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Beyond the Genome, Opportunities for a Modern Viticulture: A Research Overview

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Abstract: Grapevine is one of the world's most important fruit crops. All 60 $2n = 38$ *Vitis* species worldwide are diploids that cross easily; hybrids are fertile and advanced generation pedigrees are available. The cultivated grape species *Vitis vinifera* has the potential to become a model for fruit tree genetics. Given its cultural and economic importance, grapevine has received much attention from the scientific community in the last few years, resulting in considerable progress in genetic and genomic research. A consensus sequence of the grapevine genome was generated, providing information on overall organization, gene content, and structural components of the DNA in the 19 chromosomes of *V. vinifera*. Extensive genetic mapping has been conducted in *Vitis* ssp. based on SSR markers, including the identification of quantitative trait loci for a variety of traits. A large set of single-nucleotide polymorphisms was developed from expressed sequence tags, bacterial artificial chromosome-end sequences, and unique regions of the assembled genome of Pinot noir, providing a comprehensive grapevine genetic map. Single-nucleotide polymorphism markers represent a substantial resource for molecular breeding programs, providing a new basis for map-based gene isolation and fine-mapping quantitative trait loci by identifying candidate genes.

Key words: *Vitis vinifera,* genetic map, whole genome sequencing, candidate gene

Grapevine is one of the world's most important fruit crops. Europe grows the highest percentage of the world's grapes (50%), followed by Asia (23%), the Americas (20%), Africa (5%), and Oceania (2%). The majority of grapes produced worldwide are from cultivars of *Vitis vinifera* L. ssp. *sativa*, while the rest are other *Vitis* spp. and interspecific hybrids. Of total grape production, 70% is used for wine, 22% for table grapes, and 8% for raisins; other transformed products such as juices, jams, and jellies are only of local interest. Several commodities are by-products or derivatives of the wine industry, such as must, marc distillates, marc pulp, tartaric acid, seed oil, and vinegar. The earliest evidence of winemaking has been found in Iran and dates back to ~7400–7000 BP (before present); since that time this beverage has been present throughout the development of human culture. A traditional icon of the Mediterranean diet along with olive oil and wheat (Panagiotakos et al. 2004), winemaking has spread to the New World during recent centuries. Wine is a nutraceutical product; when the fruit is consumed as table grapes or as a transformed product (juice, wine, etc.), it helps reduce cardiovascular disease and has anticarcinogenic and extrogenic properties (Burns et al. 2000). These nutraceutical properties are due to the high concentration of resveratrol in the berry, which prevents blood platelet aggregation and elevates beneficial HDL (high density lipoprotein), the antioxidant quercitin, and ellagic acid, which scavenges carcinogens and moves them out of the body (Kharb and Singh 2004). The medicinal value of grapes and wine was known by the ancient Egyptians and by Hippocrates (2467–2384 BP) (Masquelier 1992). Grapes and wine today are part of the Ayurvedic medicine of East India (Paul et al. 1999) and the traditional medicines of South Africa, the Middle East, and China (Kalt 2001). *Vitis vinifera* is mentioned in several pharmacopeias. Novel uses of grapes and wine are becoming popular: fasting on grapes is called "ampelotherapy" (the grape cure) and is alleged to be powerfully detoxifying and alkalinizing, and cosmetic treatments with wine and its derivates are referred to as "winetherapy."

Vitis vinifera ssp. *sativa* is hermaphroditic, except for rare cases. Although most fruit and nut trees, woody ornamental trees and shrubs, herbaceous perennials, and many annuals are incompatible, cultivated grapevine is self-fertile and thus is not an obligate outcrosser. Wild grapevines are dioecious, and, due to their prevailing wind- and insect-pollinated habit, outbreeding is ensured with high gene flow. Plant breeding through controlled pollination between selected genotypes produces cultivars that are highly heterozygous and carry a heavy load of deleterious

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recessives: inbreeding depression is severe enough that, by the second or third generation, sterility often ensues. All 60 $2n = 38$ *Vitis* species worldwide are diploids that can be easily crossed; hybrids are fertile and advanced generation pedigrees are available (Olmo 1979).

Grapevine is a potential model organism for fruit trees, as poplar is for forest trees (Tuskan et al. 2006). Although genotype is a major determinant for transformation (Wang et al. 2005), *Vitis* spp. can be transformed, regenerated, and micropropagated via somatic embryogenesis of anthers (Kikkert et al. 2005). The relatively small haploid genome size of *V. vinifera* (475 Mbp; Lodhi and Reisch 1995) compared to many other perennial plant species (Arumuganathan and Earle 1991) facilitates molecular genetic study of *Vitis*. Unfortunately, grapevine breeding is a time-consuming process because of the long reproductive cycle, the large size of plants, and the fact that productivity and quality can be evaluated at best only after five years. Molecular tools may overcome these difficulties and open the way to new strategies for more efficient breeding (Morgante and Salamini 2003).

Given these features and applications, it is not surprising that grape has received much attention from the scientific community, resulting in considerable progress in genetic and genomic research and complementing advances made with the sequenced *Arabidopsis*, rice, and poplar.

