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A Cytogenetic Study of Seven Tea Clones [*Camellia sinensis* (L.) O. Kuntze]

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Abstract

Karyotype analyses in seven cultivated clones (cv. T78, T383, TV30, HV39, TeenAli17/1/54, TV29, and UPASI-26) of tea (*Camellia sinensis*) are investigated for their cytogenetic characterization. Karyotypes of the chromosomes ($2n = 30$) were grouped arbitrarily on the basis of their length and position of the centromere into four types (A-D). Centromeric index (F %), total centromeric index (TF %), disparity index (DI) and total haploid chromosome length (TCL) were calculated. Chromosomes were found to be short to medium in size varied in length from 1.24 µm to 4.20 µm. Karyotypes were gradate and asymmetric in nature with median to nearly submedium chromosome. On the basis of karyotype analysis, varietal distinction can be marked to some extent.

Keywords: *Camellia sinensis*, Chromosome complement, Karyotype and Idiogram.

Tea is caffeine containing old beverage coming from the three different varieties of species *Camellia sinensis*. The genus *Camellia* of which tea is a member belongs to the family Theaceae, tribe Gordonieae (Barua, 1989). Two main varieties of tea were grouped as *C. sinensis* var. *sensensis* (L.)(China type) and *Camellia sinensis* var. *assamica* (Masters) (Assam type). Then a third type of tea plant was found which was considered as a Cambod (Cambodiensis) or southern form as described by Planchon. This plant did not differ much from the Assam type plant. Later on, it was described as *Camellia assamica* sub sp. *lasiocalyx* (Planchon MS.). It has been suggested that the tea might have arisen from the same basic genome because most of the species of the genus *Camellia* maintain a comparable chromosomal structure and numbers (Table 1). The cultivated varieties contain diploid chromosome number 2n=30 but except a seed population in Vietnam showed triploid Chromosome constitution 2n=3x=45 (Bezbaruah, 1971). The wild species *Camellia caudata*, *C. kissi* and *C. irrawadiensis* have diploid chromosome numbers as like the cultivated varieties, 2n=30. The *C. sasanqua* is a hexaploid having 2n=6x= 90. Existing wide natural genetic variability present in the tea populations are due to free hybridization among the tea species and varieties (Singh and Bezbaruah, 1988; Singh and Bera, 1994). Elite tea clones are selected or created using the available existing genetic variability in tea germplasm. There is no scope for further genetic evolution to occur in the clones (asexual reproduction). Seeds can be used for the creation of evolution naturally through sexual reproduction. Natural as well as created genetic variability has been exploited for the development of new vegetative clones. All the three varieties of tea (*Camellia sinensis*) are highly cross-pollinated and intercrossable without any reproduction barrier, so the existing population is a mixture of three categories of tea.

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Cultivated tea (*Camellia sinensis*) has been maintained for centuries, by vegetative propagation. An immense heterogeneity is existed in the commercial tea populations, because of the polymorphic origin of the latter. Cultivated tea generally is a mixture of species of tea *Camellia sinensis* (L.) O. Kuntze (China type); *Camellia assamica* (Masters) (Assam type) and *Camellia assamica* sub-species *lasiocalyx* (Planchon MS.). (Cambod type), and other species of *Camellia*, including those fall outside the purview of *Thea* section. Most of the cultivated tea of the world are diploid 2n=30 and highly heterogeneous as a result of free natural hybridization between geographical races during cultivation. Moreover, continued development and release of cultivated varieties have added to the genomic diversity of commercial tea. There are no serious and systematic attempts have been made for gaining detailed information about the genomic constitution of cultivated tea. So, the plant breeder urgently needs to characterize the genetic variation among the available tea germplasm on cytological phenomenon to choose a right clone to be used in hybridization for the crop improvement. Except for the few natural triploids and polyploids reported by Bezbaburah (1971), the cultivated tea plant is a diploid with a chromosome number of $2n = 30$. The chromosome number in tea was determined by many investigators (Bezbaruah, 1971). Root-tip method was generally used for mitotic studies which necessitated digging up of roots. To avoid this, Bezbaruah adopted the shoot-tip method for examination of mitotic chromosomes in tea and other related species after necessary modification and standardization. Bezbaruah (1971) made detailed karyotype analysis of 30 tea clones belonging to the three races of tea, ten from each race, as well as of plants of a few allied species. The somatic chromosome number of 30 was common to all the tea clones and plants of the allied species. The chromosome of tea, as of other *Camellias*, is short but there is a gradation of size from the longest to the shortest. The chromosomes have median to sub-median

