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Genetic diversity of Chinese and Swedish rapeseed (*Brassica napus* L.) analysed by inter-simple sequence repeats (ISSRs)

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Abstract

We have compared the genetic diversity of 24 Chinese weak-winter, Swedish winter and spring *Brassica napus* accessions by inter-simple sequence repeats (ISSRs). Using cluster analysis (UPGMA) based on 125 polymorphism bands amplified with 20 primers, the 24 accessions were divided into three groups. Six Swedish winter lines and eight Chinese weak-winter lines were in group I and group II consisted of two Chinese weak-winter lines, Xiangyou15 and Bao81. The third group contained eight Swedish spring lines. Principal coordinates (PCO) analysis showed similar groupings to cluster analysis. Results from cluster analysis and PCO analysis showed very clearly that Chinese weak-winter, Swedish spring and winter accessions were distinguished from each other and Chinese weak-winter accessions in this study were genetically closer to Swedish winter accessions than to Swedish spring accessions. The Chinese weak-winter accessions had larger diversity than the Swedish spring or winter accessions. This study indicated that ISSR is a suitable and effective tool to evaluate genetic diversity among rapeseed germplasm.

Keywords: *Brassica napus* L.; genetic diversity; inter-simple sequence repeats (ISSRs)

Introduction

Hybrid rapeseed has high heterosis (Sernyk and Steffanson, 1982; Grant and Beversdorf, 1985). Typically hybrids from two parents with distant genetic background have higher heterosis (Wu, 2000). Therefore, knowing the genetic diversity of two parents would facilitate the prediction of heterosis and would be helpful in preliminary screening before field trials.

China plants about one-third of the world's rapeseed acreage and produces about one-third of the world's crop (Fu, 1999). Sweden annually plants a substantial area of rapeseed and Swedish varieties are cultivated in Canada and Germany. Swedish rapeseed varieties are strongly responsive to either temperature (winter type) or day length (spring type). They need vernalization or long-day photoperiods before flowering. These requirements are different from Chinese rapeseed, which are alternative or day-length neutral. But no study has considered the genetic diversity of both Chinese and Swedish rapeseed.

DNA markers are promising tools to evaluate genetic diversity among germplasm. The marker systems used in rapeseed include restriction fragment length polymorphism (RFLP) (Thormann *et al.*, 1994; Diers *et al.*, 1996; Meng *et al.*, 1996), random amplified polymorphic DNA (RAPD) (Mailer *et al.*, 1994; Wu *et al.*, 1997), simple sequence repeat polymorphism (SSR) (Plieske and Struss, 2001), and anchored simple sequence repeat polymorphism (ISSR) (Charters *et al.*, 1996). RFLP analysis is renowned for its reliability but is time-consuming, relatively expensive and requires

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considerable technical expertise. RAPD analysis is a simple, quick and convenient procedure requiring much smaller quantities of template DNA. However, problems of reliability and reproducibility have been noted. SSRs are locus-specific and co-dominant polymerase chain reaction (PCR)-based markers but the development of oligonucleotide primers to be used as SSR markers is time-consuming, expensive and few loci are identified per reaction. ISSRs are a new source of genetic markers that overcome many of the technique limitations of RFLP and RAPD analysis (Gupta *et al.*, 1994; Zietkiewicz *et al.*, 1994).

We analysed the genetic variation among Chinese and Swedish *Brassica napus* accessions using ISSRs in order to provide some helpful information on the application of molecular markers to the rapeseed hybrid breeding programme.

Materials and methods

Plant materials

Twenty-four *B. napus* accessions were used to assess genetic variation. They comprised 10 Chinese weak-winter accessions (including one self-incompatible line), eight Swedish spring accessions and six Swedish winter accessions (Table 1).

