Chapter 5
Grape Breeding

Phyllis Burger, Alain Bouquet, and Michael J. Striem

5.1 Introduction

Grapevine is one of the most widely grown crops in the world and covers about 7,955 million hectares. In 2003 the world wine production was 2,667 million hectolitres with France, Italy and Spain the leading producer countries. Table grape world production was in the order of 174 million metric ton. China is the leading producer country, but the bulk of the Chinese crop is consumed locally and little of the Chinese table grape industry is known to the Western world. The largest exporter of table grapes is Chile, followed by Italy (OIV statistics for 2003, http://www.oiv.int/). It is generally believed that *Vitis vinifera*, originated in the Middle East and that cultivation of the grapevine began during the Neolithic era (6,000–5,000 BC) along the eastern shores of the Black Sea in the region known as Transcaucasia in the areas south of the Caspian and the Black Sea (Mullins et al. 1992). From its centre of origin, the grapevine traveled with man to other parts of the world and in ancient Egypt mention was made of grapevine growing and wine-making. The domesticated grapevine reached European and North African Mediterranean countries, first with the Phoenician and Greek trade routes and later with the Romans, who spread it through their Empire. From Europe, the domesticated grape spread globally with settlers to the more temperate climates in the Americas, South Africa, Australia and New Zealand. *V. vinifera* grows in temperate climates and flourishes in Mediterranean climates, can be cultivated in tropical climates and with careful mangement and pruning may produce two crops a year (Possingham et al. 1990).

Of the 17 genera belonging to the family *Vitaceae*, that developed its own identity inside the Eudicots at the end of the Cretaceous period about 100 million years ago (Ingrouille et al. 2002), it is only the genus *Vitis* that produces edible fruits. Planchon (1887) divided the *Vitis* species between two sub-genera namely *Euvitis* (bunch grapes) and *Muscadinia* (muscadine grapes). *Muscadinia* has a genome of $2n = 40$,

P. Burger (✉)
ARC Infruitec-Nietvoorbij, Private Bag X 5026, Stellenbosch, 7599, South Africa
e-mail: burgerp@arc.agric.za

S.M. Jain, P.M. Priyadarshan (eds.), *Breeding Plantation Tree Crops: Tropical Species*,

similar to the one of several other genera of *Vitaceae* (*Ampelopsis*, *Ampelocissus*, *Parthenocissus*), while for *Euvitis* it is $2n = 38$. Though some researchers (Olmo 1986; Mullins et al. 1992) argued that *Muscadinia* should be elevated to generic level as is supported by morphological, anatomical and karyological characteristics, it has not achieved general acceptance. *Muscadinia* is endemic to the southeastern states of the USA and among the three species known, only *Vitis rotundifolia* Michx. is of commercial value (Olien 1990).

Great genetic diversity is found in grapevines and they are adapted to different soils and climates. The majority of the table and wine grapes grown commercially are from *V. vinifera* origin. In the USA, some other *Vitis* spp. are grown commercially and were of local economic importance (Hedrick 1908). A number of American *Vitis* spp. were used in the past and are still being used in breeding programmes, especially in developing phylloxera resistant rootstocks, but also disease resistant wine and table grape cultivars. Of these, the following are of highest importance: *V. labrusca, V. aestivalis* and *V. rupestris Vitis* spp. native to tropical America, like *V. smalliana, V. caribaea* and *V. shuttleworthii* are included in breeding programmes to develop cultivars adapted to climatic conditions of the tropics (Camargo, 2000). In the Eastern European and Asian countries, *V. amurensis* is often included in breeding programmes to introduce cold hardiness and disease resistance.

Man's interest in grape growing evolved by selection from wild vines bearing fruit with desirable eating or other superior characteristics. The origins of many of the well-known wine and some table grape cultivars are still shrouded in mystery. They probably originated from chance seedlings or as sports of old cultivars and were easily propagated by cuttings and grown for centuries. Though the use of deliberate crosses was likely since the Middle Ages (Boursiquot et al. 2004), it is only since the late nineteenth century that such crosses are well-known. Some important wine grape cultivars were developed from these crosses (e.g. Alicante Bouschet in France that was bred by Louis and Henri Bouschet between 1829 to 1855 (Snyder 1937), Müller-Thurgau in Germany that was bred by Müller-Thurgau in 1882 (Dettweiler et al. 2000) and Pinotage in South Africa that was bred by Perold in 1925 (Orffer 1979)). Although grapes for wine-making were grown from antiquity, table grape production only became an important enterprise by the end of the nineteenth century (Einset and Pratt 1975).

5.2 Flower Types and Flower Structure

Oberle (1938) published a comprehensive study on flower types and the inheritance of floral morphology. He recognized three flower types: functionally hermaphroditic, functionally pistillate and functionally staminate and found that only one type of flower normally occurred on any one individual. In staminate types, which are functionally male, the pistil is incompletely developed and non-functional, while functionally pistillate or female types have non-functional, reflexed stamens. Perfect or hermaphrodite types have flowers in which both the stamens and pistils are functional. American species bear male and female

flowers on separate vines, while most European grape cultivars of *V. vinifera* origin bear hermaphrodite flowers (Snyder 1937; Olmo 1943). Various breeders postulated different hypotheses for the inheritance of flower type (Einset and Pratt 1975).

In perfect flowers, the pistil is normally surrounded by five stamens, although the number may vary from five to more on individual flowers of the same flower cluster. The fused corolla encloses the above-mentioned flower parts. At flowering it becomes loosened at the base and comes off like a cap (Snyder 1937). After fertilisation has taken place, the ovary develops into a seed in seeded cultivars or rudimentary seed in most of the 'seedless' types. For a review on anatomy and development of the reproductive organs of the grapevine, see Pratt (1971).

5.3 Flowering and Pollination

Grapevine is either self-pollinated or pollinated by wind, but honeybees and some other insects may also be active pollinators (Bronner and Wagner 1997). Olmo (1943) found honeybees to play an active role (at least in some seasons) in the cross-pollination of the table grape Almeria with reflexed stamens. Kimura et al. (1998) showed that insects contributed more than wind in the pollination of the dioecious *V. coignetiae*.

Not all the flowers on a bunch open simultaneously and some of the varieties are cleistogamous (self-pollination takes place before opening of flowers). The percentage of flowers in which cleistogamy occurred varied greatly among cultivars and appeared to be a genetic trait (Barbagallo et al. 1988; Staudt 1999). Heazlewood and Wilson (2004) also found that anthesis occurred before cap fall and found pollen to be viable, although pollen tube growth only started after cap fall. Furthermore, pollen remained viable for several days after cap fall. Bronner and Wagner (1997) found pollination to be highly influenced by temperature, relative humidity and stigma receptivity. Breeders may find the work of Staudt (1999) on flowering cycles, on the influence of temperature on pollen germination and the tempo of pollen tube growth (Staudt 1982) helpful in deciding on the best time of day for cross-pollination. Some fungicides and other chemicals sprayed in the vineyard against *Botrytis* may have an effect on pollen germination and pollen tube growth (Heazlewood et al. 2005).

5.4 Controlled Cross Breeding

Grape breeding is based primarily on hybridisation. Self-pollination as breeding method has been poorly worked out and most of the attempts were unsuccessful due to strong inbreeding depression. However, Pospisilova (1974) crossed inbred generations (I_1) of the *V. vinifera* cultivars Traminer and Veltliner and obtained higher productivity in the progeny compared to the cross between these two cultivars.

Bronner and Oliveira (1990) isolated from the sixth self-pollinated generation of Pinot noir, a homogeneous progeny, which they considered as a 'quasi pure line' usable for genetic studies. Finally, Todorov (2000) is the only breeder who obtained a commercial table grape cultivar (Velika) by self-pollinating a genotype selected in the progeny of a Bolgar x Ribier cross.

5.4.1 Emasculation and Pollination

Since the flowers of most of the commercial grape cultivars are hermaphroditic, it is necessary for breeders to emasculate flowers before self-pollination takes place. Emasculation is done by removing the cap and stamens, usually with a fine-point forceps (Fig. 5.1). Emasculated clusters are pollinated by hand and covered with paper (or other suitable) bags to prevent contamination by 'foreign' pollen. Some breeders use a small brush and dust stigmas with pollen (Snyder 1937), while others simply put a flowering cluster in the paper bag with the emasculated cluster and shake the bag or simply tap a flowering cluster of the chosen pollen parent against the emasculated cluster; Barrett and Arisumi (1952) described a home-made 'pollen atomizer' or 'duster' used for pollination.

5.4.2 Pollen Collection and Storage

Pollen can be collected in bags in the vineyard by covering clusters before anthesis and removing these after flowering or clusters may be harvested and dried indoors. In the latter case, clusters are collected when the first flowers start opening, taken indoors and stripped from the cluster and spread in a thin layer on glass sheets. These are placed in a warm, dry area free of air currents where pollen is left to dry for approximately 24 h. Dried flowers are scraped off the glass, rubbed through a sieve and pollen collected by scraping it off the glass plate into a suitable container (Barrett and Arisumi 1952). Equipment and hands need to be cleaned with alcohol to prevent contamination when working with more than one cultivar (Barrett and Arisumi 1952). Dried pollen are stored in a desiccator at −16 °C with silica gel (Bronner and Wagner 1997). These authors also found that germination percentages of pollen exposed to UV radiation rapidly decreased.

Breeders usually prefer to use pollen as soon as they are collected or within the same season, but under optimal conditions it may be stored for a limited number of years. It is advisable to do germination tests before using stored pollen. Olmo (1942) reported that pollen showing a germination percentage of as low as 6% gave as good a set in the field as fresh pollen. Bronner and Wagner (1997) found optimum temperatures for germination to be between 22 and 26 °C and added 20% sucrose to the medium as well as boric acid (5 mg/l) and agar (20 g/l). Agarwal (1983) used various organic solvents to store pollen at 4–6 °C. Ganeshan and Alexander (1990)

Fig. 5.1 Grape flowers (*top*) and emasculation of clusters (*below*)

obtained viable progeny when pollen stored for up to 64 weeks in liquid nitrogen was used in crosses, but seed yield was reduced after 5 years in storage.

5.4.3 Seed Germination

Grapevine seeds are dormant and therefore are stratified in moistness under controlled temperatures (usually around 4 °C) for 2–3 months, followed by germination at around 25 °C (see Einset and Pratt 1975 for references).

Researchers have investigated the use of chemicals to aid in seed germination. Spiegel-Roy et al. (1987) reported the efficiency of cyanamide in overcoming dormancy. Manivel and Weaver (1974) used various treatments and only gibberellic acid was partially effective. Ellis et al. (1983) proposed the following procedure for grapevine seed germination: a 24 h soak in H_2O_2 (0,5M) a further 24 h soak in 1,000 ppm GA_3, followed by a 21 day pre-chill at 3–5 °C with germination in a diurnal alternating temperature regime of 20–30 °C (16/8 h).

5.5 Breeding for Specific Characteristics

Characteristics most desirable vary depending on whether grapes are produced for winemaking, juice, fresh consumption, raisin production or for use as rootstocks for grape propagation. For table grapes, preferences differ between markets and countries. In general, these include seedlessness, large natural berry size, good eating quality and other unique characteristics. In some grape growing countries, cold hardiness is very important and for table grape producers in countries far from the world-markets, cold storage is extremely important. With high costs incurred to protect the crop from major fungal diseases and a higher awareness of environmental issues amongst consumers, the production of disease resistant cultivars that would also comply to the other criteria for table, wine and raisin grapes are very important.

