MOLECULAR CHARACTERIZATION OF PEANUT (*ARACHIS HYPOGAEA* L.) GERMPLASMS IN BANGLADESH

Ph.D. THESIS

BY
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APRIL 2014

DEPARTMENT OF BOTANY
FACULTY OF BIOLOGICAL SCIENCES
UNIVERSITY OF DHAKA
DHAKA-1000, BANGLADESH

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

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Dedicated
To
My Beloved
Wife-Evana Reza
and
Son-S.M. Areeb Ahsan (Ishan)

Certificate

This is to certify that this thesis entitled ***"Molecular characterization of peanut (Arahcis hypogaea L.) germplasms in Bangladesh"*** *submitted by Md. Ahashan Habib has been carried out under our supervision. This is further to certify that it is an original work and suitable for submission for the award of Ph.D. in Botany.*

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Acknowledgement

At first thanks to Almighty Allah for giving me patience, strength and courage to complete my research work in due time.

I am indebted to Dr. M. Imdadul Hoque, Professor and Dean, Faculty of Biological Sciences, University of Dhaka for his supervision, valuable suggestions and care. I am obliged for the mentoring I received from him.

I feel pleasure to express my immense and heartfelt gratitude to Dr. Sheikh Shamimul Alam, Professor, Department of Botany, University of Dhaka for his intellectual guidance, constructive criticism and untiring supervision throughout the whole process.

I am grateful to Dr. Rakha Hari Sarker, Professor, Department of Botany, University of Dhaka for his generous co-operation, guiding and encouragement throughout the research period.

I would like to express my sincere thank Prof. Dr. M. Nurul Islam, Professor, Department of Botany, University of Dhaka for his valuable suggestion, support and encouragement during my research work.

I am thankful to Prof. Dr. Moniruzzaman Khondker, Chairman and Prof. Dr. Abdul Aziz, former Chairman, Department of Botany, University of Dhaka for providing the necessary facilities of the Department.

I wish to express my deepest sense of gratitude to Prof. Dr. Ahmad Ismail Mustafa, Chairman and Prof. Dr. SM Imamul Huq, former Chairman, BCSIR. I am also grateful to Md. Abu Anis Jahangir, Director, BCSIR laboratories Dhaka for their opinion, recommendation and cordial behavior.

I would like to express my sincere and heartiest gratitude to Late Prof. Abdul Matin, Prof. Dr. Masuda Khatun and Prof. Dr. Rokeya Begum, Department of Botany, University of Dhaka for their generous support, help, invaluable suggestions, warm encouragement and co-operation during the study.

I express my sincere and heartiest gratitude to Syeda Sharmeen Sultana, Lecturer, Department of Botany, University of Dhaka for her inspiration, immense assistance to my work and warm encouragement to complete the study.

I also express my sincere and heartiest gratitude to Prof. Dr. M. Mozammel Haque, Prof. Dr. M. Abul Hassan, Prof. Dr. Md. Abul Bashar, Prof. Dr. Mihir Lal Shaha, Prof. Dr. M. Zabed Hossain, Rifat Samad, Lecturer and Ms. Rita Sarah Borna, Lecturer,

Department of Botany, University of Dhaka for their encouragement and valuable suggestions.

Sharmin apu, Dollar Apu, Nasheta apu, Kamrun Apu, Rubaiyath vai, Rekha apu, Jessy, Ira, Hira, Lina, Sonia, Sharmin, Shohana, Hira, Tinny, Nahida, Asma, Zaman, Rony, Shahid, Tania, Sussan, Alamgir, Nowren, Gity, Riffat, Mumu, Poly, Himu, Nasrin, Israt (Sonia), Nusrat, Mahin, Shema, Shifa, Hridi, Prithula, Imran, Laila and other members of Cytogenetics laboratory, who have helped me in their own way as much as they could, I thank you all.

I also want to thank all the members of Plant breeding and biotechnology laboratory, Department of Botany, University of Dhaka for their generous cooperation.

I wish to express my indebtedness to Dr. Shahina Islam (CSO), Dr. Salim Khan (SSO), Shahina Akter (SSO), Tanjina Akhtar Banu (SSO), Mousona Islam (SO), Nila (RF), Aleya Ferdousi (RF), Tapan Chandra Mollick (Lab. Tech.), Moyna Begum (LA) of plant tissue culture section and also grateful to Md. Moniruzzaman (SSO), soil and environment section and all the members of biological research division, BCSIR laboratories Dhaka for their amiable behavior, co-operation and pleasant help during the research.

I specially thank to Dr. Rabeya Begum (Lipu) for her help, inspiration and invaluable suggestions to prepare my thesis paper. I am really grateful to her from the core of my heart.

I am thankful to Mr. Harun-or-Rashid (SSO, BARI), Md. Rezwan Molla (SSO, BARI) and Dr. Mirza Mofazzal Islam (PSO, BINA) for their cordial help and co-operation.

I sincerely thanks to Shuvas Da, Jashim, Shuman, Tofazzal Bhai and Nuruzzaman, Gardener, Department of Botany, University of Dhaka for providing all the garden facilities and co-operation.

I am indeed thankful to my respected parent-in-law SM Alim Reza and Farida Yesmen for their continuous help and cooperation during my research work.

And last but not the least, I am really grateful to my parents and family members for their love and support to help me complete my thesis in due time.

Finally, the thesis is dedicated to my beloved wife-Evana Reza and my dearest son-S.M. Areeb Ahsan (Ishan).

– The Author

Contents

List of Tables

List of Figures

List of Abbreviations

BARI	:	Bangladesh Agricultural Research Institute
BINA	:	Bangladesh Institute of Nuclear Agriculture
BBS	:	Bangladesh Bureau of Statistics
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 A3
DAPI	:	4'-6-Diamidino-2-Phenylindole
GC	:	Guanine-Cytocine
AT	:	Adenine-Cytocine
m	:	Minute
s	:	Second
h	:	Hour
1N	:	1 Normal
MW	:	Molecular weight
g	:	Gram
mg	:	Milligram
µg	:	Microgram
ng	:	Nanogram
µ	:	Micron
µm	:	micro meter
µM	:	Micro molar
µl	:	Micro liter
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 by volume
α	:	Alpha
β	:	Beta
γ	:	Gama
∂	:	Sigma
θ	:	Theta
Ø	:	Phi
€	:	Epsilon
λ	:	Lambda
δ	:	Delta
Ω	:	Omega
%	:	Percentage
°C	:	Degree centigrade

Abstract

Eleven varieties of peanut (*Arachis hypogaea* L.) were investigated cytogenetically and at the molecular level using Random Amplified Polymorphic DNA (RAPD) and Simple Sequence Repeat (SSR) primers for authentic characterization. All varieties were found to possess 2n= 40 chromosomes, except BARI Cheena Badam-7 and BARI Cheena Badam-8, where a small extra chromosome found in addition to 2n=40 chromosomes in several cells. The extra chromosome may be considered as B-chromosome and this is the first report of B-chromosome in *Arachis hypogaea*. One pair (XX) of small chromosomes was found in all varieties. The 11 varieties showed distinct centromeric formulae *viz*., i) 34m+6sm, ii) 36m+4sm, iii) 38m+2sm, iv) 32m+ 8sm and v) 40m. All varieties of peanut have distinct CMA- and DAPI-banding patterns. The number, location, intensity and percentage of GC- and AT-rich repeats were different in these varieties. Asymmetry banding pattern in homologue members indicated minute chromosomal alterations. Similar numbers of CMA and DAPI bands were observed in different stages of cell cycle revealed that the GC- and AT-rich repeats were very stable in each variety. Few chromosomes could be used as marker chromosomes of respective variety due to their unique banding pattern. Genetic diversity analysis among 11 peanut varieties was performed through polymerase chain reaction (PCR) using RAPD and SSR primers. Eight arbitrary oligonucleotide RAPD primer and four pairs of SSR primer were used for DNA fingerprinting. In case of RAPD, a total of 272 bands were produced in all the 11 peanut varieties with an average of 34 bands per primer. Based on the banding pattern 71.69% polymorphisms observed among the peanut varieties. The molecular size of the amplified DNA fragments ranged from 400 to 3000 bp. Eight unique bands were amplified from the genome of the 11 peanut varieties. 62.06% polymorphic fragments and a unique band of 50 bp were

found in four SSR primer pairs. The size of the amplified bands ranged from 50 to 246 bp. The values of pair-wise genetic distances ranged from 0.1226 to 0.5500, indicating the presence of wide genetic diversity. The highest genetic distance (0.5500) was found between Dhaka-1 vs BARI Cheena Badam-8 while the lowest (0.1226) between BINA Cheena Badam-2 vs BINA Cheena Badam-3 and Tridana Badam (DM-1) vs Basanti Badam (DG-2). Dendrogram based on Nei's (1972) genetic distance was constructed using Unweighted Pair Group Method of Arithmetic Means (UPGMA) segregating the 11 varieties of peanut into two major clusters C_1 and C_2. BARI Cheena Badam-7 and BARI Cheena Badam-8 were distinctly related from the rest. Therefore, each variety could be characterized by combined cytogenetical and molecular features which would be helpful for future breeding programme.

1. Introduction

The genus *Arachis* L. belongs to the family Fabaceae consisting of about 80 species. The genus includes nine sections: *Trierectoides, Erectoides, Extranervosae, Triseminatae, Caulorrhizae, Procumbentes, Rhizomatosae,* H*eteranthae* and *Arachis* (Krapovickas and Gregory 1994, Valls and Simpson 2005). *Arachis hypogaea* L. belongs to the section *Arachis.* This species is commonly known as groundnut or peanut. Based on morphological features, i.e., the ramification pattern and the presence/absence of flowers on the main axis, the cultivated peanut is divided into two subspecies *viz.,* i) *A. hypogaea* subsp. *hypogaea* Waldron and ii) *A. hypogaea* subsp. f*astigiata* Waldron, which are further classified into four botanical varieties. Botanical varieties *hypogaea* and *hirsuta* belong to subsp. *hypogaea* while varieties *fastigiata* and *vulgaris* to subsp. *fastigiata*. The botanical varieties *fastigiata* was further divided into three botanical varieties such as *fastigiata*, *peruviana* and *aequatoriana* (Krapovickas and Gregory 1994).

Peanut is considered as the world's fourth most important source of edible vegetable oil and third most important source of vegetable protein (Duke 1981) because of its multiple uses as human food, vegetable oil, feedstock and ground cover (Wynne and Halward 1989). Mature peanut seeds contain per 100 g edible portion: water 6.5 g, energy 2374 kJ (567 kcal), protein 2.5 g, fat 49.2 g, carbohydrate 16.1 g, dietary fibre 8.5 g, Ca 92 mg, Mg 168 mg, P 376 mg, Fe 4.6 mg, Zn 33 mg, vitamin A 0 IU, thiamine 0.64 mg, riboflavin 0.14 mg, niacin 12.1 mg, vitamin B6 0.35 mg, folate 240 µg and ascorbic acid 0 mg. The essential amino acid composition per 100 g edible portion is: tryptophan 250 mg, lysine 920 mg, methionine 883 mg, valine 1082 mg, leucine 1672 mg and isoleucine 907 mg. The principle fatty acids are per 100 g edible portion: oleic acid 23.7 g, linoleic acid 15.6 g and palmitic acid 5.2 g (Brink and Belay 2006). It contains mostly unsaturated fat, which has been shown to lower LDL-

cholesterol levels in human blood. In fact, recent studies indicate that frequent consumption of peanuts, as part of a healthy diet, may actually lower the risk of heart attack. It is naturally cholesterol-free and added value for health conscious consumers. A pound of peanuts is high in food energy and provides approximately the same energy value as 2 pounds of beef, 1.5 pounds of cheddar cheese, 9 pints of milk or 36 medium-size eggs (sources: wikipedia. org, banglapedia. org, nationalpeanutboard. org).

Peanut is considered as an allotetraploid (2n=4x=40) with an AABB genome constitution (Smartt *et al.* 1978). However, there are some reports about the single origin of peanut. Two diploid species belonging to the section *Arachis* were suspected as donor of A and B genomes. More than eight diploid species with either the A or B genome have been considered as the putative parents of peanut. A number of opinions regarding the origin of peanut are existed. On the basis of morphological similarities and inter-specific cross-compatibilities, it was proposed that *A. correntina*, *A. duranensis* and *A. cardenasii* (all with A genome) and *A. batizocoi* (B genome) are probable parents of *A. hypogaea* (Singh and Smartt 1998). Classical chromosome analysis suggested that *A. duranensis* and *A. ipaensis* (B genome), in case of single origin or *A. trinitensis* (A genome) and A*. williamsii* (B genome), in case of allopolyploid origin could be the genome donors of the cultigen (Fernandez and Krapovickas 1994). Restriction fragment length polymorphism (RFLP) data suggested that *A. duranensis* and *A. ipaensis* are the closest diploid relatives of *A. hypogaea* (Kochert *et al.* 1991), while randomly amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR) analysis considered *A. villosa* and *A. ipaensis* as the best candidates (Raina *et al.* 2001). On the other hand, amplified fragment length polymorphism (AFLP) data showed that at least three diploid species with the A genome and three with the B genome displayed small genetic distance when compared with the cultigen (Milla *et al.* 2005). by using fluorescent *in situ* hybridization (FISH) (Raina and Mukai 1999)

proposed that *A. villosa* as the most probable genome donors instead of *A. duranensis.* In spite of several attempts to resolve this uncertainty, no data conclusively determined the authentic origin of cultivated peanut.

Peanut was supposed to be originated about 5000 years ago in the southern Bolivia–north-western Argentina of South America. The portuguese apparently took them from Brazil to West Africa and then to south-western India in the 16th century. Africa is now regarded as a secondary centre of diversity. At the same time, the Spaniards introduced them from Mexico to the Western Pacific and later it has been spreaded to China, Indonesia and to Madagascar. The Dutch also probably took them from Brazil to Indonesia by the middle of the 17th century. They were probably introduced to the United States on slave ships from Africa, though they may have been introduced directly from the Caribbean Islands. Peanuts are now grown in most tropical, subtropical and temperate countries between 40 $^{\circ}$N and 40 $^{\circ}$S latitude, especially in Africa, Asia, North and South America. In Asia, the peanut is a major crop in India, China, Indonesia, Myanmar, Thailand and Vietnam (van der Maesen and Somaatmadja 1992).

Peanut is cultivated in more than 100 countries. According to 2011-2012, China leads in production of peanut, having a share of about 41.50% of overall world production, followed by India (18.20%), Nigeria (9.0%), the United States of America (6.8%), Myanmar (4.2%), Sudan (2.3%), Argentina (2.1%), Indonesia (2.1%), Tanjania (2.0%) and Cameroon (1.6%). Bangladesh is 40th in the world ranking and share of 0.1% (Source: en.cnagri.com)

In Bangladesh, peanuts are predominantly grown in the vast “Char” areas of Bangladesh by the marginal farmers. Peanut is very much popular as leisure time snacks. Roasted peanut is consumed most during the outside activities, such as by spectator of different sports, mass gathering in political parties meetings or other national tradition celebration. According to Bangladesh

Bureau of Statistics (BBS) during 2010-11, it was cultivated in about 35000 hectare of lands and about 40,000 MT of peanuts are produced annually in the districts of the Noakhali, Faridpur, Pabna, Kishorganj, Patuakhali, Rangpur and Dhaka.

The peanut variety growing in Bangladesh is characterized by their low yield potential. The major constraints of peanut production in our country as well as in many countries of the world are the fungal foliar diseases (Talukder 1974). Among them early and late leaf spot (commonly known as 'Tikka') caused by *Cercospora arachidicola* Hori. and *Cercosporidium personatum* Deighton respectively, are the most destructive and widely distributed diseases of peanut in Bangladesh which may cause 30-40% yield loss. Rust disease caused by *Puccinia arachidis* Speg. is also one of the major diseases, particularly appears during the wet seasons. It appears at the later stage of plant growth and may cause 20-30% yield loss. These diseases also reduced seed quality by decreasing seed size and oil content (Ghuge *et al.* 1981).

Due to its continuous popularity as food, fodder and source of N_2 breeder are attracted to this plant. Several institutes had been trying to develop improved varieties of peanut to meet the demand. As a consequences, Bangladesh Agricultural Research Institute (BARI) released nine peanut varieties, namely- Dhaka-1, Tridana Badam (DM-1), Basanti Badam (DG-2), Jhinga Badam (Acc. no.-12), BARI Cheena Badam-5, BARI Cheena Badam-6, BARI Cheena Badam-7, BARI Cheena Badam-8 and BARI Cheena Badam-9. Moreover, Bangladesh Institute of Nuclear Agriculture (BINA) also released five improved varieties *viz.*, BINA Cheena Badam-1, BINA Cheena Badam-2, BINA Cheena Badam-3, BINA Cheena Badam-4 and BINA Cheena Badam-5.

Characterizations of varieties are very important for improved breeding programs. However, the above mentioned released varieties of peanut were characterized on the basis of their morphological features. This kind of

characterization is not always reliable. Due to phenotypic plasticity any of the varieties may show morphological changes in different environments. Therefore, an authentic characterization and streamlining of these varieties are needed. Karyotype analysis is a reliable technique for characterization since it is stable and specific to each variety (Sultana *et al.* 2013).

Only a few earlier workers tried to characterize peanut with classical karyotype analysis (Husted 1936, D'Cruz and Tankasale 1961, Singh and Moss 1982, Stalker and Dalmacio 1986, Cai *et al*. 1987). In most of the cases, the analysis was confined to 2n chromosome count. In addition, an attempt was taken to determine the location of satellite in peanut chromosomes by Fernandez and Krapovickas (1994). None of the earlier workers made any comparative karyotype study among the peanut varieties. Conventional karyotype analysis is alone unable to express critically the differences among different varieties of a species since the varieties of a species possess similar 2n chromosomes numbers and even other karyotype parameters (Khantun and Alam 2010, Kahtun *et al.* 2011). Minute deletion, inversion, tandem duplication etc. could not be possible to detect by conventional karyotype analysis (Sultana and Alam 2007). Therefore, a critical karyotype analysis with modern cytogenetical and molecular techniques is necessary for comparative study among different varieties of peanut. In addition, other karyomorphological parameters should be considered to get more data about each variety. Study of the staining properties of interphase nuclei and prophase chromosomes is one such recent karyomorphological parameter. This is usually done by differential staining with orcein, CMA and DAPI (Alam and Kondo 1995, Fawzia and Alam 2011, Shahla and Alam 2011). Tanaka (1971) classified the different types of interphase nuclei and prophase chromosomes on the basis of orcien staining property. 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.

Another recent method for karyotype study is concerned to DNA-base specific banding with fluorochromes such as chromomycin A_3 (CMA) and 4′-6-diamidino-2-phenylindole (DAPI). CMA binds with GC (Guanine-Cytosine)-rich repetitive sequences of the genome and gives characteristics yellow colour bands. On the other hand, DAPI binds to AT (Adenine-Thymine)-rich repeats giving characteristic blue colour (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 GC-rich base pairs of chromosomes etc.

DNA based molecular markers have become important tools in studying 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) markers 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).

RAPD is a powerful tool for identification and monitoring pedigree breeding record of inbred parents or varieties (Baird *et al.* 1992, Echt *et al.* 1992, Struss *et al.* 1992) and determining genetic relationships among genotypes (Alam *et al.* 2012). It is an updated plant varietal identification method independent of restriction sites employing in the detection of polymorphisms by using the PCR technology (Welsh and McCleland 1990). This technique uses specific

oligonucleotide primers, which are highly sensitive in mapping traits and fingerprinting of individuals for crop improvement (Carlson *et al.* 1991, Klein-Lankhorst *et al.* 1991, Rafalsik *et al.* 1991, Rajput *et al.* 2006 and Waugh and Powell 1992). Moreover, the main advantages of RAPD over other molecular methods are the low sample DNA requirements, high frequency of detectable polymorphic DNA bands and independent from the effects of environmental factors (Kuras *et al.* 2004). RAPD markers have been used in molecular characterization of peanut varieties (Lang and Hang 2007, Raina *et al.* 2001, Subramanian *et al.* 2000) as well as other species such as chickpea (Rasool 2013), potato (Rocha *et al.* 2010) and eggplant (Biswas *et al.* 2009).

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 (Powell *et al.* 1996b, Milbourne *et al.* 1997, Russell *et al.* 1997, Crouch *et al*. 1999) 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 (Dwivedi *et al.* 2003). However, the first SSRs to be developed in groundnut detected disappointing levels of polymorphism in cultivated varieties (Hopkins *et al.* 1999). Nevertheless, in the past several years, about 600 SSR markers in genus *Arachis* were developed and a higher level of polymorphism was detected in the cultivated peanut (He *et al.* 2003, Ferguson

et al. 2004b). In addition, few genetic linkage maps have been developed in cultivated groundnut (Varshney *et al.* 2009, Sujay *et al.* 2012).

Although some conventional karyotype and PCR based marker analysis of peanut have been undertaken earlier, these were scattered not exactly used for characterization. There was no report on fluorescent banding for comparative karyotype analysis of peanut varieties. No attempt has been taken earlier to combine cytogenetical and molecular data for characterization of peanut varieties. Moreover, no cytogenetical and molecular analyses have yet been initiated for the released variety of Bangladesh.

Therefore, a combined effort of cytogenetical and molecular parameters should be undertaken for authentic characterization of peanut varieties available in Bangladesh. In the present study, a combination of cytogenetical and molecular analysis was carried out for the first time to characterize 11 peanut varieties released by BARI and BINA.

The aims of the present study were to-

i. determine the diploid (2n) chromosome number of 11 peanut varieties.

ii. compare the staining properties of the interphase nuclei and prophase chromosomes following staining with orcein, CMA and DAPI.

iii. compare the conventional,- CMA- and DAPI-karyotypes of the 11 peanut varieties.

iv. characterize the peanut varieties using RAPD and SSR primers.

v. elucidate the phylogenetic relationship among peanut varieties on the basis of their cytogenetical and molecular data.

Figs. 1-7. Pods and seeds of 7 varieties of peanut (*Arachis hypogaea* L.). 1. Dhaka-1, 2. Tridana Badam (DM-1). 3. Basanti Badam (DG-2), 4. Jhinga Badam (Acc. no.-12), 5. BARI Cheena Badam-6, 6. BARI Cheena Badam-7 and 7. BARI Cheena Badam-8.

Figs. 8-11. Pods and seeds of 4 varieties of peanut (*Arachis hypogaea* L.). 8. BINA Cheena Badam-1, 9. BINA Cheena Badam-2, 10. BINA Cheena Badam-3 and 11. BINA Cheena Badam-4.

2. Materials and Methods

2.1. Materials

2.1.1. Plant materials

The following 11 varieties of peanut (*Arachis hypogaea* L.) were collected from two national Institutes *viz.*, i) Bangladesh Agricultural Research Institute (BARI) and ii) Bangladesh Institute of Nuclear Agriculture (BINA). These 11 varieties were maintained in the Botanical Garden, Department of Botany, University of Dhaka.

BARI varieties

i. Dhaka-1 (Fig. 1)
ii. Tridana Badam (DM-1) (Fig. 2)
iii. Basanti Badam (DG-2) (Fig. 3)
iv. Jhinga Badam (Acc. no.-12) (Fig. 4)
v. BARI Cheena Badam-6 (Fig. 5)
vi. BARI Cheena Badam-7 (Fig. 6)
vii. BARI Cheena Badam-8 (Fig. 7)

BINA varieties

i. BINA Cheena Badam-1 (Fig. 8)
ii. BINA Cheena Badam-2 (Fig. 9)
iii. BINA Cheena Badam-3 (Fig. 10)
iv. BINA Cheena Badam-4 (Fig. 11)

2.1.2. Description of varieties

The brief description of each variety was given below-

i. Dhaka-1

Plant height is 30-40 cm. Leaves are light green in colour. Seed has no dormancy. Maturity in 'Rabi' season it takes 140-150 days and in 'Kharif' season it takes 120-140 days. Rabi' season pod yield is 1.8-2.0 t/ha and in 'Kharif' season pod yield is 1.6-1.8 t/ha. This variety is heavily infested by tikka and rust diseases. It is short duration variety. Pods are clustered form. Pods shell is thin and no hardy character.

ii. Tridana Badam (DM-1)

Plants are height in short. Leaves are deep green in colour. Seeds have no dormancy**.** It takes 110 to 115 days to mature**.** Per hectare yield is 2.0-2.2 ton. This variety is moderately resistant to tikka and rust diseases.

iii. **Basanti Badam (DG-2)**

Plant height is 30-35 cm. Leaves are deep green in colour. Average yield 2.0-2.2 t/ha in 'Rabi' season and 1.8-2.0 t/ha in 'Kharif' season. Seed has dormancy for about 30-40 days. This variety is resistant to tikka and rust diseases compare to Dhaka-1 variety. It is long duration variety, days to maturity 170-180.

iv. Jhinga Badam (Acc. no.-12)

Plant height is 30-35 cm. Leaves are longer and deep green in colour. It takes 130 to 140 days to mature. Per hectare yield is 2.5-3.0 ton. This variety is moderately resistant to tikka and rust diseases. Seeds have no dormancy. Seeds are very tasteful. Number of empty pods is very high compare to other variety. Shell of pods is thick. Surface of pods is very rough. Shape of the pods

is irregular.

v. BARI Cheena Badam-6

Plant height is 35-40 cm. Leaves are deep green in colour. Life cycle is 140-155 days in 'Rabi' season and 135-140 days in 'Kharif' season. Pod yield is 'Rabi' season is 2.8-3.0 t/ha and in 'Kharif' season is 1.8-2.0 t/ha. Seeds have no dormancy. This variety is moderately resistant to tikka and rust diseases. Outer surface of the pod is smooth and soft.

vi. BARI Cheena Badam-7

Plant height moderate, leaves are deep green in colour. Maturity day is 135-150 in 'Rabi' season and 120-130 days in 'Kharif' season, production rate per hectare 2.8-3.0 ton. This variety is moderately resistant to tikka and rust diseases.

vii. BARI Cheena Badam-8

Plant height is 35-42 cm. Leaves are deep green in colour. Life cycle is 140-150 days in 'Rabi' season and 125-140 days in 'Kharif' season. Average yield is 2.8-3.0 t/ha. Seeds have no dormancy. Outer surface of the pod is smooth and soft. This variety is susceptible to tikka and rust diseases

viii. BINA Cheena Badam-1

Plant height is about 32 cm. Leaves are light green in colour, stem sparsely hairy. Pods and grain size is 26% and 30% bigger than Dhaka-1. Oil and protein content is about 47% and 28%. Life time 'Rabi' 150-160 days and 'Kahrif' 125-135 days. Yield Rabi is 3.7 t/ha and Kharif is 2.4 t/ha. This variety is moderately resistant to tikka and rust diseases

ix. BINA Cheena Badam-2

Plant height is 28-30 cm. Leaves are deep green in colour, stem sparsely hairy. Outer surface of pod is smooth and soft. Pods and grain size is 24% and 28% bigger than Dhaka-1. Oil and protein content is about 50% and 28%. Life time 'Rabi' 150-160 days and 'Kahrif' 125-135 days. Production Rabi is 3.2 t/ha and Kharif is 1.7 t/ha. Seeds have no dormancy

x. BINA Cheena Badam-3

Plant height is 30-35 cm, Leaves are light green in colour, stem sparsely hairy. Outer surface of pod is smooth and soft. Pods and grain size is 27% and 40% bigger than Dhaka-1. Oil and protein content is about 52% and 29%. Life time 'Rabi' 150-160 days and 'Kahrif' 125-135 days. Yield is Rabi 3.0 t/ha and Kharif is 1.6 t/ha. Seeds have no dormancy

xi. BINA Cheena Badam-4

Plant height is 37-40 cm. Leaves are deep green in colour, stem sparsely hairy. Outer surface of pod is smooth and soft. Pods and grain size is 26% and 40% bigger than Dhaka-1. Oil and protein content is about 48.6% and 27.5%. Life time 'Rabi' season 140-150 days and 'Kahrif' season 100-120 days. Yield is Rabi 2.6 t/ha and kharif is 2.47 t/ha. Seeds have no dormancy.

2.2. METHODS -Cytogenetical studies

2.2.1. Preparation of reagents

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

0.29 gm of 8-hydroxyquinoline 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)

45 ml of glacial acetic acid was mixed with 55 ml of distilled water.

2.2.1.3. Preservative (70% alcohol)

70 ml of absolute alcohol was mixed with 30 ml of distilled water.

2.2.1.4. Hydrolyzing agent (1N HCl)

36.5 ml of HCl 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%)

1 g of orcein dye (Sigma, USA) was added to a flask containing 45 ml (100%) acetic acid. These were heated (not boiling) for about 20 h and volume 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)

1 mg of chromomycin A_3 (CMA) (Sigma, USA) was thoroughly dissolved in 10 ml of McIlvaine's buffer (McIlvaine 1921) 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)

1 mg 4'-6-Diamidino-2-Phenylindole (DAPI) (Sigma, USA) was thoroughly dissolved in 100 ml 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 mixed in 1 litre distilled water. It was then autoclaved 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 and then 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)

2.0 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)

2.0 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

A 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 of seedlings from the Botanical 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 root tips were soaked on a filter paper to remove surface water and pretreated with 8-hydroxyquinoline (0.002 M) for 1 h.

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 50 s 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 30-40 m. 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 Nikon 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 °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 slides and a clean cover glass placed on it. The slide was kept in a humid chamber for 12 m. The slides were 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 °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 with 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. The slide was washed with distilled water to remove cover glass. 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 °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.

∂= Band throughout the length of short arm.

β = Band in interstitial portion of short arm.

γ = Band in interstitial portion of long arm.

Ω = Band throughout the length.

θ = Band throughout the length of long arm.

Ø= Bands on the proximal ends of centromere.

€ = Band on the terminal portion of short arm.

λ = Bands on the terminal portion of both arms.

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 variety. 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 (5X, 3.5X). The final magnification was calculated using printed photographs.

2. 3. MOLECULAR STUDY (RAPD and SSR primer 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 varieties of peanut. 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. Stock solution of Tris-HCl (1M) 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 °C. The solution was shaken well before each use.

2.3.2.8. 70% ethanol (100 ml)

30 ml double distilled water (ddH_2O) 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 °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_2O, adjusted the final volume to 100 ml with ddH_2O 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 1. 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 of 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 up to the dissolve of CTAB.
- 700 µl β-mercaptoethanol was added prior use and mixed by glass rod under fume hood
- pH of all solutions wered 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 equipment

1. Autoclave machine (Hirayama, Japan)
2. Digital electrical balance (AND, FX-300, Japan)
3. Beaker
4. Centrifuge machine (Hettich, Germany) with 10,000-14,000 rpm
5. Conical flasks
6. Eppendorf tubes
7. Gloves
8. Ice machine (Ziegra, Germany)
9. Water bath (Julabo, Germany) capable of maintaining 65 °C
10. Micropipettes and nuclease free micropipette tips
11. Mortar and pestle (China)
12. pH meter (Consort, Belgium)
13. Refrigerator (LabTech, Korea)
14. Water de-ionizer (TKA, Germany)
15. Water distillation plant (LabTech, Korea)
16. Fume hood (LabTech, Korea)

2.3.3.2. Required chemicals

1. Liquid nitrogen
2. 100% ethanol
3. Trisbase
4. EDTA (Ethylene diamine tetra acetic 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 were 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 stored 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 equipment and chemicals were used to conduct agarose gel electrophoresis:

1. A horizontal electrophoresis chamber (Roth, Germany) 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) (Invitrogen, USA)
7. Electrophoresis buffer (TAE)
8. 6X sample loading buffer

9. Agarose (Invitrogen, USA)
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 Trisbase (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 °C.

