Original Research Report

Chapter 3

CHROMOSOME CONSTITUTION OF SOME INDIAN TEA CLONES

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ABSTRACT

Resultant of large-scale spontaneous hybridization between *Camellia* taxa, the present day commercial tea clones are highly variable and heterozygous. It is in this context that thirty two UPASI and other tea clones were investigated with regard to comparative karyomorphology and meiotic pairing properties so as to ascertain the nature and extent of intra – and interclonal differentiation. Barring one clone (UPASI 3), all other investigated clones were diploid (2n = 2x = 30) in constitution. UPASI 3 was triploid (2n = 3x = 45) in constitution. The chromosome complements in the diploid tea clones, wherever investigated, resolved into 15 median and submedian homomorphic pairs. Between clones, minor variation in the proportion of median and submedian chromosomes and (or) number and location of secondary constrictions was observed. Inspite of the fact that tea clones are considered to be highly heterozygous, the male meiosis in the diploid clones was perfectly normal resulting in regular 15 bivalents at diakinesis/metaphase I, and equal (15:15) distribution of chromosomes at anaphase I. The only feature which could indicate degree of cryptic hybridity was the occurrence of bivalents with localized chiasmata. The predominant occurrence of trivalents in the triploid UPASI 3 indicated either autopolyploid or segmental allopolyploid origin. The genomic constitution of tea clones is discussed in the light of present results.

Keywords: *Tea clones, chromosome complements, male meiosis, genomic constitution*

INTRODUCTION

The taxonomic status of the present day cultivated tea is a subject of much debate and controversy. Cultivated tea has been variously classified into 1) *C. sinensis* var *sinensis* ('China type') and *C. sinensis* var. *assamica* ('Assam type'); 2) *C. assamica, C. sinensis* and *C. assamica* ssp *lasiocalyx* ('Cambod type'); and 3) *C. sinensis* var. *sinensis* ('China type'), *C. sinensis* var. *assamica* ('Assam type') and *C. sinensis* var. *waldenae* ('Cambod type') (Sealy 1958; Wight 1962; Chang and Bartholomew 1984). In strong contrast, Chang Hung – ta (1981) and Tan *et al.* (1989) are of the opinion that "beveragial tea trees" are comprised of many more species. Almost self sterile and outbreeding nature coupled with frequent spontaneous hybridizations that takes place between different types of tea, resulting in highly heterozygous plants ranging from 'China type' to 'Assam type', has further complicated the taxonomic treatment in tea. The hybridization is so extreme that the existence of archetypical taxon is often debated (Barua 1965; Banerjee 1992). The present difficulties over correct identification and nomenclature of cultivated tea has not even been resolved by molecular markers like RAPD (Wachira *et al.* 1995; Tanaka *et al.* 1995; Mondal 2000; Tanaka and Yamaguchi 1996; Wright *et al.* 1996; Singh *et al.* 1999; Tanaka *et al.* 2001), ISSR (Mondal 2002 b), AFLP (Paul *et al.* 1997; Rajashekaran 1997; Misra and Sen-Mandi 2001), Microsatellite (Kaundun and Matsumoto 2002) and CAPS markers (Kaundun

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and Matsumoto 2003). At best, they were able to classify the tea clones to 'Assam', 'China' and 'Cambod' types or nearer to these types.

The cultivated tea is cytogenetically very poorly understood. The investigations are largely restricted to mere determination of zygotic numbers (Moringa *et al.* 1929; Simura 1935; Karasawa 1935; Janaki Ammal 1952). Even preliminary meiotic analysis have either not been conducted or the information, wherever available, is quite scanty and conflicting (Bezbaruah 1968, 1971; Chauhdhuri 1995). The present work is, therefore, concerned with the cultivated tea cytogenetics particularly with regard to comparative karyotypic analysis and meiotic pairing properties of tea clones particularly raised by United Planters Association of South India (UPASI) Tea Research Foundation. An equally important objective is to offer new aspects and to allow an insight into the extent and nature of differentiation within and between tea clones.

MATERIALS AND METHODS

Materials

A list of tea clones investigated is given in Table 1. The material was obtained from UPASI Tea Research Foundation, Valparai, Coimbatore, India. The voucher species are available in the UPASI Tea Research Foundation.