5.5.1 Fruit Characteristics

5.5.1.1 Seedlessness

Worldwide, seedless table grapes are high in demand and, therefore, many breeders focus much of their efforts on the creation of new seedless cultivars. From a consumer's perspective, seedless grapes would be those with undetectable rudimentary seeds (seed traces), while from a botanical viewpoint only parthenocarpic cultivars would be truly seedless. Breeders regard seedlessness to be of two types. Seeds are never formed in the parthenocarpic cultivars like Black Corinth, but rudimentary seeds (which may contain viable embryos) develop in the stenospermocarpic cultivars like Sultanina and are the result of embryo and/or endosperm abortion. When parthenocarpic cultivars were used as pollinators of various seeded cultivars no seedless progeny was obtained (Stout 1937), while seedless progeny could be obtained when stenospermocarpic cultivars were used to pollinate seeded cultivars (Stout 1937; Weinberger and Harmon, 1964). It is thus clear that breeders would focus on the stenospermocarpic cultivars.

Rudimentary seeds vary in size, and lignification and classification of offspring into seedless and seeded individuals varied greatly amongst researchers. Some based their classification on detectability of the rudimentary seed (Loomis and Weinberger 1979; Spiegel-Roy et al. 1990a). Ledbetter et al. (1994) used sinker frequency, relative seed mass (sinker frequency multiplied by average sinker mass) and seed ratio (total seed weight divided by total fruit weight) as parameters. Ramming et al. (1990a) viewed 25 mg fresh weight as the division between seeded and seedless genotypes, but placed the maximum seed trace size for consumer acceptance around 10 mg fresh weight. Striem et al. (1992) used four categories for seed/seed trace size and regarded hardness of the seedcoat and the degree of endosperm development as sub-traits of seedlessness. Furthermore, the perceptibility of the seed traces was not necessarily correlated to size, but rather to the hardness of the seed-coat. To complicate matters, rudimentary seed size may differ from year to year and also seem to be influenced by vine age and rootstock (Christensen et al. 1983).

The seeded:seedless ratios in progeny of crosses between seeded and stenospermocarpic seedless cultivars varied greatly and, thus, led to various hypotheses to explain the inheritance of the stenospermocarpic trait. Weinberger and Harmon (1964) and Loomis and Weinberger (1979) regarded seedlessness to be controlled by complex recessive genes. Ramming et al. (1990a) supported this view and concluded the seedless trait not to be controlled by a single recessive gene, since all seedless × seedless crosses had some seeded offspring. Spiegel-Roy et al. (1990a) postulated two complementary recessive genes. The hypothesis of Bouquet and Danglot (1996) stated that inheritance of seedlessness was based on a complex system whereby the expression of three independently inherited recessive genes was controlled by a dominant regulator gene.

5.5.1.2 Other Fruit Characteristics

The heritability of a number of quantitative traits has been studied, including berry characteristics and ripening (Fanizza and Raddi 1973; Firoozabady and Olmo 1987; Eibach 1990; Wei et al. 2002).

Some breeders investigated the inheritance of berry skin colour, a relevant trait influencing both table grape and wine making quality. Barritt and Einset (1969) proposed two pairs of genes for fruit colour inheritance. A gene for black (blue) colour (*B*---) dominant, epistatic to that for red and white fruit and with red fruit (*bbR*-) dominant to white (*bbrr*). Recently Kobayashi et al. (2004) indicated that variation for berry skin colour is associated with the presence of a retrotransposon in the promoter region of a transcription factor that is involved in skin colouration and probably corresponds to the gene B.

Researchers also reported on the inheritance of aromas, mostly the *V. vinifera* muscat flavour. Wagner (1967) proposed that three to five complementary genes and a modifying gene were involved. Eibach et al. (2003) identified three aroma terpene compounds – namely Hotrienol, Trans-p-Linalooloxide and Terpendiol II – in offspring from a cross between a muscat and non-muscat parent. With regards to the *V. labrusca* (foxy) flavour, Reynolds et al. (1982) proposed a three-gene, dominant and complementary system for methyl anthranilate (MA) and a two-gene system for total volatile esters (TVE). Fisher et al. (1990) confirmed the previous hypothesis for TVE, but suggested a more complex environmentally influenced system for MA.

5.5.2 Resistance to Pests and Diseases

V. vinifera evolved in mild climates and without the presence of the economically most damaging pathogens, like the downy mildew (*Plasmopara viticola*), powdery mildew (*Uncinula necator* Syn. *Oidium tuckeri* and *Erysiphe necator*) and insects like phylloxera (*Daktulosphaira vitifoliae*). The *vinifera* grapevine is often very susceptible to these biological factors as it became clear when, for example, it was exposed to these stress factors in the USA. By the end of the nineteenth century, pests and diseases introduced from North America, caused havoc in the European

grapevine industry. Powdery mildew appeared in 1845 in France and by 1851 it had spread to all the vineyards in Europe. Phylloxera found its way to Europe shortly hereafter in 1865 and was followed by downy mildew (1878) and black rot (*Guigardia bidwellii*) in 1885 (Krul and Mowbray 1984).

5.5.2.1 The Use of Euvitis Species in Resistance Breeding

The introduction of American species resistant to phylloxera (mainly *V. riparia*, *V. rupestris* and *V. berlandieri*) and intensive breeding programmes saved grapevine production in many regions of the world (Pouget 1990). Among the resistant species, only *V. berlandieri* was adapted to the highly calcareous soils, but could not be used in its pure form and hybridisation was necessary to develop rootstocks resistant to lime-induced chlorosis. Rootstocks were also bred for resistance against nematodes like *Meloidogyne* spp. (Cousins et al. 2003) and *Xiphinema* spp. (Meredith et al. 1982).

Although chemical sprays (sulphur and copper) were rapidly effective against the fungal diseases, these are still difficult to control and costs are high. Very early, numerous European breeders made crosses between *V. vinifera* and resistant American *Vitis* spp. like *V. rupestris, V. labrusca, V. riparia* and *V. aestivalis* to combine their resistance with the fruit quality of *V. vinifera*. Although hundreds of so-called 'direct producer hybrids' were introduced to the French wine industry during the first half of the twentieth century, they are not of commercial importance today, but some of them (Villard blanc, Chambourcin, Seyval) were intensively used in modern breeding programmes for disease resistance (Kozma 2000; Eibach and Töpfer 2003). Resistance to powdery mildew was also found in the wild Chinese species *V. bryoniifolia, V. davidii* and *V. piasezkii* (Wang et al. 1995) and to downy mildew in the Asiatic species *V. amurensis* (Korbuly 2000). Other fungal diseases apart from the mildews, that breeders endeavour to develop resistance against, include anthracnose (Mortensen 1981) and *Botrytis*, while resistance against bacterial diseases include Pierce's Disease (PD), of importance in the USA (Mortensen 1968; Krivanek et al. 2005).

In *Euvitis*, Boubals (1959) postulated resistance to downy mildew to be dependent on two genic systems: a single gene for the hypersensitive reaction at the time of infection and several genes for the inhibition of growth of the fungal mycelium. Boubals (1961) postulated resistance to powdery mildew to be dependent on a polygenic system. Li (1993) also viewed resistance to powdery mildew to be of a polygenic nature and found minor resistance genes in *V. vinifera*. Eibach (2000) found different genes to be responsible for resistance to downy and powdery mildew and that no marked linkage seemed to exist between those genes.

5.5.2.2 The Use of Muscadinia in Resistance Breeding

Muscadinia is native to the southeastern United States and is a useful source for genes of resistance to phylloxera, nematodes, PD and fungal diseases. Often, these

genes have a high degree of dominance (Olmo 1986). This was confirmed by Bouquet (1983) who found a high degree of resistance to phylloxera in *Muscadinia*. Bouquet (1981) found the muscadines resistant to *Xiphinema index* the vector of grapevine fanleaf virus (GFLV), but not to the virus itself and also confirmed a large degree of dominance of this resistance (Bouquet et al. 2000a). By back-crossing a resistant F_1 hybrid to the rootstock cultivar 140 Ruggeri, a new rootstock resistant to virus spread has been selected (Bouquet et al. 2004). In *Muscadinia*, the same author also identified a dominant gene called *Run* 1 that confers resistance to powdery mildew and which he introduced in advanced back-cross progenies with *V. vinifera* (Bouquet, 1986; Bouquet et al. 2000b). It appeared that genotypes carrying the *Run* 1 gene also showed partial resistance to downy mildew due to the *Rpv* 1 linked gene (Merdinoglu et al. 2003). Current strategies being developed in European countries aim at combining genes of resistance from *Muscadinia* and *Euvitis* (Kozma and Dula 2003).

5.5.2.3 Resistance to Abiotic Stress

In breeding programmes for some wine grapes and rootstocks, tolerance to abiotic stress factors is also important. Rootstocks of *V. rupestris* × *V. berlandieri* ancestry were found the most tolerant to drought in greenhouse conducted tests by Carbonneau (1985). Pouget (1980) bred a new rootstock cultivar highly resistant to iron chlorosis by inter-crossing rootstocks of *V. vinifera* × *V. berlandieri* ancestry. Further work, using inter-crossing rootstocks of *V. riparia, V. rupestris* and *V. berlandieri* ancestry led to another new cultivar well adapted to acid soils (Pouget and Ottenwaelter 1986).

Cold tolerance is found in *V. riparia, V. labrusca* and *V. amurensis* (Reisch and Pratt 1996) and was largely used in breeding programmes for wine grapes in the USA (Hemstad and Luby 2000) and Eastern Europe (Korbuly 2000). For discussion on resistance breeding against other fungi, bacteria, insects and abiotic stress factors, see Mullins et al. (1992) and Reisch and Pratt (1996).

5.6 Tissue Culture Techniques

The value of conventional methods in grapevine breeding is limited by long generation intervals, the highly heterozygous nature of the vine and inbreeding depression. Furthermore, few traits of viticultural importance are controlled by single genes with dominant alleles. Thus, researchers have embarked on investigating biotechnological tools in grapevine improvement. Tissue culture techniques are used for micro-propagation by shoot tip culture, production of virus-free clones, protoplast culture, embryo rescue, organogenesis, callus induction, somatic embryogenesis and genetic transformation.

5.6.1 Micro-propagation

In practice, micro-propagation is mostly applied for the production of virus-free plant material. The technology for micro-propagation of shoot tip culture is well established and the resulting plants appear to be genetically identical to the mother plant. According to Bouquet (1989) the role of tissue culture in commercial propagation is likely to be limited because standard propagation systems based on grafting are well established. However, this author identified five possible situations where tissue culture could be of benefit: (1) Rapid multiplication of newly bred or imported cultivars, rootstocks in particular, (2) propagation, maintenance and international exchange of virus-free plants, (3) maintenance of germplasm under slow-growth in vitro conditions, (4) application of thermotherapy to obtain virus-free plants and (5) testing in vitro for resistance to biotic and abiotic stress factors.

This chapter will not deal with in vitro multiplication and micro-grafting techniques and their use in the production of virus-free material, since reviews were published elsewhere (Krul and Mowbray 1984; Monette 1988; Gray and Meredith 1992; Reisch and Pratt 1996; Torregrosa et al. 2001; Bouquet and Torregrosa 2003). These publications also address other biotechnological techniques not discussed here or only touched on, like organogenesis, haploidy induction, etc.

5.6.2 Embryo Rescue

The development of embryo rescue techniques enabled breeders to develop offspring from two stenospermocarpic seedless parent cultivars. Although labour intensive, these techniques are applied routinely in the majority of table grape breeding programmes as the proportion of seedless progeny in such crosses are much higher than in conventional seeded × seedless crosses. Cain et al. (1983) reported the first successful embryo rescue of grapevine, followed soon after by Emershad and Ramming (1984) and Spiegel-Roy et al. (1985).

Genotypes, media composition, culture dates (days after pollination), effect of cold treatments and various interactions were studied in numerous publications. For instance, Gray et al. (1987) compared the effect of liquid medium to that of solid medium. In general, higher germination rates were obtained when rudimentary seeds were cultured at a later date, though some researchers found no or little correlation. Correlations between size of the rudimentary seed and the presence of viable embryos were found by Bouquet and Davis (1989) and Spiegel-Roy et al. (1990b). Although embryos are able to germinate directly from the rudimentary seeds, researchers found it necessary to dissect rudimentary seeds after two to three months in culture and remove the embryos for maximum recovery of plants (Cain et al. 1983; Ramming et al. 1990b). Gray et al. (1990) reported that embryos appeared to be dormant. Varying results were obtained when cold stratification and plant growth regulators were investigated to break dormancy of embryos (Emershad and Ramming 1984; Bouquet and Davis 1989; Gray et al. 1990). Seed

from early ripening seeded cultivars usually have low germination rates and embryo rescue techniques were successfully applied to develop seedlings from such cultivars (Ramming et al. 1990b; Goldy et al. 1989).

