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Genetic and physical mapping of the grapevine powdery mildew resistance gene, *Run1*, using a bacterial artificial chromosome library

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Abstract Resistance to grapevine powdery mildew is controlled by *Run1*, a single dominant gene present in the wild grapevine species, *Muscadinia rotundifolia*, but absent from the cultivated species, *Vitis vinifera*. *Run1* has been introgressed into *V. vinifera* using a pseudo-backcross strategy, and genetic markers have previously been identified that are linked to the resistance locus. Here we describe the construction of comprehensive genetic and physical maps spanning the resistance locus that will enable future positional cloning of the resistance gene. Physical mapping was performed using a bacterial artificial chromosome (BAC) library constructed using genomic DNA extracted from a resistant *V. vinifera* individual carrying *Run1* within an introgression. BAC contig assembly has enabled 20 new genetic markers to be identified that are closely linked to *Run1*, and the position of the resistance locus has been refined, locating the gene between the simple sequence repeat (SSR) marker, VMC4f3.1, and the BAC end sequence-derived marker, CB292.294. This region contains two multigene families of resistance gene analogues (RGA). A comparison of physical and genetic mapping data indicates that recombination is severely repressed in the vicinity of *Run1*, possibly due to divergent sequence contained within the introgressed fragment from *M. rotundifolia* that carries the *Run1* gene.

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Introduction

Grapevine powdery mildew is caused by the biotrophic pathogen, *Uncinula necator*, and is the most serious fungal disease of grapevines worldwide. The pathogen infects the leaves and berries of susceptible plants, causing a reduction in yield and berry quality. *U. necator* originated in North America and is a particular threat to cultivated grapevines as most are of the species *Vitis vinifera*, which is European in origin and lacks natural resistance to the pathogen. Powdery mildew was introduced into Europe in the mid-19th century and caused devastating losses until adequate chemical control measures were introduced (Olmo 1986). Modern grapevine cultivation relies heavily upon the use of chemical fungicides, such as sulphur and sterol biosynthesis inhibitors, to control the pathogen. However, chemical application is costly, fungal strains have evolved that are fungicide-insensitive (Pearson 1980; Erickson and Wilcox 1997) and it is now considered that widespread agrochemical use is environmentally detrimental. Consequently, it is desirable to identify natural sources of resistance to *U. necator* that might be employed to increase the resistance of cultivated vines.

One source of powdery mildew resistance is *Muscadinia rotundifolia*, a wild grapevine species originating from southeastern USA. *M. rotundifolia* is resistant to a number of pathogens known to affect cultivated grapevines, including powdery mildew, downy mildew, phylloxera and nematodes and could, therefore, provide an important source of resistance to a number of economically significant diseases (Olmo 1986). Classical genetic

studies determined that powdery mildew resistance is controlled by a single, dominant gene in *M. rotundifolia*, termed resistance to *U. necator* 1 (*Run1*) (Bouquet 1986; Pauquet et al. 2001), and a number of genetic markers have been identified that are linked to the resistance locus (Pauquet et al. 2001; Donald et al. 2002). Interestingly, the GLP1-12 and MHD145 markers identified by Donald et al. (2002) that co-segregate with *Run1* are sequences that show homology to many other plant resistance genes. The majority of plant resistance genes identified to date encode modular proteins with a central nucleotide binding site (NBS) linked to a C-terminal leucine-rich repeat (LRR) domain (Belkhadir et al. 2004). Conserved motifs within the NBS have been used to design degenerate primers capable of amplifying novel resistance gene analogue (RGA) sequences in many plants (Leister et al. 1996; Aarts et al. 1998; Collins et al. 1998) and, in the case of GLP1-12 and MHD145, the products amplified by Donald et al. (2002) were used as probes to identify restriction fragment length polymorphisms (RFLPs) linked to *Run1*. GLP1-12 is a dominant RFLP marker that hybridises a 1.6-kb *Eco*RI fragment found only in the genome of resistant plants, whereas MHD145 is a co-dominant RFLP marker that hybridises a 2.7-kb *Eco*RI fragment from the genome of susceptible plants or a 2.2-kb fragment from resistant plants (Donald et al. 2002). However, of the 17 amplified fragment length polymorphism (AFLP) and RGA-derived markers identified in previous studies, 13 co-segregated with the resistance locus in the population of approximately 160 plants tested and the remaining four adjacent markers were all located on one side of *Run1* (Pauquet et al. 2001; Donald et al. 2002). Therefore, an improved genetic map is required to define the location of the resistance locus before positional cloning can proceed.