Table 1. List of tea clones used in the present study

Clone	Accession number	Place of Collection
UPASI[a] 1	B/4/141 (Evergreen)	Brooklands Estate, The Nilgiris
UPASI 2	B/4/142 (Jayaram)	Brooklands Estate, The Nilgiris
UPASI 3	B/5/163 (Sundaram)	Brooklands Estate, The Nilgiris
UPASI 4	B/6/10	Brooklands Estate, The Nilgiris
UPASI 5	B/6/21	Brooklands Estate, The Nilgiris
UPASI 6	B/6/24 (Brooklands)	Brooklands Estate, The Nilgiris
UPASI 7	B/6/34	Brooklands Estate, The Nilgiris
UPASI 8	B/6/36 (Golconda)	Brooklands Estate, The Nilgiris
UPASI 9	B/6/61 (Athery)	Brooklands Estate, The Nilgiris
UPASI 10	B/6/62 (Pandian)	Brooklands Estate, The Nilgiris
UPASI 11	B/6/127	Brooklands Estate, The Nilgiris
UPASI 12	B/6/129	Brooklands Estate, The Nilgiris
UPASI 13	B/6/137	Brooklands Estate, The Nilgiris
UPASI 14	S/6/99 (Singara)	Singara Estate, The Nilgiris
UPASI 15	SP/4/5 (Spring field)	Springfield Estate, The Nilgiris
UPASI 16	B/6/182	Brooklands Estate, The Nilgiris
UPASI 17	B/6/203 (Swarna)	Brooklands Estate, The Nilgiris
UPASI 20	B/7/372	Brooklands Estate, The Nilgiris
UPASI 21	B/4/198	Brooklands Estate, The Nilgiris
UPASI 22	B/6/29	Brooklands Estate, The Nilgiris
UPASI 24	B/5/149	Brooklands Estate, The Nilgiris
UPASI 26	DVS/3A/39	Devarshola Estate, Nilgiri-Wynaad
UPASI 27	A/58	Anaimudi Estate, Anamallais
TRI 2024		TRI, Sri Lanka
TRI 2025		TRI, Sri Lanka
AKK		Akkamalai, Anamallais
ATK		Attikunna, Nilgiri-Wynaad
C-17		BBTC, Singampatty
CR 6017		Craigmore, The Nilgiris
SA 6		High Wayves, Tea Estates India
B/5/163		UPASI Selection
TES – 34		UPASI Selection

UPASI[a] – United Planters Association of South India

Methods

Mitosis. Actively growing root tips from the cuttings were excised and pretreated with aqueous saturated solution of para-dichlorobenzene for 4 h at room temperature. Root tips were then washed with water, and fixed in freshly prepared acetic acid: ethyl alcohol (1:3) for at least 24 h. After fixation, thoroughly washed root tips were hydrolysed in 5 N HCl for 1 h at room temperature. Hydrolysed root tips washed in water were stained with basic Fuschin in dark for ~ 1 h. The root tips were finally squashed in 1% acetocarmine.

Meiosis. The flower buds of appropriate size were collected on various visits to UPASI Tea Research

