Theor Appl Genet (2008) 116:1129–1143
DOI 10.1007/s00122-008-0741-3

ORIGINAL PAPER

A grapevine (*Vitis vinifera* L.) genetic map integrating the position of 139 expressed genes

Marzia Salmaso · Giulia Malacarne · Michela Troggio · Giorgia Faes · Marco Stefanini · M. Stella Grando · Riccardo Velasco

Received: 21 February 2007 / Accepted: 1 March 2008 / Published online: 18 March 2008

Abstract Grapevine molecular maps based on microsatellites, AFLP and RAPD markers are now available. SSRs are essential to allow cross-talks between maps, thus upgrading any growing grapevine maps. In this work, single nucleotide polymorphisms (SNPs) were developed from coding sequences and from unique BAC-end sequences, and nested in a SSR framework map of grapevine. Genes participating to flavonoids metabolism and defence, and signal transduction pathways related genes were also considered. Primer pairs for 351 loci were developed from ESTs present on public databases and screened for polymorphism in the "Merzling" (a complex genotype Freiburg 993–60 derived from multiple crosses also involving wild *Vitis* species) × *Vitis vinifera* (cv. Teroldego) cross population. In total 138 SNPs, 108 SSR markers and a phenotypic trait (berry colour) were mapped in 19 major linkage groups of the consensus map. In specific cases, ESTs with putatively related functions mapped near QTLs previously identified for resistance and berry ripening. Genes related to anthocyanin metabolism mapped in different linkage groups. A *myb* gene, which has been correlated with anthocyanin biosynthesis, cosegregated with berry colour on linkage group 2. The possibility of associating candidate genes to known position of QTL is discussed for this plant.

Communicated by E. Guiderdoni.

Marzia Salmaso and Giulia Malacarne contributed equally to the present work.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-008-0741-3) contains supplementary material, which is available to authorized users.

M. Salmaso · G. Malacarne · M. Troggio · G. Faes · M. S. Grando · R. Velasco
Genetics and Molecular Biology Department,
Istituto Agrario San Michele all'Adige,
via Mach 1, 38010 San Michele a/A (TN), Italy

M. Stefanini
Agricultural Resources Department,
Istituto Agrario San Michele all'Adige,
via Mach 1, 38010 San Michele a/A (TN), Italy

Present Address:
G. Faes
Istituto Trentino di Cultura-IRST,
Via Sommarive 18, 38050 Povo (TN), Italy

M. Salmaso (✉)
Department of Environmental Agronomy and Crop Production,
University of Padova, Agripolis, 35020 Legnaro (PD), Italy
e-mail: marzia.salmaso@gmail.com; marzia.salmaso@unipd.it

Introduction

Grapevine genomics has improved: genetic maps are available (Doligez et al. 2002; Grando et al. 2003; Doucleff et al. 2004; Fischer et al. 2004; Riaz et al. 2004; Doucleff et al. 2004; Lowe and Walker 2006; Di Gaspero et al. 2007), mainly based on microsatellites produced by the international Vitis Microsatellites Consortium and in part by the France Genomic National Program Genoplante (Adam-Blondon et al. 2004). Recently, a SSRs reference genetic linkage map based on five different segregating populations has been published by Doligez et al. (2006) and a dense SNP-based genetic linkage map anchoring Pinot Noir BAC contigs (http://genomics.research.iasma.it) has been published by Troggio et al. (2007). The grapevine community can now use concrete molecular tools (Donald et al. 2002; Barker et al. 2005; Adam-Blondon et al. 2005). In the last four years, a number of sequencing initiatives, coordinated by

the International Grapevine Genome Program (http://www.vitaceae.com and http://www.intl-pag.org/13/13-grape.html), have produced more than 316,000 grapevine ESTs deposited in international databases (http://www.ncbi.nih.nlm.gov and www.tigr.org). This large assembly of available sequences, resulting in roughly 27,000 unigenes, is a source of data for developing single nucleotide polymorphism (SNP) markers from coding sequences. Single base substitution and/or small insertion–deletion polymorphisms represent the most abundant type of DNA variation (Rafalski 2002). SNPs in grapevine are quite frequent: they occur every 47 bp when clones of different *Vitis* species are compared (Salmaso et al. 2004), or every 64 bp when the comparison is intraspecific (Lijavetzky et al. 2007). More recently, the genome sequence data from the highly heterozygous genome of Pinot Noir, and the discovery of more than 2 millions of mapped SNPs, extended the evaluation of nucleotide variation to the entire genome rather than to limited resequenced DNA regions. It was found that the SNP frequency had an average value of 4.0 per kilobase across the grape genetic map, with several regions showing SNP frequency peaks between 5 and 7.5 per 1 kb (Velasco et al. 2007). The high frequency of SNPs, both in coding and non-coding regions, enables to develop SNP based maps, including them in a SSRs reference framework to allow comparative mapping (Rieseberg et al. 1995; Lai et al. 2005a, b).

In this paper, progress towards a transcript map for grapevine, based on the localization of expressed genes and unique genomic sequences, are described. The mapping experiment is based on a F1 population (the hybrid "Merzling" × *Vitis vinifera* L, cv Teroldego) which was selected due to the large number of segregating traits, including tolerance to fungal pathogens, colour and quality of anthocyanins, resistance to *Phylloxera vastatrix*, shape and compactness of the bunch and high versus low quality of berry metabolic profiles.

Public sequence sources (Moser et al. 2005; http://www.tigr.org) were screened taking particular care to select also genes involved in three metabolic pathways: flavonoid metabolism, defence response and signal transduction. Transcription factors putatively involved in the control of agronomic traits were also considered. Particular attention was focussed on anthocyanins, secondary metabolites which constitute the major subgroup of grape flavonoids. In *V. vinifera*, besides their natural role as pigments, these compounds are associated to other putative features, such as anti-oxidant, potential anticancer and anti-arteriosclerosis (Hou 2003; Kahkonen and Heinonnen 2003; Navindra et al. 2003; Passamonti et al. 2003).

Colour segregation as a simple Mendelian trait justified a deeper analysis of the last five enzymes of the anthocyanin pathway: *chalcone isomerase* (*CHI*), *flavanone 3-hydroxylase* (*F3H*), *dihydroflavonol 4-reductase* (*DFR*), *leucoanthocyanidin dioxygenase* (*LDOX*) and *UDP glucose-flavonoid 3-o-glucosyl transferase* (*UFGT*) (Sparvoli et al. 1994) and, in addition of some *myb* transcription factors proposed as regulators of the phenylpropanoid pathway (Kobayashi et al. 2002). We have investigated the localization of these genes on the map and any correlation with the berry colour by co-localization with putative berry colour genetic loci (Doligez et al. 2002, 2006; Grando et al. 2003; Kobayashi et al. 2004; Lijavetzky et al. 2006; This et al. 2007). Concerning other traits having QTL components, we have performed QTL-gene co-localization with the possibility of generating information on QTL candidate genes. Recent proposals have adopted a similar meta-QTL approach (Arcade et al. 2004; Chardon et al. 2004).

The results provided demonstrate that comparison of genetic maps, and the detection of the SNPs in coding regions which allows to identify candidate genes, open perspectives to future grapevine genomic approaches.

Materials and methods

Plant material and DNA extraction

An interspecific F1 population of 89 individuals derived from the cross between "Merzling" (**F**) (complex hybrid of *V. vinifera* descending from *Vitis rupestris* and *Vitis lincecumii*, with a white berry and tolerant to several pathogens) and *V. vinifera* cv Teroldego (**T**) (high quality traits, susceptible to pathogens and with black berry) was used for linkage analysis. The cross was developed at the Istituto Agrario di San Michele all'Adige (IASMA). The progeny segregated for disease resistance, berry colour and other quality traits.

DNA was isolated from young leaves following the Doyle and Doyle (1990) procedure with a few modifications as in Grando et al. (2003). After RNase digestion (10 μg/μl Rnase A), samples were resuspended in sterile water.

Simple sequence repeats (SSRs)

A total of 177 primer pairs were used on the "Merzling" × Teroldego cross: 25 VVMD (Bowers et al. 1996, 1999); 5 VVS (Thomas and Scott 1993); 2 scuVV (Scott et al. 2000); 9 VrZAG (Sefc et al. 1999); 39 VVI loci (Merdinoglu et al. 2005); 3 UDV (Di Gaspero et al. 2005) and 94 markers developed by the Vitis Microsatellite Consortium (VMC), managed by Agrogene SA, Moissy Cramayel, France. The SSR markers were selected to be well-spread over the 19 linkage groups according to the last available version of the reference map of Doligez et al. (2006).

PCR was carried out in a standard reaction of 12.5 µl. Ten nanograms of template DNA were added to the reaction mixture containing 0.25 µM of each primers, 100 µM dNTPs, 1 mM $MgCl_2$, 1 U of *Taq* polymerase and 1× *Taq* polymerase buffer. Amplification was carried out on the two parents of the map using a Biometra T gradient thermocycler, programmed as follows: 4 min at 94°C followed by 30 cycles of 1 min at 94°C, 30 s at 54 or 58°C, 1 min and 30 s at 72°C followed by a final stage of 10 min at 72°C. After PCR, the presence of amplification products was tested using agarose gel electrophoresis. Once the melting temperature was optimised for primer pairs showing polymorphism, the entire population was screened at the optimal temperature. The primers were labelled with ABI fluorescent dyes at the 5′-ends of "forward" primers and analysed using capillary electrophoresis with an automatic 3100 ABI sequencer. Chromatograms were analysed using the software GENESCAN 3.7 (Applied Biosystems); allele calling was carried out using GENOTYPER (Applied Biosystems).

