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ORIGINAL PAPER

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Mapping of a locus for adult plant resistance to downy mildew in broccoli (*Brassica oleracea* convar. *italica*)

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Abstract The identification of the gene *Pp523*, conferring downy mildew resistance to adult plants of broccoli (*Brassica oleracea* convar. *italica*), led to the construction of a genetic map that included this resistance locus, 301 amplified fragment length polymorphisms, 55 random amplified polymorphic DNAs, 46 inter-simple sequence repeats, three simple sequence repeats, four other PCR markers and a flower colour locus, all gathered into nine major linkage groups. Nineteen additional molecular markers were clustered into one group of four markers, one group of three markers and six pairs of markers. The map spans over 731.9 cM, corresponding to 89.5% of the 818 cM estimated to be the total genome length. A significant number of the mapped markers, 19.3%, showed distorted segregation. The average distance between mapped adjacent markers is 1.64 cM, which places this map among the densest published to date for this species. Using bulked segregant analysis, we identified a group of molecular markers flanking and closely linked in coupling to the resistance gene and included these in the map. Two markers linked in coupling, OPK17_980 and AT. CTA_133/134, are located at 3.1 cM and 3.6 cM, respectively, at each side from the resistance gene. These markers can be used for marker-assisted selection in breeding programs aiming at the introgression of this gene in susceptible *B. oleracea* genotypes. The fine mapping of the genomic region surrounding the *Pp523* resistance gene is currently being carried out, a basic condition for its isolation via positional cloning.

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Introduction

Downy mildew incited by the oomycete *Hyaloperonospora* Constant. *parasitica* (Pers.:Fr) Fr. (Constantinescu and Fatehi 2002), formerly *Peronospora parasitica*, is a worldwide economically important disease of *Brassica oleracea* L. This disease has a severe destructive impact on young seedlings and strongly reduces the yield and quality of established crops. Multiple sources of genetic resistance to downy mildew have been identified in *B. oleracea* at both the seedling (Natti el al. 1967; Hoser-Krauze et al. 1995; Jensen et al. 1999) and adult plant stages (Natti and Atkin 1960; Dickson and Petzoldt 1993; Mahajan et al. 1995; Coelho et al. 1998), thereby generating confidence that genetic resources are available for the genetic control of this disease and, consequently, the use of pesticides can be reduced.

Coelho et al. (1998) identified a new source of resistance to downy mildew in mature broccoli plants. This resistance is dominantly inherited and controlled by a single locus (Coelho and Monteiro 2003a) and shows good potential for direct use in commercial broccoli breeding.

Map-based cloning or positional cloning, which makes use of dense maps of molecular markers, is a very efficient and systematic strategy for the isolation of plant genes exclusively identifiable by phenotypic expression, such as disease resistance genes (Martin et al. 1993; Cai et al. 1997; Botella et al. 1998; Swiderski and Innes 2001). Notwithstanding the fact that several maps of molecular markers of *B. oleracea* have been constructed (Bohuon et

al. 1996; Cheung et al. 1997; Hu et al. 1998; Lan et al. 2000; Lan and Paterson 2000, 2001; Sebastian et al. 2000; Saal et al. 2001), to our knowledge no resistance gene has yet been isolated in this species using this strategy. The construction of maps of molecular markers linked to resistance genes in *B. oleracea* has mainly focused on resistances to *Plasmodiophora brassicae*, the inciting agent of clubroot (Landry et al. 1992; Fidgore et al. 1993; Voorrips et al. 1997; Morigushi et al. 1999). Nevertheless, data on molecular markers linked to downy mildew resistance genes in broccoli have been communicated by Agnola et al. (2000) and Farinho et al. (2000). More recently, Giovannelli et al. (2002) published the first linkage group of molecular markers linked to a dominantly inherited gene that confers resistance to downy mildew at the cotyledon stage in broccoli.

The objective of the investigation reported here was to construct a genetic map based on random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), inter-simple sequence repeat (ISSR) and simple sequence repeat (SSR) markers in *B. oleracea* L. and to simultaneously map a dominantly inherited downy mildew resistance gene expressed at the adult stage, *Pp523* (Coelho and Monteiro 2003a). Additionally, a locus determining flower colour (white vs. yellow) was also located on the map.