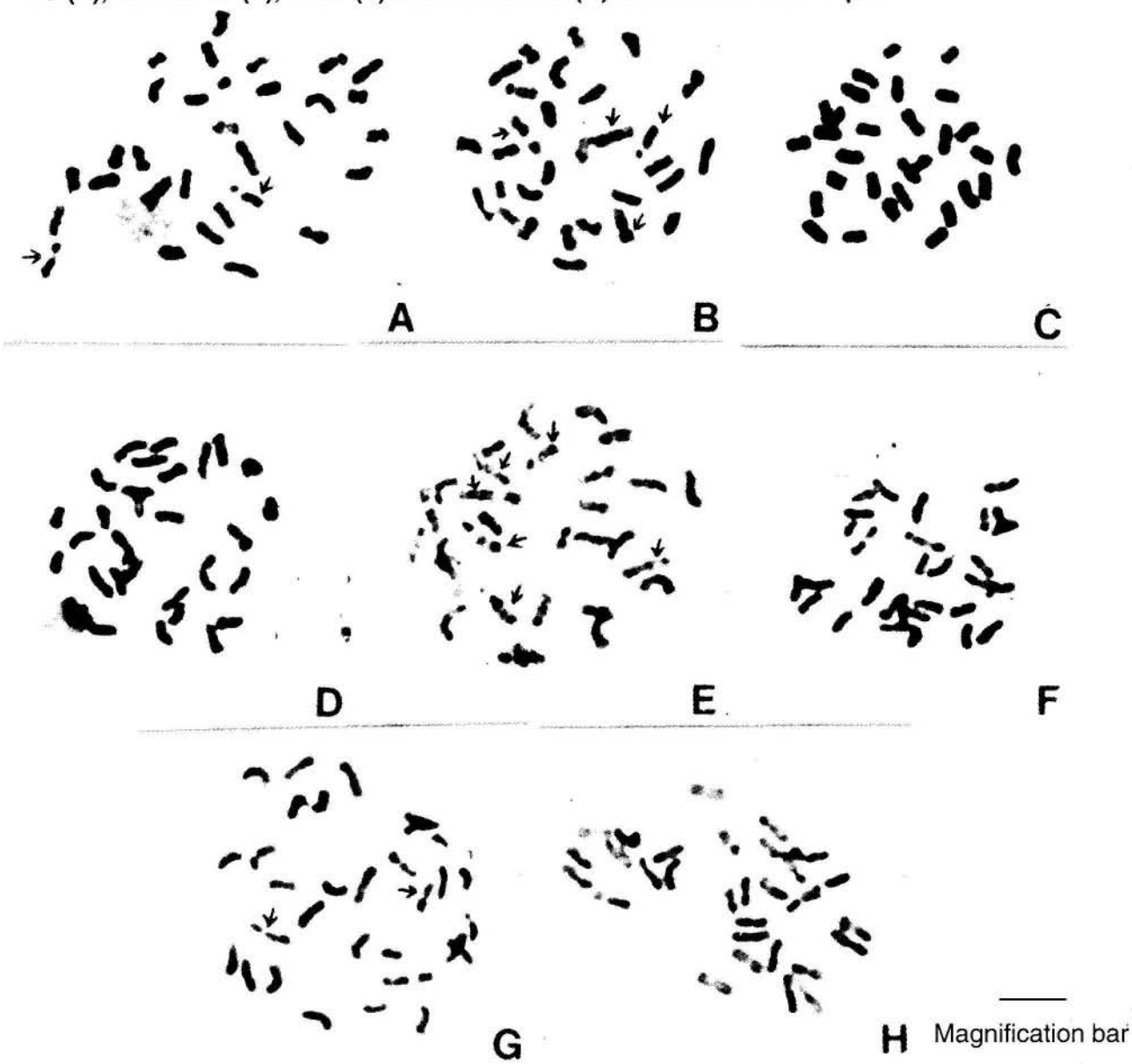
Foundation. Young flower buds were fixed in freshly prepared acetic acid: ethyl alcohol (1:3) for at least 24 h. Anthers were dissected out from the buds and squashed in 1% acetocarmine. Analysis of mitotic and meiotic chromosomes were made from temporary slides. Suitable chromosome preparations were photographed using Olympus BH-2 microscope in combination with PM-6 photographic attachment.

RESULTS

The chromosome complements

The zygotic number and chromosome morphology were determined in 32 tea clones. In 31 clones the somatic cells possessed thirty chromosomes (Fig. 1). One clone (UPASI 3) was found to be triploid (2n = 3x = 45) in constitution.

Fig. 1: Mitotic complements of the diploid ATK (A), UPASI 12 (B), TRI 2024 (C), UPASI 24 (D), UPASI 10 (E), UPASI 17 (F), AKK (F) and UPASI 26 (H) tea clones. Bar: 7 μm



Karyograms for each of the five diploid clones were prepared from at least five well spread metaphases. The 30 median (1:1) and submedian (> 1:1<3:1) chromosomes in all the five clones resolved into perfect 15 homomorphic pairs (Fig. 2, Table 2). There was, however, minor variation in the chromosomes morphology between the clones. The difference between the clones lies in the proportion of median and submedian chromosome pairs, and number and location of secondary constrictions in the nucleolar chromosomes. In clone ATK, for example, there were three pairs of nucleolar chromosomes (Fig. 2A), whereas in clone UPASI 26 the nucleolar chromosomes were represented by only two pairs (Fig. 2B), and so on.