2.3.4.1.1.3. Ethidium bromide solution

For preparing 1 ml 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 (10 mg/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 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 then off the power switch.

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, Germany) for observing the DNA bands. The DNA was observed as band and photographed using gel documentation system. The electrophoregram of DNA samples of 11 peanut varieties are shown in figure 177.

2.3.5. Quantification 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:

DNA concentration = A260 × Dilution factor × Conversion factor

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

= (ng/µl)

= (µg/ml)[since 1 µg = 10^{-3}ng i.e., µg/ml = ng/µl]

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 2. Spectrophotometric absorbance readings at 260 nm wave length and concentration of DNA of 11 varieties of *Arachis hypogaea* L.

Varieties	Absorbance reading at 260 nm	Concentration of DNA (ng/µl)
Dhaka-1	0.094	4700
BINA Cheena Badam-1	0.098	4900
BINA Cheena Badam-2	0.082	4100
BINA Cheena Badam-3	0.067	3350
BINA Cheena Badam-4	0.058	2900

Tridana Badam (DM-1)	0.083	4150
Basanti Badam (DG-2)	0.044	2200
Jhinga Badam (Acc. no.-12)	0.078	3900
BARI Cheena Badam-6	0.094	4700
BARI Cheena Badam-7	0.098	4900
BARI Cheena Badam-8	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 3. Preparation of working DNA solution (25 ng/µl) for PCR

Varieties	Working solution (25 ng/µl) for PCR	
	TE buffer/ de-ionized water (µl) required	DNA (µl) required
Dhaka-1	374	2
BINA Cheena Badam-1	390	2
BINA Cheena Badam-2	326	2
BINA Cheena Badam-3	266	2
BINA Cheena Badam-4	230	2
Tridana Badam (DM-1)	330	2
Basanti Badam (DG-2)	174	2
Jhinga Badam (Acc. no.-12)	310	2
BARI Cheena Badam-6	374	2
BARI Cheena Badam-7	390	2
BARI Cheena Badam-8	326	2

2.3.6.2. Primer test

Thirty two decamer primers (Operon Technology, USA and Sigma, USA) were tested for RAPD amplification of which eight primers exhibited good quality banding patterns and sufficient variability. These eight primers were selected for further analysis. The details of the eight primers were given in Table 4.

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

Table 4. Eight arbitrary RAPD primers used in the present study

Primer code	Sequence (5′—3′)	Annealing Temp. (°C)	G+C content (%)
OPA-1	CAG GCC CTT C	36.4	70
OPA-2	TGC CGA GCT G	40.7	70
OPA-3	AGT CAG CCA C	34.3	60
OPA-7	GAA ACG GGT G	33.2	60
OPA-8	GTG ACG TAG G	31.1	60
Primer-2	GTT GCG ATC C	34.2	60
Primer-19	GAT GAC CGC C	38.8	70
Primer-23	GTC AGG GCA A	34.7	60

Table 5. Four SSR primers were used in the present study

Primer code	Sequence (5′—3′)	Anneal Temp (°C)	G+C content (%)
BA00175669	forward-GAAAGAAATTATACACTCCAATTATG	57.8	27
BA00175670	reverse-CGGCATGACAGCTCTATGTT	62.9	50

BA00175671	forward-CCTTTTCTAACACATTCACACATGA	63.7	36
BA00175672	reverse-GGCTCCCTTCGATGATGAC	64.7	58
BA00175673	forward-ACTCGCCATAGCCAACAAAC	63.9	50
BA00175674	reverse-CATTCCACAACTCCCACAT	60.9	47
BA00175677	forward-CTCTCCTCTGCTCTGCACTG	63.2	60
BA00175678	reverse-ACAAGAACATGGGGATGAAGA	63.3	43

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-

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

$$\mathrm{v} = \frac{53.4\ \mathrm{nmol}}{100\ \mu\mathrm{mol}}$$

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

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

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

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

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

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

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

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

$$v = 534\ \mu\text{l}$$

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 100 µM 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 6, 7). The total volume of PCR cocktail was 25 µl per sample.

Table 6. 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 7. Component of PCR cocktail (12 reactions) for SSR primers

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 (0.5 ml) 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 thermal cycler 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:

2.3.6.5.1. Arbitrary primer-PCR profile

Denaturation/ Annealing/ Extension		Temperature	Time
	Initial denaturation	95 °C	5 m
45 cycles	Denaturation at	95 °C	1 m
	Annealing at	26-36 °C	30 s

Extension at	72 °C	3 m
Final extension at	72 °C	5 m

2.3.6.5.2. SSR primer-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 extension 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 1.0 g 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 (BioSciTec, Gelscan 6.0 Professional, Germany) for both the markers.

2.3.7. RAPD and SSR primer 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 primers 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 variety using computer program "POPGENE 32" (Version 1.32).

3. Results

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

Almost equal numbers of dividing cells were observed throughout the year in the root tip cells (RTCs) (about 80%). However, the number of dividing cells was 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 11 peanut (*Arachis hypogaea* L.) varieties were collected at 9.00, 9.30, 10.00, 10.30, 11.00, 11.30 a.m. and 12:00, 12:30 p.m. It was found that 12.30 p.m. was the optimum time for obtaining maximum number of dividing cells (~ 80%).

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 8-hydroxyquinoline solution (0.002 M) for 1 h gave the best result.

3.4. Karyotypes and Idiograms

On the basis of overall length and centromeric position, somatic chromosomes of peanut could be assembled in 20 pairs (2n = 40), except BARI Cheena Badam-7 and BARI Cheena Badam-8 (2n=41) (Figs. 78-88, 111-121, arrow). In later two cases, the extra chromosome placed alone in the karyotype.

3.5. Interphase nuclei

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

3.5.1. Orcein staining

The 11 varieties have more or less similar staining type of interphase nuclei with orcein. Few heterochromatic bodies were distributed throughout the nucleus (Figs. 12-22, arrow). BINA Cheena Badam-1 had relatively more and BARI Cheena Badam-6 less heterochromatin (Figs. 12, 20). Big nucleli were found in Dhaka-1, BINA Cheena Badam-1, BINA Cheena Badam-2, BINA Cheena Badam-3 and BINA Cheena Badam-4.

3.5.2. CMA-staining

Each variety showed several CMA-positive bands. The number of bands ranged from 4-8 (Figs. 23-33). A big non-staining area was observed in BINA Cheena Badam-2 and Tridana Badam (DM-1) (Figs. 25, 28, arrow).

3.5.3. DAPI-staining

Many bright DAPI-positive bands were observed in interphase nuclei in each variety. The number of bands varied from 8-24 (Figs. 34-44). Big non-staining areas were observed in BINA Cheena Badam-1 and BINA Cheena Badam-4 (Figs. 35, 38, arrow).

3.6. Prophase chromosomes

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

3.6.1. Orcein staining

Prophase chromosomes of each variety stained differently with orcein. Terminal ends of few chromosomes were darkly stained with orcein in Dhaka-1 (Fig. 45), BINA Cheena Badam-1 (Fig. 46), BINA Cheena Badam-4 (Fig. 49), Basanti Badam (DG-2) (Fig. 51), Jhinga Badam (Acc. no.-12) (Fig. 52), BARI Cheena Badam-6 (Fig. 53), BARI Cheena Badam-7 (Fig. 54) and BARI Cheena Badam-8 (Fig. 55). The rest of prophase chromosomes did not show darker regions. The prophase chromosomes of BINA Cheena Badam-3 and Tridana Badam (DM-1) were stained at the interstitial region (Figs. 48, 50). In BINA Cheena Badam-2, 7-8 chromosomes were stained along the entire length. Ten-twelve chromosomes showed darker heterochromatic regions at one terminal ends and then gradually faint to another end. The rest of the chromosomes did not have any prominent darker region (Fig. 47).

3.6.2. CMA-staining

Six to eight CMA-positive bands were observed in 11 varieties. Most of the bands were present in the terminal regions of chromosomes (Figs. 56-66).

3.6.3. DAPI-staining

The prophase chromosomes of 11 varieties were stained more or less homogeneously. Many DAPI-positive bands were distributed within the prophase chromosomes of 11 varieties (Figs. 67-77). The bands were different in number, size and location.

3.7. Metaphase chromosomes

3.7.1. Orcein staining

Total length, l/s ratio, centromeric index, relative length and centromeric type

of each chromosome of 11 peanut (*Arachis hypogaea* L.) varieties were tabulated (Tables 8-18). Except BARI Cheena Badam-7 and BARI Cheena Badam-8, all varieties possess 2n=40 chromosomes. In BARI Cheena Badam-7 and BARI Cheena Badam-8, a small extra chromosome was found in addition to 2n=40 chromosomes in many cells. The length of chromosomes was gradually decreased in each case. No satellite or secondary constriction was found. Karyotype features of each variety were given below:

Dhaka-1 was found to possess 2n=40 chromosomes (Fig. 111). The total length of 2n chromosome complement was 99.56 µm. The individual chromosome length ranged from 1.32-3.00 µm. The centromeric formula of this variety was 34m+6sm (Table 19). The relative length of chromosome was ranging from 0.01-0.03 (Table 8).

In BINA Cheena Badam-1, 2n=40 chromosomes were found. The total length of 2n chromosome complement was 74.82 µm. This length was the smallest among 11 varieties. The individual chromosome length ranged from 1.28-2.38 µm. The centromeric formula of this variety was 36m+4sm (Table 19). The relative length of chromosome was ranging from 0.02-0.03 (Table 9).

In BINA Cheena Badam-2, 2n=40 chromosomes were found (Fig. 113). The total length of 2n chromosome complement was 91.10 µm. The individual chromosome length ranged from 0.92-3.36 µm. The centromeric formula of this variety was 34m+6sm (Table 19). The relative length of chromosome was ranging from 0.01-0.04 (Table 10).

2n=40 chromosomes were found in BINA Cheena Badam-3 (Fig. 114). The total length of 2n chromosome complement was 93.78 µm. The individual chromosome length ranged from 1.20-2.76 µm. The centromeric formula of this variety was 38m+2sm (Table 19). The relative length of chromosome ranged from 0.02-0.03 (Table 11).

In BINA Cheena Badam-4, 2n=40 chromosomes were found (Fig. 115). The total length of 2n chromosome complement was 83.32 µm. The individual chromosome length ranged from 1.32-2.66 µm. The centromeric formula of this variety was 32m+8sm (Table 19). The relative length of chromosome ranged from 0.02-0.03 (Table 12).

In Tridana Badam (DM-1), 2n=40 chromosomes were observed (Fig. 116). The total length of 2n chromosome complement was 97.92 µm. Individual chromosome length ranged from 1.40-3.30 µm and the centromeric formula was determined as 32m+8sm (Table 19). The relative length of chromosome was ranging from 0.01-0.03 (Table 13).

In Basanti Badam (DG-2), 2n=40 chromosomes were found (Fig. 117). The total length of 2n chromosome complement was 96.20 µm and the individual chromosome length ranging from 2.00-3.06 µm. The centromeric formula of this variety was 38m+2sm (Table 19). The relative length of chromosome was determined between 0.02-0.03 (Table 14).

Jhinga Badam (Acc. no.-12) was found to possess 2n=40 chromosomes (Fig. 118). The total length of 2n chromosome complement was 105.84 µm. The individual chromosome length ranged from 1.52-3.28 µm and the centromeric formula of this variety was 38m+2sm (Table 19). The relative length of chromosome was determined 0.01-0.03 (Table 15).

In BARI Cheena Badam-6, 40 chromosomes were found in diploid cells (Fig. 119). The total length of 2n chromosome complement was 81.86 µm while individual chromosome length ranged from 1.32-2.64 µm. Centromeric formula of this variety was 40m (Table 19). The relative length of chromosome ranged from 0.02-0.03 (Table 16).

BARI Cheena Badam-7 was usually found to possess 2n=40 chromosomes (Fig. 120). However, 2n=41 chromosomes were also observed. The total length

of 2n chromosome complement was 91.22 µm (in case of 2n=41 chromosomes). The individual chromosome length ranged from 0.80-3.40 µm. The centromeric formula of this variety was 41m (Table 19). The relative length of chromosome was ranging from 0.02-0.04 (Table 17). The extra chromosome was much smaller than the smallest chromosome (pair XX) of other varieties. This chromosome did not have any homologue pair and thus placed alone as a member of pair XXI in the karyotype (Fig. 120, Table 17).

BARI Cheena Badam-8 had 2n=40 chromosomes. In addition, 2n=41 chromosomes were also observed in several cases (Fig. 121). The total length of 2n chromosome complement was 86.42 µm (in case of 2n=41 chromosomes) and the individual chromosome length ranged from 0.80-2.72 µm. The 41 chromosome of this variety was metacentric (Table 19). The relative length of chromosome was ranging from 0.02-0.03 (Table 18). The extra chromosome was much smaller than the smallest chromosome (pair XX) of other varieties. This chromosome did not have any homologue pair and thus placed alone as a member of pair XXI in the karyotype (Fig. 121, Table 18).

3.7.2. CMA-staining

In Dhaka-1, 8 CMA-positive bright bands were observed in seven chromosomes (Figs. 89, 133). A member of pair VIII was entirely fluoresced with CMA whereas its homologue member had CMA-band along the short arm. Both the members of pair XIII was found to possess CMA-band in upper terminal regions. A member of pair XVI had a band on each proximal end of the centromere. An upper terminal band was found in a member of pair XIX while no band present in its homologue. A member of pair XX was fluoresced entirely, however, band was totally lacking in its homologue. The total length of CMA banded region was 3.30 µm which covered about 3.31% of the total

chromatin length. The CMA banded karyotype formula of this variety was 2Ω + 1∂ + 1Ø +3€ + 33δ (Table 20).

A total of twelve CMA-positive bands was found in eleven chromosomes of BINA Cheena Badam-1 (Figs. 90, 134). Band was observed in each terminal end of a member of pair II. One member of pair V was entirely fluoresced with CMA whereas its homologue member had no CMA-band. Both the members of pair IX, X and a member of pair XII were found to possess CMA-band on terminal region of short arms. Entirely fluoresced CMA positive band was found in both members of pair XX. Both the members of pair XIII had a centromeric CMA-positive band. The total length of CMA banded region was 4.34 µm which covered about 5.80% of the total chromatin length. The CMA-positive karyotype formula of this variety was 2α + 1λ + 5€ + 3Ω + 28δ (Table 20).

In BINA Cheena Badam-2, nine CMA-positive bright bands were observed (Figs. 91, 135). Both the members of pair II and XIII had CMA-positive band at the terminal region of short arm. In contrast, one terminal band was found in a member of pair XIX. Both the members of pair XV and XVII had CMA-band along short arm. The total length of CMA banded region was 3.43 µm which covered about 3.76% of the total chromatin length. The CMA band karyotype formula of this variety was 5€ + 4∂ + 31δ (Table 20).

Four CMA-positive bands were found in three chromosomes of BINA Cheena Badam-3 (Figs. 92, 136). A member of pair VI had a thick and bright band that almost occupied the short arm. Two small dots like upper terminal bands were present on two chromatids in a member of pair XIV. One minute CMA-band was found in two terminal regions of a chromosome of pair XIII (Fig. 136). The total length of CMA banded region was 1.14 µm which covered about 1.22% of the total chromatin length. The CMA-positive karyotype formula of this

variety was 1€ + 1λ + 1∂ + 37δ (Table 20).

In BINA Cheena Badam-4, twelve chromosomes showed different CMA-positive bands (Figs. 93, 137). Heteromorphicity in respect of banding pattern was found in pair IV. In this pair a member had a terminal band on short arm and its homologue showed a centromeric band. Both the members of pair X and IX had thick CMA-bands occupied almost the short arms. Two members of pair XVII and XX flourescent entirely with CMA. Pair XII had a centromeric CMA-positive band in each member. The total length of CMA banded region was 5.14 µm which covered about 6.17% of the total chromatin length. The CMA banded karyotype formula of this variety was 3α + 1€ + 4∂ + 4Ω + 28δ (Table 20).

Eight chromosomes of Tridana Badam (DM-1) showed different CMA-positive bands (Figs. 94, 138). Members of pair III, VI and XIII had band along the short arms. A member of pair XI was entirely fluoresced with CMA while only short arm of its homologue fluoresced entirely. The total length of CMA banded region was 4.11 µm which covered about 4.19% of the total chromatin length. The CMA karyotype formula of this variety was 2β + 5∂ + 1Ω + 32δ (Table 20).

In Basanti Badam (DG-2), 7 CMA-positive bright bands were observed (Figs. 95, 139). Both the members of pair XIII had CMA band at upper terminal region. Short arm of only one member of pair XVIII was fluoresced entirely while both the members of pair XVI fluoresced entirely. A centromeric CMA-positive band was found in each member of pair III. The total length of CMA banded region was 3.20 µm which covered about 3.33% of the total chromatin length. The CMA banded karyotype formula of this variety was 2α + 1€ + 2∂ + 2Ω + 33δ (Table 20).

Only four chromosomes of Jhinga Badam (Acc. no.-12) showed CMA-positive bands (Figs. 96, 140). Both the members of pair XVIII fluoresced entirely. In

the member of pair IV, a thick and prominent band was observed that occupied entire long arms. The total length of CMA banded region was 2.51 µm which covered about 2.37% of the total chromatin length. The CMA-positive karyotype formula of this variety was 2θ + 2Ω + 36δ (Table 20).

BARI Cheena Badam-6 had in total six CMA-positive bright bands on six chromosomes (Figs. 97, 141). Both the member of pair XV and XVIII were found to possess a CMA-band on the terminal region of short arms. A CMA positive band was found almost at the centromeric region in both members of pair VIII (Figs. 141). The total length of CMA banded region was 1.83 µm which covered about 2.23% of the total chromatin length. The CMA banded karyotype formula of this variety was 2α + 4€ + 34δ (Table 20).

In BARI Cheena Badam-7, six chromosomes showed CMA bands (Figs. 98, 142). The short arms of the members in pair II and XV fluoresced entirely. On the other hand, pair XVIII fluoresced along the length. The total length of CMA banded region was 3.20 µm which covered about 3.50% of the total chromatin length. The CMA-positive karyotype formula of this variety was 4∂ + 2Ω + 34δ (Table 20).

In BARI Cheena Badam-8, six CMA-positive bright bands were observed (Figs. 99, 143). Both the members of pair VII and XI were found to possess CMA-band at the terminal region of short arm. CMA positive entirely fluoresced chromosomes were found in both members of pair XX. The total length of CMA banded region was 2.10 µm which covered about 2.51% of the total chromatin length. The CMA banded karyotype formula of this variety was 4∂ + 2Ω + 34δ (Table 20).

3.7.3. DAPI-staining

In Dhaka-1, 15 DAPI-positive bright bands were observed (Figs. 100, 155). Both the members of chromosome pair I, IX, X, XV and XX were found to

possess centromeric DAPI-positive band. A centromeric band was observed in a member of pair XVIII, whereas its homologue member had no band. Band in middle portion of short arm was found in both members of pair XVII. An interstitial long arm was observed in a member of pair XI whereas its homologue member had a centromeric band. The total length of DAPI banded region was 2.84 µm which covered about 2.83% of the total chromatin length. The DAPI banded karyotype formula of this variety was 12α + 2β + 1γ + 25δ (Table 21).

Eighteen chromosomes of BINA Cheena Badam-1 showed DAPI-positive bands (Figs. 101, 156). Both the members of chromosome pair IX, X and XV were found to possess a centromeric DAPI-positive band. Band in the interstitial portion of short arm was found in both members of pair VI, VII, XIV and XVII. One member of chromosome pair XVIII was entirely fluoresced with DAPI, whereas its homologue member had band in the interstitial portion of short arm. Both the members of chromosome pair XX were fluoresced along the length (Fig. 156). The total length of DAPI banded region was 4.46 µm which covered about 5.96% of the total chromatin length. The DAPI-positive karyotype formula of this variety was 6α + 9β + 3 Ω + 22δ (Table 21).

In BINA Cheena Badam-2, 14 DAPI-positive bright bands were observed (Figs. 102, 157). Both the members of chromosome pair VII, IX and X were found to possess a centromeric DAPI-positive band. A band in the interstitial portion of long arm was observed in a member of pair XI whereas its homologue member had a centromeric band. The short arms in both members of pair XV were fluoresced entirely with DAPI. A member of pair I had big band at the interstitial region of long arm while its homologue member had no band. In one member of pair VI band was found throughout the length of short arm, whereas its homologue member had no band. A centromeric band was observed in one member of pair XVIII, in contrast its homologue member

had band throughout the length of short arm (Fig. 157). The total length of DAPI banded region was 4.11 µm which covered about 5.51% of the total chromatin length. The DAPI banded karyotype formula of this variety was 8α + 2γ + 4∂ + 26δ (Table 21).

Eighteen DAPI-positive bands were found in BINA Cheena Badam-3 (Figs. 103, 158). Both the members of chromosome pair III, IV, VII, XI and XV had a centromeric DAPI-positive band. A centromeric band was observed in one member of pair XIII whereas its homologue member had no band. Band in interstitial portion of short arm was observed in both members of pair IX. A DAPI positive band was found at the terminal portion of short arm in one member of pair XII whereas its homologue member had no band. Both the members of chromosome pair XIX and XX were entirely fluoresced with DAPI (Fig. 158). The total length of DAPI banded region was 5.39 µm which covered about 5.75% of the total chromatin length. The DAPI-positive karyotype formula of this variety was 11α + 2β + 1€ + 4Ω + 22δ (Table 21).

In BINA Cheena Badam-4, a total of 12 DAPI-positive bright bands were observed (Figs. 104, 159). Both the members of chromosome pair VII, IX and XVII were found to possess centromeric DAPI-positive band. A centromeric band was observed in one member of pair XVI. A Band on the terminal portion of short arm was observed in one member of pair III, whereas its homologue member had a centromeric band. An upper terminal band was found in a member of pair XVIII, however, no such band was present in its homologue member. Both the members of chromosome pair XX were entirely fluoresced with DAPI (Fig. 159). The total length of DAPI banded region was 2.86 µm which covered about 3.43% of the total chromatin length. The DAPI banded karyotype formula of this variety was 8α + 2€ + 2Ω + 28δ (Table 21).

Eighteen DAPI-positive bands were found in Tridana Badam (DM-1) (Figs. 105,

160). Both the members of chromosome pair VII, X, XI, XII, XV, XVI and XVII were found to possess a centromeric DAPI-positive band. Moreover, a centromeric band was observed in one member of pair I, whereas its homologue member had no band. Both the members of chromosome pair XIX and a member of XX were fluoresced throughout the length. The total length of DAPI banded region was 4.57 µm which covered about 4.67% of the total chromatin length. The DAPI-positive karyotype formula of this variety was 15α + 3Ω + 22δ (Table 21).

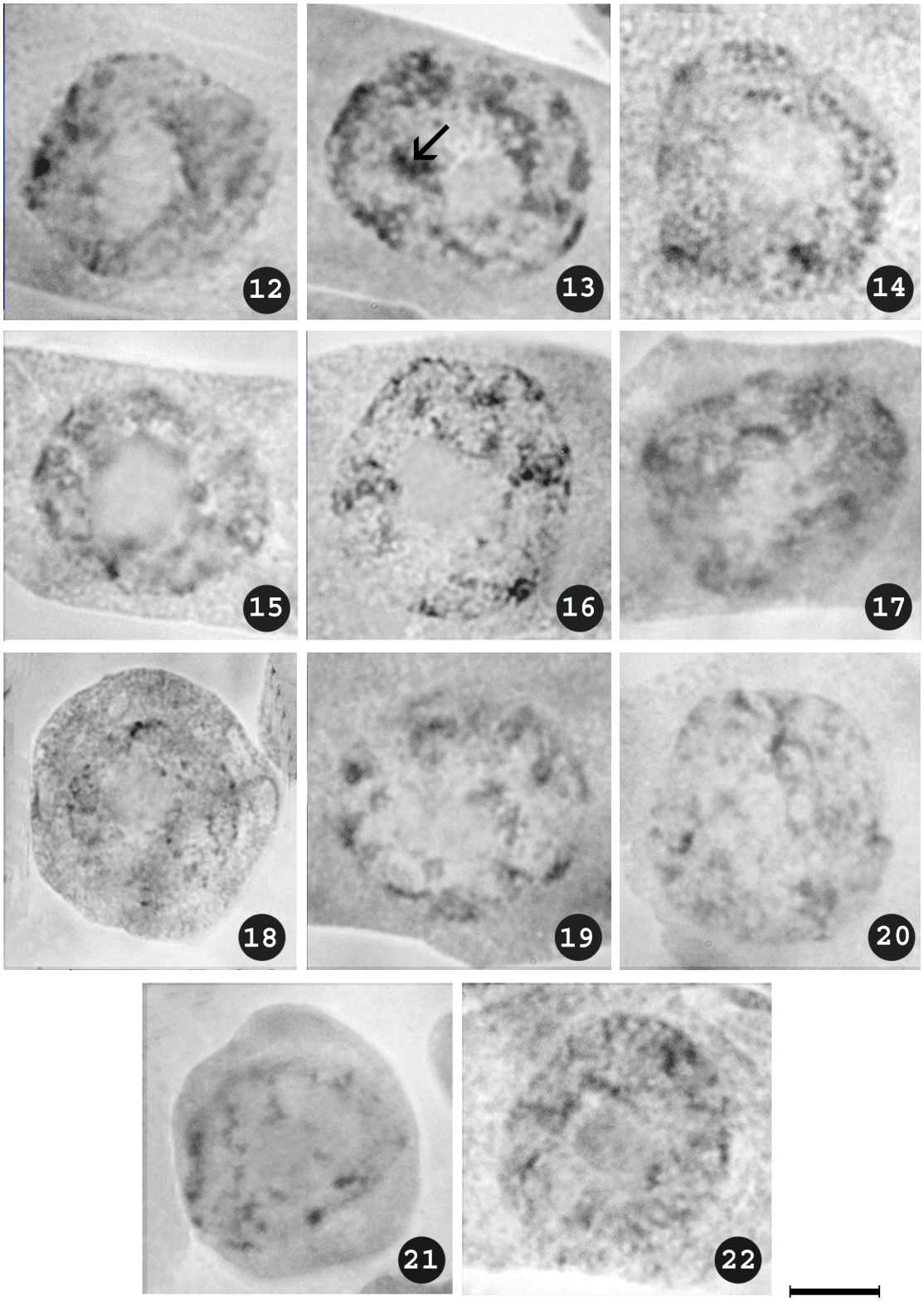
In Basanti Badam (DG-2) nine chromosomes showed bright bands (Figs. 106, 161). Centromeric bands were found in the members of chromosome pair IV, XI and XV. Both the members of chromosome pair XIX and a member of pair XVII fluoresced throughout the length. The total length of DAPI banded region was 2.61 µm which covered about 2.51% of the total chromatin length. The DAPI banded karyotype formula of this variety was 6α + 3Ω + 31δ (Table 21).

A total of five DAPI-positive bands were found in Jhinga Badam (Acc. no.-12) (Figs. 107, 162). Both the members of pair II and a member of chromosome pair XIII were found to possess a centromeric DAPI-positive band. Both the members of chromosome pair XX and a member of pair XVIII were entirely fluoresced with DAPI. The total length of DAPI banded region was 2.17 µm which covered about 2.05% of the total chromatin length. The DAPI-positive karyotype formula of this variety was 2α + 3Ω + 35δ (Table 21).

In BARI Cheena Badam-6, 10 DAPI-positive bright bands were observed (Figs. 108, 163). Both members of chromosome pair VII, XI XII, XV and a member of pair IV and XX had centromeric DAPI-positive band (Fig. 163). The total length of DAPI banded region was 2.40 µm which covered about 2.93% of the total chromatin length. The DAPI banded karyotype formula of this variety was 10α+ 30δ (Table 21).

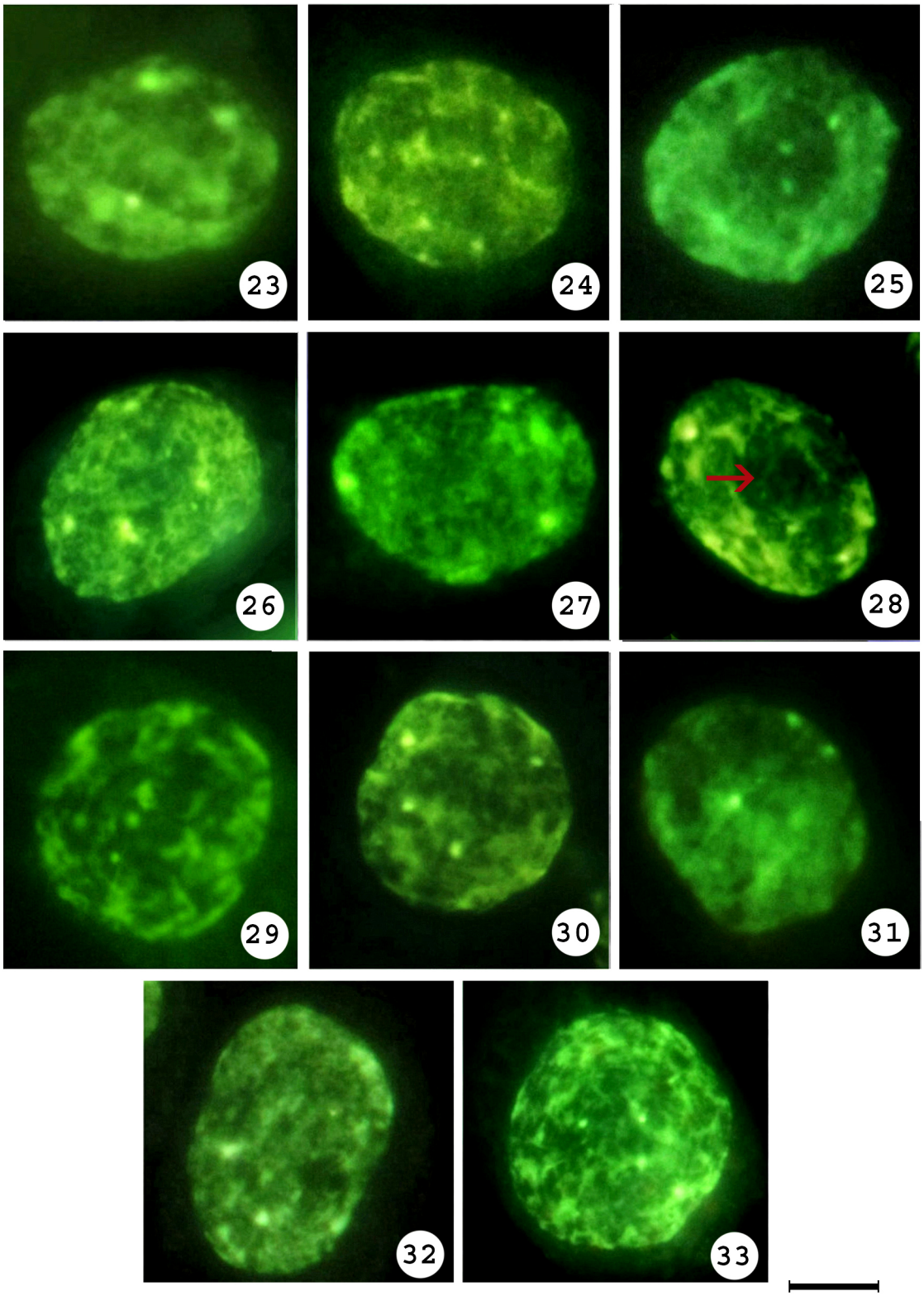
In BARI Cheena Badam-7, members of chromosome pair IV, VII, XI, XIV and a member of pair I had centromeric DAPI-positive band. Moreover, members of pair IX, XVIII and a member of pair XV had an upper terminal DAPI band (Figs. 109, 164). The total length of DAPI banded region was 4.02 µm which covered about 4.41% of the total chromatin length. The DAPI-positive karyotype formula of this variety was 9α + 5€ + 26δ (Table 21).

