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ORIGINAL ARTICLE

Study on Karyotype of Selected Tea Clones (*Camellia sinensis*) in Iran

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ABSTRACT
In order to study karyotype of selected tea clones (Camellia sinensis) in Iran, squash technique was used. Root samples were pretreated with alpha-bromonaphthalene and then were fixed in Farmer solution. It was hydrolyzed by 1 normal of chloridric acid and stained byaceto-iron-haematoxylin. Total chromosome length, long and short arm length, ratio of long to short arm, and centromere index were measured and also numbers of chromosomes were counted. In all genotypes numbers of chromosomes were 2n=30. In table of correlation coefficients, ahigh positive correlation was observed between total length ofchromosome with length of long arm ($r=0.987^{++}$), total length ofchromosome with length of short arm ($r=0.973^{++}$), and length of long arm with short arm ($r=0.925^{++}$), while there was a high negative correlation between the ratio of long to short arm with centromere index ($r=-0.990^{++}$). Based on the analysis of main components, total length (TL), long arm length (LA), and short arm length (SA) contributed in creation of the first component, and the most contribution is attributed to long arm of chromosome with coefficient of 0.984. Also, in order to create the second component, parameters such aslong arm to short arm ratio (AR) and centromere index (CI) were involved from which centromere indexwith coefficient of 0.837 played the greatest role. The results showed that in terms of %TF statistics G_1 and G_7 respectively had the most symmetric and asymmetric karyotypes, and in terms of TL,DRL, TL, and L/S statisticsG_2 and G_7respectively had the most symmetric and asymmetric karyotypes.
Keywords: *tea, Camellia sinensis, chromosome, karyotype, squash technique*

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INTRODUCTION

Tea with scientific name of *Camellia sinensis* (L) O. Kuntze is anevergreen perennial plant ofTeaceae family with many health benefits and is consumed as a refreshing drink. Free confluences between heterozygous tea plants over past years have increased genetic heterogeneity and morphological, physiological, and biochemicaldifferences among plants in tea gardens. According to the increase in population and the need to produce more tea in one hand and aging in tea plants associated with a gradual decline in performance and deficiency in quality on the other hand, it is thus essential to study the selective tea clones in order to determine the chromosome number,ploidy level, structural differences of chromosomes, cytogenetic diversity, karyotypic characteristics, and possibly identification of B chromosomes, so that an important would be taken with sufficient knowledge of cytogenetic status of the selected clones to refine and makecultivarsresistant toliving and non-living stresses leading to an increase in the quantity and quality of theproduct. First reviewed chromosomes in tea and the number of chromosomes of the plant was reported to be 2n=2x=30 [1]. It has been reported that chromosomal structure in diploid cultivars of tea are often similar and *C. japonica* species vary in number ofchromosomes and ploidy level [2]. The results of a cytogenetic study on nine genotypes of tea from imported genetic resources in Iran showed that all the genotypes were diploid (2n=2x=30) and also had two types of metacentric and sub metacentric chromosomes with varying frequency. Study of

chromosome behavior in three wild species of tea and a cultivar from tea-rich provinces of China indicated that all karyotypes have a given formula as 2n=30=26m+4Sm, but were different in terms of satellite position and length of chromosome [3].

MATERIALS AND METHODS

In this study, seven tea clonesincluding clones 13, 222, 456, 280, 63 p 85, and 100 in tea research stations (Table 1) were used to conduct cytogenetic studies by squash method [4]. After cutting the youngest roots from each clone, they were pretreated with saturated alpha-bromonaphthalene solutionat 25°Cfor 8 hours. In order to fix roots, they were placed in Carnoy solution (1 part pure acetic acid with 3 parts ethylic alcohol) in a refrigerator for 24 hour.After washing the roots with abundant distilled water, they were kept in 70% ethylic alcohol in refrigerator. Sample tissues were softened with 1 normal chloridric acidat 60 ° C for 10 minutes. Then, roots were stained with aceto-iron-haematoxylincolored solution at 30 to 35 ° C for 16 h [5,6].

