Physical Mapping in a Triplicated Genome: Mapping the Downy Mildew Resistance Locus Pp523 in Brassica oleracea L.

Jorge D. Carlier,* Claudia S. Alabaça,* Nelson H. Sousa,* Paula S. Coelho,† António A. Monteiro,§ Andrew H. Paterson,‡ and José M. Leitão*,1

*Center for Biodiversity, Functional & Integrative Genomics (BioFIG), FCT, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal, †Instituto Nacional de Recursos Biológicos, 2780-505 Oeiras, Portugal, ‡Instituto Superior de Agronomia, Universidade Técnica de Lisboa, 1349-017 Lisbon, Portugal, and §Plant Genome Mapping Laboratory, Departments of Crop and Soil Sciences, Plant Biology, and Genetics, University of Georgia, Athens, Georgia 30602, USA

ABSTRACT We describe the construction of a BAC contig and identification of a minimal tiling path that encompass the dominant and monogenically inherited downy mildew resistance locus Pp523 of Brassica oleracea L. The selection of BAC clones for construction of the physical map was carried out by screening gridded BAC libraries with DNA overgo probes derived from both genetically mapped DNA markers flanking the locus of interest and BAC-end sequences that align to Arabidopsis thaliana sequences within the previously identified syntenic region. The selected BAC clones consistently mapped to three different genomic regions of B. oleracea. Although 83 BAC clones were accurately mapped within a \(~4.6\) cM region surrounding the downy mildew resistance locus Pp523, a subset of 33 BAC clones mapped to another region on chromosome C8 that was \(~60\) cM away from the resistance gene, and a subset of 63 BAC clones mapped to chromosome C5. These results reflect the tripllication of the Brassica genomes since their divergence from a common ancestor shared with A. thaliana, and they are consonant with recent analyses of the C genome of Brassica napus. The assembly of a minimal tiling path constituted by 13 (BoT01) BAC clones that span the Pp523 locus sets the stage for map-based cloning of this resistance gene.

KEYWORDS genetic resistance, plant disease resistance, map-based cloning, BAC contig, genome triplication

Downy mildew caused by the oomycete Hyaloperonospora brassicae (Gäum.) (Göker et al. 2003) affects Brassica oleracea L. plants from seedlings in nurseries to adult plants in the field, reducing yield and severely compromising the quality of the marketable product. For some specific genotypes and environmental conditions, such as the Romanesco-type cauliflower in Brittany, losses due to this disease can even be total (Monot et al. 2010).

One of the most effective, low-cost, and ecologically benign methods for plant disease control is the use of genetically resistant plants. For downy mildew, several sources of genetic resistance have been identified at seedling and adult plant stages of B. oleracea (Natti and Atkin 1960; Natti et al. 1967; Dickson and Petzoldt 1993; Hoser-Krauze et al. 1995; Mahajan et al. 1995; Coelho et al. 1998; Jensen et al. 1999).

However, resistance to downy mildew in these two plant developmental stages is apparently determined by different genetic systems: plants that exhibit resistance at the cotyledonary phase can be susceptible at the adult phase and vice versa (Monteiro et al. 2005).

During the last few years, there were some advances in the genetic study of the inheritance of downy mildew resistance and in the isolation and cloning of resistance genes in Brassica species. One locus conferring downy mildew resistance at the cotyledon stage in broccoli (Brassica oleracea convar. italic) was genetically mapped by...
Giovannelli et al. (2002) and located in close linkage to the glucosinolate pathway gene BoGsl-elong on a dense map of R. oleracea (Gao et al. 2007). A second downy mildew resistance gene at seedling stage was recently mapped in Chinese cabbage (Brassica rapa ssp. pekinensis) (Yu et al. 2009).

A dominant and monogenically inherited resistance locus expressed at the adult plant stage was identified in broccoli by Coelho et al. (1998) and named Pp523 (after a pathogen strain). This locus was later located on a new genetic map of RAPD and AFLP markers (Farinhó et al. 2004) within a linkage group assigned to the B. oleracea chromosome C8 (Carlter et al. 2011). Five DNA markers that defined a genomic region of 8.5 cM encompassing this resistance locus were then cloned, sequenced, and remapped as SCAR and CAPS markers. BLAST queries (www.ncbi.nlm.nih.gov/blast) identified a genomic region syntenic to this B. oleracea genome segment at the extremity of the top arm of Arabidopsis thaliana L. chromosome 1 (Farinhó et al. 2007).

