

Studies on Morphological Characteristics of Chromosomes of Xinjiang Wild Rape

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Abstract

The morphological characteristics of chromosomes of seventy different number's Xinjiang wild rape and *Brassica nigra* were studied in this paper. The results showed that morphological characteristics of chromosomes can all be described as chromosomal number of Xinjiang wild rape is $2n=18$. It is different with *B. nigra* (*Juntus*) $2n=16$. The chromosomal karyotype of Xinjiang wild rape is the same with *B. nigra*, that is B type, but there is difference in chromosomal ratio between Xinjiang wild rape and *B. nigra*, Xinjiang wild rape chromosome ratio less than *B. nigra*. After chromosomes stained with Giemsa, the chromosomal banding patterns of Xinjiang wild rape are different with *B. nigra*. The most obvious difference is that there is one pair chromosome showed whole band in Xinjiang wild rape, it demonstrates constitutive heterochromatin. But there isn't one in *B. nigra*. It could therefore be considered as Xinjiang wild rape and *B. nigra* aren't the same species, but it has a close related with *B. nigra* and supposed during evolution process of *B. nigra*, the Xinjiang wild rape ($2n=18$) is the original type. Perhaps, there are a few middle types between Xinjiang wild rape and *B. nigra* have kept until present.

Key words: Xinjiang wild rape; *B. nigra*; Chromosome; Karyotype

Introduction

Xinjiang wild rape is wild rape, which is widely distributed in northwest of Xinjiang of China. It distributes from $42^{\circ}38'$ to $47^{\circ}14'$ north latitude, $80^{\circ}10'$ to $89^{\circ}54'$ east longitude. In this area, the level elevation is from 340 to 2400 feet high, Xinjiang wild rape is mainly distributed from 1600~2400 feet high. At this altitude, the annual mean temperatures is $3.1\sim 9.0^{\circ}\text{C}$, frost free period 80~180 days and annual rainfall is 160~504 mm. The soil belongs to both brown soil and chernozem soil with higher fertility. The wild rape resources were investigated in over 23 counties and 6 farms of Xinjiang by the Chinese Xinjiang wild rape investigation team. What is Xinjiang wild rape? Some breeders think, it is the same with *B. nigra* but some think differently, it is a new species. There was no general agreement on this question. In order to clarification the evolutionary location and role of Xinjiang wild rape in different rape species of *Brassica*, in this paper, we reported the chromosomal number, size, karyotype, constitute and characteristics of chromosomal bands in Xinjiang wild rape and as compare with *B. nigra*. These studies are important to determination evolutionary line of Xinjiang wild rape in *Brassica*.

1 Materials and methods

1.1 Material offering

The Xinjiang wild rape of seventy different numbers and *B. nigra* were mainly offered by China Oil Crops Institute.

1.2 Chromosome preparation

The method of chromosome preparation was modified from Prakash. The seeds were germinated on Ms medium at 25°C incubator. Germinating seeds with roots 0.3~0.5 cm in length were immersed 100ug/ml dactinomycin solution at 25°C dark incubator, then thoroughly washed in distilled water. The good root tips were excised and immersed them 0.05% colchicines solution for 30 min at 25°C, and then macerated in 2.5% of mixture enzymatic solution of cellulose and pectinase for 3-4h at 25°C. The preparations were stained in 1:9(v/v) Giemsa solution. The classified criterion of morphological of chromosomes is determined by Leven^[6]. Karyotype Classification is determined by Stebbins^[9].

2 Results

Chromosomes of different species have their own morphological characteristics, which are considered as a mark of studying relatives.

2.1 Chromosomal number

The tests were observed seventy materials of Xinjiang wild rape. Chromosomal number is $2n=18$ (Fig.1, 2), It is different with chromosomal number of *B. nigra* $2n=16$ (Fig.3).

2.2 Chromosomal size

According to Lima-De-Faria (1980) classified criterion of the chromosomal length variation in the whole bio-kingdom. It has been found out that though there is difference among different No. Xinjiang wild rapes it is yet more different in chromosomal size between Xinjiang wild rape and *B. nigra*. The small chromosomes of Xinjiang wild rape as 67% ~77% of the all Chromosomal number, while in *B. nigra*, there are only 38%. As it is showed in Table 1, the total length ratio of the chromosome of *B. nigra* is larger than that of Xinjiang wild rape.