Table 1 Chromosome numbers in *Camellia* species.

Species	Chromosome number (2n)
Camellia sinensis	
var. *assamica*	30, 45
var. *sinensis*	30
C. kissi	30
C. caudata	30
C. irrawadiensis	30
C. japonica	30,45
C. rosaeflora	45
C. sasanqua	90
Camellia spp.	30, 45

primary constrictions. In a few clones, one or two pairs of chromosomes may have secondary constrictions. Clones belonging to the three races of tea do not reveal any major differences in their karyotype, although some minor differences exist. However, none of the minor differences could be correlated with any morphological feature of the plants. No irregularities are observed in the meiosis of any of the clones. In many genera and species, the process of evolution is accompanied by different types of karyotypic changes whereas in others morphological difference and divergence occur without any visible change in chromosome morphology. This phenomenon is attributed to cryptic gene mutation.

In the present study, the genetic variation is carried out in seven tea clones on the basis of chromosomal constitution.

Materials and Methods:

Plant material

Karyotype analyses in seven cultivated tea clones [(*Camellia sinensis* (L.) O. Kuntze] were investigated for their characterization. Seven tea cultivars, T78, T383, TV30, HV39, TeenAli17/1/54, TV29, and UPASI-26, were used in the present chromosome study. Six months old tea cuttings were maintained in the earthen pot for the collection and availability of root tips. Root tips were collected by inverting the whole pot and removing the pot from soil clumps. And then fresh root tips of 5 mm size were collected in distilled water with a scissor from the surface of the soil clumps. Root tips were collected in a day light between 10.00 am to 12 noon.

Somatic chromosome technique

The actively growing apical meristem part of the roots were collected between 10 am to 12 noon and properly washed in distilled water immediately after collection. About 5 mm long cut apical root meristems were pretreated in saturated solution of p-dichlorobenzene with a little amount of Aesculine for 3.3 h at room temperature and fixed in a fixative chemical, acetic: ethanol (1:3) for at least 24 h at room temperature. Following hydrolysis in 5N HCl for 1 h at room temperature, the root tips were transferred to 45% acetic acid for 10 min. The root tips were then stained in 2% aceto-orcein by heating the sample over spirit lamp for few seconds and allowed for 40 minutes to take the proper stain by the chromosomes. The temporary squash preparations were made in 45% acetic acid on grease free slide covered by square cover slips. The slides were sealed with wax to prevent air penetration into the chromosome preparation. The chromosome karyotypes from each of the clone were drawn using Camera-Lucida at the table magnification X 1500 and photomicrographs were taken in Lieze microscope at different magnifications and suitably enlarged and analyzed.

Each of the Karyotype was analyzed by the following index-

Centromeric index (F %), Total centromeric index (TF %), Disparity index (DI) and Total Haploid Chromosome Length (TCL) were calculated according to Huziwara (1962).

F%=(short arm length of the chromosome)/(total length of the chromosome)X100

TF% = (total sum of short arm length)/(total sum of the chromosome length) X100

DI=(LCL–SCL)/(LCL+SCL)X100

Where LCL = Longest chromosome length & SCL = Shortest chromosome length

Results and Discussion:

Chromosome morphology in relation to karyotype and idiogram

Karyotypes of the chromosomes (2n=30) were grouped arbitrarily on the basis of their length and position of the centromere into four types (Type A-D). These are summarized in Table 2.