In BARI Cheena Badam-8, 18 centromeric DAPI-positive bright bands were observed in both the members of chromosome pair II, VII, XI, XII, XIII, XIV, XVI, XIX and XX (Figs. 110, 165). The total length of DAPI banded region was 4.68 µm which covered about 5.42% of the total chromatin length. The DAPI banded karyotype formula of this variety was 18α + 22δ (Table 21).



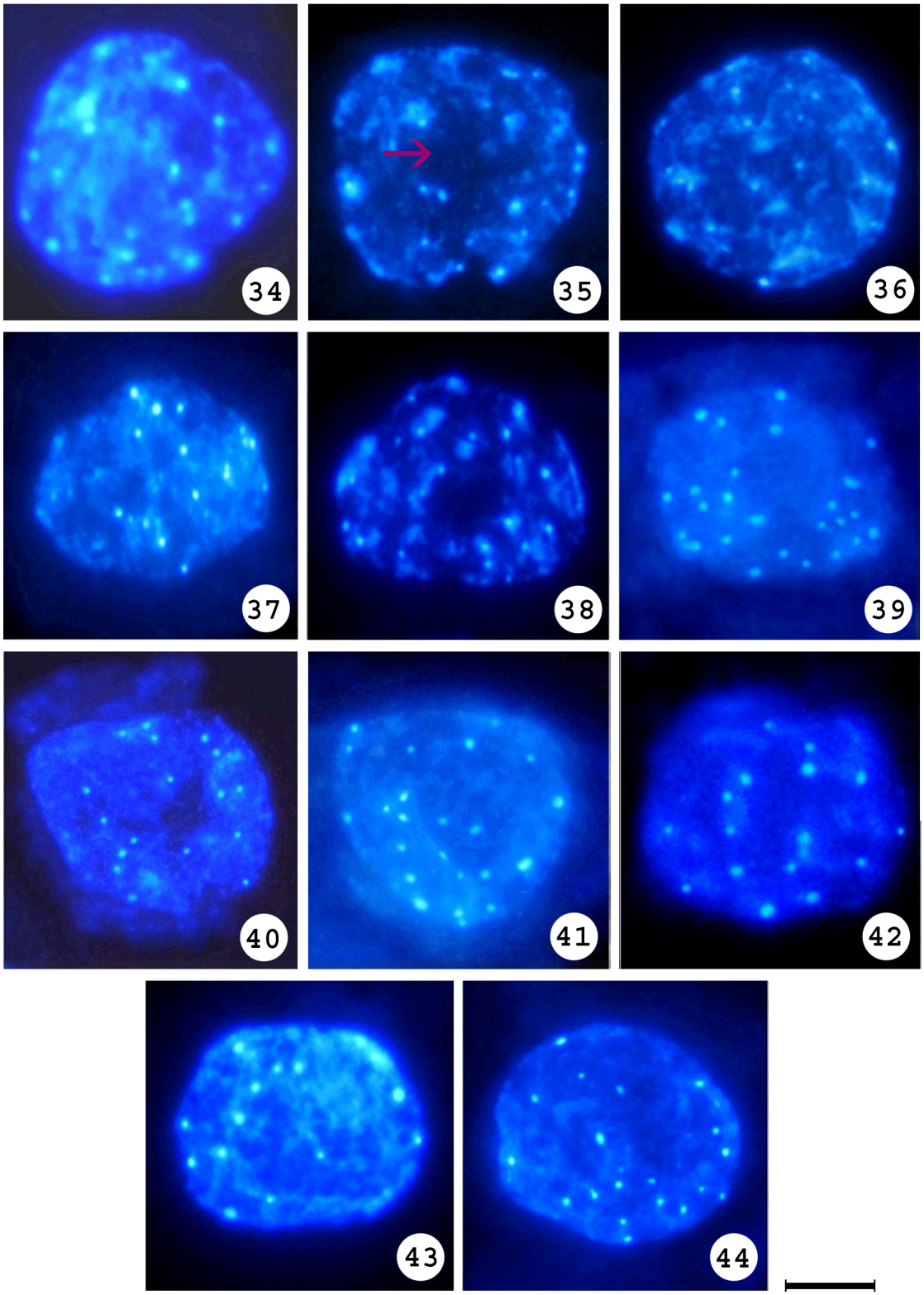
→ Heterochromatic body

Figs. 12-22. Orcein stained mitotic interphase nuclei of 11 varieties of peanut (*Arachis hypogaea* L.). 12. Dhaka-1, 13. BINA Cheena Badam-1, 14. BINA Cheena Badam-2, 15. BINA Cheena Badam-3, 16. BINA Cheena Badam-4, 17. Tridana Badam (DM-1), 18. Basanti Badam (DG-2), 19. Jhinga Badam (Acc. no.-12), 20. BARI Cheena Badam-6, 21. BARI Cheena Badam-7 and 22. BARI Cheena Badam-8. Bar = 5 µm.



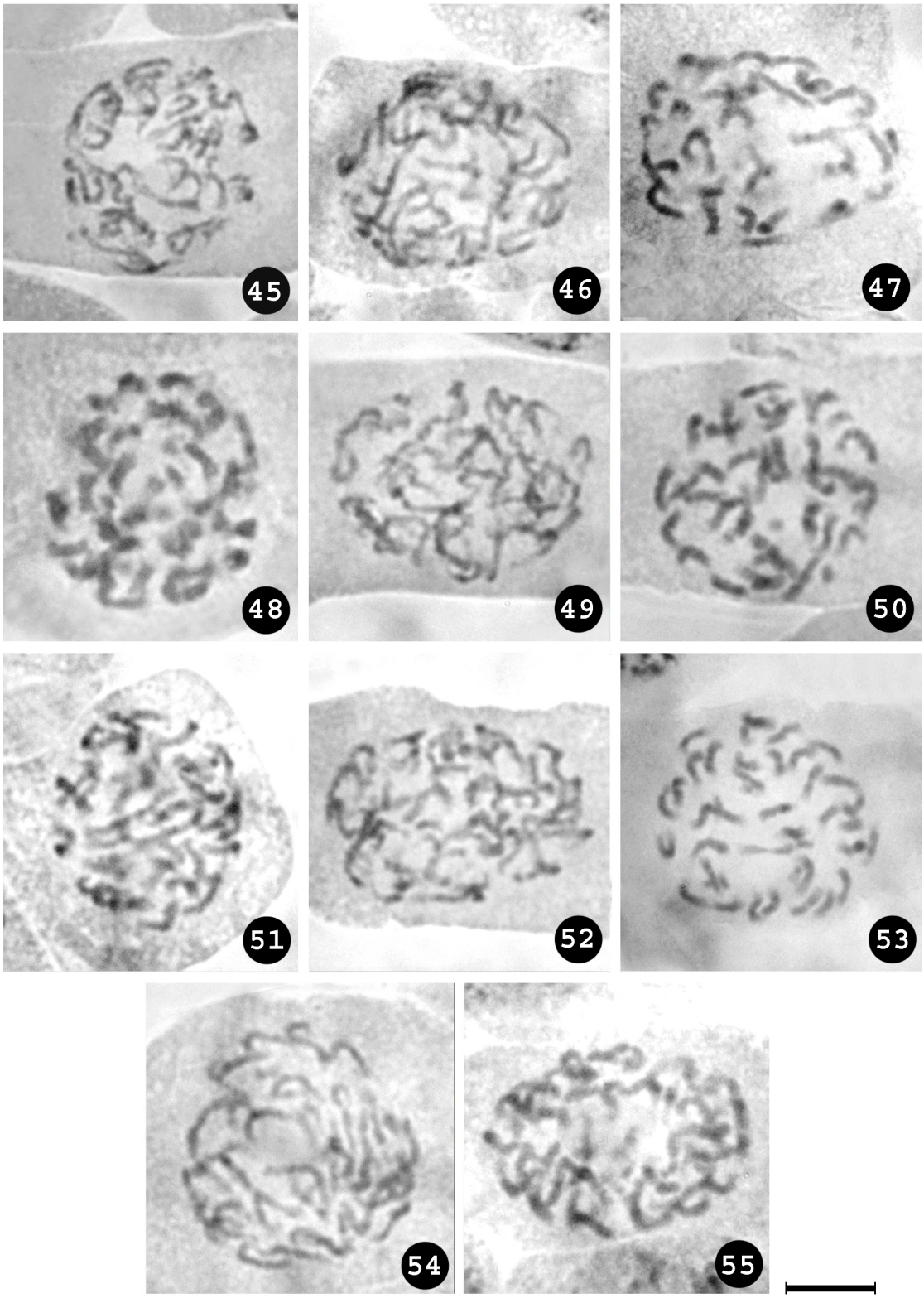
→ Big non-staining area

Figs. 23-33. CMA-stained mitotic interphase nuclei of 11 varieties of peanut (*Arachis hypogaea* L.). 23. Dhaka-1, 24. BINA Cheena Badam-1, 25. BINA Cheena Badam-2, 26. BINA Cheena Badam-3, 27. BINA Cheena Badam-4, 28. Tridana Badam (DM-1), 29. Basanti Badam (DG-2), 30. Jhinga Badam (Acc. no.-12), 31. BARI Cheena Badam-6, 32. BARI Cheena Badam-7 and 33. BARI Cheena Badam-8. Bar = 5 µm.

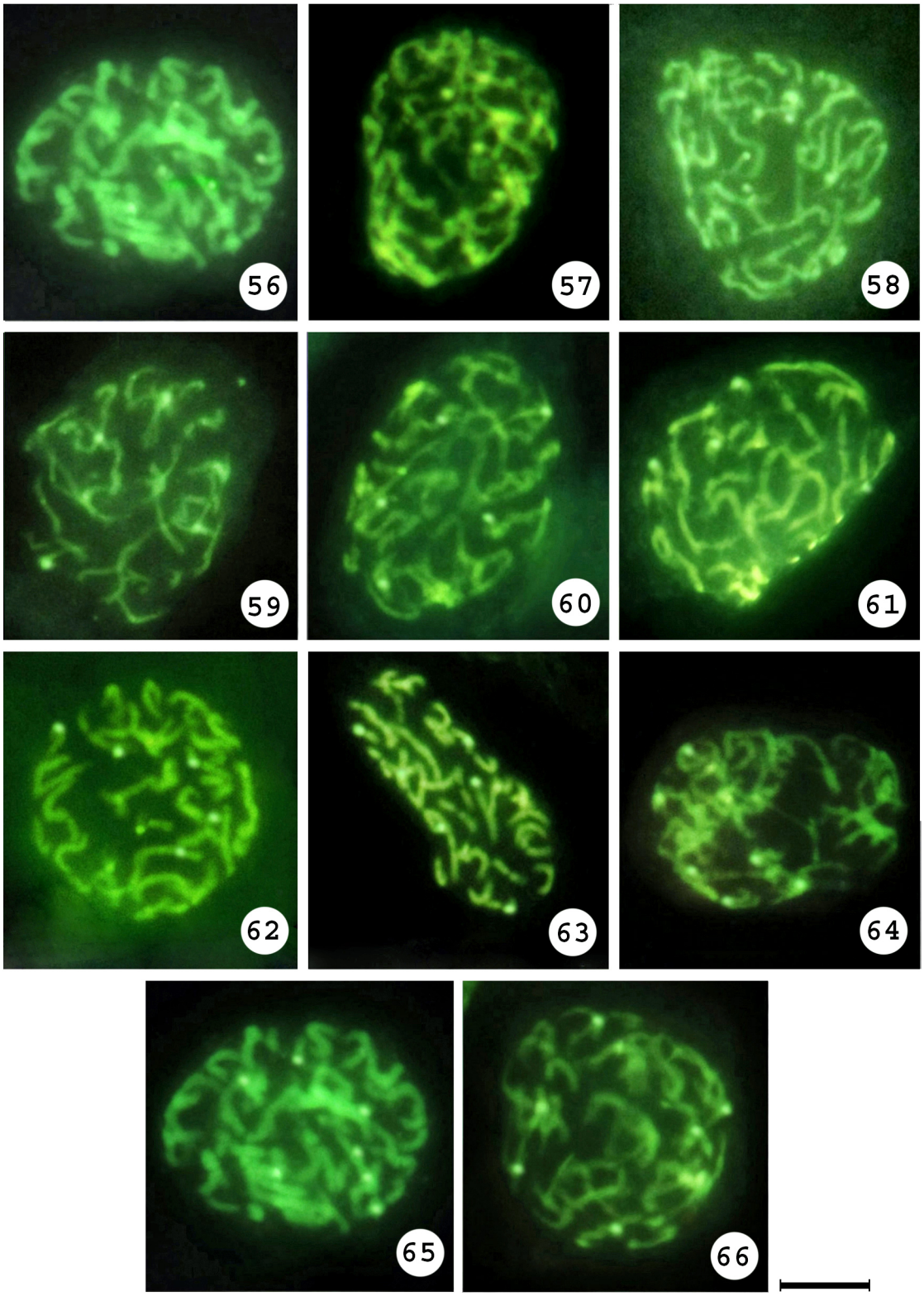


→ Big non-staining area

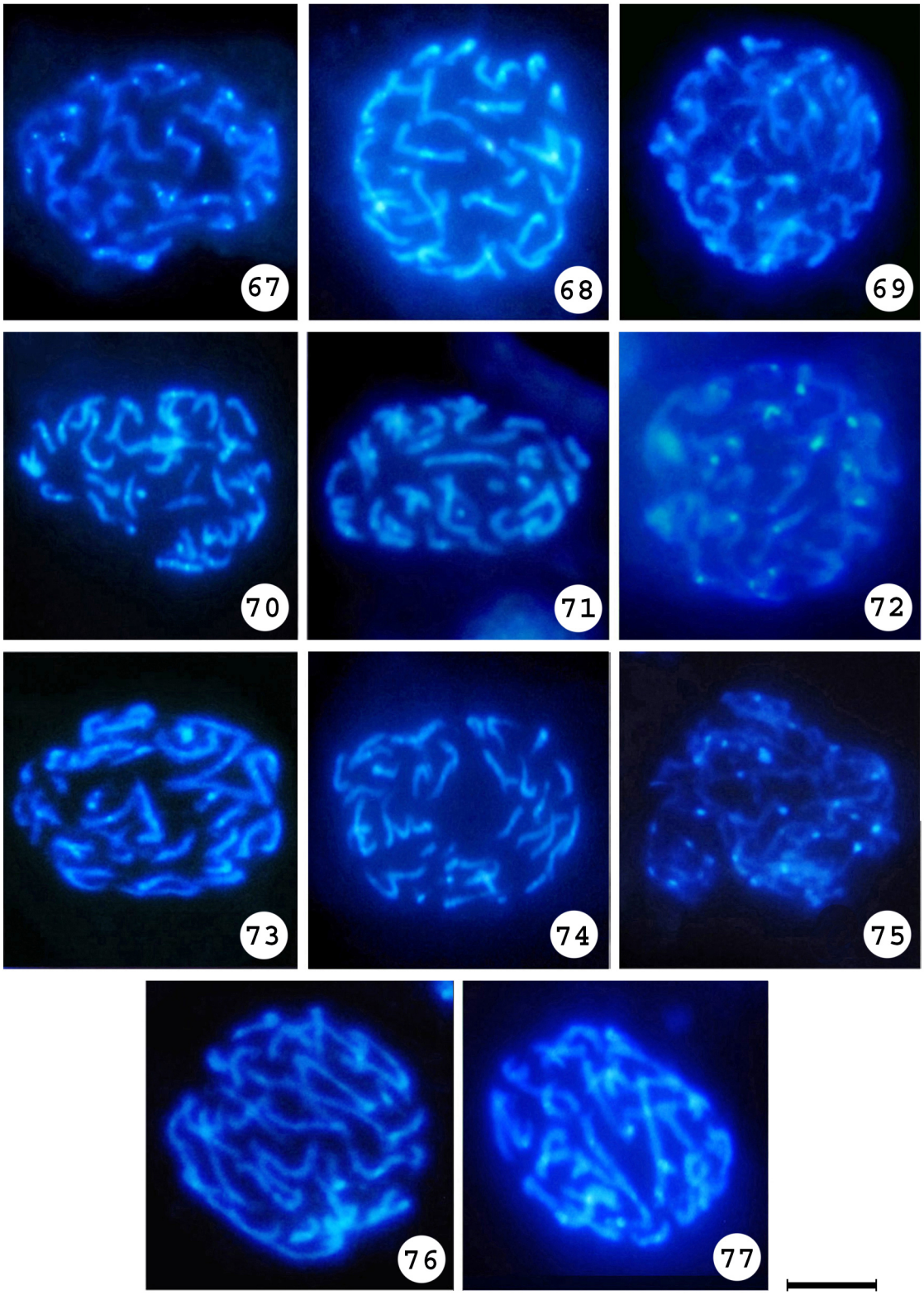
Figs. 34-44. DAPI-stained mitotic interphase nuclei of 11 varieties of peanut (*Arachis hypogaea* L.). 34. Dhaka-1, 35. BINA Cheena Badam-1, 36. BINA Cheena Badam-2, 37. BINA Cheena Badam-3, 38. BINA Cheena Badam-4, 39. Tridana Badam (DM-1), 40. Basanti Badam (DG-2), 41. Jhinga Badam (Acc. no.-12), 42. BARI Cheena Badam-6, 43. BARI Cheena Badam-7 and 44. BARI Cheena Badam-8. Bar = 5 µm.



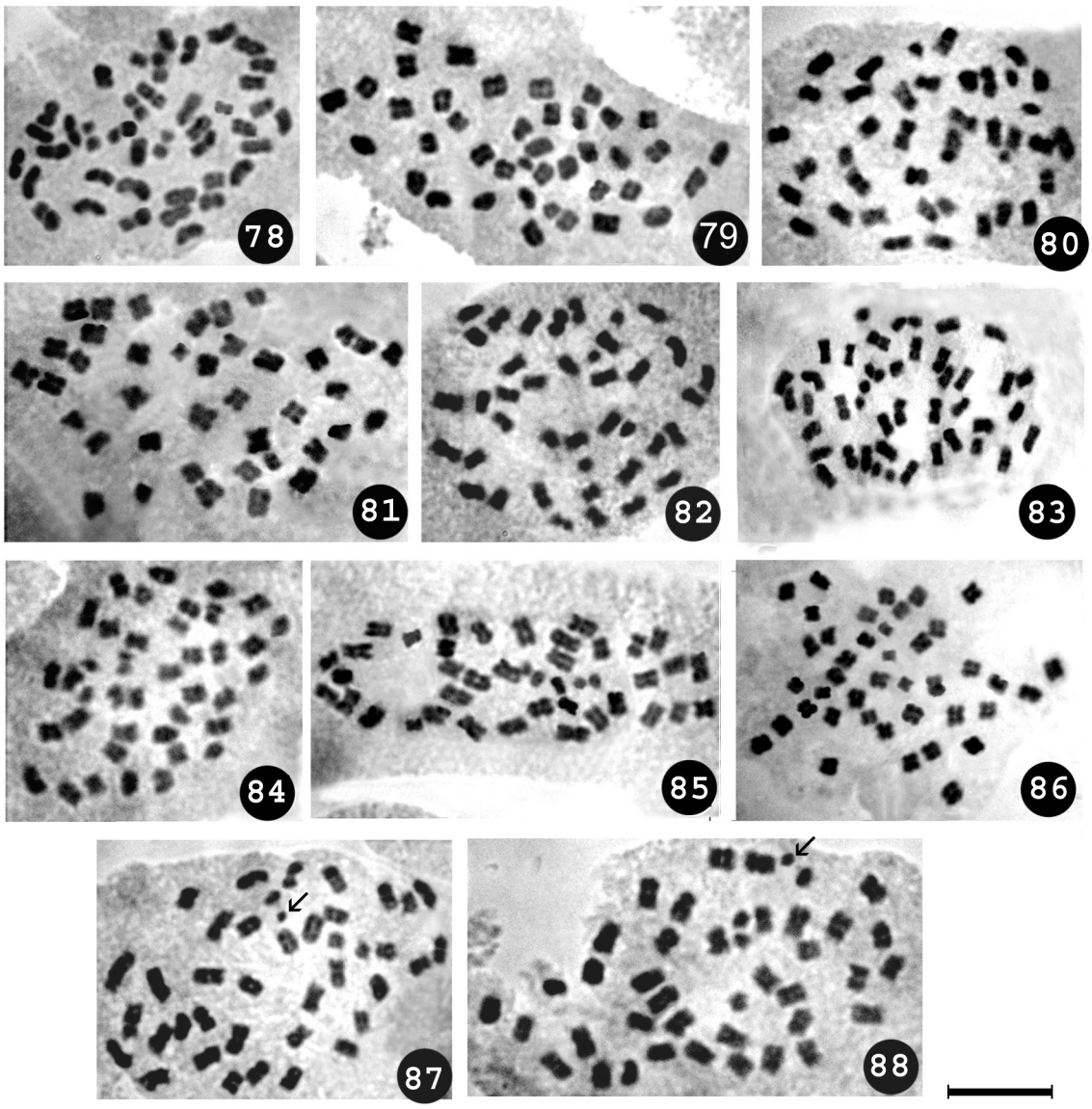
Figs. 45-55. Orcein-stained mitotic prophase chromosomes of 11 varieties of peanut (*Arachis hypogaea* L.). 45. Dhaka-1, 46. BINA Cheena Badam-1, 47. BINA Cheena Badam-2, 48. BINA Cheena Badam-3, 49. BINA Cheena Badam-4, 50. Tridana Badam (DM-1), 51. Basanti Badam (DG-2), 52. Jhinga Badam (Acc. no.-12), 53. BARI Cheena Badam-6, 54. BARI Cheena Badam-7 and 55. BARI Cheena Badam-8. Bar = 5 µm.



Figs. 56-66. CMA-stained mitotic prophase chromosomes of 11 varieties of peanut (*Arachis hypogaea* L.). 56. Dhaka-1, 57. BINA Cheena Badam-1, 58. BINA Cheena Badam-2, 59. BINA Cheena Badam-3, 60. BINA Cheena Badam-4, 61. Tridana Badam (DM-1), 62. Basanti Badam (DG-2), 63. Jhinga Badam (Acc. no.-12), 64. BARI Cheena Badam-6, 65. BARI Cheena Badam-7 and 66. BARI Cheena Badam-8. Bar = 5 µm.

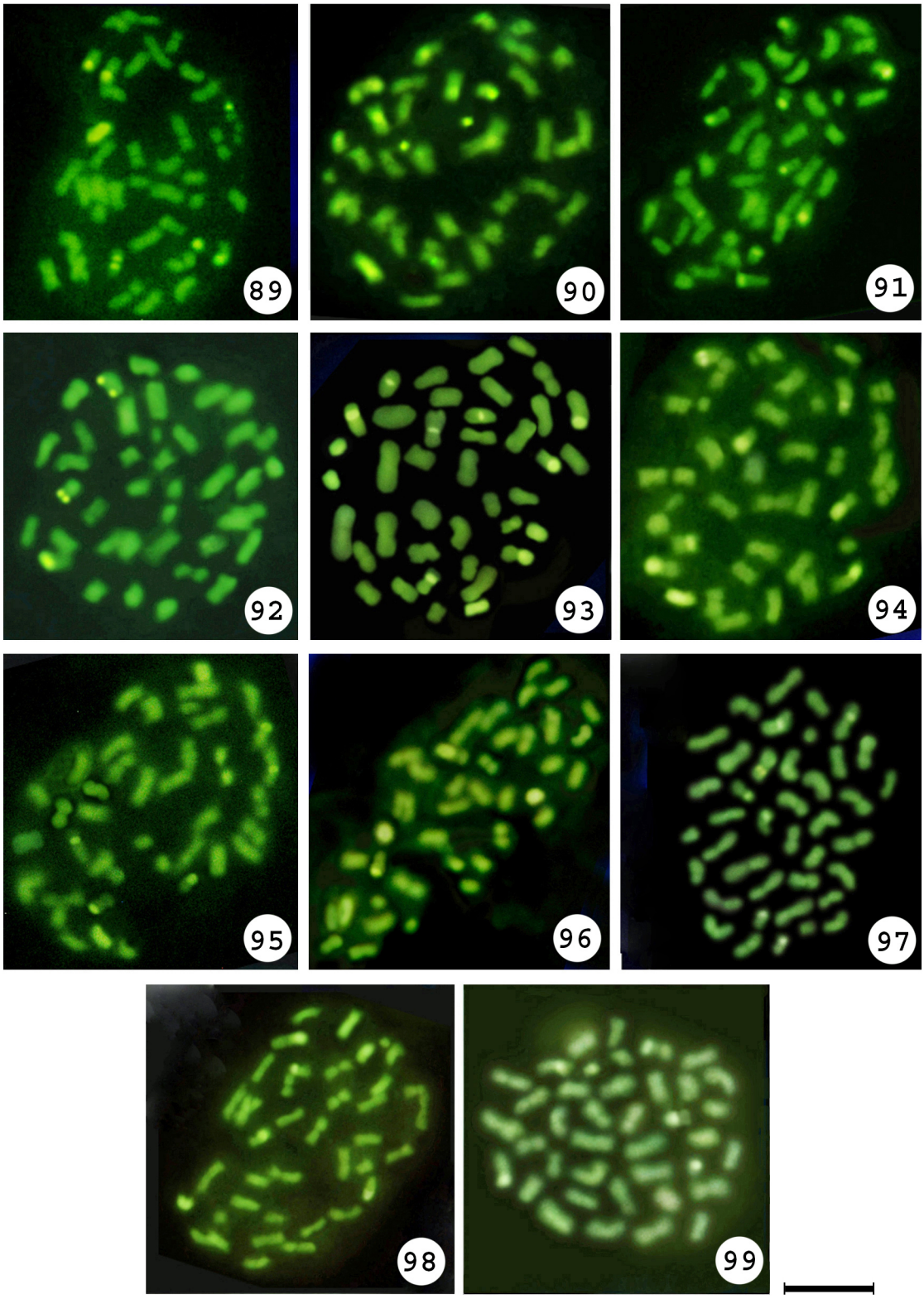


Figs. 67-77. DAPI-stained mitotic prophase chromosomes of 11 varieties of peanut (*Arachis hypogaea* L.). 67. Dhaka-1, 68. BINA Cheena Badam-1, 69. BINA Cheena Badam-2, 70. BINA Cheena Badam-3, 71. BINA Cheena Badam-4, 72. Tridana Badam (DM-1), 73. Basanti Badam (DG-2), 74. Jhinga Badam (Acc. no.-12), 75. BARI Cheena Badam-6, 76. BARI Cheena Badam-7 and 77. BARI Cheena Badam-8. Bar = 5 µm.

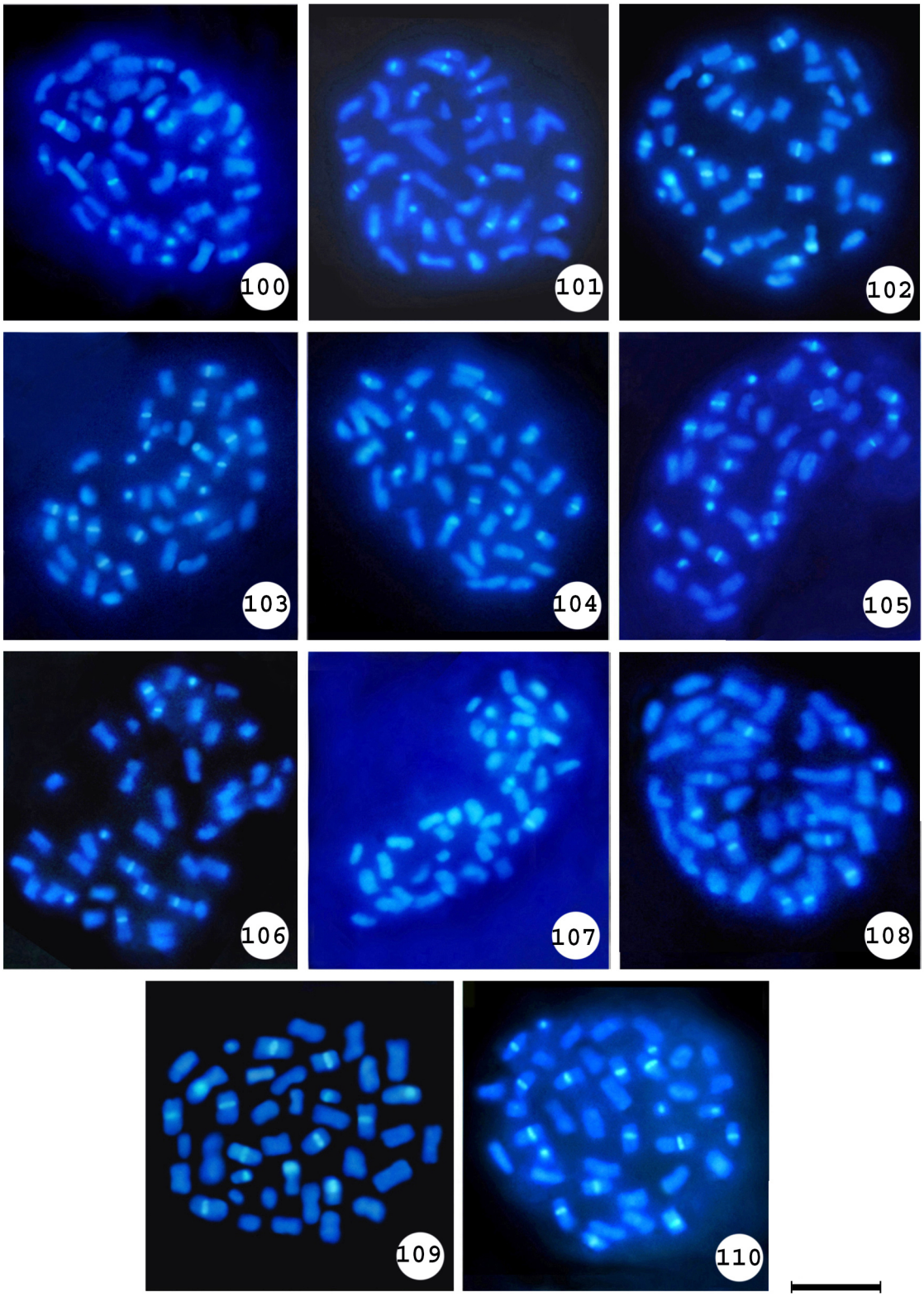


→ Extra chromosome

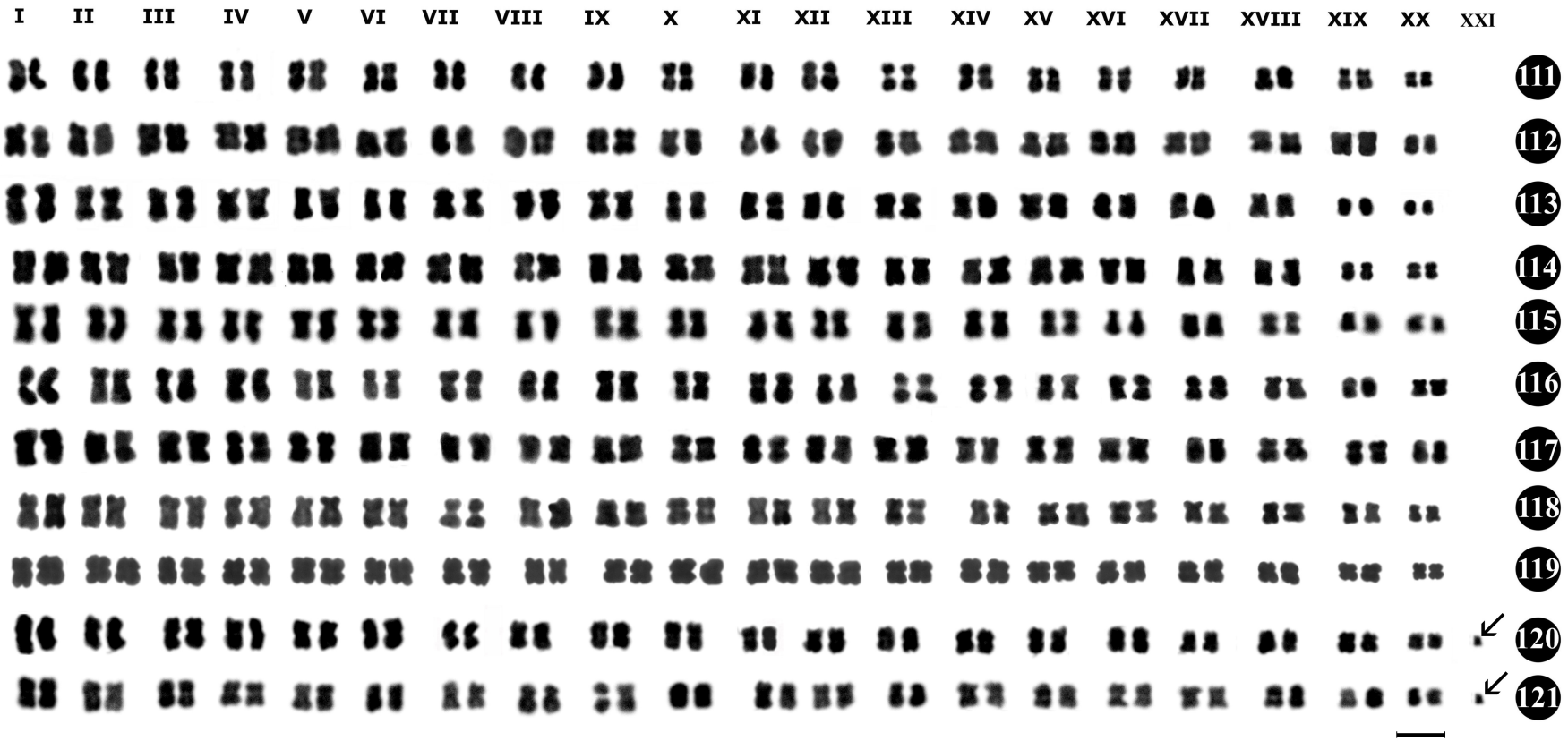
Figs. 78-88. Orcein-stained mitotic metaphase chromosomes of 11 varieties of peanut (*Arachis hypogaea* L.). 78. Dhaka-1, 79. BINA Cheena Badam-1, 80. BINA Cheena Badam-2, 81. BINA Cheena Badam-3, 82. BINA Cheena Badam-4, 83. Tridana Badam (DM-1), 84. Basanti Badam (DG-2), 85. Jhinga Badam (Acc. no.-12), 86. BARI Cheena Badam-6, 87. BARI Cheena Badam-7 and 88. BARI Cheena Badam-8. Bar = 5 µm.