DNA extraction and ISSR analysis

Leaf samples (approximately 20 mm^2) from 14-day-old plants were taken with a paper punch and placed in a microtitre plate at −70°C. The DNA extraction procedure was according to Cheung *et al.* (1993). The PCR mix for ISSR analysis consisted of 1 μl DNA (*ca* 50 ng), 2.5 μl buffer (10×, 100 mM Tris-HCl pH 8.0, 500 mM KCl, 20 mM $MgCl_2$ and 0.2% gelatin), 0.2 μl dNTPs (100 mM), 0.33 μl primer (15 mM), 0.1 μl *Taq* pol (5 U/ml, Sigma) and H_2O to a final volume of 25 μl. The 20 ISSR primers used were provided by the University of British Columbia.

The PCR programme was: 1 min at 94°C, 30 cycles of (1 min at 94°C, 2 min at 55°C, 0.5 min at 72°C), 5 min at 72°C (Charters *et al.*, 1996). PCR amplification was performed on a PTC225 peltier thermal cycler from MJ Research. The amplified fragments were resolved by sodium dodecyl sulphate–polyacrylamide gel electrophoresis using pre-cast Clean Gel 48S (Pharmacia LKB Biotechnology) and stained using a DNA silver staining kit from Amersham Pharmacia Biotech (Charters *et al.*, 1996).

Data collection and analysis

Amplified bands were recorded as present: 1 or absent: 0, and only polymorphic bands were scored. The 0–1 data set was entered into the program Popgene version 1.31

Table 1. List of materials used in the evaluation of the genetic diversity of Chinese and Swedish rapeseed

Code	Cultivar/line	Seed sources	Type	Assigned name
1	99-420395	Hubei, China	Weak-winter	C1
2	Sponsor	Svalof Weibull, Sweden	Spring	S1
3[a]	Sl1300	Hubei, China	Weak-winter	C2
4	Pastell	Svalof Weibull, Sweden	Winter	W1
5	Kvintett	Svalof Weibull, Sweden	Winter	W2
6	SW0754	Svalof Weibull, Sweden	Winter	W3
7	SW0756	Svalof Weibull, Sweden	Winter	W4
8	SW0742	Svalof Weibull, Sweden	Winter	W5
9	Banjo	Svalof Weibull, Sweden	Winter	W6
10	Maskot	Svalof Weibull, Sweden	Spring	S2
11	Senator	Svalof Weibull, Sweden	Spring	S3
12	Estrade	Svalof Weibull, Sweden	Spring	S4
13	Canyon	Svalof Weibull, Sweden	Spring	S5
14	Puma	Svalof Weibull, Sweden	Spring	S6
15	SW9522170	Svalof Weibull, Sweden	Spring	S7
16	SW9623628	Svalof Weibull, Sweden	Spring	S8
17	Shanghai9715	Shanghai, China	Weak-winter	C3
18	Huyou12	Shanghai, China	Weak-winter	C4
19	Huyou14	Shanghai, China	Weak-winter	C5
20	Xiangyou15	Hunan, China	Weak-winter	C6
21	Bao81	Hubei, China	Weak-winter	C7
22	Zhou668	Hubei, China	Weak-winter	C8
23	89008	Hubei, China	Weak-winter	C9
24	Huahuang3	Hubei, China	Weak-winter	C10

[a] A self-incompatible line.

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(C. Y. Francis and Y. Rong-cai, http://www.ualberta.ca/~fyeh/). Similarity matrix and genetic distance (GD) were calculated based on Nei's (1978) unbiased genetic identity and genetic distance and a dendrogram was drawn based on genetic distance using UPGMA (unweighted pair-group method with an arithmetic average). Similarity matrices were also used as inputs into principal coordinates (PCO) analyses, and a scatter was plotted on the first two principal components, which largely explain most of the variation.

Results

Genetic diversity

High polymorphism was observed among 24 lines (Fig. 1); 125 polymorphic bands were amplified with 20 primers with an average of 6.26 bands (Table 2).

Based on these 125 polymorphic bands, a genetic distance matrix was generated. Maximum, minimum, variance and some average pairwise genetic distances are shown in Table 3. Average genetic distance within the 10 Chinese lines is 0.387, significantly more than that within the six Swedish winter lines (0.246) and that within the eight Swedish spring lines (0.281). It showed that Chinese accessions had larger genetic variation than Swedish winter or Swedish spring lines used in this study.