Materials and methods

Plant material

A mapping population of 163 F_2 plants was produced from a cross between the white-flowered and downy mildew-susceptible, rapid-cycling *Brassica oleracea* doubled-haploid (DH) line GK97362 (G. King, Horticulture Research International (HRI), Wellesbourne, UK) and an S_4 line derived from the downy mildew adult-stage-resistant accession OL87125 of broccoli (*B. oleracea* convar. *italica*) (Coelho et al. 1998), whose resistance is determined by a single major gene (Coelho and Monteiro 2003a).

The evaluation of the downy mildew interaction-phenotype of F_2 adult plants was performed using the single-leaf inoculation method described by Coelho and Monteiro (2003b). Briefly, plants were grown in pots in the greenhouse and, after artificial inoculation with a *Hyaloperonospora parasitica* conidia suspension, the leaves were enclosed in plastic bags to stimulate infection. The disease-interaction phenotype was evaluated using a six-class scale of increasing susceptibility, taking into consideration the pathogen sporulation and the host response. Plants in the first three classes were considered to be resistant and plants in the last three classes, susceptible (Coelho and Monteiro 2003b).

DNA extraction and DNA bulking

Plant genomic DNA was extracted according to Carlier et al. (2004) with minor modifications. Briefly, approximately 1 g of leaf tissue was ground in a mortar with a pestle in the presence of liquid nitrogen. The resulting powder was resuspended in 5 ml of buffer A (200 m*M* Tris-HCL, pH 8.0, 250 m*M* NaCl, 25 m*M* EDTA, 1% sodium dodecy sulphate) containing 20 µg/ml of RNAse (Ribonuclease A; Sigma-Aldrich, St. Louis, Mo.) and incubated for 1 h at 37°C. Then, after centrifugation at 4,500 *g* for 10 min, the supernatant was collected and successively extracted with equal volumes of phenol, phenol:chloroform and chloroform and the DNA precipitated with an equal volume of isopropanol. After washing with ethanol (80%) the DNA pellet was resuspended in TE and quantified by UV-spectrophotometry and agarose gel electrophoresis. In order to perform bulked segregant analysis (Michelmore et al. 1991) for identification of markers closely linked to the downy mildew resistance gene, we made bulks of DNA aliquots of 19 resistant and 17 susceptible F_2 plants, respectively, making two independent DNA bulks, hereafter referred to as the R-bulk and S-bulk.

RAPD markers

Six hundred ten-nucleotide-long RAPD primers, corresponding to 30 kits commercialised by Operon Technologies (Alameda, Calif.), were used for the initial screening in bulked segregant analysis comparing the amplifications products of both DNA bulks. Of these primers 460 were used in the primary screening using both progenitors to identify polymorphic markers useful for map construction. The PCR amplifications and the agarose gel electrophoresis were carried out according to Williams et al. (1990) with minor modifications.

AFLP markers

AFLP markers were produced using the kits AFLP Analysis System I and II commercialised by Invitrogen (Carlsbad, Calif.). All procedures, DNA digestion with restriction enzymes, adaptors ligation, preamplification and selective amplification, were carried out following the standard protocol (Vos et al. 1995) provided by the suppliers, with minor modifications. Eighty-seven primer combinations were used in bulked segregant analysis and 83 primer combinations were used for the primary identification of AFLP markers suitable for mapping. Electrophoresis was carried out on denaturating 6% polyacrylamide (Long Ranger; FMC, Rockland, Me.) gels with 7.5 *M* urea using 1× TBE, at 45 W for 2.5 h. Gels were dried on 3 MM (Whatman) paper and autoradiographs were obtained on X-ray film (X-OMAT AR5, Kodak) exposed for 24–72 h depending on the intensity of the radioactive signal.

ISSR markers

The ISSR amplification reactions were performed using one single primer, either 12, 17 or 18 nucleotides long, consisting of a six- to eightfold repeat of a short sequence of two nucleotides and zero to three additional nucleotides, often degenerated and usually anchored to the 3′ primer end (Table 1). The 32 primers used in this study were synthesised by Invitrogen. The PCR reactions and the analysis of the amplified products by agarose gel electrophoresis were performed as previously described (Cabrita et al. 2001).

Microsatellites

In this study, we analysed ten SSR (microsatellite) markers previously identified and mapped by other research group: nga248, sORA43, BN12A, LS107, sORA21b, BN72a, MB4, nga111, BN35D, BN83b1 (Sebastian et al. 2000; Smith and King 2000; HRI, unpublished data). Sequences for primers flanking these SSR markers were obtained consulting the HRI website (http://www.hri.ac.uk).

