# Karyotype Analysis of Brassica Napus CV. Huayou No.2

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**Abstract.** Brassica napus is one of the important oil crops in the world and has important economic value, B. napus cv. Huayou No.2 has excellent characteristics of resistance to lodging and disease, and has been widely cultivated in all provinces of the Yangtze River Basin and Yunnan-Guizhou Plateau in China. In this research, we try to obtain cytological parameters on 'Huayou No.2'. Seven chromosomal parameters were measured and calculated: chromosome length, relative length, index of relative length, type of relative length, arm ratio, centromere index, centromere type. The experiment results showed that the maximum chromosome length was measured 2.70 μm and max arm ratio was determined 2.12, relative length ranged between 3.54%-7.07%. There are fout 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 47.70%, centromere type included submetacentric (sm) and metacentric (m) chromosomes. Karyotype asymmetry index was 60.52%, and the karyotype formula was 2n=38=28m+10sm (2SAT). The karyotype characteristics were type 2A according to Stebbins's classification criteria. The findings revealed its karyotypic characteristics of 'Huayou No.2' from the cytogenetic aspects.

## 1. Introduction

Brassica napus is a kind of cruciferae brassica plant, which originated from spontaneous hybridization of the ancestors of B. rapa (AA, n=10) and B.oleracea (CC, n=9) followed by diploidization[1,2]. It is one of the most important oil crops in the world and is widely used in produce edible oil[3], in addition, its dross can be used as animal feed because of its high protein content, B. napus also has the characteristics of strong resistance to disease, so it is clear that the genetic composition of B. napus can provide the basis for the improvement of Brassica species. Brassica napus cultivar 'Huayou No.2' is a new hybrid rape variety bred by Fengle Seed Industry Co., Ltd. in 2014, which has good high yield, disease resistance and adaptability, and low content of erucic acid and glucosinolate. It is widely planted in China because of its good comprehensive characters. The plant is compact in shape and suitable for mechanical harvesting, it conforms to the requirements and direction of rape development, and has high value of research and application. 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 'Huayou No.2' to reveal its chromosome composition, and to provide the basis for determining the genetic composition of B. napus cv.Huayou No.2.

## 2. Materials and Methods

### 2.1 Plant Materials.

The representative Brassica napus cv. Huayou No.2 from Anhui Province was used as experimental material.

### 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 4h, and fixed in Carnoy's solution (acetic acid: absolute ethanol, 1:3,

v/v) at 4 ℃for 24 h, subsequently, the root tips were macerated in 1 mol·L-1 hydrochloric acid at 60 ℃ for 8 min, stained with Carbol Fuchsin, and observed under microscope[4].

**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.[5]. 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.[6], and the asymmetry coefficient of karyotypes was calculated by the method of Arano[7], the karyotypes were calculated according to Stebbins' standard[8].

## 3. Results

**3.1 Chromosome Number of Brassica Napus Cv. Huayou No.2.**

Metaphase chromosomes and karyotype of B. napus cv. Huayou No.2 root tips were shown in Fig. 1, detailed karyotype parameters of chromosome were listed in Table1. The chromosome number of 'Huayou No.2' were 2n=38.

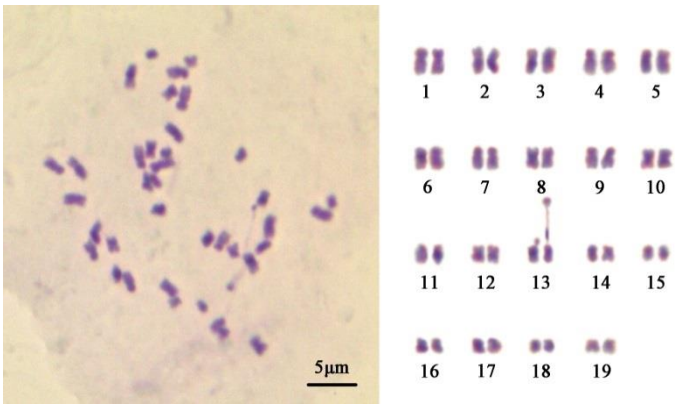


Fig. 1. Metaphase chromosomes and karyotype of Brassica napus cv. Huayou No.2 root tips
Note: The number 1-19 represent chromosome No.

**3.2 Karyotype Analysis.**

Chromosome relative length ranged from 3.54% to 7.07%, and chromosome length ratio (longest chromosome / shortest chromosome) was 2.00. 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+14M2+10M1+8S. The centromeric index ranged from 32.09% to 47.70%, and arm ratio ranked from 1.10 to 2.12. There were five pairs (the fourth, fifth, sixth, seventh and ninth chromosome) of submetacentric chromosomes (sm) and fourteen pairs (number One, two, three, eight, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen and nineteen chromosome) of metacentric chromosomes (m). Moreover, the two satellites were observed at the thirteen pair of chromosomes. The karyotype formula was 2n=38=28m+10sm (2SAT). Karyotype asymmetry index was 60.52%, and karyotype characteristics fell into type 2A according to Stebbins's classification criteria. The chromosome idiogram of B. napus cv. Huayou No.2 were shown in Fig. 2.