The average genetic distance among Chinese accessions and Swedish spring accessions was largest, even more than that among Swedish spring lines and Swedish winter lines, both are distinguished genetically. This indicated that Chinese accessions were more distinguished from Swedish spring lines than from Swedish winter lines.

Grouping

All 24 accessions were divided into three groups by UPGMA cluster analysis (Fig. 2). Group I included all six Swedish winter lines and all Chinese weak-winter lines except Xiangyou15 (C6) and Bao81 (C7). Two Chinese weak-winter lines, Xiangyou15 and Bao81, formed group II. All eight materials in the third group were Swedish spring lines. It was shown that Chinese weak-winter, Swedish spring and winter accessions were distinguished from each other, and that Chinese weak-winter *B. napus* had large genetic diversity and was nearer to Swedish winter *B. napus* than to Swedish spring *B. napus*.

A PCO analysis was also carried out on the similarity matrix. The scatter of 24 accessions based on the first principal component and the second principal component represents 18.9 and 11.0% of the total variation, respectively (Fig. 3). The results showed similar groupings to cluster analysis. Eight Swedish spring accessions formed one group, which was separated clearly from Swedish winter and Chinese weak-winter accessions. Six Swedish winter accessions were scattered together, but close to the Chinese weak-winter accessions, which were distributed loosely in one group. Results of PCO analysis showed very clearly that Chinese weak-winter accessions have larger diversity than Swedish spring or winter accessions, and that Chinese weak-winter accessions in this study are genetically closer to Swedish winter accessions than to Swedish spring accessions.

Discussion

ISSR or anchored SSR primers are complementary to genomic microsatellites (one to four nucleotides occurring in tandem repeats) and contain short oligo-nucleotide

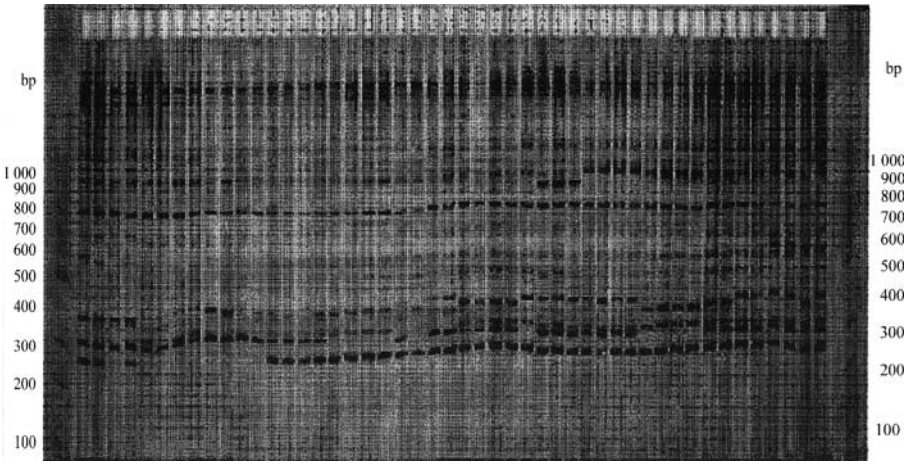

Fig. 1. PCR-amplified ISSR patterns with primer UBC841. Lanes on two sides are 100 ladder size markers.

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Table 2. Primers, their sequence and the polymorphic bands scored in the evaluation of the genetic diversity of Chinese and Swedish rapeseed