Since a white paper concerning the grapevine genomics initiative (IGGP, International Grapevine Genome Project, www.vitaceae.org) was written in Davis, California, in June 2001, researchers on grapevine have had access to most of the tools commonly used in this field, from published microsatellite (or simple sequence repeat, SSR) markers developed as international consortiums (Vitis Microsatellite Consortium) or as national projects. Thus, a second-generation set of markers based on single-nucleotide polymorphisms (SNPs), which along with insertion/deletion (in/del) events provide reliable PCR-based genetic markers, has been developed, exploiting the most frequent genetic differences within a species (Rafalski 2002). Hundreds SNP-based markers have been developed in grapevine and uploaded to public databases (NCBI, http://www.ncbi.nlm.nih.gov). Extensive genetic maps have been constructed in *Vitis* spp. based on these markers (Troggio et al. 2007), including the identification of quantitative trait loci (QTLs) for a variety of traits (Xu et al. 2008). Moreover, given the robust physical mapping information of Cabernet Sauvignon and Pinot noir (Adam-Blondon et al. 2005; http://genomics.research.iasma.it), large collections of expressed sequence tags (ESTs) (Moser et al. 2005, da Silva et al. 2005, Peng et al. 2007), and associated proteomics and metabolic profiling (Sarry et al. 2004, Pereira et al. 2005, Castro et al. 2005, Mattivi et al. 2006, Deluc et al. 2007, Deytieux et al. 2007), grapevine genome sequencing is a timely and important undertaking. To date, grapevine is the first fruit tree to have its genome deciphered (Jaillon et al. 2007, Velasco et al. 2007).

This article is an overview of the genetic and genomic tools cited and their use in molecular-based approaches aimed at gene isolation and characterization, biodiversity exploitation, and breeding applications.

Molecular marker development and genetic mapping. Molecular marker development helps clarify the genetic basis for complex traits and facilitates construction of genetic linkage maps. Linkage maps are a prerequisite for study of both qualitative and quantitative trait inheritance and for integration of the molecular information necessary for marker-assisted selection (MAS; Mazur and Tingey 1995), map-based cloning (Tanksley et al. 1992), and anchoring to physical maps (Meyers et al. 2005) and genome sequences. Thus, a key resource forming the basis of classical genetics and genomics of *V. vinifera* is the construction of a dense genetic map based on well-characterized, gene-specific molecular markers.

Most first-generation linkage maps of *V. vinifera* were based on anonymous genetic loci such as SSR and amplified fragment length polymorphism (AFLP) markers (Lodhi et al. 1995, Dalbò et al. 2000, Doligez et al. 2002, Grando et al. 2003, Adam-Blondon et al. 2004, Doucleff et al. 2004, Fischer et al. 2004, Riaz et al. 2004, Fanizza et al. 2005, Doligez et al. 2006, Lowe and Walker 2006, Riaz et al. 2006, Xu et al. 2008). Recently, resistance gene analog (RGA)-derived markers have been mapped (Di Gaspero et al. 2007, Welter et al. 2007). SNP-based markers were an improved resource, providing a new basis for map-based gene isolation, and for fine-mapping QTLs by identifying candidate genes (Troggio et al. 2007, Salmaso et al. 2008, S. Vezzulli, unpublished data, 2007). In particular, the development of integrated reference linkage maps (515 loci, Doligez et al. 2006; 1134 loci, S. Vezzulli, unpublished data, 2007) covering most of the 475 Mbp grapevine genome (Lodhi and Reisch 1995) has allowed cross-talk between maps and crossing populations developed in different parts of the world.

For several years, the development of SNP markers has been a priority at the Istituto Agrario di San Michele all'Adige (IASMA) research center and considerable investment has been made in high-tech instruments and laboratory equipment. We started with gel-based techniques such as single-strand conformational polymorphism analysis (SSCP; Orita et al. 1989) and considered fluorescence-based techniques such as minisequencing (Syvanen 2005, Troggio et al. 2008). Essentially, low-throughput genotyping systems have been replaced with mid- and high-throughput genotyping systems, culminating in the successful application of the SNPlex assay (Applied Biosystems Inc.) in grapevine (Lijavetzky et al. 2007, Pindo et al. 2008). High efficiency coupled with full automation of SNP detection and screening can generate over 200,000 data points per week.

In addition to the available IGGP SSR reference set, the development at IASMA of hundreds of SNP markers—mainly targeting genes—laid the foundations for construction of high resolution and functional linkage

maps in grapevine (Troggio et al. 2007, Salmaso et al. 2008, S. Vezzulli, unpublished data, 2007). The first haplotype analysis in *Vitis* spp. was also performed (Salmaso et al. 2004).