In order to prepare microscopic slides, a drop of 45% acetic acid was spilled on the slide and 1 to 2 millimeter apical meristems separated from the root were added to it. After placing a coverslip on it and performing squash, the sample was prepared for microscopic study.The best metaphasic cells were photographed.After counting chromosomes of each sample, morphological traits of chromosomes of each clone including total chromosome length (TL),long arm length (LA),short arm length (SA), long arm to short arm ratio (AR), and centromere index (CI) were measured by Micro measure software.Analysis of variance based on factorial tests with two factors of genotype and chromosome was done in a completely randomized design in triplicate using SAS, and SPSS was used for grouping clones. Also, a number of statistics were used to assess karyotype symmetry as follows:

1. Total formpercentage of karyotype (%TF): This isa method to assess karyotype symmetry proposed by Huziwara [7]. He used this index as a karyotype classification index.
2. Difference range of relative length (DRL): This is another index to compare karyotype symmetry [7].
3. Telomere length in micron (TL).
4. The ratio of the longest to the shortest chromosomes (L/S).

Table 1:Profile of Selected Tea Clones for Cytogenetic Studies and Their Breeding Status

Breeding Status	Origin	Genotype	Genotype Number
Under test in tea research station of Khalsar, Langerood	Iran	13	1
Under test in tea research station of Khalsar, Langerood	Iran	120	2
Under test in tea research station of Khalsar, Langerood	Iran	63p85	3
Under test in tea research station of Fshalym, Fouman	Iran	456	4
Under test in tea research station of Fshalym, Fouman	Iran	222	5
Under test in all tea research station	Iran	100	6
Under test in tea research station of Fshalym, Fouman	Iran	280	7

RESULTS AND DISCUSSION

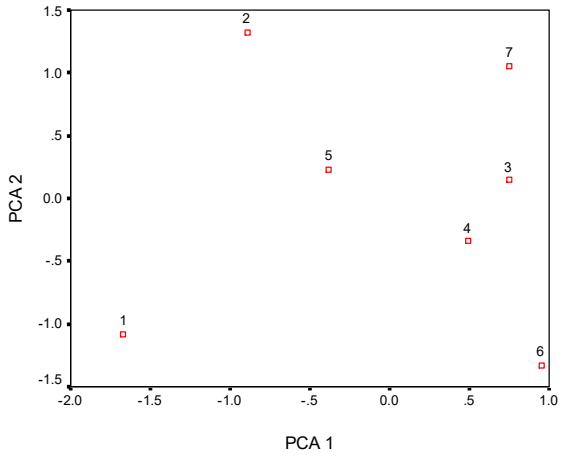
The results of principal component analysis based on morphological characteristics of genotypes are presented in Tables 2 and 3. Total length of chromosome (TL), length oflong arm (LA), and length of short arm (SA) contributed in creation of the first component, that the highest impact was related to long arm of chromosome with a coefficient of 0.984. Also, parameters such aslong arm to short arm ratio (AR) and centromic index (CI) were involved in the second component and centromic index had thehighest impact with a coefficient of 0.837. In order to separate and classify genotypes, a plot was drawn (Graph 1). Genotypes were divided into three categories, namely genotypes 3,4,6 and 7 in the first group and genotypes 2 and 5 in the second group and genotype 1 in the third group. This classification can verify the results of genotypes classification based on cluster analysis.Given the eigenvalue of 3.232, variance of 64.645, and special coefficientsfor the first component listed in Table 3 and 4, traits such as total length of chromosome (TL), length of long arm (LA), and length of short arm (SA) had the greatest variance between the traits and were effective in grouping genotypes.Given the eigenvalue of 1.758, variance of 35.167, and special coefficientsfor the first component listed in Table 2 and 3, it can be concluded that traits such as long arm to short arm ratio and centromic index had the maximum variance between other traits and were effective in grouping genotypes.

