Association Prosperity and Development in Bulgaria
Project:"SOS for endangered traditional vine varieties"
Acronym: "VineSOS"
Project No. 1829

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Genetic variation of DNA testing of local vine varieties from Bulgaria and Greece

A unique DNA study of local local grapevine varieties from Bulgaria and Greece was performed as part of the "SOS project for endangered traditional vine varieties".

The aim of this study was to estimate the genetic diversity of eight Greek and nine Bulgarian local grapevine varieties with the use of seven microsatellite markers. Statistical analysis of data showed that there is high degree of genetic heterogeneity among most of the varieties studied, as well as a close genetic relationship in three variety pairs. Based on our results, we suggest the synonymy of Greek Pamid & Bulgarian Pamid and Greek Zoumiatiko & Bulgarian Dimyat. On the other hand, Greek

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Keratsuda and Bulgarian Keratsuda varieties should be characterized as homonyms. Additional molecular work is needed for a thorough analysis of Greek and Bulgarian grapevine genepool.

The aim of this study was to analyze the genetic diversity and differentiation of various local Greek and Bulgarian grapevine varieties, using microsatellite genetic markers. Besides that, our goal was also to clarify the putative relationship of varieties coming from the above two countries and characterized as common.

Results and Discussion

Genetic variation

In total, 53 different alleles were found in the 384 samples analyzed with seven microsatellite loci. Polymorphism of the markers was low to medium, ranging from four (VVMD25) to ten (VrZAG79) alleles. Mishej Sandanski variety exhibited the highest mean number of alleles (4.71), while the lowest respective value was found in Keratsuda(G) variety (1.57). The population Ho values ranged from 0.57 to 1 with a mean of 0.79, while the respective range for He was much lower (0.3 to 0.6) and averaged 0.45, indicating a heterozygote excess in our data. Regarding the mean value for Ho (0.79) found herein, this was similar to Ho values reported in other studies analyzing grapevine cultivars with microsatellite markers (see Introduction for related references).

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Genetic differentiation

Average FST over loci and samples was 0.41, showing that nearly half of the genetic diversity is explained between populations. According to pairwise multilocus FST estimates (Supplemental Table 3), the lowest values were observed between Pamid(G) and Pamid(B) (-0.0040), Zumiatiko and Dimyat (0.0000) and Mavrud(G) & Mavrud(B) (0.0175), being statistically non-different from zero. All the rest FST values were quite high, ranging from 0.1835 to 0.6154 indicating that there is a high level of genetic differentiation between the remaining varieties. This finding is also supported by the population differentiation analysis through FSTAT software, where all population pairs were found statistically different (at least in 1% level of significance), except for the aforementioned three variety pairs that were not significantly different (Supplemental Table 4).

According to the Factorial Correspondence Analysis (Fig. 1.a), there is a clear grouping of Zumiatiko & Dimyat, Pamid(G) & Pamid(B) and Mavrud(G) & Mavrud(B) varieties. Keratsuda(G) and Mishej Sandanski are also close to each other, which is not however supported by their FST value (0.5095) and population differentiation analysis (different at 1‰ level of significance). On the other hand, Sefka, Limnio, Karnachalas, Bogiolamas, Rubin and Tamyanka populations tend to isolate more compared to all others. The UPGMA tree topology, comprising of two major clusters (I and II, Fig. 1.b), verified with high bootstrap values the close relationship of the three undifferentiated variety pairs, mentioned previously. Cluster I includes three Greek and three Bulgarian populations. The separation of the Greek ones (Pamid(G), Zumiatiko and Keratsuda(G)) from all other Greek varieties is also observed in the FCA plot, that was constructed using only Greek populations (data not shown). Cluster II is divided in two subclusters, with the first one containing a Bulgarian (Mavrud(B)) and all the rest Greek varieties, while the second one includes the remaining Bulgarian populations. The differentiation patterns observed in the FCA plot and the UPGMA

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dendrogram are, with minor exceptions, in agreement with the pairwise FST estimates and the population differentiation analysis of the studied varieties.

