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RESEARCH ARTICLE

# Estimation of nuclear DNA content and its variation among Indian Tea accessions by flow cytometry

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**Abstract** Nuclear DNA content and genome size variation among 36 Indian tea accessions were analyzed by flow cytometry. Initial standardization of protocols for isolation of nuclei, DNA staining and selection of an internal standard for tea accessions which have significantly high amount of phenolic secondary metabolites in their cytosol was carried out. Results obtained revealed that 2C DNA content of Indian tea is 7.46 pg which corresponds to 1C genome size of 3673 Mb. Inter accession variation in 2C DNA content was also observed among 35 diploid taxa ranging from 7.23 to 7.73 pg which was significant at 1% probability level. The 2C DNA content of triploid (UPASI 3) was observed to be 11.47 pg which is concurrent with the expected value. Results obtained showed that Assam and Cambod type tea accession have higher 2C DNA content of 7.73 pg whereas Assam Cambod hybrids and Assam China hybrids have reduction in DNA content with 2C amounts, 7.23 and 7.32 pg DNA respectively. The present study suggests that the species involved in origin of Indian tea must have differed in their genome sizes owing to significant inter accession variation in nuclear DNA content.

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**Keywords** Indian Tea · Internal standard · 2C DNA · Genome size

## Introduction

Tea (*Camellia* L.) (Camelliaceae) is world's one of the most widely consumed non alcoholic beverage (Charles 1981). Because of its specific agro-climatic requirements, tea is mainly cultivated in South and South East Asian countries. India and China are the two largest producers of tea (Chen et al. 2007). Taxonomy of beveragial tea is highly complex and challenging since it is an open pollinated crop involving frequent hybridization followed by stabilization of promising forms by clonal multiplication (Krahulcova and Krahulec 2000). Classification of tea species on the basis of morphology is highly variable and is also not supported by chromosome pairing property of hybrids (Kamemoto 1987), various taxa identified in beveragial tea are still debatable. Inspite of several taxonomic variants available, tea is commonly classified as *Camellia sinensis* (L.o. Kuntze), *C. assamica* (Masters) Wight ssp. *assamica* and *C. aasamica* sp *lasiocalyx* (Watt) Wight, being endemic to China, Assam (India) and Indo-China regions of South-East Asia commonly known as China, Assam and Cambod type respectively (Roboerts et al. 1958; Barua 1965a, b; Banerjee 1992; Ellis 1995). Tea was commercialized in late nineteen century after introduction of tea seeds from China into various regions of NE India followed by their subsequent spontaneous hybridization with endemic tea accessions and wild relatives leading to origin of hybrid swarms with tremendous genetic variability (Barua 1965a, b; Banerjee 1992; Wachira et al. 1995; Paul et al. 1997). At present tea is commercially planted in approximately 30 countries and all these

plantations mainly have hybrids of Assam and China type commonly called India Hybrid Tea (Ellis 1995).

Genome size and DNA C-value are often used as synonyms, but according to Greilhuber et al. (2005), C-value is defined as amount of DNA present in an un-replicated haploid chromosome set (n), while genome size is a covering term for the amount of DNA in un-replicated haploid chromosome set (n) and also in un-replicated basic (monoploid) chromosome set (x) in polyploids. Information on genome size is important to understand taxonomic relationships, to trace evolutionary changes and for genome sequencing and gene cloning projects. Genome size among angiosperms is highly variable, ranging from 1C = 0.06 pg in *Genlisea margaretae* to 1C = 152.23 pg in the *Paris japonica* (Bennett and Leitch 2011), along with extensive intraspecific variation in some species. Genome size variation at intraspecific level has been reported in many plant species including Soyabean (Graham et al. 1994; Rayburn et al. 1989), maize (Laurie and Bennett 1985) and *Linum usitatissimum* (Cullis 2005). Despite the fact that genome size is higly variable at species level, great stability in nuclear genome has been reported for geographically isolated populations of *Sesteria albicans* (Lysak et al. 2000), *Settaria* sp. (Le Thierry d'Ennequin et al. 1988), citrus (Ellul et al. 2002), capsicum (Moscone et al. 2003) and in cultivars of pea and onion (Baranyi and Greilhuber 1995; Bennett et al. 2000). The relative frequency of increase and decrease in DNA content still remains unresolved in angiosperm phylogeny (Wendel et al. 2002). In literature, several reasons have been assigned to change in genome size (Bennetzen and Kellogg 1997; Piegu et al. 2006; Wicker and Keller 2007; Gregory 2003; Bennetzen et al. 2005).