Table 2: Special Coefficients for the Two Principal Components Derived from PCA Based on Karyotypic Characteristics of Genotypes

Traits	First component	Second component
TL	0.945	0.325
LA	0.984	0.177
SA	0.845	0.533
AR	0.601	-0.797
CI	-0.544	0.837

Table 3: Eigenvalue and Variance Derived from PCA Based on Morphological Characteristics of Chromosome

Cumulative Variance	Relative Variance	Eigenvalue	Component
64.645	64.645	3.232	First component
99.812	35.167	1.758	Second component



Graph (1): The plot of the first two factors derived from PCA to classify genotypes based on karyotype data

In order to determine karyotype symmetry of the genotypes, %TF, DRL, TL, and L/S were used. Results of the statistics in Table 4 showed that in terms of %TF (total form percentage) genotypes G1 and G7 were the most symmetrical and asymmetrical karyotypes, respectively, and that in terms of DRL, TL, and L/S G2 and G7 had the most symmetric and most asymmetrical karyotypes. Since symmetrical karyotype had more primitive evolutionary status compared to asymmetric karyotype [8], G1 and G2 are more likely to have primitive evolutionary status and G7 is more likely to have a more advanced evolutionary status compared to the other genotypes. Due to the limited range of statistical changes in the genotypes, judgments about the symmetry and asymmetry of the karyotype are not quite certain. In order to determine chromosomes type and karyotype formula of the genotypes, method of Levan *et al* was used.
Results in Table 4 indicated that G1, G3 and G4 had a similar karyotype formula as 12m+3sm. This karyotype formula has been reported in a study on tea karyotype in Darjeeling Colons of 13, 35, and B-275 [9]. Genotypes of G2, G5, G6, and G7 have karyotype formula of 13m+2sm, 14m+1sm, 11m+4sm, and 10m+5sm.

Table 4: Statistics calculated from chromosomal morphologic characterizationsto measure karyotypic asymmetry of the genotypes

Karyotype Formula	L/S	TL	DRL	TF%	Genotypes
12m + 3Sm	1.69	52.32	3.6	41.9	G1
13m + 2Sm	1.40	47.06	2.26	41.67	G2
12m + 3Sm	1.73	61.038	3.5	41.62	G3
12m + 3Sm	1.80	55.75	3.9	40	G4
14m + 1Sm	1.66	56.979	3.1	41.8	G5
11m + 4Sm	1.65	59.196	3.3	40.57	G6
10m + 5Sm	1.93	65.983	4.4	39.41	G7

Total form percentage (TF): Ratio of total length of short arms of chromosomes of a genotype to its total chromosomes.
Difference range of relative length of chromosomes (DRL): Difference between the minimum and maximum relative length of chromosomes.
TL: Telomere length in micron.
L/S: The ratio of the longest to the shortest chromosomes.

Karyotype analysis of genotype 1

Average total length of chromosomes in this genotype ranged from4.64124 to 2.74047 micrometers. Based on microscopic observation and the resulting images, number of chromosomes in this genotype was 2n=2x=30 and satellite chromosomes and B chromosomes were not observed. Such conclusion was reported on Kenyan tea cultivarsby Wachira *et al* [10]. Furthermore, similar results have been reported on imported tea genotypes in Iran by Qolami [9]. Lack of satellite chromosome is due to low activity heritability of nucleolus organizer regions that results in failure of this genotype to produce secondary compaction. In terms of %TF, this genotype had the most symmetric karyotype among other genotypes, indicating its more primitive evolutionary status. According to the method of Levan *et al* [11], chromosomes were metacentric and sub-metacentric, which is consistent with results of Qolami [9] onimported tea genotypes in Iran. Also, similar results have been reported about Assam, China, and Cambodia cultivars by Mataro *et al* [12].

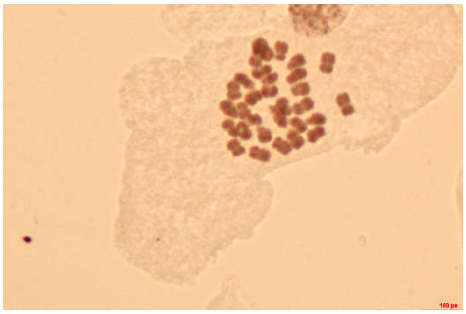
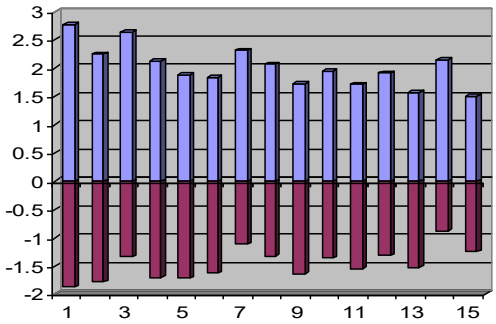