Primer	Sequence	Number of polymorphic bands among				Size range (bp)
		Total	Spring	Winter	Weak-winter	
807	AGA GAG AGA GAG AGA GT	7	6	6	7	400–1300
808	AGA GAG AGA GAG AGA GC	6	5	5	6	310–1100
814	CTC TCT CTC TCT CTC TA	3	3	0	3	480–1600
816	CAC ACA CAC ACA CAC AT	12	10	7	9	420–1700
823	TCT CTC TCT CTC TCT CC	5	4	4	5	650–1300
825	ACA CAC ACA CAC ACA CT	2	2	2	2	850–1050
834	AGA GAG AGA GAG AGA GYT	12	9	11	12	190–1200
835	AGA GAG AGA GAG AGA GYC	10	9	9	10	260–1600
840	GAG AGA GAG AGA GAG AYT	8	8	4	8	200–900
841	GAG AGA GAG AGA GAG AYC	7	7	7	7	280–1100
847	CAC ACA CAC ACA CAC ARC	2	2	2	2	740–770
866	CTC CTC CTC CTC CTC CTC	3	2	3	3	650–1500
880	GGA GAG GAG AGG AGA	1	0	1	0	400
884	HBH AGA GAG AGA GAG AG	5	5	2	5	280–1100
885	BHB GAG AGA GAG AGA GA	2	1	2	1	280–400
887	DVD TCT CTC TCT CTC TC	7	4	4	6	370–1100
888	BDB CAC ACA CAC ACA CA	7	7	6	6	440–1100
889	DBD ACA CAC ACA CAC AC	6	6	5	6	450–830
890	VHV GTG TGT GTG TGT GT	11	8	8	8	380–910
891	HVH TGT GTG TGT GTG TG	9	6	5	6	470–1000
Total		125	104	93	112	

Single letter abbreviations for mixed base positions: N = (A, G, C, T); R = (A, G); Y = (C, T); B = (C, G, T) (i.e. not A); D = (A, G, T) (i.e. not C); H = (A, C, T) (i.e. not G); V = (A, C, G) (i.e. not T).

Table 3. Maximum, minimum, variance and average genetic distance of some pairwise comparisons in the evaluation of the genetic diversity of Chinese and Swedish rapeseed

Pairwise	S + C	S + W	W + C	S + S	W + W	C + C
Maximum	0.831	0.654	0.693	0.425	0.428	0.546
Minimum	0.380	0.375	0.242	0.130	0.088	0.164
Variance	0.010	0.005	0.008	0.007	0.007	0.007
Average	0.552	0.504	0.421	0.281	0.246	0.387
N	80	48	60	28	15	45

S, Swedish spring accessions; C, Chinese weak-winter accessions; W, Swedish winter accessions.
N, number of pairwise comparisons.

'anchor' sequences that ensure the primers anneal to either the 5′ or 3′ end of the genome repeat. Microsatellite regions are abundant throughout the eukaryotic genome and are highly polymorphic in length, so SSR primers target highly variable and numerous loci. Charters *et al.* (1996) got 56 variable bands using only two primers and these bands could discriminate all the 20 cultivars that they used. Huang and Sun observed 2071 ISSR fragments with 15 primers and 62.2% of the ISSR fragments were polymorphic between 40 accessions of *Ipomoea* (Huang and Sun, 2000). High polymorphism was also revealed in our study. Between 24 lines, 125 polymorphic bands were amplified with 20 ISSR primers and the average polymorphic bands of each primer were 6.25. Primer UBC816 and UBC834 produced 12 polymorphic bands. The ISSR technique can detect high polymorphism.

Both cluster analysis and PCO analysis showed that Swedish spring accessions were separated from Swedish winter accessions. Many researchers report similar results that spring *B. napus* are quite distinct from winter *B. napus* (Charters *et al.*, 1996; Diers *et al.*, 1996; Meng *et al.*, 1996; Plieske and Struss, 2001). Spring and winter accessions in this study were bred from crossing within spring lines or within winter lines without any effort to enlarge germplasm variation by crossing spring accessions with winter accessions, and retained their distant relationship. Banjo is an F_1 hybrid and SW0742 is its restorer. They had the lowest genetic distance (0.088) and were clustered. Chinese weak-winter accession 99-420395 is also a hybrid from SI1300, and it was closest to SI1300. All the above indicated that clustering results were consistent with pedigree generally and confirmed

