2 Grape

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2.1 Introduction

The *Vitis vinifera* L. grape is one of the oldest cultivated plants, and is thought to have originated in the region between the Mediterranean basin and the Caspian Sea (Olmo 1976). Grapevines are climbing perennial plants with coiled tendrils. Under cultivation they generally require trellising to increase productivity and optimize growth and quality. They are pruned during the dormant and growing season to enable cultivation and promote fruitfulness and fruit quality. The fruit, a berry, is essentially an independent biochemical factory. It is primarily composed of water, sugars, amino acids, minerals, and micronutrients. The berry has the ability to synthesize other berry flavor and aroma components that define a particular berry or wine character. The berry is a commercial source of tartaric acid and is also rich in malic acid. Cultivation is easiest in a Mediterranean type climate with hot dry summers and cool rainy winters, however grapevines are grown throughout the world's temperate climates. *Vitis vinifera* cultivars are heterozygous and are therefore propagated clonally in order to maintain their distinctive and economically significant individual characteristics. These cultivars are typically grown on rootstocks to resist soil-borne pests and to adapt to adverse soil conditions, but there are areas of the world where they can be grown without rootstocks.

Grapes are grown in more than 80 countries of the world with a total of 7,572,237 hectares devoted primarily to wine grapes, but also including table and raisin grapes. The countries with the greatest acreage are Spain, France, Italy, Turkey, China and the United States of America (FAOSTAT data 2005). Wine production adds at least $2 for each $1 of farm gate value. The leading countries for production of table grapes consumed as fresh fruit are China, Turkey, Italy, Chili, the USA, RSA, Spain and Greece (www.fas.usda.gov/psd/complete_tables/HTP-table6-104.htm). The leading countries in the production of raisins, largely sun dried fruit of seedless cultivars, are the USA, Turkey, Greece and Australia.

2.1.1 Origin and Early History of Domestication

A single Eurasian grape species (*V. vinifera*) is the source of the estimated 10,000 cultivars that produce 99% of the world's wine and table grapes today. This species has tremendous genetic diversity and an extremely wide range of variants have been selected over the millennia. Grape cultivation is a very ancient art. Legend and tradition favor ancient Armenia as the home of the first grape (Olmo 1976). Figure 1 indicates the principle areas of the Old World where viniculture began. Levadoux (1956) summarized the distribution of wild and domesticated varieties of *V. vinifera* as follows:

- *Vitis vinifera* was in existence during the final stages of the tertiary period as evidenced by the fossils in many locations of Western Europe and the Mediterranean basin.
- During the Pleistocene period fossil evidence suggests that *V. vinifera* survived in the forests circling the Mediterranean and south shores of the Caspian Sea.
- In the Neolithic period, *V. vinifera* occupied the same distribution, as at present, however, primitive polymorphism and dioecious nature remained intact because of heterozygosity.

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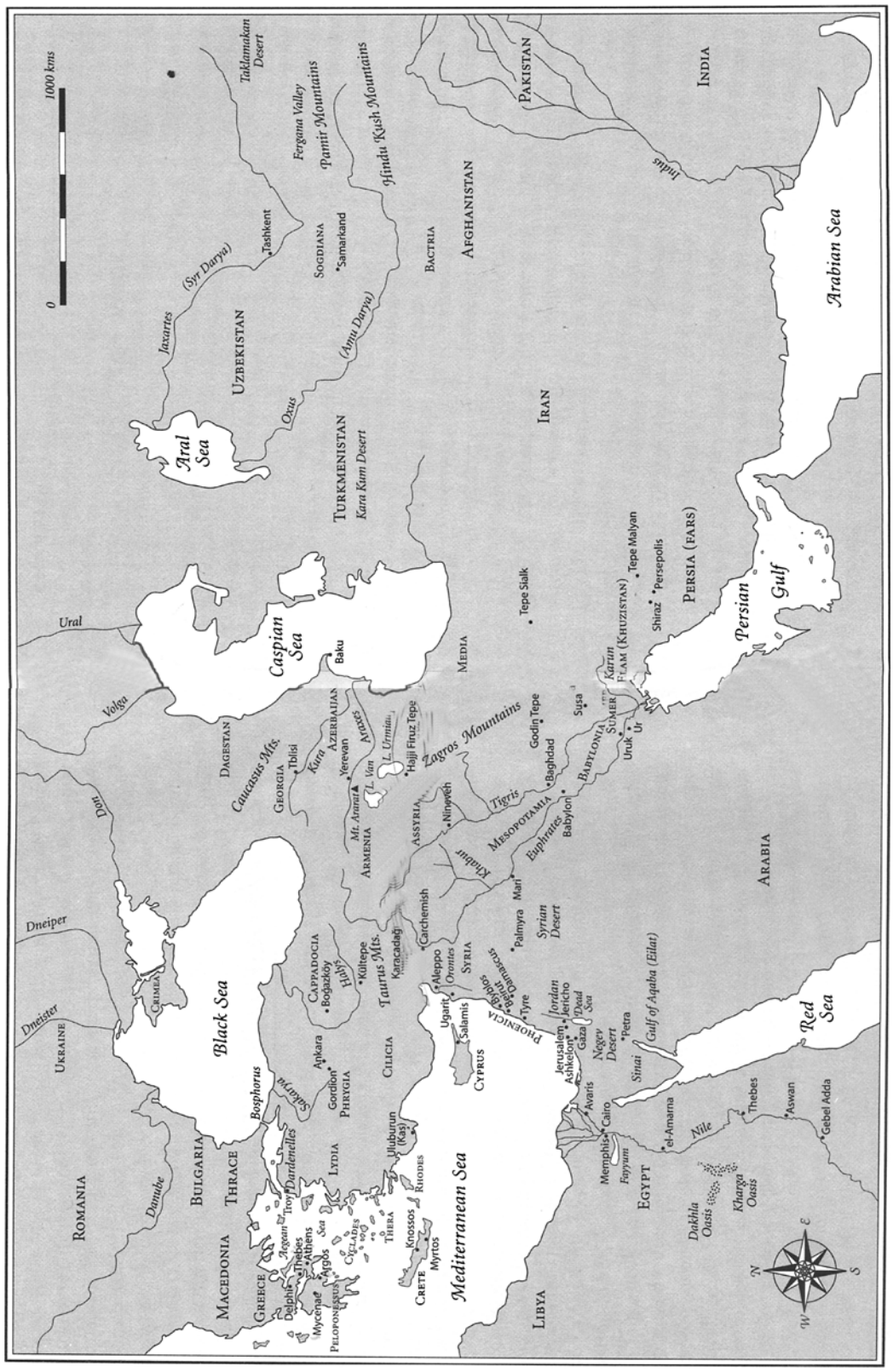


Fig. 1. The principal areas of the Old World where viniculture began

- The domestication of *V. vinifera* began ca. 8000–6000 BC in Transcaucasia.
- Toward the end of fifth millennium BC, grape culture began to spread around the Mediterranean.

Although there is no written record describing the process, there has been sufficient archaeological evidence uncovered to demonstrate the transformation from wild to domesticated forms (Olmo 1995). Domestication likely started when nomads marked forest trees that supported particularly fruitful vines. Grapevines grow most successfully in areas where water is readily available. As sedentary agriculture developed and forests were cleared, fruit trees and vines were kept in areas where water was available and plants were protected from the reach of grazing animals by building mud walls around what became vineyards and orchards. Neolithic communities of the ancient Near East and Egypt were permanent, year-round settlements made possible by domesticated plants and animals. Given a more secure food supply and a stable base of operations than nomadic groups possessed, a Neolithic culture and "cuisine" emerged. Using a variety of food processing techniques – fermentation, soaking, heating, spicing – Neolithic peoples are credited with first producing bread, beer, and an array of meat and grain entrées we continue to enjoy today. A major advance in the development of winemaking was the creation of pottery vessels about 6000 BC that allowed the production and storage of wine. Confirmation of the evolution of winemaking comes from yellowish residue inside a wine storage jar excavated by Mary M. Voigt at the site of Hajji Firuz Tepe in the northern Zagros Mountains of Iran (McGovern 2003, see Plate 1 and 2). The jar, with a volume of about 10 liters was found together with five similar jars embedded in the earthen floor along one wall of a "kitchen" of a Neolithic mud brick building, and was dated to ca. 5400–5000 BC (McGovern 2003, see Map 2). Infrared, liquid chromatographic, and wet chemical analyses were conducted and detected the presence of calcium tartrate in the jars. Grapes are the only fruit in which tartaric acid occurs in large amounts.

Archeological evidence indicates that organized cultivation of wine grapes was underway in the near east as early as fourth millennium BC and in Greece during the first millennium BC (Helbaek 1959). The westward movement of viticulture fanned out from Asia Minor and Greece, following the Phoenician sea routes. Religion was strongly associated with viticulture and winemaking. The Egyptians ascribed wine to the god Osiris, the Greeks had Dionysus, the Romans Bacchus, and the Babylonians the goddess Siduri (McGovern 2003). Wine was associated with the Christian faith as a necessary ingredient in the consecration of the Mass during the Roman period. With the decline of the Roman Empire, Europe plunged into the Dark Ages, wine's influence waned, and vineyards became relegated to monasteries and churchyard plots. Wine's influence was revived in 800 AD, and vineyards were planted along the major river valleys of the Danube, Rhône, Rhine, Tiber and Douro. Records document vineyards along the Moselle valley in Germany by 55 AD. In the fifteenth century viticulture became established in Madeira and the Canary Islands. Later it spread to South Africa, Australia and South America. The *V. vinifera* grape was introduced to the new World by Portuguese and Spanish explorers and settlers in the 1500s. The first recorded introduction of grape into the USA was in 1621 (Olmo 1976). Grapes were moved from Mexico into California in the mid 1700s, and expanded rapidly during the 1850s.

2.1.2 Genetic Diversity

The botanical family Vitaceae is made up of 15 genera (http://www.ars-grin.gov/) and about 1,000 species. Only the genus *Vitis* contains species with edible fruit. There are about 60 *Vitis* species in the world, with the greatest concentration in Asia and North America. The number of *Vitis* species is in taxonomic dispute due to the interfertility of all the species, their sympatric nature and the resulting high degree of hybridity. Some authors separate the species *V. rotundifolia* and its related subspecies and species (*V. rotundifolia* var. *munsoniana* and *V. popenoei*) into a separate genus *Muscadinia* (Small 1913). *Muscadinia* species have 40 somatic chromosomes and are restricted to the southeastern USA and northeastern Mexico (Winkler et al. 1974; Einset and Pratt 1975).

Domestication of *V. rotundifolia*, the muscadine grape, pre-dates the arrival of Europeans in the USA in the 1600s. The rest of the *Vitis* species contain 38 very small somatic chromosomes that form 19 bivalents at meiosis and fertile hybrids with the muscadine species are rare and do not occur naturally (Jelenkovic and Olmo 1969). Estimates of the number of *Vitis* species varies widely depending upon taxonomic opinion. De Lattin (1939) grouped the species

into nine sections, and included 18 North American species. Bailey (1934) included 28 American species and his grouping and designation differed from that of De Lattin. Galet (1956, Vol 1) states that about 20 species of *Vitis* can be found in America but later reports that there are 28 (Galet 1988). A literature search covering published reports from 1753 to 1940 revealed 155 species names for American grapes, adding to the confusion (Rogers and Rogers 1978). This confusion is largely based on disagreements as to what constitutes good species, extreme variants and hybrid forms (Levadoux et al. 1962; Barrett et al. 1969; Comeaux et al. 1987). Additional summaries of the family Vitaceae are found elsewhere (Galet 1988; Alleweldt et al. 1990; Mullins et al. 1992 Chap. 2). The United States Department of Agriculture Germplasm Resources Information Network (GRIN) (http://www.ars-grin.gov/) is an accepted listing of crop plant germplasm. This list describes the 15 genera and 43 species, 5 natural hybrids and 15 varieties of species in *Vitis*. Molecular techniques are being applied to taxonomic relationships within Vitaceae (Rossetto et al. 2001), but more work is needed. The North American species, including *V. aestivalis, V. cinerea var. helleri, V. labrusca, V. riparia* and *V. rupestris*, have been extensively used to produce rootstocks and fruiting cultivars with fungal resistance. Among the Asian species, only *V. amurensis* has been domesticated and used for fresh fruit, juice, wine and jelly production (Huang 1980). *Vitis vinifera* is the most successfully used grape species with thousands of wine, table and raisin grape cultivars grown throughout the world's temperate zones (Alleweldt et al. 1990).

2.1.3 Advanced Breeding Objectives

The common objectives of most breeding programs are to produce locally adapted, high yielding and quality cultivars adapted to environmental and pest stresses. In practice these objectives are complex given the different characteristics needed for table, raisin and wine grape production. In addition, other desirable qualities are considered when breeding rootstocks.

Grapes are generally grown in the Northern hemisphere between 20 and 51°N latitude. The most northern extent of *V. vinifera* cultivation is in Germany's Rhine Valley and British Columbia, Canada. The southern ranges extend into India, but most cultivation occurs between 20 and 40°S latitude. The major limiting factors to *V. vinifera* cultivation are the length of the growing season and water availability, which must allow both fruit and cane maturation, and winter cold. Tropical viticulture is practiced with both *V. vinifera* cultivars, and with hybrids based on American grape species, in areas where dormancy can be enforced by a dry season, by withholding water or by planting at high elevation. Grapes are grown beyond the winter cold limits of *V. vinifera*. These cultivars are hybrids based on northern species particularly *V. riparia*, *V. amurensis* and *V. labrusca.*

Grape is subject to an array of diseases caused by bacteria, fungi, mycoplasmas, nematodes and viruses (Pearson and Goheen 1988). The most damaging grape pests are indigenous to North America and, because *V. vinifera* cultivars have no or little inherent resistance, they created havoc when introduced into Europe during the nineteenth century. Insects and nematodes can also act as vectors for diseases such as Pierce's disease, flavescence dorée and fanleaf degeneration, and cause serious vine decline or death as in the case of grape phylloxera and root borer. The most common fungal disease in the world's grape growing regions is powdery mildew, caused by *Uncinula necator*. This fungus was unintentionally introduced to Europe from North America before the 1850s (Reisch and Pratt 1996). About 20 years later downy mildew caused by another fungus, *Plasmospora viticola*, became a serious problem. Soon after, black rot (*Guignardia bidwellii*) appeared in European vineyards. These diseases were all introduced from North America. A serious disease of warmer climate is anthracnose, *Elsinoë ampelina*, perhaps the first North American grape disease to be imported to Europe. Sources of resistance to these diseases are found in many North American grape species. Barrett (1955) reported that resistance to black rot is quantitatively controlled. A few forms of resistance to this disease have been identified. Some genotypes of *V. rupestris* and *V. cinerea* transmit high levels of resistance, however, there is great variability among different clones suggesting quantitative inheritance is likely, as reported by Barrett earlier (McGrew 1976).