Since the seedless trait is only found in *Euvitis*, embryo rescue techniques were also employed to develop plants from crosses between *Euvitis* and *Muscadinia*. Although it is difficult to regenerate progeny from such crosses, Ramming et al. (2000) reported the first *V. vinifera* × *V. rotundifolia* seedless seedling. Some researchers investigated the application of chemicals in the vineyard to aid in embryo rescue. Kender and Remaily (1970) were able to produce viable seeds from seedless cultivars by applying ethephon. Bharathy et al. (2003) found pre-bloom and bloom sprays of BA beneficial for in vitro embryo and plant recovery, while Ponce et al. (2002) found putrescine to increase the number of embryos.

5.6.3 Screening for Resistance to Biotic Stress

Classical selection methods include screening of plants in vineyards or greenhouses. Aldwinckle (1978) found a correlation between field resistance and plants selected in greenhouses for powdery mildew resistance. However, Eibach (1994) found that powdery mildew infection on leaves and berries may vary considerably and that screening for resistance by using leaves was not necessarily applicable to berries. Leaf discs were also used to evaluate various accessions of *Vitis* spp. and hybrids for downy (Staudt and Kassemeyer 1995) and powdery mildew (Peros et al. 2006). Stein et al. (1985) found a good correlation between the development of powdery mildew on leaf discs and on vines in the field and the greenhouse. With downy mildew the correlation was not as good.

In vitro dual culture gives researchers the opportunity to study host/parasite interactions without the interference of environmental factors. Heintz et al. (1985) and Klempka et al. (1984) developed in vitro dual culture methods for powdery mildew and *V. vinifera* cultivars, while Sparapano et al. (2001) determined grapevine susceptibility to esca-associated fungi in micro-propagated shoots and callus. Following earlier studies, Bessis et al. (1992) suggested the use of phytotoxic polysaccharides, produced by *Botrytis* to select in vitro for resistance. However, Fanizza et al. (1995) found a low correlation between the response of in vitro assayed cultivars to *Botrytis* and bunch susceptibility under field conditions.

Mauro et al. (1988) found that the fungus *Eutypa lata*, which causes eutypa dieback (dead arm disease), produced toxic metabolites that induced typical symptoms on in vitro grapevines. Following this research, Soulie et al. (1993) developed in vitro tests to screen in vitro propagated plants and micro-cuttings of *V. vinifera* cv. Ugni blanc for tolerant clones. Jayasankar et al. (2000) selected plants resistant to anthracnose by exposing embryogenic masses of *V. vinifera* cv. Chardonnay to culture filtrate of the fungus.

Techniques to screen potential parent cultivars and seedling populations include phytoalexin production and the stilbene oligomers, ε-viniferin and α-viniferin,

which were found to be closely associated with resistance to downy mildew (Langcake 1981; Dercks and Creasy 1989) and also *Botrytis* (Sbaghi et al. 1995). However, Barlass et al. (1987) found the production of the phytoalexin precursor, resveratrol, highly sensitive to environmental changes and its usefulness for screening limited. Kortekamp and Zyprian (2003) found a correlation between peroxidase activity and resistance to downy mildew.

Vitis and *Muscadinia* spp. were grown in in vitro dual culture with phylloxera by Forneck et al. (1996) and Grzegorczyk and Walker (1998) to study grapevine/phylloxera interaction. Kellow et al. (2002) confirmed the value of in vitro screening for resistance and determination of phylloxera biotypes. Van Mieghem and Goussard (1987) induced reproduction of the nematode *Meloidogyne javanica* on in vitro Chenin blanc plants, while Franks et al. (2003) could clearly distinguish between resistant and susceptible cultivars when grown in vitro in dual culture with *M. javanica*. Bavaresco and Walker (1994) described techniques for the in vitro dual culture of grapevine and *X. index*.

5.6.4 Screening for Resistance to Abiotic Stress

In vitro techniques were developed by Bavaresco et al. (1993) and Netzer et al. (1991) to screen grapevines for tolerance to lime-induced chlorosis. Apart from lime-chlorosis, salt-tolerant rootstocks are also important as the grapevine is easily affected by salinity. Lebrun et al. (1985) used single cells for in vitro selection of tolerance in *V. rupestris*. Barlass and Skene (1981) and Troncoso et al. (1999) found in vitro techniques suitable for the selection of salt tolerance in various rootstocks. However, Skene and Barlass (1988) stressed the need for verification under field conditions. Some rootstocks are known to be susceptible to magnesium deficiency and Bouquet et al. (1990) investigated the possibility of in vitro selection.

5.6.5 Somatic Embryogenesis

Somatic embryogenesis is the process of embryo initiation and development from cells that are not the direct product of gametic fusion and is of great importance in the improvement of grapevines. Researchers have used these techniques for the development of in vitro screening systems for resistance to stress factors, virus elimination, germplasm conservation and introduction of foreign genes by genetic transformation. Various organs, for example, unfertilised ovaries, flower clusters, anthers, tendrils, petioles and leaves from greenhouse or in vitro cultured plants, were used as explants to initiate callus. Though the response varied greatly, embryogenic cultures were established for various grapevine cultivars and species. The first reports of somatic embryogenesis were made by Mullins and Srinivasan (1976) who obtained plants via somatic embryogenesis from unfertilised ovules of Cabernet Sauvignon (*V. vinifera*) and Hirabayashi et al. (1976) who employed anther culture to develop

shoots from *V. thunbergii*. Since these early reports, numerous studies reporting successful somatic embryogenesis or organogenesis and plantlet formation were published. Successful plantlet development via somatic embryogenesis was achieved in many *Vitis* spp. and inter-species hybrids. For a review see Martinelli and Gribaudo (2001), Bouquet and Torregrosa (2003) and also references mentioned under genetic transformation.

The most recent and spectacular application of somatic embryogenesis in grapevine was the separation of the chimaeric periclinal L_1 and L_2 layers in *V. vinifera* Pinot meunier and the obtention of plants with phenotypes different from the original plant (Franks et al. 2002). Pinot meunier has a L_1 cellular layer responsible for its characteristic hairiness and also carrying a mutation in a gene homologous of the *Arabidopsis GAI* (Gibberellin inhibitor) gene. The mutation of this gene gives the plant a dwarf phenotype in which all the tendrils are converted into inflorescences, capable of flowering and bearing fruit after a few months (Boss and Thomas 2002). These characteristics confer considerable interest in future grapevine genetics and breeding on this genotype.

5.6.6 Protoplast Culture

A number of researchers studied protoplast culture, but encountered difficulties to regenerate plants. The first report of success was from Reustle et al. (1994) who regenerated plants from protoplasts derived from embryogenic material of the hybrid cultivar Seyval blanc. Zhu et al. (1997) obtained plants through somatic embryogenesis from protoplasts of cv. Koshusanjaku (*V. vinifera*). See also Papadakis et al. (2001) for a review on protoplast technology in grapevine.

5.6.7 Somaclonal Variation

In the wine grape industry, where many of the most prominent cultivars are of ancient origin, somatic mutations have undoubtedly taken place over the long period of vegetative propagation as can be seen in the many clones of established cultivars that were selected and are cultivated today. This somatic heterogeneity provides sources of variation that could be exploited by researchers to increase variability in existing cultivars, thus thwarting genetic erosion induced by clonal and sanitary selection (Bouquet 1989) and select for specific traits, particularly for resistance to biotic and abiotic stress. Schneider et al. (1996) presented the first molecular evidence for somaclonal variation in grapevine. Three plants with altered phenotype derived from protoclones of Seyval blanc showed modified RAPD profiles. Popescu et al. (2002) confirmed somaclonal variation in anther-derived grapevines at the molecular level by using AFLP (Amplified Fragment Length Polymorphism) techniques. See Predieri (2001) for a review of somaclonal variations and in vitro induced mutagenesis and see also Torregrosa et al. (2001).

5.6.8 Genetic Transformation

It is not possible to introduce specific characteristics or desirable traits into an existing cultivar by hybridisation without destroying its originality. Therefore, the alternative approach of using direct gene transfer is very promising for grapevine improvement. Researchers need to be able to select transformants from non-transformants and to confirm whether stable introduction of a foreign gene into *Vitis* has taken place. Most commonly the *npt*II and *hpt* genes conferring antibiotic (kanamycin or hygromicin) resistance have been used as selectable marker genes, while the *uid*A gene conferring β-glucuronidase (GUS) expression and the *gfp* gene conferring green fluorescence were used to confirm transformation. However, the use of antibiotics in the selection process is not widely accepted by the public. Reustle et al. (2003) investigated the possibilities of using mannose instead of antibiotics in a selectable marker system. However, none of the regenerated plants was transgenic. These results were confirmed by Kiefer et al. (2004) who found mannose as well as xylose unsuitable for use in selection systems for transformation.

Though some researchers use efficient biolistic (micro-projectile bombardment) transformation systems (Vidal et al. 2003), the majority make use of the *Agrobacterium*-mediated transformation system. This technology is based on the ability of *Agrobacterium* to insert genes into plant cells during the infection process. Although *A. tumefaciens* causes crown gall in grapevine, disarmed strains that do not induce crown gall are used for genetic transformation. The most suitable plant material for co-cultivation with *Agrobacterium* is embryogenic cell lines, of which the quality and developmental stage have a strong effect on transformation efficiency. The first report of researchers who obtained transformed plants were from Mullins et al. (1990) with *V. rupestris* 'St. George'. Please see Perl and Eshdat (1998) and Bouquet et al. (2006) for techniques and protocols for genetic transformation in grapevine.

5.6.8.1 Rootstock Transformation

GFLV is transmitted by the nematode *X. index*. Dangerous chemicals are used for soil disinfection and, therefore, many researchers put their efforts into producing transgenic plants with coat protein mediated protection. Krastanova et al. (1995) obtained *V. rupestris* and 110 Richter plants transformed with the coat protein of GFLV, while Mauro et al. (1995) obtained 41B and SO_4 plants also transformed with GFLV-CP. From 18 independent transgenic grapevine lines established in a naturally infected vineyard, 3 did not show reaction to GFLV infection 3 years after planting (Vigne et al. 2003). Bouquet et al. (2003a) found that transgenes (*npt*II, *uid*A and GFLV-CP), introduced into 110 Richter and *V. rupestris* du Lot rootstocks and transmitted by hybridisation in *X. index* resistant rootstocks developed by conventional cross-breeding, expressed normally with a mendelian segregation in offspring.

Le Gall et al. (1994) transformed 110 Richter with the coat protein of Grapevine Chrome Mosaic Virus (GCMV-CP). Torregrosa and Bouquet (1997) co-inoculated in vitro grown plantlets of the rootstock Gravesac with a mixture of wild

A. rhizogenes and *A. tumefaciens* carrying plasmids containing GCMV-CP genes. Transformed hairy root cultures were initiated from excised root tips. Plant regeneration was not achieved, but the authors mentioned the possibility to graft in vitro transgenic roots to non-transformed shoot systems. Radian-Sade et al. (2000) cloned the gene encoding the coat protein of Grapevine Virus A (GVA-CP) and used this gene to transform the rootstock 41B and tobacco. Martinelli et al. (2002) reported stable transformation of *V. rupestris* with the movement protein of GVA.

