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BRIEF COMMUNICATION

RFLP analyses of several *Brassica* representatives

S. ZELENKOVÁ

Research Institute of Plant Production, Drnovská 507, 161 06 Praha 6, Czech Republic

Abstract

Molecular variability was determined between 17 *Brassica napus* cultivars, 8 new selections, 4 F_1 hybrids, 5 cultivars of *B. oleracea*, *B. campestris* and *B. peruvianum* with 4 rDNA probes from *Lycopersicon esculentum*, 3 probes from a PstI library of *B. oleracea*, 3 probes from EcoRI libraries of *B. napus* and *B. oleracea* and the acetolactatsynthase gene from *Nicotiana tabacum*. Sporadic interspecific and rare intraspecific variability was detected.

High degree of molecular polymorphism observed between closely related representatives of the genus *Brassica* (Figdore *et al.* 1988) showed, that RFLP analysis could be very useful in genetic, taxonomic and evolutionary studies of this genus. Song *et al.* (1988a, b, 1990) elucidated with help of RFLP analysis the origin and evolution of 55 subspecies of 6 cultivated species of *Brassica*. Hosaka *et al.* (1990) confirmed by RFLP methods, that A, B and C genomes are partially homologous. A linkage map of *B. oleracea* with 258 RFLP loci was described by Slocum *et al.* (1990). Parts of linkage maps (chromosomes 1,2,3,5) of *B. oleracea* and *B. campestris* were compared by Slocum (1989) who ascertained a high degree of linkage arrangement conservation. Heterogeneity in rDNA genes of some species of *Brassica* genus was studied with probes from *Raphanus sativus* and *Brassica oleracea* by Delseny *et al.* (1990). Generated chromosome addition lines in *Brassica* (Chevre *et al.* 1991) have been mapped with RFLP and isozyme markers.

The following species, cultivars and new selections were used for the DNA isolation:
B. napus:
a) winter cultivars of 00-type: Jantar, Ceres, Arabella, Lirabon, Licantara, Rubin, Darmor, Santana, B 001, B 004, S 004

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b) spring cultivars of 00-type: Loras, Hanna, SV 2279
c) winter cultivars of 0-type: Solida, Tandem, Jet Neuf
d) new selections of 00-type from breeding stations Slapy and Opava: SL-501, 506, 509, 516, 521, 522, 523, OP-08
e) F_1 hybrids of 00-type from Slapy:
$F_1$42 - Ceres × /(R39 × L46) × Jet Neuf/
$F_1$108 - Arabella × /(R39 × L46) × Jet Neuf/
$F_1$118 - Ceres × SL-506
$F_1$175 - B 001 × Ceres
Brassica oleracea:
a) var. *gemminifera* cv. Ervin
b) var. *botrytis* - new selection FTN
c) var. *acephala* cv. Winterboor
d) var. *italica* cv. Skiff
e) var. *gongylodes* f. *alboviridis*
f) var. *gongylodes* f. *violacea*
B. campestris var.*pekinensis* cv. Nozaki
B. peruvianum

DNA was isolated by a modification (Dr. Bezděk - personal communication) of the method of Dellaporta *et al.* (1983). Young leaf tissue (1 g) was placed in a mortar and ground with liquid nitrogen into a fine powder. The powder was transferred into centrifuge tube, 5 cm^3 of lysis buffer with increased NaCl concentration (2 M) was added and lysed at increased temperature (75 °C) for 10 min. After filtration DNA was precipitated for a short period (10 - 20 min). RNA was removed and DNA was further precipitated according to original procedure. The concentration of isolated DNA was determined by comparing band intensity with known lambda DNA standards on gel. 5 - 10 µg of genomic DNA was digested with EcoRI, HindIII, BamHI, BglII, EcoRV, SacI, TaqI, BspRI and electrophoresed in a 0.8 % or 1.3 % (TaqI, BspRI) agarose gel in Tris-acetate buffer with 0.5 µg cm^{-3} ethidium bromide. After running the gels at 30 mA for 15-20 h, the DNA was transferred onto *Amersham Hybond* or *Sartorius Sartolon* nylon membranes by capillary transfer (Sambrook *et al.* 1989) or vacuum transfer on the *Vacu ™XL Blotting System* following the manufacturer's protocols. The probes were labelled with ^{32}P-dCTP using either nick-translation (Sambrook *et al.* 1989) or random prime oligolabelling with *Serva* DNA labelling kit according to manufacturer's protocol. Prehybridization was done in 200 µl hybridization solution (50 % formamid, 4× SSPE, 1% SDS, 5× Denhardt's solution and 100 µg cm^{-3} denaturated herring sperm DNA - Sambrook *et al.* 1989) per cm^2 of filter for 4-16 h at 42 °C. Hybridization was performed with 100 µl cm^{-2} of filter in hybridization solution for 24-48 h at 42 °C. The filters were washed once in 2× SSPE, 0,1 % SDS, 20 °C, twice in 0.2× SSPE, 0.1 % SDS, 42 °C and once 0.1× SSPE, 0,1 % SDS, 60 °C. Autoradiography lasted for 1 - 21 days at -136 °C using *Foma* films. To reuse blots, the hybridised probe was stripped from filters by washing three times in boiling 0.1× SSPE, 0.1 % SDS, 10 min each washing.

