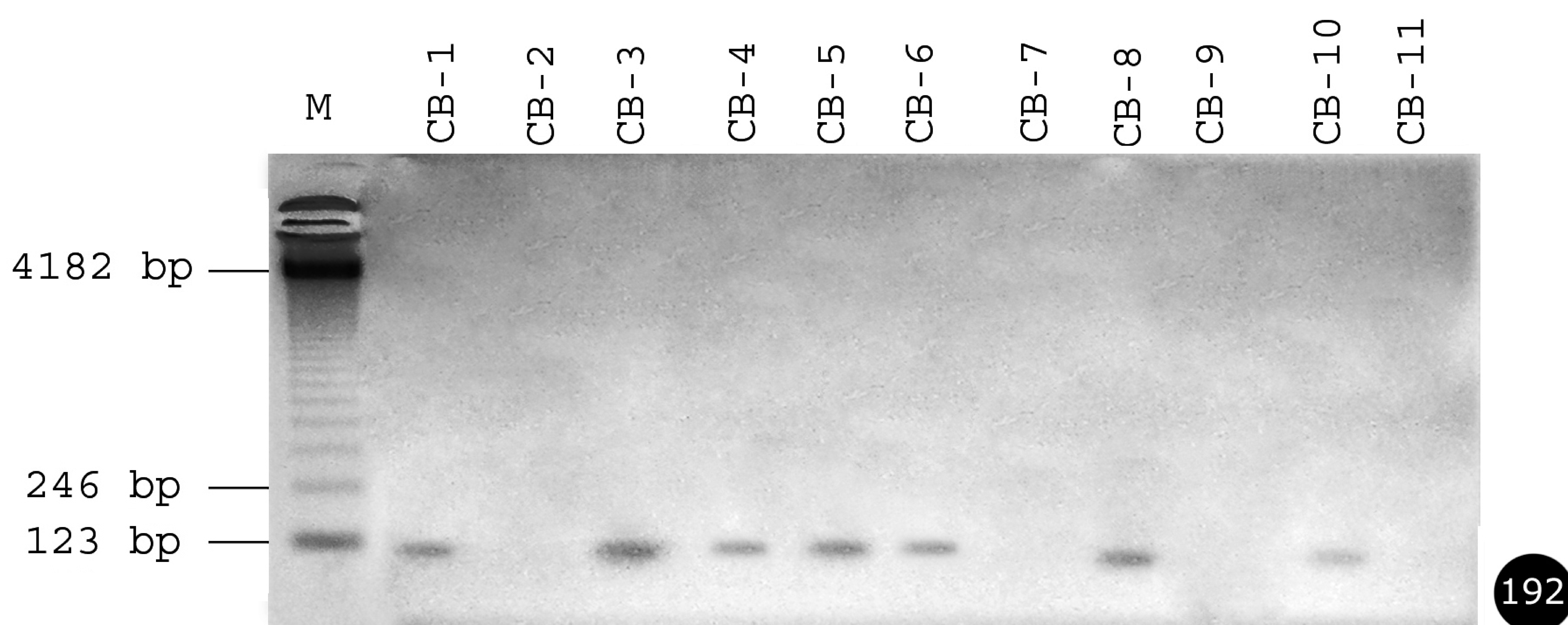

Fig. 192. SSR analysis with primer pair Forward-5′-GCCTAGGTGGAGTTCGTG-3′-Reverse-5′-CTGAACCTGCTCCTGAATC-3′ of eleven germplasms of *Gossypium hirsutum* L. M = 123 bp DNA ladder.