Single nucleotide polymorphisms (SNPs)

cDNA sequences were identified from two libraries of *V. vinifera* cv. Regent—developing inflorescence (IN: 187) and shoot tips (GR: 32); from five cDNA libraries of *V. vinifera* cv Pinot noir—berry (BA: 26); root (RA: 10); bud (GE: 7); and two leaf libraries (F1 and F2: 2) (Moser et al. 2005); from a BAC library of *V. vinifera* cv. Pinot noir (BAC-ends: 80) (Faes 2004; Adam-Blondon et al. 2005) and from public database (*CHI*, *F3H*, *LDOX*, *DFR*, *UFGT*, *MybA*, *MybB*). Primer pairs have been deduced using Primer3 software (Rozen and Skaletski 2000) and used to amplify the corresponding genomic sequences. Primers were designed to have an average length of 20 nucleotides, melting temperatures of 58–62°C, and to amplify 200–300 bp. Gene homology was deduced by comparison to known genes present in the public database.

Gene fragments belonging to three metabolic pathways (anthocyanin metabolism, defence response and signal transduction) were considered including sugar metabolism and transcription factors putatively controlling agronomic traits. Primer pairs deduced from libraries and gene putative functions are available at the web site http://genomics.research.iasma.it/iasma/marker_vite/snp.html and in Salmaso 2003 and in Faes 2004. They are reported in Table 1. Primer pairs of genes related to the anthocyanin pathway were:

CHI (*chalcone isomerase*) (primers as in Salmaso et al. 2004): primer forward AGTTCAGGTCGAGAACGTCC; primer reverse CCATCTCTCCTTCAACCACC); *F3H* (*flavanone 3-hydroxylase*), primer forward TAC AGG AGG AAG ATG AGC AA; primer reverse TTA AAG ATG GTC CAA GAT GAA C; *DFR* (*Dihydroflavonol 4-reductase*), primer forward GAT GAC CTC TGC AAT GCT CA; primer reverse CCA TGC AGA GAC CAC CTT G; *LDOX* (*leucoanthocyanidin dioxygenase*), primer forward AAG GTT CCC CAG CCT GAA T; primer reverse AGC AGG CAG AGA CAA ACA TA; *UFGT* (*UDP-glucose-flavonoid 3-o-glucosyl transferase*) (primers as in Salmaso et al. 2004: primer forward TTCTTGGA GAAGACCAGAGG; primer reverse TCCAAACAGGTG GTACAAGC); *MybA* (*VvmybA1* transcription factor) (primers from Kobayashi et al. 2004); *MybB* (*VlmybB1-2* transcription factor), primer forward GGT AAG AGC TCC TTG TTG CG; primer reverse GAG AAT TCA CTG GAG GAC GG.

PCR amplification was carried out in 25 µl reaction containing 0.2 mM dNTPs, 1.5 mM $MgCl_2$, 0.2 mM of each primer and 0.2 U *Taq* DNA polymerase. The amplification protocol consisted of 35 cycles of 45 s at 94°C, 30 s from 55 to 60°C and 90 s at 72°C, preceded by denaturation of 4 min at 94°C and followed by a 10 min extension at 72°C. After amplification in the two parents of the map, the presence of amplification products was tested on agarose gel electrophoresis. Once the melting temperature was optimised, the genes selected were screened on the entire population at the optimal temperature.

Four approaches were used to score for polymorphisms: single strand conformational polymorphism (SSCP; Orita et al. 1989); cleaved amplified polymorphic sequences (CAPS; Konieczny and Ausubel 1993; Neff et al. 2002); DNA fragment length polymorphysm (DFLP; Schneider et al. 1999); and microsequencing (Syvanen et al. 1990). SSCP was modified as in Salmaso et al. (2004). For the development of CAPS, the software dCAPS finder 2.0 (Neff et al. 2002) allowed to search for SNPs cleaveable in the parental lines. Digestion was carried out as follows: 500 ng of PCR product were added to 2 U of restriction enzymes and 1× digestion buffer. Digestion was carried out at 37° for 30 min and polymorphic patterns on 2% agarose gels were recorded. Polymorphisms of DFLP were determined in 2% agarose gel. Microsequencing was carried out as in Troggio et al. (2008).

Linkage analysis

The pseudo-testcross strategy (Weeden 1993; Grattapaglia et al. 1995) was followed to produce separate linkage maps for both parental lines. The two maps were aligned each other based on co-dominant or doubly heterozygous dominant markers present in both parental genotypes resulting into the single "Merzling" × Teroldego map. The initial linkage analysis was carried out using MAPMAKER/EXP 3.0 (Lander et al. 1987), excluding bands heterozygous in both parents (*ab* × *ab* segregation type). A framework map

Table 1 Description of the 151 mapped EST/BAC-end derived marker loci

Marker name	Hit accession number[a]	Putative function	aa alignment[b]	*E* value[c]
BA0025	Q9ZP50	FtsH-like protein Pftf precursor	671	0.00
BA0118	Q9ZTW5	GDP-mannose pyrophosphorylase	361	0.00
BA0141		No hits		
BA0168	Q9ZSZ6	DnaJ protein	178	8.00E-44
BA0179	P49729	Ubiquinol-cytochrome *c* reductase iron–sulfur subunit 1	242	1.00E-116
BA0185	Q9SJH9	*Arabidopsis* hypothetical protein	43	3.31E-29
BA0661	Q8LSK7	Auxin-regulated protein	234	2.00E-85
BA0919	Q9ZQF5	Putative RING-H2 zinc finger protein	200	9.00E-46
BA1371	Q8LLD9	BEL1-related homeotic protein 29	269	2.00E-62
BA1517	Q9FH37	Putative bHLH transcription factor	239	8.00E-70
BA1755	Q9M6N8	RPT2	174	9.00E-80
1030A15F	Q9M331	Transporter-like protein	215	8.00E-75
1031I21R	Q9SHY2	Metal–nicotianamine transporter YSL7	222	2.00E-99
1031N12R	Q8S4P9	48-kDa glycoprotein precursor	160	5.00E-20
1037J17F		No hits		
1044E06R	Q6Z6Y3	*Arabidopsis* hypothetical protein	44	8.00E-15
1044J09F		No hits		
1048E22F		No hits		
1070D05R	Q9LW61	*Arabidopsis* hypothetical protein	55	6.00E-08
1071I11F	Q9FF11	Similarity to HepA-related protein Harp	63	1.00E-17
1074L06R	Q762M8	MAP kinase phosphatase	83	2.00E-25
1075M09R	Q9FIU1	*Arabidopsis* hypothetical protein	212	4.00E-83
1076J22F	O04388	A-type cyclin	124	4.00E-36
1076O07R	Q9SSM2	Similar to (*R*)-mandelonitrile lyase isoform 1	201	9.00E-85
1077P05F	Q9C5K1	Putative ubiquitin-specific protease UBP12	39	1.00E-13
1078C16F	Q41335	Phytochrome F	229	1.00E-97
1078N04R	Q9ZTT3	Subtilisin-like protease C1	143	4.00E-34
1079N02F	Q7XB39	Class IV chitinase	134	7.00E-42
1079N03R	Q6J192	Fasciclin-like AGP 12	51	1.00E-16
1082B22R		No hits		
1082L12F	Q42884	5-Enolpyruvylshikimate-3-phosphate phospholyase 1	51	1.00E-20
1083I24R	O65555	*Arabidopsis* hypothetical protein	203	3.00E-92
1084H20R	Q71RI5	Resistance protein (fragment)	205	3.00E-79
1086H11F	Q9SIU2	Putative cap-binding protein	151	4.00E-58
1086H15F	Q9LJ64	Extensin protein-like	252	1.00E-103
1086K12R	Q8S700	Putative disease resistance protein	115	3.00E-17
1087M15F		No hits		
1089K14R	Q9SYK2	Putative ADP-glucose pyrophosphorylase, small subunit	177	1.00E-40
1089L13R	Q9FM07	Permease 1	29	6.00E-07
1089N12R	Q6PND7	Ferric reductase	90	4.00E-35
1095J09R	Q9LM59	*Arabidopsis* hypothetical protein	250	2.00E-79
1096J24R	Q9SN90	Chromosome assembly protein homolog	160	2.00E-37
1097I14F	Q9SJA1	Putative ubiquitin carboxyl terminal hydrolase	36	7.00E-09
1097N13R		No hits		
1098N17R	Q9M099	Serine carboxypeptidase II precursor	48	5.00E-16
1100N02R	Q4R0I0	Pinoresinol-lariciresinol reductase	80	5.00E-37
2001A12F	Q9SHY2	Metal–nicotianamine transporter YSL7	130	2.00E-34