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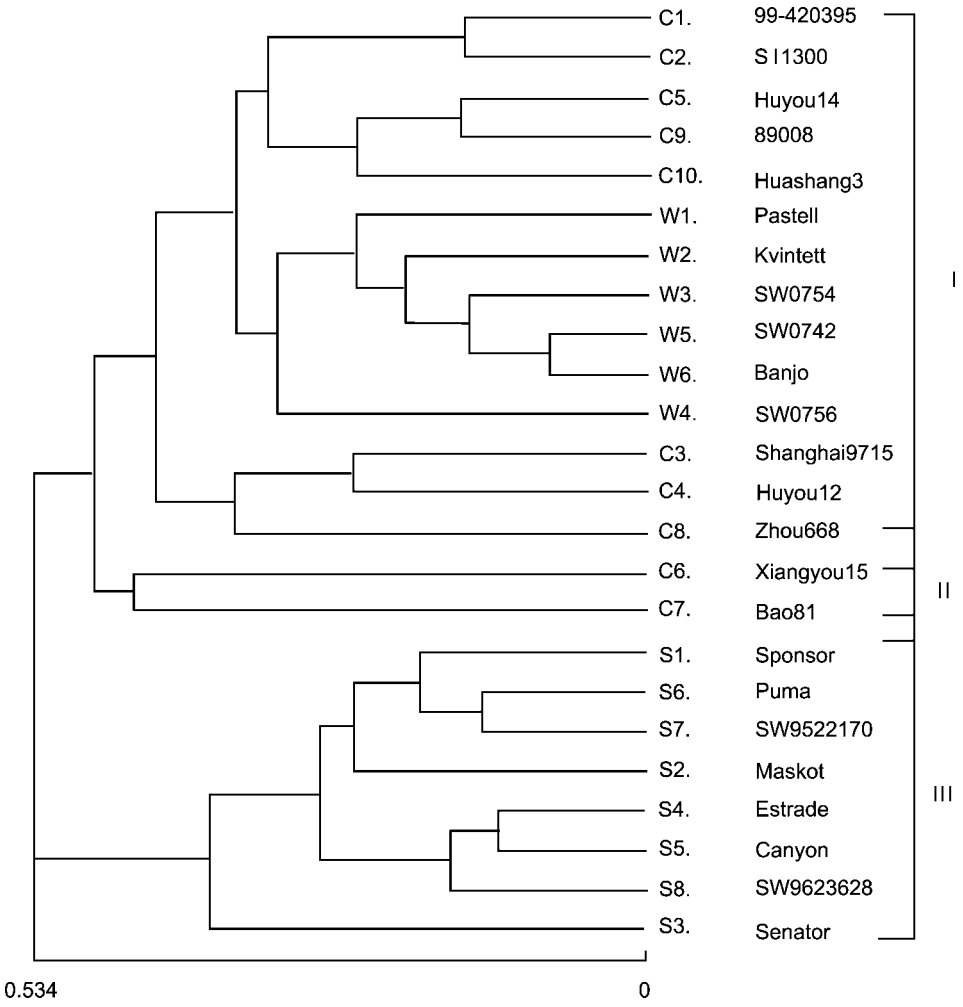

Fig. 2. Dendrogram for 24 accessions based on ISSR data using UPGMA.

that ISSR is a powerful method for revealing genetic variation in rapeseed.

Knowledge of genetic variation is useful for selecting parents for hybrid breeding programmes. Genetic variation between accessions was larger than within spring, winter and weak-winter and Chinese weak-winter accessions, so crossing between Swedish spring, Swedish winter and Chinese weak-winter accessions might be one useful way to enlarge diversity of their respective gene pool. Hybrids from crossing between Swedish spring, Swedish winter and Chinese weak-winter accessions might have high heterosis and seed yield potential. Genetic diversity within Chinese accessions was much higher than that within winter and weak-winter accessions. Large genetic variation within Chinese accessions might be one reason why hybrids have high seed yield and are widely cultivated in China.

In summary, ISSR is a suitable and effective tool to evaluate genetic diversity among rapeseed germplasm; hybrids from crossing between Swedish spring, Swedish