Figs. 89-99. CMA-stained mitotic metaphase chromosomes of 11 varieties of peanut (*Arachis hypogaea* L.). 89. Dhaka-1, 90. BINA Cheena Badam-1, 91. BINA Cheena Badam-2, 92. BINA Cheena Badam-3, 93. BINA Cheena Badam-4, 94. Tridana Badam (DM-1), 95. Basanti Badam (DG-2), 96. Jhinga Badam (Acc. no.-12), 97. BARI Cheena Badam-6, 98. BARI Cheena Badam-7 and 99. BARI Cheena Badam-8. Bar = 5 µm.

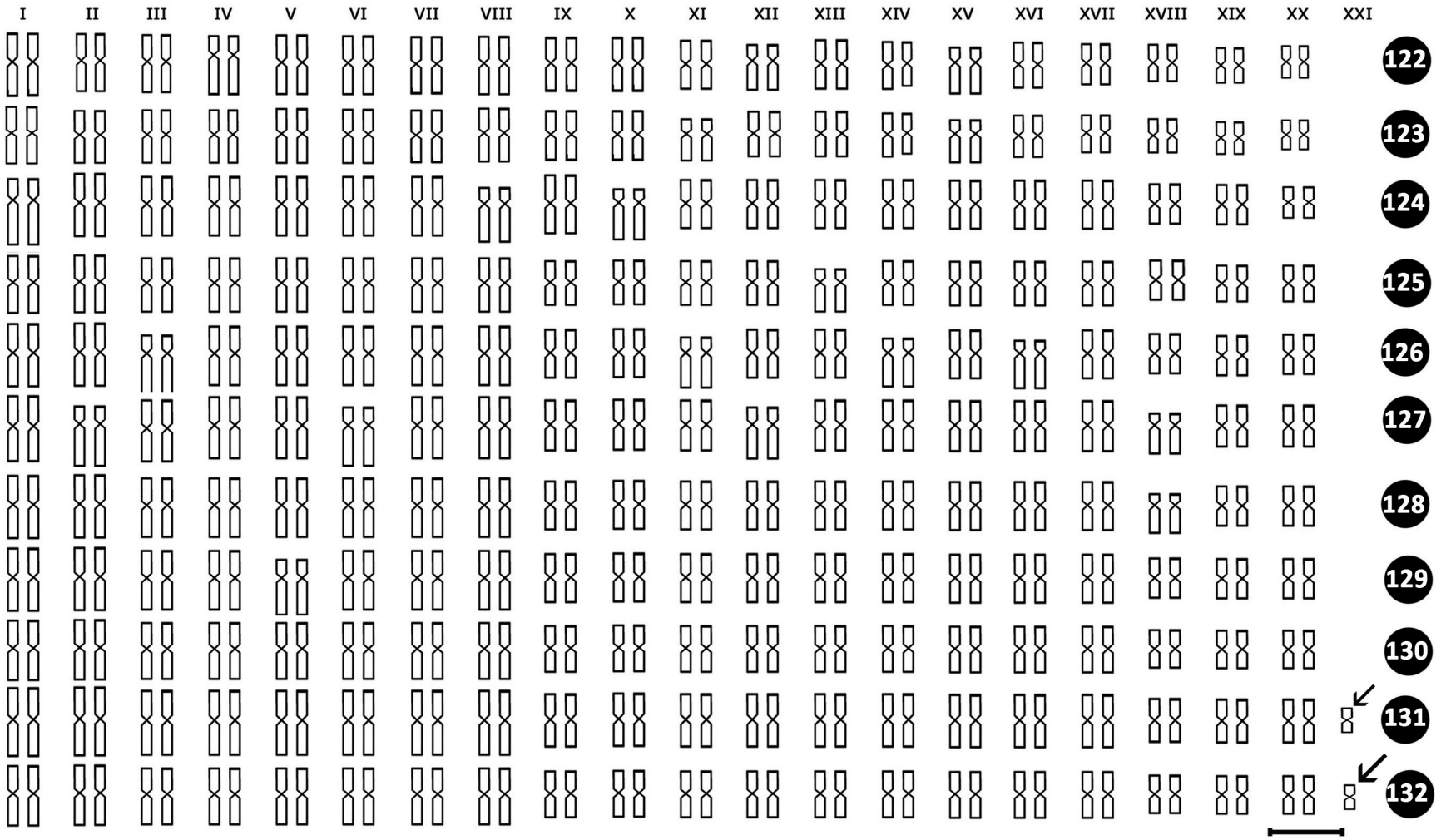


Figs. 100-110. DAPI-stained mitotic metaphase chromosomes of 11 varieties of peanut (*Arachis hypogaea* L.). 100. Dhaka-1, 101. BINA Cheena Badam-1, 102. BINA Cheena Badam-2, 103. BINA Cheena Badam-3, 104. BINA Cheena Badam-4, 105. Tridana Badam (DM-1), 106. Basanti Badam (DG-2), 107. Jhinga Badam (Acc. no.-12), 108. BARI Cheena Badam-6, 109. BARI Cheena Badam-7 and 110. BARI Cheena Badam-8. Bar = 5 µm.



→ Extra chromosome

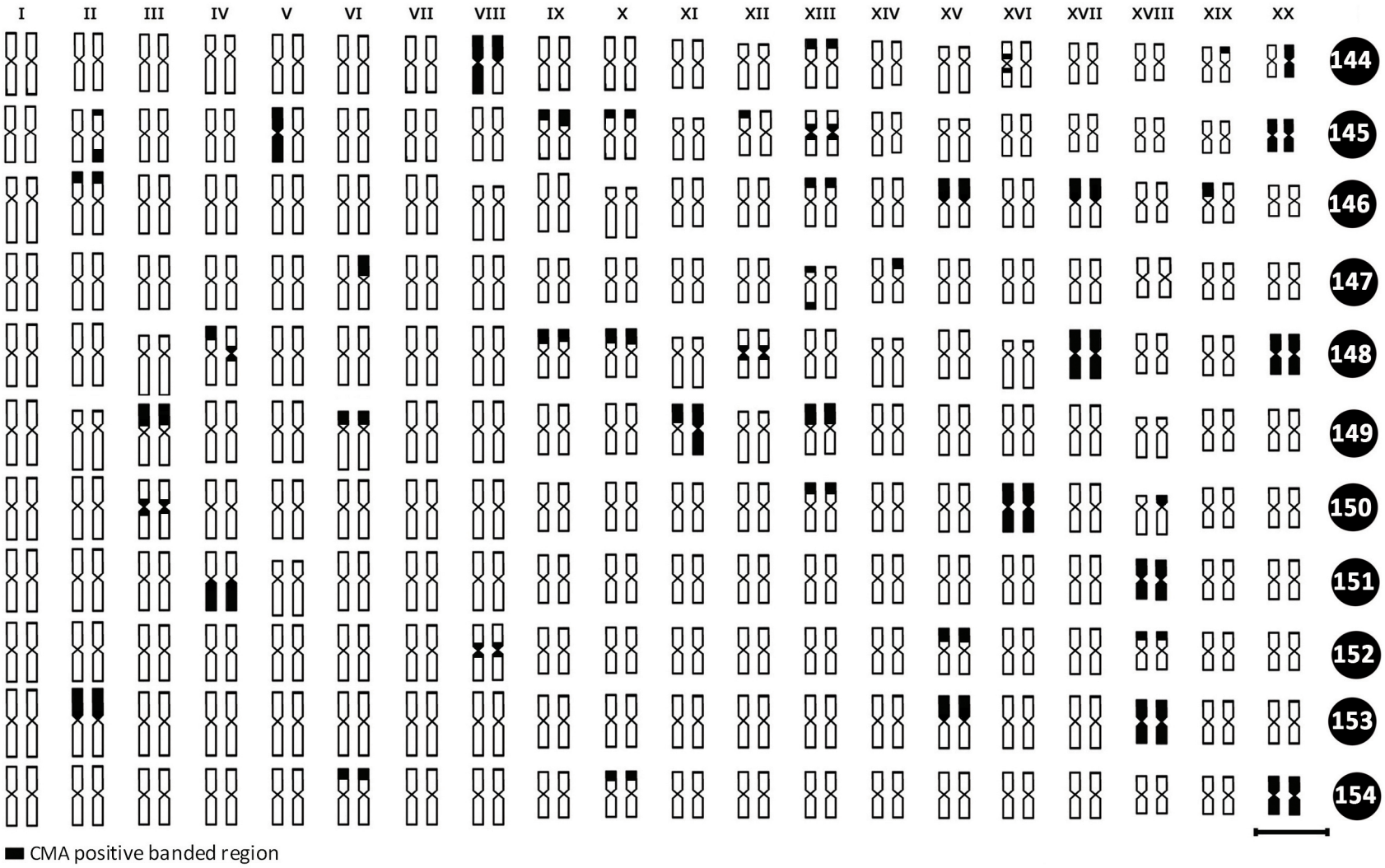
Figs. 111-121. Karyotypes prepared from orcein-stained mitotic metaphase chromosomes of 11 varieties of peanut (*Arachis hypogaea* L.). 111. Dhaka-1, 112. BINA Cheena Badam-1, 113. BINA Cheena Badam-2, 114. BINA Cheena Badam-3, 115. BINA Cheena Badam-4, 116. Tridana Badam (DM-1), 117. Basanti Badam (DG-2), 118. Jhinga Badam (Acc. no.-12), 119. BARI Cheena Badam-6, 120. BARI Cheena Badam-7 and 121. BARI Cheena Badam-8. Bar = 5 µm.



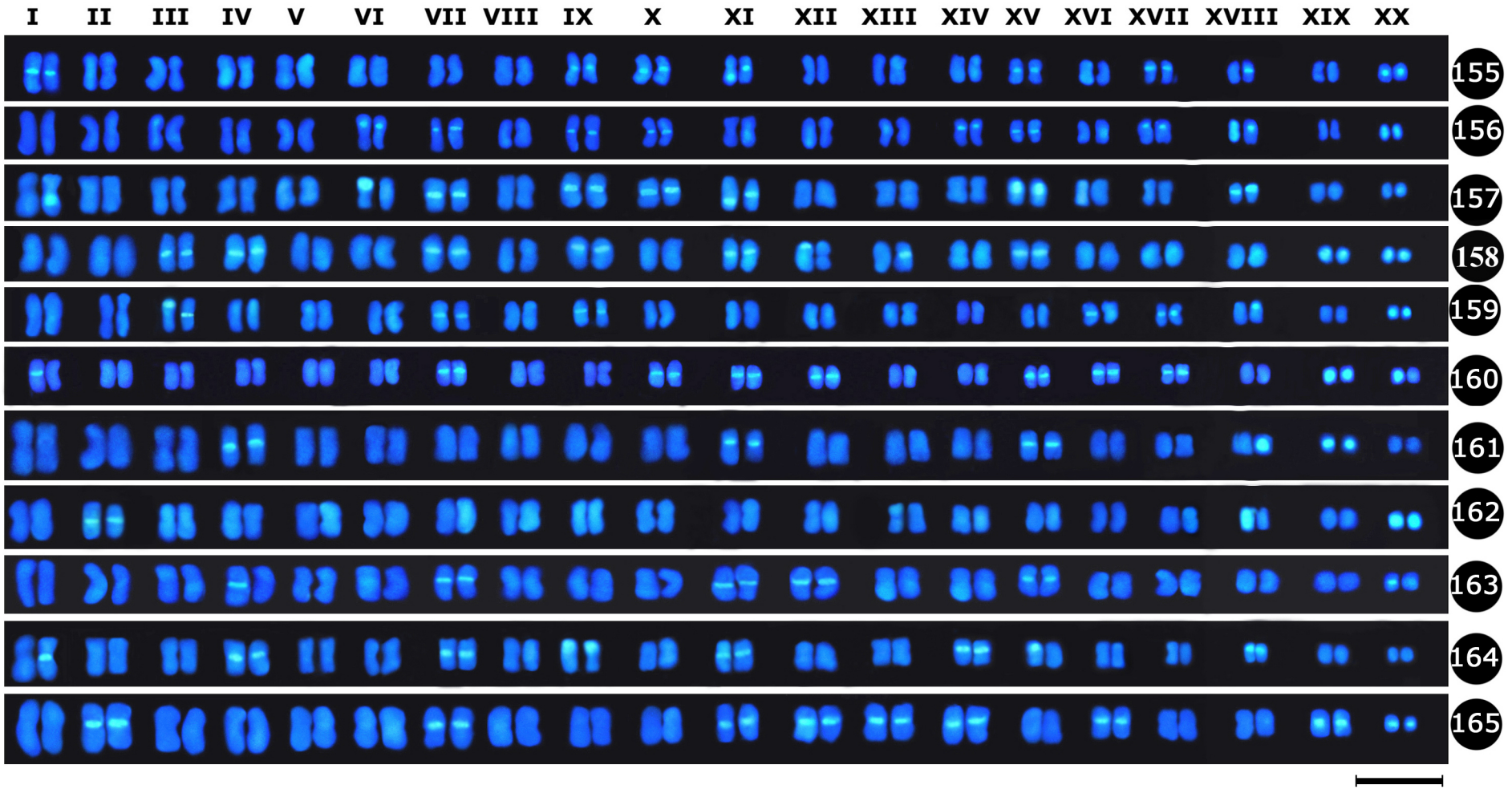
→ Extra chromosome

Figs. 122-132. Idiogram prepared from orcein-stained mitotic metaphase chromosomes of 11 varieties of peanut (*Arachis hypogaea* L.). 122. Dhaka-1, 123. BINA Cheena Badam-1, 124. BINA Cheena Badam-2, 125. BINA Cheena Badam-3, 126. BINA Cheena Badam-4, 127. Tridana Badam (DM-1), 128. Basanti Badam (DG-2), 129. Jhinga Badam (Acc. no.-12), 130. BARI Cheena Badam-6, 131. BARI Cheena Badam-7 and 132. BARI Cheena Badam-8. Bar = 3.5 µm.

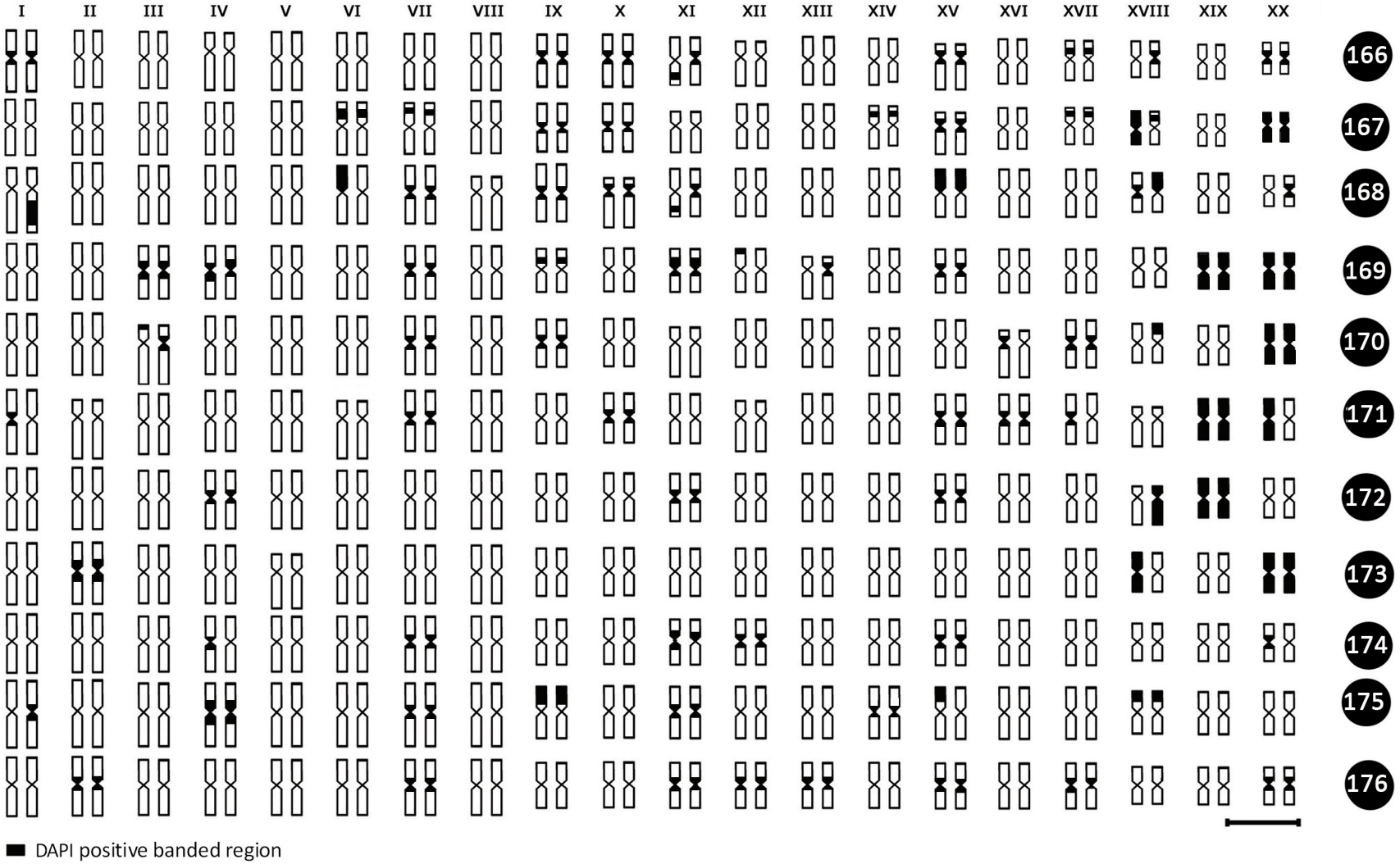
Figs. 133-143. Karyotypes prepared from CMA-stained mitotic metaphase chromosomes of 11 varieties of peanut (*Arachis hypogaea* L.). 133. Dhaka-1, 134. BINA Cheena Badam-1, 135. BINA Cheena Badam-2, 136. BINA Cheena Badam-3, 137. BINA Cheena Badam-4, 138. Tridana Badam (DM-1), 139. Basanti Badam (DG-2), 140. Jhinga Badam (Acc. no.-12), 141. BARI Cheena Badam-6, 142. BARI Cheena Badam-7 and 143. BARI Cheena Badam-8. Bar = 5 µm.



Figs. 144-154. Idiogram prepared from CMA-stained mitotic metaphase chromosomes of 11 varieties of peanut (*Arachis hypogaea* L.). 144. Dhaka-1, 145. BINA Cheena Badam-1, 146. BINA Cheena Badam-2, 147. BINA Cheena Badam-3, 148. BINA Cheena Badam-4, 149. Tridana Badam (DM-1), 150. Basanti Badam (DG-2), 151. Jhinga Badam (Acc. no.-12), 152. BARI Cheena Badam-6, 153. BARI Cheena Badam-7 and 154. BARI Cheena Badam-8. Bar = 3.5 µm.



Figs. 155-165. Karyotypes prepared from DAPI-stained mitotic metaphase chromosomes of 11 varieties of peanut (*Arachis hypogaea* L.). 155. Dhaka-1, 156. BINA Cheena Badam-1, 157. BINA Cheena Badam-2, 158. BINA Cheena Badam-3, 159. BINA Cheena Badam-4, 160. Tridana Badam (DM-1), 161. Basanti Badam (DG-2), 162. Jhinga Badam (Acc. no.-12), 163. BARI Cheena Badam-6, 164. BARI Cheena Badam-7 and 165. BARI Cheena Badam-8. Bar = 5 µm.



Figs. 166-176. Idiogram prepared from DAPI-stained mitotic metaphase chromosomes of 11 varieties of peanut (*Arachis hypogaea* L.). 166. Dhaka-1, 167. BINA Cheena Badam-1, 168. BINA Cheena Badam-2, 169. BINA Cheena Badam-3, 170. BINA Cheena Badam-4, 171. Tridana Badam (DM-1), 172. Basanti Badam (DG-2), 173. Jhinga Badam (Acc. no.-12), 174. BARI Cheena Badam-6, 175. BARI Cheena Badam-7 and 176. BARI Cheena Badam-8. Bar = 3.5 µm.

3.8. RAPD analysis

Eight primer combinations, namely, OPA-1, OPA-2, OPA-3, OPA-7, OPA-8, Primer-2, Primer-19 and primer-23 were used for RAPD analysis of 11 peanut varieties. Each primer combination showed different banding patterns. The primer-wise RAPD analysis of 11 varieties of peanut are described below:

3.8.1. Primer OPA-1 (5'-CAG GCC CTT C-3')

In Dhaka-1, BINA Cheena Badam-1, BINA Cheena Badam-2, BINA Cheena Badam-3, BINA Cheena Badam-4 and Tridana Badam, a total of five RAPD bands were found. The sizes of these bands were 2000, 1400, 1100, 1000 and 750 bp of which four were lightly stained (2000, 1400, 1100 and 1000 bp) and another one brightly stained (750 bp). In Basanti Badam and BARI Cheena Badam-8, three lightly stained bands (1400, 1100 and 1000 bp) and a bright band of 750 bp were found. In Jhinga Badam, BARI Cheena Badam-6 and BARI Cheena Badam-7, four lightly stained bands (1400, 1100, 1000 and 500 bp) and one brightly stained RAPD band (750 bp) were found. No unique band was found in these varieties, however, four common bands (1400, 1100, 1000 and 750 bp) found in the 11 varieties (Fig. 178, Table 22).

3.8.2. Primer OPA-2 (5'-TGC CGA GCT G -3')

Two bright RAPD bands (1000 and 750 bp) were found in Dhaka-1. In BINA Cheena Badam-1, four RAPD bands were found of which two lighter (1200 and 750 bp) and two brighter (700 and 600 bp). In BINA Cheena Badam-2 and BINA Cheena Badam-3, a total of five RAPD bands were found. The sizes of these bands were 1200, 1000, 750, 700 and 600 bp of which three lighter (1200, 1000 and 750 bp) and two were brighter (700 and 600 bp). Only a bright band of 700 bp was found in BINA Cheena Badam-4.Two lightly stained (1300 and 1200 bp) and another two brightly stained RAPD bands (700 and 600 bp) were found in Tridana Badam, Basanti Badam, Jhinga Badam and BARI Cheena

Badam-6. In BARI Cheena Badam-7 and BARI Cheena Badam-8, a total of three RAPD bands were founds. The size of these bands were 1200, 700 and 600 bp of which one lightly (1200 bp) and two brightly stained (700 and 600 bp) (Fig. 179, Table 23).

3.8.3. Primer OPA-3 (5'-AGT CAG CCA C-3')

No band was found in BINA Cheena Badam-1. Four light bands (1000, 800, 600 and 400 bp) were found in Dhaka-1. In BINA Cheena Badam-2, Cheena Badam-3, Cheena Badam-4, Tridana Badam and Basanti Badam, three lighter bands were observed. The sizes of these bands were 800, 600 and 400 bp. No band was found in BINA Cheena Badam-1. Two bright bands (800 and 600 bp) and a light band (400 bp) were found in Jhinga Badam, BARI Cheena Badam-6, BARI Cheena Badam-7 and BARI Cheena Badam-8. In Addition to these three bands, BARI Cheena Badam-6 had two more lighter band of 1700 bp and 1500 bp (Fig. 180, Table 25). 1000 bp fragment of Dhaka-1 and 1700, 1500 bp fragment of BARI Cheena Badam-6 were unique since absent in other varieties. These gemplasms showed three common bands at the molecular weight of 800, 600 and 400 bp except BINA Cheena Badam-1 (Fig. 180, Table 24).

3.8.4. Primer OPA-7 (5'-GAA ACG GGT G-3')

No Band was found in Jhinga Badam (Acc. no.-12) and BARI Cheena Badam-7 in this primer. In Dhaka-1 and BARI Cheena Badam-6, three lightly stained RAPD bands (2500, 2000 and 750 bp) were found. In BINA Cheena Badam-1 and BINA Cheena Badam-3, two light bands (2500 and 2000 bp) and a bright band (750 bp) were found. BINA Cheena Badam-2 had four RAPD bands of which two lighter (2500 and 600 bp) and two brighter (2000 and 750 bp). In BINA Cheena Badam-4, a total three RAPD band was found. The size of the bands were 2000, 600 bp (light) and 750 bp (bright). In Tridana Badam, three

lightly stained (2000, 600 and 400 bp) and two brightly stained (1000 and 750 bp) bands were observed. Only a band of 750 bp was found in Basanti Badam and BARI Cheena Badam-8, however, in BARI Cheena Badam-8, it was brighter. A fragment of 750 bp was common to all varieties except Jhinga Badam and BARI Cheena Badam-7. 1000 and 400 bp DNA fragments were unique in Tridana Badam since absent in other varieties (Fig. 181, Table 25).

3.8.5. Primer OPA-8 (5'-GTG ACG TAG G-3')

No band was found in BARI Cheena Badam-7 and BARI Cheena Badam-8. In Dhaka-1, Jhinga Badam (Acc. no.-12) and BARI Cheena Badam-6, two lighter (1100 and 500 bp) and one brighter RAPD band (750 bp) were found. Three lighter RAPD bands (1500, 1100 and 750 bp) were found in BINA Cheena Badam-1, BINA Cheena Badam-3 and BINA Cheena Badam-4. In BINA Cheena Badam-2, four RAPD bands were found. The size of the bands were 1500, 750 (brighter) and 1100, 500 bp (lighter). Three light (1500, 1100, 500 bp) and a bright band (750 bp) were found in Tridana Badam and Basanti Badam. No unique band was found in any variety. Two common bands of 1100 and 750 bp were found in nine varieties except BARI Cheena Badam-7 and BARI Cheena Badam-8 (Fig. 182, Table 26).

3.8.6. Primer-2 (5'-GTT GCG ATC C-3')

No band was found in BINA Cheena Badam-1. One lightly stained (600 bp) and a brightly stained RAPD band (900 bp) was found in Dhaka-1 and BARI Cheena Badam-6. In BINA Cheena Badam-2, a total of three RAPD bands were found. The sizes of these bands were 1500, 1400 and 900 bp of which two lightly stained (1500 and 1400 bp) and one brightly stained (900 bp). In BINA Cheena Badam-3 and BARI Cheena Badam-8, a brightly stained band of 900 bp was found. One lightly stained (1400 bp) and a brightly stained (900 bp) RAPD bands were found in BINA Cheena Badam-4. In Tridana Badam, three lightly

stained (1400, 600 and 500 bp) and one brightly stained RAPD band (900 bp) was found. In Basanti Badam and Jhinga Badam, a total number of four RAPD band was found. The sizes of these bands were 1400, 900, 600 and 400 bp of which three lightly stained (1400, 600 and 400 bp) and one brightly (900 bp). One unique fragment of 500 and 1500 bp were found in Tridana Badam and BINA Cheena Badama-2, respectively. One common band (900 bp) was found in 10 varieties except BINA Cheena Badam-1 (Fig. 183, Table 27).

3.8.7. Primer-19 (5'-GAT GAC CGC C-3')

Two bright RAPD bands (1100 and 800 bp) were common in eleven varieties. In addition to these bands, BARI Cheena Badam-6, BARI Cheena Badam-7 BARI Cheena Badam-8 two (3000 and 2300 bp), one (3000 bp) and three (3000, 2300 and 2000 bp) light bands, respectively. One unique fragment of 2000 bp was present only in BARI Cheena Badam-8 (Fig. 184, Table 28).

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

A bright and thick fragment of 1000 bp was found in all gemplasms. In addition to this common band, a band of 1800 bp and 500 bp were common in all varieties except BARI Cheena Badam-7 and BARI Cheena Badam-8. Moreover, fragment 400 bp was present in 8 varieties except Basanti Badama, BARI Cheena Badam-7 and BARI Cheena Badam-8 (Fig. 185, Table 29).