The second requirement for successful positional cloning is the availability of a large-insert DNA library containing the gene of interest that is suitable for chromosome walking. In recent years, bacterial artificial chromosome (BAC) vectors have become the vehicle of choice for the generation of large-insert libraries due to their increased stability and ease of manipulation in comparison to previously used vectors such as cosmids or yeast artificial chromosomes (Shizuya et al. 1992; Woo et al. 1994). We describe here the construction of a BAC library suitable for positional cloning of *Run1* and the generation of comprehensive genetic and physical maps spanning the resistance locus.

Materials and methods

Plant material and evaluation of resistance to powdery mildew

Three populations of *Vitus vinifera* were used in the mapping studies: Mtp3294 (VRH3082-1-42 × *V. vinifera* cv. Cabernet Sauvignon; 161 individuals), Mtp3322 (VRH3176-21-11 × *V. vinifera* cv. Cabernet Sauvignon; 419 individuals) and Mtp3328 (*V. vinifera* cv. Marselan × VRH3082-1-49; 416 individuals). The *V. vinifera* cultivars Cabernet Sauvignon and Marselan are susceptible to powdery mildew, whereas all other parents are resistant heterozygotes that carry the *Run1* gene (Pauquet et al. 2001). Resistance to powdery mildew in the Mtp3294 population has been described by Donald et al. (2002). The Mtp3322 and Mtp3328 populations were tested according to Pauquet et al. (2001).

Simple sequence repeat marker analysis

VMC1g3.2, VMC8g9 and VMC4f3.1 are simple sequence repeat (SSR) markers that were developed by the *Vitis* Microsatellite Consortium coordinated by Agrogène, France. The Mtp3322 and Mtp3328 populations were screened with VMC8g9 and VMC4f3.1 as described by Adam-Blondon et al. (2004). The Mtp3294 population was screened with VMC1g3.2, VMC4f3.1 and VMC8g9 essentially as described by Thomas et al. (1994).

MHD145 and GLP1-12 marker analysis

Primers GLP1-12P1 and GLP1-12P3, the analysis of MHD145 and GLP1-12 as RFLP markers and the analysis of GLP1-12 as a PCR-based marker have been previously described (Donald et al. 2002).

BAC library construction

An individual *Uncinula necator*-resistant plant from the Mtp3294 population (3294-R23) was clonally propagated to generate sufficient leaf material for construction of the BAC library. Megabase-sized plant DNA was extracted, digested with *Hin*dIII or *Bam*HI and size-fractionated essentially as described by Peterson et al. (2000). The library was constructed in pIndigoBAC-5 (Epicentre, Madison, Wis.), and individual clones were stored in 144 × 384-well plates.

The library was gridded onto three 22.5-cm^2 Hybond N+ filters (Amersham Biosciences, Buckinghamshire, UK) using the Genetix Q-Bot (Genetix, Hampshire, UK) and screened by hybridisation using standard techniques (Sambrook and Russell 2001). The PCR products were used as templates to generate [^{32}P]-labelled probes using the GIGAprime DNA labelling kit (Geneworks, Adelaide, Australia) or Rediprime II DNA labelling kit (Amersham Biosciences). The sequence of the chloroplast genome-encoded RNA polymerase B gene from grapevine was obtained by PCR using primers RPOB1 (5′-CTT CCG AAT TAT ATG TAT CCG CG-3′) and RPOB2 (5′-CGA TTC ATA TTT CGT CGA CCA AC-3′).

For PCR-based screening, we constructed 29 DNA superpools that contained equal volumes of BAC DNA extracted from 384-well plates following overnight culture. Products were initially amplified from the 29 superpools, then individually from DNAs extracted from the five plates that composed any superpool found to contain a clone of interest. Single plates were then replicated twice, enabling DNAs to be extracted from groups of four columns or four rows, respectively. The PCR using row- and column-pooled DNAs identified a group of 16 clones that were then individually tested by colony PCR to identify the clone of interest.

BAC DNA sequencing

The BAC end sequences were obtained either by using the sub-cloning procedure of Yang and Mirkov (2000) or by direct sequencing. Approximately 25 μg BAC DNA was used for sequencing using BigDye terminator V3.1 chemistry (Applied Biosystems, Foster City, Calif.) in a 40-μl volume containing 0.5 μM sequencing primer. Thermal cycling was performed in a PTC-200 thermal cycler (MJ Research, Waltham, Mass,) using 80 cycles of: 94°C for 30 s, 55°C for 30 s, 68°C for 4 min.