Several plant parasitic nematodes attack grape roots, and many commonly used commercial rootstocks are susceptible (Raski et al. 1965). The root-knot nematode (*Meloidogyne* spp.) and dagger nematode (*Xiphinema index*) cause serious damage to grape roots and reduce vigor and productivity of the

plant. *Xiphinema index* also acts as a vector for grape fanleaf virus, and this virus/nematode complex cause one of the most severe grape viral diseases – fanleaf degeneration. This disease interferes with normal fertilization of the flowers, disrupting berry set and resulting in severe yield losses. Nematode problems become more severe with time as growers replant grapes on vineyard sites without regard to fallow or crop rotation, or plant vineyards on agricultural soils with high nematode populations. Resistance to nematodes is found in a number of North American grape species particularly *V. arizonica, V. candicans* and its hybrids, *V. cinerea* and *Muscadinia rotundifolia*. Table 1 summarizes known sources of resistances to different pests and diseases in grapes.

2.1.4 Classical Breeding Efforts: Obstacles and Achievements

French scientists, nurserymen and viticulturists first initiated well-documented grape breeding, when phylloxera and fungal diseases created havoc in European grape growing regions. Table 1 presents the main genetic resources used by European and North American breeders to incorporate disease, pest and abiotic stress resistance into *V. vinifera* cultivars. Some of these hybrids, Hybrid Direct Producers or French Hybrids, are still used to combat fungal diseases and cold winter weather, however they are generally considered to have inferior fruit quality compared to *V. vinifera* cultivars. Breeding of these interspecific hybrids ceased in Europe after the creation and utilization of phylloxera resistant rootstocks took hold. Progress on limiting the expression of undesirable flavor compounds was limited because most of them are inherited as complex polygenic traits (Alleweldt and Possingham 1988). However, new *V. vinifera* cultivars continue to be developed. The most successful of these are seedless table grapes, while wine grapes have been less successful since their wide utilization is greatly limited by the demands of winemakers and marketers to have traditional varieties with well-documented quality and historical acceptance. New *V. vinifera* varieties continue to be released in a number of countries including Argentina, Australia, France, Germany, Hungary, South Africa, USA and Chile (Antcliff 1978).

There are several main constraints to grapevine improvement. Grape is a relatively long-lived perennial and requires time and space for adequate evaluation. It can also be slow to come into bloom resulting in a relatively long generation time. In the case of wine grapes vinification and wine evaluation must be carried out which further complicates and delays selection. Most wine grape cultivars are extremely heterozygous and old varieties carry deleterious alleles that exhibit pronounced inbreeding depression after selfing or sibling mating, although inbreeding affects can vary among cultivars (Winkler et al. 1974). The grape breeding efficiency depends on the screening methods used for fruit quality, yield, disease resistance, winter hardiness and tolerance to other abiotic stresses. Field and laboratory procedures are often performed in order to select for horticultural traits prior to determining enological potential. Wine grape evaluation is again more complex because single seedling vines produce very small amounts of fruit, adding to the difficulty of judging wine making potential. Finally, little is known about the inheritance of wine quality components, which are likely to be quantitatively inherited and under environmental influence.

Improvement of crops through breeding is greatly facilitated by genetic knowledge of traits under selection. Such genetic information can be used to calculate heritability estimates, which help breeders to select parents for controlled crosses. Heritability estimates could be derived from parameters of covariance among relatives. One method of covariance estimation is through factorial sib analysis, a mating system that is less biased by environmental covariances than other methods (Fehr 1991; Falconer and Mackay 1996). The design II mating system consists of a series of male parents each mated to a series of female parents. To make the calculations simple, selected females are not mated to each other, selected males are not mated to each other, and there are no reciprocal or selfing crosses. Such factorial designs are particularly well-suited to a dioecious species such as the wild species and rootstocks of grape. This design has been used with wild grape species to study the inheritance of Pierce's disease resistance (Krivanek et al. 2005) and has been used with grape rootstocks to study root-knot nematode resistance (Cousins and Walker 2002).

Table 1. Native american species as sources of resistance or tolerance to diseases and biotic stress

Stress factor	Causal agent	Sources of resistance or tolerance	References
Fungal Diseases			
Anthracnose	*Elsinoe ampelina* [de Bary] shear	*V. simpsoni* Mun.	Mortensen (1981)
		V. smalliana Bailey	Olmo (1986b)
		V. shuttleworthii House.	
		V. labrusca L.	
		V. rotundifolia Michx	
		V. munsoniana Simp ex Mun	
Botrytis bunch rot	*Botrytis cinerea* Pers.	*V. vinifera* L.	Alleweldt et al. (1990)
		V. riparia Michx	
		V. rupestris Scheele	
Black rot	*Guignardia bidwellii* [Ellis]Viala & Ravaz	*V. riparia* Michx	Alleweldt et al. (1990)
		V. rupestris Scheele	
		V. candicans Engelm	Jabco et al. (1985)
		V. rotundifolia Michx	McGrew (1976)
		V. cinerea Engelm	
Downy mildew	*Plasmopara viticola* Berl. and Toni	*V. riparia* Michx	Alleweldt et al. (1990)
		V. rupestris Scheele	Eibach et al. (1989)
		V. lincecumii Buckl.	He and Wang (1986)
		V. labrusca L.	
		V. amurennsis Rupr.	
		V. rotundifolia Michx	
		V. yenshanesis	
		V. aestivalis	
		V. cinerea Engelm	
		V. berlandieri	
Powdery mildew	*Oidium*, *Uncinula necator* (schw.) Burr.	*V. aestivalis* Michx	Alleweldt et al. (1990)
		V. cinerea Engelm	Pearson and Goheen (1988)
		V. riparia Michx	
		V. berlandieri	
		V. rotundifolia Michx	
		V. labrusca L.	
Rust	*Physopella ampelopsidis*	*V. shuttleworthii* House.	Fennell (1948)
		V. simpsoni Mun.	
		V. rotundifolia Michx	
Bacterial Diseases			
Crown gall	*Agrobacterium tumefaciens*	*V. amurennsis* Rupr.	Alleweldt et al. (1990)
		V. labrusca L.	Pearson and Goheen (1988)
Pierce's disease	*Xylella fastidiosa* Wells et al.	*V. rotundifolia* Michx	Mortensen et al. (1977)
		V. candicans Engelm	Olmo (1986b)
		V. champinii Pl	Stover (1960)
		V. vulpina L.	
		V. shuttleworthii House.	
		V. simpsoni Mun.	
		V. smalliana Bailey	
		V. arizonica	
Flavescence doree	Mycoplasma like organism suspected	*V. labrusca* L.	
		V. rupestris Scheele	Pearson and Goheen (1988)

Table 1. (continued)

Stress factor	Causal agent	Sources of resistance or tolerance	References
Viral diseases			
grapevine fan leaf virus		*V. arizonica*	
		V. rotundifolia Michx	Walker et al. (1985)
		V. vinifera L.	Walker and Meredith (1990)
		V. rufotomentosa Small	
		V. candicans Engelm	
		V. riparia Michx	
Insects			
Rootknot nematodes	*Meloidogyne* Goeldi spp	*V. champinii* Pl	Lider (1954)
		V. candicans Engelm	
		V. rotundifolia Michx	Olmo (1986b)
Dagger nematodes	*Xiphinema index*	*V. rufotomentosa* Small	Alleweldt et al. (1990)
		V. arizonica	
		V. rotundifolia Michx	Bouquet and Danglot (1983)
		V. cinerea Engelm	Meredith et al. (1982)
Phylloxera	*Dakyulosphaira vitifolia* [Fitch]	*V. riparia* Michx	Alleweldt et al. (1990)
		V. rupestris Scheele	Olmo (1986a)
		V. berlandieri	
		V. rotundifolia Michx	
		V. cinerea Engelm	
		V. champinii Pl	

2.1.5 New Genetic Tools for Grape Improvement

2.1.5.1 In Vitro Culture

Tissue culture has greatly increased our knowledge of plant biology from the cellular (metabolism, differentiation) to the plant level (organogenesis, host-parasite relationships). Successful tissue culture also led to unconventional methods for genetic improvement. Since early 1960s, grapevine has been the subject of research aimed at defining the best procedures for micropropagation.

In vitro culture starts with the excision of a small piece of contaminant-free plant tissue followed by its establishment in sterile culture. The choice of plant material and preparation of sterile explants are critical, since the tissue must be able to survive the initial culture and produce expected or experimental responses. Environmental conditions and the physiological state of the mother plant also need to be considered. Once the plant material is cleaned with surface disinfectants [common surface disinfectants and procedures are reviewed by Street (1977) and Hu and Wang (1983)], the tissue is placed in an appropriate culture media. The major functions of culture media are (i) to supply the basal nutrients for continued growth of the isolated explants and its subsequent propagules; and (ii) to manipulate growth and development through the balance of growth regulators. In vitro development is commonly controlled by the kind of growth regulator, its concentration and combination with other growth regulators, and the sequence in which growth regulators are supplied. Auxins and cytokinins are most typically used, but gibberellins and abscisic acid have also been used in specific situations.

Techniques of in vitro culture are commonly classified as standard techniques using pre-existing meristems, and those requiring neoformation of buds or meristem like structure. The standard method uses explants bearing intact apical or axillary buds cultured on a growth regulator-free media containing sucrose, macro and micronutrients with vitamins, and solidified with a gelling agent. Depending on the genotype and environmental conditions, an ax-

illary bud gives rise to a single rooted plant. Subculturing of these plants can generate yearly multiplication rates of 10^4 to 10^6. Such techniques are widely used because of their operational feasibility and ease of plantlet transfer to greenhouse conditions. In addition the culture of small meristems can often give rise to virus-free plantlets and thus these methods are specifically used for virus elimination programs.

Neo-formation techniques require the stimulation of axillary bud proliferation through the use of cytokinins, plant growth regulators with the ability to overcome the apical dominance of axillary buds. Cytokinins in the culture medium induce intense shoot proliferation by the enhanced release of axillary buds. Axillary bud proliferation is currently considered one of the most convenient and reliable regeneration techniques for shoot multiplication in many plants, herbaceous and woody crop species, and grapevine (Hu and Wang 1983). Yearly production rates can theoretically reach 10^8 buds per initial explant. Many research groups have adapted and improved these techniques with a wide range of *Vitis* genotypes (Table 2).

Progress in cell, tissue and organ culture of grapevine led to the development of other technologies with great potential for grape improvement (Mullins et al. 1992; Torregrosa and Bouquet 1993). Major advances in genetic engineering of grapevine have been made through the coupling of recombinant DNA technologies with regeneration from plant tissue cultures. A brief overview of uses of in vitro culture in grapevine is present below.

Generation of Virus-Free Grapevines Virus and virus-like entities greatly hinder grape cultivation by reducing vine vigor and yields, delaying and arresting berry ripening, changing must composition and aromatic profiles, and affecting graft compatibility (Walter and Martelli 1996). Many viruses affect grape including fanleaf (GFLV), leafroll (GLRaV), fleck (GFkV), stem pitting (RSPaV), stem grooving (GVA-closely associated) and corky bark (GVB-closely associated) and are considered to be of major importance to growers, nurseries and winemakers. In vitro meristem, shoot apex cultures, and one node explant culture were developed to eliminate viruses from grapevines (Barlass et al. 1982; Hatzinikolakis and Roubelakis-Angelakis 1993; Staudt and Kassemeyer 1994). In recent years, micrografting of scion graft meristems on hypocotyls of germinating embryos resulted in the advantage of simultaneous virus indexing (Tanne et al. 1993, 1996). Somatic embryogenesis became a useful tool to eliminate harmful viruses after methods were developed to establish long-term regeneration of somatic embryos in different grape genotypes (Torregrosa 1995). When combined with heat therapy, somatic embryogenesis successfully eliminated viruses from vascular and non-vascular tissues (Goussard and Wiid 1992). Researchers in South Africa have used somatic embryogenesis to establish *V. vinifera* cultivars since 1990. It was judged to be more effective and less expensive than conventional techniques at virus elimination and has not resulted in somaclonal variation or virus contamination, as judged by ISEM and ELISA (Goussard and Wiid 1995).

Establishment of Germplasm Repositories Grape germplasm is currently maintained in field collections where two or more plants of each genotype (species, hybrid, variety and clone) are cultivated. Management of germplasm in the filed is expensive and subject to environmental hazards and funding shortages. There are three basic types of in vitro storage modes for conservation: (i) standard micropropagation, (ii) in vitro culture combined with reduced growth rate, and (iii) suspension of growth (Withers 1992). Because of the cost and risk of genotype instability, the first method is unsuitable for long-term conservation of grapevine. Reducing the growth rate of in vitro cultures increases the time between subcultures, reducing upkeep costs and risk of subculture mistakes. Galzy et al. (1990) reported that grapevine plantlets could adapt to a number of different culture conditions. When culture conditions encourage growth, plant behavior depends on a number of variables such as nutrients, carbohydrate source and concentration, and light, but dry matter remains stable. Conversely, when growth is restricted by lowering temperature, dry matter content increases significantly in response to stress. To compensate for this effect, Galzy et al. (1990) suggested reducing the carbohydrate content of the medium. The nutrient content of media has a strong impact on growth (Torregrosa 1994), and restricting nutrients, especially nitrogen and potassium, can alter plantlet growth (Moriguchi and Yamaki 1989).

Grapevine cryopreservation studies have been conducted on latent buds taken from in situ canes. Ezawa et al. (1989) obtained high survival rate with *V. labrusca (V. X. labruscana*), and low to no success with several *Vitis* species and *V. vinifera* cv Riesling,

Table 2. Axillary bud proliferation studies in grapevine

Species	Studied factor	Reference
V. vinifera	Culture vessel size	Monette (1983)
Vitis hybrids	Vitamins, amino acids, BAP/Kin/Picloram	Chee and Pool (1985)
V. labrusca	Adenine/MS strength	Reisch (1986)
Vitis hybrids	Light spectrum, Mn and KI	Chee (1986)
Vitis hybrids	Salt formulation	Chee and Pool (1987)
V. rotundifolia	BAP/IBA	Lee and Wetztein (1990)
V. vinifera	TDZ	Gribaudo and Fronda (1991)
V. rotundifolia	BAP/TDZ/Kin/NAA/ explant length	Gray and Benton (1991)
V. vinifera and *Vitis* hybrids	MS strength, vitamins	Zlenko et al. (1995)
Vitis × *muscadinia* hybrids	Mg, Ca, BAP, salt formulation	Torregrosa and Bouquet (1995)
Vtis hybrids, *V. vinifera*	BAP/2iP/NAA, darkness	Molina et al. (1998)

respectively (Esensee et al. 1990). Plessis (1994) described the most comprehensive work while adapting cryopreservation techniques developed for pear. In this process, axillary buds from in vitro grown plantlets, composed of the prompt (lateral) bud with several leaf primordia and a rudimentary latent bud are encapsulated in calcium alginate and soaked in a liquid medium containing 1 M sucrose to reduce the water content of beads. The coated buds are then partially dehydrated under sterile airflow and frozen through two immersion steps in liquid nitrogen. Using this process, it was found that 24% of frozen buds from *V. vinifera* cv. Chardonnay were capable of producing viable plants (Plessis 1991).