3.9. SSR analysis

Four primer pairs *viz.*, i) forward- BA00175669/reverse- BA00175670, ii) forward-BA00175671/reverse- BA00175672, iii) forward- BA00175673/reverse-BA00175674 and iv) forward- BA00175677/reverse- BA00175678 were used for SSR analysis of 11 peanut varieties. Each primer pair showed different banding patterns. The pair-wise SSR analysis of 11 varieties of peanut was described below:

3.9.1. Primer pair forward- 5'-GAAAGAAATTATACACTCCAATTATG-3'/reverse-5'-CGGCATGACAGCTCTATGTT-3'

A band of 50 bp was found in all varieties except BINA Cheena Badam-4 and Tridana Badam (DM-1). In BINA Cheena Badam-1, Basanti Badam and Jhinga Badam (Acc. no.-12) the bands were lighter than the rest. Dhaka-1, BINA Cheena Badam-2, Basanti Badam (DG-2), BARI Cheena Badam-6 and BARI Cheena Badam-7 had a band of 150 bp. Bands of Dhaka-1, Basanti Badam (DG-2) and BARI Cheena Badam-7 was brighter (Fig. 186, Table 31).

3.9.2. Primer pair forward- 5'-CCTTTTCTAACACATTCACACATGA-3'/reverse-5'-GGCTCCCTTCGATGATGAC-3'

SSR band of 246 bp was common in all varieties except BINA Cheena Badam-3. All bands are lighter excluding BINA Cheena Badam-4 and Tridana Badam. One unique fragment of 50 bp was found only in BARI Cheena Badam-8 (Fig. 187, Table 32).

3.9.3. Primer pair forward- 5'-ACTCGCCATAGCCAACAAAC-3'/reverse-5'-CATTCCACAACTCCCACAT-3'

A bright band of 150 bp was found in all varieties. These bands were bright except BARI Cheena Badam-8. A lighter band of 50 bp was observed in Dhaka-1 and Jhinga Badam (Acc. no.-12) only (Fig. 188, Table 33).

3.9.4. Primer pair forward- 5'-CTCTCCTCTGCTCTGCACTG-3'/reverse-5'-ACAAGAACATGGGGATGAAGA-3'

Only one common band of 150 bp was found in 11 germplams of peanut. Except BARI Cheena Badam-6 and BARI Cheena Badam-7, all varieties showed 50 bp bands (Fig. 189, Table 34).

3.10. Genetic distances

The values of pair-wise Nei's (1972) genetic distances analyzed by using computer software "popgene32" among 11 varieties were computed from a combined data of eight RAPD and four pairs of SSR primer. The genetic distance ranged from 0.1226 to 0.5500 (Table 36). The highest genetic distance (0.5500) was found between Dhaka-1 vs BARI Cheena Badam-8. The lowest (0.1226) genetic distance was observed between BINA Cheena Badam-2 vs BINA Cheena Badam-3 and Tridana Badam (DM-1) vs Basanti Badam (DG-2).

3.11. Cluster analysis (Tree diagram)

Cluster analysis on the basis of DNA fingerprinting by RAPD and SSR was carried out. Dendrogram based on Nei's (1972) genetic distance was constructed using Unweighted Pair Group Method of Arithmetic Means (UPGMA) segregating the 11 varieties of peanut into two major clusters C_1 and C_2. BARI Cheena Badam-7 and BARI Cheena Badam-8 formed cluster 2 (C_2). On the other hand, rest of the varieties formed cluster 1 (C_1). Cluster C_1 divided into two sub-clusters SC_1 and SC_2. Tridana Badam (DM-1), Basanti Badam (DG-2), Jhinga Badam (Acc. no.-12) and BARI Cheena Badam-6 were comprised sub-cluster 1 (SC_1). Whereas, Dhaka-1, BINA Cheena Badam-1, BINA Cheena Badam-3, BINA Cheena Badam-2 and BINA Cheena Badam-4 were included in sub-cluster 2 (SC_2). Therefore, BARI Cheena Badam-7 and BARI Cheena Badam-8 were separated from rest of the varieties (Fig. 190).

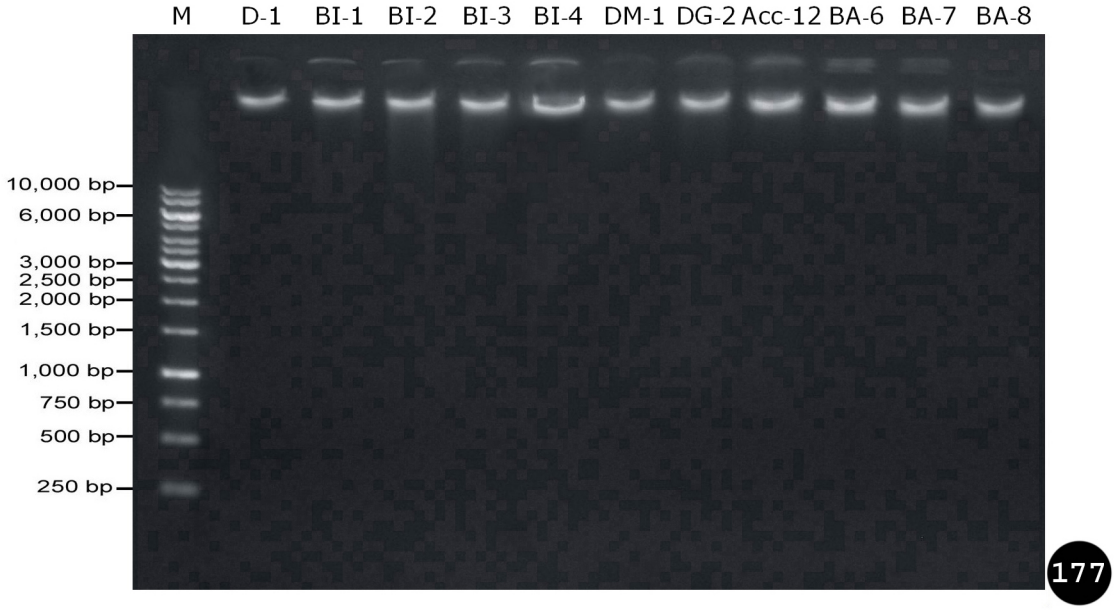


Fig. 177. Electrophoregram of ethidium bromide stained genomic DNA samples of 11 varieties of peanut (*Arachis hypogaea* L.). M=1 Kb DNA ladder, D-1=Dhaka-1, BI-1=BINA Cheena Badam-1, BI-2=BINA Cheena Badam-2, BI-3=BINA Cheena Badam-3, BI-4=BINA Cheena Badam-4, DM-1=Tridana Badam, DG-2=Basanti Badam, Acc-12=Jhinga Badam, BA-6=BARI Cheena Badam-6, BA-7=BARI Cheena Badam-7 and BA-8=BARI Cheena Badam-8.

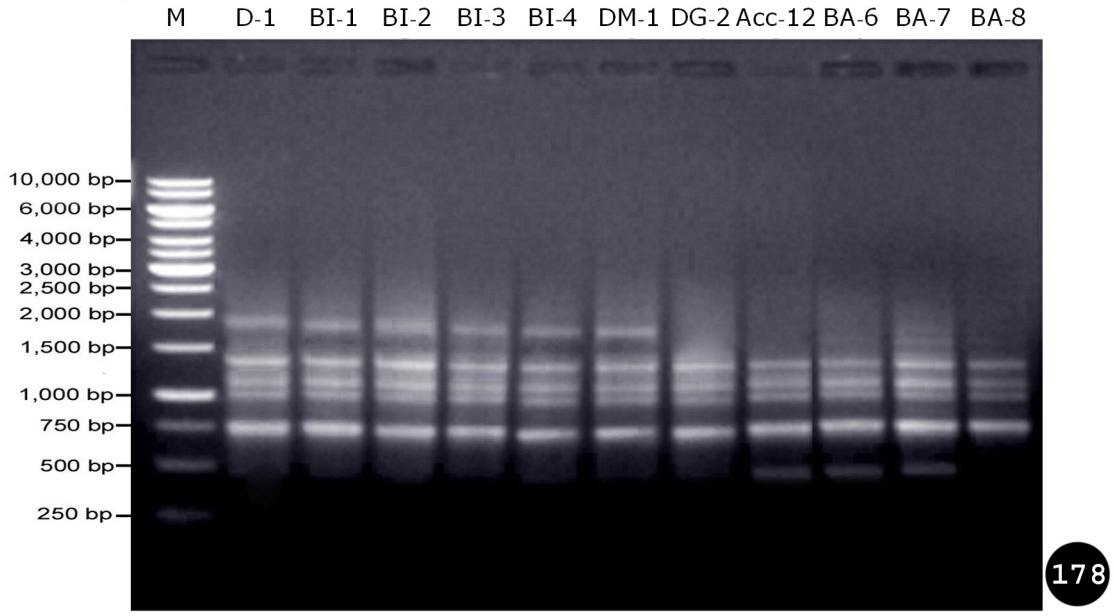


Fig. 178. RAPD analysis with primer OPA-1 (5'-CAG GCC CTT C-3') of 11 varieties of peanut (*Arachis hypogaea* L.). M=1 Kb DNA ladder, D-1=Dhaka-1, BI-1=BINA Cheena Badam-1, BI-2=BINA Cheena Badam-2, BI-3=BINA Cheena Badam-3, BI-4=BINA Cheena Badam-4, DM-1=Tridana Badam, DG-2=Basanti Badam, Acc-12=Jhinga Badam, BA-6=BARI Cheena Badam-6, BA-7=BARI Cheena Badam-7 and BA-8=BARI Cheena Badam-8.

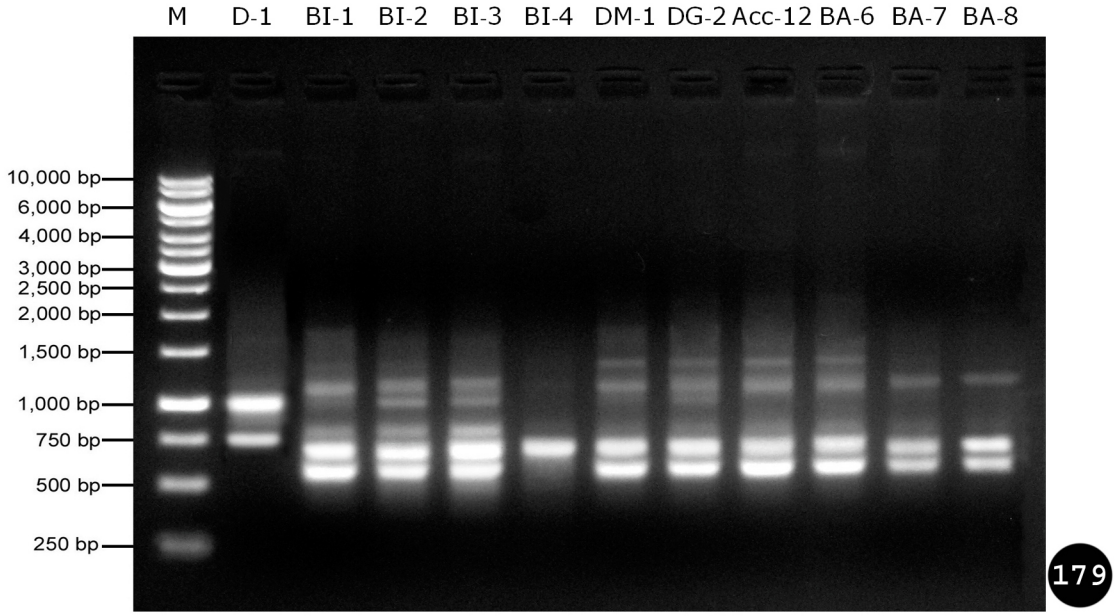


Fig. 179. RAPD analysis with primer OPA-2 (5'-TGC CGA GCT C-3') of 11 varieties of peanut (*Arachis hypogaea* L.). M=1 Kb DNA ladder, D-1=Dhaka-1, BI-1=BINA Cheena Badam-1, BI-2=BINA Cheena Badam-2, BI-3=BINA Cheena Badam-3, BI-4=BINA Cheena Badam-4, DM-1=Tridana Badam, DG-2=Basanti Badam, Acc-12=Jhinga Badam, BA-6=BARI Cheena Badam-6, BA-7=BARI Cheena Badam-7 and BA-8=BARI Cheena Badam-8.

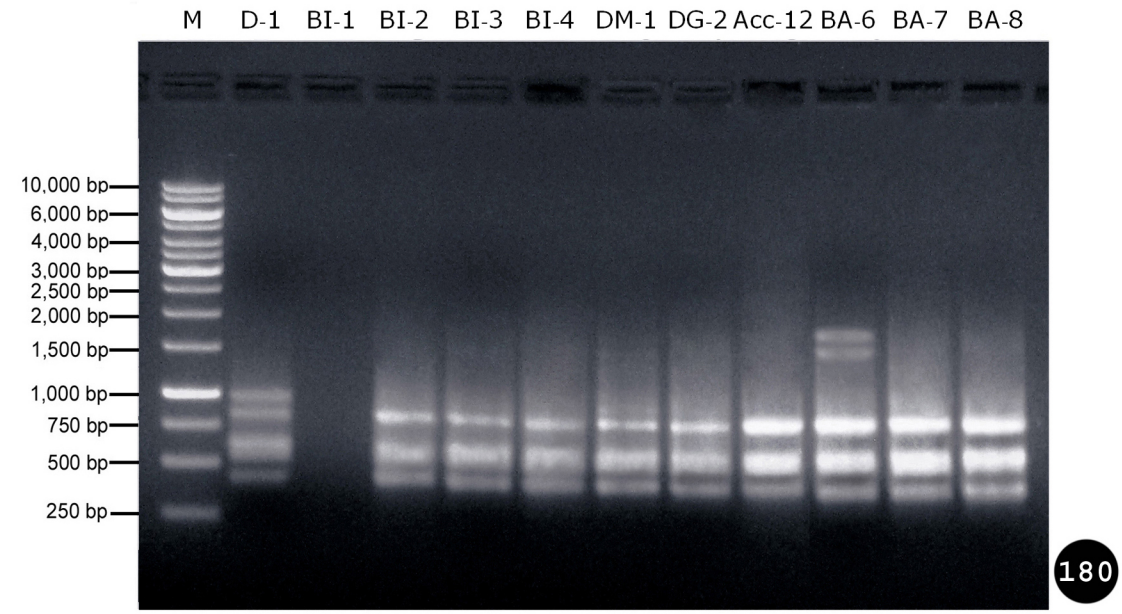


Fig. 180. RAPD analysis with primer OPA-3 (5'-AGT CAG CCA C-3') of 11 varieties of peanut (*Arachis hypogaea* L.). M=1 Kb DNA ladder, D-1=Dhaka-1, BI-1=BINA Cheena Badam-1, BI-2=BINA Cheena Badam-2, BI-3=BINA Cheena Badam-3, BI-4=BINA Cheena Badam-4, DM-1=Tridana Badam, DG-2=Basanti Badam, Acc-12=Jhinga Badam, BA-6=BARI Cheena Badam-6, BA-7=BARI Cheena Badam-7 and BA-8=BARI Cheena Badam-8.

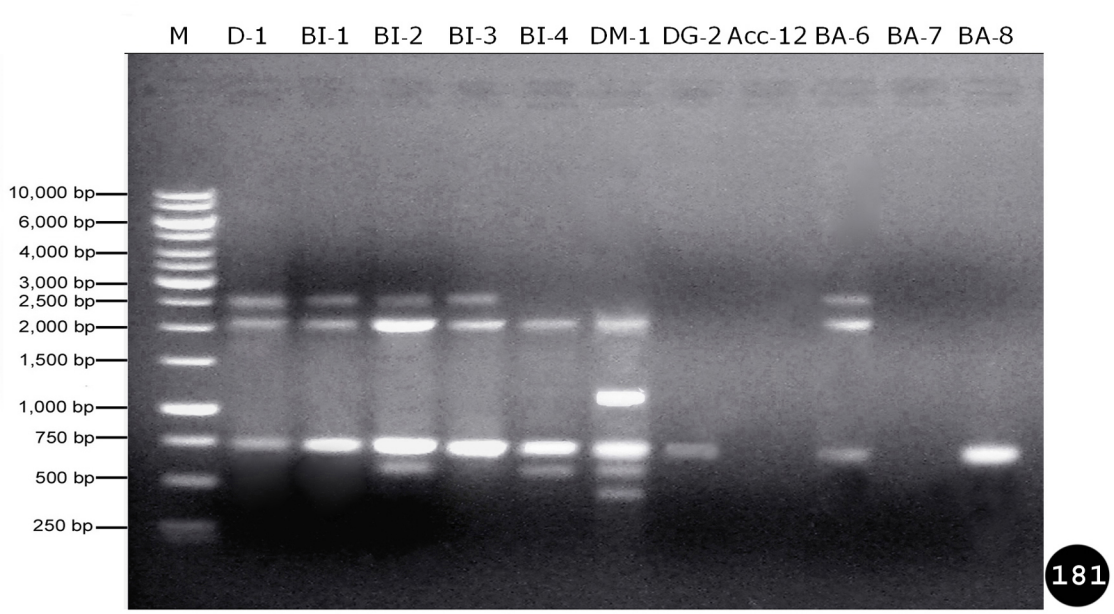


Fig. 181. RAPD analysis with primer OPA-7 (5'-GAA ACG GGT G-3') 11 varieties of peanut (*Arachis hypogaea* L.). M=1 Kb DNA ladder, D-1=Dhaka-1, BI-1=BINA Cheena Badam-1, BI-2=BINA Cheena Badam-2, BI-3=BINA Cheena Badam-3, BI-4=BINA Cheena Badam-4, DM-1=Tridana Badam, DG-2=Basanti Badam, Acc-12=Jhinga Badam, BA-6=BARI Cheena Badam-6, BA-7=BARI Cheena Badam-7 and BA-8=BARI Cheena Badam-8.

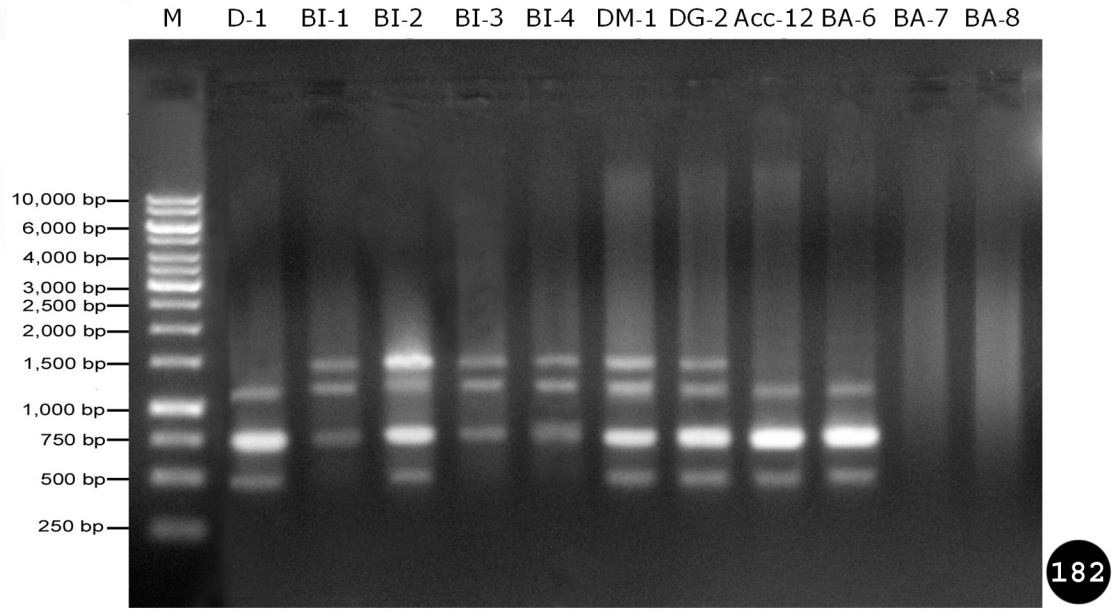


Fig. 182. RAPD analysis with primer OPA-8 (5'-GTG ACG TAG G-3') of 11 varieties of peanut (*Arachis hypogaea* L.). M=1 Kb DNA ladder, D-1=Dhaka-1, BI-1=BINA Cheena Badam-1, BI-2=BINA Cheena Badam-2, BI-3=BINA Cheena Badam-3, BI-4=BINA Cheena Badam-4, DM-1=Tridana Badam, DG-2=Basanti Badam, Acc-12=Jhinga Badam, BA-6=BARI Cheena Badam-6, BA-7=BARI Cheena Badam-7 and BA-8=BARI Cheena Badam-8.

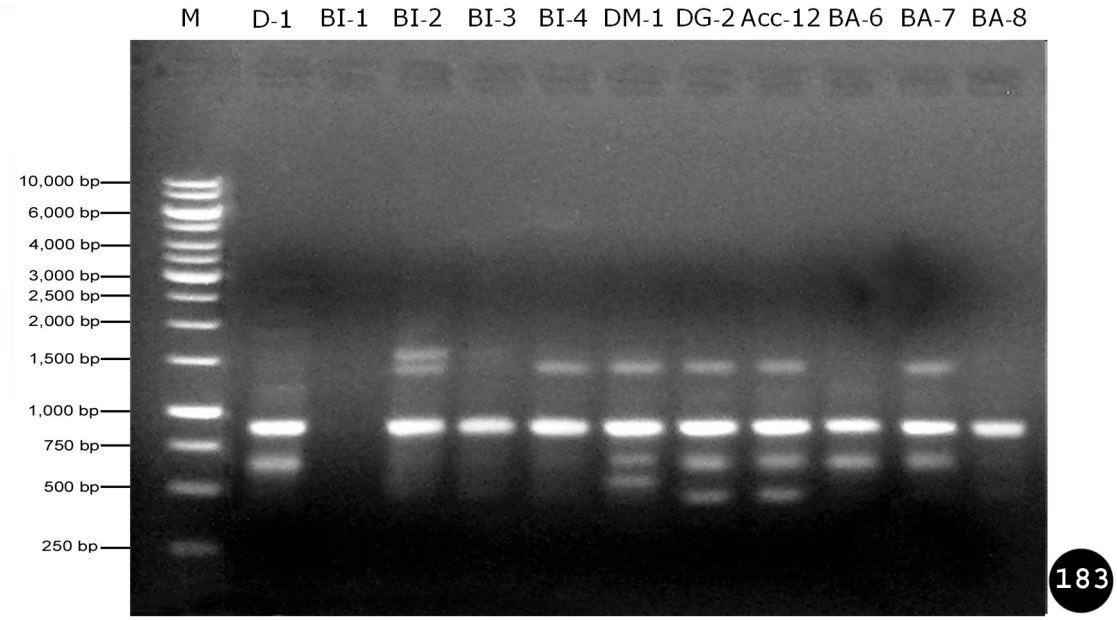


Fig. 183. RAPD analysis with primer-2 (5'-GTT GCG ATC C-3') of 11 varieties of peanut (*Arachis hypogaea* L.). M=1 Kb DNA ladder, D-1=Dhaka-1, BI-1=BINA Cheena Badam-1, BI-2=BINA Cheena Badam-2, BI-3=BINA Cheena Badam-3, BI-4=BINA Cheena Badam-4, DM-1=Tridana Badam, DG-2=Basanti Badam, Acc-12=Jhinga Badam, BA-6=BARI Cheena Badam-6, BA-7=BARI Cheena Badam-7 and BA-8=BARI Cheena Badam-8.

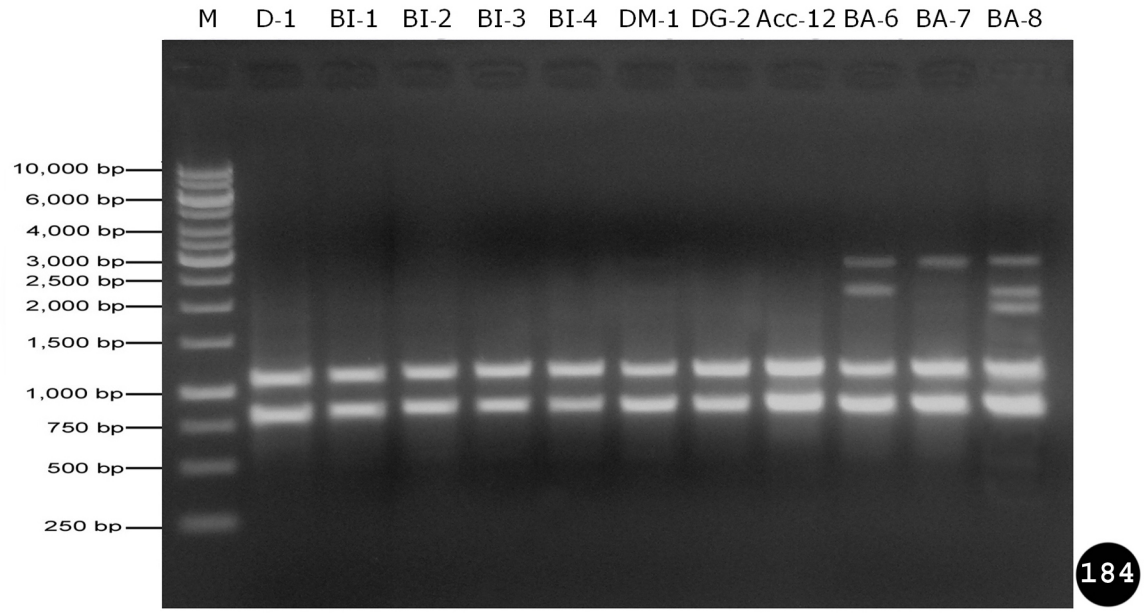


Fig. 184. RAPD analysis with primer-19 (5'-GAT GAC CGC C-3') of 11 varieties of peanut (*Arachis hypogaea* L.). M=1 Kb DNA ladder, D-1=Dhaka-1, BI-1=BINA Cheena Badam-1, BI-2=BINA Cheena Badam-2, BI-3=BINA Cheena Badam-3, BI-4=BINA Cheena Badam-4, DM-1=Tridana Badam, DG-2=Basanti Badam, Acc-12=Jhinga Badam, BA-6=BARI Cheena Badam-6, BA-7=BARI Cheena Badam-7 and BA-8=BARI Cheena Badam-8.

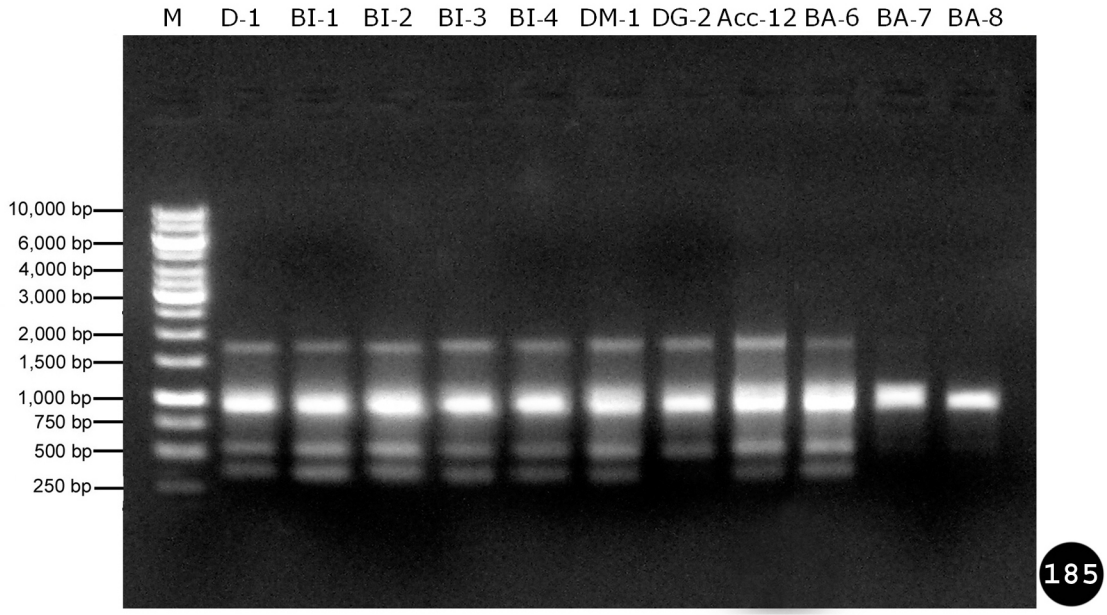


Fig. 185. RAPD analysis with primer-23 (5'-GTC AGG GCA A-3') of 11 varieties of peanut (*Arachis hypogaea* L.). M=1 Kb DNA ladder, D-1=Dhaka-1, BI-1=BINA Cheena Badam-1, BI-2=BINA Cheena Badam-2, BI-3=BINA Cheena Badam-3, BI-4=BINA Cheena Badam-4, DM-1=Tridana Badam, DG-2=Basanti Badam, Acc-12=Jhinga Badam, BA-6=BARI Cheena Badam-6, BA-7=BARI Cheena Badam-7 and BA-8=BARI Cheena Badam-8.

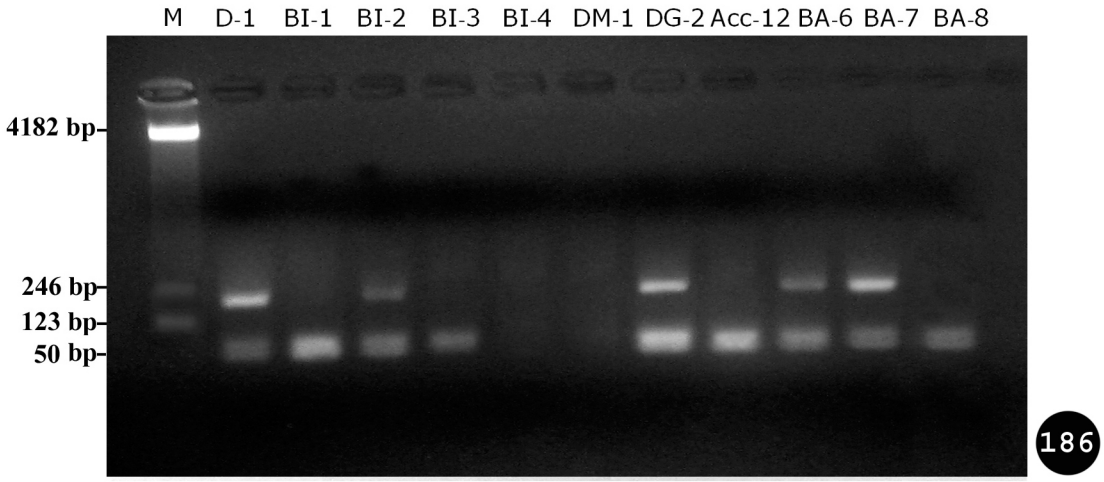


Fig. 186. SSR analysis with primer pair forward-5'-GAAAGAAATTATACACTCCAATTATG-3' /reverse-5'-CGGCATGACAGCTCTATGTT-3' of 11 varieties of peanut (*Arachis hypogaea* L.). M=123 bp DNA ladder, D-1=Dhaka-1, BI-1=BINA Cheena Badam-1, BI-2=BINA Cheena Badam-2, BI-3=BINA Cheena Badam-3, BI-4=BINA Cheena Badam-4, DM-1=Tridana Badam, DG-2=Basanti Badam, Acc-12=Jhinga Badam, BA-6=BARI Cheena Badam-6, BA-7=BARI Cheena Badam-7 and BA-8=BARI Cheena Badam-8.

