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Assessment of the degree of restriction fragment length polymorphism in *Brassica*

S. S. Figdore [1], W. C. Kennard [2], K. M. Song [2], M. K. Slocum [1] and T. C. Osborn [2]

[1] NPI, 417 Wakara Way, Salt Lake City, UT 84108, USA
[2] Department of Agronomy, University of Wisconsin, Madison, WI 53706, USA

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Summary. The feasibility of creating a restriction fragment length polymorphism (RFLP) linkage map in *Brassica* species was assessed by screening *EcoRI*-, *HindIII*-, or *EcoRV*-digested total genomic DNA from several accessions of *B. campestris, B. oleracea,* and *B. napus* using random genomic DNA clones from three *Brassica* libraries as hybridization probes. Differences in restriction fragment hybridization patterns occurred at frequencies of 95% for comparisons of accessions among species, 79% for comparisons of accessions among subspecies within species, and 70% for comparisons among accessions within subspecies. In addition, species differences in the level of hybridization were noted for some clones. The high degree of polymorphism found even among closely related *Brassica* accessions indicates that RFLP analysis will be a very useful tool in genetic, taxonomic, and evolutionary studies of the *Brassica* genus. Development of RFLP linkage maps is now in progress.

Key words: Restriction fragment length polymorphism – *Brassica campestris* – *Brassica oleracea* – *Brassica napus*

Introduction

Restriction fragment length polymorphisms (RFLPs) are heritable differences in the length of DNA fragments arising after treatment with a particular restriction endonuclease. RFLPs have been used as genetic markers, and extensive RFLP marker linkage maps have been generated in maize and tomato (Helentjaris et al. 1986) and in lettuce (Landry et al. 1987). RFLP markers can be used in varietal identification, in assessing genetic diversity, and in mapping genes of interest (see Helentjaris et al. 1985; Nienhuis et al. 1987; Osborn et al. 1987).

The development of RFLP markers in *Brassica* crops may facilitate genetic and evolutionary studies of this economically important genus. Although many morphological markers have been identified in *Brassica* crop species, detailed linkage maps have not been developed and the need for more genetic markers has been cited (James and Williams 1980). The availability of RFLP markers would allow the rapid development of comprehensive *Brassica* linkage maps. These markers would be useful for identifying and mapping economically important genes, as well as for studying the interrelationships and genome organization of *Brassica* species which has been previously based primarily on cytogenetic and biochemical data (see Song et al. 1988).

The potential usefulness of RFLP markers is dependent on the degree of polymorphism existing among *Brassica* species of interest. This study was undertaken to evaluate the degree of restriction fragment length polymorphism among and within three *Brassica* crop species, *B. campestris, B. oleracea,* and *B. napus.* Variability for RFLP patterns was assessed at three taxonomic levels: (1) among *Brassica* species, (2) among subspecies within species, and (3) among accessions within subspecies. During the course of this study, specific DNA fragments were identified which reveal differences in genome organization among the *Brassica* species studied.

Materials and methods

Preparation of clones

Genomic DNA libraries were prepared from leaf tissue DNA of three *Brassica* sources, 'Early White' cauliflower (*B. oleracea*

ssp. *botrytis*), 'Wisconsin Golden Acres' cabbage (*B. oleracea* ssp. *capitata*), and 'WR 70 Days' chinese cabbage (*B. campestris* ssp. *pekinensis*). Genomic DNA was digested with the endonuclease restriction enzyme, *PstI* (BRL), and electrophoresed in an agarose gel. DNA fragments 500 to 2,000 base pairs in length were eluted from the gel and subcloned into either the pUC19 or the pTZ18R (Pharmacia) plasmid vectors, which previously had been digested with *PstI* and treated with calf intestinal alkaline phosphatase. The resulting recombinants were used to transform either the JM83 or the DH5α bacterial hosts to drug resistance, and the resulting colonies were grown and stored in freezing media at −70 °C.

