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Karyotype Analysis of Brassica Juncea CV. Hong Kong Bamboo Mustard

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Abstract. Brassica juncea is one of the important leaf vegetables which had important edible value, and was popular with consumers. Brassica juncea cv. Hong Kong Bamboo 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 Bamboo 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 4.76 μm and max arm ratio was determined 1.99, relative length ranged between 2.85% to 7.64%. 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 47.24%, centromere type were metacentric (m) and submetacentric chromosomes (sm) chromosomes. Karyotype asymmetry index was 60.58%, and the karyotype formula was 2n=36=22m (2SAT)+14sm. The karyotype characteristics was type 1B according to Stebbins's classification criteria. The findings revealed its karyotypic characteristics of 'Hong Kong Bamboo Mustard' from the cytogenetic aspects.

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 Bamboo 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 high output. When mustard is grown to 20-30 days, it can be harvested and marketed as a young stem 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 Bamboo Mustard' to reveal its chromosome composition, and to provide the basis for determining the genetic composition of Brassica juncea cv. Hong Kong Bamboo Mustard.

MATERIALS AND METHODS

Plant Materials

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

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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. Karyotype formula referred to the standard of Levan et al. [4], and the asymmetry coefficient of karyotypes was calculated by the method of Arana [5], the karyotypes were calculated according to Stebbins's standard [6].

RESULTS

Chromosome Number of Brassica Juncea Cv. Hong Kong Bamboo Mustard

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

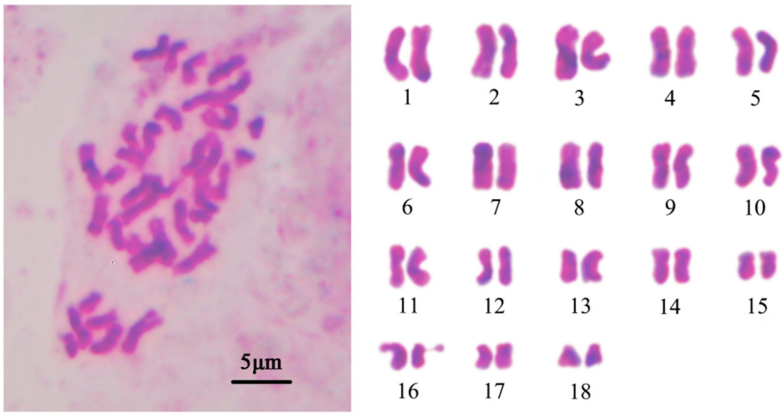

FIGURE 1. Metaphase chromosomes and karyotype of Brassica juncea cv. Hong Kong Bamboo Mustard root tips

Note: The number 1-18 represent chromosome no.

Karyotype Analysis

Chromosome relative length ranged from 2.85 % to 7.64%, and chromosome length ratio (longest chromosome / shortest chromosome) was 2.68. 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 6L+16M2+6M1+8S. The centromeric index ranged from 33.49% to 47.24%, and arm ratio ranked from 1.12 to 1.99. There were seven pairs (the second, sixth, seventh, tenth, eleventh, twelfth and thirteenth chromosome) of submetacentric chromosomes (sm) and eleven pairs (number one, three, four, five, eight, nine, fourteen, fifteen, sixteen, seventeen and eighteen chromosome) of metacentric chromosomes (m). Moreover, two satellites were observed at the sixteenth pair of chromosomes. The karyotype formula was 2n=2x=36=22m(2SAT)+14sm. Karyotype

asymmetry index was 60.58%, and karyotype characteristics fell into type 2B according to Stebbins's classification criteria. The chromosome idiogram of Brassica juncea cv. Hong Kong Bamboo Mustard were shown in Fig. 2.

TABLE 1. Karyotype parameters of chromosome of Brassica juncea cv. Hong Kong Bamboo 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	3.38	4.27	7.64	1.38	L	1.26	44.19	m
2	2.55	4.65	7.20	1.30	L	1.83	35.38	sm
3	3.18	4.00	7.18	1.29	L	1.26	44.28	m
4	3.06	3.81	6.87	1.24	M2	1.25	44.53	m
5	2.81	3.76	6.57	1.18	M2	1.34	42.80	m
6	2.30	4.04	6.34	1.14	M2	1.76	36.28	sm
7	2.22	4.01	6.22	1.12	M2	1.81	35.62	sm
8	2.32	3.88	6.19	1.11	M2	1.67	37.40	m
9	2.69	3.48	6.18	1.11	M2	1.29	43.60	m
10	2.18	3.76	5.94	1.07	M2	1.72	36.76	sm
11	2.02	3.70	5.72	1.03	M2	1.84	35.27	sm
12	1.88	3.28	5.16	0.93	M1	1.75	36.41	sm
13	1.58	3.15	4.73	0.85	M1	1.99	33.49	sm
14	1.89	2.82	4.71	0.85	M1	1.49	40.10	m
15	1.39	2.29	3.69	0.66	S	1.65	37.79	m
16*	1.62	1.81	3.44	0.62	S	1.12	47.24	m
17	1.28	2.09	3.37	0.61	S	1.63	37.97	m
18	1.07	1.78	2.85	0.51	S	1.67	37.49	m