Recently, a large set of SNPs was mapped in a mapping population of 94 F_1 individuals derived from a *V. vinifera* cross of the cultivars Syrah and Pinot noir. SNP-based markers were developed from both gene sequences derived by cDNA libraries (dbEST at NCBI) and genomic sequences derived by sequencing of bacterial artificial chromosome (BAC)-ends (BESs; Cinzia Segala 2005, unpublished data) with similar efficiency rates of 38.3% of polymorphic markers from 454 selected EST sequences and 35% from 903 selected BESs, respectively. Spanning 1,245 cM over 19 linkage groups (LGs), the map was generated from the segregation of 483 SNP-based markers, 132 SSRs, and 379 AFLP markers (994 loci) and represents the first fine grapevine genetic map produced with transferable markers (Troggio et al. 2007). To build the two parental and the consensus maps, a recently developed mapping program TMAP (Cartwright et al. 2007) that considers genotyping errors and reduces the inflationary effect of increasing the number of markers was used. Errors inflate the number of recombinations and considerably expand map intervals (Harald et al. 2000). In this respect, the Syrah x Pinot noir map is more reliable in marker order and marker distance estimation (Cartwright et al. 2007). The accuracy of marker order estimated by meiotic methods was also verified using physical distance information for genetically mapped markers contained in the anchored BAC contigs (http://genomics.research.iasma.it) and using anchoring on the genome sequence of Pinot noir (accession numbers AM423240-AM489403 at the EMBL/GenBank/DDBJ databases; Velasco et al. 2007). Based on this recent achievement, the Pinot noir map has been improved with an additional 800 SNPs (dbSNP accession numbers at the NCBI SNP database from 76900200 to 76900755) on specific regions of the grapevine genome poorly covered by previous markers (Pindo et al. 2008) and an additional 94 individual progeny screened with the SNPlex genotyping system (Michela Troggio 2007, unpublished data). Since it is much easier to generate a high resolution genetic map in the presence of high recombination values, in regions of suppressed recombination more progeny size are needed to recover the number of crossovers necessary for constructing detailed genetic maps (Tanksley et al. 1992).

An integrated genetic map of the five elite *V. vinifera* L. cultivars Syrah, Pinot noir, Grenache, Cabernet Sauvignon, and Riesling, parents of 275 individuals from three crosses, was thus developed with 33.3% common markers per pair of crosses. Spanning 1,443 cM over 19 linkage groups, the complete integrated map (also built by TMAP) comprises 1134 markers (350 AFLPs, 332 BESs, 169 ESTs, and 283 SSRs) and shows a mean distance between neighbor markers of 1.27 cM. This is therefore the densest genetic map developed so far in grapevine. Marker order was mainly conserved between the integrated map and the very dense Syrah x Pinot noir consensus map, except for a few inversions. Moreover, marker order was proved reliable since this integrated linkage map partially anchors the genome sequence of Pinot noir through 671 markers (Velasco et al. 2007). These markers anchor 623 contigs assembled into 178 metacontigs for a total genome coverage of 360.4 Mb (S. Vezzulli, unpublished data, 2007). This "species consensus map" will serve as a fundamental tool for molecular breeding in *V. vinifera* and related species.

Physical mapping and marker integration. Development of physical maps and their integration with genetic maps is needed to isolate and clone genes of interest efficiently (Barker et al. 2005, Troggio et al. 2007). In grapevine, a growing resource of BAC libraries is available (Adam-Blondon et al. 2005, Lamoureux et al. 2006; http://genomics.research.iasma.it; www.vitaceae.com), mainly for well-known *V. vinifera* cultivars but also for *V. vinifera* genotypes introgressed with disease resistance genes. A further step will be to develop BAC libraries on other *Vitis* species that are used as sources for resistance genes for grapevine breeding (Pauquet et al. 2001). These BAC libraries can be used for development of local physical maps or whole genome physical maps.

Physical maps consist of the assembly into contigs of overlapping large insert clones based on fingerprint similarities and the presence of common markers. These assemblies are made using the software FPC (Soderlund et al. 2000) and the distance is in base pairs. Most physical maps developed for plants are now based on assembly of BAC clones (Meyers et al. 2004).

The first BAC-based physical map of the grape genome and its integration with the genetic map has been reported for the cultivar Pinot noir, which is highly heterozygous at the sequence level (Velasco et al. 2007), using FPC (http://genomics.research.iasma.it) and high information content fingerprinting of 49,536 BAC clones from Pinot noir. The FPC program that assembles contigs obtained by BAC fingerprinting is designed to assemble highly homozygous genomes. Assembly could be improved using new map assembly algorithms that explicitly deal with the presence of two haplotypes regardless of the frequency and patterns of heterozygosity (Dustin Cartwright 2006, unpublished data).

Two complementary strategies have been adopted to help integrate the Syrah x Pinot genetic map. The first strategy involved construction of BAC pools (Barillot et al. 1991) as described (Klein et al. 2000). 24,576 BAC clones with a mean insert size of 100 kb (five genome equivalents) contained in 64 384-well microtiter plates were arranged in a stack and sampled in six distinct ways. Three of these were according to the Cartesian coordinates (first configuration): plate pool (PP), side pool (SP), and front pool (FP). The three remaining pool types were sections taken at an angle through the stack (second

configuration): row pool (RP), column pool (CP), and diagonal pool (DP). In total, the six pool types resulted in 184 BAC pools. Five of the six configurations (PP, FP, RP, CP, and DP) were composed of 32 pools, each containing 768 BACs. The sixth configuration (SP) was composed of 24 pools each containing 1024 BACs. Thus, each clone in the stack was present in exactly one pool of each configuration.

The BAC pools were then screened with EST, SSR, and AFLP primers to identify mapped fragments. SSR and EST amplification products from BAC pools were run on agarose gel. BAC clones hosting SSR and EST markers were identified by a Unix-based application with a web interface. AFLP amplification products from BAC pools were analyzed on acrylamide gels along with amplification products from the two parents and the mapping population as a control (AFLP Quant-Pro, Keygene, Wageningen, Netherlands). BACs containing AFLPs were identified in the same way as the other markers.

