Karyotype Analysis of Three Varieties of Red Leaf Beet

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Karyotype Analysis of Three Varieties of Red Leaf Beet

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Abstract. Red leaf beet was one of the most important leaf vegetables and an ornamental with rich pigment. However, there is a lack of information on genetic variability. In this study, cytological parameters on three varieties of red leaf beet were obtained. The results displayed that the average arm ratio of RR1 was determined 1.52 and relative length was ranged from 9.26% to 13.10%. The karyotype asymmetry index was 59.93%, and the karyotype formula was 2*n*=2*x*=18=14m+4sm (2SAT). The average arm ratio of RR2 was determined 1.50 and relative length was ranged from 8.57% to 13.11%. The karyotype asymmetry index was mensurated as 59.60%, and the karyotype formula was 2*n*=2*x*=18=14m (2SAT) +4sm. The average arm ratio of RR3 was determined 1.33 and relative length was ranged from 9.44% to 12.56%. The karyotype asymmetry index was 56.85%, which was much lower than RR1 and RR2, and the karyotype formula was 2*n*=2*x*=18=18m (2SAT). Although RR3 had an obvious difference with RR1 and RR2, the karyotype characteristics of three varieties of red leaf beet were all type 1A. This research revealed the karyotypic characteristics of red leaf beet. The results indicated that there was an obvious difference among the three varieties of red leaf beet in karyotypic characteristics, and the evolution of red leaf beet was relatively primitive.

1. Introduction

Leaf beet (*Beta vulgaris* var. *cicla*) belongs to the Chenopodiaceae family, and it is the variety of beet. Leaf beet is origin from the shores of Mediterranean, whose climate is warm and humid [1]. And it is the common leaf vegetable consumed in summer, for containing abundant nutrients, such as protein, minerals and dietary fibre. Besides, leaf beet is so easy to plant, and had a good high yield, disease resistance and adaptability. It is widely planted in China because of its good comprehensive characters. Besides, red leaf beet was a new developmental ornamental plant. However, there were little information on leaf beet especially on karyotype analysis, and they mainly focused on agronomic traits, nutrient content and mineral element content [2]. Chromosome is the most important and stable component in the nucleus of cells and also is the carrier of hereditary material. 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 cultivars vary widely. In this experiment, the karyotype analysis was carried out on three varieties of red 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
Three varieties of red leaf beet were used as experimental material, including the representative Beta vulgaris cv. Tezhong numbering RR1, Beta vulgaris cv. Fangxuanyuan numbering RR2 and Beta vulgaris cv. Fengmingyashi numbering RR3.

2.2. Chromosome preparation
The seeds were stored at -20 °C for 4 d, then cultured in dark in petri dishes with moist filter paper at the incubator with a temperature of 25 °C /16 h and 18 °C /8 h. Waiting for about 4 d when the root length of red leaf beet grown to 1 cm. And then cut those root tips to 0.002 mol·L^{-1} 8-hydroxyquinoline at 4 °C for 9 h. After that, fixed the chromosomes 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. Five different visions with clear cell, complete chromosome and good shape were selected to take a picture. The measure was performed using Image-Pro plus 6.0. 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], the asymmetry coefficient of karyotypes was calculated by the method of Arano [6], and the karyotypes were calculated according to Stebbins' standard [7].

3. Results

3.1. Chromosome number of three varieties of red leaf beet.
Metaphase chromosomes and karyotype of three varieties of red leaf beet are shown in Figure 1. The chromosome number of the three varieties of red leaf beet were 2*n*=18. None abnormal chromosome were determined, which indicating the number of the red leaf beet is stable.

