

CYTOMORPHOLOGICAL CHARACTERIZATION OF TEA CULTIVARS

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Abstract

Cytomorphological characterization was performed on tea cultivars, three each of *Camellia sinensis* and *Camellia assamica* species. For plant morphological study, one and a half year old healthy shoots were obtained from the selected mother bushes of the six tea cultivars. The field experiment conducted in randomized complete block design having four replications was aimed at evaluating plant height, number of leaves plant⁻¹, number of branches plant⁻¹, number of flowers plant⁻¹, fresh and dry leaf weight plant⁻¹. The data indicated significant difference between the two species with narrow leaved cultivars having increased plant height, number of leaves and branches plant⁻¹ than the broad leaved cultivars, but less number of flowers plant⁻¹, fresh and dry leaf weight. Karyotype analysis indicated that both the groups are diploid with $2n = 30$. On the basis of chromosome morphology, *C. assamica* had larger chromosomes (3-10.5µm) as compared to *C. sinensis* (3.9-8µm). *C. assamica* has relatively advanced features as compared to *C. sinensis*. However, both the groups possessed mostly median to sub-median centromeres with no secondary constrictions which possibly indicates that little or no evolutionary changes have taken place in tea and that the karyotype is still at a primitive stage, with *C. sinensis* being more primitive than *C. assamica*. Our results suggest that both the groups are different from each other in morphological as well as cytological attributes and could therefore generate more germplasm if the two species could be involved in tea breeding programs.

Introduction

The present day commercial tea population comprises three species and their derivatives (Wood & Barua, 1958). China type (*Camellia sinensis* (L.) O.Kuntze), Assam type (*Camellia assamica* (M.) Wight) and the Cambod type (*Camellia ssp. lasiocalyx* planch.ex. watt Weight). The cultivated China type is a slow growing shrub, 1-3 m high with a number of stems arising from the ground, having small, thick, deep green erect leaves. The plant is hardy and may grow in high latitudes, where winters are cold or at high altitudes in tropics. The China type is grown in subtropical regions such as Japan, China, Pakistan, Turkey and Georgia. The Assam type is more of a small tree, growing upto a height of 10-15 m having a ramifying branch system and a distinct trunk with large, glossy, horizontal and light green leaves. It is high yielding but is less tolerant to extreme weather and is well adapted to tropical conditions such as in India, Bangladesh, Sri Lanka and East Africa. Due to high content of polyphenols in tender leaves, the crop harvest of Assam type can be processed in black tea only. Cambod type reaches a height of about 6-10 m, with more or less equally developed ascending main stems. Leaves are semi-erect and size varies between extreme China and Assam types.

The out breeding characters of tea species have led to a wide natural hybridization resulting in considerable heterogeneity in the existing populations. Therefore, it is difficult to assign a definite varietal status for a crop grown in a particular region. Bezbaruah (1968) established the basic chromosome number of different *Camellia* species

to be $n = x = 15$, suggesting a monophyletic origin of all tea species and even some wild *Camellia* (*C. caudata*, *kissi* and *irrawadiansis*). The diploid chromosome number is the same in cultivated tea varieties ($2n = 2x = 30$). In Japanese taxa, more derivation from normal chromosome number is known. *C. sasanqua* is a hexaploid having $2n = 6x = 90$. *C. rosaeflora*, a native of Sri Lanka, is triploid with $2n = 3x = 45$.

Interspecific hybrids produced, naturally or artificially, by chromosomal compatibility may not always have all the attributes of high yielders. Yet increasing the number of ploidy could provide wealth of genetic stocks for the breeders and the geneticists for exploitation and use in basic as well as applied science. Out of different types of tea polyploids produced so far, dry weights, leaf size and rooting ability of triploids were higher by 14 and 109%, respectively, over diploids. Pentaploids and aneuploids were, however, poor rooters and had smaller leaves than diploids, triploids and tetraploids. Consequently breeding may have to be concentrated mostly on the production of vigorous triploids ($3n = 3x = 45$) or perhaps tetraploids ($2n = 4x = 60$), provided that the quality aspects do not deteriorate (Singh, 1980).

