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From *Arabidopsis thaliana* to *Brassica napus*: development of amplified consensus genetic markers (ACGM) for construction of a gene map

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Abstract The evolution of genomes can be studied by comparing maps of homologous genes which show changes in nucleic acid sequences and chromosome rearrangements. In this study, we developed a set of 32 amplified consensus gene markers (ACGMs) that amplified gene sequences from *Arabidopsis thaliana* and *Brassica napus*. Our methodology, based on PCR, facilitated the rapid sequencing of homologous genes from various species of the same phylogenetic family and the detection of intragenic polymorphism. We found that such polymorphism principally concerned intron sequences and we used it to attribute a *Brassica oleracea* or *Brassica rapa* origin to the *B. napus* sequences and to map 43 rapeseed genes. We confirm that the genetic position of homologous genes varied between *B. napus* and *A. thaliana*. ACGMs are a useful tool for genome evolution studies and for the further development of single nucleotide polymorphism suitable for use in genetic mapping and genetic diversity analyses.

Keywords Amplified consensus genetic markers (ACGM) · *Arabidopsis thaliana* · *Brassicaceae* · Single nucleotide polymorphism (SNP)

Introduction

Genome evolution involves changes in DNA sequences due to nucleotide substitutions/deletions and changes in DNA organization due to chromosome rearrangements. By comparing the chromosomal location of orthologous genes in different species, the extent of chromosome conservation and rearrangement can be determined, even for distantly related species (Ehrlich et al. 1997).

In mammalian evolution, the number of homologous genes identified is much larger (tens of thousands) than the number of chromosome rearrangements that is likely to have occurred (hundreds). Even in this well-documented domain, limitations must be taken into account when considering published analytical measures of conservation of synteny: gene loci on the same chromosome in different species (Renwick 1971). Systematic errors tend to overestimate the degree of conservation. The key elements for the analysis of genome organization and evolution are the identification of orthologous genes, the identification of conserved segments, the estimation of the length of conserved linkage domains and the determination of the number of conserved syntenies to estimate the rate of chromosome rearrangement (Nadeau and Sankoff 1998). The major source of error and ambiguity is the incorrect identification of orthologous genes (Ehrlich et al. 1997). The second most important error is poor estimation of the frequency of recombination, resulting in the mapping of genes to the wrong location (Nadeau and Sankoff 1998).

Comparative maps have been made for many plants: for example, between grass genera (Bennetzen et al. 1998; Gale and Devos 1998), tomato and potato (Tanksley et al. 1992), pea and lentil (Weeden et al. 1992), and *Arabidopsis* and *Brassica* (Kowalski et al. 1994; Lagercrantz and Lydiate 1996; Lagercrantz et al. 1996; Lagercrantz 1998). These comparisons all involved the use of hybridization-based mapping procedures, which, with variable stringency conditions, made it possible to detect similar but imperfectly matched DNA sequences. In grasses, a set of 152 anchor RFLP probes for comparative mapping has been established (Van Deynze et al. 1998). The colinearity of these probes is well established but may be disrupted by insertions, deletions and inversions (Foote et al. 1997). Various papers have recently been published concerning advances and future prospects in studies of macro- and micro-syn-

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teny in plant genomes (Bennetzen 2000; Devos et al. 2000; Schmidt 2000).

To-date, PCR technology has rarely been used in comparative studies in plants due to the difficulty of designing PCR primers for one genome that also function efficiently in another genome, and to the lack of conservation of microsatellites between species. In addition, the identification of homologous genes is certainly complicated in many plants by the polyploidization of genomes. The applicability of consensus PCR primers across species and genera has been demonstrated by the use of wheat Em sequences to develop markers for orthologs in rye (Van Campenhout et al. 2000) and by using consensus primers between *Arabidopsis thaliana* and *Brassica napus* (Brunel et al. 1999). However, progress lags well behind that in mammalian work, in which genetic markers such as comparative anchored tagged sites (CATS, Lyons et al. 1997) have been designed by comparing 410 mammalian sequences. The conserved primer sequences identified in this way are then used for anchor locus amplification from the DNA of any mammalian species.

Another way of comparing genome organization was developed by Grant et al. (2000), who compared soybean and *Arabidopsis* genomes by means of tBlastx conceptual translation of soybean DNA sequences from already mapped RFLP probes and using information currently available from the Arabidopsis Genome Initiative (A.G.I.) program.

Comparative mapping between *Arabidopsis* and *Brassica oleracea* (Kowalski et al. 1994; Lan et al. 2000) or *Brassica nigra* (Lagercrantz 1998; Sadowki and Quiros 1998) has indicated that linkage zones are disrupted by a considerable number of chromosome rearrangements, although colinear regions can usually be found (Lagercrantz et al. 1996; Sadowski et al. 1996; Cavell et al. 1998). Analysis of 186 corresponding loci detected by hybridization in *B. oleracea* and *A. thaliana* has shown that at least 19 chromosome structure rearrangements differentiate the *B. oleracea* and *A. thaliana* genomes. Chromosomal duplication in the *B. oleracea* genome is strongly suggested by parallel arrangements of duplicated loci on different chromosomes accounting for 41% of the loci mapped in *Brassica* (Lan et al. 2000).

We developed a set of 32 amplified consensus gene markers (ACGM) between *A. thaliana* and three different *Brassica* species to initiate the construction of a gene map. We studied various genes thought to be linked to agronomic traits, including the fatty acid biosynthetic chain, plant defense mechanisms (induced by pathogen attack or involved in the biosynthesis of lignin or flavonoid compounds), disease resistance, flowering time and floral morphology. The results obtained confirmed the efficiency of the method previously described for primer determination (Brunel et al. 1999). We discuss here the sequences of homologous genes in different species of the *Brassicaceae* family, their position on a *B. napus* genetic map and the polymorphism observed in four rapeseed lines.

