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## APPLICATION OF MICROSATELLITE MARKERS FOR VARIETAL IDENTIFICATION GRAPE ROOTSTOCKS: PRELIMINARY ANALYSIS

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In this study the possibility of application of SSR marker sets for grape rootstock identification in diverse genetic material was assessed. 65 rootstocks of 7 table grape varieties were investigated. The results of preliminary study prove the usefulness SSR marker sets for grape rootstocks identification and might be further optimized and studied in more details.

### *Grape – rootstock – microsatellite marker – genetic profile*

Ուսումնասիրվել է միկրոսատելիտային մարկերների կիրառելիությունը խաղողի տնկի-ների մաքրաստրուկտուրայի որոշման համար: Ուսումնասիրվել է խաղողի 7 սորտի 65 նմուշ: Նախ-նական հետազոտությունների արդյունքները ցույց են տվել միկրոսատելիտային մարկերների կիրառելիությունը տնկիների նույնականացման համար և կարիք ունեն օպտիմացման ու հետագա ուսումնասիրության:

### *Խաղող – տնկի – միկրոսատելիտային մարկեր – գենետիկական պրոֆիլ*

Изучена применимость микросателлитных маркеров для сортовой идентификации посадочного материала винограда. Проанализировано 65 образцов 7-и сортов винограда. Предварительные результаты показали применимость микросателлитных маркеров для сортовой идентификации посадочного материала винограда и требуют дальнейшего изучения и оптимизации.

### *Виноград – посадочный материал – микросателлитный маркер – генетический профиль*

Grapevine (*Vitis vinifera* L.) is one of the economically important crops cultivated around the world. According to certain data sources, there are some 5,000-6,000 cultivars of *Vitis vinifera* are known globally (Alleweldt and Dettweiler 1994).

The fast vegetative propagation has supported widespread distribution of different cultivars in the world (Dion1977; Fregoni 1991). As a result some cultivars have more than 100 synonyms, and numerous homonyms also exist (<http://www.genres.de/idb/vitis/>). In addition, the different languages used in the east European countries produce different spellings of the same variety name, which may have caused double registering. Simultaneously accurate identification of accessions is a most important issue for the sustainable conservation, management and use of germplasm, the clarification of synonymy, homonymy, and misnaming is a significant problem in the grapevine collections that exist worldwide (Dettweiler et al. 2000a).

The identification of grape cultivars has traditionally been based on ampelography, which is the analysis and comparison of morphological characters of leaves, shoot tips, fruit clusters, and berries (Boursiquot and This 1996; IPGRI UPOV OIV 1997; Galet

2000). Some genetically related cultivars are morphologically very similar and difficult to differentiate by visual comparison (Aradhya et al. 2003). On the other hand, intravarietal clones can differ considerably in phenotype even though they have virtually identical DNA profiles (Vignani et al. 1996; Franks et al. 2002; Riaz et al. 2002). To overcome these limitations molecular biology approaches have been used as molecular markers and DNA barcodes to differentiate, characterize, and identify grapevine accessions. Microsatellite markers (Sefc et al. 2000; Aradhya et al. 2003; This et al. 2004) are favored among RFLP, AFLP or RAPD markers because of their combination of polymorphism, reproducibility, and their codominant nature (Sefc et al. 2001). GENRES081 was a European Union research project focused on the compilation, standardization, and exchange of information concerning grapevine genetic resources (Dettweiler et al. 2000b; This and Dettweiler 2003; <http://www.genres.de/vitis/>) was developed reference microsatellite profiles for true-to-type identification of grapevine accessions.

The aim of this study was to assess applicability of microsatellite markers for grape variety identification within genetically diverse rootstocks.

**Materials and methods.** Within collaboration with “Maran” wine making company and Scientific Center of Viticulture, Fruit-growing and Wine-making and under the project financed by Armenian Harvest Promotion Centre 65 mixed rootstocks of 7 raisin grape varieties were analyzed. The leaf samples were collected from Dalaril, Eraskhahun, Tandzut, Bmbakashat, Mec Armair, Vrein Artashat, Arevik regions. The leaves of grape varieties from living collection were used as references (Deghin Yerevni, Sev Kishmis, Karmir Icaptuk, Parvana, Marmari, Caradguyn Yerevni). DNA from leaves were extracted by using modified by us cetyl trimethylammonium bromide (CTAB) (Doyle & Doyle, 1987) with addition of 2% PVP to remove polyphenols. 50-100 mg of fresh tissue were homogenized using homogenizer Tissue Lyzer LT (Qiagen) to avoid cross contamination among samples. Extracted DNA quantity and quality were evaluated using Multiskan Microplate Spectrophotometer (Thermo Scientific). In addition, 10 µl of each DNA extract will be loaded and visualized on a 2% agarose gel. The following SSR primers were analyzed for grapevine cultivars genetic profiling and identification: VVS2 (Thomas and Scott, 1993), VVMD5, 7 (Bowers et al. 1996), VVMD25, 27, 28, 32 (Bowers et al. 1999), ZAG62 and ZAG79 (Sefc et al. 1999). These are di-nucleotide repeat sequence motifs with allele sizes ranging from 129 bp (the smallest with VVS2) through to 315 bp (the largest for VVMD36). Amplification were performed on standard PCR (Techne, gradient thermal cycler) in 20 µl reactions in 96 well plates. For PCR the Type It Microsatellite Kit (Qiagen) was used added with 0.25 µM of each primer and 50 ng genomic DNA. PCR products will be analyzed in Qiaxcel system using DNA high resolution kit, by method OM700 by with injection time 5-7 minutes.

**Results** The preliminary results of the comparison of genetic profiles allowed dividing studied rootstocks into the three groups. The first group comprises 85% of the studied rootstocks and includes genetically identical to the reference varieties rootstocks.

The second group comprises around 15% where genetic profiles of studied rootstocks are differing from those of reference varieties. This proves that these rootstocks might be the clones of the varieties and cannot be further used for planting and production purposes, but might be interesting sources for breeding programmes. Within the third group the rootstocks with absolutely different genetic profiles were included.

So, the results of preliminary study proves the usefulness of SSR marker sets for grape rootstocks identification and might be further optimized and studied in more details.

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