Colony lifts from the cauliflower library were probed with ^{35}S-radiolabelled total genomic DNA to screen for highly repetitive sequences (Grunstein and Hogness 1975; Maniatis et al. 1982), which were not used in assessing polymorphism within *Brassica*. Recombinant plasmids from the cabbage and chinese cabbage libraries were screened by hybridizing slot blots of plasmid mini-preparations with ^{32}P-radiolabelled chloroplast, mitochondrial, and total genomic *Brassica* DNAs. Recombinant plasmids which hybridized to chloroplast or mitochondrial DNAs or which hybridized at very high levels to total genomic DNAs were not used in assessing polymorphism within *Brassica*.

Plant DNA preparation, restriction enzyme digestion, and blotting

Procedures for the preparation of plant total genomic DNA, digestion by restriction enzymes, agarose gel electrophoresis, and Southern blotting have been described previously (Helentjaris and Gesteland 1983; Helentjaris et al. 1985, 1986). Plant DNA extracted from *Brassica* germplasm assessions was digested with either *EcoRI, HindIII,* or *EcoRV*. Nylon membrane, obtained from either AMF CUNO or Schleicher and Schuell, was used as the blotting matrix.

Nick translation of probes and hybridization conditions

Procedures for plasmid mini-preparation and nick translation of probes and for the prehybridization, hybridization, washing, and autoradiography of blots were as previously described (Helentjaris and Gesteland 1983; Helentjaris et al. 1985, 1986).

Brassica germplasm used in assessing polymorphism and measurement of polymorphism

Thirty-seven different *Brassica* germplasm accessions, representing *B. campestris, B. oleracea,* and *B. napus* species, were used in assessing the degree of restriction fragment length polymorphism in *Brassica* (Table 1). Accessions from several different subspecies of *B. campestris* and *B. oleracea* were chosen, so that polymorphism could be assessed: (1) among accessions of different species, (2) among accessions of different subspecies within the same species, and (3) among accessions within subspecies. Several types of blots were made, each containing only a subset of the 37 total accessions which were screened for polymorphism.

A clone-enzyme combination was considered informative in detecting polymorphism if different hybridization patterns were observed between accessions being compared. The frequency of polymorphism between any two accessions being compared was calculated as the number of informative clones divided by the total number of clones tested for a given restriction enzyme, expressed on a percent basis. Accessions were grouped according to subspecies and species, and cumulative frequencies of informativeness for all comparisons were calculated to assess polymorphism at each of the three taxonomic levels.

Table 1. List of the accessions used in assessing the level of restriction fragment length polymorphism in the *Brassica* genus

I. *B. campestris*	II. *B. oleracea*
A. ssp. *chinensis*	A. ssp. *italica*
1. Gai Choi	20. Atlantic
2. Canton Dwarf	21. Bonanza
3. Takii no. 1	22. Surfer
4. Best Seed	23. Expt. B19[a]
5. China Pak Choi	24. Gem
6. Milky Way	25. Di Cicco
7. Expt. CC419[a]	26. Cruiser
8. Hon Tsai	27. Green Top
9. Tsai Sai	28. Premium Crop
B. ssp. *pekinensis*	29. Expt. B18[a]
10. Michihili	30. Packman
11. Dynasty	31. OSU CR-7
12. Jade Pagoda	32. OSU CR-8
13. Expt. CC25[a]	B. ssp. *capitata*
14. Green Rocket	33. Wisconsin Golden Acres
15. Hakuran	34. Brunswick
16. WR 70 Days	C. ssp. *botrytis*
C. ssp. *utilis*	35. Early White
17. Spring Broccoli	D. ssp. *oleracea*
D. ssp. *oleifera* and ssp. *campestris*	36. Rapid-cycling oleracea
18. Rapid-cycling campestris	III. *B. napus*
19. UCD 77-4 Turnip Rape	A. ssp. *oleifera*
	37. Westar

