Arabidopsis thaliana: A source of candidate disease-resistance genes for *Brassica napus*

D. Sillito, I.A.P. Parkin, R. Mayerhofer, D.J. Lydiate, and A.G. Good

Abstract: Common structural and amino acid motifs among cloned plant disease-resistance genes (R genes), have made it possible to identify putative disease-resistance sequences based on DNA sequence identity. Mapping of such R-gene homologues will identify candidate disease-resistance loci to expedite map-based cloning strategies in complex crop genomes. *Arabidopsis thaliana* expressed sequence tags (ESTs) with homology to cloned plant R genes (R-ESTs), were mapped in both *A. thaliana* and *Brassica napus* to identify candidate R-gene loci and investigate intergenomic collinearity. *Brassica* R-gene homologous sequences were also mapped in *B. napus*. In total, 103 R-EST loci and 36 *Brassica* R-gene homologous loci were positioned on the N-fo-61-9 *B. napus* genetic map, and 48 R-EST loci positioned on the Columbia × Landsberg *A. thaliana* map. The mapped loci identified collinear regions between *Arabidopsis* and *Brassica* which had been observed in previous comparative mapping studies; the detection of syntenic genomic regions indicated that there was no apparent rapid divergence of the identified genomic regions housing the R-EST loci.

Key words: RFLP mapping, candidate R genes, R-gene homologues, genomic collinearity, *Arabidopsis* ESTs.

Résumé : Plusieurs éléments de structure et motifs d'acides aminés sont conservés chez les gènes de résistance (gènes R) clonés chez les plantes et ceci rend possible l'identification de gènes de résistance candidats par homologie de séquence. La cartographie de tels homologues de gènes R permet d'identifier de potentiels loci de résistance afin d'accélérer le clonage positionnel chez des espèces dont le génome est complexe. Des EST (étiquettes de gènes exprimés) d'*Arabidopsis thaliana* présentant de l'homologie avec des gènes R clonés (R-EST) ont été cartographiés à la fois chez *A. thaliana* et *Brassica napus* afin d'identifier des loci R candidats et d'explorer la colinéarité des génomes. Des séquences de *Brassica* homologues à des gènes R ont également été cartographiés chez *B. napus*. Au total, 103 R-EST et 36 séquences de *Brassica* homologues à des gènes R ont été positionnés sur la carte génétique N-fo-61-9 de *B. napus* et 48 R-EST ont été situés sur la carte Columbia × Landsberg d'*Arabidopsis*. Ces loci identifient des régions colinéaires chez *Arabidopsis* et *Brassica* qui avaient été observées précédemment dans le cadre d'études de cartographie comparée. La détection de régions génomiques synténiques indique qu'il n'y aurait pas eu de rapide divergence des régions génomiques portant des loci R-EST.

Mots clés : cartographie RFLP, gènes R candidats, homologues de gènes R, colinéarité génomique, EST d'*Arabidopsis*.

[Traduit par la Rédaction]

Introduction

One of the greatest challenges in plant breeding has been to introduce durable disease resistance into crop plants. Flor (1956), studying rust in flax, determined that disease resistance was the result of a "gene for gene" relationship between disease-resistance genes (R genes) in the plant host and complementary avirulence genes (Avr genes) in the pathogen. The introduction of single R genes into a crop monoculture places the pathogen population under tremendous evolutionary pressure, which generally results in the emergence of avirulence genes that are no longer recognized by the host R gene, nullifying its effect (Crute and Pink 1996). In response, the breeder must introduce a new resistance gene with specificity corresponding to an alternate Avr gene product. Plant breeders have typically utilized resistance sources from wild relatives of crop species, but the introduction of R genes from such sources necessitates lengthy backcrossing programs to remove deleterious genes concomitantly transferred with the resistance phenotype. It is now possible to identify and clone R genes, thus allowing the relatively easy introduction of resistance sources into crops using transformation techniques. Cloned genes will also facilitate pyramiding of multiple resistance genes in an elite background, in an attempt to generate more durable resistance in future cultivars.

Brassica napus is an important oilseed crop species that is prone to attack by a number of pathogens, including *Sclerotinia sclerotiorum*, *Albugo sativa*, and the most damaging worldwide, the fungus *Leptosphaeria maculans*, which

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Table 1. Genomic locations and estimated copy number of loci detected with *Arabidopsis thaliana* EST probes selected by homology to cloned disease-resistance genes (R-ESTs).