Male meiosis

Male meiosis was studied in 31 tea clones. Twenty and ten cells for each clone were analysed at metaphase I and anaphase I, respectively for relevant details. The plants representing diploid clones did not show appreciable variation in meiotic details at metaphase I and anaphase I. All the PMCs analysed had 15II (Fig. 3B, C, F, G, H), of which the average frequency per cell of ring and rod bivalents ranged from 10.0 to 12.8 and 2.2 to5.0, respectively. The chiasmata were characteristically localized at proximal region in most of the bivalents. The chiasmata frequency per cell ranged from 24.8 in TRI 2025 to 23.5 in UPASI 22. Terminalization

Fig. 2: Karyograms of ATK (A) and UPASI 26 (B) clones. Bar: 2.9 µm

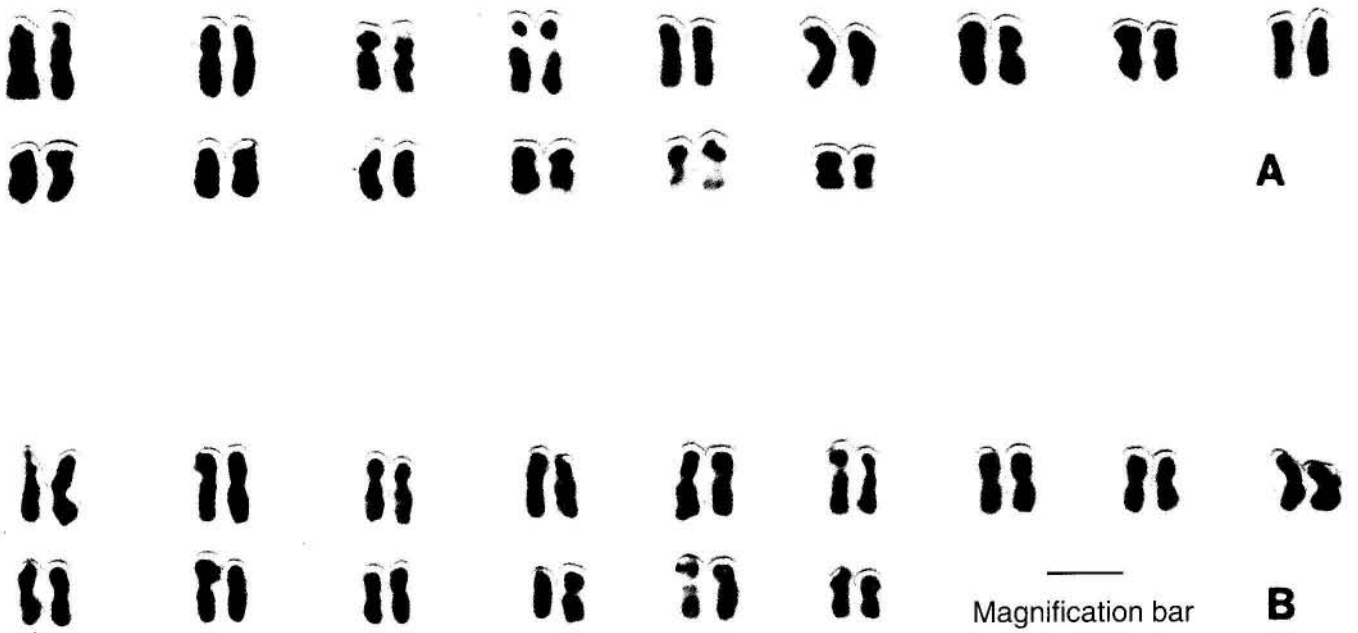
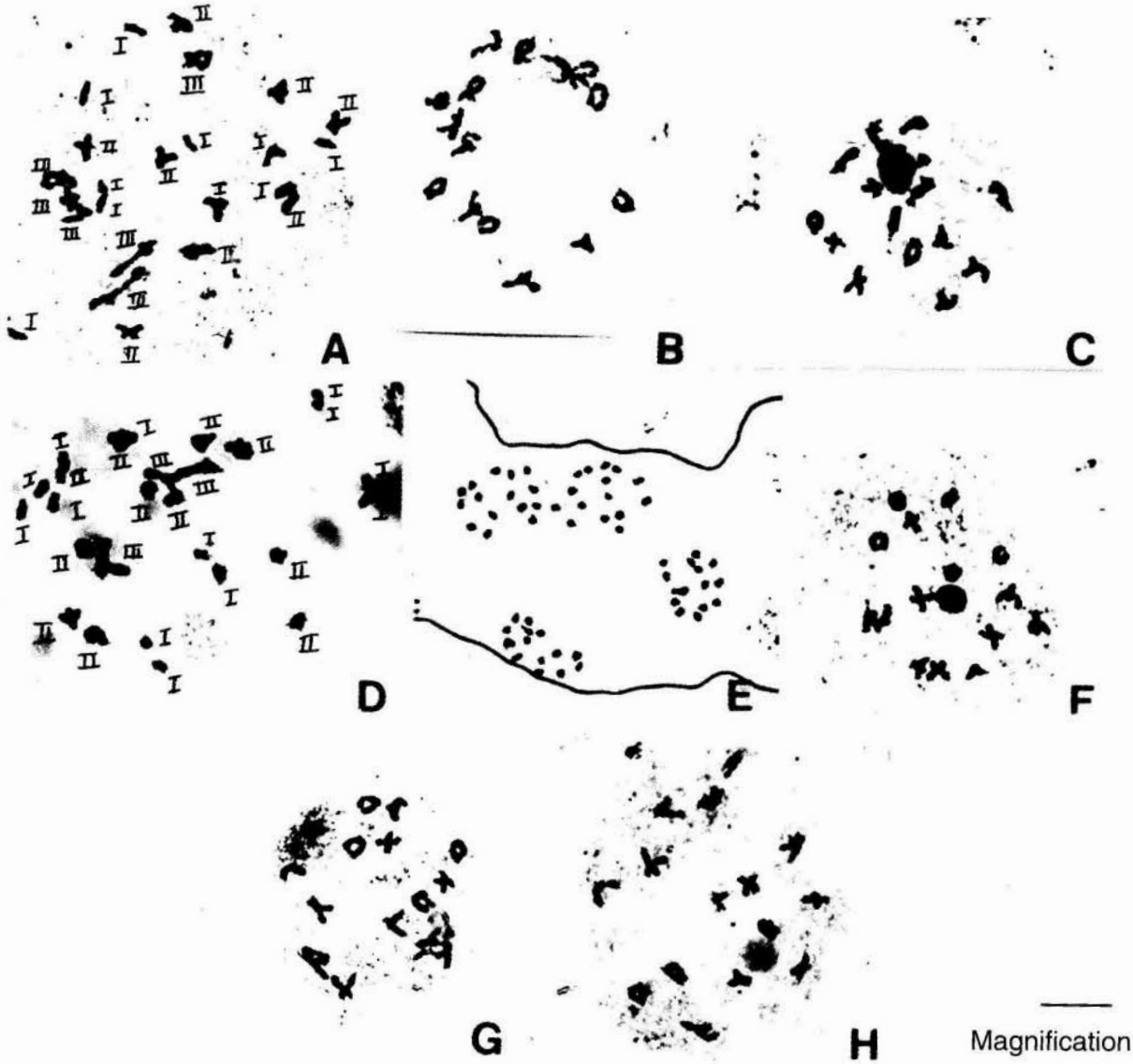


Table 2. Arm ratio and karyomorphology in the diploid tea clones

Clone	2n	L/S ratio															Karyotypic formula
		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	
TES-34	30	2.0	1.2	1.5	1.5	1.7	1.4	1.8	1.5	1.5	1.6	1.2	1.5	1.6	1.2	1.0	1V + 14L
UPASI 12	30	1.9	1.1	1.6	1.5	1.6	1.4	1.4	1.5	1.6	1.5	1.5	1.0	1.4	1.6	1.0	2V + 13L
ATK	30	2.0	1.4	1.3	1.8	1.5	1.7	1.4	1.3	1.6	1.2	1.1	1.6	1.4	1.0	1.0	2V + 13L
UPASI 26	30	2.0	1.5	1.2	1.8	1.3	1.5	1.4	1.2	1.0	1.7	1.5	1.0	1.6	1.8	1.0	3V + 12L
TRI 2024	30	1.8	1.4	1.2	1.2	1.2	1.2	1.4	1.2	1.0	1.5	1.5	1.0	1.2	1.6	1.0	3V + 12L