Guillen et al. (1998) purified a NADPH-dependent aldehyde reductase from *Vigna radiata* that converts eutypine, the toxin that is involved in eutypa dieback, into non-toxic eutypinol. Grapevine (*V. vinifera*) cells transformed with the gene (*Vr-ERE*) encoding the eutypine-reducing enzyme showed in vitro resistance to the toxin. Transformed plants were established only for the rootstock cultivar 110R (Legrand et al. 2003) and were not affected by relatively high concentrations of eutypine, whereas growth of untransformed plants were highly inhibited.

5.6.8.2 Scion Transformation

Mauro et al. (1995) reported the first transformed plants of a scion cultivar (*V. vinifera* cv. Chardonnay) with a gene of agricultural value, namely the GFLV-CP gene, while Gambino et al. (2005) reported transformation of cv. Nebbiolo with the same gene. Scorza et al. (1996) reported that by combining particle bombardment of somatic embryos with *Agrobacterium* co-cultivation produced Thompson seedless plants transformed with the lytic peptide Shiva-1 or the tomato ringspot virus (TomRSV) coat protein genes.

Chitinase is one of the hydrolytic enzymes, which can degrade fungal cell wall components. Because of this characteristic, hydrolytic enzyme coding genes are very attractive for researchers in their efforts to improve disease resistance. Neo Muscat (*V. vinifera*) plants transformed with a rice chitinase gene showed enhanced disease resistance to powdery mildew and anthracnose (Yamamoto et al. 2000). Harst et al. (2000) obtained Riesling plants transformed by antifungal genes (glucanase and chitinase), while Bornhoff et al. (2005) obtained transgenic Seyval blanc plants carrying genes for chitinase and RIP (ribosome inactivating protein). Although the foreign DNA was stably integrated, there was no visible improvement of field resistance against downy and powdery mildew. Agüero et al. (2005) obtained Chardonnay and Thompson Seedless plants transformed with the pear polygalacturonase inhibiting protein (pPGIP) gene. Plants were evaluated for tolerance to PD and *Botrytis* and delay in the development of PD was observed in some transgenic lines with increased pPGIP activity. Chardonnay plants, transformed with an antimicrobial peptide gene by using biolistics, were evaluated for resistance to crown gall and powdery mildew (Vidal et al. 2006).

Mezetti et al. (2002) used a novel approach based on organogenesis to introduce the gene *DefH9-iaaM* that conferred parthenocarpic fruit in tomato, eggplant, strawberry and raspberry in grapevine. See also Perl and Eshdat (1998), Thomas et al. (2000), Kikkert et al. (2001), Martinelli and Mandolino (2001), Colova-Tsolova

et al. (2001) and Bouquet et al. (2003b) for applications and prospects of genetic transformation in grapevine.

5.7 Molecular Studies

Since about fifteen years, the availability of inexpensive and easy-to-use molecular markers has considerably facilitated research in *Vitis* genetics and breeding. It is now possible to create unique DNA profiles for each genotype, to map the grapevine genome, to tag specific genes for breeding purposes, marker-assisted selection and gene cloning. For a general review on molecular markers, see Reisch (2000).

5.7.1 Fingerprinting and Diversity Assessment

Cultivar naming is a major problem in viticulture. Often the same cultivar is grown under different denominations or different cultivars are sometimes grown under the same denomination. Molecular markers have been widely applied in grapevine for cultivar identification. They include successively random fragment length polymorphisms (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphisms (AFLP) and single sequence repeats (SSR), also called micro-satellites. Standard sets of micro-satellite markers have been proposed (This et al. 2004) and they are now commonly applied to solve problematic naming (Dangl et al. 2001), for genetic diversity assessment (Sefc et al. 2000) and for parentage analysis (Bowers et al. 1999). For a review on micro-satellite markers, see Sefc et al. (2001). Other techniques for DNA fingerprinting are currently in development, such as retrotransposon-based molecular markers (Pelsy et al. 2003) or single nucleotide polymorphisms (SNP) (Salmaso et al. 2004).

Bowers and Meredith (1997) determined the parentage of Cabernet Sauvignon, while Bowers et al. (1999) investigated the origin of Chardonnay, Gamay noir and other important cultivars. Boursiquot et al. (2004) identified a number of synonyms for Gouais. Though not of importance today, this cultivar was widespread in Europe in the past and the authors underline the important role it played in the parentage of many of modern-day prestigious French and European cultivars.

However, the relationships between cultivated vines and wild vines have not yet been clearly established. Some results indicate the absence of links (This et al. 2001; Carreño et al. 2004). In contrast, other results suggested that local domestication of wild vines predominated over introduction of cultivars from other regions (Sefc et al. 2003; Grassi et al. 2003). Vine domestication has resulted in a radical change in vine biology, such as the change from dioecism to hermaphroditism. In their wild state, due to a strict allogamy, all the species of the *Vitis* genus are dioecious and, therefore, have a high level of heterozygosity that meets in cultivars or wild populations of *V.vinifera* and is revealed by micro-satellite studies (Sefc et al. 2000; Aradhya et al.

2003). The high heterozygosity of *V. vinifera* cultivars was also confirmed by the first studies investigating SNPs (Salmaso et al. 2004).

5.7.2 Genetic Mapping and Marker-Assisted Selection

Suitable screening methods for fruit quality and disease resistance are necessary for maintaining efficient breeding programmes. Some of these screening techniques were already discussed, but one of the most exciting techniques is the fairly recent development of Marker Assisted Selection (MAS). These techniques enable breeders to make sophisticated decisions regarding which parents to choose for crosses and also to pre-screen seedling populations for certain traits and discard those individuals with the undesirable characteristic, before establishment in the vineyard. Strong linkage between molecular markers and the genes (alleles) responsible for the expression of these traits has to be established. Genotyping the gene-pool of potential parents and identifying those carrying the desired alleles to be combined would enhance the efficiency of the breeding process.

Since many agronomical important traits are quantitatively inherited in grapevine, it is difficult to control them in breeding programmes. By establishing associations of these traits with linked molecular markers, molecular maps may be produced and genetic factors involved localized as quantitative trait loci (QTL). Once the correlation between a specific phenotype and molecular marker has been established, the inheritance of a trait can be scored in the progeny at very early stages of plant development. To find good correlation between molecular markers and the measured trait, a combination of several markers will be necessary (Striem et al. 1996).

In 1997, grapevine researchers founded the International Grape Genome Programme (IGGP) (http://www.vitaceae.org). Among the interests of the IGGP was development of a reference linkage map resulting from individual mapping projects and as a resource for physical mapping. Such a map is also useful for targeting genomic regions for more intensive mapping efforts, such as for localizing QTLs or for gene cloning. Researchers combined various markers in developing genetic maps, using essentially and successively RAPD, AFLP, and SSR markers. Twenty research groups of 10 countries worked cooperatively in the Vitis Microsatellite Consortium (VMC) to develop a large number of micro-satellite markers. Several linkage maps were constructed from inter-specific hybrid populations (Lodhi et al. 1995; Dalbó et al. 2000; Grando et al. 2003; Fischer et al. 2004; Doucleff et al. 2004; Lowe and Walker 2006) or *V. vinifera* populations (Doligez et al. 2002; Riaz et al. 2004 and Adam-Blondon et al. 2004). An integrated SSR map based on five mapping populations was recently published (Doligez et al. 2006).

Several large-insert comprehensive genomic libraries have also been developed for the grape genome using the bacterial artificial chromosome system (Tomkins et al. 2001; Adam-Blondon et al. 2005). The connection between genetic maps and a physical map based on a Cabernet Sauvignon BAC library is currently under way (Lamoureux et al. 2006).

5.7.2.1 Mapping for Seedlessness and Other Berry Characteristics

Striem et al. (1994; 1996) described a number of sub-traits of seedlessness and found RAPD markers with significant effects on several of these sub-traits. These authors could exclude most of the seeded individuals of the progeny by using a two-step process of marker assisted selection. As was already discussed, Bouquet and Danglot (1996) proposed three complementary recessive genes regulated by a dominant inhibitor gene, which they identified as the *Sd*I gene. This work was followed-up by a number of publications on marker assisted selection for seedlessness (Lahogue et al. 1998; This et al. 2000; Adam-Blondon et al. 2001; Doligez et al. 2002). Lahogue et al. (1998) used a bulk segregant analysis with RAPD markers, and developed a SCAR (sequence characterised amplified region) marker linked to the *Sd*I gene. This marker (SCC8) explained a large part of the phenotypic variation of seedlessness components traits. Doligez et al. (2002) constructed parental and consensus genetic maps from a F_1 population derived from a cross between two rudimentary seedless genotypes. They found QTLs for several sub-traits of seedlessness and berry weight. In an independent study Meija and Hinrichsen (2003) developed another highly assertive SCAR marker potentially useful to assist selection for seedlessness.

The gene controlling sex in grapevine was placed by Dalbó et al. (2000) on a linkage group corresponding to the LG 2 of the *V. vinifera* consensus map. Placement of the sex locus was confirmed by Lowe and Walker (2006). A major gene for berry colour was also placed on LG 2 (Doligez et al. 2002). Fischer et al. (2004) identified QTLs for véraison (onset of berry ripening) and berry size. Fanizza et al. (2005), using a table grape progeny Italia (seeded) × Big Perlon (seedless), detected QTLs for different components of fruit yield such as berry and cluster weight. Marino et al. (2003) detected several QTLs for free aroma volatile components on the map derived from the cross *V. vinifera* Moscato Bianco × *V. riparia* (Grando et al. 2003). Eibach et al. (2003) identified QTLs for three aroma terpene compounds in a progeny from a cross between a muscat and non-muscat parent.

5.7.2.2 Mapping for Disease Resistance

As mentioned Bouquet (1986) introduced powdery mildew resistance from *Muscadinia* into *V. vinifera* and found resistance to be conferred by a dominant allele at a single locus designated *Run*1. Pauquet et al. (2001) developed genetic markers tightly linked to the *Run*1 locus. Donald et al. (2002) described the mapping of resistance gene analogs (RGAs) to the *Run*1 locus. Barker et al. (2005) using a bacterial artificial chromosome (BAC) library described the construction of comprehensive genetic and physical maps spanning the *Run*1 locus, that enabled cloning of the resistance gene. Fischer et al. (2004) developed a map from progeny of a cross between a disease resistant and a susceptible cultivar. Regent, derived from the French hybrid Chambourcin, has a complex parentage, involving a number of wild *Euvitis* species and shows high field resistance to powdery and downy mildews, while Lemberger is susceptible. The authors found that resistance to the two fungal

pathogens relied on independent genetic factors. One major QTL region was identified for powdery mildew resistance, while a major QTL and a less pronounced "minor" QTL for resistance to downy mildew were found in Regent. In another preliminary study, Zyprian et al. (2003) constructed a partial map by using two downy mildew resistant parents (Villard blanc and Ga-47-42, derived from the French hybrid Seyval). The authors postulated that resistance to downy mildew in the two segregating populations was determined by different genetic factors. Marino et al. (2003) also identified two different QTLs for resistance to downy mildew in the *V. vinifera* × *V. riparia* map constructed by Grando et al. (2003).

Dalbó et al. (2001) constructed a genetic map from inter-specific cultivars and developed a marker for powdery mildew resistance. Doucleff et al. (2004) constructed linkage maps from a cross between two half-sib genotypes *V. rupestris* × *V. arizonica* that carry resistance to Pierce's Disease (PD) and *X. index*. A primary resistance gene to PD, *Pdr1* was identified and mapped by (Krivanek et al. 2006). Lowe and Walker (2006), using a progeny from a cross between Ramsey (*V. champini*) and Riparia Gloire (*V. riparia*) developed the first linkage map of rootstocks and presented it as a valuable tool for studying the genetics of many rootstock traits including nematode resistance, lime and salt tolerance, and ability to induce vigor.

5.8 Conclusions

Biotechnological techniques made great impact on grapevine improvement in the recent past, especially the past decade by the development of successful genetic transformation systems and stable insertion of foreign genes of agricultural importance into grapevine. The use of molecular markers and the creation of genetic maps have contributed to a better understanding of grapevine genetics. Though biotechnological and molecular tools are making a big impact on the efficiency of breeding programmes, the grapevine community should appreciate the great history and culture of the grapevine.