Table 1. Karyotype parameters of chromosome of *Brassica napus* cv. Huayou No.2

| 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 | 2.69 | 4.38 | 7.07 | 1.34 | L | 1.63 | 37.99 | m |
| 2 | 2.74 | 4.23 | 6.96 | 1.32 | L | 1.55 | 39.29 | m |
| 3 | 2.80 | 4.00 | 6.80 | 1.29 | L | 1.43 | 41.23 | m |
| 4 | 2.04 | 4.31 | 6.35 | 1.21 | M2 | 2.12 | 32.09 | sm |
| 5 | 2.29 | 4.04 | 6.34 | 1.20 | M2 | 1.76 | 36.18 | sm |
| 6 | 2.06 | 3.86 | 5.93 | 1.13 | M2 | 1.87 | 34.84 | sm |
| 7 | 2.14 | 3.77 | 5.91 | 1.12 | M2 | 1.76 | 36.18 | sm |
| 8 | 2.46 | 3.26 | 5.72 | 1.09 | M2 | 1.33 | 42.99 | m |
| 9 | 2.09 | 3.60 | 5.70 | 1.08 | M2 | 1.72 | 36.76 | sm |
| 10 | 2.29 | 3.00 | 5.29 | 1.01 | M2 | 1.31 | 43.34 | m |
| 11 | 1.99 | 3.27 | 5.26 | 1.00 | M1 | 1.64 | 37.85 | m |
| 12 | 1.86 | 2.71 | 4.57 | 0.87 | M1 | 1.46 | 40.69 | m |
| 13* | 2.18 | 2.39 | 4.56 | 0.87 | M1 | 1.10 | 47.70 | m |
| 14 | 1.79 | 2.59 | 4.38 | 0.83 | M1 | 1.45 | 40.82 | m |
| 15 | 1.72 | 2.48 | 4.20 | 0.80 | M1 | 1.44 | 40.96 | m |
| 16 | 1.62 | 2.32 | 3.94 | 0.75 | S | 1.43 | 41.10 | m |
| 17 | 1.76 | 2.16 | 3.92 | 0.74 | S | 1.22 | 44.98 | m |
| 18 | 1.51 | 2.04 | 3.55 | 0.67 | S | 1.35 | 42.49 | m |
| 19 | 1.44 | 2.10 | 3.54 | 0.67 | S | 1.45 | 40.76 | m |

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

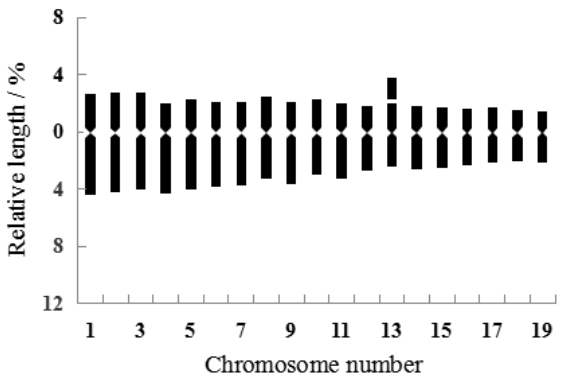


Fig. 2. Chromosome idiogram of Brassica napus cv. Huayou No.2

## 4. Summary

The results of karyotype analysis of Brassica napus cv. Huayou No.2 were significantly different among different studies. For example, in this experiment, the karyotype formula of 'Huayou No.2' was 2n=38=28m+10sm(2SAT). And Du et al. observed that its karyotype formula was 2n=38= 20m+4sm(SAT)+10sm+4st [9], Marina et al. reported that the karyotype formula was 2n=38=20m+10sm+8st(2SAT) [10]. Although the results of different studies show that the chromosome types are mostly m-type chromosomes, there are still differences in the presence of st-type chromosomes. The number of chromosomes with satellites also varies, with two pairs or one pair of satellites. In terms of chromosome relative length, the results of this experiment were more similar to Du et al. (2.68%-7.54%) and was far from the result of Marina et al. (1.49%-4.04%). 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: firstly, it's may be due to the very small size of chromosomes, which vary in length from 1 to 3μm, during squashed preparations, the 'Huayou No.2' metaphase chromosomes are very condensed, making it difficult to analyze precisely their morphology. Furthermore, different varieties of B. napus 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. napus during the long period of cultivation and planting process and with the influence of human activities and the natural environment.

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