The nuclear DNA content can be a highly reliable marker, in some cases delineating disputed taxa and has a predictive value to determine evolutionary relationship (Suda et al. 2007). The knowledge of genome size variation in Indian Hybrid Tea may help to understand the evolution and also to the trace events involved in evolution like hybridization among tea accession. Therefore, in the present study, genome size of 36 Indian Hybrid Tea accession is determined using flow cytometric analysis. The data obtained in this study can be utilized to infer taxonomic relationship in Hybrid tea.

## Materials and methods

### Plant material

The germplasm investigated in present study were obtained from United Planters Association of South India (UPASI), Tea Research Foundation, Valparai, Coimbatore, India. Most of these clones are the progenitors plants and (or) seed stocks brought from Assam, China and other tea growing regions of the world and were planted for research purpose in Hills of Nilgiris (Table 1). Among the 36 clones, twenty-seven are commercially released and they have revolutionized the tea industry in South India due to their color, aroma and various other agronomic traits (UPASI bulletin).

### Sample preparation

Nuclei were isolated following the protocol of Arumugnathan and Earle (1991) with slight modification in the constituents of nuclei isolation buffer which was done to exclude inhibitory effect of phenolic compounds present in tea leaves on staining of nuclei with Propidium Iodide (PI). Nuclei isolation buffer used in the present study included 10 mM $MgSo_4 \cdot 7H_2O$, 50 mM KCl, 5 mM HEPES (*N*-2-hydroxyethyl piperdazine–N′-2-ethanosulphonic acid), 1.0 mg/ml dithiothreitol(DTT), 10% Triton X-100 (w/v), 1% PVP, 10 mM EDTA, 15 mM β-mercaptoethanol. One hundred milligram (fresh weight) leaves of tea clones were chopped with 150 mg leaves of Internal Standard *Hordeum vulgare* cv. Sultan (1C = 5.56 pg, Johnston et al. 1999) in 2 ml of ice cold nuclei isolation buffer with the help of a sharp razor blade. The isolated nuclear suspension was filtered through a 40 µm nylon mesh. The filtrate was centrifuged at 300 g for 4 min at 4 °C. The pellet was resuspended in 100 µl of nuclear isolation buffer and to this 50 µl (5 mg/ml) of PI was added and microfuge tubes were incubated on ice for 40 min. Consequently, DNAase free RNAase (1.25 µg/ml) was added to the tube and was further incubated at 37 °C for 30 min. RNAase was added to avoid staining of double stranded RNA with PI as the dye is not specific to DNA and therefore may result in erroneous estimation of nuclear DNA content.

### Flow cytometric analysis

Nuclear samples were analyzed by BD FacSort (USA) Flow Cytometer. The instrument was equipped with an argon ion laser operating at 488 nm. The PI fluorescence was collected by 620 nm FL2 filter. Parameters for data acquisition were kept constant for all samples. Sample flow rate was set at about 100 nuclei/s and at least 10,000 nuclei were acquired for each sample. The results acquired were later analysed using Cell Quest software. Densely gathered nuclei region in dot plot was gated and considered for final analysis to avoid unwanted counts. The average of coefficient of variation values (CV) for G1 peaks was used to evaluate the results. The results with CV $< 5\%$ were considered as reliable. The DNA content in absolute amount of each clone was calculated through a linear relationship