References

Adam-Blondon, A.F., Lahogue-Esnault, F., Bouquet, A., Boursiquot, J.M., and This, P. (2001) Usefulness of two SCAR markers for marker-assisted selection of seedless grapevine cultivars. Vitis 40, 147–155.

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

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

Agarwal, P.K. (1983) Effect of storage in organic solvents on the germination of grapevine pollen. J. Hort. Sci. 58(3), 389–392.

Agüero, C.B., Uratsu, S.L., Greve, C., Powell, A.L.T., Labavitch, J.M., Meredith, C.P., and Dandekar A.M. (2005) Evaluation of tolerance to Pierce's disease and *Botrytis* in transgenic plants of *Vitis vinifera* L. expressing the pear PGIP gene. Mol. Plant Pathol. 6, 43–51.

Aldwinckle, H.S. (1978) Screening grape seedlings for resistance to powdery mildew (*Uncinula necator*), pp. 259–264. In: *Génét. Amélior. de la Vigne. IIe Symp. Intern*. R. Pouget and J.P-. Doazan (Eds.), Bordeaux, Publ. INRA, Paris.

Aradhya, M.K., Dangl, G.S., Prins, B.H., Boursiquot, J.M., Walker, M.A., Meredith C.P., and Simon C.J. (2003) Genetic structure and differentiation in cultivated grape, *Vitis vinifera* L. Genet. Res. Camb. 81, 179–192.

Barbagallo, M.G., Crescimanno, F.G., De Michele, A., Di Lorenzo, R., and Sottile, I. (1988) Osservazioni sulla cleistogamia nella vite (*Vitis vinifera* L.). Vignevini, Bologna 15, 51–54 (Eng. abstr.).

Barker, C.L., Donald, T., Pauquet, J., Ratnaparkhe, M.B., Bouquet, A., Adam-Blondon, A.F., Thomas, M.R., and Dry, I. (2005) Genetic and physical mapping of the grapevine powdery mildew resistance gene, *Run*1, using a bacterial artificial chromosome library. Theor. Appl. Genet. 111, 370–377.

Barlass, M. and Skene, K.G.M. (1981) Relative NaCl tolerances of grapevine cultivars and hybrids *in vitro*. Z. Pflanzenphysiol. 102, 147–161.

Barlass, M., Miller, R.M., and Douglas, T.J. (1987) Development of methods for screening grapevines for resistance to infection by downy mildew. II. Resveratrol production. Am. J. Enol. Vitic. 38, 65–68.

Barrett, B.H. and Arisumi, T. (1952) Methods of pollen collection, emasculation and pollination in fruit breeding. Proc. Amer. Soc. Hort. Sci. 59, 259–262.

Barritt, B.H. and Einset, C. (1969) The inheritance of three major fruit colours in grapes. J. Amer. Soc. Hort. Sci. 94, 87–89.

Bavaresco, L. and Walker, M.A. (1994) Techniques for successfully establishing *Xiphinema index* in dual culture with grape. Am. J. Enol. Vitic. 45, 273–277.

Bavaresco, L., Fregoni, M., and Gambi, E. (1993) *In vitro* method to screen grapevine genotypes for tolerance to lime-induced chlorosis. Vitis 32, 145–148.

Bessis, R., Vannel, D., and Barbier, M. (1992) *In vitro* bioassays to evaluate the relationship between grapevines and *Botrytis cinerea*, pp. 104–108. *In*: *Recent Advances in* Botrytis *Research*. K. Verhoeff, N. Malathrakis, and B. Williamson (Eds.), Purdoc Scientific Publisher, Wageningen.

Bharathy, P.V., Karibasappa, G.S., Biradar, A.B., Kulkarni, D.D., Solanke, A.U., Patil, S.G., and Agrawal, D.C. (2003) Influence of pre-bloom sprays of benzyladenine on *in vitro* recovery of hybrid embryos from crosses of Thompson Seedless and 8 seeded varieties of grape (*Vitis* spp.). Vitis 42, 199–202.

Bornhoff, B.A., Harst, M., Zyprian, E., and Töpfer, R. (2005) Transgenic plants of *Vitis vinifera* cv. Seyval blanc. Plant Cell Rep. 24, 433–438.

Boss, P.K. and Thomas M.R. (2002) Association of dwarfism and floral induction with a grape 'green revolution' mutation. Nature 416, 847–850.

Boubals, D. (1959) Contribution à l'étude des causes de la résistance des vitacées au mildiou (*Plasmopara viticola* (B. et C;) Berl. et T.) et de leur mode de transmission (English summary). Ann. Amélior. Plantes 9, 225–233.

Boubals, D. (1961) Études des causes de la résistance des Vitacées à l'oïdium de la vigne - *Uncinula necator* (SCHW.)BURR. - et de leur mode de transmission (English summary). Ann. Amélior. Plantes 11, 401–500.

Bouquet, A. (1981) Resistance to grape fanleaf virus in Muscadine grape inoculated with *Xiphinema index*. Plant Dis. 65, 791–793.

Bouquet, A. (1983) Étude de la résistance au phylloxera radicole des hybrides *Vitis vinifera* × *Muscadinia rotundifolia* (English abstract). Vitis 22, 311–323.

Bouquet, A. (1986) Introduction dans l'espèce *Vitis vinifera* L. d' un caractère de résistance à l'oïdium (*Uncinula necator*) issu de l'espèce *Muscadinia rotundifolia* (Michx.) Small. Vignevini 13 (suppl. to n° 12), 141–146.

Bouquet, A. (1989) Intérêt des techniques de culture *in vitro* pour l' amélioration génétique de la vigne (English abstract). Bull.O.I.V. 697–698, 179–192.

Bouquet, A. and Davis, H.P. (1989) Culture *in vitro* d'ovules et d'embryons de vigne (*Vitis vinifera* L.) appliquée à la sélection de variétés de raisins de table sans pépins (English abstract). Agronomie 9, 565–574.

Bouquet, A. and Danglot, Y. (1996) Inheritance of seedlessness in grapevine (*Vitis vinifera* L.). Vitis 35, 35–42.

Bouquet, A. and Torregrosa, L. (2003) Micropropagation of the grapevine (*Vitis* spp.). *In*: *Micropropagation of Woody Trees and Fruits*. S.M. Jain and K. Ishii (Eds.), Kluwer Academic Publishers, Dordrecht, 75, 319–352.

Bouquet, A., Chabbert, A.M., and Danglot, Y. (1990) *In vitro* selection for tolerance to magnesium deficiency in grapevine rootstocks. *Vitis* (special issue) Proc. 5th Intern. Symp. Grape Breeding, St. Martin/Pfalz, Germany, 12–16 September 1989, p. 445.

Bouquet, A., Chatelet, P., and Torregrosa, L. (2003b) Grapevine genetic engineering : Tool for genome analysis or plant breeding method? Which future for transgenic vines? AgBiotechnet, vol. 5, ABN 116, 1–10.

Bouquet, A., Torregrosa, L., and Chatelet, P. (2004) Combinaison des approches biotechnologiques et conventionnelles dans la sélection de porte-greffes présentant une résistance durable à la transmission de la maladie du court-noué (Eng. abstr.). Bull. O.I.V. 77(879–880), 361–376.

Bouquet, A., Danglot, Y., Torregrosa, L., Bongiovani, M., Castagnone-Sereno, P., Esmenjaud, D., and Dalmasso, A. (2000a) Breeding rootstocks resistant to grape fanleaf virus spread using *Vitis* × *Muscadinia* hybridization. Acta Hortic 528, 517–526

Bouquet, A., Pauquet, J., Adam-Blondon, A., Torregrosa, L., Merdinoglu, L., and Wiedemann-Merdinoglu, D. (2000b) Vers l'obtention de variétés de vigne résistantes à l'oïdium et au mildiou par les méthodes conventionnelles et biotechnologiques (Eng. abstr.). Bull. O.I.V. 73(833–834), 445–452.

Bouquet, A., Marck, G., Pistagna, D., and Torregrosa, L. (2003a) Transfer of grape fanleaf virus coat protein gene through hybridization with *Xiphinema index* resistant genotypes to obtain rootstocks resistant to virus spread. Acta Hortic 60, 325–334.

Bouquet, A., Torregrosa, L., Iocco, P., and Thomas, M.P. (2006) Grapevine (*Vitis vinifera* L.). *In: Methods in Molecular Biology*, Vol. 344 : *Agrobacterium* Protocols, Vol. 2, Ch. 24, 273–285.

Boursiquot, J.M., Lacombe, T., Bowers, J., and Meredith, C. (2004) Le Gouais, un cépage clé du patrimoine viticole européen (Eng. abstr.). Bull. O.I.V. 77(875–876), 5–19.

Bowers, J.E. and Meredith, C.P. (1997) The parentage of a classic wine grape, Cabernet Sauvignon. Nat. Genet. 16, 84–87.

Bowers, J.E., Boursiquot, J.M., This, P., Chu, K., Johansson, H., and Meredith, C.P. (1999) Historical genetics: The parentage of Chardonnay, Gamay and other wine grapes of northeastern France. Science 285, 1562–1565.

Bronner, A. and Oliveira, J. (1990). Creation and study of the Pinot noir variety lineage. *Vitis* (special issue) Proc. 5th Intern. Symp. Grape Breeding, St. Martin/Pfalz, Germany, 12–16 September 1989, pp. 69–80.

Bronner, A. and Wagner, R. (1997) Pollen et floraison chez *Vitis vinifera* L. Techniques de contrôle du pouvoir germinatif du pollen (Eng. abstr.). Progr. Agric.Vitic. 114, 130–139.

Cain, D.W., Emershad, R.L., and Tarailo, R.E. (1983) *In-ovulo* embryo culture and seedling development of seeded and seedless grapes (*Vitis vinifera* L.). Vitis 22, 9–14.

Camargo, U.A. (2000) Grape breeding for the subtropical and tropical regions of Brazil. Acta Hortic 528, 473–477.

Carbonneau, A. (1985) The early selection of grapevine rootstocks for resistance to drought conditions. Am. J. Enol. Vitic. 36, 195–198.

Carreño, E., Lopez, M.A., Labra, M., Rivera, D., Sancha, J., Ocete, R., and Martinez de Toda, F. (2004) Genetic relationship between some spanish *Vitis vinifera* L. *ssp. sativa* cultivars and wild grapevine populations (*Vitis vinifera* L*ssp. sylvestris*): a preliminary study. Plant Genetic Resources Newsletter 137, 42–45.

Christensen, P., Ramming, D., and Andris, H. (1983) Seed trace content of Fiesta grapes. Am. J. Enol. Vitic. 34(4), 257–259.

Colova-Tsolova, V., Perl, A., Krastanova, S., Tsvetkov, I., and Atanassov, A. (2001) Genetically engineered grape for disease and stress tolerance, pp. 411–431. *In*: *Molecular Biology and Biotechnology of Grapevine*. K.A. Roubelikas-Angelakis (Ed.), Kluwer Publish Co., Amsterdam.

Cousins, P., Lauver, M., and Boyden, L. (2003) Genetic analysis of root-knot nematode resistance derived from *Vitis mustangiensis*. Acta Hortic 603, 149–155.

Dalbó, M.A., Ye, G.N., Weeden, N.F., Steinkellner, H., Sefc, K.M., and Reisch, B.I. (2000) A gene controlling sex in grapevines placed on a molecular marker-based genetic map. Genome 43, 333–340.

Dalbó, M.A.,Ye, G.N., Weeden, N.F., Wilcox, W.F., and Reisch, B.I. (2001) Marker-assisted selection for powdery mildew resistance in grapes. J. Amer. Soc. Hort. Sci. 126, 83–89.

Dangl, G.S., Mendum, M.L., Prins, B.H., Walker, M.A., Meredith, C.P., and Simon, C.J. (2001) Simple sequence repeat analysis of a clonally propagated species: A tool for managing a grape germplasm collection. Genome 44, 432–438.