R-EST Probe[a]	Copy No. *B. napus*[b]	Mapped loci in *B. napus*[c]	Copy No. *A. thaliana*[d]	Mapped loci in *A. thaliana*[c]	Map Pos. (Chrom.)[e]
F20107/F20108	4	4–N13(a/b), N3(c), N5(eNM)	2	1–3(a)	2
H36320	6	3–N4(b), N7(eNP), N18(d)	1	1–3(a)	
H36821	6	4–N3(d), N13(aNP), N10(b)**, N19(c)**	2	1–5(c)	5
H36913	6	4-N12(aNP), N19(c), N13(b), N3(e)	1	no polymorphism	5
H37061	1	no polymorphism			
H77224	6	1–N7(a)	2	1–1(b)**	1
N65549	6	2–N11(bNP), N5(dNM)	3	2–1(b)**, 2(a)	
N65692		no polymorphism			
N95848	2	2–N3(a), N13(b)	2	1–1(a)	2
N96078	3–6	1–N13(aNP)	2	no polymorphism	4
N96307	2	2–N5(a), N15(b)	1	1–3(a)	
N96493	4–6	4–N10(a), N3(bNP), N19(d), N11(eNP)	1	1–5(a)	5
N96711	2	no polymorphism	2	1–5(a)	
N97067	6–8	3–N6(d), N7(bNP/eNP)	1–2	b(NM) unlinked	5
R29891		no polymorphism			
R30025	2	1–N9(a)	1	1–3(a)	3
R30624	7–9	3–N9(dNP), N10(bNP), N18(a)	2	2–4(b), 5(a)**	5
R64749		not R-EST			
R89998/T04109/T21150	6	5–N12(cNP), N3(gNP), N13(aNP), N10(fNP), N19(bNM)	3	2–3(b), 5(a)	5
R90150	2	1–N4(a)	2	1–2(a)	1
T04135	4	2–N6(a), N15(b)	1	1–1(a)	1
T04362/T20493	4	2–N5(cNM), N9(a)	3	2–1(b)**, 2(a)	5
T13648	6–8	3–N12(bNM), N5(aNP), N18(c)	2	2–1(a)**, 5(b)**	
T14233	6	5–N1(cNM), N11(dNM), N14(bNP)**, N4(a)**, N8(eNP)	1	1–4(a)**	4
T20671	2	1–N7(aNP)	2	2–3(a), 4(b)	
T20808	6–8	5–N3(eNP), N4(c), N7(fNP), N19(aNM/dNP)	3	2–5(a/b)**	5
T21447	2	1–N10(a)	1	no polymorphism	1
T22090	3–4	2–N6(b)**, N15(a)	2	2–1(a), 4(b)	
T41629	2–4	1–N12(bNP)	1	1–1(a)	1
T41662	1	1–N17(a)	1	1–4(a)**	4
T42294	2–4	2–N3(b), N17(aNP)	1	1–1(a)**	
T43968	6	6–N2(hNP), N14(f/bNP), N5(e), N4(a), N19(c)	3	2–4(b), 5(a)**	5
T44979	4	3–N1(a), N3(d), N11(c)	1	no polymorphism	
T45845	4	3–N14(a/b), N4(c)	1–2	1–2(a)	2
T45996	5	3–N1(d), N19(c), N18(b)	1	1–3(a)	
T46145	2	2–N6(b), N15(a)**	1	1–1(a)	1
T46379	2	2–N14(c), N7(bNP)	1	1–2(a)	2
T46721	4	2–N13(a), N8(bNM)	1	1–4(a)	4
T75662	3	3–N5(a), N9(c), N15(d)**	1	1–1(a)	5
Z17798	4	4–N2(aNP/dNM), N12(cNP/bNM)	1	no polymorphism	5
Z17993	4–6	3–N6(bNP), N15(cNP), N10(aNM)	1	1–1(a)**	5
Z18443	4	2–N6(b), N17(a)	3	2–3(a), 1(dNM)	5
Z26226	4	2–N3(a), N13(b)	1	no polymorphism	5
Z30800	5–6	3–N13(dNM), N5(cNP), N8(b)	4	4–1(a/bNP/c), 4(dNP)	
Z33873	6	3–N3(dNP), N10(a), N19(b)	1	1–5(a)	5
Z34772	1	no polymorphism	2	1–1(a)	

Note: Loci with multiple segregating RFLP bands are indicated by **.

[a]GenBank accession number for *A. thaliana* ESTs.

[b]Estimated from the number of hybridizing fragments in *Eco*RI- or *Eco*RV-digested genomic *B. napus* DNA.

[c]Locus information presented as: number of loci–linkage group (specific locus designation).

[d]Estimated from the number of hybridizing fragments from *A. thaliana* genomic DNA digested with *Cla*I, *Cfo*I, *Dra*I, *Eco*RI, *Eco*RV, *Hpa*II, or *Sca*I.

[e]*A. thaliana* chromosomal map position as determined by Botella et al. (1997).

Table 2. Copy number and linkage group assignment of *Brassica* R-gene homologous sequences.

