

Use of genetic diversity for grapevine resistance breeding

RUDOLF EIBACH, REINHARD TÖPFER and LUDGER HAUSMANN

Julius Kuehn Institute, Federal Research Centre for Cultivated Plants
Institute for Grapevine Breeding Geilweilerhof
D-76833 Siebeldingen
E-mail: Ludger.Hausmann@jki.bund.de

Compared to most other agricultural crops grapes need a substantial higher impact on plant protection measurements mainly for the protection against the mildew diseases. Therefore since decades breeding activities in several countries have been focused on the development of new varieties with fungus resistance. Sources for resistance are preferably the genetic resources of American and Asian wild species. Recent success in research on grapevine genetics led to the identification of resistance related quantitative trait loci (QTL) which are linked with molecular markers. An example is given for pyramiding resistance loci by the application of marker assisted selection (MAS). Also for establishing introgression lines with valuable traits from wild species the application of MAS is a highly powerful tool. It allows to reduce the number of generation cycles considerably.

Keywords: grapevine, resistance, wild species, MAS

Nutzung der genetischen Biodiversität im Rahmen der Resistenzzüchtung bei Rebe. Verglichen mit anderen landwirtschaftlichen Nutzpflanzen braucht die Weinrebe einen deutlich intensiveren Pflanzenschutz, hauptsächlich gegen Mehltaubefall. Deshalb konzentrierten sich seit Jahrzehnten die züchterischen Aktivitäten in vielen Ländern auf die Entwicklung pilzresistenter Sorten. Die genetischen Ressourcen für diese Resistenz findet man bevorzugt in amerikanischen und asiatischen Wildarten. Jüngste Forschungserfolge im Bereich der Rebengenetik führten zur Identifizierung der mit Resistenz in Zusammenhang stehenden Quantitative Trait Loci (QTL), die mit Molekularen Markern verbunden sind. Ein Beispiel für die Pyramidisierung von Resistenz-Loci mittels Marker-unterstützter Selektion (MAS) wird dargestellt. MAS ist auch eine sehr wirkungsvolle Methode für die Entwicklung von Introgressionslinien mit wertvollen Eigenschaften aus Wildarten und erlaubt eine beträchtliche Reduzierung der Anzahl von Generationszyklen.

Schlagwörter: Rebe, Resistenz, Wildarten, MAS

Among the family of Vitaceae the genus *Vitis* is the only one with economical importance. According to VAVILOV (1926) three gene centers can be distinguished: the Eurasian gene center with the only one species *Vitis vinifera* and the Asian and North American gene centers, each of both containing about 35 different species. Because of its distinguished quality traits the varieties belonging to *Vitis vinifera* are by far the most important ones for the production of wine, table grapes or raisins. On the other hand the varieties belonging to *Vitis vinifera* are highly susceptible against a range of fungus diseases, among them also powdery and downy mildew, the diseases with the highest economical impact. Depending on the climatic conditions of the vine

growing area about six to twelve treatments per year are necessary to protect the grapes properly against these mildews. For this reason grapes belong to the crops with the highest demand of plant protection measurements (ANONYMOUS, 2003).

On the other hand due to quality deficiencies American or Asian wild species are not suitable for wine or grape production but exhibit a range of resistance characteristics. This is why in different grape breeding programs initiated around the world the main goal is directed in the development of new varieties with combined quality and resistance characteristics by using the genetic resources of the wild species. These time-consuming and exhausting efforts that have

been going on for over more than one hundred years have led to reasonable success resulting in new varieties with good quality profiles and a considerable degree of resistance against the mildews. Nevertheless the achieved success is somewhat disappointing in relation to the enormous efforts. This is mainly due to the large generation cycle of grapes and the labor-, space- and time-consuming growing of grapes as well as the lack of knowledge about the inheritance of important traits. Recent successes in grapevine research like the development of various genetic maps, the identification of quantitative trait loci (QTL) for important traits, the development of molecular markers linked to these traits as well as the sequencing of various genomes raise hope for an improved and more efficient use of the genetic resources of wild species within breeding programs.