Figure (1): chromosomal image of genotype 1



Graph (2): ideogram of genotype 1

Karyotype analysis of genotype 2

Average total length of chromosomes in this genotype ranged from 3.703763 to 2.636580 micrometers. Based on microscopic observation and the resulting images, number of chromosomes in this genotype was 2n=2x=30 and satellitechromosomes and B chromosomes were not observed.Such conclusion was reported on Kenyan tea cultivars by Wachira *et al* [10]. Lack of satellite chromosome is due to low activity heritability of nucleolus organizer regions that results in failure of this genotype to produce secondary compaction or disability of used methods. Karyotypic formula and some statistical measures of karyotypic asymmetry are given in Table 5. In terms of statistics for DRL, TL, and L/S, this genotype had the most symmetric karyotype among other genotypes, indicating its more primitive evolutionary status. Chromosomes were metacentric and sub-metacentric, which is consistent with results of Mataro *et al* [12] on Assam, China, and Cambodia cultivars.

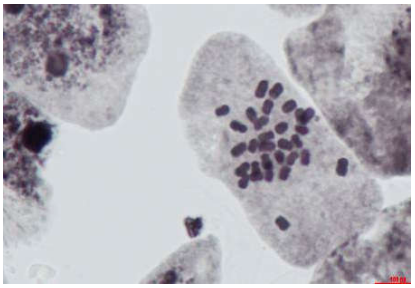
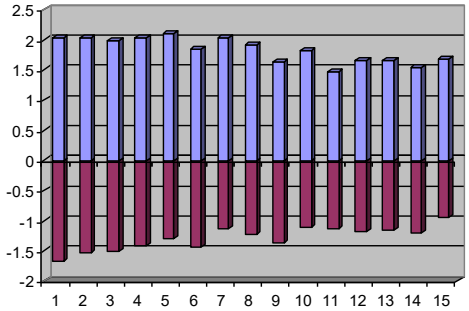


Figure 2: chromosomal image of genotype 2



Graph (3): ideogram of genotype 2

Karyotype analysis of genotype 3

Average total length of chromosomes in this genotype ranged from 5.173072 to 2.99383 micrometers.Based on microscopic observation and the resulting images,number of chromosomes in this genotype was 2n=2x=30. No B chromosomes were observed in this genotype.Chromosomes were metacentric and sub-metacentric, which is consistent with results of Qolami *et al* [9] on imported tea genotypes in Iran.

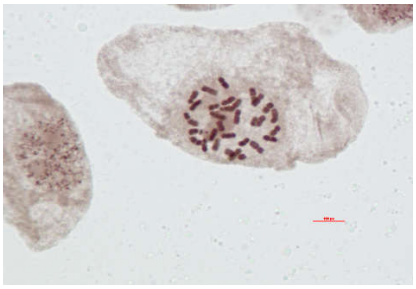
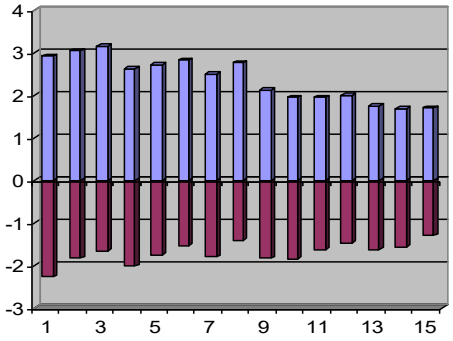


Figure (3): chromosomal image of genotype 3



Graph (4): ideogram of genotype 3

Karyotype analysis of genotype 4

Average total length of chromosomes in this genotype ranged from 4.83182 to 2.68230 micrometers. Based on microscopic observation and the resulting images, number of chromosomes in this genotype was 2n=2x=30. No satellitec hromosomes and B chromosomes were observed. Such conclusion was reported on Kenyan tea cultivars by Wachira *et al* [10]. Also, similar results have been reported on imported tea genotypes in Iran by Qolami 9]. Lack of satellite chromosome is due to low activity

heritability of nucleolus organizer regions that results in failure of this genotype to produce secondary compaction. Karyotype formula of this genotype was similar to genotype 1 and chromosomes were metacentric and sub-metacentric, which is consistent with results of Qolami [10] onimported tea genotypes in Iran. Furthermore, similar results have been reported on tea genotypes by Mataro *et al* [12].