Materials and methods

Plant material

PCR was performed with 50 ng of DNA extracted from *A. thaliana* leaves (ecotype: Columbia accession N1092), as well as four cultivars of *B. napus* (Darmor-*bzh*: French winter line; Drakkar: French spring line; Stellar: Canadian spring line; Yudal: Korean spring line), *B. oleracea* (line: Rapid Cycling) and *B. rapa* (line: R500). We chose to work with these rapeseed lines because they are representative of the observed genetic variability (Diers and Osborn 1994). They have been used to construct a *B. napus* genetic map (Foisset et al. 1996; Lombard and Delourme 2001) and to map various major traits concerning plant development (dwarfism, early flowering), seed quality (erucic acid, linolenic acid and glucosinolate content), and resistance to different diseases (*Leptposphaeria maculans* and *Cylindrosporium concentricum*). Both populations were derived from isolated microspore cultures. A total of 152 doubled-haploid lines from "Darmor-*bzh*" × "Yudal" were previously used to establish a genetic map with 266 segregating markers (Foisset et al. 1996). The second population (94 DH lines) was obtained from the cross "Stellar" × "Drakkar" (Jourdren et al. 1996a).

Laboratory protocols

The protocols used have been described elsewhere (Brunel et al. 1999). We employed the Internet to search various databases (GenBank-EMBL, AATDB, KAOS). Sequences were aligned with the GCG sequence analysis software package (University of Wisconsin). PCR primers were determined as previously described (Brunel et al. 1999) using the OLIGO program for Mac v4.0 (Rychlik et al. 1990).

Genetic mapping was carried out by looking for polymorphism in segregating populations (Lombard and Delourme 2001) employing electrophoresis in non-denaturing gels. The concentration of polyacrylamide used was between 4% and 6%, depending on the size of the anticipated PCR product, to optimize the detection of sequence differences (Kirkpatrick et al. 1993).

In one case (BN-RPS2-2), a single nucleotide polymorphism was detected by sequencing and scored in offspring populations by mass spectrometry, using the protocol described by Sauer et al. (2000).

Loci were mapped with MAPMAKER/EXP version 3.0 (Lincoln et al. 1992) on the individual maps constructed by Lombard and Delourme (2001). A maximum recombination fraction of 0.4 and a threshold LOD score of 3.0 were used. Loci positions on the consensus linkage groups were deduced from their relative positions to neighbouring markers. Centimorgan distances were determined with the Kosambi function.

Results

We selected 32 genes for the development of ACGMs (Table 1): 25 sequences originating from *A. thaliana* "mother" sequences and seven from *Brassica* species. The method used to isolate homologous sequences from any *Arabidopsis* or *Brassica* gene was 100% effective for all genes tested.

Number of identified genes

The patterns observed on the polyacrylamide gels are displayed at the following web site: http://www.inra.fr/Internet/Produits/acgm/ under the heading project:

Table 1 Consensus primer sequences. Each ACGM was chosen from an *Arabidopsis* or *Brassica* gene, identified by its Genebank accession number and biological function. The consensus primer pairs are used for the positive amplification of homologous sequences from *Arabidopsis* and the three *Brassica* species

