

# Crop wild relatives as genetic resources – the case of the European wild grape

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<sup>1</sup>Molecular Cell Biology, Botanical Institute, Karlsruhe Institute of Technology, Kaiserstrasse 2, 76128 Karlsruhe, Germany; <sup>2</sup>Department of Plant Sciences, North Dakota State University, Dept. 7670, Fargo, ND 58108-6050, USA; and <sup>3</sup>DLR, Dienstleistungszentrum Ländlicher Raum, Breitenweg 71, 67435 Neustadt, Germany.

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Schröder, S., Kortekamp, A., Heene, E., Daumann, J., Valea, I. and Nick, P. 2015. **Crop wild relatives as genetic resources – the case of the European wild grape.** *Can. J. Plant Sci.* **95**: 905–912. *Vitis vinifera* L. ssp. *sylvestris* (Gmelin) Hegi, the European Wild Grape and ancestor of cultivated grapevine varieties (*V. vinifera* L. ssp. *vinifera*) is the sole wild grapevine species existing in Europe. This important crop wild relative (CWR) species is almost extinct, and persists only in residual habitats. Since these habitats are close to vineyards, this CWR species is endangered by hybridisation with its descendant crop and naturalised rootstocks that originate from viticulture. For this reason, we addressed two questions: To what extent have the remaining South German European Wild Grape accessions escaped hybridisation and preserved genetic identity? Second, what is the potential of this CWR species as a genetic resource for breeding in relation to several grapevine diseases? Using a set of highly resolving genetic markers, we were able to exclude introgression of autochthonous *sylvestris* accessions by cultivated grapevine. However, we detected introgression mostly from wild American species used as rootstocks in viticulture. The autochthonous accessions can be grouped into clusters. Comparative inoculation studies with the grapevine pathogens powdery mildew (*Erysiphe necator*), downy mildew (*Plasmopara viticola*), and black rot (*Guignardia bidwellii*) revealed relatively high levels of resistance in some of the ssp. *sylvestris* accessions and represents a valuable genetic resource for resistance breeding.

**Key words:** European wild grape, *Vitis sylvestris*, crop wild relative, *Erysiphe necator*, *Plasmopara viticola*, *Guignardia bidwellii*

Schröder, S., Kortekamp, A., Heene, E., Daumann, J., Valea, I. et Nick, P. 2015. **Les espèces sauvages apparentées aux cultures en tant que ressource génétique – cas du raisin sauvage européen.** *Can. J. Plant Sci.* **95**: 905–912. *Vitis vinifera* L. ssp. *sylvestris* (Gmelin) Hegi, le raisin sauvage européen, ancêtre de toutes les variétés de vigne (*V. vinifera* L. ssp. *vinifera*) cultivées, est la seule vigne sauvage qui existe en Europe. Cette importante espèce sauvage apparentée à la vigne cultivée est presque éteinte, et on ne la retrouve que dans des habitats résiduels voisins des vignobles. L'hybridation avec la descendance et les porte-greffes naturalisés issus de la viticulture menace l'espèce sauvage d'extinction. C'est pourquoi les auteurs se sont posé deux questions : dans quelle mesure les obtentions de raisin sauvage européen du sud de l'Allemagne ont-elles échappé à l'hybridation et préservé leur identité génétique, et quel potentiel cette ESA présente-t-elle en tant que ressource génétique pour l'hybridation, face à diverses maladies de la vigne? En recourant à un jeu de marqueurs génétiques à haute résolution, les chercheurs sont parvenus à exclure les introgressions venant de la vigne cultivée des obtentions indigènes de *sylvestris*. Ils ont néanmoins décelé des introgressions venant essentiellement des espèces américaines employées comme porte-greffe. Les obtentions indigènes peuvent être regroupées. Les études comparatives avec inoculation des agents pathogènes que sont l'oïdium de la vigne (*Erysiphe necator*), le mildiou (*Plasmopara viticola*) et la pourriture noire (*Guignardia bidwellii*) révèlent une résistance relativement élevée chez quelques obtentions de la sous-espèce *sylvestris*. Celles-ci constituent donc une intéressante ressource génétique en vue d'inculquer la résistance par hybridation.

**Mots clés:** Raisin sauvage européen, *Vitis sylvestris*, espèce sauvage, *Erysiphe necator*, *Plasmopara viticola*, *Guignardia bidwellii*

Crop wild relatives (CWRs) have shifted to the centre of attention of plant breeding and evolution biology (Ellstrand et al. 2010) because they represent valuable genetic resources for breeding on the one hand and still entertain gene flow with the respective cultivated crops. This gene flow may have unwanted consequences; for instance, when advantageous traits from the crop are transferred into the CWR species that often share similar

ecological niches to the related crop species and by this gene flow acquire undesired weed traits. On the other hand, the population of rare CWR species might be brought to extinction by introgression from the crop (Ellstrand et al. 2010). For instance, in Taiwan the wild subspecies *Oryza rufipogon* ssp. *formosana* has been almost eliminated by gene flow from cultivated rice, *O. sativa* ssp. *japonica* (Kiang et al. 1979) and the wild

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**Abbreviation:** CWR, crop wild relative

cotton of Galapagos (*Gossypium darwinii*) has suffered extensive introgression by *G. hirsutum* (Wendel and Percy 1990).