Type A: Comparatively long chromosomes (4.2 µm to 2.8 µm) each with two constrictions, primary and secondary, one of them nearly median (nM) and the other nearly sub-terminal (nST).

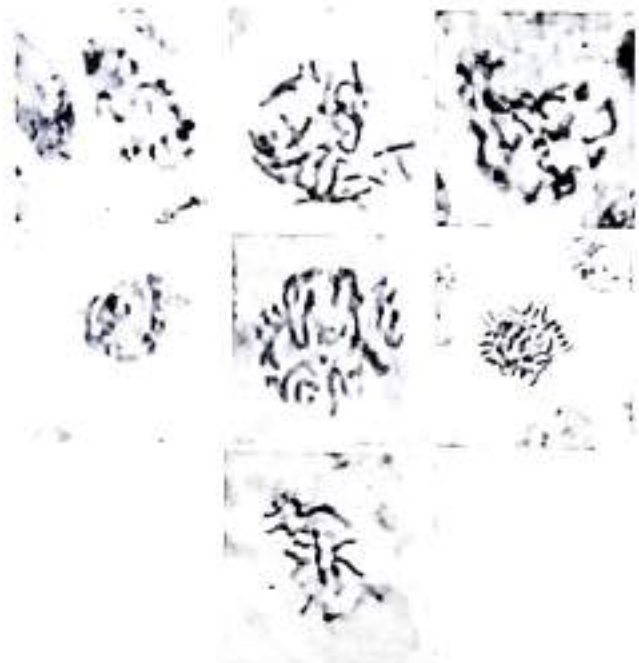

Fig. 1: Photomicrographs of somatic chromosome complements in seven tea cultivars showing 2n=30