Biological function	Protein	Arabidopsis gene name	Gene accession	ACGM number	Primer names	Primer sequences
Fatty acid synthesis	3-OXOacyl-ACP Reductase	OXOR	X64464	ACGM 1	ATBORED-454	GTGGCCAGGCTATCACNTTYGG
					ATBORED-744	AGTCGCTGTGTAGTTTGCYTGNCC
	Acetyl CoA Carboxylase (cytosolic form)	ACC	L27074	ACGM 2	ATHCOACAR-2115	GCTGTACGTGTGACAWGNGARGA
					ATHCOACAR-2741	CTACTATCCAACCANCCNGTRTG
	Acyl Carrier Protein	ACP	X70336[a]	ACGM 3	BCACPSFI-332	ACGAGGATTAGTTTYCARAARCC
					BCACPSFI-706	CTTCCTCTAAACCCATNACDATYTC
	Enoyl-ACP-Reductase	ENAR	X95462[a]	ACGM 4	BNENOYLRD-740	CTACCTCGCTGCTATHWCNGC
					BNENOYLRD-1062	TCATCTGCGGTCARNGTYTT
	Fatty Acid Elongase	FAE1	U50771[a]	ACGM 5	BNU50771-990	CGGAGCTGACGACAARWCNTT
					BNU50771-1513	TCGATGCAGTGYTSCCANGG
	Ketoacyl ACP-Synthase II	KASIII	L31891	ACGM 6	ATHKASIII-690	GATATCACAGCTGCTTGYAGNGG
					ATHKASIII-1047	GCAAAGACCTCTTTNCCRTTCAT
	Omega 3 Desaturase (plastid form)	FAD7	L22961	ACGM 7	ATHCPFADD-193	TCTCAGACATAGGRTGCCANGA
					ATHCPFADD-594	GTATACGACCTCTCCCNAGNAT
	Omega 3 Desaturase (cytosolic form)	FAD3	L01418[a]	ACGM 8	BNALINDES-385	GTGGACATGGGAGTTTYTCNGA
					BNALINDES-1020	TGGCATCGACCAARTGRTARTG
	Omega 6 Desaturase (plastid form)	FAD6	U09503	ACGM 9	ATU09503-312	CAGTGCAGAAGACAGNGARCA
					ATU09503-1030	GCCGTATGATGAACNATNGTRAA
	Omega 6 Desaturase	FAD2	L26296	ACGM 10	ATHD12AAA-431	CGACTACCAATGGCTNGAYGA
					ATHD12AAA-1183	CTTCCCTGTCCGGTTCNACRT
	Stearoyl-ACP-Desaturase (cytosolic form)	DELTA 9	X74782[a]	ACGM 11	BNSACPDI-2243	AGATCTCCCAAGTTYCTNTG
					BNSACPDI-3619	TTAGGGGGAGTGAATGGSTT
Disease	Acid Endochitinase	CHIA	M34107	ACGM 12	ATCHIA-1787	AACTTGGCTCTCCTCARCAYTG
					ATCHIA-2221	TGAACACGGTGGATTRTTRTARAA
	Basic chitinase	CHIT25	M95835[a]	ACGM 13	BNCH25A-693	AGGAAGAAAGAAATCGCNGCNTT
					BNCH25A-1445	TCTTGTTTGAAACARTANCCCA
	Chitinase	CHITIN	X61488[a]	ACGM 14	BNCHITIN-372	CCGCAAATACTTTYCCNAAYTT
					BNCHITIN-683	CTTACGCTATTCATCCARAACCA
	Beta 1,3 glucanase	BG2	M58462	ACGM 15	ATHBG2A-1299	ACGTCTCCTTCCGWCNCARGG
					ATHBG2A-1596	TCCCTCTSTNGGCCANCC
	Chalcone Isomerase	CFI	M86358	ACGM 16	ATHCFI-761	CCTCCTCCAATCCATTNTTYCT
					ATHCFI-1199	CCACACAATTCTCCGTNACYTT
	Chalone Synthase	CHS	M20308	ACGM 17	ATHCHS-2117	CATCTTGGCTATTGGNACNGC
					ATHCHS-2594	CTTGACGGAAGGACGNARNCC
	Cinnamate 4 Hydroxylase	C4H	U71080	ACGM 18	ATU71080-3347	CATGACGGTTCCTTTYTTYAC
					ATU71080-3901	CATTGTCCTCGTTDATYTCNCC
	Cinnamyl Alcohol Dehydrogenase	CAD2	Z31715	ACGM 19	ATCIACDE-2342	GCACGAGGTAGTAGGNGARGT
					ATCIACDE-3228	AAAGCCAACACTTCTTCNGTYTC
	Cinnamyl Alcohol Dehydrogenase	CAD3	L37883	ACGM 20	ATHCAD1B-914	GAGGCAGGGAAGCAYYTNGG
					ATHCAD1B-1551	AGCCTCTCCATCGCNGTRTT
	Coumarate CO Ligase	C4L	U18675	ACGM21	U18675-1307	CGGTTGGCTTCATACNGGNGA
					U18675-1635	TCCTCAATATCTTCCCNGWNGG
	Dihydroflavonol 4 reductase	DFR	M86359	ACGM22	ATHD4R-527	GTCAGAAAGAGACCGTNTGYGTNAC
					ATHD4R-987	TTCCATTCACTGTCGGYTTDAT
	EL13	EL13	X67816	ACGM23	ATELI3-1-254	TTGTGGGCGTGGTGACNGA
					ATELI3-1-985	CCGCCGTGATGTTRTGYTT

Table 1 (continued)

Biological function	Protein	Arabidopsis gene name	Gene accession	ACGM number	Primer names	Primer sequences
Disease defense	Phenylalanine Ammonia Lyase	PAL	L33677	ACGM24	ATHPAL-2301 ATHPAL-3296	GTGTTACTACTGGTTTTGGNGCNAC GCTTACCACTCATCACYTCNGC
Disease resistance	Disease resistance protein	RPS2	U14158	ACGM25	U14158-64 U14158-1150	GCGGAGAGAAGAGGNCAYAARAC AGGGCAAATACATARTTCATNCC
Floral meristem	Agamous	AG	X53579	ACGM 26	ATAGAMSG-892 ATAGAMSG-1185	ATTGATGGGTGAGACNATHGG ATGCCGCGACTTGGAARTART
	Apetala 3	AP3	M86357	ACGM 27	ATHAPETALA-132 ATHAPETALA-444	AATGGTTTATTCAAGAARGCNCA CGAACGAGTTTGAAAGTRTTYT
	Leafy	LFY	M91208	ACGM28	ATHLEAFY-3135 ATHLEAFY-3852	GACGCCGTCATTTNCTNCT CCCGTCGTCATCCTCNCCYTC
Flowering time	CONSTANS	CO	X94937	ACGM 29	ATCONSTAN-623 ATCONSTAN-970	ACGGGGGAGATAGAGTNGTNCC CCTCGAAGCATACCTGATNGTYTT
	Lumini Dependens	LD	U03456	ACGM30	ATLUMINIDESPENDES ATLUMINIDESPENDES	AAGCTATGAAGAAACAAAAYCGNAA GTTTCTTGGTCGAATCRTCNGC
Other	Phytochrome A	PHYA	L21154	ACGM 40	ATHPHYTOA-4919 ATHPHYTOA-5201	GCATTTCCTCACACTNGGTNGARG GTCTTGATCTCAAAYTGNACRTT
	Alcohol Dehydrogenase	ADH	M12196	ACGM 41	ATHADH-1941 ATHADH-2481	TTTTGGTCTTGGTGCNGTNG GTCAGTTTTGGGTTTGTARTTNC

[a] *Brassica* "mother" sequence

"Known gene function". Table 2 presents the number of homologous genes identified by sequencing for the four genomes analyzed. With the 32 consensus primer pairs, 37 sequences were obtained from *A. thaliana*, 102 from *B. napus*, 50 from *B. oleracea* and 54 from *B. rapa*. Most (79 *B. napus*, 47 *B. oleracea*, 43 *B. rapa*) are registered in the GenBank database (Table 2), the other sequences are presented on the web site.

For most of the primer pairs, only one sequence was amplified in *A. thaliana*. In two cases (ATHCPFADD and BNCHITIN) two sequences were amplified and, in one case (U18675), three sequences. In these cases, the positions of sequences were determined *a posteriori* from systematic sequencing data (AGI program).

