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New Horizons for Grapevine Breeding

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ABSTRACT

The introduction of fungi – particularly powdery and downy mildew – and of phylloxera during the second half of the 19th century was the catalyst to initiate enormous grapevine breeding activities in several European countries. These efforts aimed at the combination of resistance traits found e.g. in American *Vitis* species and quality traits found in the cultivated *Vitis vinifera* L. subsp. *vinifera*. It became evident that grapevine breeding is a huge challenge due to the complexity of traits and long breeding cycles of about 25 years. Despite some major drawbacks, at the onset of the 20th century rootstocks became available solving the phylloxera crisis. In contrast to the progress in rootstock breeding for some decades, it was believed that the aim for scions of combining resistance against the mildew diseases and quality can not be achieved. By the end of the 20th century, however, first cultivars were introduced into the market showing high wine quality and good field resistance against powdery and downy mildew. Simultaneously new technologies were developed to genetically dissect traits e.g. by QTL analysis and molecular markers were introduced into breeding research. Genetic fingerprints characterizing cross parents, marker assisted selection, and marker assisted backcrossing recently initated a paradigm shift in grapevine breeding from a purely empirical work to the strictly goal-oriented design of crosses and of gene management. These new tools and next generation sequencing technologies will reduce the breeding cycle by up to 10 years. In addition, genetic engineering opens the door to improve existing cultivars, for which otherwise any improvement of resistance is utterly impossible.

Keywords: breeding, genome analysis, grapevine, genetic mapping, genetic resources, marker assisted selection, transgenic plants, *Vitis*
Abbreviations: BAC, bacterial artificial chromosome; **bp**, base pair; **GC**, gas chromatography; **GM**, genetically modified; **GMO**, genetically modified organism; **ha**, hectares; **hl**, hectolitre; **LC**, liquid chromatography; **MABC**, marker-assisted backcrossing; **MAS**, marker-assisted selection; **Mb**, mega base pair; **MS**, mass sprectrometry; **pBC**, pseudo backcross; **RGA**, resistance gene analogue; **SCAR**, sequence characterized amplified region; **SNP**, single nucleotide polymorphism; **SSR**, simple sequence repeat; **t**, ton

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INTRODUCTION

Grapevine (*V. vinifera* L. subsp. *vinifera*) is one of the oldest cultivated plants tightly linked to the cultural development of mankind as no other crop plant. The primary centre of domestication from the wild Eurasian grapevine *Vitis vinifera* L. subsp. *sylvestris* (C.C. Gmelin) Hegi is most likely the Transcaucasian region (Vavilov 1930; Myles *et al*.

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Invited Review

2010). Therefrom grapevine moved via Mesopotamia, Egypt, with the Phoenicians, Greeks and the Romans around the Mediterranean basin and northwards. Secondary hybridisation events have been proposed for the western Mediterranean region (Grassi *et al.* 2003; Arroyo-Garcia *et al.* 2006; Lopes *et al.* 2009; Cunha *et al.* 2010). Originally grapevine surely has attracted humans for its tasty fruit when consumed either fresh or as a dried fruit which can be stored for some time. But later in development of human culture fermented beverages became highly desired for religious, social, and military purposes. They were microbiologically rather safe and storable and provided also valuable nutritives. Wine making from grapes is documented by artefacts dating back to the Neolithic period about 7000 – 7400 years ago in northern Iraq (McGovern 1996). Grapevine cultivation most widely spread over Europe before Christ and after that during Christianisation until the late Middle Ages and was disseminated around the world in the course of colonisation from the beginning of the 15th century.

It is anticipated that worldwide 8,000 to 12,000 grapevine cultivars exist, mainly used for wine production (56.8%) but also for table grapes (27.0%), a mixed utilisation for both wine and table grapes (7.3%), and finally dried fruits (0.7%). Other genotypes are used as rootstocks (www.vivc.de). Plenty of former cultivars may be extinct and others survived only in grapevine repositories. Romans like Virgil (70-19 B.C.), Columella (4-70 A.D.), and Pliny the Elder (23-79 A.D.) were the first mentioning around 100 different varieties. Their names mostly referred to the regions of origin or described properties and up to now can – except for speculations – not be assigned to currently existing varieties. One of the oldest known genotypes is the cultivar 'Gouais Blanc' having dozens of synonyms like 'Gwäss' or 'Weisser Heunisch'. It was first mentioned by Philippe de Beaumanoir in 1283. 'Gouais Blanc' together with the 'Pinots', a family of also very old cultivars, forms the parentage of numerous cultivars of present importance (Bowers *et al.* 1999; Boursiquot *et al.* 2004). How these cultivars emerged remains unclear. It is tempting to speculate that they originated from occasional selections rather than from planned breeding activities. The first clear cut evidence for controlled grapevine breeding efforts is found in America during the late 18th century.

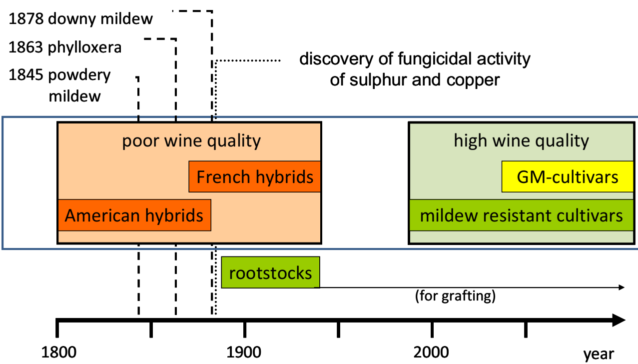

Fig. 1 Milestones in grapevine resistance breeding on the time scale. Red: American and French Hybrids did not succeed in the market due to poor wine quality. Green: phylloxera tolerant or resistant rootstocks saved viticulture in Europe. Newly bred wine grape cultivars showing good field resistance and high wine quality entered the market around the turn of the millennium. Decoupling of resistance and quality could be proven in the 1960th but these cultivars were not accepted in the market (see text). Yellow: Genetically modified cultivars will become available at the earliest in about two decades if consumer acceptance will be given. Appearance of mildew fungi and phylloxera in Europe and the discovery of sulphur and copper as fungicides are indicated.

HISTORY OF GRAPEVINE BREEDING

Wine grapes

At the end of the 18th century the origin of grapevine breeding arose from the insight of two hundred years of unsuccessful trials to cultivate the Old World grape, *V. vinifera* L. subsp. *vinifera*, in eastern America (Hedrick 1908). To make a long story short, unfavourable conditions, pests and climatic factors, had caused the failure. "In comparing the vines, those of the Old World grape are more compact in habit, make a shorter and stouter annual growth, and therefore require less pruning and training. The roots are fleshier and more fibrous. The species, taken as a whole, is adapted to far more kinds of soil, and much greater differences in environment, and is more easily propagated from cuttings, than most of the species of American grapes" (Hedrick 1908). Bolling in his *Sketch of Vine Culture* (1765), was probably the first suggesting to raise "new varieties, by marrying our native [American] with foreign [European] vines". He gave a plan to plant vines as to "interlock their branches as that they shall be completely blended together" and expected from the offspring that, "it is probable that we shall obtain other varieties better adapted to our climates and better for wine and table, than either of those kinds from which they sprung" (Hedrick 1908). The first cultivar successfully grown in the New World was 'Alexander', a native grape originating from *Vitis labrusca* L. It was selected around 1800 by the Frenchman Peter Legaux (Hedrick 1908). First documented cultivars and defined crossings are 'Sage' (H.E. Sage, 1811[1]), 'Cunningham' (J. Cunningham, 1812), 'Isabella' (N.N., 1816), 'Catawba' (Scholl, 1819), and 'Flowers' (B. Flowers, 1819) (www.vivc.de). These and other cultivars are well known as American hybrids (**Fig. 1**).

In European countries and first in France major breeding activities emerged as a consequence of the introduction of powdery mildew (1845, *Erysiphe necator* (formerly *Uncinula necator*, Braun and Takamatsu 2000), anamorph: *Oidium tuckeri*, Berk.), phylloxera (1863, *Daktulosphaira vitifoliae* Fitch), and downy mildew (1878, *Plasmopara viticola* (Berk. & Curt. *ex.* De Bary)). These pathogens changed dramatically the many thousand years old tradition of viticulture in Europe (see **Fig. 1**). The use of sulphur and copper as first found to possess useful fungicide activity in the Bordeaux mixture (Millardet 1885) became inevitable to combat the mildew fungi, and still in our days an extraordinarity intense plant protection is necessary (Phytowelt *et al.* 2003). In 1878 Millardet suggested to combine the fruit quality of *V. vinifera* L. subsp. *vinifera* and the resistance against powdery and downy mildew found in American wild species. A biological trick was found rather soon against phylloxera, which nevertheless took decades to be acceptable for the market: the use of grafted vines (scions of traditional cultivars (with leaf-resistance to phylloxera) on phylloxera root-tolerant rootstocks (see below)). An acceptable solution of the mildew problem by breeding took about 120 years to become reality and first cultivars showing good field resistance and high wine quality were introduced at the turn of the millennium (**Fig. 1**).

In addition to the activities initiated at public instituteons in France at the end of the 19th century to combat the pests also various dedicated private viticulturists started their own breeding programmes in order to combine "European wine quality" with "American resistance". The resulting hybrids were called "direct producers" indicating that they could be grown on their own roots. Private French breeders like Albert Seibel (1844-1936), Georges Couderc (1850-1928), Eugene Kuhlmann (1858-1932), Bertille Seyve (1864-1939), Seyve-Villard (1895-1959) and others made thousands of crosses resulting in tens of thousands of seedlings from which the best grape genotypes where selected. Some of these showed quite mediocre wine quality

[1] year of crossing

Table 1 Grapevine cultivars derived from resistance breeding, which are listed in the official German variety list. The year of crossing and admission, respectively, indicates the time required for breeding. Prior to admission, growing a new cultivar is only permitted as an experimental planting.

Cultivar	Parentage	Year of Crossing/ Admission	Breeder	Institution
Rondo	Zarya Severa x Saint Laurent	1964/1999	Becker, Helmut	FA Geisenheim
Hibernal	(Seibel 7053 x Riesling)F2	? /1999	Becker, Helmut	FA Geisenheim
Saphira	Arnsburger x Seyve Villard 1-72	1978/2004	Becker, Helmut	FA Geisenheim
Principal	Geisenheim 323-58 x Ehrenfelser	1971/1999	Becker, Helmut	FA Geisenheim
Bolero	(Rotberger x Reichensteiner) x Chancellor	1982/2008	Becker, Helmut	FA Geisenheim
Orion	Optima x Villard Blanc	1964/1994	Alleweldt	JKI Geilweilerhof
Phoenix	Bacchus x Villard Blanc	1964/1992	Alleweldt	JKI Geilweilerhof
Regent	Diana x Chamboucin	1967/1995	Alleweldt	JKI Geilweilerhof
Sirius	Bacchus x Villard Blanc	1964/1995	Alleweldt	JKI Geilweilerhof
Staufer	Bacchus x Villard Blanc	1964/1994	Alleweldt	JKI Geilweilerhof
Felicia	Sirius x Vidal Blanc	1984/ -	Eibach & Töpfer	JKI Geilweilerhof
Villaris	Sirius x Villard Blanc	1984/ -	Eibach & Töpfer	JKI Geilweilerhof
Reberger	Regent x Lemberger	1986/ -	Eibach & Töpfer	JKI Geilweilerhof
Calandro	Domina x Regent	1984/ -	Eibach & Töpfer	JKI Geilweilerhof
Johanniter	Riesling x Freiburg 589-54	1968/2001	Zimmermann	WBI Freiburg
Merzling	Seyval Blanc x (Riesling x Pinot Gris)	1960/1995	Zimmermann	WBI Freiburg
Baron	Cabernet Sauvignon x Bronner	1983/ -	Becker, Norbert	WBI Freiburg
Bronner	Merzling x (Zarya Severa x Saint Laurent)	1975/1999	Becker, Norbert	WBI Freiburg
Cabernet Cantor	Chancellor x Solaris	1989/ -	Becker, Norbert	WBI Freiburg
Cabernet Carbon	Cabernet Sauvignon x Bronner	1983/2008	Becker, Norbert	WBI Freiburg
Cabernet Carol	Merzling x Solaris	1982/2008	Becker, Norbert	WBI Freiburg
Cabernet Cortis	Cabernet Sauvignon x Solaris	1982/2008	Becker, Norbert	WBI Freiburg
Helios	Merzling x Freiburg 986-60	1973/2005	Becker, Norbert	WBI Freiburg
Monarch	Solaris x Dornfelder	1988/2008	Becker, Norbert	WBI Freiburg
Prior	(Joannes Seyve 234-16 x Pinot Noir) x Bronner	1987/2008	Becker, Norbert	WBI Freiburg
Solaris	Merzling x (Severnyi x Muscat Ottonel)	1975/2004	Becker, Norbert	WBI Freiburg

combined with a high expression of resistance characteristics. They were recognized as the so-called "French Hybrids" (**Fig. 1**). In 1929 the plantation surface of these French Hybrids covered about 250,000 hectares (ha) and it reached its peak in 1958 with about 500,000 ha. Due to the limited wine quality and political decisions their area decreased later on. Nowadays the "French Hybrids" are almost totally removed from production. In retrospective, the bad image of the French Hybrids prevented any continuation of the breeding programmes in France. While the breeding efforts stopped in France, countries like Germany, Hungary, or others used the valuable French material for their own pursuing breeding activities.

To introduce resistances into the gene pool of *V. vinifera* L. subsp. *vinifera* breeders generated F1-plants by interspecific crosses. This strategy was quite successful for rootstock breeding, but for wine grapes it yielded only unacceptable genotypes. Consequently, Erwin Baur (1922) suggested to create in a first step a small number of interspecific hybrids between *V. vinifera* L. subsp. *vinifera* and a wild species as a resistance donor to generate an F1 generation selected for resistance, vigour, and yield (10-12 plants). Following multiplication of these F1 plants, in a second step the selection should be performed at the level of large populations (about 100,000 plants) of the F2 generation generated from sister pollination. The outline was the consequent application of Mendel's laws re-discovered in 1900. To generate large numbers of seeds derived from defined crosses always remained a challenge. It finally turned out that it requires more than two generations from the wild to select acceptable genotypes and even more crosses to obtain really elite lines and new quality cultivars.