Improvement in tea quality and yield as in other long lived cross pollinated crops through conventional breeding is time consuming and beset with practical difficulties. For any genetic improvement program aimed at production of superior genotypes and understanding of the genetics basis of variation, growth, yield and quality parameters of tea, the knowledge of karyotype is useful to determine the chromosome morphology and other diagnostic features of the chromosomal component. This information on cytological behavior of different tea cultivars in Pakistan is lacking. The present research was therefore, initiated with the objectives to carry out cytomorphological study of existing tea cultivars and to determine the association of chromosome morphology with morphological parameters.

Materials and Methods

Morphological study of field plants: This experiment was conducted at National Tea Research Institute (NTRI), Shinkiari, Mansehra (Pakistan) during April-October 2002. Sixteen years old mother bushes of three tea cultivars of each *C. sinensis* (Qi-men, Roupi and Jue king) and *C. assamica* (Indonesian, High grown Sri Lankan and Hung shun) were selected for the purpose of obtaining cuttings. Healthy shoots were obtained from the bushes after seven months of pruning of the last year. Semi-hardwood cuttings with green and red portion were taken. The size per cutting was 4 cm (single node). The cuttings were immersed in water having Dithane-M-45 fungicide under shade for 30 minutes before planting in polythene sleeves of 8 x 3 cm in the nursery, to keep the cuttings moist, and free from fungus attack. The pH of the soil was 5.5 with mean temperature of 13-32°C and humidity 45-73% during the growing season. All cultural practices were kept constant. Single stem plants of uniform heights were selected after one and a half year to start the experiment.

The six tea cultivars were evaluated in a randomized complete block design with four replications. Row to row and plant to plant distance was 10 cm each. Data were recorded on five randomly selected plants of each cultivar for plant height, branches plant⁻¹, leaves plant⁻¹, flowers plant⁻¹, fresh and dry leaf weight plant⁻¹. The data were analysed using statistical software Mstat-C following the standard linear model for randomized complete block design. Least significant difference (LSD) test was used for mean comparison among the cultivars. Genotypic correlations were calculated to determine the strength of association among the morphological parameters (Arshad *et al.*, 2002).

Cytological study

Root initiation for karyotype study: Softwood cuttings from the mother bushes of the same 6 cultivars of *C. sinensis* and *C. assamica* were taken in July, 2002 from NTRI, Shinkiari. Cuttings were rooted in greenhouse at Horticultural Research Programme (HRP), NARC, Islamabad. Eighteen cuttings of each cultivar were planted for rooting following the method reported by Hafeez *et al.*, (1991). Leaves from the base of each cutting were removed and top four leaves were left intact. Before treating, each cutting was wounded at the base with two equidistant 2.5 cm long vertical incisions through the bark and cambium. Basal side of the cuttings was subsequently dipped in 2% Benlate solution (fungicide) to prevent rotting and then in 3000 ppm of Indole butyric acid (IBA) in talc powder. Following IBA treatment, cuttings were planted 5-6 cm deep, keeping 10 cm distance between rows in sand beds covered with polythene sheets in the greenhouse with 50% shading, 85% relative humidity and 25°C day/night temperature. Intermittent mist was applied for 30 seconds after an hour interval during day time. Cuttings were evaluated after 3-4 weeks for the formation of callus. Cuttings started rooting in 6-8 weeks. Healthy, whitish roots were collected hourly during 0600-1800 hrs, to get cells in active cell division stage of mitosis for karyotype analysis.

Karyotype analysis: Chromosome preparation for the sample of each type of tea was carried out following Arun & Sharma (1980). This involved collection of young roots of 1-2 cm and kept in colchicine/5m M8 hydroxyquoniline at room temperature for 3-4 hours, followed by washing with distilled water. The specimens were fixed in 3 ethanol:1 acetic acid solution at room temperature for 24 hours, followed by washing with distilled water and citrate buffer (0.01 M Citric acid monohydrate + 0.01 M Trisodium citrate dihydrate) having 4.6 pH. Meristematic portion of 1-2 mm root tips were excised which were subjected to enzymatic maceration with 3% cellulase (onozuka R 10) + 2% pectolyase (Y-23) at 37°C for 4-5 hours, followed by a thorough wash with citrate buffer and distilled water. Cells were gently tapped and spread on the slide in a drop of fixative and air dried. A drop of aceto-carmin was put on the slide and covered with coverslip. Slides were examined under microscope and photographs of 10-15 good cells with well spread chromosomes were taken. Chromosomes were classified according to centromere position as median (M), submedian (SM), subterminal (ST) and terminal (T) types, following the nomenclature given by levan *et al.*, (1964). Data were recorded for the following parameters relevant to chromosome morphology.

