Chromosomal Karyotype Analysis of Four Varieties of Green Petioles Leaf Beet

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Abstract. Chromosome analysis of four varieties of green petioles leaf beet was performed by pressing plate's method. The results revealed the chromosome numbers of all four varieties of green petioles leaf beet was $2n=2x=18$. And their centromere type was mainly composed by metacentric chromosomes (m) and sub metacentric chromosomes (sm), while the number of m was more than sm. However, evident variation among varieties was presented on their karyotype formula, karyotype type, satellitic position, and arm ratio, ratio of chromosome length (L/S) and index of karyotypic asymmetry. Although they all had one pair of satellites, the location of the satellites was different. The karyotype type contained 1A from 'Zhongnong', 'Huaxianzi' and 2A from 'Lifeng', 'Huaguan'. Statistically ratio of chromosome length for tested varieties ranged from 1.20 in 'Lifeng' to 1.52 in 'Zhongnong', and average arm ratio ranged from 1.42 in 'Zhongnong' to 1.62 in 'Huaguan'. The index of karyotypic asymmetry of 'Zhongnong', 'Huaxianzi', 'Lifeng', 'Huaguan' were 58.50%, 59.21%, 58.79% and 61.32%, respectively. This study revealed the karyotypic characteristics of leaf beet from the cytogenetic aspect, and provided a reference for variety breeding of leaf beet.

1. Introduction

Leaf beet (*Beta vulgaris* var. *cicla*) belongs to Chenopodiaceous, and it is the variety of beet. Leaf beet is origin from Mediterranean coast, so it prefer warm and humid climate [1]. And it is the common leaf vegetable consumed in summer, for containing abundant nutrients. Besides, leaf beet had good high yield, disease resistance and adaptability. It is widely planted in China because of its good comprehensive characters. However, there were few studies on leaf beet especially on karyotype analysis, and they mainly focused on agronomic traits, nutrient content and mineral element content [2]. Green petioles leaf beet which had many varieties was the type of most planted. Karyotype analysis is a basic method to study chromosomes, and it is a basic work in cytogenetics research. However, the chromosomes of different types of plants, even different cultivars vary widely. In this experiment, the karyotype analysis was carried out on four varieties of green petioles leaf beet to reveal their chromosome composition and diversity, and to provide the basis for determining the genetic composition of leaf beet.

2. Materials and methods

2.1. Plant materials

There are four varieties of green petioles leaf beet were used as experimental material, including the representative Beta vulgaris cv. Zhongnong numbering G1, Beta vulgaris cv. Huaxianzi numbering G2, Beta vulgaris cv. Lifeng numbering G3, and Beta vulgaris cv. Huaguan numbering G4.

2.2. Chromosome preparation

The seeds were soaked for 2 h, then cultured in dark in petri dishes with moist filter paper at 25°C incubator 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 9 h, 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 12 min, stained with Carbol Fuchsin, and observed under microscope [3].

2.3. 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].

3. Results

3.1. Chromosome number of four varieties of green petioles leaf beet.

Metaphase chromosomes and karyotype of four varieties of green petioles leaf beet are shown in Fig. 1, detailed karyotype parameters of chromosome are listed in Table 1. The chromosome number of the four varieties of green petioles leaf beet were 2*n*=18. Abnormal chromosome number and morphology were not observed, indicating the chromosome number of green petioles leaf beet was stably.

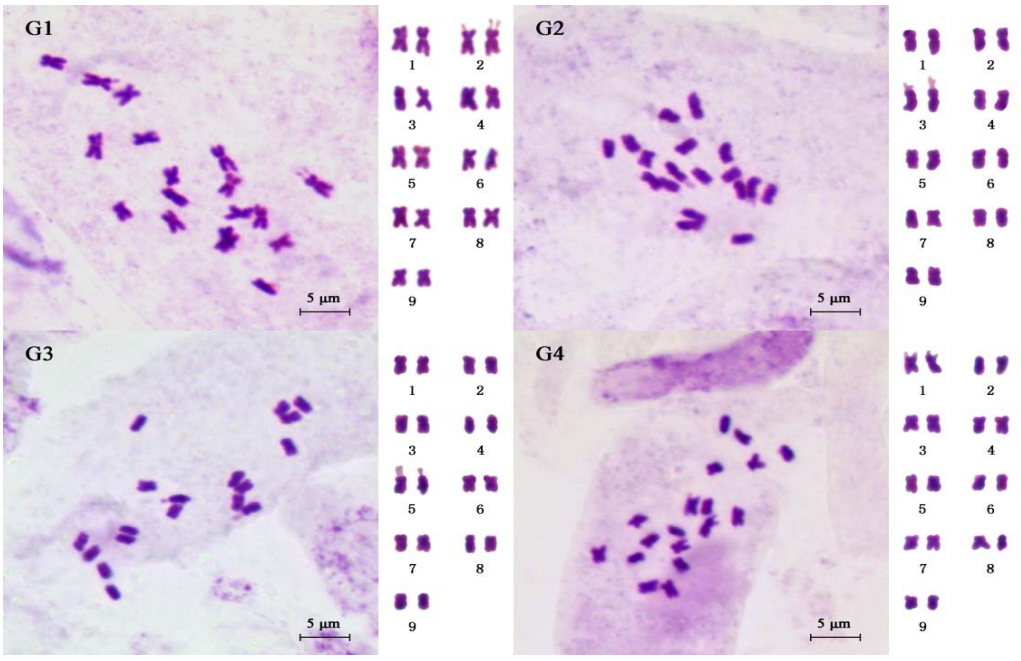

Figure 1. Metaphase chromosomes and karyotype of four varieties of green petioles leaf beet root tips
Note: The number 1-9 represent chromosome no.