Utilization of cryopreservation techniques to conserve germplasm is an appealing alternative to field culture. However, cryopreservation of large collections of genotypes is expensive and time consuming. Moreover the possibility, even if remote, of propagating plants with genotypic alterations undetectable under in vitro conditions is problematic. The primary goal of cryopreservation is to back-up working collections for short and long terms, but they are not likely to replace field collections.

In Vitro Embryo Rescue In many table-grape growing countries, consumers favor seedless table grapes. In the USA, seedless cultivars make up more than 80% of the total table grape production, and only one seeded table grape, Redglobe, is a commercial success. Table grape breeding has been pursued intensively for more than 70 years in California, and a large number of new seedless cultivars have been released (Ledbetter and Ramming 1989). Traditional breeding methods are based on hybridization between seeded female parents and seedless male parents. The seedlessness is stenospermocarpic (where fertilization occurs, embryo is viable, but seed development aborts at various stages, leading to quantitative variation of seed trace size) with low proportion of seedless plants in the progenies. Since seedlessness is only one of a number of important traits, the selection process requires growing a large number of plants. In addition, since grape seedlings often take 3–4 years to produce fruit after planting, selection for seedless progeny is further delayed.

Through the use of in ovulo and in vitro culture techniques, it is possible to rescue viable embryos from seedless × seedless crosses and greatly increase the number of seedless progeny (Emershad and Ramming 1984, Spiegel-Roy et al. 1985; Bouquet and Davis 1989; Gray et al. 1990; Gribaudo et al. 1993; Garcia et al. 2000; Ponce et al. 2000). Fertilized ovules are extracted and placed on media with $GA_3$and IAA followed (although not in all cases) by the excision of the embryos. The success of embryo rescue depends on many factors, the most important being the variety used as the female parent, and the harvest time of the berries and ovules after pollination (Bouquet and Davis 1989; Ponce et al. 2000). Low temperatures and treatments with growth retardants have been shown to improve embryo germination (Agüero et al. 1995, 1996).

Emershad and Ramming (1994a) showed that proliferative somatic embryogenesis could be initiated from in ovulo cultured zygotic embryos of seed-

less grapes. This phenomenon was later shown to be a demonstration of direct somatic embryogenesis occurring from epidermal cells of larger embryos (Margosan et al. 1994), and was proposed as a system to facilitate gene transfer technology in seedless grapes (Emershad and Ramming 1994b). However, the seedless character cannot be controlled in the genotypes of such embryos. Higher proportions of seedless plants can be recovered through in ovulo embryo culture (Ramming et al. 1990; Spiegel-Roy et al. 1990; Bouquet and Danglot 1996). The limitation of these procedures is their labor-intensive nature, and the size of progeny populations must therefore be limited.

2.1.5.2 Genetic Engineering

Over the last 20 years, advances in plant biotechnology have produced new tools for genetically improving crops. The general aim of molecular grapevine breeding programs is to develop and apply novel gene technologies capable of introducing genes in a careful targeted manner. The transfer of a single trait into a grape variety is almost impossible by classical methods due to grape's heterozygous nature. The potential of genetic engineering would be to make directed and specific changes in existing grape cultivars, thus modifying disease or pest resistance and perhaps regulating fruit and wine quality factors. The use of genetic engineering in the wine, table and raisin grape industries has high potential because grapevines are vegetatively propagated. Thus, modifications to established cultivars by genetic transformation should, in theory, leave intact the essential characteristics that make each cultivar unique. This is especially important in the wine industry, due to the dependence of wine sales on the use of established and historic cultivars names. New cultivars resulting from classical breeding are assigned new names, which contributes to their slow acceptance in the marketplace.

Agrobacterium-mediated transformation of grape began with the use of leaf disks, petioles, and other shoot/root explants in the 1980s (reviewed by Grey and Meredith 1992; Reisch and Pratt 1996). These efforts produced transformed cells, but not transgenic plants, due to the tissue type used, the competency of the cells, and difficulties with regeneration. However, by the mid 1990s, many groups had reported development of transgenic grapes including rootstocks and scion cultivars (Table 3). These successes derived from advances in embryogenesis, regeneration, and transformation and biolistic methods. The production of transgenic vines has now become routine in both public and private laboratories (Table 3).

Many projects have focused upon pest resistance including fungal resistance in scion varieties (powdery mildew: Kikkert et al. 2000) and virus resistance in rootstocks (fanleaf degeneration: Mauro et al. 1995). Other studies have also focused on product quality: changing seeded grapes into seedless grapes (Perl et al. 2000a, b), and reducing the browning of raisins (Thomas et al. 2000). While potentially improved forms of important cultivars have been produced, years of field and product testing are still required before genetically engineered grapes will reach the marketplace. Although it may become possible to target gene incorporation and expression, at this point transformation events are independent of each other and require the same evaluation strategies, as would classically bred grapes.

Field trials in most countries require approval from the relevant authorities. In France, transgenic research is controlled by two authorities: the Commission de Génie Génétique (CGG), which oversees research in confined environments such as laboratories and glasshouses; and the Commission d'étude de la dissémination des produits issus du Génie Biomoléculaire (CGB), which is responsible for field releases. In Germany, license from the Robert Koch Institute is required for field trials and the "Gene Technology law" controls transgenic research. In Australia, the office of the Gene Technology Regulator (OGTR) established by the Federal Government oversees the deliberate release of transgenic plants for field trials. A legislative basis for the regulation of GMO's in Australia came into force following passage of the *Gene Technology Bill 2000*. In the USA, authority to regulate transgenic plant research resides within the Animal and Plant Health Inspection Service (APHIS)– Biotechnology Permits Unit of the United States Department of Agriculture (USDA). The web database of field releases in the United States (http://www.nbiap.vt.edu/cfdocs/fieldtests1.cfm) lists 33 separate release notifications and permits for grape from both private companies and universities dating from 1995. Most of these were later withdrawn. Any plant engineered for fungal, viral or herbicide resistance would also undergo a required review by the Environmental Protection Agency, which assesses the impact upon the environment.

Table 3. Summary of transgenic plants of Vitis scion and rootstocks

	Cultivar	Selectable marker	Trait of interest	Reference
Rootstocks	110R	NPTII	Coat protein (GCMV resistance)	Le Gall et al. 1994
	110R	NPTII	Coat protein (GFLV)	Krastanova et al. 1995
	41B	NPTII	Coat protein, replicase protein	Mauro et al. 1995
	SO4	NPTII	Coat protein (GFLV)	Mauro et al. 1995
	V. rupestris	NPTII	Coat protein (GFLV)	Krastanova et al. 1995
	Freedom	NPTII	GNA (homopeteran insect resistance)	Viss and Driver 1996
	MGT101-14			
	5C Teleki			
	V. rupestris, 110R	NPTII	Coat protein, antifreeze protein	Tsvetkov and Atanassov 2000
	V. rupestris	NPTII	Anti-sense movement protein	Martinelli et al. 2000
	110R	NPTII	Coat protein	Gölles et al. 2000
		NPTII	Replicase (virus resistance)	Barbier et al. 2000
		na	Eutypa toxin resistance	Legrand et al. 2000
	3309C	NPTII	Virus resistance	Krastanova et al. 2000
	V. riparia	NPTII	Virus resistance	Krastanova et al. 2000
	MGT101-14	NPTII	Virus resistance	Krastanova et al. 2000
	5C Teleki	NPTII	Virus resistance	Krastanova et al. 2000
Scion cultivars	Chardonnay	NPTII	Coat protein (GFLV)	Mauro et al. 1995
	Sultana	NPTII	Shiva-1 (disease resistance)	Scorza et al. 1996
	Superior seedless	Bar	Basta herbicide resistance	Perl et al. 1996
	Cabernet Franc	NPTII	Fe-superoxide dismutase (freeze tolerance)	Rojas et al. 1997
	Chardonnay	NPTII	Chitinase (disease resistance)	Kikkert et al. 2000
	Sultana	NPTII, HPT	Silencing of polyphenol oxidase to reduce browning	Thomas et al. 2000
	Merlot	NPTII	Chitinase (disease resistance)	Kikkert et al. 2000
	Riesling, Dornfelder	NPTII	Glucanase, chitinase (disease resistance)	Harst et al. 2000a
	Red Globe	na	Barnase gene (seedlessness)	Perl et al. 2000a
	Red Globe	NPTII, HPT	Seedlessness	Perl et al. 2000b
	Neo Muscat	NPTII	Class I chitinase (disease resistance)	Yamamoto et al. 2000

Public perception Education about the environmental and health benefits likely to derive through the use of gene technology for crop improvement appears to be the key to public acceptance of transgenic plants. The year 1999 saw increased media attention paid to consumer and environmentalist groups opposed to the use of genetic engineering for the production of food items. This opposition was particularly strong in Europe where the matter quickly became a political and economic issue. Most of ongoing field trials were discontinued or put on halt in France and Germany. However, in Australia and the USA, public opposition to field trials of transgenic grapevines has been much less vocal. Perhaps an advantage of working on transgenic grapes, at least wine grapes is that many years of field evaluation and wine quality tests are required before release. Thus, there will be more time for public education and awareness before transgenic grapes are used commercially, compared with transgenic cereals, grains and vegetables.

2.2 Genome Mapping

The genome size of *Vitis* is 475 Mbp, 96% of which is non-coding (Lodhi et al. 1995a). It is about half the size of the tomato genome (950 Mbp) and equivalent to the rice genome (450 Mbp). There is no significant variation for DNA content among cultivars of *V. labrusca, V. vinifera* and diploid *Vitis* hybrids (Lodhi et al. 1995b). Knowledge of an organism's DNA content is essential to allow correlation of genetic and physical mapping distances. In grapes, 1 cM represents on average 300 kb in physical distance.

A genetic linkage map of an organism is an abstract model of the linear arrangement of a group of genes and markers. The gene can be a traditionally defined Mendelian factor or a piece of DNA identified by a known function or by means of a biochemical assay. The marker can be a cytological marker, a protein, or a piece of DNA without known function. Because a genetic map is based on homologous recombination during meiosis, this map is also a meiotic map.

In plants, some traits are controlled by a single gene (major gene). The location of the gene controlling a trait of interest is deduced by following the inheritance of the trait relative to the inheritance of linked molecular markers. Markers that are located very close to the DNA region controlling the trait are identified by virtue of co-inheritance with the trait in the progeny of a cross between two plants differing in the trait (but not necessarily in heterozygote species). By identifying two such markers that are very close and flank the trait of interest (fine-mapping), a small DNA fragment that contains the gene can be isolated (positional or map-based cloning). Once isolated, the DNA sequence can be determined and the function and organization of the gene can be studied.

Map-based cloning has been used to isolate disease resistance genes in many crop plants, for example the gene controlling resistance to bacterial pathogen, *Pseudomonas syringae*, in tomato (Martin et al. 1993). This gene product was determined to be a protein kinase, and when this gene was transferred to susceptible plants, they became resistant. A rice gene controlling resistance to *Xanthomonas oryza* was also identified with the map-based cloning approach (Song et al. 1995). Genome maps have also been used to find genes controlling various aspects of plant composition and development that have not been previously described or isolated. For example, map-based cloning of *Arabidopsis* has been used to find a gene controlling fatty acid composition, as well as several genes controlling developmental response to ethylene and abscisic acid (Arondel et al. 1992; Chang et al. 1993; Leung et al. 1994).

In plants, many traits exhibit continuous variation resulting from the action of multiple genes that are subject to environmental modification, a quantitative trait loci (QTL). Determining the location and number of genes that condition such quantitative traits and estimating the magnitude of individual gene effects is the focus of quantitative geneticists. Before interval mapping, QTL detection could be done by variance analysis at individual markers: Lander and Botstein (1989) provided the theoretical basis for QTL analysis. The availability of detailed linkage maps composed of molecular markers and major genes for traits of interest provided the framework for manipulation of QTL. Once a large number of markers are available, segregating loci can be chosen to mark most regions of a genome. QTL mapping has been used to locate genomic regions controlling aroma in corn (Azanza et al. 1996) and clone sugar content QTLs from the wild tomato species *Lycopersicon pennellii* (Zamir et al. 2000). In the latter case, the *L. pennellii* introgression IL9-2-5 improves sugar content by 22% by increasing fructose and glucose compared to the controls. This partially dominant QTL (designated as Brix9-2-5) enhanced total soluble solids of the fruit in different years, environments and genetic backgrounds. In a similar study, another QTL fw2.2 was found to be responsible for approximately 30% of the difference in fruit size between large, domesticated tomatoes and their small-fruited wild relatives. The gene underlying this QTL was cloned and shown to be associated with altered cell division in ovaries (Frary et al. 2000). Many QTLs were detected but only few identified.

Genome maps also provide tools to plant breeders for marker-aided selection (MAS), allowing them to optimize selection for a desirable trait. If seedlings are screened for the presence of a closely linked molecular marker, there is high probability that the seedlings carrying the marker will also carry the desirable trait, allowing them to be selected at a much earlier stage than would otherwise have been possible.

2.2.1 History and Current Status of Grape Genetic Linkage Mapping

Linkage maps in most plants are obtained from segregating populations derived from crosses between pure or inbred lines. Because grapes are extremely heterozygous, their mapping populations are usually F_1 and the pseudo-testcross mapping strategy is used to construct genetic linkage maps of both parents, which can be then be integrated into a consensus map with the use of multialleleic codominant markers with alleles that segregate in both parents (Grattapaglia and Sederoff 1994). In the last decade, several groups have initiated programs to develop molecular marker linkage maps in grapes. Table 4 summarizes all published maps in grapes. Initial efforts by Weeden et al. (1988) and Mauro et al. (1992) reported linkage groups in grape using isozyme and RFLP (restriction fragment length polymorphism) markers. However, these molecular markers are limited; isozymes are restricted to genes encoding soluble proteins, and RFLP markers are mostly limited to coding regions of the genome. In 1995, Lodhi et al. reported the first detailed genetic linkage map of grape based on a seedling population from a cross of 'Cayuga White' (a complex hybrid of *V. vinifera, V. labrusca, V. rupestris* and *V. aestivalis*) and 'Aurore' (a complex hybrid of *V. vinifera, V. rupestris* and *V. aestivalis*). The parental maps were based on 422 randomly amplified polymorphic DNA (RAPD) and 16 RFLP and isozyme markers. These maps were developed by using the double pseudo-testcross strategy with an average distance of 6.1 cM between markers. The 'Cayuga White' map consisted qof 20 linkage groups with 214 markers covering 1,196 cM and that of 'Aurore' map consisted of 22 linkage groups with 255 markers spanning 1,477 cM. This mapping population segregated for disease resistance and other important traits.

A second grape map utilizing interspecific hybrids was developed by Dalbò et al. (2000), using the progeny from 'Horizon' ('Seyval' × 'Schuyler') × Illinois 547-1 (*V. cinerea* B9 × *V. rupestris* B38). Parental maps were constructed with 277 RAPD, 25 microsatellite, 4 CAPS (cleaved amplified polymorphic sequences), and 12 amplified fragment length polymorphism (AFLP) markers This map also used the double pseudo-testcross strategy, and consisted of 153 markers covering 1,199 cM, with an average distance of 7.6 cM between markers on the Horizon map and 179 markers covering 1,470 cM with an average distance of 8.1 cM on the Illinois 547-1 map.

