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Karyotype Analysis of Brassica Juncea CV. Hong Kong Hakka Mustard

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Abstract. Brassica juncea was one of the important leaf vegetables which had important edible value, and was popular with consumers. Brassica juncea cv. Hong Kong Hakka Mustard had characteristics of flesh crispy, tasted sweet and delicious, and had been widely cultivated in many places of China. In this research, we try to obtain cytological parameters on 'Hong Kong Hakka Mustard'. 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 maximum chromosome length was measured 4.10 μm and max arm ratio was determined 1.66, relative length ranged between 2.48% to 7.94%. 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 46.49%, centromere type were metacentric (m) chromosomes. Karyotype asymmetry index was 58.81%, and the karyotype formula was $2n=36=36m$ (2SAT). The karyotype characteristics was type 1B according to Stebbins's classification criteria. According to the above information, we can draw a conclusion that 'Hong Kong Hakka Mustard' is a relatively primitive mustard variety. The findings revealed its karyotypic characteristics of 'Hong Kong Hakka Mustard' from the cytogenetic aspects.

Key words: Brassica juncea cv. Hong Kong Hakka Mustard, Chromosome, Karyotype.

INTRODUCTION

Brassica juncea is a kind of cruciferae brassica annual herbaceous plant, which originated from spontaneous hybridization of the ancestors of *B. rapa* (AA, $n=10$) and *B. nigra* (BB, $n=8$) [1]. Brassica juncea is origin from Asia and are cultivated throughout China which contain abundant nutrients. The mustard variety 'Hong Kong Hakka Mustard' is a leaf mustard variety produced by Hong Kong Choi Hing Lee International Co., Ltd., which is native to Australia. It has the characteristics of wide stalks and thick mesophyll, the flesh quality is sweet and delicious, no bitter taste and good yield. When mustard is grown to 20-30 days, it can be harvested and marketed as a young vegetable, and it can also grow to mature to harvest which has high edible and market value.

Karyotype analysis is a basic method to study chromosomes, it is a basic work in cytogenetics research. In this experiment, the karyotype analysis was carried out on the typical rape variety 'Hong Kong Hakka Mustard' to reveal its chromosome composition, and to provide the basis for determining the genetic composition of Brassica juncea cv. Hong Kong Hakka Mustard.

MATERIALS AND METHODS

Plant Materials

The representative Brassica juncea cv. Hong Kong Hakka Mustard from Australia was used as experimental material.

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 4h, 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 8 min, 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 Brassica Juncea Cv. Hong Kong Hakka Mustard

Metaphase chromosomes and karyotype of Brassica juncea cv. Hong Kong Hakka Mustard root tips were shown in Fig. 1, detailed karyotype parameters of chromosome were listed in Table 1. The chromosome number of 'Hong Kong Hakka Mustard' was 2n=36.

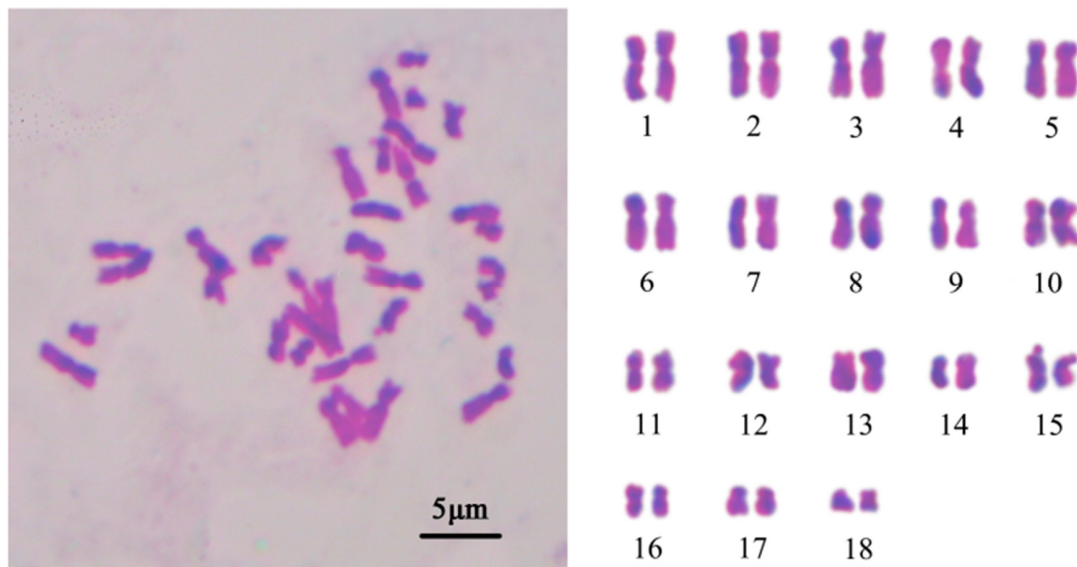


FIG 1. Metaphase chromosomes and karyotype of Brassica juncea cv. Hong Kong Hakka Mustard root tips

Note: The number 1-18 represent chromosome No.