The huge efforts in France prepared the ground for the break through though the "French Hybrids" failed. In Germany for example where resistance breeding was initiated in the early 1920th the development took a different direction. While in France first private breeders retired, Erwin Baur and others initiated publicly funded breeding programmes and took advantage of the breeding material and cultivars developed in France. As a consequence of the continuation of breeding activities for decades and despite the poor image of "French Hybrids" concerning quality, Husfeld was the first who proved that resistance and quality can be combined (Alleweldt 1977). His cultivars 'Aris' ((Oberlin 716) F1 x 'Riesling', cross 1937) and 'Siegfriedrebe' ((Oberlin 595) F1 x 'Riesling', cross 1936) showed a convincing wine quality and high mildew resistance. Unfortunately, these two cultivars could not satisfy the wine growers due to insufficient yield and virus susceptibility (Alleweldt 1977). A next generation cultivars like 'Phoenix' ('Bacchus' x 'Villard Blanc', cross 1964) or 'Regent' (('Silvaner' x 'Müller-Thurgau') x 'Chambourcin', cross 1967) was developed by Alleweldt. Husfeld and Alleweldt used a breeding scheme similar to that given in **Fig. 4** except for MAS which is a recent development. 'Regent', 'Phoenix', and other cultivars gained access to the market (see **Table 1**) and it is just a matter of time to review their success and recognize their overall value. Up to now the most successful cultivar derived from resistance breeding in Germany is cv. 'Regent' being grown on more than 2,200 ha (2008). The numerous cultivars selected (see **Table 1**) at various breeding stations in Germany are the outcome of continuation and the use of step-wise improved breeding material. They are today's basis of prosperous breeding which will result in further improvements in regard to pathogen resistance and quality of grapevines.

Rootstocks

In 1868 phylloxera (introduced in 1863) was identified as the devastating pest destroying the vineyards in France. Its rapid spread throughout France eliminated within 15 years about 800,000 ha of vineyards. Its subsequent spread throughout Europe was a serious threat for the survival of viticulture. No treatment whatsoever (e.g. removal of vines and/or various chemical treatments or flooding of vineyards with water) could stop the pest from dissemination which was spread rapidly by planting material, wind, and surface water. Observations in the grape collection in Bordeaux showed that some American hybrids exhibited a certain resistance against phylloxera on their roots. In 1869 Laliman first suggested to use phylloxera resistant American vines as rootstocks for the traditional European grapevine varieties. In 1872 Bazille performed the first successful graftings.

Table 2 Important rootstock cultivars and their parentage.

Cultivar	Parentage	Year of crossing/selection	Breeder	Institution
Riparia Gloire de Montpellier	*Vitis riparia*	1880	Viala & Michel	
Rupestris du Lot	*Vitis rupestris*		Sijas	private
Rupestris St George	*Vitis rupestris*	1860s		
Millardet et Grasset 101- 14	*Vitis riparia* x *Vitis rupestris*	1882	Millardet & de Grasset	private
Couderc 3309	*Vitis riparia* x *Vitis rupestris*	1881	Couderc & Georges	private
Ruggeri 140	*Vitis berlandieri** x *Vitis rupestris*	1897	Ruggeri	
Richter 99	*Vitis berlandieri x Vitis rupestris*	1889	Richter	private
Richter 110	*Vitis berlandieri x Vitis rupestris*	1889	Richter	private
Paulsen 1103	*Vitis berlandieri x Vitis rupestris*	1895	Paulsen & Federico	Vivaio Governativo di Viti Americane di Palermo (V.G.V.A.)
Selektion Oppenheim SO4	*Vitis berlandieri* x *Vitis riparia*	1896	Oppenheim	private
Kober 5 BB	*Vitis berlandieri* x *Vitis riparia*	1896	Kober & Teleki	private
Kober 125 AA	*Vitis berlandieri* x *Vitis riparia*	1896	Kober & Teleki	private
Teleki 5 C	*Vitis berlandieri* x *Vitis riparia*	1922	Teleki	private
Börner	*Vitis riparia* x *Vitis cinerea*	1930s	Börner	FA Geisenheim

* new nomenclature: *Vitis berlandieri* = *Vitis cinerea* Engelm. var. *helleri*

American cultivars like 'Clinton', 'Jaquez' and others were recommended as rootstocks. But the degree of resistance of these cultivars proved to be not high enough. Hence Millardet recommended in 1878 to use pure American *Vitis* species like *Vitis riparia* Michx., *Vitis rupestris* Scheele, *Vitis cinerea* Engelm. var. *cinerea*, *Vitis vulpina* L., or *Vitis aestivalis* Michx. (**Table 2**). However, soon it became evident that the tolerance of these species to lime soils is rather poor. In 1887 Viala conducted an expedition through North America. In Texas he found *Vitis berlandieri* Planch. (today called *V. cinerea* Engelm. var. *helleri*) which grows very well on calcareous soils. But because of the poor rooting ability of this species crosses with other *Vitis* species, mainly with *V. riparia* Michx., were performed in several research institutes in France. This was the beginning of a target oriented rootstock breeding leading in the end to a series of rootstock cultivars with good rooting ability and good adaptation to calcareous soils (**Table 2**).

A major impact came from the Hungarian winegrower Zsigmond Teleki when he received about 10 kg of seeds of open pollinated *V. cinerea* Engelm. var. *helleri* in 1896 from Rességuier, a French viticulturist. Teleki grew about 40,000 seedlings and selected them first according to their morphology. Later he tested them in various calcareous soils. The best growing genotypes were propagated and multiplied. Some of the most promising genotypes were transferred to Franz Kober in Austria for further selection and finally distributed to various locations in Europe where very important rootstock cultivars like 'Kober 5 BB' could be selected (**Table 2**) (Manty 2006).

There is no doubt about the vital importance of the development of rootstocks to rescue viticulture from phylloxera crisis. It is the greatest success breeders could have achieved. However, genetic analyses done in the past were less successful. One of the most important objectives for rootstock breeding was the resistance against phylloxera. Therefore, great emphasis was given to elucidate the genetics of phylloxera resistance, however, without any final conclusion (Börner 1943; Breider 1969; Manty 2006). This might be due to the material analysed which originates from a small number of genotypes representing a limited genetic basis (Schmid *et al*. 2007). Almost all of this material shows rather tolerance than resistance. Since rootstocks became available at the beginning of the 20th century (see **Fig. 1**) and brought the solution of the phylloxera disaster, rootstock breeding activities declined. Nevertheless rootstock breeding programmes are continued and research is directed to elucidate the genetics of certain traits (see below).

BOTANICAL DESCRIPTION AND GENETIC RESOURCES

The genus *Vitis* consists of about 70 species which are endemic to the northern hemisphere. *Vitis* species are found in North and Central America (*ca*. 30 species), Asia (*ca*. 40 species), as well as in Europe and Asia Minor (1 species) (**Fig. 2A**). *Vitis* plants are dioecious liana usually growing up to the top of supporting trees (**Fig. 3A**). Their pollen is rather small thus being disseminated predominantly by wind. *Vitis* species are principally cross-fertile and interspecific hybrids may occur naturally. However, *in situ* the species are kept apart probably due to geographic isolation and different timing of flowering.

In general the so-called European wine grape, *V. vinifera* L. subsp. *vinifera* is cultivated (**Fig. 3B**) for wine grape, table grape, and dried fruit production, while its wild European relative *V. vinifera* L. subsp. *sylvestris* (C. C. Gmelin) Hegi is endangered to become extinct. Almost all cultivated vines are hermaphroditic and normally need three years from planting to first fruit-set. They are propagated vegetatively by hard wood cuttings and are grown between 52° latitude north and 40° latitude south. Though cultivated vines are self-fertile, high inbreeding depression occurs maintaining high heterozygosity and preventing recurrent backcrosses with the same cultivar. The only nearly homozygous genotype is a Pinot noir inbred line (F8) which was used for genome sequencing and development of the reference genome sequence (Jaillon *et al*. 2007). Thus, for breeding purposes pseudo-backcrossing (pBC) is required changing the (recurrent) *V. vinifera* L. subsp. *vinifera* parent at each crossing step to develop introgression lines. Despite of self-fertility out-crossing occurs in the vineyard which, as determined in a pilot study, was found to be in a low percentage range within a distance of up to 20 m (Harst *et al*. 2009).

Depending on the cultivar unfavourable weather conditions during bloom result in a failure of berry development and reduced yield. This phenomenon is known as "millerandage". Generally berries might contain up to 5 seeds but on average between two to three seeds are found. A reduced seed set has a significant impact on the yield since berry size in grapevine is positively correlated with seed formation: the smaller the seed number the smaller the berry. As peculiarity seedlessness does occur which is the most important trait for table grape breeding. Two forms of seedlessness do exist: parthenocarpy and stenospermocarpy (Ledbetter and Ramming 1989). Fruit development after pollination but without fertilization (parthenocarpy) appears with 'Corinth' cultivars. Abortion of embryo development during early fruit growth after fertilization (stenospermocarpy) is found e.g. in 'Sultanina' (='Thompson Seedless' or 'Kishmish belyi').

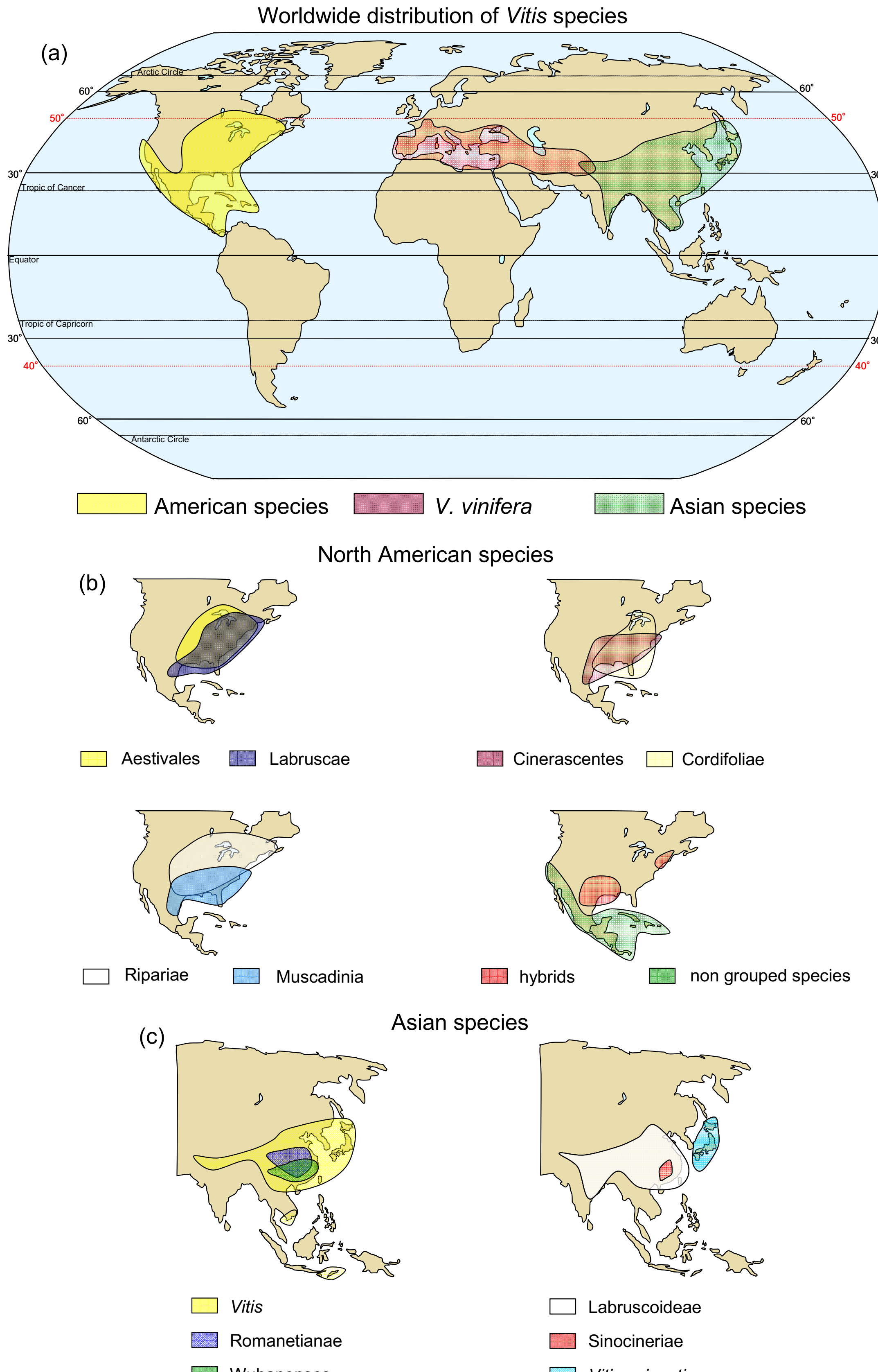

Fig. 2 Distribution of *Vitis* species around the world. The cultivated vine *V. vinifera* L. subsp. *vinifera* originates from Europe and Asia Minor. The most widely used source of resistance is the American gene pool, while the Asian gene pool is barely accessible. Geographical distribution according to Moore (1991), Tso and Yuan (1986), Galet (1988), and Wan *et al.* (2008b).

The genome of *Vitis* species is diploid and organized into 2 × 19 chromosomes. The chromosomes are very small and of similar size which makes it very difficult to distinguish them cytologically (Haas *et al*. 1994). Recent progress in molecular analysis of the grapevine genome revealed a rather small genome size for *V. vinifera* L. subsp. *vinifera* of about 500 Mb, roughly comparable to rice. This figure is based on investigations of Lodhi and Reisch (1995) calculating 475 Mb from flow cytometry. More recent data from whole genome sequencing published by Jaillon *et al*. (2007) and Velasco *et al*. (2007) calculate 487 Mb and 504 Mb, respectively.