Table 1 Code and sequences of the ISSR primers used

Code	Sequence[a]
ISSR.01	$(CA)_8RG$
ISSR.02	$(CA)_8RY$
ISSR.03	$(GA)_8YT$
ISSR.04	$(GA)_8YC$
ISSR.05	$(GA)_8YG$
ISSR.06	$(AG)_8YT$
ISSR.07	$(AG)_8YC$
ISSR.08	$(AC)_8YA$
ISSR.09	$(AC)_8YG$
ISSR.10	$(GT)_8YC$
ISSR.11	$(GT)_8YG$
ISSR.12	$(AG)_8YG$
ISSR.13	$(AC)_8YT$
ISSR.14	$(AG)_8T$
ISSR.15	$(AG)_8C$
ISSR.16	$(AG)_8G$
ISSR.17	$(GA)_8T$
ISSR.18	$(GA)_8C$
ISSR.19	$(GA)_8A$
ISSR.20	$(GT)_8C$
ISSR.21	$(TC)_8C$
ISSR.22	$(TC)_8G$
ISSR.23	$(CT)_8RC$
ISSR.24	$(CT)_8RG$
ISSR.25	$(TG)_8RC$
ISSR.26	$(CT)_6$
ISSR.27	$BDB(CA)_7$
ISSR.28	$DBD(AC)_7$
ISSR.29	$VHV(GT)_7$
ISSR.30	$HVH(TG)_7$
ISSR.31	$(AG)_8$ VC
ISSR.32	$CCC(GT)_7$

[a] *R*, A+G; *Y*, C+T; *H*, A+T+C; *B*, T+C+G; *D*, A+T+G; *V*, A+C+G

SSR reactions were performed in a 10-μl final volume of the reaction mixture: 10 m*M* Tris-HCL, pH 9.0, 50 m*M* KCl, 1.5 m*M* MgCl, 0.2 m*M* of each dNPT, 0.5 μ*M* of each primer, 1 U of *Taq* DNA polymerase (Amersham Pharmacia Biotech, Piscataway, N.J.) and 20 ng of genomic DNA. For each amplification reaction one of the primers was 5′ end labelled with γ-[^{33}P] ATP using T4 polynucleotide kinase. The PCR programme was as follows: an initial step of 1 min and 10 s at 94°C, followed by 30 cycles of 20 s at 94°C, 2 min at 55–65°C (depending on the primer pair), 1 min and 30 s at 72°C, and a final extension step of 3 min and 30 s at 72°C. The electrophoresis and the autoradiography of SSR markers were performed as described for the AFLP markers.

Marker designation

RAPD markers were labelled using the prefix OP (Operon Technologies) followed by the letter(s) designating the kit, the number of the primer and the length (in basepairs) of the DNA fragment. The AFLP markers were identified by two or three letters corresponding to the random nucleotides of primers *Eco*RI (E) and *Mse*I (M) followed by the length of the amplified fragment. ISSR markers were identified with the letters ISSR followed by primer number (Table 1) and fragment length. For microsatellite markers, the original designations were maintained. For example, OPA11_650 specifies a RAPD marker 650 bp long amplified with primer 11 from kit A of Operon Technologies; AC.CTT_250 stands for the AFLP marker 250 bp long amplified by primers E+AC and M+CTT; ISSR03_950 designates an ISSR marker 950 bp long amplified by primer 03.

Map construction

The segregation data for molecular markers, resistant phenotype and flower colour among 163 F_2 plants were recorded and subjected to a χ^2-test in order to determine concordance to a 3:1 or 1:2:1 Mendelian segregation ratio. The analysis of linkage between markers, the ordering of markers within each linkage group and the drawing of the linkage groups were carried out using the JOINMAP 3.0 software (Van Ooijen and Voorrips 2001) set for the Kosambi's mapping function. Linkage groups were assembled for LOD scores greater than 4.4. Map coverage was calculated as the fraction of the sum of the map distances covered by all linkage groups enlarged at both ends by the average distance between two adjacent markers, as suggested by Marques et al. (1998), divided by the genome size estimated using method 3 of Chakravarti et al. (1991).

