J. Phytol. Res. 19(2) : 203-207, 2006

KARYOTYPE ANALYSIS IN THREE CULTIVATED VARIETIES OF TEA [*CAMELLIA SINENSIS* (L) O. KUNTZE] FOR THEIR CHARACTERIZATION

SUBHAS CHANDRA ROY
Department of Botany, University of North Bengal, PO-NBU-734013, Siliguri, West Bengal, India.
Subhasr@rediffmail.com

Karyotype analysis in three cultivated varieties (cv.TV23, TV-25 and TV-26) of tea (*Camellia sinensis*) has been investigated for their characterization. Cultivated *Camellia sinensis* form a stable polyploid series with a basic chromosome number of 15. The majority of the cultivated *Camellia* species are diploid (2n=30). The taxa are characterized by a gradate karyotype with median to nearly terminal medium sized chromosome. The chromosomes varies in length ranging from 2.1μm to 4.2μm. Microchromosome or B-chromosome is found in TV-25 among the three cultivars. The presence of 3 pairs of nucleolar chromosomes is the characteristics of the two out of three cultivars of *C. sinensis* namely TV-23, and TV-25. As many as 5 pairs of such chromosome characterize the cultivar TV-26, which also has the highest value of total chromatin material.

Keywords : *Camellia sinensis*; Genomic constitution; Idiogram; Karyotype.

Introduction

Cultivated tea (*Camellia sinensis*) has been maintained for centuries, by vegetative propagation. An immense heterogeneity is existing 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* Shneider (Assam type) and *Cammellia assamica* sub-species *lasiocalyx* (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. A survey of literature clearly indicates the highly inadequate state of knowledge regarding cytogenetic situation in tea species and varieties. Except some stag reports of chromosome number[1-3] no serious and systematic attempts have been made for gaining detailed information about the genomic constitution of cultivated teas.

Camellia sinensis in wild condition is an evergreen tree attaining a height of 15-25m, but under cultivation the plants are maintained as a much branched shrubby bush, about 0.5 m to 1.6 m in height. Stem internodes length varies between 2.1 to 6.5 cm. Leaves evergreen simple, alternate, exstipulate; elliptic, oblong, serrate, acute at both ends or cuspidate acuminate at apex. Flowers are solitary, axillary, often in a cluster of 2-4 bisexual, about 3 cm in diameter, white in colour, bracteate, bracts 2-3, small. Sepals are 5-6 in number, unequal orbicular, glabrous, with membranous ciliate margin. Petals white, 5-6, broadly obovate, slightly coherent at a base, about 1.5 cm long and 1 cm in diameter. Stamens many in several whorls, 5-12 of the inner most whorl free, others unite to form a monadellonous ring, adnate to the base of the petals, about 1 cm long, yellow in colour. Ovary superior, 3-5 celled with 4-5 pendulous ovules on each cell, styles 3-5, united upto 2/3rd of their length. Fruit a loculicidal capsule, trigonous, with leathery pericarp. Seeds 1-2 in each cell of the fruit, 1.5 – 2 cm in diameter, globose or obtusely angled, pale-brown and smooth.

Recently a detailed and comprehensive survey of genomic constitution in the existing commercial cultivars of tea growing in this Northern part of India has been initiated by so many researchers. The importance of karyotype study in establishing phyletic relationship and evolutionary trends is well recognized[4-7]. With the aid of improved chromosome techniques it has been possible to work out the chromosomal basis of intervarietal or even inter-strain differences[5,8,9]. In the present investigation chromosomal analysis in the somatic chromosome complement has been undertaken involving three cultivated varieties of tea to characterize the tea cultivars on the basis of karyotype analysis.

Materials and Methods

The present investigation deals with some high yielding clonal varieties of Assam tea developed and released by Tea Research Association. The clones such as TV-23,

TV-25, TV-26 are Tocklai released high yielding vegetative clones considered for chromosome analysis in the present report.

Somatic chromosome technique: - The actively growing youngest leaf about 1 cm in length, light green in colour, with less epidermal hairs and calcium oxalate crystals were taken for chromosome studies. The leaf cut into suitable pieces were pretreated in aqueous saturated solution of p-dichlorobenzene for 3h at 12°C – 16°C with an initial treatment at 0°C–4°C for 10 min, followed by washing in distilled water. The materials were transferred to a mixture of propionic acid, chloroform and ethanol in the proportion of 1:3:6 for fixation and removal of chlorophyll pigments.