To improve integration between genetic and physical maps, a second strategy used markers derived from collections of BESs (Cinzia Segala 2005, unpublished data; Troggio et al. 2007) with subsequent determination of their genetic position on the linkage map. 30,832 Pinot noir BESs were characterized and used to integrate the Syrah x Pinot noir genetic map with the Pinot noir physical map; an additional 68,000 BESs from the Pinot noir sequencing project were used to assemble the Pinot noir genome (Velasco et al. 2007).

A total of 623 markers from a Syrah x Pinot noir genetic map were anchored on 367 BAC contigs covering 352 Mb (Troggio et al. 2007). Based on contigs with two or more genetically mapped markers, regions of both increased and reduced recombination were identified within the grape genome, although the results are not conclusive because of significant uncertainties in both genetic and physical distances. The genome sequencing data can help resolve the problem (Figure 1). Variations in the correspondence between physical and genetic distance along chromosomes have already been well documented in several species (Tanksley et al. 1992, Chen et al. 2002, King et al. 2002). When available, such variations add important informa-

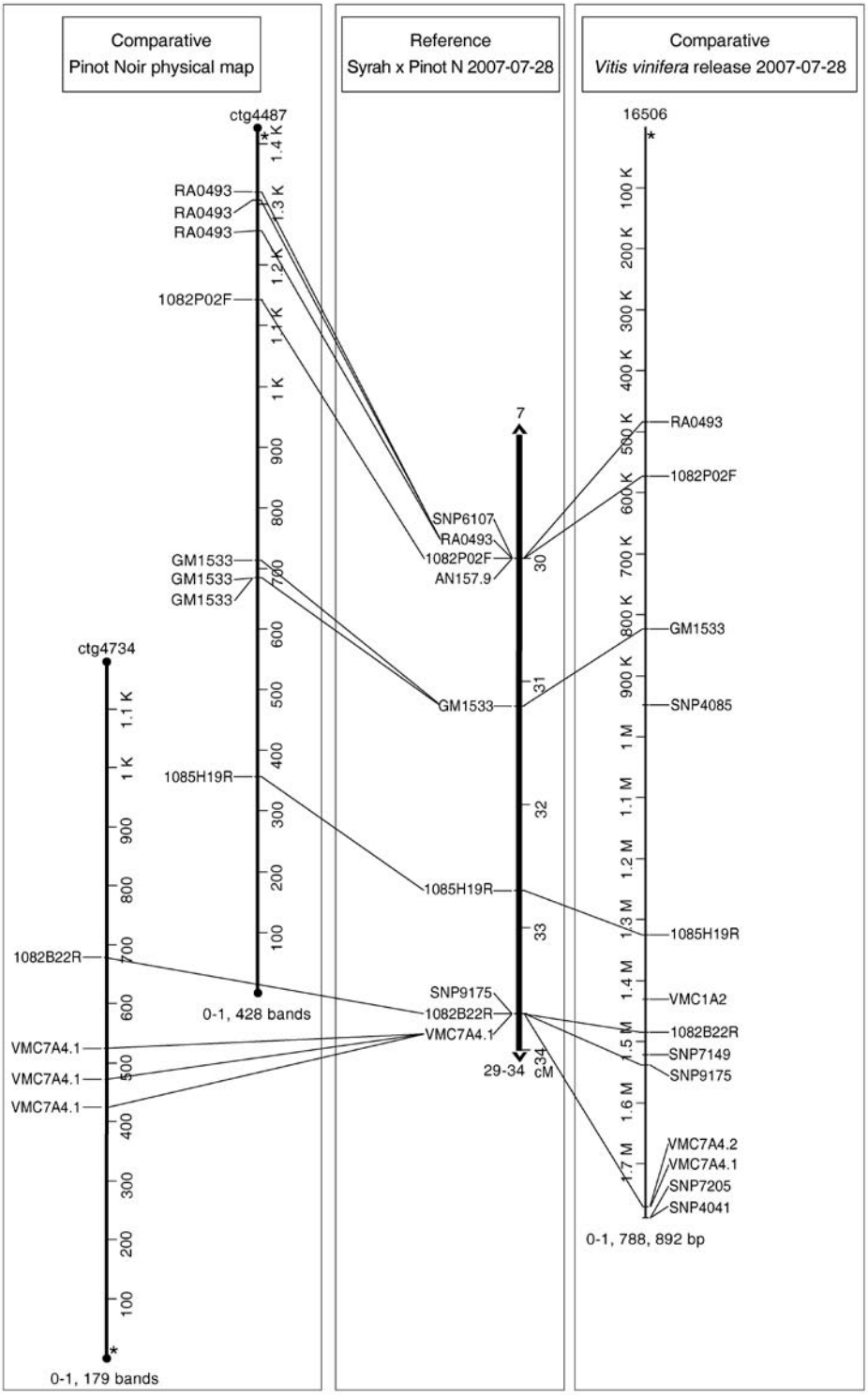

Figure 1 The image displayed by the comparative map tool CMap of LG 7 (29-34 cM interval), the BAC contigs 4487 and 4734, and the anchored metacontig 16506 of the assembled *V. vinifera* genome. Correspondences between marker loci in the genetic map, the BAC contig, and the assembled metacontig are shown with solid lines.

tion to map-based cloning projects. The BAC pooling strategy was also used as a high-throughput method to identify positive clones for a corresponding target region within the thousands of BAC clones of a genomic library (Pindo et al. 2006).