Fig. 3: Male meiosis in the triploid UPASI 3(A,D) and the diploid UPASI 24 (B), UPASI 9 (C), UPASI 27 (E), SA6 (F), UPASI 8 (G), AKK (H) tea clones. Bar: 7 μm



coefficient for all the 30 diploid clones studied, ranged from maximum 0.38 in UPASI 15 to minimum 0.34 in UPASI 12 (Table 3). Anaphase I, and anaphase II (wherever observed) showed equal distribution at the poles (Fig. 3E).

The PMCs in the triploid clone (UPASI 3) were characterized by the presence of mixture of trivalents, bivalents and univalents (Fig. 3A, D). The average (11 III) number of trivalents per cell, however, outnumbered both bivalents (4II) and univalents (4I). The trivalents were typically of pan, chain and ring shaped (Fig. 3A, D).

DISCUSSION

It is explicitly clear from the present studies that chromosome complements in the diploid clones resolved into homomorphic pairs. No complement of a particular clone exhibited any kind of heteromorphicity within the 'pairs' of chromosomes. Between complements of various clones also, there

Table 3. Male meiosis in the tea clones

Clone	2n	No. of cells analysed	Association mean/cell	Ring II		Rod II		Xta frequency/cell	Terminalization coefficient
				Mean	Range	Mean	Range		
UPASI 1	30	20	15 II	12.3	9 – 15	2.7	0 – 6	24.3	0.37
UPASI 2	30	20	15 II	11.0	10 – 12	4.0	3 – 5	24.5	0.35
UPASI 3	45	20	11 III + 4 II + 4 I	10.3	4 – 11	4.7	0 – 5	26.0	0.45
UPASI 4	30	20	15 II	11.5	11 – 12	3.5	3 – 4	24.7	0.37
UPASI 5	30	20	15II	10.0	8 – 11	5.0	4 – 7	24.0	0.37
UPASI 6	30	20	15II	11.0	9 – 12	4.0	3 – 6	24.1	0.36
UPASI 7	30	20	15II	12.3	9 – 14	2.7	1 – 3	24.5	0.36
UPASI 8	30	20	15II	12.8	11 – 15	2.2	0 – 4	23.7	0.36
UPASI 9	30	20	15II	11.0	10 – 12	4.0	3 – 5	24.2	0.37
UPASI 10	30	20	15II	12.5	12 – 13	2.5	2 – 3	24.4	0.38
UPASI 11	30	20	15II	12.2	10 – 15	2.8	0 – 5	24.5	0.37
UPASI 12	30	20	15II	10.8	9 – 11	4.2	4 – 6	24.6	0.34
UPASI 13	30	20	15II	12.5	11 – 13	2.5	2 – 4	24.1	0.35
UPASI 14	30	20	15II	11.6	8 – 14	3.4	1 – 7	24.0	0.36
UPASI 15	30	20	15II	10.6	10 – 12	4.4	3 – 5	24.5	0.38
UPASI 16	30	20	15II	12.5	11 – 13	2.5	2 – 4	24.7	0.37
UPASI 17	30	20	15II	10.8	9 – 11	4.2	3 – 6	24.3	0.36
UPASI 20	30	20	15II	12.5	11 – 13	2.5	2 – 4	24.6	0.36
UPASI 21	30	20	15II	11.9	10 – 14	3.1	1 – 5	24.5	0.35
UPASI 22	30	20	15II	12.6	9 – 15	2.4	0 – 6	23.5	0.37
UPASI 24	30	20	15II	11.3	10 – 12	3.7	3 – 5	24.6	0.34
UPASI 26	30	20	15II	10.0	8 – 11	5.0	4 – 7	24.0	0.36
UPASI 27	30	20	15II	12.3	9 – 14	2.7	1 – 6	24.4	0.35
TRI 2024	30	20	15II	12.3	10 – 13	2.7	2 – 5	24.6	0.35
TRI 2025	30	20	15II	11.5	8 – 15	3.5	0 – 7	24.8	0.35
ATK	30	20	15II	10.2	9 – 11	4.8	4 – 6	24.3	0.36
AKK	30	20	15II	12.0	11 – 14	3.0	1 – 4	24.0	0.38
SA 6	30	20	15II	12.5	10 – 15	2.5	0 – 5	24.4	0.37
B/5/163	30	20	15II	11.8	9 – 12	3.2	3 – 6	23.9	0.38
CR 6017	30	20	15II	11.6	9 – 12	3.4	3 – 6	24.5	0.38
C-17	30	20	15II	11.8	11 – 15	3.2	0 – 4	24.0	0.37