Results

Genetic mapping

The previous studies of Pauquet et al. (2001) and Donald et al. (2002) indicated that *Run1* co-localised with 11 AFLP and two RGA-derived markers in a Mtp3294 population of 160 plants derived from a cross between the resistant parent, VRH3082-1-42 and the susceptible parent, *V. vinifera* cv. Cabernet Sauvignon. In an attempt to identify further markers that were linked to the *Run1* locus, we examined SSR markers to identify those that showed polymorphism between the resistant and susceptible parents of the Mtp3294 population. Three such markers were identified (VMC1g3.2, VMC4f3.1 and VMC8g9), and analysis of these markers in the Mtp3294 population indicated that all three were genetically linked to *Run1* (Fig. 1). VMC8g9 completely co-segregated with *Run1* in this population, whereas VMC4f3.1 and VMC1g3.2 were located 0.6 cM or 4.4 cM away from the resistance locus, respectively.

To facilitate scoring of the AFLP markers identified by Pauquet et al. (2001), amplified products corresponding to EMfd3 and EMhb1 were cloned, sequenced and used as probes in RFLP experiments or as PCR-based markers. Sfd3 and Shb1 were markers derived from Emfd3 and Emhb1, respectively, and both completely co-segregated with *Run1* in a sample of 160 plants tested from the Mtp3294 population (data not shown).

As a large number of markers co-segregated with *Run1* in the Mtp3294 population, we sought additional recombinant plants to enable markers in this region to be ordered. Two alternative populations were examined: Mtp3322, which was derived from a cross between the resistant parent, VRH3176-21-11 (Pauquet et al. 2001), and the susceptible parent, *V. vinifera* cv. Cabernet Sauvignon, and Mtp3328, which was derived from a cross between the resistant parent, VRH3082-1-49 (Pauquet et al. 2001), and the susceptible parent, *V. vinifera* cv. Marselan. Plants were initially scored using the PCR-based markers GLP1-12, VMC4f3.1, VMC8g9 and Shb1. Once recombinant plants had been identified, they were tested for powdery mildew resistance, and additional markers were analysed. This resulted in the isolation of a number of recombinant plants, two of which showed recombination between VMC8g9 and *Run1* and four of which showed recombination between Shb1 and *Run1*. This allowed a marker order to be assigned in which VMC8g9 and Shb1 are located on the opposite side of *Run1* to VMC4f3.1 (Fig. 1). Thus, *Run1* is located in the interval defined by the closest flanking markers, VMC4f3.1 and VMC8g9.

Generation of BAC library

To facilitate positional cloning of the *Run1* gene, a BAC library was generated using the genomic DNA of a resistant individual from the Mtp3294 population (3294-R23) as the DNA source. In total, the 3294-R23 BAC library contained 55,295 clones, of which 49,920 were *Hin*dIII-derived and 5,376 were *Bam*HI-derived. An analysis of 38 randomly selected clones indicated that insert sizes ranged between 40 kb and 160 kb, with an average insert size of 93 kb. Less than 5% of the clones did not contain an insert, and approximately 1.7% of the clones contained chloroplast-derived DNA, as determined by screening one-third of the library with the grapevine RNA polymerase B chloroplast gene (data

Plant	VMC1g3.2	**MHD98**	VMC4f3.1	**GLP1-12**	*Run1*	Sfd3	**MHD145**	VMC8g9	Shb1
3294-R11	+	-	-	-	-	-	-	-	-
3294-R102	+	-	-	-	-	-	-	-	-
3294-S100	+	-	-	-	-	-	-	-	-
3294-R149	+	+	-	-	-	-	-	-	-
3294-R5	-	-	+	+	+	+	+	+	+
3294-R104	-	-	+	+	+	+	+	+	+
3294-R37	-	-	-	+	+	+	+	+	+
3328-245	-	-	-	-	-	-	-	+	+
3322-42	-	-	-	-	-	-	-	+	+
3328-174	-	-	-	-	-	-	-	-	+
3328-422	-	-	-	-	-	-	-	-	+

Fig. 1 Genotype of informative recombinant plants isolated from the *Vitus vinifera* populations Mtp3294, Mtp3322 and Mtp3328. The presence of either the resistant (+) or susceptible (−) allele of each marker is shown. Markers shown in *bold* are RGA markers previously described by Donald et al. (2002)

not shown). Taking into account average insert size, percentage of empty clones, percentage of clones containing chloroplast DNA and a genome size of 511 Mb (Thomas et al. 1993), the library is calculated to contain 9.4-fold coverage of the haploid grapevine genome.

