Research Note

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

S. MIEßNER, F. ROCKEL, E. MAUL and E. ZYPRIAN

Julius Kühn-Institut (JKI), Federal Research Centre for Cultivated Plants, Institute for Grapevine Breeding Geilweilerhof, Siebeldingen, Germany

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Introduction: The unfortunate introduction of the pathogen Erysiphe necator (Schwein.) Burr. (Oidium, powdery mildew), Daktulosphaira 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 resistance against these diseases. 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 'Pe-

tra' 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 H2O 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 H2O (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 H2O. 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 frane' 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 (ITVC, Koleda, 1975) with 'Afus Ali' as a parent.
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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Figure: Pedigree of 'Kunbarat', 'Kunleany' and its descendants. The interspecific breeding 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 'Issai 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.

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