Table 1 continued

Marker name	Hit accession number[a]	Putative function	aa alignment[b]	*E* value[c]
2001H06R		No hits		
2002G02F	Q9LZA6	*Arabidopsis* hypothetical protein	130	8.00E-62
2002P11R	Q6IDB3	*Arabidopsis* hypothetical protein	89	2.00E-10
2003A19R	Q7X5X9	*Arabidopsis* hypothetical protein	110	1.00E-36
2004K11F	Q60DJ5	Expressed protein	31	3.00E-06
2005P20F	Q56WB9	Putative leucyl-tRNA synthetase	110	5.00E-33
2006H03R	Q9XIR2	*Arabidopsis* hypothetical protein	249	2.00E-66
2017D24R	Q6L464	Putative disease resistance complex protein I2C-1	276	5.00E-21
2017H02F	Q4R1J3	SEL-1	75	3.00E-29
2017M20F		No hits		
2018N06R		No hits		
2019A20F		No hits		
2019C20F		No hits		
GM0971	Q9SJM6	Zinc finger A20 and AN1 domains containing protein	161	6.00E-42
GM1026	Q9SYQ4	Scarecrow-like 6	76	4.00E-17
GR0015	Q9FY79	Laccase-like protein	162	2.00E-54
GR0074	Q9FEL7	Putative AUX1-like permease	179	3.00E-77
GR0169	Q712P2	Calmodulin 3 protein	275	3.00E-73
GR0176	Q4F8J0	Putative endo-1,4-beta-glucanase	216	2.00E-90
GR0188	Q6SS00	YABBY-like transcription factor GRAMINIFOLIA	107	4.00E-32
GR0220	Q4KP31	CRT/DRE binding factor 1	144	2.00E-41
GR0244	Q02166	Anthranilate phosphoribosyltransferase, chloroplast precursor	94	3.00E-23
GR0255	Q84NG3	Flavonoid 3′,5′-hydroxylase	451	0.00
GR0354	Q9FVD6	Ser/Thr specific protein phosphatase 2A A regulatory subunit beta isoform	375	0.00
GR0381	O04870	Pectinesterase	145	1.00E-63
GR0397	Q8RWQ7	*Arabidopsis* hypothetical protein	338	1.00E-145
GR0436	Q943K1	Putative photosystem II subunit PsbS	177	3.00E-76
GR0442	Q8L9J9	*Arabidopsis* hypothetical protein	87	2.00E-28
GR0456	Q9S7M6	Glutathione *S*-transferase 2	218	1.00E-75
GR0466	P93092	Acyl carrier protein 1, chloroplast precursor	95	5.00E-38
GR0576	Q6RZW9	Putative WRKY4 transcription factor	307	1.00E-76
GR0618	Q9M309	*Arabidopsis* hypothetical protein	382	0.00
GR0641	Q9FGX1	ATP-citrate lyase subunit B	608	0.00
IN0010 (IB02)	Q1SL70	Leucine-rich repeat	121	8.00E-27
IN0014	Q7XZU2	SAC domain protein 3	329	2.00E-95
IN0031	Q9CA28	Putative reductase	320	1.00E-116
IN0036 (ID05)		putative prot		
IN0049	Q9LLS5	Inorganic phosphate transporter	380	1.00E-151
IN0050	Q9LPW0	Putative calcium-binding protein, calreticulin	404	1.00E-172
IN0055	Q5Z9P6	Putative SSR alpha subunit	242	5.00E-85
IN0058	Q9LK94	Probable monodehydroascorbate reductase	202	4.00E-88
IN0062	Q6DBE3	*Arabidopsis* hypothetical protein	170	4.00E-36
IN0100	Q8L6S5	IAA16 protein	213	9.00E-62
IN0105	Q9FPK7	Inositol-3-phosphate synthase	141	7.00E-33
IN0109 (IIE02)	Q9M6E0	DNA-binding protein 4	206	8.00E-41
IN0129 (IIA05)	Q67Y01	Putative glycerate dehydrogenase	146	1.00E-45

Table 1 continued

Marker name	Hit accession number[a]	Putative function	aa alignment[b]	*E* value[c]
IN0155 (IIC08)	Q9XEU0	Zinc-finger protein 1	253	1.00E-57
IN0160 (IIH08)	Q9SP48	Homeodomain-leucine zipper protein 56	311	7.00E-80
IN0169	Q9SPL2	Ubiquitin ligase protein CIP8	150	3.00E-46
IN0185 (IIA12)	P51110	Dihydroflavonol-4-reductase	122	6.00E-46
IN0208	P26205	Cyanogenic beta-glucosidase precursor	180	3.00E-78
IN0210	P93075	BvcDNA-205 protein	256	7.00E-66
IN0211 (IIIC03)	Q6TKQ3	Putative ethylene response factor ERF3b	220	2.00E-88
IN0251	Q71BZ1	Type-B response regulator	44	4.00E-12
IN0283 (IIIC12)	Q20BD3	Pathogenesis-related protein	268	4.00E-71
IN0320 (IVH04)		No hits		
IN0353	Q9ZPX7	*Arabidopsis* hypothetical protein	123	1.00E-42
IN0356 (IVD09)	Q9FS43	Pathogenesis-related protein 10	158	3.00E-88
IN0357 (IVE09)	Q652R9	Finger-containing phosphoinositide kinase	188	5.00E-47
IN0360 (IVH09)	Q9SRQ6	Phosphatidylglycerol specific phospholipase C	311	1.00E-120
IN0396	O82161	Phi-1 protein	317	1.00E-118
IN0415	Q94HJ7	Putative RING-H2 finger protein	160	1.00E-32
IN0417	Q9SH64	*Arabidopsis* hypothetical protein	165	2.00E-93
IN0425	Q9LRA7	Putative trehalose 6-phosphate synthase	154	9.00E-57
IN0432	Q9SMN1	Unknown mitochondrial protein	254	1.00E-108
IN0498	Q9M899	*Arabidopsis* hypothetical protein	180	3.00E-70
IN0523	O49952	Phosphoinositide-specific phospholipase C	311	1.00E-139
IN0564	Q9AVG7	Isopentenyl diphosphate isomerase 2	222	1.00E-121
IN0596	Q9SRK9	*Arabidopsis* hypothetical protein	189	2.00E-45
IN0599	Q8W3P5	Chalcone synthase	393	0.00
IN0607	P32811	Alpha-glucan phosphorylase, H isozyme	306	1.00E-161
IN0620	Q8LAJ7	Transfactor, putative	250	5.00E-67
IN0662	Q49RB3	Gip1-like protein	100	3.00E-20
IN0671		No hits		
IN0672	Q9XEE6	Hypothetical Cys-3-His zinc finger protein	670	1.00E-156
IN0732	Q8VX73	Cyclophilin	171	4.00E-79
IN0763	Q70XK1	ADP-ribosylation factor 1-like protein	181	3.00E-99
IN0764	Q70AB2	Ethylene transcription factor	386	1.00E-125
IN0767	Q9FX54	Putative glyceraldehyde-3-phosphate dehydrogenase	318	1.00E-159
IN0779	Q9LXM2	Putative CCR4-associated factor 1	269	1.00E-113
IN0780	Q9SRW8	*Arabidopsis* hypothetical protein	54	3.00E-23
IN0844	Q9SL29	Putative cyclic nucleotide-gated ion channel 15	165	4.00E-80
IN0850	Q5Z8T3	Putative myo-inositol oxygenase	120	2.00E-55
IN0860	Q9SWP6	Hypersensitive reaction associated Ca^{2+}-binding protein	160	5.00E-50
IN0873	Q6L4Q9	Putative casein kinase	213	2.00E-54
IN0875	Q8VZG5	*Arabidopsis* hypothetical protein	276	1.00E-119
IN0886	Q59J81	Cyclo-DOPA 5-*O*-glucosyltransferase	148	3.00E-38
IN0907	Q8S862	Putative epimerase/dehydratase	360	0.00
IN0912	Q38908	Probable xyloglucan endotransglucosylase/hydrolase	290	1.00E-129
IN0945	Q564G6	Galactomannan galactosyltransferase		
IN0951	Q9ZQP4	Putative glycogenin	128	6.00E-45
IN0954	P29057	3-Hydroxy-3-methylglutaryl-coenzyme A reductase 1	114	2.00E-45
RA0332	Q9FXS1	WRKY transcription factor NtEIG-D48	181	1.00E-44

Table 1 continued

Marker name	Hit accession number[a]	Putative function	aa alignment[b]	*E* value[c]
RA0421	Q9SUS1	Probable WRKY transcription factor 29	183	2.00E-32
RA0493	Q8L5N7	Myb-related transcription factor VlMYBB1-2	126	8.00E-11
RA0561	Q9M4Y9	AP2-related transcription factor (Ethylene responsive)	155	2.00E-22
RA1742	O22731	*Arabidopsis* hypothetical protein	170	1.00E-63
CHI	X75963	Chalcone isomerase		
DFR	AF280768	Dihydroflavonol 4-reductase		
F3H	X75965	Flavanone 3-hydroxylase		
LDOX	X75966	Leucoanthocyanidin dioxygenase		
MybA	AB111101	VvmybA1 transcription factor		
MybB	AB073017	VlmybB1-2 transcription factor		
UFGT	AF000372	UDP-glucose: flavonoid 3-*O*-glucosyltransferase		

Primers sequences available from http://genomics.research.iasma.it/iasma/marker_vite/snp.html. (Primers in parenthesis are published in Salmaso 2003, Faes 2004)

[a] Accession number of homologous sequences identified in Genbank/Uniprot

[b] Number of aminoacids included in the homology alignment

[c] *E* value of the alignment between the marker sequence and the homologous sequence in Genbank/Uniprot

for each parent was obtained using first the "SUGGEST SUBSET" command (LOD 3.0, minimum distance 2.0, minimum individuals 50) to define linkage groups and then the "COMPARE" command to determine the probable order of all markers in each linkage group (minimum LOD 2.0). Markers shown to depart from Mendelian segregation were also used for map construction, but their position was defined after establishing the order for other markers. Markers with distorted segregations have an *asterisk* indicating the level of distortion ($*P < 0.05$, $**P < 0.01$, $***P < 0.005$). Integrated linkage analysis was carried out with Joinmap 3.0 (Van Ooijen and Voorrips 2001), using the Kosambi function for the estimation of map distances (Kosambi 1944), LOD $\geq$4.0 as a thresholds for determination of linkage groups, and 0.4 recombination fractions. Three rounds of mapping were carried out for six of the 20 linkage groups identified in "Merzling", and for four of the 21 linkage groups identified in Teroldego. Homologous chromosome pair maps were integrated into the consensus map: the "FIXED SEQUENCE" command was used to determine the order of markers relative to the order obtained from the initial MAPMAKER analysis.