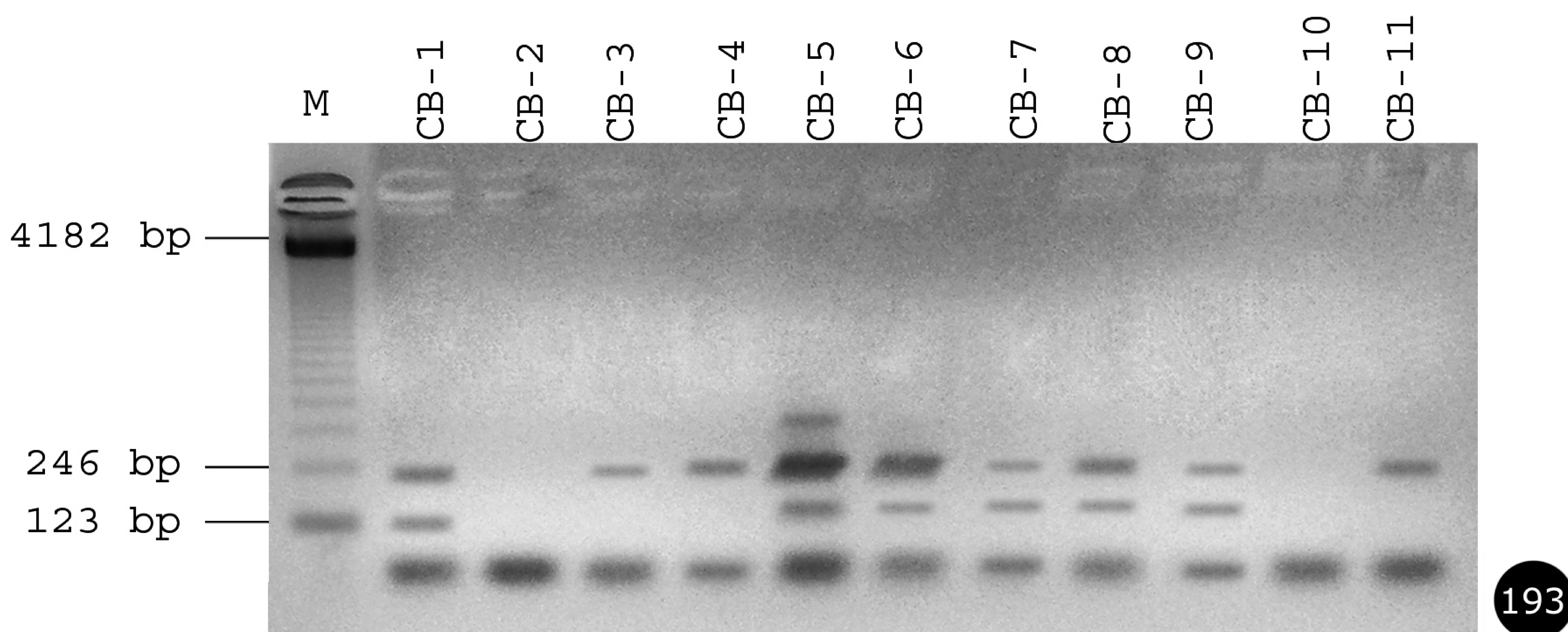

Fig. 193. SSR analysis with primer pair Forward-5′-CATCGGAAAACTCTGAAC-3′-Reverse-5′-GTAGCAGTACAGATGAAAGAG-3′ of eleven germplasms of *Gossypium hirsutum* L. M = 123 bp DNA ladder.