Results

Phenotype segregation

The 163 F_2 plants segregated 120 (resistant):43 (susceptible), which is very close to the expected segregation of 3:1 ($\chi^2=0.2<\chi^2_{0.05}$), thereby confirming the very good fit to a 3:1 ratio obtained in similar crosses (Coelho and Monteiro 2003b).

Flower colour, a trait that showed a distinct 3:1 Mendelian segregation in a number of other crosses (Monteiro et al., unpublished results) exhibited in this particular F_2 population a distorted segregation of 110 (white):53 (yellow), ($\chi^2_{0.01}>\chi^2=4.9>\chi^2_{0.05}$).

Bulked segregant analysis

Among the 600 RAPD primers used to discriminate between the two DNA bulks (R-bulk and S-bulk), 499 (83%) amplified 2,306 markers, of which only four markers were reproducibly polymorphic. Markers OPJ19_550, OPR15_920 and OPK17_980 mapped relatively clustered at 5.6, 3.6 and 3.1 cM, respectively, from the downy mildew resistance gene (*Pp523*). The fourth marker, OPAE15_980, mapped 10 cM to the other side of the resistance gene.

Eighty-seven AFLP primer combinations amplified 5,544 markers, and although some apparent polymorphisms were revealed, only one codominant AFLP marker, AT.CTA 133/134, was later confirmed to be closely co-segregating with the resistance phenotype, mapping at 3.6 cM from the respective locus. One marker, AAG.CTA_113, which was present in the susceptible progenitor, was mapped at 2.7 cM from the resistance locus. Four hundred and seventy-two ISSR markers were amplified with the 32 primers (Table 1), but not one of these was found to be polymorphic between the DNA bulks.

Overall, five molecular markers showed close linkage in coupling to the resistance gene and, therefore, can be used in marker-assisted selection to accelerate the incorporation of resistance in susceptible genotypes of *B. oleracea*. Markers OPK17_980 and OPAE15_980 are particularly suitable for this purpose because of the ease in performing RAPD analysis and because the simultaneous break of the linkage between both markers and the resistance gene requires an improbable double crossover to occur.

Map construction

Simultaneous to screening the DNA bulks, we used genomic DNA of both parents to identify polymorphic markers suitable for the construction of a linkage map spanning the entire genome that could be used for mapping new genes of interest, particularly new downy mildew resistance genes. Two hundred and fifty-nine RAPD primers revealed polymorphisms between the female and the pollen progenitor. Taking into consideration the number and quality of polymorphic bands and the general quality of the amplified products, we selected 23 primers for further analysis and recorded segregation data among the F_2 population for 59 RAPD markers.

Based on similar criteria of quantity and quality of the amplified products, 20 AFLP primer combinations were selected for map construction. Segregation data were recorded for 330 AFLP markers and, despite the dominant nature of these markers, six pairs of markers were identified as allelic since they segregate codominantly: AAG.CAC_153/140, ACC.CTT_144/142, ACC.CTT_155/157, AT.CTA_133/134, AT.CTA_480/470 and AAG.CTC_480/488. Fifteen ISSR primers were also selected, thereby contributing 51 segregating markers to map construction.

With a view toward rendering the genetic map more robust and at the same time creating the conditions for further comparison and integration with other maps, we proceeded to the analysis and mapping of ten microsatellite (SSR) loci previously mapped by other research teams (Sebastian et al. 2000; Smith and King 2000; HRI unpublished data). Unexpectedly, only three SSR loci exhibited polymorphism: two of these, sORA43 and nga111, segregated codominantly, while the third locus, BN83b1, exhibited a dominant type of segregation. Nevertheless, four unexpected additional markers (BN12A_A, sORA21b_A, sORA21b_B and sORA21b_C) were amplified by the primers for the microsatellite loci BN12A and sORA21b.

Our analysis of the segregation data for 448 molecular markers and two morphological (resistant phenotype and flower colour) loci revealed that 357 of these showed a segregation pattern complying with the expected 3:1 (dominant markers) or 1:2:1 (codominant markers) Mendelian segregation. Ninety-three markers (20.6%) exhibited distorted segregation ($\chi^2>\chi^2_{0.05}$), while 61 showed slight distortion ($\chi^2_{0.05}<\chi^2<\chi^2_{0.01}$). By successively increasing the LOD threshold to between 4.4 and 5.4 and using a maximum recombination fraction of 0.3, we constructed a map grouping 430 markers (Fig. 1) that gathered 411 loci (301 AFLP, 55 RAPD, 46 ISSR, three SSR, four other PCR marker loci, and the resistance locus and flower colour locus) into nine major linkage groups, putatively corresponding to the nine chromosomes of *B. oleracea* L. The 19 other molecular markers were assembled into one group of four markers, one group of three markers and six pairs of markers. Twenty markers remained unlinked.