Absolute length = Length of individual chromosome

$$\text{Relative length} = \frac{\text{Length of individual chromosome}}{\text{Total length of haploid complement}} \times 100$$

$$\text{Arm ratio} = \frac{\text{Long arm length}}{\text{Short arm length}}$$

Results

Plant height: Significant differences ($p < 0.05$) were exhibited among the tea cultivars for plant height, which ranged from 11.32 to 16.66 cm (Table 1). Narrow leaved cultivars i.e., Roupi, Jue king and Qi-men were taller with somewhat similar plant heights of 16.6, 16.3 and 15.0 cm, respectively. Broad leaved tea cultivars viz., Hung Shun, High grown Sri Lankan and Indonesian had short plants of 11.3, 12.2 and 12.7 cm, respectively. Plant height had significant positive genetic correlation with number of leaves ($r_G = 0.86$, $p < 0.05$) and number of branches ($r_G = 0.77$, $p < 0.05$) (Table 2). However, plant height had negative genetic correlation with number of flowers ($r_G = 0.82$, $p < 0.05$), fresh leaf weight plant⁻¹ ($r_G = 0.92$, $p < 0.05$) and dry leaf weight plant⁻¹ ($r_G = 0.87$, $p < 0.05$).

Leaves plant⁻¹: The six tea cultivars differed significantly ($p < 0.05$) for leaves plant⁻¹ which ranged from 8.6 to 20.2 (Table 1). Maximum number of leaves was recorded for narrow leaved cultivars i.e., Qi-men, Roupi and Jue king with leaves of 20.24, 19.07 and 18.05, respectively. Broad leaved cultivars of Indonesian, High grown Sri Lankan and Hung Shun had significantly less number of leaves of 13.60, 15.24 and 8.62, respectively. The data revealed that number of leaves had significant positive genetic correlation with number of branches ($r_G = 0.92$), however, a significant but negative genotypic correlation of leaves number was found with number of flowers ($r_G = -0.90$), fresh leaf weight ($r_G = -0.85$) and dry leaf weight ($r_G = -0.87$) (Table 2).

Branches plant⁻¹: Data pertaining to number of branches of tea cultivars showed significant difference for branches plant⁻¹ and ranged from 7.53 to 15.35 (Table 1). Maximum number of branches were recorded for narrow leaved cultivars, Qi-men, Roupi and Jue King with the highest count of branches of 15.35, 15.05 and 15.28, respectively. Broad leaved cultivars, Indonesian, High grown Sri Lankan and Hung Shun had significantly low count of branches of 9.20, 14.20 and 7.53, respectively. The data showed that number of branches had significant negative genotypic correlation with fresh leaf wt. ($r_G = -0.86$) and dry leaf wt. ($r_G = -0.93$) (Table 2).

Flowers plant⁻¹: The six tea cultivars exhibited significant difference ($p < 0.05$) for flowers plant⁻¹ ranging from 0.86 to 5.2 (Table 1). The highest number of flowers plant⁻¹ was recorded for broad leaved cultivars viz., Indonesian, High grown Sri Lankan and Hung Shun, with flowers of 2.8, 4.2 and 5.2, respectively. The narrow leaved cultivars viz., Qi-Men, Roupi and Jue King, had significantly low count of flowers of 0.86, 1.7 and 2.3, respectively. A non significant genotypic correlation of the number of flowers plant⁻¹ was manifested with leaf area ($r_G = 0.64$) (Table 2).

