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A 'Regent' pedigree update: ancestors, offspring and their confirmed resistance loci

F. Röckel[1)], O. Trapp[1)], E. Zyprian[1)], L. Hausmann[1)], D. Migliaro[2)], S. Vezzulli[3)], R. Töpfer[1)] and E. Maul[1)]

[1)] Julius Kühn Institute (JKI) - Federal Research Centre for Cultivated Plants, Institute for Grapevine Breeding Geilweilerhof, Siebeldingen, Germany
[2)] CREA - Research Centre for Viticulture and Enology, Conegliano (Treviso), Italy
[3)] Research and Innovation Centre, Edmund Mach Foundation, San Michele all'Adige, Italy

Summary

'Regent' is the fungal resistant grapevine cultivar with the highest acreage in Germany and an important resistance donor in international breeding programs. It carries the resistance loci *Rpv3.1* as well as *Ren3* and *Ren9* against downy and powdery mildew, respectively. As the parents of 'Chambourcin', the resistant paternal ancestor of 'Regent', did not coincide with the breeder's information, the germplasm repository of JKI Geilweilerhof was screened to find the missing ancestors. SSR marker analysis revealed that 'Joannes Seyve 11369' and 'Plantet' are the true parents of 'Chambourcin' and not 'Seyve Villard 12-417' and 'Chancellor'. Furthermore, the origin of the resistance loci *Ren3* and *Ren9* could be traced back to the genotypes 'Seibel 4614' and 'Munson'. Since the breeder Hermann Jaeger mentioned 'Munson' as a direct descendant of *Vitis aestivalis* Michx. var. *linsecomii* (Buckley) L. H. Bailey and *Vitis rupestris* Scheele, one of these wild species might have been the donor of the loci.

K e y w o r d s : pedigree analysis; *Rpv* loci; *Ren* loci; SSR marker genotyping.

Introduction

During the 19th century, three major grapevine pests, *Daktulosphaïra vitifoliae* (phylloxera), *Plasmopara viticola* (downy mildew) and *Erysiphe necator* (anamorph *Oidium tuckeri*, powdery mildew) were unintentionally introduced from the U.S. to Europe by the import of vines derived from crosses of native North American *Vitis* species and Eurasian *Vitis vinifera* L. cultivars (Töpfer *et al.* 2011). The arrival of these pathogens and the susceptibility of European grapevine varieties led to severe damages and finally almost the collapse of wine production in France, Spain and other European countries about 150 years ago.

While the problem of phylloxera infestation on the roots was solved by grafting *V. vinifera* scions onto phylloxera-resistant/tolerant *Vitis* hybrid rootstocks, the control of downy and powdery mildew remains a challenging task. For efficient plant protection, sulphur and copper or synthetic antifungal protectants are applied up to 12 times each growing season (Chen *et al.* 2020) making viticulture in general one of the biggest consumers of fungicides worldwide (European Commission 2007). The best way to reduce these tremendous amounts of fungicides in viticulture is the breeding and use of novel resistant grapevine varieties carrying resistance loci (R-loci) against downy (*Rpv*) and powdery (*Ren*/*Run*) mildew while maintaining the high wine quality of their *V. vinifera* ancestors (Töpfer *et al.* 2011, Reisch *et al.* 2012, Delrot *et al.* 2020). However, for breeders it is crucial to know the particular resistance alleles a genotype posseses in order to select the best candidates for combination. The pyramiding of multiple R-loci with supposedly different resistance mechanisms is currently considered as the most appropriate approach to prevent the rapid breakdown of resistance.

The most prominent and widely planted fungal-resistant cultivar in Germany is 'Regent'. It was generated by a cross in 1967 at JKI Geilweilerhof, Germany, and inherited the *Rpv3.1* locus (most likely from *V. rupestris*) against downy mildew (Fischer *et al.* 2004, Welter *et al.* 2007, Di Gaspero *et al.* 2012) as well as the *Ren3* and *Ren9* loci against powdery mildew (Welter *et al.* 2007, Zendler et al. 2017 and 2021). The parents of 'Regent' are 'Diana' and 'Chambourcin'. 'Diana' is an early ripening *V. vinifera* cultivar with excellent wine quality and 'Chambourcin' is a French cultivar that served as the resistance donor (http://www.vivc.de/). Previous SSR marker analysis at the *Rpv3* locus had already shown that 'Chancellor', one of the putative parents of 'Chambourcin' mentioned by the breeder Joannès Seyve, did not match (Di Gaspero et al., 2012). Therefore, the grape germplasm repository of JKI Geilweilerhof was screened to elucidate the true parents of 'Chambourcin' and the origin of the important R-loci *Ren3* and *Ren9*. Furthermore, presumed offspring of 'Regent' were characterized for trueness-to-type and the inheritance of resistance loci to supply wine growers and breeders with appropriate information.