More than one-quarter of the distorted markers (26.5%) mapped in linkage group 6 (LG_6), most of them clustered in a region of about 15 cM. Also located in this linkage group are two-thirds of the markers with a more skewed segregation. In contrast, only one distorted marker was mapped in linkage group 7. Ten of these distorted markers remained unlinked, while the remainder were distributed among the linkage groups in numbers oscillating between the above two extreme situations.

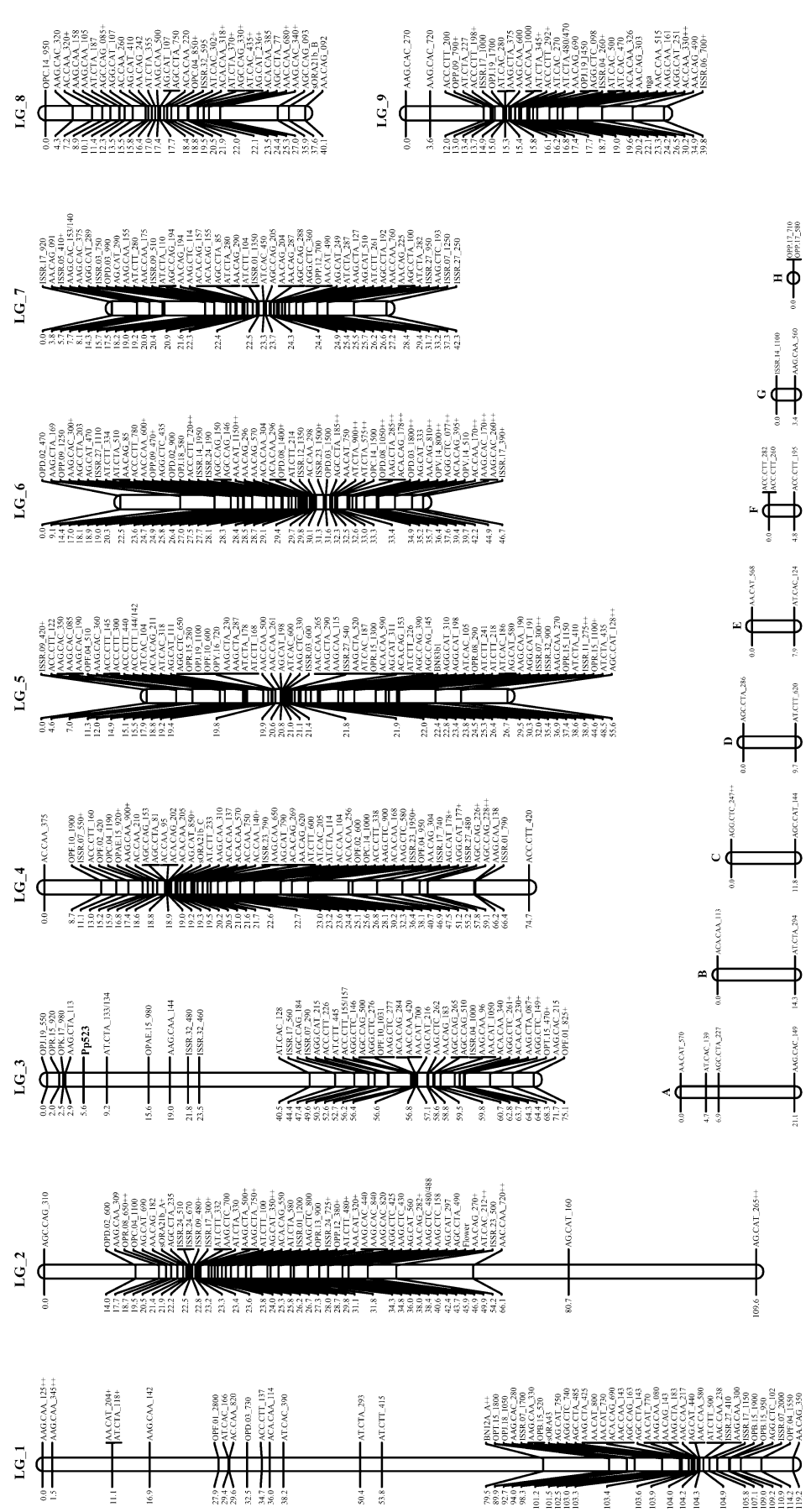

Fig. 1 Genetic map of *Brassica oleracea*. Major linkage groups (*LG*) are indicated by *numbers* (*1–9*) and minor groups by *letters* (*A–H*) at the *top* of each LG. The linkage groups are ordered according to their length (centiMorgans). Absolute map distances (in centiMorgans) are indicated on the *left*; locus names are indicated on the *right*. Markers showing a segregation distortion at $P \leq 0.05$ and $P \leq 0.001$ are identified by + and ++, respectively

Genome coverage

Using the method 3 of Chakravarti et al. (1991), the total length of the genome (G) was computed to be 818 cM. The sum of all linkage groups constituting the map is 676.1 cM, which corresponds to 82.7% genome coverage. When the average distance between adjacent markers, 1.64 cM, is added to the extremities of each linkage group, the total length spanned by the map increases to 731.9 cM, corresponding to 89.5% genome coverage.

Discussion

To our knowledge, this is the first downy mildew adult plant resistance gene mapped in *B. oleracea* L. that is surrounded relatively closely by molecular markers.

With respect to the number of mapped marker loci (430) and map length (731.9 cM), the map presented here is within the range of other maps previously constructed, which vary from 92 marker loci spanning over 615 cM (Voorrips et al. 1997), 124 marker loci over 823.6 cM (Morigushi et al. 1999), 547 marker loci over 893 cM (Sebastian et al. 2000) to the 310 or 167 markers constituting uncommonly large maps of 1,606 or 1,738 cM, respectively (Cheung et al. 1997; Hu et al. 1998). Nevertheless, the average distance between adjacent markers (1.64 cM) is one of the smallest reported to date, placing this map amongst the densest maps published so far.