Results

SSRs

Out of the 177 primer pairs used to amplify DNA loci, 117 resulted polymorphic (13 VVMD, 4 VVS, 1 scuVV, 9 VrZAG, 29 VVI, 3 UDV and 58 VMC). Eight SSR primer pairs amplified two DNA fragments per genotype, and two SSR primer pairs amplified three, thus increasing the number of polymorphic loci to 129. These extra loci were distinguished with the letters *a*, *b* or *c*. Marker segregations were tested against expected segregation ratios using a chi-square test. 31 SSR loci segregated in "Merzling" (P1), 28 in Teroldego (P2) and 70 in both parents, these representing bridges anchoring homologous linkage groups of the two genetic maps. Of 89 markers showing distorted segregation ratios, ten were discarded due to the high distortion at $P < 0.001$ and eight because affecting the order of neighbour marker loci. Eighty SSRs used in this study have also been placed in the framework-integrated grape map of Doligez et al. (2006), while 66 SSRs are in common with the Syrah × Pinot Noir genetic map of Troggio et al. (2007) (Table 3, Supplementary Fig. 1).

SNPs and phenotypic traits

A total of 351 primer pairs were tested for amplification on the two parents of the map. Of these 259 yielded single PCR product and were further considered in SNP discovery, while 92 failed to amplify any fragment. Seven primers pairs amplified two DNA fragments of identical size identified as unique for a specific genotype by the SSCP analysis and were considered in the analysis for multilocus SNP, loci distinguished with the letters *a* and *b*. The 259 primer combinations were assayed for SNP discovery on the two parents with different technique. Of these, 76 markers were polymorphic at SSCP analysis, 100 at microsequencing analysis, four at CAPs and two at DFLP.

Eighty-four were discarded because monomorphic at the four techniques.

A total of 182 markers were evaluated for segregation by a chi-square test. A total of 70 SNP markers segregated in P1, 76 in P2 and 36 in both parents, the last representing bridges anchoring homologous linkage groups of the two genetic maps. Of 92 markers showing distorted segregation ratios, seven were discarded due to the high distortion at $P < 0.001$ and 14 because affecting the order of neighbour marker loci.

Because of our interest in specific genes, seven primer pairs were used to amplify the flavonoid related genes *CHI*, *DFR*, *LDOX*, *F3H*, *UFGT*, *VvmybA1* and *VlmybB1-2*. Berry colour segregated as a simple mendelian trait and scoring data were also included in the marker matrix.

Ninety-five out of 139 SNPs are in common with the highly dense SNP-based grape genetic map of Troggio et al. (2007) (Table 3, Supplementary Fig. 1).

Linkage maps

Assembly of the maps by linkage/recombination analysis was carried out with Mapmaker/EXP 3.0 and JoinMap 3.0. Following a pseudo-testcross strategy, marker sets from both parents were processed separately and the maps aligned with each other based on markers segregating in both parents (Table 2).

After a first round of calculation at LOD ≥4, eight SSR markers and 14 SNPs were excluded because affecting the order of neighbouring marker loci or excessively increased the linkage group end distances.

A total of 26 SSRs and 58 SNPs were mapped only in the "Merzling" map, 25 SSRs and 61 SNPs only in the Teroldego map, whereas and 56 SSRs and 26 SNPs in both maps (Table 2). The "Merzling" map consisted of 166 loci (82 SSRs and 84 SNPs) covering 20 linkage groups and 914 cM, with an average marker interval of 5.5 cM. The Teroldego map consisted of 168 loci (81 SSRs, 87 SNPs and one morphological marker) distributed on 21 linkage groups covering 1,173.7 cM, with an average marker interval of 7.0 cM. There were 17 and 18 unlinked loci, respectively for the "Merzling" and Teroldego maps.

A consensus map was produced based on integrated dataset and using the Joinmap v3.0 program (Fig. 1). A total of 247 loci (108 SSRs and 139 SNPs) were mapped to 20 linkage groups (linkage group 18 in fact splitted in groups 18a and 18b), covering 1,309.2 cM, with an average marker distance of 5.4 cM (Table 2). Linkage groups were numbered according to the map of Doligez et al. (2006) and Troggio et al. (2007). A small group was identified because

Table 2 Number of polymorphic loci identified by SSR and SNP markers and mapped in the maternal, paternal and consensus maps, together with map statistics

Type of marker	Statistic	"Merzling" maternal map (F)	*V.vinifera* cv Teroldego paternal map (T)	Both "Merzling" and Teroldego maps	Consensus map (FT)	Total
SSR	Amplified polymorphic loci	31	28	70		129
	Loci used for linkage analysis	30	28	70		128
	Interference of phase[a]	5	0	3		8
	Unlinked markers	1[b]	2[b]	2		5
	Total number of loci	26	25	56		
SNP	Amplified polymorphic loci	70	76	36		182
	Loci used for linkage analysis	70	76	36		182
	Interference of phase[1]	3	5	2	4	14
	Unlinked markers	8 + 1[b]	9	5		23
	Total number of loci	58	61	26		
Linkage maps						
	Total number of loci in the map	166	168 (with colour trait)		247 (including the colour trait)	
	Number of linkage groups	20	21		20	
	Average size of linkage groups (cM)	45.7	55.9		65.5	
	Average distance between markers (cM)	5.5	7.0		5.4	
	Total map lenght (cM)	914.0	1173.7		1309.2	

[a] Markers showing distorted segregation affecting the order of neighbour marker loci

[b] Loci segregating in both parents but unlinked or affecting the order of neighbour loci in only one parent map. These loci are present in the consensus map

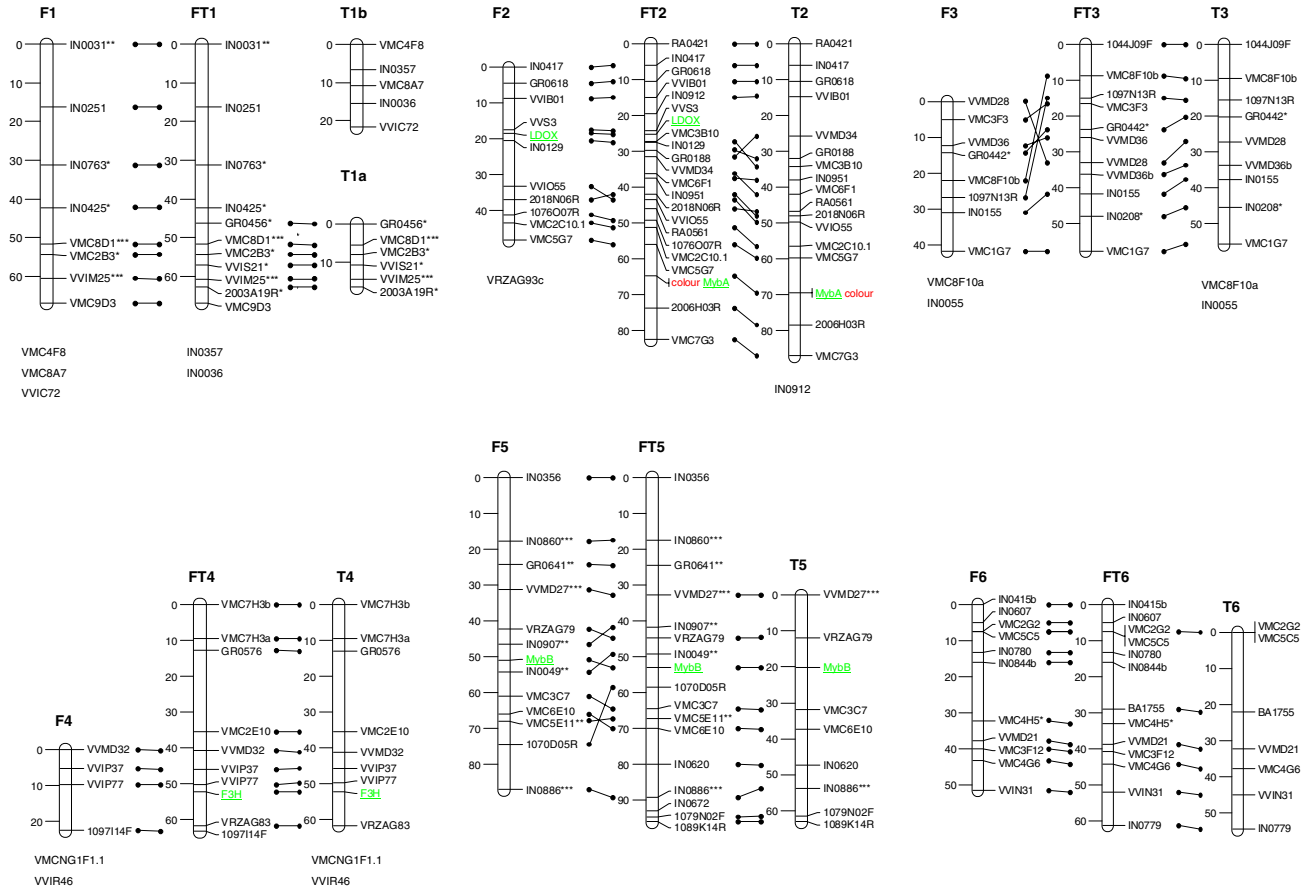

Fig. 1 "Merzling" (F), Teroldego (T) and consensus "Merzling" × Teroldego (F × T) maps. Linkage groups of the "Merzling", Teroldego and consensus map are numbered from 1 to 19 with the prefixes F, T and FT. Markers with distorted segregations have *asterisks* indicating the level of distortion (*$P < 0.05$, **$P < 0.01$, ***$P < 0.005$). Marker positions are reported based on recombination distances (cM). Genes belonging to anthocyanin biosynthesis are *underlined*. Linked markers which excessively increased the linkage group end distances or which affected the order of neighbours in the group were not included in the map and listed below each linkage group

