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**Research Note**

# 'Kunbarat' and 'Kunleany' – full not half-siblings

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**Introduction**: The unfortunate introduction of the pathogens *Erypsiphe necator* (Schwein.) Burr. (*Oidium*, powdery mildew), *Daktulosphaïra vitifoliae* Fitch (Phylloxera) and *Plasmopara viticola* (Berk. & Curt.) Berl. & de Toni (downy mildew) to European viticulture during the 19th century caused West European grapevine breeders to seek for resistance traits. Initially, they investigated interspecific crosses between *Vitis vinifera* and American *Vitis* species like *V. rupestris* or *V. riparia* (Maul *et al.* 2019) in searching for resistant hybrids.

In Eastern Europe, e.g. Serbia and Hungary, breeders used different genetic resources in their efforts to improve disease resistance. Especially the Asian species *V. amurensis* Rupr., the Amur grape, was a popular choice for interspecific crosses. Up to date, 71 different *V. amurensis* descendants and interspecific hybrids between *V. amurensis* and *V. vinifera* are reported (Maul *et al.* 2019). The Amur grape was preferred for breeding not only because of its valuable downy mildew resistance, but also due to its frost tolerance. *V. amurensis* is adapted to the cold climate of the East Asian region, ranging from Siberia via China to Japan (Wan *et al.* 2008, Koleda 1975). Three different *P. viticola* resistance loci originating from *V. amurensis* germplasm (*Rpv8*, *Rpv10*, *Rpv12*) have recently been identified (Blasi *et al.* 2011, Schwander 2012, Venuti *et al.* 2013). In eastern grapevine breeding the *Rpv12*-carrying genotype 28/19# (Hungary) was crossed and the hybrids were backcrossed with *V. vinifera* genotypes (Koleda 1975, Venuti *et al.* 2013).

A combination of several loci (stacking of loci) is desirable for sustainable maintenance of the resistance trait (Zini *et al.* 2019). The *P. viticola* resistance factor *Rpv12* is therefore combined with *Rpv10* and *Rpv3* alleles (the latter from American sources) in grapevine breeding. To accelerate this process, the pedigree of *Rpv12* resistance carriers was checked based on molecular (SSR) markers.

**Material and Methods**: Plant material and DNA extractions: Plant material of the genotypes reported in the pedigrees of 'Kunbarat' 'Kunleany' and 'Petra' were sampled from the Grape Germplasm Repository at Geilweilerhof, Germany. For DNA extraction, young, healthy leaves were collected, lyophilized and crushed. The DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) was applied to extract genomic DNA.

Simple Sequence Repeat-Marker Analysis: In total, 81 simple sequence repeat (SSR)-flanking primer pairs were combined in 15 multiplex PCR reactions. All applied primer pairs flanking SSR markers are published and available in the repository of the National Centre for Biotechnology Information (NCBI, https://www.ncbi.nlm.nih.gov/probe). Primers were purchased from Metabion International, Planegg, Germany.

All SSR-loci were amplified in a reaction mixture with a total volume of 5 µL containing 1 ng genomic DNA, 2.5 µL Kapa2G Multiplex-Mix Kit (Kapa Biosystems, VWR, USA), 0.2 µM of the fluorescently labelled forward primer (FAM, HEX, TAMRA, ROX) 0.2 µM of the unlabelled reverse Primer, and $H_2O_{deion}$ to adjust the volume. The PCR reactions were performed on an ABI 9700 Thermocycler (Applied Biosystems, Germany) as follows: 3:00 min at 95.0 °C, followed by 30 cycles of 0:15 min at 95.0 °C, 0:30 min at 60.0 °C and 0:30 min at 72 °C. Final elongation extended for 7:00 min at 72 °C, followed by a cool-down to 4 °C. Up to nine different primer pairs were mixed in the same PCR reaction, depending on the expected fragment sizes and the fluorescent primer tag. After PCR, the solution was diluted by addition of $H_2O_{deion}$ (10 µL) and 1 µL of diluted PCR-Product was added to a mixture of 0.5 µL Liz-labelled size standard (based on the commercially available GeneScan™ 500 LIZ®) and 12 µL $H_2O_{deion}$. The mix was denatured for 5 min at 95°C and loaded onto a capillary gel electrophoresis system. Size determination used the ABI 3130xl Genetic Analyzer (Applied Biosystems, Germany) equipped with a 36 cm-long 16 channel capillary. Fragment lengths were determined employing the software GeneMapper® 5.0 (Applied Biosystems). For allele sizing the reference genotypes 'Muscat à petit grains blanc' and 'Cabernet franc' were employed.