The cultured grape *Vitis vinifera* L. ssp. *vinifera* has played an important role with respect to economy and culture over many centuries. It represents one of the most important crops worldwide considering its global distribution and its high economic yield of about US\$4500 t<sup>-1</sup> (Cooper et al. 2012). However, its ancestor and CWR species, the European wild grape, *V. vinifera* L. ssp. *sylvestris* (C. C. Gmel.) Hegi is close to extinction.

Due to human interference, such as river regulation and drainage, this habitat has been destroyed progressively over centuries, and the European wild grape has survived only in small residual and dissociated populations (Arnold et al. 2005). The situation became worse when American rootstocks (*V. riparia* Michx., *V. labrusca* L. and *V. rupestris* Scheele) were introduced to Europe in the 19th century (Bodor et al. 2010) as a strategy to control insect pests such as *Phylloxera*. Thus, the genetic identity of the European wild grape is not only endangered by introgression from its cultivated descendant, the cultured grape, but also from American wild species of *Vitis*. Additionally, these American rootstocks frequently escape from abandoned vineyards into the natural habitats of *V. vinifera* ssp. *sylvestris*. These neophytic *Vitis* species have developed into strong competitors of the European wild grape (Arnold et al. 1998).

Along with the *Phylloxera* resistant North American rootstocks, new diseases, such as powdery mildew (*Erysiphe necator*), downy mildew (*Plasmopara viticola*), and black rot (*Guignardia bidwellii*) were introduced into Europe. Since the grapevine species *V. vinifera* had not been previously exposed to these new diseases, both the cultivated grapevine as well as the wild European grape were expected to be naive hosts, in contrast to their wild relatives from North America that had co-evolved with these pathogens (Arnold et al. 2005).

The residual *V. vinifera* ssp. *sylvestris* populations at the Upper Rhine Valley represent the largest occurrence in Germany, but suffer from extensive habitat fragmentation (Arnold et al. 2005). The largest contiguous autochthonous population with more than 40 individuals is found in the Ketsch peninsula between Mannheim and Karlsruhe in Baden-Württemberg, Germany, and a second population in the vicinity of Ketsch, on the Reiss peninsula. The current study focused on two main objectives:

1. To elucidate the genetic relationship among the ssp. *sylvestris* accessions from southern Germany and populations from Hördt (Germany), and Austria. For this purpose, eight SSR markers commonly used to discriminate wild European grape from grapevine cultivars (Arrigio and Arnold 2007; Zecca et al. 2008) were used in addition to morphological markers. In addition to the wild grapes, we also

compared our results to data from cultured grapes common in regional viticulture.

2. To determine to what extent this CWR species has potential as a genetic resource for resistance breeding. Although ssp. *sylvestris* is expected to be a naive host for pathogens introduced from North America, a Europe-wide sanitary study on plant health in wild European grapes uncovered a surprisingly low and only sporadic incidence of diseases and pests, which was explained with a high degree of genetic variation in the wild populations (Ocete et al. 2000). To get more insight into potential sources of pathogen resistance in ssp. *sylvestris*, we conducted a comparative infection study in 10 selected accession of wild European grape with powdery mildew (*Erysiphe necator*), downy mildew (*Plasmopara viticola*), and black rot (*Guignardia bidwellii*).

## MATERIALS AND METHODS

### Plant Material

Forty *V. vinifera* ssp. *sylvestris* plants collected from natural sites on the Ketsch Peninsula on the Rhine River, Southern Germany, the largest natural population in Germany, were analysed in this study. Additionally, 25 *Vitis vinifera* ssp. *sylvestris* plants originating from the Reiss Peninsula near Mannheim, and 18 plants from different isolated sites in the Upper Rhine Valley were included into the study, as well as five plants from the Lobau (Danube region, Vienna), eight cultivars common in the vineyards adjacent to the Ketsch Peninsula, and three wild *Vitis* species for reference (*V. rupestris* and *V. riparia* from North America and *V. amurensis* from Siberia) commonly used as genetic source of rootstocks along with three Chinese wild species of *Vitis* (*V. jaquemontii*, *V. yenshanensis*, and *V. quinquangularis*). All accessions were located by GPS, photographically documented, then redetermined using morphological keys and ampelographic descriptors of the *Organisation Internationale de Vigne et du Vin* (Olmo 1976) with the help of an ampelograph (Dr. Erika Maul, Julius-Kühn-Institute Institute for Grape Breeding Geilweilerhof). All accessions are maintained as living specimens at the Botanical Garden of the Karlsruhe Institute of Technology. Herbarium vouchers are deposited at the herbarium of the Botanical Garden of the Karlsruhe Institute of Technology. The SSR marker lengths for the grapevine cultivars Cabernet, Chardonnay, Müller-Thurgau, Pinot Blanc, Pinot Noir, Riesling, Silvaner and Traminer were obtained from *Organisation Internationale de la Vigne et du Vin*, 2nd edition of the OIV descriptor list for grape varieties and *Vitis* species database (OIV, <http://news.reseau-concept.net/pls/news>).