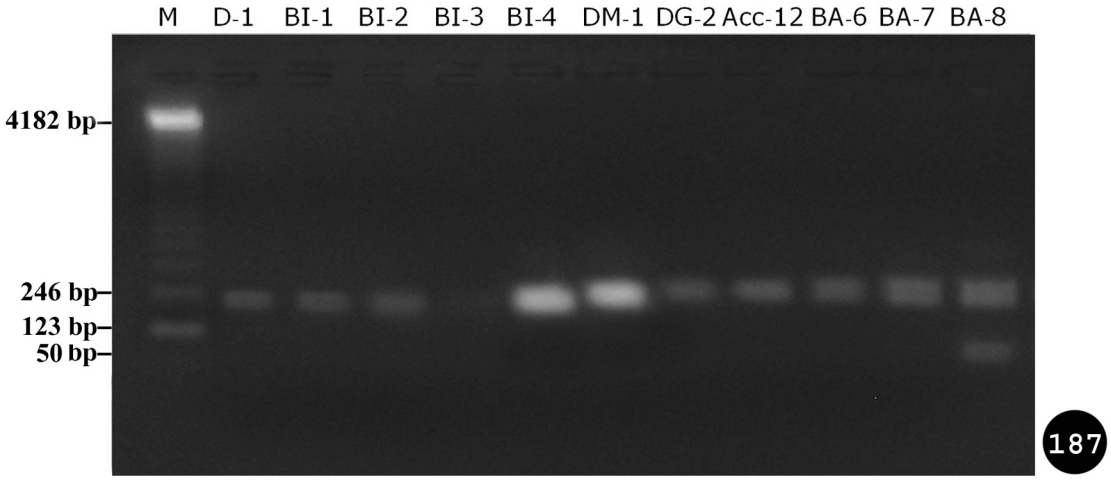


Fig. 187. SSR analysis with primer pair forward-5'-CCTTTTCTAACACATTCACACATGA-3'/ reverse-5'-GGCTCCCTTCGATGATGAC-3' of 11 varieties of peanut (*Arachis hypogaea* L.). M=123 bp DNA ladder, D-1=Dhaka-1, BI-1=BINA Cheena Badam-1, BI-2=BINA Cheena Badam-2, BI-3=BINA Cheena Badam-3, BI-4=BINA Cheena Badam-4, DM-1=Tridana Badam, DG-2=Basanti Badam, Acc-12=Jhinga Badam, BA-6=BARI Cheena Badam-6, BA-7=BARI Cheena Badam-7 and BA-8=BARI Cheena Badam-8.

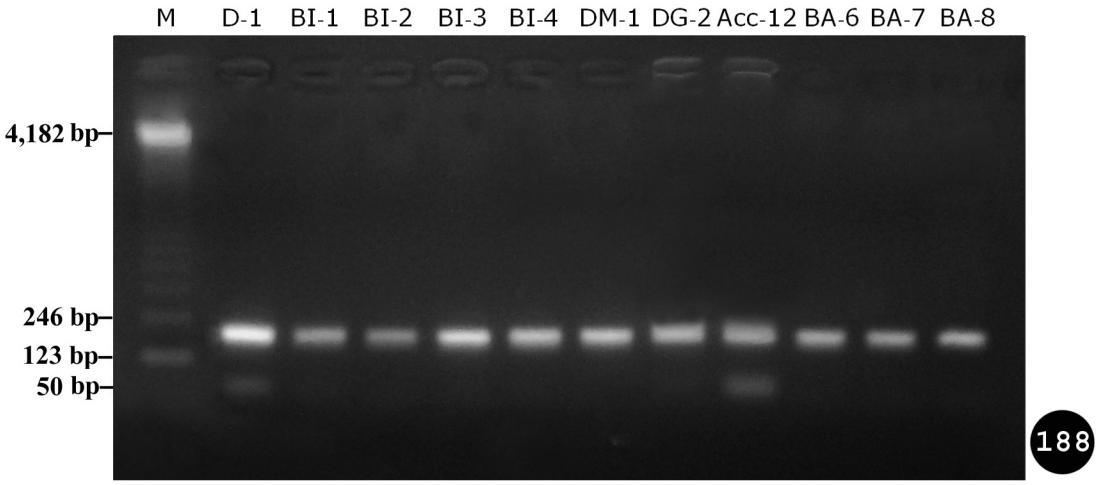


Fig. 188. SSR analysis with primer pair forward-5'-ACTCGCCATAGCCAACAAAC-3'/ reverse-5'-CATTCCACAACTCCCACAT-3' of 11 varieties of peanut (*Arachis hypogaea* L.). M=123 bp DNA ladder, D-1=Dhaka-1, BI-1=BINA Cheena Badam-1, BI-2=BINA Cheena Badam-2, BI-3=BINA Cheena Badam-3, BI-4=BINA Cheena Badam-4, DM-1=Tridana Badam, DG-2=Basanti Badam, Acc-12=Jhinga Badam, BA-6=BARI Cheena Badam-6, BA-7=BARI Cheena Badam-7 and BA-8=BARI Cheena Badam-8.

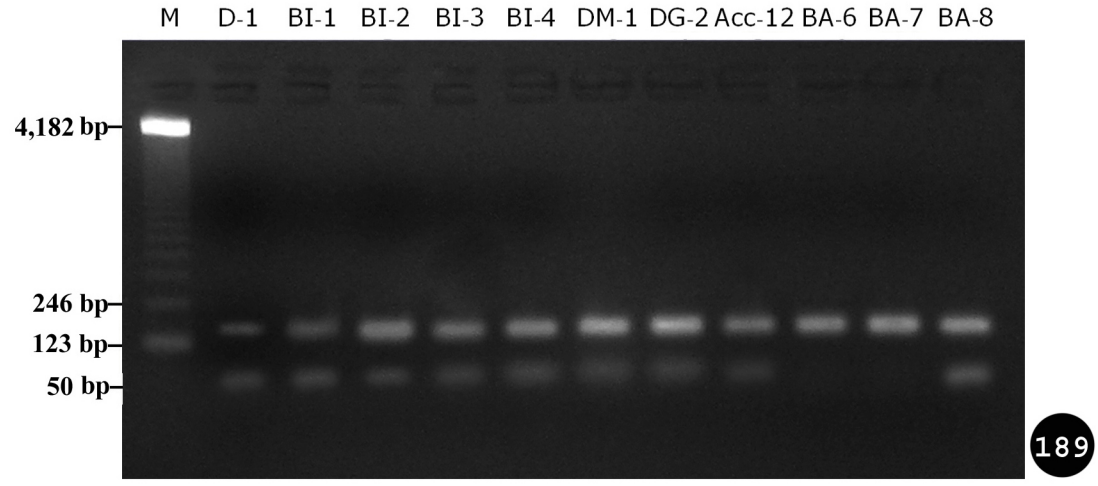


Fig. 189. SSR analysis with primer pair forward-5'-CTCTCCTCTGCTCTGCACTG-3'/ reverse-5'-ACAAGAACATGGGGATGAAGA-3' of 11 varieties of peanut (*Arachis hypogaea* L.). M=123 bp DNA ladder, D-1=Dhaka-1, BI-1=BINA Cheena Badam-1, BI-2=BINA Cheena Badam-2, BI-3=BINA Cheena Badam-3, BI-4=BINA Cheena Badam-4, DM-1=Tridana Badam, DG-2=Basanti Badam, Acc-12=Jhinga Badam, BA-6=BARI Cheena Badam-6, BA-7=BARI Cheena Badam-7 and BA-8=BARI Cheena Badam-8.

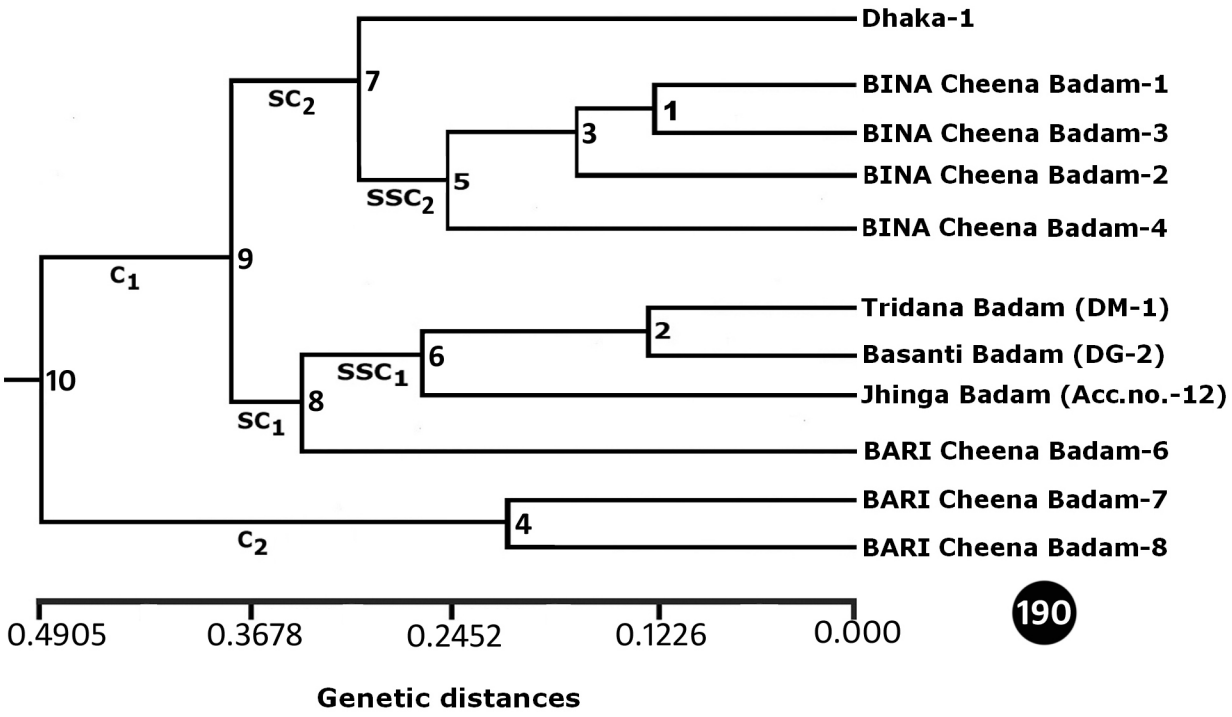


Fig. 190. UPGMA dendrogram constructed based on Nei's (1972) genetic distance summarizing the data on differentiation among 11 varieties of peanut (*Arachis hypogaea* L.) by RAPD and SSR analysis.

Table 8. Length (in µm), arm ratio, centromeric index, relative length and centromeric type of metaphase chromosomes of Dhaka-1

Chromo-some pair	Long arm (l) µm	Short arm (s) µm	Total length (T) µm	Arm ratio (l/s)	Relative length (RL)	Centrom-eric index (CI)	Centrom-eric type (CT)
I	1.66	1.34	3.00	1.24	0.03	44.67	m
	1.66	1.34	3.00	1.24	0.03	44.67	m
II	1.66	1.30	2.96	1.28	0.03	43.92	m
	1.50	1.46	2.96	1.03	0.03	49.32	m
III	1.50	1.46	2.96	1.03	0.03	49.32	m
	1.50	1.46	2.96	1.03	0.03	49.32	m
IV	1.84	1.10	2.94	1.67	0.03	37.41	sm
	1.84	1.10	2.94	1.67	0.03	37.41	sm
V	1.46	1.46	2.92	1.00	0.03	50.00	m
	1.46	1.46	2.92	1.00	0.03	50.00	m
VI	1.50	1.18	2.68	1.27	0.03	44.03	m
	1.50	1.18	2.68	1.27	0.03	44.03	m
VII	1.34	1.34	2.68	1.00	0.03	50.00	m
	1.34	1.34	2.68	1.00	0.03	50.00	m
VIII	1.34	1.26	2.60	1.06	0.03	48.46	m
	1.34	1.26	2.60	1.06	0.03	48.46	m
IX	1.34	1.26	2.60	1.06	0.03	48.46	m
	1.34	1.26	2.60	1.06	0.03	48.46	m
X	1.34	1.26	2.60	1.06	0.03	48.46	m
	1.34	1.26	2.60	1.06	0.03	48.46	m
XI	1.34	1.16	2.50	1.16	0.03	46.40	m
	1.34	1.16	2.50	1.16	0.03	46.40	m
XII	1.56	0.94	2.50	1.66	0.03	37.60	sm
	1.56	0.94	2.50	1.66	0.03	37.60	sm
XIII	1.34	1.16	2.50	1.16	0.03	46.40	m
	1.34	1.16	2.50	1.16	0.03	46.40	m
XIV	1.34	1.16	2.50	1.16	0.03	46.40	m
	1.16	1.16	2.32	1.00	0.02	50.00	m
XV	1.50	0.80	2.30	1.88	0.02	34.78	sm
	1.50	0.80	2.30	1.88	0.02	34.78	sm
XVI	1.34	0.96	2.30	1.40	0.02	41.74	m
	1.34	0.96	2.30	1.40	0.02	41.74	m
XVII	1.06	0.96	2.02	1.10	0.02	47.52	m
	1.06	0.96	2.02	1.10	0.02	47.52	m
XVIII	1.06	0.96	2.02	1.10	0.02	47.52	m
	1.00	1.00	2.00	1.00	0.02	50.00	m
XIX	1.06	0.92	1.98	1.15	0.02	46.46	m
	1.06	0.92	1.98	1.15	0.02	46.46	m
XX	0.66	0.66	1.32	1.00	0.01	50.00	m
	0.66	0.66	1.32	1.00	0.01	50.00	m

m = metacentric, sm = sub-metacentric chromosome

Table 9. Length (in µm), arm ratio, centromeric index, relative length and centromeric type of metaphase chromosomes of BINA Cheena Badam-1

Chromo-some pair	Long arm (l) µm	Short arm (s) µm	Total length (T) µm	Arm ratio (l/s)	Relative length (RL)	Centrom-eric index (CI)	Centrom-eric type (CT)
I	1.34	1.04	2.38	1.29	0.03	43.70	m
	1.34	1.04	2.38	1.29	0.03	43.70	m
II	1.34	1.00	2.34	1.34	0.03	42.74	m
	1.34	1.00	2.34	1.34	0.03	42.74	m
III	1.34	1.00	2.34	1.34	0.03	42.74	m
	1.16	1.00	2.16	1.16	0.03	46.30	m
IV	1.16	1.00	2.16	1.16	0.03	46.30	m
	1.16	1.00	2.16	1.16	0.03	46.30	m
V	1.16	1.00	2.16	1.16	0.03	46.30	m
	1.16	1.00	2.16	1.16	0.03	46.30	m
VI	1.16	0.90	2.06	1.29	0.03	43.69	m
	1.16	0.90	2.06	1.29	0.03	43.69	m
VII	1.16	0.90	2.06	1.29	0.03	43.69	m
	1.16	0.90	2.06	1.29	0.03	43.69	m
VIII	1.14	0.80	1.94	1.43	0.03	41.24	m
	1.14	0.80	1.94	1.43	0.03	41.24	m
IX	1.14	0.76	1.90	1.50	0.03	40.00	m
	1.14	0.76	1.90	1.50	0.03	40.00	m
X	1.14	0.76	1.90	1.50	0.03	40.00	m
	1.14	0.76	1.90	1.50	0.03	40.00	m
XI	1.16	0.72	1.88	1.61	0.03	38.30	sm
	1.16	0.72	1.88	1.61	0.03	38.30	sm
XII	1.06	0.74	1.80	1.43	0.02	41.11	m
	1.06	0.74	1.80	1.43	0.02	41.11	m
XIII	1.06	0.74	1.80	1.43	0.02	41.11	m
	1.06	0.74	1.80	1.43	0.02	41.11	m
XIV	0.86	0.74	1.60	1.16	0.02	46.25	m
	0.86	0.74	1.60	1.16	0.02	46.25	m
XV	1.00	0.60	1.60	1.67	0.02	37.50	sm
	1.00	0.60	1.60	1.67	0.02	37.50	sm
XVI	0.86	0.74	1.60	1.16	0.02	46.25	m
	0.86	0.74	1.60	1.16	0.02	46.25	m
XVII	0.86	0.74	1.60	1.16	0.02	46.25	m
	0.86	0.74	1.60	1.16	0.02	46.25	m
XVIII	0.84	0.70	1.54	1.20	0.02	45.45	m
	0.84	0.70	1.54	1.20	0.02	45.45	m
XIX	0.84	0.70	1.54	1.20	0.02	45.45	m
	0.84	0.70	1.54	1.20	0.02	45.45	m
XX	0.66	0.66	1.32	1.00	0.02	50.00	m
	0.64	0.64	1.28	1.00	0.02	50.00	m

m = metacentric, sm = sub-metacentric chromosome

Table 10. Length (in µm), arm ratio, centromeric index, relative length and centromeric type of metaphase chromosomes of BINA Cheena Badam-2

Chromo-some pair	Long arm (l) µm	Short arm (s) µm	Total length (T) µm	Arm ratio (l/s)	Relative length (RL)	Centrom-eric index (CI)	Centrom-eric type (CT)
I	2.20	1.16	3.36	1.90	0.04	34.52	sm
	2.16	1.16	3.32	1.86	0.04	34.94	sm
II	1.54	1.46	3.00	1.05	0.03	48.67	m
	1.54	1.20	2.74	1.28	0.03	43.80	m
III	1.54	1.20	2.74	1.28	0.03	43.80	m
	1.54	1.20	2.74	1.28	0.03	43.80	m
IV	1.54	1.10	2.64	1.40	0.03	41.67	m
	1.54	1.10	2.64	1.40	0.03	41.67	m
V	1.54	1.06	2.60	1.45	0.03	40.77	m
	1.44	1.16	2.60	1.24	0.03	44.62	m
VI	1.44	1.16	2.60	1.24	0.03	44.62	m
	1.44	1.16	2.60	1.24	0.03	44.62	m
VII	1.34	1.26	2.60	1.06	0.03	48.46	m
	1.34	1.26	2.60	1.06	0.03	48.46	m
VIII	1.60	0.94	2.54	1.70	0.03	37.01	sm
	1.60	0.94	2.54	1.70	0.03	37.01	sm
IX	1.34	1.16	2.50	1.16	0.03	46.40	m
	1.34	1.16	2.50	1.16	0.03	46.40	m
X	1.56	0.86	2.42	1.81	0.03	35.54	sm
	1.54	0.80	2.34	1.93	0.03	34.19	sm
XI	1.16	1.14	2.30	1.02	0.03	49.57	m
	1.16	1.14	2.30	1.02	0.03	49.57	m
XII	1.36	0.96	2.32	1.42	0.03	41.38	m
	1.36	0.96	2.32	1.42	0.03	41.38	m
XIII	1.14	0.84	1.98	1.36	0.02	42.42	m
	1.14	0.84	1.98	1.36	0.02	42.42	m
XIV	1.14	0.84	1.98	1.36	0.02	42.42	m
	1.14	0.84	1.98	1.36	0.02	42.42	m
XV	1.14	0.84	1.98	1.36	0.02	42.42	m
	1.14	0.84	1.98	1.36	0.02	42.42	m
XVI	1.14	0.84	1.98	1.36	0.02	42.42	m
	1.14	0.84	1.98	1.36	0.02	42.42	m
XVII	1.14	0.84	1.98	1.36	0.02	42.42	m
	0.86	0.84	1.70	1.02	0.02	49.41	m
XVIII	1.10	0.74	1.84	1.49	0.02	40.22	m
	1.10	0.74	1.84	1.49	0.02	40.22	m
XIX	0.84	0.76	1.60	1.11	0.02	47.50	m
	0.84	0.76	1.60	1.11	0.02	47.50	m
XX	0.46	0.46	0.92	1.00	0.01	50.00	m
	0.46	0.46	0.92	1.00	0.01	50.00	m

m = metacentric, sm = sub-metacentric chromosome

Table 11. Length (in µm), arm ratio, centromeric index, relative length and centromeric type of metaphase chromosomes of BINA Cheena Badam-3

Chromo-some pair	Long arm (l) µm	Short arm (s) µm	Total length (T) µm	Arm ratio (l/s)	Relative length (RL)	Centrom-eric index (CI)	Centrom-eric type (CT)
I	1.50	1.26	2.76	1.19	0.03	45.65	m
	1.36	1.32	2.68	1.03	0.03	49.25	m
II	1.36	1.30	2.66	1.04	0.03	48.87	m
	1.32	1.32	2.64	1.00	0.03	50.00	m
III	1.32	1.32	2.64	1.00	0.03	50.00	m
	1.32	1.32	2.64	1.00	0.03	50.00	m
IV	1.32	1.30	2.62	1.01	0.03	49.62	m
	1.32	1.30	2.62	1.01	0.03	49.62	m
V	1.32	1.30	2.62	1.01	0.03	49.62	m
	1.32	1.30	2.62	1.01	0.03	49.62	m
VI	1.32	1.30	2.62	1.01	0.03	49.62	m
	1.32	1.30	2.62	1.01	0.03	49.62	m
VII	1.32	1.26	2.58	1.04	0.03	48.84	m
	1.32	1.26	2.58	1.04	0.03	48.84	m
VIII	1.32	1.26	2.58	1.04	0.03	48.84	m
	1.32	1.26	2.58	1.04	0.03	48.84	m
IX	1.32	1.26	2.58	1.04	0.03	48.84	m
	1.30	1.26	2.58	1.03	0.03	49.22	m
X	1.30	1.26	2.58	1.03	0.02	49.22	m
	1.32	1.16	2.48	1.13	0.02	46.77	m
XI	1.36	1.04	2.40	1.30	0.02	43.33	m
	1.36	1.04	2.40	1.30	0.02	43.33	m
XII	1.36	1.04	2.40	1.30	0.03	43.33	m
	1.36	1.04	2.40	1.30	0.03	43.33	m
XIII	1.45	0.90	2.35	1.61	0.02	38.79	sm
	1.45	0.90	2.35	1.61	0.02	38.79	sm
XIV	1.16	1.04	2.20	1.11	0.02	47.27	m
	1.16	1.04	2.20	1.11	0.02	47.27	m
XV	1.16	1.04	2.20	1.11	0.02	47.27	m
	1.16	1.04	2.20	1.11	0.02	47.27	m
XVI	1.16	1.04	2.20	1.11	0.02	47.27	m
	1.16	1.04	2.20	1.11	0.02	47.27	m
XVII	1.16	1.04	2.20	1.11	0.02	47.27	m
	1.16	1.00	2.16	1.16	0.02	46.29	m
XVIII	1.10	1.06	2.16	1.03	0.02	49.07	m
	1.10	1.06	2.16	1.03	0.02	49.07	m
XIX	0.80	0.66	1.46	1.21	0.02	45.20	m
	0.80	0.66	1.46	1.21	0.02	45.20	m
XX	0.70	0.50	1.20	1.40	0.02	41.67	m
	0.70	0.50	1.20	1.40	0.02	41.67	m

m = metacentric, sm = sub-metacentric chromosome

Table 12. Length (in µm), arm ratio, centromeric index, relative length and centromeric type of metaphase chromosomes of BINA Cheena Badam-4

Chromo-some pair	Long arm (l) µm	Short arm (s) µm	Total length (T) µm	Arm ratio (l/s)	Relative length (RL)	Centrom-eric index (CI)	Centrom-eric type (CT)
I	1.40	1.26	2.66	1.11	0.03	47.37	m
	1.40	1.26	2.66	1.11	0.03	47.37	m
II	1.44	1.18	2.62	1.22	0.03	45.04	m
	1.44	1.18	2.62	1.22	0.03	45.04	m
III	1.46	0.96	2.42	1.52	0.03	39.67	sm
	1.46	0.96	2.42	1.52	0.03	39.67	sm
IV	1.30	1.08	2.38	1.20	0.03	45.38	m
	1.30	1.08	2.38	1.20	0.03	45.38	m
V	1.30	1.08	2.38	1.20	0.03	45.38	m
	1.30	1.08	2.38	1.20	0.03	45.38	m
VI	1.10	1.10	2.20	1.00	0.03	50.00	m
	1.10	1.10	2.20	1.00	0.03	50.00	m
VII	1.20	1.00	2.20	1.20	0.03	45.45	m
	1.20	1.00	2.20	1.20	0.03	45.45	m
VIII	1.26	0.96	2.22	1.31	0.03	43.24	m
	1.26	0.96	2.22	1.31	0.03	43.24	m
IX	1.06	1.06	2.12	1.00	0.03	50.00	m
	1.06	1.06	2.12	1.00	0.03	50.00	m
X	1.04	1.02	2.06	1.02	0.02	49.51	m
	1.04	1.02	2.06	1.02	0.02	49.51	m
XI	1.28	0.78	2.06	1.64	0.02	37.86	sm
	1.28	0.78	2.06	1.64	0.02	37.86	sm
XII	1.10	0.92	2.02	1.20	0.02	45.54	m
	1.10	0.92	2.02	1.20	0.02	45.54	m
XIII	1.16	0.86	2.02	1.35	0.02	42.57	m
	1.16	0.86	2.02	1.35	0.02	42.57	m
XIV	1.22	0.76	1.98	1.61	0.02	38.38	sm
	1.22	0.76	1.98	1.61	0.02	38.38	sm
XV	1.00	0.96	1.96	1.04	0.02	48.98	m
	1.00	0.96	1.96	1.04	0.02	48.98	m
XVI	1.18	0.74	1.92	1.59	0.02	38.54	sm
	1.18	0.74	1.92	1.59	0.02	38.54	sm
XVII	1.00	0.86	1.86	1.16	0.02	46.24	m
	1.00	0.86	1.86	1.16	0.02	46.24	m
XVIII	0.96	0.90	1.86	1.07	0.02	48.39	m
	0.96	0.90	1.86	1.07	0.02	48.39	m
XIX	0.70	0.70	1.40	1.00	0.02	50.00	m
	0.70	0.70	1.40	1.00	0.02	50.00	m
XX	0.66	0.66	1.32	1.00	0.02	50.00	m
	0.66	0.66	1.32	1.00	0.02	50.00	m

m = metacentric, sm = sub-metacentric chromosome

Table 13. Length (in µm), arm ratio, centromeric index, relative length and centromeric type of metaphase chromosomes of Tridana Badam (DM-1)

Chromo-some pair	Long arm (l) µm	Short arm (s) µm	Total length (T) µm	Arm ratio (l/s)	Relative length (RL)	Centrom-eric index (CI)	Centrom-eric type (CT)
I	1.70	1.60	3.30	1.06	0.03	48.48	m
	1.70	1.60	3.30	1.06	0.03	48.48	m
II	1.90	1.14	3.04	1.67	0.03	37.50	sm
	1.90	1.14	3.04	1.67	0.03	37.50	sm
III	1.70	1.30	3.00	1.31	0.03	43.33	m
	1.70	1.30	3.00	1.31	0.03	43.33	m
IV	1.50	1.42	2.92	1.06	0.03	48.63	m
	1.50	1.42	2.92	1.06	0.03	48.63	m
V	1.42	1.36	2.78	1.04	0.03	48.92	m
	1.42	1.36	2.78	1.04	0.03	48.92	m
VI	1.70	1.00	2.70	1.70	0.03	37.04	sm
	1.70	1.00	2.70	1.70	0.03	37.04	sm
VII	1.36	1.32	2.68	1.03	0.03	49.25	m
	1.36	1.32	2.68	1.03	0.03	49.25	m
VIII	1.42	1.24	2.66	1.15	0.03	46.62	m
	1.42	1.24	2.66	1.15	0.03	46.62	m
IX	1.34	1.26	2.60	1.06	0.03	48.46	m
	1.34	1.26	2.60	1.06	0.03	48.46	m
X	1.38	1.14	2.52	1.21	0.03	45.24	m
	1.38	1.14	2.52	1.21	0.03	45.24	m
XI	1.40	1.04	2.44	1.35	0.02	42.62	m
	1.40	1.04	2.44	1.35	0.02	42.62	m
XII	1.50	0.90	2.40	1.67	0.02	37.50	sm
	1.50	0.90	2.40	1.67	0.02	37.50	sm
XIII	1.14	1.14	2.28	1.00	0.02	50.00	m
	1.14	1.14	2.28	1.00	0.02	50.00	m
XIV	1.14	1.14	2.28	1.00	0.02	50.00	m
	1.14	1.14	2.28	1.00	0.02	50.00	m
XV	1.10	1.04	2.14	1.06	0.02	48.60	m
	1.10	1.04	2.14	1.06	0.02	48.60	m
XVI	1.10	1.04	2.14	1.06	0.02	48.60	m
	1.10	1.04	2.14	1.06	0.02	48.60	m
XVII	1.04	0.96	2.00	1.08	0.02	48.00	m
	1.04	0.96	2.00	1.08	0.02	48.00	m
XVIII	1.24	0.64	1.88	1.94	0.02	34.04	sm
	1.24	0.64	1.88	1.94	0.02	34.04	sm
XIX	0.94	0.86	1.80	1.09	0.02	47.78	m
	0.94	0.86	1.80	1.09	0.02	47.78	m
XX	0.76	0.64	1.40	1.19	0.01	45.71	m
	0.76	0.64	1.40	1.19	0.01	45.71	m

m = metacentric, sm = sub-metacentric chromosome

Table 14. Length (in µm), arm ratio, centromeric index, relative length and centromeric type of metaphase chromosomes of Basanti Badam (DG-2)

Chromo-some pair	Long arm (l) µm	Short arm (s) µm	Total length (T) µm	Arm ratio (l/s)	Relative length (RL)	Centrom-eric index (CI)	Centrom-eric type (CT)
I	1.66	1.40	3.06	1.19	0.03	45.75	m
	1.66	1.40	3.06	1.19	0.03	45.75	m
II	1.50	1.36	2.86	1.10	0.03	47.55	m
	1.50	1.36	2.86	1.10	0.03	47.55	m
III	1.50	1.34	2.84	1.12	0.03	47.18	m
	1.50	1.34	2.84	1.12	0.03	47.18	m
IV	1.50	1.16	2.66	1.29	0.03	43.61	m
	1.50	1.16	2.66	1.29	0.03	43.61	m
V	1.50	1.16	2.66	1.29	0.03	43.61	m
	1.50	1.16	2.66	1.29	0.03	43.61	m
VI	1.50	1.06	2.56	1.42	0.03	41.41	m
	1.50	1.06	2.56	1.42	0.03	41.41	m
VII	1.50	1.04	2.54	1.44	0.03	40.94	m
	1.50	1.04	2.54	1.44	0.03	40.94	m
VIII	1.34	1.16	2.50	1.16	0.03	46.40	m
	1.30	1.16	2.46	1.12	0.03	47.15	m
IX	1.30	1.16	2.46	1.12	0.03	47.15	m
	1.20	1.16	2.36	1.03	0.02	49.15	m
X	1.20	1.16	2.36	1.03	0.02	49.15	m
	1.34	1.16	2.50	1.16	0.03	46.40	m
XI	1.16	1.16	2.32	1.00	0.02	50.00	m
	1.16	1.16	2.32	1.00	0.02	50.00	m
XII	1.24	1.06	2.30	1.17	0.02	46.09	m
	1.24	1.06	2.30	1.17	0.02	46.09	m
XIII	1.24	1.00	2.24	1.24	0.02	44.64	m
	1.24	1.00	2.24	1.24	0.02	44.64	m
XIV	1.24	1.00	2.24	1.24	0.02	44.64	m
	1.24	1.00	2.24	1.24	0.02	44.64	m
XV	1.16	1.04	2.20	1.12	0.02	47.27	m
	1.16	1.04	2.20	1.12	0.02	47.27	m
XVI	1.10	1.04	2.14	1.06	0.02	48.60	m
	1.10	1.04	2.14	1.06	0.02	48.60	m
XVII	1.06	1.04	2.10	1.02	0.02	49.52	m
	1.06	1.04	2.10	1.02	0.02	49.52	m
XVIII	1.30	0.74	2.04	1.76	0.02	36.27	sm
	1.30	0.74	2.04	1.76	0.02	36.27	sm
XIX	1.16	0.86	2.02	1.35	0.02	42.57	m
	1.16	0.86	2.02	1.35	0.02	42.57	m
XX	1.00	1.00	2.00	1.00	0.02	50.00	m
	1.00	1.00	2.00	1.00	0.02	50.00	m

m = metacentric, sm = sub-metacentric chromosome

Table 15. Length (in µm), arm ratio, centromeric index, relative length and centromeric type of metaphase chromosomes of Jhinga Badam (Acc. no.-12)