rDNA probes (1.1; 1.7; 2.5; 3.7 kb) from *Lycopersicon esculentum* (Kiss *et al.* 1989) were supplied kindly by Dr. B. Koukalová from the Biophysical Institute of Czechoslovak Academy of Sciences, probes from PstI library of *Brassica oleracea* (T 3-16, T 4-9, T 4-44) by Dr. H. Dobrovolná and Dr. M. Bezděk from the same institute. Probes pB185, pB485 and pB547 from EcoRI genomic library of *B. napus* and *B. oleracea* are a gift of Dr. F.C. Quiros from University of California and acetolactatsynthase gene of *Nicotiana tabacum* from Dr. B. Mazur, DuPont.

Low inter- and intraspecific variability was observed after hybridization with rDNA probes.

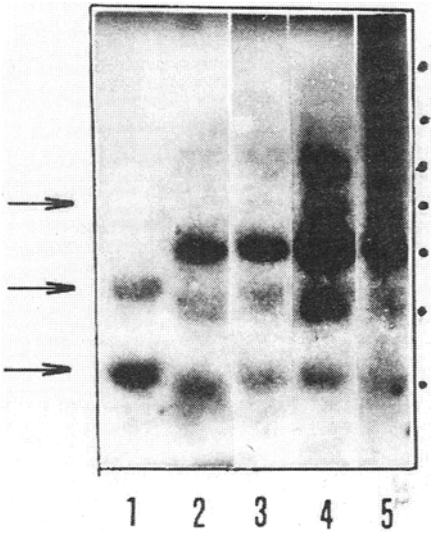

Fig. 1. RFLP analysis of *Brassica* accessions. Hybridization of DNA from *B. napus* and *B. oleracea* digested by EcoRI with 1, 1rDNA probe: *1* - *B. oleracea* var. *italica* , *2* - var. *acephala*, *3* - *B. napus* cv. Santana, 4 - cv. Arabella, *5* - $F_1$118.

No EcoRI polymorphism with 1,1 rDNA probe was detected between band position (4.3; 3.0; 2.2; 1.3; 0.6; 0.5; 0.1 kb) of cultivars and F_1 hybrids of *B. napus*, varieties of *B. oleracea* and *B. peruvianum*, but strong differences in intensity of bands were observed. Variability was found only by *B. oleracea* var. *acephala* (Fig. 1). All representatives of *B. napus* and *B. oleracea* showed 2 HindIII fragments (9.4; 6.6 kb), polymorphism was present in *B. napus* cultivars B 001 (8.2; 5.9 kb) and B 004 (only 9.4 kb). No differences were found in 3 BamHI (3.0; 2.2; 1.4 kb) and 3 Bg1II (6.6; 2.3; 1.1 kb) fragments.

The same autoradiography pattern of 8 EcoRI (6.6; 4.3; 3.3; 2.1; 2.0; 1.6; 1.4; 0.5 kb) and 2 HindIII (9.4; 6.6 kb) fragments of *B. napus* genotypes was observed with the 1,7 rDNA probe. Bands in different positions were found by *B. oleracea* var. *italica* (8.0; 4.3; 2.5; 2.1; 0.5 kb), *B. oleracea.* var. *gongylodes* (6.6; 4.3; 3.3; 1.5; 0.5 kb) and *B. o.* var. *acephala* (6.6; 4.3; 1.7; 0.7; 0.5 kb). *B. peruvianum* has in

comparison with *B. napus* (BamHI - 9.6; 4.3; 3.7; 1.5; BglII - 9.5; 6.6; 3.1; 1.4 kb) one BglII (0.5 kb) and one BamHI (0.2 kb) fragment more.

Differences solely in the intensity of hybridization signals of *B. napus* and *B. oleracea* materials were observed with the 2.5 rDNA probe. A specific BamHI fragment (1.4 kb) appears by *B. peruvianum* in comparison with *B. napus*, on the other hand 1 BglII fragment (2.9 kb) is lacking by *B. napus* cv. Arabella (Fig. 2).

The 3.7 rDNA probe detected by *B. napus* and *B. oleracea* no changes in patterns of 6 EcoRI fragments (5.4; 4.3; 2.4; 1.8; 0.7; 0.5 kb). Only one HindIII band (8.2 kb) was observed. No differences were found in 4 BamHI (5.4; 4.3; 1.8; 1.2 kb) and 3 BglII (8.2; 4.3; 3.4 kb) fragments of *B. napus* and *B. peruvianum*.

Sporadic variability in representatives of *B. napus*, *B. oleracea* and *B. peruvianum* corresponds with data of Cordesse *et al.* (1990) found in a wide spectrum of *Oryza* accessions. Usefulness of these probes might be for tracing of introgression DNA from wild species into cultivated varieties after distant hybridization or as a possibility to distinguish between different species and also in characterization of plants regenerated from somatic protoplast fusion. Delseny *et al.* (1990) observed a variability in the length of spacer fragment. In the case of our probes we found the highest variability by the 1.7 rDNA probe, which includes only short spacer sequence, although higher variability with 3.7 rDNA, with a longer spacer sequence, would be expected.