As the European grape *V. vinifera* L. subsp. *vinifera* evolved in an environment without pests like powdery mildew (*E. necator*), downy mildew (*P. viticola*) or black rot (*Guignardia bidwellii*), the species does carry barely any resistance against these fungi[2]. Similarly against phylloxera (*D. vitifoliae*) high root susceptibility is observed resulting in a root rot within a few years due to secondary infections at the insects feeding sites. Though susceptible at the root, *V. vinifera* L. subsp. *vinifera* fortunately shows very high resistance to leaf attack of phylloxera. Thus, for continuation of viticulture the European grape can be grafted on tolerant or resistant rootstocks. As *V. vinifera* L. subsp. *vinifera* does not carry resistances against the pests mentioned, the entire primary gene pool has to be used for resistance breeding. In particular American species have been used as donors of resistances as outlined above. Species like *V. labrusca* L., *V. riparia* Michx., *V. rupestris* Scheele, and others are well known for resistance traits (Alleweldt and Possingham 1988). But also the Asian gene pool which, however, is poorly accessible can be used to improve resistances. In particular *Vitis amurensis* Rupr. has been applied in breeding programmes but also other species carry resistances (He and Wang 1986, Wan *et al*. 2007). Strong resistances have been found in the American species *Muscadinia rotundifolia* Michx., a relative ordered in a different genus, which carries 20 chromosomes in the haploid genome (Branas 1932; Patel and Olmo 1955). As it turned out *M. rotudifolia* Michx. can be used only with great difficulties to develop hybrids with *Vitis* species due to frequently sterile F1 plants. Irrespective of these problems a few very valuable introgression lines have been developed (Olmo 1986; Pauquet *et al*. 2001).

The distribution of *Vitis* species has been first summarized by de Lattin (1939). Most *Vitis* species of North America occur in the south and east. The Asian species are found predominantly in the Far East. Due to their relatedness the borders between species and subspecies are somewhat unclear and remain in the debate. Moore (1991) placed the *Vitis* species of central and east America in a new order. Based on thorough studies on similarity of morphological characteristics and geographical occurrence, sections and series have been built for both American aand Asian species (Moore 1991; Wan *et al.* 2008a). Thus, considering the International Code of Botanical Nomenclature, the well known species *V. berlandieri* Planch. became *V. cinerea* (Engelm.) Engelm. ex Millardet var. *helleri* (L.H. Bailey) M.O. Moore (Moore 1991). Species excluded in Moore's study are found beyond the non grouped species. **Fig. 2B** illustrates the distribution of the North American species (USDA; Galet 1988). Also the taxonomy of the Asian species is called into question. **Fig. 2C** presents the distribution of the Asian species (Tso and Yuan 1986; Galet 1988; Wan *et al.* 2008b). The summary of the current taxonomic view is given in **Table 3**.

ECONOMIC IMPORTANCE

Grapevine is one of the most important fruit crops which in 2008 was cultivated worldwide on approximately 7.7 Million ha (OIV 2009). On this basis 58% of grapes are cultivated in Europe, 21% in Asia, 13% in America, 5% in Africa, and 3% in Oceania. In 2008 grape production reached 67.8 million metric tonnes (t): For wine production 45.9 million t resulting in 269 million hectolitres (hl) of wine, 20.6 million t for table grapes and 1.3 million t for dry fruits (raisins, Corinth's). Details of the production per country for wine grapes, table grapes and raisins are given in **Table 4**. The largest wine producer with 3.5 million ha and 179 million hl is the EU with Italy, France, and Spain as the largest producers. Major table grape producers are China, Iran, Turkey, India, Egypt, and Italy and for dry fruits Turkey, USA, Iran, Greece, Chile, and South Africa. The vast majority of wines are produced from about 260 cultivars exceeding an acreage of 1,000 ha each (Eibach, unpublished data).

[2] Up to now only the *Ren*1 locus found in cv. 'Kishmish vatkana' is known as resistance factor in *V. vinifera* against powdery mildew (Hoffmann *et al*. 2008).

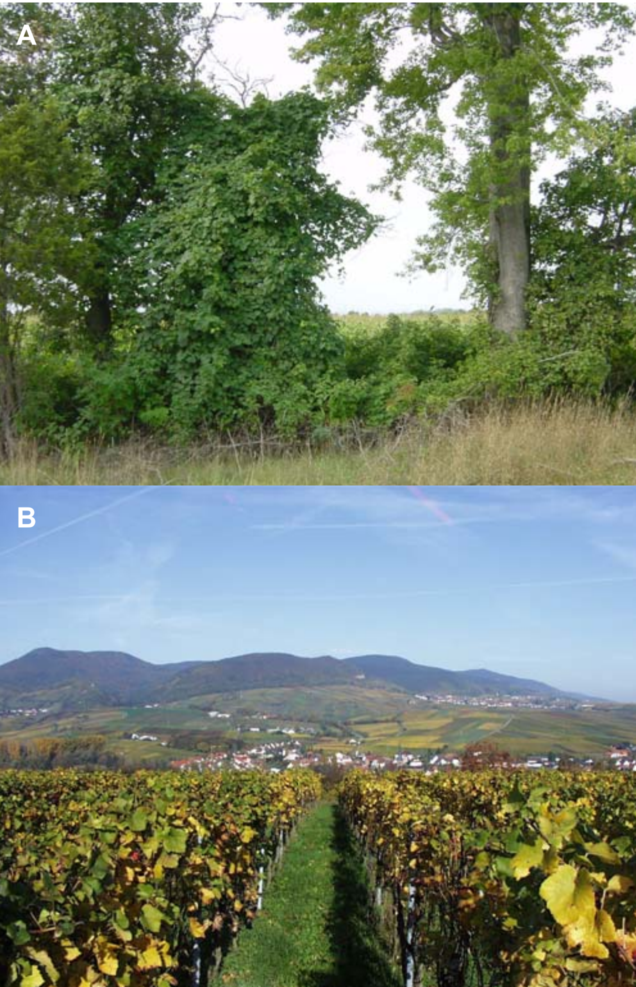

Fig. 3 Habitus of *Vitis* plants. (**A**) Wild grapevine in a natural habitat. (**B**) *V. vinifera* subsp. *vinifer*a in culture.

GENERAL BREEDING OBJECTIVES

Grapevine breeding is time consuming due to a long generation cycle, the requirement of several repetitions caused by environmental impact on the traits to get sufficient evaluation data for selection, limited plant material and slow propagation rates through hard wood cuttings (compare **Fig. 4**). Furthermore breeding goals need to be diversified according to the grapes/plants uses (see **Table 5**):

- **Clonal selection** is performed within existing cultivars in order to keep the cultivar phytosanitarily healthy and morphologically stable. Clonal selection makes use of the limited genetic variation given within a vegetatively propagated genotype (a cultivar) to select for variants (mutants) of certain traits. These may be loose clusters, higher sugar accumulation, aroma variants etc. Sometimes clonal variants have become independent cultivars. For example berry color mutants of 'Pinot noir' are 'Pinot gris', 'Pinot blanc' and a mutant with earlier ripening time is 'Pinot précoce noir'.
- In contrast to clonal selection controlled sexual reproduction is required for **cross breeding** allowing genetic segregation through meiosis and generating a wide genetic variation within the offspring. Depending on the utilisation, rootstocks being tolerant or resistant against phylloxera need to be distinguished from scions with

Table 3 Taxonomic classification of *Vitis* and *Muscadinia* species around the world.

North and Central America	**Europe**	**Asia**
Genus *Vitis* **Subgenus Euvitis** **Series**	**Genus *Vitis*** **Subgenus Euvitis** **Series**	**Genus *Vitis*** **Subgenus Euvitis** **Section**
Aestivales (*Vitis aestivalis* Michx. var. *aestivalis*, *Vitis aestivalis* Michx. var. *bicolor* Deam, *Vitis aestivalis* Michx. var. *lincecumii* (Buckley) Munson) **Cinerescentes** (*Vitis cinerea* (Engelm.) Engelm. ex Millardet var. *baileyana* (Munson) Comeaux, *Vitis cinerea* (Engelm.) Engelm. ex Millardet var. *cinerea, Vitis cinerea* (Engelm.) Engelm. ex Millardet var. *floridana* Munson*, Vitis cinerea* (Engelm.) Engelm. ex Millardet var. *helleri* (L.H. Bailey) M.O. Moore), *Vitis cinerea* (Engelm.) Engelm. ex Millardet var. *tomentosa* (Planch.) Comeaux) **Cordifoliae** (*Vitis vulpina* L., *Vitis palmata* Vahl, *Vitis monticola* Buckl.) **Labruscae** (*Vitis labrusca* L., *Vitis shuttleworthii* House, *Vitis mustangensis* Buckl.) **Ripariae** (*Vitis acerifolia* Raf., *Vitis riparia* Michx., *Vitis rupestris* Scheele) **Hybrids** (*Vitis* x *champinii* Planch. (pro sp.) [*mustangensis* x *rupestris*], *Vitis* x *doaniana* Munson ex Viala (pro sp.) [*acerifolia* x *mustangensis*], *Vitis* x *novae-angliae* Fernald (pro sp.) [*labrusca* x *riparia*]) **Non grouped species:** *Vitis arizonica* Engelm., *Vitis californica* Benth., *Vitis girdiana* Munson, *Vitis tiliifolia* Humb. & Bonpl. ex Schult.	**Viniferae** (*Vitis vinifera* L.) **Subspecies** *Vitis vinifera* L. subsp. *sylvestris* (C. C. Gmelin) Hegi *Vitis vinifera* L. subsp. *vinifera*	**Labruscoideae** (*Vitis pentagona* Diels et Gilg, *Vitis heyneana* subsp. *ficifolia* (Bunge) C. L. Li, *Vitis bellula* (Rehd.) W. T. Wang, *Vitis bellula* var. *pubigera* C. L . Li, *Vitis retordii* Roman. ex Planch., *Vitis hui* Cheng, *Vitis longquanensis* P. L.Qiu, *Vitis bashanica* P. C. He, *Vitis menghaiensis* C. L. Li.) **Sinocineriae** (*Vitis sinocinerea* W.T. Wang) **Vitis** **Series** **Vitis** (*Vitis amurensis* Rupr., *Vitis amurensis* Rupr. var. *dissecta* Skvorts, *Vitis betulifolia* Diels et Gilg, *Vitis wilsonae* Veitch, *Vitis flexuosa* Thunb., *Vitis pseudoreticulata* W. T. Wang, *Vitis yunnanensis* C. L. Li, *Vitis mengziensis* C. L. Li, *Vitis fengqinensis* C. L. Li, *Vitis balanseana* Planch., *Vitis chunganensis* Hu, *Vitis piloso-nerva* Metcalf, *Vitis chungii* Metcalf, *Vitis luochengensis* W. T. Wang, *Vitis luochengensis* var. *tomentoso-nerva* C. L. Li, *Vitis hekouensis* C. L. Li) **Piasezkianae** (*Vitis piasezkii* Maxim., *Vitis piasezkii* var. *pagnucii* (Planch.) Rehd., *Vitis lanceolatifoliosa* C. L. Li) **Davidianae** (*Vitis davidii* (Roman.) Föex, *Vitis davidii* (Roman.) Föex var. *ferruginea* Merr. et Chun, *Vitis davidii* (Roman.) Föex var. *cyanocarpa* (Gagnep.) Gagnep. **Adstrictae** (*Vitis bryoniaefolia* Bunge, *Vitis bryoniaefolia* var. *ternate* (W. T. Wang) C. L. Li, *Vitis zhejiang-adstricta* P.L. Qiu **Romanetianae** (*Vitis romanetii* Roman. ex Planch., *Vitis romanetii* Roman. var. tomentosa Y. L. Cao et Y. H. He, *Vitis shenxiensis* C. L. Li **Wuhanenses** (*Vitis wuhanensis* C. L. Li, *Vitis silvestrii* Pamp., *Vitis wenchouensis* C. Ling ex W. T. Wang, *Vitis tsoii* Merr. *Vitis ruyuanensis* C. L. Li, *Vitis jinggangensis* W. T. Wang, *Vitis erythrophylla* W. T. Wang, *Vitis hancockii* Hance) *Vitis coignetiae* Pulliat ex Planch.
Genus Muscadinia		
Muscadinia rotundifolia Michx. var. *rotundifolia* *Muscadinia rotundifolia* Michx. var. *munsoniana* (Simpson ex Munson) M. O. Moore *Muscadinia rotundifolia* Michx. var. *popenoei* Fennell		

fungal disease resistances and high berry quality for either table or wine grape.

The general breeding objectives for cross breeding are listed in **Table 6**. Achievement of the specific breeding goals for table or wine grapes respectively rootstocks requires totally independent breeding programmes and makes use of different kinds of genetic resources.

Rootstocks

For **rootstock improvement** mainly non-*vinifera* vines from the North American gene pool have been used for interspecific crosses. Despite of phylloxera resistance agronomical performance is the major issue in rootstock breeding since the grafted vine is influenced by many factors (**Table 7**) as yet poorly understood. Since *V. vinifera* is considered to be rather lime tolerant growing well on calcareous soils in Europe rootstocks need to be equally tolerant. The failure of the first generation of rootstocks was mostly due to insufficient adaptation to this kind of soil. Thus, first rootstocks were poor mediators of iron and mineral uptake into the vine. Consequently, rootstock breeding aims at lime tolerance which prevents iron chlorosis on calcareous soils.

Similarly rootstocks should tolerate drought to guarantee high quality berry development even during hot and dry weather periods. A source known for drought tolerance is e.g. *V. rupestris* Scheele. The quality of the tissue connection between scion and rootstock, so-called “affinity” is another characteristic, which is of crucial importance for the production of grafted vines. Also the ability to establish a good root system is of major importance in order to obtain a well and equally rooted grafted vine that can be established easily in the vineyard. The genetics of these traits still need to be investigated.

Table 4 Top 15 countries in grape production in 2008 (Source: OIV 2009). Corresponding figures for wine grapes, table grapes, and dry fruits are given, too.