Map-based, or positional, cloning is a common strategy for isolation of genes responsible for phenotypic differences. This strategy was used for the isolation of most of the >100 reference R-Genes so far included in the Plant Resistance Genes database (http://prgdbs.cbm.fvg.it); Sanseverino et al. 2010). Map-based cloning, with specific variations, was also in the central procedure used for the isolation of the A. thaliana genes RPP5 (Parker et al. 1997), RPP8 (McDowell et al. 1998), RPP1 (Botella et al., 1998), RPP4 (Van Der Biezen et al. 2002), and RPP2A/RPP2B (Sinapidou et al. 2004), the single downy mildew resistance genes so far isolated in the Brassicaceae family.

One of the major steps in map-based cloning is the physical identification of the genomic region where the gene is located. For genomes still not fully sequenced, this implies the physical mapping of the gene of interest via construction of a contig of large insert DNA clones, usually BACs. Here we report the construction of a physical map of a genomic region of 2.9 cM that encompasses the downy mildew resistance locus Pp523 in B. oleracea, carried out by exploiting the conserved synteny between B. oleracea and A. thaliana (Farinhó et al. 2007). One major obstacle to overcome was the triplicated nature of B. oleracea genome (O’Neill and Bancroft 2000; Lysak et al. 2005; Town et al. 2006).

MATERIAL AND METHODS

Plant material and DNA marker analyses

The B. oleracea mapping population (163 F2 plants), the evaluation of plant response to downy mildew, and the procedures for plant DNA extraction and molecular marker analyses have been previously described (Coelho and Monteiro 2003; Farinhó et al. 2004, 2007).

BAC selection by overgo hybridization

Two gridded B. oleracea BAC libraries (BoT01 and BoCig) constructed at the Plant Genome Mapping Laboratory, University of Georgia, were used for identification of BAC clones located at the genomic region that spans the Pp523 locus.

Overgo probes hybridization analysis was carried out for markers OPK17_980, SCR15, SCJ19/Pagl, and SCAFB1/Bful, which define a 4.8 cM genomic region encompassing the Pp523 locus (Farinhó et al. 2007), and for 28 A. thaliana sequences (At1g01090 to At1g07360; Figure 1 and File S1) within the syntetic region defined by the most external B. oleracea markers OPK17_980 (At1g01220) and SCAFB1/Bful (At1g07420). Sequences of 40 bp were selected within the DNA-marker sequences for design of 24 bp forward and reverse overgo primers, which shared an overlapping terminal sequence of 8 bp. Two overgo probes were designed for each marker sequence so that the forward primer of the first overgo and the reverse primer of the second overgo could generate a PCR product for confirmation of hybridizing BACs.

Overgo probes labeling was performed at 37°C for 2 hr in a total volume of 15 µl containing 0.0067 nM forward and reverse primers denatured at 94°C for 5 min and cooled on ice, 1 µg BSA, 2.5 U of Taq polymerase, 1 µl of [α-32P]dATP (6000 Ci/mmol) (MP Biomedicals), 1 µl of [α-32P]dCTP (6000 Ci/mmol) (MP Biomedicals), and 3 µl OLB [oligo-labeling buffer without dATP or dCTP, and random hexamers (Ross et al. 1999)]. The labeled probes were filtered through Sephadex minicolumns to remove the unincorporated radioactive nucleotides.

Nylon membranes separated with a nylon mesh were incubated at 55°C for 2 hr in a hybridization oven at 4.5 RPM in hybridization buffer [0.5 M sodium phosphate (pH 7.2), 7% (w/v) SDS, 1 mM EDTA, and 0.01% (w/v) BSA]. Radioactive overgo probes were hybridized at 55°C for 18 hr, and membranes were washed thrice at 55°C for 30 min with constant shaking, successively in buffer A [1x SSPE, 1% (w/v) SDS], buffer B [0.5 x SSPE, 1% (w/v) SDS], and again in buffer A. Membranes were blot-dried with filter paper, placed between two sheets of cellophane paper, and autoradiographed using two intensifying screens (L-Plus; Optonix) on X-ray film (Blue Medical, Source One) for 2 weeks at −80°C.

The hits on X-ray films were scored manually using grid overlays that were scanned and read by the software ABBYY FineReader 5.0. The hit scores were manually corrected and converted to individual BAC clone addresses using the BACeater software (http://bacman.sourceforge.net/program/BACeater.html).

