Karyotype Analysis of White, Green and Red Petioles Leaf Beet

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Abstract. Leaf beet is one of the important leaf vegetables which has important ornamental value, and is popular with consumers. Green leaves leaf beet includes three kinds, white petioles, green petioles and red petioles. In this research, we try to obtain cytological parameters on these three leaf beet. The results showed that the relative length of white petioles leaf beet was ranged from 8.47% to 12.98% and max arm ratio was determined 1.53. The karyotype asymmetry index was 55.25%, and the karyotype formula was 2n=2x=18=18m (2SAT). The relative length of green petioles leaf beet was ranged from 9.95% to 12.44% and max arm ratio was determined 1.77. The karyotype asymmetry index was 57.42%, and the karyotype formula was 2n=2x=18=16m (2SAT)+2sm. The relative length of red petioles leaf beet was ranged from 9.58% to 12.66% and max arm ratio was determined 1.66. The karyotype asymmetry index was 58.57%, and the karyotype formula was 2n=2x=18=18m (2SAT). The karyotype characteristics of three kinds of leaf beet were all type 1A. The findings revealed the karyotypic characteristics of leaf beet from the cytogenetic aspects.

1. Introduction

Leaf beet (Beta vulgaris var. cicla) belongs to Chenopodiaceae, 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 is a common ornamental plant. Leaf beet has good high yield, disease resistance and adaptability. It is widely planted in China because of its good comprehensive characters. Varieties of leaf beet exist in the world, and they present significant difference in appearance. The difference in plant phenotype is mainly influenced by genetic material and environment, in which genetic material is the main factor. Karyotype analysis is a basic method to study chromosomes, it is a basic work in cytogenetics research. It has been reported that sugar beet has diploid, triploid and tetraploid [2]. However, the chromosomes of different types of plants, even different cultivars vary widely. In this experiment, the karyotype analysis was carried out on three kinds of green leaves leaf beet (white petioles, green petioles and red petioles) 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 kinds of green leaves leaf beet were used as experimental materials, including the representative *Beta vulgaris* cv. Jinxing with white petioles numbering W, *Beta vulgaris* cv. Sitong with green petioles numbering G, and *Beta vulgaris* cv. Lvjinlan with red petioles numbering R.

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 9h, 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 white petioles, green petioles and red petioles leaf beet
Metaphase chromosomes and karyotype of three kinds of green leaves leaf beet root tips were shown in Fig. 1, detailed karyotype parameters of chromosome were listed in Table 1. The chromosome number of the three kinds of green leaves leaf beet all were 2n=18.

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

3.2. Karyotype analysis

The chromosome relative length of white petioles leaf beet ranged from 8.47% to 12.98%, green petioles leaf beet ranged from 9.95% to 12.44% and red petioles leaf beet ranged from 9.58% to 12.66%, and chromosome length ratio of white, green and red petioles kinds of green leaves leaf beet were 1.53, 1.25 and 1.32, respectively. The relative length constitution of white petioles was 10M2+8M1, green petioles kind and red petioles kind were 8M2+10M1. The centromeric index of white petioles kind ranged from 39.51% to 48.14%, and arm ratio ranked from 1.08 to 1.53. The centromeric index of green petioles kind ranged from 36.04% to 48.23%, and arm ratio ranked from 1.07 to 1.77. The centromeric index of red petioles kind ranged from 37.53% to 48.38%, and arm ratio ranked from 1.07 to 1.66. There were one pair (the fourth chromosome) of submetacentric chromosomes (sm) and other eight pairs of metacentric chromosomes (m) in green petioles kind of leaf beet, while white petioles kind and red petioles kind of leaf beet only consisted by metacentric chromosomes (m). Moreover, the three kinds of green leaves leaf beet all had one pairs of satellites, and the two satellites were observed at the eighth, second, third pair of chromosomes in white, green and red petioles kind leaf beet, respectively. The karyotype formula of white and red petioles kind of leaf beet was \( n = 2x = 18 = 16m(2SAT) \), green petioles kind was \( 2n = 2x = 18 = 16m(2SAT) + 2sm \). Karyotype asymmetry index of white petioles kind was 55.25%, green petioles kind was 57.42%, and red petioles kind was 58.57%. The karyotype characteristics of three kinds of green leaves leaf beet fell into type 1A according to Stebbins’s classification criteria. The chromosome idiogram of white, green and red petioles leaf beet were shown in Fig. 2.

