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Molecular linkage maps of Vitis vinifera L. and Vitis riparia Mchx

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Abstract Two linkage maps for grape (*Vitis* spp.) have been developed based on 81 F_1 plants derived from an interspecific cross between the wine cultivar Moscato bianco (*Vitis vinifera* L.) and a *Vitis riparia* Mchx. accession, a donor of pathogen resistance traits. The double pseudotest-cross mapping strategy was applied using three types of molecular markers. The efficiency of SSRs to anchor homologous linkage groups from different *Vitis* maps and the usefulness of AFLPs in saturating molecular linkage maps were evaluated. Moreover, the SSCP technique was developed based on sequence information in public databases concerning genes involved in flavonoid and stilbene biosynthesis. For the maternal genetic map a total of 338 markers were assembled in 20 linkage groups covering 1,639 cM, whereas 429 loci defined the 19 linkage groups of the paternal map which covers 1,518 cM. The identification of 14 linkage groups common to both maps was possible based on 21 SSR and 19 AFLP loci. The position of SSR loci in the maps presented here was consistent with other published mapping experiments in *Vitis*.

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Introduction

Species assigned to the genus *Vitis* subgenera *Euvitis* Planch. (2n = 38) are populations of inter-fertile grapes which are distinguished on the basis of morphology and geographical distribution (Small 1903). Several of them are valuable sources of genes for resistance to diseases, insects and abiotic stresses (Mullins et al. 1992).

When phylloxera and fungal diseases began to decimate the European vineyards in the second half of the 19th century, a great impulse was given to resistant wine grape breeding. Epidemic fungal infections of *Uncinula necator* and *Plasmopara viticola* on leaves and fruits of *Vitis vinifera* were reported starting from 1845 and 1878 respectively. The first attacks of the root system by the aphid *Phylloxera vastatrix* were recorded in 1860. Despite the successful development of phylloxera-tolerant rootstocks, breeding programs to combine fungal resistance with the high wine quality of *V. vinifera* were drastically reduced in most countries soon after the turn of the century. This pitfall was mainly due to undesirable flavour compounds introduced from American *Vitis* species, resulting in unpalatable wines (Alleweldt and Possingham 1988).

Today, the introduction of traditional varieties grafted on phylloxera-resistant rootstocks combined with improved agronomic technique and chemical protection, are central to modern grape cultivation. However, there is an increasing environmental concern about chemical treatments and their residues, and in their use the allowed levels become more and more restrictive. Genetic improvement of grapevine cultivars is impaired by its plant size and long life cycle, combined with clone heterozygosity and the presence of inbreeding depression. Moreover, the few morphological genetic markers available have not yet been used to produce genetic linkage maps.

In recent years, the rapid development of molecular genetics has provided the tools to allow for grape as well

as for other perennial species, an efficient approach to genetic analysis and breeding. Molecular markers have been widely applied in grape for cultivar identification and parentage analysis (see Sefc et al. 2001 for a review), but only recently the development of DNA markers was applied to the optimization of breeding strategies (Lahogue et al. 1998; Pauquet et al. 2001; Donald et al. 2002). The first grape linkage map was generated by Lodhi et al. (1995) and was mostly based on RAPD markers. A number of mapping experiments were subsequently started, based on different crosses and designed to exploit the informativeness of markers, such as the codominant SSR (Dalbò et al. 2000; Doligez et al. 2002; Riaz et al. 2002). In the frame of a grapevine breeding program for resistance to downy mildew (*P. viticola*), the work presented in this paper describes the creation of a genomic map of *Vitis* as a first step towards the production of QTL information to be used in practical breeding for disease resistance and quality.

Materials and methods

Plant material and DNA extraction

The mapping population consisted of 81 F1 individuals derived from the cross between the cv Moscato bianco of *V. vinifera* L., used as female parent (P1), and the *Vitis riparia* Mchx. accession Wr 63 from the Ampelographic Collection of the Istituto Agrario San Michele all'Adige – IASMA, which bears only male flowers (P2). The female parent, also known as "Muscat blanc à petits grains", is a grapevine cultivar producing aromatic wines. Like the large majority of *V. vinifera* varieties, Moscato b. is susceptible to important fruit and foliar pathogens as gray mold (*Botrytis cinerea* Pers.), downy mildew (*P. viticola* (Berk. & Curt.) Berl. & de Toni) and powdery mildew (U. *necator* (Schw.) Burr.). The male parent is resistant or tolerant to several fungal diseases, including downy mildew.

DNA was isolated from young leaves following the Doyle and Doyle (1990) procedure with few modifications: 2 g of fresh or frozen tissue were ground in the presence of liquid nitrogen to obtain a fine powder. The powder was scraped into 5 ml of preheated (60 °C) CTAB buffer (CTAB (Sigma) 3% (w/v), 2 M NaCl, 0.2% (v/v) of 2-mercaptoethanol, 25 mM EDTA, 1 M Tris HCl (pH 8.0) and 1% (w/v) polyvinylpyrrollidone (PVP-40). Samples were incubated at 60 °C for 40 min, then extracted with chloroform-isoamylalcol 24:1. DNA was precipitated with cold isopropanol, recovered by centrifugation (5,000 rpm, 15 min), washed with a solution of ethanol (76% v/v) containing 10 mM of ammonium acetate, air dried and resuspended in TE (10 mM Tris HCl pH 7.4, 1 mM EDTA). Thereby RNase digestion followed (10 µg/ml RNase A).

Molecular marker development and fragment analysis

Simple sequence repeats (SSR)

Forty nine primers pairs were used to amplify the DNA of the mapping panel at the microsatellite loci reported in Table 1. VMC primers were designed in the flanking regions of microsatellite motifs from genomic clones provided by IACR-Long Ashton Research Station, University of Bristol, on behalf of AGROGENE, Moissy Cramayel, France. These microsatellites were developed in the framework of the Vitis Microsatellite Consortium. Ten types of SSR were included: repetitions of CA, GA, CG, CAA, CATA, GATA, GAA, GTC, GGT and CCT.