Leaf area plant⁻¹: Analysis of variance showed significant ($p < 0.05$) differences among the tea cultivars for leaf area plant⁻¹ with a range of 22.1 to 62.5 cm² (Table 1). As expected, maximum leaf area was observed for broad leaved cultivars i.e., Indonesian, High grown Sri Lankan and Hung Shun with leaf area of 62.5, 50.5 and 48.6 cm², respectively. The narrow leaved cultivars i.e., Qi-Men, Roupi and Jue King had significantly less leaf area of about 22.1, 36.8 and 42.87 cm², respectively. There was a significant positive genotypic correlation ($p < 0.05$) of leaf area with fresh weight ($r_G = 0.82$) and dry weight of leaves ($r_G = 0.79$) (Table 2).

Fresh and dry leaf weight: Differences among the six tea cultivars were significant ($p < 0.05$) for fresh leaf weight which ranged from 0.6 to 1.6 g (Table 1). Maximum fresh leaf weight was observed for broad leaved cultivars, Indonesian, Hung Shun and High grown Sri Lankan, with fresh leaf weight of 1.7, 1.5, 1.3 g, respectively. Narrow leaved cultivars viz., Qi-Men, Roupi and Jue King had significantly less fresh leaf weight of about 0.64, 0.58 and 0.61 g, respectively. Highly significant positive genotypic correlation ($p < 0.01$) was observed for fresh leaf weight with dry leaf weight ($r_G = 0.98$) (Table 2). Average dry leaf weight ranged from 0.16 to 0.47 g among the 6 tea cultivars. Maximum dry leaf weight was observed for broad leaved cultivars viz., Indonesian, High grown Sri Lankan and Hung Shun with a dry leaf weights of 0.46, 0.43 and 0.30 g, respectively. The narrow leaved cultivars viz., Qi-Men, Roupi, and Jue King had significantly less dry leaf weight i.e., 0.18, and 0.16 g, respectively.

Karyotype analysis of broad leaved cultivars (*C. assamica*): The chromosomes were numbered from 1 to 15 according to their lengths; the longest being number 1, the next longest being number 2, and so on, while the shortest being number 15. The homologous chromosomes were tentatively paired on the basis of their morphology and size as proposed by Levan *et al.*, (1964). Chromosomes were mostly of medium size but one pair of small chromosome was also recorded (chrom. # 15). The absolute length ranged from 3 to 10.5 μm , and the relative length ranged from 2.91 to 10.2% (Table 3a). The arm ratio varied from 1.0 to 3.66. Based on the arm ratio, the chromosome complement comprises 3 submetacentric, 1 subtelocentric and 11 metacentric including one satellited chromosome which was chrom. # 6. Chromosomes 7, 8, 9 and 10 did not show much difference in lengths (6.9, 6.8, 6.6 and 6.4), respectively. However, they could be differentiated based on the positions of centromere and staining ability (Fig. 1). Chromosome 7 is submetacentric and chromosome 8 is subtelocentric, while chromosome 9 and 10 are metacentric. Chromosome 10 is uniformly stained while the long arm of chromosome 9 is darkly stained, which can be easily differentiated from chromosome 10. Chromosomes 4, 5 and 6 also did not show considerable difference in lengths (7.77, 7.58 and 7.28 μm). Chromosome 5 is submetacentric while chromosomes 4 and 6 are metacentric. Chromosome 4 is uniformly stained, while chromosome 6 has a nucleolar organizer.

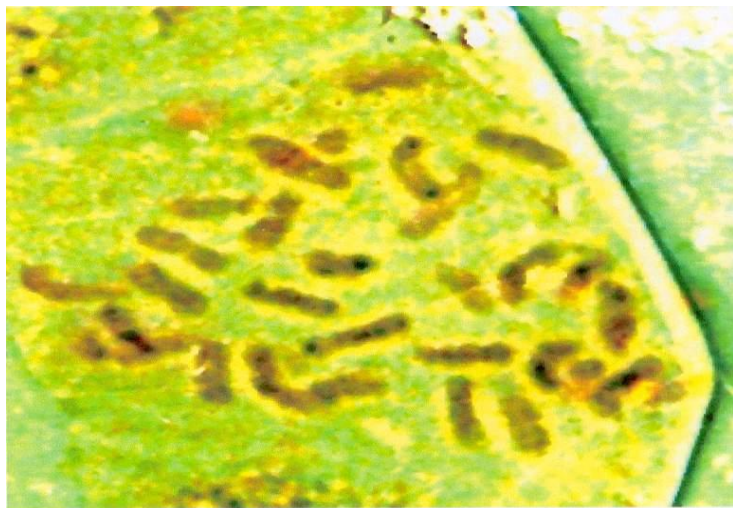


Fig. 1. Chromosomes of Broad Leaved cultivars of tea (*Cammellia assamica*).

