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The potential for cross-taxa simple-sequence repeat (SSR) amplification between *Arabidopsis thaliana* L. and crop brassicas

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Abstract Simple-sequence repeats (SSRs) may prove to be valuable descriptors of plant genetic variation, but developing markers for each of many taxa is costly. The applicability of SSR markers may be increased if they can be amplified across taxa. In this study, we examined whether 30 primer pairs designed to amplify single-locus SSRs in *Arabidopsis thaliana* could be used to amplify marker loci in six *Brassica* crop species. Most of the pairs amplified multiple fragments in *Brassica*. In initial assays, 17 pairs generated products that were identified as likely to contain SSRs. Most of these products were similar in size to *A. thaliana* SSRs; many were variable for product length, and many of the variants were species- or genome-specific. In subsequent hybridization assays, however, not all of the identified *Brassica* products hybridized to SSR probes. A *Brassica* product's length variation and its size similarity to *A. thaliana* SSRs were not good predictors of whether the product contained repeats. In addition, some products of two-primer reactions hybridized to SSR probes but were the same size as single-primer reaction products, so they may have been amplified by only one primer. In summary, *Brassica* products amplified by *A. thaliana* SSR primer pairs may be useful as markers, but additional tests (hybridization, DNA sequencing, and single-primer assays) are necessary in order to determine whether the products contain SSRs and whether they are amplified by one or two primers. The results of this study are consistent with conservation, duplication, and rearrangement between *Arabidopsis* and *Brassica* genomes.

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Introduction

Plant genetic variation is best described with markers that are cost-effective, easily measured, and discriminate between taxa or individuals. In many cases such criteria are met by simple-sequence repeat (SSR) loci, or microsatellites. These arrays of short (1–6 bp) tandem DNA repeats are abundant in plant genomes (Condit and Hubbell 1991). When SSRs are amplified by the polymerase chain reaction (PCR), using primer pairs that complement the flanking sequences, variations in repeat number are detected as co-dominantly inherited differences in fragment length (Akkaya et al. 1992). SSRs can be highly polymorphic; in barley, the number of alleles per locus ranged to 37 among 207 genotypes (Saghai Maroof et al. 1994).

Many laboratories have the resources and expertise to conduct SSR marker assays, but developing new markers can be demanding: the process involves detecting repeats in genomic DNA libraries, isolating and sequencing SSR loci, and designing and synthesizing primers (Brown et al. 1996). Developing markers for each taxon may be practical for research programs focused on a few species, but not for programs involving hundreds of taxa. Alternatively, SSRs can be identified within DNA database entries (Akkaya et al. 1992). However, few plant taxa are well represented in the major databases (Wang et al. 1994). In addition, most entries are from genomic DNA coding regions or cDNA (Broun and Tanksley 1996), and SSRs in these entries may be biased descriptors of variation.

In a third approach, primer pairs designed for SSRs in a source taxon may be used to amplify SSRs in related (non-source) taxa. SSR conservation across

many plant genera and species has been documented (Lagercrantz et al. 1993; Thomas and Scott 1993; Röder et al. 1995), but not all SSR primer pairs amplify across taxa; even when they do, results are easily misinterpreted. The genomes of related taxa may differ by rearrangements, mutations, and duplications, and thus differ for the presence, copy number, and sequence of repeat and flanking regions. Amplification across these taxa may generate false-positive results, with products that contain no repeats (Röder et al. 1995), or false-negative results, with repeats present but not amplified (Devos et al. 1995). If SSR flanking sequences have been duplicated, a primer pair designed for one locus in the source taxon may amplify multiple products in a non-source taxon (Provan et al. 1996). If one flanking sequence has been duplicated and copies are inverted, only one primer may be needed to amplify between the copies. As a result, single-primer products may be generated in a two-primer reaction (Hiltunen et al. 1994). They may be the same size as two-primer products but differ in sequence and inheritance, and they may contain SSRs. Even when two-primer products containing SSRs are amplified across taxa, sequence differences can make analysis difficult: repeat number may be identical for products that differ in size, or may differ between same-sized products (Kayser et al. 1995).

To determine whether cross-taxa SSR amplification products actually contain repeats, either SSR probe hybridization or DNA sequencing is necessary (e.g., Röder et al. 1995; Provan et al. 1996). However, these techniques are rarely practical for large studies. In many surveys of SSR amplification across plant species and genera, products are selected as useful markers if they amplify consistently over replicates; have good staining intensity (a measure of product amplification); and are similar to SSRs in the source taxon in terms of size and polymorphism (Cato and Richardson 1996; Szewc-McFadden et al. 1996; Smulders et al. 1997). However, the relationships between these characteristics and the presence of SSRs may not be known.

Duplication and rearrangement are extensive in the genomes of six crucifer crop species: the diploids *Brassica rapa* L. (genomes AA, $2n = 2x = 20$), *B. nigra* (BB, $2n = 2x = 16$), and *B. oleracea* L. (CC, $2n = 2x = 18$) and amphidiploids *B. juncea* (L.) Czern. (AABB, $2n = 4x = 36$), *B. napus* L. (AACC, $2n = 4x = 38$), and *B. carinata* Braun (BBCC, $2n = 4x = 34$) (McGrath et al. 1990). Genetic variation in these species has been described using restriction fragment and randomly amplified polymorphic DNA (RAPD) markers (Quiros et al. 1991; Teutonico and Osborn 1994). SSRs have been identified in *B. napus*, and primer pairs designed for some of them can amplify in other *Brassica* crop species, but not all of the cross-taxa products are polymorphic (Lagercrantz et al. 1993; Kresovich et al. 1995; Szewc-McFadden et al. 1996).