[a] Experimental accessions provided by NPI AgServices

Results

Screening genomic libraries for low copy DNA inserts

A total of 480 random genomic clones from the 'Early White' cauliflower library were screened via colony hybridization using total genomic DNA from 'Early White' as a probe, and 418 of these clones (87.1%) appeared to contain low copy number DNA sequences. A total of 161 random genomic clones from the 'Wisconsin Golden Acres' cabbage library were screened via slot bot hybridization, and 123 of these clones (76.4%) appeared to contain low copy nuclear DNA sequences. Twenty-five of the 161 clones from the cabbage library contained chloroplast DNA sequences. A total of 140 random genomic clones from the 'WR 70 Days' chinese cabbage library were screened via slot blot hybridization, and 105 of these clones (75.0%) appeared to contain low copy nuclear DNA sequences. Seventeen of the 140 clones from the chinese cabbage

library contained chloroplast DNA sequences, and five of the chinese cabbage library clones contained mitochondrial DNA sequences.

A total of 391 random genomic clones containing suspected low copy number DNA sequences (163 clones from the caulifower library, 123 clones from the cabbage library, and 105 clones from the chinese cabbage library) were screened against Southern blots of genomic DNA from various *Brassica* sources (Table 1). Twenty of the cauliflower library clones (12.3%), five of the cabbage library clones (4.1%), and five of the chinese cabbage library clones (4.8%) had hybridization patterns characteristic for repeated sequences, but were not detected as such in the colony or slot blot hybridization procedures. A total of 361 random genomic clones containing low copy number DNA sequences (143 clones from the cauliflower library, 118 clones from the cabbage library, and 100 clones from the chinese cabbage library) were used subsequently in determining the degree of restriction fragment length polymorphism within *Brassica.* These clones yielded strong hybridization to a relatively small number of fragments, thus fulfilling the criteria for selection as useful clones, as outlined by Helentjaris et al. (1986).

The frequency of detectable polymorphism within *Brassica* was assessed for genomic DNA digested with each of three different restriction enzymes (*EcoRV, HindIII,* and *EcoRI*). Since estimates of the degree of polymorphism were similar regardless of which enzyme was used in making comparisons (data not shown), only the results from *EcoRI* analyses are presented.

Degree of polymorphism

The occurrence of RFLPs can be due to base pair changes in an enzyme recognition site, rearrangements encompassing the site, or rearrangements localized between enzyme recognition sites. In addition, overall differences in the hybridization pattern of a given clone to various endonuclease-digested DNAs can result from differences in the number of chromosomal regions sharing sequence homology with the cloned sequence, and from differences in the degree of shared homology, as reflected by differences in hybridization signal strength (Fig. 1).

When DNA from various *Brassica* sources was digested with *EcoRI* and probed with random clones from the three genomic DNA libraries, differences in hybridization patterns were observed in 95% of the total number of comparisons made between accessions of different species (Table 2). The frequency of hybridization pattern differences detected in comparisons made between subspecies from different species ranged from 88.2% in comparisons of *B. oleracea* ssp. *capitata* vs. *B. napus* ssp. *oleifera* to 98.4% in comparisons of *B. campestris* ssp. *oleifera* vs. *B. oleracea* ssp. *capitata.* Differences in hybridization patterns were observed in 79% of the total number of comparisons made between accessions of different subspecies within the same *Brassica* species (Table 3). The frequency of hybridization pattern differences detected in comparisons of particular subspecies within species ranged from 67.8% in comparisons of *B. oleracea* ssp. *capitata* vs. ssp. *oleracea* to 88.4% in comparisons of *B. oleracea* ssp. *oleracea* vs. ssp. *botrytis.* Differences in hybridization patterns were observed in 70% of the total number of comparisons made between accessions within the same subspecies (Table 3). The frequency of hybridization pattern differences detected in comparisons of accessions within particular subspecies ranged from 46.4% in comparisons of accessions within cabbage to 81.8% in comparisons of accessions within pak choi, *B. campestris* ssp. *chinensis.*