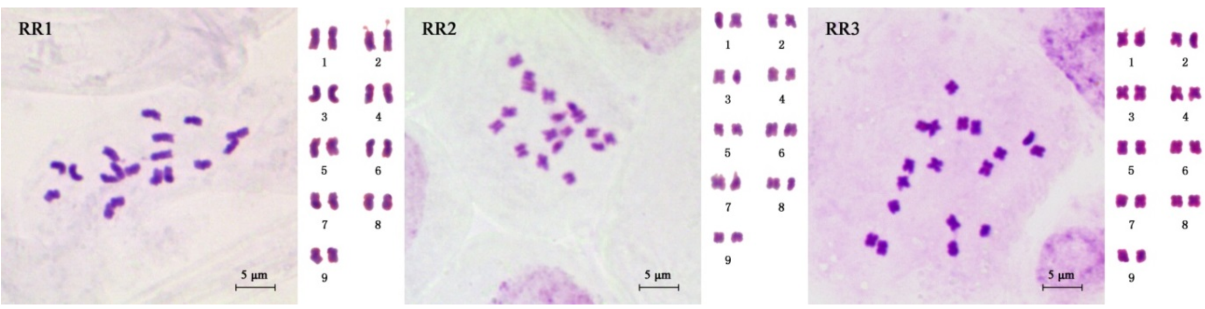

Fig. 1. Metaphase chromosomes and karyotype of red leaf beet root tips.
Note: The number 1-9 represent chromosome No.

3.2. Karyotype analysis
The detailed karyotype parameters of chromosome are listed in Table 1 and Table 2. The chromosome relative length of RR1 ranged from 9.26% to 13.10%, while RR2 ranged from 8.57% to 13.11% and RR3 ranged from 9.44% to 12.56%. Chromosome length ratio of RR1, RR2, and RR3 was 1.42, 1.53 and 1.33, respectively. The relative length constitution of RR1 and RR2 was 10M2+8M1, and RR3 was 8M2+10M1. The centromeric index of RR1 ranged from 33.31% to 42.21%, and arm ratio ranked from 1.37 to 2.00. The centromeric index of RR2 ranged from 35.48% to 45.96%, and arm ratio ranked from 1.18 to 1.82. The centromeric index of RR3 ranged from 37.32% to 48.70%, and arm

ratio ranked from 1.05 to 1.68.There were two pair of submetacentric chromosomes (sm) and other seven pairs of metacentric chromosomes (m) in RR1 and RR2, while RR3 only composed by metacentric chromosomes (m). Moreover, the three varieties of red leaf beet all had one pair of satellites, and the two satellites were observed at the, second, seventh and first pair of chromosomes respectively in RR1, RR2 and RR3. Besides, the satellites of RR1 were located in submetacentric chromosomes (sm). Therefore, the karyotype formula of RR1 was $2n=2x=18=14m+4sm$ (2SAT). However, the satellites of RR2 and RR3 were located in metacentric chromosomes (m), so the karyotype formula of RR2 was $2n=2x=18=14m$ (2SAT) +4sm, and RR3 was $2n=2x=18=18m$ (2SAT). Karyotype asymmetry index of RR1 was 59.93%, RR2 was 59.60% and RR3 was 56.85%. The karyotype characteristics of three varieties of red leaf beet fell into type 1A according to Stebbins's classification criteria. The chromosome idiogram of RR1, RR2 and RR3 are shown in Figure 2.

Table 1. Karyotype parameters of chromosome of red leaf beet.

