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Genetic characterization of grape varieties in Armenia

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Summary

Historically, grapes have been an important crop in Armenia. The world's earliest known wine-making facility has been discovered in Armenia during excavation of Areni-1 cave between 2007-2010, and analysis has confirmed the discovery of the oldest complete wine production facility ever discovered dated between 6,000 B.C. and 8,000 B.C. Having thousands of years history, Armenian native grape varieties are characterized with high genetic diversity and variability. The study has evaluated the genetic diversity of the Armenian grapevine cultivars within the *Vitis* collection of the Scientific Center of Fruit Growing, Viticulture and Wine-making (Merdzavan, Armenia) and analysed the relationships of this genetic pool with the international varieties registered in European *Vitis* Database. The analysis of 59 accessions of grapevines from Armenia at 23 microsatellite markers generated 336 alleles. The most informative locus turned out to be VVS2 (21 alleles, PI = 0.016). Twelve cases of identical genotypes and five cases of homonymy among studied genotypes were identified. The genetic profiles of 28 accessions were unique. Most of them belonged to autochthonous varieties. Genetic analyses tools are highly contributing to the identification and inventory of existing grape varieties. The data generated proves the importance of molecular characterization of grapevines in Armenia especially old ones to support effective preservation of rich diversity of Armenian grape varieties and clones.

Key words: clones; microsatellite markers; homonyms.

Introduction

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 (Dallakyan *et al*. 2014). Historically, grapes have been an important crop in Armenia. The world's earliest known wine-making facility has been discovered in Armenia during excavation of Areni-1 cave between 2007-2010, and analysis has confirmed the discovery of the oldest complete wine production facility ever discovered dated between 6,000 B.C. and 8,000 B.C (Barnard *et al*. 2010). The study of autochthonous grapevine cultivars is not only of theoretical interest, but also has great practical significance, as the importance of grapevine genetic resources goes beyond national borders. It was reiterated that without this diversity, viticulture and oenology would be endangered and the consequence of genetic erosion would be a uniform viticulture, which would be susceptible to any kind of biotic or abiotic stress (Maul *et al.* 2003).

The aim of the present research was to evaluate the genetic diversity of the Armenian grapevine cultivars within Vitis collection of the Scientific Center of Fruit Growing, Viticulture and Wine-making (Merdzavan, Armenia) and to study the relationship of this genetic pool with the international varieties registered in European Vitis Database.

Material and Methods

Plant material used for nucleic acid extraction was obtained from Vitis collection of the Scientific Center of Fruit Growing, Viticulture and Wine-making, Yerevan. Samples of 59 accessions were analyzed in triplicate. The genetic analyses were implemented at Yerevan State University (Yerevan, Armenia) and JKI laboratories (Siebeldingen, Germany). Genomic DNA was isolated according to the protocol for DNeasy Plant Mini Kit (Qiagen, Hilden,Germany) or for peqGold Plant Mini Kit (PEQLAB Biotechnologie GmbH, Erlangen, Germany). 23 polymorphic microsatellites considered as the most appropriate to evaluate grapevines (European project GENRES081, http://www.genres.de/vitis) were used. VMC1B11 (Zyprian and Töpfer 2005); VMC4F3.1 (Di Gaspero *et al*. 2000); VrZAG62, VrZAG67 and VrZAG79 (Sefc *et al.*1999); VVIB01, VVIH54, VVIN16, VVIN73, VVIP31, VVIP60, VVIQ52, VVIV37, VVIV67 (Merdinoglu *et al.* 2005); VVMD5, VVMD7, VVMD21, VVMD24, VVMD25, VVMD27, VVMD28 and VVMD32 (Bowers *et al.*1996, 1999); VVS2 (Thomas and Scott 1993). All forward primers were 5' end-labeled with fluorescent dyes (FAM, HEX, TAMRA or ROX). The combination of markers, optimized in JKI, using different labels and diverse fragment lengths allowed performing multiplex polymerase chain reactions (PCR) with up to 5 markers.

The KAPA2G™ Fast Multiplex PCR Kit (2x) (PEQLAB Biotechnologie GmbH) and Type IT Microsatellite Kit (Qiagen) were used to set up reaction mixtures containing master mix, 100 pmol of each primer and about

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1 ng of template DNA. Amplification was performed in ABI 9700 thermal cyclers (Applied Biosystems) and TC 5000 Thermal Cyclers (Techne), using the following program: 3 min initial denaturation at 95 °C, followed by 30 cycles of denaturation at 95 °C (15 s), annealing at 60 °C (30 s) and extension at 72 °C (30 s). A final extension was performed at 72 °C for 7 min. DNA of two certified reference varieties of 'Muscat á petits grains' and 'Cabernet franc' were amplified and used for data comparison.

An aliquot of 1 μL of multiplex PCR product was used for fragment length determination and analyzed by capillary electrophoreses (ABI 3130xl Genetic Analyzer: Applied Biosystems, Foster City, California, USA) and Qiaxcel genetic Analyzer (Qiagen). Peaks were identified by size and height with GeneMapper 4.0 software (Applied Biosystems) and Biocalculator Software (Qiagen). The mean number of alleles per locus (Na), number of effective alleles (Ne), levels of observed (Ho), and expected (He) heterozygosity, as well as probability index were calculated using GenAlEx 6.5 (Peakall and Smouse 2006, 2012).

Results and Discussion

The analysis of 59 accessions of grapevines from Armenia at 23 microsatellite markers generated 336 alleles. The number of alleles per locus ranged from five (VVIN16 and VVIN73) to 20 (VrZAG62) with a mean number of 14.6 (Tab. 1). The same value for 12 nuclear microsatellite markers: VVMD5, VVMD7, VVMD24, VVMD28, VVMD31, VVMD32, VrZAG62, VrZAG79, VVS2, VMC2C3, VMC2H4 and VMC5A markers reported by Vouillamoz *et al* (2006) including 13 Armenian grape varieties is lower at 11.9.