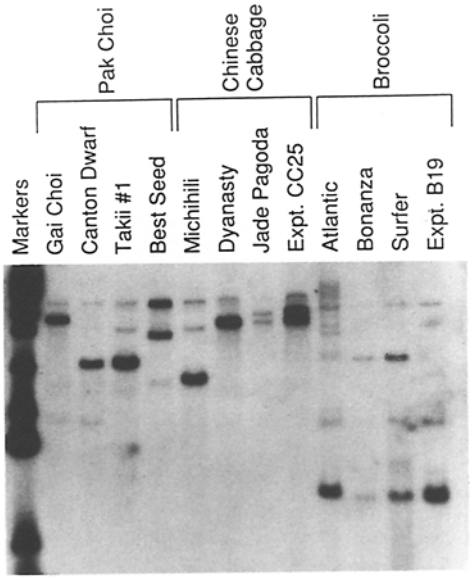

Fig. 1. Autoradiograph of a Southern blot of *EcoRI*-digested total genomic DNA from several *Brassica* accessions, hybridized to radiolabelled DNA from a random clone isolated from the cauliflower genomic library. Differences among hybridization patterns of accessions are detected as differences in restriction fragment length as well as differences in hybridization signal strength

Calculated frequencies of polymorphism between *Brassica* accessions were notably dependent to some extent upon the library source and upon the number of varietal comparisons made. For example, 53 out of 65 clones from the cauliflower library (81.5%) were useful in detecting polymorphism between 'Packman' broccoli (*B. oleracea* ssp. *italica*) and 'Brunswick' cabbage cultivars, whereas only 56 out of 94 clones from the other two libraries (59.6%) were useful in detecting polymorphism between the same two cultivars. Therefore, only general conclusions on the degree of polymor-

Table 2. Frequency of hybridization pattern differences among *Brassica* species for pairwise comparisons of *Brassica* accessions using *EcoRI*-digested DNA

Level of comparison	No. of informative comparisons	No. of total comparisons	Frequency of informative comparisons (%)
Among species	6332	6671	94.9
B. campestris vs. *B. oleracea*	5493	5759	95.4
ssp. *chinensis* vs. ssp. *italica*	1162	1216	95.6
ssp. *pekinensis* vs. ssp. *italica*	1696	1789	94.8
ssp. *pekinensis* vs. ssp. *capitata*	641	670	95.7
ssp. *pekinensis* vs. ssp. *oleracea*	235	242	97.1
ssp. *pekinensis* vs. ssp. *botrytis*	192	200	96.0
ssp. *utilis* vs. ssp. *italica*	325	348	93.4
ssp. *utilis* vs. ssp. *capitata*	316	337	93.8
ssp. *utilis* vs. ssp. *oleracea*	120	129	93.0
ssp. *utilis* vs. ssp. *botrytis*	95	100	95.0
ssp. *oleifera* vs. ssp. *italica*	310	318	97.5
ssp. *oleifera* vs. ssp. *capitata*	239	243	98.4
sp. *oleifera* vs. ssp. *oleracea*	57	59	96.6
ssp. *oleifera* vs. ssp. *botrytis*	105	108	97.2
B. campestris vs. *B. napus*	346	368	94.0
ssp. *pekinensis* vs. ssp. *oleifera*	159	171	93.0
ssp. *utilis* vs. ssp. *oleifera*	82	89	92.1
ssp. *oleifera* vs. ssp. *oleifera*	105	108	97.2
B. oleracea vs. *B. napus*	493	544	90.6
ssp. *italica* vs. ssp. *oleifera*	225	245	91.8
ssp. *capitata* vs. ssp. *oleifera*	157	178	88.2
ssp. *oleracea* vs. ssp. *oleifera*	32	33	97.0
ssp. *botrytis* vs. ssp. *oleifera*	79	88	89.8

Table 3. Frequency of hybridization pattern differences among subspecies within species and among accessions within subspecies for pairwise comparisons of *Brassica* accessions using *EcoRI*-digested DNA