### DNA Extraction and Analysis

DNA was extracted from leaf tissue by a slightly modified CTAB method (Doyle and Doyle 1987). For DNA amplification, 25 ng of genomic DNA was used as

template. The PCR preparation contained: 2  $\mu$ L: rTaq buffer (10  $\times$ ), 2  $\mu$ L dNTPs, 12 pmol of each primer, template, 1.5 Units rTaq DNA (Takara Bio Inc., Otsu, Japan), and water ad 20  $\mu$ L.

Samples were genotyped at eight microsatellite loci located on different chromosomes (<http://www.genres.de/eccdb/vitis/>) using the SSR-markers: VVS2 (Thomas and Scott 1993), VVMD07 (Bowers et al. 1996), VVMD25, VVMD27, VVMD28, VVMD32 (Bowers et al. 1999), VrZag62, and VrZag79 (Sefc et al. 1999). The PCR parameters were 5 min at 95°C, followed by 36 cycles of 15 s 95°C, 30 s annealing, and 30 s synthesis at 72°C. Annealing occurred at 53°C (for VVMD7, VVMD32 and VrZag79), 58°C (for VVS2, VVMD27 and VrZag62), or at 60°C (for VVMD25 and VVMD28). Amplification products were separated on a 2% agarose gel. Lengths of microsatellite markers were determined by a commercial provider (GATC, Konstanz, Germany). Fragment lengths of the random repeats were evaluated by GeneMarker<sup>®</sup>.

### Construction of Genetic and Geographic Relationships

The software Structure 2.2 (Pritchard et al. 2000) was used to find a model-based (Bayesian clustering) genetic structure in the SSR data. This method is used to cluster individuals into  $K$  distinct populations by minimizing Hardy–Weinberg disequilibrium and linkage disequilibrium between loci within groups. The admixture model was used setting Pop IDs for each location and grapevine variety respectively. A series of five independent runs for each value of  $K$  (from  $K=1$  to  $K=10$ ) was performed, each with a burn-in phase of 50 000 iterations followed by 500 000 MCMC repetitions.

For a tree-based approach to analyse the SSR data, the software Identity 1.0 (Wagner and Sefc 1999) was used, and a Microsat input file was created. With this input file, a distance matrix was calculated using the software MICROSAT (Minch 1997) using “chord distance”, a strictly geometric view of the distances between multi-dimensional points on a hypersphere (a sphere with more than three dimensions). The distance matrix was used in Mega 4.0 (Tamura et al. 2007) to reconstruct a Neighbour Joining Tree and formatted using the software FigTree v1.3.1 (<http://tree.bio.ed.ac.uk/software/figtree/>).

The principal component analysis was performed using GenAlEx 6.41 (Peakall and Smouse 2006).

### Assay for Sensitivity to Powdery Mildew, Downy Mildew, and Black Rot

In order to evaluate the response to important and widespread grapevine diseases, 10 autochthonous ssp. *sylvestris* accessions (nine from the Ketsch, and one from an isolated stand at Hördt), along with three cultigen accessions (‘Riesling’, ‘Müller-Thurgau’, and ‘Regent’) were tested. For the assay on powdery mildew and black rot susceptibility, entire plants were inoculated with the respective pathogen and incubated for 14 d in a

phytochamber at 21°C with a 12:12 dark:light cycle. For the assay on downy mildew susceptibility, the 4th to the 6th leaf from the shoot tip was harvested and put upside down in a petri dish on wet filter paper (3 mL water). Five leaves from five different plants for each grapevine species were tested. The leaves were sprayed five times with a mist of a *Plasmopara viticola* suspension (50 000 sporangia mL<sup>-1</sup>). The petri dish was sealed and placed in a dark room over night at 21°C. The next day, excess water was removed from each sample under the clean bench and the petri dish was resealed. Then, the samples were incubated in a phytochamber at 21°C with a 12:12 dark:light cycle for 6 further days. To quantify disease incidence for the respective pathogen, the leaves were classified into seven classes depending on the proportion of affected leaf surface (0%, <5%, <10%, <25%, <50%, <75%, <100%). The data represent the results from three independent experimental series.

## RESULTS

### Genetic Structure of *Vitis sylvestris*

The genetic structure reveals two populations of *V. vinifera* ssp. *sylvestris*, analysed using a set of eight SSR-markers, which are widely used as probes for the genotyping of *V. vinifera* (Thomas and Scott 1993; Bowers et al. 1996, 1999; Sefc et al. 1999) (Table 1). All eight analysed loci were polymorphic in the panel of wild and cultivated accessions tested with 8 (VrZAG62) to 17 (VVMD25) alleles per individual locus. With the exception of locus VVMD25, the number of alleles was significantly higher in the autochthonous Ketsch population as compared with the secondary Reiss population (Supplementary Table 1). A third cluster was formed by non-*V. sylvestris* species. *V. sylvestris* specific alleles were detected for markers VVS2, VVMD25 and VVMD28, as well as non-*V. sylvestris* alleles for all markers analysed (Supplementary Table 1).