of common SSRs with the published maps (group T1b). In general, marker distribution was fairly even: no pronounced clustering of any marker type was evident, also when genes participating to the same biochemical pathway were considered. Few markers with segregation distortion (*$P < 0.05$, **$P < 0.01$, ***$P < 0.005$) were mapped to linkage groups 1, 3, 5, 6, 7, 8, 10,13, 14, 16, 17, 18a, 18b, 19 (Table 3). Large gaps of 20 or more cM were present on three linkage groups of the consensus map (FT4, FT12, FT18a, FT19). Marker order and marker intervals were generally consistent between homologs from the parental and the consensus maps, with local inversions of closely linked markers more evident when "Merzling" and the consensus map are compared (F3, F5, F7, F8, F10, F14, T10, T13).

Marker order in the two parental maps (based on 66 SSR and 95 SNP markers) was in general consistent with the one observed in the consensus linkage map of *V. vinifera* Syrah × Pinot Noir cross (Troggio et al. 2007), expect for few local inversions, mainly in the "Merzling" map (Supplementary Fig. 1).

Genes related to the flavonoid pathway did not position in one or few clusters: *F3H* and *VvmybA1* mapped in the Teroldego map in two different linkage groups (LG 4 and LG 2, respectively). *CHI* (LG 13), *DFR* (LG 18b) and *LDOX* (LG 2) were assigned to the "Merzling" map. *UFGT* (LG 16) and *VlmybB1-2* (LG 5) were assigned both in Teroldego and "Merzling" maps. Berry colour mapped to linkage group 2 in the Teroldego map at the same locus as *VvmybA1* (Fig. 1).

Discussion

SSR markers made possible to create a marker framework including SNPs related to functional genes. In rare cases, SSRs clustered (e.g. LG 1), but in general they were well distributed along linkage groups and maintained the position occupied in the recently published grapevine map (for instance Doligez et al. 2006; Troggio et al. 2007) with few exceptions.

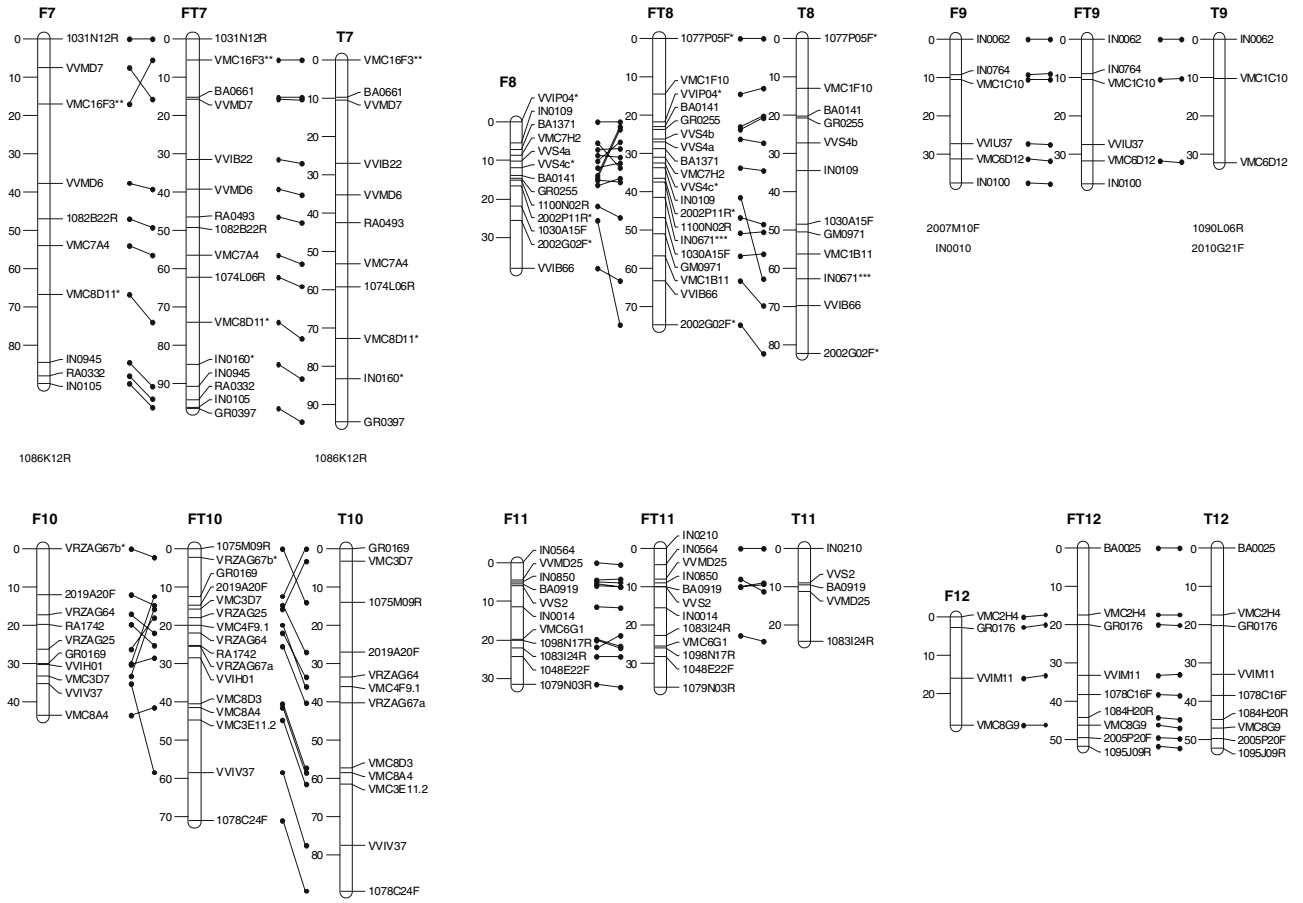

Fig. 1 continued

The map contains 139 new functional gene markers which represents an average of 7.3 new markers per each of the 19 linkage groups. Marker order in the consensus map differed from the parental maps due to local inversions and to the mapping position of 20 loci. Marker order differences may depend from local variation in recombination frequency, from the segregation of specific markers in only one parent and from synteny disruption in the two parents of the map. The maps in fact derive from a complex *V. vinifera* hybrid parent ("Merzling"), such that a possible chromosomal rearrangement of the *Vitis* spp. genomes should not be excluded (Doucleff et al. 2004; Lowe and Walker 2006). Although discussion on grape genome duplication remain still open, a possible explanation could be find in duplications of loci in *Vitis* spp. genomes followed by mutations in primer regions (Jaillon et al. 2007; Velasco et al. 2007). Microsatellite sequences resulted randomly distributed throughout the map. Random distribution of SSR loci has also been reported by other authors for grapevine fruit-tree maps (Testolin et al. 2001; Doligez et al. 2006; Troggio et al. 2007). In contrast to microsatellite markers, the SNP markers, selected in this study from expressed genome regions, were supposed to map in high-coding euchromatic regions. We have not found evidence of a particular clustering of SNPs, to the point of suggesting the presence, in grapevine, of gene-rich chromosomal regions. The low density of our SNP markers may have, in this sense, contributed. Gaps on linkage groups 4, 12, 18a and 19 were noted, besides in this paper also in the Syrah × Grenache map (Adam-Blondon et al. 2004), supporting, for the *Vitis* spp. genome, the possible presence of local heterogeneity in recombination. Differences in recombination rates, either global or restricted to particular genomic regions, have been reported for several plant and animal species (Karp and Jones 1983; Causse et al. 1996; Simianer et al. 1997) and recently addressed in grapevine during the construction of a dense linkage map anchoring Pinot Noir BAC-contig (Troggio et al. 2007; http://genomics.research.iasma.it).