**Table 1** Tea accessions used for genome size estimation by flow cytometry

| Serial no. | Clone | Source | Remarks |
|---|---|---|---|
| 1 | UPASI[a]-1 | Brookland Estate, The Nilgiris | A, B, F |
| 2 | UPASI-2 | Brookland Estate, The Nilgiris | A, B, N |
| 3 | UPASI-3 | Brookland Estate, The Nilgiris | B, C, E, N |
| 4 | UPASI-4 | Brookland Estate, The Nilgiris | A, B, F |
| 5 | UPASI-5 | Brookland Estate, The Nilgiris | B |
| 6 | UPASI-6 | Brookland Estate, The Nilgiris | A, B |
| 7 | UPASI-7 | Brookland Estate, The Nilgiris | A, B, E |
| 8 | UPASI-8 | Brookland Estate, The Nilgiris | B, C |
| 9 | UPASI-9 | Brookland Estate, The Nilgiris | G, N |
| 10 | UPASI-10 | Brookland Estate, The Nilgiris | A, F, J |
| 11 | UPASI-11 | Brookland Estate, The Nilgiris | B |
| 12 | UPASI-12 | Brookland Estate, The Nilgiris | |
| 13 | UPASI-13 | Brookland Estate, The Nilgiris | B |
| 14 | UPASI-14 | Singara Estate, The Nilgiris | B |
| 15 | UPASI-15 | Springfield Estate, The Nilgiris | A, B |
| 16 | UPASI-16 | Brookland Estate, The Nilgiris | A, B |
| 17 | UPASI-17 | Brookland Estate, The Nilgiris | B, C, N |
| 18 | UPASI-18 | Brookland Estate, The Nilgiris | A, B |
| 19 | UPASI-19 | Springfield Estate, The Nilgiris | A, D, K |
| 20 | UPASI-20 | Brookland Estate, The Nilgiris | B, E |
| 21 | UPASI-21 | Brookland Estate, The Nilgiris | B |
| 22 | UPASI-22 | Brookland Estate, The Nilgiris | A |
| 23 | UPASI-24 | Brookland Estate, The Nilgiris | A, B |
| 24 | UPASI-25 | Anamallaias, Valparai, Coimbatore | B |
| 25 | UPASI-26 | Davershola, Gudalur, The Nilgiris | A, B, F, L, |
| 26 | UPASI-27 | Anamallaias, Valparai, Coimbatore | A, B, L, N |
| 27 | TRI 2024 | TRI Sri Lanka | A, M, N |
| 28 | TRI 2025 | TRI Sri Lanka | |
| 29 | ATK 1 | Attikunna, the Nilgiris - wayanad | A, B |
| 30 | C-17 | BBTC, Singampatti | |
| 31 | CR-6017 | Craigmore, The Nilgiris | C |
| 32 | SA-6 | UPASI | D |
| 33 | SMP | Munnar, Idukki district, kerala | D, N |
| 34 | TES-34 | Tea Experimant Station, Tocklai | |
| 35 | B/5/163 | Brookland Estate, The Nilgiris | |
| 36 | BSB I | UPASI | N |

Remarks Key: A: Drought tolerant; B: good rooter; C: susceptible to drought; D: highly resistant to blister blight; E: susceptible to blister blight; F: susceptible for wind prone areas; G: moderately resistant to blister blight; J: excellent rooter; k: slow rooter; L: frost resistant; M: contain high amount of anthocyanin pigment; N: high yielding (average quality but high yield, with yield potential of 4000 kg made tea/ha)

between ratio of 2C value peaks of the sample and internal standard (*H. vulgare* cv. Sultan, 2C DNA content 11.12 pg). For each accession, two plants were taken for analysis and for each plant, three independent replicates were prepared, acquired and analyzed for DNA estimation. Data was acquired at low PMT voltage channel on X-axis as per manufacturer recommendations to avoid background noise and signal anomalies.