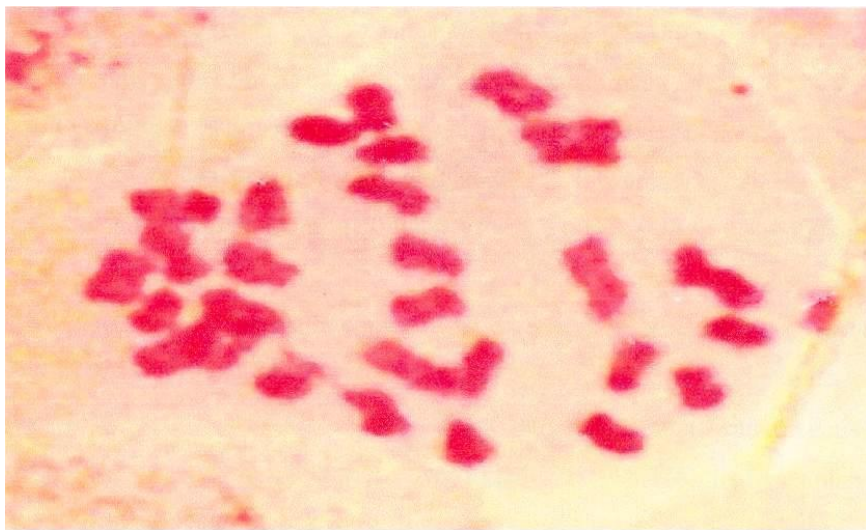


Fig. 2. Chromosomes of Narrow Leaved cultivars of tea (*Camellia sinensis*).

Karyotype analysis of narrow leaved cultivars (*C. sinensis*): The absolute length ranged from 3.9 to 8.0, (Table 3b) and the relative length varied from 5 to 10%, while the arm ratio ranged from 1.10 to 3.5. Based on the arm ratio, the chromosome complement comprised two submetacentric, two subtelocentric and 11 metacentric chromosomes (Fig. 2). Chromosome 1 showed the maximum length of 8 μm followed by chromosome 2 and 3 which did not show considerable difference in lengths (6.3 and 6.1 μm), but their staining ability made them distinctive from each other. Short arm of Chromosome 2 is darkly stained while distal parts of both the arms of chromosome 3 are densely stained. Chromosomes 4, 5 and 6 are not distinctively different in their length (5.5, 5.4 and 5.3 μm , respectively). Chromosome 5 is subtelocentric and chromosomes 4 and 6 are metacentric. Distal end of long arm of chromosome 4 is darkly stained while short arm of chromosome 6 is darkly stained. Chromosome 7 to 14 could be differentiated in having absolute lengths of 4.9, 4.6, 4.5, 4.5, 4.4, 4.3, 4.2 and 4.1 μm , respectively. Chromosome 8 and 9 are submetacentric, while chromosome 10 is subtelocentric. Chromosome 8 is uniformly stained while both the arms of chromosome 9 are darkly stained which can be easily discriminated from chromosome 8. Chromosome 11 to 14 are metacentric but their respective staining ability differentiate them from each other. Short arm of chromosome 11 is darkly stained. Distal part of the short arm of chromosome 13 is darkly stained. Distal parts of both the arms of chromosome 14 are darkly stained. Chromosome 15 is metacentric and is uniformly dark stained similar to chromosome 12 but their respective absolute lengths make them separate pairs.

Discussion

Plant morphological study: Tea is an important perennial crop. The vegetative parts i.e. two leaf and a bud is economically important and it can be propagated by seed. A species valued for its vegetative parts but propagated by seed would offer the greatest chance of success to induce polyploidy. The aim of tea breeding is to develop high yielding tea per unit area of bush surface with acceptable quality under different agroclimatic conditions. Clones are selected from tea fields in production farms, seedling nurseries and from

special plantings of selected seeds. Ideally selection should be possible during the nursery stages to avoid unwanted clones in advanced yield trials. Studies of morphological and physiological characteristics of clones in relation to the components of yield should aid selection efficiency (Squire, 1985) and hence reduce the proportion of poor clones involved in the clonal trials. Productivity in tea is mainly related to the production of dry matter and its partitioning to the part of plant which makes up the commercial yield.