All microsatellite loci were amplified in a 12.5 µl-vol reaction mixture containing 0.25 µM of each primer, 100 µM of each dNTP, 1, 1.5 or 2 mM of $MgCl_2$, 0.25 U of *Taq* Polymerase and 40 ng of template DNA. A three-steps protocol consisting of denaturation (5 min at 95 °C) annealing (30 s at specific primer T_m) and extension (1 min 30 s at 72 °C) for 30 cycles, followed by a fill-in step of 10 min at 72 °C was applied for PCR amplification. Fragment analysis was carried out either by capillary electrophoresis on an automated laser fluorescence sequencer (ABI Prism 310), as described in Grando and Frisinghelli (1998), or on a standard sequencing gel (6% polyacrylamide, 19:1 acrylamide:bis; 7.5 M Urea; 0.5 × TBE buffer) with a 2-h run at 58 W and visualization by silver staining, according to the Promega silver sequencing kit (Promega, Madison, Wis., USA).

Amplified fragment length polymorphism (AFLP)

The AFLP analysis established by Vos et al. (1995) was slightly modified as follows: genomic DNA (0.5 µg) was incubated for 2 h at 37 °C with 5 U of *Eco*RI and 5 U of *Mse*I in 50 µl of 10 mM Tris-HAc pH 7.5, 10 mM of MgAc, 50 mM of KAc, 5 mM of DTT and 50 ng/µl of BSA. Next, a 10 µl of a solution containing 5 pmol of *Eco*RI adapters, 50 pmol of *Mse*I-adapters, 1 U of T4 DNA ligase, 1 mM of ATP in 10 mM Tris-HAc pH 7.5, 10 mM of MgAc, 50 mM of KAc and 5 mM of DTT were added, and the incubation was continued for 5 h at 37 °C.

A primer pair based on the sequences of the *Eco*RI and *Mse*I adapters with one additional selective nucleotide at the 3′ end (*Eco*RI + A, *Mse*I + C) was used for the pre-selective PCR step (30 s DNA denaturation at 94 °C, 1 min annealing at 60 °C, 1 min extension at 72 °C, for 20 cycles). After the preamplification step, reaction mixtures were diluted 20-fold with 10 mM Tris-HCl, 1.1 mM EDTA pH 8.0, and used as templates for the second amplification performed with primer pairs having three additional se-

Table 1 List of the SSR loci analysed in the present study together with their origin

SSR locus	Origin	Reference
VVS1, VVS2, VVS3, VVS4, VVS5	*V. vinifera* cv Sultana	Thomas and Scott (1993)
VVMD5, VVMD6, VVMD7, VVMD8	*V. vinifera* cv Pinot noir	Bowers et al. (1996)
VrZAG25, VrZAG47, VrZAG62, VrZAG67, VrZAG79, VrZAG93, VrZAG112	*V. riparia*	Sefc et al. (1999)
VVMD24, VVMD25, VVMD28, VVMD31, VVMD34, VVMD36	*V. vinifera* cv Pinot noir	Bowers et al. (1999)
VMC1E11, VMC2A7, VMC2A9, VMC2A10, VMC2A12, VMC2B11, VMC2C10.1, VMC2D9, VMC2F10, VMC2H3, VMC2H9, VMC2H10, VMC3B7.2, VMC3C11.1, VMC3D4, VMC4D9, VMC4F8, VMC4F9.1, VMC4H5, VMC4H6, VMC5A1, VMC5H11, VMC6B11, VMC7F2	*V. vinifera* cv Syrah	Vitis Microsatellite Consortium
scu05VV, scu06VV, scu11VV	*V. vinifera* cv Chardonnay	Scott et al. (2000)

Table 2 Sequences of the adapters and primers used to amplify AFLP fragments. The M02 and E01 primer pair was adopted for the pre-selective amplification step and the listed *Eco*RI+3 primers and *Mse*I+3 primers were used to generate 63 pair combinations for the second amplification step

Adapters/Primers	*Mse*I side	*Eco*RI side
Adapters	5′ GACGATGAGTCCTGAG	5′ CTCGTAGACTGCGTACC
	3′ TACTCAGGACTCAT	3′ CTGACGCATGGTTAA
Universal primer	*MU* 5′GATGAGTCCTGAGTAA	*EU* 5′GACTGCGTACCAATTC
+1 primer	*M02* MU + C	*E01* EU + A
+3 primers	*M48* MU + CAC	*E34* EU + AAT
	M49 MU + CAG	*E35* EU + ACA
	M50 MU + CAT	*E36* EU + ACC
	M51 MU + CCA	*E37* EU + ACG
	M52 MU + CCC	*E38* EU + ACT
	M53 MU + CCG	*E39* EU + AGA
	M54 MU + CCT	*E40* EU + AGC
	M55 MU + CGA	*E41* EU + AGG
	M56 MU + CGC	*E43* EU + ATA
	M57 MU + CGG	
	M58 MU + CGT	
	M59 MU + CTA	
	M60 MU + CTC	
	M61 MU + CTG	
	M62 MU + CTT	

lective nucleotides at both *Eco*RI and *Mse*I adapter sides. A total of 63 primer combinations, which have been previously tested for the ability to generate AFLPs using grapevine DNA as template (Bellin et al. 2001), were screened for polymorphisms on the full mapping panel (Table 2). Primer labelling was performed with [γ-^{33}P] ATP (Amersham). PCR products were separated on 4.5% denaturing polyacrylamide gels (7.5 M Urea; 0.5 × TBE buffer) run at 58 W constant power for 1.5 h. Presence or absence of informative AFLP fragments was visually scored. Each polymorphic marker segregating as heterozygous in one parent and null in the other (expected segregation ratio 1:1), or as heterozygous in both parents (expected segregation ratio 3:1), was given a suffix according to the primer combination and the position from the top of the gel. Only clear-cut fragments were recorded and markers with more than 25% missing data were not included in further analyses.