Evaluation of wild species

Various research activities have been carried out in recent decades for evaluating the genetic resources of wild species in order to identify new sources of resistance and making them accessible for grape breeding. Table 1 summarizes the activities focused on the evaluation of fungus resistance traits. Results demonstrate the broad range of wild species possessing fungus resistance

characteristics. On the other hand literature describes also considerable variations in the degree of resistance between different accessions within the same species. This fact clearly points out that individual accessions have to be evaluated thoroughly before using them as a resistance source in breeding programs, a precondition for an effective way for getting access to this source.

It is obvious that not only the American wild species located in the origin centers of the spread of the mildews exhibit resistance characteristics but also Asian wild species. Whereas it is likely that the resistance in American species evolved by co-evolution of grapes and fungus, this cannot be the reason for unidentified resistance sources against the mildews in Asian species because no co-evolution with the pathogens happened there. To some extent this may be an indication for different resistance mechanisms of American and Asian species. RAMMING et al. (2010) describe an accession of the Asian species *Vitis romanetii* as source of powdery mildew resistance and guess that the resistance mechanism may be a kind of non-host resistance. In terms of resistance management in grapevine breeding this might be a very favorable option because the probability of overcoming this resistance by evolving new races of the fungus is considerably reduced.

Table 1: Survey on evaluation of wild species for resistance against fungus diseases

Disease	Wild species		Reference
	American	Asian	
Downy mildew	+		GALET, 1956
		+	HE and WANG, 1986
	+	+	STAUDT et al., 1995
		+	WAN et al., 2007a
		+	WAN et al., 2007b
	+	CADLE-DAVIDSON, 2008	
Powdery mildew	+		GALET, 1956
	+		BOUQUET, 1980
		+	STAUDT, 1980
		+	GOLODRIGA et al., 1981
		+	WANG et al., 1995
	+	+	STAUDT, 1997
	+	WAN et al., 2007a	
Downy mildew, Powdery mildew	+		WAGNER, 1965
<i>Pseudopeziza tracheiphila</i>		+	KOZMA, 1994
<i>Elsinoe ampelina</i>		+	WANG et al., 1998
<i>Guignardia bidwellii</i>	+		GALET, 1956
	+		BARRETT, 1953 und 1955
	+		BOUQUET, 1980
	+		JABCO et al., 1985
	+		HAUSMANN et al. (unpublished)

Table 2: Identified resistance loci for various pests and diseases (source: www.vivc.de)

Trait	Symbol	Original species trait derived from	Reference
Plasmopara viticola	Rpv1	<i>M. rotundifolia</i>	MERDINOGLU et al., 2003
	Rpv2	<i>M. rotundifolia</i>	WIEDEMANN-MERDINOGLU et al., 2006
	Rpv3	American wild species	WELTER et al., 2007; BELLIN et al., 2009
	Rpv4	American wild species	WELTER et al., 2007
	Rpv5	<i>V. riparia</i>	MARGUERIT et al., 2009
	Rpv6	<i>V. riparia</i>	MARGUERIT et al., 2009
	Rpv7	American wild species	BELLIN et al., 2009
	Rpv8	<i>V. riparia</i>	MOREIRA et al., 2010
	Rpv9	<i>V. riparia</i>	MOREIRA et al., 2010
	Rpv10	<i>V. amurensis</i>	SCHWANDER et al. (in preparation)
Erysiphe necator	Run1	<i>M. rotundifolia</i>	BARKER et al., 2005
	Ren1	<i>V. vinifera</i>	HOFFMANN et al., 2008
	Ren2	<i>V. rupestris</i> , <i>V. cinerea</i>	DALBO et al., 2001
	Ren3	American wild species	WELTER et al., 2007
	Ren4	<i>V. romanetii</i>	RAMMING et al. (in preparation)
Daktulosphaera vitifoliae	Rdv1	<i>V. cinerea</i>	ZHANG et al., 2009
Xiphinema index	Xir1	<i>V. arizonica</i>	XU et al., 2008