The plasmid DNA of the selected BAC clones were isolated, the BAC ends were sequenced, and the sequences were submitted to GenBank.

BAC fingerprinting (restriction analysis)

Plasmid DNA was isolated from BAC clones using a standard alkaline-lysis protocol and digested with 40 U of HindIII for 4.5 hr. The digestion products were run on 1% agarose gel electrophoresis for 16 hr at 95 V. The gel images were analyzed with IMAGE (Sulston et al. 1989), and the overlapping contigs were assembled using the software FPC V 4.7 and a cutoff E-value of e−7 (Soderlund et al. 2000).

Selection of additional BAC clones

Two-hundred thirty additional B. oleracea BAC clones were selected in silico by exploiting the B. oleracea/A. thaliana syntenic relationship at the genomic region of the locus Pp523. The search for BAC-end sequences (BoT01 BAC library) exhibiting high level of similarity to A. thaliana sequences was performed using the Brassica oleracea BLAST search at the JCVI Institute (http://blast.jcvi.org/er-blast/index.cgi?project=bog1) against 5000 nucleotides sequences consecutively retrieved from the A. thaliana chromosome 1 between At1g01770 and At1g07200 [Arabidopsis Information Resource (TAIR), www.arabidopsis.org/]. Sequence similarities with E-values greater than 0.001 were assumed nonsignificant (Table 1).

Genetic and physical mapping of BAC clones

Specific primers were designed to convert BAC-end sequences into sequence-tagged site (STS) markers (see File S1). Polymorphic (BAC-end-derived) STS markers were genetically mapped using the
JoinMap 3.0 software (Van Ooijen and Voorrips 2001) set for the Kosambi function. The same software was used for drawing the linkage groups. Both polymorphic and monomorphic STS markers were used to establish and stabilize the physical map establishing the BAC-to-BAC ligation by PCR. The PCR products of the anchorage points between the BAC clones of the minimal tiling path were sequenced to confirm their similarity to the original BAC-end sequence.

RESULTS

The screening of BoT01 and BoCig BAC libraries resulted in the identification of 58 BoT01 BAC clones and 12 BoCig BAC clones (Figure 1), putatively surrounding the downy mildew resistance gene.

The fingerprinting (restriction) analysis of this set of BAC clones allowed their grouping into 11 small groups of at least two overlapping clones; nine BAC clones remained ungrouped (Figure 1). The BAC clones were assembled into a putative contig following the linear order of the Arabidopsis loci used to design the overgo probes (Figure 1).

Excluding the cases of absence of significant similarity and of similarity to transposable element-like sequences, the order of the end sequences of these BAC clones appeared collinear to the A. thaliana genome (Table 1). However, genetic mapping associated the BAC clones in the second genomic region (in chromosome C8) and in the third genomic region (in chromosome C5) was not done.

The BAC clones that remained at the premapped stage are clearly discriminated (horizontally displayed) in Figures 2 and 3 and File S1.

Multiple BAC clones were anchored to the genetically mapped ones, either by inferring overlap (established by restriction analysis and confirmed by PCR), or by BAC-to-BAC ligation through PCR (using STS markers derived from BAC-end sequences), or in some cases, by alignment of identical end sequences. Anchored BAC clones were accepted as being genetically mapped, and they are displayed vertically in the above-cited figures.

In total, 83 BAC clones were accurately mapped in the region (~4.6 cM in the present map) surrounding the downy mildew resistance locus Pp523 in chromosome C8 (Figure 2). A relatively smaller group of 33 BAC clones were mapped at the other end of the chromosome C8 (Figure 2), while a large group of 63 BAC clones mapped to chromosome C5, where they are scattered throughout 18.5 cM (Figure 3).

The distribution of the selected BAC clones by more than one location was not completely surprising because the triplicate nature of Brassica genomes has been extensively documented both at the genetic map (Cavell et al. 1998; Lagercrantz 1998; Parkin et al. 2005) and the microsynteny levels (O’Neill and Bancroft 2000). The triplication of the Brassica genomes, despite multiple chromosome rearrangements, gene loss, and insertions (Town et al. 2006), is accompanied by extensive conservation of macro- and microsynteny (Kowalski et al. 1994; Lan et al. 2000; O’Neill and Bancroft 2000; Parkin et al. 2005; Kaczmarek et al. 2009) with A. thaliana, a feature that seems also to be valid for the genomic region that surrounds the Pp523 locus in B. oleracea.