Another crucifer, *Arabidopsis thaliana* L. ($2n = 2x = 10$), is a model species for genetic analysis (Goodman et al. 1995). *Arabidopsis* and *Brassica* are in different tribes (Price et al. 1994), but some regions are conserved between their genomes (Kowalski et al. 1994; Teutonico and Osborn 1994). When primer pairs designed for 30 *A. thaliana* SSRs (Bell and Ecker 1994) were tested in a small array of genotypes from three *Brassica* species, limited amplification in the expected size range was reported (Barret et al. 1995). In the present study, we have evaluated the 30 primer pairs for amplification and polymorphism in a larger array of genotypes representing six *Brassica* crop species. We selected products likely to be useful markers, then utilized probe hybridization to determine whether they contained SSRs. We also examined whether single-primer products were generated in two-primer SSR reactions. Finally, we asked whether relationships exist between the presence of SSRs in the *Brassica* products and their staining intensity, length variation, and size similarity to *A. thaliana* SSRs.

Materials and methods

Plant materials

The test array included one plant from each of 13 *Brassica* entries (Table 1), plus two closely related *A. thaliana* strains (C24 and Columbia-98) as positive controls. Tissue grinding and DNA extraction procedures were modified from Hillis et al. (1990) and Colosi and Schaal (1993). DNA concentration was measured on a fluorometer (Hoefer TKO 100, 365 nm excitation, 460 nm emission) by fluorescence with bisbenzimide (Hoechst dye 33258).

Amplification using SSR primer pairs

Thirty oligonucleotide primer pairs designed to amplify SSR loci in *A. thaliana* (Bell and Ecker 1994) were used to amplify genomic DNA from *Brassica* and *A. thaliana* entries. Reactions lacking DNA were included as negative controls. Reaction mix and cycling profile were modified from Bell and Ecker (1994). Each 10-µl reaction contained 30 ng of DNA, 5 pmol of each primer, each dNTP at 200 µM, 1 × PCR buffer, 2 units of *Taq* polymerase, and 2 mM of $MgCl_2$ (Perkin Elmer reagents). Amplifications were carried out in a Perkin Elmer 9600 thermocycler, with a 2 min denaturation at 94°C followed by 45 cycles of 94°C denaturation for 15 s, 55°C annealing for 15 s, and 72°C extension for 30 s. For three pairs, the protocol variations described by Bell and Ecker (1994) were used (see Table 2). Three microliters of 6 × loading dye (0.25% bromophenol blue, 0.25% xylene cyanol FF, 15% Ficoll) (Sambrook et al. 1989) were added to each completed reaction, and the PCR products were separated by electrophoresis on 4% agarose gels (FMC NuSieve 3 : 1) with 1 × TBE buffer. Each gel included lanes of a DNA size-standard (GIBCO/BRL, 100 bp). Gels were stained with ethidium bromide (0.5 µ g/ml) after electrophoresis, and the products were visualized under UV light and sized relative to the DNA standard. Amplifications were confirmed in replicate assays.

Analysis of amplification products

The size ranges and polymorphism of the SSRs in *A. thaliana* were determined, using the results of Bell and Ecker (1994) and Todokoro

Table 1 Crop *Brassica* test array

Entry[a]	*Brassica* species	Cultivar group	Description	Origin
RA1	*rapa* var. *oleifera*	Turnip rape	cv Span	Canada
RA2	*rapa* var. *narinosa*	Taatsai	cv Tatsoi	Japan
RA3	*rapa* var. *pekinensis*	Chinese cabbage	cv Michihili	USA
RA4	*rapa* var. *rapifera*	Vegetable turnip	cv Purple Top White Globe	USA
NI1	*nigra*	Black mustard	Market sample	India
NI2	*nigra*	Black mustard	Market sample	Ethiopia
NI3	*nigra*	Black mustard	NY weed population	USA
NI4	*nigra*	Black mustard	VA weed population	USA
OL1	*capitata* var. *capitata*	White/green cabbage	cv Wisconsin Golden Acre	USA
OL2	*capitata* var. *capitata*	White/green cabbage	cv Jersey Wakefield	USA
OL3	*capitata* var. *costata*	Portuguese cole	Couve Nabica	Portugal
OL4	*capitata* var. *selenesia*	Borecole	cv Westland Winter	Netherlands
JU1	*juncea* var. *oleifera*	Brown mustard	cv Cutlass	Canada
JU2	*juncea* var. *oleifera*	Brown mustard	Commercial brown	Canada
JU3	*juncea* var. *rugosa*	Leaf mustard	cv Southern Giant Curled	USA
JU4	*juncea* var. *rapifera*	Root mustard	Duck root mustard	China
NA1	*napus* var. *oleifera*	Winter oil rape	cv Jet Neuf	Belgium
NA2	*napus* var. *oleifera*	Spring oil rape	cv Westar	Canada
NA3	*napus* var. *rapifera*	Rutabaga	cv Laurentian	Canada
CA1	*carinata*	Ethiopian mustard	cv Dodolla	Canada
CA2	*carinata*	Ethiopian mustard	Breeding line	Canada
CA3	*carinata*	Ethiopian mustard	Breeding line	Canada
CA4	*carinata*	Ethiopian mustard	Breeding line	Canada