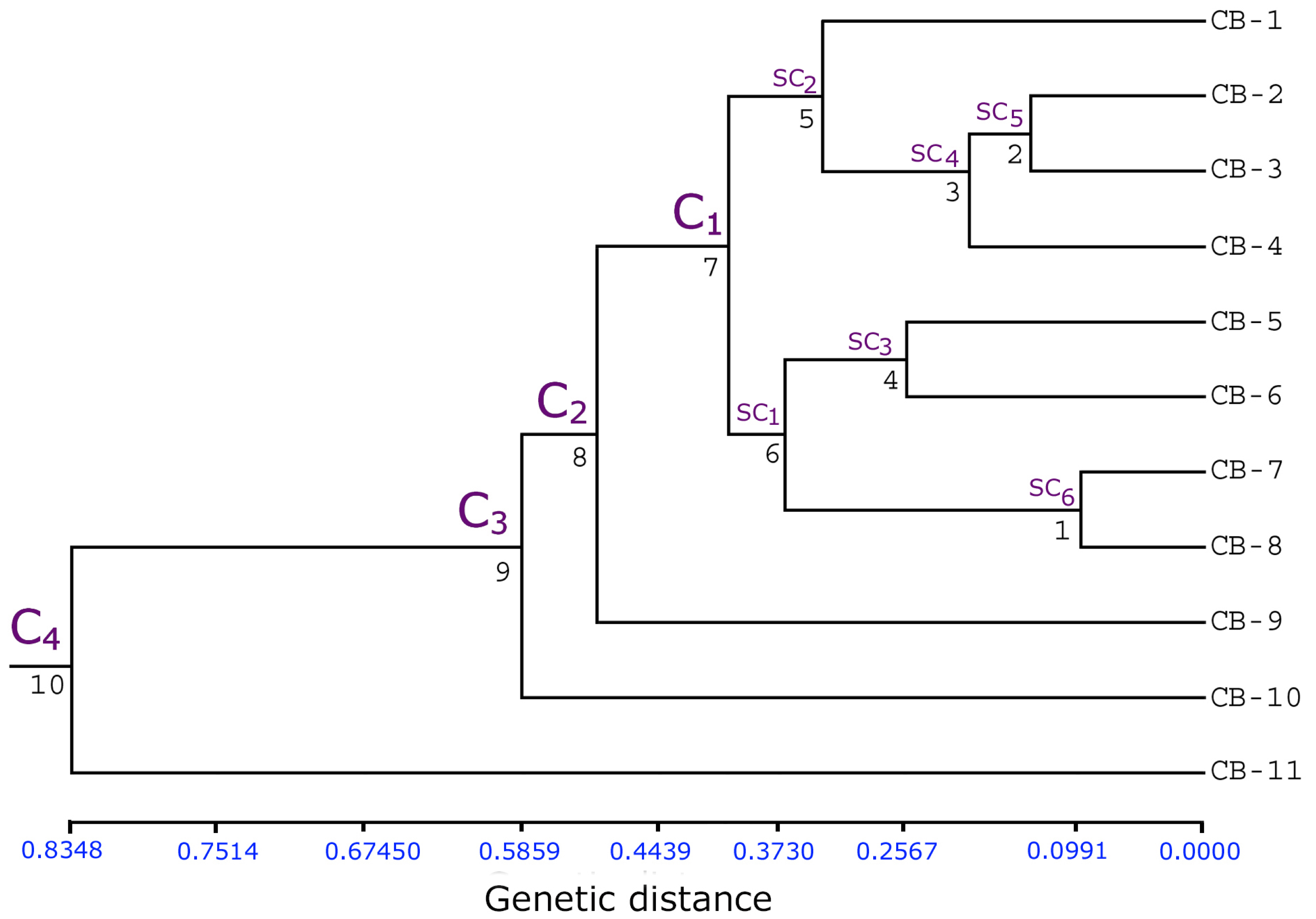

Fig. 194. UPGMA dendrogram based on Nei's (1972) genetic distance summarizing the data on differentiation between 11 germplasms of *Gossypium hirsutum* L. based on RAPD and SSR analysis.

4. DISCUSSION

4. Discussion

Eleven germplasms 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 were cytogenetically studied after orcein, CMA- and DAPI-staining. The nature of differential staining property of interphase nuclei and prophase chromosomes were accomplished to get additional information. Besides, DNA fingerprinting by using RAPD and SSR markers were also carried out for molecular characterization.

4.1. Orcein-staining properties of interphase nuclei and prophase chromosomes

The staining properties of interphase nuclei and prophase chromosomes sometimes provide karyomorphological features that help to characterize different germplasms. 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 germplasms 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. 13, 15, 17, 18, 20, 21, 22,

23, Table 11). 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. 14, 16, 19, Table 11). In these germplasms, few heterochromatic regions were aggregated together forming bigger heteropycnotic regions in the interphase nuclei.

The prophase chromosmoes 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. 47, 48, 49, 50, 54, Table 11). According to Tanaka (1971), this type of prophase chromosomes were regarded as "Gradient Type". The germplasms CB-1, CB-6, CB-7, CB-8, CB-10 and CB-11 were found to possess "Continuous Type" of prophase chromosomes where most of the chromosomes stained homogenously along the entire length (Figs. 46, 51, 52, 53, 55, 56, Table 11).