was little variation in the proportion of median and submedian chromosomes, and number and location of secondary constrictions. It is well known that chromosome morphology between species within a particular genus may be so similar to each other that on the basis of karyomorphology, it is not possible to discriminate one species from the other. There are, however, many genera wherein the chromosome morphology between species is quite distinct to serve as diagnostic character for species distinction. In the absence of complete lack of information with regard to chromosome morphology of various *Camellia* species, it is difficult to ascertain true nature of chromosomes not only which form (as seen from outside) homologues within the clones but also between chromosomes of various clones. The associated taxa is more or less indistinguishable on the basis of karyomorphology so that we are not able to identify hybridity, if any, within or between complements.

Genetic differentiation, as assessed from the extent and nature of meiotic pairing in an hybrid, is one of

the criteria for determining degree of relationship between two different taxa. The occurrence of bivalents with localized chiasmata in the 31 clones presently investigated indicates explicitly that in general, there seems to be chromosomal homoeology between analysed genomes of 'highly heterozygous' tea clones. The lower chiasmata frequency by the predominant occurrence of localized (provided it is not a characteristic feature for the *Camellia* species) chiasmata indicates a reasonable degree of nonhomology among the associated taxa chromosomes, which is usually true for inter – taxon hybrids (Stebbins 1945, 1950).

With the well authenticated background information that the tea clones are highly heterozygous hybrids between the taxa vaguely named as 'China type' and 'Assam type', the present results are best understood when it is assumed that the involved taxa genomes are not sufficiently differentiated from each other to display distinctive chromosome repatterning and/or occurrence of univalents but exhibit cryptic hybridity. Such hybridity of varying nature is sufficient enough to make distinction between clones not only from the point of view of morphology but agronomic traits as well.

The predominant occurrence of trivalents in the triploid UPASI 3 indicates either autotriploid or segmental allotriploid nature of polyploid. Based on the meiotic analysis of 30 diploid clones as above, it might be considered to be segmental allotriploid in origin. More studies are, however, needed.

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REFERENCES

Banerjee, B. (1992). Botanical classification of tea. Tea cultivation to consumption. R.C. Willson and M.N. Clifford. Chapman and Hall. London.

Barua, P.K. (1965). Classification of the tea plant species hybrids. Two and a Bud. Tocklai Experimentation Station. 12: 13-27.

Bezbaruah, H.P. (1971). Cytological investigations in the family Theaceae –I. Chromosome number in some *Camellia* species and allied genera. Caryologia. 24(4): 421-426.

Bezbaruah, H.P. (1968). Cytology of Wilson's *Camellia* (*C. irrawadiensis* Barua). Current Science 37(21): 624-625.

Chaudhuri, T.C. (1995). Chromosomal complexes in tea (aneulploids and polyploids). Chapter 3 "Tea-culture, Processing and Marketing". Edts: Mulky and Sharma. 17-18.

Chang, H.; Bartholomew, B. (1984). *Camellias.* Timber Press. Portland. Oregon. (An edited translation of Hung – ta Chang 1981. A taxonomy of the genus *Camellia.* Acta Sci. Nat. Univ. Sunyatseni, monograph ser. 1: 1-180).

Chang Hung-ta. (1981). A taxonomy of the genus *Camellia.* Acta Sci. Nat. Univ. Sunyatseni, monograph ser. 1: 1-180.

Janaki Ammal, E.K. (1952). Chromosome relationship in cultivated species of *Camellia.* American *Camellia* Year Book. 106-114.