Single-strand conformation polymorphism (SSCP)

SSCPs (Slabaugh et al. 1997) of PCR products were based on *V. vinifera* cDNA sequences coding for flavanone 3-hydroxylase (F3H), leucoanthocyanidin dioxygenase (LDOX), stilbene synthase (StSy), phenylalanine ammonia-lyase (PAL) and UDP glucose:flavonoid 3-*O*-glucosyl transferase (UFGT) (Sparvoli et al. 1994). These genes are involved in the flavonoid and stilbene biosynthesis, and play a role in many plant functions. In grapevine, StSy, like other flavonoid-related enzymes, is induced by a variety of environmental stimuli including fungal elicitors (Liswidowati et al. 1991). The five forward and reverse primer sequences (5′ → 3′) listed below were designed and used first to amplify parental genomic DNA:

F3H	ACGACACTGACGGCTCTTG	TCACCACCGCTTGGTGATC,
LDOX	AATGGTGACTTCAGTGGCTC	GCCTTCAGGATGATCTTCTC,
StSy	ACCATCCTAGCCATTGGCAC	CCTGCAATGGCACCTGCAG,
PAL	GACTTCACCTCAATGGCTTG	ACCGTTCCAAGCATCGAGAC,
UFGT	TTCTTGGAGAAGACCAGAGG	TCCAAACAGGTGGTACAAGC.

PCR fragments were digested with restriction enzymes for 1 h at 37 °C and denaturated for 5 min at 95 °C before SSCP analysis. MDE solution (FMC BioProducts) was used as described by Schneider et al. (1999) to obtain gels 1-mm or 0.4-mm thick and 20-cm long. Four microliters of each digestion were mixed with 9 µl of loading buffer, and 6 µl of the resulting samples were run for 16 h at 2 W. Gels were silver stained as in Sanguinetti et al. (1994). SSCP polymorphisms between the two parents were analysed on the 81 DNAs of the mapping population.

Linkage analysis and map integration

The molecular results were analysed according to a double pseudotest-cross model (Grattapaglia and Sederoff 1994). Data from the SSR, AFLP and SSCP analyses were divided into three files (Lodhi et al. 1995). The first contained data of the 1:1 segregating bands present in the parental P1but absent in P2; the second file contained data of 1:1 segregating bands present in P2 but not in P1; and a third file contained data concerning bands present in both parents and segregating 3:1 in the progeny. Segregating bands were evaluated by chi-square tests for goodness-of-fit to expected segregation ratios (P = 0.05). The initial linkage analysis was performed excluding bands heterozygous in both parents using the program MAPMAKER 3.0 (Lander et al. 1987; UNIX version/EXP 3.0b). Because of the lack of phase information in pseudotest-cross populations, each segregating locus was "inverted" (e.g. homozygous codes changed to heterozygous and viceversa) and merged with the normally coded data in order to detect repulsion-phase linkages. This data set resulted in two linkage maps for each parent, one in coupling and the other in repulsion phase. Only one map was randomly selected for further analysis with MAPMAKER 3.0. In the case of doubly heterozygous SSR markers, only homozygous null genotypes were used in linkage analysis. SSR markers with alleles segregating from both parents were present in both maps and provided a set of common loci that served as bridges to align homologous linkage groups.

The existence of singletons (Castiglioni et al. 1999) was assessed by computer analysis and the presence of the related polymorphism was checked again in the autoradiograms.

A framework of markers was defined, using SSR and AFLP markers assigned to linkage groups at LOD 9.0. The ORDER command was given to find marker order in linkage groups with more than six markers. In the other cases, COMPARE and MAP commands were used without the "error detection" option to set group orders and distances. Subsequently, associated markers were found at LOD 3.5 and new linkage groups were defined respecting the markers order imposed at LOD 9.0. As accessory markers are defined here those markers which were associated to the linkage groups, but with unknown orientation with respect to the framework, obtained at LOD 3.5.

MAPMAKER results were verified using JoinMap 3.0 (Stam 1993; Van Ooijen and Voorrips 2001) at LOD 4.0, which allowed the additional mapping of the AFLPs segregating 3:1 and SSR markers not included in the data sets used by MAPMAKER.

Table 3 Summary of the linkage analyses carried out independently for the two parents and resulting from the use of SSR, AFLP and SSCP markers

	V. vinifera cv Moscato bianco maternal parent	*V. riparia* paternal parent
SSR		
Amplified loci	49	49
Segregating markers	39	37
Distorted segregations	0	11
Loci used for the linkage analysis	39	26
Framework loci	25	19
Accessory markers	11	7
Unlinked markers	3	0
AFLP		
Amplified loci	2,085	2,145
Segregating markers recorded	341	513
Distorted segregations	29	84
Loci used for the linkage analysis	312	429
Framework loci	171	221
Accessory markers	131	179
Unlinked markers	10	29
SSCP		
Amplified loci	5	5
Segregating markers	0	3
Distorted segregations	–	2
Loci used for the linkage analysis	0	3
Framework loci	0	1
Accessory markers	0	2
Linkage maps		
Number of linkage groups	20	19
Average size of linkage groups (cM)	81.9 +/– 50.1	79.9 +/– 39.3
Average distance between markers (cM)	9.3 +/– 9.1	6.9 +/– 6.5
Total map distance (cM)	1,639	1,518

Results

Polymorphism rate of molecular markers

SSR

Twenty two of the 49 PCR primers were used to amplify SSR DNA loci from the parental and the 81 F1 lines belonging to the public DNA sequences of *V. vinifera* and *V. riparia*. Other 24 primer pairs were developed by members of the Vitis Microsatellite Consortium (VMC). The *scu* primers were from Chardonnay ESTs (Scott et al. 2000), and represent AT repeats (scu05VV and scu06VV) or CTT repeats (scu11VV) located respectively in 3′ or 5′ untranslated gene regions. Loci amplified with several SSR primer pairs used in this study are placed also in other recently published grape maps. The linkage map of *V. vinifera* produced at U.C. Davis by Riaz et al. (2002) contains 43 out of the 49 SSR loci considered in the present analysis, and 20 SSRs are in common with the maps produced at INRA Montpellier by Doliguez et al. (2002) for table grape. Thirteen of these loci are also present in the interspecific *Vitis* maps produced at Cornell University by Dalbó et al. (2000).

