

Study and parentage analysis of old Albanian grapevine cultivars by ampelography and microsatellite markers

F. CARKA¹⁾, E. MAUL²⁾ and R. SEVO¹⁾

¹⁾Genetic Resources Centre, Agricultural University of Tirana, Albania

²⁾JKI - Julius Kühn-Institut, Institut für Rebenzüchtung Geilweilerhof, Siebeldingen, Germany

Summary

The number of old grapevine cultivars in Albania, originated in the country or introduced over many centuries and adapted to the conditions of the country, is huge. Because numerous cases of homonymy and synonymy exist, the interest for variety recognition is high. Investigation of these cultivars aptitudes is related to identification of desired characteristics and their utilization. In the framework of COST Action FA1003, trueness to type assessment of 13 old Albanian cultivars was undertaken including morphologic description and genetic fingerprinting. Parentage relationships were studied as well. The selected cultivars are of interest for their antiquity, for high production and distinctive organoleptic features. The ampelographic study of these cultivars supported confirmation of the results obtained by genetic profile comparison with seven SSR-marker databases and the European *Vitis* Database. In summary 12 unique genotypes, four parent-offspring relationships and one full parentage were found.

Key words: grapevine; characterization; fingerprinting; identification; relationship; biodiversity.

Introduction

Albania is placed in the west of the Balkan Peninsula. High diversity of relief and climate is preserving an extremely rich grapevine biodiversity (CARKA *et al.* 2010). Albania has a long viticulture tradition. Grapevine has been one of the most important crops. About 50 Albanian cultivars were described by SOTIRI *et al.* (1973) and CARKA (2006) published an ampelography presenting 13 autochthonous grape varieties. Today the Gene Bank of Albania maintains 106 old and adapted grapevine accessions/cultivars. Further 98 old individuals are under study and were not yet introduced into the Gene Bank collection. Conservation, characterization, evaluation and utilization of the old cultivars are one of the priority tasks of the Gene Bank. Recently, Albanian grapevine genetic resources were described using morphological descriptors (CARKA *et al.* 2010) and molecular markers (LADOUAKIS *et al.* 2005, ZULJ MIHALJEVIC *et al.* 2013). The aim of these studies was the characterization of old Albanian, respectively South

East European (SEE) germplasm and the detection of genetic relationships between neighboring countries. Neither LADOUAKIS *et al.* (2005) nor ZULJ MIHALJEVIC *et al.* (2013) found synonyms of old Albanian cultivars in Greece and South East Europe respectively. During the long period of cultivation the names of old Albanian grapevine cultivars were often changed and several synonyms and homonyms exist. The aim of this work is to contribute to the assessment of trueness to type of old Albanian cultivars using morphologic description and genetic fingerprinting and to study genetic relatedness.

Material and Methods

During the vegetative period of years 2012-2013 the ampelographic description was done for eleven old grapevine cultivars (Tab. 1) maintained in the Genetic Resources Center, Agricultural University of Tirana (Albania). Forty-eight OIV descriptors suggested by MAUL *et al.* (2012) were investigated. They were recorded by the same person to obtain comparable results and to avoid discrepancies due to subjectivity. Description comprised the following organs: young shoot, young and mature leaves, inflorescence, bunch and berry. The genetic characterization was done for thirteen accessions (Tab. 1) through nuclear microsatellite analysis according to MAUL *et al.* (2015). DNA was extracted from frozen young leaves. For genotyping, the following SSR-markers were used: VrZAG62, VrZAG79, VVMD5, VVMD7, VVMD25, VVMD27, VVMD28, VVMD32 and VVS2. These nine markers were recommended as an outcome of two projects funded by the European Commission: Genres 081 and GrapeGen06 (THIS *et al.* 2004, MAUL *et al.* 2012). For investigation on parent-offspring relationships by an excel macro application further 12 SSR-markers (VMC1B11, VMC4F3.1, VVIB01, VVIH54, VVIN16, VVIN73, VVIP31, VVIP60, VVIV37, VVIV67, VVMD21 and VVMD24) were analyzed. Genetic profiles of the thirteen Albanian cultivars were compared with (1) fingerprints recorded in seven large SSR-marker databases: Italy: CRA-VIT Conegliano, CNR Grugliasco and IASMA San Michele all' Adige; Spain: IMIDRA Alcalá de Henares and ICVV Logroño; France: INRA Montpellier and Germany: JKI Geilweilerhof and (2) fingerprints registered in the European *Vitis* Database (www-eu-vitis.de, BACILIERI and THIS 2010).