Material and Methods

For identification of the grandparents on the pathogen-resistant, paternal side of 'Regent', the JKI-internal SSR-marker database containing approximately 8,000 ge-

Correspondence to: Dr. F. Röckel, Julius Kühn Institute (JKI) - Federal Research Centre for Cultivated Plants, Institute for Grapevine Breeding Geilweilerhof, 76833 Siebeldingen, Germany. E-mail: franco.roeckel@julius-kuehn.de

netic profiles of varieties from the genus *Vitis* for the nine GrapeGen06 markers VVS2, VVMD5, VVMD7, VVMD25, VVMD27, VVMD28, VVMD32, VrZAG62 and VrZAG79 (Maul and Töpfer 2015) was used for pre-screening of putative ancestors. The selected candidates and further relatives (suppl. Tab. 1) in the pedigree tree were genotyped with an additional 16 genome-widely distributed SSR markers (Sefc *et al.* 1999, Laucou *et al.* 2011, Fechter *et al.* 2014) to validate kinships. Leaf samples were taken from young shoots of accessions maintained in the germplasm field repository at JKI Geilweilerhof. DNA extraction, PCR amplification and DNA fragment length determination were conducted according to Maul *et al.* (2015). Marker mismatches due to monoallelic patterns were considered as null alleles as the presence of null alleles was already concluded for hybrids and rootstocks for some of the used markers (Laucou *et al.* 2008 and 2011). The extensive SSR marker dataset published by Lacombe *et al.* (2013) consisting of 1,130 *V. vinifera* varieties, each genotyped with 20 SSR markers, was used to estimate allele frequencies for statistical validation of relationships with Cervus v 3.0 (Marshall *et al.* 1998, Kalinowski *et al.* 2007) and, for additional evidence, Colony2 (Jones and Wang 2010). In Cervus, LOD statistics (likelihood of the odds) for each parent-offspring pair were calculated based on a pairwise parentage analysis of a simulated population with the allele frequencies of the input genotypes (1162 candidate parents and 10,000 offspring). Parentage assignments were only accepted at the strict 95 % confidence level or higher and candidate parents with the highest LOD score for an offspring were selected. The remaining five SSR markers used in this study but not in Lacombe *et al.* (2013), could not be taken into account for the statistical validation of relationships, but were manually evaluated for all accepted parent pairs and trios.

Furthermore, the presence of the resistance loci *Rpv1*, *Rpv3.1*, *Rpv3.2*, *Rpv10* and *Rpv12* against *Plasmopara viticola* as well as *Run1*, *Ren1*, *Ren3* and *Ren9* against *Erysiphe necator* were determined (marker overview and modifications in suppl. Tab. 2). Berry color and flower sex of the ancestors of 'Regent' were evaluated during the 2016 season at JKI Geilweilerhof. One InDel marker (APT3Indel: CGTATTCTTGACAAAATGTTGCTT, AAACTCAGCCCTCCCTCAGT) was additionally employed to validate the flower sex genetically (Fechter *et al.* 2012). The basic pedigree trees were drawn with Pedimap (Voorrips *et al.* 2012) and berry color, flower sex as well as the R-loci were added manually.

Results and Discussion

After preliminary selection of putative pedigree candidates of 'Regent' within the JKI SSR-marker database based on nine SSR-marker allele size patterns, the true ancestors of 'Regent' were identified and some offspring cultivars were confirmed using an additional 16 SSR markers (Figure; suppl. Tabs 3 and 4).

The two putative parents for 'Chambourcin' indicated by the breeder Joannès Seyve - 'Seyve Villard 12-417' and 'Chancellor' - did not match the marker inheritance patterns and were excluded. However, the putative parents of 'Seyve Villard 12-417' ('Seibel' 6468 x 'Subereux') and 'Chancellor' ('Seibel 880' x 'Seibel 5163') given by the breeders were confirmed, as well as their progenitors (suppl. Figure).