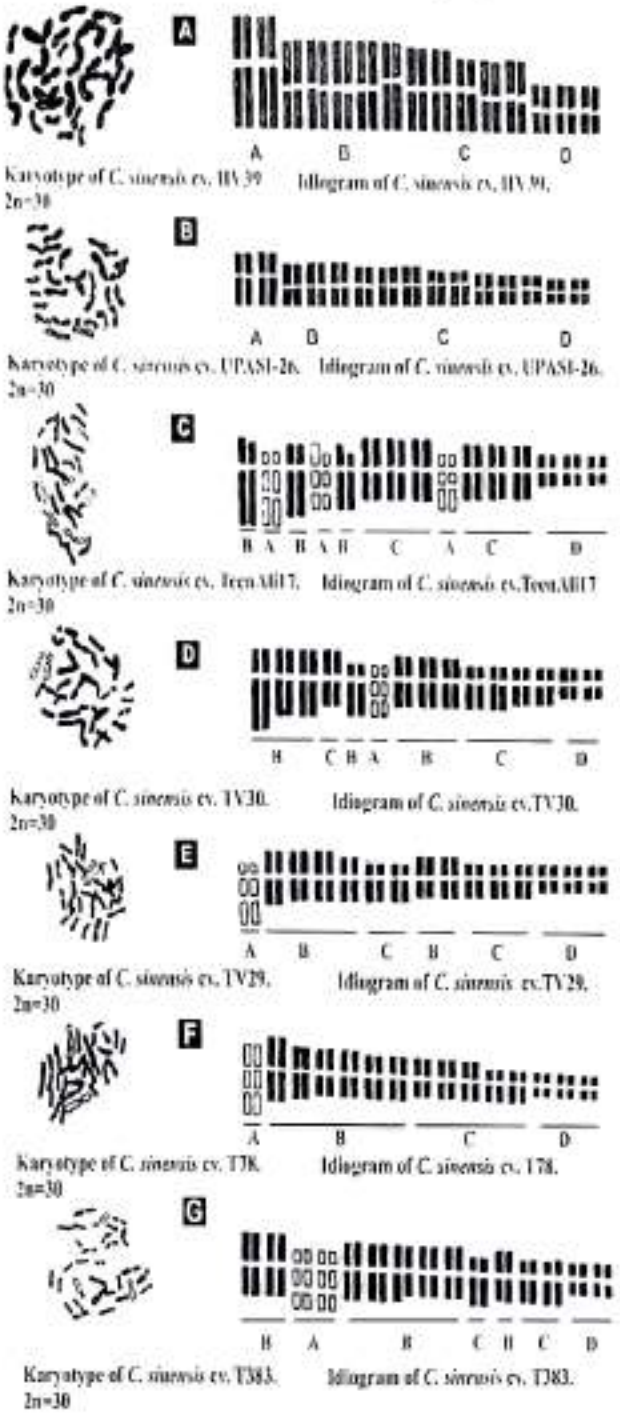

Fig. 2: Karyotype and Idiogram of seven tea cultivers

Type B: Medium to small chromosomes (2.8 µm) with median (M) centromeric constriction.

Type C: Medium sized chromosomes (3.54 µm) with nearly submedian (nSM) centromeric constriction.

Type D: Medium to small chromosomes (1.24 µm) with nearly subterminal (nST) centromeric constriction.

The detailed karyotype analyses of the seven cultivated varieties of tea *viz*- T78, T383, TV30 HV39 TeenAli17/1/54, TV29, and UPASI-26, were done according to Huziwara (1962).

Camellia sinensis cv.TV30

Karyotype formula: 2n = 30 = A4 + B5 + C4 + D2

The somatic complement of the taxon posses 2 pair of chromosomes with secondary constriction. The chromosomes are medium to small sized varying in length 1.24 µm – 3.12 µm (Fig. 1.). Total centromeric index (TF%), disparity index (DI) and total haploid chromosome length (TCL) are 45.12, 43.11 and 64.44 µm, respectively. The camera lucida drawing and the ideogram of the karyotype was represented in figure 2 D.

Camellia sinensis cv.T78

Karyotype formula: 2n=30= A1 + B6 + C5 + D3

The somatic complement of the taxon posses 3 pair of chromosomes with secondary constriction. The chromosomes are medium to small sized varying in length 1.24 µm – 3.75 µm (Fig. 1). Total centromeric index (TF%), disparity index (DI) and total haploid chromosome length (TCL) are 37.35, 50.30 and 35.36 µm, respectively. The camera lucida drawing and the ideogram of the karyotype was represented in figure 2 F.

Camellia sinensis cv.T383

Karyotype formula: 2n = 30= A2 + B8 + C3 + D2

The somatic complement of the taxon posses 4 pairs of chromosomes with secondary constriction. The chromosomes are medium to small sized varying in length 1.24 µm – 3.75 µm (Fig. 1). Total centromeric index (TF%), disparity index (DI) and total haploid chromosome length (TCL) are 38.25, 50.30 and 33.33 µm, respectively. The camera lucida drawing and the ideogram of the karyotype was represented in figure 2 G.

Camellia sinensis cv.TV29

Karyotype formula: 2n = 30 = A1 + B6 + C5 + D3

The somatic complement of the taxon posses one pair of chromosomes with secondary constriction. The chromosomes are medium to small sized varying in length 1.24 µm – 3.12 µm (Fig. 1). Total centromeric index (TF%), disparity index (DI) and total haploid chromosome length (TCL) are 41.87, 43.12 and 28.94 µm, respectively. The camera lucida drawing and the ideogram of the karyotype was represented in figure 2 E.

Camellia sinensis cv.TeenAli17/1/54

Karyotype formula: 2n = 30 = A3 + B3 + C6 + D3

The somatic complement of the taxon posses one pair of chromosomes with secondary constriction. The chromosomes are medium to small sized varying in length 1.24 µm – 3.75 µm (Fig. 1). Total centromeric index (TF%), disparity index (DI) and total haploid chromosome length (TCL) are 45.60, 50.30 and 32.06 µm, respectively. The camera lucida drawing and the ideogram of the karyotype was represented in figure 2 C.

