Analysis of genetic diversity in rapeseed[1]

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Abstract

Genetic diversity of 64 rapeseed varieties of *Brassica napus* L. was analyzed by RAPD. 11 polymorphic primers out of 180 random primers were selected. 120 bands were amplified and 65 bands were polymorphic (54.2%). The number of polymorphic bands of Primer 1946 was the maximum (8 bands). That of Primer 1956 was the minimum (4 bands). The 64 materials were divided into five groups by cluster analysis. The majority of materials in the first, the fourth and the fifth groups were landraces from Sichuan. The materials in the second group were landraces from Sichuan and varieties bred in Sichuan. The materials in the third group were varieties introduced from other countries and landraces from Sichuan. The genetic relationship among different materials displayed in the cluster tree indicated the genetic difference between the landraces of *B.napus* L. from Sichuan and varieties of *B.napus* L. introduced from other countries was obvious. That was the same between the landraces from Sichuan and approved varieties in China. The genetic diversity in the landraces from Sichuan was very abundant.

Key words: *Brassica napus* L., Landraces, Introduced varieties, Genetic diversity, RAPD, Cluster

Introduction

Brassica napus L.(AACC, 2n=38), *Brassica campestris* L. (AA, 2n=20) and *Brassica juncea* L.(AABB, 2n=36) are the main three rapeseed types in the world (Liu, 1984, 1985; Qian, 1996). Compared to the other two types, *B. napus* L. is the most important cultivated type, which have the good agronomic traits, such as high-yield, wide-adaptability, strong-resistance and middle-growth-period (Liu,2000).

B. napus L. originated from the west seacoast of Europe. The original species of *B. napus* L. was the heteropolyploid of natural crossing between *Brassica oleracea ssp.oleracea*(CC, 2n=18) and the original species of *B. campestris* L., cultivated for edible oil from the 16th century and introduced to Hukuoka and Hokkaido in Japan in the 19th century (Liu, 1984, 1985). The first cultivated variety of *B. napus* L. in China was the *Brassica napella Chaix* introduced by the Japanese army to Zhejiang province during the war period (Liu, 1985, 2000).

The " *victory rapeseed* " of *B. napus* L. was bred by Sichuan Academy of Agricultural Sciences (SAAS) in 1953, which was the disease-resistant line of *Brassica napella Chaix* and cultivated in Jianyang experimental farm in Sichuan. Then, the variety was cultivated widely in China (Jiang, 1998; Liu, 2000). The more ecotypes adapting to different environment were selected by man or nature from the progenies of " *victory rapeseed* " , crossing with plentiful landraces of *B. campestris* L. and *B.juncea* L. or vegetable and wild-plants in Cruciferae . The different ecotypes were cultivated more widely. Then , the varieties of *B. napus* L. were the main cultivated types, indeed of the others (Jiang, 1996, 1998) . It could be concluded that not only the most landraces , but also some new hybrids of *B. napus* L. were from the "*victory rapeseed*" of *B. napus* L.. The yield, disease-resistance and quality of the cultivated varieties of *B. napus* L. were not developed much more, although varieties of *B.napus* L. were the main cultivated types in China now. The main problems were narrow-genetic-background and short-genetic-distance among the parents and no different genes from the landraces of the cultivated varieties of *B. napus* L.. Yang (1996) and Jiang (1996) studied the landraces of *B. napus* L. from Sichuan province systematically. But they only identified the landraces on agronomic traits and phenotypic characters, never on the molecular level. In this paper, the authors selected some landraces and introduced varieties of *B. napus* L.. The genetic-distance among the materials was studied by RAPD marker. It could provided the evidence for developing the varieties of *B. napus* L. in Sichuan.

Materials and Methods

Plant Materials

The 64 accessions were 49 landraces(lines), 2 restorers and 3 varieties from Sichuan , 1 variety from Hubei, 1 landrace from Henan in China and 8 introduced varieties from the other countries. All were stored in super-low-temperature-bank in Sichuan Academy of Agricultural Sciences (SAAS).

Reagents and Apparatuses

180 RAPD primers were synthesized by Beijing Tianwei Time Biotechnology Company. Taq polymerase, Buffer, dNTPs,

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$MgCl_2$, EB and Bromophenol blue were bought from Chengdu Boreker Biotechnology Company. Agarose was produced by Spain and imported by Chengdu Gene Biotechnology Company. Molecular marker was DL2000 , provided by Shanghai Bioengineer Company.

Mycycler™ Thermal Cycler580BR2871 and the electrophoresis apparatus PAC300 were made by BIO-RAD Company. The UV Gel Image Analysis System GDS8000 was made by UVP in USA.

DNA Isolation

10 young plants per accession growing 15 days after germinating were used for DNA extraction. The method of DNA isolation was the modified CTAB procedure (Sambrook et al., 1989).