Table 1. The parameters of chromosome of four varieties of green petioles leaf beet

No.	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	5.34	7.95	13.29	1.20	M2	1.49	40.19	m
	2*	4.57	7.89	12.46	1.12	M2	1.73	36.67	sm
	3	5.45	6.80	12.24	1.10	M2	1.25	44.49	m
	4	5.57	6.27	11.84	1.07	M2	1.13	47.04	m
G1	5	4.74	6.26	11.00	0.99	M1	1.32	43.09	m
	6	4.04	6.25	10.29	0.93	M1	1.55	39.28	m
	7	4.23	5.96	10.19	0.92	M1	1.41	41.51	m
	8	3.91	6.02	9.93	0.89	M1	1.54	39.40	m
	9	3.65	5.10	8.75	0.79	M1	1.40	41.70	m
	1	4.89	7.76	12.65	1.14	M2	1.59	38.63	m
	2	5.15	7.16	12.31	1.11	M2	1.39	41.87	m
	3*	4.87	7.14	12.01	1.08	M2	1.47	40.55	m
	4	4.33	6.98	11.31	1.02	M2	1.61	38.25	m
G2	5	4.55	6.63	11.18	1.01	M2	1.46	40.66	m
	6	4.92	5.95	10.87	0.98	M1	1.21	45.26	m
	7	3.50	6.56	10.07	0.91	M1	1.87	34.80	sm
	8	3.82	6.09	9.91	0.89	M1	1.59	38.58	m
	9	4.76	4.93	9.69	0.87	M1	1.04	49.13	m
	1	5.10	6.93	12.02	1.08	M2	1.36	42.39	m
	2	5.10	6.62	11.71	1.05	M2	1.30	43.50	m
	3	4.56	6.86	11.43	1.03	M2	1.50	39.93	m
	4	4.95	6.45	11.39	1.03	M2	1.30	43.42	m
G3	5*	4.13	7.00	11.14	1.00	M1	1.70	37.10	m
	6	5.03	6.09	11.12	1.00	M1	1.21	45.25	m
	7	4.37	6.47	10.83	0.97	M1	1.48	40.31	m
	8	3.30	7.05	10.34	0.93	M1	2.14	31.87	sm
	9	4.68	5.33	10.02	0.90	M1	1.14	46.75	m
	1*	4.39	8.23	12.62	1.14	M2	1.88	34.76	sm
	2	5.60	6.55	12.16	1.09	M2	1.17	46.09	m
	3	4.95	6.81	11.76	1.06	M2	1.38	42.09	m
	4	4.31	7.16	11.47	1.03	M2	1.66	37.56	m
G4	5	4.34	6.65	10.99	0.99	M1	1.53	39.51	m
	6	4.05	6.79	10.84	0.98	M1	1.68	37.37	m
	7	4.07	6.70	10.77	0.97	M1	1.65	37.80	m
	8	3.94	6.21	10.15	0.91	M1	1.58	38.83	m
	9	3.02	6.21	9.23	0.83	M1	2.05	32.75	sm

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

3.2. Karyotype analysis

The major karyotype parameters of four varieties of green petioles leaf beet are shown in Table 1 and Table 2. The chromosome relative length of G1 ranged from 8.75% to 13.29%, G2 ranged from 9.69% to 12.65%, G3 ranged from 10.02% to 12.02% and G4 ranged from 9.23% to 12.62%, and chromosome length ratio of G1, G2, G3 and G4 were 1.52, 1.31, 1.20 and 1.37, respectively. The relative length constitution of G1, G3 and G4 was 8M2+10M1, and G2 was 10M2+8M1. The centromeric index of G1

