

# Analysis of the grapevine genetic diversity existing in Montenegro using ICVV-SNP and *VIVC* databases

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Montenegro has a long winemaking tradition dated back to Greek and Illyrian period times. Current Montenegrin wine-growing regions have evolved following globalization rules to fit international market needs. Nevertheless, this modern system co-exists with another Montenegrin viticulture that still maintains most of its traditional practices, including the cultivation of autochthonous cultivars. This traditional way is particularly important in Montenegro's viticulture because of the significant number of small grape growers that keep it alive, converting this region in an invaluable opportunity to study traditional ways to produce new varieties and genetic diversity which are currently extinct in Western European countries. Here, we performed the widest prospection of grapevine genetic resources carried out in Montenegro so far, which involved the collection of 419 grapevine leaf samples from old vines found in traditional vineyards across the different viticulture regions of the country. These samples were analyzed together to 57 accessions from the *ex situ Vitis* collection of the Biotechnical Faculty of the University of Montenegro (BTF *Vitis* collection), which was created in 1960 to preserve local grapevine genetic resources. Samples were genotyped by a combination of single nucleotide polymorphisms (SNP) and simple sequence repeats (SSR), and unique genetic profiles were compared with international databases (ICVV-SNP and *VIVC* databases, respectively) for proper grapevine varietal identification and for the detection of synonymies (different names for the same genotype) and homonymies (different genotypes with the same name). SNP genotyping at 48 loci revealed 144 different genetic profiles, of which 68 corresponded to prospected cultivated plants in ancient vineyards and 43 to plants prospected as wild vines. In addition, 33 genetic profiles were exclusively found in the *ex situ* BTF *Vitis* collection. The comparison of the SNP and SSR profiles obtained from old vineyards with those stored in the ICVV-SNP and *VIVC* databases allowed the full identification of 32 grapevine cultivars, including autochthonous cultivars from the Western Balkans (such as cvs. Kratošija (found 106 times), Vranac (76), Lisica (35) or Krstac (22)), others from Eastern countries (such as cvs. Razaklija (27), Kadarun (5) or Chaouch blanc (4)) and others from Western countries (such as cvs. Vulpea (3), Muscat Hamburg (2) or Merlot (1)), cultivated in many cases under synonym names. We did not find any match for up to 33 unique profiles, six of which were found at least twice across Montenegrin vineyards, indicating they are likely true, old autochthonous varieties on the edge of extinction. The high genetic diversity found in one of smallest European countries (13.812 km<sup>2</sup>) reflects historic reports that indicate multiple introductions of plant material in the country from diverse European viticultural regions in different times and with different purposes. In addition, we found a complex parentage network linking multiple autochthonous cultivars, in which two varieties (Razaklija and Kratošija) played a leading role on the generation of local genetic resources. Our findings demonstrate that isolated, local niche-selection can represent an important mechanism contributing to the generation of current grapevine varietal diversity.