A general similarity can be observed between the distribution of marker density on this map and that on other maps published by other research teams (Cheung et al. 1997; Sebastian et al. 2000; Saal et al. 2001). The more highly dense regions, corresponding to regions more polymorphic between the parent lines, far from being randomly dispersed along the genome tend to map in the interior of the linkage groups while the extremities are, in general, less dense. This phenomenon, apparently common to several genotypes of *B. oleracea* L., is likely to reflect specific aspects of genome organisation and genome evolution within this species.

Nevertheless, the different types of molecular markers are interspersed in the map apparently at random—in regions of higher marker density, in regions of less density or in regions where markers with distorted segregation are congregated. A significant fraction of the markers, 20.6% (19.3% of mapped markers), showed segregation distortion, which is slightly higher than that found in other maps: 5–12% (Kianin and Quiros 1992) and 15% (Cheung et al. 1997). On the other hand, using a population of DH lines, Voorrips et al. (1997) observed a much higher fraction (65%) of markers with distorted segregation. The presence of advantageous or disadvantageous alleles linked to molecular markers, otherwise assumed as selection neutral, which drive the competition among gametes and the adaptability of the new genotypes, is generally assumed to be the main cause for the frequently observed segregation ratio distortions (Moriguchi et al. 1999).

To date, only one other downy mildew resistance gene has been mapped in *B. oleracea* L. This gene, which confers resistance to plants at the cotyledon stage, was mapped into a partial map constituted by a single linkage group 69 cM long (Giovanelli et al. 2002). In the present map, five molecular markers linked in coupling to the resistance gene encompass a region of approximately 15 cM. One of these markers, AT.CTA_133/134, is codominant and, consequently, the other allele (134) is linked in repulsion. One additional marker, AAG.CTA_113, identified in the susceptible progenitor GK97362 also mapped within this region linked in repulsion to the resistance gene.

All of the markers in coupling phase, particularly those pairs of markers that flank the resistance gene on both sides—for example, OPK17_980 or OPR15_920 and OPAE15_980 or AT.CTA_133/134—can be used as selective markers in breeding programmes aimed at the introgression of this gene into susceptible genotypes of *B. oleracea*. Currently, sequenced characterised amplified regions are being developed for these markers to make the PCR analysis more specific when tracking down the resistance gene without inciting and analysing the disease interaction phenotype in the progeny plants.