SNPs are the most frequent polymorphisms present in an eukaryotic genome. This would suggest to concentrate mapping efforts on SNPs markers. One target of the present study was to map genes participating to anthocyanin metabolism responsible for the colour of the berry. *CHI*, *F3H*, *DFR*, *LDOX* and *UFGT*, involved in this metabolic pathway, mapped to different linkage groups. Only *LDOX* was

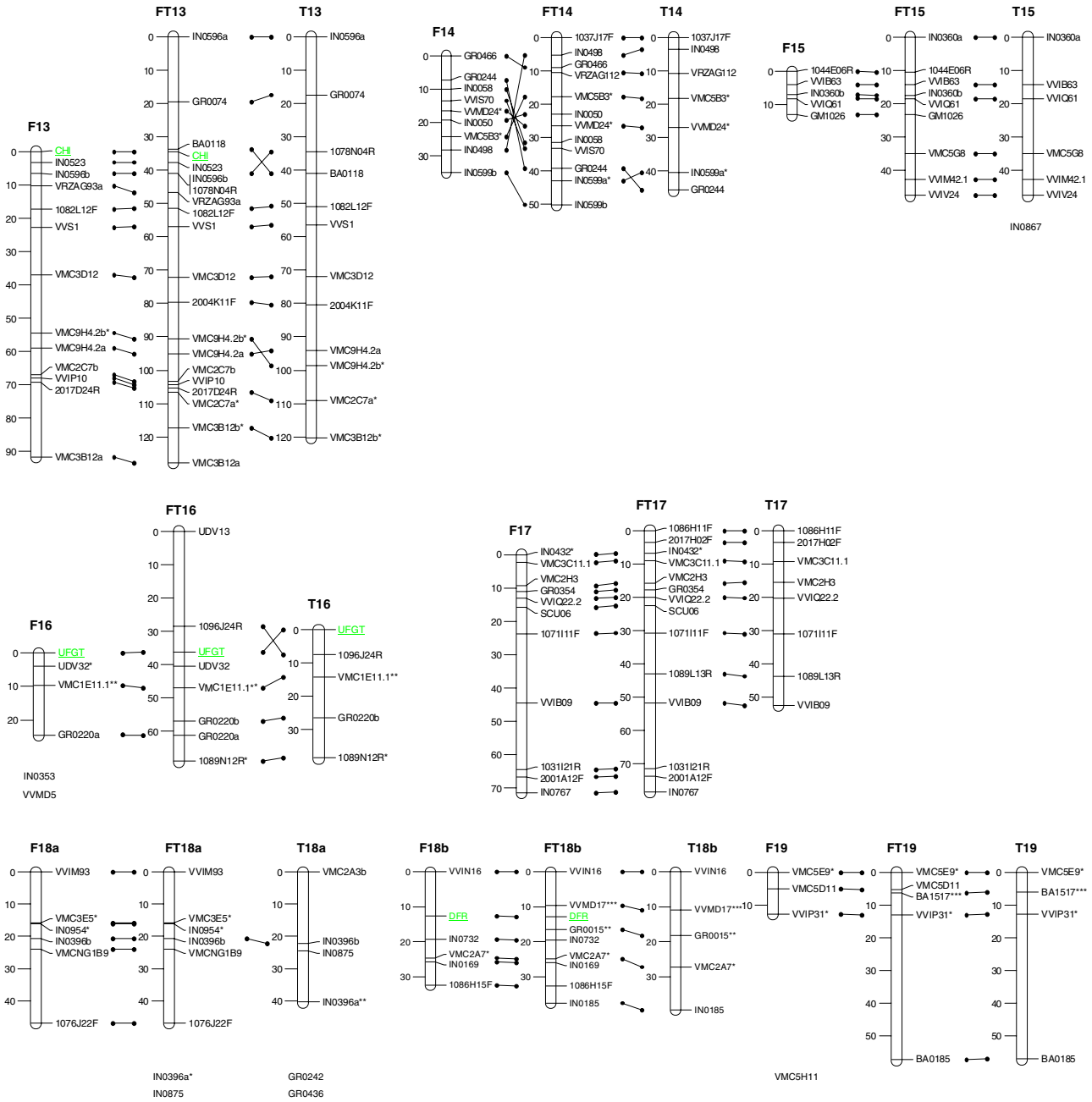

Fig. 1 continued

positioned in the linkage group to which belong the genetic factor supporting berry colour (LG 2) (see also Grando et al. 2003). However, the distance of 39.0 cM between the two loci excludes a direct functional correlation between *LDOX* and LG 2. The same locus supporting berry colour has been mapped to the same genomic region by Fischer et al. (2004), Doligez et al. (2002) and Doligez et al. (2006), revealing a coincidence with the location we propose. In the "Merzling" × Teroldego segregating population, berry colour co-segregated with a *myb* gene mapping to linkage group 2. Evidence of cosegregation between the expression of *VvmybA1* and skin colour in the grapevine has been demonstrated by Kobayashi et al. (2004) analysing *Vitis* species. In the work of Kobayashi (2004), white-skinned genotypes had an insertion of the retrotransposon Gret1 in the 5′-flanking region of *VvmybA1*. The Gret1 insertion inhibits the expression of the *myb* transcription factor and, consequently, do not induce the expression of *UFGT*, the last gene of the phenylpropanoid pathway. The genetic link between this myb transcription factor and anthocyanin biosynthesis, as proposed at molecular level by Kobayashi et al. (2004), has been demonstrated here in

Table 3 Main characteristics of the linkage groups in the three maps F, T and FT

Linkage group no.	"Merzling"			Teroldego			"Merzling" × Teroldego			Reference maps	
	Number of markers	Map length (cM)	Number of markers with distorted segregation	Number of markers	Map length (cM)	Number of markers with distorted segregation	Number of markers	Map length (cM)	Number of markers with distorted segregation	Common markers (Troggio et al. 2007)	Common markers (Doligez et al. 2006)
1*a*	8	66.9	6	7	16.5	6	11	66.9	11	5	5
1*b*	–	–	–	5	21.7	0	–	–	–		
2	11	48.2	0	19	87.0	0	23	82.5	0	17	8
3	8	41.8	1	7	55.7	2	11	57.6	2	8	3
4	4	22.6	0	−9	61.6	0	10	63.2	0	7	5
5	13	87.0	7	9	63.1	2	17	96.0	7	9	5
6	11	51.4	1	7	54.6	0	13	61.1	1	8	6
7	10	89.9	2	11	94.5	3	16	96.3	3	12	5
8	13	38.1	4	12	82.1	3	19	74.7	5	13	4
9	6	37.4	0	3	32.1	0	6	37.7	0	2	2
10	10	43.5	1	12	89.3	0	16	71.0	1	9	8
11	11	31.7	0	5	24.2	0	12	36.3	0	10	3
12	4	28.1	0	9	52.1	0	9	51.6	0	7	3
13	13	91.6	1	12	120.3	3	20	127.8	3	11	4
14	9	34.9	2	7	45.8	3	12	50.1	3	7	4
15	5	12.9	0	6	47.5	0	9	47.5	0	8	3
16	4	24.5	2	7	38.5	3	9	69.0	3	3	2
17	11	71.5	1	8	52.4	0	14	78.4	1	9	5
18*a*	6	46.9	2	4	40.4	1	6	46.9	2	5	3
18*b*	6	32.4	1	5	37.5	3	9	37.5	3	7	2
19	3	12.7	2	4	57.0	3	5	57.2	3	4	3
Total	166	914.0	33	168	1173.7	32	247	1309.2	48	161	83

A comparison of our consensus map to the consensus linkage map of *Vitis vinifera* from the Syrah × Pinot Noir cross of Troggio et al. (2007) and to the framework integrated SSR map of Doligez et al. (2006) is available in the last two columns. "Common markers" indicates markers mapped both in "Merzling" × Teroldego, in Syrah × Pinot Noir and on the five mapping population. Linkage groups are numbered as in Troggio et al. (2007) and in Doligez et al. (2006); linkage groups identified by SSRs, but not present in the consensus map, are indicated with *a* and *b*. Distances are in cM

V. vinifera in agreement with recently published papers (Lijavetzky et al. 2006; This et al. 2007). Our work is the first demonstration of co-localization of Myb with the colour starting from a cross population segregating for the berry colour.

Our map provides a resource for the identification of candidate genes supporting known QTL positions. In fact, co-segregation of SNPs derived from coding genes and a marker linked to QTLs is a logic strategy for the identification of genes underlying important traits (Wright et al. 2005). Data reporting the position of grapevine QTLs (Doligez et al. 2002; Fischer et al. 2004; Fanizza et al. 2005), when interpreted on the light of our map, show cases where ESTs, credited to have a specific function, map in proximity to QTLs (Fig. 2). However, in most cases the exercise did not reveal an obvious match between the trait and the putative function of the concerned gene. We identified two genes (*RA0493* and *BE1074L06R*) that may contribute to the berry ripening. Kobayashi et al. (2002) have previously shown that *mybB* transcripts are present in all stage of grape berry development, but they increase noticeably at the colouring stage. *RA0493*, homologue to *mybB* transcription factor, maps coincident with a QTL controlling begin of berry ripening (OIV code 303, http://www.genres.de) in the interval defined by markers VVMD6 and VMC7A4 (Fischer et al. 2004). While the grape has been classified as a non-climacteric fruit whose ripening is thought to be ethylene independent, it has been showed that a transient increase of endogenous ethylene production occurs just before veraison (i.e. inception of ripening). The observation that ethylene perception is required for the increase of berry diameter, the decrease of berry acidity and for anthocyanin accumulation implies that grape contains a functional network of ethylene signalling at the onset of ripening, in part necessary for the ripening process (Chervin et al. 2004). *BE1074L06R* is homologous to a kinase/phosphatase MAP