Dercks, W. and Creasy, L.L. (1989) The significance of stilbene phytoalexins in the *Plasmopara viticola*-grapevine interaction. Physiological and Molecular Plant Pathol. 34, 189–202.

Dettweiler, E., Jung, A., Zyprian, E., and Töpfer, R. (2000) Grapevine cultivar Müller-Thurgau and its true to type descent. Vitis 39, 63–65.

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

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

Donald, T.M., Pellerone, F., Adam-Blondon, A.F., Bouquet, A., Thomas, M.R., and Dry, I.B. (2002) Identification of resistance gene analogs linked to a powdery mildew resistance locus in grapevine. Theor. Appl. Genet. 104, 610–618.

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

Eibach, R. (1990) Investigations about the influence of some physiological and phenological characteristics on quality and their heritability. Vitis (special issue) Proc. 5th Intern. Symp. Grape Breeding, St. Martin/Pfalz, Germany, 12–16 September 1989, 149–158.

Eibach, R. (1994) Investigations about the genetic resources of grapes with regard to resistance characteristics to powdery mildew (*Oidium tuckeri*). Vitis 33, 143–150.

Eibach, R. (2000) Investigations on the inheritance of resistance features to mildew diseases. Acta Hortic 528, 455–465.

Eibach, R. and Töpfer, R. (2003) Success in resistance breeding: “Regent” and its steps into the market. Acta Hortic 603, 687–692.

Eibach, R., Hastrich, H., and Töpfer, R. (2003) Inheritance of aroma compounds. Acta Hortic 603, 337–344.

Einset, J. and Pratt, C. (1975). Grapes. *In: Advances in Fruit Breeding*. J. Janick and J. N. Moore (Eds.), Purdue University Press, West Lafayette, Indiana, pp. 130–153.

Ellis, R.H., Hong, T.D., and Roberts, E.H. (1983) A note on the development of a practical procedure for promoting the germination of dormant seed of grape (*Vitis* spp.). Vitis 22, 211–219.

Emershad, R.L. and Ramming, D.W. (1984) *In-ovulo* embryo culture of *Vitis vinifera* L. cv. ‘Thompson Seedless’. Amer. J. Bot. 71, 873–877.

Fanizza, G. and Raddi, P. (1973) The heritability of fruit ripening in *Vitis vinifera* L. Vitis 12, 93–96.

Fanizza, G., Bisignano, V., Pollastro, S., Miazzi, M., and Faretra, F. (1995) Effects of polysaccharides from *Botryotinia fuckeliana (Botrytis cinerea)* on *in vitro* culture of table and wine grapes (*Vitis vinifera*). Vitis 34, 41–44.

Fanizza, G., Lamaj, F., Costantini, L., Chaabane, R., and Grando, M.S. (2005) QTL analysis for fuit yield components in table grapes (*Vitis vinifera*). Theor. Appl. Genet. 111, 658–664.
Firoozabady, E. and Olmo, H.P. (1987) Heritability and correlation studies of certain quantitative traits in table grapes, *Vitis* spp. Vitis 26, 132–146.
Fisher, K.H., Fuleki, T., and Reynolds, A.G. (1990) The heritability of methyl anthranilate and total volatile esters in *Vitis* spp. Hybrids. Vitis (special issue) Proc. 5th Intern. Symp. Grape Breeding, St. Martin/Pfalz, Germany, 12–16 September 1989, pp. 159–168.
Fischer, B.M., Salakhutdinov, I., Akkurt, M., Eibach, R., Edwards, K.J., Töpfer, R., and Zyprian, E.M. (2004) Quantitative trait locus analysis of fungal disease resistance factors on a molecular map of grapevine. Theor. Appl. Genet. 108, 501–515.
Forneck, A., Walker, M.A., and Merkt, N. (1996) Aseptic dual culture of grape (*Vitis* spp.) and grape phylloxera (*Daktulosphaira vitifoliae* FITCH). Vitis 35, 95–98.
Franks, T., Botta, R., and Thomas, M.R. (2002) Chimerism in grapevines : implications for cultivar identity, ancestry and genetic improvement. Theor. Appl. Genet. 104, 192–199.
Franks, T.K., Savocchia, S., and Van Heeswijck, R. (2003) *In vitro* systems for studying the interaction of root-knot nematode with grapevine. Nematology 5, 235–242.
Gambino, G., Gribaudo, I., Leopold, S., Schartl, A., and Laimer, M. (2005) Molecular characterization of grapevine plants transformed with GFLV resistance genes. Plant Cell Rep. 24, 655–662.
Ganeshan, S. and Alexander, M.P. (1990) Fertilizing ability of cryopreserved grape (*Vitis vinifera* L.) pollen. Vitis 29, 145–150.
Goldy, R.G., Ramming, D.W., Emershad, R.L., and Chaparro, J.X. (1989) Increasing production of *V. rotundifolia* hybrids through embryo rescue. HortScience 24, 820–822.
Grando, M.S., Bellin, D., Edwards, K.J., Pozzi, C., Stefanini, M., and Velasco, R. (2003) Molecular linkage maps of *Vitis vinifera* L. and *Vitis riparia* Michx. Theor. Appl. Genet. 106, 1213–1224.
Grassi, F., Labra, M., Imazio, S., Spada, A., Sgorbati, S., Scienza, A., and Sala, F. (2003) Evidence of a secondary grapevine domestication centre detected by SSR analysis. Theor. Appl. Genet. 107, 1315–1320.
Gray, D.J. and Meredith, C.P. (1992). Grape. *In: Biotechnology in Agriculture, No. 8. Biotechnology of Perennial Fruit Crops*. F. Hammerschlag and R.E. Litz (Eds.), CAB International, Wallingford, pp. 229–262.
Gray, D.J., Fischer, L.C., and Mortensen, J.A. (1987) Comparison of methodologies for *in ovulo* embryo rescue of seedless grapes. HortScience 22, 1334–1335.
Gray, D.J., Mortensen, J.A., Benton, C.M., Durham, R.E., and Moore, G.A. (1990) Ovule culture to obtain progeny from hybrid seedless bunch grapes. J. Amer. Soc. Hort. Sci. 115, 1019–1024.
Grzegorczyk, W. and Walker, M.A. (1998) Evaluating resistance to grape phylloxera in *Vitis* spp. with an *in vitro* dual culture assay. Am. J. Enol. Vitic. 49(1), 17–22.
Guillen, P., Guis, M., Martinez-Reina, G., Colrat, S., Dalmayrac, S., Deswarte, C., Bouzayen, M., Roustan, J.P., Fallot, J., Pech, J.C., and Latche, A. (1998) A novel NADPH-dependent aldehyde reductase gene from *Vigna radiata* confers resistance to the grapevine fungal toxin eutypine. The Plant J. 16, 335–343.
Harst, M., Bornhoff, B.A., Zyprian, E., and Töpfer, R. (2000) Influence of culture technique and genotype on the efficiency of *Agrobacterium*-mediated transformation of somatic embryos (*Vitis vinifera*) and their conversion to transgenic plants. Vitis 39, 99–102.
Heazlewood, J.E. and Wilson, S. (2004) Anthesis, pollination and fruit set in Pinot Noir. Vitis 43, 65–68.
Heazlewood, J.E., Wilson, S., Clark, R.J., and Gracie, A. (2005) Pollination of *Vitis vinifera* L. cv. Pinot noir as influenced by *Botrytis* fungicides. Vitis 44, 111–115.
Hedrick, U.P. (1908) The Grapes of New-York. N.Y. Agr. Exp. Sta. Report, 564pp.
Heintz, C., Stein, U., and Blaich, R. (1985) Recherches sur la résistance de nouvelles variétés de vigne a l'oidium (English abstract). Progr. Agric. Vitic. 102, 585–590.
Hemstad, P.R. and Luby, J.J. (2000) Utilization of *Vitis riparia* for the development of new wine varieties with resistance to disease and extreme cold. Acta Hortic 528, 487–490.

Hirabayashi, T., Kozaki, I., and Akihama, T. (1976) *In vitro* differentiation of shoot from anther callus in *Vitis*. HortScience 11, 511–512.

Ingrouille, M.J., Chase, M.W., Fay, M.F., Bowmann, D., Van der Bank, M., and Bruijn, D.E. (2002) Systematics of *Vitaceae* from the point of view of plastid *rbcL* DNA sequence data. Bot. J. Linnean Soc. 138, 421–432.

Jayasankar, S., Zhijian, L., and Gray, D.J. (2000) *In vitro* selection of *Vitis vinifera* 'Chardonnay' with *Elsinoe ampelina* culture filtrate is accompanied by fungal resistance and enhanced secretion of chitinase. Planta 211, 200–208.

Kellow, A.V., McDonald, G., Corrie, A.M., and Van Heeswijck, R. (2002) *In vitro* assessment of grapevine resistance to two populations of phylloxera from Australian vineyards. Australian J. Grape Wine Res. 8, 109–16.

Kender, W.J. and Remaily, G. (1970) Regulation of sex expression and seed development in grapes with 2-chloroethylphosphonic acid. HortScience 5, 491–492.

Kiefer, F., Triouleyre, C., Bertsch, C., Farine, S., Leva, Y., and Walter, B. (2004) Mannose and xylose cannot be used as selectable agents for *Vitis vinifera* L. transformation. Vitis 43, 35–39.

Kikkert, J.R., Thomas, M.R., and Reisch, B.I. (2001) Grapevine genetic engineering, pp. 393–410. *In*: *Molecular Biology and Biotechnology of the Grapevine* K.A. Roubelakis-Angelakis (Ed.), Kluwer Publish. Co, Amsterdam.

Kimura, P.H., Okamoto, G., and Hirano, K. (1998) The mode of pollination and stigma receptivity in *Vitis coignetiae* Pulliat. Am. J. Enol. Vitic. 49, 1–5.

Klempka, K.C., Meredith, C.P., and Sall, M.A. (1984) Dual culture of grape powdery mildew (*Uncinula necator* Burr.) on its host (*Vitis vinifera* L.). Am. J. Enol. Vitic. 35, 170–174.

Kobayashi, S., Goto-Yamamoto, N., and Hirochika, H. (2004) Retrotransposon-induced mutations in grape skin colour. Science 304, 982–984.

Korbuly, J. (2000) Results of breeding for resistance to winter frosts and different pathogens using *Vitis amurensis*. Acta Hortic 528, 551–557.

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

Kozma, P. Jr. (2000) Winegrape breeding for fungus disease resistance. Acta Hortic 528, 505–510.

Kozma, P. Jr. and Dula, T. (2003) Inheritance of resistance to downy mildew and powdery mildew of hybrid family *Muscadinia* × *V. vinifera* × *V. amurensis* × Franco-Amercan hybrid. Acta Hortic 603, 457–463.

Krastanova, S., Perrin, M., Barbier, P., Demangeat, G., Cornuet, P., Bardonnet, N., Otten, L., Pinck, L., and Walter, B. (1995) Transformation of grapevine rootstocks with the coat protein gene of grapevine fanleaf nepovirus. Plant Cell Rep. 14, 550–554.

Krivanek, A.F., Famula, T.R., Tenscher, A., and Walker, M.A., (2005) Inheritance of resistance to *Xyllela fastidiosa* within a *Vitis rupestris* × *Vitis arizonica* hybrid population. Theor. Appl. Genet. 111, 110–119.

Krivanek, A.F., Riaz, S., and Walker, M.A. (2006) Identification and molecular mapping of *Pdr1*, a primary resistance gene to Pierce's disease in *Vitis*. Theor. Appl. Genet. 112, 1125–1131.

Krul, W.R. and Mowbray, G.H. (1984) Grapes, pp. 396–434. *In: Handbook of Plant Cell Cultures*, Vol. 2. W.R. Sharp, D.A. Evans, P.V. Ammirato, and Y. Yamada (Eds.), Macmillan, New York.