RFLP Probe[a]	Copy No. *B. napus*[b]	Mapped loci in *B. napus*[c]
P1–3A	7	1–N1(f)
P1–3C	6	2–N3(aNP), N17(cNP)
P1–7B	7–10	2–N8(dNM), N3(cNM)
P1–9B	7–10	7–N9(a), N7(cNP), N8(bNP), N6(hNP), N13(fNM), N17(d), N14(e)
P1–14A	6	1–N11(eNM)
P1–14C	10	4–N9(j), N8(kNM), N13(cNM), N17(e)
P1–15B	8	5–N9(d), N8(f), N13(bNM), N17(c), N14(eNP)
P1–17B	4	2–N3(a), N13(b)
P1–19C	4	3–N1(d), N8(b), N11(e)
P1–31A	5	2–N9(bNM), N10(aNM)
P2–13B	6	4–N1(d), N8(fNP), N6(b), N11(c)
H-3	1	1–N10
HS1	2	2–N14(a), N4(b)

[a]P1– and P2– probes are cloned *B. napus* sequences amplified via PCR using primers designed from the *A. thaliana RPS2* resistance gene (Joyeux et al. 1999); H-3 is a *Brassica Pto* homologue; and HS1 is a *Brassica HS1^{pro-1}* homologue.
[b]Estimated from the number of hybridizing fragments in *Eco*RI- or *Eco*RV-digested genomic DNA.
[c]Locus information presented as: number of loci–linkage group (specific locus designation).

causes blackleg (Gugel and Petrie 1992). A number of blackleg resistance sources have been identified from *B. napus*, and some have been genetically positioned within the genome (Dion et al. 1995; Ferreira et al. 1995*a*; Mayerhofer et al. 1997), however none have been cloned. In fact, no resistance genes have yet been cloned from the amphidiploid *B. napus* or its diploid progenitors, probably due to the technical difficulties imposed by a relatively large and highly duplicated genome. In comparison, a rapidly growing set of disease-resistance genes has been cloned from the model dicot *Arabidopsis thaliana*, which is also in the family Brassicaceae (Cruciferae) (Kunkel 1996; Warren et al. 1999). A number of researchers have shown regions of conserved gene content and gene order between *A. thaliana* and *B. napus* (Cavell et al. 1998; Scheffler et al. 1997) and it has been suggested that cloned genes from *A. thaliana* may have similar functions in *B. napus* (Fray et al. 1997; Lagercrantz et al. 1996). For example, the *Arabidopsis* fatty acid elongase gene *FAE1* has been mapped coincidentally with a locus controlling variation in erucic acid content in *B. napus* (Barret et al. 1998). Cloned disease-resistance genes from wide ranging taxa and against various pathogenic organisms (i.e., bacteria, fungi, viruses, nematodes) have been shown to share structural or amino acid motifs such as leucine-rich repeat regions (LRRs), nucleotide binding sites (NBS), leucine zippers (LZ), or protein kinase domains (Bent 1996) allowing classes of these genes to be defined (Jones and Jones 1997). Such conservation of protein structure suggests commonality in disease-resistance pathways among angiosperms, and the mapping of *Arabidopsis* R genes in *B. napus* may prove to be an efficient method for identifying possible functional resistance gene candidates for some of the more devastating *Brassica* diseases.

To identify large numbers of *A. thaliana* sequences with similarity to disease-resistance genes, Botella et al. (1997) searched the *Arabidopsis* expressed sequence tag (EST) database for sequences homologous to any of 8 cloned resistance genes *Cf-9*, *Xa21*, *RPM1*, *RPS2*, *L6*, *N*, *RPP5*, and *Pto* (R-ESTs). Although no cDNA libraries have been constructed from pathogen-infected tissues, R genes are known to be expressed in uninfected tissues, and are well represented in the EST database (Botella et al. 1997). Ninety-four *A. thaliana* EST sequences representing 62 nonredundant clones were identified from the R-gene homology search, and 42 were mapped in *A. thaliana* by assignment to physically mapped yeast artificial chromosomes (YACs) using PCR, or by molecular marker segregation in *A. thaliana* recombinant inbred lines (RILs).

In this research, we mapped 44 of 50 R-ESTs identified by Botella et al. (1997) as restriction fragment length polymorphism (RFLP) markers in both *A. thaliana* and *B. napus.* We also mapped 11 of 22 cloned *Brassica* R-gene homologues amplified with primers corresponding to the NBS and LRR regions of the *RPS2* gene in *B. napus* (Joyeux et al. 1999), and 2 *Brassica* homologues of the resistance genes *Pto* and *HS1^{pro-1}*. The combined *B. napus* and *A. thaliana* R-EST map information was used to investigate possible clustering and collinearity of R-gene homologues between the two genomes, and to infer candidate disease-resistance loci in economically valuable *B. napus*.

Materials and methods

Parental plant material

The *B. napus* mapping population consisted of 30 doubled-haploid (DH) lines of the highly polymorphic N-fo-61-9 population derived from a cross between a DH British winter cultivar (*B. napus* cv. N-o-9) and a newly resynthesized *B. napus* (SYN1) line (Parkin et al. 1995). The *Arabidopsis thaliana* mapping population consisted of 30 RILs derived from a cross between the Columbia and Landsberg ecotypes (Lister and Dean 1993).

DNA extraction and Southern hybridization

Genomic DNA was extracted from 0.5 g of freeze-dried leaf tissue as described by Sharpe et al. (1995). For the mapping populations, 15 µg of genomic *B. napus* DNA was digested with either *Eco*RI or *Eco*RV, and 4 µg of genomic *A. thaliana* DNA with one of *Cla*I, *Cfo*I, *Dra*I, *Eco*RI, *Eco*RV, *Hpa*II, or *Sca*I. Gel electrophoresis, alkaline transfer, and Southern hybridization were carried out according to Sharpe et al. (1995). Filters were washed twice in 2×

SSC, 0.1% SDS at 65°C, and once at room temperature, and placed against film (Kodak X-OMAT AR) for autoradiography at –80°C.