Country	Grape production	Wine grape		Table grapes	Dry fruits
	Mio. [t]	Mio. [hl]	Mio. [t]	Mio. [t]	Mio. [t]
Italy	8.1	48.6	6.8	1.3	
China	7.2	12.0	2.4	4.8	0.01
USA	6.7	19.2	5.4	0.9	0.36
Spain	5.7	34.6	5.7		
France	5.7	41.4	5.7		
Turkey	3.9		1.8	1.7	0.37
Iran	3.0		1.0	1.8	0.23
Argentina	2.8	14.7	2.8		0.02
Chile	2.5	8.7	1.6	0.8	0.07
Australia	2.0	12.4	2.0		0.01
South Africa	1.8	10.3	1.5	0.2	0.04
India	1.7		0.1	1.6	
Egypt	1.5			1.5	
Brazil	1.4			0.7	
Germany	1.4	10.0			
others	12.4	57.1	9.1	5.1	0.20
World	67.8	269.0	45.9	20.6	1.30

Wine grapes

High wine quality combined with high disease resistances and good climatic adaptation summarize the major objectives in wine grape breeding since the initial breeding activities. These roughly formulated objectives of course need to be specified, but they describe certainly the main direction and the major demand (**Table 6**) which in more detail is given in **Table 8**. Depending on the climatic conditions, cool climate viticulture or hot climate viticulture, the kind of disease resistances required may vary. In any way the motivation for grapevine breeding around the world came from pests which are a continuous threat for a safe production. In recent times environmental concerns of the public are an additional driving force to get improved grapevine cultivars requiring less pesticide applications. A major difficulty in grapevine breeding was and still is the lack of knowledge about the genetics of major traits. However, already at the beginning of the 20th century when Mendel's laws could be applied in breeding programmes, first attempts were undertaken to systematically elucidate the inheritance of important traits.

Hedrick and Anthony, summarizing work with *Vitis* species in 1915, provided some data for inheritance of self-sterility, sex of the flower, colour of berry skin, berry size, berry shape, berry quality, and berry ripening time (Hedrick and Anthony 1915). In terms of genetics the only reliable conclusion which could be drawn was that berry colours black and red are dominant over white and white is homozygous recessive. Further details of colour formation could not be resolved indicating the complexity of this and other traits. However, Hedrick and Anthony already recognized inbreeding depression as a problem in grapevine breeding. They described that certain cultivars turned out to be rather poor parents to achieve vigorous and resistant F_1 plants essentially free of off-flavours and yielding good wine quality.

Further analyses were made during the last decades and several scientists contributed to our understanding of inheritance in the genus *Vitis* as cited by de Lattin (1957): leaf colour (Husfeld, de Lattin, Müller-Thurgau and Kobel, Rasmuson, Seeliger), berry colour (Hedrick and Anthony, Husfeld, de Lattin, Müller-Thurgau and Kobel, Satorius, Seeliger), berry juice colour (Branas, Bernon and Levadoux, Seeliger), leaf morphology (Negrul, Rasmuson), positioning of shoot tip (Husfeld), hairiness of shoot tip (Seeliger), growth habit (Husfeld), panaschure (Husfeld, Rasmuson, Seeliger) and parthenocarpy (Harmon and Snyder). For most of the traits data were not as clear as desired and not all of the variation could be explained. De Lattin resumed that breeders established large F1-progenies and selected desired genotypes being unable to resolve the genetic pattern of trait inheritance (de Lattin 1957). Aside from the complexity of the traits, one explanation for the difficulty to unravel their genetics could have been the problem of unrecognized selfings which might have occurred accidentally in crosses of monoecious parents resulting in apparently distorted segregation patterns. Generally speaking, during the 20th century some insights were gained but in most cases breeders remained far from a clear understanding of the genetics of the traits of interest. In 1962 Husfeld resumed that the manifold failure of early resistance breeding and genetic dissection of the traits was largely due to their complexity and to the insufficient knowledge of the plant material used (Husfeld 1962). Many traits in grapevine are polygenic and are subjected to environmental influences, thus being difficult to be resolved by classical approaches.

1. Berry and wine quality

A first attempt to elucidate berry quality genetically was reported by Hedrick and Anthony (1915). The authors analysed results of various crosses with different parental combinations. Most noticeable was the very low percentage of seedlings whose quality was good or above good even when parents of the best quality were used. The authors observed a tendency for the proportion of seedlings giving good quality to decrease with the use of parents showing poorer quality. They concluded that for breeding only high quality parents should be used. Thousands of years of selection of grapevine during domestication have raised the quality in *V. vinifera* subsp. *vinifera* to a point that it has become a powerful factor in transmitting high quality (Hedrick and Anthony 1915).

Berry quality and hence wine quality is by far the most complex trait in grape breeding. It relies on complex sensory perceptions including taste, smell, and mouthfeel. Selection of good quality genotypes depends on the organoleptic perception of a tasting panel thus being rather subjective. Berry quality is difficult to evaluate for table grapes and even more difficult for wine grapes since must fermentation by yeasts increases the complexity of the trait through metabolic conversions. The amounts of sugars, acids, fermentable nitrogen (amino acids), minerals (e. g. potassium), bal-

Table 5 Categories of grapevine breeding and the currently estimated period for developing a clone/cultivar. MAS is expected to reduce duration of breeding see **Fig. 4** and text.

Method	Breeding category	Years to breed a clone resp. cultivar	Reproduction and gene pool
clonal selection			**asexual reproduction**
	phytosanitary selection for keeping cultivars healthy and stable in yield	10 - 15	*Vitis vinifera*
	selection of variants within a cultivar (aroma, sugar content, lose clusters etc.)	random	*Vitis vinifera*
cross breeding			**sexual reproduction**
	rootstock breeding	30 - 50*	*Vitis* spec. (and *Vitis vinifera* introgression lines)
	breeding for table grapes	15 - 20*	*Vitis vinifera* (and *Vitis* spec.)
	breeding for wine grapes	25 - 30*	*Vitis vinifera* and *Vitis* spec.

* Counting from the cross to the introduction into the market

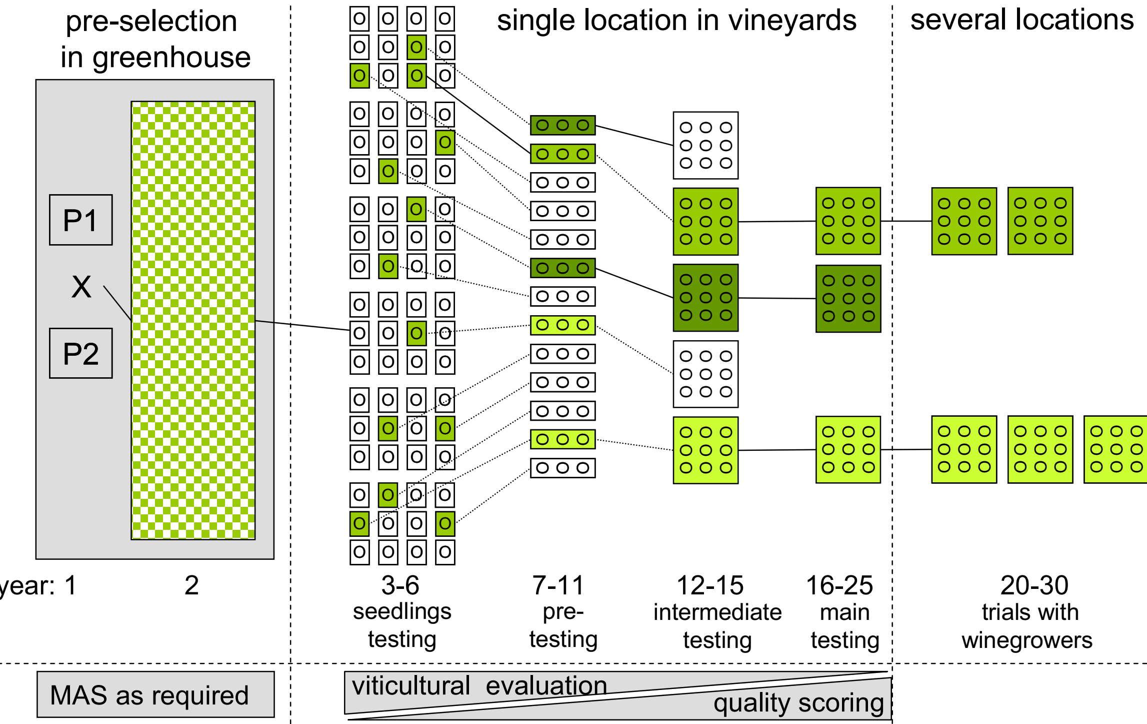

Fig. 4 Steps and timescale of a typical wine grape breeding programme. A pre-selection eliminating e.g. highly mildew susceptible vines is conducted in the greenhouse followed by MAS for traits difficult to evaluate prior to planting in the vineyard. MAS will receive increasing importance during the next couple of years. The various stages of testing, seedlings- (1 vine), pre- (10 vines), intermediate- (50 vines) and main testing (500 vines), with increasing numbers of vines are followed by trials in viticultural practise. Usually developing a new cultivar requires 25 to 30 years. Acceleration of the breeding process for up to 10 years is expected by the use of MAS and by merging pre- and intermediate testing to one testing phase as planting material becomes available.

Table 6 Comparison of the general objectives in cross breeding according to different utilisation of the plant/grape.

Major trait	Wine grapes	Table grapes	Rootstocks
Quality			
	high wine quality (e.g. high sugar, balanced acidity, flavours, colour, body of a wine)	seedlessness	
	taste	taste	
	free of off-flavours	free of off-flavours	
		berry texture	
		berry colour	
Resistance/tolerance (biotic)			
	Phylloxera resistance leaf	Phylloxera resistance leaf	phylloxera tolerance or resistance of roots
	Phylloxera resistance root (with perspective for own rooting)		nematode resistance
	powdery mildew resistance	powdery mildew resistance	
	downy mildew resistance	downy mildew resistance	
	Botrytis resistance	Botrytis resistance	
	Black root resistance	Black root resistance	
Resistance/tolerance (abiotic)			
	frost resistance		
	drought tolerance	drought tolerance	lime tolerance
	sun burn resistance	sun burn resistance	rooting ability
Maturity / Yield			
	balanced, stable yield	high, stable yield	
	maturity (preferably medium to late)	variation in time of ripening according to market demand	
Others			
	climate adaptation	climate adaptation	callus formation and affinity for grafting
	viticultural properties (i.e upright growth, medium vigour)		growth to support scion

anced (positive) aroma compounds, and lack of off-flavours in the must are major components to estimate berry quality. In particular the concentration, the balance, and the interactions of up to 800 different aroma compounds (Rapp 1994) – not all are relevant for sensory perception and most are formed during fermentation – are crucial for the appraisal of quality. In a wine, which is free of sugar after fermentation, any inharmonious taste can easily be recognized and off-flavours quickly emerge. Changes during storage and aging of wine need to be evaluated to uncover sensory deficits which are attributed to the breeding line. Within a breeding programme berry respectively must quality can be recorded only 4 to 5 years after a cross and it is strongly influenced by environmental factors. Furthermore, the amount of grapes available for experimental micro-vinification for assessment of wine quality is limited. The number of vines available impairs the scale of fermentation and hence a quality evaluation. Thus, the assessment of berry quality is direfully complex, most time consuming, and the most important trait to be evaluated. Up to now the trait

Table 7 Objectives in rootstock breeding.

Breeding goal	Range of characteristics		
1. Pest resistance			
root phylloxera	tolerance	resistance	
nematodes			
- damage by feeding	tolerance	resistance	
- vector for virus diseases	resistance		
2. Grafting properties			
affinity to scion	good callus formation		
rooting capability	high		
3. Agronomic performance			
vigour	low	medium	high
adaptation to calcareous soils	high		
salt tolerance	medium	high	
drought tolerance	medium	high	

Table 8 Objectives in wine grape breeding.

Breeding goal	Range of characteristics		
1. wine quality			
white	fruity	neutral	muscat/aromatic
red	dark colour	moderate colour	
rich in various components	tannins, flavonols	amino acids	potassium
sugar (hot or cold climate)	medium	high	
acidity (hot or cold climate)	high	medium	
off-flavours	none	none	none
other wine taste characters	well balanced taste	wine with rich body	long lasting wine
aging potential	medium aging potential	high	
2. agronomical performance			
resistances – fungi	*Erysiphe necator* (syn. *Uncinula necator*)	*Plasmopara viticola*	*Botryotinia fuckeliana* (syn. *Botrytis cinerea*)
	Black rot	*Anthracnose*	*Phomopsis viticola*
resistance - bacteria	*Pierce`s disease*	*Agrobacterium*	
resistances – insects	*Daktulosphaira vitifoliae*	*Xiphinema index* (*vector for viruses*)	
resistances – abiotic factors	frost	drought	sunburn
growth	upright		
berry ripening	early	middle	late
wood maturation	early	middle	
fruit characters	loose cluster	thickness of berry skin	
3. yield traits	**< 1 kg/m²**	**≤ 1.5 kg /m²**	**> 1.5 kg/m²**
berry size	small	medium	high
berries per cluster	< 200	200-300	> 300
cluster per cane	2	3	4

"quality" was treated mostly empirically with the help of trained tasting panels and analytical measurements of major must components.

2. Berry colour formation

Berry colour varies in a wide range from green/yellow (considered as white) to many shades of red and purple to black. Several authors found berry colour as a dominant trait (Hedrick and Antony 1915) though the variation in colour expression is influenced by additional factors. Genetic studies during the years could not resolve further details. Genetic maps produced by applying molecular markers (see below) localized the ability to form dark-coloured berries as a single qualitative trait on chromosome 2 (Doligez *et al*. 2006a; Welter *et al*. 2007). Using molecular tools a transposon integration in a regulatory *myb* gene (a transcription factor regulating the gene for the last enzymatic conversion in anthocyanin biosynthesis) was identified as causal for the white phenotype (Kobayshi *et al*. 2004; Lijavetzky *et al.* 2006; This *et al*. 2007; Walker *et al.* 2007). The expression of the *Myb* factor could widely explain the phenotypes qualitatively. The gene was found to co-segregate with the colour locus on chromosome 2 (Salmaso *et al*. 2008). The regulation of colour formation was further elucidated by Yamane *et al*. (2006) as well as by Castellarin and Di Gaspero (2007) providing further insights into gene regulation and genes involved in modulating colour formation. This knowledge will be useful for the development of cultivars yielding colour-intense red wines under various climatic conditions.