Following overnight fixation a part of the material was stored in 70% ethanol for further studies. Following pre-treatment and fixation, the materials were warmed to steaming over a small flame for 10-15 min in a mixture of 1 N HCl 1 part and 2% orcein in 45% propionic acid 9 parts, kept as such for 45 min, and then separated small leaf tissues were squashed on dry grease free and clean slides in 1% carmine in 45% propionic acid solution. The slides were made permanent on the following day by detachment of the cover glass and dehydration in alcohol grades and mounted in DPX.

Results and Discussion

Somatic chromosome analysis has been made in three cultivars of Tea. The cultivars TV-23, TV-25, TV-26, consist of 30 chromosomes in their somatic complements. The taxa are characterized by a gradate karyotype with median to nearly terminal medium sized chromosome varying in length from 2.1µm to 4.2µm. Micro-chromosome or B chromosome is found in cultivar TV-25 only.

Centromeric index (F%), total centromeric index (TF%), disparity index (DI) and total haploid chromosome length (TCL) were calculated as given by Huziwara[10]:

$$F\% = \frac{\text{Short arm length of the chromosome}}{\text{Total length of the chromosome}} \times 100$$

$$TF\% = \frac{\text{Total sum of short arm lengths}}{\text{Total sum of chromosome lengths}} \times 100$$

$$DI = \frac{\text{Longest chromosome length (–) shortest chromosome length}}{\text{Total length of largest and shortest chromosome}} \times 100$$

On the basis of the position of centromere/ secondary constriction as indicated by their R_2 ratio, where R_2 = (Long arm length/short arm length), and length of the chromosomes, different chromosome types have been characterized. For precise determination of centromeric location and position of secondary constriction the method of Adhikary[11] has been adopted in general. The chromosome types encountered in the taxa studied have been described under the following main groups-

Karyotype Analysis:

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).

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

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

Type D: Medium to small chromosomes (2.8µm) with nearly subterminal (nST) centromeric constriction.

Type E: Medium to small chromosomes (2.1µm) with nearly terminal (nT) centromeric constriction.

The detailed karyotype analysis of the three cultivated varieties of tea viz-TV-23, TV-25 and TV-26 are summarized in the Table-1a, b, and c, respectively.

Camellia sinensis cv TV-23

Karyotype formula, 2n = 30=A3 + B3 + C4 + D3 + E2.

The somatic complement of the taxon possesses 3 pair of chromosomes with secondary constructions (Fig.2). The chromosomes are medium sized varying in length from 2.8µm to 2.1µm, Total centromeric index (TF %), disparity index (DI) and total haploid chromosome length (TCL) are 51.08, 29.41, 39. 90µm respectively.

Camellia sinensis cv. TV-25

Karyotype formula, 2n = 30= A3 + B5 + C2 + D2 + E3.

The somatic complement of the taxon possesses 3 pair of chromosomes with secondary constrictions (Fig.3). The chromosomes are median to small sized with nearly terminal in lengths 2.1µm to 3.1µm. Total centromeric indix (TF%), disparity index (DI) and total haploid chromosome length (TCL) are 38.67 ,20.00, 35.35 µm respectively.

Camellia sinensis cv.TV-26

Karyotype formula, 2n = 30= A5, B5, C2, D3, E0.

The somatic complement of the taxon posses 5 pair of chromosomes with secondary constrictions (Fig. 1). The chromosomes are medium to small chromosome sized varying in lengths 3.15µm to 2.8µm. Total centromeric index (TF%), disparity index (DI) and total haploid chromosome length (TCL) are 37.03, 20.00, 48.3µm respectively.

The chromosomal analysis in tea has been carried out with a view to gaining insight into the cytogenetic situation in three cultivars, TV-23, TV-25, and TV-26 of *Camellia sinensis*. The taxon reveals 30 chromosomes in their somatic complements[1-3]. The present observation strengthens the concept of numerical uniformity in the chromosome complement in so far cytological investigated diploid members of *Camellia sinensis*[1].

A distinct similarity is noted in general morphology of chromosome of the investigated cultivars. The homogeneity is 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.

Fig. 1. Photomicrograph showing 2n=30 chromosomes in the somatic complement of *Camellia sinensis* cv.TV-26.