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

4.2. Nucleolus

A nucleolus was found in almost every interphase, prophase and metaphase stages in these cotton germplasms (Figs. 13-23, 46-56). Usually the nucleolus disappears at late prophase of mitosis. There is considerable evidence that it is not unusual for plant nucleoli to 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 entities, usually rounded in shape. 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 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), 16 species of *Cassia* (D'Amato Avanzi 1953), 10 species of *Reseda* (Gori 1956) and 45 species of the family Gramineae (Walter and Emery 1957). However, no such report was found in any species of *Gossypium.* Therefore, the persistant nature of nucleolus is a salient feature of *G. hirsutum.*

4.3. Orcein-karyotype

4.3.1. 2n chromosome number

In this study, 11 germplasms of cotton (*Gossypium hirsutum* L.) were found to possess 2n= 52 chromosomes (Figs. 79-89, 112-122, 145-155, Tables 12-23). Similar chromosome number for *G. hirsutum* L. was reported earlier by different scientists (Beasly 1942, Kammacher 1959, Mehetre *et al*. 1980, Nei and Li 1985, Wang *et al.* 1996). Thus the present

report on 2n chromosome number of 11 cotton germplasms correlate with the earlier reports.

Besides, different chromosome numbers were also reported for *G. hirsutum.* Such as 2n = 26 (Mehetre and Thombre 1877, 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 condition was 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 this study, no numerical annomalies was observed in the 11 germplasms of *G. hirsutum.* Therefore, the 11 germplasms have strict 2n chromosome number.

4.3.2. Centromeric formulae

Out of 11 cotton germplasms, 8 were found to possess all metacentric chromosomes (52m) (Figs. 79-89, 112-122, 145-155, Tables 12-23). Only three germplasms (CB-1, CB-2, CB-5) were found to possess few submetacentric chromosomes (sm) *viz.* 10 sm in CB-1, 2 sm in CB-2 and 6 sm in CB-5 (Figs. 79, 80, 83, 112, 113, 116, Tables 12, 13, 16, 23). In these 11 cotton germplasms, the range of chromosomal length was almost negligible i.e. distance between small and large chromosomes was about 1 µm (Table 23). As a result, no gradual decrease of chromosomal length was observed in their karyotypes (Table 23). 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.

4.3.3. Probable reasons for the origin of submetacentric chromosomes

In this study, out of 11 cotton germplasms , only three germplasms were found to possess some sub-metacentric chromosomes (CB-1 : 10 sm + 42 m, CB-2 : 2 sm + 50 m and CB-5 : 6 sm + 46 m) (Figs. 79, 80, 83, 112, 113, 116, Tables 12, 13, 16, 23). Nie (1985) observed 16 submetacentric chromosomes and 2 subtelocentric chromosomes in *G. hirsutum.* The submetacentric chromosomes might be originated from metacentric chromosomes by some chromosomal aberration *viz.* terminal deletion, pericentric inversion, non reciprocal or unequal translocation between fragments of chromosomes within *G. hirsutum*.

4.4. Fluorescent-Banding

Chromomycin A_3 (CMA) and 4´-6-Diamidino-2-Phenylindole (DAPI) are two fluorochromes specific to GC- and AT-rich base specific segments, respectively (Schweizer 1976). Fluorescent banding gives decisive analysis of karyotype, even chromosome having similar morphology and other

conventional karyotypic features. In this study, these two fluorochromes were used for critical analysis of karyotypes of 11 cotton germplasms.

No report on CMA and DAPI banding in *Gossypium hirsutum* was found in the available literatures and internet sources. Therefore, characterization of cotton germplasms by CMA and DAPI was the pioneer attempt.

4.4.1. Satellite

In CMA-staining, satellites were found in all germplasms except CB-5 (Figs. 90-100, 123-133, 156-166, Table 24). The satellites were confined to chromosome pair XII, XIX and XXVI only. Satellites in some germplasms were extended (CB-1, CB-2, CB-3, CB-4, CB-6, CB-7, CB-8 and CB-10) while contracted at the terminal region in the rest (CB-9 and CB-11). The germplasms CB-2, CB-3, CB-8 and CB-10 were found to possess total six satellites, two in each chromosome pair of XII, XIX and XXVI. In CB-9, there were total five satellites of which two were observed in both the members of pair XII, XIX and a member of pair XXVI. Four CMA-positive satellites were found in CB-1 and CB-11 on chromosome pair XII and XIX. Three satellites were found in CB-6 of which two in both the chromosomes of pair XII and one in a member of chromosome pair XIX. CB-4 and CB-7 had only 2 satellites on chromosome pair XII. The above data suggested that satellites on chromosome pair XII was common and more stable for all germplasms. In contrast, satellites on chromosome pair XIX and XXVI were unstable (Figs. 90-100, 123-133, 156-166, Table 24).