In 2002, Doligez et al. reported the first *V. vinifera*-based genetic linkage map. The map was constructed using a F_1 progeny of 139 individuals from a cross between two partially seedless genotypes [MTP2223-2 (Dattier de Beyrouth × Pirovano 75) × MTP2121-30 (Alphonse Lavallée × Sultanina)]. All the progeny were produced via embryo rescue (Bouquet and Davis 1989). This consensus map consisted of 301 markers [AFLP, simple sequence repeat (SSR), RAPD, SCAR (sequence characterized amplified region)]. This map consisted of 20 linkage groups and covered 1,002 cM. In 2003, Grando et al. reported on a map of a *Vitis* inter-specific hybrid population from 81 progeny of *V. vinifera* 'Moscato bianco' × *V. riparia* Wr63. Three types of markers were used to construct this map, AFLP, SSR and SSCP (single strand conformation polymorphism). A total of 338 markers were assembled in 20 linkage groups covering 1,639 cM for the maternal map, and 429 loci defined the 19 linkage groups of the paternal map, which covered 1,518 cM.

In 1998, the grape genetics research community formed the International Grape Genome Program (IGGP) for the purpose of cooperation and coordination in increasing knowledge of the grape genome (http://www.vitaceae.org). The cooperative effort resulted in the Vitis Microsatellite Consortium (VMC), established to generate a large number of codominant SSR markers, an effort coordinated by Agro-Gene S.A. in France. Among the goals of the IGGP is the creation of reference linkage maps to harmonize linkage groups resulting from individual mapping projects, and to serve as a resource for physical mapping. This map would also be useful for targeting genomic regions for more intensive mapping efforts, such as localizing QTLs and optimizing MAS.

The first reference map was based on only codominant SSR markers and used *V. vinifera* 'Riesling' (prime name 'Riesling weiss') × *V. vinifera* 'Cabernet Sauvignon'. Riesling is one of the world's most important white wine varieties and is especially important in cool climates, such as Germany, Canada and the northeastern United States. Cabernet Sauvignon is the world's most widely distributed red wine variety; it has also been selected by the IGGP as the target cultivar for cooperative efforts on physical mapping. This reference mapping population consisted of 153

Table 4. A list of all published maps in grapes

Population	Marker system	No. of Genotypes	Average marker distance (cM)	Reference
Cayuga White (Hybrid of *V. vinifera*, *V. labrusca*, *V. rupestris* and *V. aestivalis*) × Aurora (Hybrid of *V. vinifera*, *V. rupestris* and *V. aestivalis)*	RAPD, RFLP, Isozyme	60	6.1	Lodhi et al. 1995
Horizon ('Seyval' × 'Schuyler') × Illinois 547-1 (*V. rupestris* × *V. cinerea*)	RAPD, SSR, CAPS, AFLP	58	7.8	Dalbo et al. 2000
MTP2223-2 (Dattier de Beyrouth × Pirovano 75) × MTP2121-30 (Alphonse Lavallée × Sultanina)	AFLP, SSR, RAPD, SCAR, Isozymes	139	6.2	Doligez et al. 2002
Moscato bianco (*V. vinifera* L.) × *V. riparia* Mchx	SSR, AFLP, SSCP	81	8.1	Grando et al. 2003
Riesling × Cabernet Sauvignon	SSR, EST	153	11	Riaz et al. 2004
V. rupestris and *V.arizonica* hybrids	AFLP, SSR, RAPD, ISSR	116	10.2	Doucleff et al. 2004
Syrah × Grenache	SSR	96	6.4	Adam-Blondon et al. 2004
Regent × Lemberger	AFLP, RAPD, SSR, SCARs/CAPS	153	5.9	Fisscher et al. 2004
Riesling Self	SSR	96	6.4	Adam-Blondon et al. 2004

progeny plants. A total of 152 SSR markers and one polymorphic expressed sequence tag (EST) marker mapped to 20 linkage groups (Riaz et al. 2004). An integrated linkage analysis was performed to obtain the consensus map. The map covered 1,728 cM with an average distance of 11.0 cM between markers (Fig. 2).

As part of the IGGP an international grape genomics initiative (IGGI) was proposed to generate an international consensus genetic linkage map to integrate the codominant marker data from different mapping populations. This effort will combine information from different genetic backgrounds into one framework map for use in MAS and the physical mapping of genes. Five different populations have been chosen for this purpose. The first population (A1) of 95 full-sib progeny is the Syrah × Grenache map mentioned above (Adam-Blondon et al. 2004). The second population (A2) is the population of 114 progeny obtained by selfing Riesling as mentioned above (Adam-Blondon et al. 2004). The third population of 46 full-sib progeny (DG) is from a cross between Chardonnay and Bianca cultivars (Di Gaspero et al. 2005). The fourth population (D) consists of 139 full-sib progeny from the cross MTP2223-27 × MTP2121-30 mentioned above (Bouquet and Danglot 1996). The fifth population (R) consists of 153 full-sib progeny from the Riesling × Cabernet Sauvignon cross, mentioned above (Riaz et al 2004). The first two and the fourth population are being maintained at INRA, France, the third population is maintained at the University of Udine (Italy), and the National Clonal Germplasm Repository, Davis, USA, maintains the last population.

2.2.2 Mapping and Tagging of Major Genes

Relatively few genes have been isolated in grapes compared to the other major agronomic crop plants and model organism *Arabidopsis thaliana*. Two strategies from "phenotype to gene" and from "gene to phenotype" (reverse genetics) have been used to isolate

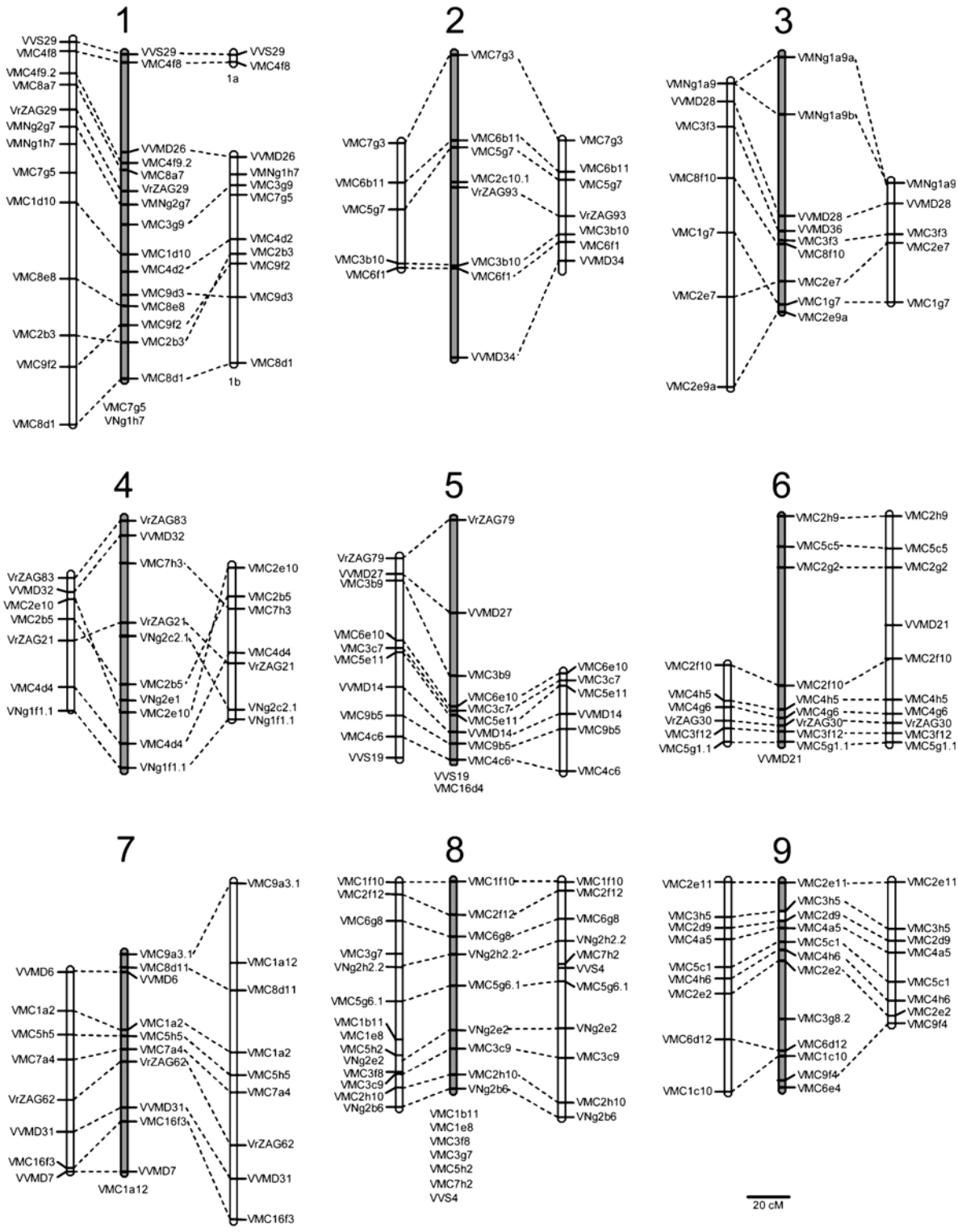


Fig. 2. Linkage map of *Vitis vinifera* 'Riesling' × 'Cabernet Sauvignon'. For each linkage group, parental maps are shown on the *left* ('Riesling') and *right* ('Cabernet Sauvignon') and consensus map is in the *center* (Riaz et al. 2004)

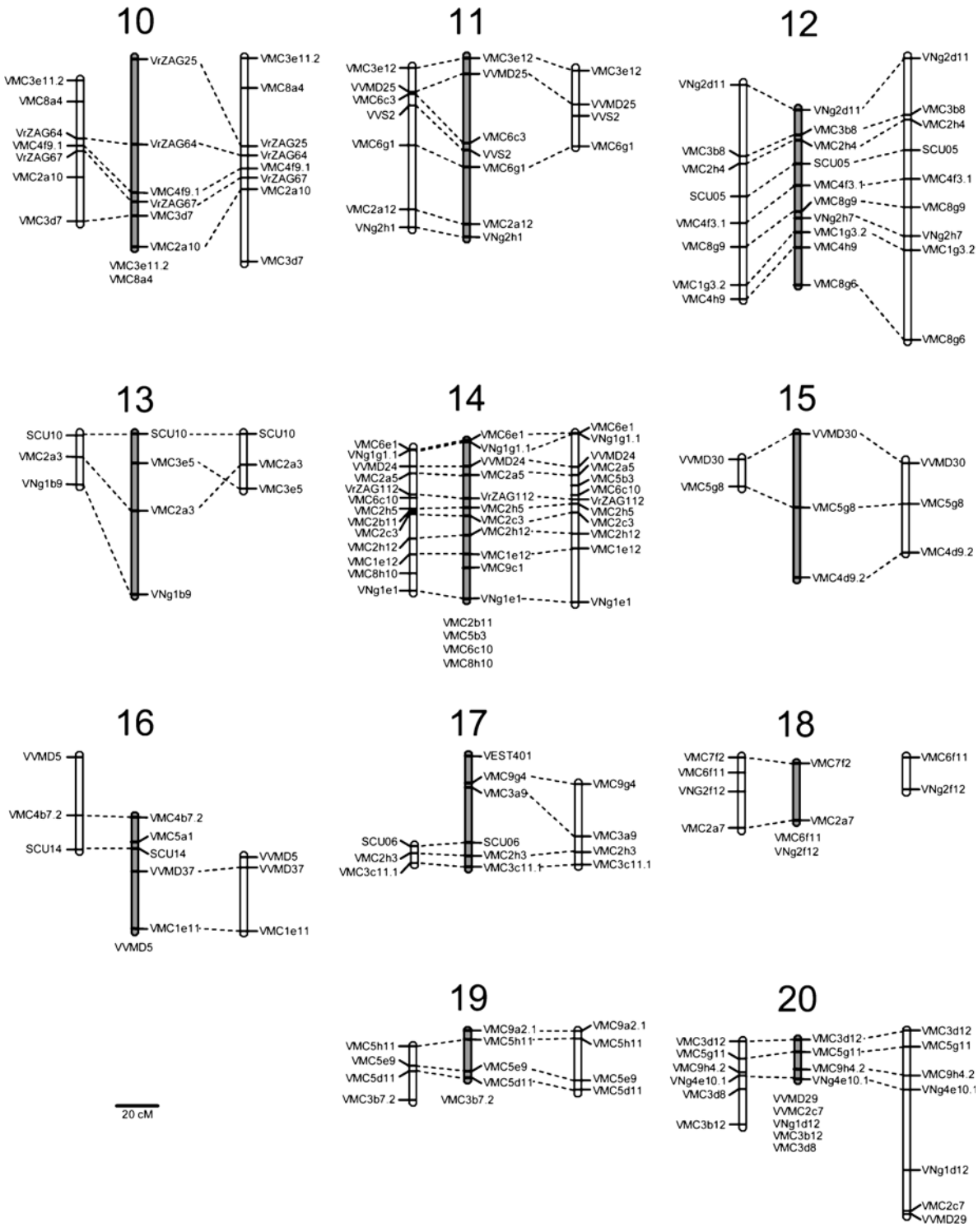


Fig. 2. (continued)

and characterize genes. In *Arabidopsis*, several combined approaches, such as positional cloning, candidate gene approach, and insertional mutagenesis with either transposons or T-DNA vectors have been used successfully to isolate genes identified by the phenotype of their mutant alleles. In grape, it is very difficult to use reverse genetic approach to tag and isolate genes. Multiple genes control most horticultural traits and no information is available about gene function and expression. With the availability of molecular markers, it became possible to map traits of interest on genetic linkage maps of segregating populations. The main focus is on disease resistance for different pests and diseases.

2.2.2.1 Fungal Diseases

Bouquet (1986) introduced a dominant resistance gene for powdery mildew from *Muscadinia rotundifolia*, *Run1*, into the *Vitis vinifera* genome over five backcross generations (Bouquet 1986). *Run1* confers total resistance to the populations of this fungus naturally occurring in Montpellier, France. The segregating population was created in 1995 by crossing a resistant hybrid carrying *Run1* in the heterozygous state (VRH 3082-1-42) with Cabernet Sauvignon. They used the bulked segregant analysis (BSA) approach with AFLP markers tightly linked to the *Run1* locus to develop a local map around the gene. Pauquet et al. (2001) later reported a local map of AFLP markers around the *Run1* gene (Fig. 4a). A BC_5population of 157 genotypes was used to select AFLP markers linked to the resistance gene. A total of 13 markers were used to develop this local map and 10 of them co-segregated with the resistance gene. They also studied the usefulness of these 13 AFLP markers in a wider set of resistant and susceptible genotypes. Three markers out of 13 analyzed were absent in all susceptible genotypes and present in all resistant genotypes.

