RFLP CHARACTERIZATION OF BRASSICA NAPUS-BRASSICA NIGRA ADDITION LINES

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INTRODUCTION

Wild or cultivated Brassica species related to B.napus carrying useful traits may be used to improve rapeseed through interspecific crosses or protoplast fusion. In order to transfer disease resistance genes from B.nigra to B.napus, an interspecific hybrid B.napus (2n=38) x B.nigra (2n=16) was backcrossed to B.napus for extraction of alien addition lines. Addition lines are usefull tools for genetic analysis and evolutionary studies. Crops of economical importance such as wheat, maize and tomato have extensive cytological stocks (addition, substitution or translocation lines) and markers and therefore their genomes are well characterized. In a previous paper, we reported the characterization of B.napus-B.nigra addition lines by molecular markers (Chèvre et al. 1991). More recently, we have developed new chromosome markers by amplification of DNA sequences using the polymerase chain reaction (PCR) (Quiros et al. submitted). We present in this report additional molecular markers (RFLP and PCR) mapped on the B.napus-B.nigra addition lines.

MATERIAL AND METHODS

Plant Material

Two plants from six monosomic or disomic addition lines constructed by Jahier et al. (1989) and previously characterized (Chèvre et al. 1991) were used in the present study. In addition, five unmarked plants carring alien B.nigra chromosomes were used in an attempt to find the two missing B genome chromosomes not represented in the addition series. The two parental varieties "tandem" (B.napus) and "Junius" (B.nigra) were used as controls. Plants originating from the same crosses than the addition lines but presenting only 38 chromosomes were used as additional controls.

DNA Extraction

DNA was extracted from young leaves of single plants according to the protocol described by This et al. (1990).

RFLP Analysis

RFLP analysis was performed according to the protocol described by Chèvre et al. (1991). Probes were extracted from cDNA libraries from mRNA of immature or dry seed of Raphanus.sativum (Raynal et al. 1991) or a partial genomic library prepared using DNA extracted from B.nigra leaves (Grellet, unpublished)

PCR Amplification

PCR reactions were performed using Taq-polymerase (Promega) following the technique of William et al. (1991). One unit of the enzyme was used in each 25µl of reaction mix containing 1x reaction buffer, 0.8 mM of each dNTP, 0,2 µmol of each primer and 50 ng of DNA (Quiros et al. submitted). For amplification, we used

arbitrary 10-mer oligonucleotides purchased from Operon (Kit B) and specific 20-mer oligonucleotides corresponding to cruciferin gene sequence (Depigny et al. in preparation) and isocitrate lyase gene sequences (Quiros, unpublished). The samples were subjected to forty cycles of amplification at 92 °C for 2min, 35°C for 1min 30 and 72°C for 2min in a Hybaid (Middless, UK) thermal cycler for the arbitrary primers. For the specific oligonucleotides, the hybridization step was performed at 55°C. After amplification, the samples were run in a 1.2 % agarose gel containing 1µg/ml ethidium bromide and the gel photographed under UV light.

RESULTS

RFLP markers

Table 1 summarizes the isozymes and RFLP markers assigned on each of six B.nigra chromosomes by following B genome specific markers in the addition lines.

Table 1. RFLP and Isozymes markers mapped on six *B.napus*-*B.nigra* addition lines

group 1	group 2	group 3	group 4	group 5	group 6
6pgdh2*	Tpi1*	Pgm1*	Pgm2*	Adh1*	pBN128-2*
Got5*	Pgm3*	pBN7*	Pgi2*	pBN6*	
pBG9*	pB488*	pAF7*	pB850*	pBN14*	
pBN128-1*				pBN27*	
BnBH35	BnBH35	BnBH35	BnBH35	BnBH35	
				p8B6*	

* previously described markers (Chèvre et al. 1991)

Six enzymes and eleven probes disclosed markers distributed on the 6 addition lines. Nine DNA probes (pBG9, pB488, pAF7, pB850,pBN6, pBN7. pBN14, pBN27,p8B6), disclosed single markers located on independent chromosomes Probe pBN128 disclosed two markers of different size on two chromosomes. Probe BnBH35 revealed identical fragments dispersed on 5 chromosomes.

PCR markers

Amplification of genomic DNA using specific oligonucleotides corresponding to radish cruciferin sequences produced very simple patterns: one intense band of 410 bp and one weak band of 700 bp for B.napus and two intense bands for B.nigra, one of 410 and an other one of 700bp. The latter marker was mapped on group 5. Hybridization with two radish cruciferin cDNA clones,(pAF7 and pAE10) (Depigny et al. in preparation) revealed that the 700 bp band correspond to pAE10 sequences which is 97% homologous to a rapeseed cruciferin cDNA clone (Simon and al. 1985).

Amplification using oligonucleotides corresponding to an isocitrate lyase gene produced 3 nigra specific products of approximately 850, 750 and 450 bp. The largest product (IL9-1) was mapped to group 3 whereas the other 2 products (IL9-2) mapped to group 2.