3. Mildew resistances

For a long time resistance breeding was dominated by selecting genotypes resistant to powdery mildew (*Erysiphe necator*, an ascomycete) and downy mildew (*Plasmopara viticola*, an oomycete) combined with high wine quality. In the 19th century breeders used resistant genotypes which were available and breeding material carrying some beneficial gene combinations, thus taking advantage of the breeding progress. Furthermore, at that time they aimed at direct producers being resistant against both phylloxera and the mildew pathogens. A survey of the genetic resources used for early resistance breeding made evident, that just a limited number of resistance donors provided the basis of today's elite lines for wine grapes (Eibach 1994). A systematic approach to take advantage of genetic resources is the introgression of resistance traits from wild *Vitis* species followed by consecutive pseudo backcrosses with *V. vinifera* L. subsp. *vinifera*. An exceptionally good but also rare example is the introgression of the *run* 1 locus of *M. rotundifolia* conferring resistance to powdery mildew by Bouquet *et al*. (2000). Recurrent pseudo backcrosses e.g. for 6 generations can be estimated to last about 25 to 30 years and result statistically in less than 1% of genetic material from the wild species remnant in the introgression line. Due to this huge time span it does not surprise that such an endeavour has rarely been taken during the last 200 years. New techniques put this strategy into a new light and new time frame (see below).

Table grapes

In contrast to wine grape breeders, table grape breeders mainly performed crosses within *V. vinifera* L. subsp. *vinifera*, though recently the entire *Vitis* gene pool in particular breeding strains developed thereof became of increasing relevance in order to extent the genetic basis for the introduction of resistances. Breeding for seedlessness, taste, sweetness, colour, uniformity of colour, crispness, berry size (large but not more than 10 g), symmetric cluster architecture, *Botrytis* resistance, time of ripening (very early to very late for an extended availability on the market), shelf-life (transport stability, no release of berries form the peduncle) are important criteria for table grape breeding (Truel 1982). Details concerning table grape breeding are given by Clingeleffer (1995) and Clingeleffer *et al*. (2003).

Classical breeding of wine grapes

A typical breeding programme consists of several consecutive steps decreasing the number of individuals in each selection step. Burger *et al*. (2009) describe several practical aspects of grape breeding. The most important traits are summarized in **Table 6**. The illustration of **Fig. 4** shows the various breeding steps and gives an idea about the number of individuals of a particular breeding strain available at each step. Assuming a current breeding programme for wine grapes starts with 50,000 seedlings a year, greenhouse testing and screening for mildew resistances results in about 5,000 plants to be planted in a seedling plot (requiring about one hectare). Beyond the seedling stage, all further breeding steps require five to eight years of growth: year one to three to get the vine established and year four to eight for a full crop. By far most time consuming is the evaluation of wine quality. Grapes from breeding lines showing good viticultural performance including sufficiently high levels of resistance will be used for wine making. This starts already from a single vine yielding frequently no more than one litre of wine. This so called “micro-vinification” is crucial in wine grape breeding. Wines need to be made in a comparable standardized manner for evaluation. Reducing the time required to enable a thorough evaluation of wine quality could be the major step to accelerate breeding. This can be achieved only by the development and application of markers monitoring distinct aspects of wine quality like sugars, acids, flavours, off-flavours, etc. or which are correlated to important quality and yield traits like berry size, berry number, cluster size, cluster architecture, ripening time, ripening duration, etc. At the beginning of the 21st century the tools become available. This marks the beginning of a paradigm shift from empirical to a knowledge-based and much more target-oriented grapevine breeding.

MOLECULAR MARKERS AND GENOME SEQUENCING

From a genetic point of view a new chapter is being opened, based on recent progress in the development and application of molecular markers, genetic mapping and whole genome sequencing (Jaillon *et al*. 2007; Velasco *et al*. 2007) combined with high throughput technologies forthcoming. One hundred years ago due to non existence of suitable technologies Hedrick and Anthony (1915) were unable to dissect the genetic base of traits. For roughly a decade now we have started to learn more about where traits are located in the genome, how they are inherited, and how they are molecularly organized. For some traits valuable knowledge is accumulating that is relevant for breeding: Most important is the development of molecular markers.

Marker-assisted selection (MAS)

The rapid development of molecular techniques and genome sequencing capacities will accelerate plant breeding. Entirely new tools, in particular molecular markers, showed up in the 1990th permitting a new endeavour to dissect grapevine genetics. While in other crops marker techniques like isoenzyme analysis (Shiraishi *et al*. 1994; Dzheneev *et al*. 1998) or DNA-based markers as RFLP (restriction fragment length polymorphism) (Zyprian 1998) were introduced in the breeding process, the break through for grapevine came with PCR-based DNA amplification techniques. First genetic mapping studies using RAPD markers (randomly amplified polymorphic DNA, Williams *et al*. 1993) were described by application of a double pseudo testcross strategy (Grattapaglia and Sederoff 1994) suitable for highly heterozygous plants such as grapevine (Weeden *et al.* 1994) and the first genetic map of grapevine was published shortly thereafter (Lodhi *et al*. 1995).

Most successful was the development and application of DNA microsatellite analysis using STMS, sequence tagged microsatellite sites (Beckmann and Soller 1990), also called SSR (simple sequence repeats). This type of molecular markers proved to be reliable, comparable, and robust permitting a more detailed analysis of genetically determined traits in grapevine. Many sets of SSR markers became available over the last decade (Thomas and Scott 1993; Bowers *et al*. 1996, 1999; Sefc *et al*. 1999; Scott *et al*. 2000; Arroyo-Garcia and Martínez-Zapater 2004; Di Gaspero *et al*. 2005; Merdinoglu *et al*. 2005; Di Gaspero *et al*. 2007; Welter *et al*. 2007; Cipriani *et al*. 2008). Microsatellites were used first for genotyping studies to unravel the descent of cultivars (e.g. Bowers and Meredith 1997; Sefc *et al.* 1998; Bowers *et al.* 1999; This *et al.* 2004) and were soon introduced into genetic mapping (e.g. Adam-Blondon *et al*. 2004; Grando *et al*. 2004; Fischer *et al*. 2004; Riaz *et al*. 2004). Meanwhile several genetic maps have been developed using SSR or other marker types and combinations thereof (**Table 9**) providing the genetic framework required for QTL (quantitative trait locus) mapping combining genotypic and phenotypic information. This biostatistic analysis permits the dissection of complex traits that are polygenic and governed by several factors as QTL into a genetic map (Costantini *et al*. 2009). It provides a rough localisation of the underlying genes and an orientation in the grapevine genome (compare **Fig. 5**). Single nucleotide polymorphism (SNP) based markers will present the next generation of markers for applications in grapevine breeding. SNPs in grapevine have already been found to be frequent and useful for genetic analysis (Salmaso *et al*. 2004; Troggio *et al*. 2007; Vezzulli *et al*. 2008a, 2008b; Salmaso *et al*. 2008; Myles *et al*. 2010); their future, however, relies on high throughput analysis. SNP markers proved to be very useful for linkage analysis and could also be transferred within the genus *Vitis* (Vezzulli *et al*. 2008b). Their versatility for whole genome association studies, however, is in question since a rapid decay of linkage disequilibrium (LD) was found in grapevine (Myles *et al*. 2010). The LD drops down to background levels at an inter SNP distance of around 10 kb. Even in a small inter SNP distance of 50 bp LD is found to be very low (Myles *et al*. 2010). In this situation SNP analysis with very high marker numbers are necessary to detect any association to neighbouring alleles determining trait expression. It may be more productive to use cost efficient SNP genotyping for genetic mapping of segregating populations followed by QTL analysis rather than expensive high number SNP analyses for whole genome spanning association mapping. An alternative strategy may rely on whole genome sequencing approaches on now emerging 2nd generation and 3rd generation sequencing platforms (see below). Such approaches will become standard once the bioinformatic tools for rapid and correct genome sequence assembly from “2nd generation” sequencing reads become generally available and data management of huge datasets will be quickly possible.

1. Markers for resistance

An allele specific marker for powdery mildew resistance was used by Dalbo *et al*. (2001) to monitor inheritance in a

Table 9 Loci/QTL relevant for breeding: Associated markers, their chromosomal localisation, and the donor genotype are given. Genome position [Chr/Mb] = chromosome number and position in megabases according to the 12 x genome sequence of PN40024 (http://www.genoscope.cns.fr/vitis). (According to Töpfer *et al.* 2010, modified). A similar table is being updated at www.vivc.de section "data on breeding and genetics".

Symbol	Resistance / Trait	Associated marker	Genome Position [Chr/Mb]	Authors	Mapping population (population size)	Source (origin)
be size[(1)]	berry size (berry weight)	SCC8 VMC7f2	18/25.9 18/26.9	Doligez *et al.* 2002; Cabezas *et al.* 2006; Mejia *et al.* 2007; Costantini *et al.* 2008	MTP2223-27 x MTP2121-30 (139); 'Dominga' x 'Autumn Seedless' (118); 'Ruby Seedless' x 'Thompson Seedless' (144); 'Italia' x 'Big Perlon' (163)	*Vitis vinifera*
	monoterpene content	DXS1	5/3.8	Battilana *et al.* 2009; Duchene *et al.* 2009	'Italia' x 'Big Perlon' (163); 'Moscato Bianco' x V. riparia (174); 'Muscat Ottonel' x S.P. (121); 'Gewürztraminer' x S.P. (115)	*Vitis vinifera*
	Linalool content	cnd41 VrZAG64 VMC3d7	10/ 10/13.4 10/10.8	Battilana *et al.* 2009; Duchene *et al.* 2009	'Italia' x 'Big Perlon' (163); 'Moscato Bianco' x V. riparia (174); 'Muscat Ottonel' x S.P. (121); 'Gewürztraminer' x S.P. (115)	*Vitis vinifera*
flb	Fleshless berry	VMC2A3	18/0.9	Fernandez *et al.* 2006	'Chardonnay' x 'Ugni Blanc' Mutant (71)	*'Ugni Blanc' Mutant*
mybA	berry skin colour		2/14.2			*Vitis vinifera*
*Pdr*1	Pierce's disease	VMCNg3h8 VVIn64 UDV-095	14/25.3 14/26.6 14/26.1	Riaz *et al.* 2006; Riaz *et al.* 2008	*V. rupestris* x *V. arizonica* (181)	*Vitis arizonica*
*rdv*1	*Daktulosphaira vitifoliae*	Gf13_9 VMC8e6	13/21.9 13/22.5	Zhang *et al.* 2009	Gf.V3125 x 'Börner' (188)	*Vitis cinerea*
rpv1	*Plasmopara viticola*	VMC72 VVIb32	12/ - 12/10.3	Merdinoglu *et al.* 2003	'Syrah' x 22-8-78	*Muscadinia rotundifolia*
rpv2	*Plasmopara viticola*		18	Wiedemann-Merdinoglu *et al.* 2006; Bellin *et al.* 2009	'Cabernet Sauvignon' x 8624 (129)	*Muscadinia rotundifolia*
rpv3	*Plasmopara viticola*	UDV-112 VVIn16[(2)] UDV-305 VMC/F2	18/ - 18/23.4 18/24.9 18/26.9	Welter *et al.* 2007 Bellin *et al.* 2009	'Regent' x 'Lemberger' (153) 'Chardonnay' x 'Bianca' (116)	'Regent' 'Bianca'
rpv4 [(3)]	*Plasmopara viticola*	VMC7h3 VMCNg2e2.1	4/4.7 4/5.2	Welter *et al.* 2007	'Regent' x 'Lemberger' (153)	'Regent'
rpv5 [(3)]	*Plasmopara viticola*	VVIo52b	9/4.0	Marguerit *et al.* 2009	'Cabernet Sauvignon' x 'Gloire de Montpellier' (138)	*Vitis riparia*
rpv6 [(3)]	*Plasmopara viticola*	VMC8G9	12/20.4	Marguerit *et al.* 2009	'Cabernet Sauvignon' x 'Gloire de Montpellier' (138)	*Vitis riparia*
rpv7 [(3)]	*Plasmopara viticola*	UDV-097	7/11.4	Bellin *et al.* 2009	'Chardonnay' x 'Bianca' (116)	'Bianca'
*ren*1	*Erysiphe necator*	UDV-020 VMC9h4-2 VMCNg4e10.1	13/ - 13/18.4 13/18.4	Hoffmann *et al.* 2008	'Nimrang' x 'Kishmish vatkana' (310)	'Kishmish vatkana'
*ren*3	*Erysiphe necator*	UDV-015b VVIv67	15/7.1 15/10.9	Welter *et al.* 2007	'Regent' x 'Lemberger' (153)	'Regent'
*run*1	*Erysiphe* (*Uncinula*) *necator*	VMC1g3.2 VMC4f3.1	12/10.0 12/13.1	Barker *et al.* 2005	VRH3082-1-42 x 'Cabernet Sauvignon' (161)	VRH3082-1-42 (*Muscadinia rotundifolia*)
sdI	seed development inhibitor	SCC8	18/25.9	Doligez *et al.* 2002	MTP2223-27 x MTP2121-30 (139)	
	seedlessness	VMC7f2 VMC6f11	18/26.9 18/23.2	Cabezas *et al.* 2006	'Dominga' x 'Autumn Seedless' (118)	'Autumn Seedless'
sex	sex	VVMD34 VVS3 VVIb23	2/3.7 2/4.2 2/4.9	Dalbó *et al.* 2000; Lowe and Walker 2006; Riaz *et al.* 2006	'Horizion' x Illinois 547-1 (58); 'Ramsey' (*Vitis champinii*) x 'Riparia Gloire' (*Vitis riparia*) (188); *V. rupestris* x *V. arizonica* (181)	
ufgt		SCAR	16/2.3	Fischer *et al.* 2004	'Regent' x 'Lemberger' (153)	
ver[(4)]	*véraison*	VMC1E11	16/13.7	Fischer *et al.* 2004; Constantini *et al.* 2008	'Regent' x 'Lemberger' (153); 'Italia' x 'Big Perlon' (163)	'Regent'
*xir*1	*Xiphinema index*	VMC5a10	19/20.9	Xu *et al.* 2008	*V. rupestris* x *V. arizonica* (185)	*Vitis arizonica*
5-gt	*anthocyanin 3,5-diglucosides*	Gf09_01	9/6.5	Hausmann *et al.* 2009; Hausmann *et al.* unpublished	'Regent' x 'Lemberger' (153)	'Regent'

(1) Only one major QTL for berry size is indicated. There are several other QTLs described in the literature.
(2) VVIn16 according to Merdinoglu *et al.* (2005)
(3) In publication symbol not yet assigned. Symbol according to www.vivc.de
(4) For véraison (begin of ripening) several QTL loci are published but the QTL locus on LG 16 is the only one which was found in two independent mapping populations.

segregating population. Eibach *et al*. (2007) gave an example of pyramiding resistance loci, two for resistance against *E. necator* and two for resistance against *P. viticola* (see below). The examples show that for grapevine breeding programmes, which still in our days are operating empirically, marker assisted selection (MAS) is at the onset of utilisation.

