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Karyotype Analysis of *Camellia Sinensis* Cv. Chuannong-Huangyazao

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Abstract. Chuannong- Huangyazao' is a new yellow tea variety bred by Sichuan Agricultural University, and approved by Sichuan Crop Variety Validation Committee in 2009. In this research, we try to obtain cytological parameters on 'Chuannong-Huangyazao'. Seven chromosomal parameters were measured and calculated: chromosome length, relative length, index of relative length, type of relative length, arm ratio, centromere index and centromere type. The experiment results showed that the max arm ratio was determined 5.54, relative length ranged between 4.69% to 6.83%. There are four types of relative length, including long (L), medium long (M2), medium short (M1) and short (S) chromosomes. In addition, the maximum centromeric index was measured in 43.11%, there were seven pairs of submetacentric chromosomes (sm), six pairs of centromere types were metacentric chromosomes (m) and two pairs of centromere types were subterminal chromosomes (st). Karyotype asymmetry index was 64.902%, and the karyotype formula was $2n=2x=30=12m+14sm(2SAT)+4st$. The karyotype characteristics was type 2C according to Stebbins's classification criteria. According to the above information, we can draw a conclusion that 'Chuannong- Huangyazao' is a relatively evolved tea variety. The findings revealed its karyotypic characteristics of 'Chuannong-Huangyazao' from the cytogenetic aspects.

Key words: *Camellia sinensis*, Chuannong- Huangyazao, Chromosome, Karyotype.

INTRODUCTION

Tea plants (*Camellia sinensis*) are used to produce the most widely consumed beverage. They contains a variety of beneficial ingredients which include free amino acids (FAAs), caffeine, and catechins etc . Many laboratory experiments have demonstrated that tea has good preventive effects for multiple diseases, including cardiovascular disease ,metabolic syndrome, cancer, and neurodegenerative diseases [1]. 'Chuannong-Huangyazao' is a new variety of tea varieties of Sichuan Agricultural University, and approved by Sichuan Crop Variety Validation Committee in 2009. Its population is shrub type, middle lobe type and special early seed. It has smooth leaf, green leaves, soft leaves and medium hairs. It's good quality and its bud-shape suitable for making famous quality yellow tea, especially dry tea for its great leaf bottom color and high aroma. Also its resistance and adaptability are strong [2].

Karyotype analysis is a basic method to study chromosomes, it is a basic work in cytogenetics research [3]. In this experiment, the karyotype analysis was carried out on the yellow tea variety 'Chuannong-Huangyazao' to reveal its chromosome composition, and to provide the basis for determining the genetic composition of *Camellia sinensis* cv. Chuannong-Huangyazao.

MATERIALS AND METHODS

Plant Materials

The representative yellow tea variety 'Chuannong-Huangyazao' from Sichuan was used as experimental material.

Chromosome Preparation

The cutting seedling of 'Chuannong- Huangyazao' were cultured in plastic flower pots at greenhouse. The root tips were cut about 1-1.5 cm length. Then they were pretreated in 0.002 mol·L⁻¹ 8-hydroxyquinoline at 4°C for 24h, and fixed in Carnoy's solution (acetic acid: absolute ethanol, 1:3, v/v) at 4°C for 24 h, subsequently, the root tips were macerated in 1 mol L⁻¹ hydrochloric acid at 60°C for 16.5min, stained with Carbol Fuchsin, and observed under microscope [3].

Karyotype Analysis

Chromosome counts were performed on 30 well-spread metaphase chromosomes from five different root tips. Karyotype analysis referred to the standard of Li et al. [4]. Following parameters were calculated: chromosome relative length, arm ratio, type of chromosomes, index of chromosomes relative length and centromere index. karyotypic formula referred to the standard of Levan et al. [5], and the asymmetry coefficient of karyotypes was calculated by the method of Arano [6], the karyotypes were calculated according to Stebbins' standard [7].

RESULTS

Chromosome Number of Yellow Tea Variety 'Chuannong-Huangyazao'

Metaphase chromosomes and karyotype of yellow tea variety 'Chuannong-Huangyazao' root tips were shown in Fig. 1, detailed karyotype parameters of chromosome were listed in Table1. The chromosome number of ' Chuannong-Huangyazao' was 2n=30.



FIG 1. Metaphase chromosomes and karyotype of yellow tea variety 'Chuannong-Huangyazao' root tips

Note: The number 1-15 represent chromosome No.

Karyotype Analysis

Chromosome relative length ranged from 4.69% to 6.83%, and chromosome length ratio (longest chromosome / shortest chromosome) was 2.394. The chromosome types included long chromosomes (L), medium long chromosomes2 (M2), medium short chromosomes1 (M1) and short chromosome (S), the constitution of the relative length was 2L+14M2+12M1+2S. The centromeric index ranged from 15.28% to 43.11%, and arm ratio ranked from

1.32 to 5.54. There were seven pairs (the third, fifth, eighth, ninth, tenth, eleventh and fourteenth chromosome) of submetacentric chromosomes (sm), six pairs (number one, two, four, six, thirteen and fifteen) of centromere types were metacentric chromosomes (m) and two pairs (the seventh and twelfth) of centromere types were subterminal chromosomes (st). Moreover, two satellites were observed at the fourteenth pair of chromosomes. The karyotype formula was $2n=2x=30=12m+14sm(2SAT)+4st$. Karyotype asymmetry index was 64.902%, and karyotype characteristics fell into type 2C according to Stebbins's classification criteria. The chromosome idiogram of yellow tea variety 'Chuannong-Huangyazao' were shown in Fig. 2.