Assembly of BAC contigs containing genetic markers

To initiate a physical map of the region containing *Run1*, we screened the 3294-R23 BAC library to identify clones containing three genetic markers that co-segregated with resistance (GLP1-12, MHD145 and Sfd3) and two flanking markers (VMC8g9 and VMC4f3.1). For VMC4f3.1, VMC8g9 and Sfd3, between seven and nine clones were isolated from the library, close to the expected representation of a single-copy sequence in a library of predicted 9.4-fold haploid genome coverage. In contrast, over 60 clones were identified that contained MHD145-hybridising sequences, and over 50 hybridised the GLP1-12 probe. This indicated that GLP1-12 and MHD145 might represent repeated elements, or gene families, present within the grapevine genome.

Once clones containing genetic markers had been identified, they were assessed to determine the allele present. The BACs were then grouped into "resistant" and "susceptible" contigs representing the chromosome containing *Run1* derived from the resistant parent, VRH3082-1-42, or the chromosome lacking *Run1* derived from the susceptible parent, *V. vinifera* cv. Cabernet Sauvignon, respectively. A comparison of restriction digest patterns was used to determine overlap between BAC clones, and alignments were confirmed by PCR using primers designed from BAC end sequences. To determine if primers designed using BAC end sequences could be used as PCR-based markers for powdery mildew resistance, we amplified products from a selection of resistant and susceptible genotypes from the Mtp3294 population. This enabled a large number of dominant and co-dominant markers to be identified. Primers that amplified products of identical size from both resistant and susceptible genotypes were used to directly sequence BAC clones assigned to contigs representing each chromosome that were known to contain the region of interest. Alignment of sequences enabled polymorphic nucleotides to be identified that could be utilised to generate cleaved amplified polymorphic sequence (CAPS) markers. Primers were then used to isolate overlapping clones that could be used to extend BAC contigs (Fig. 2).

For all three single-copy genetic markers (VMC4f3.1, Sfd3 and VMC8g9), BAC contigs were assembled that represented the resistant and susceptible chromosome surrounding the marker. The haplotypic nature of corresponding contigs was confirmed in each case as BAC end sequences could be used to generate new co-dominant markers. The largest BAC contig assembled was initiated from Sfd3, and the minimum tiling path consisted of five clones representing the resistant chromosome. The contig was estimated to span over 400 kb, but could not be extended beyond CB298.299 as 86E16 was the only clone in the library that contained this sequence.

Assembly of BAC contigs containing RGA families

BACs containing MHD145-hybridising and GLP1-12-hybridising sequences were examined by RFLP analysis, which enabled them to be grouped into contigs representing the resistant or susceptible chromosomes (Fig. 2). Of 49 clones examined, only two overlapping BACs were identified that contained the 2.2-kb MHD145-hybridising *Eco*RI fragment characteristic of resistant plants, and of 43 GLP1-12-hybridising BACs examined, five contained the 1.6-kb *Eco*RI fragment characteristic of resistant plants.

The two overlapping BACs that contained the 2.2-kb MHD145-hybridising *Eco*RI fragment contained additional fragments that hybridised the probe, indicating that more than one homologous sequence was present.

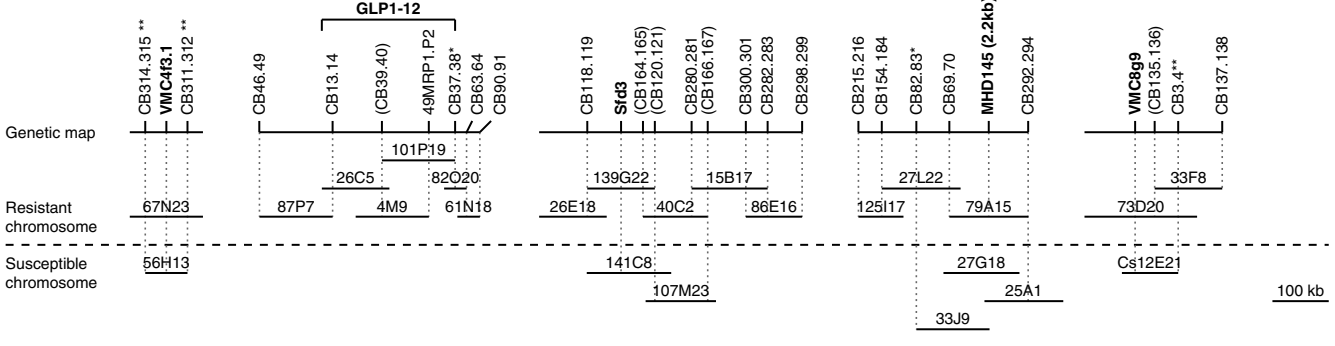

Fig. 2 A physical map of the region surrounding *Run1*. The BAC clones were identified that contained genetic markers linked to *Run1*. Clones were assembled into contigs representing the resistant or susceptible chromosome and extended by the identification of overlapping BACs. Genetic markers used to initiate contigs are shown in *bold*, and the location of multiple GLP1-12-hybridising sequences is shown by *square brackets*. PCR products used to align BACs are shown, and their presence in individual BAC clones is indicated by *dotted lines*. The PCR products that could not be used as genetic markers, but were informative in contig assembly, are shown in *parenthesis*. All other PCR products are dominant, co-dominant (*single asterisk*) or CAPS (*two asterisks*) markers for powdery mildew resistance