The resulting SSR profiles are shown in suppl. Tab. 1. The data were processed using the software ML Relate (Kalinowski *et al.* 2006). The likelihoods for the pedigree of 'Kunbarat' are represented in suppl. Tab. 2.

**Results and Discussion**: A set of 81 SSR-markers was employed to recheck the pedigree of 'Kunbarat' and 'Kunleany' (suppl. Tab. 1). This revealed two inconsistencies compared to literature descriptions:

(1) 'Italia' (Italy, 1911), previously described as a parent of 'Kunbarat', has no direct genetic relationship to this cultivar. In contrast, 'Afus Ali' showed 100 % identity to parentage (suppl. Tab. 2), as reported for 'Kunleany'. Therefore, 'Kunbarat' and 'Kunleany', both crossed 1960 in Hungary, are full siblings (*V*IVC, Koleda, 1975) with 'Afus Ali' as a parent.

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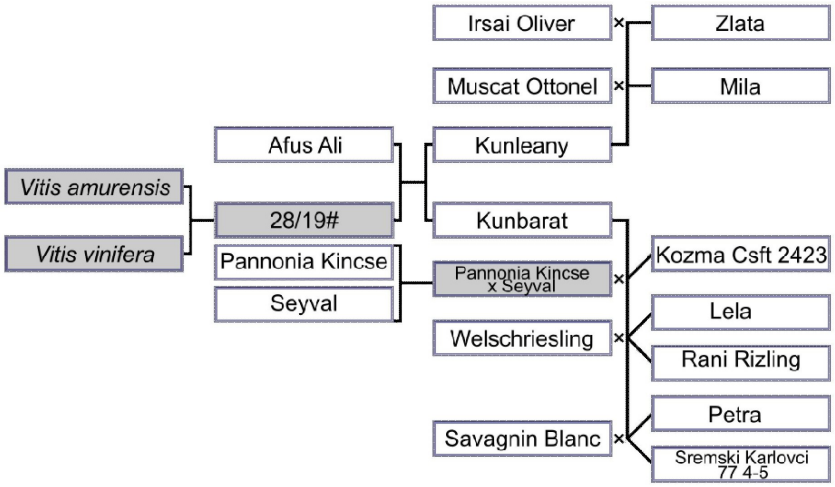


Figure: Pedigree of 'Kunbarat', 'Kunleany' and its descendants. The interspecific breedig line 28/19# between *Vitis amurensis* and *Vitis vinifera* was backcrossed with 'Afus Ali'. The resulting cultivars are 'Kunleany' and 'Kunbarat'. 'Kunleany' on the one hand was further crossed with 'Muscat Ottonel' and on the other hand with 'Irsai Oliver'. 'Mila' and 'Zlata' respectively were confirmed as F1 progeny. 'Kunbarat' was crossed with a breeding line from 'Pannonia Kincse' x 'Seyval' ('Seyval' is also known as 'Seyve Villard 5276'), 'Welschriesling' and 'Savagnin Blanc' ('Savagnin Blanc' also known as 'Traminer weiß') respectively. The admitted descendants are 'Kozma Csft 2423', 'Lela' and 'Rani Rizling', 'Petra' and 'Sremski Karlovci 77 4-5' respectively. The samples presented in grey shaped background were unavailable for genotyping. All the others were analysed with 81 SSR markers as detailed in suppl. Tables.

(2) A second allelic mismatch was detected in the backcrosses of the interspecific genotype 28/19#: 'Petra' (Serbia, 1977) proved not to be an offspring of 'Pinot Noir' and 'Kunbarat'. It turned out that it resulted from a cross between 'Kunbarat' and 'Savagnin Blanc' ('Traminer'). 'Petra' may thus be a full sibling of 'Sremski Karlovci 77 4-5' (Serbia, 1977). Progeny cultivars of 'Kunbarat' and 'Kunleany' were found to be correctly assigned to their parentage. The results of this study are summarized in the Figure.

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