P18 – Chloroplasts and targeted nuclear DNA based genetic diversity among grapevine (*Vitis* sp.)

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Abstract

As for grapevine, there are many genetic resources in the Balkan region that have not yet been evaluated at the molecular level. Conventional molecular markers used for genotyping of grapevines have been largely replaced in recent years by HTS techniques that have dramatically expanded the possibilities for identifying allelic variations used in the application for genotyping by multiplexing. Multigene panels can be easily performed with gene-specific target enrichment probes. In this project we developed probes to capture information on key sites in the grapevine nuclear genome: 2271 highly variable SNP loci, 943 sites of the sex locus, 96 GAI1 loci associated with berry traits and phenology, 51 loci associated with resistance, 59 random loci, 312 MYB loci associated with color, and 47 TFL1 loci associated with flowering and phenology. For each polymorphic site, a 120-mer probe was designed with the expected variant in the central position. The panel was designed to genotype most of the diversity in grapevine from Balkan region (370 *Vitis vinifera* genotypes; 124 from Slovenia, 28 from Serbia, 76 from Croatia, 16 from Montenegro, 55 from BIH, 6 from Macedonia, 26 from Greece, 39 from Albania) and 23 *V. vinifera* references from France and 27 *Vitis* sp. species. The obtained data will allow to determine the exact calling of the variants for the evaluation of Balkan grapevines: their true-to-typness, important traits and kinships in the grapevine gene-pool.

For the same group of samples, we performed whole-genome shotgun sequencing to detect DNA variation in chloroplasts and performed sequence alignment and phylogenetic analyses. Analyses were performed at inter- and intra-specific levels to improve previous phylogenetic work that was limited in taxonomic scope or marker choice (Peros *et al.* 2011, Wan *et al.* 2013, Trondle *et al.* 2010,

Lozsa *et al.* 2015) and to improve parental analysis, particularly of grapevine varieties from the Balkans (Stajner *et al.* 2015), using maternally inherited chloroplast variation. Using low coverage DNA-seq, we were able to sequence a grapevine genome with an average coverage of 0.17, while the chloroplast genome reached up to 60-fold coverage, which was high enough to determine reliable SNPs.

Keywords: variability, chloroplast, targeted nuclear DNA, high-troughput sequencing