Comparative Analysis of Chromosome Karyotype of Three Varieties of the Characteristic Tea plants

Zhen Jin¹, †, Bo Sun¹, †, Jiacheng Huang¹, Liqiang Tan¹, and Qian Tang¹, *

¹College of Horticulture, Sichuan Agricultural University, Chengdu 611130, China
† Zhen Jin and Bo Sun contributed equally to this work.
*)Corresponding author: tangqi2008@126.com

Abstract. ZY, HYZ and CH-1 are three characteristic tea varieties with different shoot colors. However, there is a lack of karyotype analysis of these characteristic tea varieties. In this experiment, the differences of cytological parameters of three characteristic tea varieties, and the control variety FDDB, were analyzed. The results showed that significant differences of the average arm ratio, relative length, karyotype asymmetry index and the karyotype formula were found among the three characteristic tea varieties as well as the control variety FDDB. Moreover, only ZY’s karyotype type was 2B, while the three other varieties all were 2C. Among them, HYZ was the most evolved and closed to FDDB, while CH-1 and ZY were very different from FDDB and evolved more primitive than HYZ. HYZ was the most evolved, and ZY was relatively primitive. PCA analysis indicated that the notable differences were found between FDDB and two characteristic tea varieties (ZY and CH-1), while HYZ is relatively close to FDDB. This findings revealed the variation of chromosomes among different color varieties at the cytological level, and provided a certain theoretical basis for the study of genetic variation and diversity of tea resources.

1. Introduction

As a plant for leaf, the tea tree is one of the most vigorous parts of tea tree metabolism. It is unique in secondary metabolism, which is manifested in the characteristic secondary metabolites such as catechin, caffeine and theanine[1]. ZY is a new tea variety with dark purple young shoots, which has abundant anthocyanin[2]. CH-1 and HYZ are characteristic tea varieties with yellow shoots, which have unique quality, ornamental when the new shoots grow, and the dry tea made from them is of good quality[3-4].

Chromosome is the most important and stable component in the nucleus of organisms, and also is the carrier of genetic material that determines the reproduction of species. Karyotype analysis is a basic method to study chromosomes, and it is a basic work in cytogenetics research. However, the chromosomes of different species of plants, even different varieties vary widely. In this experiment, FDDB tea, a national improved variety, was used as control to analyze the karyotype of three characteristic tea varieties. The variation law of chromosomes among different color varieties was revealed through the analysis of karyotype evolution trend, in order to provide some theoretical basis for the study of genetic variation of tea plants and diversity of germplasm resources.

2. Materials and methods
2.1. Plant materials
The representative ZY, CH-1, HYZ and FDDB from Mingshan County, Sichuan Province was used as the material. Among them, ZY, CH-1 and HYZ are three characteristic tea varieties with colorful leaves, and FDDB is as the control variety.

2.2. Chromosome preparation
The cutting seedlings of ZY, HYZ, CH-1 and FDDB were cultured in plastic flowerpots in greenhouse to the root length of 1-1.5 cm and cut root tips of about 1 cm. Pretreated in 0.002 mol·L⁻¹ 8-hydroxyquinoline at 4 °C for 24 h, and fixed in Carnoy’s solution at 4 °C for 24 h, subsequently, the root tips were macerated in 1 mol·L⁻¹ hydrochloric acid at 60 °C for 16 min, stained with Carbol Fuchsin, and observed under microscope[5].

2.3 Karyotype analysis
Chromosome counts were performed on 30 well-spread metaphase chromosomes. Five different visions with clear cell, complete chromosome and good shape were selected to take a picture. The measure was preformed using Image-Pro Plus 6.0. Karyotype analysis referred to the standard of Li et al.[6]. Following parameters were calculated: chromosome number (counter in each cell), MAR (mean of long arm length/short arm length ratio), and karyotypic formula referred to the standard of Levan et al[7], percentage of chromosomes with an arm ratio > 2 in chromosome set, As.K (index of the karyotypic asymmetry: total long arm length in chromosome set/ total chromosome length in chromosome set), and karyotype (the classification of karyotype in relation to their degree of asymmetry) was calculated by the method of Arano[8] and followed Stebbins' standard[9]. Based on the karyotype parameters, principal component analysis (PCA) was completed by Simca-P11.5 software[10].

3. Results
3.1. Chromosome number of four varieties of tea plants
The chromosome idiogram, metaphase chromosomes and karyotype of four varieties of tea plants are shown in Figure 1. The chromosome number of the four varieties of tea plants all were 2n=30. None abnormal chromosome were determined, which indicating the number of these tea varieties is stable.