A fine genetic map of the 4.8 cM region that encompasses locus Pp523 was assembled by the inclusion of 25 new STS markers derived from BAC-end sequences (Figure 2). This allowed defining a shorter genomic region of ~2.9 cM spanning the downy mildew resistance locus Pp523 for the construction of a robust physical map for which a minimal tiling path of 13 BAC clones (BoT01 library) was established (Figure 4). Because of possible errors due to the triplication of the genome, which can originate multiple PCR products similar in length but with relatively different sequences and from different

Figure 1 BAC clones (BoT01 and BoCig libraries) selected via hybridization against overgo probes derived from four Brassica oleracea markers that flank the locus Pp523 and 28 loci of the corresponding syntenic genomic region of Arabidopsis thaliana.
<table>
<thead>
<tr>
<th>BAC</th>
<th>BLAST A. thaliana</th>
<th>Mapping</th>
<th>BAC</th>
<th>BLAST A. thaliana</th>
<th>Mapping</th>
<th>BAC</th>
<th>BLAST A. thaliana</th>
<th>Mapping</th>
</tr>
</thead>
<tbody>
<tr>
<td>87O2</td>
<td>At1g01190</td>
<td>NS</td>
<td>167K22</td>
<td>At1g04860</td>
<td>Map F</td>
<td>C8a</td>
<td>172M11</td>
<td>NS</td>
</tr>
<tr>
<td>11K10</td>
<td>NS</td>
<td>Map F</td>
<td>C8a</td>
<td>162C6</td>
<td>Map F</td>
<td>C8a</td>
<td>31N6</td>
<td>NS</td>
</tr>
<tr>
<td>68L</td>
<td>At1g25120</td>
<td>Map R</td>
<td>C8a</td>
<td>91K18</td>
<td>Map R</td>
<td>C8a</td>
<td>90D1r</td>
<td>Not Seq</td>
</tr>
<tr>
<td>153J15</td>
<td>At1g01600</td>
<td>PCR</td>
<td>C8a</td>
<td>1E21</td>
<td>Map R</td>
<td>C8a</td>
<td>63O14</td>
<td>Map F</td>
</tr>
<tr>
<td>122G28</td>
<td>At1g01777</td>
<td>NS</td>
<td>C8a</td>
<td>183P3</td>
<td>NS</td>
<td>C8a</td>
<td>84D2</td>
<td>PCR</td>
</tr>
<tr>
<td>35G16</td>
<td>At1g01448</td>
<td>PCR</td>
<td>C8a</td>
<td>162K16</td>
<td>PCR</td>
<td>C8a</td>
<td>47L11</td>
<td>PCR</td>
</tr>
<tr>
<td>181K21</td>
<td>At1g01610</td>
<td>Not Seq</td>
<td>PCR</td>
<td>39E3</td>
<td>Not Seq</td>
<td>C8a</td>
<td>129M11</td>
<td>Not Seq</td>
</tr>
<tr>
<td>105A5</td>
<td>TnLs</td>
<td>PCR</td>
<td>C8a</td>
<td>107D22</td>
<td>PCR</td>
<td>C8a</td>
<td>20N12</td>
<td>PCR</td>
</tr>
<tr>
<td>35H15</td>
<td>At1g01777</td>
<td>NS</td>
<td>C8a</td>
<td>33N5</td>
<td>PCR</td>
<td>C8a</td>
<td>12E22</td>
<td>PCR</td>
</tr>
<tr>
<td>46P13</td>
<td>At1g01777</td>
<td>NS</td>
<td>C8a</td>
<td>159G23</td>
<td>PCR</td>
<td>C8a</td>
<td>87O21</td>
<td>TnLs</td>
</tr>
<tr>
<td>6P17</td>
<td>At1g01777</td>
<td>NS</td>
<td>C8a</td>
<td>171O18</td>
<td>PCR</td>
<td>C8a</td>
<td>63M20</td>
<td>PCR</td>
</tr>
<tr>
<td>161N21</td>
<td>Not Seq</td>
<td>Not Seq</td>
<td>PCR</td>
<td>49K12</td>
<td>PCR</td>
<td>C8a</td>
<td>76N24</td>
<td>PCR</td>
</tr>
<tr>
<td>35E22</td>
<td>Not Seq</td>
<td>TnLs</td>
<td>PCR</td>
<td>83K19</td>
<td>Map F</td>
<td>C8a</td>
<td>20L6</td>
<td>PCR</td>
</tr>
<tr>
<td>47P19</td>
<td>TnLs</td>
<td>PCR</td>
<td>C8a</td>
<td>85O24</td>
<td>PCR</td>
<td>C8a</td>
<td>150N21</td>
<td>Not Seq</td>
</tr>
<tr>
<td>191C7</td>
<td>Not Seq</td>
<td>Not Seq</td>
<td>PCR</td>
<td>111O21</td>
<td>PCR</td>
<td>C8a</td>
<td>185F9</td>
<td>Not Seq</td>
</tr>
<tr>
<td>1P13</td>
<td>Not Seq</td>
<td>Not Seq</td>
<td>PCR</td>
<td>15M10</td>
<td>PCR</td>
<td>C8a</td>
<td>120K18</td>
<td>Not Seq</td>
</tr>
<tr>
<td>53G16a</td>
<td>NS</td>
<td>At1g02100</td>
<td>PCR</td>
<td>9O6</td>
<td>At1g0540</td>
<td>PCR</td>
<td>151G12</td>
<td>NS</td>