Level of comparison	No. of informative comparisons	No. of total comparisons	Frequency of informative comparisons (%)
Among subspecies within species	2835	3596	78.8
Among *B. campestris* ssp.	1578	1920	82.2
ssp. *chinensis* vs. ssp. *pekinensis*	967	1192	81.1
ssp. *pekinensis* vs. ssp. *utilis*	285	346	82.4
ssp. *pekinensis* vs. ssp. *oleifera*	221	255	86.7
ssp. *utilis* vs. ssp. *oleifera*	105	127	82.7
Among *B. oleracea* ssp.	1257	1676	75.0
ssp. *italica* vs. ssp. *capitata*	541	716	75.6
ssp. *italica* vs. ssp. *oleracea*	147	199	73.9
ssp. *italica* vs. ssp. *botrytis*	206	265	77.7
ssp. *capitata* vs. ssp. *oleracea*	173	255	67.8
ssp. *capitata* vs. ssp. *botrytis*	152	198	76.8
ssp. *oleracea* vs. ssp. *botrytis*	38	43	88.4
Among accessions within subspecies	2011	2862	70.3
Among accessions of *B. campestris*	823	1150	71.6
Within ssp. *chinensis*	454	555	81.8
Within ssp. *pekinensis*	369	595	62.0
Among accessions of *B. oleracea*	1188	1712	69.4
Within ssp. *italica*	1103	1529	72.1
Within ssp. *capitata*	85	183	46.4

Table 4. Frequency of hybridization pattern differences among accessions for pairwise comparisons of *Brassica* accessions using *EcoRI*-digested DNA for 65 clones from the 'Early White' cauliflower library

Level of comparison	Accessions in comparison	No. of informative clones (out of 65 total)	Frequency of informative clones (%)
Among species			
B. campestris vs. *B. oleracea*			
ssp. *pekinensis* vs. ssp. *italica*	'Michihili' vs. 'Packman'	62	95.4
	'Michihili' vs. OSU CR-7	60	92.3
	'Michihili' vs. OSU CR-8	61	93.8
	'WR 70 Days' vs. 'Packman'	63	96.9
	'WR 70 Days' vs. OSU CR-7	63	96.9
	'WR 70 Days' vs. OSU CR-8	63	96.9
ssp. *pekinensis* vs. ssp. *capitata*	'Michihili' vs. 'Wis. Golden Acres'	62	95.4
	'Michihili' vs. 'Brunswick'	62	95,4
	'WR 70 Days' vs. 'Wis. Golden Acres'	63	96.9
	'WR 70 Days' vs. 'Brunswick'	63	96.9
Among subspecies within species			
B. oleracea			
ssp. *italica* vs. ssp. *capitata*	'Packman' vs. 'Wis. Golden Acres'	56	86.2
	'Packman' vs. 'Brunswick'	53	81.5
	OSU CR-7 vs. 'Wis. Golden Acres'	54	83.1
	OSU CR-7 vs. 'Brunswick'	53	81.5
	OSU CR-8 vs. 'Wis. Golden Acres'	54	83.1
	OSU CR-8 vs. 'Brunswick'	50	76.9
Among accessions within subspecies			
B. campestris ssp. *pekinensis*	'Michihili' vs. 'WR 70 Days'	52	80.0
B. oleracea ssp. *italica*	'Packman' vs. OSU CR-7	47	72.3
	'Packman' vs. OSU CR-8	49	75.4
	OSU CR-7 vs. OSU CR-8	39	60.0
ssp. *capitata*	'Wis. Golden Acres' vs. 'Brunswick'	40	61.5

phism should be drawn from the results of the entire data set (Tables 2 and 3), and interpretation of taxonomic differences among species and subspecies based on the frequencies of polymorphism shold be limited to comparisons made using the same clones from the same library source.

To more accurately assess the genomic variability among a subset of *Brassica* accessions, frequencies of polymorphism were calculated based on comparisons of hybridization patterns of 65 clones from the cauliflower library to *Eco RI*-digested DNA from two chinese cabbage, three broccoli and two cabbage accessions (Table 4). Frequencies of polymorphism were similar to the frequencies calculated for the entire data set (Tables 2 and 3), ranging from 92.3% to 96.9% for comparisons between accessions of different species, from 76.9% to 86.2% for comparisons between accessions of different subspecies within *B. oleracea*, and from 60.0% to 80.0% for comparisons between accessions within subspecies. As expected, frequencies of polymorphism were higher for comparisons made between 'Packman' vs. OSU CR-7 and 'Packman' vs. OSU CR-8 broccoli than for the comparison between the two related inbreds, OSU CR-7 vs. OSU CR-8, which were both developed by Baggett and Kean (1985).