Camellia sinensis cv.UPASI-26

Karyotype formula: 2n = 30 = A3 + B6 + C5 + D1

Table 2 Salient karyotypic features of seven cultivars of *Camellia sinensis*

Cultivar	Type	No. of Pair	Chromosome length (µm)			F%	Special features.
			Long arm	Short arm	Total length		
TV30							
	A	4	1.25-1.87	1.25-1.25	2.50-3.12	40.06	nM Sat+
	B	5	1.25-1.25	0.93-1.25	2.18-2.50	48-50	M
	C	4	0.62-1.25	0.62-0.93	1.24-1.87	33.15	nSM
	D	2	0.62-0.62	0.82-0.82	1.44-1.44	43.05	nM
T78							
	A	1	1.87-2.50	0.62-1.25	2.49-3.75	33.33	nSM Sat+
	B	6	1.87-2.50	0.62-0.62	2.49-3.12	33.21	nSM
	C	5	0.62-1.25	0.62-1.25	1.24-2.50	50-50	M
	D	3	1.25-1.25	0.93-0.93	2.18-2.18	42.66	nM
T383							
	A	2	1.25-2.50	0.62-1.25	1.87-3.75	33.33	nSM Sat+
	B	8	0.93-1.87	0.62-1.25	1.55-3.12	42.66	nM
	C	3	0.62-1.25	0.62-1.25	1.24-2.50	50-50	M
	D	2	1.87-1.87	0.62-0.62	2.49-2.49	24.89	nST
TV29							
	A	1	2.50-2.50	0.62-0.62	3.12-3.12	19.87	nSM Sat+
	B	6	0.93-1.56	0.62-1.25	1.55-2.82	42.66	nM
	C	5	0.62-1.25	0.62-1.25	1.24-2.50	50.00	M
	D	3	1.25-1.25	0.62-0.62	1.87-1.87	33.15	nSM
TeenAli17/1/54							
	A	3	2.50-2.50	1.25-1.25	3.75-3.75	33.33	nSM Sat+
	B	3	0.93-1.87	0.93-1.87	1.87-3.74	50.00	M
	C	6	0.62-0.93	0.62-0.93	1.24-1.86	50.00	M
	D	3	0.93-1.56	0.62-1.25	1.55-2.18	44.48	nM
UPASI-26							
	A	3	0.62-1.25	0.62-1.25	1.24-2.50	50.00	M Sat+
	B	6	1.87-1.87	0.60-0.62	2.49-2.49	24.89	nSM
	C	5	0.62-0.93	0.93-1.25	1.55-2.18	42.7-50	nM
	D	1	1.25-1.25	0.62-0.62	1.87-1.87	33.15	nSM
HV39							
	A	4	1.25-1.87	1.25-1.25	2.25-3.12	40.06	nSM Sat+
	B	6	1.25-1.83	0.62-1.25	1.87-2.50	48-50	M
	C	3	0.93-0.93	0.62-0.93	1.55-1.87	33.81	nSM
	D	2	1.25-1.25	0.62-0.62	1.87-1.87	33.15	nSM

The somatic complement of the taxon posses three pairs of chromosomes with secondary constriction. The chromosomes are medium to small sized varying in length 1.24 µm – 2.50 µm (Fig. 1). Total centromeric index (TF%), disparity index (DI) and total haploid chromosome length (TCL) are 42.20, 33.68 and 29.39 µm, respectively. The camera lucida drawing and the ideogram of the karyotype was represented in figure 2 B.

Camellia sinensis cv. HV39

Karyotype formula: 2n = 30 = A4 + B6 + C3 + D2

The somatic complement of the taxon posses four pairs of chromosomes with secondary constriction. The chromosomes are medium to small sized varying in length 1.55 µm – 3.12 µm (Fig. 1). Total centromeric index (TF%), disparity index (DI) and total haploid chromosome length (TCL) are 43.51, 43.11 and 62.92 µm, respectively. The camera lucida drawing and the ideogram of the karyotype was represented in figure 2 A.