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

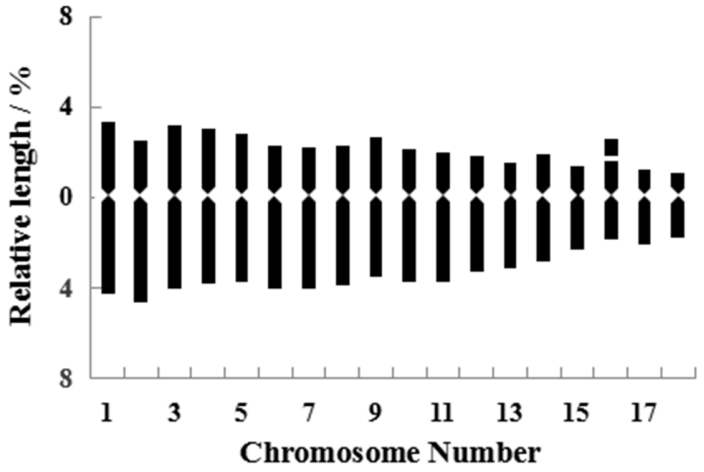

FIGURE 2. Chromosome idiogram of Brassica juncea cv. Hong Kong Bamboo Mustard

SUMMARY

The results of karyotype analysis of Brassica juncea cv. 'Hong Kong Bamboo Mustard' were significantly different among different studies. For example, in this experiment, the karyotype formula of 'Hong Kong Bamboo Mustard' was 2n=2x=36=22m (2SAT) +14sm, but some researchers observed that karyotype formula were 2n=2x=30m+4sm+2st or 2n=2x=22m+12sm+2st, which centromere type contain st-type, besides m-type and sm-type [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 were more similar to Brassica juncea var. foliosa (58.21%) and Brassica juncea var. leucanthas (61.61%), and was far from the result of Brassica juncea var. rugosa (66.33%). 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 Bamboo Mustard' should be a relatively primitive mustard variety in all mustard varieties. In addition, there were differences in arm ratio range, chromosome length ratio, karyotype asymmetry coefficient, karyotype type and so on.

The reasons for these differences may be as following three aspects: first, it's may be due to the very small size of chromosomes, which vary in length from 1 to 4 μm, during squashed preparations, the 'Hong Kong Bamboo Mustard' metaphase chromosomes are very condensed, making it difficult to analyze their morphology. Furthermore, different types of B. juncea have differences in some traits, which may also be reflected in karyotypic results. In addition, it probably owing to there be a certain degree of variation in B. juncea during the long period of cultivation and planting process and with the influence of human activities and the natural environment.

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REFERENCES

1. J. L. Wang, Y. He, Y. Q. Zhang, et al. Chinese Agri. Sci. Bull. 22, 489–494 (2006).
2. X. R. Wang, H. R. Tang, J. Duan, et al. J. Sys. And Evol. 46, 505–515 (2008).
3. M. X. Li and R. Y. Chen, J. Wuhan Botanic. Res. 3, 297–302 (1985).
4. A. Levan, K. Fradga and A. A. Sandberg, Hereditas 52, 201–220 (1964).
5. H. Arano, Japanese J. Bot. 19, 31–67 (1965).
6. G. L. Stebbins, Chromosomal evolution in higher plants (Edward Arnold Ltd. Press, London, 1971), pp. 87–123.
7. P. Fang, F. B. Chen, Q. L. Yao, et al. J. Changjiang Veg. 12, 13–17 (2014).
8. D. M. Xu, F. B. Chen, Q. Q. Jiang, et al. J. Henan Agri. Sci. 43, 111–115 (2014) .
9. W. Du, Q. X. Chen, H. Mo, et al. J. August 1st Agri. Coll. 16, 26–31 (1993).