Doucleff et al. (2004) reported on a map of *V. rupestris* × *V. arizonica*. This mapping population segregates for resistance to the dagger nematode (*Xiphinema index*) and to Pierce's disease (PD), a bacterial disease caused by *Xylella fastidiosa*. A total of 475 DNA markers [mostly AFLP, inter simple sequence repeat (ISSR), RAPD and SSR)] were used to construct the parental maps with PGRI (Plant Genome Research Initiative) mapping program. Maternal and paternal maps covered 756 and 1,082 cM, respectively. Currently, this population is being re-mapped with SSR, EST-SSR and EST markers. A total of 240 markers have been mapped to 19 linkage groups. The main focus is to develop a high density linkage map around the nematode and PD resistance loci, and use these tightly linked markers for MAS in an ongoing grape scion and rootstock breeding program and initiate map-based positional cloning of resistance genes.

Fischer et al. (2004) reported on a map of full sib F_1 population consisting of 153 genotypes from the cross of 'Regent' × 'Lemberger'. Parental maps were constructed with AFLP, RAPD, SSR and SCARs/CAPS markers. The Regent map covered a total of 1,277.3 cM with an average marker distance of 4.8 cM. The Lemberger map extends over 1,157.7 cM with an average marker distance of 7.0 cM.

A second international grape reference map solely based on SSR markers was published in 2004 (Adam-Blondon et al. 2004). It mapped 96 progeny from *V. vinifera* 'Syrah' × *V. vinifera* 'Grenache'. The Syrah map was constructed from 177 markers (many VMC, and newly developed VVI within Genoplante, see Merdinoglu et al 2005) into 19 linkage groups (1,172.2 cM) and the Grenache map was constructed of 178 markers into 18 linkage groups (1,360.6 cM). The consensus map consisted of 220 markers ordered in 19 linkage groups covering 1,406.1 cM. This was the first published map that represented the 19 chromosomes of genus *Vitis* (Fig. 3). In the same study, a map based on progeny from a selfed Riesling population consisting of 110 SSR and covering 1,191.7 cM was also reported. Using these maps, the genome length was estimated to be around 2,200 cM, which was comparable to genome length estimates from the first published reference map (Riaz et al. 2004).

A new PCR-based approach for rapid generation of genetic markers capable of tagging disease resistance genes has been developed and effectively used in other crops. This approach is based on the observation that genes conferring resistance from a diverse range of host-pathogen interactions have a high degree of structural and amino acid sequence conservation. In particular, the majority of cloned resistant genes, "R genes", contain a nucleotide binding site (NBS) and a leucine rich repeat (LRR) domain (Meyers et al. 1999; Young 2000). The NBS sequences of these genes are characterized by the presence of up to seven conserved domains including the P-loop, Kinase-2, and GLPL motifs. The presence of these conserved domains has facilitated the cloning of resistance gene

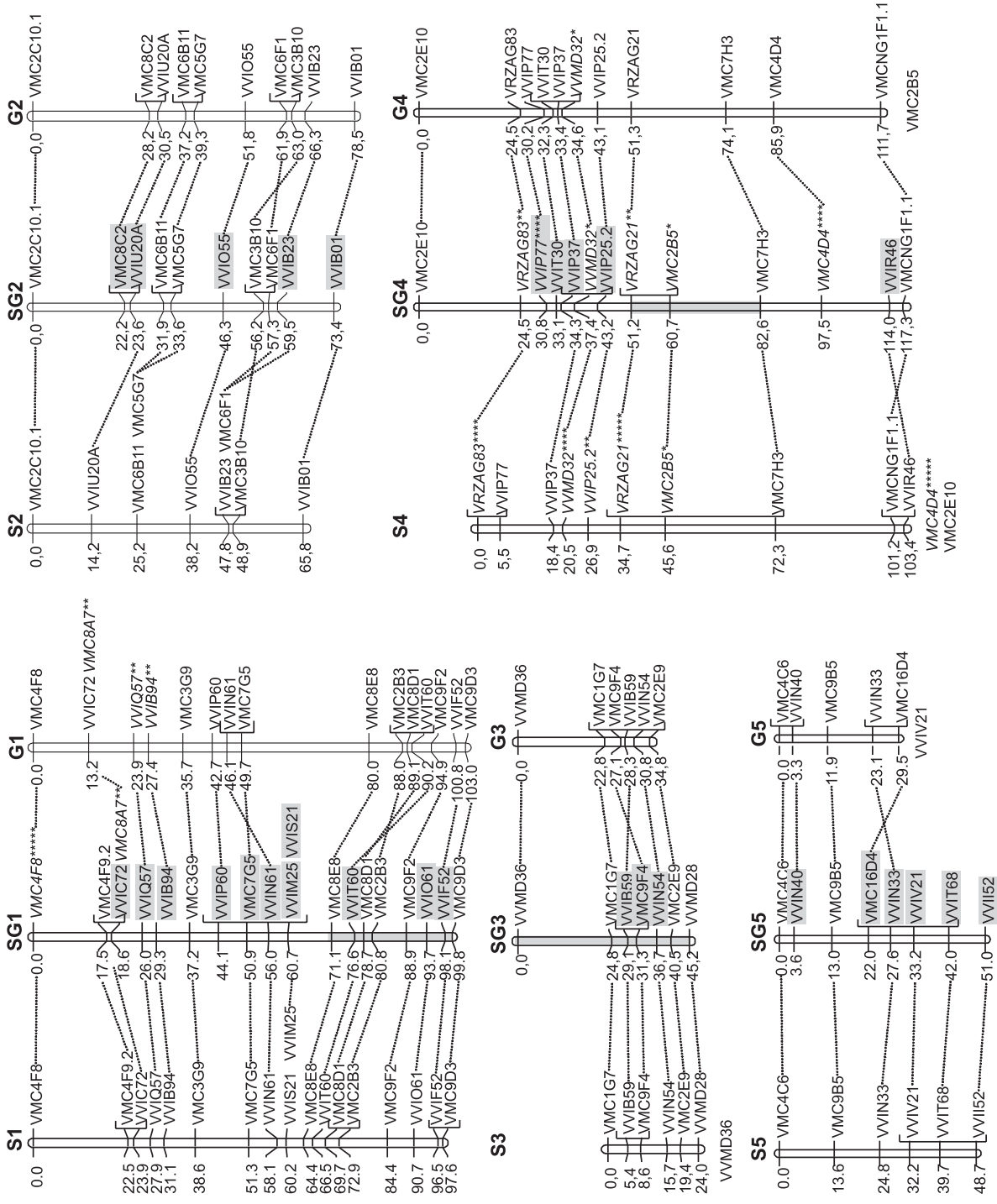
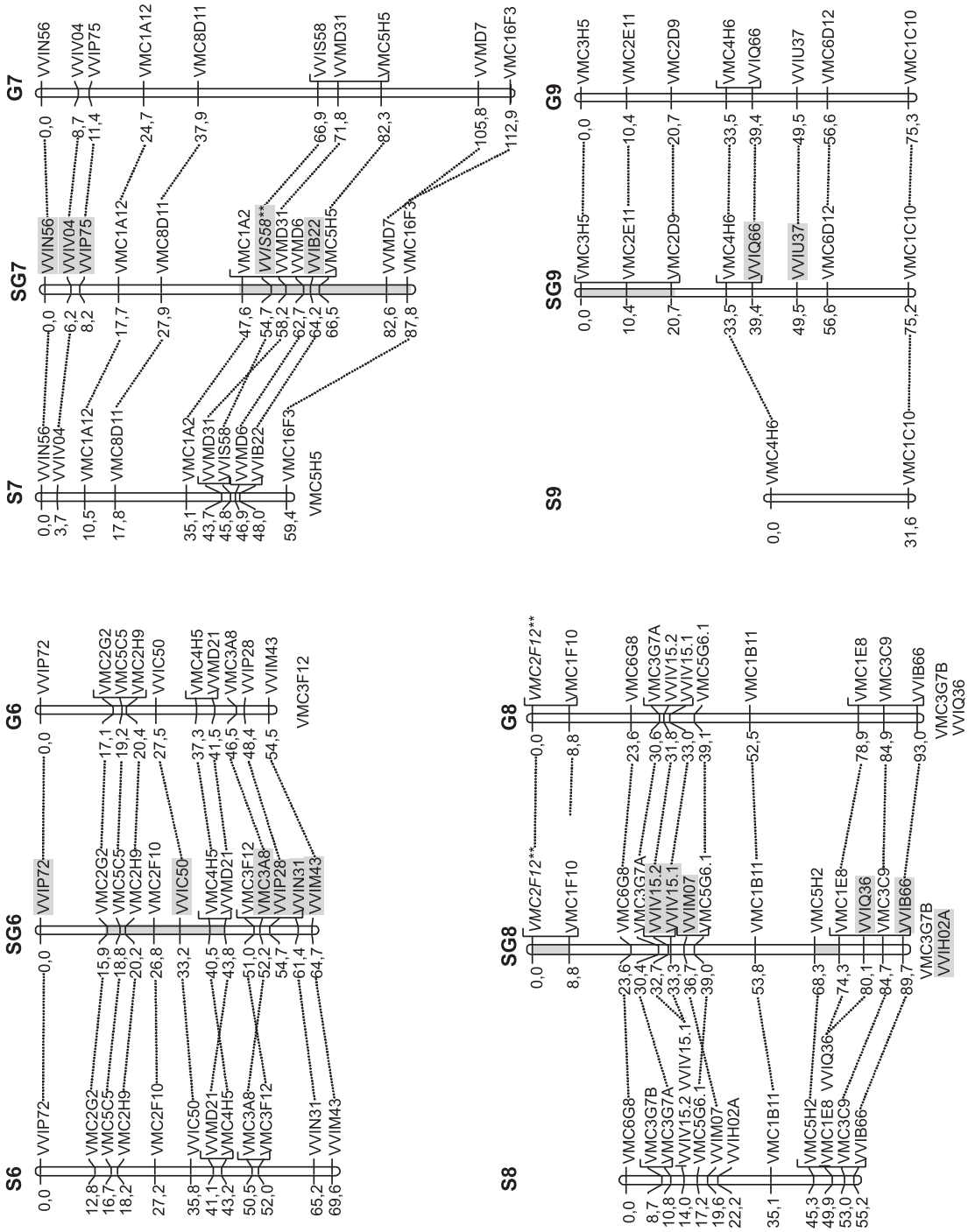


Fig. 3. Syrah, Grenache and consensus S × G maps. Linkage groups are respectively numbered from 1 to 19 with the prefix *S*, *G* and *SG*. Distorted markers are in *italics* with an *asterisk* indicating the level of distortion (* $P < 0.05$; ** $P < 0.02$; *** $P < 0.01$; **** $P < 0.001$; ***** $P < 0.0001$). Markers that were ordered at LOD = 2.0 are indicated with the *bracket* on the *left*. Markers present only in S × G map and not in Riaz et al. (2004) are indicated with *grey boxes*. *Grey zones* in the linkage groups are indicating the markers with a different order in this map and the three maps published by Riaz et al. (2004). (Adam-Blondon et al. 2004)



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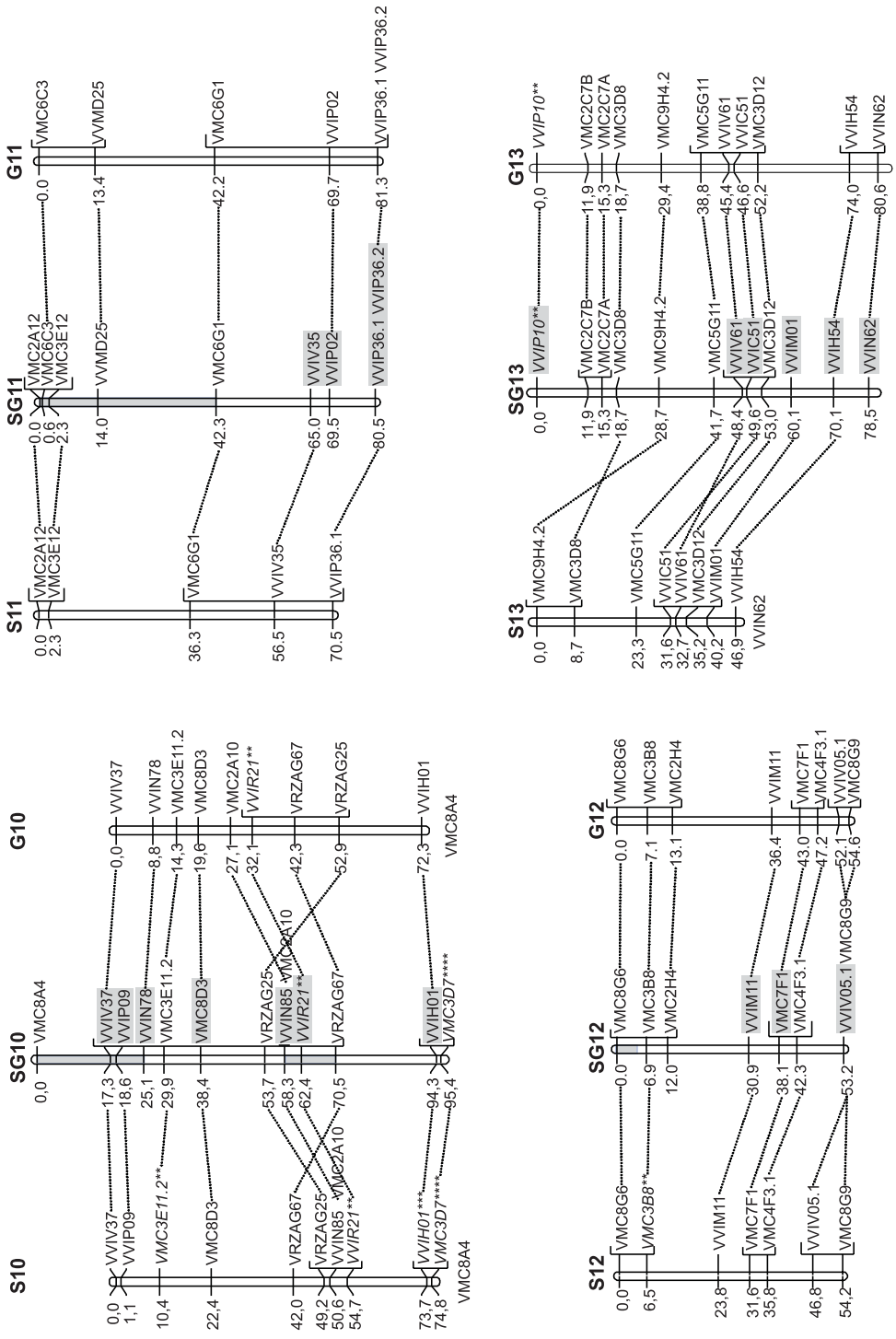