RFLP probes

R-EST probes used for RFLP mapping were a subset of ESTs selected by Botella et al. (1997) and are listed in Table 1. Probes were obtained from the *Arabidopsis* Biological Resource Centre in Ohio, and from Drs. P. Dao and G. Philipps of Laboratoire de Gênêtique Moleculaire des Plantes, Grenoble, France. *Brassica* R-gene homologous probes were cloned from *B. napus* cv. Quantum using primers homologous to LRR and NBS protein motifs (Joyeux et al. 1999) (Table 2). *B. napus Pto* (H-3) and *HS1^{pro-1}* (HS1) homologues were provided by B. Kennedy, University of Alberta and Dr. D. Hegedus, Agriculture and Agri-Food Canada, Saskatoon Research Centre, respectively (Table 2).

Linkage analysis

RFLP loci detected by the R-EST probes were assigned to the 19 *B. napus* linkage groups (Parkin et al. 1995) and the five linkage groups of *A. thaliana* (Chang et al. 1988; Nam et al. 1989) using MAPMAKER/EXP v. 3.0 (Lander et al. 1987), LOD score 3.0.

Results

Genetic mapping of *Arabidopsis* R-ESTs

Of 50 *Arabidopsis* R-ESTs chosen as RFLP probes: five (H37061, N65692, N96711, R29891, and Z34772) hybridized to *B. napus* genomic DNA but detected no polymorphisms between the parental lines, 'N-o-9' and SYN1; one (R64749) proved to be an incorrect clone (leaving a total of 49 R-ESTs); and four (F20108, T04109, T21150, and T20493) proved to be redundant since they detected RFLP patterns identical to probes previously used in the study (Table 1). Botella et al. (1997) had previously indicated that F20107 and F20108 were from the same clone and had placed T21150 in a contig with R89998, however T04109 and T20493 were considered to be non-redundant clones. Redundancy is a common problem when dealing with the short error-prone sequences of ESTs, however resistance genes frequently show tandem duplications, some of which are functional, and it is possible that the redundancy found within these clones is a reflection of this type of gene organization.

Forty-four of 49 R-ESTs were mapped in the N-fo-61-9 *B. napus* population. R-ESTs were mapped to every linkage group except N16 (no markers could be mapped to N16 due to monosomy in the 61–9 F_1 plant (Parkin et al. 1995); and ranged in number from 3 (N1, N2, N8, and N17) to 11 (N3) loci per linkage group. In total, 103 loci detected by the R-ESTs were placed on the *B. napus* genetic linkage map (Fig. 1).

In *A. thaliana*, 38 of 46 R-ESTs were mapped to 48 loci positioned on all 5 chromosomes, with loci numbers ranging from 5 (chromosome 2) to 17 (chromosome 1). For most R-ESTs, the chromosomal location determined by Botella et al. (1997) was confirmed by our mapping, however for seven of the R-ESTs we did not map any loci to the previously determined linkage group. In the case of F20107, N95848, R90150, T04362, and Z18443, we had unmapped loci that could potentially correspond to the previously mapped position; but for each of T75662 and Z17993, we only detected one locus on a different linkage group than was determined by Botella et al. (1997)(Table 1). This was not unexpected, as it was acknowledged by Botella et al. (1997) that some map positions may be ambiguous due to high similarity among members of multigene families, or the presence of chimeric YAC clones. We mapped the majority of R-ESTs in *A. thaliana* to more that one location, in contrast to the findings of Botella et al. (1997), where only one locus was mapped per R-EST probe (Fig. 2). This latter discrepancy is as a result of Botella et al. (1997) mapping many of the probes by PCR-based screening of the *Arabidopsis* CIC (CEPH/INRA/CNRS) YAC library. Many duplicate loci that can be detected by Southern hybridiation would not be observed by amplification from primers specific to individual EST probes.

Based on the minimum number of bands identified with each enzyme, the R-ESTs had estimated copy numbers ranging from 1 to >9 in *B. napus*, and from 1–4 in *A. thaliana* (Table 1). Many probes detected loci that could not be mapped due to lack of polymorphism between the parental lines or difficulty in resolving the banding patterns on autoradiographs (data not shown).

Genetic mapping of *Brassica* R-gene homologues

Eleven cloned R-gene like sequences from *B. napus* detected 33 loci on 11 linkage groups, ranging from 1 (N7 and N10) to 6 (N8) loci per linkage group. Many of the clones isolated by Joyeux et al. (1999) produced identical banding patterns in Southern hybridization, and were assumed to be from redundant sequences but it could be that these sequences represent tandem copies at the same locus. Joyeux et al. (1999) mapped only 14 loci with 6 polymorphic RFLP markers in the Major × Stellar *B. napus* mapping population, whereas we were able to map 33 loci with 11 markers in the highly polymorphic N-fo-61-9 population (Table 2). Copy numbers of these *Brassica* R-gene-like sequences were generally high, ranging from 4 to 10, however, the *Brassica* homologue of the tomato *Pto* gene conferring resistance to *Pseudomonas syringae* (H-3), and the *Brassica* homologue of the sugar beet nematode resistance gene, *Hs1^{pro-1}* (HS1) detected one and two loci, respectively.