A specific primer pair was designed on the *MybA1* sequence (AB111100) to develop a new EST marker to position on the genetic map of the hybrid Merzling (the complex genotype 'Freiburg 993-60' derived from multiple crosses also involving wild species such as *V. rupestris* and *V. lincecumii)* x *V. vinifera* cv. Teroldego cross (FxT). *MybA1* co-segregated with the phenotypic trait "color" on LG 2 (Salmaso et al. 2008). The BAC pools were screened with the *MybA1* primer combination and two positive clones, 1044_B05 and 1085_L05, were found. Based on SNPs found within the corresponding BESs, a new SNP-based marker (1044B05) was developed. The polymorphisms in the FxT mapping population and in the *V. vinifera* cv. Syrah x Pinot noir cross (SxP) were assessed using the minisequencing technique (Troggio et al. 2008). As the SxP population does not segregate for the "color trait," its position was indirectly located on the map (Figure 2).

Whole genome sequencing. Given its cultural and economic importance, winegrape was an obvious first candidate among woody fruit crops to have its genome deciphered. Two projects were aimed at sequencing the grapevine genome. One was a collaboration between IASMA and two private companies, Myriad Genetics, Incorporated, and the 454 Life Science (Velasco et al. 2007). The other was a consortium of French and Italian public laboratories (Jaillon et al. 2007). The latter project selected a near-homozygous line, originally derived from Pinot noir, that has been bred close to full homozygosity (estimated at ~ 93%) by successive selfings to facilitate assembly of 12X shotgun sequences.

The IASMA project focused on the elite cultivar Pinot noir, with the multiple goals of genome assembly, gene identification and annotation, and identification of the most possible polymorphisms. Of special interest to biologists and breeders are polymorphisms in and around coding regions. Pinot noir is highly polymorphic, with