The true parents of 'Chambourcin', which solely match on all tested marker allele sizes, were identified as the cultivars 'Joannes Seyve 11369' and 'Plantet'. Based on the historical information given by their breeders, both parents seem plausible regarding the availability at the crossing time and resistance heritage. 'Joannes Seyve 11369' is a full sibling of the falsely mentioned parent 'Seyve Villard 12-417' resulting from the cross of 'Seibel 6468' and 'Subereux'. It carries *Rpv3.1/Rpv3.2/Ren3/Ren9* and the shouldered bunches and ovate berries of the cultivar match the description given by Galet (1988). The second parent 'Plantet' is an offspring of 'Seibel 867' as specified by the breeder Albert Seibel and carries *Rpv3.2/Ren3/Ren9*. Its other parent, 'Seibel 2524', is most likely extinct.

When tracing back the origins of the resistance loci *Ren3* and *Ren9* of 'Regent', the earliest proven carriers are 'Seibel 4614' and 'Munson' from the maternal and paternal side of 'Chambourcin', respectively. All further ancestors that could indicate a wild species resistance donor unambiguously could not be included in the study, because most of them are likely to have become extinct (http://www.vivc.de/). The pedigree of 'Seibel 4614' given by the breeder is relatively complex, with several possible resistance gene donors. In contrast, the pedigree of 'Munson' leads directly to *Vitis aestivalis* Michx. var. *linsecomii* (Buckley) L. H. Bailey (accession: 'Jaeger 43') and *Vitis rupestris* Scheele (accession: 'Jaeger 60 O.P.') (http://www.vivc.de/) as the possible resistance donors for *Ren3* and *Ren9*.

Before marker-assisted selection became possible, breeders had already successfully selected offspring with strong and combined resistances for downy and powdery mildew (Töpfer *et al.* 2011). While analysing breeding lines and new varieties of various European institutions, Zini *et al.* (2019) demonstrated that the presence of *Rpv3.1/Ren3/Ren9* is the most frequently occurring combination of resistance loci in individual genotypes. In the pedigree of 'Regent', the continuous transmission and selection of this combination can be traced back over six generations. It was detected four generations earlier in 'Seibel 4614' and was then transferred *via* 'Seibel 6468', 'Joannes Seyve 11369', and 'Chambourcin' to 'Regent' by different breeders. Since 'Regent' itself was used frequently as a resistance donor parent with high wine quality characteristics, the combination of *Rpv3.1/Ren3/Ren9* was transmitted to seven new varieties, bred in the last three decades: 'Calandro', 'Cabernet Blanc', 'Pinotin', 'Cabernet Colonjes', 'Julius', 'Artaban', and 'Vidoc'. In addition, the combination of *Rpv3.2/Ren3/Ren9* seemed to convince previous breeders, too. As shown in the Figure, this second combination first occurred in 'Munson' and was continuously passed on and selected over four further generations from 'Vivarais' to 'Seibel 867', 'Plantet' and finally 'Chambourcin'. Breeders like Bertille Seyve-Villard and Joannès Seyve obviously realized the strength of these combined mildew resistances and hybridized 'Seibel 6468' with 'Subereux' or 'Joannes Seyve 11369' with 'Plantet' in crosses to get even higher resistances. Stacked breeding lines with two R-loci