**Table 1 Analysis on morphological characteristics of chromosome of
Xinjiang wild rape and *B. nigra***

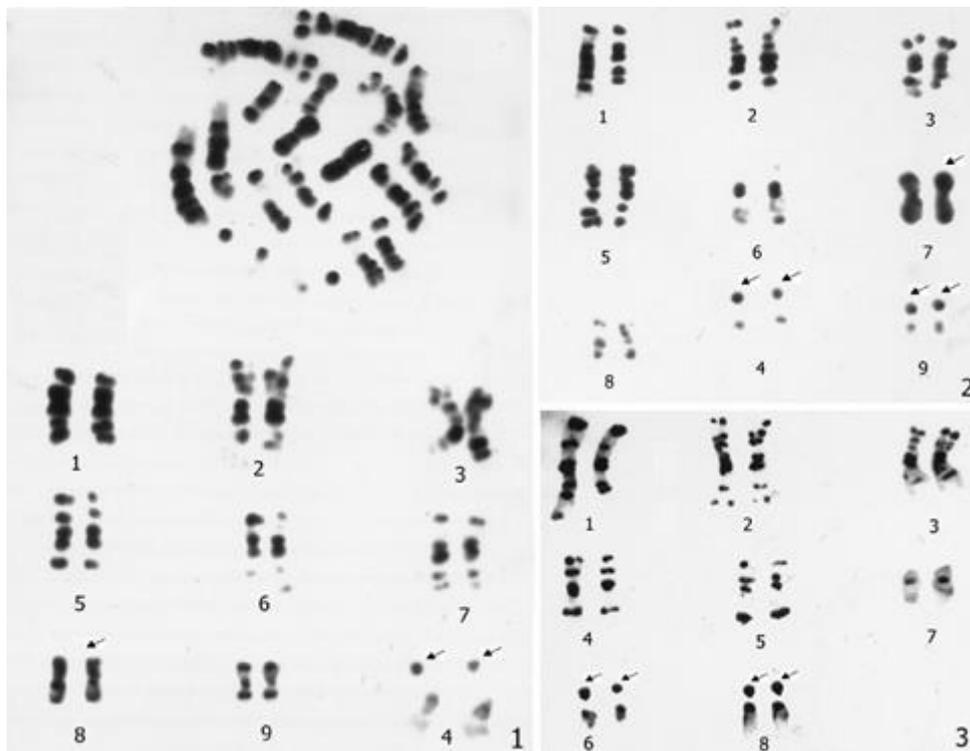
Material	Wild rape No.231	Wild rape No.235	Wild rape No.246	<i>B. nigra</i>
No. of cell	100	100	100	100
Total length of Chromosome (μm)	31.95	32.35	34.20	35.50
Variant range of chromosomal length (μm)	5.01-2.37	3.61-1.54	4.97-1.87	6.61-2.27
Ratio of Chromosome (L/S)	2.12	2.34	2.66	2.91
Karyotype	1B	1B	1B	1B
Chromosomal constitute	$2n=18=16M+$ $2M^{\text{sat}}$	$2n=18=16M+$ $2M^{\text{sat}}$	$2n=18=14M+$ $2SM+2M^{\text{sat}}$	$2n=16=12M+$ $2M^{\text{sat}}+2M^{\text{sat}}$
Position of satellite	The fourth pairs are sat-chromosomes	The fourth pairs are sat-chromosomes	The fourth and ninth pairs are sat-chromosomes	The sixth and eighth pairs are sat-chromosomes

2.3 Chromosomal karyotype

According to Stebbins (1971) karyotype classification, who proposed two indexes : The length ratio of chromosome in karyotype (the length of the longest chromosome divided by that of the shortest chromosome) and arm ratio (the long arm divided by the short ones), and the karyotype is divided 12 groups. There exists less difference among the ratio between the largest chromosome and the smallest ones in the Xinjiang wild rapes, the chromosomal average ratio of Xinjiang wild rape is 2.37, but in *B. nigra*, it is 2.91. So both belong to 2:1-4:1 this catalogue. In addition, because both of arm ratios of Xinjiang wild rape and *B. nigra* are smaller than 2:1, thus the karyotype of Xinjiang wild rape and *B. nigra* fall into 1B type, that is karyotype of symmetry.