One to seven sequences were amplified in rapeseed. One primer pair amplified one gene, 13 pairs amplified two homologous sequences, six amplified three genes and 12 amplified four genes or more. The number of homologous rapeseed sequences for each ACGM was the exact sum of those obtained from the *B. oleracea* and *B. rapa* amplifications in most cases, but with a few exceptions (ACGM 4, ACGM 6, ACGM 16 and ACGM 23).

A unique sequence was amplified in the two parental species for 50% of the ACGMs but two to four sequences were found for the other 50 %.

Sequence conservation

Sequences were aligned systematically (http://www.inra.fr/Internet/Produits/acgm/ under the heading project: "Known gene function"). The mean lengths of exons and introns were estimated at 115 and 132 base pairs respectively for the three Brassica species, similar to the data obtained for *Arabidopsis*.

A very high level of conservation was observed between coding sequences in the three *Brassica* species and in *A. thaliana*. There was often no evident sequence alignement between the introns of *Brassica* and *Arabidopsis*. However, it was possible to match a part of each *B. napus* sequence to the sequence present in one of the parental species on the basis of similarities in intron sequence: of the 102 *B. napus* sequences, 23 were found to be related to *B. oleracea* and 20 to *B. rapa* sequences, without ambiguity (Table 2). These sequences made it possible to identify a putative origin for the various linkage groups on the rapeseed genetic map. This was more difficult to achieve based on exon sequence information due to the very high level of sequence conservation. For the other 59 rapeseed sequences, it was not possible to determine the correspondence unambiguously, either due to the high level of similarity between intron sequences in *B. rapa* and *B. oleracea* or because the parental sequence was not available. In a few cases (BN-AG-3 and BN-ADH-3 for example), differences in intron sequences between rapeseed and the parental species were so great that no relationship could be identified.

Table 2 *Arabidopsis* and *Brassica* sequences obtained by amplification with the ACGM primers. The *B. napus* (BN) sequence and its related parental sequence (*B. oleracea* BO, *B. rapa* BR) are written on the same line. The column "other loci" corresponds to loci with an undefined phylogenetic correspondence

ACGM number	Amplified *Arabidopsis* sequence	*B. napus* locus	Genbank accessions	Related parental species locus	Genbank accessions	Other loci	Genbank sequences
ACGM 1	X64464	BN-BOXAR-1	AF179856			BR-BOXAR-1	AF179863
		BN-BOXAR-2	–			BO-BOXAR-1	AF179860
		BN-BOXAR-3	AF179857			BR-BOXAR-2	AF179864
		BN-BOXAR-4	AF179858			BO-BOXAR-2	AF179861
		BN-BOXAR-5	AF179859	BO-GOXAR-3	AF179862		
ACGM 2	L27074	BN-ACC-1	AF179851			BO-ACC-1	AF179852
		BN-ACC-2	AF179850			BR-ACC-1	–
		BN-ACC-3	–			BR-ACC-2	–
ACGM 3	X13708	BN-ACP-1	AF229418	BR-ACP-2	AF229423	BR-ACP-1	AF229422
		BN-ACP-2	AF229419	BO-ACP-1	AF229425	BR-ACP-3	AF229424
		BN-ACP-3	–			BO-ACP-3	AF229427
		BN-ACP-4	AF229420				
		BN-ACP-5	AF229421	BO-ACP-2	AF229426		
		BN-ACP-6	–				
ACGM 4	Y13860	BN-ENAR-1	AF181723	BO-ENAR-1	AF181725	BR-ENAR-1	–
		BN-ENAR-2	AF181724			BR-ENAR-2	–
ACGM 5	U29142	BN-FAE1-1	AF054497	BR-FAE1-1	AF054500		
		BN-FAE1-2	AF054498	BO-FAE1-1	AF054499		
ACGM 6	L31891	BN-KASIII-1	AF179854			BO-KASIII-1	AF179855
		BN-KASIII-2	AF179853			BR-KASIII-1	–
		BN-KASIII-3	–				
ACGM 7	AF056564	BN-FAD7-1	AF056558	BO-FAD7-1	AF056562		
		BN-FAD7-2	AF056559	BR-FAD7-1	AF056561		
		BN-FAD7-3	–			BR-FAD7-1	–
		BN-FAD7-4	AF056560	BO-FAD7-2	AF056563		
	U08216	BN-FAD8-1	AF056567			BO-FAD8-1	AF056566
						BR-FAD8-1	AF056568
ACGM 8	D26508	BN-FAD3-1	AF056569	BR-FAD3-1	AF056572		
		BN-FAD3-2	AF056570	BO-FAD3-1	AF056571		
ACGM 9	U09503	BN-FAD6-1	–	BR-FAD6-1	AF229390		
		BN-FAD6-2	AF229391	BR-FAD6-2	–		
		BN-FAD6-3	AF229388	BO-FAD6-1	AF229389		
ACGM 10	L26296	BN-FAD2-1	–			BO-FAD2-1	AF181726
		BN-FAD2-2	–			BR-FAD2-1	
ACGM 11	X93461	BN-DELTA9-1	AF230688-9			BR-DELTA9-1	AF230695-6
		BN-DELTA9-2	AF230690-1			BO-DELTA9-1	AF230693-4
		BN-DELTA9-3	–			BR-DELTA9-3	AF230696
		BN-DELTA9-4	X74782, AF230692			BO-DELTA9-2	–
ACGM 12	M34107	BN-CHIA-1	AF207560	BO-CHIA-1	AF207562		
		BN-CHIA-2	AF207561	BR-CHIA-1	AF207563		
ACGM 13	M38240	BN-CHIT25-1	M95835, AF230685			BO-CHIT25-1	AF230686
						BR-CHIT25-1	AF230687
ACGM 14	AC002333	BCHITIN-1	X61488, AF230681			BR-BCHITIN-1	AF230684
		BCHITIN-2	AF230682			BO-BCHITIN-1	AF230683
	AL132955	BCHITlike1	AF261095			BO-CHITlike-1	AF261098
		BCHITlike2	AF261096			BR-CHITlike-1	AF261097
		BCHITlike3	–			BO-CHITlike-2	–
ACGM 15	M58462	BN-1.3-GLUCAN-1	AF229403			BR-GLUCAN-1	AF229404
		BN-1.3-GLUCAN-2	–			BR-GLUCAN-2	AF229405
		BN-1.3-GLUCAN-3	–			BO-GLUCAN-1	–