Analysing the genetics of cv. 'Regent', Fischer *et al.*

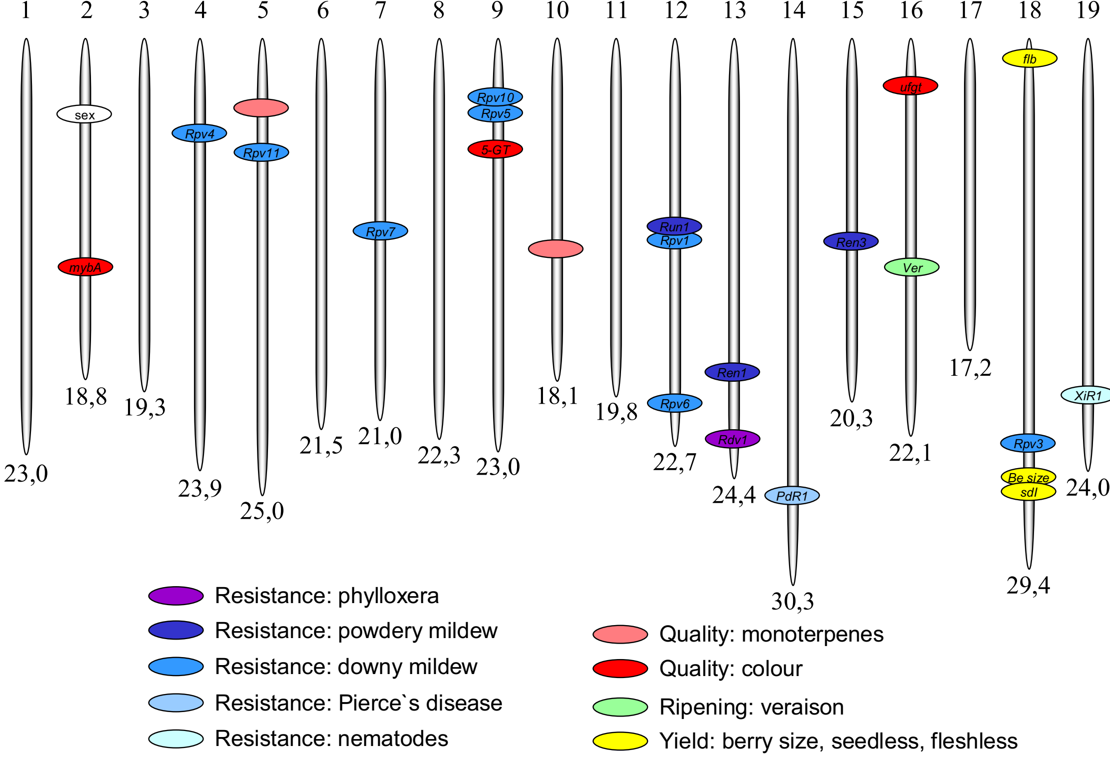

Fig. 5 Chromosomal map of *Vitis* and location of some relevant traits. For details see **Table 9**.

(2004) and Welter *et al*. (2007) identified one major QTL for powdery mildew (chromosome 15) and two QTLs for downy mildew (on chromosomes 4 and 18, see **Table 9; Fig. 5**). Further two loci for powdery mildew resistance are available. Bouquet *et al*. (2000) and Pauquet *et al*. (2001) characterized the *run*1 locus, which was molecularly dissected by Donald *et al*. (2002), Barker *et al*. (2005) and is located on chromosome 12 (**Table 9; Fig. 5**). Closely associated with the *run*1 locus, a resistance against *P. viti-cola* assigned as *rpv*1 was found which is partially lost in line VRH3082-1-42 (Wiedemann-Merdinolgu *et al*. 2006). A further locus for resistance against powdery mildew, *ren*1, could be identified on chromosome 13 in cv. 'Kishmish vatkana' (Hoffmann *et al*. 2008) (**Table 9; Fig. 5**). Finally Marguerit *et al*. (2009) described downy mildew resistances from *V. riparia* on chromosomes 9 and 12 which can be used in addition. Further markers for other traits which are applicable for MAS are listed in **Table 9**.

2. Markers for berry and wine quality

With respect to wine quality a considerable lack of knowledge and methodology has to be stated. However, insights into the complex trait of wine quality will be gained during the forthcoming years. A method of choice will be the use of SNP markers in canalising diverse and expensive analytical methods like GC, GC/MS, LC, LC/MS. Concerning positive aroma compounds (e.g. monoterpenes) first QTLs have been described (Eibach *et al*. 2003; Grando *et al*. 2004; Doligez *et al*. 2006b) and a good candidate gene (1-deoxy-D-xylulose 5-phosphate synthase) for terpenol content was identified on chromosome 5 (Battilana *et al*. 2009; Duchene *et al*. 2009). But the data still need to form a clearer picture to become useful for MAS of berry quality. In contrast it could be much easier to develop markers to monitor off-flavours. They would be very useful to eliminate undesirable flavour compounds (e.g. furaneol or methylantranilate) very rapidly from the gene pool while introducing new resistance genes into *V. vinifera*.

Recently the biosynthesis of tartaric acid contributing to taste, mouthfeel, and aging potential received some interest, since too low acidity in hot climate viticulture is a major quality issue. DeBolt *et al*. (2004, 2006) gained major insights in the biosynthetic pathway of tartaric acid synthesis and the underlying enzymes. Hypothesized for a long time the authors gave convincing evidence that tartaric acid in grapevine is a product of vitamin C (ascorbate) catabolism. In a recent report about ascorbate metabolism first regulatory aspects could be elucidated (Melino *et al*. 2009). The accumulating knowledge will be used to unravel the regulation of the pathway opening the possibility to build up new selection schemes for cultivars showing an appropriate acid balance.

As indicated above an important trait is the colour of the grapevine berries which is caused by the synthesis of anthocyanins in the berry skin of red and black genotypes in the second ripening phase after véraison (for review see Boss and Davis 2009). The key biosynthetic enzyme for anthocyanin formation, UDP-glucose:flavonoid 3-O-glucosyltransferase (UFGT), has been mapped on chromosome 16 (Fischer *et al*. 2004) by using a SCAR marker deduced from sequence information provided by Sparvoli *et al*. (1994). More important for colour formation is the transcription factor M*ybA* that controls UFGT gene expression. The *mybA* gene is located on chromosome 2. Due to a transposon-based mutation within the promoter of one allele of the *myb*A gene the development of a molecular marker is now possible correlating very tightly with berry skin colour (Kobayashi *et al*. 2004; Walker *et al*. 2007). This transposon insertion was tightly correlated with white berry colour. Colour variants could be explained in 95% of the cases by different alleles of the *mybA*1 gene showing molecular fingerprints of transposon excision (Lijavetzky *et al*. 2006; This *et al*. 2006). Further modulation of colour can be explained by different expression of genes for anthocyanin modifying enzymes (Castellarin and DiGaspero 2007).

In terms of genetic understanding another modification which has been introgressed into *V. vinifera* L. subsp. *vinifera* has been much easier to be accomplished. Among the anthocyanins two major types exist: anthocyanin 3-glucosides and anthocyanin 3,5-diglucosides (mainly malvin). Anthocyanin 3-glucosides are found in all coloured grapes

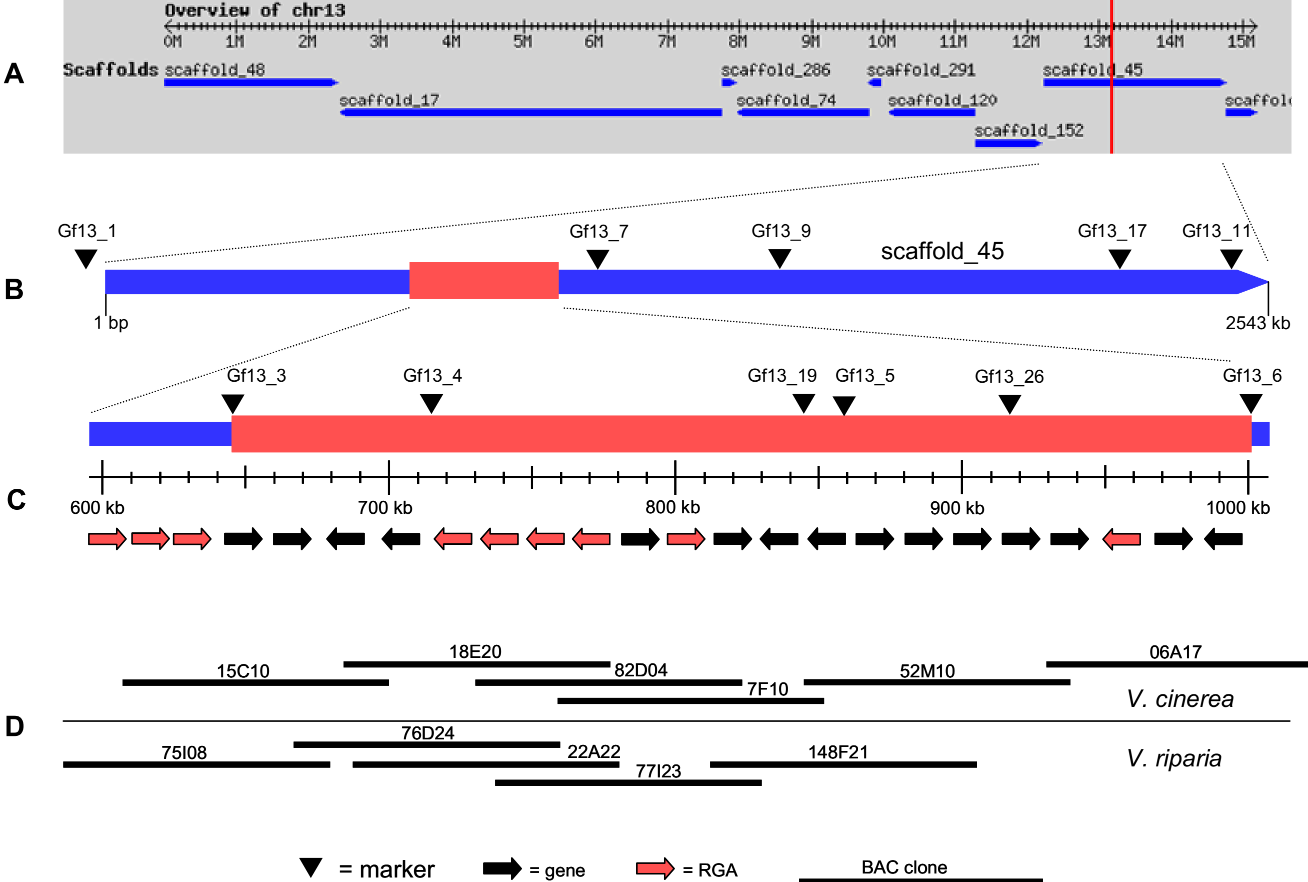

Fig. 6 Elucidation of the structure of the phylloxera locus of rootstock cv. 'Börner' (*V. riparia* 183 G x *V. cinerea* Arnold). (**A**) chromosome 13 of the reference genotype PN40024. (**B**) Scaffold 45 of PN40024 and relevant SSR markers for orientation. (**C**) Structure of the region of PN40024 corresponding to the resistance locus of 'Börner'. Red bars in (**B**) and (**C**) indicate regions of the PN40024 genome syntenic to the region of resistance against phylloxera from 'Börner'. Red arrows indicate resistance gene analogous (RGA) and black arrows correspond to open reading frames found in the sequence of PN40024. (**D**) Minimal tiling path of both haplotypes of 'Börner'. The *V. cinerea* haplotype carries the resistance locus. Black bars indicated BAC clones derived from 'Börner' according to both parental haplotypes.

whereas anthocyanin 3,5-diglucosides occur in most wild *Vitis* species and in derivatives of crosses of *V. vinifera* L. subsp. *vinifera* with wild *Vitis* species. They are absent on very low level in traditional *V. vinifera* L. subsp. *vinifera* cultivars. Anthocyanin 5-glucosyltransferase (5-GT) is the responsible enzyme catalyzing the glycosylation reaction from anthocyanin 3-glucoside to anthocyanin 3,5-diglucoside. Expression of the 5-gt gene correlates positively with anthocyanin 3,5-diglucoside formation in berry skins of different grape genotypes (Hausmann and Töpfer 2006). Therefore the gene encoding 5-GT was cloned and sequenced from different *Vitis* genotypes. The 5-gt alleles from traditional *V. vinifera* genotypes showed mutations leading to non-functional gene products in contrast to a functional 5-GT originally descended from a wild *Vitis* species (Hausmann *et al.* 2009; Jánváry *et al.* 2009). Based on the sequence differences in the 5-gt alleles a molecular marker was developed. Using this 5-gt sequences characterized amplified region (SCAR) marker the 5-gt gene was mapped on chromosome 9 at the same site where the trait 'malvin' has been previously localized (Welter *et al.* 2007). Since malvin is very intense in colour and quite stable it may be used to develop cultivars with dark coloured berries.