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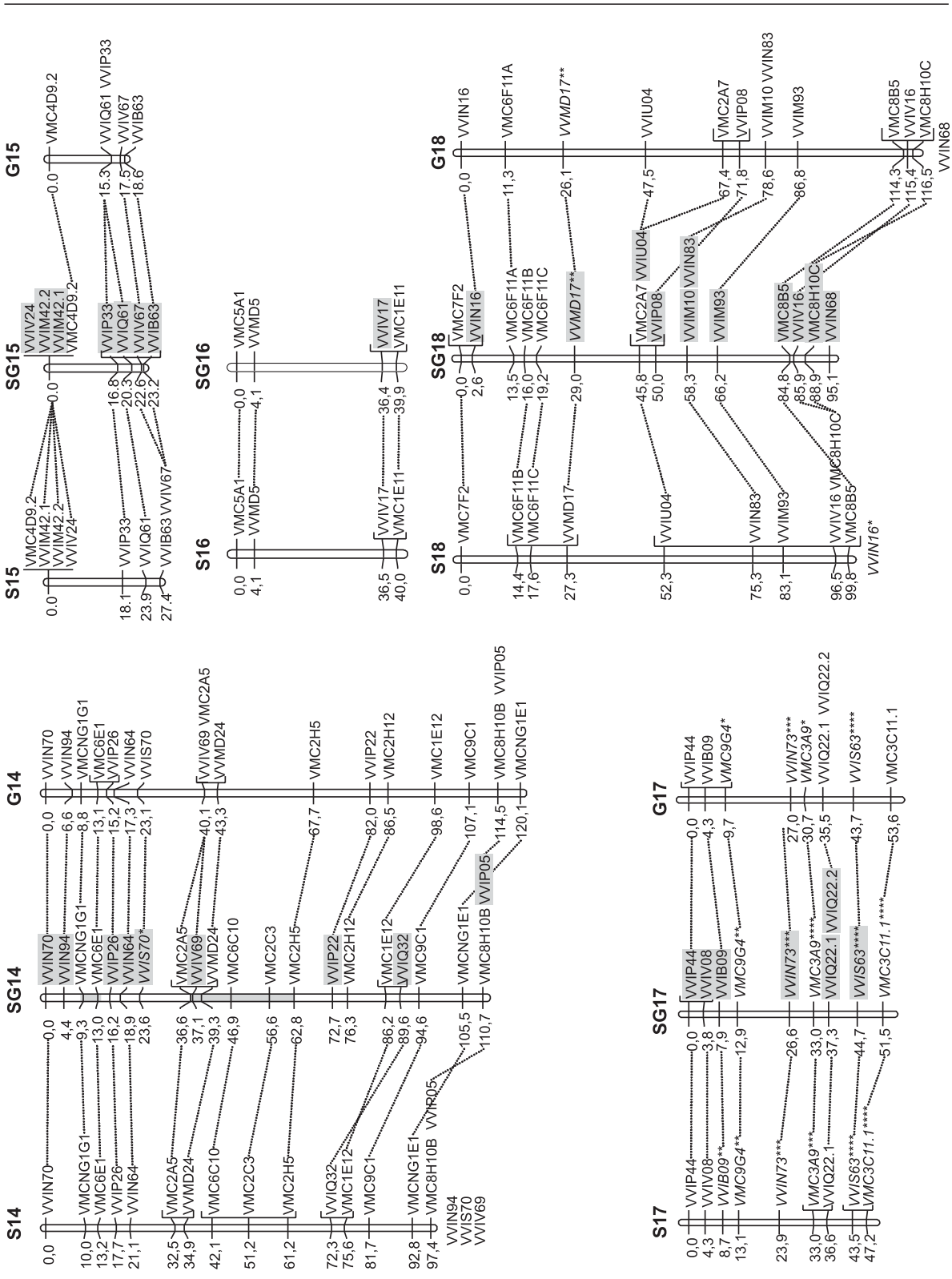

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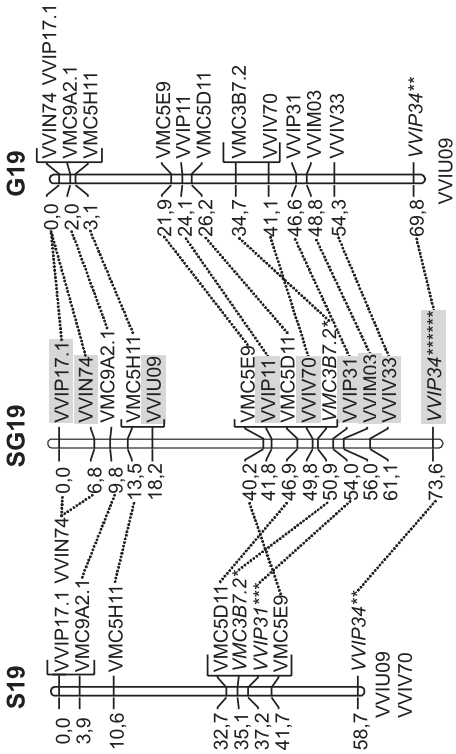

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analogs (RGA) from diverse species using PCR and degenerate oligonucleotide primers. NBS encoding sequences tend to be clustered in the genome and, in accordance with this, isolated RGAs are frequently genetically located at, or near, previously identified resistance loci (Aarts et al. 1998; Collins et al. 1998; Leister et al. 1999; Mago et al. 1999; Pan et al. 2000). Therefore, the identification of RGAs represents a potentially powerful strategy to develop new markers around resistance genes and a good aid for map-based positional cloning of genes.

In a continuation of the previous work on the *Run1* locus, Donald et al. (2002) were the first grape researchers to utilize the RGA approach in grapes. They used degenerate primers designed to conserved regions of NBS motifs within previously cloned pathogen resistance genes, to amplify RGAs from grape. Twenty-eight unique grapevine RGA sequences were identified and subdivided into 22 groups on the basis of a nucleic acid sequence identity of approximately 70% or greater. Three RGA markers were tightly linked to the *Run1* locus. Of these markers, two RGA (GLP1-12 and MHD 145) co-segregated with the resistance phenotype in the 167 tested genotypes of BC5 population, and the RGA marker MHD98 was mapped to a position 2.4 cM from the *Run1* locus (Fig. 4b). As part of the continuing effort to tag the *Run1* gene, Barker et al. (2005) recently published a genetic and physical map of the gene using a BAC library constructed using genomic DNA from a resistant *V. vinifera* individual carrying *Run1* within an introgression. This is the first published report of physical mapping of any gene in grape. The BAC contig assembly also allowed the generation of new genetic markers that are closely linked to the *Run1* gene. Initial analysis indicates that region containing *Run1* gene contains two multigene families of RGA.

Luo et al. (2001) also employed BSA with RAPD and sequence characterized amplified region (SCAR) molecular markers to tag the downy mildew-resistance genes of grape derived from *V. quinquangularis*. The parents and 60 selected progeny were tested. Among 280 Operon RAPD primers tested, 160 gave distinct banding patterns and one marker, OPO06-1500, was tightly linked to a major gene for resistance to *Plasmopara viticola* (RPv-1). Linkage analysis with Mapmaker determined the distance between RPv-1 and OPO06-1500 to be 1.7 cM. Marker OPO06-1500 was cloned and sequenced to develop a SCAR marker (SCO06-1500),

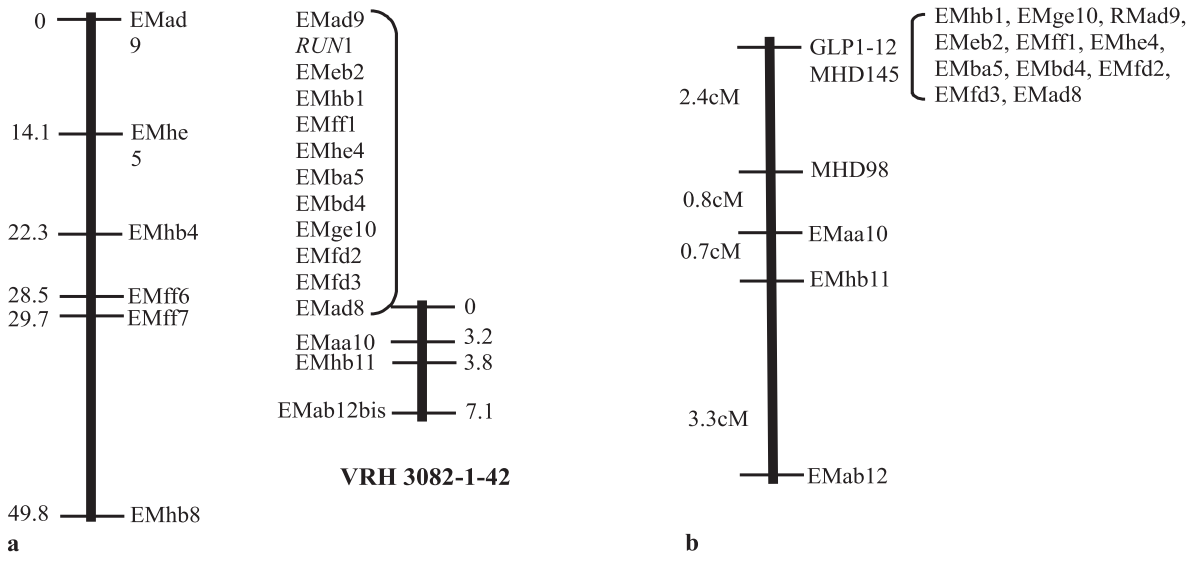

Fig. 4. Local map of the resistant genotype VRH3082-1-42 and in Cabernet Sauvignon of the RUN1 region. The 11 loci in *brackets* together with ***RUN***1 at the top of the VRH3082-1-42 linkage groups are all co-segregate. Figure 3b shows linkage map of the resistant locus RUN1 and RGA markers GLP1-12, MHD145 and MHD98 (Pauquet et al. 2001; Donald et al. 2002)

which produces a single band only in resistant plants.

2.2.2.2 Bacterial Diseases

In spite of the fact that bacterial diseases cause serious losses in grape (Pierce's disease and bacterial blight of grape), there has been little information available for incorporation of bacterial resistance from wild species into *V. vinifera* except for the case of Pierce's disease (PD). All *V. vinifera* varieties are highly susceptible to PD, which is caused by the bacterium *Xylella fastidiosa*. In grape growing areas, where the bacterium is endemic, the disease severely limits the cultivation of *V. vinifera* cultivars. Symptoms of PD include: leaf scorching, fruit cluster dehydration, uneven maturation of infected canes, stunting and death within 3–4 years. Resistance to PD exists in American *Vitis* species and has been introgressed into many hybrid cultivars, but very little is known about the genetics of resistance. Krivanek et al. (2005) reported that a single gene *PdR1* with a dominant allele is responsible for PD resistance originating from a *V. arizonica* background. An extensive, grape-breeding program is underway to incorporate this resistance gene into improved wine, table and raisin grapes. The *PdR1* locus has been localized on chromosome 14 of a genetic linkage map resulting from a cross of D8909-15 [*V. rupestris* 'A. de Serres' × 'b42-26' (*V. arizonica*)] and F8909-17 [*V. rupestris* 'A. de Serres' × b43-17 (*V. arizonica*/*V. candicans*)] (Krivanek et al. 2006).

2.2.2.3 Insects and Nematodes

Grape phylloxera (*Daktulosphaira vitifoliae* Fitch) is the most important insect pest of grape and continues to impact the world's vineyards. It is native to North America but has spread to every region where grapes are grown and caused billions of dollars in damage by its destructive feeding on *V. vinifera* roots. Native American grape species are resistant to phylloxera and rootstocks have been bred and used to control phylloxera for over 100 years. Very little is known about the mechanism of resistance or the number of resistance genes available from the Native American grape species.

Researchers at the Department of Viticulture and Enology, University of California, Davis, are attempting to position phylloxera resistance on a genetic linkage map of a population from a cross between the resistant *V. rupestris* and the susceptible *V. vinifera* grape (Roush et al. 2004). It has been hypothesized that the number and type of root galls formed in response to phylloxera should be associated with a plant's level of resistance. In this study, plants from

the F_1 generation were selected for a series of sibling crosses among resistant and susceptible individuals as well as backcrosses to *V. vinifera* 'Aramon' and *V. rupestris* 'Ganzin'. A subset of the resulting F_2 progeny was selected from these crosses and infested to identify resistant and susceptible plants and to determine the inheritance of gall formation. Preliminary data suggests that more than one gene is likely responsible for gall formation, and hence resistance or susceptibility.

The Department of Viticulture and Enology is also studying resistance to root-knot (*Meloidogyne incognita*) and dagger (*Xiphinema index*) nematodes. Two mapping populations have been developed from progeny segregating for resistance to these pests. The first is a *V. riparia* 'Riparia Gloire' × *V. champinii* 'Ramsey' population created to map resistance to root-knot nematode and locate QTLs for salt tolerance and rooting angle (Lowe and Walker 2004). The second is the 9621 population mentioned above on which *X. index* resistance is being mapped. Both nematode resistances seem to map as a single dominant gene, but to different linkage groups.

2.2.2.4 Other Morphological Traits

Grape, being relatively large, perennial, and heterozygous, is not a good system for classical developmental genetic efforts to map and tag important horticultural traits that affect plant form, cluster architecture, factors affecting fruit composition and yield. Thus, only a few morphological traits have been mapped. Dalbo et al. (2000) mapped a locus controlling flower type on linkage group 14 of parental map of 'Illinois 547-1' that corresponds to linkage groups 2 of reference map (Adam-Blondon et al. 2004; Riaz et al. 2004). The probable parental genotypes were homozygous hermaphrodite, HH ('Horizon'), and heterozygous male, MF (III.547-1). The resulted progeny indicated 1:1 segregation of male and hermaphrodite types. This confirmed that a single gene controls sex expression in grapes as previously suggested by Olmo (1976).

In another study, Doligez et al. (2002) mapped a major gene for berry color to linkage group 3 that now corresponds to linkage group 2 of the international reference maps (Adam-Blondon et al. 2004; Riaz et al. 2004). Fischer et al. (2004) also reported that berry color segregated as a simple trait and it mapped on the linkage group 13 of their population, which also corresponded to linkage group 2 of international consensus map.

Seedlessness is another important trait that was tagged in a study by Bouquet and Danglot (1996). Analysis of progenies obtained by crossing seedless genotypes led to a proposed model for the inheritance of seedlessness (Bouquet and Danglot 1996) and to the identification of a SCAR marker linked to the seedless character (Lahogue et al. 1998). The efficiency of obtaining seedless progeny can be greatly improved by the use of molecular markers tightly linked to seedlessness and these markers will also help in optimizing parental selections.

2.2.3 Detection of QTLs

Genetic linkage maps have facilitated mapping of agriculturally important QTLs in grapes, including QTLs for disease resistance, seedlessness and berry weight. Using QTL mapping, resistance loci whose alleles exert smaller effects on the phenotype may be manipulated more effectively (Young 1996). In the case of disease resistance, an obvious goal would be to develop grape cultivars with resistance alleles at all QTLs of interest. Establishment of generalized genomic regions that affect a particular trait within inter- and intra-species grape mapping populations with common markers will help to clarify the relationships of QTLs in different genetic backgrounds, and promote marker assisted selection and breeding.