Collinearity between the *Arabidopsis* and *Brassica* genomes detected by the R-EST loci

As expected, because of the dispersed nature of the mapped R-EST loci considered in this study only limited regions of collinearity could be detected. R-EST loci mapped on *A. thaliana* chromosomes 1, 3, 4, and 5 detected collinear regions on multiple *B. napus* linkage groups; consistent with the hypothesized evolution of *Brassica* by duplication and rearrangement of an ancestral genome similar in size to that of *A. thaliana* (Lagercrantz 1998). The patterns of collinearity uncovered for chromosomes 3, 4, and 5 were confined to previously defined blocks of synteny described in Scheffler et al. (1997), Cavell et al. (1998), and Osborn et al. (1997), respectively. In this study, *A. thaliana* chromosome 1 had the highest number of mapped R-EST loci, making collinear relationships more apparent (Fig. 3). The upper part of chromosome 1 was duplicated on N6 and N15, and a lower region of *Arabidopsis* chromosome 1 was duplicated on N5 and N15. Distances between loci on collinear chromosomal stretches varied considerably. For example, the group of R-

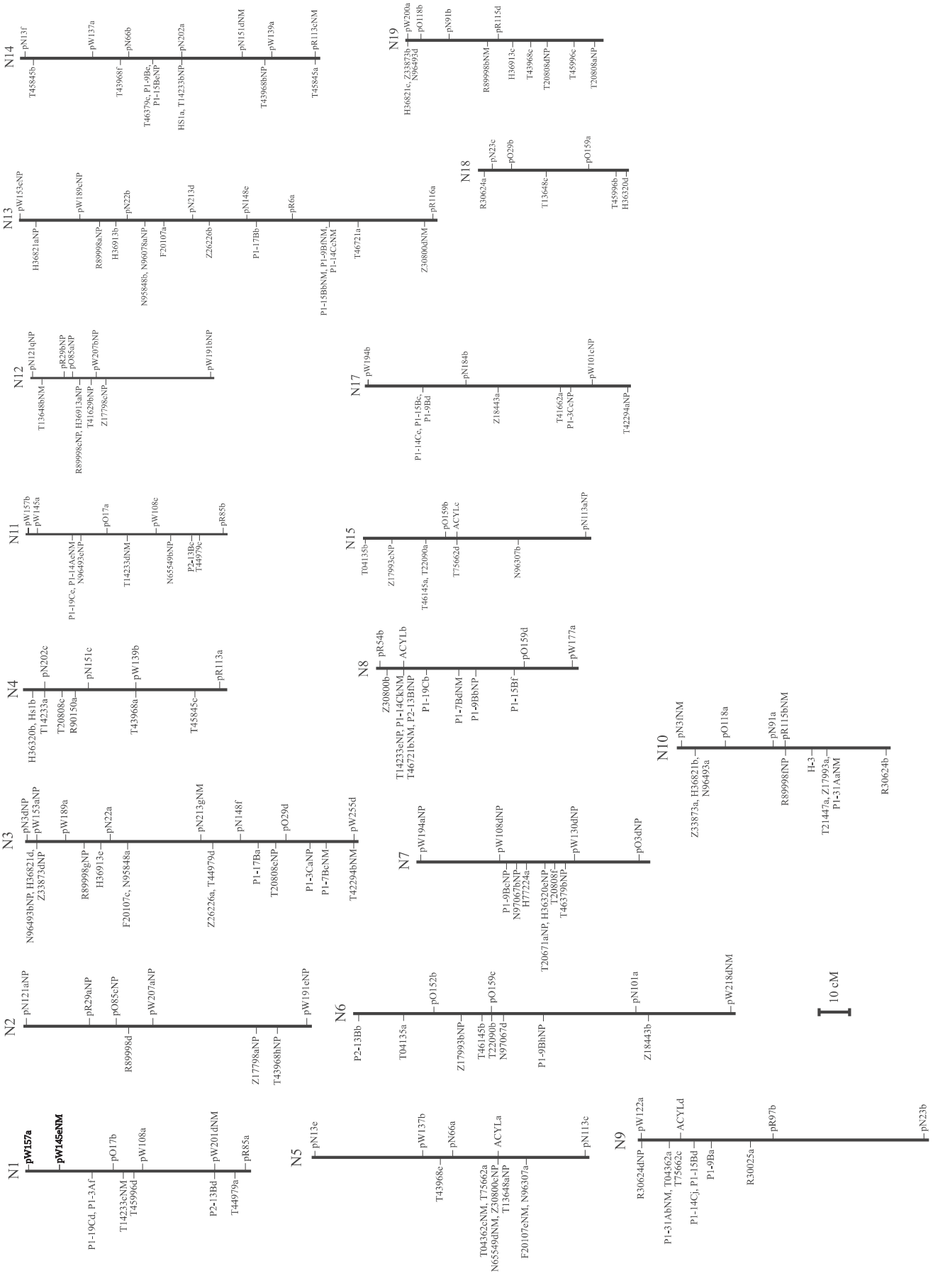