3. Markers for other traits

Despite these perspectives current markers have been assigned to traits such as seedlessness or resistances and can be used for selection of particular traits. Seedlessness could be scored easily by markers developed by Striem *et al.* (1992, 1996) or Adam-Blondon *et al.* (2001). Similarly, Doligez *et al.* (2002) developed markers for seedlessness and berry weight. Several publications identified the locus for sex of the flowers on chromosome 2 (Dalbo *et al.* 2000; Lowe and Walker 2006; Marguerit *et al.* 2009) a trait of interest e.g. for developing introgression lines. A major QTL for begin of berry ripening (veraison) was found on chromosome 16 as described by Fischer *et al.* (2004) for 'Regent' x 'Lemberger', Costantini *et al.* (2008) for 'Italia' x 'Big Perlon', and Zyprian *et al.* (unpublished data) for Gf.Ga-47-42 x 'Villard blanc'. Markers for resistance against Pierce's disease are available (Riaz *et al.* 2006, 2008) as well as makers for phylloxera resistance (Zhang *et al.* 2009a) (**Table 9; Fig. 5**). A QTL influencing Magnesium-update was identified on LG 11 (Mandl *et al.* 2006).

Pyramiding mildew resistance loci

In order to avoid breakdown of resistance in a crop such as grapevine growing in the vineyard for 30 or more years and considering the utilization of cultivars for hundreds of years, a resistance trait must be durable. A single resistance gene might quickly be overcome by a pathogen. For a long time the existence of different isolates for the two mildews of grapevine were not known, though expected. This might be due to the fact that both mildews are obligate parasites and single spore isolates are difficult to be kept separately. Recently Merdinoglu (2009) reported that isolates of *P. viticola* show a different pathogenic potential on certain grapevine cultivars indicating the occurrence of races at least of different mildew populations. Similar results were obtained with American isolates of powdery mildew (Frenkel *et al.* 2010). Genetic evidence for pathogen diversity has been provided (Stark-Urnau *et al.* 2000; Delmotte *et al.* 2006) and inter-isolate variation of virulence (Kast *et al.* 2000) has been shown. Therefore it becomes very important to create

durable resistance which could be achieved by combining resistance loci from various sources, potentially representing different defense mechanisms. Since molecular markers are available for several resistance loci a combination of these loci becomes feasible. A first example is given by Eibach *et al*. (2007) combining the resistances of VRH3082-1-42 (*run*1/*rpv*1) locus and the resistance found in 'Regent' (*ren3*/*rpv*3/*rpv*4) employing linked markers. F1-plants showing already the combination (*run*1/*rpv*1/*rpv*3) were found to be essentially free from mildew infection. For further breeding purposes plants showing the complete set of resistance-linked markers (*run*1/*ren3*/*rpv*1/*rpv*3) were selected (**Fig. 6**). A combination with *ren*1 (from 'Kishmish vatkana') and a downy mildew resistance from 'Solaris' (*rpv10)* (**Table 1**) which is expected to be derived from *V. amurensis* Rupr., is envisaged creating lines which have even more resistance loci (Schwander *et al*. 2011). Introducing the resistances into the gene pool in various combinations (**Fig. 6**) permits a broad range of crosses resulting in an offspring segregating for multiple resistances. MAS can simply be used to select at the seedling stage genotypes having a desired pattern of markers linked to resistance loci. From that point of view the mildew pathogens could be considered as a problem which might be solved with a good chance of getting durable and stable mildew resistance. Despite that it may be necessary to keep spraying chemicals for plant protection at a minimal level since other pathogens currently also covered by the intense fungicide treatments might emerge. Such an example is black rot (*G. bidwellii*) which became a problem in Germany a few years ago due to false management strategies (Kast and Schiefer 2004; Lipps and Harms 2004) though it is not a general threat. Minimal sprayings will also affect the mildew pathogens thus supporting the resistance properties of the plant to a certain extent and contributing to durability.

Marker-assisted backcrossing (MABC)

The evaluation of genetic resources permits the identification of new sources of resistance. Due to the long lasting process of introgression of new resistance alleles from a wild species, breeders hesitated to take this effort. MABC, however, opens up the possibility at each generation to select for a maximum of *V. vinifera* L. subsp. *vinifera* genome while maintaining the trait of interest (Di Gaspero and Cattonaro 2010). Using the pseudo backcross approach in pBC5 theoretically 1.6% of the non-recurrent (wild ancestor) genome remains in the introgression line. This can be accelerated by background selection (Collard and Mackill 2008) identifying those genotypes in a progeny that, due to recombination in meiosis, received a higher proportion of the *V. vinifera* L. subsp. *vinifera* genome. Selecting against the wild ancestor about two generations i.e. eight years might be saved calculating with 4 years generation time and cultivation in the vineyard. Based on this calculation introgression requires 16 instead of 24 years. Reducing the generation time due to greenhouse cultivation the goal might be achieved already within 8-10 years. For such an approach five to ten markers per chromosome should be sufficient i.e. about 200 markers equally distributed throughout the genome (Frisch *et al*. 2005). Preliminary analysis in a MABC population of 300 pBC1 seedlings subjected to background selection revealed three plants showing more than 85% of *V. vinifera* genome when 75% are statistically expected. These three plants were found in the 50% of plants carrying the locus of interest. Thus, as long as a single locus is concerned population sizes of at least 300 plants give a reasonable basis for running a MABC programme to find desirable recombinants. New marker techniques based on SNP analysis will permit the investigation of 300 plants with 200 markers (60,000 data points) within a few days leaving sufficient time to integrate such analyses in breeding programmes and their tight time schedule.

Map-based cloning approaches

To understand the mechanism of how a trait is expressed the responsible gene needs to be isolated. Having genetic maps this can be achieved by map-based cloning approaches (Gibson and Somerville 1993; Zhu and Zhao 2007). In principle molecular markers are to be identified successively reducing the distance between markers and the trait locus down to a distance permitting cloning of the locus. Two close markers are required flanking the locus of interest. A straight forward approach takes advantage of the reference genome sequence of PN40024 (Jaillon *et al*. 2007) and requires co-linearity between the two genome regions (PN40024 and the locus of interest). Around the desired locus e.g. an SSR based marker can be deduced from PN40024 and placed on the genetic map moving towards the locus of interest. For grapevine an ideal distance would be around 1 cM (statistically *ca*. 300 kb) or below. Isolation of marker-carrying BACs (bacterial artificial chromosomes) followed by identifying overlapping clones from both sides of a locus will reveal at a certain point in time an overlap of clones and thus a continuous physical map, a BAC contig, spanning the locus (**Fig. 7**). In a final step the BACs will be sequenced and candidate genes for the trait can be identified. One example is the cloning of the *run*1 locus (Donald *et al*. 2002; Barker *et al*. 2005) derived form introgression from *M. rotundifolia* (Bouquet *et al*. 2000; Pauquet *et al*. 2001). Recently Walker and coworkers mapped a Pierce's resistance locus from *Vitis arizonica* Engelm. (Riaz *et al*. 2008). Another example is a phylloxera resistance locus from *V. cinerea* Arnold (Zhang *et al*. 2009a; Hausmann *et al*. unpublished).

As phylloxera resistance was a breeding goal since the beginning of grapevine breeding this trait became less important with the introduction of vines grafted on tolerant or resistant rootstocks. Subsequently the breeding goal of phylloxera resistance was given up due to the complexity of the overall goals of fungal disease resistance, wine quality etc. Using the molecular tools available and in spite of the achievements in wine grape breeding, a revival of the breeding for phylloxera root resistance by MAS becomes feasible.

Recently Roush *et al*. (2007) analysed phylloxera resistance in a F2 progeny from a remake of AXR1 (*V. vinifera* x *V. rupestris*) for inheritance of nodosities and tuberosities. The genetic analysis revealed two loci involved in formation of nodosities and one or two loci for tuberosity formation, being recessive in each case. A different picture was obtained for rootstock cv. 'Börner' (*V. riparia* 183 G x *V. cinerea* Arnold), which is a phylloxera resistant rootstock showing a hypersensitive response (Schmid *et al*. 1998; 2003). The resistance against phylloxera root infection was discovered by Börner in the 1930s in *V. cinerea* Arnold (Börner 1943). Using a mapping population of Gf.V3125 ('Schiava Grossa' x 'Riesling') x 'Börner' the *rdv*1 locus could recently be identified on chromosome 13 (Zhang *et al*. 2009a). New SSR markers deduced from the reference genome sequence of PN40024 (Jaillon *et al*. 2007) were found to be generally in a co-linear order in the genetic map of Gf.V3125 x 'Börner' (see **Fig. 7**). Thus, following this procedure of using "synteny-derived" markers the *rdv*1 resistance locus of chromosome 13 could be narrowed down to less than 0.5 Mb. With a genome sequence based marker development and BAC screening clones were isolated covering the entire region for both haplotypes: *V. riparia* 183G and *V. cinerea* Arnold. Sequening the BACs quickly provided the information of the complete locus. Despite a high sequence density in the core region of *rdv*1 it turned out to be difficult to reconstruct the contig arrangement and thus to identify candidate genes due to repetitive RGA sequences (Hausmann *et al*. unpublished data). Based on this detailed information MABC was initiated to make the *rdv*1 locus accessible for grapevine breeding.

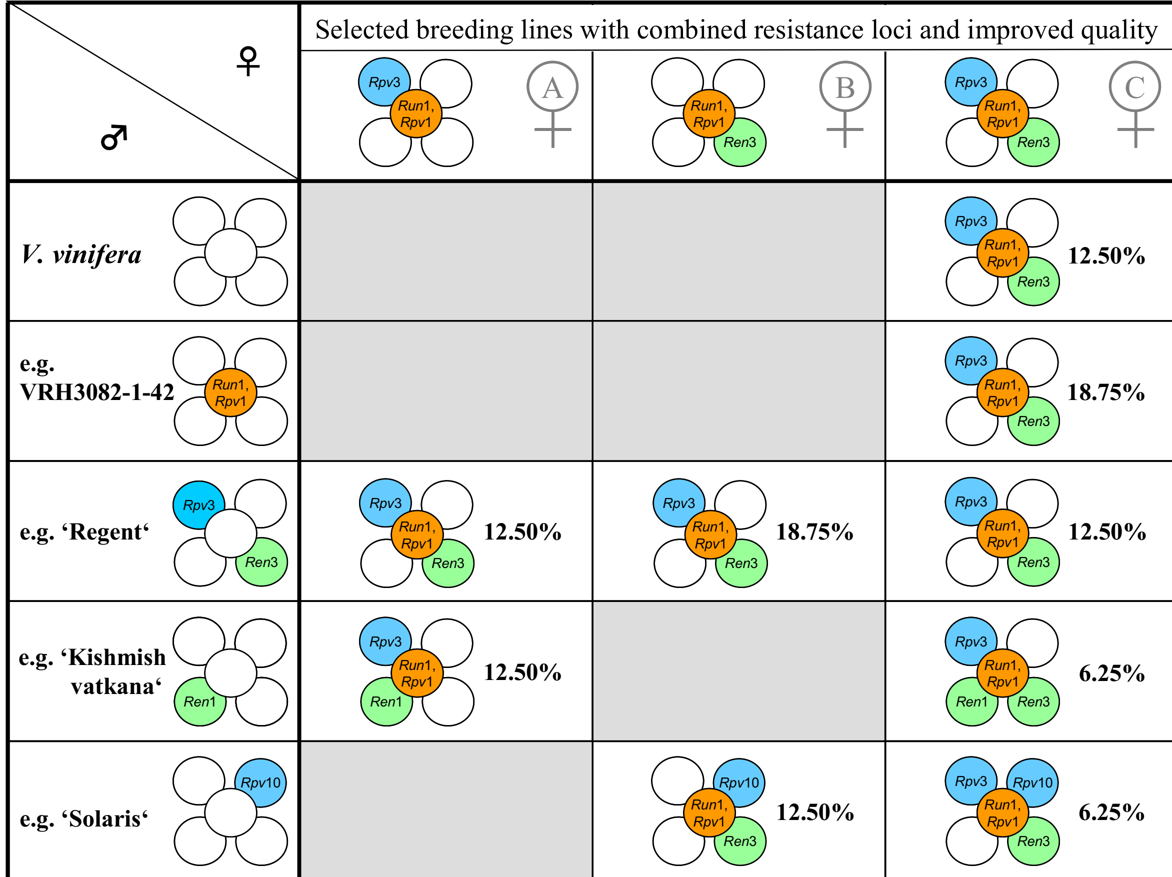

Fig. 7 Scheme for the construction of pyramided mildew resistance loci from a running breeding programme. Mother plants A, B, and C show first combinations of milderw resistance (coloured cirles: orange = resistance against *E. necator* and *P. viticola*, blue = resistance against *P. viticola*, green = resistance against *E. necator*). As farther plants representative genotypes are indicated carrying individual loci. The expected frequencies are given to find a F1-genotype with the desired combination of resistance loci. Combination of female parent C and any *V. vinifera* cultivar show 12.5% F1-plants two resistance loci for each mildew. A crossing using female parent C and 'Kishmish vatkana' or 'Solaris' can add on additional resistance loci. In a final cross all the resistance loci can be pyramided by using 'Kishmish vatkana' or 'Solaris', respectively, with a frequency of 3.125%.

Genome sequencing

The best marker is a marker identifying the desired allele of the corresponding gene. Map-based cloning approaches as described above successively reduce the distance between markers and the gene of interest down to a distance permitting cloning of a locus. The new sequencing options in terms of efficiency and low costs open novel possibilities.

Since the first grapevine genome sequence was published (Jaillon *et al.* 2007; Velasco *et al.* 2007) dozens of cultivars and accessions including *Vitis* species. have been re-sequenced (Myles *et al.* 2010; Morgante *et al.* unpublished data; Töpfer *et al.* unpublished data) or their re-sequencing is in progress (Adam-Blondon pers. comm.; Weisshaar and Töpfer unpublished data) giving rise to thousands of SNP markers opening a huge potential of applications such as genotyping or high resolution gene mapping (Martínez-Zapater *et al.* 2010). Progress in sequencing technologies and decreasing costs for sequencing will permit within the next few years to sequence any genotype of choice. The "1000 dollar human genome" (3000 Mb = 6x grapevine genome) is currently the key word of this development and is expected to come within the next few years. Thus, a genome sequence like that of grapevine (500 Mb) will be obtained easily and markers are coming not only for a locus but for the desired allele or haplotype. Further map-based approaches will no longer rely on BAC clones to get the gene. The genome sequence and a fine genetic map will permit identification of the corresponding gene and its alleles.