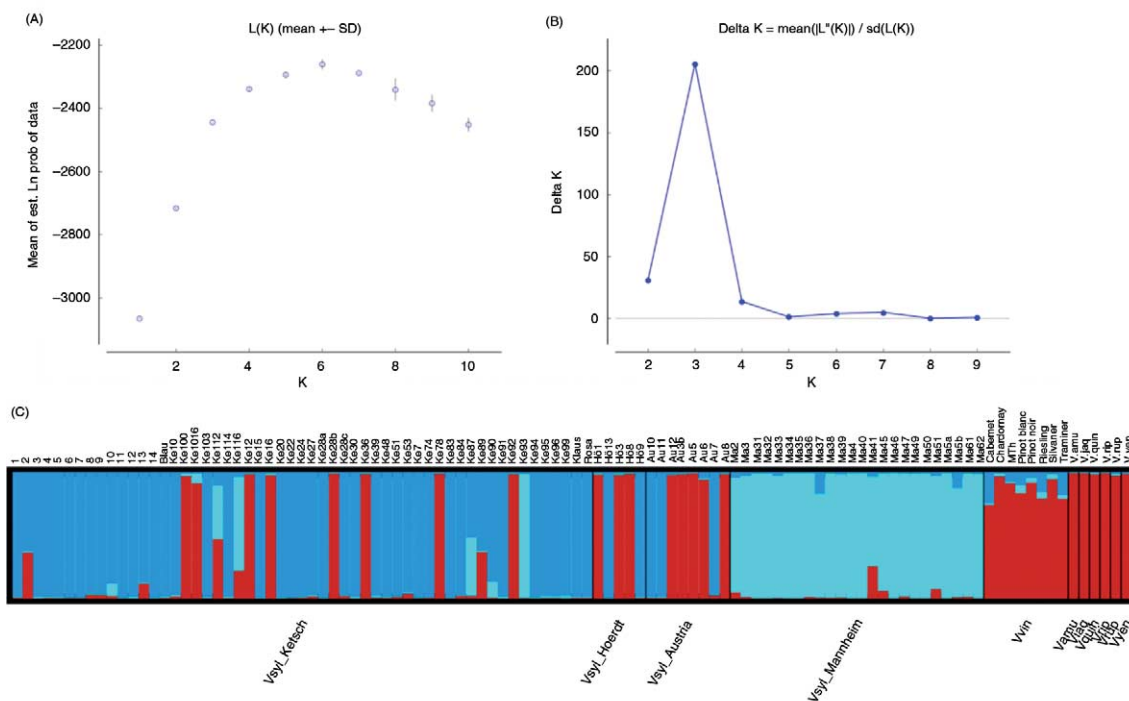
The population structure manifest as polymorphisms in the SSR marker set was analysed by Bayesian structuring using Structure 2.2 (Evanno et al. 2005), clustering the individuals into  $K$  distinct populations such that Hardy–Weinberg and linkage disequilibria between loci within groups are minimal (Pritchard et al. 2000). To estimate  $K$ , the number of  $K$ s is increased until a plateau is reached, reporting that increasing  $K$  will not extract additional information from a given data set (Garnier et al. 2004). In this data set, the Ln P(D) was increased from  $K=1$  to  $K=10$ . Structure results were analysed using the online software “structure harvester” (v0.6.94). DeltaK (mean(|L'(K)|)/sd(L(K))) peaks at  $K=3$ , assigning the individuals into three populations (Fig. 1). The first cluster (red), marks our controls/outgroups, and contains all cultured *V. vinifera* ssp. *vinifera* varieties, the Chinese wild species *V. quinquangularis* and *V. yenshanensis*, as well as the Siberian *V. amurensis*, the Pakistani *V. Jacquemontii*, and the North American *V. rupestris* and *V. riparia*, which are commonly used as rootstocks in

**Table 1.** Designations, sequences, and reference for the primers used for SSR analysis. Fam 6-FAM-phosphoramidite, Cy3 cyanine 3 analogue NED, Hex hexachlorofluorescein are conjugated to the forward primer as fluorescent dyes for multiplexing