Table 1

List of investigated Albanian grapevine accessions. Identification results are displayed. They include: matching accessions, prime name and variety number in *VVC*, remark to the accession name and bibliographical reference. Descriptor notations of five bunch and berry descriptors of 11 accessions are given

Accession name / berry color	Accession- number	Identical profiles (institution-accession number-accession name)	Prime name- <i>VVC</i>	Variety number - <i>VVC</i>	Remark to the accession name	Bibliographical reference, describing or illustrating morphology	Utilization	Berry: color of skin (OIV 225)	Berry: shape (OIV 223)	Bunch: density (OIV 204)	Sugar content of must (OIV 505)	Time of full physiological maturity of the berry (OIV 304)
Caush B	ALB017-08	FRA139-Mtp-1673-Chaouch blanc, DEU098-1990-121-Chauch blanc, ROM045-11-Ceaus alb, UKR050-N33-Chaush, BG013-P15#1458-Chaush, ITA388-(492,06) Tchaouch, FRA139-1673Mtp8-Chaouch de Bulgarie	CHAOUCH BLANC	10196	alternative spelling	FROLOV-BAGREEV et al. (1956), vol. 6, p. 230	T	1	7	5	3	3
Dhelper RG	ALB017-10	BGR013-P15#751-Marach Cherven	MARAS CERVEN	7370	synonym	none	W					
Gomaresh N	ALB017-09	Not found	GOMARESH	23801	true name	none	T	5	7	7	5	7
Kosinjot N	ALB017-06	DEU098-1990-030-Servin Cernyi, ITA388-Rosso di Lecce (111,01), ITA362-V. silvestris Pioppeto	KOSINJOT	23803	true name	none	T	5	7	5	5	7
Kumbullor i zi N	ALB017-02	ITA388-14.13 Kumbullor, ITA388-89.13 Argvetuli Sapere, (misnomer)	KUMBULLOR I ZI	23805	true name	none	T	6	4	7	3	3
Manakuq RG	ALB017-05	Not found	MANAKUQ	23806	true name	none	T	3	4	5	5	5/7
Muskat i bardhe B	ALB017-11	Not found	MUSKAT I BARDHE	24698	true name	none	T	1	7	5	5	5/7
Serine e zeze N	ALB017-13	ITA 388-Pules 2 (213,13), ITA 388-Pules Bylysh (214,13), ITA362-3086-Cetcepesci	PULEZI	23809	misnomer	SOTIRI et al. (1973), p. 549	W					
Sulltanine B	ALB017-12	Not found			misnomer	none	T	1	7	5	5	7
Tajka e bardhe B	ALB017-01	Not found	TAJKA E BARDHE	12212	true name	none	T	1	6	7	5	7
Tajka e kuqe RG	ALB017-04	DEU098-1980-183-Drenak orven, ITA362-1193-Malvasia Rei, ITA360-527-Pupa 'd Cagna	PARMAK CERVEN	8945	synonym	CONSTANTINESCU (1966), vol. 7, p. 317	T	3	6	7	5	7
Tajka e zeze N	ALB017-07	BGR013-P15#1532-Shiroka Melnishka, DEU098-1980-298-Pamid (misnomer), FRA139-2771Mtp1-Chiroka Melnichka	SIROKA MELSINSKA	11838	synonym	DEL ZAN et al. (2004), p. 429	T	6	6	5	5	7
Tajka roze RS	ALB017-03	DEU098-1980-183-Drenak orven, ITA362-1193-Malvasia Rei, ITA360-527-Pupa 'd Cagna	PARMAK CERVEN	8945	synonym	CONSTANTINESCU (1966), vol. 7, p. 317	T	2	6	5	5	7

Notes: T = table grape; W = wine grape; color of berry skin: 1 = green-yellow; 2 = rose; 3 = red; 4 = red-grey; 5 = dark red-violet; 6 = blue-black; berry shape: 3 = elliptic; 4 = round; 5 = oblate; 6 = ovate; 7 = obtuse-obovate; bunch density: 5 = medium; 7 = dense; sugar content of must: 3 = low (~ 15 % sugar); 5 = medium (~ 18 % sugar). Time of full physiological maturity of the berry: 3 = early; 5 = medium; 7 = late.

Results and Discussion

The morphological characterization of eleven old grapevine cultivars showed a relatively high level of diversity. The evaluation of the data showed that most of the old grapevine cultivars are clearly distinct. Only 'Tajka e kuqe' and 'Tajka roze' showed matching expression levels at 82 % of the descriptors and 'Tajka e bardhe' and 'Tajka roze' 78 %. In Tab. 1 the results of five bunch and berry characteristics are given. Further characterization data will be uploaded in the European *Vitis* Database.