2.4 Chromosomal constitute

The chromosomal constitute of Xinjiang wild rape has something in common with that of the *B. nigra*. The types of the chromosomal centromere both belong to localized uni-centromere, which is having a permanent localized centromeric region. Both have satellites, which are small satellites. But Xinjiang wild rape has two kinds of satellites. One kind has a pair of satellites. For example, Xinjiang wild rape 231 (Fig.1) 235. Another kind has two pairs of satellites. For example Xinjiang wild rape 246 (Fig.2). *B. nigra* also has two pair of satellites, only they are with different chromosomes (Fig.3). There is a little varieties among chromosomal constitute of Xinjiang wild rape, in which some one differs larger from *B. nigra* and others don't. For example, the chromosomal constitute of Xinjiang wild rape 231 is identical with that of 235. Both are $2n=18=16M+2M^{sat}$, metacentric chromosome, not submetacentric chromosome.



But the constitute of the chromosome of Xinjiang wild rape 246 is $2n=18=14M+2SM+2M^{sat}$. In all the nine pairs of chromosomes eight pairs of them are metacentric chromosomes; one of them is submetacentric chromosome (No.5 chromosome). The chromosomal constitute of *B. nigra* is $2n=16=12M+2M^{sat}+2SM^{sat}$. In eight pairs of chromosomes, seven pairs of them is metacentric chromosome, one of them is submetacentric chromosome (No.6 chromosome). From above indicated that the difference of the chromosomal constitute between the Xinjiang wild rape 231, 235 and *B. nigra* is large, while the difference of chromosomal constitute between the Xinjiang wild rape 246 and *B. nigra* is small.

2.5 Characteristics of chromosomal bands

The chromosome of seventy different numbers Xinjiang wild rape and *B. nigra* are stained with Giemsa after being treated. The chromosomes showed band constitutes of dark segment and light segment along the entire length of chromosomes. The region with dark segment indicates the distribution of constitutive heterochromatin; some of its characteristics are shown in Table 2. The types of chromosomal bands and different kinds of banding distribution can be seen in Table 2.

Table 2 Distribution of chromosomal bands in Xinjiang wild rape and *B. nigra*

	Material	No. of chromosome									Total
		K ₁	K ₂	K ₃	K ₄	K ₅	K ₆	K ₇	K ₈	K ₉	
Wild Rape No.231	Centromeric band	1	1	1	1	1	1	1			7
	Intercalar band	3	3	4	1	2	2	1			16
	Telomeric		2	1		1	2	2			8
	Whole band in one arm									1	1
	Whole band in two arms								1		1
	Satellite chromosome			*							1
Wild rape No.246	Centromeric band	1	1	1	1	1	1	1	1	1	9
	Intercalar band	4	3	4		2	1		1		15
	Telomeric band		1	1			1		1		4
	Whole band in one arm					1					1
	Whole band in two arms								1		1
	Satellite chromosome				*					*	2
<i>B. nigra</i>	Centromeric band	1	1	1	1	1	1	1	1		8
	Intercalar band	2	3	3	1						9
	Telomeric band	2	2	1	2	2			1		10
	Satellite chromosome						*		*		2

The characteristics of chromosomal bands of Xinjiang wild rape and *B. nigra* have similarities as well as great difference, The similar points are that both has centromeric bands, intercalary bands and telemetric bands. The different points are that banding pattern of Xinjiang wild rape are more plentiful than that of *B. nigra*, Xinjiang wild rape 231 has 33 bands (Fig.1) Xinjiang wild rape 246 has 30 bands (Fig.2), yet *B. nigra* has only 27 bands (Fig. 3). Second, the telemetric bands of Xinjiang wild rape are weaker than that of *B. nigra*. The most obvious difference is that Xinjiang wild rape has chromosomes of whole band in two arms or that of whole band in one arm, yet *B. nigra* has not.

3 Discussion

The analysis of the number of the chromosome of Xinjiang wild rape.

