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Karyotype Analysis of the Dark-Purple Tea Cultivar ‘Ziyan’

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Abstract. Tea plants (*Camellia sinensis*) are used to produce the most widely consumed beverage. ‘Ziyan’ is a new tea cultivar with dark purple young shoots at an altitude of approximate 1500 m in Sichuan province, China. There are abundant anthocyanins in ‘Ziyan’. In this research, we try to obtain cytological parameters on the dark-purple tea cultivar ‘Ziyan’. 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 max arm ratio was determined 2.50, relative length ranged from 3.56% to 5.39%. There are two types of relative length, including medium long (M2) and medium short (M1). In addition, the maximum centromeric index was measured in 49.02%, centromere type were metacentric (m) and submetacentric chromosomes (sm) chromosomes. Two satellites were observed at the tenth pair of chromosomes. Karyotype asymmetry index was 59.92%, and the karyotype formula was $2n=2x=30=22m(2SAT)+8sm$. The karyotype characteristics was type 2B according to Stebbins’s classification criteria. The findings revealed its karyotypic characteristics of the dark-purple tea cultivar ‘Ziyan’ from the cytogenetic aspects.

INTRODUCTION

Tea plants (*Camellia sinensis*) are used to produce the most widely consumed beverage. Many laboratory experiments have demonstrated that tea has good preventive effects for multiple diseases, including cardiovascular disease, metabolic syndrome, cancer, and neurodegenerative diseases. The common bioactive components of tea include free amino acids (FAAs), caffeine, and catechins. ‘Ziyan’ is a new tea cultivar with dark purple young shoots at an altitude of approximate 1500 m in Sichuan province, China. Most tea cultivars do not contain abundant anthocyanins in their leaves. However, there are abundant anthocyanins in ‘Ziyan’. Anthocyanins are beneficial to human health because they are powerful antioxidants and anti-inflammatory agents [1].

Karyotype analysis is a basic method to study chromosomes, it is a basic work in cytogenetics research [2]. In this experiment, the karyotype analysis was carried out on the dark-purple tea cultivar ‘Ziyan’ to reveal its chromosome composition, and to provide the basis for determining the genetic composition of the tea cultivar ‘Ziyan’.

MATERIALS AND METHODS

Plant Materials

The representative the dark-purple tea cultivar ‘Ziyan’ from Sichuan was used as the experimental material.

Chromosome Preparation

The cutting seedlings of ‘ziyan’ were cultured in plastic pots at greenhouse. The root tips were cut about 1-1.5 cm. 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 17min, stained with Carbol Fuchsin, and observed under microscope [2].

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. [3]. 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. [4], and the asymmetry coefficient of karyotypes was calculated by the method of Arano [5], the karyotypes were calculated according to Stebbins's standard [6].

RESULTS

Chromosome Number of the Dark-Purple Tea cultivar 'Ziyan'

Metaphase chromosomes and karyotype of 'Ziyan' root tips were shown in Fig. 1, detailed karyotype parameters of chromosome were listed in Table 1. The chromosome number of 'Ziyan' were $2n=30$.

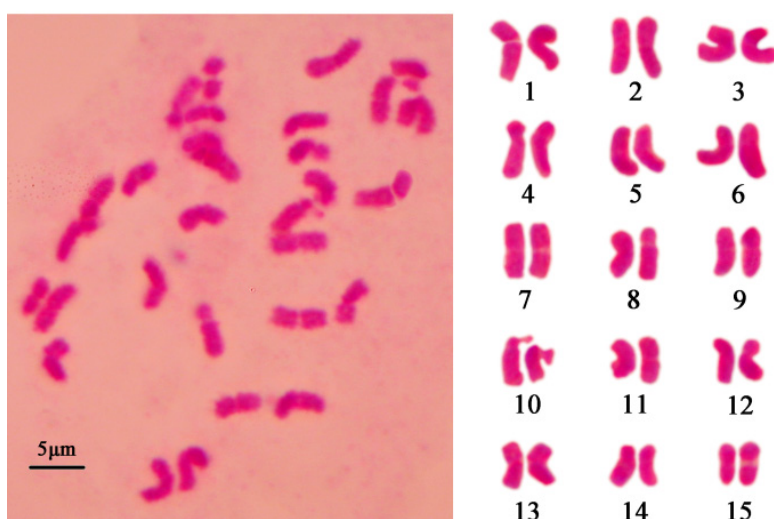


FIGURE 1. Metaphase chromosomes and karyotype of the dark-purple tea cultivar 'Ziyan' root tips

Note: The number 1-15 represent chromosome no.