In CB-6 and CB-9, only one member of pair XIX and XXVI had a satellite, respectively (Figs. 128, 131). No satellite was found in their homologue

members showing heteromorphicity regarding the presence and absence of satellite on the homologue member. The lack of satellite in a member of homologous chromosome might be due to deletion of the satellited portion from the respective chromosomes. This observation suggested that deletion took place in the satellited portions of both the members of chromosome pair XIX and XXVI of CB-1, CB-4, CB-6, CB-7, CB-9 and CB-11. As a result, no satellite was observed in these pair of the six germplasms.

After DAPI-staining no satellite was found in pair XIX (except CB-9 and CB-11) and pair XXVI in all germplasms (Figs. 90-100, 123-133, 156-166, Table 24). The CMA-positive and the DAPI-negative feature, i.e., the reversible staining pattern of the satellites revealed that the satellites were made fully of GC-rich base sequences (Kondo and Hizume 1992, Alam and Kondo 1995, Lubna *et al*. 2004, Ruma *et al*. 2006, Sumner 1990, Schweizer 1976). On the other hand, satellites were observed in pair XIX of CB-9 and CB-11 after staing with both CMA- and DAPI. This features suggested the tandem existance of GC- and AT-rich repeats (Alam and Kondo 1996).

Moreover, after orcein staining only a pair of satellite was observed in CB-1 on the short arm of chromosome pair XII (Figs. 79, 112, Table 12, 23). Satellites of this pair were also observed in CMA-staining. However, no satellite was found after DAPI-staining in this pair. Except this germplasms, no satellite was found in any chromosomes of rest 10 germplasms after orcein staining. Therefore, the satellites showed stain specificity. Stain specific satellites was reported earlier by different scientists. Alam and Kondo (1995) found a satellite in *Drosera echinoblasta* at metaphase in orcein staining. It was however, absent in

C-, CMA- and DAPI-banding. Alam and Kondo (1995) also reported on the nature of a small chromosome in *Drosera ericksonea*. They carried out sequential staining of the same metaphase plate with Giemsa, CMA and DAPI and found a small chromosome in Giemsa and DAPI, whereas it was not observed in CMA. From these observations, they suggested that there were stain specific satellites and chromosomes. In the present study, the pair of satellites that was not observed after staining with CMA might possess some kind of DNA sequences which made it stain-specific. However, no report about stain specific satellite was available for *G. hirsutum.*

4.4.2. CMA-banding pattern

4.4.2.1. Nature of CMA-bands

Most of the CMA-bands were present at the terminal regions of respective chromosomes in 11 cotton germplasms (Figs. 90-100, 123-133, 156-166, Table 24). The presence of terminal CMA bands indicated a tendency of accumulating GC-rich repetitive sequences at the chromosomal ends (Zaman and Alam 2009). In addition to terminal bands, few CMA bands were found at centromeric regions of the respective chromosomes in CB-7, CB-9 and CB-11 germplasms revealing the presence of GC repeats in those regions (Figs. 129, 131, 133). Few chromosomes of CB-1, CB-2, CB-4, CB-7, CB-8 and CB-10 were entirely fluoresced with CMA. In these entirely fluoresced chromosomes, GC-rich repeats were not confined to the terminal or centromeric region rather distributed along the chromosomes. The possible reason for these entirely fluoresced chromosomes was tandem duplication of GC-rich repeats (Schweizer

1976, Khatun and Alam 2010, Sultana and Alam 2007, Hiron *et al.* 2006, Mahbub *et al.* 2007).

4.4.2.2. CMA karyotype

The karyotypes of 11 cotton germplasms were compared critically after CMA-staining. The 11 cotton germplasms used in this study have distinct CMA-banding pattern. The number, location, distribution and intensities of CMA-bands varied in different germplasms. The number of CMA-bands varied from 2 (CB-5) to 22 (CB-8) (Figs. 90-100, 123-133, 156-166, Table 24). The percentage of GC-rich repeats was ranging from 0.40 (CB-5) to 31.73 (CB-8) (Table 24). On the basis of number and location of CMA-bands, karyotypes formulae were prepared for each germplasms. It has seen that each germplasm has distinct CMA-banded karyotype formula (Tables 24).