By comparing *Eco*RI digests and RFLP patterns, other MHD145-hybridising BACs could be aligned with those containing the fragments characteristic of the co-dominant marker (Fig. 3), and overlap between clones was confirmed by PCR amplification using primers designed to BAC end sequences. Three BACs containing MHD145-hybridising sequences were assembled into each of the resistant and susceptible contigs (Fig. 2), and additional co-dominant markers designed using BAC end sequence information confirmed that the two contigs were haplotypes. The resistant contig contained three MHD-145 hybridising sequences, and the susceptible contig contained six, as determined by RFLP analysis (Fig. 3).

In contrast, the five clones identified that contained the 1.6-kb *Eco*RI fragment that hybridised the GLP1-12 probe did not completely overlap, suggesting that the hybridisation signals observed upon RFLP analysis of genomic DNA might be contributed by multiple members of a gene family. Three BACs containing the characteristic 1.6-kb *Eco*RI fragment formed the minimum tiling path through the resistant contig (26C5, 4M9 and 101P19), and a further GLP1-12-hybridising BAC (87P7) could be added to the contig using the dominant marker, CB13.14, to confirm overlap with 26C5 (Figs. 2, 4a). Two BAC clones that did not contain GLP1-12-hybridising sequences (82O20 and 61N18) were also added to the contig using the BAC end-derived markers, CB37.38 and CB63.64 (Figs. 2, 4a). However, the contig could not be extended beyond CB90.91 due to a lack of additional clones present in the library that contained this dominant marker sequence.

To confirm that multiple GLP1-12-hybridising sequences were present in the BAC contig, products were amplified from GLP1-12-containing BACs using primers GLP1-12P1 and GLP1-12P3. Following RFLP

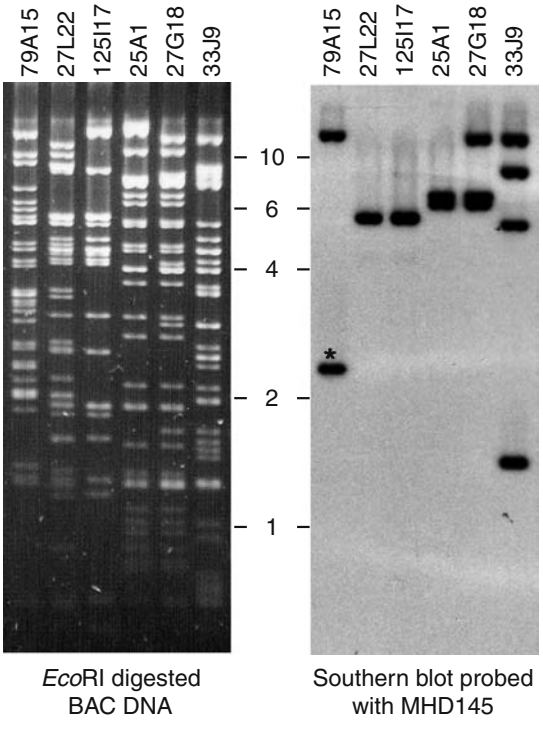

Fig. 3 MHD145 hybridises to a multigene family of RGAs. BAC DNAs containing MHD145-hybridising sequences were digested with *Eco*RI, and DNA fragments were separated by agarose gel electrophoresis. Ethidium bromide-stained DNA fragments were visualised using a UV transilluminator (*left*) before preparation of a Southern blot (*right*). Approximate sizes are given in kilobase pairs, and the location of the 2.2-kb *Eco*RI fragment that co-segregates with powdery mildew resistance upon RFLP analysis of genomic DNA is indicated by an *asterisk*