Each of the SSR PCR primer pairs amplified one or two DNA fragments per genotype. Twelve SSR markers segregated in P1, 10 in P2 and 27 microsatellites in both parents, thus representing potential bridges to anchor homologous linkage groups in the two genetic maps. Based on a chi-square test, 11 cases of segregation distortion were identified, all concerning alleles from P2. Two of these loci, VVS3 and VMC6B11, were polymorphic only in P2, while the nine loci, VMC4D9, VMC2C10, VMC2H3, VMC5H11, VMC3C11, VMC4H6, scu06VV, VrZAG93 and VVMD24, were heterozygous in both parents. Finally, the data sets used by MAPMAKER contained 18 SSR loci for mapping in both P1 and P2, 21 SSR loci for mapping only in P1 and 8 SSR loci for mapping only in P2 (Table 3).

AFLP

Sixty three primer pairs were tested in selective AFLP amplifications of P1, P2 and 81 F1 DNAs. Forty nine primer combinations (78%) were retained and scored. In total, ca 4,300 bands were generated. Each primer com-

Fig. 1 Genetic linkage maps of the cross *V. vinifera* Moscato bianco × *V. riparia*. Accessory markers are indicated to the right of the related LG frameworks and are grouped into clusters when numerous. Markers mapped based on the JoinmMap analysis allowing the identification of further homologies between P1 and P2 linkage groups are indicated by an *asterisk* and written *in italics*. *Dashed lines* connecting bars linkage groups transversely point to common markers. In the linkage map maternal and paternal linkage groups are positioned at left and right respectively ▶

1

E436013 31.4 E395929
34.0
E355423
4.1 E386110
3.8 E39522 0.0 BE38614
2.6 E355436 12.5 E40583 0.0 *E385910
14.7
0.0 *E355424
3.6 E385011
BE34551 3.0 BE395920
5.0 SCU05VV 0.0 E36525 0.1 BE36526 0.0 *E38505
31.5
E365420
17.5
E394911
7.2 E345420 41.6 E39512
47.2
0.0 *E344814
3.2 E395215
2.7 E43578
BE34527 3.2 E355213
9.9 BE34488
11.1 3.5 E435216
BE40587 8.5 E40588
4.4 BE436115 6.5 VVMD24
10.7
E40609
5.2 BE37482
7.4 VMC2B11
8.6 E36497 1.6 E34483

a

BE395217 3.9 E435221 0.0 E395219 1.7 BE43609
5.3 BE365210
6.7 BE40485 5.3 BE395116 0.1 BE375010
4.5 E40486 0.0 E345226 5.3 E34481
6.1 BE38484 3.5 BE36493 5.3 E43617
6.7 E38489 2.4 E38607
9.6
E436028 5.2 BE436016
6.6 E406017 0.0 BE375018 0.1 E36545
5.3 E395134 0.0 *E385910
10.2
E40487 0.0 SCU05VV 0.0 *E355424
4.1 BE43521
3.1 BE375023 0.0 *E38505
8.6 E355429
13.6
BE38498
16.0
E406115

b

E415913
22.0
BE35486
13.4
E40581 0.0 *E344814
9.4
BE36549 0.0 *VVMD24
2.6 BE395923 0.6 E395133
2.9 BE36594 0.6 E36593
7.6 VRZAG112 0.1 E395921
1.3 BE40616 0.1 E39594
1.3 BE386113 0.0 BE38499
2.6 0.6 E36527
5.5 E395123 0.0 E436119
3.6 BE345414 3.9 E384917
E386225 3.9 BE345118
8.0 3.5 E35517
E435210 3.9 E354916
5.2 BE395310 3.7 BE38608
8.1 BE34565
27.7
E41595

2

BE364912 15.1 E386120 11.1 BE374810 15.3 E385113
18.4
BE43526 4.1 E39517
47.7
E41597
19.5
BE384916
7.4 VMC2A7
6.8 E43601
4.4 BE436022 3.6 BE395212
1.3 E364913 3.1 E40482
3.9 E35516 0.8 BE345422
2.6 BE37523 0.0 E38612
5.2 E37504
4.4 BE345513 2.4 E37488
BE40617
7.5 E386017
5.2 BE38503
2.5 BE395117 11.1 E34521
12.6
E345227 0.0 *E36512
11.2
BE395312

BE385222 30.9 BE395924
34.4
E386012 17.1 BE395916
21.0
VMC7F2
8.0 E39536 2.4 E40614 2.4 E40489
4.3 BE385227 0.0 *E36512
3.9 BE395913 0.0 BE374812
14.4
E43524
8.2 E35525 1.1 E39526
5.2 E355433
7.0 BE385016
5.9 BE43604
15.9
E38601
10.3
BE395111 1.9 E435217
7.8 E34567
1.5 E43525
3.2 BE385017 1.8 BE39534
3.2 E354811
3.9 BE395216
2.5 E386125 1.6 BE37521
6.6 E395210
6.3 BE405816 0.1 BE39555
1.5 BE345112 0.0 E39554
2.8 E345111

3

E34511 1.5 BE345517 3.1 E43528
31.7
E385023
4.3 E355212
13.3 E39598
9.1 E405810 1.3 BE395220
4.7 BE34546 0.0 BE364914
3.1 BE36548
3.9 E384919 15.4 VVMD31 3.6 E35547 3.6 BE35548
17.6
BE39527
9.6 VVMD6
14.4
BE43602 7.4 E355416
9.6 VRZAG62
5.5 VVMD7

BE38581 15.1 E36542
18.5
VVMD6 1.6 E345114 8.5 E40483 1.6 E395125 1.3 E395218
10.8
E37491 0.0 BE345121
6.7 BE345512 1.3 E435212
1.3 BE355431 1.3 E37501
1.3 BE344815 1.3 E38589
3.9 E436112 1.4 E384914
1.3 BE355118 3.1 BE385914
5.0 BE34549 3.1 E38521
8.5 BE384911 3.4 BE38511
1.5 E354915 0.0 E38512
6.3 E35521 0.0 E345232
5.3 BE354911 1.5 E374910
8.0 BE38626 1.3 E39523
9.6 E406015 1.1 BE38488