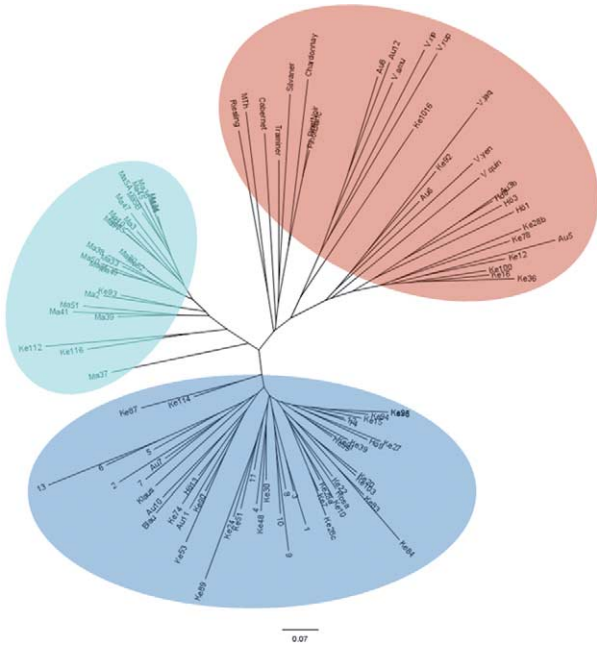
Designation	Sequence	Source
VVMD7(f)	Cy3 – AGAGTTGCGGAGAACAGGAT	Bowers et al. (1996)
VVMD7(r)	CGAACCTTCACACGCTTGAT	Bowers et al. (1996)
VVMD25(f)	Fam – TTCCGTTAAAGCAAAGAAAAGG	Bowers et al. (1999)
VVMD25(r)	TTGGATTTGAAATTTATTGAGGGG	Bowers et al. (1999)
VVMD27(f)	Fam – GTACCAGATCTGAATACATCCGTAAGT	Bowers et al. (1999)
VVMD27(r)	ACGGGTATAGAGCAACGGTGT	Bowers et al. (1999)
VVMD28(f)	Hex – AACAATTCATGAAAAGAGAGAGAGAGA	Bowers et al. (1999)
VVMD28(r)	TCATCAATTCGTATCTCTATTTGCTG	Bowers et al. (1999)
VVMD32(f)	Cy3 – TATGATTTTTTAGGGGGGTGAGG	Bowers et al. (1999)
VVMD32(r)	GGAAAGATGGGATGACTCGC	Bowers et al. (1999)
VVS2(f)	Fam – CAGCCCGTAAATGTATCCATC	Thomas and Scott (1993)
VVS2(r)	AAATTCAAAATTCATTCAACTGG	Thomas and Scott (1993)
VrZag62(f)	Hex – GGTGAAATGGGACCCGAACACACGC	Sefc et al. (1999)
VrZag62(r)	CCATGTCTCTCCTCAGCTTCTCAGC	Sefc et al. (1999)
VrZag79(f)	Hex – AGATTGTGGAGGAGGGAACAAACCG	Sefc et al. (1999)
VrZag79(r)	TGCCCCCATTTCAAACCTCCCTTC	Sefc et al. (1999)

viticulture. It also contains several plants which, due to their deviant morphology to *V. sylvestris*, were already doubted as being true European wild grapes (Au3b, Au5, Au6, Au8, Au12, Hö1, Hö3, Hö8, Ke12, Ke16, Ke28b,

Ke36, Ke78, Ke92, Ke100, and Ke1016). The second population (light blue) contains mainly individuals originating from the Reiss Peninsula, but also Ke93, Ke112, and Ke116. The third population (blue) represents mostly



**Fig. 1.** Genetic structure of the sampled populations based on eight SSR-loci of autochthonous *Vitis vinifera* ssp. *sylvestris* plants collected from natural sites at the Ketsch Peninsula (Ke), the largest natural population in Germany, ssp. *sylvestris* plants originating from the Reiss Peninsula near Mannheim (Ma), and additional ssp. *sylvestris* plants from different isolated sites in the Upper Rhine Valley, the Lobau (Danube region, Vienna, Austria), cultivars common in the vineyards adjacent to the Ketsch Peninsula, and wild non-*vinifera* species for reference (*V. rupestris*, *V. riparia* from North America, *V. amurensis* from Siberia, *V. jaquemonitii* from Pakistan, *V. quinqueangularis* and *V. yenshanensis* from China). The genetic structure was analysed by Bayesian structuring (using Structure 2.2, with a burn-in Phase of 50 000 at 500 000 repeats). (A) Log likelihood for each *K* (population number), (B)  $\Delta K$  showing the true value of *K*, and (C) distribution of the three populations derived from Bayesian clustering.



**Fig. 2.** Neighbour-joining tree calculated over the genetic distances of the taxa analysed in Bayesian clustering.

individuals from the Ketsch Peninsula, but also includes plants from Austria (Au7, Au10 and Au11) and Hördt (Hö9 and Hö13).

The Bayesian clustering by Structure 2.2 also indicates several hybridisation events within the autochthonous *spp. sylvestris* population from Ketsch, such as plants 2, Ke89 and Ke112, which share >20% of their alleles with non-European *Vitis* species, sometimes accompanied by alleles from grapevine cultivars. Ke87 shares >20% of alleles with the Reiss population.