Karyotype Analysis

Chromosome relative length ranged from 2.48% to 7.94%, and chromosome length ratio (longest chromosome / shortest chromosome) was 3.20. 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 8L+12M2+8M1+8S. The centromeric index ranged from 37.53% to 46.49%, and arm ratio ranked from 1.15 to 1.66. Eighteen pairs of centromere types were metacentric chromosomes (m). Moreover, two satellites were observed at the fifteenth pair of chromosomes. The karyotype formula was $2n=2x=36=36m(2SAT)$. Karyotype asymmetry index was 58.81%, and karyotype characteristics fell into type 1B according to Stebbins's classification criteria. The chromosome idiogram of *Brassica juncea* cv. Hong Kong Hakka Mustard were shown in Fig. 2.

TABLE 1. Karyotype parameters of chromosome of *Brassica juncea* cv. Hong Kong Hakka Mustard

| 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 | 3.22 | 4.71 | 7.94 | 1.43 | L | 1.46 | 40.63 | m |
| 2 | 3.19 | 4.57 | 7.76 | 1.40 | L | 1.43 | 41.14 | m |
| 3 | 2.75 | 4.58 | 7.33 | 1.32 | L | 1.66 | 37.53 | m |
| 4 | 3.06 | 4.15 | 7.21 | 1.30 | L | 1.36 | 42.45 | m |
| 5 | 2.60 | 4.13 | 6.73 | 1.21 | M2 | 1.59 | 38.67 | m |
| 6 | 2.45 | 4.05 | 6.49 | 1.17 | M2 | 1.66 | 37.66 | m |
| 7 | 2.81 | 3.49 | 6.30 | 1.13 | M2 | 1.24 | 44.62 | m |
| 8 | 2.37 | 3.80 | 6.16 | 1.11 | M2 | 1.60 | 38.40 | m |
| 9 | 2.32 | 3.52 | 5.84 | 1.05 | M2 | 1.52 | 39.67 | m |
| 10 | 2.38 | 3.24 | 5.62 | 1.01 | M2 | 1.36 | 42.34 | m |
| 11 | 2.31 | 2.66 | 4.98 | 0.90 | M1 | 1.15 | 46.49 | m |
| 12 | 2.15 | 2.63 | 4.78 | 0.86 | M1 | 1.23 | 44.90 | m |
| 13 | 1.91 | 2.85 | 4.76 | 0.86 | M1 | 1.49 | 40.08 | m |
| 14 | 1.75 | 2.46 | 4.20 | 0.76 | M1 | 1.41 | 41.56 | m |
| 15* | 1.79 | 2.33 | 4.12 | 0.74 | S | 1.30 | 43.52 | m |
| 16 | 1.70 | 2.19 | 3.89 | 0.70 | S | 1.29 | 43.69 | m |
| 17 | 1.36 | 2.05 | 3.41 | 0.61 | S | 1.51 | 39.88 | m |
| 18 | 1.07 | 1.41 | 2.48 | 0.45 | S | 1.32 | 43.05 | m |

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

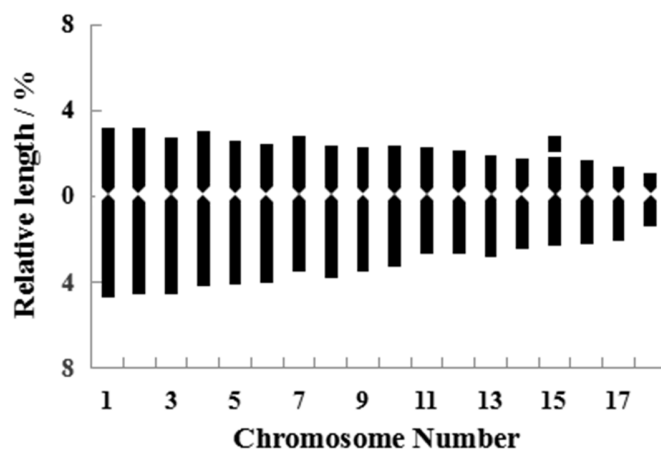


FIG 2. Chromosome idiogram of *Brassica juncea* cv. Hong Kong Hakka Mustard

SUMMARY

The results of karyotype analysis of *Brassica juncea* cv. Hong Kong Hakka Mustard were significantly different among different studies. For example, in this experiment, the karyotype formula of 'Hong Kong Hakka Mustard' was

$2n=2x=36=36m(2SAT)$, all chromosome types in this study were m-type, no sm-type and st-type chromosomes were observed, but Fang et al. observed that its karyotype formula was $2n=2x=30m+4sm+2st$ or $2n=2x=22m+12sm+2st$ [7, 8]. The number of chromosomes with satellites also varies, in this experiment, mustard has a pair of satellites, but some studies have shown two pairs or one pair of satellites or none [9]. In terms of chromosome karyotype asymmetry index, the results of this experiment was more similar to *Brassica juncea* var. *foliosa* (58.21%), and was far from the result of *Brassica juncea* var. *rugosa* (66.33%) and *Brassica juncea* var. *Leucanthas* (61.61%). 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, 'Hong Kong Hakka Mustard' should be a relatively primitive mustard variety in all mustard varieties.

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