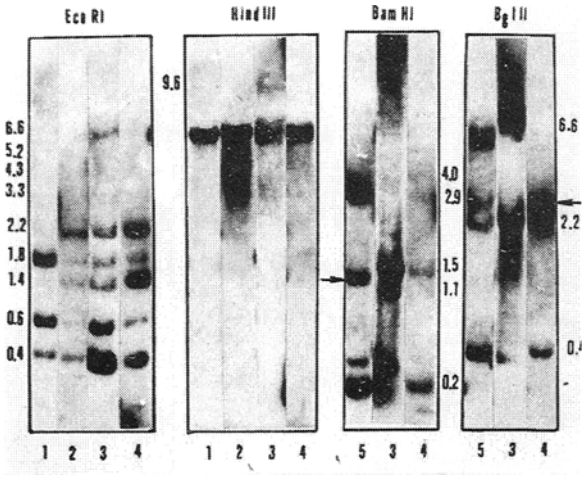

Fig. 2. Hybridization of DNA from *B. napus, B. oleracea* and *B. peruvianum* with 2.5 rDNA probe: *1* - *B. oleracea* var. *italica*, *2* - var. *gongylodes*, *3* - *B. napus* cv. Arabella, *4* - cv. Jantar, *5* - *B. peruvianum*

EcoRI fragment polymorphism was observed in *B. oleracea* var. *gongylodes* (3.3; 1.0; 0.2 kb) and *B. peruvianum* (3.3; 2.3 kb) after hybridization with the acetolactatsynthase gene from *Nicotiana tabacum*, no variability was detected in the cultivars and new selections of *B. napus* (3.3; 1.0 kb).

Spring Loras and winter B 001 cultivars of *B. napus, B. oleracea* var. *gongylodes* and var. *italica* and *B. campestris* were compared after restriction by EcoRI, HindIII and EcoRV and hybridization with 3 probes from PstI library of *B. oleracea*. Two of these probes (T 4-44/EcoRI, T 3-16/EcoRI) can be used to distinquish *B. campestris* (T 44-4/4.0 kb; T 3-16/4.0; 3.0 kb) from *B. napus* and *B. oleracea* (T 44-4/1.8 kb, T 3-16/3.0; 1.5 kb).

RFLP was detected after hybridization with probes from *B. napus* and *oleracea* EcoRI libraries. Some representatives of *B. napus* (SV 2279, Loras, Hanna, Rubin, Jet Neuf, B 001, Ceres, OP-08, SL-521, $F_1$118) and *B. campestris* differed in their EcoRI, HindIII and SacI fragments with the pB 485 probe, some of the *B. oleracea* varieties (*botrytis, gongylodes, italica*) in the EcoRI fragments using the pB 547 probe. All representatives of *B. napus* showed 3.7 and 2.3 kb EcoRI fragments after hybridization with pB 485, variability was observed in the number (0 - 5) of weakly intensive bands of higher molecular weight (4.3 - 9.8 kb). We noted 2.9 and 4.4 kb HindIII fragments and differences in the presence of 2 - 6 weakly intensive bands in the area of 2.9 - 9.8 kb, further 2 - 7 SacI fragments as very weakly intensive bands in the range of 6.6 - 9.9 kb. *B. oleracea* varieties differed after digestion of EcoRI and hybridization with pB 547 in the presence of 7.2; 4.9 and 2.3 kb bands. No differences were observed in TaqI, BspRI and EcoRV fragments of *B. napus* using the pB 485 probe, variability was seen by *B. campestris* (Fig. 3). *B. napus* HindIII fragments hybridised with the pB 185 probe and EcoRI fragments with the pB 547 probe correspond to each other. *B. oleracea* does not hybridize with the pB 485 probe, *B. campestris* with the pB 547.

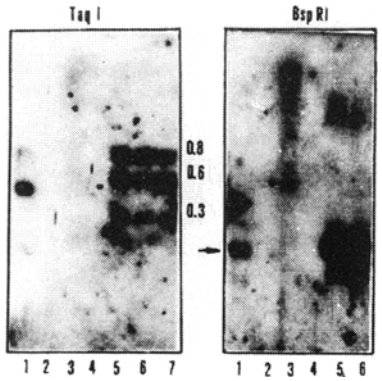

Fig. 3. Hybridization of DNA from *B. campestris, B. oleracea* and *B. napus* with pB 485 probe: *1* - *B. campestris*, *2* - *B. oleracea* var. *gemminifera*, *3* - var. *botrytis*, *4* - var. *italica*, *5* - *B. napus* cv. Hanna, *6* - SL-506, *7* - cv. Lirabon.

Hosaka *et al.* (1990) demonstrated the presence of genome specific markers in the comparison of various *Brassica* species using these probes. The polymorphisms, which were detected by us between several varieties of *B. napus* and *B. oleracea*, are probably hardly suitable for distinquishing of cultivars because of variability in

weakly intensive fragments. Therefore these markers will be usefull only using much more sensitive detection, then the system reported here.

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