Fig. 1. Genetic linkage map based on 30 doubled-haploid (DH) lines of the N-fo-61-9 *Brassica napus* population, showing disease-resistance gene-related sequences mapped in this study. Vertical bars represent linkage groups N1–N19, with selected framework markers from Parkin et al. (1995) on the right, and resistance-related markers on the left. Lower-case letters following the locus designate multiple loci detected with the same RFLP probe. NP or NM after a locus name signify that only alleles from the 'N-o-9' or SYN1 parent, respectively, could be scored. R-ESTs are designated by GenBank accession numbers (e.g., R89998), random *Brassica* R-gene homologues by names beginning with P1- or P2- (e.g., P1-17B), the *Brassica Pto* homologue by H-3, and the *Brassica HS1^{pro-1}* homologue by HS1. Map distances between markers are drawn to scale.

Fig. 2. Genetic linkage map of *Arabidopsis thaliana* based on segregation in 30 RILs of the Lister and Dean (1993) Columbia × Landsberg mapping population. The 5 chromosomes are represented by vertical bars, with markers on the right and map distances indicated on the left. R-ESTs are designated by boldface GenBank accession numbers. Major recognition complex regions (MRC) (Holub 1997) have been found to contain multiple disease-resistance loci and are indicated by vertical bars to the left of chromosomes 1, 4, and 5.

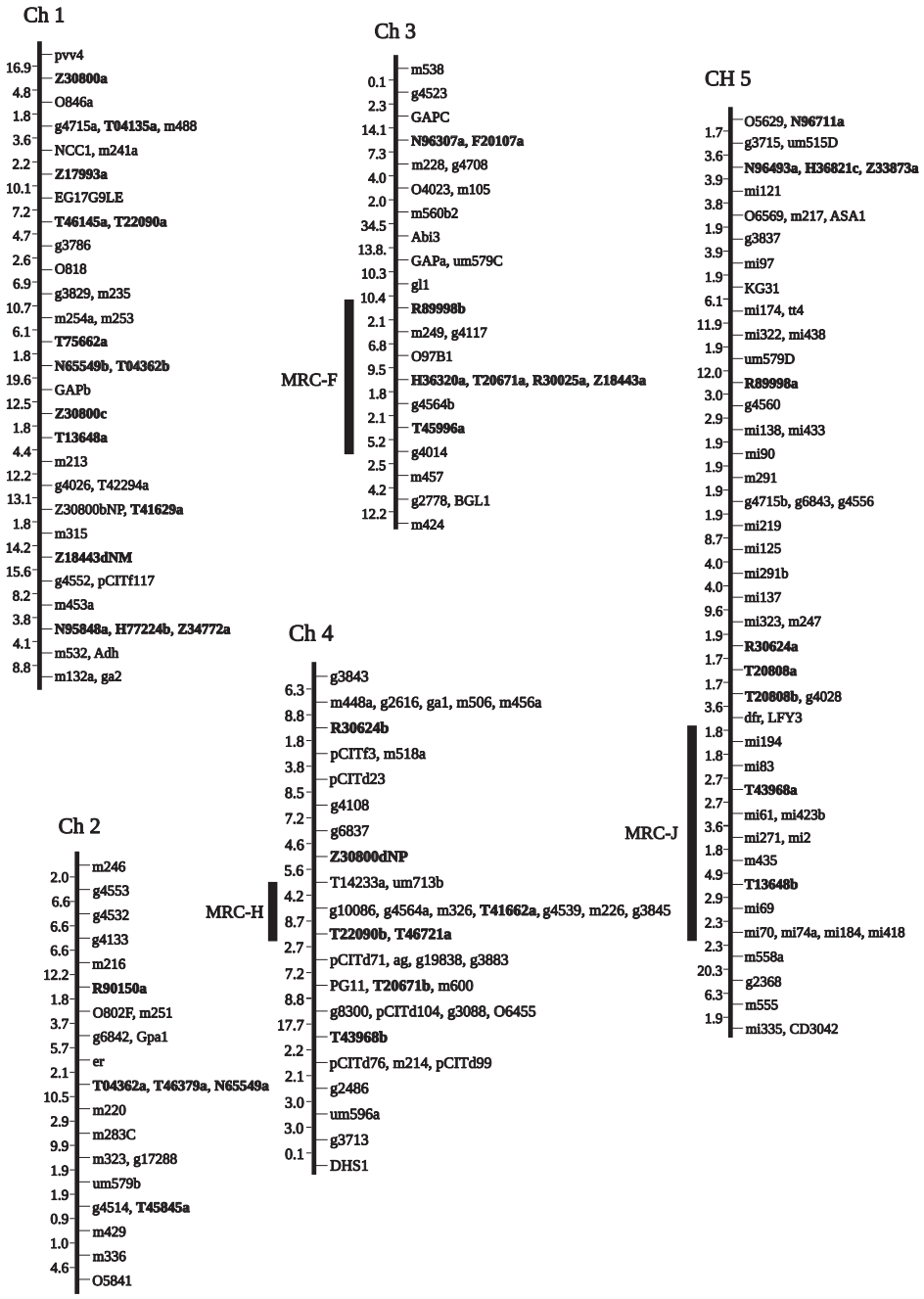
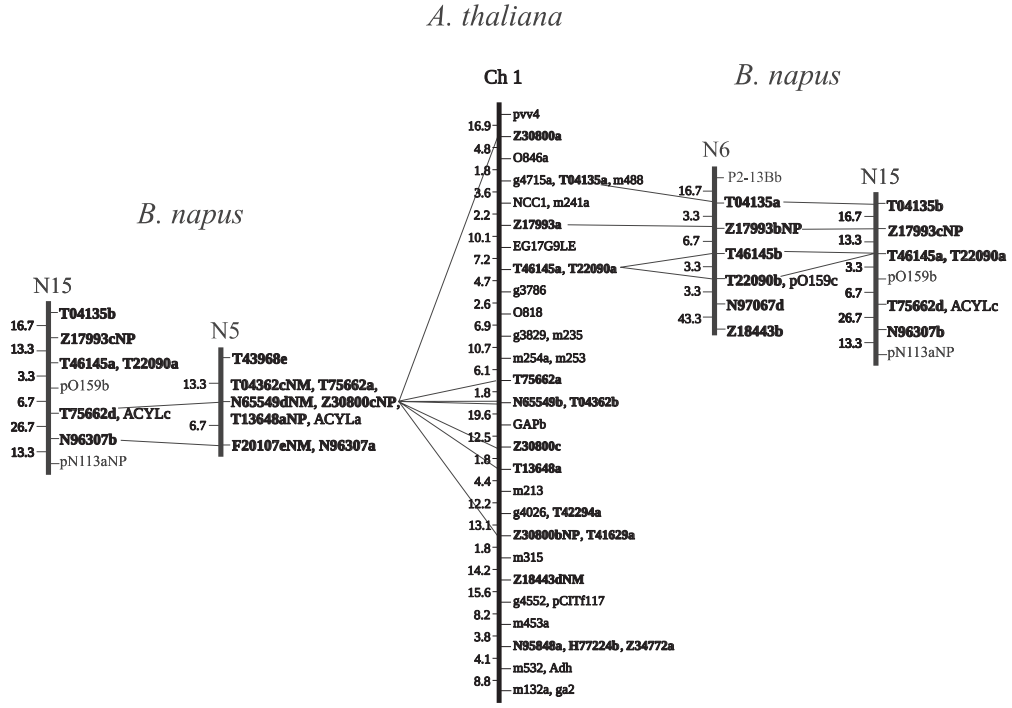


