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Chromosomal Karyotype Analysis of 'Four Seasons Shuidong Sweet Crisp' 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. four seasons shuidong sweet crisp mustard had characteristics of flesh crispy, tasted sweet and delicious. And have high resistance to disease, rain and heat. It also have advantages of not easy to bolting in the growth process, high yield, and can be planted throughout the year. Therefore it had been widely cultivated in many places of China. In this study, we measured and calculated seven chromosome parameters of 'four seasons shuidong sweet crisp mustard': chromosome length, relative length index, relative length type, arm ratio, centromere index and centromere type. Chromosomal analysis was carried out. The experiment results showed that the karyotype formula was $2n=36=24m+12sm$ and the maximum chromosome length was measured 3.70 μm and max arm ratio was determined 2.39, relative length ranged between 3.28% to 7.84%. 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 45.82%, centromere type were metacentric (m) and submetacentric chromosomes (sm), while the number of m was more than sm. Karyotype asymmetry index was 62.04%, and the karyotype characteristics was type 2B according to Stebbins's classification criteria. According to the above information, we can draw a conclusion that 'four seasons shuidong sweet crisp mustard' is a relatively primitive mustard variety. The findings revealed its karyotypic characteristics of 'four seasons shuidong sweet crisp mustard' from the cytogenetic aspects.

Key words: Brassica juncea, four season's shuidong sweet crisp mustard, Chromosome, Karyotype analysis.

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 'four seasons shuidong sweet crisp mustard' is a leaf mustard variety produced by Nanxing Seed Industry Co., Ltd. The newly selected mustard variety with vegetable ball shaped like lantern type. The quality is sweet and no bitter taste, and the taste is crisp and tender which has high edible value. The plant have high resistance to disease, rain and heat. It also have short growth cycle, from sowing to first harvest spent about 45 days. 'Four seasons shuidong sweet crisp mustard' also have advantages of not easy to bolting in the growth process, high yield, and can be planted throughout the year which has high 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 variety 'four seasons shuidong sweet crisp mustard' to reveal its chromosome composition, and to provide the basis for determining the genetic composition of Brassica juncea cv. four seasons shuidong sweet crisp mustard.

MATERIALS AND METHODS

Plant Materials

The representative *Brassica juncea* cv. four seasons shuidong sweet crisp mustard from Gansu Province 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. Four Seasons Shuidong Sweet Crisp Mustard

Metaphase chromosomes and karyotype of *Brassica juncea* cv. four seasons shuidong sweet crisp mustard root tips were shown in Fig. 1, detailed karyotype parameters of chromosome were listed in Table 1. The chromosome number of 'four seasons shuidong sweet crisp mustard' was $2n=36$.

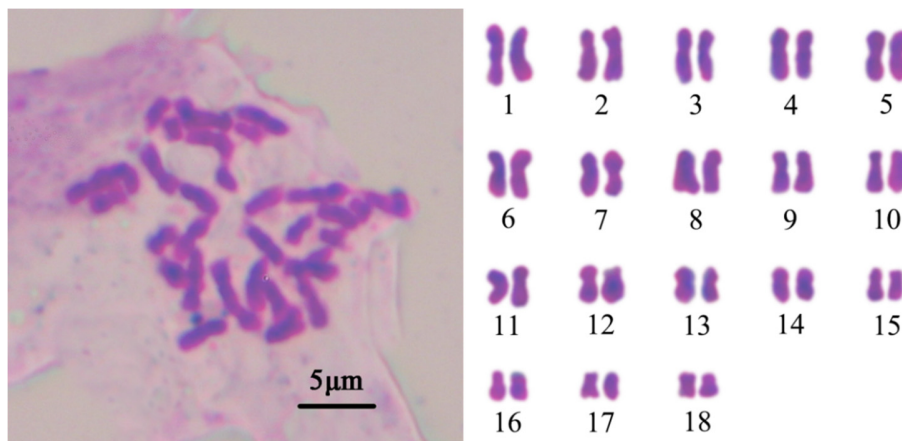


FIG 1. Metaphase chromosomes and karyotype of *Brassica juncea* cv. four seasons shuidong sweet crisp mustard root tips

Note: The number 1-18 represent chromosome No.