Table 2 (continued)

ACGM number	Amplified *Arabidopsis* sequence	*B. napus* locus	Genbank accessions	Related parental species locus	Genbank accessions	Other loci	Genbank sequences
ACGM 16	M86358	BN-CFI-1	AF258293			BO-CFI-1	AF258295
		BN-CFI-2	AF258294			BR-CFI-1	–
						BR-CFI-2	–
ACGM 17	M20308	BN-CHS-1	AF229393			BO-CHS-1	AF229396
		BN-CHS-2	***			BO-CHS-2	AF229397
		BN-CHS-3	AF229394			BR-CHS-1	AF229398
		BN-CHS-4	AF229395			BR-CHS-2	AF229399
ACGM 18	U71080	BN-C4H-1	–			BO-C4H-1	AF230674
		BN-C4H-2	–			BO-C4H-2	AF230675
		BN-C4H-3	–			BO-C4H-3	AF230676
		BN-C4H-4	–			BO-C4H-4	AF230677
		BN-C4H-5	–			BR-C4H-1	AF230678
		BN-C4H-6	AF230672			BR-C4H-2	AF230679
		BN-C4H-7	AF230673			BR-C4H-3	AF230680
ACGM 19	Z31715	BN-CADa-1	AF207552	BR-CADa-1	AF207555		
		BN-CADa-2	AF207553	BO-CADa-1	AF207554		
ACGM 20	L37883	BN-CADb-1	AF207556	BR-CADb-1	AF207559		
		BN-CADb-2	AF207557	BO-CADb-1	AF207558		
ACGM 21	AF106084.1	BN-4CLa-1	AF207564-65	BR-4CLa-1, BR-4CLa-2	AF207570-71		
		BN-4CLa-2	AF207566	BO-4CLa-1	AF207572		
	AF106085.1	BN-4CLb-1	AF207567	BR-4CLb-1	AF207573		
	AL021711	BN-4CLc-1	AF207568	BR-4CLc-1	AF207574		
	AF106085.1	BN-4CLd-1	AF207569	BO-4CLd-1	AF207575		
ACGM 22	M86359	BN-DFR-1	AF229383			BO-DFR-1	AF229385
		BN-DFR-2	–			BR-DFR-1	AF229384
ACGM 23	X67815	BN-ELI3-1	AF229406			BR-ELI3-1	AF229411
	X67816	BN-ELI3-2	AF229407			BR-ELI3-2	AF229412
		BN-ELI3-3	AF229408			BO-ELI3-1	AF229410
		BN-ELI3-4	AF229409				
ACGM 24	L33677	BN-PAL-1	AF2294228			BO-PAL-1	AF229429
		BN-PAL-2	–			BR-PAL-1	AF229430
ACGM 25	U14158	BN-RPS2-1	AF181727	BO-RPS2-1	AF181727		
		BN-RPS2-2	AF181728	BR-RPS2-1	AF181730		
ACGM 26	AF056540	BN-AG-1	AF056525	BR-AG-1	AF056530		
		BN-AG-2	AF056526	BO-AG-1	AF056529		
		BN-AG-3	AF056527	BR-AG-2	AF056539		
		BN-AG-4	AF056528	BO-AG-2	AF056531		
ACGM 27	AF056541	BN-AP3-1	AF056532	BO-AP3-1	AF056536		
		BN-AP3-2	AF056533	BR-AP3-1	AF056538		
		BN-AP3-3	AF056534	BO-AP3-2	AF056542		
		BN-AP3-4	AF056535	BR-AP3-2	AF056537		
ACGM 28	AF056550	BN-LFY-1	AF056543	BO-LFY-1	AF056547		
		BN-LFY-2	AF056544	BO-LFY-2	AF056548		
		BN-LFY-3	AF056545	BR-LFY-1	–		
		BN-LFY-4	AF056546	BR-LFY-2	AF056549		
ACGM 29	X94937	BN-CONSTANS-1	AF230668			BO-CONSTANS-1	AF230670
		BN-CONSTANS-2	AF230669			BRCONSTANS-1-	AF230671
ACGM 30	U03456	BN-LUMINI-1	AF229401			BR-LUMINI-1	AF229400
		BN-LUMINI-2	–			BO-LUMINI-1	–
ACGM 40	L21154	BN-PHYA-1	AF229413	BO-PHYA-1	AF229417		
		BN-PHYA-2	AF229414, AF229415	BR-PHYA-1	AF229416		
		BN-PHYA-3	–	BR-PHYA-2	–		
ACGM 41	AF056557	BN-ADH-1	AF056551	BO-ADH-1	AF056554		
		BN-ADH-2	AF056552	BR-ADH-1	AF056556		
		BN-ADH-3	AF056553	BO-ADH-2	AF056555		

Table 3 Polymorphism within the four *B. napus* genotypes. Analysis of polymorphism at the level of exons, introns and complete sequences by pairwise comparison of the rapeseed lines. The means were calculated for all the sequences available (mean 1) or after elimination of the FAD6-2 and PHYTO-1 sequences for the Stellar-Drakkar pair and of LFY-1 for the Darmor-Yudal pair (mean 2)

