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Karyotype Analysis of Tuber Mustard (*Brassica juncea* Var. Tumida Tsen & Lee) in China

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ABSTRACT

Tuber mustard (*Brassica juncea var. tumida* Tsen & Lee), a variation in the species *B. juncea* belonging to the Cruciferae family, is an agriculturally and economically important crop cultivated along the Yangtze River in China. In this study the karyotypic characteristics of tuber mustard has been investigated by examining metaphase chromosome spreads obtained from root tip. The results showed that the karyotype formula of tuber mustard was 2n=2x=36=18 m (2SAT)+12sm+6st. The total haploid length of the species was 27.85 μm. The chromosome lengths ranged from 1.47 μm to 2.10 μm with an average of 1.73. The relative lengths of chromosomes ranged from 5.28 to 7.54. The ratio between the longest chromosome and the shortest chromosome was 1.43. The arm ratio ranged from 1.12 to 4.21. The karyotypic asymmetry of tuber mustard was 70.10%, of which belonged to "2A". We can differentiate tuber mustard and the other species in brassica juncea by their karyotype characteristics. These results may help to carry out germplasm protection, research and improving tuber mustard in future. © 2011 Friends Science Publishers

Key Words: Chromosome number; Karyotype analysis; Karyotype formula; Tuber mustard

INTRODUCTION

Tuber mustard (*Brassica juncea var. tumida Tsen & Lee*), a variation in the species Brassica juncea belonging to the genus *Brassica* in the Cruciferae family, is an agriculturally and economically important crop merely cultivated along the Yangtze River in China. Fuling hot pickled tuber mustard, a product of mustard tuber, as well as European pickled vegetable and Japanese pickles, are hailed as the three most famous pickled vegetables in the world (Liu, 1996). Currently, there is no scientific evidence to prove where or when people began to cultivate the plant.

Historical origin of tuber mustard is presumably in the Sichuan Basin along the Yangtze River of China before the mid-18th century, where the first record about the plant was in the year 1786 in the Qing Dynasty (Liu, 1996). Zeng and Li named the plant *Brassica juncea var. tumida Tsen* and *Lee* as Latin name in 1942 (Liu, 1996). Generally, the origin of tuber mustard was presumed to be from the wild mustard (Yang *et al*., 1989). Chen *et al.* (1992) studied the origin and evolution of mustard in China. They investigated the distribution and variation of wild mustard, the original parental species and the cultivated mustards. It was indicated that one of the origin center of mustard was the northwest of China and the cultivated mustard originated from their wild progenitors. Additionally, the tuber mustard was first discovered in the Sichuan Basin.

Since 1950s, researchers have studied the growth characteristics(Chen *et al.*, 1997a; Liu *et al.*, 2004; Tang *et al.*, 2004; Lin *et al*., 2005), the mechanism of swelling characteristics and hollow heart (Liu *et al.*, 2005; Zhang, 2005), transfer and change of endogenous hormones (Zhang, 1996), inheritance of major characters (Liu, 2006, 2007) and resistance to diseases and insect pests (Zhang *et al.*, 1990; Xiao *et al.*, 2004) in tuber mustard. In 1990s, breeders began to use heterosis and develop hybrids in tuber mustard. Chen *et al.* (1995; 1997b) studied the cytoplasmic male sterile lines and the ways to use hesterosis in tuber mustard. Chen *et al.* (2001) and Hu *et al*. (2001) studied some morphological, physiological, and biochemical characteristics of the cytoplasmic male sterile lines in tuber mustard. Nonetheless, none of these reports haves described the karyotype characteristics in tuber mustard.

Karyotype characteristics playd a vital role in improvement and comprehension of the phylogenetic relationships between the related species (Cao, 2003; Lavia *et al.*, 2009). In addition, cytogenetic studies are also relevant in the study of plant evolution and diversification (Stebbins, 1971; Ropiquet *et al.*, 2008). In this study, Karyotype analysis was applied to analyze the karyotype characteristics of tuber mustard in order to increase the knowledge and accelerate the future study in the plant.

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MATERIALS AND METHODS

Materials: The materials used in this study were five tuber mustard accessions (Fig. 1) obtained from Fuling Institute of Agricultural Sciences of Chongqing (Table I).

Separation of chromosomes: The method of root tip squash was majorly according to the method of Li and Chen (1985) method. The seed of tuber mustard were germinated in humidified Petridishs covered with gauze at 25°C in electro-heating standing-temperature cultivator. The root tips of tuber mustard with different length were dipped in five kinds of chemicals (ice water, colchicine, 8-Hydroxyquinoline, saturated 1-Bromonaphthalene & P-Dichlorobenzene) in different pretreatment time (1, 2, 3, 4, 5 & 6 h). Afterwards, the root tips were fixed in a 3:1 absolute alcohol: glacial acetic acid mixture for 24 h. Until the root tips were analyzed, they were stored in 70% alcohol in a refrigerator. The root tips were hydrolysis by HCL and enzymes to study the suitable squash technique of root tip. Then the root tips were stained with Schiff's reagent until the root tips become red. And the meristematic tissue of root tips were dyed by carbol fuchsin and used for cytological observation.