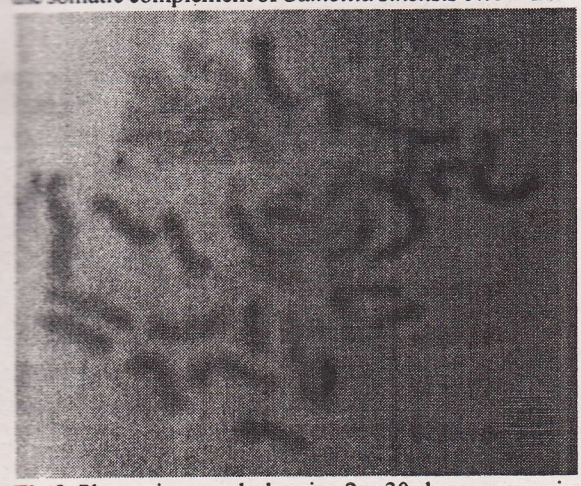

Fig.2. Photomicrograph showing 2n=30 chromosomes in the somatic complement of *Camellia sinensis* cv.TV-23.

Fig.3. Photomicrograph showing 2n=30 chromosomes in the somatic complement of *Camellia sinensis* cv.TV-25.

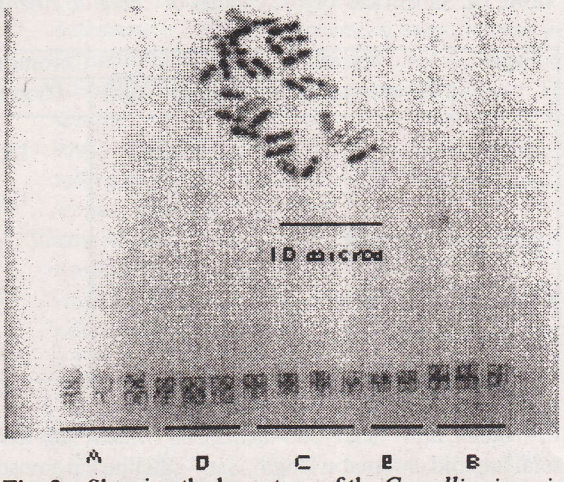

Fig. 2a. Showing the karyotype of the *Camellia sinensis* cv. TV-23, 2n=30 (above) and Idiogram of the same (below).

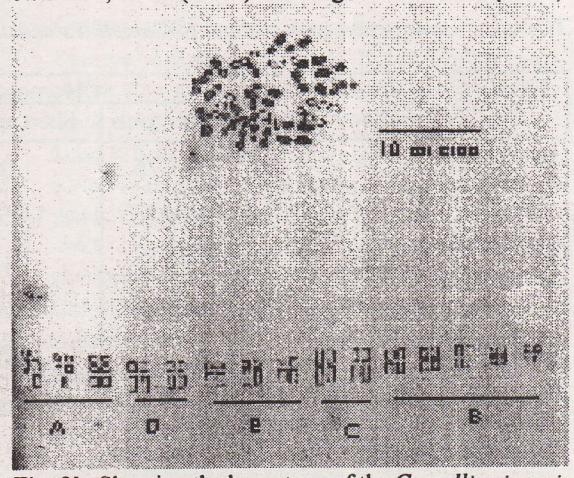

Fig. 2b. Showing the karyotype of the *Camellia sinensis* cv. TV-25, 2n=30 (above) and Idiogram of the same (below).

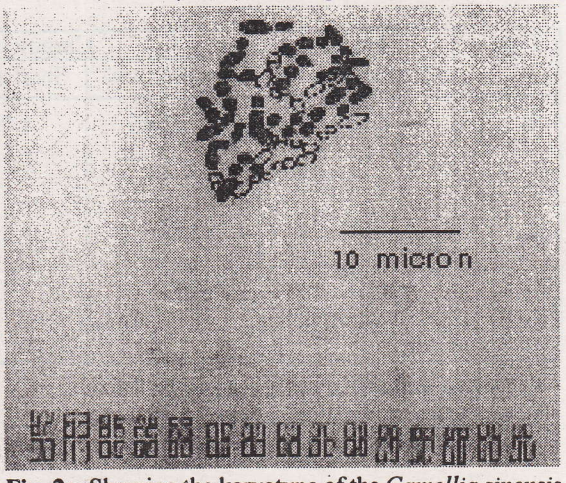

Fig. 2c. Showing the karyotype of the *Camellia sinensis* cv. TV-26, 2n=30 (above) and Idiogram of the same (below).

Table 1a. Salient karyotypic features of *Camellia sinensis* cv. TV-23.

