Abstract The monogenic dominant genetic determinism of total resistance to powdery mildew, introduced from *Muscadinia rotundifolia* into *Vitis vinifera*, was further assessed in BC$_5$ and BC$_6$ full-sib families. A BC$_5$ population of 157 individuals was used to select AFLP markers linked to the resistance gene, *Run*$_1$. Thirteen AFLP markers were selected and a local map was constructed around the *Run*$_1$ gene. Ten markers among the 13 were found to co-segregate with the resistance gene. The usefulness of these 13 AFLP markers for the selection of *Run*$_1$-carrying genotypes was further assessed through their analysis in a set of 22 *Run*$_1$-carrying resistant genotypes and 16 susceptible genotypes. Three markers out of the 13 analysed were found to be absent in all susceptible genotypes and present in all resistant individuals, and may thus represent good tools for the marker-assisted selection of grapevine varieties resistant to powdery mildew. A recombination event among the markers that were originally found to co-segregate was observed in one of the resistant individuals, showing that recombination is possible in this region and may therefore be observed in larger populations.

Keywords *Vitis vinifera* · *Uncinula necator* · Resistance · AFLP · Introgression

Introduction

The most-widely cultivated grapevine is the *Vitis vinifera* species which originated in middle Asia and subsequently spread over all of occidental temperate Asia, southern Europe and northern Africa (Olmo 1976). *V. vinifera* is valued for the quality of its berries for wine or table consumption; however, it is susceptible to a number of potentially damaging pathogens. Among the most threatening are the powdery and downy mildews which were introduced during the 19th century together with accessions of the American wild *Vitis* species in which they were endemic (Galet 1977). Powdery mildew is caused by the fungus *Uncinula necator* (Schw. Burr.) which is able to attack species in all the *Vitaceae* family (Boubals 1961). It was first described in 1845 in a greenhouse in Great-Britain and it rapidly spread into Europe, causing severe yield losses (Galet 1977). It is now a world-wide problem in viticulture. Despite the fungicidal effect of sulphur discovered in 1850 and considerable progress made in the development of new organic fungicides, the parasite is still difficult to control for a number of reasons including the appearance of strains resistant to systemic fungicides (Steva et al. 1988; Debieu et al. 1995), the existence of two sources of primary inoculum (hyphae in dormant buds or cleistothecia in bark; Boubals 1961; Diehl and Heintz 1987; Pearson and Gadoury 1987; Gadoury and Pearson 1988; Delye and Corio-Costet 1998), and difficulties in establishing epidemiological models efficient in any climate (Thomas et al. 1994). From a more general point of view, the limitation of systematic chemical sprayings would be of significant benefit to environment and health.

At the turn of the 20th century, the first attempts to select resistant varieties to downy and powdery mildew were conducted by private breeders (Olmo 1976; Galet 1988). Many accessions of American *Vitis* species (*Vitis labrusca*, *Vitis rupestris*, *Vitis riparia*, *Vitis aestivalis*) are partially or totally resistant to powdery mildew. In contrast, *V. vinifera* is classified as susceptible, although different cultivars do show varying levels of susceptibility (Boubals 1961) and an ontogenetic partial resistance has been reported in developing grape berries (Gadoury et al. 1997; Ficke et al. 1999). The resistant varieties that were bred were thus interspecific complex hybrids between several resistant *Vitis* accessions and *V. vinifera* (Galet 1988). However, good fruit quality was not fully recovered, mainly because of the quantitative inheritance of resistance and quality traits (Boubals...
The muscadine grape \([M.\ rotundifolia\ (Michx.)\ Small]\), which originated from South-East USA, is highly resistant to most of the \(V.\ vinifera\) pathogens (Olmo 1971; Bouquet 1986) including \(Uncinula\ nectar\). The species was first classified by Planchon (1887) in the genus \(Vitis\), section \(Muscadinia\), but, due to its anatomical and morphological characteristics, Small (1903) proposed that the section \(Muscadinia\) be classified as a distinct genus. The discovery that the chromosome numbers were different in \(Vitis\ (2n=38)\) and \(Muscadinia\ (2n=40)\) gave new support to this proposal (Branas 1932). Programs aimed at the introgression of resistance genes from \(M.\ rotundifolia\) into \(V.\ vinifera\) were developed (Wylie 1871; Detjen 1919a, b) but have been limited by the high sterility of the hybrids (Patel and Olmo 1955; Nesbitt 1966; Olmo 1971; Bouquet 1986). A total resistance to powdery mildew derived from \(M.\ rotundifolia\) was demonstrated to be controlled by a single dominant locus by Bouquet (1986). This locus, called \(Run1\) (for \(Resistance\ to\ Uncinula\ nectar\ 1\)), was introduced into the \(V.\ vinifera\) genome using a pseudo-backcross strategy (different \(V.\ vinifera\) genotypes are used at each backcross step to avoid inbreeding) aimed at the creation of new good quality grape varieties which are resistant to powdery mildew (Bouquet 1986).