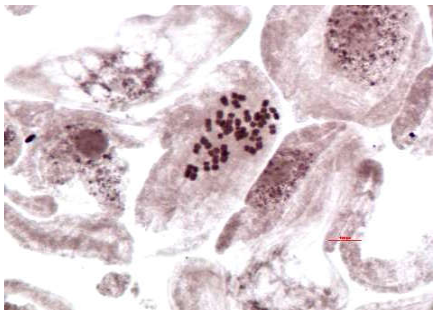
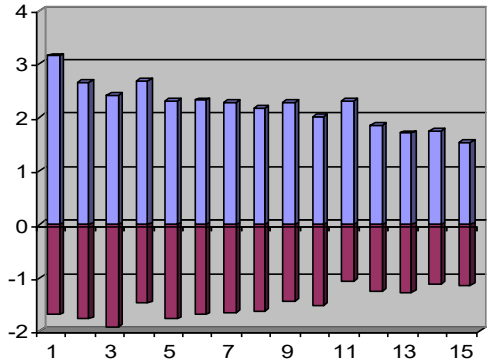


Figure (4): chromosomal image of genotype 4



Graph (5): ideogram of genotype 4

Karyotype analysis of genotype 5

Average total length of chromosomes in this genotype ranged from 2.895598 to 4.811100 micrometers. Based on microscopic observation and the resulting images, number of chromosomes in this genotype was 2n=2x=30. Nosatellite chromosomes and B chromosomes were observed. Such conclusion was reported on Kenyan tea cultivars by Wachira *et al* [10]. Furthermore, similar results have been reported on imported tea genotypes in Iran by Qolami [9]. Lack of satellite chromosome is due to low activity heritability of nucleolus organizer regions that results in failure of this genotype to produce secondary compaction. Chromosomes were metacentric and sub-metacentric, which is consistent with results of Tong *et al* [3] ontea genotypes.

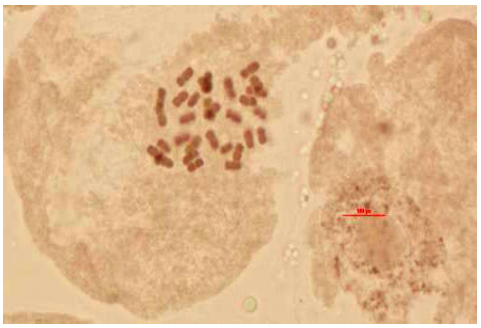
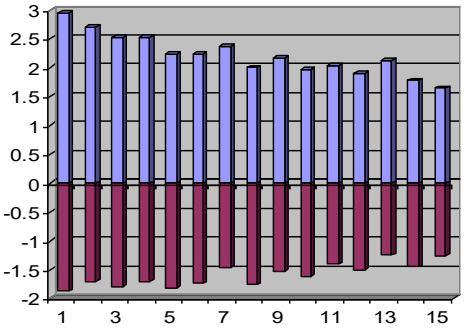


Figure (5): chromosomal image of genotype 5



Graph (6): ideogram of genotype 5

Karyotype analysis of genotype 6

Average total length of chromosomes in this genotype ranged from 2.997610 to 4.955496 micrometers. Based on microscopic observation and the resulting images,number of chromosomes in this genotype was 2n=2x=30 and satellite chromosomes and B chromosomes were not observed. Such conclusion was reported on Kenyan tea cultivars by Wachira *et al* [10]. Lack of satellite chromosome is due to low activity heritability of nucleolus organizer regions that results in failure of this genotype to produce secondary compaction. Chromosomes were metacentric and sub-metacentric, which is consistent with results of Qolami [9] onimported tea genotypes in Iran. Also, similar results have been reported by Tong *et al* [3].