2.2.3.1 QTLs for Disease and Pest Resistance

There are only a few published reports of QTL studies in grape and the main focus is powdery (*Uncinula necator*) and downy mildew (*Plasmopora viticola*). Dalbo et al. (1997) studied the inheritance of powdery mildew resistance in the cross Horizon × Illinois 547-1. Genetic maps based on RAPD markers were constructed for each parent with a mean distance between markers of 5.5 cM. A major QTL was found in the resistant parent Illinois 547-1. BSA was used to screen 203 primers to find additional linked RAPD markers. A single marker (S25b; LOD = 6.9) explained 44% of the variation. The same marker was present in *V. cinerea* B9, one of the parents of Illinois 547-1 and the likely source of resistance. Two other regions on the Horizon map were associated with powdery mildew resistance. The markers S25b (from Illinois 547-1) and S35a (from Horizon) could be used to correctly classify resistance in all but 9 of 60 seedlings.

Zyprian et al. (2002) reported on the tagging of resistance to powdery and downy mildew from the cultivar Regent. An F_1 population on about 153 individuals was derived from the cross of the fungus-resistant Regent × the fungus-susceptible Lemberger. This population segregates for resistance to both diseases as well as other agronomic traits in a quantitative manner and AFLP, RAPD and SSR markers were used in the map. In continuation of this work, Fisher et al. (2004) reported major QTLs for resistance to powdery and downy mildew on linkage groups 9, 10 and 16 of the Regent map that corresponds to group 7 and 11 of international consensus map. These QTLs explained up to 69% variation in the tested population.

2.2.3.2 QTLs for Other Traits

Doligez et al. (2002) reported on the detection of QTLs for traits involved in seed production with the goal of characterizing seedlessness sub-traits (seed number, seed total fresh and dry weights, seed percent dry matter and seed mean and fresh dry weights) and berry weight in an F_1 progeny obtained by crossing two partially seedless genotypes (MTP2223-2 × MTP2121-30, mentioned above) and embryo rescuing the progeny. QTL detection was performed with two methods: the non-parametric Kruskal-Wallis rank-sum test, and composite interval mapping. QTLs with large effects (R^2 up to 51%) were detected for all traits and years at the same location on linkage group X (which now corresponds with linkage group 18 of the international reference map, Riaz et al. 2004). Three QTLs with small effects (R^2 from 6% to 11%) were found on three other groups.

Riaz (2001) genetically analyzed different components of the grape cluster. Compact cluster architecture is closely associated with bunch rot (Vail et al. 1998), and small berries contribute to loose clusters. Small berries are also desirable for red wine production because the higher skin to pulp ratio is thought to increase wine color intensity. Cluster form was divided into different components (rachis length, number of laterals, length of laterals, total cluster weight, number of berries, berry weight, and cluster density) in order to study their individual contribution to cluster architecture and compactness and their relationship to each other. The QTL analysis was carried out on a consensus framework linkage map based on 154 SSR and one EST marker on 153 progeny of Riesling × Cabernet Sauvignon. QTLs were identified with two different methods (Interval mapping and Kruskal-Wallis rank sum test). They were identified for total cluster weight, average weight of one berry, berry number per cluster, rachis length, number of laterals per cluster, average lateral length and cluster density, as well as for fruit composition and young shoot morphology. Most of the traits that markedly affected cluster architecture showed strong correlation to each other and QTLs were identified with overlapping intervals. These were preliminary results based on three years of data on a single plant of each genotype and it is very important to validate data with multi-vine replicates of progeny and parents in different environments. Thus far, three studies on QTLs associated with berry size have been reported (Riaz 2001; Doligez et al. 2002; Fischer et al. 2004), however they mapped to different linkage groups in first two studies (group 17 and group, 18 respectively) and mapped to linkage groups 3 and 10 of Regent map (Fischer et al. 2004). Neither of these linkage groups had SSR markers common to the international reference map (Riaz et al. 2004). Fischer et al. (2004) also reported QTLs for veraison and axillary shoot growth.

2.3 Whole Genome Projects

The completion of the human, *Arabidopsis* and rice genome sequences in the last five years stimulated rapid development of genomic technologies and applications. The functional information accumulating in *Arabidopsis* also offers a model system for the functional analyses of grape genes. These possibilities provide a framework for a concerted effort to efficiently identify and functionally analyze important grape genes. The International Grape Genome Program was formally announced in January 2002 at the Plant, Animal, and Microbe Genome X Conference, in San Diego, California. The main objectives are: (1) Coordinate the Grape Genome Program. (2) Facilitate exchange of information and collaboration with the wider viticulture and enology research communities. (3) Monitor, summarize and communicate progress of scientific activities of participating laboratories. (4) Identify research areas of benefit to grape improvement and plant biology and communicate them to funding agencies of participating nations. (5) Periodically up-date the goals of the program. (6) Serve as a primary contact with other plant genome projects. (7) Interact with an Industry Advi-

sory Committee to ensure relevance of the research to industry problems. (8) Act on recommendations received from the various working groups.

In addition to the International Grape Genome Initiative, individual genomic efforts are also underway in grape growing countries and are briefly described below.

2.3.1 Australia

Current research involves a wide range of techniques from functional characterization of single genes to genomic approaches including genetic mapping, physical mapping, gene discovery using ESTs, and gene expression analysis using microarrays and transgenic plants. Beneficial outcomes from this research are expected to increase our knowledge of grape biology, improved berry and wine quality, and provide resistant or tolerant plants to powdery mildew, botrytis, nematodes and phylloxera.

Recently, Dupont Genome Sciences in conjunction with Southern Cross University initiated a large-scale grape DNA sequencing project. The main focus of this project is to obtain genetic information to allow research in areas such as dormancy and bud burst; fruit quality including sugar content, flavor and color, and tendril development. The technologies include large-scale expressed sequence tag (EST) analysis (Ablett et al. 2000), a 16× BAC library (Tomkins et al. 2001), and functional analysis of grape genes in *Arabidopsis* to advance gene discovery in these areas. The BAC library was constructed from the cultivar Syrah and consisted of 55,296 clones with average insert size of 144 kb.

To date, this project has produced over 45,000 grape (*V. vinifera*) ESTs or partial gene sequences from a range of tissues and cultivars. These represent nearly 19,000 distinct ESTs covering an estimated two-thirds to three-quarters of the grape genes (based on an estimated number of 25,000 to 28,000 genes). New SSR markers with a high degree of transferability have been developed from the ESTs (Scott et al. 2000). This was the first report of SSR identification from grape ESTs. This approach has been used widely in other plant species. Phenotypic changes produced by over-expression of novel grape transcription factors in *Arabidopsis*, are being analyzed, and cDNA grids are being used to study gene expression during budburst and berry development.

2.3.2 France

The development of grape genomic resources in France has been greatly aided since 1999 through financial support from the Génoplante consortium (www.genoplante.org) and INRA (www.inra.fr). BAC libraries have been constructed and will be used in the development of a physical map of the *V. vinifera* genome in collaboration with members of the IGGP (Chalhoub et al. 2002; Adam-Blondon et al. 2005). The URGV (Unité de recherche en génomique Végétale) has been set-up at INRA to work on several BAC libraries from different cultivated plants of importance to France and Europe. They have developed three grape BAC libraries: Cabernet Sauvignon (13×, International Grape Genome Program reference library, www.vitaceae.org), Syrah (8×) and Pinot noir (15×), with about 70,000 BAC clones each. The average size of inserts is 150 Kb. Further work was carried out to develop physical map with the Cabernet Sauvignon BAC library (http://www.evry.inra.fr/public/projects/genome/grape). It involved development of 3D pools from a 6× subset of the Cabernet Sauvignon BAC library to anchor with PCR 237 SSR markers (Adam-Blondon et al. 2004) and 565 ESTs (from the unigene set used in the Génoplante project CI2001003). An additional set of 592 ESTs from the NCBI Vitis Unigene set # 11 was anchored in silico on the BAC end sequences. These results are providing access to regulatory regions of genes of interest and to the position of about 50 new genes on the genetic map. The fingerprinting of 30,000 BAC clones is now underway in collaboration with Génoscope and the University of Udine (M. Morgante).

Recently, emphasis was put on the development of SSR markers and on a reference genetic map as a tool for QTL detection of traits such as berry characters and pathogen resistance. The production of ESTs by Terrier et al. (2001) will contribute to the development of microarrays for the study of the expression, regulation and signaling control of berry development genes. In parallel, INRA has been developing methods for grapevine transformation (transient & stable). A database for grape genetic resources is available at INRA and at the European level and several other databases are under development (EST management and processing, genetic maps, BAC) (See lists below). This knowledge should help in the development of high quality grape varieties resistant to pathogens and also lead to a better understanding and management

of grape-environment interactions and their effect on fruit and wine quality.

2.3.3 Germany

In Germany grapevine genomics started in the early 1990s with the application of molecular marker technology to questions of cultivar identification, pedigree analysis, evaluation of genetic resources, and genetic mapping (also in France). The major focus is on localization and long-term molecular characterization of genes involved in pest resistance and fruit quality traits with the aim of understanding their complex genetic basis. Different marker systems are being employed, including SSR markers developed by the Vitis Microsatellite Consortium (VMC) allowing integration with the results from other international mapping projects.

2.3.4 Italy

Since the early 1990s molecular biologists have been using molecular tools for variety characterization, disease diagnosis, phylogenetic studies, and genetic transformation of *Vitis* species. In the last few years, the interest in grape genomics has increased enormously and research involves marker-assisted selection, molecular mapping, and large EST sequencing, establishing BAC libraries for map-based positional cloning of genes of economical interest, pest resistance, and fruit quality. Italy has two large genomic projects: the first is headed by a public institution, The University of Udine, focused on developing tools for molecular breeding and map-based positional cloning of genes approaches; and the second is a collaborative project among several universities and headed by S. Grando, with a focus on the functional genomics of berry maturation phases.

2.3.5 South Africa

Grapevine genomics research in South Africa started with participation in the Vitis Microsatellite Consortium (VMC) in 1998. The Institute for Wine Biotechnology (IWBT) and the Institute for Plant Biotechnology (IPB), are the two major sites for grape genomic research. Genomics efforts include genetic transformation, and development of cDNA libraries. The IWBT generated genomic libraries for the *V. vinifera* cultivars Sultana (correctly Sultanina) and Pinotage, and cDNA libraries from young expanded leaves of the same two cultivars. Genomic libraries for Chardonnay and Merlot as well as cDNA libraries from early and late berry developmental stages of these cultivars were made at the IPB. A consortium including the Genetics Department, the IWBT, the Department of Molecular and Cellular Biology at the University of Cape Town, and the Biotechnology Department of the University of the Western Cape, have an interest in studying molecular interactions between grape and fungal pathogens using microarray technology.

2.3.6 USA

In the USA, grape genomics work commenced in the early 1990s. Several groups have developed molecular marker based maps in both *V. vinifera* and interspecific hybrid populations (Lodhi et al. 1995; Dalbo et al. 2000; Doucleff et al. 2004; Riaz et al. 2004). There are several labs involved with research on functional genomics of *V. vinifera*. The main focus of research at the University of Nevada, Reno (GR Cramer and JC Cushman) is to study the effect of abiotic stresses (e.g. cold, heat, salinity, drought) on grape. They have initiated an EST-based gene discovery program focused solely on stressed vines by constructing cDNA libraries from mRNA isolated from leaf, root, and berry tissues of *V. vinifera* cv. Chardonnay. The growing database of EST sequence information will allow large-scale gene expression profiling using microarray technology.

At the Department of Plant Pathology, University of California, Davis (DR Cook) another EST project is focused on identifying the transcriptional pathways correlated with susceptibility or resistance in *V. vinifera* to Pierce's disease (PD) and with genes involved in berry ripening (http://cgf.ucdavis.edu/). This database contains an analysis of all public ESTs from *Vitis*, and ESTs are grouped as contigs or singletons and analyzed for homology to the NCBI Non-Redundant (NR) database by means of BLASTX. All contigs and singleton ESTs were also analyzed for the presence of SSRs and 1000 EST-SSR markers were developed that are available to grape genetic research community. The GeneChip® *Vitis vinifera* Genome

Array developed by Affymetrix is the first commercially available array to provide comprehensive coverage of the *V. vinifera* genome. Convenient one-array views of 14,000 *V. vinifera* transcripts and 1,700 transcripts from other *Vitis* species can be examined (http://www.vitaceae.org/).

A collaborative research project between the USDA/ARS – Parlier, CA and the Department of Viticulture and Enology, University of California, Davis (H. Lin and M.A. Walker) is studying resistance to PD and developing new tools for grape improvement (Lin and Walker 2004). The goal of this project is to characterize the molecular events in grape/*Xylella fastidiosa* interaction and develop a functional genomic approach to specifically identify the PD-related gene expression profiles from susceptible and resistant responses. About 5000 expressed genes have been sequenced from PD resistant and susceptible grape plants. These expression profiles derived from stem, leaf and shoot of resistant and susceptible genotypes throughout the course of disease development will provide informative details of molecular basis of PD responses.

Lin et al. (2005) used a cDNA-AFLP technique to analyze the gene expression profile of PD infected grapevines. In this study, they compared gene expression of highly susceptible and resistant siblings selected from a segregating population of *V. rupestris* × *V. arizonica*. Comparing the profiles of resistant and susceptible genotypes identified fragments representing up- and down-regulated genes. About 100 differentially expressed cDNA-AFLP fragments were collected, sequenced and annotated. These fragments reflect the differentially expressed genes from various tissues at different stages of PD development. To further study the genes involved in the host-pathogen interaction at different stages of disease development, a Taq-Man gene expression assay was developed to analyze selected genes for their spatial and temporal expression in response to PD infection. This study will help identify genes involved in the defense response and signaling/recognition cascade in PD susceptible genotypes.

2.4 Marker-Aided Selection and Breeding

In the last 15 years, the development of molecular markers has stimulated advances in breeding, since these markers directly reveal genetic variability through DNA analysis (Staub et al. 1996), and environmental effects do not influence their detection. The primary use of these molecular markers is in marker-assisted selection (MAS) (Paterson et al. 1991). The main objective of crop breeding is to obtain new cultivars exhibiting better yield, quality traits, and resistance to biotic and abiotic stresses. In many cases, these useful traits come from wild and distantly related species. The traditional approach is based on interspecific hybridization to transfer genes from wild to cultivated species, followed by selection of hybrids that combine the "new wild" trait with the cultivated genetic background. This breeding strategy is primarily achieved by generational backcrossing in which the selected hybrids at each generation are crossed back to the cultivated genotype (although the cultivated genotype may vary in grape to avoid inbreeding depression) with the aim of reducing the wild genome and its undesirable traits.

Marker-aided selection is one of the most efficient applications of biotechnology to plant breeding. It is an effective and efficient breeding tool for detecting, tracking, retaining, combining, and pyramiding disease resistance genes in crop species (Kelly and Miklas 1998, 1999). The essential requirements for MAS in a plant breeding program are: 1) the marker(s) should be closely linked (1 cM or less is probably sufficient for MAS) with the desired trait; 2) an efficient means of screening large populations for the molecular marker(s) is key; 3) the screening technique should have high reproducibility across laboratories, be economical to use and user-friendly. Molecular markers closely linked to the gene controlling the trait to be transferred allow precocious screening on the DNA extracted from young leaves without waiting for the specific developmental stage at which the trait is expressed. This precocious screening results in large savings in time and space, and becomes far more valuable when multiple traits and many progeny are under testing. Choosing the most suitable markers for MAS depends on the ease of their detection, the possibility of revealing single or multiple loci, their dominant or co-dominant nature, and their expense. The most widely used markers for MAS are RFLP, RAPD, AFLP, and SSR. Their polymorphic nature is based on point mutations or chromosome rearrangements that accumulate during the evolution of the species without negatively influencing survival and reproduction. The recent surge of development of grape genetic linkage maps with molecular markers has the potential to greatly expand use of MAS in grape breeding pro-

grams. However, until additional work is completed that tags phenotypic traits with molecular markers, progress will be limited.