The usefulness of a clone for RFLP analysis of several accessions simultaneously is dependent on the number of accessions of interest which can be differentiated when that clone is used as a probe on Southern blots. Thus, a clone which is useful in detecting four distinct hybridization patterns when probed against four broccoli accessions of interest would be considered of more value than a clone which is useful in detecting only two distinct patterns when probed against the same four accessions. The frequency of clones of use in differentiating several accessions of a given subspecies simultaneously was assessed by screening 80 random genomic DNA clones from the cauliflower library using blots containing *EcoRI*-digested DNA from four pak choi, four chinese cabbage, and four broccoli accessions. Hybridization with 23 out of 69 clones (33.3%) yielded unique pattern differences for all four pak choi cultivars, while hybridization with only 7 clones (10.1%) failed to give detectable pattern differences for any of the four pak choi cultivars

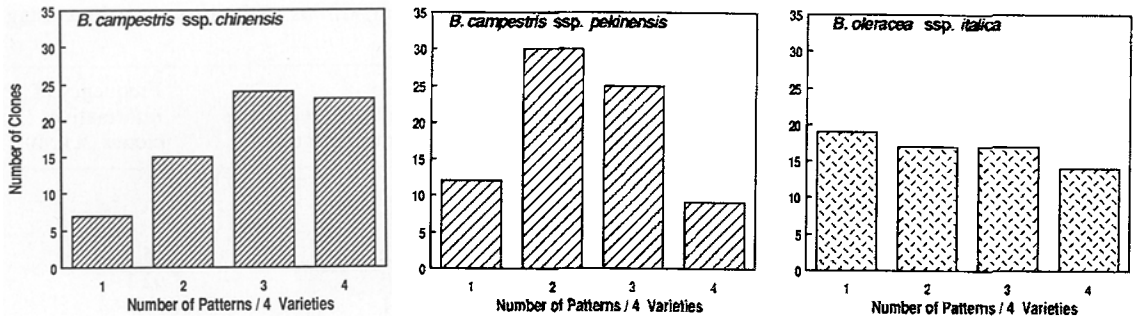

Fig. 2. Frequency distribution of clones useful in simultaneously detecting differences among *EcoRI*-digested DNA hybridization patterns of four accessions within pak choi (*B. campestris* ssp. *chinensis*). within chinese-cabbage (*B. campestris* ssp. *pekinensis*), and within broccoli (*B. oleracea* ssp. *italica*)

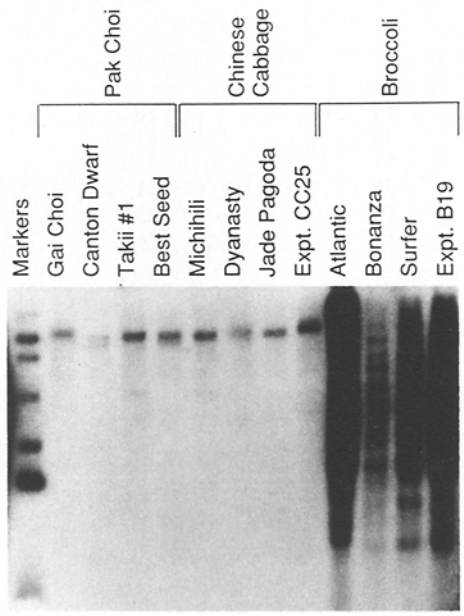

Fig. 3. Autoradiograph of a Southern blot of *EcoRI*-digested total genomic DNA from several *Brassica* accessions, hybridized to radiolabelled DNA from a random clone isolated from the cauliflower genomic library. The probe hybridizes to a DNA sequence of single or low copy number in pak choi and chinese cabbage accessions and of much higher copy number in broccoli accessions

(Fig. 2). Hybridization with 9 out of 76 clones (11.8%) yielded unique pattern differences for all four chinese cabbage accessions, while hybridization ith 12 clones (15.8%) failed to give detectable pattern differences for any of the four chinese cabbage accessions. Hybridization with 14 out of 67 clones (20.9%) yielded unique pattern differences for all four broccoli accessions, while hybridization with 19 clones (28.4%) failed to give detectable pattern differences for any of the four broccoli accessions.