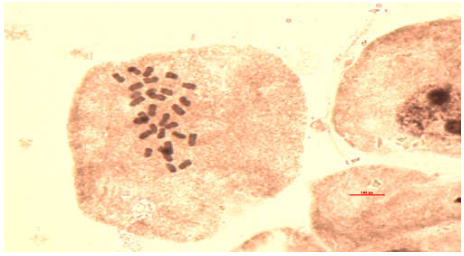
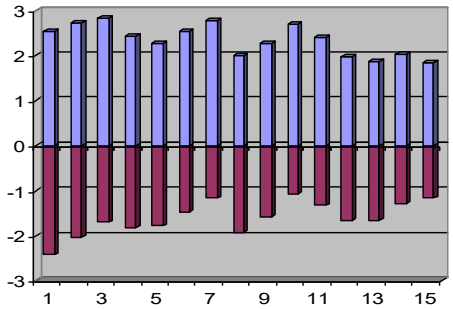


Figure (5): chromosomal image of genotype 6



Graph (8-3): ideogram of genotype 6

Karyotype analysis of genotype 7

Average total length of chromosomes in this genotype ranged from 3.115294 to 6.014745 micrometers. Based on microscopic observation and the resulting images,number of chromosomes in this genotype was 2n=2x=30. No satellite chromosomes were observed. Such conclusion was reported on Kenyan tea cultivars by Wachira *et al* (1999). In terms of statistics for %TF, DRL, TL, and L/S, this genotype had the most asymmetric karyotype among other genotypes, indicating its more advanced evolutionary status than pther genotypes. The total length ofchromosome represents DNA amount in nucleus and smaller chromosomes have less DNA [8]. This genotype hastotal length of chromosome longer than other genotypes, indicating that the amount of DNA in the nucleus is higher. DNA content and chromosome size are associated with the degree of species specificity [8]. Therefore, this advantage can be used to combine cultivars and breed.

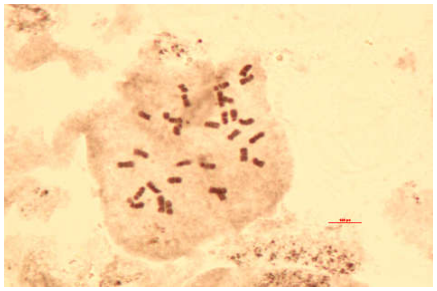
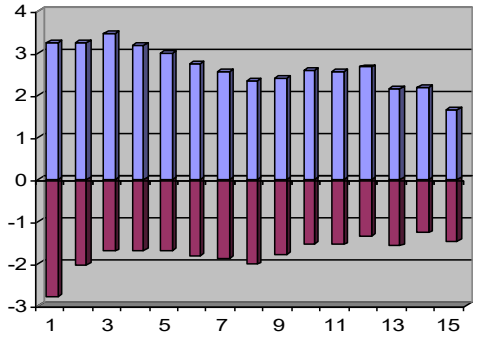


Figure (7): chromosomal image of genotype 7



Graph (8): ideogram of genotype 7

Correlation coefficients of chromosomal morphology in Table 5 show that there is high correlation between the length of long arm and chromosome length (0.987) at 1% probability level. There is correlation between short arm of chromosome and chromosome length (0.973) at 1% probability level. Moreover, there is correlation between short arm and long arm (0.925). There is no significant correlation between long arm to short arm ratio (AR) with total length of chromosome and long and short arms. Furthermore, there is a high negative correlation between long arms to short arm ratio with centromeric index. High correlation between total length of chromosomes and length of long arm and short arm indicates that since total length of each chromosome is derived from the sum of long and short arms' length, therefore the more length of long arm and short arm is, the more total length of chromosome is and vice versa.

Table 5: The Correlation Coefficient between Chromosomal Characteristics

Trait	LA	SA	AR	CI
TL	0.987^{++}	0.973^{++}	^{ns}0.306	^{ns}-0.24
LA		0.925^{++}	0.45 ^{ns}	-0.388 ^{ns}
SA			0.083 ^{ns}	-0.014 ^{ns}
AR				-0.99^{++}
CI				

In other words, changes in total length of chromosome are affected by variations in length of long arm and short arm. On the other hand, in total length of chromosome indicates amount of DNA in the cell nucleus [8], which means quantitative changes in the genetic material of total length of chromosome, is affected by changes in genetic material of length of long arm and short arm. High negative correlation between centromeric index and long arm to short arm ratio indicates that the more this ratio is closer to 1, the higher centromeric index becomes. We can guess that chromosome types are approaching metacentric state.

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