Expected heterozygosity for each locus ranged from 63.18 % (VrZAG83) to 90.37 % (VVS2), with mean 80.91 %, while observed heterozygosity varied from 40.98 % (VVIN73) to 93.44 % (VVIP31). The high rate of heterozygosity may be explained as a result of hybridization during grape domestication process (Lamboy and Alpha 1998) and is considered as commonly observed among clonally propagated, outbreeding species (Aradhya *et al.* 2003). The most informative locus turned out to be VVS2 (21 alleles, PI = 0.016), and the least informative with highest value of identity probability (0.312) was the locus VMC1B11 (Tab. 1). The 'Tozot', 'Karmrahyut', 'Areni Vankapatkan' exhibited three alleles at the same loci, especially VVS2. This three-allele status probably could be due to periclinal chimerism, which was reported by Franks *et al.* 2002 and Vouillamoz *et al*. 2006 and which gives an idea that cultivars might be very old (Vouillamoz *et al*. 2006).

The distribution of allele frequences for each locus allows to assess identification ability of the markers, which might be considered as more informative if this distribution is equitable (Sefc *et al.* 1999; Tessier *et al.* 1999). The most frequent alleles in this study were VrZAG83-190 (40.16 %), VVIN16-151 (50 %), VVIN73-268 (68.85 %)

Table 1

Genetic parameters for 23 SSR loci analyzed for 59 Armenian grape cultivars

Locus	Number of alleles (Na)	Number of effective alleles (Ne)	Expected Heterozygosity (He)	Observed Heterozygosity (Ho)	Probability of identity (PI)
VMC1B11	12	5.720	0.825	0.902	0.050
VMC4f3.1	33	9.290	0.892	0.767	0.018
VrZAG62	20	8.187	0.878	0.721	0.025
VrZAG67	17	8.187	0.878	0.902	0.026
VrZAG79	14	6.910	0.855	0.883	0.035
VrZAG83	7	2.716	0.632	0.590	0.207
VVIB01	12	5.233	0.809	0.607	0.060
VVIH54	19	8.603	0.884	0.639	0.025
VVIN16	5	2.972	0.664	0.590	0.161
VVIN73	5	1.927	0.481	0.410	0.312
VVIP31	17	7.464	0.866	0.934	0.031
VVIP60	12	3.613	0.723	0.557	0.114
VVIQ52	7	4.056	0.753	0.705	0.103
VVIV37	19	9.629	0.896	0.847	0.019
VVIV67	19	7.240	0.862	0.491	0.033
VVMD5	15	7.156	0.860	0.685	0.033
VVMD7	19	8.922	0.888	0.717	0.022
VVMD21	11	5.231	0.809	0.755	0.060
VVMD24	12	6.671	0.850	0.789	0.040
VVMD25	18	7.962	0.874	0.776	0.028
VVMD27	9	4.456	0.776	0.852	0.086
VVMD32	13	4.002	0.750	0.650	0.094
VVS2	21	10.379	0.904	0.754	0.016
Mean	14.61	6.371	0.809	0.718	0.070
Cumulated	336				

Table 2

Identical genotypes and homonyms of grape cultivars from Armenia

Identical pairs	
Areni (clone Ekheghisy) (46)*	Areni (41), Areni (clone- high yield) (45), Areni (clone) (44), Areni clone (43), V-1 (Vankapatkan) (73)
Black Kishmish (65)	Vard Yerevani (31), Vardagouyn (32), Deghin Yerevanian clone-1 (35), Marmary (38), House grown Black Kishmish (64)
Black Kishmish (69)	Black Kishmish (19), Sev Kishmish (33)
House grown Black Kishmish (63)	Nor Itsaptouk (68), Karmir Itsaptuk (34)
Karmrahyout (9)	Tozot (29)
Khishrau (11)	Vagahas areni (4)
Mouscat Dessertain (22)	Mouscat Tskha (6)
Sev kishmish (clone) (19)	Sev Kishmish (33), Black Kishmish (69)
Homonym pairs	
House grown Black Kishmish (63)	House grown Black Kishmish (64)
Parvana (1)	Parvana (30), Parvana (47)
Hadis (7)	Hadis (77)
Areni (clone) (43)	Areni (clone) (44)

* Accession number

and VVIP60-319 (42.62 %). The analyzed genotypes were compared against the European Vitis Database (http://www.eu-vitis.de/index.php). Twelve cases of identical genotypes and five cases of homonymy among studied genotypes were identified (Tab. 2). The genetic profiles of 28 accessions were unique. Most of them belonged to autochthonous varieties (e.g. 'Vardabuyir', 'Kakavik', 'Karmir Kakhany', 'Voskehat', 'Arevik', 'Chilar' and 'Tozot'). Genetic differences between some clones were identified ('Deghin Yrevanian' (36) and 'Deghin Yrevanian clone-1' (35), 'Nazeli clone' (67) and 'Nazeli' (66)). In some cases the clones of the same variety might be discriminated by microsatellite markers. This is happening, for example, in case of polyclonal origin of a variety derived by a breeding event (like Nazeli) (Kozjak *et al.* 2003) or mutations as slipped strand mispairing and/or polymerase slippage in the repetitive motif (Riaz *et al.* 2002). It has to be mentioned that the molecular identification of clones still remains a challenge for many varieties.

Conclusion

Genetic analyses tools are highly contributing to the identification and inventory of existing grape varieties. The data generated proves the importance of molecular characterization of grapevines in Armenia especially old ones to support effective preservation of rich diversity of Armenian grape varieties and clones.

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