### Tests for cytosolic inhibitors

Tea leaves are rich in secondary metabolites such as phenolics, polyphenols and tannins. These secondary metabolites may interfere with staining of nuclei. To determine inhibitory effect of these cytosolic compounds, samples were prepared in two different ways: in one method leaves from both tea and *Hordeum* were chopped

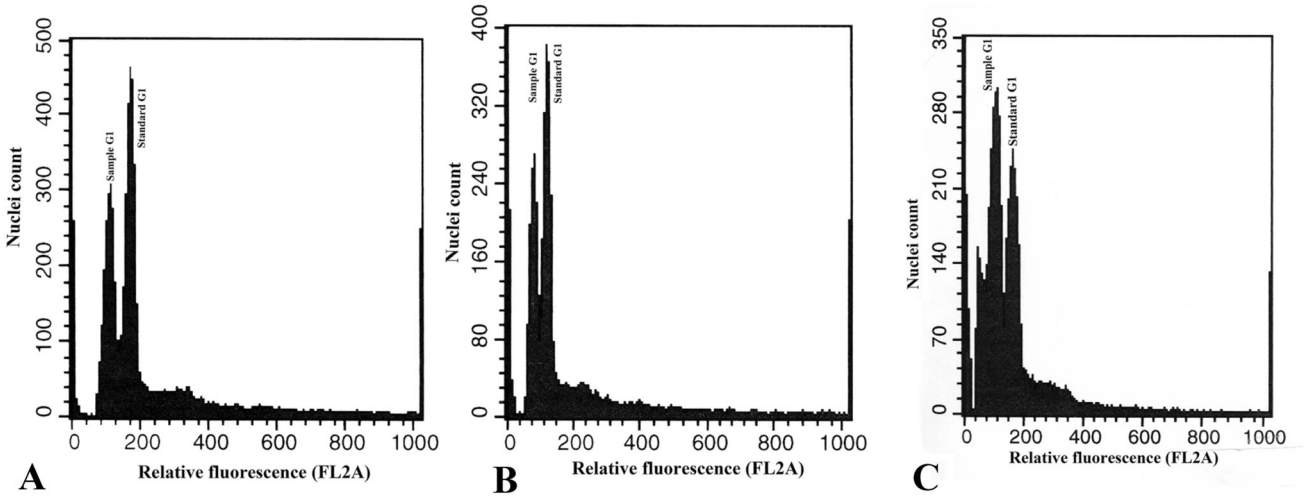


**Fig. 1** Histogram showing estimation of nuclear DNA content in absolute units. Standard G1: Peak shown by internal standard *H. vulgare* cv. sultan (2C DNA = 11.12 pg) nuclei at G1 stage. Sample G1: **a** UPASI 9, **b** UPASI 26, **c** ATK

together while in another method leaves were processed separately. The comparison of the G1 peaks of *Hordeum* from both methods revealed that peaks were sharper and showed less CV when processed independently, thereby confirming the inhibitory effect of cytosolic compounds present in tea leaves. Essential modifications were done in chopping buffer to minimize the effect of these inhibitory compounds (discussed later). All subsequent analysis were done by chopping tea and *Hordeum* leaves together for all the accessions analyzed in the present study.

### Statistical analysis

Differences and correlation obtained among different tea clones and their replicates were statistically analyzed using one-way ANOVA implemented with the software SPSS (Sigma start for Windows Version 3.1 SPSS Inc. Richmond, CA, USA). Significance of data was determined at 5 and 1% probability level.

## Results

### Optimization of DNA flow cytometry for tea clones

In the present study, three isolation buffers (Galbraith et al. 1983; Dolezel et al. 1989; Arumugnathan and Earle 1991) were compared. Armugnathan and Earle's $MgSo_4$ buffer produced best results as this buffer had shown high nuclei count and less debris. Initially, results obtained showed wider peaks and large CV with Armugnathan's method but when slight modification were done by addition of β-mercaptoethanol and EDTA to this buffer, peaks became sharper and CV was reduced significantly. Although, results acquired for tea samples showed only a single peak corresponding to the 2C value (Fig. 1). The coefficient of variation for G1 peaks among tea clones varied from 1.92 to 5.28. Unlike several other protocols, PI was not included in the chopping buffer. The nuclei were stained after they were isolated and pelleted down. This modification helped the analysis by increasing the number of intact stained nuclei and hence reduced noise and amount of debris (Dickson et al. 1992; Costich et al. 1993). RNAase was added after staining nuclei following the protocol of Lee et al. (1997). Uncoupling of PI staining and RNAase treatment also helped in reducing the CV values. Since PI was added later in protocol, therefore incubation period after adding PI was increased to 40 min followed by 30 min of RNAase treatment to avoid under-staining of nuclei.