Chromo-some pair	Long arm (l) µm	Short arm (s) µm	Total length (T) µm	Arm ratio (l/s)	Relative length (RL)	Centrom-eric index (CI)	Centrom-eric type (CT)
I	1.84	1.44	3.28	1.28	0.03	43.90	m
	1.80	1.44	3.24	1.25	0.03	44.44	m
II	1.76	1.40	3.16	1.26	0.03	44.30	m
	1.72	1.44	3.16	1.19	0.03	45.57	m
III	1.76	1.40	3.16	1.26	0.03	44.30	m
	1.76	1.40	3.16	1.26	0.03	44.30	m
IV	1.68	1.42	3.10	1.18	0.03	45.81	m
	1.68	1.42	3.10	1.18	0.03	45.81	m
V	1.92	1.18	3.10	1.63	0.03	38.06	sm
	1.92	1.18	3.10	1.63	0.03	38.06	sm
VI	1.50	1.30	2.80	1.15	0.03	46.43	m
	1.50	1.30	2.80	1.15	0.03	46.43	m
VII	1.50	1.30	2.80	1.15	0.03	46.43	m
	1.50	1.30	2.80	1.15	0.03	46.43	m
VIII	1.50	1.30	2.80	1.15	0.03	46.43	m
	1.50	1.30	2.80	1.15	0.03	46.43	m
IX	1.50	1.30	2.80	1.15	0.03	46.43	m
	1.50	1.30	2.80	1.15	0.03	46.43	m
X	1.50	1.30	2.80	1.15	0.03	46.43	m
	1.50	1.30	2.80	1.15	0.03	46.43	m
XI	1.44	1.30	2.74	1.11	0.03	47.45	m
	1.44	1.30	2.74	1.11	0.03	47.45	m
XII	1.44	1.30	2.74	1.11	0.03	47.45	m
	1.44	1.30	2.74	1.11	0.03	47.45	m
XIII	1.44	1.30	2.74	1.11	0.03	47.45	m
	1.44	1.30	2.74	1.11	0.03	47.45	m
XIV	1.44	1.24	2.68	1.16	0.03	46.27	m
	1.44	1.24	2.68	1.16	0.03	46.27	m
XV	1.40	1.20	2.60	1.17	0.02	46.15	m
	1.40	1.20	2.60	1.17	0.02	46.15	m
XVI	1.24	1.04	2.28	1.19	0.02	45.61	m
	1.24	1.04	2.28	1.19	0.02	45.61	m
XVII	1.20	1.04	2.24	1.15	0.02	46.43	m
	1.20	1.04	2.24	1.15	0.02	46.43	m
XVIII	1.04	0.80	1.84	1.30	0.02	43.48	m
	1.04	0.80	1.84	1.30	0.02	43.48	m
XIX	0.88	0.88	1.76	1.00	0.02	50.00	m
	0.88	0.88	1.76	1.00	0.02	50.00	m
XX	0.76	0.76	1.52	1.00	0.01	50.00	m
	0.76	0.76	1.52	1.00	0.01	50.00	m

m = metacentric, sm = sub-metacentric chromosome

Table 16. Length (in µm), arm ratio, centromeric index, relative length and centromeric type of metaphase chromosomes of BARI Cheena Badam-6

Chromo-some pair	Long arm (l) µm	Short arm (s) µm	Total length (T) µm	Arm ratio (l/s)	Relative length (RL)	Centrom-eric index (CI)	Centrom-eric type (CT)
I	1.32	1.32	2.64	1.00	0.03	50.00	m
	1.30	1.30	2.60	1.00	0.03	50.00	m
II	1.32	1.26	2.58	1.05	0.03	48.84	m
	1.30	1.22	2.52	1.07	0.03	48.41	m
III	1.30	1.16	2.46	1.12	0.03	47.15	m
	1.30	1.16	2.46	1.12	0.03	47.15	m
IV	1.32	0.90	2.22	1.47	0.03	40.54	m
	1.32	0.90	2.22	1.47	0.03	40.54	m
V	1.26	0.90	2.16	1.40	0.03	41.67	m
	1.26	0.90	2.16	1.40	0.03	41.67	m
VI	1.26	0.90	2.16	1.40	0.03	41.67	m
	1.26	0.90	2.16	1.40	0.03	41.67	m
VII	1.26	0.90	2.16	1.40	0.03	41.67	m
	1.26	0.90	2.16	1.40	0.03	41.67	m
VIII	1.26	0.90	2.16	1.40	0.03	41.67	m
	1.26	0.90	2.16	1.40	0.03	41.67	m
IX	1.24	0.90	2.14	1.38	0.03	42.06	m
	1.24	0.90	2.14	1.38	0.03	42.06	m
X	1.22	0.90	2.12	1.36	0.03	42.45	m
	1.22	0.90	2.12	1.36	0.03	42.45	m
XI	1.00	1.00	2.00	1.00	0.02	50.00	m
	1.00	1.00	2.00	1.00	0.02	50.00	m
XII	1.00	1.00	2.00	1.00	0.02	50.00	m
	1.00	1.00	2.00	1.00	0.02	50.00	m
XIII	1.00	0.96	1.96	1.04	0.02	48.98	m
	1.00	0.96	1.96	1.04	0.02	48.98	m
XIV	1.00	0.94	1.94	1.06	0.02	48.45	m
	1.00	0.94	1.94	1.06	0.02	48.45	m
XV	1.00	0.94	1.94	1.06	0.02	48.45	m
	1.00	0.94	1.94	1.06	0.02	48.45	m
XVI	1.00	0.94	1.94	1.06	0.02	48.45	m
	1.00	0.86	1.86	1.16	0.02	46.24	m
XVII	0.94	0.94	1.88	1.00	0.02	50.00	m
	1.00	0.86	1.86	1.16	0.02	46.24	m
XVIII	1.00	0.86	1.86	1.16	0.02	46.24	m
	0.94	0.90	1.84	1.04	0.02	48.91	m
XIX	0.70	0.70	1.40	1.00	0.02	50.00	m
	0.70	0.70	1.40	1.00	0.02	50.00	m
XX	0.66	0.66	1.32	1.00	0.02	50.00	m
	0.66	0.66	1.32	1.00	0.02	50.00	m

m = metacentric

Table 17. Length (in µm), arm ratio, centromeric index, relative length and centromeric type of metaphase chromosomes of BARI Cheena Badam-7

Chromo-some pair	Long arm (l) µm	Short arm (s) µm	Total length (T) µm	Arm ratio (l/s)	Relative length (RL)	Centrom-eric index (CI)	Centrom-eric type (CT)
I	1.74	1.66	3.40	1.05	0.04	48.82	m
	1.44	1.66	3.10	0.87	0.03	53.55	m
II	1.40	1.36	2.76	1.03	0.03	49.28	m
	1.40	1.36	2.76	1.03	0.03	49.28	m
III	1.36	1.34	2.70	1.01	0.03	49.63	m
	1.36	1.34	2.70	1.01	0.03	49.63	m
IV	1.36	1.34	2.70	1.01	0.03	49.63	m
	1.36	1.34	2.70	1.01	0.03	49.63	m
V	1.30	1.16	2.46	1.12	0.03	47.15	m
	1.30	1.16	2.46	1.12	0.03	47.15	m
VI	1.26	1.20	2.46	1.05	0.03	48.78	m
	1.26	1.20	2.46	1.05	0.03	48.78	m
VII	1.30	1.16	2.46	1.12	0.03	47.15	m
	1.30	1.16	2.46	1.12	0.03	47.15	m
VIII	1.30	1.12	2.42	1.16	0.03	46.28	m
	1.30	1.12	2.42	1.16	0.03	46.28	m
IX	1.26	1.12	2.38	1.13	0.03	47.06	m
	1.26	1.12	2.38	1.13	0.03	47.06	m
X	1.26	1.12	2.38	1.13	0.03	47.06	m
	1.26	1.12	2.38	1.13	0.03	47.06	m
XI	1.12	1.06	2.18	1.06	0.02	48.62	m
	1.12	1.06	2.18	1.06	0.02	48.62	m
XII	1.06	1.00	2.06	1.06	0.02	48.54	m
	1.06	1.00	2.06	1.06	0.02	48.54	m
XIII	1.06	1.00	2.06	1.06	0.02	48.54	m
	1.06	1.00	2.06	1.06	0.02	48.54	m
XIV	1.12	0.94	2.06	1.19	0.02	45.63	m
	1.12	0.94	2.06	1.19	0.02	45.63	m
XV	1.16	0.94	2.10	1.23	0.02	44.76	m
	1.16	0.94	2.10	1.23	0.02	44.76	m
XVI	1.02	1.00	2.02	1.02	0.02	49.50	m
	1.02	1.00	2.02	1.02	0.02	49.50	m
XVII	1.02	0.84	1.86	1.21	0.02	45.16	m
	1.02	0.84	1.86	1.21	0.02	45.16	m
XVIII	1.00	0.84	1.84	1.19	0.02	45.65	m
	1.00	0.84	1.84	1.19	0.02	45.65	m
XIX	0.98	0.68	1.66	1.44	0.02	40.96	m
	0.98	0.68	1.66	1.44	0.02	40.96	m
XX	0.70	0.70	1.40	1.00	0.02	50.00	m
	0.70	0.70	1.40	1.00	0.02	50.00	m
XXI	0.40	0.40	0.80	1.00	0.02	50.00	m

m = metacentric

Table 18. Length (in µm), arm ratio, centromeric index, relative length and centromeric type of metaphase chromosomes of BARI Cheena Badam-8

Chromo-some pair	Long arm (l) µm	Short arm (s) µm	Total length (T) µm	Arm ratio (l/s)	Relative length (RL)	Centrom-eric index (CI)	Centrom-eric type (CT)
I	1.46	1.26	2.72	1.16	0.03	46.32	m
	1.46	1.26	2.72	1.16	0.03	46.32	m
II	1.46	1.26	2.72	1.16	0.03	46.32	m
	1.46	1.26	2.72	1.16	0.03	46.32	m
III	1.50	1.16	2.66	1.29	0.03	43.61	m
	1.50	1.16	2.66	1.29	0.03	43.61	m
IV	1.46	1.00	2.46	1.46	0.03	40.65	m
	1.46	1.00	2.46	1.46	0.03	40.65	m
V	1.30	1.00	2.30	1.30	0.03	43.48	m
	1.30	1.00	2.30	1.30	0.03	43.48	m
VI	1.30	1.00	2.30	1.30	0.03	43.48	m
	1.30	1.00	2.30	1.30	0.03	43.48	m
VII	1.30	1.00	2.30	1.30	0.03	43.48	m
	1.30	1.00	2.30	1.30	0.03	43.48	m
VIII	1.30	1.00	2.30	1.30	0.03	43.48	m
	1.30	1.00	2.30	1.30	0.03	43.48	m
IX	1.16	1.00	2.16	1.16	0.03	46.30	m
	1.16	1.00	2.16	1.16	0.03	46.30	m
X	1.16	1.00	2.16	1.16	0.03	46.30	m
	1.16	1.00	2.16	1.16	0.03	46.30	m
XI	1.28	0.86	2.14	1.49	0.02	40.19	m
	1.28	0.86	2.14	1.49	0.02	40.19	m
XII	1.16	0.84	2.00	1.38	0.02	42.00	m
	1.16	0.84	2.00	1.38	0.02	42.00	m
XIII	1.16	0.84	2.00	1.38	0.02	42.00	m
	1.16	0.84	2.00	1.38	0.02	42.00	m
XIV	1.16	0.84	2.00	1.38	0.02	42.00	m
	1.16	0.84	2.00	1.38	0.02	42.00	m
XV	1.00	0.84	1.84	1.19	0.02	45.65	m
	1.00	0.84	1.84	1.19	0.02	45.65	m
XVI	1.00	0.84	1.84	1.19	0.02	45.65	m
	1.00	0.84	1.84	1.19	0.02	45.65	m
XVII	1.00	0.84	1.84	1.19	0.02	45.65	m
	1.00	0.84	1.84	1.19	0.02	45.65	m
XVIII	1.00	0.76	1.76	1.32	0.02	43.18	m
	1.00	0.76	1.76	1.32	0.02	43.18	m
XIX	1.00	0.76	1.76	1.32	0.02	43.18	m
	1.00	0.76	1.76	1.32	0.02	43.18	m
XX	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
XXI	0.40	0.40	0.80	1.00	0.02	50.00	m

m = metacentric

Table 19. Comparative orcein karyotype analysis of 11 germplasms of peanut (*Arachis hypogaea* L.)

Germplasms	2n	Range of chromosomal length (μm)	Total length of 2n chromosome complements (μm)	Centromeric formulae
Dhaka-1	40	1.32-3.00	99.56	34m+6sm
BINA Cheena Badam-1	40	1.28-2.38	74.82	36m+4sm
BINA Cheena Badam-2	40	0.92-3.36	91.10	34m+6sm
BINA Cheena Badam-3	40	1.20-2.76	93.78	38m+2sm
BINA Cheena Badam-4	40	1.32-2.66	83.32	32m+8sm
Tridana Badam (DM-1)	40	1.40-3.30	97.92	32m+8sm
Basanti Badam (DG-2)	40	2.00-3.06	96.20	38m+2sm
Jhinga Badam (Acc. no.-12)	40	1.52-3.28	105.84	38m+2sm
BARI Cheena Badam-6	40	1.32-2.64	81.86	40m
BARI Cheena Badam-7	40 (in few cells 41)	0.80-3.40	91.22	41m
BARI Cheena Badam-8	40 (in few cells 41)	0.80-2.72	86.42	41m

m = metacentric chromosome, sm = sub-metacentric chromosome

Table 20. Comparative CMA-banding analysis of 11 germplasms of peanut (*Arachis hypogaea* L.)

Germplasms	CMA-bands			CMA-banded karyotype formulae
	No.	Total length (μm)	%	
Dhaka-1	8	3.30	3.31	2Ω + 1∂ + 1Ø +3€ + 33δ
BINA Cheena Badam-1	12	4.34	5.80	2α + 1λ + 5€ + 3Ω + 28δ
BINA Cheena Badam-2	9	3.43	3.76	5€ + 4∂ + 31δ
BINA Cheena Badam-3	4	1.14	1.22	1€ + 1λ + 1∂ + 37δ
BINA Cheena Badam-4	12	5.14	6.17	3α + 1€ + 4∂ + 4Ω + 28δ
Tridana Badam (DM-1)	8	4.11	4.19	7∂ + 1Ω + 32δ
Basanti Badam (DG-2)	7	3.20	3.33	2α + 1€ + 2∂ + 2Ω + 33δ
Jhinga Badam (Acc. no.-12)	4	2.51	2.37	2θ + 2Ω + 36δ
BARI Cheena Badam-6	6	1.83	2.23	2α + 4€ + 34δ
BARI Cheena Badam-7	6	3.20	3.50	4∂ + 2Ω + 34δ
BARI Cheena Badam-8	6	2.10	2.51	4∂ + 2Ω + 34δ

Classification of CMA-positive bands:

α = Band in centromeric region

∂= Band throughout the length of short arm

Ω = Band throughout the length

θ = Band throughout the length of long arm

Ø= Bands on the proximal ends of centromere

€ = Band on the terminal portion of short arm

λ = Bands on the terminal portion of both arms

δ =No band

Table 21. Comparative DAPI-banding analysis 11 germplasms of peanut (*Arachis hypogaea* L.)

Germplasms	DAPI-bands			DAPI-banded karyotype formulae
	No.	Total length (μm)	%	
Dhaka-1	15	2.84	2.83	12α + 2β + 1γ + 25δ
BINA Cheena Badam-1	18	4.46	5.96	6α + 9β + 3Ω + 22δ
BINA Cheena Badam-2	14	4.11	5.51	8α + 2γ + 4∂ + 26δ
BINA Cheena Badam-3	18	5.39	5.75	11α + 2β + 1€ + 4Ω + 22δ
BINA Cheena Badam-4	12	2.86	3.43	8α + 2€ + 2Ω + 28δ
Tridana Badam (DM-1)	18	4.57	4.67	15α + 3Ω + 22δ
Basanti Badam (DG-2)	9	2.61	2.51	6α + 3Ω + 31δ
Jhinga Badam (Acc. no.-12)	5	2.17	2.05	2α + 3Ω + 35δ
BARI Cheena Badam-6	10	2.40	2.93	10α + 30δ
BARI Cheena Badam-7	14	4.02	4.41	9α + 5€ + 26δ
BARI Cheena Badam-8	18	4.68	5.42	18α + 22δ

Classification of DAPI-positive bands:

α = Band in centromeric region

β = Band in interstitial portion of short arm

γ = Band in interstitial portion of long arm

∂= Band throughout the length of short arm

Ω = Band throughout the length

€ = Band on the terminal portion of short arm

δ =No band

Table 22. RAPD analysis with primer OPA-1 (5'-CAG GCC CTT C-3') of 11 germplasms of peanut (*Arachis hypogaea* 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)
Dhaka-1	5 (2000, 1400, 1100, 1000, 750)	4 (2000, 1400, 1100, 1000)	1 (750)	---	
BINA Cheena Badam-1	5 (2000, 1400, 1100, 1000, 750)	4 (2000, 1400, 1100, 1000)	1 (750)	---	
BINA Cheena Badam-2	5 (2000, 1400, 1100, 1000, 750)	4 (2000, 1400, 1100, 1000)	1 (750)	---	
BINA Cheena Badam-3	5 (2000, 1400, 1100, 1000, 750)	4 (2000, 1400, 1100, 1000)	1 (750)	---	
BINA Cheena Badam-4	5 (2000, 1400, 1100, 1000, 750)	4 (2000, 1400, 1100, 1000)	1 (750)	---	4 (1400, 1100, 1000, 750)
Tridana Badam (DM-1)	5 (2000, 1400, 1100, 1000, 750)	4 (2000, 1400, 1100, 1000)	1 (750)	---	
Basanti Badam (DG-2)	4 (1400, 1100, 1000, 750)	3 (1400, 1100, 1000)	1 (750)	---	
Jhinga Badam (Acc. no.-12)	5 (1400, 1100, 1000, 750, 500)	4 (1400, 1100, 1000, 500)	1 (750)	---	
BARI Cheena Badam-6	5 (1400, 1100, 1000, 750, 500)	4 (1400, 1100, 1000, 500)	1 (750)	---	
BARI Cheena Badam-7	5 (1400, 1100, 1000, 750, 500)	4 (1400, 1100, 1000, 500)	1 (750)	---	
BARI Cheena Badam-8	4 (1400, 1100, 1000, 750)	3 (1400, 1100, 1000)	1 (750)	---	

Table 23. RAPD analysis with primer OPA-2 (5'-TGC CGA GCT G -3') of 11 germplasms of peanut (*Arachis hypogaea* 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)
Dhaka-1	2 (1000, 750)	---	2 (1000, 750)	---	
BINA Cheena Badam-1	4 (1200, 750, 700, 600)	2 (1200, 750)	2 (700, 600)	---	
BINA Cheena Badam-2	5 (1200, 1000, 750, 700, 600)	3 (1200, 1000, 750)	2 (700, 600)	---	
BINA Cheena Badam-3	5 (1200, 1000, 750, 700, 600)	3 (1200, 1000, 750)	2 (700, 600)	---	
BINA Cheena Badam-4	1 (700)	---	1 (700)	---	
Tridana Badam (DM-1)	4 (1300, 1200, 700, 600)	2 (1300, 1200)	2 (700, 600)	---	---
Basanti Badam (DG-2)	4 (1300, 1200, 700, 600)	2 (1300, 1200)	2 (700, 600)	---	
Jhinga Badam (Acc. no.-12)	4 (1300, 1200, 700, 600)	2 (1300, 1200)	2 (700, 600)	---	
BARI Cheena Badam-6	4 (1300, 1200, 700, 600)	2 (1300, 1200)	2 (700, 600)	---	
BARI Cheena Badam-7	3 (1200, 700, 600)	1 (1200)	2 (700, 600)	---	
BARI Cheena Badam-8	3 (1200, 700, 600)	1 (1200)	2 (700, 600)	---	

Table 24. RAPD analysis with primer OPA-3 (5'-AGT CAG CCA C- 3') of 11 germplasms of peanut (*Arachis hypogaea* 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)
Dhaka-1	4 (1000, 800, 600, 400)	4 (1000, 800, 600, 400)	---	1 (1000)	
BINA Cheena Badam-1	---	---	---	---	
BINA Cheena Badam-2	3 (800, 600, 400)	3 (800, 600, 400)	---	---	
BINA Cheena Badam-3	3 (800, 600, 400)	3 (800, 600, 400)	---	---	
BINA Cheena Badam-4	3 (800, 600, 400)	3 (800, 600, 400)	---	---	3 (800, 600, 400) except BINA Cheena Badam-1
Tridana Badam (DM-1)	3 (800, 600, 400)	3 (800, 600, 400)	---	---	
Basanti Badam (DG-2)	3 (800, 600, 400)	3 (800, 600, 400)	---	---	
Jhinga Badam (Acc. no.-12)	3 (800, 600, 400)	1 (400)	2 (800, 600)	---	
BARI Cheena Badam-6	5 (1700, 1500, 800, 600, 400)	3 (1700, 1500, 400)	2 (800, 600)	2 (1700, 1500)	
BARI Cheena Badam-7	3 (800, 600, 400)	1 (400)	2 (800, 600)	---	
BARI Cheena Badam-8	3 (800, 600, 400)	1 (400)	2 (800, 600)	---	

Table 25. RAPD analysis with primer OPA-7 (5'-GAA ACG GGT G-3') of 11 germplasms of peanut (*Arachis hypogaea* 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)
Dhaka-1	3 (2500, 2000, 750)	3 (2500, 2000, 750)	--	---	1 (750) except Jhinga Badam and BARI Cheena Badam-7
BINA Cheena Badam-1	3 (2500, 2000, 750)	2 (2500, 2000)	1 (750)	---	
BINA Cheena Badam-2	4 (2500, 2000, 750, 600)	2 (2500, 600)	2 (2000, 750)	---	
BINA Cheena Badam-3	3 (2500, 2000, 750)	2 (2500, 2000)	1 (750)	---	
BINA Cheena Badam-4	3 (2000, 750, 600)	2 (2000, 600)	1 (750)	---	
Tridana Badam (DM-1)	5 (2000, 1000, 750, 600, 400)	3 (2000, 600, 400)	2 (1000, 750)	2 (1000, 400)	
Basanti Badam (DG-2)	1 (750)	1 (750)	---	---	
Jhinga Badam (Acc. no.-12)	---	---	---	---	
BARI Cheena Badam-6	3 (2500, 2000, 750)	3 (2500, 2000, 750)	---	---	
BARI Cheena Badam-7	---	---	---	---	
BARI Cheena Badam-8	1 (750)	---	1 (750)	---	

Table 26. RAPD analysis with primer OPA-8 (5'-GTG ACG TAG G-3') of 11 germplasms of peanut (*Arachis hypogaea* 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)
Dhaka-1	3 (1100, 750, 500)	2 (1100, 500)	1 (750)	---	
BINA Cheena Badam-1	3 (1500, 1100, 750)	3 (1500, 1100, 750)	---	---	
BINA Cheena Badam-2	4 (1500, 1100, 750, 500)	2 (1100, 500)	2 (1500, 750)	---	
BINA Cheena Badam-3	3 (1500, 1100, 750)	3 (1500, 1100, 750)	---	---	
BINA Cheena Badam-4	3 (1500, 1100, 750)	3 (1500, 1100, 750)	---	---	2 (1100, 750) except BARI Cheena Badam-7 and BARI Cheena Badam-8
Tridana Badam (DM-1)	4 (1500, 1100, 750, 500)	3 (1500, 1100, 500)	1 (750)	---	
Basanti Badam (DG-2)	4 (1500, 1100, 750, 500)	3 (1500, 1100, 500)	1 (750)	---	
Jhinga Badam (Acc. no.-12)	3 (1100, 750, 500)	2 (1100, 500)	1 (750)	---	
BARI Cheena Badam-6	3 (1100, 750, 500)	2 (1100, 500)	1 (750)	---	
BARI Cheena Badam-7	---	---	---	---	
BARI Cheena Badam-8	---	---	---	---	

Table 27. RAPD analysis with primer-2 (5'-GTT GCG ATC C-3') of 11 germplasms of peanut (*Arachis hypogaea* 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)
Dhaka-1	2 (900, 600)	1 (600)	1 (900)	---	
BINA Cheena Badam-1	---	---	---	---	
BINA Cheena Badam-2	3 (1500, 1400, 900)	2 (1500, 1400)	1 (900)	1 (1500)	
BINA Cheena Badam-3	1 (900)	---	1 (900)	---	
BINA Cheena Badam-4	2 (1400, 900)	1 (1400)	1 (900)	---	1 (900) except BINA Cheena Badam-1
Tridana Badam (DM-1)	4 (1400, 900, 600, 500)	3 (1400, 600, 500)	1 (900)	1 (500)	
Basanti Badam (DG-2)	4 (1400, 900, 600, 400)	3 (1400, 600, 400)	1 (900)	---	
Jhinga Badam (Acc. no.-12)	4 (1400, 900, 600, 400)	3 (1400, 600, 400)	1 (900)	---	
BARI Cheena Badam-6	2 (900, 600)	1 (600)	1 (900)	---	
BARI Cheena Badam-7	3 (1400, 900, 600)	2 (1400, 600)	1 (900)	---	
BARI Cheena Badam-8	1 (900)	---	1 (900)	---	

Table 28. RAPD analysis with primer-19 (5'-GAT GAC CGC C-3') of 11 germplasms of peanut (*Arachis hypogaea* 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)
Dhaka-1	2 (1100, 800)	---	2 (1100, 800)	---	
BINA Cheena Badam-1	2 (1100, 800)	---	2 (1100, 800)	---	
BINA Cheena Badam-2	2 (1100, 800)	---	2 (1100, 800)	---	
BINA Cheena Badam-3	2 (1100, 800)	---	2 (1100, 800)	---	
BINA Cheena Badam-4	2 (1100, 800)	---	2 (1100, 800)	---	
Tridana Badam (DM-1)	2 (1100, 800)	---	2 (1100, 800)	---	2 (1100, 800)
Basanti Badam (DG-2)	2 (1100, 800)	---	2 (1100, 800)	---	
Jhinga Badam (Acc. no.-12)	2 (1100, 800)	---	2 (1100, 800)	---	
BARI Cheena Badam-6	4 (3000, 2300, 1100, 800)	2 (3000, 2300)	2 (1100, 800)	---	
BARI Cheena Badam-7	3 (3000, 1100, 800)	1 (3000)	2 (1100, 800)	---	
BARI Cheena Badam-8	5 (3000, 2300, 2000, 1100, 800)	3 (3000, 2300, 2000)	2 (1100, 800)	1 (2000)	

Table 29. RAPD analysis with Primer-23 (5'-GTC AGG GCA A-3') of 11 germplasms of peanut (*Arachis hypogaea* 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)
Dhaka-1	4 (1800, 1000, 500, 400)	3 (1800, 500, 400)	1 (1000)	---	
BINA Cheena Badam-1	4 (1800, 1000, 500, 400)	3 (1800, 500, 400)	1 (1000)	---	
BINA Cheena Badam-2	4 (1800, 1000, 500, 400)	3 (1800, 500, 400)	1 (1000)	---	
BINA Cheena Badam-3	4 (1800, 1000, 500, 400)	3 (1800, 500, 400)	1 (1000)	---	
BINA Cheena Badam-4	4 (1800, 1000, 500, 400)	3 (1800, 500, 400)	1 (1000)	---	1 (1000)
Tridana Badam (DM-1)	4 (1800, 1000, 500, 400)	3 (1800, 500, 400)	1 (1000)	---	
Basanti Badam (DG-2)	3 (1800, 1000, 500)	2 (1800, 500)	1 (1000)	---	
Jhinga Badam (Acc. no.-12)	4 (1800, 1000, 500, 400)	3 (1800, 500, 400)	1 (1000)	---	
BARI Cheena Badam-6	4 (1800, 1000, 500, 400)	3 (1800, 500, 400)	1 (1000)	---	
BARI Cheena Badam-7	1 (1000)	---	1 (1000)	---	
BARI Cheena Badam-8	1 (1000)	---	1 (1000)	---	

Table 30. Compilation of RAPD analysis in 11 germplasms of peanut (*Arachis hypogaea* 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	CAG GCC CTT C	53	500-2000	09		16.98
OPA-2	TGC CGA GCT C	39	600-1300	39		100
OPA-3	AGT CAG CCA C	33	400-1700	33	Dhaka-1 (1000) BN-6 (1700, 1500)	100
OPA-7	GAA ACG GGT G	26	400-2500	26	DM-1 (1000, 400)	100
OPA-8	GTG ACG TAG G	30	500-1500	30		100
Primer-2	GTT GCG ATC C	26	400-1500	26	BN-2 (1500) DM-1 (500)	100
Primer-19	GAT GAC CGC C	28	800-3000	06	BA-8 (2000)	21.42
Primer-23	GTC AGG GCA A	37	400-1800	26		70.27
Grand total		**272**		**195**		**71.69**

Here

BN-1 = BINA Cheena Badam-1

BN-2 = BINA Cheena Badam-2

DM-1 = Tridana Badam

BA-8 = BARI Cheena Badam-8

Table 31. SSR analysis with Primer pair forward-5'-GAAAGAAATTATACACTCCAATTATG-3'/reverse-5'-CGGCATGACAGCTCTATGTT-3' of 11 germplasms of peanut (*Arachis hypogaea* 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)
Dhaka-1	2 (150, 50)	1 (50)	1 (150)	---	1 (50) except BINA Cheena Badam-4 and Tridana Badam
BINA Cheena Badam-1	1 (50)	---	1 (50)	---	
BINA Cheena Badam-2	2 (150, 50)	2 (150, 50)	---	---	
BINA Cheena Badam-3	1 (50)	1 (50)	---	---	
BINA Cheena Badam-4	---	---	---	---	
Tridana Badam (DM-1)	---	---	---	---	
Basanti Badam (DG-2)	2 (150, 50)	---	2 (150, 50)	---	
Jhinga Badam (Acc. no.-12)	1 (50)	---	1 (50)	---	
BARI Cheena Badam-6	2 (150, 50)	1 (150, 50)	---	---	
BARI Cheena Badam-7	2 (150, 50)	1 (50)	1 (150)	---	
BARI Cheena Badam-8	1 (50)	1 (50)	---	---	

Table 32. SSR analysis with Primer pair forward-5'-CCTTTTCTAACACATTCACACATGA-3'/reverse-5'-GGCTCCCTTCGATGATGAC-3' of 11 germplasms of peanut (*Arachis hypogaea* 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)
Dhaka-1	1 (246)	1 (246)	---	---	1 (246) except BINA Cheena Badam-3
BINA Cheena Badam-1	1 (246)	1 (246)	---	---	
BINA Cheena Badam-2	1 (246)	1 (246)	---	---	
BINA Cheena Badam-3	---	---	---	---	
BINA Cheena Badam-4	1 (246)	---	1 (246)	---	
Tridana Badam (DM-1)	1 (246)	---	1 (246)	---	
Basanti Badam (DG-2)	1 (246)	1 (246)	---	---	
Jhinga Badam (Acc. no.-12)	1 (246)	1 (246)	---	---	
BARI Cheena Badam-6	1 (246)	1 (246)	---	---	
BARI Cheena Badam-7	1 (246)	1 (246)	---	---	
BARI Cheena Badam-8	2 (246, 50)	1 (246, 50)	---	1 (50)	

Table 33. SSR analysis with Primer pair forward-5'-ACTCGCCATAGCCAACAAAC-3'/reverse-5'-CATTCCACAACTCCCACAT-3' of 11 germplasms of peanut (*Arachis hypogaea* 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)
Dhaka-1	2 (150, 50)	1 (50)	1 (150)	---	
BINA Cheena Badam-1	1 (150)	---	1 (150)	---	
BINA Cheena Badam-2	1 (150)	---	1 (150)	---	
BINA Cheena Badam-3	1 (150)	---	1 (150)	---	
BINA Cheena Badam-4	1 (150)	---	1 (150)	---	1 (150)
Tridana Badam (DM-1)	1 (150)	---	1 (150)	---	
Basanti Badam (DG-2)	1 (150)	---	1 (150)	---	
Jhinga Badam (Acc. no.-12)	2 (150, 50)	1 (50)	1 (150)	---	
BARI Cheena Badam-6	1 (150)	---	1 (150)	---	
BARI Cheena Badam-7	1 (150)	---	1 (150)	---	
BARI Cheena Badam-8	1 (150)	1 (150)	---	---	

Table 34. SSR analysis with Primer pair forward-5'-CTCTCCTCTGCTCTGCACTG/reverse-5'-ACAAGAACATGGGGATGAAGA-3' of 11 germplasms of peanut (*Arachis hypogaea* 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)
Dhaka-1	2 (150, 50)	2 (150, 50)	---	---	
BINA Cheena Badam-1	2 (150, 50)	2 (150, 50)	---	---	
BINA Cheena Badam-2	2 (150, 50)	1 (50)	1 (150)	---	
BINA Cheena Badam-3	2 (150, 50)	1 (50)	1 (150)	---	
BINA Cheena Badam-4	2 (150, 50)	1 (50)	1 (150)	---	
Tridana Badam (DM-1)	2 (150, 50)	1 (50)	1 (150)	---	1 (150)
Basanti Badam (DG-2)	2 (150, 50)	1 (50)	1 (150)	---	
Jhinga Badam (Acc. no.-12)	2 (150, 50)	1 (50)	1 (150)	---	
BARI Cheena Badam-6	1 (150)	---	1 (150)	---	
BARI Cheena Badam-7	1 (150)	---	1 (150)	---	
BARI Cheena Badam-8	2 (150, 50)	1 (50)	1 (150)	---	

Table 35. Compilation of SSR analysis in 11 germplasms of peanut (*Arachis hypogaea* L.)