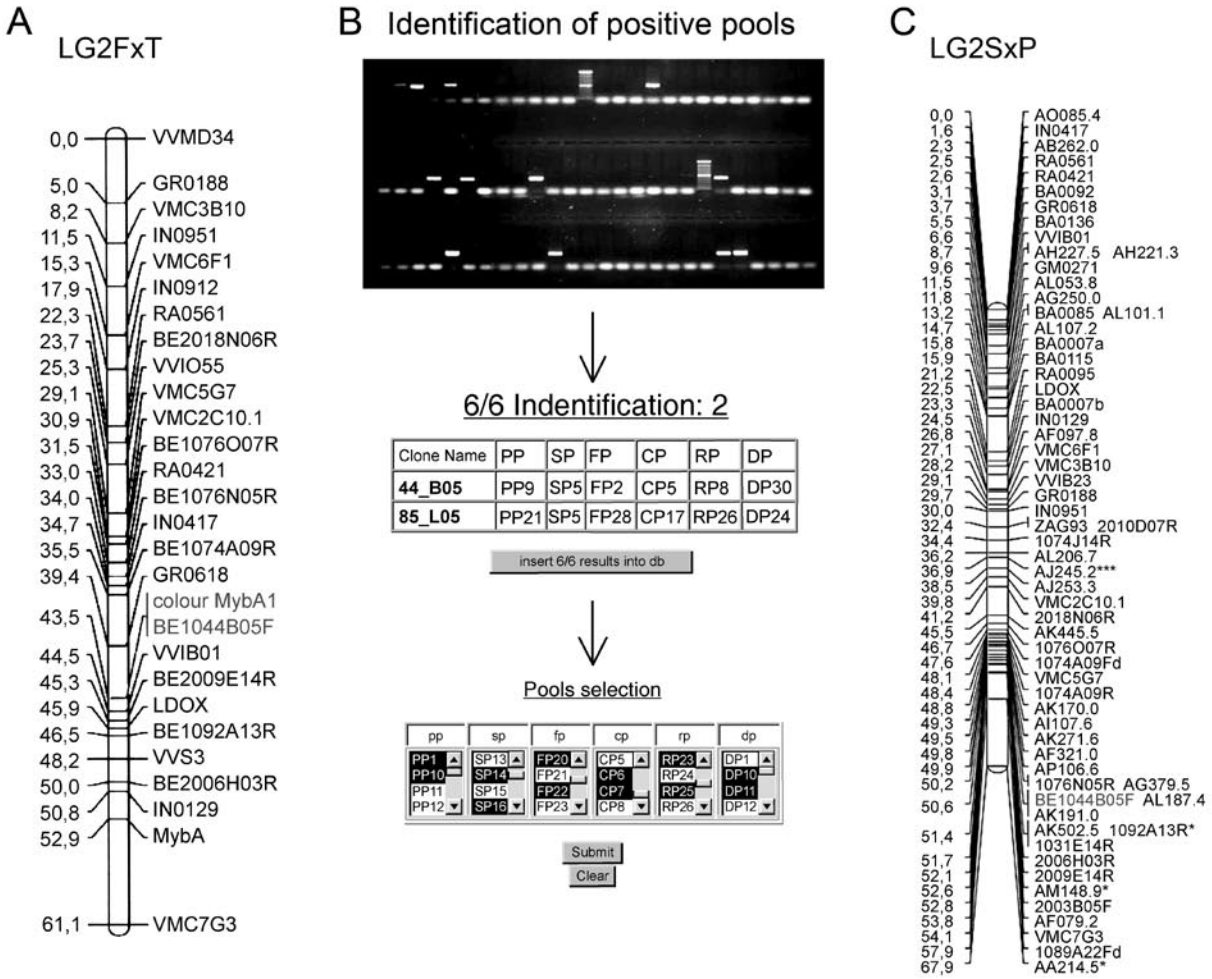

Figure 2 (**A**) Mapping of "color trait" and *MybA1* markers on LG2 on the genetic map Merzling (the complex genotype 'Freiburg 993-60' derived from multiple crosses also involving wild species such as *V. rupestris* and *V. lincecumii*) x *V. vinifera* cv. Teroldego (FxT) cross. (**B**) Identification of BAC clones 1044_B05 and 1085_L05 by *MybA1* primers combination. (**A** and **C**) Mapping of 1044B05F marker on LG2 on the genetic maps FxT and Syrah x Pinot noir (SxP) crosses.

two clearly distinguishable haplotypes revealing several million SNPs and small indels. This represents a substantial resource for molecular breeding programs and trait and QTL marker association.

Three main strategies are currently used for sequencing complex genomes: a clone-by-clone approach, a whole-genome shotgun approach, and a combination of both (Green 2001). The first and last strategies need a very precise positioning of BAC clones on a physical map. The strategy for whole-genome sequencing was suggested by the large set of markers available for Pinot noir and the troubleshooting developed during physical mapping experiments.

Two sequencing techniques were adopted. The Sanger 6.5X sequencing approach was integrated with a scalable, highly parallel sequencing by synthesis (SBS) system with throughput significantly greater than that of capillary electrophoresis instruments. The 4.2X coverage of SBS was crucial for identifying polymorphic sites and closing most gaps between DNA contigs, both by providing increased coverage and by using a method free of biases introduced by cloning before sequencing. This is the first project which used both Sanger and SBS shotgun sequencings for a large eukaryotic genome.

Existing software and strategies were not adequate to assemble this highly heterozygous genome. A novel approach to genomic alignment was developed to generate a single final sequence representing both chromosomes of Pinot noir. The result is a mosaic of two Pinot noir haplotypes which includes haplotype-specific gaps (sequences inserted in one haplotype but not in the other) and in which all identified SNP-type polymorphisms are represented in the final consensus by IUPAC ambiguous base notations. Around two million highly validated SNPs and more than a million in/dels were identified in the assembled sequences.

Two BAC libraries and a fosmid library were end-sequenced to assemble large meta-contigs. Contigs were oriented and ordered on appropriate chromosomes by high-throughput marker development and genotyping in an F_1 cross of Syrah x Pinot noir (Figure 3). The SNP-based markers were essential to improve the metacontig assembly. Many adjacent metacontigs that were not initially merged because of nonsignificant links between them were associated with neighboring genetic markers and could therefore be safely merged into a single larger metacontig. On the other hand, if a metacontig was associated with several markers from different LGs or with distant markers from the same LG, it was considered chimeric and was split into two separate metacontigs by a semi-automated procedure.

The assembled contigs were used to predict gene content. Various gene prediction methods were used: FgenesH homology-based FgenesH+ (Solovyev et al. 2006), Twinscan (Korf et al. 2001), GlimmerHMM (Majoros et al. 2004), and Tentative Consensus (TC) transcripts derived from 320,000 ESTs deposited in public databases. Gene annotation followed a consensus approach. BLAST searches were performed against UniProt and plant protein databases annotated with GO terms of various domain libraries (Prints, HMMPIR Pfam, and SMART).

The sequence provides information on overall organization, gene content, and structural components of the DNA in the 19 chromosomes of *V. vinifera*. 44,179 contigs merged into 2,093 meta-contigs covering 477.1 Mb of genomic DNA. Of these, 435.1 Mb were anchored to the 19 LGs using 1,356 markers. More than 80% of the anchored metacontigs were oriented by two or more markers. The number of predicted genes is 29,585, of which 96.1% were assigned to LGs. Of around 2,000,000 SNPs, 1,751,176 were mapped to chromosomes and one or more of them were identified in 86.7% of anchored genes.

The enormous set of data generated by the two projects will constitute a useful resource for marker-assisted selection and breeding, once QTLs and monogenic traits are assigned to well-defined regions.

Candidate gene isolation for modern breeding. Although more than 5,000 *V. vinifera* cultivars exist, the global market is dominated by a few wine and table grape cultivars. Ancient cultivars and germplasm are disappearing as a consequence, even though they represent an important source of genetic variability and traits of interest (This et al. 2006). The need for a new approach to breeding that exploits the great diversity within the *Vitis* genus is widely recognized. Dense integrated genetic and physical maps are a key step in map-based cloning projects for genes of interest. The long generation time and the space needed to grow large progenies means this approach is more problematic in perennial species than in annual species, although some success has already been obtained (Patocchi et al. 1999, Claverie et al. 2004). Integrated genetic and physical maps are very efficient in accelerating gene mapping, as no polymorphism is required for anchoring them on BAC clones. Such integrated maps are thus invaluable resources for the quick development of new markers in targeted regions using BESs (Barker et al. 2005, Castellarin et al. 2006, Troggio et al. 2007), for candidate gene approaches by establishing links between genetic maps—where QTLs for traits of interest have been located—and gene-containing BACs, and for preparing and accelerating map-based cloning projects.

