

Genetic diversity in 200 years old Serbian grapevine herbarium specimens

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Abstract

A grapevine herbarium dated from 1812-1824, prepared by botanist Andreas Wolny Slovak, has recently been found in Sremski Karlovci (wine region of Vojvodina, Serbia). This collection should represent local cultivated grapevine diversity before *Phylloxera* was introduced to these areas. The herbarium collection comprises more than 100 samples, organized in two subcollections: red-berried varieties and white-berried varieties, totalling 47 different grape varieties. The goal here was to study the history of cultivated grapevines in the Balkans and Pannonia wine-growing areas with a long viticulture tradition. The obtention of DNA from plant remains older than 100 years requires the use of procedures of ancient DNA (aDNA) extractions in specific clean rooms, with positive pressure to avoid external contamination with modern DNA. Though, internal contamination from other organism is expected, such as bacteria and fungi associated to the living plant or herbarized samples. To avoid any cross-contamination with exogenous grapevine DNA, this work was performed following a protocol for recovering ultra-short DNA molecules from 10 mg of herbarized leaves in specific facilities for aDNA extraction at the University of Tübingen. In 80 samples, DNA could be quantified. In part of the samples, *Vitis psaeA* chloroplast gene amplification was checked and confirmed the presence of grapevine DNA in these extractions. Furthermore, genotyping using standard DNA markers was performed in a specific laboratory at the ICVV where grapevine DNA had never been amplified. Different degrees of success were achieved in the genotyping analyses, from samples that did not produce any positive result to other that worked fine, like modern DNA samples. The genetic profiles obtained from the herbarized samples were compared to those stored in international databases (ICVV and VIVC). This task allowed us to successfully identify some of the herbarized samples as known varieties from the Western Balkans and neighbouring regions, such as Kadarka Kek, indicating their uninterrupted cultivation for more than 200 years. The joint analysis of ancient and modern samples allows establishing possible relationships among them, elucidating the historic evolution of the crop in Serbia.

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