Karyotype Analysis

Chromosome relative length ranged from 3.28% to 7.84%, and chromosome length ratio (longest chromosome / shortest chromosome) was 2.39. The chromosome types included long chromosomes (L), medium long chromosomes (M2), medium short chromosomes (M1) and short chromosome (S), the constitution of the relative length was 6L+14M2+8M1+8S. The centromeric index ranged from 29.53% to 45.82%, and arm ratio ranked from 1.18 to 2.39. There were six pairs (the first, second, eighth, tenth, eleventh and twelfth chromosome) of submetacentric chromosomes (sm) and twelve pairs (number three, four, five, six, seven, nine, thirteen, fourteen, fifteen, sixteen, seventeen and eighteen chromosome) of metacentric chromosomes (m). The karyotype formula was $2n=2x=36=24m+12sm$. Karyotype asymmetry index was 62.04%, and karyotype characteristics fell into type 2B according to Stebbins's classification criteria. The chromosome idiogram of *Brassica juncea* cv. four seasons shuidong sweet crisp mustard were shown in Fig. 2.

TABLE 1. Karyotype parameters of chromosome of *Brassica juncea* cv. four seasons shuidong sweet crisp 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	2.66	5.18	7.84	1.41	L	1.95	33.90	sm
2	2.34	4.76	7.10	1.28	L	2.03	32.98	sm
3	2.91	4.17	7.08	1.27	L	1.44	41.06	m
4	2.64	4.22	6.85	1.23	M2	1.60	38.47	m
5	3.08	3.64	6.71	1.21	M2	1.18	45.82	m
6	2.61	4.04	6.65	1.20	M2	1.55	39.29	m
7	2.74	3.28	6.01	1.08	M2	1.20	45.53	m
8	1.75	4.19	5.94	1.07	M2	2.39	29.53	sm
9	2.23	3.38	5.61	1.01	M2	1.52	39.69	m
10	1.68	3.91	5.59	1.01	M2	2.32	30.09	sm
11	2.00	3.45	5.45	0.98	M1	1.72	36.71	sm
12	1.77	3.24	5.01	0.90	M1	1.83	35.31	sm
13	1.84	2.85	4.70	0.85	M1	1.55	39.24	m
14	1.73	2.89	4.62	0.83	M1	1.67	37.48	m
15	1.57	2.59	4.16	0.75	S	1.66	37.65	m
16	1.54	2.26	3.80	0.68	S	1.46	40.61	m
17	1.50	2.08	3.58	0.65	S	1.39	41.87	m
18	1.38	1.91	3.28	0.59	S	1.39	41.89	m

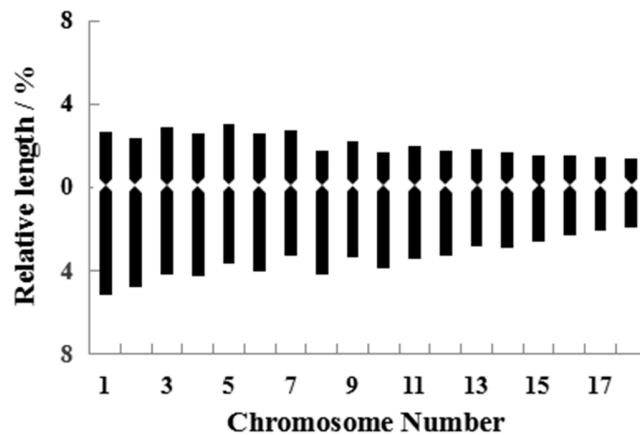


FIG 2. Chromosome idiogram of *Brassica juncea* cv. four seasons shuidong sweet crisp mustard

SUMMARY

The results of karyotype analysis of *Brassica juncea* cv. four seasons shuidong sweet crisp mustard were significantly different among different studies. For example, in this experiment, the karyotype formula of 'four seasons shuidong sweet crisp mustard' was $2n=2x=36=24m+12sm$, chromosome types were m-type and sm-type chromosomes, no st-type chromosome 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 none satellites, but some studies have shown one or two pairs of satellites [9]. In terms of chromosome karyotype asymmetry index, the results of this experiment was more similar to *Brassica juncea* var. *Leucanthas* (61.61%), and was far from the result of *Brassica juncea* var. *rugosa* (66.33%) and *Brassica juncea* var. *foliosa* (58.21%). 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, 'four season's shuidong sweet crisp mustard' should be a relatively primitive mustard variety in all mustard varieties.

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