Marker assisted selection (MAS) for combining different resistance sources

Recent research in grapevine genetics has led to remarkable success. The development of various genetic maps resulted in the identification of quantitative trait loci (QTL) for important traits like mildew resistance (DALBÓ et al., 2001; MERDINOGLU et al., 2003; BARKER et al., 2005; WELTER et al., 2007; BELLIN et al., 2009; MARGUERIT et al., 2009; MOREIRA et al., 2010). The development of molecular markers linked to these traits, which was strongly supported by sequencing of various genomes, allows the application of MAS in grapevine breeding as a new tool for increasing breeding efficiency. Table 2 gives an overview about the so-far identified molecular markers linked to various pests and diseases (source: www.vivc.de). The existence of different resistance related loci on different linkage groups for powdery mildew as well as for downy mildew can be recognized. Pyramiding of different resistance loci in new varieties is highly desired because a higher degree and/or sustainability of resistance can be expected. Especially in these cases the replacement of traditional phenotypic selection for mildew resistance by MAS is of special interest because genotypes with combined re-

sistance loci may be difficult to differentiate phenotypically. EIBACH et al. (2007) identified in the progeny of a cross between VRH3082-1-42 and 'Regent' genotypes

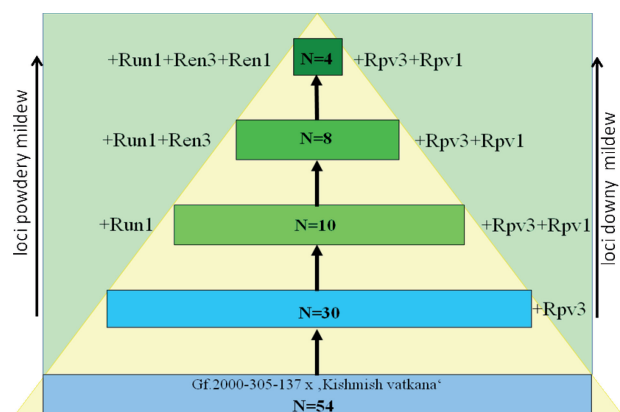


Fig. 1: Results for pyramiding different mildew resistance loci in a population derived from the cross Gf.2000-305-137 x 'Kishmish vatkana'. Gf.2000-305-137 exhibits loci Run1, Ren3 (related to powdery mildew resistance) and the loci Rpv1, Rpv3 (related to downy mildew resistance). 'Kishmish vatkana' exhibits the locus Ren1 (related to powdery mildew resistance).

carrying the resistance locus Run1/Rpv1 for powdery and downy mildew resistance inherited from VRH3082-1-42 as well as the resistance loci Ren3 (powdery mildew) and Rpv3 (downy mildew) inherited from 'Regent'. These by MAS selected genotypes were used for further crossings with 'Kishmish vatkana'. 'Kishmish vatkana' is a variety indigenous in Uzbekistan carrying the powdery mildew resistance related locus Ren1 (HOFFMANN et al., 2008). Figure 1 shows the scheme of the marker assisted selection procedure resulting in four genotypes with the pyramided resistance loci of both parents. These genotypes feature now three powdery mildew related loci and two downy mildew related loci. It is intended to add another resistance related downy mildew locus in a further breeding step in order to achieve a preferably high degree of resistance as well as a preferably high sustainability of resistance for both mildews.

Establishing of introgression lines with valuable traits from wild species

Historical review confirms that the introgression of valuable traits from wild species into the gene pool of *Vitis vinifera* lasts very long. In fact in Germany for example targeted resistance breeding started in 1926 and the first varieties derived from this breeding program and approved for the production of quality wine were released in 1995 ('Phoenix'; white variety) and 1996 ('Regent'; red variety), respectively. As already mentioned one reason for this time-consuming procedure is the long generation cycle of grapes. Another reason is the necessity to execute a backcross and pseudo-backcross (pBC) program, resp., because of the quality characteristics. So far very little knowledge has been gained about the genetics of the complex of compounds influencing quality. It is likely that there exist a range of loci within the genome, which account for different compounds affecting quality. Therefore it is appropriate to establish introgression lines possessing a preferably high percentage of the *Vitis vinifera* genome besides the target locus for resistance. The application of MAS for the establishing of such introgression lines can increase efficiency substantially as well as speeding up marker assisted backcrossing (MABC) significantly. As an example for establishing introgression lines by the use of MAS the course of action is demonstrated for the introgression of a resistance locus for phylloxera into *Vitis vinifera* varieties. ZHANG et al. (2009) established a genetic map for the population derived from a cross between the *Vitis vinifera* variety Gf.V3125 and the phylloxera