Karasawa, A.K. (1935). On the somatic chromosome number of triploid Thea. Japanese Journal of Genetics. 11: 320.

Kaundun, S.S.; Matsumoto, S. (2002). Hetrologous nuclear and chloroplast microsatellite amplification and variation in tea, *Camellia sinensis.* Genome. 45: 1041-1048.

Kaundun, S.S.; Matsumoto, S. (2003). Development of CAPS markers based on three key genes of the phenylpropanoid pathway in Tea, *Camellia sinensis* (L.) O. Kuntze, and differentiation between *assamica* and *sinensis* varieties. Theoretical and Applied Genetics. 106: 375-383.

Misra, R. K; Sen-Mandi, S. (2001). DNA fingerprinting and genetic relationship study of tea plants using amplified fragment length polymorphism (AFLP) technique. Indian Journal of Plant Genetic Resources. 14(2): 148-149.

Mondal, T.K. (2000). Studies on RAPD marker for detection of genetic diversity, in vitro regeneration and *Agrobacterium* mediated genetic transformation of tea (*Camellia sinensis*). Ph.D. thesis Utkal University. India.

Mondal, T.K.; Bhattacharya, A.; Sood, A.; Ahuja, P.S. (2002b). Factors effecting germination and conversion frequency of somatic embryos of tea. Journal of Plant Physiology. 159(12): 1317-1321.

Moringa, T.; Fukushima, E.; Kano, T.; Maruyama, Y.; Yamasaki, Y. (1929). Chromosome numbers in cultivated plants. Botanical Magazine of Tokyo. 43: 589-594.

Paul, S.; Wachira, F.N.; Powell, W; Waugh, R. (1997). Diversity and genetic differentiation among population of Indian and Kenyan tea (*Camellia sinensis* L. O. Kuntze) revealed by AFLP markers. Theoretical and Applied Genetics. 94: 255-263.

Rajasekaran, P. (1997). Development of molecular markers using AFLP in tea. In: Molecular Approaches to Crop Improvement. Varghese, J.P. (ed). Proceedings of National Seminar on molecular approaches to Crop improvement 29-31st Dec. Kottayam, Kerala, India. 54-58.

Sealy, J.B. (1958). A revision of the genus *Camellia*. Royal Horticultural Society of London.

Simura, T. (1935). Cytological investigations in tea plants (a preliminary report). Proceedings Crop Science Society of Japan. 7: 121-133.

Singh, M; Bandana.; Ahuja, P.S. (1999). Isolation and PCR amplification of genomic DNA from market samples of dry tea. Plant Molecular Biology Reporter. 17: 171-178.

Stebbins, G.L. (1945). Cytological analysis of species hybrids. 2- Botanical Review. 11: 463-486.

Stebbins, G.L. (1950). Variation and evolution in plants. Columbia University Press. New York.

Tanaka, J.; Yamaguchi, N.; Nakamura,Y. (2001). Polllen parent of tea cultivar "Sayamakori with insect and cold resistance may not exist". Breeding Research. 3: 43-48.

Tanaka, J.I.; Yamaguchi, S. (1996). Use of RAPD markers for the identification of parentage of tea cultivars. Bulletin of National Research Institute for Vegetational and Ornamental Plant Tea. 9: 31-36.

Tanaka, J.I.; Sawai, Y.; Yamaguchi, S. (1995). Genetic analysis of RAPD markers in tea. Journal of Japanese Breeding. 45(2): 198-199.

Tan, Y.J. *et al.* (1989). New species and new varieties of tea trees. International *Camellia* Journal. 21: 65-76.

Wachira, F.N.; Waugh, R.; Hackett, C.A.; Powell, W. (1995). Detection of genetic diversity in tea (*Camellia sinensis*) using, RAPD markers. Genome. 38: 201-210.

Wight, W. (1962). Tea classification revised. Currrent Science 31: 298: 299.

Wright, L.P.; Apostolides, Z.; Louw, A.I. (1996). DNA fingerprinting of tea clones. In: Whittle AM and Khumalo FRB (eds.) Proceedings of the 1st Regional Tea Research seminar Blantyre, Malawi 22-23rd March. (1995): 44-50.