RAPD Reaction System and Program

RAPD reaction volume was 21.8μl containing 10mmol/l Tris-HCl, 50mmol/l KCl, 0.001% Gelatin, 2.5mmol/l $MgCl_2$, 0.16mmol/l each of dNTPs, 10% glycerol, 0.3μmol/l RAPD primer, 1 Unit Taq DNA enzyme, and 50ng or so DNA template. The reaction mix was overlaid with 28μl of mineral oil. The amplification program was one cycle of initial denaturation at 95°C for 10 min; 35 cycles of 94°C for 1 min, 36°C for 1 min, and 72°C for 2 min; and one cycle of a final extension at 72°C for 10 min. The amplified products were kept at 4°C until electrophoresis.

Detection of Amplified Products

3-4 μl 5×Bromophenol blue was added to each amplified product. The amplicin were separated by electrophoresis in a Model 10cm×15cm×0.1cm ATT0AE-6220 vertical gel system using 1× TAE on a 1.5% Agarose gel with 16 lanes. DL2000 used as DNA ladder marker was loaded into lane 1 per comb. The gels ran at 100 consistent voltages for 50 min. After electrophoresis, the gel was stained by EB for 3 min. The gel was carefully slided onto the UV transillminator GDS8000 and photographed automatically.

Data Analysis

For the RAPD markers, bands were scored as "1" if presents or "0" if absents, and the data were transferred to a binary (1/0) matrix. The Data Processing System(DPS) and UPGMA method were used. The genetic distance (GD) were counted (Tang, 1997). The results were analyzed through clustering.

Results and Analysis

Polymorphic primers selected and RAPD amplified

11 polymorphic primers out of 180 random primers were selected (Table 1). 120 amplified bands and 65 polymorphic bands were obtained. The ratio of polymorphic sites was 54.2%. The number of polymorphic bands of Primer 1946 was the maximum(8 bands), but that of Primer 1956 was the minimum(4 bands)(Fig 1, Fig 2).

Table 1 The polymorphic RAPD primers

Code of primer	Primer sequence	Annealing temperature	Total bands	Different bands	Ratio of polymorphic sites(%)
1946	TGCTCTGCCC	36°C	15	8	53.3
1947	GGGGGTCTTT	36°C	10	6	60.0
1948	GATGACCGCC	36°C	12	7	58.3
1949	TGGACCGGTG	36°C	9	5	55.6
1950	TTATCGCCCC	36°C	11	6	54.5
1951	CCGATATCCC	36°C	14	7	50.0
1952	GGGATATCGG	36°C	11	6	54.5
1953	CTACGGAGGA	36°C	12	6	50.0
1954	ACGCATCGCA	36°C	9	5	55.6
1955	CTGCATCGTG	36°C	10	5	50.0
1956	ACGCGCATGT	36°C	7	4	57.1

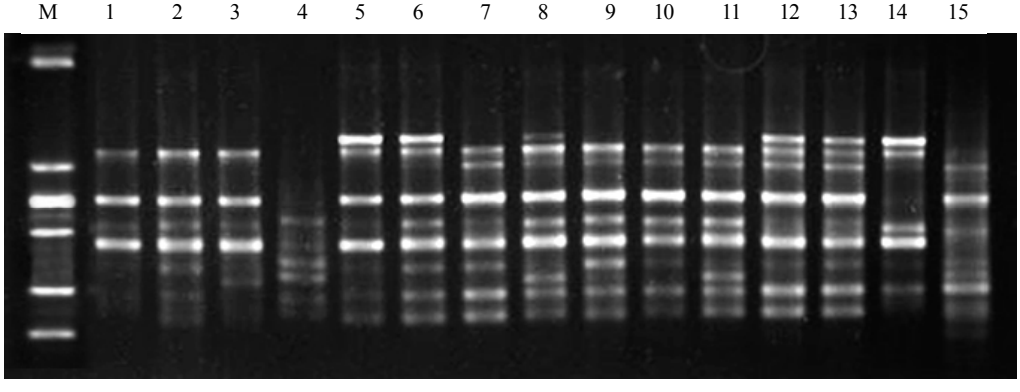

Fig 1 DNA fragments amplified by Primer 1946 in DNA samples of the accessions No.33 to No.47

Note: Lane M was Molecular marker DL2000; Lanes 1 to 15 were DNA samples of the accessions No.33 to No.47, respectively.