[a] Sources: (JU3-4, CA1-4) Agriculture and Agri-Food Canada, Research Station, Oilseeds Section, Saskatoon SK; (NI3-4) collected populations; (RA1-4, OL1-4, NA1-3, JU3-4) Plant Genetic Resources Unit, USDA-ARS, Geneva, NY; (NI1-2) North Central Regional Plant Introduction Station, USDA-ARS, Ames, IA

Table 2 *A. thaliana* SSR primer pairs classified by their amplification in *Brassica*

Amplified products $\leqslant$ 750 bp in size, consistent amplification, moderate to dark staining intensity								
Reject			Accept					
			Amplified in $\geqslant$ 2 entries, length variants within a 200-bp size range					
			Reject			Accept for further analysis		
Primer pair	Repeat[a]	Chr[a]	Primer pair	Repeat	Chr	Primer pair	Repeat	Chr
ATHCHIB	$(AT)_{14}$	3	ATHACS + [c]	$(A)_{36}$	1	ca72 + [b]	$(CA)_{18}$	5
ATEAT1	$(AT)_{11}$	1	nga6	$(GA)_{31}$	3	ATHCTR1 + [b]	$(GA)_{16}$	5
ATHGENEA	$(A)_{39}$	1	nga126 +	$(GA)_{31}$	3	nga12 +	$(GA)_{16}$	4
ATHATPASE	$(GA)_{18}$	1	nga128 +	$(GA)_{16}$	1	nga32 +	$(GA)_{13}$	3
nga8	$(GA)_{27}$	4	nga158	$(GA)_{13}$	5	nga59 +	$(GA)_{19}$	1
nga76	$(GA)_{22}$	5				nga63 +	$(GA)_{23}$	1
nga112[b]	$(GA)_{16}$	3				nga106 +	$(GA)_{26}$	5
nga225	$(GA)_{18}$	5				nga111	$(GA)_{16}$	1
						nga129 −	$(GA)_{20}$	5
						nga139	$(GA)_{29}$	5
						nga151	$(GA)_{31}$	5
						nga162	$(GA)_{21}$	3
						nga168	$(GA)_{25}$	2
						nga172 +	$(GA)_{29}$	3
						nga248	$(GA)_{24}$	1
						nga249	$(GA)_{15}$	5
						nga280	$(GA)_{15}$	1

[a] Core repeat sequence, number of core repeats in the original cloned sequence, and chromosome assignment of the corresponding *A. thaliana* SSR (Bell and Ecker 1994)

[b] Protocol variations (from Bell and Ecker 1994) were 61°C and 56°C annealing temperatures for ca72 and ATHCTR1 respectively, and 3 mM $MgCl_2$ for nga112

[c] Twelve pairs were evaluated in single-primer assays; + or − indicate the presence or absence of two-primer reaction products that were the same size as single-primer reaction products

et al. (1995) and the results for control entries in the present study. All *A. thaliana* SSRs were $\leqslant 500$ bp in size, and most had length variants within a 200-bp size range.

Brassica amplification products were evaluated in two stages, in order to select products for further analysis as markers (see Table 2). First, primer pairs were identified that generated one or more products $\leqslant 750$ bp in size, with consistent amplification over replicates and moderate-to-dark staining intensity. Twenty two pairs were selected. Next, for these pairs, products were selected that amplified in at least two entries and had length variants within a 200-bp size range. For 12 pairs, reactions with both primers were compared to reactions with only one primer. Some two-primer reaction products were the same size as single-primer products, and thus may have been amplified by only one primer; products in these size ranges were rejected as possible markers. Thirty five products (amplified by 17 pairs) were selected and are referred to subsequently as SSR amplification products.

SSR probe preparation and blot hybridization

Sixteen of the seventeen pairs that amplified the selected *Brassica* products were originally designed to amplify $(GA)_n$ SSRs in *A. thaliana* (Table 2). For each of these pairs, hybridization assays were conducted to determine whether the selected products actually contained SSRs. In order to detect the presence of both perfect and modified SSRs in *Brassica*, relatively non-stringent hybridization conditions were used. Following the procedures of Ausubel et al. (1994), synthetic oligonucleotide $(CT)_{10}$ probes were 5′ end-labeled in 20-µl reactions containing 100–200 ng of DNA, 10 U of T4 polynucleotide kinase (Promega), and 50 µCi of [γ-^{32}P]ATP ($\geqslant 3000$ Ci/mol; Dupont NEN). Unincorporated [γ-^{32}P]ATP was removed by column chromatography on Sephadex G-50. For each primer pair, DNA from one *A. thaliana* strain and 11 *Brassica* entries was amplified. Products were electrophoresed, stained, and transferred to Magna Charge™ nylon membranes (MSI) per manufacturer's instructions. Membranes were pre-hybridized ($6\times$ SSPE, 1% SDS, $5\times$ Denhardt's solution); hybridized overnight with the probe (in $6\times$ SSPE, 1% SDS); washed two to three times ($1\times$ or $2\times$ SSPE, 0.1% SDS, 10–15 min); and autoradiographed (Kodak X-Omat AR film) at -70°C for 1–6 days, using intensifying screens. Each membrane was assayed at least once with pre-hybridization, hybridization, and wash temperatures at 50°C (low stringency) and at least once at 55°C (high stringency). Between assays, membranes were washed three times (0.1% SDS at 70°C, 10–60 min) to remove the probe. For each pair, two to three replicate membranes were assayed.