Genetic characterization of the thirteen old grapevine cultivars (Tab. 1) was carried out to identify homonyms and synonyms, to discover migration of the studied grapevine germplasm and to investigate parentage relationships. Microsatellite markers used in this study proved to be very useful for that purpose. Comparison of the allelic profiles of the studied cultivars showed that twelve of them are distinct. 'Tajka e kuqe' and 'Tajka roze' displayed the same SSR profile (Tab. 2) and a great overlap of morphological traits such as: large bunches (more or less dense), no erect or prostate hairs on the lower side of the leaves and the berry skin color ranging from rose ('Tajka roze') to red ('Tajka e kuqe'). Hence, they were identified as berry color mutants. 'Tajka e bardhe' and 'Tajka roze' showing certain morphological similarities displayed different profiles. It turned out that both cultivars are connected by parent-offspring relationship, sharing one allele at each of the 21 studied loci (Tab. 2).

Via comparison of the obtained genetic fingerprints with those recorded in the European *Vitis* Database, in seven large SSR-marker databases from France, Germany, Italy and Spain and in various databases in the web, for eight profiles matches were found. Identification results are listed in Tab. 1 and the genetic fingerprints in Tab. 2. 'Caus' displayed the same profile as 'Chaouch Blanc' preserved in a large number of collections: INRA - Unité Expérimentale du Domaine de Vassal & Montpellier SupAgro, Marseillan Plages, France (FRA139), Institut für Rebenzüchtung Geilweilerhof, Germany (DEU098), Research and Development Station for Viticulture and Oenology Dragasani-Valcea, Dragasano, Romania (ROM045), National Institute of Vine and Wine "Magarach", Yalta, Crimea, Ukraine (UKR050), Institut de Viticulture et d'Oenologie, Pleven, Bulgaria (BGR013) and CRA - Centro di Ricerca per la Viticoltura, Conegliano, Italy (ITA388). The accession names are very si-

Table 2

Allele sizes of 13 old Albanian grapevine accessions. Allele sizes of Heumisch weiss and Coarna alba were added to illustrate parent-offspring relationship with Kosinjot and Dhelpel respectively. Kumbullor i zi was placed in-between its progenitors and Muskati i bardhe and Tajka e bardhe beside Tajka e kuqe / Tajka roze with which they share at least one allele at each of the 21 analyzed loci. Allele sizes of Tajka e kuqe / Tajka roze showing identical profiles are given only once

	Heumisch weiss	Kosinjot	Kumbullor i zi	Tajka e kuqe / Tajka roze	Muskati i bardhe	Tajka e bardhe	Dhelpel	Coarna alba	Caus	Gomaresh	Manakuq	Serime e zeze	Sulltamine	Tajka e zeze
VVS2	133-143	143-143	139-143	139-143	143-143	143-143	143-143	133-143	135-151	135-151	133-139	145-155	143-145	133-133
VVMD5	236-242	230-236	230-248	234-248	230-234	234-236	228-240	240-248	230-240	234-236	240-248	228-234	234-234	236-248
VVMD7	239-249	239-249	239-239	239-247	245-247	247-249	239-239	239-239	247-249	239-249	247-249	239-249	239-239	239-239
VVMD21	250-250	250-250	250-257	244-257	257-257	244-257	250-257	257-259	250-257	md-md	md-md	244-250	250-257	250-257
VVMD24	206-206	206-215	206-206	206-210	206-215	206-210	206-210	206-206	204-215	206-210	206-206	206-210	206-210	md-md
VVMD25	239-255	255-255	241-249	241-249	249-255	249-255	249-255	239-239	249-255	239-255	239-239	239-239	241-255	239-239
VVMD27	180-182	182-186	182-182	182-186	186-186	180-186	180-180	180-182	180-184	182-195	180-182	182-182	182-190	180-182
VVMD28	228-246	228-246	246-258	236-258	246-258	246-258	234-258	234-236	258-258	236-236	234-258	236-246	236-246	246-246
VVMD32	250-272	250-272	250-252	252-272	272-272	250-252	272-272	264-272	252-272	250-272	252-252	252-272	250-268	272-272
VrZAG62	196-204	186-204	186-188	186-188	186-202	186-204	188-188	188-188	188-204	188-194	186-200	196-196	186-188	188-196
VrZAG79	237-243	243-251	243-259	251-259	251-251	237-251	237-251	251-259	247-249	239-259	249-251	243-259	243-259	243-251
VMC1B11	171-185	185-185	185-185	185-189	185-189	185-189	175-185	183-185	167-183	185-185	189-189	171-185	171-185	171-183
VMC4B3.1	171-171	171-171	165-171	165-171	165-171	165-171	165-171	165-208	181-187	206-208	171-175	167-171	167-208	171-208
VVIB01	295-299	291-295	291-295	291-295	291-295	291-295	291-295	295-295	291-295	295-295	295-295	291-295	295-299	295-295
VVIH54	149-167	165-167	165-165	165-177	167-177	165-177	165-165	165-165	159-165	165-177	163-179	165-165	163-163	149-165
VVIN16	149-149	149-149	147-149	147-149	147-149	149-149	149-149	149-149	149-151	149-149	147-151	149-151	149-149	149-149
VVIN73	266-266	266-266	266-266	266-266	266-268	266-266	266-266	266-266	258-266	258-266	266-266	258-266	266-266	266-268
VVIP31	173-181	173-181	173-181	177-181	177-181	175-193	175-193	181-193	181-185	181-187	177-187	181-187	181-185	md-md
VVIP60	322-324	306-322	322-328	326-328	318-326	318-318	318-318	318-328	322-322	322-332	322-326	318-322	306-318	328-332
VVIP37	160-168	160-168	160-160	160-168	160-160	160-168	156-168	156-168	150-173	156-156	158-160	160-168	160-168	md-md
VVIV67	365-366	365-376	362-365	362-365	362-365	362-376	365-376	358-365	358-358	358-365	358-362	365-365	365-365	358-365