TABLE 1. Karyotype parameters of chromosome of yellow tea variety 'Chuannong-Huangyazao'

Chromosome No.	Relative length / %			Index of relative length	Type of relative length	Arm ratio	Centromere index / %	Centromere type
	Short arm	Long arm	Total length					
1	2.88	3.95	6.83	1.31	L	1.37	42.11	m
2	2.80	3.70	6.50	1.24	M2	1.32	43.11	m
3	2.10	3.96	6.06	1.16	M2	1.89	34.59	sm
4	2.42	3.56	5.97	1.14	M2	1.47	40.47	m
5	2.08	3.62	5.71	1.09	M2	1.74	36.49	sm
6	2.20	3.24	5.44	1.04	M2	1.47	40.40	m
7	0.83	4.58	5.41	1.04	M2	5.54	15.28	st
8	1.94	3.37	5.31	1.02	M2	1.74	36.54	sm
9	1.54	3.69	5.23	1.00	M1	2.39	29.47	sm
10	1.61	3.33	4.94	0.95	M1	2.06	32.69	sm
11	1.47	3.33	4.80	0.92	M1	2.26	30.66	sm
12	1.07	3.61	4.69	0.90	M1	3.37	22.91	st
13	1.97	2.55	4.52	0.87	M1	1.30	43.52	m
14*	1.47	2.67	4.14	0.79	M1	1.82	35.52	sm
15	1.14	1.72	2.85	0.55	S	1.51	39.87	m

Note: * means the chromosomes with satellites, and the length of satellites is not included in the chromosome length.

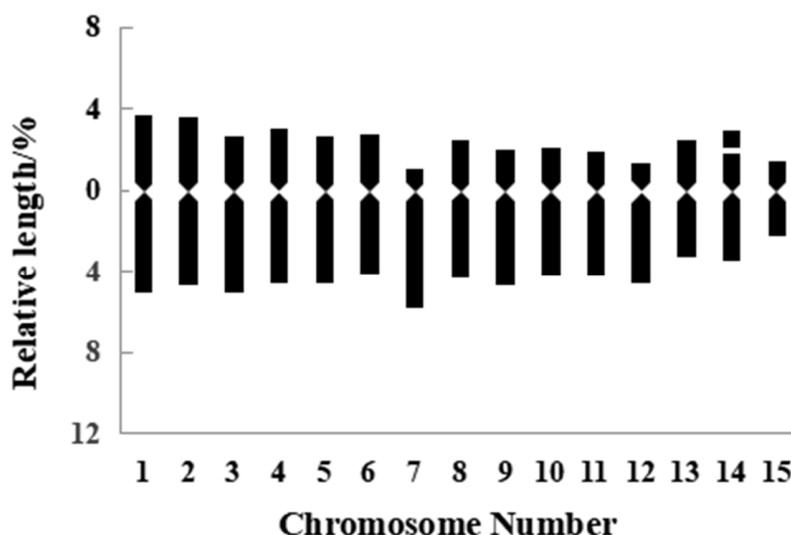


FIG 2. Chromosome idiogram of yellow tea variety 'Chuannong-Huangyazao'

SUMMARY

The results of karyotype analysis of yellow tea variety 'Chuannong- Huangyazao' were demonstrated in this study. Four chromosome types were observed in Chuannong- Huangyazao, including long chromosomes (L), medium long chromosomes (M2), medium short chromosomes (M1) and short chromosome (S), and the constitution of the relative length was $2L+14M2+12M1+2S$. Gulin- Niupicha also has the four chromosome types [8], whereas Chuancha and Yaan- dayecha were only owned M1 and M2 [9]. Karyotype asymmetry index of Chuannong- Huangyazao was 64.902%, which is higher than those of Gulin- Niupicha (60.93%) [8]. And six kinds of 'Chongzhou loquat' tea plants (58.28% to 59.66%) [10]. The karyotype characteristics of Chuannong- Huangyazao was type 2C according to Stebbins's classification criteria, whereas those of Gulin- Niupicha and six kinds of 'Chongzhou loquat' tea plants were 2A or 2B [9, 10]. The basic evolutionary trend of plant karyotypes is from symmetry to asymmetry. Thus, primitive plants have symmetrical karyotypes. And the more asymmetric the plant karyotype is, the higher its degree of evolution [7]. Therefore, the yellow tea variety 'Chuannong- Huangyazao' should be in the relatively evolved level in tea plants.

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