</tr>
<tr>
<td>88O13a</td>
<td>At1g01820</td>
<td>TnLs</td>
<td>PCR</td>
<td>2M20</td>
<td>At1g0523</td>
<td>PCR</td>
<td>151G12</td>
<td>TnLs</td>
</tr>
<tr>
<td>64F16a</td>
<td>Not Seq</td>
<td>Not Seq</td>
<td>PCR</td>
<td>101N4</td>
<td>PCR</td>
<td>C8a</td>
<td>147J13</td>
<td>Not Seq</td>
</tr>
<tr>
<td>19M21</td>
<td>At1g02070</td>
<td>Map R</td>
<td>C8a</td>
<td>63E7</td>
<td>At1g0523</td>
<td>NS</td>
<td>151M16</td>
<td>Not Seq</td>
</tr>
<tr>
<td>117M5</td>
<td>At1g01950</td>
<td>Map F</td>
<td>C8a</td>
<td>115C6</td>
<td>At1g3201</td>
<td>PCR</td>
<td>181P7</td>
<td>At1g02860</td>
</tr>
<tr>
<td>65H5</td>
<td>At1g01950</td>
<td>PCR</td>
<td>C8a</td>
<td>23K23</td>
<td>At1g05950</td>
<td>PCR</td>
<td>35L20</td>
<td>At1g25440</td>
</tr>
<tr>
<td>65L14</td>
<td>At1g02010</td>
<td>NS</td>
<td>C8a</td>
<td>24H17</td>
<td>TnLs</td>
<td>C8a</td>
<td>142E19</td>
<td>Not Seq</td>
</tr>
<tr>
<td>96L11</td>
<td>At1g01960</td>
<td>PCR</td>
<td>C8a</td>
<td>12L14</td>
<td>NS</td>
<td>Map R</td>
<td>32P20</td>
<td>Map F</td>
</tr>
<tr>
<td>96L11</td>
<td>At1g02580</td>
<td>Map R</td>
<td>C8a</td>
<td>12L14</td>
<td>Map R</td>
<td>C8a</td>
<td>111J21</td>
<td>Map R</td>
</tr>
<tr>
<td>104H17</td>
<td>At1g02660</td>
<td>PCR</td>
<td>C8a</td>
<td>28N8</td>
<td>At1g07920</td>
<td>PCR</td>
<td>18G3</td>
<td>At1g05020</td>
</tr>
<tr>
<td>97K22</td>
<td>At1g02750</td>
<td>NS</td>
<td>PCR</td>
<td>117B1</td>
<td>At1g06490</td>
<td>PCR</td>
<td>149J21</td>
<td>Not Seq</td>
</tr>
<tr>
<td>53O21</td>
<td>At1g02660</td>
<td>NS</td>
<td>PCR</td>
<td>114F8</td>
<td>At1g06590</td>
<td>PCR</td>
<td>19M3</td>
<td>At1g05300</td>
</tr>
<tr>
<td>6D7</td>
<td>NS</td>
<td>At1g02270</td>
<td>Map F</td>
<td>11A22</td>
<td>At1g06680</td>
<td>PCR</td>
<td>89C6</td>
<td>At1g05577</td>
</tr>
<tr>
<td>92O1</td>
<td>NS</td>
<td>At1g02990</td>
<td>PCR</td>
<td>38E20</td>
<td>At1g06490</td>
<td>PCR</td>
<td>16F14</td>
<td>At1g05577</td>
</tr>
<tr>
<td>68M7</td>
<td>NS</td>
<td>At1g03010</td>
<td>NS</td>
<td>84E23</td>
<td>At1g07110</td>
<td>NS</td>
<td>54F20</td>
<td>At1g06510</td>
</tr>
<tr>
<td>97P4</td>
<td>NS</td>
<td>At1g03140</td>
<td>NS</td>
<td>88A18</td>
<td>At1g06780</td>
<td>PCR</td>
<td>90L6</td>
<td>At1g06510</td>
</tr>
<tr>
<td>97K11</td>
<td>At1g33910</td>
<td>NS</td>
<td>PCR</td>
<td>82H2</td>
<td>At1g06740</td>
<td>PCR</td>
<td>13D22</td>
<td>Map R</td>
</tr>
<tr>
<td>10C19</td>
<td>At1g03080</td>
<td>Map R</td>
<td>C8a</td>
<td>6K18</td>
<td>At1g07110</td>
<td>Map R</td>
<td>85G23</td>
<td>Not Seq</td>
</tr>
<tr>
<td>7L14</td>
<td>At1g03140</td>
<td>NS</td>
<td>Map F</td>
<td>51P12</td>
<td>At1g06930</td>
<td>Map F</td>
<td>159P2</td>
<td>Not Seq</td>
</tr>
<tr>
<td>111P15</td>
<td>At1g03475</td>
<td>PCR</td>
<td>C8a</td>
<td>76C8</td>
<td>At1g07510</td>
<td>PCR</td>
<td>62B20</td>
<td>Map F</td>
</tr>
<tr>
<td>13N3</td>
<td>NS</td>
<td>At1g03890</td>
<td>Map R</td>
<td>18P4</td>
<td>At1g07720</td>
<td>PCR</td>
<td>52A2</td>
<td>At1g07570</td>
</tr>
<tr>
<td>40J10</td>
<td>At1g04210</td>
<td>NS</td>
<td>PCR</td>
<td>27D24</td>
<td>At1g07485</td>
<td>PCR</td>
<td>40G18</td>
<td>At1g07230</td>
</tr>
<tr>
<td>112O12</td>
<td>At1g04470</td>
<td>PCR</td>
<td>C8a</td>
<td>120I20</td>
<td>At1g07570</td>
<td>PCR</td>
<td>37G4</td>
<td>At1g07560</td>
</tr>
<tr>
<td>69I2</td>
<td>At1g04440</td>
<td>Map R</td>
<td>C8a</td>
<td>37G4</td>
<td>At1g07560</td>
<td>A33g13445</td>
<td>PCR</td>
<td>887O24</td>
</tr>
</tbody>
</table>