The various cultivars used in this study indicated significant differences for fresh and dry leaf weight. Cultivars of narrow leaved group had less fresh and dry leaf weight (0.16-0.18g) as compared to cultivars of broad leaved group (0.30-0.47g). Similar results have been found by Waheed *et al.*, (2000) who reported that narrow leaved group had high number of leaves but less fresh and dry leaf weight. This is justified by the study of Kumar *et al.*, (1993) who reported that clones with high yield of green leaf do not always necessarily project the high content of dry matter since clonal differences exists in partitioning of dry matter. Leaf area for cultivars of narrow leaved group was significantly smaller (22.12 - 42.40 cm²) than leaf area for broad leaved group (48.64-62.49 cm²) as manifested by the nomenclature of each group. This is in confirmation to the study of Banerjee (1987). Narrow leaved group has more number of leaves but smaller leaf size. Barua & Dutta (1971) also advocated use of leaf size as a selection criterion for yield in areas where the population consists mainly of China and China-hybrid types of tea. However, Murty & Sharma (1988) observed positive correlation between surface area, leaf size, accumulation of dry matter in foliage and wood in the top 20 cm and yield.

The size of bush in tea with well established frames has a definite bearing on selection. Data regarding number of branches among cultivars of two tea groups was significant at 5 % level of probability. Cultivars of narrow leaved group had more number of branches (15.05-15.35 plant⁻¹) as compared to cultivars of broad leaved group (7.53-14.20 plant⁻¹). This is in confirmation with the results of Waheed *et al.*, (2000). Significant correlations were observed between yield and bush area and between number of branches and bush area in Assam (Barua & Dutta, 1971). Results on number of flowers among the cultivars were significant at 5% level of probability. Cultivars of narrow leaved group had less number of flowers (0.86-2.28), while cultivars of broad leaved group had more number of flowers (3.0-5.0). The reproductive phase is always energy demanding and low count of flowers in a species would mean that most of the photosynthates and stored food is utilized in the development of plant vigour with more plant height, number of leaves and number of branches as has been observed for cultivars of narrow leaved group. Significant difference for plant height was found among the cultivars of two tea species i.e., *C. sinensis* and *C. assamica*. Narrow leaved varieties had greater plant heights (15-16 cm) as compared to broad leaved varieties (11-12 cm). The data indicated that plant height was positively correlated with number of leaves, number of branches and negatively correlated with number of flowers, leaf area, fresh and dry leaf weights as observed by Bakhsh *et al.*, (2006) in chickpea.

Karyotype analysis: Karyotype is referred to as group of characteristics that identifies a particular chromosome set. Karyotype suggests primitive or advance features of an organism. The absolute chromosome lengths ranged from 2.91 to 10.2 µm in *C. assamica* and 3.9 to 8 µm in *C. sinensis*. Similar results with varying chromosomal lengths of tea cultivars were reported by Mutharoo *et al.*, (1996). The difference between the largest and the smallest chromosome in *C. assamica* was 7.29 µm as compared to *C. sinensis*

which had a difference of 4.10 μm . This indicates that *C. assamica* has relatively advanced features as compared to *C. sinensis*. However, both the groups possessed mostly median to sub-median centromeres with no secondary constrictions which possibly indicates that little evolutionary changes have taken place in tea and that the karyotype is still at a primitive stage with *C. sinensis* being more primitive than *C. assamica*. In flowering plants there is predominant trend towards asymmetric karyotype, however, vegetatively propagated plants have mostly symmetric karyotype (Lewitsky, 1931). The karyotype analysis showed that both the species have heterochromatin flanking the centromeres, however, it is more prominent in *C. Sinensis*. It appears that majority of the repeat DNA sequences are localized around the edges in *C. sinensis* unlike *C. assamica* where it could be scattered through out the entire length of chromosomes.

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