To get further insight into the genetic fine structure of these clusters, we constructed a neighbour-joining tree (Fig. 2). As to be expected, the grapevine cultivars formed one contiguous cluster together with the non-*vinifera* accessions (red background). The *V. sylvestris* accessions form two clusters. Again, all *sylvestris* accessions from the Reiss population were grouped together, also includ-

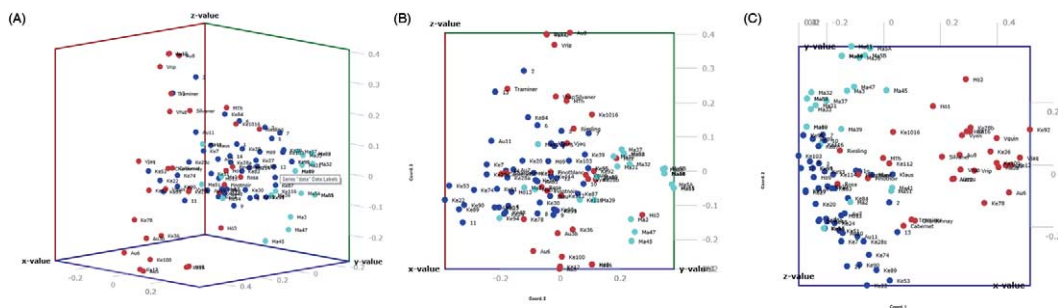
ing Ke93, Ke112 and, Ke116 (light blue background). The accessions from the autochthonous Ketsch population, excluding Au3b, Au5, Au6, Au8, Au12, Hö1, Hö3, Hö8, Ke12, Ke16, Ke28b, Ke36, Ke78, Ke92, Ke100, and Ke1016, which could be found in the non-*vinifera* cluster, form a third cluster (blue background).

The results of the principal component analysis reflect the structure and neighbour-joining analysis, whereas the first component explains 22.01% of the variance, the second accounts for 14.24% and the third component for 8.41%, totaling 44.66% of the variance (Fig. 3).

***Vitis vinifera* ssp. *sylvestris* harbours Pathogen-resistance Factors**

We investigated the potential of European wild grapes as a genetic resource for resistance breeding. We excised leaf discs from accessions belonging to the Ketsch population, and conducted a comparative infection study with downy mildew (*Plasmopara viticola*, Fig. 4A), powdery mildew (*Erysiphe necator*, Fig. 4B), and black rot (*Guignardia bidwellii*, Fig. 4C). As references, we used the susceptible grapevine cultivars ‘Müller-Thurgau’ and ‘Riesling’ along with the mildew-resistant cultivar ‘Regent’ originating from a complex pedigree involving different North American wild *Vitis* species.

As to be expected, the cultivars ‘Riesling’ and ‘Müller-Thurgau’ were fully susceptible to infection with both downy and powdery mildew as well as with black rot. The cultivar ‘Regent’ was strongly resistant to downy and powdery mildew, but remained completely susceptible to black rot. In contrast, the majority of the tested *spp. sylvestris* genotypes were strongly resistant to both downy and powdery mildew and performed equally as well as the mildew-resistant cultivar ‘Regent’ (Fig. 4A, B). Genotype Ke20, strongly resistant to powdery mildew, was only partially resistant to downy mildew and genotype Ke39, which was strongly resistant to Downy Mildew, showed only a partial resistance to powdery mildew. For black rot, the genotypes did not perform significantly better compared with the three tested cultivars with exception of the highly resistant Ke99 (Fig. 4C). This indicates genetic variation within the *V. sylvestris* population with respect to disease resistance.



**Fig. 3.** (A) Three-dimensional principal component analysis. Different coloured dots represent affiliation with structure populations. (B) First two coordinates of PCA. (C) Coordinates two and three of PCA.

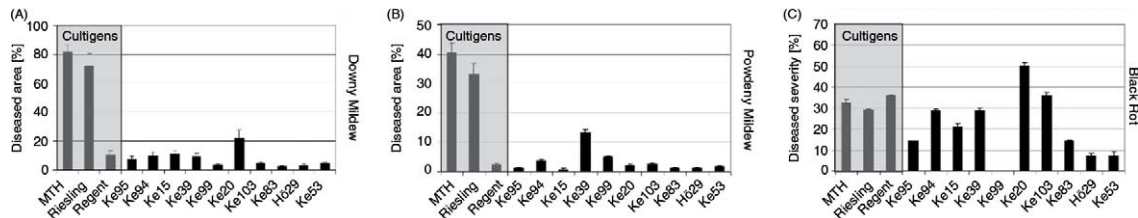


Fig. 4. Response of different *V. vinifera* genotypes to downy mildew (A), powdery mildew (B), and black rot (C). Data represent three independent experimental series each using five leaves from five different plants per genotype. Cultivars include the cultivars ‘Müller-Thurgau’ (MTh), ‘Riesling’, and the mildew-resistant hybrid cultivar ‘Regent’.

## DISCUSSION

### *Vitis vinifera* ssp. *silvestris* is under Pressure by Introgression

In the present work, the genetic structure of the largest autochthonous and contiguous German population of *ssp. silvestris* at the Ketsch Peninsula was investigated, and compared with a secondary population (originating from material propagated *ex situ*), using a set of highly resolving SSR markers. More than 50 putative autochthonous accessions of the European wild grape along with the regional grapevine cultivars and several non-*vinifera* species, commonly used as source for rootstocks, were analysed. Gene flow could be detected in several accessions, usually manifesting as introgression events of either non-*vinifera* or cultivar alleles.