TnLs, transposable element-like sequence; NS, not significant similarity; Not Seq, not sequenced; PCR, anchored by PCR; Map F, mapped forward BAC end; Map R, mapped reverse BAC end; TF, forward terminus; TR, reverse terminus; C8a, chromosome C8 (PpS23 region); C8b, chromosome C8 (second mapping region); C5, chromosome C5.

* BoCig library.
Figure 2 BAC clones mapped in chromosome C8. (Right) BAC clones mapped near the locus Pp523. (Left) BAC clones mapped apart from the resistance locus. Accurately mapped clones are represented vertically. Premapped clones are represented horizontally, ordered according to their collinearity with A. thaliana. The forward and reverse end of BAC clones are represented by a triangle and a lozenge, respectively. Black-filled triangles and lozenges indicate sequence identity between overlapping BAC ends. BAC-to-BAC (PCR) ligations are indicated by intersecting dotted lines.
genomic loci, the PCR products that confirm the BAC-to-BAC linkages within the minimal tiling path were sequenced and carefully compared with the original BAC-end (STS) sequences used to design the primers. In all cases, they were identical.

DISCUSSION
Exploitation of the genetic similarity and syntenic relationship between *A. thaliana* and *B. oleracea* has guided the construction of a physical map surrounding the downy mildew resistance locus *Pp523*,
by integration of genetic mapping with probe hybridization to BAC libraries and in silico selection of BAC clones using end-sequence information.

Two main obstacles have slowed, but not compromised, the accomplishment of this task: a) the large amount of transposable element-like sequences in the *B. oleracea* genome; and 2) the triplicate nature of the *B. oleracea* genome.