Based on the information provided by the map, new DNA bulks have been prepared. One bulk reunites DNA of F_2 resistant plants that exhibit OPK17_980 and AT.CTA_133/134, the markers that flank the *Pp523* locus the most closely . A second bulk joins up DNA of susceptible plants that lack these molecular markers. Using a larger F_2 population, we are at present carrying out a second round of bulked segregant analysis in order to obtain a fine map of the genomic region encompassed by these two markers, thereby rendering it highly dense in molecular markers tightly linked to *Pp523*, a condition essential for the isolation of this resistance gene via positional cloning.

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References

Agnola B, Silue D, Boury S (2000) Identification of RAPD markers of downy mildew (*Peronospora parasitica*) resistance gene in broccoli (*Brassica oleracea* var. *italica*). In: (King GJ) Abstr 3rd Int Symp Brassicas. Horticulture Research International, Wellesbourne, UK, poster 64

Bohuon EJR, Keith DJ, Parkin IAP, Sharpe AG, Lydiate DJ (1996) Alignment of the conserved C genomes of *Brassica oleracea* and *Brassica napus*. Theor Appl Genet 93:833–839

Botella MA, Parker JE, Frost LN, Bittner-Eddy OD, Beynon JL, Daniels MJ, Holub EB, Jones JDG (1998) Three genes of the *Arabidopsis* RPP1 complex resistance *locus* recognize distinct *Peronospora parasitica* avirulence determinants. Plant Cell 10:1847–1860

Cabrita LF, Aksoy U, Hepaksoy S, Leitao JM (2001) Suitability of isozyme, RAPD and AFLP markers to assess genetic differences and relatedness among fig (*Ficus carica* L.) clones. Sci Hortic 87:261–273

Cai D, Kleine M, Kifle S, Harloff HJ, Sandal NN, Marcker KA, Klein-Lankhorst RM, Salentijn EMJ, Lange W, Stiekema WJ, Wyss U, Grundler FMW, Jung C (1997) Positional cloning of nematode resistance in sugar beet. Science 275:832–834

Carlier JD, Reis A, Duval MF, d'Eeckenbrugge GC, Leitão JM (2004) Genetic maps of RAPD, AFLP and ISSR markers in *Ananas bracteatus* and *A. comosus* using the pseudo-testcross strategy. Plant Breed 123:186–192

Chakravarti A, Lasher LA, Reefer JE (1991) A maximum likelihood method for estimating genome length using genetic linkage data. Genetics 128:175–182

Cheung WY, Champagne G, Hubert N, Landry BS (1997) Comparison of the genetic maps of *Brassica napus* and *Brassica oleracea*. Theor Appl Genet 94:569–582

Coelho PS, Monteiro A (2003a) Inheritance of downy mildew resistance in mature broccoli plants. Euphytica 131:65–69

Coelho PS, Monteiro A (2003b) Expression of resistance to downy mildew at cotyledon and adult plant stages in *Brassica oleracea* L. Euphytica 133:279–284

Coelho PS, Leckie D, Bahcevandziev K, Valério L, Astley D, Boukema I, Crute IR, Monteiro A (1998) The relationship between cotyledon and adult plant resistance to downy mildew (*Peronospora parasitica*) in *Brassica oleracea*. Acta Hortic 459:335–342

Constantinescu O, Fatehi J (2002) *Peronospora*-like fungi (*Chromista peronosporales*) parasitic on *Brassicaceae* and related hosts. Nova Hedwigia 74:291–334

Dickson MH, Petzoldt R (1993) Plant age and isolate source affect expression of downy mildew resistance in broccoli. Hortic Sci 28:730–731

Farinho MJ, Coelho PS, Monteiro AA, Leitão JM (2000) RAPD and AFLP markers linked to *Peronospora parasitica* resistance genes in broccoli. In: King (GJ) Abstr 3rd Int Symp Brassicas. Horticulture Research International, Wellesbourne, UK poster 75

Fidgore SS, Ferreira ME, Slocum MK, Osborn TC (1993) Association of RFLP markers with trait *loci* affecting clubroot resistance and morphological characters in *Brassica oleracea*. Euphytica 69:33–44

Giovannelli JL, Farnham MW, Wang M (2002) Development of sequence characterized amplified region markers linked to downy mildew resistance in broccoli. J Amer Soc Hortic Sci 127:597–601