ranged from 36.67% to 44.49%, and arm ratio ranked from 1.25 to 1.73. The centromeric index of G2 ranged from 34.80% to 49.13%, and arm ratio ranked from 1.04 to 1.87. The centromeric index of G3 ranged from 31.87% to 46.75%, and arm ratio ranked from 1.14 to 2.14. The centromeric index of G4 ranged from 32.75% to 46.09%, and arm ratio ranked from 1.17 to 2.05. There were one pair (the second, seventh, eighth chromosome) of sub metacentric chromosomes (sm) and other eight pairs of metacentric chromosomes (m) in G1, G2 and G3, and two pairs of sub metacentric chromosomes (sm) and seven pairs of metacentric chromosomes (m) in G4. Moreover, the four varieties of green petioles leaf beet all had one pair of satellites, and the two satellites were observed at the second, third, fifth and first pair of chromosomes, respectively, in G1, G2, G3 and G4. Besides, the satellites of G1, and G4 were all located in sub metacentric chromosomes (sm), while the satellites of G2 and G3 were located in metacentric chromosome (m). Therefore, the karyotype formula of G1 was 2*n*=2*x*=18=16m+2sm (2SAT), G2 and G3 was 2*n*=2*x*=18=16m (2SAT) +2sm, and G4 was 2*n*=2*x*=18=14m+4sm (2SAT). Karyotype asymmetry index of G1 was 58.50%, G2 was 59.21%, G3 was 58.79%, and G4 was 61.32%. The karyotype characteristics of G1, G2 fell into type 1A, while G3, G4 fell into 2A according to Stebbins's classification criteria. The chromosome idiogram of G1, G2, G3 and G4 are shown in Fig. 2.

Table 2. The karyotypes of four varieties of green petioles leaf beet

No	Karyotype fomula	Sat		L/S	Arm ratio	Karyotype type	As.K/%
		Number	Chromsome number				
G1	2n=2x=18=16m+2sm	2	2	1.52	1.42	1A	58.50
G2	2n=2x=18=16m+2sm	2	3	1.31	1.47	1A	59.21
G3	2n=2x=18=16m+2sm	2	5	1.20	1.46	2A	58.79
G4	2n=2x=18=14m+4sm	2	1	1.37	1.62	2A	61.32

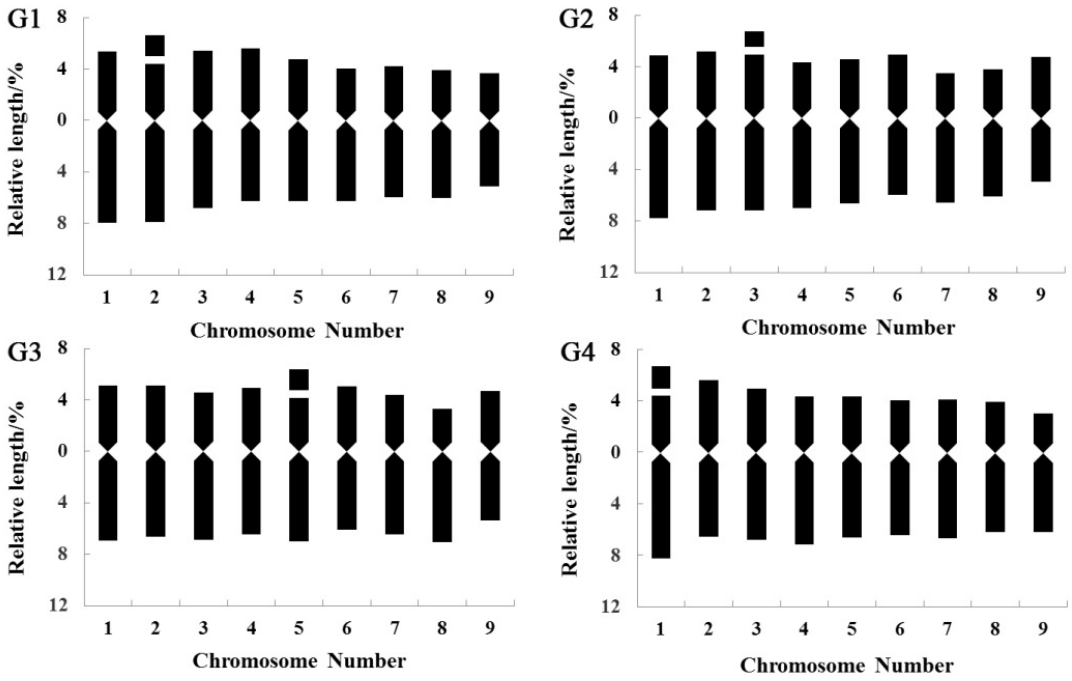

Figure 2. Chromosome ideogram of four varieties of green petioles leaf beet

4. Discussion

All of the four varieties of green petioles leaf beet exhibited a diploid chromosome number 2*n*=2*x*=18, with a basic number *x*=9, and they have one pair of satellites. The relative length type of four varieties of green petioles leaf beet was all consisted by M1 and M2, and the chromosomes type of them was

mainly composed by metacentric chromosomes (m). Besides, the karyotype characteristics of G1, G2 fell into type 1A, and G3, G4 fell into type 2A. Green petioles leaf beet was reported the most primitive leaf beet [8]. In our study, the karyotype asymmetry index was from 58.50% to 61.32%. Although they all have green petioles, however, other diversities were also existence among them. For example, the satellites were observed at different chromosomes. Therefore, they may have diversities in many other economical characters. As many research reported there were evidently diverse among different varieties [9, 10]. The difference, which resulted from long terms of environmental options, confirmed their distinct origin. And the different environment resulted in their different evolution level. 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 [11]. In our study, G4 had a relatively high evolutionary degree and it was senior than G2, G3 and G1 according to the karyotype asymmetry index and average arm ratio. Our research provides a reference for the genetic evolution of green petioles leaf beet.

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