### A Crop Wild Relative as Genetic Resource

Whereas, in the past, breeding was targeted towards high-yield elite cultivars, the limited and progressively disappearing arable land and consumer emphasis towards reduction of chemical plant protection has shifted the focus towards stress and pathogen resistance. The yield increases during domestication were often accompanied by a loss of secondary compounds and stress-resistance traits. Crop wild relative species can be used to reintroduce these valuable traits back into the crop cultivar. In the case of grapevine, a long history of traditional resistance breeding by crossing *V. vinifera* with North American wild *Vitis* species, and nowadays by the support of advanced molecular genetics, has allowed us to obtain new cultivars that are resistant to both downy and powdery mildew (Eibach et al. 2007). Traditional viticulture employs the use of copper-containing products against downy mildew, charging the soil with ecologically highly problematic copper compounds (up to a yearly Cu load of 6 kg ha<sup>-1</sup> per year). Resistance breeding provides alternatives to the use of copper products. However, the spread of *P. viticola* strains that are able to grow on resistant grapevine cultivars such as ‘Regent’ (Schröder et al. 2011) illustrates that continuous breeding efforts are required to ensure the sustainability of this approach.

The cause of black rot, *Guignardia bidwellii*, has been observed to cause epidemics in Germany since 2002, and resistance factors for this disease have not yet been

identified. The finding that the tested genotype Ke 99 is resistant to black rot represents the first evidence for resistance factors against this pathogen. These factors provide valuable starting material for resistance breeding. We have therefore launched a new approach to introduce the black rot resistance present in Ke 99 into grapevine cultivars. By a combination of cell biological and morphological investigations with inoculation studies and genetic mapping we hope to identify genetic factors that contribute to black rot resistance.