Data analysis

The *Brassica* SSR amplification products were classified by: (1) size range (as $\leqslant 100$ bp or > 100 bp from the size range of the corresponding *A. thaliana* SSR); (2) staining intensity (dark or moderate); (3) presence/absence of length variation between entries; (4) presence and intensity of hybridization (see Table 4 for description of classes); (5) number of genomes with amplification; and (6) chromosome assignment of the corresponding *A. thaliana* SSR. Chi-square tests were used to assess relationships between each pair of characteristics, e.g., between the number of products in each size class and the number in each hybridization class. Kruskal-Wallis tests were used to determine whether the number of species amplified differed between size, staining, or hybridization classes.

Each product was classified as present/absent in each of the *Brassica* species and genomes, and by whether it had species- or genome-specific variants. Chi-square tests were used to evaluate whether the number of products that amplified, the number with taxon-specific variants, and the percentage in each size, staining intensity, and hybridization class differed between species or between genomes. Pairwise similarity between *Brassica* species and between genomes was calculated as the proportion of shared SSR amplification products.

Significance at the 5% probability level was determined for all statistical tests, and SAS Version 6.08 (SAS Institute 1989) was used for most tests. Cochran-Mantel-Haenszel tests were used instead of chi-square tests when cell frequencies were low.

Results

Amplification in *A. thaliana* and *Brassica*

As a control, we confirmed that all primer pairs generated products in *A. thaliana*. Most products had moderate-to-dark staining intensity, and all pairs except *ca72* had products $\leqslant 100$ bp from published size ranges (Bell and Ecker 1994; Todokoro et al. 1995). Eight pairs had products outside these ranges; this may be related to differences between studies in the *A. thaliana* genotypes assayed.

In *Brassica*, 22 pairs met the first set of criteria (consistent amplification, products $\leqslant 750$ bp in size, moderate-to-dark staining intensity) and were selected for further analysis (Table 2). For each of the 12 pairs tested for single-primer amplification, products were generated in one or both single-primer reactions (Table 2). Most of these products were $\geqslant 300$ bp in size. For 11 pairs, some two-primer reaction products were the same size as single-primer reaction products and thus may have been amplified by only one primer (Figs. 2 c, 3 a).

Thirty five *Brassica* products, generated by 17 pairs, met our second set of criteria (amplified in $\geqslant 2$ entries, length variants within 200 bp) and were selected as SSR amplification products (Table 3 and Fig. 1). Twenty eight of the products were $\leqslant 100$ bp from *A. thaliana* SSR size ranges, and one-third had dark-staining intensity. Eighty percent had length variation between entries; most had fewer than five length variants, but *nga129-1* had eight variants (Fig. 1 c). Length variation was not related to product size or staining intensity. No relationships were found between characteristics of *Brassica* SSR amplification products (size, staining intensity, length variation, and number of species and genomes amplified) and chromosome assignment of the corresponding *A. thaliana* SSRs (data not shown).

Variation between *Brassica* species and genomes

Each of the SSR amplification products was present in at least two *Brassica* species (Table 3). Most were present in at least four species, and most were polymorphic in at least one species. Two products were specific to B-genome species and two were specific to C-genome species. Twenty three had species-specific variants, and 25 had genome-specific variants. Sixteen