4.4.2.3. Heteromorphicity

After CMA-staining, heteromorphicity was found in chromosome pair VII of CB-1, CB-2, CB-7 and CB-10. In these pairs, one chromosome fluoresced entirely while its homologue member had upper terminal CMA-positive bands. In this case, GC-rich repeats of one member may tandemly duplicated along the length of the respective chromosomes. On the other hand, GC-rich repeats of its homologue member were confined at terminal region without duplication (Figs. 123, 124, 129, 132). Long arms of a member of chromosome pair I fluoresced entirely with CMA in both CB-2 and CB-4 whereas no band was found in its homologue member. Heteromorphicity was also observed in chromosome pair X and XI of CB-9 and CB-11, respectively (Figs. 131, 133). In these pairs, a chromosome had centromeric bands and no band was observed in their homologue

suggesting deletion of the banded region from the respective chromosomes. In CB-7, a terminal band was found in a member of pair XI, while the other member had an interstitial band. These banding features indicated a paracentric inversion either from terminal to interstitial or interstitial to terminal region (Figs. 129).

Therefore, the pair wise comparison revealed the occurrence of structural aberration such as deletion, tandem duplication and inversion. Generally GC-rich repeats are heterochromatic in nature (Schweizer 1976). As a result, the changes in heterochromatic region did not have acute impact on the morphology of a germplasms. Although the 11 cotton germplasms possess strict symmetric karyotypes, a number of genomic alterations have been taken place within their karyotypes.

4.4.2.4. Marker chromosomes

A pair of dot like CMA-bands was found in two chromosomes of CB-5 (Figs. 94, 127). In CB-9, four such chromosomes were observed after CMA-banding (Figs. 98, 131). These chromosomes could easily be isolated from the rest due to their unique CMA-banding pattern. Agronomically these two germplasms were Jassid insect resistant. It is not clear whether these chromosomes were somehow linked with Jassid insect resistant property. What is clear that these chromosomes could be used as marker chromosomes for these two germplasms.

4.4.3. DAPI-banding pattern

4.4.3.1. Nature of DAPI-bands

Unlike CMA-staining, most of the banded chromosomes fluoresced entirely with DAPI fluorochrome. This banding nature makes these chromosomes

isolated from the other. The probable reason for entirely florescence was i) either these chromosomes were completely AT-rich by nature or ii) due to successive duplication of AT-rich repeats (Hiron *et al.* 2006, Mahbub *et al.* 2007). In addition, some terminal and few centromeric bands were also observed in 11 cotton germplasms. Schweizer (1976) first reported that DAPI bands revealed the presence of AT-rich repeats. Therefore, the AT-rich repeats were less confined rather distributed throughout the length of banded chromosomes.

4.4.3.2. DAPI-karyotypes

The DAPI-banding patterns helped to construct karyotype formulae. Each germplasms has characteristic DAPI-banded karyotype formula. The most striking feature was that entirely fluoresced chromosomes are frequent in each germplasm. An average of 6.5 entirely fluoresced chromosomes was found in each germplasms. These chromosomes have been used to characterize each karyotype. The percentage of AT-rich repeats was another parameter for distinguishing each karyotype.

A pair of centromeric DAPI bands was found one in each member of pair IX in CB-1, CB-2 and CB-5 (Figs. 101, 102, 105, 134, 135, 138). However, no such band was found in this pair in the rest germplasms. This data indicated the occurrence of deletion of the banded region from this pair in the rest germplasms.

Therefore, with the help of DAPI-staining, it was possible to characterize the 11 germplasms of *Gossypium hirsutum* L.

4.4.3.3. Heteromorphic bands

Heteromorphicity was found in chromosome pair VIII of CB-2, CB-4, CB-5, CB-6, CB-7, CB-8 and CB-9 with DAPI-staining. In these pairs, one chromosome had centromeric bands and an upper terminal DAPI-positive band was found in its homologue member (Figs. 135, 137, 138, 139, 140, 141, 142). Since these are homologous, should have similar banding pattern. This kind of heteromorphicity indicated the occurrences of inversion. There might be two modes of inversion such as i) from terminal end to centromeric region and ii) from centromeric region to terminal end (Akter and Alam 2005, Zaman and Alam 2009). Centromeric band was found in same chromosome pair of CB-10. These two homologous chromosomes showed similar banding pattern as expectation. The pair wise comparison easily concluded that an inversion from centromeric region to terminal end had been taken place in this pair of respective germplasm (Figs. 134-144).