3.2. Karyotype analysis
The karyotype parameters of chromosome are listed in Table 1. The max arm ratio of ZY, HYZ, CH-1 and FDDB were 2.50, 5.54, 4.06 and 5.46, respectively, and their chromosome length ratio were 1.513, 2.394, 2.972 and 1.967, respectively. Their mean arm ratio were 1.90, 2.08, 1.70, and 1.56, respectively. HYZ, CH-1 and FDDB contained median (m), submedian (sm), and subterminal (st) regions, whereas ZY just has m and sm. Moreover, a pair of satellites were observed in ZY, HYZ and CH-1, and they were located at the tenth, fourteenth and ninth chromosome, respectively. Besides, all the satellites of the three tea plants were located in submetacentric chromosomes (sm). However, no satellite was observed in FDDB. The karyotype formula of ZY was 2n=2x=30=22m+8sm(2SAT). The karyotype formula of HYZ was 2n=2x=30=12m+14sm(SAT)+4st. The karyotype formula of CH-1 was 2n=2x=30=18m+10sm(2SAT)+2st. The karyotype formula of FDDB was 2n=2x=30=18m+10sm+2st. There were 13.3%, 33.3%, 26.7% and 33.3% of chromosomes of ZY, HYZ, CH-1 and FDDB with an arm ratio > 2 in chromosome set. Based on the ratio of longest and shortest chromosome and percentage of chromosome with an arm ratio in chromosome set, the karyotype types of the tea varieties were belonged to 2C except that ZY was 2B.
3.3. Karyotype evolution trend analysis
In this study, karyotype asymmetry indexes of ZY, HYZ, CH-1 and FDDB were 59.920%, 64.902%, 61.549% and 62.501%. All these values were higher than 50%, indicating that these degrees of karyotype asymmetry were high. Therefore, it is inferred that the evolution degree of these four kinds of tea plants is high, among which the evolution degree of HYZ is the highest, and that of ZY is the most primitive.

In our experiment, the scattered map of the degree of karyotype asymmetry of four characteristic tea plants was made with the average arm ratio as the transverse coordinate and the karyotype asymmetry coefficient as the longitudinal coordinate, which was shown in Figure 2. HYZ was located in the upper right corner of Figure 2, indicating that it was the most evolved. ZY was located at the bottom left of HYZ, which means it was relatively primitive.

3.4 Principal component analysis
In order to reflect the karyotype differences among the characteristic tea varieties more intuitively, the karyotype parameters of the tea varieties were analyzed by PCA as shown in Figure 3. Two principal components were obtained by dimension reduction treatment, in which PC 1 accounted for 48.9%, PC 2 accounted for 32.5%, and the sum of the two components was much more than 80%. As can be seen
from figure 3, the notable differences were found between FDDB and two characteristic tea varieties (ZY and CH-1), while HYZ is relatively close to FDDB.

Table 1. The karyotypes of four varieties of tea plants

| No. | Karyotype formula | Satellites | L/S | Arm ratio | Karyotype type | As.K/% |
|-----|------------------|------------|-----|-----------|----------------|--------|
|     |                  | Number     | Chromosome number |       |               |        |
| ZY  | 2n=2x=30=22m+8sm(2SAT) | 2          | 10  | 2.50      | 1.90           | 2B     | 59.920 |
| HYZ | 2n=2x=30=12m+14sm(SAT)+4st | 1          | 14  | 5.54      | 2.08           | 2C     | 64.902 |
| CH-1| 2n=2x=30=18m+10sm(2SAT)+2st | 2          | 9   | 4.06      | 1.70           | 2C     | 61.549 |
| FDDB| 2n=2x=30=18m+10sm+2st | 0          |      | 5.46      | 1.56           | 2C     | 62.501 |

Figure 2. Scattered map of karyotype asymmetry of ZY, HYZ, CH-1 and FDDB

Figure 3. Principal component analysis map of karyotype parameters of four tea plants
4. Discussion
Phenotypic differences among varieties may be caused by different genetic backgrounds. In this experiment, there were significant phenotypic differences among the four varieties, especially in the color of new shoots. In our study, ZY's chromosomes are more uniform in length, no particularly long or short chromosomes, and the maximum arm ratio is not much different from the average arm ratio, which is different from FDDB. Moreover, only ZY's karyotype characteristics was type 2B, the others were 2C. Therefore, ZY was the most different from the control FDDB and the other two tea varieties. In angiosperms, asymmetrical karyotypes are generally derived from symmetrical karyotypes[11]. It is widely accepted that there is an evolutionary trend toward increasing chromosome sizes and karyotype diversity[12]. The karyotype asymmetry coefficient and the average arm ratio can represent the asymmetry among different provenances. The larger the value is, the more asymmetric the karyotype is. The karyotype of plant boundary develops from symmetry to asymmetry. According to the size of karyotype asymmetry coefficient, the degree of plant evolution in the system can be judged. In this study, the evolutionary degree of HYZ was the highest, CH-1 was the second, and ZY was relatively primitive, which might be due to the influence of different environmental conditions and artificial selection on their genetic materials. These evolutionary differences may be reflected in plant traits such as new shoot color, germination period, seed setting rate, and so on. Although the degree of evolution of HYZ is much higher than that of FDDB, its karyotype parameters are close to those of FDDB. HYZ and FDDB are close in appearance, but ZY and CH-1 are quite different from FDDB in appearance. The results of karyotype analysis were consistent with the differences of these appearances. To sum up, this findings revealed the variation of chromosomes among different color varieties at the cytological level, and these differences need to be studied at the molecular level in the future to provide a certain theoretical basis for the study of genetic variation and diversity of tea resources.

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