4

7.0 BE405913
2.6 E36482 3.2 E34561 0.0 E375013
8.1 E40484 0.1 E375014
2.8 BE345214 1.8 BE38486
E436015 1.8 BE435211 0.0 *E386213
13.9
1.3 E345210 0.0 E38584
6.7 E40589 0.1 E39496
E38605
10.5
E355123
7.3 BE385010 0.0 *E36518
2.6 BE406114 0.0 *E344812
3.2 BE436120 0.9 VRZAG25
3.2 BE345230 0.0 BE43574
8.1 VRZAG67 0.1 VMC2A10 0.0 *E386218
12.0 BE355430 0.0 BE365411
33.8
VMC4F9.1

E39588 0.0 *E386213
4.1 BE395113
5.3 BE38624
2.5 BE37487
6.6 E345231
5.2 BE34489 0.0 *E344812
2.6 BE365414 0.1 BE 38615
1.3 E355119 0.0 E38616
4.4 E365419 2.4 E406010 0.0 *E36518
9.1 E345125
2.5 E395127 0.0 E365413 0.1 BE 38483
6.7 E374811 1.9 VRZAG25
7.4 VRZAG67 0.0 *E386218 7.8 BE38481
8.9 E386117
1.3 E345415
2.3 E34562
2.5 E345222
4.3 E404811 0.0 *VMC4F9.1
9.1 E34553 0.1 BE36513 1.5 BE 37492 0.0 BE395211

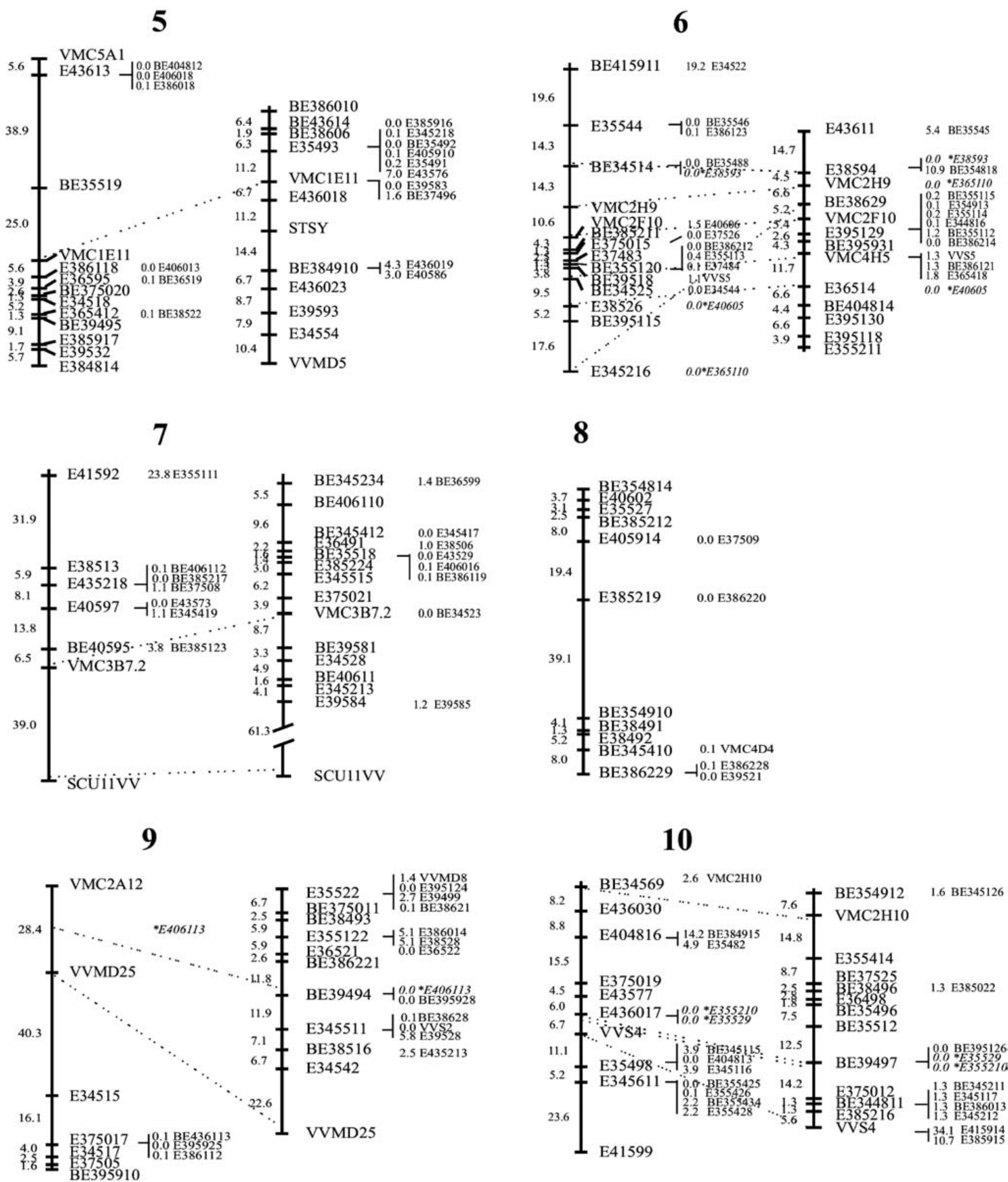

Fig. 1 (continued)

bination amplified from 24 to 65 bands per gel in P1 (average of 43) and from 21 to 68 in P2 (average of 44). From 14 to 52 fragments resulted polymorphic per primer combination corresponding to 1,412 AFLPs. More than 60% of these bands were segregating in the progeny. The unambiguous markers which segregated from P1 were 341, 513 from P2 and 43 from both parents (Table 3). The most effective primer combinations resulted E34 M51, E34 M52, E35 M54, E37 M50, E38 M52, E39 M51, E43 M60 and E38 M61, which generated more