The genetic similarity of the variety pairs Zumiatiko & Dimyat, Pamid(G) & Pamid(B) and Mavrud(G) & Mavrud(B), is supported by all methods of statistical analysis conducted in the present study. Looking at their genotypes, Zumiatiko and Dimyat varieties have identical alleles in all loci examined and, therefore, might be considered as synonyms. The same goes for Pamid(G) and Pamid(B) populations, which is also reported in HVARLEVA et al. (2004), where Bulgarian and Greek Pamid were found to be synonyms having identical allele profiles. Regarding Mavrud(G) and Mavrud(B) varieties, there is a close genetic relationship between them, however, they don't share common alleles in all microsatellite loci and should not be characterized as synonyms. On the other hand, Keratsuda(G) and Keratsuda(B) which are supposed to be common varieties, they are found to be genetically different (at 1‰ level of significance), having a quite high FST value (0.4266) as well. In that case, we suggest that these two varieties should be considered as homonyms. Despite the discriminative power of the chosen set of markers, we strongly believe that additional molecular work is needed in order to safely suggest any synonym or homonym annotation.

The genetic analysis of Greek and Bulgarian grapevine samples, in the frame of VineSOS project, is published online https://www.mdpi.com/1424-2818/12/7/273/htm.

Table 1. Greek (1-8) and Bulgarian (9-17) populations-varieties used in the present study. Letters G and B in parentheses stand for Greek and Bulgarian, respectively.

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Population	Variety	Number of samples
Population 1	Pamid(G)	14
Population 2	Keratsuda(G)	10
Population 3	Zumiatiko	10
Population 4	Limnio	10
Population 5	Sefka	10
Population 6	Mavrud(G)	9
Population 7	Bogiolamas	10
Population 8	Karnachalas	10
Population 9	Tamyanka	26
Population 10	Mavrud(B)	26

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Population 11	Rubin	20
Population 12	Pamid(B)	8
Population 13	Dimyat	19
Population 14	Ruen	20
Population 15	Shizoka Melnishkaloza	36
Population 16	Mishej Sandanski	27
Population 17	Keratsuda(B)	119
Total		384

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Supplemental Table 1. Locations and varieties of collected samples in Greece and Bulgaria.

BULGARIAN LOCATION	**GRAPE VARIETY**
Shishmanovo	Tamyanka, Rubin, Mavrud
Kolarovo	Pamid
Dimitrovche	Mavrud, Tamyanka, Dimyat
Vranya	Ruen, Shizoka Melnishkaloza, Keratsuda
Mitino	Shizoka Melnishkaloza
Stazchero	Mishej Sandanski
Slivnitsa	Keratsuda
Kzesha	Keratsuda
Gzadeshnitza	Keratsuda
GREEK LOCATION	**GRAPE VARIETY**
Alexandroupoli	Keratsuda
Avdira	Pamid, Zumiatiko
Soufli	Mavrud, Bogialamas, Karnachalas
Thermi	Limnio, Sefka

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Supplemental Table 2. Information for used microsatellite markers.

MARKERS	PRIMERS	FLUORESCENT DYE	REFERENCE
VVS2	F:CAGCCCGTAAATGTATCCATC R:AAATTCAAAATTCTAATCACTGG	FAM	THOMAS and SCOTT, 1993
VVMD7	F:AGAGTTGCGGAGAACAGGAT R:CGAACCTTCACACGCTTGAT	FAM	BOWERS *et al.*, 1996
VVMD25	F:TTCCGTTAAAGCAAAAGAAAAAGG R:TTGGATTTGAAATTTATTGAGGGG	HEX	BOWERS *et al.*,1999
VVMD27	F:GTACCAGATCTGAATACATCCGTAAGT R:ACGGGTATAGAGCAAACGGTGT	HEX	BOWERS *et al.*, 1999
VrZAG47	F:GTTCTTGGTCTGAATACATCCGTAAGT R:ACGGTGTGCTCTCATTGTCATTG	TAMRA	DRABEK *et al.*, 2016
VrZAG62	F:GGTGAAATGGGCACCGAACACACGC R:CCATGTCTCTCCTCAGCTTCTCAGC	FAM	SEFC *et al.*, 1999
VrZAG79	F:AGATTGTGGAGGAGGGAACAAACCG R:TGCCCCCATTTTCAAACTCCCTTCC	TAMRA	SEFC *et al.*, 1999

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Supplemental Table 3. Pairwise F_{ST} values between all possible pairs of the studied populations.