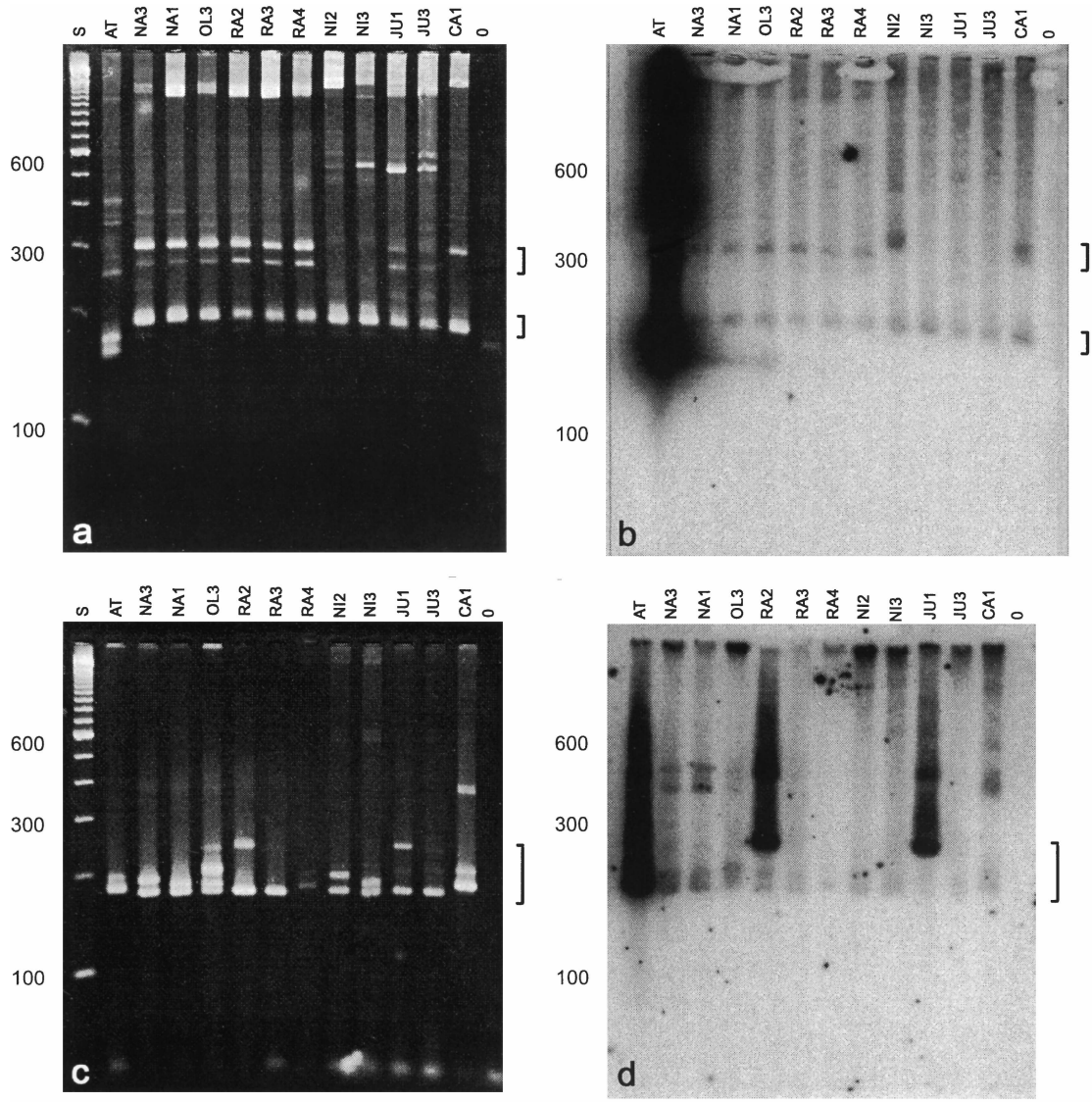

Fig. 1a–d Two-primer reaction products, stained with ethidium bromide (**a, c**) and hybridized to $(CT)_{10}$ at high (**b**) or low (**d**) stringency. **a, b** nga151; **c, d** nga129. *Lane 0*, negative control; *AT*, *A. thaliana*; *S*, size standard; *Brassica* entries labeled as in Table 1. Numbers on left indicate product size (bp). Brackets indicate size ranges of *Brassica* products selected for analysis

products were present in all three genomes. A product's size and staining intensity were not related to the number of species or genomes in which it amplified.

The number of products and the percentage in each size class did not differ between *Brassica* species or genomes (compiled from Table 3). However, *B. oleracea* and *B. nigra* had more polymorphic products than did *B. carinata* or *B. juncea*. *B. nigra* and *B. rapa* had the most species-specific variants; none were specific for *B. napus* or *B. carinata*. Diploids were more polymorphic and had more species-specific variants than did amphidiploids. The B genome had the most genome-specific variants. Pairwise similarities ranged from 0.67 between *B. napus* and *B. nigra*, which share no genomes, to 0.94 between *B. napus* and one of its putative parents, *B. oleracea* (data not shown). Similarity between diploids was highest for *B. rapa*-*B. oleracea* and lowest for *B. nigra*-*B. oleracea*; likewise, similarity between genomes was highest for A-C and lowest for B-C.

Hybridization to the $(CT)_{10}$ probe

Thirty three *Brassica* products, amplified by 16 primer pairs, were classified by their hybridization results (Table 4). Thirteen products gave negative results: they did not hybridize or else hybridized only at low stringency, and most likely either lack SSRs or have highly modified SSRs (classes 1 and 2 in Table 4). Twenty products had positive results: they hybridized at high

Table 3 Selected *Brassica* SSR amplification products, generated by *A. thaliana* SSR primer pairs

Product	Size (bp)[a]	SSR hybridization[b]	Staining intensity[c]	# Length variants	Amplification in *Brassica* species[d]						
					Genomes	*rapa*	*nigra*	*oleracea*	*juncea*	*napus*	*carinata*
ca72-1	160–210	–	Mod	3	ABC	SM +	SM	AM	AM	AM	AM
ca72-2	(420–550)	–	Dark	5	AC	AM	–	AP +	AM	AP	AM
ATHCTR1-1	80	1	Mod	1	BC	–	AM	AM	–	AM	SM
ATHCTR1-2	150–180	2	Mod	3	AB	SP +	AM	–	AM	–	AM
ATHCTR1-3	(300–380)	3	Dark	4	ABC	SM	SP +	AM	SM	AP	AM
nga12-1	260–340	3	Mod	4	ABC	SM	AP +	AP	AM	SM	AM
nga12-2	(550–650)	3	Dark	2	ABC	AM	SM	AM	AM	AM	AM
nga32-1	180	1	Mod	1	B	–	AM	–	AM	–	SM
nga32-2	310	2	Dark	1	AC	AM	–	SM	–	–	–
nga59-1	110–190	2	Mod	4	ABC	AP +	SM	SP	SM	SM	SM
nga63-1	150–170	2	Dark	2	AB	AM +	AM	–	AM	–	AM
nga63-2	220–250	2	Mod	2	AC	AP	–	AP	–	AP	–
nga63-3	330–420	3	Mod	3	AC	AP +	–	AM	AM	AM	–
nga106-1	140	1	Mod	1	B	–	AM	–	SM	–	AM
nga106-2	240–330	3	Mod	5	AB	SM +	AP +	–	AP +	–	AM
nga111-1	130–160	4	Mod	4	ABC	AM	AP +	AP	AP	AM	AP
nga111-2	(320–450)	3	Mod	4	BC	–	AP +	AP	AM +	AP	AM
nga129-1	175–250	4	Dark	8	ABC	AP	AP +	AP	AP	AP	AM
nga139-1	120–190	4	Mod	6	ABC	AP +	AP +	SP	AM	AP	AM
nga151-1	180	4	Dark	1	ABC	AM	AM	AM	AM	AM	AM
nga151-2	240–280	4	Mod	3	ABC	AM	SM +	AP	SM	AM	AM
nga162-1	75–90	3	Mod	3	AB	SM	AP	–	AP	–	AM
nga162-2	180	3	Mod	1	AC	SM	–	AM	–	AM	–
nga168-1	150–170	1	Dark	3	AC	AP +	–	AM	SM	AM	AM
nga168-2	330–480	3	Mod	5	ABC	AM	AP +	SM	AP +	–	AM
nga172-1	170–180	2	Mod	2	BC	–	SM	SM +	SM	–	SM
nga172-2	270–380	3	Mod	7	ABC	AP	SP +	SP +	AP +	SM	AM
nga172-3	(600–700)	4	Dark	4	ABC	AM	AP +	AP	AM	AM	AM
nga248-1	230–290	3	Mod	3	C	–	–	AP	–	AP	AM
nga248-2	(600–700)	3	Dark	3	C	–	–	AP +	–	SM	AM
nga249-1	80	1	Dark	1	AC	AM	–	SM	AM	AM	AM
nga249-2	120–150	4	Mod	4	ABC	SM	SP +	AP +	–	AM	–
nga249-3	(310–330)	3	Mod	2	ABC	AM	SM	SM	SP	AM	–
nga280-1	80–210	1	Mod	5	AC	SM +	–	SM +	AM +	SM	–
nga280-2	270–340	2	Mod	4	ABC	SM +	SM	AP	SM	SP	SM