4.4.3.4. Marker chromosomes

In CB-11, a member of chromosome pair XIII had terminal DAPI-positive bands on both long arm and short arm (Figs. 144, 177). No band was found in its homologue. This feature may indicate unequal exchange of AT-rich region between the homologous members during crossing over. This chromosome is unique since absent in the rest germplasms and thus could be used as a marker for this germplasm.

4.5. DNA fingerprinting

The 11 cotton germplasms selected for the present study represented a broad spectrum of variation for several phenotypic traits. DNA from the 11 cotton germplasms was studied with 10 oligonucleotide primers and 5 microsatellite primer pairs for RAPD and SSR assay, respectively.

4.5.1. DNA Fingerprinting by Random Amplification of Polymorphic DNA (RAPD)

RAPD is a PCR based marker technique that has been used for estimation of genetic diversity of populations and for studying the genetic relationships among different genotypes (Esmail *et al.* 2008).

4.5.1.1. Polymorphism as detected by RAPD analysis

In this study, a total of 25 RAPD primers were screened on template DNA of eleven cotton germplasms. Only 10 out of 25 were selected because they revealed multiband fingerprinting, which easily scorable and reproducable. The primer sequence, band size and banding pattern of 11 cotton germplasms were shown in Table (Tables 26-35, 41). The ten primers generated 335 distinct bands of which all were considered as polymorphic. Hundred percent (100%) polymorphisms indicated the high level of polymorphisms in 11 cotton germplasms. Band size ranging from 200-7000 bp of PCR amplification products scored for all primers. Light and bright bands were produced in the RAPD reactions. Light bands produced from low homology between the primer and the pairing site on the DNA strand (Thormann *et al.* 1994).

A diverse level of polymorphism in different crops have been reported earlier such as Chickpea 98.14% (Rasool 2013), Brassica 98.03% (Ghosh *et al.* 2009), Eggplant 57.89% (Biswas *et al*. 2009) and Chilli 90% (Paran *et al.* 1998). Wide range of RAPD polymorphism in cotton germplasms was reported. Esmail *et al.* (2008) scored a high degree of polymorphism in 21 cotton genotypes from Egypt using RAPD markers. The molecular

weight of bands analysed by them ranged from 100 and 1500 bp of which 84.95% were polymorphic. Maleia *et al.* (2010) reported 90.96% polymorphism among 21 cotton cultivars. Saravanan *et al.* (2006) scored a high degree of polymorphism in 10 cotton genotypes using RAPD markers and observed 69.37% polymorphism. Hussain *et al.* (2007) reported 63.20% polymorphism among 11 cotton genotypes.

In this study, 100% polymorphism was recorded for the first time in cotton. Therefore, the 11 germplasms used in this study were highly diversed from each other. On the other hand, low level of DNA polymorphism in cotton germplasms was not observed by any previous workers. Therefore, broad range of polymorphism revealed wide diversity in cotton germplasm. The diversification would be useful for improved breeding programme of cotton.

4.5.1.2. Unique RAPD markers

In addition to polymorphism, 29 unique RAPD sequences were identified in 11 cotton germplasms using ten different primer combinations. The term unique sequence means that the sequence found in a germplasms with a certain primer was absent in other germplasms (Figs. 179-188, Table 41). In the earlier literature, there was no information about unique band (Esmail *et al.* 2008, Maleia *et al.* 2010, Saravanan *et al.* 2006). The earlier authors considered all bands as polymorphic band. In this study, mentioning unique band was a new parameter for RAPD analysis. The unique bands were stable and specific for the respective germplasms and thus could be used as a tool for characterization.

4.5.2. DNA fingerprinting by Simple Sequence Repeats (SSRs)

Five SSR primer pairs were used in this study of which all generated well-defined and reproducible polymorphic bands. The primer sequence, band size and banding pattern of 11 cotton germplasms were given in Tables 36-40 and in Figs. 189-193.

4.5.2.1. Polymorphism as detected by SSR analysis

The 5 primer pairs generated 69 distinct bands of which 39 were considered as polymorphic and thus showed 56.52% polymorphisms which indicated the moderate level of polymorphisms (Tables 36-40, 42). The size of band was ranging from 50 to 2000 bp. Out of five, the primer pair (BA00175683/ BA00175684) produced highest number (20) of polymorphic bands followed by 16 polymorphic bands in the primer pair (BA00175687/ BA00175688) (Figs. 191, 193, Tables 38, 40, 42). In contrast, the primer pair (BA00175685/ BA00175686) did not generate any polymorphic band (Fig. 192, Tables 39, 42).