The report on the number of the chromosome of Xinjiang wild rape is not in agreement. Wangling Zhong (1982) first reported its number as $2n=16$ and he thought that it is just *B. nigra*^[10]. Chen Qing Xiang (1985) reported that the number of the chromosome of Xinjiang wild rape has the two kind of type, that is $2n=18$ and $2n=16$ ^[11]. Li Xun (1988) reported that its number is $2n=18$ and she concluded that this type of $2n=18$ is a stable species through the observation of the behavior of the chromosome and the analysis of meiosis index of 96.2 percent^[7,8]. It is still found in the experiment that the chromosome number of Xinjiang wild rape is $2n=18$ by further expanding the collection number of specimen to seventy. From above the conclusion may be drawn that the chromosome number of Xinjiang wild rape is different from that of the *B. nigra*. Besides according to Guan Chanyun's (1988) reported on the analysis of the morphological characteristics of Xinjiang wild rape and *B. nigra* they two share many similarities. But Xinjiang wild rape differs from *B. nigra* that it has many thorny down on its stem and leaves, as well as on its ovary and purple specks may be found at this base of its branches and its pot do not stick to the inflorescence^[3]. Another based on their morphology dissecting structural. He Fengxian (1998) reported the morphology and dissecting structures Xinjiang wild rape and *B. nigra* are similar, but obvious difference can be found in the number of the breathing pores in the leaf cuticulae and the fibrovascular bundle of mid rib^[5]. According to the analysis of the seeds, Guan Chunyun (1988) points out that the seed of Xinjiang wild rape are similar to those of *B. nigra* in shape, size, color, the obvious degree of the net of seed coat, omphalode Chalaza, character of the spine of embryonic root and the quality of sticky substance of seed coat, but are obvious different from those of other types of rapes^[4]. But the picture of the electron microscope scanning of Xinjiang wild rape is completely different from that of *B. nigra*. According to the above research results, it can be said that close related as well as obvious differences can be found between Xinjiang wild rape and *B. nigra*. Thus a hint can be obtained that Xinjiang wild rape is not of the same species of *B. nigra*, probably, it is the originalest type in the systematic evolution of *B. nigra*. The morphological characteristics of different number of Xinjiang wild rape have some variation, it can be shown diversity at number of chromosome, that is the most of Xinjiang wild rape is originalest type,

$2n=18$. But further researches have to be done to find whether intermediate types, this is the chromosome number being $2n=17$ and $2n=16$, exist or not.

The analysis of the band shape of Xinjiang wild rape.

According to the analysis of the band shape of Xinjiang wild rape and *B. nigra*, similarities as well as distinguished differences can be found. Similarities being that both have Centromeric band, Intercalary band, Telomeric band. Differences being the band of Xinjiang wild rape is more than that of *B. nigra* in band type and number, and telemetric band of Xinjiang wild rape is weaker than that of *B. nigra*, in which the most obvious differences being of Xinjiang wild rape has chromosome of two arm completely banding or an arm completely banding, while *B. nigra* doesn't have. One pair of chromosome of the nine pairs of the former is the high content of heterochromatin, it must be connected with their genetical ineffectiveness, and the latter doesn't have such a pair. Thus it is supposed that in the systematic evolution of *B. nigra*, Xinjiang wild rape is its original type. When the unequal mutual exchange of two chromosome segments between one stip of the pair of heterochromatin chromosome and another one stip of the pair of nonhomologous origin, translocation products are a long chromosome and a very small chromosome that can pass to the same pole in anaphase I. Thus it can be formed one gametes with small chromosome and lost it in the normal gametes. Whether the gametes of lost small chromosome are effective depend on the genetic effective of small chromosome. The small chromosome of Xinjiang wild rape may be no effective, because the regions close to the centromere in the two arms of chromosome are all heterochromatin which is inert substances in genetic. If so, the gamete without this kind of small chromosome is effective and the syngamy of two such gamete, female gamete and male gamete, will produce a line that is lost a chromosome in basis number. The number of chromosome will change from $2n=18$ to $2n=16$. Therefore, it can be regarded if Xinjiang wild rape is original type in the systematic evolution of *B. nigra*, the reduction of the basic number of chromosome must be provided with two conditions: (1) through unequal translocation, (2) the most part of chromosome consists of heterochromatin, especial is that whether the substance around centromere is active or inert.

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Explanation of plates

1. Chromosomal bands of Xinjiang wild rape No. 231. Arrows indicate a pair of whole band chromosomes and a pair of sat-chromosomes;
2. Chromosomal bands of Xinjiang wild rape No.246. Arrows indicate a pair of whole band chromosomes and two pairs of sat-chromosomes;
3. Chromosomal bands of *B. nigra*. Arrows indicate two pairs of sat-chromosomes.