Hoser-Krauze J, Lakowska-Ryk E, Antosik J (1995) The inheritance of resistance of some *Brassica oleracea* L. cultivars and lines to downy mildew, *Peronospora parasitica* (Pers) ex. Fr. J Appl Genet 36:27–33

Hu J, Sadowski J, Osborn TC, Landry BS, Quiros CF (1998) Linkage group alignment from four independent *Brassica oleracea* RFLP maps. Genome 41:226–235

Jensen BD, Hockenhull J, Munk L (1999) Seedling and adult plant resistance to downy mildew (*Peronospora parasitica*) in cauliflower (*Brassica oleracea* convar *botrytis* var *botrytis*). Plant Pathol 48:604–612

Kianian SF, Quiros CF (1992) Generation of a *Brassica oleracea* composite RFLP map: linkage arrangements among various populations and evolutionary implications. Theor Appl Genet 84:544–554

Lan TH, Paterson AH (2000) Comparative mapping of quantitative trait loci sculpting the curd of *Brassica oleracea*. Genetics 155:1927–1954

Lan TH, Paterson AH (2001) Comparative mapping of QTLs determining the plant size of *Brassica oleracea*. Theor Appl Genet 103:383–397

Lan TH, Delmonte TA, Reischmann KP, Hyman J, Kowalski S, McFerson J, Kresovich S, Paterson AH (2000) EST-enriched comparative map of *Brassica oleracea* and *Arabidopsis thaliana*. Genome Res 10:776–788

Landry BS, Hubert N, Crete R, Chang MS, Lincoln SE, Etoh T (1992) A genetic map of *Brassica oleracea* based on RFLP markers detected with expressed DNA sequences and mapping resistance genes to race 2 of *Plasmodiophora brassicae* (Woronin). Genome 35:409–419

Mahajan V, Gill HS, More TA (1995) Inheritance of downy mildew resistance in Indian cauliflower (group III). Euphytica 86:1–3

Marques CM, Araujo JA, Ferreira JG, Whetten R, O'Malley DM, Liu BH, Sederoff R (1998) AFLP genetic maps of *Eucalyptus globulus* and *E-tereticornis*. Theor Appl Genet 96:727–737

Martin GB, Brommonschenkel SH, Chunwongse J, Frary A, Ganal MW, Spivey R, Wu T, Earle EA, Tanksley SD (1993) Map-based cloning of a protein kinase gene conferring disease resistance in tomato. *Science* 262:1432–1436

Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proc Natl Acad Sci USA 88:9828–9832

Morigushi K, Kimizuka-Takagi C, Ishii K, Nomura K (1999) A genetic map based on RADP, RFLP, isozyme, morphological markers and QTL analysis for clubroot resistance in *Brassica oleracea*. Breed Sci 49:257–265

Natti JJ, Atkin JD (1960) Inheritance of downy mildew resistance in broccoli. Phytopathology 50:241

Natti JJ, Dickson MH, Atkin JD (1967) Resistance of *Brassica oleracea* varieties to downy mildew. Phytopathology 57:144–147

Saal B, Plieske J, Hu J, Quiros CF, Struss D (2001) Microsatellite markers for genome analysis in *Brassica*. II. Assignment of rapeseed microsatellite to the A and C genomes and genetic mapping in *Brassica oleracea* L. Theor Appl Genet 102:695–699

Sebastian RL, Howell EC, King GK, Marshall DF, Kearsey MJ (2000) An integrated AFLP and RFLP *Brassica oleracea* linkage map from two morphologically distinct double haploid mapping populations. Theor Appl Genet 100:75–81

Smith LB, King GJ (2000) The distribution of BoCAL-a alleles in Brassica oleracea is consistent with a genetic model for curd development and domestication of the cauliflower. Mol Breed 6:603–613

Swiderski MR, Innes RW (2001) The *Arabidopsis* PBS1 resistance gene encodes a member of a novel protein kinase subfamily. Plant J 26:101–112

Van Ooijen JW, Voorrips RE (2001) JOINMAP 3.0, Software for the calculation of genetic linkage maps. Plant Research International, Wageningen

Voorrips RE, Jongerius MC, Kanne HJ (1997) Mapping of two genes for resistance to clubroot (*Plasmodiophora brassicae*) in a population of double haploid lines of *Brassica oleracea* by means of RFLP and AFLP markers. Theor Appl Genet 94:75–82

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23:4407–4414

Williams JGK, Kubelik AR, Livak JK, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18:6531–6535