| Table 1. Karyotype parameters of chromosome of white, green and red petioles leaf beet |
|---|---|---|---|---|---|---|---|
| No. | Chromosome No. | Relative length / % | Index of relative length | Type of relative length | Arm ratio | Centromere index / % | Centromere type |
|---|---|---|---|---|---|---|---|
| W | 1 | 6.14 | 6.84 | 12.98 | 1.17 | M2 | 1.11 | 47.32 | m |
| 2 | 6.00 | 6.46 | 12.47 | 1.12 | M2 | 1.08 | 48.14 | m |
| 3 | 5.44 | 6.79 | 12.23 | 1.10 | M2 | 1.25 | 44.66 | m |
| 4 | 5.39 | 5.96 | 11.34 | 1.02 | M2 | 1.11 | 47.48 | m |
| 5 | 5.05 | 6.27 | 11.32 | 1.02 | M2 | 1.24 | 44.61 | m |
| 6 | 4.23 | 6.48 | 10.71 | 0.96 | M1 | 1.53 | 39.51 | m |
| 7 | 4.12 | 6.19 | 10.31 | 0.93 | M1 | 1.50 | 39.96 | m |
| 8 | 4.39 | 5.79 | 10.18 | 0.92 | M1 | 1.32 | 43.15 | m |
| 9 | 3.99 | 4.48 | 8.47 | 0.76 | M1 | 1.12 | 47.10 | m |
| 1 | 5.60 | 6.84 | 12.44 | 1.12 | M2 | 1.22 | 44.99 | m |
| 2 | 4.56 | 7.52 | 12.08 | 1.09 | M2 | 1.65 | 37.73 | m |
| G | 3 | 5.06 | 6.41 | 11.47 | 1.03 | M2 | 1.27 | 44.09 | m |
| 4 | 4.03 | 7.16 | 11.19 | 1.01 | M2 | 1.77 | 36.04 | sm |
| 5 | 4.75 | 6.36 | 11.11 | 1.00 | M1 | 1.34 | 42.76 | m |
| 6 | 5.34 | 5.73 | 11.06 | 1.00 | M1 | 1.07 | 48.23 | m |
| 7 | 4.31 | 6.10 | 10.41 | 0.94 | M1 | 1.41 | 41.42 | m |
| 8 | 4.44 | 5.83 | 10.27 | 0.92 | M1 | 1.31 | 43.27 | m |
| 9 | 4.48 | 5.47 | 9.95 | 0.90 | M1 | 1.22 | 45.05 | m |
| 1 | 4.95 | 7.71 | 12.66 | 1.14 | M2 | 1.56 | 39.10 | m |
| 2 | 5.64 | 6.54 | 12.18 | 1.10 | M2 | 1.16 | 46.31 | m |
| R | 3* | 4.83 | 7.09 | 11.91 | 1.07 | M2 | 1.47 | 40.50 | m |
| 4 | 4.37 | 6.87 | 11.24 | 1.01 | M2 | 1.57 | 38.87 | m |
| 5 | 4.39 | 6.74 | 11.14 | 1.00 | M1 | 1.54 | 39.43 | m |
| 6 | 4.71 | 6.01 | 10.72 | 0.96 | M1 | 1.28 | 43.95 | m |
| 7 | 4.08 | 6.29 | 10.37 | 0.93 | M1 | 1.54 | 39.34 | m |
| 8 | 3.83 | 6.38 | 10.21 | 0.92 | M1 | 1.66 | 37.53 | m |
| 9 | 4.63 | 4.95 | 9.58 | 0.86 | M1 | 1.07 | 48.38 | m |
Note: * means the chromosomes with satellites, and the length of satellites is not included in the chromosome length.

**Fig 2.** Chromosome idiogram of white, green and red petioles leaf beet

### 4. Discussion

The results of karyotype analysis of white petioles kind of green leaves leaf beet have consistent with green and red petioles kind at some aspects. All of them are diplont with 18 chromosomes, and they have a pair of satellites. The relative length type of three kinds of leaf beet was all consisted by M1 and M2, and the chromosomes type of three kinds of leaf beet was mainly composed by metacentric chromosomes (m), only green petioles kind leaf beet have a pair of submetacentric chromosomes (sm). Besides, the karyotype characteristics of three kinds of leaf beet all fell into type 1A. However, diversities were also existence between three kinds of green leaves leaf beet. The satellites were observed at different chromosomes. Furthermore, the karyotype asymmetry index of white petioles kind, green petioles kind and red petioles kind was 55.25%, 57.42% and 58.57%, respectively. 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 [8]. Our results infer that the leaf beet is relatively primitive, and the red petioles leaf beet is more evolution than green petioles kind than white petioles kind. However, that is inconsistent with the findings of Frese, who consider the green petioles leaf beet is the most primitive type [9]. We speculate that the reason is the variety and origin of plants will have influence on karyotype, so the evolutionary comparison of three kinds of leaf beet without many varieties may have an impact in our study. Our research provides a reference for the genetic evolution of green leaves leaf beet.
Acknowledgments
This work was supported by key project of Department of Education of Sichuan Province (14ZA0016), and Student Innovation Training Program of Sichuan Province (201510626060).

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