Amplification of DNA using 10-mer oligonucleotides showed patterns ranging from one fragment for each parent (primer B20, Fig. 1) to 10 fragments. Fragment sizes ranged from 300 to 2500 bp. Complex patterns, usually showed a few intense fragments along with many weak ones. On the addition lines profiles, intense fragments specific to B.nigra were easy to locate (Fig. 1). The weak ones were difficult to score and therefore were excluded as markers.

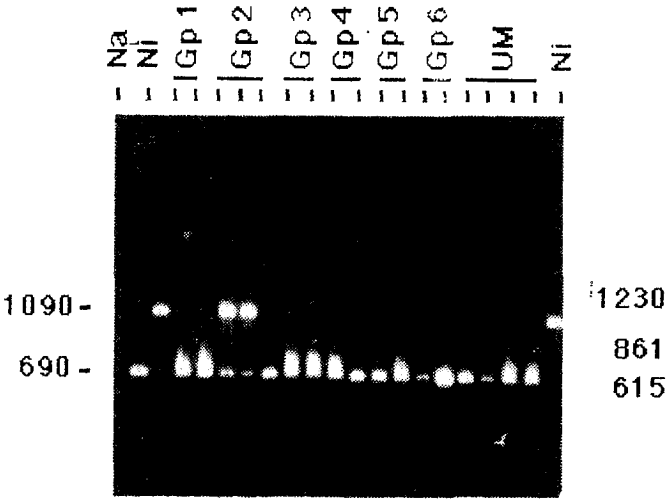

Fig. 1. DNA fragments amplified by PCR from DNA of B.napus (Na), B.nigra (Ni), addition lines from group 1 to 6 and unmarked plants (UM) using B20 primer. Plants from group 2 display the B.nigra specific band. The third plant from group 2 is a control with 38 chromosomes originating from the same crosses.

Table 2. PCR generated markers mapped on B.napus-B.nigra addition lines

group	plant #	2n	PCR markers					
			B20	B15	B13	B10	AE10	IL9
gp 1	90 v43-3	40		-	B13+	-	-	-
	90 25-1	40	-	-		-	-	-
gp 2	90 v45-4	40	B20+		-	B10+	-	IL9-2+
	90 v45-5	40	B20+		-	B10+	-	IL9-2+
gp 3	90 v57-19	39	-	-	-	-	-	IL9-1+
	90 v57-20	39	-	-	-	-	-	IL9-1+
gp 4	90 64-2	39	-	-	-	-	-	-
	90 74-3	40	-	-	-	-	-	-
gp 5	90 vl00-2	40	-	-	-	-	AE10+	-
	90 v100-3	40	-	-	-	-	AE10+	-
gp 6	90 43-2	40	-	-	-	-	-	-
	90 v111-35	39	-	-	-	-	-	-
unmarked plants	90 v27-12	40	-	-	-	-	-	-
	90 33-1	39	-	-	-	-	-	-
	90 95-21	40	-	-	-	-	-	-
	90 100-1	40	-	B15+	-	-	-	-
	90 v100-32	40	-	-	-	-	-	

Table 2 sumarizes the PCR markers located on the addition lines. Since there is little probability that two bands on the amplification profile are the same sequences, each particular fragment was considered as a different marker when present in more than one chromosome.

The amplification patterns were reproductible, that is, B.napus and all plants from the addition lines displayed the same profile except for those having B.nigra specific markers. Not all the fragments amplified from B.nigra DNA were found in the addition lines.

B.nigra specific products amplified by primer B20, B15, B13, B10 and AE10 produced single markers mapping single lines. Primer IL9 amplified products of different size marking two different lines.

DISCUSSION

In the B.napus-B.nigra addition lines, all three genomes A,B and C are represented. B genome specific markers were readily disclosed in these lines due to the extensive polymorphism among Brassica genomes. Nevertheless, some markers like the cruciferin PCR fragments were displayed by the two parents. Althrough the cruciferin700bp product was also present in B.napus, it was much less intense than in B.nigra thus it was possible to map it in the lines by band intensity.

Only two RFLP markers showed duplication in the addition lines pBN128 and BnBH35, BnBH35 showing duplications dispersed on 5 chromosomes of the B genome correspond to a higly repeated B.nigra specific sequence (Grellet personnal communication). The duplication in the B genome confirm the results previously described for B.oleracea and B.campestris by McGrath et al. (1990).

Concerning the PCR markers, they behaved like RFLP markers in the sense that most of them identified independent chromosomes. Since we used a mix of several plants of B.nigra, it explains why not all the fragments amplified from B.nigra DNA were found in the addition lines. The absence of some fragments is most likely a reflection of B.nigra polymorphism.

On the basis of isozyme and RFLP markers, the 37 plants analysed in the present study were classified in 7 groups. It was hoped that the whole set of lines representing the 8 chromosomes of the B genome would be represented (Chèvre et al.,1991). However, only 7 lines were detected. PCR marker B15 identified plant 90 100-1 as the previously unrepresented 7th line.

Analysis of the addition lines allowed us to define chromosome specific markers assigned on each of the B.nigra chromosomes. They will be further mapped on B.nigra chromosomes by segregation analysis in F2 and BC2 populations. The markers will also enable us to identify the B.napus plants introgressed with B genome chromosome fragments.

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