Fig. 3. Collinearity of R-ESTs between *Arabidopsis thaliana* chromosome 1 and *Brassica napus* linkage groups N5, N6, and N15. Markers are given to the right of the linkage groups, and map distances in cM to the left. *B. napus* linkage groups are condensed for clarity, and R-ESTs are printed in bold face. Thin diagonal lines denote collinear locations of R-EST loci between the two genomes. The top half of N15 is known to be homoeologous to a region on N6, and the lower half to a region on N5 (Parkin et al. 1995).



EST loci coincident on N5 (*T04362cNM*, *T75662a*, *N65549dNM*, *Z30800cNP*, and *T13648aNP*) are each separated by a number of recombination events on *A. thaliana* chromosome 1. Markers detecting loci on A. *thaliana* chromosome 1, and *B. napus* N6 and N15 show more conserved spacing. This region of collinearity between *A. thaliana* and *B. napus* shows a characteristic pattern, which is as a result of the duplicated nature of the A genome (derived from *B. rapa*) and C genome (derived from *B. oleracea*) within *B. napus*. The top half of the C-genome linkage group N15 is homoeologous with a region of the A-genome linkage group N6, and the bottom half of the C-genome linkage group N15 is homoeologous to a region on linkage group N5 of the A genome (Parkin et al. 1995). Only 4 R-ESTs mapped to chromosome 2, and showed no clear collinearity to any specific regions of the *B. napus* genome.

Discussion

In this study, 44 *Arabidopsis* ESTs that show significant homology to previously cloned disease-resistance loci were mapped in the amphidiploid genome of *B. napus*. The loci detected by the R-ESTs were relatively evenly distributed over 18 linkage groups with apparent clustering only on N5, N7, and N8 (Fig. 1). Botella et al. (1997) had previously positioned loci corresponding to a number of the R-ESTs on the *A. thaliana* genome, predominantly by using PCR-based screening of the CIC YAC library. In this study we positioned an additional 17 loci in *A. thaliana* corresponding to 10 previously unmapped R-EST clones, using RFLP mapping in a recombinant inbred population (Lister and Dean 1993) (Table 1). It was apparent from our study that a number of the R-EST clones were present in multiple copies in the *Arabidopsis* genome; additionally, we positioned 12 duplicate loci for 11 previously mapped R-ESTs.