Karyotypic characteristics measurements: More than 30 cells of each material were took for statistics analysis. If 85% of the cells consistent with a constant number of chromosomes, it could be considered to be the chromosome number of the plant. Afterwards, the procedures for the location of the centromer, determination of the arm index, chromosome arms and total length, were conducted after the transfer of the images for the computer using Image Analysis System. Relative chromosome length (%) = (chromosome length/total chromosome lengths)×100. Arm ratio = length of long arm/length of short arm (Levan et al., 1964). The position of centromeric constriction was recorded as median (m: 1.0-1.7), submedian (sm: 1.7-3.0) and subtermina (st: 3.01-7.0) by the arm ratio (Stebbins, 1971). Asymmetrical karyotype coefficient (%) = total length of long arm/total chromosome length×100 (Arano, 1965).

The optimized squash technique for tuber mustard: The suitable squash technique of root tip for tuber mustard was optimized as fellow: The seed of tuber mustard were germinated in humidified Petri dishs covered with gauze at 25°C in electro-heating standing-temperature cultivator. The root tips were placed into saturated 1-bromonaphthalene and kept for 4 h at 4°C when the root grow to 0.5-1.0 cm. Afterwards, the root tips were fixed in a 3:1 absolute alcohol: glacial acetic acid mixture for 24 h. Until the root tips were analyzed, they were stored in 70% alcohol in a refrigerator. The root tips were soaking in 0.075 mol/L KCL for 30 min and hydrolyzed in 2.5% mixed enzymes (5% pectinase & 5% cellulase) for 15 min at room temperature. The root tips were soaking in distilled water for 30 min. Then the root tips were stained with Schiff's reagent until the root tips become red. And the meristematic tissue of root tips (about 2 mm length of the root tips) were dyed by carbol fuchsin and used for cytological observation. It was indicated that the squash technique was successful for cytological observation in tuber mustard.

RESULTS

Karyotype analysis: Mitotic metaphase cell of tuber mustard with dispersed chromosomes and the clear centromere were obtained. Mitotic chromosomes, chromosome karyotype and idiograms are shown in Fig. 2, Fig. 3 and Fig. 4, respectively. The detailed features of the somatic metaphase chromosomes were given in Table II.

The karyotype analysis of tuber mustard revealed that the diploid chromosome number is 2n = 36 and 18 pairs of homologous chromosomes could be obtained. The karyotype formula of tuber mustard was 2n=2x=36=18 m (2SAT)+12sm+6st. According to Levan *et al.* (1964) karyotypic classification standard, the chromosome types of the 1st, 2nd, 4th, 5th, 6th, 10th, 13th, 15th and 16th chromosomes were median. The chromosome types of the 3rd, 7th, 8th, 11th, 17th and 18th were submedian, whereas those of 9th, 12th and 14th were subtermina. The total haploid length of the species was 27.85 μm. The chromosome lengths ranged from 1.47 μm to 2.10 μm with an average of 1.73, which were small

Table I: Materials used in this study and their sources

Name	Source
Yangjiaocai1	Huairen City of Guizhou Province
Suosuocai	Hanzhong City of Shanxi Provice
Bazhong	Bazhong County of Sichuan Provice
Fuzha1	Fuling District of Chongqing City
Zhetong1	Yuyao City of Zhejiang Provice

Table II: The Chromosome measurements of metaphase chromosomes in tuber mustard

Chromosome number	chromosome length (μm) = Long arm (L)+Short arm (S)	Relative length (%)	Arm ratio (L/S)	Chromosome type
1	1.14+0.96=2.10	7.54	1.19	m
2	1.10+0.87=1.97	7.07	1.26	m
3	1.25+0.60=1.85	6.64	2.08	sm
4	1.02+0.80=1.82	6.54	1.28	m
5	1.00+0.81=1.81	6.5	1.23	m
6	0.93+0.83=1.76	6.32	1.12	m
7	1.12+0.63=1.74	6.25	1.78	sm
8	1.14+0.59=1.72	6.18	1.93	sm
9	1.32+0.38=1.70	6.1	3.47	st
10	0.91+0.78=1.69	6.07	1.17	m
11	1.22+0.46=1.68	6.03	2.65	sm
12	1.35+0.32=1.67	6	4.21	st
13	1.03+0.63=1.66	5.96	1.63	m
14	1.26+0.40=1.66	5.96	3.15	st
15	0.87+0.78=1.65	5.92	1.12	m
16	0.90+0.69=1.59	5.71	1.3	m
17	1.02+0.51=1.53	5.49	2	sm
18	0.94+0.53=1.47	5.28	1.77	sm

m: median
sm: submedian
st: subtermina

Fig. 1: The plant of tuber mustard (left) and Zhacai, the product of tuber mustard (right)