Primer codes	Sequences (5'—3')	Total bands	Size ranges (bp)	Polymorphic bands	Polymorphisms (%)
BA00175669	forward-GAAAGAAATTATACACTCCAATTATG	14	50-150	14	100
BA00175670	reverse-CGGCATGACAGCTCTATGTT				
BA00175671	forward-CCTTTTCTAACACATTCACACATGA	11	50-246	11	100
BA00175672	reverse-GGCTCCCTTCGATGATGAC				
BA00175673	forward-ACTCGCCATAGCCAACAAAC	13	50-150	2	15.38
BA00175674	reverse-CATTCCACAACTCCCACAT				
BA00175677	forward-CTCTCCTCTGCTCTGCACTG	20	50-150	9	45
BA00175678	reverse-ACAAGAACATGGGGATGAAGA				
Grand total		**58**		**36**	**62.06**

Table 36. Summary of Nei's (1972) genetic distances of 11 germplasms of peanut (*Arachis hypogaea* L.)

Germplas-ms	D-1	BI-1	BI-2	BI-3	BI-4	DM-1	DG-2	Acc. no.-12	BA-6	BA-7	BA-8
D-1	****										
BI -1	0.3137	****									
BI -2	0.2136	0.2136	****								
BI -3	0.2136	0.1226	**0.1226**	****							
BI -4	0.2877	0.2377	0.1900	0.1900	****						
DM-1	0.3677	0.3137	0.2624	0.2624	0.1900	****					
DG-2	0.3677	0.3677	0.2624	0.3137	0.2877	**0.1226**	****				
Acc. no. -12	0.3403	0.3959	0.3403	0.3403	0.3137	0.2377	0.1900	****			
BA -6	0.3403	0.3959	0.3403	0.3403	0.4249	0.3403	0.2877	0.2624	****		
BA -7	0.5173	0.5173	0.4547	0.4547	0.4249	0.4547	0.3403	0.2624	0.3137	****	
BA -8	**0.5500**	0.4249	0.4855	0.3677	0.3959	0.4855	0.4249	0.3959	0.3959	0.1900	****

Here-

D-1= Dhaka-1

BI-1= BINA Cheena Badam-1

BI-2= BINA Cheena Badam-2

BI-3= BINA Cheena Badam-3

BI-4= BINA Cheena Badam-4

DM-1= Tridana Badam (DM-1)

DG-2= Basanti Badam (DG-2)

Acc. no. -12= Jhinga Badam

BA-6= BARI Cheena Badam-6

BA-7= BARI Cheena Badam-7

BA-8= BARI Cheena Badam-8

4. Discussion

Eleven varieties of peanut *viz.*, Dhaka-1, BINA Cheena Badam-1, BINA Cheena Badam-2, BINA Cheena Badam-3, BINA Cheena Badam-4, Tridana Badam (DM-1), Basanti Badam (DG-2), Jhinga Badam (Acc. no.-12), BARI Cheena Badam-6, BARI Cheena Badam-7 and BARI Cheena Badam-8 were studied cytogenetically. The nature of differential staining property of interphase nuclei and prophase chromosomes were accomplished to obtain additional information. Besides, DNA fingerprinting using RAPD and SSR markers were also carried out for molecular characterization.

4.1. The properties of orcein-stained interphase nuclei and prophase chromosomes

The eleven varieties showed more or less similar orcein stained properties of interphase nuclei. Presence of few scattered darkly stained regions indicated that heterochromatins were present throughout the nucleus (Figs. 12-22). However, the maximum numbers of heterochromatins were present in BINA Cheena Badam-1 and the minimum in BARI Cheena Badam-6. This type of staining properties may be recognized as 'Complex Chromocenter Type' as has been classified by Tanaka (1971).

Big and prominent nucleoli were found in Dhaka-1, BINA Cheena Badam-2, BINA Cheena Badam-3 and BINA Cheena Badam-4 suggested the late transcription of rDNA. Although no satellite and secondary constriction was observed in these five varieties, the presence of nucleolus revealed that ribosomal genes were scattered among chromosomes (Figs. 12, 14, 15, 16). Alam *et al.* (2012a) also observed similar results in case of *Trichosanthes anguina* L.

The terminal ends of few prophase chromosomes of Dhaka-1, BINA Cheena Badam-1, BINA Cheena Badam-4, Basanti Badam (DG-2), Jhinga Badam (Acc. no.-12), BARI Cheena Badam-6 and BARI Cheena Badam-8 were darkly stained (Figs. 45, 46, 49, 51, 52, 53, 55). The prophase chromosomes of BINA Cheena Badam-3 and Tridana Badam (DM-1) stained at the interstitial regions (Figs. 48, 50). On the other hand, in BINA Cheena Badam-2, 7-8 chromosomes were stained along the entire length. Ten-twelve chromosomes showed darker heterochromatic regions at one terminal ends, however, the other ends were faintly stained. The rest of the chromosomes did not have any prominent darker region. Tanaka (1971) found correlation between the staining properties of interphase nuclei and prophase chromosomes. According to him the 'Complex Chromocenter Type' of staining interphase nuclei generally showed interstitial type prophase chromosome. It means that the heterochromatic regions were scattered interstitially within the prophase chromosomes.

According to Tanaka's (1971) report, the interphase staining properties of the chromosomes of all varieties may be considered as 'Complex Chromocenter Type'. Based on the hypothesis it was expected that the prophase chromosomes should show interstitial type of staining. However, prophase chromosomes of some varieties stained entirely, gradually from one end to another, interstitially and one terminal end. It indicated that the heterochromatin in the interphase nuclei became uncoil and arranged in different ways among the prophase chromosomes of different varieties. Similar result was obtained by Alam *et al.* (2012b) in case of *Vigna unguiculata* ssp. *sesquipedalis* (L.) Verdc. Based on this assumption it may be considered that, the 11 peanut varieties studied in the present investigation possessed some facultative heterochromatins.

4.2. Diploid (2n) Chromosome number

All varieties of *Arachis hypogaea* used in this experiment were found to possess 2n=40 chromosomes. The findings of the present investigation confirmed the reports of the previous workers (Husted 1936, D'Cruz and Tankasale 1961, Singh and Moss 1982, Stalker and Dalmacio 1986, Cai *et al*. 1987, Fernandez and Krapovickas 1994, Ines and Fernandez 2004).

However, in BARI Cheena Badam-7 and BARI Cheena Badam-8, a small extra chromosome in addition to 2n=40 chromosomes was found in several cells. Available literature showed no such reports on the presence of small extra chromosome (2n=41) in the peanut varieties. Interestingly flower formation and seed set in the field condition was normal like the mother varieties. In this study, the extra chromosome was so small that could not place in chromosome pair XX (Figs. 111-132), means it was smaller than the smallest 'A' type chromosome. Their presence or absence did not affect the pollen fertility and yield production. However, this small extra chromosome was not found in all cells and not fixed. Now the question arisen about the nature of the extra chromosome. This chromosome may be considered as B-chromosome.

It is well known fact that B-chromosomes have some special characteristics such as i) it is smaller than 'A' type chromosomes, ii) the number of B-chromosomes is not fixed. In some cells they may present and in some absent. Their presence and absence do not affect normal activity and iii) most of the B-chromosomes are heterochromatic in nature (Akhtaruzzaman 2001, Alam and Begum 2001)).

Based on the above characteristics it is considered that the extra chromosomes found in BARI Cheena Badam-7 and BARI Cheena Badam-8 was B-chromosome and which is the first report of B-chromosome in *Arachis hypogaea*.

4.3. The conventional karyotypes

The karyotype formulae and other features were summarized in Table 19. Mitotic karyotypes and the respective idiograms were presented in Figures 111-132.

The chromosomes of 11 varieties of *Arachis hypogaea* were mostly metacentric. The eleven varieties were found to possess five types of centromeric formulae i.e., i) 34m+6sm, ii) 36m+4sm, iii) 38m+2sm, iv) 32m+ 8sm and v) 40m. Husted (1936) and Fernandez and Krapovickas (1994) determined centromeric formula of 38m+2sm for this species. Ines and Fernandez (2004) mentioned different centromeric formulae for different varieties which were collected from different provenance, such as 36m+4sm (PC 558, Guaycuru, Argentina), 36m+4sm (BP 687, Colorado Rastrero, Ecuador), 38m+2sm (BPZ 86, Chaucha Morado, Bolivia) and 38m+2sm/st (RCM 1457, Sopachuy, Bolivia). The earlier centromeric formulae correlated with the present findings.

In this study, the average chromosome length of 11 varieties was found 2.28 µm and the individual chromosome length ranged from 0.80-3.36 µm (Table 19). Almost similar results were reported earlier (Ines and Fernandez 2004, Husted 1936, Stalker and Dalmacio 1986, Cai *et al.* 1987). Earlier and present report on 2n chromosome number, chromosome length and centromeric formulae suggested that the conventional karyotypes of *Arachis hypogaea* was very stable and conserved even in specimen from far abroad.

Therefore, other karyotypic parameters *viz*., fluorescent bandings and molecular marker analysis are necessary to elucidate minute alteration in karyotype and DNA level.

4.4. Chromosome pair XX

In this study, it was found that chromosome pair XX was much smaller than other even remarkably shorter than pair number XIX (Figs.120-121, Table 17-18). Husted (1933, 1936) at first reported a pair of small chromosomes. He named these chromosomes as 'A' chromosome. Later Lavia (2000) was supported Husted (1933) idea.

Generally euchromosomes are of two types i) 'A' type chromosomes /normal chromosome and ii) special type chromosomes (Akhtaruzzaman 2001). Usually all chromosomes of an organisms are 'A' chromosomes. Why Husted named this chromosome as 'A' chromosomes and other worker supported his idea was not clear. It might be that either they wanted to separate this chromosome from the rest or nomenclature of their own way.

4.5. Fluorescent banding analysis

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 peanut varieties.

4.5.1. CMA fluorochromes

4.5.1.1. Karyotypes

The 11 varieties of peanut have distinct CMA banding pattern. The number, location, distribution, intensities and percentage of GC-rich repeats varied in different varieties (Figs. 89-99, 133-143). Most of the CMA-bands were present at the terminal regions of respective chromosomes (Figs. 133-154).

The presence of terminal CMA bands indicated a tendency of accumulating GC-rich repetitive sequences at the chromosomal ends as has been reported earlier by Zaman and Alam (2009). In addition to terminal bands, few CMA bands were present at centromeric regions of the respective chromosomes in four varieties (Figs. 134, 137, 139, 141) revealing the presence of GC repeats of those regions. The percentage of GC-rich region ranged from 1.22-6.17 (Table 20). It indicated that these heterochromatic regions were rich in GC repeats. The highest percentage of GC-rich was found in BINA Cheena Badam-4 (6.17%) while the lowest in BINA Cheena Badam-3 (1.22%) (Table 20). The reason for the variation in the percentage of GC-rich region probably due to the degree of tandem duplication of GC-rich repeat.

Therefore, each variety has its own characteristics CMA-banded karyotype.

4.5.1.2. Marker chromosomes

Few chromosomes could be used as marker chromosomes of respective varieties due to their unique banding pattern. One chromosome of BINA Cheena Badam-1 (pair II) and BINA Cheena Badam-3 (pair XIII) had one CMA band at both terminal ends (Figs. 134, 136, arrow). In addition, two CMA-positive bands were found at the proximal ends of the centromere in a member of pair XVI of Dhaka-1 (Fig. 133). The chromosomes were unique since having two CMA bands on a chromosome. Other banded chromosomes had only one CMA band. It was surprising that the homologue members of above 3 chromosomes did not show any band. This feature may indicate unequal exchange of GC-rich region between the homologous members during crossing over.

Chromosomes pair XX in BINA Cheena Badam-1, XVII and XX in BINA Cheena Badam-4, XVI in Basanti Badam (DG-2), XVII in Jhinga Badam (Acc. no.-12), XVIII in BARI Cheena Badam-7 and XX in BARI Cheena Badam-8 were

fluoresced entirely (Figs. 134, 137, 139, 140, 142, 143). This banding nature makes these chromosomes isolated from the other. The probable reason for entirely florescence may be i) either these chromosomes were completely GC-rich by nature or ii) due to successive duplication of GC-rich repeats (Hiron *et al.* 2006, Mahbub *et al.* 2007).

A member of pair XX in Dhaka-1 and V in BINA Cheena Badam-1 were fluoresced entirely whereas no such band was found in their homologues. These features revealed the tandem duplication of the GC-rich repeats along the chromosomal length. Similar results were also reported earlier workers (Khatun and Alam 2010, Sultana and Alam 2007).

4.5.1.3. Stable bands

Four very prominent CMA bands were observed at metaphase of Jhinga Badam (Acc. no.-12) (Fig. 140). Four bands were also found at interphase and prophase stages of this variety (Figs. 30, 36). In addition, 12 and 8 CMA bands were found in metaphase chromosomes of BINA Cheena Badam-1 and Tridana Badam (DM-1), respectively (Figs. 134, 138). Almost similar numbers of CMA bands were observed in their respective interphase nuclei and prophase chromosomes (Figs. 24, 28, 57, 61). Presence of similar number of CMA bands in interphase, prophase and metaphase revealed that the GC-rich repeats kept their domain intact throughout the cell cycle. Schweizer (1976) mentioned that GC-rich repeats were usually heterochromatic in nature. Heterochromatins are two types *viz.*, i) Facultative and ii) Constitutive. Constitutive heterochromatins are much conserved and never change to euchromatins like facultative heterocrhomatins. Therefore, the CMA bands of the three varieties were made up of constitutive heterochromatins.

4.5.1.4. Heteromorphicity in CMA banding patterns

A member of pair XIX in Dhaka-1, XII in BINA Cheena Badam-1, XIX in BINA Cheena Badam-2, VI and XIV in BINA Cheena Badam-3 and XVIII in Basanti Badam (DG-2) had terminal CMA band which were absent in their respective homologue members. Generally homologue members should show similar banding pattern. Lack of CMA band from the terminal end of these chromosomes might be due to a small deletion of CMA band (Mahbub *et al.* 2007).

In BINA Cheena Badam-4, a member of pair IV had a terminal band on short arm and its homologous showed a centromeric band. This kind of heteromorphicity indicated the probable occurrences of peracentric inversion either from terminal end to centromeric region or vice versa (Akter and Alam 2005, Zaman and Alam 2009).

4.5.2. DAPI fluorochromes

4.5.2.1. Nature of DAPI bands

Except a few, most of the DAPI bands were located at the cetromeric regions of the respective chromosomes in the 11 peanut varieties. Schweizer (1976) for the first time reported that DAPI bands revealed the presence of AT-rich repeats. Later many workers supported this idea (Alam and Khanam 2005, Alam and Zerin 1998, Hizume *et al.* 1988, Alam and Kondo 1995). Silva *et al.* (2010) reported that centromeric DAPI band was AT-rich. They also mentioned that the DAPI banded areas were heterochromatic in nature. The present investigation revealed heterochromatic nature of the centromeric regions of the respective chromosomes. Unlike SAT and telomeric sequence, centromeric sequences were not conserved. Moreover, the centromeric

sequence may be euchromatic or heterochromatic (King 1980). In 11 peanut varieties, the DAPI banded centromeres of some chromosomes were heterochromatic and the non-banded centromere euchromatic in nature. Normally in one species either euchromatic or heterochromatic centromere should be found. However, in the present investigation both euchromatic and heterochromatic centromeres were found in all the peanut varieties studied which are rare. In that respect, peanut may be considered as rare materials for DAPI banding patterns.

4.5.2.2. Karyotypes

Although most of the DAPI bands were the centromeric regions, each variety differed from each other regarding the size, number, position and intensity of DAPI bands. The number of DAPI band ranged from 5 [Jhinga Badam (Acc. no.-12)] to 18 (BINA Cheena Badam-1) (Table 21). The percentage of AT-rich region was also variable (Figs. 155-165). The DAPI banded karyotype formulae were sharply differed from each other. Few varieties could be identified by unique DAPI banded chromosomes. For example, one member of pair VI and I in BINA Cheena Badam-3 had thick terminal band on upper and lower arms, respectively (Fig. 158). In BINA Cheena Badam-1, BINA Cheena Badam-3, BINA Cheena Badam-4, Tridana Badam (DM-1), Basanti Badam (DG-2) and Jhinga Badam (Acc. no.-12) few chromosomes were entirely fluoresced (Figs. 156, 158-163). Two chromosomes of pair IX in BARI Cheena Badam-7 had thick terminal bands (Fig. 164). These chromosomes could easily be identified from the rest and may be used as marker for the respective varieties. Available literature reveals that the previous workers did not use both CMA and DAPI banding to characterize *Arachis hypogaea*. Therefore, the present work on the characterization of varieties by CMA and DAPI may be considered as the pioneering attempt.

4.5.2.3. Conserved bands

Several prominent DAPI bands were observed in interphase nuclei, prophase chromosomes and metaphase chromosomes of 11 peanut varieties (Figs. 23-33, 56-66). Numbers of DAPI bands were more or less similar in interphase nuclei, prophase chromosomes and metaphase chromosomes of all varieties. The presence of similar number of DAPI bands in different stages of cell cycle revealed that the AT-rich repeats were very stable in each variety as earlier reported by Ahmed *et al.* (2004). In contrast, stable CMA bands were found in only Jhinga Badam (Acc. no.-12), BINA Cheena Badam-1 and Tridana Badam (DM-1) (Figs. 134, 138, 140). This feature indicated that AT-rich repeats were more conserved than GC-rich repeats and made up of completely constitutive heterochromatins.

4.5.2.4. Heteromorphicity in DAPI banding patterns

A member of pair VI in BINA Cheena Badam-2, XII in BINA Cheena Badam-3, XVIII in BINA Cheena Badam-4 and XV in BARI Cheena Badam-7 had terminal DAPI bands which were absent in their respective homologue members. The probable reason of this heteromorphicity may be due to a minute deletion of the banded area since the terminal end of the non banded chromosomes looked smaller than its homologue members. Terminal deletions of CMA bands were reported in other species of Fabaceae (Khandaker *et al.* 2007, Mahbub *et al.* 2007, Akter and Alam 2005). Therefore, the terminal deletions of GC-rich repeats are frequent observed in the members of Fabaceae.

A member of pair XX in Tridana Badam (DM-1), XVIII in Basanti Badam (DG-2) and Jhinga Badam (Acc. no.-12) were fluoresced entirely whereas no such band was found in their homologues. These features revealed unequal

crossing over of AT-rich segment followed by the tandem duplication along the chromosomal length.

A member of pair XI in Dhaka-1 and BINA Cheena Badam-2 and a member of pair III in BINA Cheena Badam-4 had a terminal band. On the other hand, their homologue members showed a centromeric band. This may be due to an occurrence of peracentric inversion either from terminal to centromers or vice versa.

A member of pair XVIII in Dhaka-1, XX in BINA Cheena Badam-2, XIII in BINA Cheena Badam-3, XVI in BINA Cheena Badam-4, IV and XX in BARI Cheena Badam-6, I in Tridana Badam (DM-1) and BARI Cheena Badam-7 showed centromeric band which was absent in their respective homologue members (Figs. 155, 157, 158, 159, 163, 160, 164). It has been discussed earlier that the centromeric DAPI-bands of peanut were heteromorphic in nature (Silva *et al.* 2010). The centromeres of non-banded chromosomes should have heterochromatin. In this study, it has observed that one member of above pairs did not possess DAPI band. However, the centromeric regions of non-banded chromosomes were very prominent and clear. Thus it may not be a case of deletion. Sumner (1991) proposed a hypothesis “euchromatin transformation” where he mentioned that some heterochromatin may transfer to euchromatin. In peanut varieties, the centromeric region of non-banded chromosome might go “euchromatic transformation” and therefore, no DAPI-band was observed at the centromeric regions.

4.5.2.5. Co-localization in respect of CMA-and DAPI bands

Two chromosomes in each BINA Cheena Badam-2 and BINA Cheena Badam-4 showed CMA and DAPI fluorescence at the same location. In BINA Cheena Badam-2, short arms of pair XV (Figs. 135, 157) had CMA- and DAPI bands at the same location. Both the members of pair XX fluoresced entirely with CMA

and DAPI in BINA Cheena Badam-4 (Figs. 137, 159). Alam and Kondo (1996) reported similar banding pattern in *Drosera* species. They mentioned that this banding nature was due to presence of GC- and At-rich repeats at the same position of chromosomes. The present banding patterns indicated the tandem presence of GC- and AT-rich repeats on those portions of the respective chromosomes. These chromosomes could be marked by their co-localization of GC- and AT-rich nature.

4.6. DNA fingerprinting

The 11 peanut varieties selected for the present study represent a broad spectrum of variation for several phenotypic traits and in their provenance. DNA from the 11 peanut varieties was studied with 8 oligonucleotide primers and four microsatellite primer pairs for RAPD and SSR assay, respectively.

4.7.1. DNA fingerprinting by random amplification of polymorphic DNA (RAPD)

Only 8 common bands of different sizes were observed in three primer combinations (OPA-1, primer-19 and primer-23). The different sized common band indicated the sharing of similar DNA fragments among 11 varieties. Afroz *et al.* (2013) found common band with primer OPA-1 and Primer-19 in three morphological forms of *Alocasia fornicata* and Shema *et al.* (2013) also reported common band with same primer in four varieties of *Capsicum frutescens*. These results indicated that the sequences of CAG GCC CTT C (OPA-1) and GAT GAC CGC C (primer-19) are available in different species.

Although these varieties had some common RAPD bands, sufficient polymorphisms regarding RAPD fragments were observed in 11 peanut varieties. The primer sequence, band size and banding pattern of 11 peanut varieties were shown in Table 30. The eight primers generated 272 distinct bands of which 195 were considered as polymorphic. An average of 34

countable bands and 24.38 polymorphic RAPD bands generated per primer showing 71.69% polymorphisms which indicated the high level of polymorphisms. Band size ranging from 400 to 3000 bp of PCR amplification products scored for all primers. Among the eight primers OPA-2, OPA-3, OPA-7, OPA-8, primer-2 and primer-23 produced highest number of polymorphic bands. In contrast, the primer OPA-1 and primer-19 generated the least number of polymorphic bands (Table 30). 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) (Table 22-29).

A diverse level of polymorphism in different crops have been reported 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 polymorphism in peanut varieties was reported earlier using RAPD markers. Lang and Hang (2007) scored a high degree of polymorphism in 29 peanut cultivars using RAPD markers. The molecular weight of bands analysed by them ranged from 200 and 2800 bp of which 96% were polymorphic. Raina *et al.* (2001) reported 42.7% polymorphism among cultivated peanut and wild species of the genus *Arachis*. On the other hand, low level of DNA polymorphism in peanut varieties was observed by previous workers. Subramanian *et al.* (2000) found 6.68% polymorphisms in 70 groundnut genotypes with seven primers. He and Prakash (1997) reported 21% polymorphisms in six divergent genotypes of cultivated peanut. The results of the present investigation showed high level of polymorphisms (71.69%). Broad range of polymorphism revealed wide variability in peanut variety. This observed variability would be useful towards undertaking breeding programmes in peanut.

In addition to polymorphism, eight unique sequences were identified in 11 peanut varieties using eight different primer combinations. Primer OPA-3 produced 3 unique bands of which a unique band of 1000 bp in Dhaka-1 and other two (1700 and 1500 bp) in BINA Cheena Badam-1 (Fig. 180). Two unique bands (1000 and 400 bp) in primer OPA-7 and one (500 bp) in primer-2 were found in Tridana Badam (DM-1) (Figs. 181,183). BINA Cheena Badam-2 and BARI Cheena Badam-8 were found to possess a unique band of 1500 bp with primer-2 and 2000 bp with primer-19, respectively (Figs. 183, 184, Table 30). The unique bands were stable and specific for the respective varieties and thus could be used as a tool for characterization.

4.6.2. DNA fingerprinting by simple sequence repeats (SSRs)

Five SSR primer pairs were used in this study of which four generated well-defined and reproducible polymorphic bands. The primer sequence, band size and banding pattern of 11 peanut varieties were given in Table 35 and Figs. 186-189. With an average of 9 (nine) polymorphic fragments per primer, 36 (62.06%) polymorphic fragments were amplified. The size of band was ranged from 50 to 246 bp. Among the four primer pairs BA00175669/BA00175670 and BA00175671/BA00175672 were produced highest number of polymorphic bands against the primer pairs BA00175673/BA00175674 and BA00175677/BA00175678 those generated the least number of polymorphic bands (Table 35). Tang *et al.* (2007) studied genetic diversity of 96 accessions of cultivated groundnut. A total of 159 fragment were amplified and 152 (95%) were polymorphic. Raina *et al.* (2001) revealed 54.4% polymorphism among cultivated peanut and wild species of the genus *Arachis*. In contrast, Shoba *et al.* (2010) reported 29% polymorphism among the 11 genotypes of groundnut. In this study, the SSR markers revealed high polymorphism levels (62.06%) among the 11 peanut varieties analyzed. Therefore, like RAPD markers SSR also showed high polymorphism. Both the experiments indicated

possibility of developing better variety through successful breeding programmed since a large number of variations existed in different peanut varieties. Besides, BARI Cheena Badam-8 was found to possess a unique band of 50 bp with primer pair BA00175671/BA00175672 (Fig. 188, Table 33). The unique band was conserved and specific for the respective varieties and could be used as marker.

4.7.3. Combined genetic distances and phylogenetic relationships

The values of pair-wise comparison Nei's (1972) genetic distance among 11 peanut varieties computed from combined data from the eight RAPD and four pairs of SSR primer ranged from 0.1226 to 0.5500 (Table 36). The highest genetic distance (0.5500) was found between Dhaka-1 vs BARI Cheena Badam-8. The lowest (0.1226) genetic distance was observed between BINA Cheena Badam-1 vs BINA Cheena Badam-3 and Tridana Badam (DM-1) vs Basanti Badam (DG-2). The difference between the highest and the lowest value of genetic distance revealed the wide range of variability persisting among the 11 peanut varieties. High genetic distance values between varieties pair were found due to difference in genetic constituent. The varieties of lowest genetic distance can be used as parental source for breeding line to improve peanut varieties. Molla *et al.* (2010) reported wide genetic diversity ranging from 0.0 to 2.079 among 10 peanut varieties in Bangladesh. The lowest genetic distance between Tridana Badam (DM-1) and Basanti Badam (DG-2) are in agreement with findings of Molla *et al.* (2010).

Dendrogram constructed based on RAPD banding pattern indicated that BARI Cheena Badam-7 and BARI Cheena Badam-8 were distinctly related from the rest two (Fig. 190). The lowest genetic distance was found between Tridana Badam (DM-1) vs Basanti Badam (DG-2) and BINA Cheena Badam-1 vs BINA Cheena Badam-3. This result will be useful for designing future breeding programmes.

The main objective of the present investigation was to characterize the peanut variety of Bangladesh using different cytogenetical and molecular techniques. For this purpose in addition to conventional karyological investigations, different molecular cytogenetical techniques, namely, CMA- and DAPI banding patterns and DNA based molecular markers, *viz.*, RAPD and SRR were used. Although the conventional karyotypes of different varieties were apparently similar, probable occurrence of a number of alterations of chromosomal segment (deletion, tandem duplication, inversion etc.) were found in 11 peanut varieties. Each variety showed distinct CMA and DAPI banding pattern. These varieties also showed unique RAPD and SSR DNA fingerprinting which is variety specific. The number, location, intensity and percentage of GC- and AT-rich repeats were different in these varieties. High levels of polymorphisms were observed with RAPD and SSR primers. Besides the above findings through the present study it has been possible to report B-chromosomes for the first time in peanut.

From foregoing discussion it is concluded that for an authentic characterization of peanut varieties combinations of cytogenetical and molecular analysis were very effective. This information would be helpful for future breeding programme as well as patenting each variety to prevent varietal piracy.

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*Original not seen