Three different studies illustrate integrated use of genetics and genomic tools. The first (Barker et al. 2005) is a recently published local fine map in grapevine for a region containing a major gene for resistance to powdery mildew, *Run*1 (Pauquet et al. 2001, Donald et al. 2002). A BAC library developed from a resistant genotype was screened with two of the closest markers and contigs were built, mainly for the *Run*1-carrying haplotype, amplifying a band in the introgressed chromosome and nothing in the susceptible haplotype. The BESs generated from the BACs in this region allowed development of new markers and the extension of the contigs. Large progeny screening for refinement allowed a family of

candidate genes to be localized in this region (Barker et al. 2005).

The second study is the linkage map from the hybrid Merzling x *V. vinifera* cv. Teroldego cross based on localization of expressed genes and unique genomic sequences (Salmaso et al. 2008). This mapping experiment was based on an F_1 population selected for many segregating traits including tolerance to fungal pathogens, color and quality of anthocyanins, resistance to *Daktulosphaira vitifoliae*, bunch shape and compactness, and high- versus low-quality berry metabolic profiles. The aims of this study were to map berry color genes participating in anthocyanin metabolism, such as 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)—and some *myb* transcription factors proposed as regulators of the phenylpropanoid pathway (Kobayashi et al. 2002) and to study their correlation with berry color. In the Merzling x Teroldego segregating population, berry color co-segregated with a *myb* gene mapping to LG2, in accordance with other recently published papers (Lijavetzky et al. 2006, This et al. 2007). The study described above, however, is the first demonstration of co-localization of *Myb* with color, starting from a cross- population segregating for berry color.