Type	No. of Chromosome Pair	Chromosome length			F%	Special features
		Long arm (μm)	Short arm (μm)	Total length (μm)		
A	3	2.80-2.45	1.05-0.7	3.85-3.15	27.27–22.22	nST $^{Sat+}$
B	3	1.4-1.05	1.4-1.05	2.8–2.1	50.00	M
C	4	1.4	1.05	2.45	42.85	nM
D	3	1.75	1.05	2.80	37.50	nSM
E	2	1.4	0.7	2.10	33.33	nSM

Karyotype formula: A3 + B3 + C4 + d3 + E2,
M-median, nSM-nearly sub-median, nST-nearly sub-terminal, nT-nearly terminal chromosome on the basis of F%; TCL-total haploid chromatin length; Sat+- satellite chromosome with secondary constriction.
TF%=38.95, D. I. =29.41, and TCL=40.25μm.

Table 1b. Salient karyotypic features of *Camellia sinensis* cv.TV-25.

Type	No. of Chromosome Pair	Chromosome length			F%	Special features
		Long arm (μm)	Short arm (μm)	Total length (μm)		
A	3	2.45-1.75	0.7	3.15-2.45	22.22-28.57	nST $^{Sat+}$
B	5	1.05-0.7	1.05-0.7	2.1-1.40	50.00	M
C	2	1.75	1.4	3.15	44.44	nM
D	2	1.75-1.4	1.05	2.8-2.45	37.50-42.85	nSM
E	3	1.75-1.4	0.78	2.45-2.1	28.57	nST

Karyotype formula: A3 + B5 + C2 + D2 + E3
TF%=38.67, D. I. =20.00, and TCL=35.35μm.

Table1c. Salient karyotypic features of *Camellia sinensis* cv.TV-26.

Type	No. of Chromosome Pair	Chromosome length			F%	Special features
		Long arm (μm)	Short arm (μm)	Total length (μm)		
A	5	3.15-2.45	1.05-0.7	4.2-3.15	25.00	nSM $^{Sat+}$
B	5	1.4	1.4.0	2.8	22.22m	nST
C	2	2.14	1.40	3.54	39.54m	nSM
D	3	2.14	1.05	3.19	32.91	nSM
E	0					0

Karyotype formula: A5 + B5 + C2 + D3+E0
TF%=27.03, D. I. =20.00, and TCL=48.94μm.

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 the two out of three cultivars of *C. sinensis* namely TV-23, and TV-25.

As many as 5 pairs of such chromosome characterize the cultivar TV-26, which has also the highest value of total chromatin material. 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. Similar important role of chromosomal alternations in interspecific and intervarietal diversification of *Trichosanthes* have been emphasized by De Sarkar *et al.*[12]

References

1. Bezbaruah H P1968, An evaluation of preparatory procedures for leaf tip Chromosome spreads of the tea plan (*Camellia sinensis*). *Stain Tech.* **43** 279-282.
2. Bezbaruah H P1971, Cytogenetical investigations in the family theaceae. I. Chromosome number in some *Camellia* species and allied genera. *Caryologia* **24** 425-426.
3. Janaki Ammal E K 1952, Chromosome relationship in cultivated species of plantlets from tea stem callus. *Japan J. Breed.* **35** 317-322.
4. Sharma AK and Sharma A 1959, Chromosomal alterations in relation to speciation. *Bot. Rev.* **25** 514-544.
5. Sen R and Datta KB 1978,Cytological studies in some Indian cultivated varieties *Cucumio* L. *Cytol. Genet.* **113** 16-22.
6. Datta S and Datta KB 1971, Biosystematics of Cassia. I. Cytological observations on some commonly growing cassias of Bengal. *J. Cytol. and Genet. Congr. Suppl.* 55-59.
7. Katsuhiko Kondo 1977, Chromosome numbers in the Genus *Camellia*. *Biotropica* **9** 86-94.
8. Sharma A K and Datta K B 1961,Inter strain differences in Karyotypic of *Raphanus sativas* Linn. *Agric.* **5** 151-159.
9. Datta K B 1974, Chromosome studies in *Iberis* L. with a view to find out the mechanism of speciation of the genus. *Cytologia* **391** 543-551.
10. Yukio Huziwara 1962, Karyotype analysis in some genera of compositae. VIII. Further Studies on the Chromosomes of Aster. *Amer. J. Bot.* **49** 116-119.
11. Adhikary A K 1974, Precise determination of centromere locations.*Cytologia* **39** 11-16.
12. De Sarkar D, Datta K B and Sen R 1987, Cytomorphology of some wild and cultivated member of *Trichosanthes* L. *Cytologia* **52** 405 – 417.