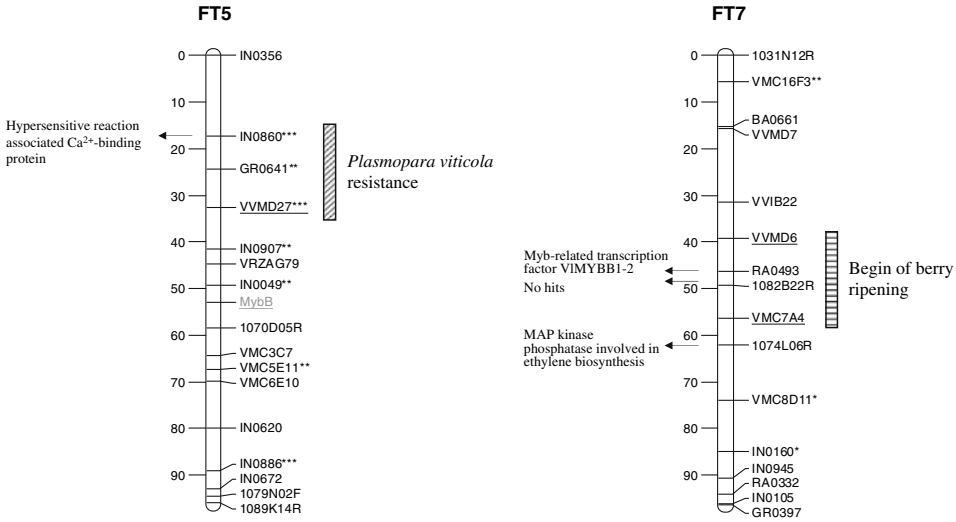

Fig. 2 Summary of associations between previously mapped QTLs (Fischer et al. 2004) and ESTs markers with similar candidate functions. QTLs are drew on the right of the linkage group around the closest SSR (*underlined*) in QTL mapping population. On the left of the linkage groups there is the candidate function of the EST mapped based on homology

putatively involved in ethylene biosynthesis (Kim et al. 2003). This is consistent with the specificity of the QTL associated with it. An additional gene, *IN0860*, encoding a putative Ca^{2+}-binding protein associated with the hypersensitive reaction of the plant to a pathogen (Jakobek et al. 1999), maps close to the VVMD27 SSR locus, in a position coincident with a "minor" QTL for downy mildew resistance (Fischer et al. 2004). The hypersensitive reaction (HR) is an inducible plant response associated with disease resistance. It is characterized by rapid and localized cell death at the site of infection. Although the mechanisms by which grapevine cells operate to reduce disease incidence caused by the downy mildew fungus *Plasmopara viticola* are not fully elucidated, an accumulation or conversion of phenolic compounds and a hypersensitive response seems to be associated with an enhanced disease resistance of the plant (Kortekamp and Zyprian 2003). This Ca^{2+}-binding protein, mapped in this population, provides a functional link with the co-mapping QTL.

The associations we have highlighted need to be functionally proved using proper tools. In any case, as observed in the sunflower (Lai et al. 2005a, b), identified EST/QTL associations represent an important step in identifying genes underlying important traits.

Acknowledgments This work has been supported by the Cass di Risparmio di Trento e Rovereto Foundation. We thank Rosalba Grillo for providing technical support and Cinzia Segala for processing ESTs. Particular thanks to Francesco Salamini and Silvia Vezzulli for the critical reading of the manuscript.

References

Adam-Blondon AF, Roux C, Claux D, Butterlin G, Merdinoglu D, This P (2004) Mapping 245 SSR markers on the *Vitis vinifera* genome: a tool for grape genetics. Theor Appl Genet 109(5):1017–1027

Adam-Blondon AF, Bernole A, Faes G, Lamoureux D, Pateyron S, Grando MS, Caboche M, Velasco R, Chalhoub B (2005) Construction and characterization of BAC libraries from major grapevine cultivars. Theor Appl Genet 110(8):1363–1371

Arcade A, Labourdette A, Falque M, Mangin B, Chardon F, Charcosset A, Joets J (2004) BioMercator: integrating genetic maps and QTL towards discovery of candidate genes. Bioinformatics 20:2324–2326

Barker CL, Donald T, Pauquet J, Ratnaparkhe MB, Bouquet A, Adam-Blondon AF, Thomas MR, Dry I (2005) Genetic and physical mapping of the grapevine powdery mildew resistance gene, *Run1*, using a bacterial artificial chromosome library. Theor Appl Genet 111:370–377

Bowers JE, Dangl GS, Vignani R, Meredith CP (1996) Isolation and characterization of the new polymorphic simple sequence repeat loci in grape (*Vitis vinifera* L.). Genome 45:1142–1149

Bowers JE, Dangl GS, Meredith CP (1999) Development and characterization of additional microsatellite DNA markers for grape. Am J Enol Vit 50:243–246

Causse M, Santoni S, Damerval C, Maurice A, Charcosset A, Deatrick J, de Vienne D (1996) A composite map of expressed sequences in maize. Genome 39:418–432

Chardon F, Virlon B, Moreau L, Falque M, Joets J, Decousset L, Murignaux A, Charcossot A (2004) Genetic architecture of flowering time in maize as inferred from quantitative trait loci meta-analysis and synteny conservation with the rice genome. Genetics 168:2169–2185

Chervin C, El-Kereamy1 E, Roustan JP, Latché A, Lamon J, Bouzayen M (2004) Ethylene seems required for the berry development and ripening in grape, a non-climacteric fruit. Plant Sci 167:1301–1305

Di Gaspero G, Cipriani G, Marrazzo MT, Andreetta D, Prado Castro MJ, Peterlunger E, Testolin R (2005) Isolation of (AC)*n*-microsatellites in *Vitis vinifera* L. and analysis of genetic background in grapevines under marker assisted selection. Mol Breed 15:11–20

Di Gaspero G, Cipriani G, Adam-Blondon A-F, Testolin R (2007) Linkage maps of grapevine displaying the chromosomal locations of 420 microsatellite markers and 82 markers for *R*-gene candidates. Theor Appl Genet 114(7):1249–1263

Donald TM, Pellerone F, Adam-Blondon AF, Bouquet A, Thomas MR, Dry IB (2002) Identification of resistance gene analogs linked to a powdery mildew resistance locus in grapevine. Theor Appl Genet 104:610–618

Doucleff M, Jin Y, Gao F, Riaz S, Krivanek AF, Walker MA (2004) A genetic linkage map of grape, utilizing *Vitis rupestris* and *Vitis arizonica*. Theor Appl Genet 109(6):1178–1187

Doligez A, Bouquet A, Danglot Y, Lahogue F, Riaz S, Meredith CP, Edwards KJ, This P (2002) Genetic mapping of grapevine (*Vitis vinifera* L.) applied to the detection of QTLs for seedlessness and berry weight. Theor Appl Genet 105:780–795

Doligez A, Adam-Blondon AF, Cipriani G, Di Gaspero G, Laucou V, Merdinoglu D, Meredith CP, Riaz S, Roux C, This P (2006) An integrated SSR map of grapevine based on five mapping populations. Theor Appl Genet 113:369–382

Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. Focus Biotech 12:13–15

Faes G (2004) Analisi della struttura del genoma di *Vitis vinifera*. Ph.D. dissertation, University of Udine, Italy, 100 pp

Fanizza G, Lamaj F, Costantini L, Chaabane R, Grando MS (2005) QTL analysis for fruit yield components in table grapes (*Vitis vinifera*). Theor Appl Genet 111:658–664

Fischer B, Salakhutdinov I, Akkurt M, Eibach R, Edwards KJ, Töpfer R, Zyprian EM (2004) Quantitative trait locus analysis of fungal disease resistance factor on a molecular map of grapevine. Theor Appl Genet 108:501–515

Grando MS, Bellin D, Edwards KJ, Pozzi C, Stefanini M, Velasco R (2003) Molecular linkage maps of *Vitis vinifera* L. and *Vitis riparia* Mchx. Theor Appl Genet 106:1213–1224

Grattapaglia D, Bertolucci FL, Sederoff RR (1995) Genetic mapping of QTLs controlling vegetative propagation in *Eucalyptus* grandis and *E. urophylla* using a pseudo-testcross strategy and RAPD markers. Theor Appl Genet 90:933–947

Hou DX (2003) Potential mechanisms of cancer chemoprevention by anthocyanins. Curr Mol Med 3(2):149–159

Jaillon O, Aury J, Noel B, Policriti A, Clepet C, Casagrande A, Choisne N, Aubourg S, Vitulo N, Jubin C, Vezzi A, Legeai F, Hugueney P, Dasilva C, Horner D, Mica E, Jublot D, Poulain J, Bruyère C, Billault A, Segurens B, Gouyvenoux M, Ugarte E, Cattonaro F, Anthouard V, Vico V, Del Fabbro C, Alaux M, Di Gaspero G, Dumas V, Felice N, Paillard S, Juman I, Moroldo M, Scalabrin S, Canaguier A, Le Clainche I, Malacrida G, Durand E, Pesole G, Laucou V, Chatelet P, Merdinoglu D, Delledonne M, Pezzotti M, Lecharny A, Scarpelli C, Artiguenave F, Pè M, Valle G, Morgante M, Caboche M, Adam-Blondon A, Weissenbach J, Quétier F, Wincker P, The French–Italian Public Consortium for Grapevine Genome Characterization (2007) The grapevine genome sequence suggests ancestral hexaploidization in major angiosperm phyla. Nature 449:463–467