	Pamid(G)	Keratsuda(G)	Zumiatiko	Limnio	Sefka	Mavrud(G)	Bogiolamas	Karnachalas	Tamyanka	Mavrud(B)	Rubin	Pamid(B)	Dimyat	Ruen	Snzoka Melnishkaloza	Mishej Sandanski	Keratsuda(B)
Pamid(G)	**0.0000**	0.4566	0.4107	0.4940	0.5047	0.4502	0.4785	0.4866	0.4298	0.3593	0.5326	-0.0040	0.4105	0.4860	0.4979	0.4449	0.2794
Keratsuda(G)		**0.0000**	0.4118	0.6000	0.6154	0.4913	0.5627	0.4730	0.3814	0.4139	0.4988	0.4582	0.3978	0.5957	0.5838	0.5095	0.4266
Zumiatiko			**0.0000**	0.5200	0.4783	0.3374	0.5089	0.4991	0.3835	0.2871	0.5073	0.3986	0.0000	0.5241	0.5091	0.3811	0.4429
Limnio				**0.0000**	0.4286	0.3553	0.4086	0.3150	0.3617	0.2754	0.4931	0.4640	0.5205	0.5797	0.5828	0.4464	0.5398
Sefka					**0.00**	0.35	0.51	0.34	0.41	0.27	0.50	0.48	0.47	0.52	0.54	0.40	0.54

	00	53	36	92	32	27	83	37	83	31	07	72	07
Mavrud(G)		**0.0000**	0.3259	0.3208	0.2906	0.0175	0.3948	0.4057	0.3447	0.3987	0.3258	0.2693	0.4310
Bogiolamas			**0.0000**	0.3857	0.3660	0.2600	0.5007	0.4620	0.5045	0.5527	0.5056	0.4729	0.5158
Karnachalas				**0.0000**	0.2761	0.2784	0.3501	0.4620	0.5052	0.4844	0.5081	0.4235	0.4940
Tamyanka					**0.0000**	0.2701	0.2612	0.3977	0.3969	0.3859	0.3908	0.1835	0.4205
Mavrud(B)						**0.0000**	0.3536	0.3192	0.2987	0.3392	0.2741	0.2267	0.3720
Rubin							**0.0000**	0.5107	0.5089	0.4338	0.4813	0.3924	0.4701
Pamid(B)								**0.0000**	0.4031	0.4541	0.4647	0.4026	0.2507
Dimyat									**0.0000**	0.5184	0.5002	0.3885	0.4412

Ruen	**0.0000**	0.2902	0.3325	0.3576
Shizoka Melnishkaloza		**0.0000**	0.2881	0.3469
Mishej Sandanski			**0.0000**	0.3787
Keratsuda(B)				**0.0000**

Supplemental Table 4. Population differentiation analysis implemented in FSTAT 2.9.3.2 software.

	Pamid(G)	Keratsuda(G)	Zumiatiko	Limnio	Sefka	Mavrud(G)	Bogiolamas	Karnachalas	Tamyanka	Mavrud(B)	Rubin	Pamid(B)	Dimyat	Ruen	Shizoka Melnishkaloza	Mishej Sandanski	Keratsuda(B)
Pamid(G)		***	***	***	***	***	***	***	***	***	***	NS	***	***	***	***	***

Keratsuda(G)	**	**	***	**	**	***	***	***	***	**	***	***	***	***	***
Zumiatiko		***	**	**	**	***	***	***	***	**	NS	***	***	***	***
Limnio			**	**	**	***	***	***	***	**	***	***	***	***	***
Sefka				**	**	***	***	***	***	**	***	***	***	***	***
Mavrud(G)					**	***	***	NS	***	**	***	***	***	***	***
Bogiolamas						**	***	***	***	**	***	***	***	***	***
Karnachalas							***	***	***	**	***	***	***	***	***
Tamyanka								***	***	***	***	***	***	***	***
Mavrud(B)									***	***	***	***	***	***	***
Rubin										***	***	***	***	***	***
Pamid(B)											***	***	***	***	**
Dimyat												***	***	***	***
Ruen													***	***	***
Shizoka Melnishkaloza														***	***

Mishej Sandanski	***
Keratsuda(B)	

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

NS: non-significant

Figure legends

Figure 1. Factorial Correspondence Analysis of all Greek and Bulgarian grapevine varieties under study, using Genetix 4.05.2 software (a); UPGMA dendrogram of all studied varieties constructed with POPTREEW package, comprising of two major clusters (I and II). Numbers in nodes represent bootstrap values (b).

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