[a] Parentheses indicate products > 100 bp larger than the *A. thaliana* SSR size range
[b] Product hybridization to the $(CT)_{10}$ probe, rated from Class 1 (poor) to Class 4 (very good) (see Table 4). Products of primer pair ca72, which amplifies a $(CA)_n$ SSR in *A. thaliana*, were not assayed for hybridization
[c] Dark or moderate (mod) staining intensity
[d] Amplification in all (A), some (S), or no (−) entries of the species; monomorphic (M) or polymorphic (P) between entries. + indicates the presence of species-specific product length variants

stringency, and most likely contain perfect or slightly modified SSRs (classes 3 and 4 in Table 4; Fig. 1). As a control, we confirmed that the probe hybridized to *A. thaliana* products of all assayed primer pairs.

Comparisons were made between products with positive and negative hybridization results (Table 5). In general, products that hybridized had more length variants and amplified in more species. However, no relationship was found between staining intensity and hybridization. Some of the products that hybridized well were larger than *A. thaliana* SSRs, and all of the products with poor hybridization were the same size as *A. thaliana* SSRs. The percentage of products that hybridized well did not differ between *Brassica* species (data not shown).

Some *Brassica* products had not been selected as possible markers, but they hybridized well. Some of these products had been rejected because they were much larger than *A. thaliana* SSRs, were not well-defined, and/or had light staining intensity (Fig. 2a, b and entry CA1 in Fig. 3). For *nga129-1*, one of the selected products, hybridization was more intense for large, infrequent variants than for common variants that were similar in size to the *A. thaliana* SSR (Fig. 1c, d).

Eight of the pairs that were assayed for hybridization had previously been assayed for single-primer amplification. For each pair, some of the two-primer products that hybridized were the same size as single-primer products in the previous assays, so they may have been

Table 4 *Hybridization of Brassica* SSR amplification products to the $(CT)_{10}$ probe[a]

Type	Class 1	Class 2	Class 3	Class 4
Low-stringency hybridization	No entries or some entries (light)	All entries (light)	Some or all entries (light to moderate)	All entries (moderate to dark)
High-stringency hybridization	No entries	No entries	Some entries (light)	Some or all entries (moderate)
	ATHCTR-1* nga32-1* nga106-1* nga168-1 nga249-1* nga280-1	ATHCTR1-2 nga32-2* nga59-1 nga63-1 nga63-2 nga172-1 nga280-2	(ATHCTR1-3) nga12-1 (nga12-2) nga63-3 nga106-2 (nga111-2) nga162-1 nga162-2* nga168-2 nga172-2 nga248-1 (nga248-2) (nga249-3)	nga111-1 nga129-1 nga139-1 nga151-1* nga151-2 (nga172-3) nga249-2

[a] Presence and intensity of hybridization in none, some, or all of the entries that had amplification. Asterisks indicate products with no length variation between entries; parentheses indicate products $>$ 100 bp larger than the *A. thaliana* SSR size range

Table 5 Characteristics of hybridizing and non-hybridizing *Brassica* SSR amplification products[a]

Hybridization	# Products	Percentage of products			Average number of	
		Within *A. thaliana* size range	With dark staining intensity	With length variation between entries	Length variants	Species amplified
Positive	20	70	30	90	3.8	5.0
Negative	13	100	38	62	2.3	4.1
χ^{2}[b]		4.8*	0.002	3.8*	5.6*	4.2*

[a] From Table 4, positive (classes 3 and 4) or negative (classes 1 and 2) results for hybridization to $(CT)_{10}$. Products ca72-1 and ca72-2 not included

[b] Chi-square or Kruskal-Wallis statistic for H_0: differences between products with positive and negative results are not significant

* Denotes statistical significance at the 0.05 probability level

products of only one primer (Fig. 2, entry CA1). In a single-primer/hybridization assay for *nga106*, in some size ranges both single-primer and two-primer reaction products hybridized to the probe (Fig. 3).