The first published effort to utilize MAS with grape was tagging QTLs for powdery mildew and black rot resistance with RAPD and AFLP markers (Dalbo et al. 2001). As mentioned above this effort utilized a segregating population from a cross of Horizon × Illinois 547-1. A major QTL (LOD 6.6) was found for powdery mildew resistance in the Illinois 547-1 (resistant parent) map and two other QTLs with a smaller effect were found in the Horizon map. When black rot resistance was mapped, four QTLs were detected, two in each parent. The three most important QTLs were located in the same linkage groups as the ones for powdery mildew. One was also associated with a QTL for production of the phytoalexin resveratrol. Two markers (a RAPD and an AFLP) linked to this QTL were obtained by BSA and then converted into CAPS markers for testing in four different crosses. In all cases, the markers were strongly associated with resistance to powdery mildew. A similar approach was used to find markers that are tightly linked to the powdery mildew resistance gene *Run1* in a BC_5population with AFLP markers (Pauquet et al. 2001).

In another study, Lahogue et al. (1998) used BSA to identify two RAPD markers tightly linked to the seedlessness gene *SdI*, a dominant gene that controls three recessive complementary genes for seedlessness in the Sultanina (Thompson Seedless in California, and Sultana in Australia) grape. The closest marker was used to develop a co-dominant SCAR named SCC8. This latter marker was used to exclude seeded progeny (scc8-scc8-) and to select seedless individuals (SCC8+SCC8+). The SCC8 marker accounted for at least 65% of the phenotypic variation of the seed fresh weight, and for at least 79% of the phenotypic variation of the seed dry matter. SCC8 was further checked by Adam-Blondon et al. (2001) in a grape germplasm collection and in other seedling populations and found out that seeded individuals can be heterozygous at SSC8 marker. This observation indicated that more understanding of the genetic determination of stenospermocarpic seedlessness is required to allow use of molecular markers for efficient MAS for seedlessness.

Mejía and Hinrichsen (2003) also chose the BSA approach with RAPD markers to identify markers linked to seedlessness. They studied a Ruby (Ruby Seedless) × Sultanina population for different stenospermocarpy sub-traits. Of the 336 RAPD primers tested, six fragments were seedless-specific and one was related to the seeded phenotype. A RAPD fragment named WF27-2000 was cloned and sequenced, and then converted into a SCAR marker. This SCAR, designated SCF27, generated a specific amplicon of 2.0 kb that was present in all of the seedless individuals, and segregated 3:1 in the population suggesting both parents were heterozygous for this locus.

2.5 Cultivar Identity

Ampelography is the traditional method of identifying grape cultivars based on morphological differences of the foliage and fruit. It is accurate and reliable, but requires years of training and practice, and few individuals are sufficiently skilled. Ampelography is also influenced by environmental conditions, which can alter leaf and cluster size and influence characters such as the degree of tomentum, vine vigor and shoot tip coloration. In addition, the most reliable leaves for identification are formed in the mid-shoot region and they may not be available for observation or shipment. In the case of rootstocks, once they are grafted they rarely form shoots from below the union. Finally, there are many thousands of cultivars in germplasm collections around the world and few have been described in readily accessible forms, and ampelographers tend to be experts on cultivars grown within the region they reside. Thus, alternative identification methods based on tissue DNA have been developed to overcome these limitations and produce DNA fingerprints of grape cultivars.

Molecular identification efforts began with the use of isozymes (Stavrakakis and Loukas 1983; Benin et al. 1988; Calo et al. 1989; Walker and Liu 1995), but this system had limitations. The primary disadvantage was that expression of certain enzymes was dependent on developmental and environmental influences, which restricted the number of available markers and the consistency of their polymorphisms.

In the early 1990s, it became possible to analyze grape DNA. The main advantage of the techniques that were developed was that DNA could be obtained from all plant material, in any environment and at any time of year. Restriction fragment length polymorphism (RFLP) analysis was used successfully to detect cultivar specific DNA fingerprints for grapevine and rootstock varieties (Bourquin et al. 1991, 1992, 1993,

1995; Thomas and Scott 1993). However, the RFLP fingerprinting method was limited by the nature of their complex banding patterns, low level of polymorphism in the coding regions of the genome, and time consuming and costly development of probes for analysis. With the advent of PCR technology, RAPD and later AFLP systems became popular in efforts to fingerprint grape cultivars (Jean-Jacques et al. 1993; Moreno et al. 1995; Xu and Bakalinsky 1996; This et al. 1997; Hinrichsen et al. 2000). Finally, the development of co-dominant SSR markers surpassed all other marker systems. The establishment and development of SSR markers was expensive and time consuming because of the construction and screening of the required genomic libraries, prior to design and optimization of PCR primers. However reproducibility, standardization, and transfer and comparison of data among different labs made SSR markers the choice for fingerprinting and cultivar identification.

Thomas and Scott (1993) were the first to report on the use of SSR markers to identify grape cultivars. Their work demonstrated that SSR sequences are abundant in the grape genome and primer sequences are conserved among *Vitis* species and other genera in Vitaceae. These results generated immense interest in grapevine SSR markers, leading to the development of many more markers (Bowers et al. 1996, 1999b; Sefc et al. 1999) culminating in the development of the Vitis Microsatellite Consortium (VMC) consisting of 21 different grape research groups from 12 countries. The VMC effort resulted in development of 333 new markers from SSR enriched genomic libraries. Most of these markers were later used to develop genetic linkage maps (Doligez et al. 2002; Adam-Blondon et al. 2004; Riaz et al. 2004).

Many studies made use of SSR markers to fingerprint and genotype wine, table, raisin grape and rootstock cultivars (Thomas and Scott 1993; Cipriani et al. 1994; Thomas et al. 1994; Botta et al. 1995; Bowers et al. 1996; Sefc et al. 1998a, 1998b, 1998c, 1998d, 1999; Grando and Frisinghelli 1998; Lin and Walker 1998; Bowers et al. 1999; Lefort et al. 2000; Sefc et al. 2000). SSR-based fingerprinting has been used to correct synonyms (Cipriani et al. 1994; Bowers et al. 1996; Sefc et al. 1998a; Lopes et al. 1999; Lefort et al. 2000), detect clonal polymorphism (Riaz et al. 2002), and construct pedigrees for old grape cultivars (Bowers and Meredith 1997; Sefc et al. 1998; Bowers et al. 1999a; Lopes et al. 1999; Meredith et al. 1999; Vouillamoz et al. 2004). There are three public databases that provide information of grapevine genetic fingerprint with SSR markers: the grape microsatellite collection (GMC) database (http://relay.ismaa.it:12164/genetica/gmc.html) was developed to permit an easy retrieval of grape nuclear microsatellite profiles and related information, the Greek Vitis database (http://www.biology.uch.gr/gvd/) contains nuclear as well as chloroplast SSR profiles of Greek grapevine cultivars, rootstocks, *Vitis* species and hybrids used as rootstocks.

2.6 Conclusions and Future Prospects

To date grape improvement has been based on classical breeding and the incorporation of advances in viticulture and enology to optimize vine growth and wine production. However, we are now poised to make rapid advances in grape improvement through the utilization of molecular genetic tools. The development of genomic technologies and their application in other crops like *Arabidopsis* and rice is now providing the necessary tools and comparative information for grape biologists to begin understanding the genetic and molecular basis of pest resistance, tolerance to abiotic stresses, and fruit ripening and quality. The potential of grape genomic research has been recognized by both the public and private sector in many countries of the European Union, Australia, the USA, South Africa, and many other grape growing countries. The coordinated efforts of the Vitis Microsatellite Consortium have generated a large set of SSR markers, which continues to be expanded, refined and utilized. Research groups in many countries are involved in developing genetic linkage maps focused on resistance and tolerance to biotic and abiotic stress and fruit and vine quality. These efforts have resulted in two international reference genetic maps based on SSR markers, and efforts are underway to develop consensus map utilizing populations of different backgrounds. Coordination of these maps will greatly aid researchers to identify set of markers linked to traits of interests (single major genes and QTLs) for use in MAS breeding programs and gene identification efforts.

The next phase of genetic research will be the initiation of the grape genome project to identify key grapevine genes and understand their functions. Grape researchers in Europe, Australia, Canada and

the USA have begun this effort with public and private sector funding. Most of these projects are combining a number of technologies including large-scale EST analysis, BAC libraries, physical map construction, and functional analysis of grape genes in *Arabidopsis*. The development of EST libraries will greatly aid the characterization of genes and allow researchers to study gene expression profiles. Finally, sequencing of the grape genome would be a quantum leap for the grape research community and is critical for the realization of molecular genetics potential on grape and wine production.

2.7 Grape Research Resources on the Web

1. The American Vineyard Foundation (AVF): (http://www.avf.org/).
2. Bioinformatics.Org: (http://bioinformatics.org/). Bioinformatics.Org is a non-profit, academe-based organization committed to opening access to bioinformatics research projects, providing Open Source software for bioinformatics by hosting its development, and keeping biological information freely available.
3. Grapevine Genomics at the Centre for Plant Conservation Genetics: (http://bioinformatics.org/). Grapevine Genome database is a result of a large-scale sequencing project carried out at the Centre for Plant Conservation Genetics.
4. The Cooperative Research Centre for Viticulture (CRCV): (http://www.crcv.com.au/). The Cooperative Research Centre for Viticulture is a joint venture between Australia's viticulture industry and leading research and education organizations
5. Grapevine Breeding and Genetics Program: (http://www.nysaes.cornell.edu/hort/faculty/reisch/grapeinfo.html)
6. CSIRO Plant Industry, Australia - Research Programs: (http://www.csiro.au/). CSIRO applies strategic research in the plant sciences to promote profitable and sustainable agri-food, fiber and horticultural industries, develop novel plant products and improve natural resource management.
7. French Institute for Agrononomical Research: (http://www.inra.fr/gap/departement/especes/vigne.htm). INRA (Institut National de Recherche Agronomique) (*site is in French*)
8. Grapevine Biotechnology at the Institute for Wine Biotechnology (IWBT) (http://academic.sun.ac.za/wine_biotechnology/research_programmes.htm): University of Stellenbosch, South Africa – The IWBT is a member of the "Vitis Microsatellite Consortium" consisting of 20 laboratories world-wide to develop genetic markers, primers and probes for the genetic fingerprinting of *Vitis vinifera* varieties.
9. International Grape Genomics Initiative (http://grapegenomics.ucdavis.edu) – The site (utilizing frames) provides information in the categories: Meetings and Conferences, Grape Experts, Grape Websites, and the Phone Book.
10. Institute for Grapevine Breeding, Geilweilerhof, Germany (http://www.bafz.de/baz99_e/baz_orte/sdg/irz/irz_frmd.htm): The institute's research concentrates on: Development of disease-resistant grapevine varieties in consideration of the wide diversity of varieties in German viticulture; Selection methods to assess characteristics such as resistance to noxious agents, resistance to stress factors (e.g. drought, frost), and the flavor and taste-determining aroma components.
11. International Grape Genome Program (http://www.vitaceae.org/): The primary research focus is grapevine genomics carried out within the framework of the International Grape Genome Program (IGGP).
12. National Clonal Germplasm Repository for Fruit and Nut Crops at Davis, California (http://www.ars-grin.gov/ars/PacWest/Davis/): is one of over two dozen facilities in the National Plant Germplasm System (NPGS) which collect, maintain, characterize, document and distribute plant germplasm from all over the world.
13. Pomology & Viticulture Program at the University of Udine, Italy (http://www.dpvta.uniud.it/arb/Arb_ric.htm#grape): The grape research group manages a grape germplasm repository, which includes wild species, international and local cultivars and breeding lines carrying disease resistance genes.
14. The Institute for Genomic Research (TIGR) (http://www.tigr.org/): The TIGR databases are a collection of curated databases containing DNA and protein sequence, gene expression, cellular role, protein family, and taxonomic data for microbes, plants and humans.
15. Vitis Gene Discovery Program: A Mission to Explore the Genetic Resources of Native North

American Grape Species. (http://mtngrv.smsu.edu/vgdp/). Wild grapes (*Vitis* species) are able to thrive in harsh environments and under high disease and pest pressure conditions. They are natural sources for genes that confer tolerance to adverse biotic and environmental conditions.

2.8 Databases

1. EST Database of Grape from Genomics Facility, College of Agricultural and Environmental Sciences, University of California, Davis. - This database contains an analysis of all public expressed sequence tags (ESTs) from grape. ESTs are grouped as contigs or singletons and analyzed for homology to the NCBI Non-Redundant (NR) database by means of BLASTX.
2. European Network for Grapevine Genetic Resources Conservation and Characterization (http://www.genres.de/vitis/vitis.htm): The database is collection-oriented, i.e. the same cultivar/variety appears in the database as many times as there are participating collections containing it. Data (IPGRI passport data, primary and secondary descriptor data) refer to an individual accession (cultivar) only.
3. Grape Microsatellite Collection (GMC) – A web-backed database of genotypes at SSR loci obtained from IASMA analysis and literature. GMC is a database developed to permit an easy retrieval of grape nuclear microsatellite profiles and related information. Each record has 8 fields: locus (name of the locus), allele 1 and allele 2 (allele size in bp), cultivar (name of the accession) and finally 3 fields providing information about authors, references and fragment analysis method of collected data.
4. Grapevine Genome Database (http://www.scu.edu.au/research/cpcg/genomics/index.php): The Grapevine Genome database is a result of a large-scale sequencing project carried out at the Centre for Plant Conservation Genetics. A number of objectives were achieved including the development of SSR markers from grape ESTs, micropropagation of table and wine grape varieties and an analysis of the grape genome based on 5000 EST sequences.
5. The Greek Vitis Database (http://www.biology.uch.gr/gvd/contents/index.htm): A multimedia web-backed genetic database for germplasm management of *Vitis* resources in Greece. By Francois Lefort and Kalliopi A. Roubelakis-Angelakis, Laboratory of Plant Physiology and Biotechnology, Department of Biology, University of Crete, Haralson, Crete, Greece.
6. TIGR *Grape* Gene Index (VvGI) (http://www.tigr.org/tigr-scripts/tgi/T_index.cgi?species=grape): The TIGR *Grape* Gene Index integrates research data from international *Grape* EST sequencing and gene research projects. The ultimate goal of the TIGR Gene Index projects, including VvGI, is to represent a non-redundant view of all *Grape* genes and data on their expression patterns, cellular roles, functions, and evolutionary relationships.
7. Vitis International Variety Catalogue (http://www.genres.de/idb/vitis/): All available information has been condensed for each cultivar/variety, i.e. each variety makes a single data set. Data (IPGRI passport data, bibliography, morphological and resistance characteristics

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