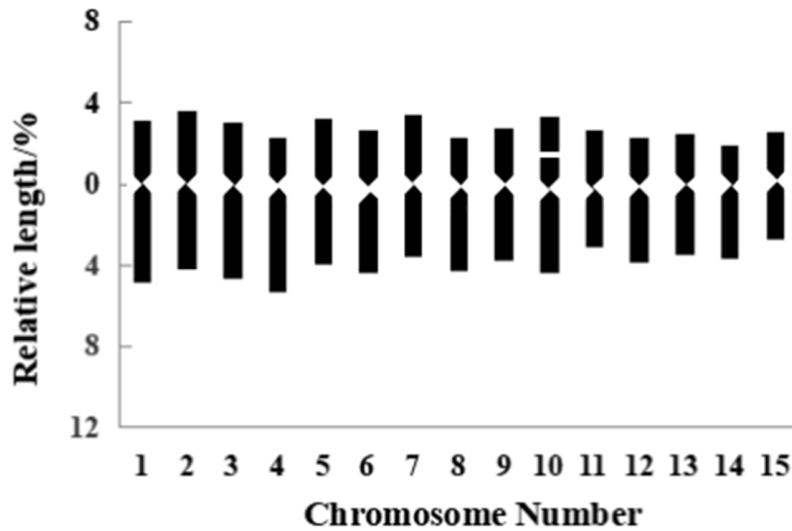
Karyotype Analysis

Chromosome relative length ranged from 3.56% to 5.39%, and chromosome length ratio (longest chromosome / shortest chromosome) was 1.513. The chromosome types included medium long chromosomes2 (M2) and medium short chromosomes1 (M1), the constitution of the relative length was 14M2+16M1. The centromeric index ranged from 28.60% to 49.02%, and arm ratio ranked from 1.04 to 2.50. There were four pairs (the fourth, eighth, tenth and fourteenth chromosome) of submetacentric chromosomes (sm) and eleven pairs (number one, two, three, five, six, seven, nine, eleven, twelve, thirteen and fifteen chromosome) of metacentric chromosomes (m). Moreover, two satellites were observed at the tenth pair of chromosomes. The karyotype formula was $2n=2x=30=22m(2SAT)+8sm$. Karyotype asymmetry index was 59.92%, and karyotype characteristics fell into type 2B according to Stebbins's classification criteria. The chromosome idiogram of 'Ziyan' were shown in Fig. 2.

TABLE 1. Karyotype parameters of chromosome of the dark-purple tea cultivar ‘Ziyan’

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	2.11	3.28	5.39	1.19	M2	1.56	39.10	m
2	2.44	2.83	5.27	1.17	M2	1.16	46.34	m
3	2.05	3.16	5.21	1.15	M2	1.55	39.29	m
4	1.54	3.64	5.18	1.15	M2	2.37	29.71	sm
5	2.20	2.66	4.87	1.08	M2	1.21	45.30	m
6	1.79	2.97	4.76	1.05	M2	1.66	37.64	m
7	2.31	2.42	4.73	1.05	M2	1.05	48.74	m
8	1.56	2.92	4.47	0.99	M1	1.87	34.79	sm
9	1.85	2.50	4.35	0.96	M1	1.35	42.53	m
10*	1.19	2.98	4.17	0.92	M1	2.50	28.60	sm
11	1.81	2.06	3.87	0.86	M1	1.13	46.86	m
12	1.57	2.58	4.16	0.92	M1	1.64	37.87	m
13	1.70	2.32	4.02	0.89	M1	1.36	42.33	m
14	1.27	2.44	3.70	0.82	M1	1.92	34.20	sm
15	1.75	1.82	3.56	0.79	M1	1.04	49.02	m

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

**FIGURE 2.** Chromosome idiogram of the dark-purple tea cultivar ‘Ziyan’

SUMMARY

The results of karyotype analysis of *Camellia sinensis* were significantly different among different studies. For example, in this experiment, the karyotype formula of the dark-purple tea cultivar ‘Ziyan’ was $2n=2x=30=22m(2SAT)+8sm$, but some researchers observed that karyotype formula of ‘Gulin-Niupicha’ were $2n=2x=30=18m(2SAT)+10sm+2st$ [7]. The number of chromosomes with satellites also varies, in this experiment, ‘Ziyan’ has a pair of satellites, but some studies have shown two pairs or one pair of satellites or none in other tea cultivars [8]. In terms of chromosome karyotype asymmetry index, the results of this experiment were more similar to six kinds of ‘Chongzhou loquat’ tea plants (58.28% to 59.66%) [9] and ‘Gulin-Niupicha’ (60.93%) [7]. 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 [6]. Therefore, the dark-purple tea cultivar ‘Ziyan’ should be in the middle of evolution level in all tea plants. In addition, there were differences in arm ratio range, chromosome length ratio, karyotype asymmetry coefficient, karyotype type and so on [8].

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