IN VITRO CULTURE AND GENETIC ENGINEERING

Grapevine marketing strongly sticks to the cultivar name, in particular in the case of wine cultivars since wines are frequently marketed by their varietal names. High heterozygosity and inbreeding depression prevent an improvement of existing cultivars by classical cross breeding techniques. Thus, for marketing and from a biological point of view improvements of traditional cultivars are exclusively possible by genetic modifications. Only in this case the cultivar name eventually could be maintained and the characteristics of a cultivar like quality traits will be preserved while deficiencies like disease susceptibility can be improved. Thus, primary genetic modifications within a grape breeding programme should be focussed on the improvement of traditional cultivars for tolerance or resistance against biotic (e.g. fungus, insect, virus resistance) or abiotic stress factors (e.g. heat, drought, cold tolerance).

Development of transformation methods

First reports of genetic transformation of grapevine tissue resulting in transgenic callus date back to the beginnings of transgenic research (Meredith *et al.* 1987, 1989). Shortly after that first transgenic plants were obtained (Mullins *et al.* 1990). Since then substantial progress has been made to improve transformation protocols (for review see Scott 1993; Perl and Eshdat 1998; Vivier and Pretorius 2000, 2002).

Somatic embryogenic tissue, mainly raised from different flower organs like anthers, ovaries, or total flowers proved to be most suited for regeneration and gene transfer purposes (Perl and Eshdat 2004). In addition, some rare cases of transformation and regeneration originating from

leaf tissue have been reported (e.g. Meredith *et al.* 1990; Das *et al.* 2002; Mezzetti *et al.* 2002; Bornhoff *et al.* 2005). For efficient transformation somatic embryogenic tissue needs to be provided in the appropriate developmental stage and in sufficient quantity. Since excision of flower explants as a source for initiation of somatic embryos is highly laborious and time-consuming and generally results in asynchronously growing cultures, somatic embryogenic suspensions have been established (e.g. Mauro *et al.* 1995; Bornhoff and Harst 2000; Jayasanakar *et al.* 2002; Ben-Amar *et al.* 2007, Vidal *et al.* 2009). Due to a rapid multiplication of homogeneous pro-embryogenic calli and to the season-independent availability of suitable starting material for gene transfer purposes embryogenic suspension cultures have proved to be the ideal culture system (Harst *et al.* 2000; Wang *et al.* 2005; Vidal *et al.* 2009).

Transformation is most frequently performed using *Agrobacterium*-mediated gene transfer (review of Perl *et al.* 2007; Li *et al.* 2008; Dhekney *et al.* 2009), but there are also successful reports concerning biolistic transformation (Hébert *et al.* 1993; Kikkert *et al.* 1996; Torregrosa *et al.* 2002a; Reisch *et al.* 2003; Vidal *et al.* 2003, 2006). Various parameters have been optimized like *Agrobacterium* strains (Berres *et al.* 1992; Torregrosa *et al.* 2002b) as well as their optimal density during the co-cultivation step (Lopéz-Pérez *et al.* 2008), the culture media (Torregrosa *et al.* 2002b), the plant genotype-specific effects of the transformation (Iocco *et al.* 2001) or the effect of antioxidants to avoid browning of tissue during the transformation procedure (Perl *et al.* 1996a, 1996b; Dan 2008).

For an early selection of transformed tissue different selectable marker systems have been tested (Peros *et al.* 1998; Colby and Meredith 1990). The antibiotic kanamycin found wide application for selection of transformed tissue using the neomycinphosphotransferase II (*nptII*) gene from *Escherichia coli*. Still today it is one of the best selectable marker systems in view of application and biosafety. Other antibiotics used are paramomycin (Vigne *et al.* 2004; Wang *et al.* 2005) and hygromycin (Perl *et al.* 1996b; Torregrosa *et al.* 2000). In a few cases the herbicide phosphinotricin was tested as a selectable marker (Perl *et al.* 1996a; Levenko and Rubtsova 2000; Reustle *et al.* 2003; Jadark-Jamoussi *et al.* 2008). As a screenable marker the β-glucuronidase (*gus*) gene from *Escherichia coli* was used as a reporter gene system (Baribault *et al.* 1990). With increasing success in transformation of grapevine a new generation of non-destructive visible marker genes like *gfp* (Thomas *et al.* 1998; Li *et al.* 2001; Nakajima *et al.* 2006; Wang *et al.* 2007) or *myb* (Cutanda-Perez *et al.* 2009) became alternatives. In the light of the public debate concerning antibiotic resistance genes containing transgenics, work was initiated to develop genetically modified (GM) grapevines free of antibiotic resistance genes for selection (Reustle *et al.* 2003; Kieffer *et al.* 2004; Dutt *et al.* 2008; Jadark-Jamoussi 2008); however, the problem remains to be solved.

Limitations of grapevine transformation

As outlined, classical breeding proved to be very difficult and likewise grapevine transformation turned out to be similarly recalcitrant. Though most grapevine cultivars are a host for *Agrobaterium vitis* infection, highly efficient transformation protocols are restricted to specific cultivars like 'Thompson Seedless' as a table grape, or the wine grapes 'Cabernet Sauvignon', 'Chardonnay', 'Chancellor' or 'Merlot' as well as the rootstock cultivars '41B' or '3309 C' (Iocco *et al.* 2001; Perrin *et al.* 2001; Gribaudo *et al.* 2004; Kikkert *et al.* 2005; Gambino *et al.* 2007; Dhekney *et al.* 2009; Oláh *et al.* 2009; Vidal *et al.* 2009). Thus, generally speaking transformation suffers from insufficient regeneration systems (Chen *et al.* 2006; Zhang *et al.* 2009b). This particularly includes the crucial differentiation step from a somatic embryo to an entire plant, the so called "conversion" of the germinating embryo to intact rooted plantlets (Harst *et al.* 2000; Lopez-Perez *et al.* 2006; Vidal *et al.* 2009).

Currently no GM-vine has reached the market. Public concern seems to be a more general retarding aspect but, except for a few examples, good genes for traits are the other missing issue. Since virus resistance was not found in *Vitis*, GM-vines could be an interesting solution and have attracted researches since the early 1990s (Le Gall *et al.* 1994; Krastanova *et al.* 1995). Rootstocks showing virus resistance have been obtained (for review see Laimer *et al.* 2009) but a cultivar is not yet available.

Vitis does not carry resistances against the wood disease eutypa dieback caused by *Eutypa lata*. In a transgenic approach Legrand *et al.* (2003) developed rootstock plants expressing a gene from *Vigna radiata* encoding a NADP-dependent aldehyde reductase (*Vr-ERE*), an enzyme converting eutypine, a toxin from *Eutypa lata*, into its corresponding non-toxic alcohol. Transgenic plants cultivated *in vitro* showing a high *VrERE* expression were not affected by relatively high concentrations of eutypine whereas growth and development of untransformed control plants were substantially retarded. Several attempts have been made to improve grapevine for mildew resistance (Bornhoff *et al.* 2005; Vidal *et al.* 2006). Field resistance has not yet been observed. A promising approach could be the expression of the *run*1 gene identified by Barker *et al.* (2005). Results of a transgenic approach are pending. Quality aspects for particular purposes have been addressed. Franks and Thomas (1997) reported on blocking the polyphenol oxidase (PPO) activity in transgenic 'Sultana' resulting in light-skinned 'Sultana' raisins. Though the principle has been shown, the improved cultivar is not yet available.

Gene function analysis

Genome analysis carried out around the world aims at resolving the molecular basis of important traits ending in the question of how to elucidate gene function. Within the next few years plenty of candidate genes for interesting traits will become available. Transformation is highly important to elucidate their function but time consuming as it requires about one year to get a transgenic plant for analysis (e.g. Legrand *et al.* 2003; Gambino *et al.* 2005, Zok *et al.* 2010). If berry traits are to be studied it takes even longer. Thus, fast systems for functional studies become more and more important. Recently transient gene expression system based on agroinfiltration in homologous (Zottini *et al.* 2008; Santos-Rosa *et al.* 2008; Xu *et al.* 2010) or heterologous systems (Le Henanff *et al.* 2009; Xu *et al.* 2010) have been used. The methods need to be refined. However, transient gene expression analysis will provide a shortcut only for some gene function studies. It will not replace stable transformation and field testing.

Practical issues of GM-grapevine and field trials

Worldwide numerous field trials of GM-grapevines (see review of Pazzi 2008) were carried out testing of transgenic plants mainly harbouring genes for fungal, bacterial or virus resistance or quality traits. These trials provide data of the first GM-grapevines in a natural environment to show the level of resistance and the behaviour of a trait in uncontrolled conditions. Furthermore, these trials prove the stability of expression of introduced foreign gene(s) over years, e.g. in USA (Gray *et al.* 2006) and France (Fuchs *et al.* 2007).

The political debate concerning GMOs in several countries around the world is a major aspect in terms of pushing GM-vines to the market. From a bio-safety point of view GM-vines have to be considered as rather uncomplicated. It is evident that vegetative propagation of planting material minimises an eventual risk of dissemination of vines. Rootstocks neither do form leaves nor flowers during the normal cultivation. However, growing transgenic scions will result in a dispersal of transgenic pollen (Harst *et al.* 2009). Since natural occurrence of wild vines in regions of viticulture is

very limited out-crossing into wild species will not be of major importance. Studies concerning the investigation of out-crossing aspects were only carried out in Australia (see field trial Application No. DIR 031/2002) and Germany (Harst *et al*. 2009). In a pilot study with transgenic 'Dornfelder' vines as pollen donor plants harbouring the *gus* gene transgenic pollen flow and out-crossing events were monitored and were found to be in the low percentage range. Further detailed studies are required to quantify the data under usual viticultural conditions. The available data do not permit any recommendation for a cultivation of GM-grapevines in the future (Harst *et al.* 2009).

The range of out-crossing needs to be known to evaluate potential risks and an eventual impact on viticulture. From grapevine biology it is evident that out-crossing can not affect the quality of the receptor cultivar since the berry flesh is formed solely from maternal tissue. Transgenes might only be found in the seeds which are usually discarded in the case of wine grapes. From a scientific point of view table grapes and raisins are the only form of production which might need a further and detailed consideration, though principal risks are not to be expected.

FUTURE WORK, PERSPECTIVES

Since immemorial time wines are highly estimated products made from superior cultivars. With the dissemination of the two mildew pathogens, other fungi, and phylloxera around the world the old tradition of viticulture experienced major changes: cultivation of grafted vines and intense chemical plant protection became necessary. Environmental concerns and new threats coming along with climatic change enforce adaptations on the plant itself. On the long term the only solution will be a genetic improvement of the grapevine plant to face the major threats either by growing newly selected cultivars or bringing existing cultivars to perfection. As the history of grapevine breeding taught us, continuous and sustainable efforts of breeders will provide the solutions even if it requires decades. However, there is considerable room for the expectation of reaching solutions much faster than in previous decades: (1) we face about 200 years of progress within the breeding material and (2) molecular genetic technologies offer unprecedented possibilities for selection. Soon breeding will no longer require 25 to 30 years to get a new cultivar. This time span is expected to be reduced by up to 10 years. In spite of all expectations for acceleration of grapevine breeding there are some biological restrictions (Töpfer *et al.* 2010): simply the availability and propagation of the plant material will become a limiting factor within the breeding process (see **Fig. 4**). Thus, irrespective of the shortening of time, sustainability of improvements will become more relevant. Modern breeding tools make this challenge accomplishable.

- Today it is possible to address single loci by molecular markers (MAS) and to combine (pyramide) several resistance loci acting against a disease in a plant to achieve a better chance of durability of resistance. Several loci for powdery mildew and for downy mildew resistance are known (compare **Fig. 5, Table 9**) other loci will follow as well as resistance loci directed against other pathogens. Moreover, resistances against various threats can be easily combined by MAS. The next generation of grapevine cultivars will have multiple resistances against several biological stress factors.
- Today it is possible to run marker assisted backcrossing (MABC) programmes to introgress traits of interest into the *V. vinifera* gene pool within a reasonable time frame.
- Today high throughput techniques are available for genotyping and for genome sequencing upgrading the breeder's toolbox. A description of the haplotype is possible and desired alleles can be addressed and monitored within a breeding programme.
- Today genes can be isolated and their function and the underlying mechanisms can be elucidated.
- Today existing cultivars can be improved though some technical difficulties need to be overcome. GM-grapevines are a current possibility. Fungus resistance for environmentally friendy viticulture could be an argument in the public debate for acceptance.
- Today phylloxera resistance as breeding goal can be reconsidered directing viticulture on the long term back to own-rooted cultivation of *Vitis vinifera*.

  Several developments are very much advanced and their contribution or their output will become soon visible. However, there are still some missing links which require further research and development and some more time:
- Major missing links are highly efficient phenotyping tools. Today phenotyping possibilities for grapevine are far behind the genotyping options.
- Markers describing quality are required. The marker description of positive and negative characters will surely be developed. Markers can be imagined for sugars, acids, certain aroma compounds, off-flavours, tannins, etc. It is an open question how deep a quality description can go. Unknown minor aroma compounds can have a major impact on sensory perception. The bouquet of a wine is influenced by the matrix of the wine. The body of a wine is not described in terms of compounds. Quality is probably the trait most deeply influenced by the environment. In order to reduce it to a genotypic description requires a very deep evaluation.
- Elucidation of the various mechanisms of resistance in order to pyramide the best suited resistances.
- Genetic resources – more precisely wild species – should be evaluated in an internationally complementary manner. Core collections for a species could be developed based on genetic distance determined by markers to maintain and manage that gene pool efficiently (Le Cunff *et al*. 2008). This would provide the opportunity to make important traits accessible on the long term within a minimal set of individuals and eventually to develop introgression line. Otherwise breeders will select their material at a given time for a particular trait and discard plants valuable from a different perspective material.

Finally, if the appropriate methods are established, a cost-benefit calculation will show what will be accomplishable in the breeder's hands and what will remain a dream. The shift from empirical to a systematic knowledge based breeding is taking place. As a consequence the chances of success in grapevine breeding have become more promising since ever.

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