Genotypes pairs	Exons						Introns						Complete sequence				
	Number of sequenced exons	Number of base pairs = le	Number of		Sum of mutation = me	me/le	Number of sequenced introns	Number of base pairs = li	Number of		Sum of mutation = mi	mi/li	Number of sequences	Percentage polymophism	Sequence length = lt	Sum of mutations = mt	mt/lt
			Substitutions	Insertions/deletions					Substitutions	Insertions/deletions							
Stellar/Yudal	73	9,186	73	5	78	0.85%	58	8,297	210	242	452	5.45%	26	73%	17,483	530	3.03%
Stellar/Yudal (except FAD6-2 and PHYTO-1)	64	8,527	40	5	45	0.53%	53	7,354	68	31	99	1.35%	24	74%	15,881	144	0.91%
Drakkar/Yudal	71	6,910	28	4	32	0.46%	50	6,972	70	41	111	1.59%	22	64%	13,882	143	1.03%
Darmor bzh/Drakkar	84	8,226	23	6	29	0.35%	59	8,421	57	34	91	1.08%	27	56%	16,647	120	0.72%
Darkkar/Stellar	94	10,964	26	6	32	0.29%	69	7,995	65	39	104	1.30%	32	41%	18,959	136	0.72%
Darmor bzh/Stellar	76	8,121	28	0	28	0.34%	55	7,191	45	36	81	1.13%	25	56%	15,312	109	0.71%
Darmor bzh/Yudal	90	11,163	24	2	26	0.23%	93	10,281	48	48	96	0.93%	35	51%	21,444	122	0.57%
Darmor bzh/Yudal (except LFY-1)	88	11,038	23	2	25	0.23%	92	9,794	32	16	48	0.49%	34	50%	20,832	73	0.35%
Mean 1	79	9,014	35	4	39	0.44%	62	8,073	80	67	148	1.83%	27	59%	17,087	186	1.10%
Mean 2	80	8,964	28	4	32	0.37%	63	7,955	56	33	89	1.16%	27	57%	10,919	121	0.74%

Observed polymorphism

The polymorphism between the four rapeseed sequences was analyzed in two ways. The easier of the two methods involved the comparison of electrophoretic mobility on non-denaturing polyacrylamide gels of PCR fragments from the different genotypes. Forty five of the 102 (45%) sequences displayed electrophoretic polymorphism between the four genotypes.

The other, more effective, method was the sequencing of PCR products for each of the rapeseed lines. However, PCR products were not systemically sequenced for *B. napus* because the amphidiploid structure of this species rendered this technique very laborious. Only 58 of the 102 genes were sequenced for more than one rapeseed genotype. We compared 22 to 35 sequences for each available pair (Table 3) and found that on average 59% of the pairs were polymorphic.

In each case, the nature of the polymorphism was analyzed precisely by noting the number of substitutions and insertions/deletions in the exons and introns for each pair of rapeseed lines (Table 3). As expected, there were many more (about four-times more) changes in intron than in exon sequences. The mean for all the sequences considered was between 0.5 and 3 bases mutated per 100 bases, depending on the pair of lines considered. Pairs of lines including the Asian line, Yudal, displayed higher levels of polymorphism. Fine analysis showed that in the case of the Darmor/Yudal pair, the Yudal sequence BN-LFY1 was very different to the equivalent sequence in the other lines. Similarly, the alleles of Yudal for sequences BN-FAD6-2 and BN-PHYTO-1 were very dif-

Table 4 Position of homologous sequences on the *B. napus* and *Arabidopsis* maps. The genetic positions in cM of the rapeseed sequences are given relative to the map produced by Lombard and Delourme (2001). The parental origin is indicated: *B. o* for *B. oleracea*, *B. r* for *B. rapa* and – for an undefined origin. Those for *Arabidopsis* are available at the TAIR web site: http://www.arabidopsis.org/

Brassica napus				*Arabidopsis thaliana*		
Name	Chromosome	Position cM	Parental origin	Name	Chromosome	Position cM
BN-FAD6-3	LG1a	41	*B.o*	FAD6	4	77
BN-RPS2-1	LG1a	63	*B.o*	RPS2	4	75
BN-FAD2-1	LG1a	110	–	FAD2	3	19
BN-ACP-4	LG1a	110	–	ACP	3	9
BN-RPS2-2	LG1b	44	*B.r*	RPS2	4	75
BN-LFY-1	LG2	2	*B.o*	LFY	5	118
BN-FAD8-1	LG2	127	–	FAD8	5	13
BN-PAL-2	LG3	19	–	ATHPAL	2	68
BN-FAD3-2	LG3	55	*B.o*	FAD3	2	56
BN-FAD7-3	LG4	54	–	FAD7	3	15
BN-ACP-1	LG4	66	*B.r*	ACP	3	9
BN-C4H-2	LG4	100	–	C4H	2	56
BN-CHS-4	LG4	135	–	CHS	5	30
BN-PHYA-2	LG5	6	*B.r*	PHYA	1	11
BN-KASIII-1	LG5	102	–	ATHKASIII	1	88
BN-ACC-3	LG6	3	–	ATHCOACAR	1	60
BN-CHIA-2	LG6	63	*B.r*	ATCHIA	5	46
BN-FAEI-2	LG17	53	*B.o*	FAE1	4	84
BN-ELI-3	LG17	56	–	EL13-1	4	95
BN-FAD-4	LG17	108	*B.o*	FAD7	3	15
BN-DELTA9-1	LG17	165	–	DELTA9	2	79
BN-PHYA-1	LG8	0	*B.o*	PHYA	1	11
BN-1.3 GLUCAN-2	LG8	77	–	BG2	3	77
BN-AP3-1	LG8	85	*B.o*	AP3	3	75
BN-ACC-2	LG9	14	–	ATHCOACAR	1	60
BN-FAEI-1	LG9	20	*B.r*	FAEI	4	84
BN-ADH-3	LG10	52	*B.o*	ADH	1	118
BN-LFY-3	LG11	102	–	LFY	5	118
BN-AG-3	LG12	31	*B.r*	AG	4	60
BN-CHIA-1	LG12	61	*B.o*	ATCHIA	5	46
BN-CFI-2	LG13	49	–	CHI	3	76
BN-AP3-3	LG14	0	*B.r*	AP3	3	75
BN-FAD3-1	LG14	25	*B.r*	FAD3	2	56
BN-CONSTANS-1	LG16	26	–	ATCONSTAN	5	32
BN-CHS-1	LG16	31	–	CHS	5	30
BN-ACP-6	LG18	8	–	ACP	3	9
BN-BOXAR-4	LG18	71	–	ATBORED	1	37
BN-DELTA9-4	LG19	13	–	DELTA9	2	79
BN-4CLa-1	LG19	63	*B.r*	4CLc	1	75
BN-CADa-1	LG19	74	*B.r*	ATCIACDE	3	26
BN-CHIT25-1	LG19	92	–	ATCHIB	3	19
BN-FAD2-2	LG19	102	–	FAD2	3	19
BN-FAD6-1	LG19	121	*B.r*	FAD6	4	77