The objectives of the present work were to further assess the monogenic dominant determinism of the resistance, to develop markers tightly linked to the \(Run1\) locus and to address the usefulness of these markers for breeding purposes. Such markers would be useful for a number of reasons including: (1) in order to select individuals with the smallest introgressed \(Muscadinia\)-originated genomic fragment, (2) to be able to monitor its association with partial resistance in breeding programs, (3) to screen other \(M.\ rotundifolia\) accessions, and (4) to facilitate isolation of the gene by map-based cloning.

Materials and methods

Plant material

Resistant plants and segregating populations were derived from different steps of the breeding program aimed at the development of resistant grapevine cultivars through the introgression of the \(Run1\) gene into \(V.\ vinifera\). The name and origin of populations is summarised in Table 1 and the names and origin of resistant individuals are described in Fig. 1. Throughout this paper, a backcross (BC) is used to define a pseudo-backcross as described in the introduction. It should be noted that the BC1, individual VRH12–4-80 was named VRH12-4-89 in Bouquet (1986). The search for \(Run1\)-linked molecular markers, was carried out through the analysis of the population Mtp3294 (VRH13082–1–42xCabernet-Sauvignon N) derived from the \(M.\ rotundifolia\) accession GS2 after five pseudo-backcrosses (Bouquet 1986). The susceptible \(V.\ vinifera\) cultivars that were used for molecular analysis were: Aranel B, Arriola B, Aubun N, Baroque B, Cabernet franc N, Cabernet-Sauvignon N, Chasan B, Cot N, Fer N, Grenache N, Marselan N, Merlot N, Pinot N, Riesling B, Semillon B and Syrah N. A partially resistant complex hybrid, Villard blanc, of different \(Vitis\) species including \(V.\ vinifera\) was also analysed (Galet 1988). All plants were grown at the experimental stations of Chapitre and Vassal near Montpellier (France).

Pathogenicity tests

In vivo greenhouse pathogenicity tests were performed in July, by spraying the seedlings with a suspension of conidia made from natural field isolates. The sprays were repeated two or three times at 1-week intervals until the infestation was homogeneous. Resistant and susceptible plants were scored 1 month later as described by Bouquet (1986). In the case of the tests made in 1987, the results obtained in the greenhouse were checked the next year in a nursery field with high disease pressure. In vitro pathogenicity tests were performed using a \(U.\ nectar\) mononoclonal isolate (Mtp1) obtained from an infested grapevine in a greenhouse at Montpellier (France). Cabernet-Sauvignon young leaves were detached from 3-month-old cuttings and surface-sterilised by soaking for 1 min in a 1% v/v sodium hypochlorite solution. The leaves were then rinsed two times with sterilised water, dried with sterilised absorbent paper and placed onto medium containing agar (2% w/v) and Benzimidazol (0.003% w/v) in a Petri dish. The upper side of the leaves was then dry inoculated with spores from the Mtp1 isolate as described by Cartolaro and Steva (1990). The leaves were used as sources of inoculum 10 to 15 days after inoculation. Plants were screened for resistance to powdery mildew, as described by Cartolaro and Steva (1990), by infection of leaf disks (three per genotype) using Cabernet-Sauvignon as a susceptible control. About 8–10 days after inoculation, sporulations on leave disks were observed and scored in two classes: resistant (no conidia or conidiophores) and susceptible (production of conidia).

Preparation of genomic DNA

Total DNA was extracted using the protocol of Lodhi et al. (1994) modified as follows. Grapevine leaves (1.5 g) were harvested and ground in the presence of liquid nitrogen. Ten microliters of extraction buffer (Tris HCl 0.1 M, EDTA 0.02 M, NaCl 1.4 M, CTAB 2%, β-mercaptoethanol 1%) were added. The homogenate was incubated at 65°C for 30 min. Most of the proteins and polysaccharides were removed by centrifugation following the addition of 10 ml of chloroform/isoamyl alcohol (24:1). Thirty micro-liters of cold 95% ethanol was added to the supernatant in the presence of 1.5 M NaCl. After 5 min, the DNA pellet was extracted with a Pasteur pipette, dried on absorbent paper, dissolved in TE buffer pH 8.0 (Maniatis et al. 1989) and stored at 4°C. DNA concentration was estimated by comparison with known quantities of phage λ DNA (GIBCO-BRL, Gaithersburg, Md., USA).

AFLP analysis

AFLP (amplified fragment length polymorphism) is a molecular marker technique based on selective PCR amplification of restriction fragments first described by Vos et al. (1995). Two hundred and fifty nanograms of genomic DNA were digested during 3 hours at 37°C with 2.5 units of EcoRI (Boehringer Mannheim, Germany) and Msel (GIBCO-BRL). The ligation of double-stranded adapters to the ends of the restriction fragments was performed at 20°C for 2 h according the instructions of the AFLP Analysis System I kit (GIBCO-BRL). Pre-amplification and amplification steps were performed using Promega Tag polymerase (Madison, Wisconsin, USA) according to the instructions of the AFLP Analysis System I kit (GIBCO-BRL). The pre-amplification step was performed with primers specific for the EcoRI and Msel adapters, including the selective nucleotides A and C respectively (EcoRI+A: 5'-GAC TGC GTA CCA ATT CA-3'; Msel+C: 5'-GAT GAG TCC TGA GTA AC) and the selective amplification step using the same primers with two additional selective nucleo-