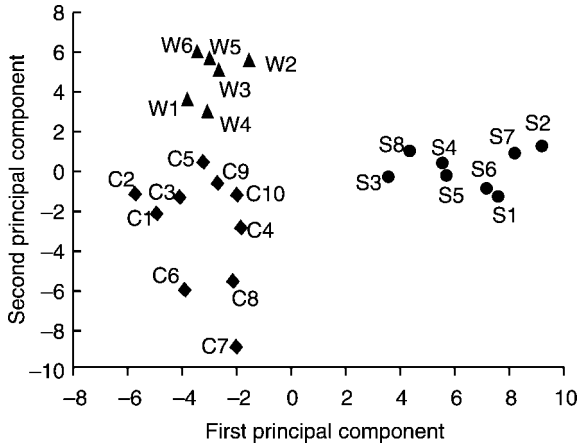

Fig. 3. Scatter of 24 accessions by PCO analysis based on ISSR data. (●) Swedish winter accessions (W); (▲) Swedish spring accessions (S); (◆) Chinese weak-winter accessions (C). The first and second coordinate axes represent 18.9 and 11.0% of the total variation, respectively.

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winter and Chinese weak-winter accessions might have high heterosis and seed yield potential.

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References

Charters YM, Robertson A, Wilkinson MJ and Ramsey G (1996) PCR analysis of oilseed rape cultivars (*Brassica napus* L. ssp. oleifera) using 5′-anchored simple sequence repeat (SSR) primers. *Theoretical and Applied Genetics* 92: 442–447.

Cheung WY, Hubert N and Landry BS (1993) A simple and rapid DNA micro-extraction method for plant, animal, and insect suitable for RAPD and other PCR analysis. *PCR Methods and Application* 3: 69–70.

Diers BW, McVetty PBE and Osborn TC (1996) Relationship between heterosis and genetic distance based on restriction fragment length polymorphism markers in oilseed rape (*Brassica napus* L). *Crop Science* 36: 79–83.

Fu TD (1999) *Breeding and Utilization of Rapeseed Hybrid*. Wuhan: Hubei Science and Technology Press (in Chinese).

Grant I and Beversdorf WD (1985) Heterosis and combining ability estimates in spring planted oilseed rape (*Brassica napus* L.). *Canadian Journal of Genetics and Cytology* 27: 472–478.

Gupta M, Chyi YS, Romero-Severson J and Owen JL (1994) Amplification of DNA markers from evolutionarily diverse genomes using single primers of simple-sequence repeats. *Theoretical and Applied Genetics* 89: 998–1006.

Huang JC and Sun M (2000) Genetic diversity and relationships of sweetpotato and its wild relatives in *Ipomoea* series Batatas (Convolvulaceae) as revealed by inter-simple sequence repeat (ISSR) and restriction analysis of chloroplast DNA. *Theoretical and Applied Genetics* 100: 1050–1060.

Mailer RJ, Scarth R and Fristensky B (1994) Discrimination among cultivars of rapeseed (*Brassica napus* L.) using DNA polymorphisms amplified from arbitrary primers. *Theoretical and Applied Genetics* 87: 697–704.

Meng JL, Lydiate D, Sharpe A, Bowman C, Tian ZH, Fu TD, Qian XZ and Lydiate D (1996) Analysis of genetic diversity by RFLP markers. *Acta Genetica Sinica* 23: 293–306 (in Chinese).

Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89: 583–590.

Plieske J and Struss D (2001) Microsatellite markers for genome analysis in *Brassica*. I. Development in *Brassica napus* and abundance in Brassicaceae species. *Theoretical and Applied Genetics* 102: 689–694.

Sernyk BR and Steffanson JL (1982) Heterosis in summer rape (*Brassica napus* L.). *Canadian Journal of Plant Science* 63: 407–413.

Thormann CE, Ferreira ME, Camargo LEA, Tivang JG and Osborn TC (1994) Comparison of genetic relationship estimates within and among cruciferous species based on RFLP and RAPD markers. *Theoretical and Applied Genetics* 88: 973–980.

Wu JF (2000) *Engineering Techniques on Crop Genetics and Breeding*. Zhengzhou: Henan Science and Technology Press (in Chinese).

Wu NF, Li NG, Wu XM, Zhu L, Fan YL and Qian XZ (1997) RAPD molecular markers and genetic diversity among 40 cultivars of *Brassica napus* in China. *Chinese Biodiversity* 5: 246–250 (in Chinese).

Zietkiewicz E, Rafalski A and Labuda D (1994) Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. *Genomics* 20: 176–183.