In the present investigation, cytological study is carried out in seven tea cultivars, T78, T383, TV30, HV39, TeenAli17/1/54, TV29, and UPASI-26, for their characterization at the chromosomal level. The chromosomal analysis in tea has been investigated with a view to gaining insight into the cytogenetic situation in these cultivars of *Camellia sinensis*. The taxon reveals 30 chromosomes in their somatic complements (Bezbaruah,1971). The present observation strengthens the concept of numerical uniformity in the chromosome complement 2n=30 in cytological investigation. A distinct similarity was noted in general morphology of chromosome of the investigated seven tea cultivars. The homogeneity was represented not only in the numerical uniformity and gross structural similarities of chromosomes, but also in the significant coincidence of total chromatin material between the members. Karyotypes of the chromosomes (2n = 30) were grouped arbitrarily on the basis of their length and position of the centromere into four types (A-D). Centromeric index (F %), total centromeric index (TF %), disparity index (DI) and total haploid chromosome

length (TCL) were calculated. Chromosomes were found to be short to medium in size varied in length from 1.24 µm to 4.20 µm. Karyotypes were gradate and asymmetric in nature. On the basis of karyotype analysis, varietal distinction can be marked to some extent.

The taxa bearing such striking resemblance in cytological features, however, differ in details of karyotype features, especially with regard to the number of chromosomes with secondary constrictions. The presence of 3 pairs of nucleolar chromosomes is the characteristics of *C. sinensis* namely TV-23, and TV-25 and TV26 (Roy, 2006). Total chromosome length ranged from 1.24 µm to 4.20 µm in the investigated cultivars. Other minor differences in many karyotype involving the absence or variable number of a given chromosome type constitute chromosomal basis of further intervarietal differentiation in *C. sinensis*. The disparity index which is significantly high in all the investigated cultivars further indicates heterozygous constitution of the varieties which have probably arisen during long cultivation, selection, and maintenance through vegetative propagation. The significance of structural alternations of chromosomes in evolution and speciation had often been underestimated in the past due to over emphasizing the role of mutation in evolution. In the recent past with the aid of improved chromosome techniques, it has been possible to work out the chromosomal basis of intervarietal and even inter strain differences in a number of cruciferous taxa mentioned earlier. Very recently similar important role of chromosomal alternations in interspecific and intervarietal diversification of *Trichosanthes* have been emphasized by De Sarkar *et al.* (1987).

Close morphological similarity of the tea chromosomes suggests that the observed differences in growth and form of plants of the different races of tea are the results of mutative changes of the genes as found in *Pinus* and *Quercus*, where the karyotype is closely similar although they are different species. Karyotype analyses of different cultivars of tea showed a smooth decrease in size of chromosome from the longest to the shortest. Naturally evolved polyploids in tea are very rare. Only a few natural triploids (2n=45) have so far been reported from Japan, South India (Venkataramani and Sharma, 1974). Ackerman (1977), and Bezbaruah (1971) mentioned that the ploidy level in the genus *Camellia* ranged up to hexaploid with 2n=90 chromosomes. Ackerman (1977) proposed that segmental allopolyploid might have originated during the course of evolution in diploid level and continued in the polyploids through inter-crossing among them. Bezbaruah (1971) reported that the chromosomes of tea plant were primitive but other classified them as of advanced status depending on the symmetry and asymmetry of chromosomes respectively. The length of tea chromosomes ranged from 1.24µm to 4.20µm. The 'r' value (ratio of long arm to short arm) for all the 15 pairs of chromosomes ranged from 1.00 to 1.91. The chromosomes were classified morphologically on the basis of relative lengths, position of the centromere and presence or absence of secondary constriction. The meiotic division in Assam and hybrid tea were regular with 15 bivalents at diakinesis and telophase I and the segregation was regular (Bezbaruah 1971). Bezbaburah (1971, 1976) observed two satellite pairs of chromosomes in the complement of *C. assamica*. Lack of predominant satellites indicated tea as being inherent in low activity of the nucleolus organizer, which restricts the chromosomes to produce secondary constrictions.

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