Figure 1 illustrates the results obtained for a few representative tea clones. Results were displayed as histograms showing relative linear fluorescence (FL2 filter channel number on X-axis) and the number of fluorescent events (total nuclei counted, on the Y-axis). The nuclear DNA content estimated for each clone was expressed as ratios relative to internal standard using the position of the respective peaks. These ratios were calculated by dividing the channel number of the sample's peak by the channel number of control peak. Knowing the DNA content of the control in picograms, the relative arbitrary values for each clone was converted into absolute amounts in picogram (Table 2). The 2C peak of UPASI 3 coincided with *H. vulgare* peak, indicating that they had almost similar DNA content. In order to determine genome size of UPASI 3, its leaves were chopped along with one of the diploid clone ATK, whose genome size was estimated using *H. vulgare* as internal standard.

**Table 2** Nuclear DNA amount in Indian tea accessions estimated by flow cytometry

| Clone | Chromosome number (2n) | Ploidy (ploidy ref[a]) | 2C-value (pg) | Standard deviation (SD) | Coefficient of variation of tea G1 peak (%) |
|---|---|---|---|---|---|
| UPASI 1 | 30 | 2n = 2x | 7.44 | 0.017 | 3.87 |
| UPASI 2 | 30 | 2n = 2x | 7.51 | 0.016 | 3.78 |
| UPASI 3 | 45 | 2n = 3x | 11.46 | 0.015 | 3.19 |
| UPASI 4 | 30 | 2n = 2x | 7.54 | 0.017 | 1.92 |
| UPASI 5 | 30 | 2n = 2x | 7.69 | 0.017 | 2.91 |
| UPASI 6 | 30 | 2n = 2x | 7.23 | 0.016 | 3.80 |
| UPASI 7 | 30 | 2n = 2x | 7.53 | 0.012 | 3.99 |
| UPASI 8 | 30 | 2n = 2x | 7.51 | 0.020 | 2.29 |
| UPASI 9 | 30 | 2n = 2x | 7.50 | 0.017 | 5.22 |
| UPASI 10 | 30 | 2n = 2x | 7.23 | 0.016 | 3.54 |
| UPASI 11 | 30 | 2n = 2x | 7.42 | 0.008 | 4.62 |
| UPASI 12 | 30 | 2n = 2x | 7.73 | 0.012 | 5.01 |
| UPASI 13 | 30 | 2n = 2x | 7.33 | 0.016 | 5.51 |
| UPASI 14 | 30 | 2n = 2x | 7.42 | 0.008 | 5.28 |
| UPASI 15 | 30 | 2n = 2x | 7.63 | 0.008 | 4.71 |
| UPASI 16 | 30 | 2n = 2x | 7.53 | 0.012 | 5.48 |
| UPASI 17 | 30 | 2n = 2x | 7.42 | 0.008 | 4.83 |
| UPASI 18 | 30 | 2n = 2x | 7.73 | 0.012 | 4.35 |
| UPASI 19 | 30 | 2n = 2x | 7.62 | 0.008 | 4.37 |
| UPASI 20 | 30 | 2n = 2x | 7.33 | 0.016 | 4.85 |
| UPASI 21 | 30 | 2n = 2x | 7.32 | 0.012 | 5.02 |
| UPASI 22 | 30 | 2n = 2x | 7.73 | 0.008 | 4.88 |
| UPASI 24 | 30 | 2n = 2x | 7.71 | 0.012 | 4.71 |
| UPASI 25 | 30 | 2n = 2x | 7.23 | 0.016 | 5.95 |
| UPASI 26 | 30 | 2n = 2x | 7.53 | 0.012 | 3.30 |
| UPASI 27 | 30 | 2n = 2x | 7.32 | 0.012 | 4.73 |
| TRI 2024 | 30 | 2n = 2x | 7.73 | 0.012 | 4.88 |
| TRI 2025 | 30 | 2n = 2x | 7.62 | 0.012 | 5.13 |
| TES-34 | 30 | 2n = 2x | 7.52 | 0.012 | 4.23 |
| B/5/163 | 30 | 2n = 2x | 7.34 | 0.008 | 4.93 |
| SA 6 | 30 | 2n = 2x | 7.53 | 0.012 | 4.68 |
| CR 6017 | 30 | 2n = 2x | 7.52 | 0.009 | 5.05 |
| SMP | 30 | 2n = 2x | 7.54 | 0.012 | 4.29 |
| C-17 | 30 | 2n = 2x | 7.62 | 0.004 | 4.85 |
| ATK | 30 | 2n = 2x | 7.63 | 0.016 | 4.14 |
| BSB 1 | 30 | 2n = 2x | 7.51 | 0.004 | **5.67** |