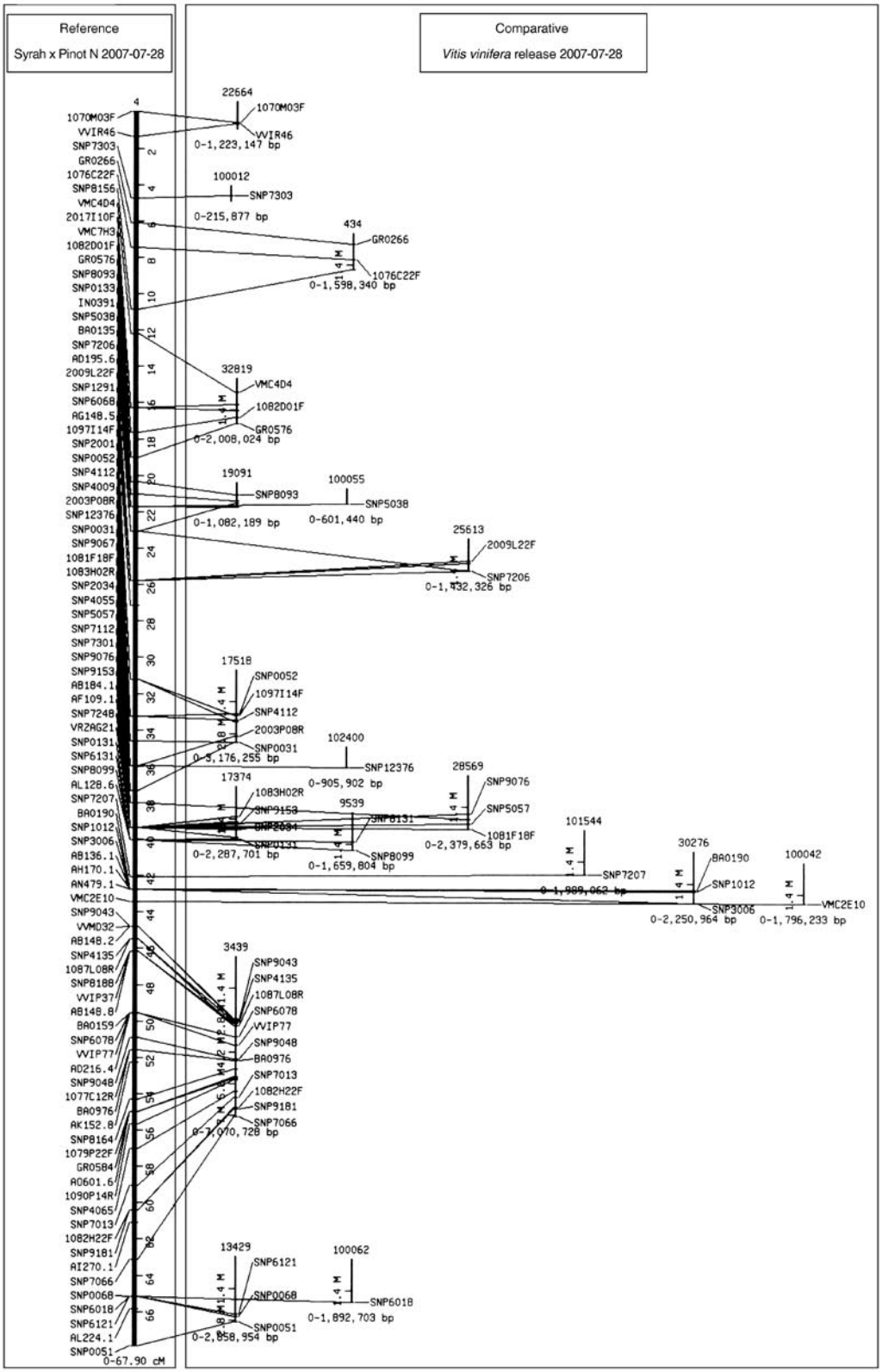

Figure 3 Image produced by the comparative map program tool CMap available at http://genomics.research.iasma.it, showing LG4 of the *V. vinifera* genetic map (on the left) and anchored metacontigs of the assembled *V. vinifera* genome.

seedless varieties in the table grape market. A better understanding of grapevine phenology could allow wider control of ripening time, thus offering the possibility of staggering the harvest over the growing season, expanding production into periods when the fruit has a higher market value, and ensuring optimal adaptation to climatic and geographic conditions (Jones 2006).

In the above work, the genetic determinism of grapevine flowering, fruit maturation timing, berry size, and seed content was investigated by performing a quantitative analysis in combination with phenotypic data collected over three years (Costantini et al. 2008). Complete linkage maps containing microsatellites, AFLPs, and candidate genes were developed from Italia x Big Perlon, a table grape segregating F_1 progeny (Fanizza et al. 2005), and used to detect QTLs.

Phenotypic data distributions (Figure 4) were very similar over three years. A continuous variation, typical of quantitative traits, and a transgressive segregation were observed for all traits. Several associations between traits within each year were found using the Spearman rank-order correlation test. Many concerned component variables of the same character, but correlations between different traits were also detected.

QTL analysis revealed the existence of several regions regulating the variation of phenological traits, namely on LG1 (flowering time), LG2 (flowering time, veraison time, veraison period, flowering-veraison interval, and veraison-ripening interval), LG6 (flowering time, veraison time, ripening date, flowering-veraison interval, and flowering-ripening interval), LG12 (veraison-ripening interval), and finally LG16 (veraison time, veraison period, and flowering-veraison interval). No QTL could be identified for flowering period. The existence of a major QTL on LG18 regulating berry size and seed content was confirmed.

QTL analysis indicates the regions of a genome which contribute to trait variation. The following step is to narrow down these regions so effects can be ascribed to specific genes. To this purpose, the candidate gene approach was adopted on two levels (Costantini et al. 2008). First, "functional candidate genes" selected according to their hypothetical biological role were mapped and tested for linkage with QTLs. Second, the genomic sequence of Pinot noir was used to identify "positional candidate genes" in proximity to molecular markers underlying QTLs. Gene prediction and protein similarity searches suggested some interesting proteins with known roles in flower and fruit development in other plant species may be involved in the studied phenotypes.

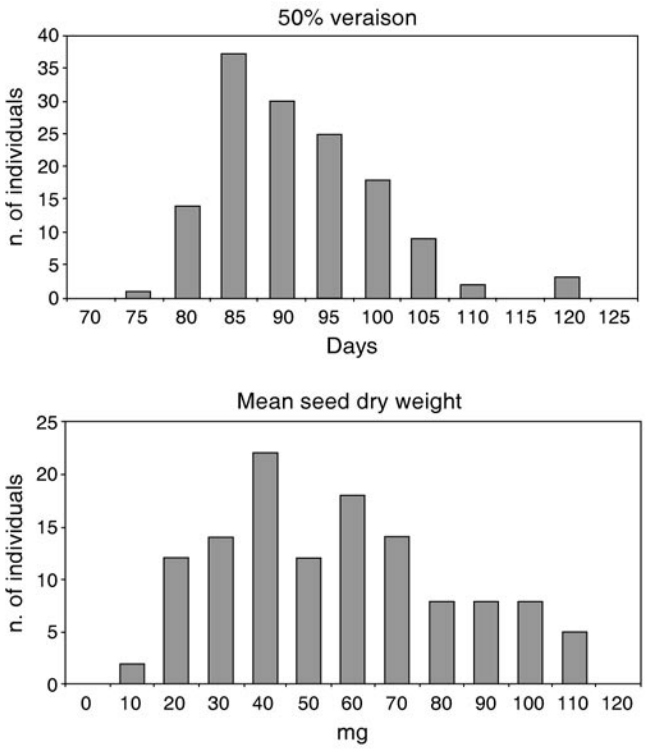

Figure 4 Distribution of veraison time and mean seed dry weight in the Italia x Big perlon progeny in 2004.

Conclusion

The grape genome sequence is fully deciphered. Decoding the full sequence information provides candidate genes implicated in traits relevant to grape cultivation, such those influencing wine quality via secondary metabolites and those connected with the extreme susceptibility of grape to pathogens. A new era in grapevine breeding is now opened. The huge number of SNPs revealed by sequencing the Pinot noir genome will allow screening of thousands of new genotypes obtained by appropriate breeding programs. Mapping of fruit quality and disease-resistance genes enables genomewide association studies. In the near future, this information will make possible a new viticulture.

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