Isolation of DNA sequences which were able to detect differences in genome organization among Brassica species

Most clones derived from any one of the library sources hybridized to some extent with genomic DNA from other *Brassica* species or subspecies. Hybridization of several genomic clones, however, resulted in differences among species in the degree of hybridization, suggesting that differences in genome organization or genetic diversity exist among *Brassica* species.

One genomic clone from the cauliflower library hybridized to a DNA sequence of single or low copy number in pak choi and chinese cabbage accessions, but hybridized to a sequence of much higher copy number in broccoli accessions (Fig. 3). When this clone was rescreened against other *Brassica* sources, it appeared that the clone hybridized to a DNA sequence of low copy number in all accessions of *B. campestris* tested and of higher copy number in all accessions of *B. oleracea* and *B. napus* tested (data not shown). Other clones from the cauliflower and cabbage libraries hybridized strongly to *B. oleracea* and *B. napus* accessions and weakly or not at all to *B. campestris* accessions (Fig. 4). The opposite situation was observed for several clones from the chinese cabbage library. In addition, several clones were useful in detecting hybridization patterns in *B. napus* cv. Westar which were the combination of hybridization patterns in *B. campestris* and *B. oleracea* accessions (Fig. 5).

Discussion

In this study, we used low copy number genomic DNA sequences as probes for assessing polymorphism in *Brassica*. When genomic DNA from several *Brassica* sources was digested with the methylation-sensitive enzyme, *PstI*, a high percentage of random clones (greater

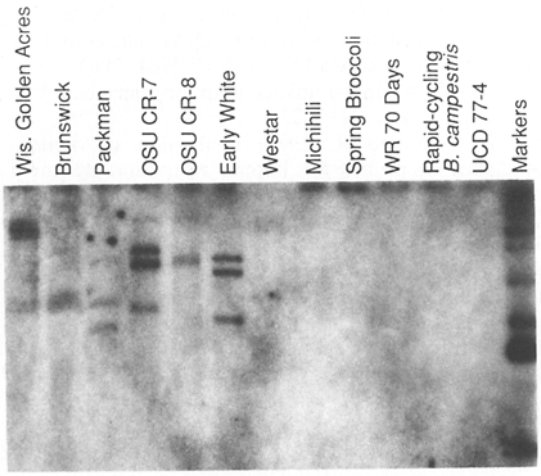

Fig. 4. Autoradiograph of a Southern blot of *EcoRI*-digested total genomic DNA from several *Brassica* accessions, hybridized to radiolabelled DNA from a random clone isolated from the cauliflower genomic library. The probe hybridizes detectably to DNA from *B. oleracea* accessions (*lanes 1–6*) and *B. napus* cv. Westar (*lane 7*), but not to DNA from *B. campestris* accessions (*lanes 8–12*)

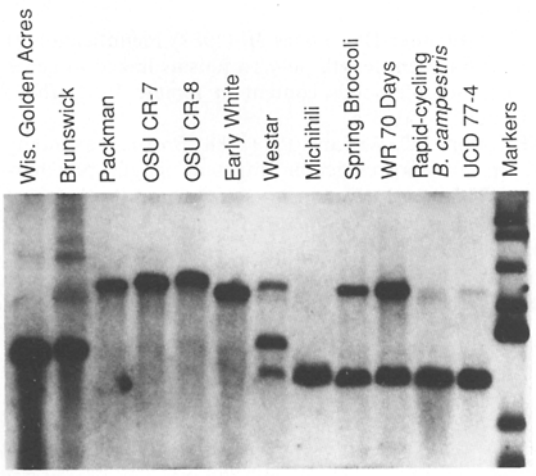

Fig. 5. Autoradiograph of a Southern blot of *EcoRI*-digested total genomic DNA from several *Brassica* accessions, hybridized to radiolabelled DNA from a random clone isolated from the cauliflower genomic library. The hybridization pattern of the amphidiploid, *B. napus* cv. Westar consists of a lower band similar to *B. campestris* accessions (*lanes 8–12*), a *middle band* similar to *B. oleracea* ssp. *capitata* accessions (*lanes 1–2*), and an *upper band* similar to both *B. oleracea* (*lanes 1–6*) and *B. campestris* accessions