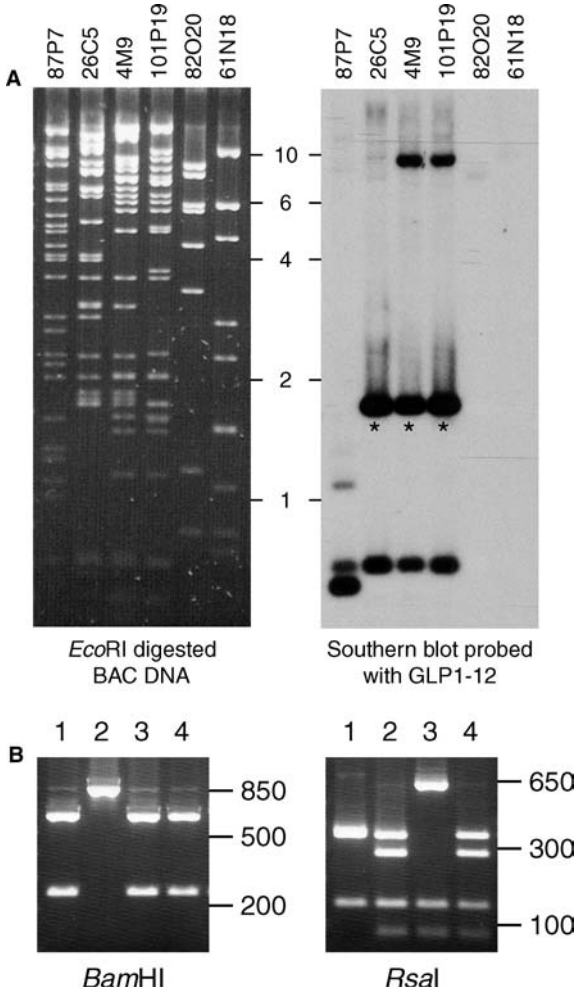

Fig. 4 GLP1-12 hybridises to a multigene family of RGAs. **A** BAC DNAs containing GLP1-12-hybridising sequences were digested with *Eco*RI, and the fragments were separated by agarose gel electrophoresis. Ethidium bromide-stained DNA fragments were visualised using a UV transilluminator (*left*) before preparation of a Southern blot (*right*). Approximate sizes are given in kilobase pairs, and the location of the 1.6-kb *Eco*RI fragment that co-segregates with powdery mildew resistance upon RFLP analysis of genomic DNA is indicated by an *asterisk*. **B** PCR products were amplified from BAC DNAs containing GLP1-12-hybridising sequences using primers GLP1-12P1 and GLP1-12P3. Products were cloned and used as templates for RFLP analysis. Four clones displaying distinct restriction patterns are shown (*numbered 1–4*). Approximate sizes are given in base pairs

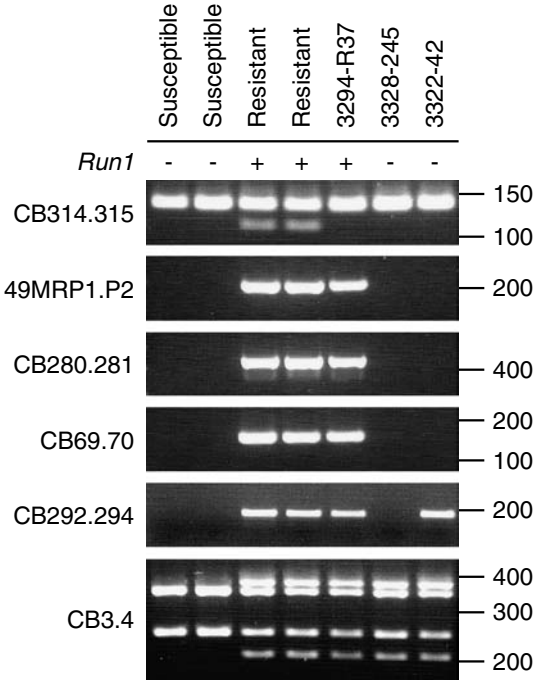

Fig. 5 Analysis of recombinant plants using BAC-derived markers identifies the recombination breakpoint in 3322-42. Two resistant and two susceptible, non-recombinant genotypes from the Mtp3294 population were analysed using BAC-derived markers along with the recombinant genotypes 3294-R37, 3328-245 and 3322-42. For CB314.315, *Afl*II-digested products are shown; for CB3.4, *Taq*I-digested products are shown. All other markers are dominant PCR-based markers for resistance. Approximate product sizes are indicated in base pairs