Lahogue, F., This, P., and Bouquet, A. (1998) Identification of a codominant scar marker linked to the seedlessness character in grapevine. Theor. Appl. Genet. 97, 950–959.

Lamoureux, D., Bernole, A., Le Clainche, I., Tual, S., Thareau, V., Paillard, S., Legeai, F., Dossat, C., Wincker, P., Oswald, M., Merdinoglu, D., Vignault, C., Delrot, S., Caboche, M., Chalhoub, B., and Adam-Blondon, A.F. (2006) Anchoring of a large set of markers onto a BAC library for the development of a draft physical map of the grapevine genome. Theor. Appl. Genet. 113, 344–356.

Langcake, P. (1981) Disease resistance of *Vitis* spp. and the production of the stress metabolites resveratrol, ε-viniferin, α-viniferin and pterostilbene. Physiol. Plant Path. 18, 213–226.

Lebrun, L., Rajasekaran, K., and Mullins, M.G. (1985) Selection *in vitro* for NaCl-tolerance in *Vitis rupestris* Scheele. Ann. Bot. 56, 733–739.

Ledbetter, C.A., Burgos, L., and Palmquist, D. (1994) Comparison of methods used for determining the stenospermic trait in *Vitis vinifera* L. Vitis 33, 11–13.

Le Gall, O., Torregrosa, L., Danglot, Y., Candresse, T., and Bouquet, A. (1994) *Agrobacterium*-mediated genetic transformation of grapevine somatic embryos and regeneration of transgenic plants expressing the coat protein of grapevine chrome mosaic nepovirus (GCMV). Plant Sci. 102, 161–170.

Legrand, V., Dalmayrac, S., Latché, A., Pech, J.C., Bouzayen, M., Fallot, J., Torregrosa, L., Bouquet, A., and Roustan, J.P. (2003) Constitutive expression of *Vr-ERE* gene in transformed grapevines confers enhanced resistance to eutypine, a toxin from *Eutypa lata*. Plant Sci. 164, 809–814.

Li, H. (1993) Studies on the resistance of grapevine to powdery mildew. Plant Pathol. 42, 792–796.

Lodhi, M.A., Ye, G.N., Weeden. N.F., and Reisch, B.I. (1995) A molecular marker-based linkage map of *Vitis*. Genome 38, 786–794.

Loomis, N.H. and Weinberger, J.H. (1979) Inheritance studies of seedlessness in grapes. J. Amer. Soc. Hort. Sci. 104, 181–184.

Lowe, K.M. and Walker, M.A. (2006) Genetic linkage map of the interspecific grape rootstock cross Ramsey (*Vitis champinii*) × Riparia Gloire (*Vitis riparia*). Theor. Appl. Genet. 112, 1582–1592.

Manivel, L. and Weaver, R.J. (1974) Effect of growth regulators and heat on germination of Tokay grape seeds. Vitis 12, 286–290.

Marino, R., Sevini, F., Madini, A., Vecchione, A., Pertot, I., Serra, A.D., Versini, G., Velasco, R., and Grando, M.S. (2003) QTL mapping for disease resistance and fruit quality in grape. Acta Hortic 603, 527–533.

Martinelli, L. and Gribaudo, I. (2001) Somatic embryogenesis in grapevine, pp. 327–351. *In: Molecular Biology and Biotechnology of the Grapevine*. K.A. Roubelakis-Angelakis (Ed.), Kluwer Publish. Co., Amsterdam.

Martinelli, L. and Mandolino, G. (2001) Transgenic grapes (*Vitis* species), pp. 325–338. *In: Biotechnology in Agriculture and Forestry*, Vol. 47, *Transgenic crops* II. Y.P.S. Bajaj (Ed.), Springer-Verlag, Berlin, Heidelberg.

Martinelli, L., Candioli, E., Costa, D., and Minafra, A. (2002) Stable insertion and expression of the movement protein gene of Grapevine Virus A (GVA) in grape (*Vitis rupestris* S.). Vitis 41, 189–193.

Mauro, M.C., Vaillant, V., Tey-Rulh, P., Mathieu, Y., and Fallot, J. (1988) *In vitro* study of the relationship between *Vitis vinifera* and *Eutypa lata* (Pers.:Fr) Tul. I. Demonstration of toxic compounds secreted by the fungus. Am. J. Enol. Vitic. 39, 200–204.

Mauro, M.C., Toutain, S., Walter, B., Pinck, L., Otten, L., Coutos-Thévenot, P., Deloire, A., and Barbier, P. (1995) High efficiency regeneration of grapevine plants transformed with the GFVL coat protein gene. Plant Sci. 112, 97–106.

Meija, N. and Hinrichsen, P. (2003) A new, highly assertive scar marker potentially useful to assist selection for seedlessness in table grape breeding. Acta Hortic 603, 559–564.

Merdinoglu, D., Wiedemann-Merdinoglu, S., Coste, P., Dumas, V., Haetty, S., Butterlin, G., Greif, C., Adam-Blondon, A.F., Bouquet, A., and Pauquet, J. (2003) Genetic analysis of downy mildew resistance derived from *Muscadinia rotundifolia*. Acta Hortic 603, 451–456.

Meredith, C.P., Lider, C.A., Raski, D.J., and Ferrari, N.L. (1982) Inheritance of tolerance to *Xiphinema index* in *Vitis* species. Am. J. Enol. Vitic. 33, 154–158.

Mezetti, B., Pandolfini, T., Navacchi, O., and Landi, L. (2002) Genetic transformation of *Vitis vinifera* via organogenesis. BMC Biotechnology 2, 18 (http://www.biomedcentral.com/1472-6750/2/18).

Monette, P.L. (1988) Grapevine (*Vitis vinifera* L.), pp. 3–37. *In: Biotechnology in Agriculture and Forestry* Vol. 6, *Crops* II. Y.P.S. Bajaj (Ed.), Springer, Berlin.

Mortensen, J.A. (1968) The inheritance of resistance to Pierce's disease in *Vitis*. J. Amer. Soc. Hort. Sci. 92, 331–337.

Mortensen, J.A. (1981) Source and inheritance of resistance to anthracnose (*Elsinoe ampelina* Shear) in *Vitis*. J. Hered. 72, 423–426.

Mullins, M.G. and Srinivasan, C. (1976) Somatic embryos and plantlets from an ancient clone of the grapevine (cv. Cabernet Sauvignon) by apomixis *in vitro*. J. Exp. Bot. 27, 1022–1030.
Mullins, M.G., Bouquet, A., and Williams, L.E. (1992) Biology of the Grapevine. Cambridge University Press, Cambridge, UK.
Mullins, M.G., Tang, F.C.A., and Facciotti, D. (1990) *Agrobacterium*- mediated genetic transformation of grapevines: Transgenic plants of *Vitis rupestris* Scheele and buds of *Vitis vinifera* L. Bio/Technology 8, 1041–1045.
Netzer, M.H., Guellec, V., and Branchard, M. (1991) Étude du comportement, au cours de l'embryogenese somatique, de divers porte-greffe de vigne cultivés *in vitro* en conditions chlorosantes. Agronomie 11, 125–131. (Eng. abstr.).
Oberle, G.D. (1938) A Genetic Study of Variations in Floral Morphology and Function in Cultivated Forms of *Vitis*. N.Y. State Agric. Exp. Sta., Geneva, Technical Bulletin 250.
Olien, W.C. (1990) The Muscadine grape: botany, viticulture, history and current industry. HortScience 25, 732739.
Olmo, H.P. (1942) Storage of grape pollen. Proc. Amer. Soc. Hort. Sci. 41, 219–224.
Olmo, H.P. (1943) Pollination of the Almeria Grape. Proc. Amer. Soc. Hort. Sci. 42, 401–406.
Olmo, H.P. (1986) The potential role of (*vinifera* × *rotundifolia*) hybrids in grape variety improvement. Experientia 42, 921–926.
Orffer, C.J. (1979) Pinotage. *In: Vine Cultivars of South Africa*. Human and Rousseau (Publ.), Cape Town, South Africa.
Papadakis, A., Reustle, G., Roubelakis-Angelakis, K.A. (2001) Protoplast technology in grapevine, pp. 353–392. *In*: *Molecular Biology and Biotechnology of the Grapevine*. K.A. Roubelakis-Angelakis (Ed.), Kluwer Publish. Co., Amsterdam.
Pauquet, J., Bouquet, A., This, P., and Adam-Blondon, A.F. (2001) Establishment of a local map of AFLP markers around the powdery mildew resistance gene *Run*1 in grapevine and assessment of their usefulness for marker assisted selection. Theor. Appl. Genet.103, 1201–1210.
Pelsy, F., Schehrer, L., and Merdinoglu, D. (2003) Development of grapevine retrotransposon-based molecular markers (S-SAP). Acta Hortic. 603, 83–87.
Perl, A and Eshdat, Y. (1998) DNA transfer and gene expression in transgenic grapes. *In: Biotechnology and Genetic Engineering Reviews*. M.P. Tombs (Ed.), 15, 365–386.
Peros, J.P., Nguyen, T.H., Troulet, C., Michel-Romiti, C., and Notteghem, J.L. (2006) Assessment of powdery mildew resistance of grape and *Erysiphe necator* pathogenicity using a laboratory assay. Vitis 45, 29–36.
Planchon, J.E. (1887) Monographie des *Ampelideae* vraies in Monographia Phanerogamarum A. et C. de Candolle, 5, 305–364.
Ponce, M.T., Guińazü, M., and Tizio, R. (2002) Effect of putrescine on embryo development in the stenospermocarpic grape cvs. Emperatriz and Fantasy. Vitis 41, 53–54.
Popescu, C.F., Falk, A., and Glimelius, K. (2002) Application of AFPLs to characterize somaclonal variation in anther-derived grapevines. Vitis 41, 177–182.
Pospisilova, D. (1974) Heterosiszüchtung bei *Vitis vinifera* L. Vitis 13, 89–97.
Possingham, J.V., Clingeleffer, P.R., and Kerridge, G.H. (1990) Breeding grapevines for tropical environments, pp. 169–177. *In: Vitis* Special edition, Proc. 5th Intern. Symp. Grape Breeding, St. Martin/Pfalz, Germany, 12–16 September 1989.
Pouget, R. (1980) Breeding grapevine rootstocks for resistance to iron chlorosis, pp. 191–197. *In: Proc. 3rd Intern. Symp. Grape Breeding*, Davis, California, UC Davis.
Pouget, R. (1990) Histoire de la lutte contre le Phylloxéra de la Vigne en France. INRA-OIV (Eds) 157pp.
Pouget, R. and Ottenwaelter, M. (1986) Recherche de porte-greffes adaptés aux sols acides : une nouvelle variété, le Gravesac. In: *Proc. 4th Intern. Symp. Grape Genetics*, April 1985, Verona (Italy), Vignevini, 13 (suppl. to n° 12), 134–137.
Pratt, C. (1971) Reproductive anatomy in cultivated grapes – A review. Am. J. Enol. Vitic. 22, 92–109.

Predieri, S. (2001) Mutation induction and tissue culture in improving fruits. Plant Cell Tiss. Org. Cult. 64, 185–210.

Radian-Sade, S., Perl, A., Edelbaum, O., Kuznetsova, L., Gafny, R., Sela, I., and Tanne, E. (2000) Transgenic *Nicotiana benthamiana* and grapevine plants transformed with grapevine virus A (GVA) sequences. Phytoparasitica 28, 79–86.

Ramming, D.W., Emershad, R.L., and Tarailo, R. (2000) A stenospermocarpic, seedless *Vitis vinifera x Vitis rotundifolia* hybrid developed by embryo rescue. HortScience 35, 732–734.

Ramming, D.W., Ledbetter, C.A., and Tarailo, R. (1990a) Hybridization of seedless grapes. In*: Vitis* Special edition, *Proc. 5th Intern. Symp. Grape Breeding*, St. Martin/Pfalz, Germany, 12–16 September 1989, pp. 439–444.