than 75% of all clones screened), corresponding to undermethylated sequences digested by this enzyme, contained the low copy number DNA inserts which we desired. These results were in agreement with those obtained from screening a maize genomic library made from *PstI*-digested DNA (Helentjaris 1987). Screening for low copy DNA inserts via slot blot hybridization was slightly more effective than screening via colony hybridization, as a higher percentage of clones with repetitive DNA inserts escaped detection in the colony hybridization as compared to in the slot blot screenings.

Helentjaris et al. (1985) observed that different restriction enzymes all possessed similar potential for revealing polymorphism among maize inbreds. Similarly, there was no real advantage in the choice of restriction enzyme used to assess polymorphism among *Brassica* accessions, as *EcoRI, HindIII,* and *EcoRV* gave similar results (data not shown). The increase in RFLP frequency gained by using information from two enzymes instead of one was relatively small (approximately a 20% increase in frequency was observed for some comparisons between accessions within species). Thus, it was concluded that screening *Brassica* germplasm for RFLPs using only one enzyme (e.g. *EcoRI*) and many clones would be more economical than using several enzymes and fewer clones.

A high degree of restriction fragment length polymorphism was observed for comparisons at all three taxonomic levels of *Brassica* when *EcoRI*-digested DNAs were probed with low copy number genomic DNA clones. The highest levels of polymorphism were found between accessions of different *Brassica* species (95% of all comparisons). Levels of polymorphism among accessions within species were slightly lower, with polymorphism observed in 79% of all comparisons among accessions of different subspecies and in 70% of all comparisons among accessions within subspecies. Frequencies of polymorphism as high as 60% were noted for comparisons between OSU CR-7 and OSU CR-8, inbred broccoli lines of similar pedigree (Baggett and Kean 1985). In addition, many clones were useful in detecting polymorphism among several accessions within a given subspecies simultaneously. These high levels of restriction fragment length polymorphism are comparable to those observed among commercial inbreds of *Zea mays* L., and are in contrast to the low levels of polymorphism found among accessions of the cultivated tomato, *L. esculentum* Mill. (Helentjaris et al. 1985). We conclude that a relatively large amount of genetic variability resides at the molecular level in the commercial *Brassica* germplasm screened.

The results of this study demonstrate the potential usefulness of RFLPs for studies on *Brassica* taxonomy, evolution, and genome organization. Several clones revealed hybridization patterns in *B. napus* that were the combination of patterns observed in the presumptive diploid progenitors, *B. campestris* and *B. oleracea,* providing preliminary evidence at the DNA level for the hypothesized origin of the amphidiploid *B. napus* (U, 1935). Using other clones, differences in levels of

hybridization were observed between species which were indicative of differences in sequence copy number or homology. These clones should be useful in studying the evolution and organization of divergent regions in the *Brassica* genomes. A more detailed study of *Brassica* taxonomy and evolution using RFLPs is presented elsewhere (Song et al. 1988).

The high levels of polymorphism detected in the *Brassica* germplasm screened, even between accessions of similar pedigree, indicates the feasibility of using these molecular-based markers as tools in taxonomy, in the study of genome organization and diversity, in the location of loci influencing economically important traits, and in the breeding of *Brassica* crops for these traits. To facilitate the efficient use of these polymorphisms as molecular markers, we are now constructing RFLP linkage maps within the *Brassica* genus, similar to those developed in maize and tomato (Helentjaris et al. 1986). It will be possible to create a RFLP linkage map in *B. campestris* (2n = 20) and a map in *B. oleracea* (2n = 18) using the same set of clones, such that comparisons can be made between the chromosomal organization of these two species.

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