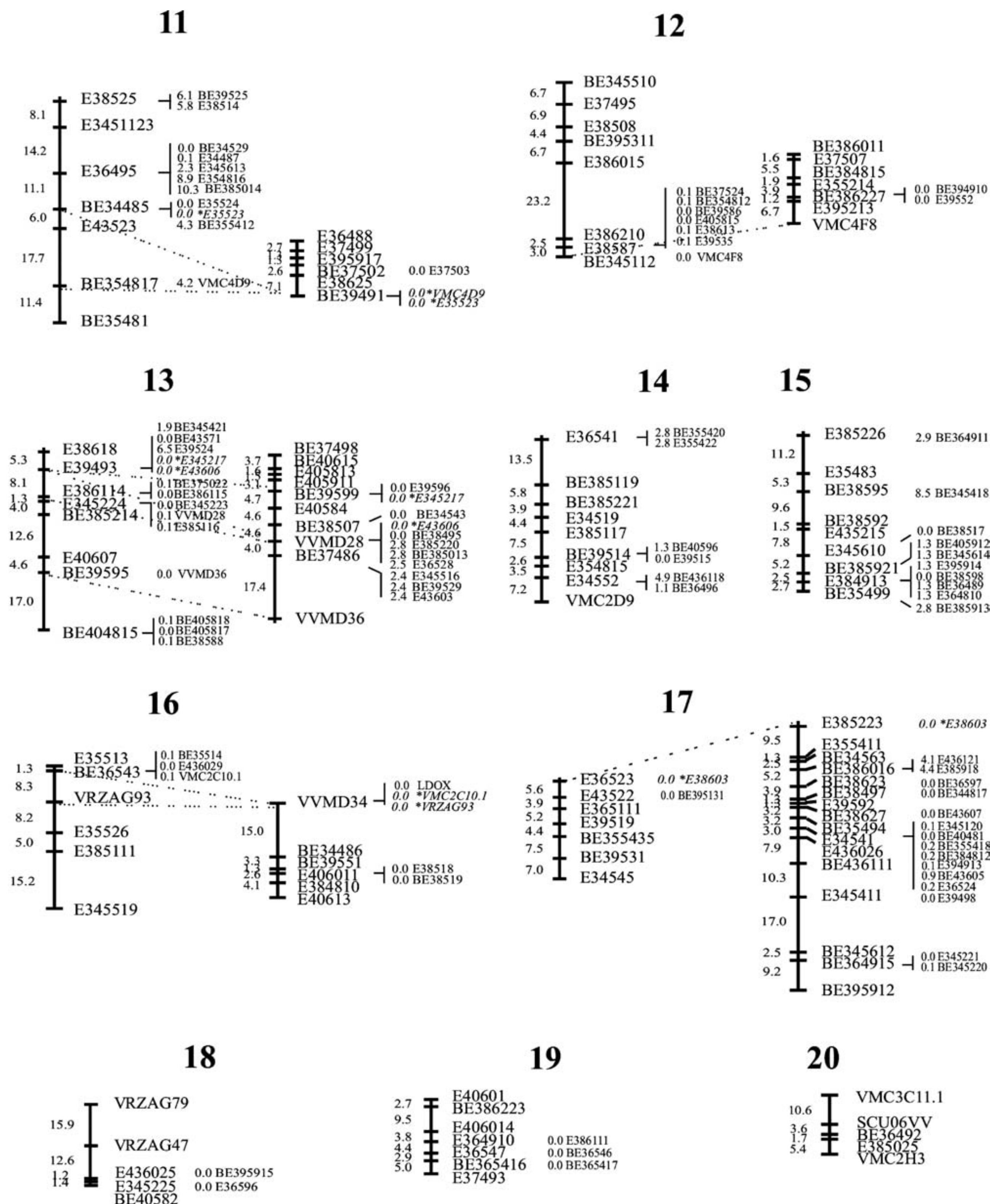

Fig. 1 (continued)

than ten segregating AFLPs per gel from each parent. Most of the AFLP markers followed a Mendelian segregation. According to chi-square analysis, 29 (8.5%) markers inherited from P1 and 84 (16.4%) markers from P2 deviated from the expected 1:1 ratio, and 6 (14%) common to both parents did not fit the 3:1 segregation in the progeny. These markers were not considered in map construction.

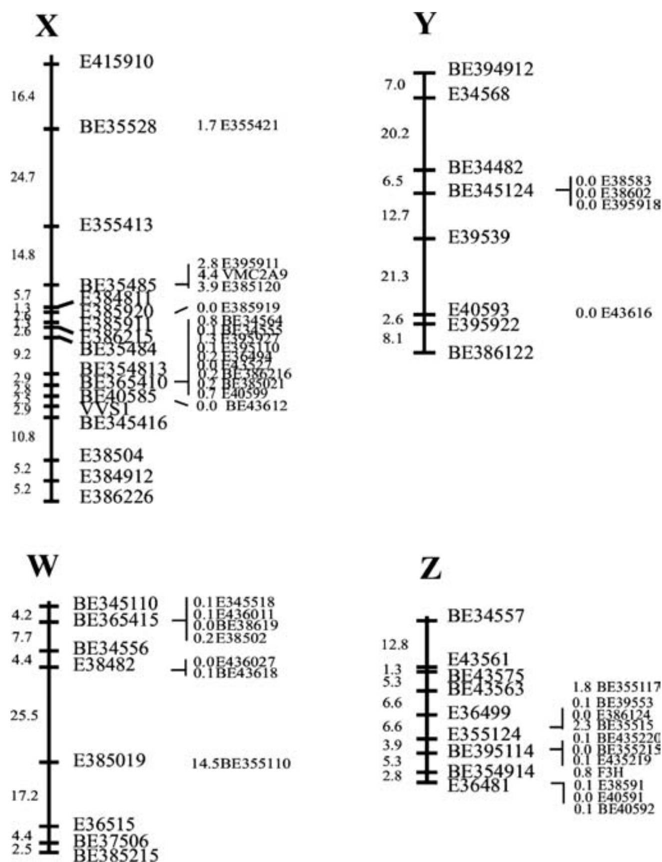

Fig. 1 (continued)

SSCP

The five primer pairs used to amplify flavonoid and stilbene related genes produced well defined PCR products. Among the restriction enzymes tested, *Alu*I and *Dde*I cut DNA fragments that showed SSCP between P1 and P2 in the case of F3H, LDOX and StSy, whereas PAL and UFGT did not. Polymorphisms at the three loci were present in P2 allowing them to map only in the male parent. LDOX and StSy polymorphism distributions slightly deviated from the expected segregating ratios. In spite of this they were included in the P2 map because they did not affect the order of surrounding markers.