As previously observed in *Arabidopsis*, duplicate loci were found on separate chromosomes, or distantly spaced on the same chromosome (McGrath et al. 1993) with the exception of T20808, which detected two loci (*T20808a* and *T20808b*) on *A. thaliana* chromosome 5 separated by only one recombination event. Of the five *B. napus* loci that could be positioned using T20808, two were positioned on N19. It is possible that these two loci are members of a tandemly repeated gene family in *A. thaliana* (McGrath et al. 1993) and that chromosomal rearrangement of a common ancestral chromosome has further separated these two loci in the *B. napus* genome.

As previously noted by Botella et al. (1997) a number of R-EST loci in *A. thaliana* (12 of a total 52 loci: 23%) appear to be present as tandemly repeated sequences, based on the presence of multiple co-segregating bands (Table 1). Multiple tandem repeat sequences have been observed in many well-characterized disease-resistance loci such as *Cf4* (Thomas et al. 1997) and *Cf9* (Jones et al. 1994) from tomato, *M* from flax (Anderson et al. 1997), *N* from tobacco (Whitham et al. 1994), and *Xa21* from rice (Song et al. 1995). It has been proposed that tandem gene repeats, caused by unequal crossing-over between sister chromatids or homologous chromosomes in meiosis (Ohno 1970) is a mechanism by which new disease-resistance specificities evolve (Bennetzen and Hulbert 1992; Song et al. 1997). Although only two restriction enzymes were used to analyze the *B. napus* genomic

DNA, very few of the detected loci (7 of a total 103 loci: 7%) showed co-segregating bands. Interestingly, T41662, which Botella et al. (1997) indicated as a putative EST for the downy mildew disease-resistance gene *RPP5*, only detected a single locus in *B. napus*, inherited from *B. oleracea*, with a single band detected in each parent, 9 and 15 kb in length. *RPP5* is known to be part of a resistance gene cluster in *A. thaliana*, with 4 linked homologues present in the Landsberg ecotype spanning a distance of 50 kb (Parker et al. 1997). It would be anticipated that if a similar organization was found in *B. napus* at the *RPP5* locus, numerous co-segregating bands, or a single band of high molecular weight would be observed in the mapping population. Recent work on the organization of the *A. thaliana RPM1* locus in *B. napus* has shown there are six genomic regions homologous to the *RPM1* region present in the *B. napus* genome, but in four of these regions, the *RPM1* gene copy has been deleted (Grant et al. 1998). It may be that the deletion of duplicate gene copies is a common occurrence in the evolution of the *Brassica* diploid genomes, and that certain genes place an unnecessary genetic burden on the survival of the plant. Only as more genomic regions are compared at the physical level between *A. thaliana* and *Brassica* species will it be possible to distinguish between deletion of a gene, or extensive divergence of the DNA nucleotide sequence.

In contrast to cereals, in which has been suggested that there has been rapid reorganization of resistance gene homologues over evolutionary time (Leister et al. 1998), we found no evidence of such a predilection in the evolution of *B. napus* and *A. thaliana* from a common Cruciferae ancestor. Duplicate loci detected by each of the R-EST probes mapped to homologous regions in the A and C genomes and the limited regions of collinearity between *Arabidopsis* and *Brassica* which could be observed with such a dispersed set of probes showed conserved gene content and gene order.

Only one copy of Z17993 was detected in the *A. thaliana* genome, which we mapped to the top of chromosome 1 (mapped to chromosome 5 by Botella et al. 1997). Previously, Z17993 was found to co-segregate with *RPS5* on chromosome 1 in 97 RILs (Warren et al. 1998) supporting our map position, and was used to clone the *RPS5* gene from a BAC (bacterial artificial chromosome) library. The hybridization pattern of Z17993 suggests it is present as a tandem repeat, which is consistent with the genomic organization of the *RPS5* locus (Aarts et al. 1998). It is encouraging that a R-EST was used successfully to clone a functional R gene, thus demonstrating the plausibility of locating as yet unidentified R genes coincident with other R-EST loci.

If as suggested by a number of researchers (Aarts et al. 1998; Botella et al. 1997; Kanazin et al. 1996; Leister et al. 1996; Spielmeyer et al. 1998; Yu et al. 1996), DNA sequences identified via homology to conserved R-gene motifs are likely to represent disease-resistance genes, then the present study will provide a useful resource for *Brassica* researchers who are attempting to clone such genes. Only a small number of disease-resistance loci have been placed on the *B. napus* genome (Dion et al. 1995; Ferreira et al. 1995*a*; Ferreira et al. 1995*b*; Mayerhofer et al. 1997) and these will be integrated into the present study. If *Arabidopsis* R-ESTs prove ineffective in directly identifying novel disease-resistance candidates, they will at least suggest homologous regions of the *A. thaliana* genome, thus providing access to the genomic sequence, markers, and physical libraries which are already available from the *Arabidopsis* community (http://genome-www.stanford.edu/Arabidopsis/).

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