Fig. 2: Mitotic metaphase chromosomes of tuber mustard

according to the classification of Lima de Faria (1980). The relative lengths of chromosomes ranged from 5.28 to 7.54. The ratio between the longest chromosome and the shortest chromosome was 1.43. The arm ratio ranged from 1.12 to 4.21. The asymmetrical karyotype coefficient of tuber mustard was 70.10%, of which belonged to "2A" based on Stebbin (1971) classification of karyotypic asymmetry and two satellites were observed.

DISCUSSION

Under the long time selections imposed by nature and human, Chinese vegetable mustards have evolved from original dwarf shape into great variations in root, leaf, stem and seed stalk forms(Qi *et al.*, 2007). Tuber mustard is one of the variations, which is cultivated only in China. However, to date, scant information is available concerning its karyotype. These result indicated that the chromosomes of tuber mustard were small and the chromosome number was 36, like those generally reported for *brassica juncea*(Mukherijea, 1975; Chen & Yang, 1986; Chen *et al*., 1992; Wang & Li, 1992; Chen *et al.*, 2003).

Wang and Li (1992) studied the karyotype of seven variations in *Brassica juncea* The result indicated the karyomorphology for seven mustard were: *B. juncea var. megarrhiza* [2n = 36 = 24 m+10sm (2SAT)+2st]; *B. juncea var. carassicaulis* [2n = 36 = 20 m+16sm (2SAT)]; *B. juncea Var. utilis Li* [2n = 36 = 24 m+12sm (2SAT)]; *B. juncea Var. capitata Hort* [2n = 36= 24 m+10sm+2st (SAT)]; *B. juncea Var. latipa Li* [2n = 36 = 22 m+12sm+2st (2SAT)]; *B. juncea Var. rugosa Bailey* [2n = 36 = 20 m+14sm+2st (SAT)]; *B. juncea Var. leucanthus Chen et Yang* [2n = 36 = 20 m+16sm (2SAT)]. Consequently, in a detailed analysis of karyotypes, we found that the chromosome mean length, karyotype formula and the asymmetry indices of tuber mustard were different to the other variations in *B. juncea*, which allowed us to differentiate tuber mustard and the other species in *B. juncea*. As concerned to the asymmetrical karyotype coefficient, the karyotypes were all symmetrical in *B. juncea*. But when compared with those of other species in *B. juncea* previously reported (Mukherijea, 1975; Chen & Yang, 1986; Chen *et al.*, 1992; Wang & Li, 1992; Chen *et al.*, 2003). The asymmetrical karyotype coefficient of tuber mustard was bigger, which indicated that tuber mustard was more evolutive (Stebbins, 1971).

Fig. 3: Chromosome karyotype of tuber mustard

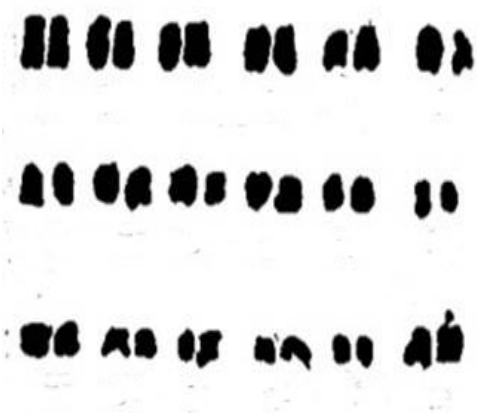
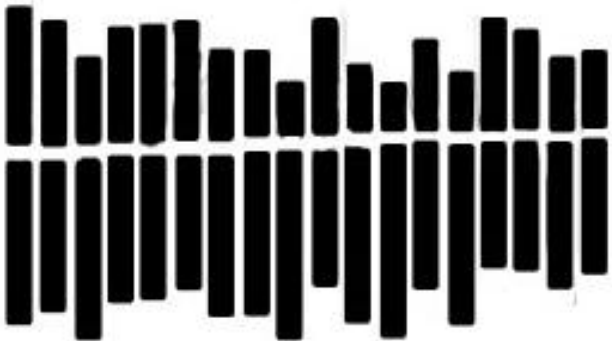


Fig. 4: Idiogram of tuber mustard (2*n* =36)



In conclusion, the karyotype formula of tuber mustard was 2n = 2x = 36 = 18 m (2 SAT) + 12 sm + 6st, belonging to"2A" with the karyotypic asymmetry was 70.1%. We can differentiate tuber mustard and the other species in *B. juncea* by their karyotype characteristics.

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