The linkage maps

SSR, AFLP and SSCP segregating markers were used to generate the P1 and P2 maps based on the segregation data obtained from 81 F1 plants. In P1, 338 markers were assigned to 20 linkage groups. These markers identified 196 framework loci represented by 25 SSR and 171 AFLP loci or loci clusters covering a total map length of 1,639 cM (Table 3). For P2, 429 markers were assigned to 19 linkage groups, hosting 241 loci represented by 19 SSR, 221 AFLP loci or loci clusters and one SSCP, covering 1,518 cM (Table 3). Few additional groups represented by two or three linked markers in both data sets were generated as well and accounted for about half of the 42 segregating markers not included in the maps. The SSR loci VVMD5, VMC4H6 and VMC5H11 were not mapped in the P1 linkage groups. Forty two per cent (142) of the markers were placed in the P1 map besides the framework loci. These accessory markers were grouped in 78 clusters. For the P2 map, 88 clusters of accessory markers accounted for the 43.8% (188) of the total mapped fragments (Fig. 1).

For the P1 map, the average size of linkage groups was 81.9 cM, ranging from 21.3 to 215.0 cM. For the P2 map, the average size was 79.9 cM, ranging from 15.0 to 177.8 cM. The total number of markers per linkage group (LG), including framework and accessory markers, was between 5 (LG 20) and 33 (LG 1) for P1 and between 7 (LG 11) and 34 (LG 2) for P2. The average distance between two framework markers was 9.3 cM in the P1 map and 6.9 cM in the P2 map. Marker-free regions longer than 20 cM were found in nine P1 linkage groups and in seven linkage groups of P2. For P2, a consistent gap of 61.3 cM was present in LG 7. Among 11 SSR and 37 AFLP markers not included in the MAPMAKER data sets, the P2 SSR loci VMC2C10.1, VMC4D9, VrZAG93, VVMD24 and 19 AFLPs segregating 3:1, were assigned to established linkage groups following JoinMap results. Their relative positions were exploited as additional anchor loci only (Fig. 1).

Map integration

The integration of the P1 and P2 maps was possible based on markers segregating in both data sets. Twenty one out of the 27 SSR markers heterozygous in both parents and the 19 AFLPs segregating 3:1 allowed us to define 14 homologous linkage groups. The homology of LG 2, 11, 16 and 17 was based on SSR and AFLP markers mapped by the JoinMap software. Homology of P1 LG 8, 14, 15, 18, 19 and 20 with the remaining four P2 linkage groups could not be defined, so they were named by the letters X, W, Y and Z (Fig. 1). The presence of two or more bridging loci allowed us to define map orientation in nine cases, while the LGs 2, 3, 5, 12 and 17 shared a single common marker.

Most of the SSR loci were placed in similar positions as compared to other mapping experiments in grape (Table 4). Forty microsatellite loci were finally in common with the framework SSR map developed by Riaz et al. (2002) and the present ones. The position of SSR markers VMC4H6, VMC5H11 and VMC6B11 could not be compared as the three loci resulted unlinked in our analysis. Only the position of SSR scu05VV was different in the two studies. No significant difference was recorded when comparing the order of markers in the eight linkage groups sharing more than two SSR markers. The comparison allowed us to identify 17 homologous linkage groups out of the 20 forming the two grapevine maps (Table 4). In the same way, the 19 SSR loci in common

Table 4 Number of the linkage group where SSR loci have been placed in the present and in other *Vitis* experiments using different software. The total distance covered by each map is indicated. Linkage groups are numbered as reported by the Authors

SSR locus	Linkage groups				
	V. vinifera 1,639 cM	*V. riparia* 1,518 cM	*V. vinifera* 1,728 cM Riaz et al. (2002)	*V. vinifera* 1,002 cM Doligez et al. (2002)	*Vitis* hybrids 1,199 cM–1,470 cM Dalbò et al. (2000)
VrZAG67	4	4	10		
VrZAG25	4	4	10	11	V
VMC2A10	4		10	11	
VMC4F9.1	4	4	10		
VVMD6	3	3	7	2	II
VVMD31	3		7	2	II
VVMD7	3		7		II
VrZAG62	3		7		II
VMC1E11	5	5	16		
VMC5A1	5		16		
VVMD5		5	16	10	VI
VMC2H9	6	6	6		
VMC2F10	6	6	6	9	
VMC4H5		6	6	9	
VVS5	6	6			
VMC3B7.2	7	7	19	15	
scu11VV	7	7			
VMC2A12	9		11	12	
VVS2		9	11	12	
VVMD25	9	9	11	12	
VVMD8		9			Maternal 17
VMC2H10	10	10	8	1	
VVS4	10	10	8		I
VVMD28	13	13	3	13	IV
VVMD36	13	13	3	13	IV
VMC4F8	12	12	1		
VMC2A7	2		18		
VMC7F2		2	18		
VMC2C10.1	16	16[a]	2		
VrZAG93	16	16[a]	2		
VVMD34		16	2	3	Paternal 14
VMC2B11	1		14		
VVMD24	1	1b[a]	14	4	
VrZAG112		1b	14		
scu05VV	1	1a	5		
VrZAG79	18		5	5	
VrZAG47	18		5	5	III
VMC2H3	20		17		
VMC3C11.1	20		17	16	
scu6VV	20		17		
VMC4D9	11	11[a]	15		
VMC4D4	8		4		
VMC2D9	14		9		
VMC2A9		X			
VVS1		X			

[a] In the linkage group the SSR locus was placed using JoinMap (see Materials and methods)

with the *V. vinifera* maps produced by Doligez et al. (2002) enabled us to establish homology between 12 linkage groups. Totally consistent results were also found when SSR locus positions in our maps were verified in the "Horizon" and Illinois 547-1 individual or homologous linkage groups from the maps obtained by Dalbó et. al (2000). In this case, 12 common codominant markers enabled the identification of eight homologous linkage groups (Table 4).