Only 1 common band of 50 bp was observed in two primer pairs *viz.* BA00175681/ BA00175682 and BA00175687/ BA00175688 (Figs. 190, 193, Tables 37, 40, 42). The common band indicated the sharing of similar DNA fragments among 11 cotton germplasms.

4.5.2.2. Unique SSR markers

In addition to polymorphism, 4 unique SSR sequences were identified in 11 cotton germplasms using 5 different primer pair combinations. Primer pair BA00175681/ BA00175682 produced 1 unique band of 738 bp in CB-9 (Table 42). With primer pair BA00175683/ BA00175684, the germplasms CB-9 showed 1 unique band of 1968 bp (Table 42). Primer

pair BA00175687/ BA00175688 produced 1 unique band of 123 bp in CB-1 and 492 bp in CB-5 (Table 42). The unique bands were stable and specific for the respective germplasms and thus could be used as marker. Except polymorphic band no reports of unique SSR band was available for cotton (Esmail *et al.* 2008, Maleia *et al.* 2010, Saravanan *et al.* 2006). Therefore, this is first time report about unique SSR band for cotton germplasms.

4.6. Genetic relationships among 11 cotton germplasms

The values of pair-wise Nei's (1972) genetic distances analyzed by using computer software "popgene32" among 11 germplasms of cotton were computed from combined data obtained from ten RAPD primer and five SSR primer pairs. The value was ranging from 0.0991 to 0.8348 (Table 43). The highest genetic distance (0.8348) was found between CB-11 with CB-5 and CB-9. On the other hand, the lowest (0.0991) genetic distance was observed between CB-7 and CB-8. The difference between the highest and the lowest value of genetic distance revealed the wide range of variability persisting among the 11 cotton germplasms. High genetic distance values between germplasm pairs were found due to difference in genetic constituent. The germplasms of lowest genetic distance can be used as parental source for breeding line to improve cotton germplasm.

4.7. CB-11 is distinct from the rest

The germplasm CB-11 is different from the rest 10 germplasms in various morphological and agronomical aspects. Flowers and fruits come faster in CB-11 compared to other germplasms. This plant is about 8 feet high whereas other 10 germplasms about 4 feet. The shapes of leaves are totally different from the rest cotton germplasms (Figs. 11). CB-11 showed the highest yielding capacity (2.10-3.50 ton cotton/hectare) from the rest (1.32-2.50 ton cotton/hectare) (Table 1).

CB-11 was also distinct from the rest in respect of DAPI-banding pattern. This germplasm have a chromosome with DAPI-band on both terminal ends. A chromosome with 2 DAPI-bands was absent in other germplasms (Figs. 111, 144).

This germplasm is different in respect of RAPD fingerprinting. Several unique sequences were found in CB-11 with various primers (Table 41, Figs. 179-188). The combined RAPD and SSR dendrogram placed CB-11 alone in a separate cluster (C_4) (Fig. 194)

The above data made CB-11 distinct from the rest 10 germplasms of *Gossypium hirsutum.*

4.8. Conclusions

In the available literatures and internet sources, report on the nature of mitotic interphase and prophase chromosomes, CMA and DAPI banding pattern, unique RAPD and SSR bands and combined cytogenetical and molecular analysis of *Gossypium hirsutum* was not found. Therefore, in this study, characterization of 11 germplasms of *Gossypium hirsutum* by the above parameters was the pioneer attempt.

Alterations of chromosomal segments were found in 11 cotton germplams. In spite of similar 2n chromosome number, diversification and reshuffling of CMA- and DAPI-positive banded regions were observed in these eleven germplasms of cotton. The number, location and distributions of GC- and AT-rich repeats were specific for each germplasm. Therefore, each germplasm has its characteristic CMA- and DAPI-banding pattern. Jassid insect resistant germplasms could be identified by CMA banded marker

chromosomes. The 11 germplasms showed unique RAPD and SSR DNA fingerprinting useful for authentic characterization.

Therefore, the 11 germplasms of *Gossypium hirsutum* L. could be characterized authentically by combined cytogenetical and molecular analysis.

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* Original not seen.