Table 5 Conservation of genetic linkage. Analysis of the conservation of genetic distances between *B. napus* and *A. thaliana*

B. napus loci	*B. napus* chromosome	Distance (cM) in *B. napus*	*Arabidopsis* loci	*Arabidopsis* chromosome	Distance (cM) in *Arabidopsis*
BN-FAD2-1/BN-ACP-4	LG1a	0	FAD2/ACP	3	10
BN-FAD6-3/BN-RPS2-1	LG1a	22	FAD6/RPS2	4	2
BN-LFY-1/BN-FAD8-1	LG2	125	LFY/FAD8	5	105
BN-PAL-2/BN-FAD3-2	LG3	36	PAL/FAD3	2	12
BN-FAD7-3/BN-ACP-1	LG4	11	FAD7/ACP	3	6
BN-PHYA-2/BN-KASIII-1	LG5	96	PHYA/KASIII	1	77
BN-FAE1-2/BN-ELI3-3	LG717	5	FAE1/ELI3	4	9
BN-1,3 GLUCAN-2/BN-AP3-1	LG8	8	BG2/AP3	3	2
BN-CONST-1/BN-CHS-1	LG16	5	CONST/CHS	5	2
BN-CHIT25-1/BN-FAD2-2	LG19	10	ATCHIB/FAD2	3	0
BN-CADa-1/BN-CHIT25-1	LG19	18	ATCIACDE/ATCHIB	3	7
BN-CADa-1/BN-FAD2-2	LG19	28	ATCIACDE/FAD2	3	7

ferent from the alleles carried by the other lines. If these three "out-type" sequences were eliminated from the calculation, the number of mutations per 100 bases was between 0.3 and 1.

A. thaliana and *B. napus* maps

The positions of the *Arabidopsis* genes were determined in most cases by amplification with the ACGM primers on the CIC YAC library (Camilleri et al. 1998). This method has now been replaced by "*in silico*" searches on the GENBANK database.

The polymorphism observed on polyacrylamide gels was used to map 43 sequences in the *B. napus* genome. The detection of single nucleotide polymorphisms (SNPs) by means of restriction enzymes (BN-FAD3-1) or MALDI-TOF technology (BN-RPS2-1) determined the positions of a few other genes.

These sequences were widely distributed on 18 of the 19 linkage groups (Table 4). The number of mapped genes on a same chromosome varies from none (LG15) to six genes (LG19). For nine chromosomes, two genes were positioned.

A *B. oleracea* or *B. rapa* origin was attributed to most of the *B. napus* linkage groups in Table 4, based on the information generated by sequence alignment (Table 2). In most of the cases the genes found on a same chromosome had the same parental origin, except for the linkage group LG12 which presented two genes (BN-AG-3 and BN-ChiA-1) from the two parental origins.

Conservation of genetic linkage

Genes on the same linkage group in *B. napus* were homologous to genes mapped on the different *Arabidopsis* chromosomes: for example LG4, LG17 and LG19 with three or four *Arabidopsis* chromosomes (Table 4).

The number of homologous sequences mapped in *Arabidopsis* and in rapeseed is too small for full identification of the areas in which synteny is conserved. From the 45 possible gene pairs, 12 indicated a conservation of the linkage between homologous genes in the two species (Table 5). We have considered that the observed synteny between the loci LFY/FAD8 and PHYA/KASIII was found fortuitously due to the small number of Arabidopsis chromosomes, and thus we eliminated these pairs in the estimation of the average conserved distance in the two species. With the ten pairs we found that the mean distance between two linked loci was 14.3 cM (standard error 11.4) in *B. napus* and 5.7 cM (SE 4.0) in *A. thaliana* with the correlation coefficient 0.29.

Discussion

This is the first comparison of plant genomes based on amplified consensus gene markers. These data confirm the results of a previous study (Brunel et al. 1999) on six *A. thaliana* genes. We describe here 32 consensus primer pairs that amplified 37 *A. thaliana* genes and 102 homologous *B. napus* sequences. The methodology used made it possible to "sequence without cloning" gene sequences from *Arabidopsis* in the *Brassica* genome (and *vice versa*). Further, it made possible to validate the homology of a sequence to the "mother sequence", to compare the different sequences present in *B. napus* and to identify correspondences of individual sequences with sequences in one of the parental species.