Discussion

Genetic markers useful to assist the breeding of grapevine for resistance against downy mildew are not available at present. Because of the complexity of factors controlling the response to *P. viticola* attack (Eibach 2000), a mapping strategy was applied to an interspecific hybrid population obtained by crossing the *V. vinifera* cultivar Moscato b. and the wild species *V. riparia* carrying the resistance trait. Besides fungal-disease tolerance, which is under evaluation for seedlings and cuttings

grown in the field and under controlled conditions in the greenhouse, other segregating traits in this progeny (such as the muscat flavour and flowers sex) are currently under investigation.

Three types of molecular markers were used to construct one linkage map from each parent and to identify homologous linkage groups. Codominantly inherited microsatellite markers were chosen for their ability to amplify homologous PCR products of expected size in different *Vitis* species. Within the *Vitaceae*, a high degree of conservation in the microsatellites flanking regions from *V. vinifera* is observed across a number of taxa (Sefc et al. 1999; Di Gaspero et al. 2000; Rossetto et al. 2002). The versatility of grape SSRs (Thomas and Scott 1993) has been widely explored for typing grapevine cultivars in order to facilitate the management of grapevine collections and control the trade of plant material (Grando and Frisinghelli 1998; Lamboy and Alpha 1998; Franks et al. 2002). Currently, the development of new microsatellite sequences and the increasing number of mapping projects are exploiting the potential of SSRs to integrate genetic maps and to generate a common reference map for grape. Most of the SSR loci placed in our Moscato b. and *V. riparia* maps have been recently mapped in other *V. vinifera* genotypes by Doligez et al. (2002) and Riaz et al. (2002). A preliminary evaluation of homology between linkage groups independently generated resulted in a good agreement of the SSR locations in the maps of different laboratories.

Only a few pairs of linked SSR loci were available on both P1 and P2 individual maps to allow a reliable comparison of recombination rates between parents. Similarly, a comparison with other maps would be speculative here, because of the different total map coverage achieved and the different linkage analysis software and stringency conditions applied. In our case, the linkage analysis using the MAPMAKER program at LOD 3.5 produced maps of 1,639 cM and 1,518 cM for P1 and P2 respectively, allowing the recognition of ten homologous linkage groups. The use of the JoinMap program assigned 23 additional anchor markers to the P2 map and allowed the identification of four new homologous linkage groups.

Markers having similar positions in different maps (Table 4) allow the direct comparison of the information derived from different crosses and thereby to assess QTL stability across different genetic backgrounds and environments. Moreover, as microsatellites are considered among the most-efficient DNA markers for grapevine-variety identification and have been used to evaluate genetic relationships among grapevines (Regner et al. 2000; Sefc et al. 2000), a comparison based on these markers should also provide a map-based subset of unlinked SSRs which could be more informative for these applications than random chosen markers. The use of unlinked markers should also reduce the bias in estimates of genetic distances.

To detect quantitative loci affecting a character in a particular cross, it is necessary to have molecular markers evenly distributed throughout the genome (Tanksley et al. 1992). AFLPs, although dominant, have greatly facilitated the genotyping step in mapping experiments, because of the simultaneous recovery of numerous polymorphic markers in the same assay. In this study the AFLP analysis has been used to generate genetic linkage maps having an average of one marker every 10 cM. The high level of polymorphism observed is consistent with the different genetic background of the parents and the high heterozygosity existing in grape species and populations. However, due to the density of bands in the AFLP patterns produced with the majority of primer combinations used, the number of polymorphisms chosen for scoring the whole progeny was restricted to the most reliable ones. AFLP marker clustering was observed in both linkage maps with the majority of groups composed of two to four markers around a single locus and few larger groups with five to eight markers. Clustering observed in these maps was only sporadically related to primer combinations generating the markers; thus, in these cases it could result from the limited resolution of the mapping population. Nevertheless, as discussed in Tanksley et al. (1992), marker clustering might have a biological basis reflecting suppressed genetic recombination in heterochromatin around the centromeres and or telomeric regions. The potential of using comigrating AFLP bands which reliably represent homologous markers between mapping populations (Sebastian et al. 2000) was explored in this study in order to improve the efficiency of homologous linkage-group identification. Further investigations will consider sequence homology and the development of SCAR markers.

The present linkage maps will be used as a framework for expressed sequence tag (EST) mapping. The increase of the size of the mapping panel has been also planned to allow the mapping of QTLs (Visscher et al. 1996). This will make it possible to select a subset of AFLP markers to optimize the use of available genetic resources as outlined in Charmet et al. (2000).

To-date, PCR-based markers used in linkage analysis of grape include RAPDs (Lodhi et al. 1995), SSRs (Riaz et al. 2002) and AFLPs (Doligez et al. 2002), whereas Dalbò et al. (2000) described the conversion of some cDNA sequences to CAPS for mapping in an interspecific hybrid population. Identifying efficient strategies for conversion of cDNA sequences to the PCR-based assay for genetic analysis in *Vitis* is desirable to facilitate exploitation of emerging grape EST data (Ablett et al. 2000; Terrier et al. 2001). ESTs will be particularly important for QTL mapping, because markers such as AFLPs, SSRs and RAPDs may predominantly target non-coding regions. To provide more informative markers for subsequent mapping, the SSCP technique has been explored in this study starting from a few genes of grape involved in the biosynthesis of flavonoids and stilbenes. As this technique relies on conformational differences between DNA molecules, it does not require the knowledge of the nucleotide sequence of the different alleles to allow a rapid detection of the polymorphism. In

fact, SSCP variants between parental PCR products were detected, and mapping of three genes was achieved. Extensive sets of polymorphic sequences are now being identified in the course of genome research from model, and economically significant, plant species (Somerville 1999). Taking into account the poor knowledge of the genetic factors affecting traits relevant for the crop, the integration of genes into a functional map of the grape genome seems to be a realistic strategy to characterize QTLs using the candidate-gene approach (Byrne and McMullen 1996).

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