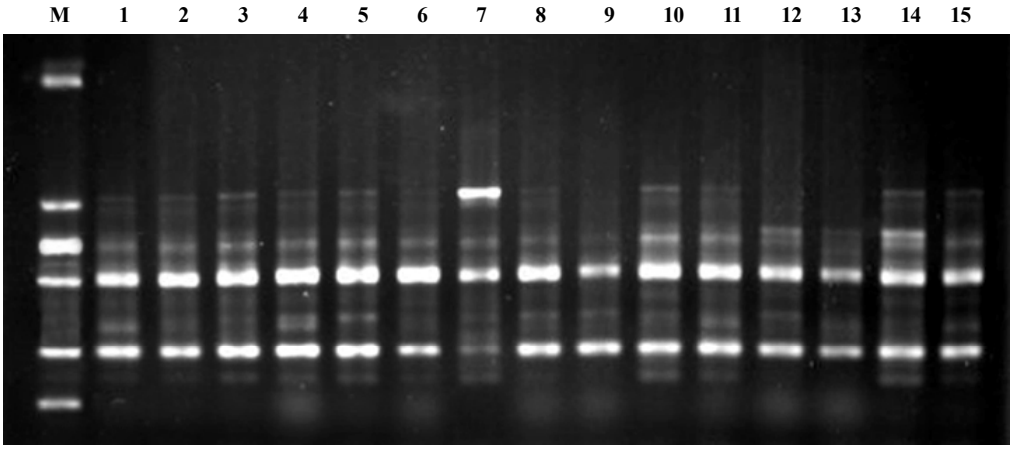

Fig 2 DNA fragments amplified by Primer 1956 in DNA samples of the accessions No.1 to No.15

Note: Lane M was Molecular marker DL2000; Lanes 1 to 15 were DNA samples of the accessions No.1 to No.15, respectively.

The cluster analysis of RAPD marker

The 64 materials were divided into five groups at GD=6.58. The majority of materials in the first , the fourth and the fifth groups were landraces from Sichuan. The materials in the second group were landraces from Sichuan and varieties bred in Sichuan. The materials in the third group were varieties introduced from other countries and landraces from Sichuan.

The first group was divided into two subgroups at GD=6.04. The one included 9 landraces(lines) from Sichuan and 1 restorer bred in Sichuan, *Mianhui No.2*. The genetic distance between No.15 and No.23 was 0.94, the nearest among 64 materials. The other one included 6 landraces(lines) from Sichuan and 1 variety introduced from other country, *Santana*.

The second group was divided into two subgroups at GD=5.98. In the first subgroup, the genetic distance between *III-228* , the systematic-selected line of SAAS and *Yu22R* , the restorer bred by SAAS was very short. That was the same between *Rongyou No.4* and *Chuanyou No.18*. *Nongpingshengliyoucai*, systematic-selected from the original variety *"Victory rapeseed"* introduced from Japan was in the second subgroup.

The third group was divided into two subgroups at GD=4.25. The first subgroup were the majority of varieties introduced from other countres. The majority of materials in the second subgroup were landraces from Sichuan.

The fourth group included 7 landraces from Sichuan and 1 variety introduced from other country, *Keleisu*. The genetic distance among No.48, No.59 and the other accessions was very long.

The fifth group was divided into three subgroups at GD=5.64. The first one included 4 landraces from Sichuan and 1 variety introduced from other country, *Hj282470*. The longest genetic distance among 64 accessions was between *Anxiandahuacazi*, the landrace from Sichuan and the other materials. *Ruishiyoucai*, introduced from other country, *Zhongyouza No.2*, bred in Hubei and *Leyou No.3*, bred in Sichuan were in the second subgroup. *Henan 1135*, landrace from Henan was in the third subgroup(Table 2 , Fig 3).

Table 2. Groups of the varieties in *Brassica napus* L. based on RAPD-marker cluster analysis

Group	Code of the varieties	subgroup	Code of the varieties	Origin of the varieties
I	1,2,3,4,5,9,11,15,17,18, 19,22,23,34,38,45,52	A	1,2,4,11,15,19,23,45,52	Landraces(lines) from Sichuan
			22	Restorer bred in Sichuan
		B	5,9,17,18,34,38	Landraces from Sichuan
			3	Introduced from other country
II	6,16,26,27,28,29,30, 31,51,55,58	A	6,16, 55,58	Landraces from Sichuan
			26,30,31	Varieties(restorer) bred in Sichuan
		B	27,28,29,51	Landraces from Sichuan
III	7,8,10,13,14,20,21,25, 35,37,60,62,63	A	7,8, 60, 63	Introduced from other countries
			20, 62	Landraces from Sichuan
		B	10,13,14,21,25, 37	Landraces(lines) from Sichuan
			35	Introduced from other country
IV	12,36,47,48,54,56,57,59	A	12,36, 56	Landraces(lines) from Sichuan
			47	Introduced from other country
		B	54,57	Landraces from Sichuan
		C	59	Landraces from Sichuan
		D	48	Landraces from Sichuan
V	24,32,33,39,40,41,42,43, 44,46,49,50,53,61,64	A	24,33,40,42	Landraces(lines) from Sichuan
			43	Introduced from other country
		B	32	Introduced from other country
			49,53	Varieties bred in China
			61,64	Landraces from Sichuan
		C	39,41,44,46,50	Landraces from Sichuan