It could be argued that only a fraction of the gene sequence is obtained with this methodology, but this sequence fragment is a tag that can easily be used to validate the possible cosegregation of a candidate gene with an agronomic trait of interest. Other molecular techniques such as Tail-PCR (Liu et al. 1995) could be used to complete the sequence data.

Although only limited results are currently available, this is a useful way of comparing the genetic information available at different phylogenetic levels (intra- and inter-specific, inter-generic). Direct sequencing rapidly provides the exact sequences (exons and introns) from all the protein-encoding genes common to different genomes. This methodology should greatly increase our understanding of the phylogenetic evolution of complex tribes such as the *Brassicaceae*. The consensus primer

pairs used in this study also amplified sequences from other species (*Brassica juncea* and *Raphanus sativus*, data not shown). In addition, the origin of "exotic" alleles in our restricted sample could probably be identified by studying a larger sample of *Brassica* genotypes. This would provide information about interspecific outcrossing events in natural populations.

In most cases, we observed the strict addition of the two parental genomes in *B. napus*. Neither *B. oleracea* nor *B. rapa* is a strict diploid, as half of the amplification by ACGM primers produced more than one sequence homologous to one *Arabidopsis* gene but neither seems to be more "polyploid" than the other. For one specific ACGM, the number of sequences was not strictly equivalent in *B. oleracea* and *B. rapa*, revealing differences in the degree of gene duplication, although the hypothesis that a nucleotide subtitution could prevent amplification can not be eliminated.

One to seven homologous sequences were identified in *B. napus*, but two to four homologous sequences were most frequently found. The presence of six copies for one *Arabidopsis* gene, as suggested by Lagercrantz and Lydiate (1996), does not seem to be the predominant situation. The "PCR protocol" may minimize the number of homologous sequences detected due to possible mismatches between the sequences of the targeted gene and the primer. However, the ability of the consensus primers to amplify sequences from six species of three genera of the *Brassicaceae* family is not consistent with this hypothesis as a general explanation. The presence of duplicated areas in *Arabidopsis* (Blanc et al. 2000) complicates the autoradiographs obtained for Southern blots because this technique is not precise enough to distinguish between very closed related sequences. This is likely to have resulted in the overestimation of homologous rapeseed sequences in previous papers. O'Neill and Bancroft (2000) indicated also a wide variation of the number of homologous genes (2 to 11) detected by comparative physical mapping of segments of the genome of *B. oleracea* and *A. thaliana*.

Nevertheless, it is clear that the number of homologous sequences to one *A. thaliana* "mother sequence" differs between genes in the genome of *B. napus* in a manner more complex than the term "amphidiploid" suggests. Further investigation is required to obtain information about the expression and function of this overabundance of related sequences in the evolution of the *Brassicaceae*.

We confirm that the rapeseed chromosomes are constituted as a "patchwork" of the homologous regions of different chromosomes of *Arabidopsis*, as already observed for *B. nigra* (Lagercrantz 1998) and *B. oleracea* (Lan et al. 2000). Unfortunately, due to the lack of precise information on the *A. thaliana* probes used in previous studies, we were unable to assess the correspondence between the maps of the three *Brassica* species. But we think that the correlation coefficient between the distances observed in *A. thaliana* and *B. napus* is too low to permit an easy cloning strategy by synteny information.

Both results concerning variation in the number of homologous sequences, and the large numbers of chromosome rearrangements provide a less-promising vision of an easy shuttle between *B. napus* and *A. thaliana* for the cloning of genes controlling agronomic traits. Translocations between *B. napus* genotypes (Lombard and Delourme 2001) is another element making it more difficult to compare different maps. This emphasizes the need for rapid protocols for obtaining homologous sequences from any candidate gene (or from any precise chromosomal region) from any plant species.

Intragenic polymorphism was used to map 43 of the 102 sequences studied, primarily by electrophoretic migration. However, other detection methods involving single nucleotide polymorphism (SNP) are becoming available (Schafer and Hawkins 1998; Sauer et al. 2000). Mass spectrometry was used for efficient analysis of the BN-RPS2-2 locus (Brunel, unpublished results).

The presence of intragenic polymorphisms was confirmed by sequencing of the PCR products from four lines of rapeseed. Estimates are still crude and will require refining in analyses of a larger number of lines, but the estimated value of 0.3 to 1 base mutated per 100 bases is close to the estimate of 0.1% between the Columbia and Landsberg ecotypes reported by Cho et al. (1999). The major difficulty in exploiting this type of polymorphism for genotyping is the amphidiploid structure of the *B. napus* genome, which prevents the direct sequencing of PCR products. Specific primers must be identified for each homologous sequence for the detection of single nucleotide polymorphism (SNP) in different individuals. This will be facilitated by the preliminary step described in the ACGM protocol. Homologous sequences coamplified with consensus primers have provided us with an indication of the number of homologous sequences in rapeseed. DNA fragments corresponding to these homologous sequences can then be isolated from acrylamide gels for the sequencing and sequence alignment required to identify differences.

The ACGM protocol permits the rapid and convenient transfer of information on qualitative and quantitative traits from *Arabidopsis* to other species. Its efficiency has been validated by our group in several cases of traits under mono or oligo-genic control in *B. napus* (linolenic content: Jourdren et al. 1996b; erucic content: Fourmann et al. 1998).

This new approach to the development of genetic markers by the sequencing of gene sequences makes it possible to accumulate precious information that can be used to increase our understanding of the evolution of plant genomes by chromosome rearrangements and allelic variability within species and populations. This information will be useful in the future, whatever the technology available.

Finally, one of the principal contributions of this approach to plant breeding is the possibility of using the same set of genetic markers for comparative mapping studies and the detection of QTLs. This gene mapping will certainly facilitate the comparison of information acquired from different cultivated species.

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