[a]Ploidy reference: Sharma and Raina (2006)

The 2C DNA content of tea accessions varied from 7.23 to 7.73 pg which is 1.1 fold increase among different tea clones. Based on the mean DNA content, the 1Cx genome size of tea accessions was estimated to be 3673 Mb by using relation 1 pg DNA = 978 Mb (Dolezel et al. 2003). Analysis of variance confirmed significant ($p \leq 0.01$) differences in DNA content between the diploid clones. Variation between the replicates for each accession was not significant (Table 3). The DNA content of triploid (UPASI-3) accession was observed to be 11.47 pg concurrent with the expected value.

## Discussion

Tea plants are known to have very high amount of cytosolic compounds like tannins and phenolics which interfere with staining of isolated nuclei with PI which may

**Table 3** Analysis of variance (ANOVA) of 2C nuclear DNA content

| | | 1 | 2 | 3 | Total |
|---|---|---|---|---|---|
| N | | 35 | 35 | 35 | 105 |
| Sum | | 262.72 | 262.82 | 262.65 | 788.19 |
| Mean | | 7.5063 | 7.5091 | 7.5043 | 7.5066 |
| Sum of square | | 1972.817 | 1974.2718 | 1971.8015 | 5918.8903 |
| Variance | | 0.0225 | 0.0211 | 0.0236 | 0.022 |
| SD | | 0.1501 | 0.1454 | 0.1535 | 0.1483 |
| Source | d.f | Sum of squares | Mean square | F | *p* |
| Clones | 34 | 2.2676 | 0.0667 | 254.78 | $p \leq 0.001$ |
| Replicates | 2 | 0.0004 | 0.0002 | 0.8 | ns |
| Error | 68 | 0.0178 | 0.00026 | | |

result in higher CV values. Moreover stability of nuclei and DNA is also affected by these compounds. In order to overcome these problems, Armugnathan and Earle's nuclei isolation buffer was modified by adding PVP and β-mercaptoethanol which can bind to phenolics and tannins and keep them in reduced state (Loureiro et al. 2006, 2007) thereby reducing the CV values significantly. The presence of antioxidant, dithiothreitol (DTT) in buffer maintains DNA integrity in nucleus and therefore lowers down stoichiometric errors in DNA staining.

Based on the mean DNA content of all tea clones analyzed, 1Cx genome size was estimated to be 3673 Mb. Our results apparently showed higher values than the amount previously reported by Huang et al. (2013) but are almost concurrent with that of 4000 Mb amount reported by Tanaka and Taniguchi (2006). This difference in genome size can be attributed to choice of internal reference standard, modification in protocol by adding PVP and β-mercaptoethanol to chopping buffer or due to longer incubation in PI staining solution to avoid understaining, as short incubation time of 15 min in PI may not be sufficient for optimum staining. DNA amount of reference and the target should be close but should not be overlapping with 2C and 4C peaks of target species (Johnston et al. 1999). Considering this, *H. vulgare* is a good internal standard. Moreover in *H. vulgare,* nuclei can be isolated with ease; they show stability in DNA content on repeated runs and have low CV for G1 peaks (Johnston et al. 1999).