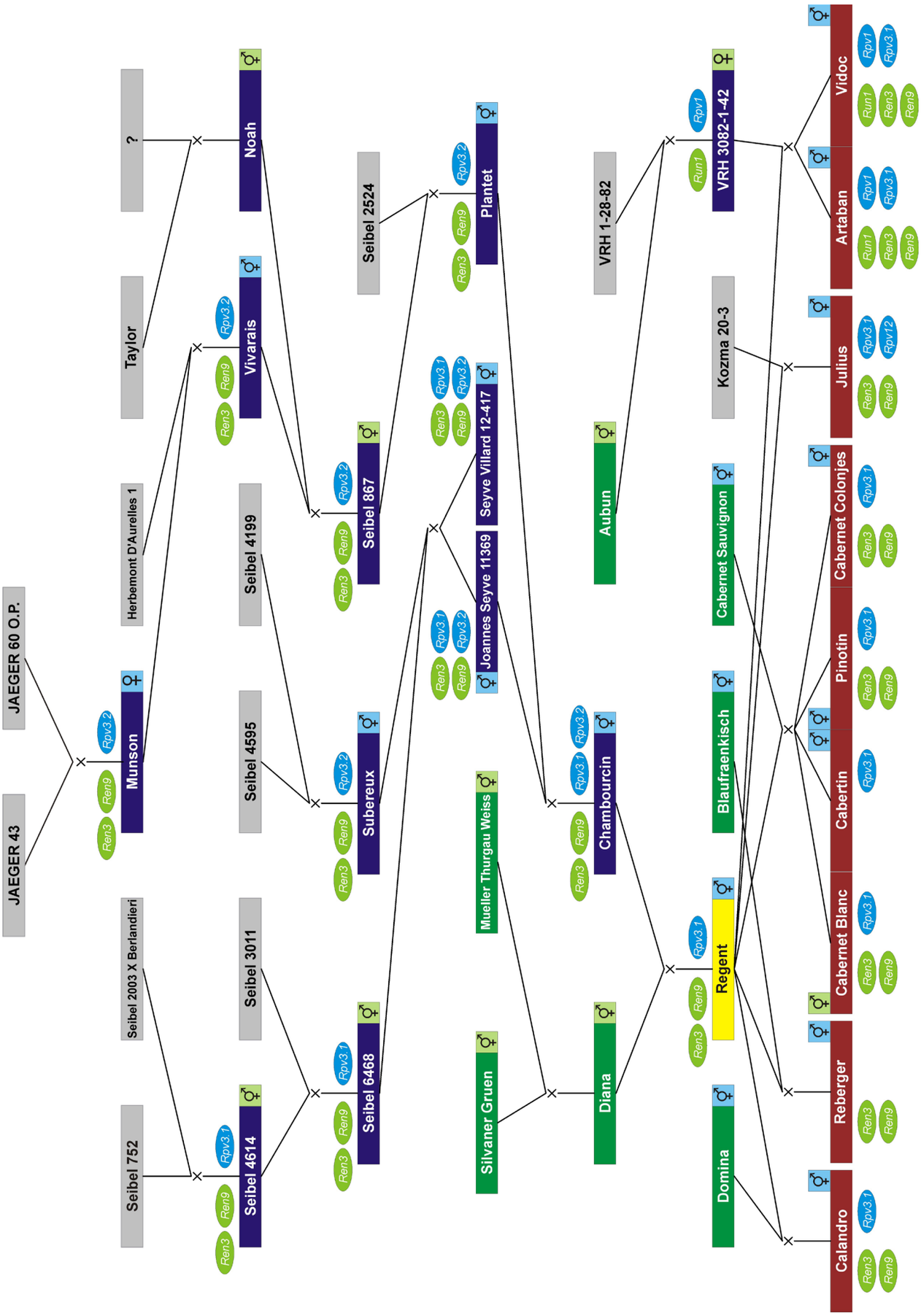

Figure: Pedigree tree of 'Regent' and its progeny. Purple: tested genotypes; green: tested traditional *Vitis vinifera* varieties; red: tested offspring of 'Regent'; grey: most likely extinct or not available for analysis. Berry color (background) and flower sex are indicated in squares. Confirmed resistance loci against *Erysiphe necator* (light green) and *Plasmopara viticola* (light blue) are indicated in ellipses. Loci *Rpv10* and *Ren1* were not found.

against downy mildew (*Rpv3.1*/*Rpv3.2*) combined with two R-loci against powdery mildew (*Ren3*/*Ren9*) were selected with 'Joannes Seyve 11369', 'Seyve Villard 12-417' and 'Chambourcin', the latter of which is planted in North America, Australia and several countries in Europe. With the possibility to genetically map resistances and trace them with molecular markers in grapevine breeding programs, it is easier to understand and explain pedigrees and the strategies of former breeders. *Rpv3.1* and *Rpv3.2* are allelic resistances (Di Gaspero *et al.* 2012, Zyprian *et al.* 2016) and can only be stacked if both parents carry at least one of the alleles, but not if one parent carries both. In contrast, today we know that *Ren3* and *Ren9*, although they are independent resistance genes, are genetically tightly linked and therefore mostly transmitted together to the next generation (Zendler *et al.* 2017 and 2021). Unfortunately, the resistance loci *Ren3* and *Ren9* have been broken in Eastern North American vineyards, as shown by inoculation experiments (Teh *et al.* 2017), but not elsewhere, so far. A similar fate might await further regions around the globe, but the possible different mechanisms of pathogen perception and the established tracking in breeding programs make *Ren3* and *Ren9* still very interesting for breeders (Zendler *et al.* 2021).

Nevertheless, genetic fingerprinting and marker assisted selection initiated a paradigm shift in grapevine breeding and breeding-related research from a purely empirical work of former breeders to a precise and systemic introduction of gene loci into the breeding material. With increasing knowledge and all the new tools at hand, the success in grapevine breeding will become more and more evident.

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