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ACCENAME	VVS2:1	VVS2:2	VVMD07:1	VVMD07:2	VVMD25:1	VVMD25:2	VVMD27:1	VVMD27:2	VVMD28:1	VVMD28:2	VVMD32:1	VVMD32:2	VrZAG62:1	VrZAG62:2	VrZAG79:1	VrZAG79:2
T	151	151	249	249	239	255	180	190	234	264	240	256	194	194	251	251
2	151	151	243	243	236	241	184	190	264	264	240	244	194	194	251	251
3	151	151	249	249	239	255	190	190	228	236	240	256	194	194	251	251
4	151	151	239	263	249	267	190	190	236	236	240	252	194	194	243	251
5	151	151	249	263	239	255	190	190	264	264	240	240	186	194	243	251
6	151	151	249	263	239	267	184	190	264	264	250	250	186	194	243	251
7	151	151	263	263	249	255	184	190	264	264	256	256	194	194	243	251
8	151	151	239	263	255	255	190	190	220	236	248	256	194	194	251	251
9	151	151	239	263	239	239	180	190	220	236	256	256	194	194	245	251
10	151	151	263	263	239	255	180	190	236	264	256	272	194	194	251	251
11	151	151	239	239	255	267	190	190	236	264	240	240	194	194	251	251
12	151	151	263	263	255	255	190	190	236	236	240	256	194	194	251	251
13	151	151	249	259	239	267	184	190	234	234	250	250	186	194	243	243
14	151	151	249	263	255	267	190	190	236	236	240	256	194	194	251	251
Au10	131	131	239	239	255	255	184	190	236	236	250	256	194	196	251	251
Au1	131	131	249	263	255	267	184	190	236	236	240	250	186	194	251	251
Au7	151	151	239	243	249	255	184	190	236	264	240	250	194	194	243	251
Bla	151	151	239	239	255	255	184	184	228	264	240	240	194	196	251	251
H13	151	151	239	263	255	267	184	190	264	264	240	256	186	194	251	251
H9	151	151	249	263	255	255	190	190	230	236	240	250	194	196	251	251
Kc10	151	151	263	263	255	255	190	190	236	236	240	250	186	194	239	251
Kc103	139	151	263	263	239	255	190	190	236	236	240	250	194	194	247	251
Kc114	151	151	239	263	249	255	184	190	234	236	240	240	194	194	251	251
Kc15	151	151	239	249	255	255	190	190	236	236	240	256	186	194	251	251
Kc20	139	151	237	263	239	267	190	190	236	236	240	250	194	194	251	251
Kc22	151	155	239	249	255	267	190	190	236	236	240	250	194	194	251	251
Kc24	151	151	239	239	249	255	190	190	236	264	242	256	194	194	245	251
Kc27	151	151	249	263	255	255	190	190	236	236	246	250	194	194	247	251
Kc28a	151	151	257	263	255	267	190	190	236	264	240	250	186	194	251	251
Kc28b	151	151	243	239	249	267	183	190	236	236	240	250	186	194	251	251
Kc29	151	151	239	263	267	267	190	190	228	236	240	256	194	194	251	251
Kc39	151	151	257	263	249	255	190	190	228	236	240	250	194	194	251	251
Kc48	151	151	239	263	267	267	190	190	236	264	242	252	186	194	251	251
Kc51	151	151	239	249	239	239	190	190	236	264	244	256	194	194	251	251
Kc53	157	157	239	257	255	267	184	190	236	236	240	240	186	194	243	251
Kc7	151	151	251	239	239	267	190	190	236	236	240	250	186	194	251	251
Kc74	131	151	239	239	255	267	184	190	234	264	240	256	186	194	251	251
Kc83	139	151	239	263	239	249	190	190	234	264	240	250	194	194	251	251
Kc84	139	151	239	263	239	249	190	190	234	264	250	250	186	194	251	251
Kc87	141	151	239	263	239	255	186	190	234	236	240	240	194	194	241	251
Kc89	151	157	239	239	239	267	190	190	236	264	238	254	194	194	249	249
Kc90	151	151	239	239	255	267	184	190	236	236	240	240	186	194	241	251
Kc91	151	151	239	263	255	255	190	190	228	236	240	250	186	194	251	251
Kc94	151	151	239	263	255	267	190	190	236	236	240	256	186	194	247	251
Kc95	151	151	263	263	255	255	190	190	228	236	240	256	186	194	247	251
Kc96	151	151	263	263	255	255	190	190	228	236	240	256	186	194	247	251
Kc99	151	151	239	263	255	255	190	190	228	236	240	240	194	194	251	251
Kbus	151	157	263	263	267	267	184	184	236	264	240	250	194	194	251	251
Rosa	151	153	239	263	239	255	190	190	230	236	240	250	186	194	251	251
Kc116	143	151	239	263	239	255	186	190	244	244	252	252	192	200	251	251
Kc93	143	151	239	263	239	239	186	190	236	236	240	240	192	200	241	251
Mu2	143	143	239	263	241	255	184	190	236	236	240	240	192	200	241	251
Mu3	141	151	239	263	239	255	186	186	228	236	240	256	192	200	241	251
Mu31	143	151	263	263	245	255	186	190	226	236	240	240	192	200	241	251
Mu32	151	151	263	263	245	255	186	190	228	236	240	240	192	200	241	251
Mu33	143	151	263	263	239	255	186	190	226	236	240	256	192	200	241	251
Mu34	143	151	263	263	239	255	186	186	228	236	240	240	192	200	241	251
Mu35	151	151	263	263	247	255	186	186	228	236	240	272	192	200	241	251
Mu36	143	151	263	263	239	259	186	186	228	236	240	240	192	200	241	251
Mu37	151	151	263	263	249	249	186	190	228	236	272	272	194	200	241	251
Mu38	143	151	263	263	239	255	186	190	228	236	262	262	192	200	241	251
Mu39	151	157	239	263	239	255	186	190	228	236	240	272	192	200	241	251
Mu4	143	151	263	263	239	255	186	186	228	236	240	240	192	200	241	251
Mu40	151	151	263	263	239	255	186	186	228	236	272	272	192	200	241	251
Mu41	151	157	249	261	255	257	186	190	226	236	240	272	192	200	241	251
Mu45	143	151	239	263	237	263	186	186	228	236	240	240	192	200	241	251
Mu46	143	151	263	263	239	255	186	186	228	236	240	240	192	200	241	251
Mu47	143	151	239	263	243	255	186	186	228	236	240	272	192	192	241	251
Mu49	143	151	263	263	245	255	186	190	236	236	240	240	192	200	241	251
Mu50	143	151	263	263	245	255	186	190	236	236	272	272	192	200	241	251
Mu51	151	157	239	263	239	263	184	190	226	236	272	272	192	200	241	251
Mu5A	143	151	263	263	247	247	186	186	228	236	240	240	200	200	241	251
Mu5B	143	151	263	263	249	249	186	186	228	236	240	240	194	200	241	251
Mu61	151	151	263	263	247	255	186	186	228	236	240	266	192	200	241	251
Mu62	151	151	263	263	239	255	186	190	228	236	240	240	192	200	241	251
Kc16	153	157	239	263	263	271	180	186	240	240	240	240	188	188	239	251
Au12	131	141	243	245	239	241	184	204	230	244	250	250	188	192	243	259
Au3b	153	157	239	239	255	263	180	186	240	240	233	233	180	188	243	251
Au5	137	137	239	239	263	263	180	186	240	268	233	233	188	196	239	255
Au6	151	153	239	239	263	263	180	180	240	240	240	240	186	194	249	251
Au8	131	141	243	243	293	241	184	202	228	242	240	250	188	192	243	259
Cabronet	139	139	239	239	239	249	178	190	234	236	240	258	194	204	247	259
137	143	239	243	243	259	259	182	190	218	228	240	272	188	196	243	245
H1	137	157	263	263	247	263	187	194	234	242	244	244	180	188	243	251
H3	157	157	263	263	263	271	180	186	240	268	246	246	180	188	243	251
H8	153	157	239	263	253	263	180	186	240	240	233	233	180	188	243	251