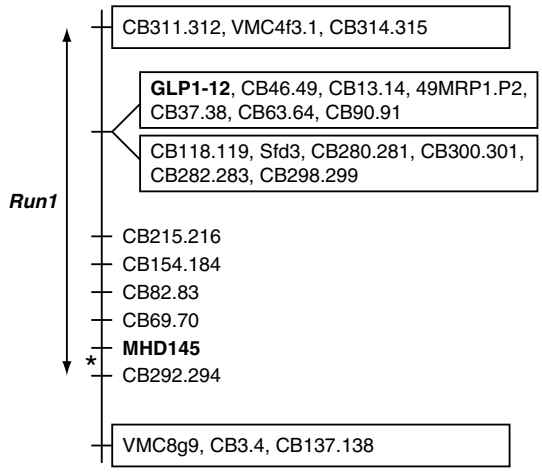

Fig. 6 A schematic representation of marker order as determined by a combination of genetic and physical mapping. As a result of contig assembly, 20 new BAC-derived genetic markers have been identified that are closely linked to *Run1*. The location of these markers is shown with respect to previously characterised genetic markers. An *arrow* indicates the predicted location of *Run1*, and an *asterisk* marks the recombination breakpoint in 3322-42 that defines the position and orientation of the MHD145 contig with respect to other markers. Markers shown in *bold* are RGA markers previously described by Donald et al. (2002). Markers belonging to BAC contigs that cannot be orientated by available recombinant plant data are grouped and *boxed*

analysis of individually cloned products, we were able to identify four distinct family members (Fig. 4b).

Genetic mapping using BAC-derived markers

All Mtp3322, Mtp3294 and Mtp3328 recombinant plants were rescreened with BAC-derived genetic markers obtained as a result of physical mapping. This allowed the recombination breakpoint in 3322-42 to be located within the MHD145 contig as between the BAC-derived markers, CB292.294 and CB69.70 (Fig. 5). These two markers were generated using sequence data obtained from the two ends of a single BAC clone, 79A15, which also contains MHD145 (Fig. 2). As 3322-42 possesses the resistant allele of CB292.294 (Fig. 5) but the susceptible alleles of MHD145 (Fig. 1) and CB69.70 (Fig. 5), the recombination breakpoint is located in an interval predicted to be less than 100 kb, between markers MHD145 and CB292.294. Significantly, the identification of the recombination breakpoint in 3322-42 defines the position and orientation of the MHD145 contig with respect to other genetic markers, placing MHD145 between VMC8g9 and GLP1-12/Sfd3 (Fig. 6). It also allows the position of *Run1* to be refined, placing the resistance locus between VMC4f3.1 and CB292.294.

Discussion

Genetic and physical mapping of the *Run1* locus

Utilising the wealth of information now available through the construction of *Vitis* linkage maps, we identified three SSR markers that were genetically linked to the resistance locus. *Run1* was located in the interval between the closest flanking SSR markers, VMC4f3.1 and VMC8g9 and, as such, is located in a region equivalent to linkage group 12 of the *V. vinifera* consensus maps of Riaz et al. (2004) and Adam-Blondon et al. (2004). Interestingly, our analysis of recombinant plants in the Mtp3322, Mtp3328 and Mtp3294 populations indicated that, of the three SSR markers linked to *Run1*, VMC4f3.1 was located between VMC1g3.2 and VMC8g9. This order differs from the map published by Riaz et al. (2004), who place VMC1g3.2 between VMC4f3.1 and VMC8g9. The reason for this discrepancy is unknown. However, the identification of markers that flanked *Run1* on both sides was of crucial importance, as it delimited the locus and allowed the production of a physical map spanning the locus to proceed.

The production of a comprehensive BAC library suitable for chromosome walking allowed contigs containing five genetic markers that co-localised or flanked the *Run1* locus to be constructed. Interestingly, for four

out of five of these markers, contigs representing homologous regions could be assembled that were derived either from the resistant chromosome that carried *Run1* or from the susceptible chromosome contributed by *V. vinifera* cv. Cabernet Sauvignon. The haplotypic nature of all four pairs of contigs was confirmed by the discovery of numerous co-dominant markers designed using BAC end sequences, indicating that the introgression from *M. rotundifolia* that carries the *Run1* gene shows co-linearity with the corresponding region of the *V. vinifera* genome. The only contig for which an equivalent region representing the susceptible chromosome could not be constructed was initiated from the RGA-marker, GLP1-12. GLP1-12 is a dominant marker for resistance, as were all six new genetic markers designed using BAC end sequences from the GLP1-12 contig. Consequently, these markers could not be used to identify BAC clones representing the susceptible chromosome. The inability to identify co-dominant markers might indicate significant sequence divergence in this region between the *V. vinifera* and *M. rotundifolia* chromosomes.