Discussion

Brassica napus L. originated from Europe and introduced to China from Japan (Liu, 1985; Jiang, 1998). Then, the variety named "*Victory rapeseed*"was bred and popularized in the area of Yangze river in China widely. The landraces of *B. napus* L. from Sichuan were the different ecotypes from the progenies of "*Victory rapeseed*". The genetic relationships among them were far or near because of complexity of nature environment and selection of man. The approved varieties were good-quality, high-yield and disease-resistant ones adapted to different climate selected by man, through crossing, backcrossing, testing and line-selecting between landraces and introduced varieties (Jiang, 1998; Liu, 2000). The breeders attached great importance to the agronomic and economic traits of the bred variety itself and ignored the genetic background, far or near of genetic relationships and some special genes of the parent materials, which were the main reasons leading to many of approved varieties but no high-yield and disease-resistant supervariety (Shen, 2002; Ma, 2003; .Zhou, 2004).

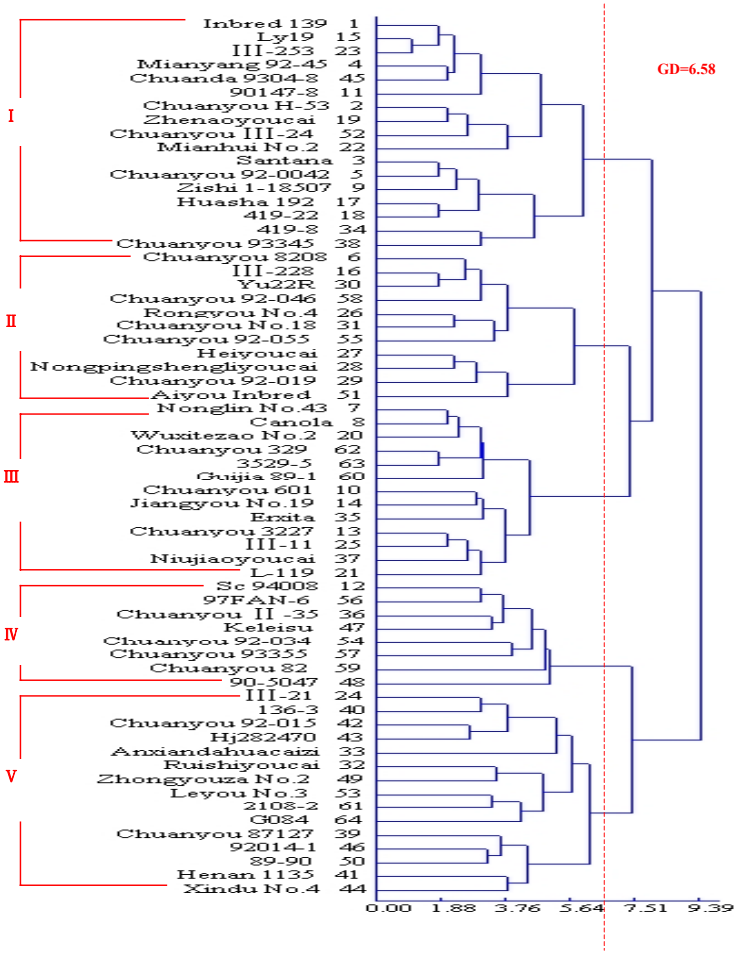

Fig 3. The cluster-tree for the varieties in *Brassica napus* L. based on RAPD markers

It was imposable to identify the genetic relationships among the materials through observing and measuring of the phenotypes. RAPD markers were more accurate for studying on molecular level (Wu, 1997; Hu, 2001; Song, 2004). In this study , the 64 materials were divided into five groups using clustering of RAPD marking results. The five groups were subdivided. The genetic relationships among the materials were demonstrated on the clustering tree.The genetic distance between No.15 and No.23 was the nearest among 64 materials. That between *III-228* , the systematic-selected line of SAAS and *Yu22R* , the restorer bred by SAAS was very short. The same was between *Rongyou No.4* and *Chuanyou No.18*. The longest genetic distance among 64 materials was between *Anxiandahuacazi*, the landrace from Sichuan and the other materials.

Conclusions

The genetic difference between the landraces of *B.napus* L. from Sichuan and varieties of *B.napus* L. introduced from other countries was obvious. That was the same between the landraces from Sichuan and approved varieties in China. The genetic diversity of the landraces from Sichuan was very abundant. RAPD marker could identify the genetic relationships among different materials more accurate than phenotypic character.

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