Ramming, D.W., Emershad, R.L., Spiegel-Roy, P., Sahar, N., and Baron, I. (1990b) Embryo culture of early ripening seeded grape (*Vitis vinifera)* genotypes. HortScience 25, 339–342.

Reisch, B.I. (2000) Molecular markers: the foundation for grapevine genetic mapping, DNA fingerprinting and genomics. Acta Hortic 528, 85–89.

Reisch, B.I. and Pratt, C. (1996) Grapes, pp. 297–369. *In: Fruit Breeding*. J. Janick and J. N. Moore (Eds.), Volume II: Vine and Small Fruit Crops. John Wiley and Sons, Inc.

Reustle, G., Harst, M., and Alleweldt, G. (1994) Regeneration of grapevine (*Vitis* sp.) protoplasts. Vitis 33, 173–174.

Reustle, G.M., Wallbraun, M., Zwiebel, M., Wolf, R., Manthey, T., Burkhardt, C., Lerm, T., Vivier, M., and Krczal, G. (2003) Experience with different selectable marker systems for the genetic engineering of grapevine. Acta Hortic 603, 485–490.

Reynolds, A.G., Fuleki, T., and Evans, W.D. (1982) Inheritance of methyl anthranilate and total volatile esters in *Vitis* spp. Am. J. Enol. Vitic. 33, 14–19.

Riaz, S., Dangl, G.S., Edwards, K.J., and Meredith, C.P. (2004) A microsatellite marker based framework linkage map of *Vitis vinifera* L. Theor. Appl. Genet. 108, 864–872.

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

Sbaghi, M., Jeandet, P., Faivre, B., Bessis, R., and Fournioux, J.C. (1995) Development of methods using phytoalexin (resveratrol) assessment as a selection criterion to screen grapevine *in vitro* cultures for resistance to grey mould *Botrytis cinerea*. Euphytica 86, 41–47.

Schneider, S., Reustle, G., and Zyprian, E. (1996) Detection of somaclonal variation in grapevine regenerants from protoplasts by RAPD-PCR. Vitis 35, 99–100.

Scorza, R., Cordts, J.M., Gray, D.J., Gonsalves, D., Emershad, R.L., and Ramming, D.W. (1996) Producing transgenic 'Thompson seedless' grape (*Vitis vinifera* L.) plants. J. Amer. Soc. Hort. Sci. 121, 616–619.

Sefc, K.M., Lopes, M.S., Lefort, F., Botta, R., Roubelakis-Angelakis, K.A., Ibanez, J., Pejic, I., Wagner, H.W., Glössl, J., and Steinkellner, H. (2000) Microsatellite variability in grapevine cultivars from different European regions and evaluation of assignment testing to assess the geographic origin of cultivars. Theor. Appl. Genet. 100, 498–505.

Sefc, K.M., Lefort, F., Grando, M.S., Scott, K.D., Steinkellner, H., and Thomas, M.R. (2001) Microsatellite markers for grapevine: a state of the art, pp. 433–463. *In: Molecular Biology and Biotechnology of the Grapevine*. K.A. Roubelakis-Angelakis (Ed.), Chapter 17. Kluwer Academic Publishers, Amsterdam.

Sefc, K.M., Steinkellner, H., Lefort, F., Botta, R., Da Câmara Machado, A., Borrego, J., Maleti, E., and Glössl, J. (2003) Evaluation of genetic contribution of local wild vines to European grapevine cultivars. Am. J. Enol. Vitic. 54, 15–21.

Skene, K.G.M. and Barlass, M. (1988) Effect of vine rootstocks on chloride concentration in sultana scions. Am. J. Enol. Vitic. 39, 125–128.

Snyder, E. (1937) Grape Development and Improvement. United States Department of Agriculture, Yearbook, Separate No 1587, 631–664.

Soulie, O., Roustan, J-P., and Fallot, J. (1993) Early *in vitro* screening of eutypine-tolerant plantlets. Application to screening of *Vitis vinifera* cv. Ugni blanc somaclones. Vitis 32, 243–244.

Sparapano, L., De Leonardis, S., Campanella, A., and Bruno, G. (2001) Interaction between esca-associated fungi, grapevine calli and micropropagated shoot cultures of grapevine. *Phytopathol. Mediterr*. 40, Supplement, S423–S428.

Spiegel-Roy, P., Sahar, N., Baron, J., and Lavi, U. (1985) *In vitro* culture and plant formation from grape cultivars with abortive ovules and seeds. J. Amer. Soc. Hort. Sci. 110, 109–112.

Spiegel-Roy, P., Shulman, Y., Baron, I., and Ashbel, E. (1987) Effect of cyanamide in overcoming grape seed dormancy. HortScience 22, 208–210.

Spiegel-Roy, P., Baron, Y., and Sahar, N. (1990a) Inheritance of seedlessness in seeded X seedless progeny of *Vitis vinifera* L. Vitis 29, 79–83.

Spiegel-Roy, P., Sahar, N., and Baron, J. (1990b) Seedless × seedless grape progeny: Technique, results and perspective. *In Vitis* Special edition, Proc. 5th Intern. Symp. Grape Breeding, St. Martin/Pfalz, Germany, 12–16 September 1989, pp. 432–438.

Staudt, G. (1982) Pollenkeimung und Pollenschlauchwachstum *in vivo* bei *Vitis* und die Abhängigkeit von der Temperatur (Eng. abstr.). Vitis 21, 205–216.

Staudt, G. (1999) Opening of flowers and time of anthesis in grapevines, *Vitis vinifera* L. Vitis 38, 15–20.

Staudt, G. and Kassemeyer, H.H. (1995) Evaluation of downy mildew resistance in various accessions of wild *Vitis* spp. Vitis 34, 225–228.

Stein, U., Heintz, C., and Blaich, R. (1985) Die *in vitro* Prüfung von Rebsorten auf *Oidium* und *Plasmopara* Resistenz (Eng. abstr). *Z*. Pflantzenkrank. Pflanzenschutz 92, 355–369.

Stout, A.B. (1937) Breeding for hardy seedless grapes. Proc. Amer. Soc. Hort. Sci. 34, 416–420.

Striem, M.J., Spiegel-Roy, P., Baron, J., and Sahar, R. (1992) The degree of development of the seed-coat and the endosperm as separate sub-traits of stenospermocarpic seedlessness in grapes. Vitis 31, 149–155.

Striem, M.J., Ben-Hayyim, G., and Spiegel-Roy, P. (1994) Developing molecular genetic markers for grape breeding, using polymerase chain reaction procedures. Vitis 33, 53–54.

Striem, M.J., Ben-Hayyim, G., and Spiegel-Roy, P. (1996) Identifying molecular genetic markers associated with seedlessness in grape. J. Amer. Soc. Hort. Sci. 121, 758–763.

This, P., Lahogue, F., Adam-Blondon, A.F., Doligez, A., and Bouquet, A. (2000) Towards marker-assisted selection for seedlessness in grapevine. Acta Hortic 528, 221–229.

This, P., Roux, C., Parra P., Siret, R., Bourse, T., Adam, A.F., Yvon, M., Lacombe, T., David, J., and Boursiquot, J.M. (2001) Characterization of genetic diversity in a population of wild grapes from Pic Saint Loup area and its relationship with the cultivated grapes. Genet. Select. Evol. 33, 289–304.

This, P., Jung, P., Boccacci, P., Borrego, J., Botta, R., Costantini, L., Crespan, M., Dangl, G.S., Eisenheld, C., Ferreira-Monteiro, F., Grando, S., Ibáñez, J., Lacombe, T., Laucou, V., Magalhães, M., Meredith, C.P., Milani, N., Peterlunger, E., Regner, F., Zulini, L., and Maul, E. (2004) Development of a standard set of microsatellite reference alleles for identification of grape cultivars. Theor. Appl. Genet. 109, 1448–1458.

Thomas, M.R., Franks, T., and Iocco, P. (2000) Transgenic grapevines: status and future. Acta Hortic 528, 279–288.

Todorov, I. (2000) The inbreeding in grapevine (*Vitis vinifera* L.) genetic improvement. Acta Hortic. 528, 631–639.

Tomkins, M.R., Peterson, D.G., Yang, T.J., Ablett, E.R., Henry, R.J., Lee, L.S., Holton, T.A., Waters, D., and Wing R.A. (2001) Grape (*Vitis vinifera* L.) BAC library construction, preliminary STC analysis and identification of clones associated with flavonoid and stilbene biosynthesis. Am. J. Enol. Vitic. 52, 287–291.

Torregrosa, L. and Bouquet, A. (1997) *Agrobacterium rhizogenes* and *A. tumefaciens* co-transformation to obtain grapevine hairy roots producing the coat protein of grapevine chrome mosaic nepovirus. Plant Cell Tiss. Org. Cult. 49, 53–62.

Torregrosa, L, Bouquet, A., and Goussard, P.G. (2001) *In vitro* culture and propagation of grapevine, pp. 281–326. *In: Molecular Biology and Biotechnology of the Grapevine*. K.A. Roubelakis-Angelakis (Ed.), Kluwer Publish. Co., Amsterdam, The Netherlands.

Troncoso, A., Matte, C., Cantos, M., and Lavee, S. (1999) Evaluation of salt tolerance of *in vitro* grown grapevine rootstock varieties. Vitis 38, 55–60.

Van Mieghem, A.P. and Goussard, P.G. (1987) The *in vitro* reproduction of *Meloidogyne javanica* on grapevine roots (*Vitis vinifera* L., cv. Chenin blanc). Phytophylactica 19, 339–340.

Vidal, J.R., Kikkert, J.R., Wallace, P.G., and Reisch, B.I. (2003) High efficiency biolistic co-transformation and regeneration of Chardonnay (*Vitis vinifera* L.) containing *npt*II and antimicrobial peptide genes. Plant Cell Rep. 22, 252–260.

Vidal, J.R., Kikkert, J.R., Malnoy M.A., Wallace, P.G., Barnard J., and Reisch, B.I. (2006) Evaluation of transgenic 'Chardonnay' (*Vitis vinifera* L.) containing magainin genes for resistance to crown gall and powdery mildew. Transgenic Res. 15, 69–82.

Vigne, E., Komar, V., and Fuchs, M. (2003) Field safety assessment of recombination in transgenic grapevines expressing the coat protein gene of grapevine fanleaf virus. Transgenic Res. 13, 165–179.

Wagner, R. (1967) Étude de quelques disjonctions dans les descendances du Chasselas et du Muscat à petits grains. Vitis 6, 353–363.

Wang, Y., Liu, Y., He, P., Chen, J., Lamikanra, O., and Lu, J. (1995) Evaluation of foliar resistance to *Uncinula necator* in Chinese wild *Vitis* species. Vitis 34, 159–164.

Wei, X., Sykes, S.R., and Clingeleffer, P. (2002) An investigation to estimate genetic parameters in CSIRO's table grape breeding programme. 2. Quality characteristics. Euphytica 128, 343–351.

Weinberger, J.H. and Harmon, F.N. (1964) Seedlessness in *vinifera* grapes. Proc. Amer. Soc. Hort. Sci. 85, 270–274.

Yamamoto, T., Iketani, H., Ieki, H., Nishizawa, Y., Notsuka, K., Hibi, T., Hayashi, T., and Matsuta, N. (2000) Transgenic grapevine plants expressing a rice chitinase with enhanced resistance to fungal pathogens. Plant Cell Rep. 19, 639–646.

Zhu, Y.M., Hoshino, Y., Nakano, M., Takahashi, E., and Mii, M. (1997) Highly efficient system of plant regeneration from protoplasts by grapevine (*Vitis vinifera* L.) through somatic embryogenesis by using embryogenic callus culture and activated charcoal. Plant Sci. 123, 151–157.

Zyprian, E., Eibach, R., and Töpfer, R. (2003) Comparative molecular mapping in segregating populations of grapevine. Acta Hortic 603, 73–77.