No.	Chromosome No.	Relative length / % Short arm	Long arm	Total length	Index of relative length	Type of relative length	Arm ratio	Centromere index / %	Centromere type
	1	5.53	7.57	13.10	1.18	M2	1.37	42.21	m
	2*	4.09	8.18	12.27	1.10	M2	2.00	33.31	sm
	3	4.15	7.53	11.68	1.05	M2	1.82	35.51	sm
	4	4.64	6.95	11.59	1.04	M2	1.50	40.06	m
RR1	5	5.61	5.94	11.55	1.04	M2	1.06	48.56	m
	6	4.50	6.31	10.80	0.97	M1	1.40	41.61	m
	7	4.12	5.94	10.06	0.91	M1	1.44	40.99	m
	8	3.72	5.96	9.69	0.87	M1	1.60	38.42	m
	9	3.71	5.54	9.26	0.83	M1	1.49	40.11	m
	1	6.03	7.09	13.11	1.18	M2	1.18	45.96	m
	2	5.10	7.27	12.37	1.11	M2	1.42	41.25	m
	3	4.03	7.34	11.37	1.02	M2	1.82	35.48	sm
	4	5.19	6.18	11.36	1.02	M2	1.19	45.64	m
RR2	5	4.05	7.17	11.22	1.01	M2	1.77	36.08	sm
	6	4.25	6.84	11.09	1.00	M1	1.61	38.35	m
	7*	4.10	6.82	10.92	0.98	M1	1.66	37.54	m
	8	4.01	5.97	9.99	0.90	M1	1.49	40.20	m
	9	3.64	4.93	8.57	0.77	M1	1.35	42.48	m
	1*	5.29	7.27	12.56	1.13	M2	1.37	42.13	m
	2	5.20	7.28	12.49	1.12	M2	1.40	41.66	m
	3	5.67	6.41	12.08	1.09	M2	1.13	46.94	m
	4	4.48	7.52	12.00	1.08	M2	1.68	37.32	m
RR3	5	4.85	6.24	11.08	1.00	M1	1.29	43.73	m
	6	5.12	5.40	10.52	0.95	M1	1.05	48.70	m
	7	4.32	6.04	10.35	0.93	M1	1.40	41.70	m
	8	4.29	5.20	9.49	0.85	M1	1.21	45.21	m
	9	3.94	5.50	9.44	0.85	M1	1.40	41.73	m

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

Table 2. The karyotypes of three varieties of red leaf beet

No	Karyotype fomula	Sat		L/S	Arm ratio	Karyotype type	As.K/%
		Number	Chromosome number				
RR1	2n=2x=18=14m+4sm(2SAT)	2	2	1.42	1.52	1A	59.93
RR2	2n=2x=18=14m(2SAT)+4sm	2	7	1.53	1.50	1A	59.60
RR3	2n=2x=18=18m(2SAT)	2	1	1.33	1.33	1A	56.85

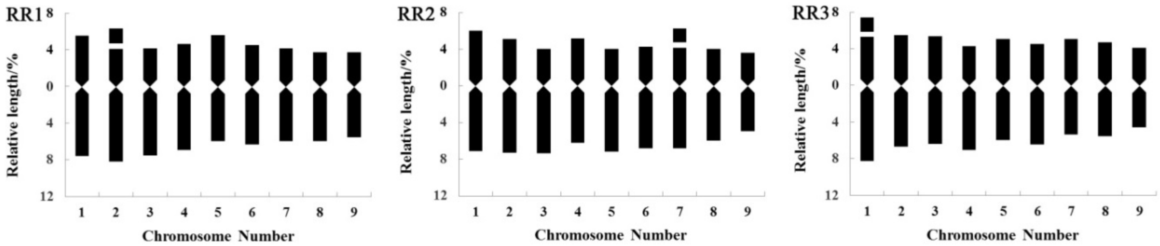

Fig 2. Chromosome idiogram of red leaf beet

4. Discussion

In our study, the karyotype asymmetry index of the chromosomes of red leaf beet was relatively low. Therefore, red leaf beet is primitive species. Leaf beet was reported the hybrid of leaf subspecies of common sugar beet and coastal sugar beet subspecies. And leaf beet was artificially selected as leaf vegetable dating back to the 2000 BC [8]. All subspecies of common sugar beet were observed as $2n=2x=18$ chromosomes, and they were mainly composed by metacentric chromosome and submetacentric chromosome, besides two of them were accompanied by satellites, The karyotypic characteristics of common sugar beet were the same as those of three varieties of red leaf beet in our study, which indicated that there was little variation on chromosome level and no ploidy change during the subsequent evolution of leaf beet. However, many other diversities were also existence among different varieties of red leaf beet. For example, the satellites located at different chromosomes. As known to all, the basic evolutionary trend of plant karyotypes is from symmetry to asymmetry. Therefore, a primitive plant will have a symmetrical karyotype [9]. In this study, the evolutionary degree of RR1 and RR2 were very closely but obviously higher than RR3, which determined by the karyotype asymmetry index and average arm ratio. The karyotype asymmetry of the three varieties of red leaf beet was highly dispersed, which might be due to the influence of different environmental conditions and artificial selection on their genetic material. These evolutionary differences may be reflected in plant traits such as plant height, petioles width, seed number, and so on.

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