Although our BAC library was theoretically comprehensive, contig extension revealed that in at least two locations there are gaps in the coverage of the library. The extension of contigs beyond markers CB90.91 and CB298.299 was impossible due to a lack of overlapping clones. Two restriction enzymes were used to digest high-molecular-weight genomic DNA to produce BAC clones. However, the majority of clones (91.3%) were generated as a result of *Hin*dIII digestion. The existence of gaps in the library might represent bias in the distribution of *Hin*dIII sites in the grapevine genome, as over-representation or under-representation of *Hin*dIII sites would lead to the production of *Hin*dIII fragments too small or too large for cloning, respectively. This problem may be overcome by using high-molecular weight DNA digested with a number of different restriction enzymes, ensuring that the library constructed is completely representative of the genome.

Two different families of RGAs co-segregate with *Run1*

The 20 BAC-derived genetic markers that resulted from physical mapping were used to refine the location of *Run1* to within an interval between markers CB292.294 and VMC4f3.1. This region contains two families of RGA sequences, both of which are currently candidates for containing the *Run1* gene. The MHD145 probe hybridises to at least three homologous genes present on the resistant chromosome and six on the susceptible. The multigenic nature of this locus supports the previous observations of Donald et al. (2002), who noted that multiple restriction fragments hybridised MHD145 in RFLP studies. In contrast, Donald et al. (2002) observed only one RFLP fragment that hybridised GLP1-12 in mapping studies, whereas our physical mapping data now suggests that this locus is also multigenic, comprising at least four members on the resistant chromosome.

Many disease resistance genes are members of multigene families, and these vary in size, both in terms of number of homologues present and the region they span. One of the largest RGA families is the *Dm3* cluster found in lettuce that confers resistance to the downy mildew pathogen, *Bremia lactucae*. This locus contains 24 NBS-LRR encoding genes in a region spanning 3.5 Mb, with an average intergenic distance of 145 kb (Meyers et al. 1998). In contrast, the tomato *I2* locus contains seven NBS-LRR encoding genes in a region of 90 kb, with an intergenic distance of 8–10 kb (Simons et al. 1998), the potato *Gpa2* locus contains four NBS-LRR encoding genes in a region of 115 kb (van der Vossen et al. 2000) and the tomato *Mi* locus contains three NBS-LRR encoding genes in a region of 52 kb (Milligan et al. 1998). Consequently, the multigenic nature of the MHD145 and GLP1-12-hybridising sequences linked to *Run1* is not unusual for an RGA locus.

The introgression from *M. rotundifolia* is associated with repressed recombination

The pronounced clustering of markers identified by Pauquet et al. (2001), Donald et al. (2002) and in the current study suggests that either these markers are physically close to *Run1* or that recombination rates are unusually low in the vicinity of the resistance locus. Two lines of evidence now suggest that the latter is true. First, both Riaz et al. (2004) and Adam-Blondon et al. (2004) used the SSR markers, VMC4f3.1 and VMC8g9, during the construction of linkages maps spanning the *V. vinifera* genome and placed these two markers 12.85 cM or 10.9 cM apart in consensus maps, respectively. In the Mtp3294 mapping population studied, VMC8g9 and VMC4f3.1 were placed 0.6 cM apart in the map of the resistant female parent and 14.1 cM apart in the susceptible male map (Adam-Blondon unpublished), indicating at least an 18- to 23-fold reduction in recombination frequency in genotypes heterozygous for powdery mildew resistance. Second, based on the genome size of grapevine being between 475 Mb (Lodhi and Reisch 1995) and 511 Mb (Thomas et al. 1993) and the total linkage map of *V. vinifera* being between 1,728 cM (Riaz et al. 2004) and 2,200 cM (Adam-Blondon et al. 2004), on average 1 cM should be equivalent to approximately 216–296 kb. However, we have assembled three contigs that represent the resistant chromosome surrounding genetic markers GLP1-12, Sfd3 and MHD145 that together span over 1 Mb, but have not yet observed physical linkage between contigs, despite complete co-segregation of these markers in over 900 plants studied from three mapping populations.

Reduced recombination frequencies have been observed around resistance loci in wheat, barley and poplar where resistance has been introduced via an introgressed

region from a related species (Wei et al. 1999; Stirling et al. 2001; Neu et al. 2002). Therefore, the dramatically reduced recombination rate in the vicinity of *Run1* might be due to the presence of divergent DNA contributed by the introgression from *M. rotundifolia*. The implication of low recombination frequencies in this region for future isolation of the *Run1* gene is that large populations may be required in order to obtain sufficient recombinant individuals to delineate the resistance locus with suitable precision.

Conclusion

The generation of comprehensive genetic and physical maps spanning the *Run1* locus has greatly improved our ability to localise this agriculturally significant disease resistance gene and will provide an excellent foundation for future map-based cloning efforts. Once identified, *Run1* will provide a natural means to improve the resistance of cultivated grapevines to *U. necator* worldwide without the need for extensive agrochemical use.

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