A large percentage (62 out of 429; 14.5%) of the BAC clones of the BoT01 library exhibit transposable element-like sequences at one or at both ends. By creating artifactual similarities between BAC-end sequences and between these and Arabidopsis genome sequences, this genome feature significantly reduced the number of BAC-end sequences suitable for mapping purposes and constrained our ability to employ Brassica/Arabidopsis synteny. The total length of transposable elements in *B. oleracea* has been calculated to be ~15 times that of *A. thaliana* and to represent ~120 Mb or 20% of the genome, leading to the suggestion that amplification of RNA and DNA transposable elements significantly contributed to the genome expansion of this crop species (Zhang and Wessler, 2004).

Nevertheless, the triplication of the genomic region of interest was the major constraint to a more efficient exploitation of the *A. thaliana/B. oleracea* genetic relatedness during the construction of the present physical map.

Besides the region in the *B. oleracea* chromosome C8 where the *Pp523* locus was previously mapped (Farinhó et al. 2004, 2007; Carlier et al. 2011), the BAC clones mapped in two additional regions, one at ~60 cM in the same chromosome (C8) and another, apparently larger, in chromosome C5, evidencing a triplication of this Arabidopsis genomic region in *B. oleracea*. Today is largely accepted that the diploid *Brassica* species are paleohexaploids (Schmidt et al. 2001; Parkin et al. 2003; Lysak et al. 2005). With the support of various other studies that highlighted the Brassica genome triplication (Cavell et al. 1998; Lagercrantz 1998; Lan et al. 2000; O’Neill and Bancroft 2000; Parkin et al. 2005) and their own data, Lysak et al. (2005)
proposed that after the Arabidopsis and Brassica lineages split, ~114–
24 Mya (millions of years ago) according to Yang et al. (1999) and
Koch et al. (2000), an hexaploitation event occurred 7.9–14.6 Mya
that gave rise to an ancestral triplicated Brassicaceae genome, a feature
that remained distinctive of all species of this tribe.

The early findings of Kowalski et al. (1994) and the comparative
genetic mapping of over one thousand RFLP loci in A. thaliana and B.
napus carried out by Parkin et al. (2005) suggested the existence of
~20–25 conserved genomic units within the A. thaliana genome
which duplication and rearrangement could generate the present B.
napus genome. The majority of the conserved units were found in six
copies, and 81% of the loci used for comparison were mapped to the
triplicated regions by Parkin et al. (2005), consistent with the hypoth-
esis of a hexaploid ancestor for the diploid Brassica progenitors. Nev-
nevertheless, the mechanism of formation of the present structure of the
Brassica genomes is assumed to include multiple rearrangements via
insertions, deletions, and translocations (Parkin et al. 2005; Town
et al. 2006). The comparative mapping study of Parkin et al. (2005)
and, specifically, the block of markers A (C1A) at the terminus of the
top arm of A. thaliana chromosome 1 identified by these authors are
of particular interest. This block corresponds to the genome block A
defined by Schranz et al. (2006) in the “ancestral karyotype” of Lysak
et al. (2006), which is delimited by the A. thaliana sequences At1g01560
and At1g19330, clearly spanning the A. thaliana genome segment
between loci At1g01570 and At1g07420 syntenic to the
Pp523 region enclosed by the homologous B. oleracea markers
SCJ19/PagI and SCAB1/Bfu (Farinhó et al. 2007). This genome block
(C1A or A) was found by Parkin et al. (2005) to have: i) a counterpart
in the extremity of the linkage group/chromosome N18 (C8) appar-
cently corresponding to the B. oleracea chromosome C8 region where
the downy mildew resistance gene Pp523 is embedded and part of
the selected BAC clones map to (in the present work); ii) a second ho-
logous region in the same chromosome (N18/C8), which appar-
cently corresponds to the second region of BAC mapping; and iii)
a large homologous region in the chromosome (N15/C5) correspond-
ing to the B. oleracea third genome region to which a large group of
the BAC clones also map. No other counterparts for this Arabidopsis
genomic C1A/A segment were identified among the other B. napus C
genome chromosomes (N11–N19).

