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## ARMENIAN ABORIGINE GRAPE VARIETIES IDENTIFICATION AND GENETIC DIVERSITY ASSESSMENT BY 48 SNP MARKERS SET FOR GRAPEVINE

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Armenia is home to many hundreds of indigenous grape varieties, many of which have invaluable genetic potential, supposedly emerged as a result of natural hybridization, mutation, and selections over years. Genetic resources/diversity of the grapevine cultivars and wild relatives have not been studied sufficiently in the territory of Armenia and detailed study on molecular genetics of the Armenian vineyards is of high priority. Genotyping of 64 grape varieties and 14 *Vitis sylvestris* populations from Armenia by using 48 SNP markers set was performed.

### *Grape- SNP-cultivar identification-genetics*

Հայաստանի տարածքը բնորոշվում է խաղողի տեղական սորտերի հարուստ բազմազանությամբ, որը ձևավորվել է երկարամյա բնական հիբրիդացման, մուտացիաների և սելեկցիայի արդյունքում: Բազմաթիվ սորտեր ունեն արժեքավոր գենետիկական պոտենցիալ: Հայաստանի խաղողի սորտերի և դրանց վայրի ցեղակիցների գենետիկական բազմազանությունը բավարար ուսումնասիրված չէ, այդ իսկ պատճառով հատկապես կարևորվում է վերջիններիս մոլեկուլային գենետիկական ուսումնասիրությունը: Իրականացվել է խաղողի 64 սորտերի և *Vitis sylvestris* 14 պոպուլյացիաների գենետիկական 48 SNP մարկերների կիրառմամբ:

### *Խաղող- SNP-սորտային նույնականացում-գենետիկա*

Территория Армении характеризуется большим разнообразием местных сортов винограда, появившихся в результате многолетней естественной гибридизации, мутаций и селекции. Многие сорта имеют бесценный генетический потенциал. Генетическое разнообразие сортов и диких сородичей винограда Армении изучено недостаточно, в связи с чем, особенно актуальным становится проведение детального молекулярно генетического анализа. Было проведено генотипирование 64 сортов винограда и 14 популяций *Vitis sylvestris* Армении с помощью 48 SNP маркеров.

### *Виноград-SNP-идентификация сортов- генетика*

Armenia is home to many hundreds of indigenous grape varieties, many of which have invaluable genetic potential, supposedly emerged as a result of natural hybridization, mutation, and selections over years. Armenia have centuries of grapevine cultivation and winemaking history. The world's earliest known wine-making facility has

been discovered in Armenia during excavation of Areni-1 cave between 2007-2010, and analysis by a UCLA-led team of scientists has confirmed the discovery of the oldest complete wine production facility ever discovered dated between 6000 B.C. and 8000 B.C., at least 1000 years older than the next comparable discovery. Rich history of grapevine cultivation brings/leads to a large number of synonyms, homonyms and misnameings. Genetic resources/diversity of the grapevine cultivars and wild relatives have not been studied sufficiently in the territory of Armenia. A detailed molecular genetics study of the Armenian vineyards is of high importance, with special attention to old varieties. Especially the most important indigenous ones such as (Itsaptouk, Nazeli, Voskehat, Khrdy chakat, Vardagouyn Yerevani, Degin Yerevani, Areni, Marmary, Tozot, Mskhaly, Karmir Kakhnay etc.) and their clones were studied.

Individual fingerprinting based on molecular markers has become a popular tool for studies of existing confusion in grapevine nomenclature including the solution of synonymy/homonymy, population genetics and analysis of genetic diversity in germplasm collections [2, 3].

After the whole grapevine genome was sequenced and published, scientific community have developed a new DNA marker type known as SNPs (Single Nucleotide Polymorphisms) for grapevine identification. SNP markers provide enhanced possibilities for genetic and breeding applications such as cultivar identification, construction of genetic maps, the assessment of genetic diversity, the detection of genotype/phenotype associations, or marker-assisted breeding. SNP markers are bi-allelic and very frequent in genomes.

The aim of the present study was to perform high throughput SNP genotyping of 64 grape varieties and 14 *Vitis sylvestris* populations from Armenia by using 48 SNP markers. 48 of this SNP genotyping assays come from a publication of Zapater on a general use set [1] for grapevine cultivar identification.

## MATERIALS AND METHODS

**Plant material:** This study is based on 64 cultivated and 14 wild grapevine accessions from Armenia. Plant material used for nucleic acid extraction was obtained from *Vitis* collection of the Scientific Center of Fruit Growing, Viticulture and Wine-making, Yerevan.

**DNA extraction:** Genomic DNA was isolated from fine powdered 1-2 g dried leaf tissues, frozen in the liquid nitrogen at  $-196^{\circ}\text{C}$  and homogenized in Tissue LyserLT (QIAGEN RETSCH MM301) to avoid cross contamination among samples. The extraction was performed according to the protocol for peqGold Plant Mini Kit (PEQLAB Biotechnologie GmbH, Erlangen, Germany). Isolated DNA quantity and quality were evaluated spectrophotometrically on NanoPhotometer® (Implen GmbH, Germany). In addition, 1  $\mu\text{l}$  of each DNA extract was loaded and visualized on a 0,8% agarose gel, non-carcinogenic fluorescence stain was used (Serva DNA Stain Clear G). Gel documentation was performed using Felix 6050 system with sensitive CCD camera and Biostep ArgusX1 (control and documentation software incl. database, user administration and GLP module) for fluorescent stained samples (Biostep®).

**SNP genotyping:** STA (Specific Target Amplification) was performed by using forward and reverse (specific target amplification primer and locus specific primer) primers (10X) in GeneAmp PCR System 9700. Conditions of the PCR amplification were as follows:  $95^{\circ}\text{C}$  (15 min), 14 cycles at  $95^{\circ}\text{C}$  (15 sec), and a final extension at  $60^{\circ}\text{C}$  for 4 min.

For SNPs analyses FR 48.48 Dynamic Array IFCs was used [1]. Which can analyzed 47 samples (plus one control without the DNA) with 48 assays at one run. SNP genotyping was carried out by BioMark™ HD System (Fluidigm®, South San Francisco, California, USA). SNPs Data analysis have been done by using Fluidigm® SNP Genotyping Analysis Software v3.

## RESULTS

A total of 64 grapevine accessions and 14 wild forms were genotyped with 48 SNP set. The preliminary results of the genetic identification confirmed that 59% of studied samples are homozygous and 41% heterozygous.

V-1 (Vankapatkan) presented a heterozygous genotype AG for 0070\_SNP579\_187e marker, while the other samples of the group were homozygous TT. For 0080\_SNP1399\_81e marker all samples show homozygous AA and heterozygous AG, while V-1 shows homozygous TT.

So, the results of preliminary study proves that 48 SNP marker set is informative and useful for Armenian grape cultivars and wild forms genetic identification and might be used further research.

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