Jakobek JL, Smith-Becker JA, Lindgren PB (1999) A bean cDNA expressed during a hypersensitive reaction encodes a putative calcium-binding protein. Mol Plant Microbe Interact 12:712–719

Karp A, Jones RN (1983) Cytogenetics of *Lolium perenne*. Part 2. Chiasma distribution. Theor Appl Genet 64:137–145

Kahkonen MP, Heinonen M (2003) Antioxidant activity of anthocyanins and their aglycons. J Agric Food Chem 51(3):628–633

Kim CY, Liu Y, Thorne ET, Yang H, Fukushige H, Gassmann W, Hildebrand D, Sharp RE, Zhang S (2003) Activation of a stress-responsive mitogen-activated protein kinase cascade induces the biosynthesis of ethylene in plants. Plant cell (11):2707–2718

Kobayashi S, Ishimaru M, Hiraoka K, Honda C (2002) *Myb*-related genes of Kyoho grape (*Vitis labruscana*) regulate anthocyanin biosynthesis. Planta 215:924–933

Kobayashi S, Yamamoto NG, Hirochika H (2004) Retrotransposon-induced mutations in grape skin color. Science 14;304(5673):982

Konieczny A, Ausubel FM (1993) A procedure for mapping *Arabidopsis* mutations using co-dominant ecotype-specific PCR-based markers. Plant J 4:403–410

Kortekamp A, Zyprian E (2003) Characterization of Plasmopara-resistance in grapevine using in vitro plants. J Plant Physiol 160(11):1393–1400

Kosamby DD (1944) The estimation of map distance from recombination values. Ann. Eugen 12:172–175

Lai Z, Livingstone K, Zou Y, Church SA, Knapp SJ, Andrews J, Rieseberg LH (2005a) Identification and mapping of SNPs from ESTs in sunflower. Theor Appl Genet 111(8):1532–1534

Lai Z, Nakazato T, Salmaso M, Burke JM, Tang S, Knapp SJ, Rieseberg LH (2005b) Extensive chromosomal repatterning and the evolution of sterility barriers in hybrid sunflower species. Genetics 171:291–303

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newberg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Lijavetzky D, Ruiz-Garcia L, Cabezas JA, De Andres MT, Bravo G, Ibanez A, Martinez-Zapater J (2006) Molecular genetics of berry colour variation in table grape. Mol Genet Genomics 276(5):427–435

Lijavetzky D, Cabezas J, Ibanez A, Rodriguez V, Martinez-Zapater J (2007) High throughput SNP discovery and genotyping in grapevine (*Vitis vinifera* L.) by combining a re-sequencing approach and SNPlex technology. BMC Genomics 8:424

Lowe KM, Walker MA (2006) Genetic linkage map of the interspecific grape rootstock cross Ramsey (*Vitis champinii*) × Riparia Gloire (*Vitis riparia*). Theor Appl Genet 112(8):1582–1592

Merdinoglu D, Butterlin G, Bevilacqua L, Chiquet A, Adam-Blondon A-F, Decroocq S (2005) Development and characterization of a large set of microsatellite markers in grapevine (*Vitis vinifera* L.) suitable for multiplex PCR. Mol Breed 15:349–366

Moser C, Segala C, Fontana P, Salakhudtinov I, Gatto P, Pindo M, Zyprian E, Toepfer R, Grando MS, Velasco R (2005) Comparative analysis of expressed sequence tags from different organs of *Vitis vinifera* L. Funct Integr Genomics 5:208–217

Navindra PS, Yanjun Z, Muraleedharan GN (2003) Inhibition of proliferation of human cancer and cyclooxygenase enzymes by anthocyanidins and chatechins. Nutr Cancer 46(1):101–106

Neff MM, Turk E, Kalishman M (2002) Web-based primer design for single nucleotide polymorphism analysis. Trends Genet 18:613–615

Orita M, Iwahana H, Kanazawa H, Hayashi K, Sekiya T (1989) Detection of polymorphisms of human DNA by gel electrophoresis as single strand conformational polymorphisms. Proc Natl Acad Sci USA 86:2766–2770

Passamonti S, Vrhovsek U, Vanzo A, Mattivi F (2003) The stomach as a site for anthocyanins absorption from food. FEBS Lett 544:210–213

Rafalski A (2002) Applications of single nucleotide polymorphisms in crop genetics. Curr Opin Plant Biol (2):94–100

Riaz S, Dangl GS, Edwards KJ, Meredith CJ (2004) A microsatellite marker based framework linkage map of *Vitis vinifera* L. Theor Appl Genet 108:864–872

Rieseberg LH, Van Fossen C, Desrochers AM (1995) Hybrid speciation accompanied by genomic reorganization in wild sunflower. Nature 375:713–727

Rozen S, Skaletsky HJ (2000) Primer3 on the WWW for general users and for biologist programmers. In: Krawetz S, Misener S (eds) Bioinformatics methods and protocols: methods in molecular biology. Humana Press, Totowa, pp 365–386

Salmaso M (2003) Analysis of genome diversity and construction of a functional map in *Vitis* spp. Ph.D. Dissertation, University of Padova, Italy, 108 pp

Salmaso M, Faes G, Segala C, Stefanini M, Salakhutdinov I, Zyprian E, Toepfer R, Grando MS, Velasco R (2004) Genome diversity and gene haplotypes in the grapevine (*Vitis vinifera* L.), as revealed by single nucleotide polymorphisms. Mol Breed 14:385–395

Schneider K, Borchardt DC, Schafer-Pregl R, Nagl N, Glass C, Jeppsson A, Gebhardt C, Salamini F (1999) PCR-based cloning and segregation analysis of functional gene homologues in *Beta vulgaris*. Mol Gen Genet. 262(3):515–524

Scott KD, Eggler P, Seaton G, Rossetto M, Ablett EM, Lee LS, Henry RJ (2000) Analysis of SSR derived from grape ESTs. Theor Appl Genet 100:723–726

Sefc KM, Regner F, Turetschek E, Glössl J, Steinkellner H (1999) Identification of microsatellite sequences in *Vitis riparia* and their applicability for genotyping of different *Vitis* species. Genome 42:367–373

Simianer H, Szyda J, Ramon G, Lien S (1997) Evidence for individual and between-family variability of the recombination rate in cattle. Mamm Genome 8:830–835

Sparvoli F, Martin C, Scienza A, Gavazzi G, Tonelli C (1994) Cloning and molecular analysis of structural genes involved in flavonoid and stilbene biosynthesis in grape (*Vitis vinifera* L.). Plant Mol Biol. 24(5):743–755

Syvanen AC, Aalto-Setala K, Harju L, Kontula K, Soderlund H (1990) A primer-guided nucleotide incorporation assay in the genotyping of apolipoprotein E. Genomics 8:684–692

Testolin R, Huang WG, Lain O, Messina R, Secchione A, Cipriani G. (2001) A kiwifruit (*Actinidia* spp.) linkage map based on microsatellites and integrated with AFLP markers. Theor Appl Genet 103:30–36

This P, Cadle-Davidson M, Lacombe T, Owens CL (2007). Wine grape (*Vitis vinifera* L.) color associates with allelic variation in the domestication gene VvmybA1. Theor Appl Genet 114:723–730

Thomas MR, Scott NS (1993) Microsatellite repeats in grapevine reveal DNA polymorphisms when analysed as sequence-tagged sites (STSs). Theor Appl Genet 86:985–991

Troggio M, Malacarne G, Coppola G, Segala C, Cartwright DA, Pindo M, Stefanini M, Mank R, Moroldo M, Morgante M, Grando MS, Velasco R (2007) A dense single-nucleotide polymorphism-based genetic linkage map of grapevine (*Vitis vinifera* L.) anchoring Pinot Noir bacterial artificial chromosome contigs. Genetics 176(4):2637–2650

Troggio M, Malacarne G, Vezzulli S, Faes G, Salmaso M, Velasco R (2008) Methods for polymorphism detection and genotyping within expressed regions in grapevine genome. Vitis 47(1):21–30

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

Velasco R, Zharkikh A, Troggio M, Cartwright DA, Cestaro A, Pruss D, Pindo M, FitzGerald LM, Vezzulli S, Reid J, Malacarne G, Iliev D, Coppola G, Wardell B, Micheletti D, Macalma TM, Facci M, Mitchell JT, Perazzolli M, Eldredge G, Gatto P, Oyzerski R, Moretto M, Gutin N, Stefanini M, Chen Y, Segala C, Davenport C, Demattè L, Mraz A, Battilana J, Stormo K, Costa F, Tao Q, Si-Ammour A, Harkins T, Lackey A, Perbost C, Taillon B, Stella A, Solovyev V, Fawcett JA, Sterck L, Vandepoele K, Grando MS, Toppo S, Moser C, Lanchbury J, Bogden R, Skolnick M, Sgaramella V, Bhatnagar SK, Fontana P, Gutin A, Van de Peer Y, Salamini F, Viola R (2007). High quality draft consensus sequence of the genome of a heterozygous grapevine variety. PLoS ONE 2(12):e1326

Weeden NF (1993) Approaches to mapping in horticultural crops. Plant genome analysis. CRC Press Inc., Boca Raton, pp 7–68

Wright SI, Bi IV, Schroeder SG, Yamasaki M, Doebley JF, McMullen MD, Gaut BS (2005) The effects of artificial selection on the maize genome. Science 308:1310–1314