The inter accession variation observed for genome size in tea correspond to intraspecific level variation reported for many plant taxa (Laurie and Bennett 1985; Rayburn et al. 1985; Rayburn and Auger 1990; Greilhuber 2005; Moscone et al. 2003; Smarda and Bures 2010; Huang et al. 2013). The nuclear DNA variation within species has been attributed to either fluctuation within highly repetitive DNA such as retrotransposons (Bennetzen et al. 2005; Smarda and Bures 2006) or variation in chromosome number or because of amplification/deletion of DNA sequences independent of change in chromosome number (Price et al. 1983a, b). Since the chromosome number ascertained in most of presently investigated clones (Sharma and Raina 2006) were found to be diploid; the inter accession variation in tea is therefore result of amplification/deletion of DNA sequences within chromosomes and not due to alteration in chromosome number. In past, the species involved in origin of Indian Hybrid tea moved eastward and westward to spread through much of southern Asia and westward to Europe, probably bringing about secondary and tertiary centers of diversity. All these regions harbor ecologically distinct environments due to which very high variation is observed in habit, fruit and seed characteristics, and leaf size. It is, therefore, possible that these *Camellia* species underwent changes in genome size within. Such type of intraspecific DNA variation which is correlated with environmental changes is very common (Price et al. 1981; Bennett and Bennett 1992; Bennett 1985; Rayburn and Auger 1990; Singh et al. 1996). Based on the assumption that *Camellia* species involved in origin of Indian Hybrid tea might have undergone intraspecific DNA variation, it is not surprising to find such variation in presently investigated hybrid accessions. Further, the accession will exhibit variation in genome size depending upon the relative introgression of each taxa in particular accession.

Apart from rapid DNA loss on the synthesis of polyploids (Raina and Ogihara 1994; Walbot and Cullis 1985; Evans 1968; Cullis 1983), there are several reports of DNA loss in long-established polyploids (Christensen 1996; Becak et al. 1967; Southern 1967; Grant 1969; Dowrick and El Bayoumi 1969; Yamaguchi and Tsunoda 1969; Pai and Swaminathan 1960; Bhaskaran and Swaminathan 1960). Further, there are evidences suggesting that many polyploids have acquired small chromosome size of adaptive value by losing some amount of DNA within their chromosomes during evolution (Darlington 1958, 1963; Stebbins 1950; Bennett 1985; Webster and Buckner 1971; Mc William 1974; Cauderon 1977). Reduction in genome size in the presently investigated triploid accession (UPASI 3) was, however, not observed which may implicate recent origin of this polyploid. The DNA content in this accession approximated to the three times mean value of the haploid complement of 35 diploid UPASI accession.

All tea accessions used in present study were previously analyzed for genetic diversity using AFLP markers (Balasaravanan et al. 2003; Sharma et al. 2010). In both studies, AFLP markers delineated all accessions as Assam, China and Cambod morphotypes with few intermediates which were supposed to be highly heterogenous and might have originated due to hybridization between the already existing and newly introduced accessions. Similarly, in

present study, nuclear DNA content among most of the accession is comparable with few exceptions. A few accessions have shown significant variation from the expected value of DNA estimation. All these accessions were Assam Cambod or Assam China hybrids having different allelic constitution or genetic structure (Raina et al. 2011). Two accessions UPASI 6 and UPASI 21 which are Assam Cambod type (Raina et al. 2011) exhibited 2C DNA amount of 7.23 pg but at the same time two accessions TRI 2024 and UPASI 22 which are Assam type and other two accessions UPASI 18 and UPASI 12 which are Cambod type (Sharma et al. 2010) have shown larger genome size to the magnitude of 7.73 pg/2C. Similarly accessions, B/5/163, UPASI 27 which are Assam China hybrids (Raina et al. 2011) comprise of small nuclear genome size of 7.3 pg/2C in comparison to UPASI 15 which is a China type with nuclear genome of 7.63 pg/2C suggesting thereby that pure Assam, Cambod and China accessions have bigger genome size and there is reduction in genome size during hybridization. Since there is extensive introgression in past during origin of Indian Hybrid Tea, most of the hybrid accession are merely classified on the basis of morphological characteristics, therefore further investigations are needed to correlate the genome size information obtained in the present study with more purelines of Assam, Cambod and China type within the Indian Hybrid Tea swarm.

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