resistant rootstock variety 'Boerner' out of a cross *Vitis riparia* x *Vitis cinerea*. Based on this map a quantitative trait locus (QTL) for phylloxera resistance on linkage group 13 could be identified. Further research by HAUSMANN et al. (unpublished) resulted in narrow flanking markers around the resistance locus (Rpd1). F1-plants carrying the Rpd1-locus were used as male parents for a further cross with 'Pinot blanc' in order to establish a pBC₁. In a first step this population was screened with narrow flanking markers for the presence and absence of the Rpd1-locus, respectively. For those genotypes exhibiting the Rpd1-locus (N = 157) in a second step a marker assisted background selection was carried out. 99 markers more or less equally distributed over the whole genome were analyzed. Including the parents and grandparents of this population in the analysis allowed the conclusion whether the individual alleles were inherited by the recurrent *Vitis vinifera* parent or by the wild species used as parents in the pedigree. The results presented in Table 3 show that there is a considerable variation for the percentage of *Vitis vinifera*-inherited alleles in the pBC₁-population. Even most genotypes confirm the expectation that 75 % of the alleles are inherited from the recurrent parent in a pBC₁-generation, six plants could be identified with a distinct higher frequency in the range between 82.5 % and 87.5 %. Presuming the tested markers are equally distributed throughout the whole genome this means that the *Vitis vinifera* parent of the pBC₁-population inherited 65 % to 75 % of its genome on these seedlings. Using these seedlings for further crossings and doing this kind of background selection in every pBC-generation accelerates the development of introgression lines considerably. Making the assumption that in every pBC-generation plants can be selected where the recurrent *Vitis vinifera* parent inherits 65 % of its genome then the *Vitis vinifera* proportion goes up to 97.9 % in the pBC₃-generation. This is close to the percentage of 98.4 % which will be reached in the pBC₅-generation assuming

Table 3: Classification of the pBC₁-plants according to their percentage of alleles inherited from the recurrent *Vitis vinifera* varieties

V. vinifera-marker (%)	Individuals	
	(N)	(%)
62.5 - 67.5	11	7.0
67.5 - 72.5	38	24.2
72.5 - 77.5	63	40.1
77.5 - 82.5	39	24.8
82.5 - 87.5	6	3.8

50 % of the genome from the recurrent parent is inherited in every generation. Results clearly show that a reduction of up to two generation cycles can be achieved when applying marker assisted background selection consequently in every generation. Taking into consideration that the seed to seed cycle which is normally around four years can be reduced up to two years by optimized cultivation of the selected genotypes then the availability of an introgression line with around 98 % *Vitis vinifera* genome portion can be reached already eight years after establishing the F1-population. Compared to traditional approaches the application of MAS in the context of background selection is a kind of breakthrough for making valuable traits of wild species in breeding programs accessible in a manageable time frame.

Conclusions

Wild species are a highly valuable source for a range of resistance characteristics. The introgression of these traits into the genetic background of quality grapes belonging to *Vitis vinifera* succeeded to some extent in the past. New varieties with high quality and improved resistance characteristics - mainly against the mildews - could be developed and are on their way to the market. Nevertheless the huge potential of the wild species is by far not exhausted. New breeding tools like the application of marker assisted selection (MAS) offer new possibilities for introducing resistance characteristics from the wild species into the gene pool of *Vitis vinifera* in a very efficient way as well as in a manageable time frame. Pyramiding resistance loci by the application of MAS promises the combination of quality and a high degree of resistance resp. a high sustainability of resistance. It can be stated that the breeding strategies based on empirical experiences in the past are on the way to be replaced by knowledge based breeding strongly supported by marker assisted selection.

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