The analysis of an integrated map of B. napus that includes the
map of Parkin et al. (2005) recently published by Wang et al. (2011)
allows the above observations to be clearly confirmed, as this map
shares common reference SSR markers with our map (Carlier et al.
2011). Nevertheless, note that chromosome C8 of our map and those
of Wang et al. (2011) and Parkin et al. (2005) are inverted relative to
one another. The analysis of the alignment of B. napus markers with
their homology BLAST hits within the Arabidopsis chromosomes
(Wang et al. 2011) shows that the C1A/A block presents two main
concentration plots of collinear hits in opposite directions at the
expected positions on chromosome C8 and a large third concentration
plot of hits on chromosome C5. Some hits can be observed on chro-
mosome C7, whereas the other C genome chromosomes exhibit al-
most no hits. These results coincide and are confirmed by our BAC
mapping results. Except for 2 BACs mapped to chromosome 2 and 1
BAC mapped to chromosome C6, the other (179) BACs mapped to
two different regions on chromosome C8 and one region on chro-
mosome C5.

One might expect the levels of identity between the B. oleracea
BAC-end sequences and a specific Arabidopsis DNA sequence to
exhibit some kind of pattern or tendency according to the Brassica
genome region where they map. However, this is not the case. For
example, the BAC-end sequences 49K12TR (C8, Pp523 region),
106H20TR (C8, distant from Pp523), and 19M3TF (C5) show, re-
spectively, 94%, 87%, and 90% of identity to a sequence stretch of
genome At1g05180, whereas the BAC ends 121A8TR (C8, Pp523 region),
76A16TR (C8, distant from Pp523), and 89G6TF (C5) show, re-
spectively, 85%, 87%, and 90% identity to a sequence stretch of gene
At1g05630. In other words, the location of a specific DNA sequence
in the B. oleracea genome cannot be inferred from its level of identity
to a specific A. thaliana sequence.

So far, a 2.0x BAC genome library from a downy mildew
resistant S4 line derived from the original resistant genotype has
been constructed at the University of Algarve, and a replica of
this map of Parkin et al. (2005) is currently being assembled
using this BAC library. The identification of polymorphisms be-
tween the two BAC contigs, in particular regarding disease resis-
tance gene-like sequences, is expected to produce significant
information to foster our research toward the isolation of the
downy mildew resistance gene Pp523.

ACKNOWLEDGMENTS

This work was funded by the European Social Fund and the
Portuguese National Science Foundation (project PTDC/AGR-GPL/

LITERATURE CITED

Botella, M. A., J. E. Parker, L. N. Frost, O. D. Bittner-Eddy, J. L. Beynon et
al., 1998 Three genes of the Arabidopsis RPP1 complex resistance locus
recognize distinct Peronospora parasitica avirulence determinants. Plant

Carlier, J. D., C. A. Alabaça, P. S. Coelho, A. A. Monteiro, and J. M. Leitão,
2011 The downy mildew resistance locus Pp523 is located on chro-
mosome C8 of Brassica oleracea L. Plant Breeding (DOI: 10.1111/j.1439-
0523.2011.01904.x).

Cavell, A. C., D. J. Lydiate, I. A. Parkin, C. Dean, and M. Trick, 1998 Collinearity
between a 30-centiMorgan segment of Arabidopsis thaliana chromosome 4
and duplicated regions within the Brassica napus genome. Genome 41: 62–69.

Coelho, P. S., D. Leckie, K. Bahcevandziev, L. Valério, D. Astley et al.,
1998 The relationship between cotyledon and adult plant resistance to
459: 335–342.

Coelho, P. S., and A. A. Monteiro, 2003 Inheritance of downy mildew

Dickson, M. H., and R. Petzoldt, 1993 Plant age and isolate source affect

Van Der Biezen, E. A., C. T. Freddie, K. Kahn, J. E. Parker, and J. D. Jones,
2002 Arabidopsis RPP4 is a member of the RPP5 multigene family of
TIR-NB-LRR genes and confers downy mildew resistance through multi-

Farinhó, M., P. Coelho, J. Carlier, D. Svetleva, A. Monteiro et al.,
2004 Mapping of a locus for adult plant resistance to downy mildew

Gao, M., G. Li, B. Yang, D. Qiu, M. Farnham et al., 2007 High-density
Brassica oleracea linkage map: identification of useful new linkages.

Göker, M., H. Voglmayr, A. Riethmüller, M. Weiß, and F. Oberwinkler,
2003 Taxonomic aspects of Peronosporaceae inferred from Bayesian


Lagercrantz, U. 1998 Comparative mapping between Arabidopsis thaliana and Brassica nigra indicates that Brassica genomes have evolved through extensive genome replication accompanied by chromosome fusions and frequent rearrangements. Genetics 150: 1217–1228.


Communicating editor: D. Zamir