Discussion

Twenty two of the thirty *A. thaliana* SSR primer pairs had consistent amplification in *Brassica* crop species. Most of the pairs amplified multiple products, evidence that SSR flanking regions are conserved and duplicated within and between genera. Genome duplication in *Brassica* species is estimated at 30–60% (Kianan and Quiros 1992; Ferreira et al. 1994), and duplication between *A. thaliana* and *B. oleracea* genomes is extensive (Kowalski et al. 1994). Amplification of multiple products by a single SSR primer pair has also been observed for potato (Provan et al. 1996) and for maize primers used in sorghum (Brown et al. 1996). Barret et al. (1995) reported that fewer *A. thaliana* primer pairs amplified DNA fragments in *Brassica* but, for many of the pairs, our results were consistent with theirs. We examined a larger size range of products than did Barret et al. (1995), and we screened more *Brassica* species; differences in results between the studies may be related to these factors.

For analysis as possible markers, in initial assays we selected *Brassica* products that had moderate-to-good staining intensity. In subsequent assays, however, many of these products did not hybridize well to the SSR probe. Conversely, some products that hybridized well had been rejected because they stained poorly. Thus some *A. thaliana* SSRs appear to be modified or lost between genera, although their flanking regions are conserved; other SSRs are conserved between genera, but have modified flanking regions. In addition, some

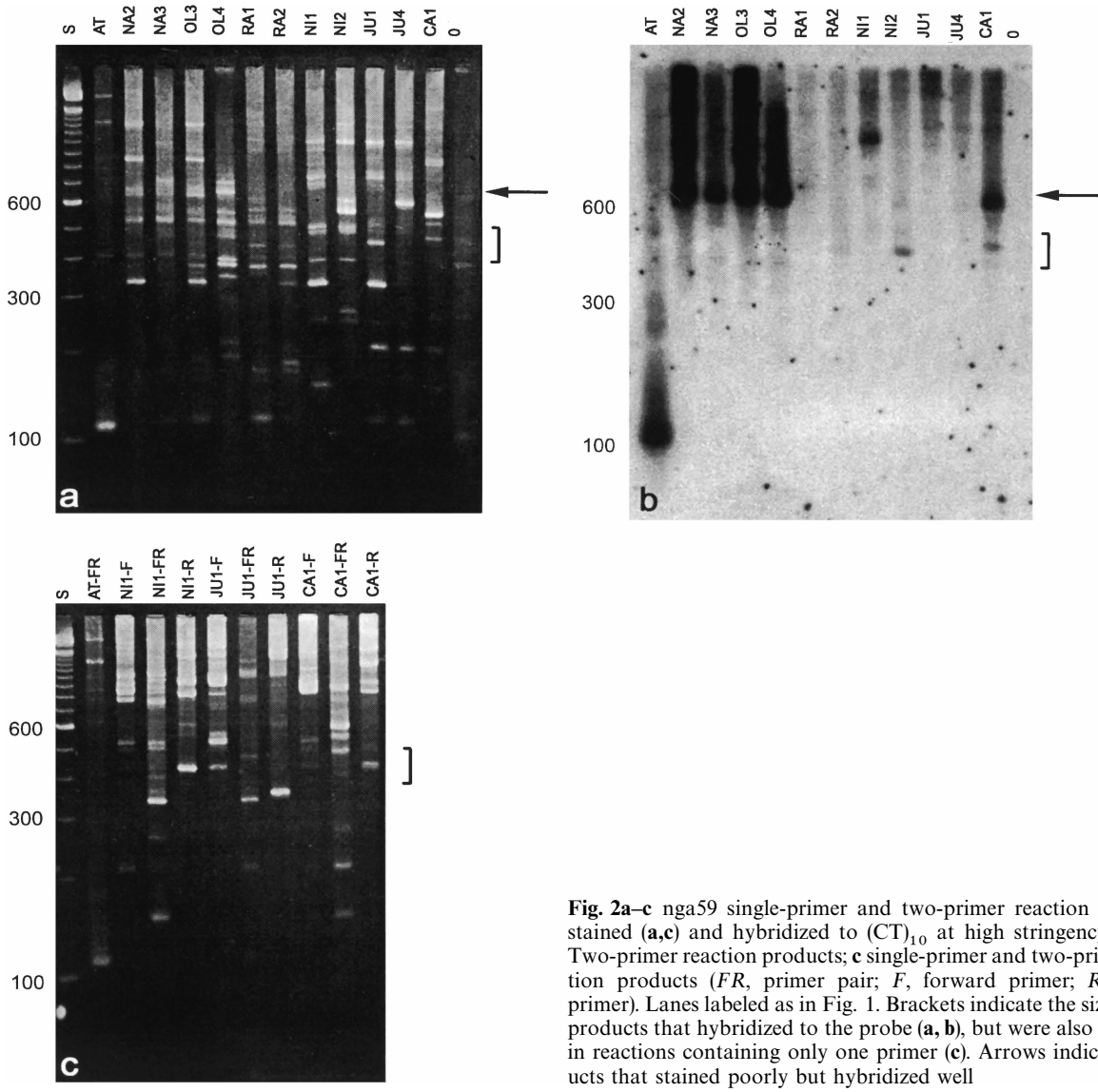

Fig. 2a–c nga59 single-primer and two-primer reaction products, stained (**a,c**) and hybridized to $(CT)_{10}$ at high stringency (**b**). **a, b** Two-primer reaction products; **c** single-primer and two-primer reaction products (*FR*, primer pair; *F*, forward primer; *R*, reverse primer). Lanes labeled as in Fig. 1. Brackets indicate the size of CA1 products that hybridized to the probe (**a, b**), but were also amplified in reactions containing only one primer (**c**). Arrows indicate products that stained poorly but hybridized well

products had been rejected because they were much larger than *A. thaliana* SSRs or appeared to be amplified by a single primer (similar to products in RAPD assays); however, some of these products hybridized well.

For 33 of the selected *Brassica* products, we examined whether product size and length variation differed between products that hybridized well and those that did not. We expected that products with good hybridization would have the most length variation and be similar in size to *A. thaliana* SSRs. However, many of the larger products hybridized well (Tables 4 and 5). In addition, the products most likely to hybridize were those that had length variation, but this relationship was not absolute: 2 of 20 products that hybridized well were monomorphic. Monomorphic products containing SSRs have also been reported in other studies (Broun and Tanksley 1996). As in many cross-taxa SSR studies of plants, however, in our study only a few genotypes from each species were assayed, so relationships between product length variation and presence of SSRs can only be estimated. The results of our study are evidence of mutation, duplication, and rearrangement between *Arabidopsis* and *Brassica* genomes. For cross-taxa SSR studies in general, these results are a caution against using a product's size and length variation as predictors of whether the product actually contains repeats. To identify products containing SSRs, hybridization assays and/or DNA sequencing are required. However, hybridization assays alone could be misleading, since single-primer products containing SSRs might be identified as two-primer SSR products.

Seven of the *Brassica* products contained SSRs, and 13 most likely contained modified SSRs (classes 4 and

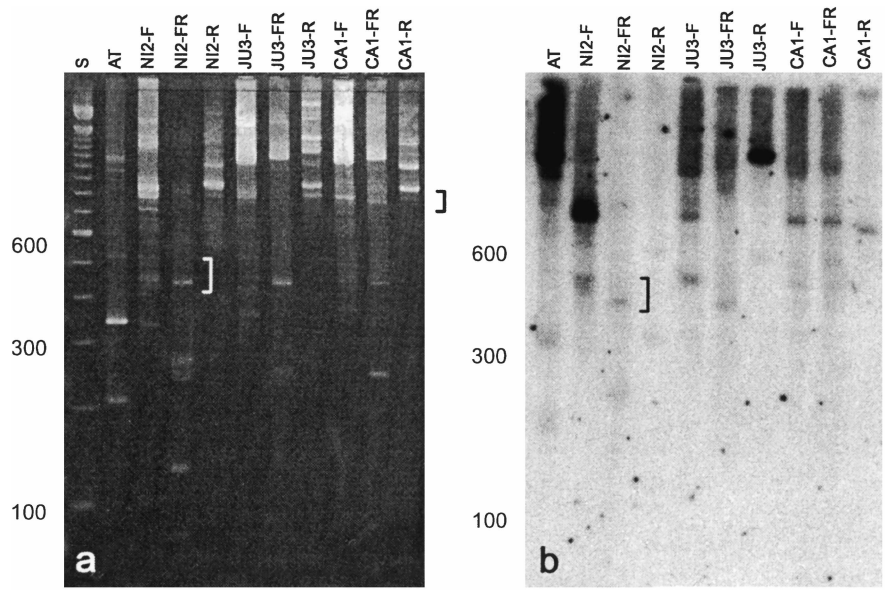

Fig. 3a, b nga106 single-primer and two-primer reaction products, stained **a** and hybridized to $(CT)_{10}$ at low stringency **b**. Lanes labeled as in Figs. 1 and 2. For NI2 and CA1, brackets indicate size ranges in which both forward-primer and two-primer reaction products hybridized to the probe

3 respectively, Table 4). These products may be useful as markers for genotyping, mapping, diagnostics, and quantifying variation within *Brassica* species. Single-primer products and those lacking repeats have more limited value as markers: since their sequences are seldom known, and same-sized products may not be homologous, they are not appropriate for genotyping or for phylogenetic analyses based on models of SSR mutation. However, if the nature and inheritance of these markers is determined, they may be useful in selected breeding applications. Most single-primer SSR products have dominant inheritance, so they have limited value for mapping and pedigree analysis, but they may be informative as descriptors of intraspecific variation. Two-primer products lacking repeats generally have low variability (Provan et al. 1996), so they may not discriminate between close relatives. However, some of these fragments may be species- or genome-specific, and primers can be designed to amplify them as diagnostic markers. In our study, the B genome and *B. nigra* (BB) had the most genome- and species-specific products. This is consistent with accepted phylogenies: the B genome is considered to be in a different lineage than the A and C genomes (Attia and Röbbellen 1986).

In summary, *A. thaliana* SSR primer pairs amplified both polymorphic and species-specific products in a diverse *Brassica* test array. However, many pairs amplified multiple products in *Brassica*, and many of the products did not contain repeats. Our results are consistent with previous reports of duplication and rearrangement in crucifer genomes, and they highlight the necessity of using hybridization, DNA sequencing, and single-primer assays to differentiate two-primer products containing SSRs, products lacking repeats, and single-primer products. Cross-taxa SSR studies do not necessarily generate large numbers of variable, co-dominant SSRs markers. They may provide other useful markers, however, plus valuable information about plant genome variation and evolution.

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