miliar confirming that finding as well. 'Dhelper' with red berry color matched with 'Maras Cerven' maintained by BGR013. A parent-offspring relationship with 'Coarna alba' was discovered (Figure). The Albanian cultivar 'Dhelper' with green berries is a distinct cultivar. 'Kosinjot' showed an allelic profile identical to a non referenced cultivar, respectively the accessions 'Servin Chernyi' maintained in the collections of DEU098 and Vyskumná stanica Vinohradnick, Senkvice, Slovakia (SVK 01), Rosso di Lecce from ITA388 and *V. sylvestris* Pioppeto in the repository of Istituto Agrario di S. Michele all' Adige, Italy (ITA362). 'Kosinjot' showed a first degree relationship with 'Heunisch Weiss'/'Gouais blanc' (Tab. 2.) (MAUL *et al.* 2015). 'Kosinjot' is an old Albanian grapevine cultivar and very rare. It has taken the name of the village where it is spread. 'Kumbullor i zi', whose historical traces need to be further investigated, is most likely the progeny of 'Kosinjot' and 'Tajka e kuqe'/'Tajka roze' (Figure). 'Serine e zeze' is a misnomer. It matches the accessions 'Pules' and 'Pules Bylysh' in the collection of ITA388, 'Cetcipesci' ('Chetchipeshi' in the *Vitis* International Variety Catalogue) in the repository of ITA362 and 'Pules y Bylyshit 2' of LADOUAKIS *et al.* (2005). The fingerprint is distinct from 'Pulez' given by ZULJ MIHALJEVIC *et al.* (2013). Bibliographical references from 'Chetchipeshi' (KARTAVCENKO *et al.* 1966) and 'Pulezi' (SOTIRI *et al.* 1973) show similar leaf architecture but distinct bunch and berry shape. For clarification of trueness to type further studies are needed. 'Sultanine' is not identical with the famous 'Sultanina/Thompson Seedless' and therefore a misnomer. 'Tajka e kuqe' and 'Tajka roze' showed the same profile as 'Parmak Cerven', a Turkish cultivar existing in numerous grapevine collections under most diverse synonyms. The designations 'Tajka e kuqe' and 'Tajka roze' were unknown before and were considered as synonyms. Besides, the involvement in the parentage of 'Kumbullor i zi', 'Tajka e kuqe'/'Tajka roze' is linked in a first degree relationship to 'Muskat i bardhe' and 'Tajka e bardhe'. 'Tajke e zeze' turned out to be 'Shiroka Melnishka' conserved in the repositories of BGR013, DEU098 and FRA139.

Conclusion

From thirteen old Albanian accessions eight matched with accessions maintained in European collections. Five accessions turned out to be unique, requiring further investigation and safety duplication. Four first degree parentages and one full parentage were detected. With respect to the small number of cultivars included in this study this result was unexpected. The analysis of more germplasm is planned to further elucidate Albanian grapevine genetic diversity. This work is considered as a start in the verification of Albanian cultivars identities, which is necessary for their recovery and preservation. The study is representing an important step in increasing the knowledge on old Albanian grapevine cultivars. Further research is necessary to provide evidence of the synonymy of all the accessions maintained in the Albanian Gene Bank, most of which are at present greatly neglected.

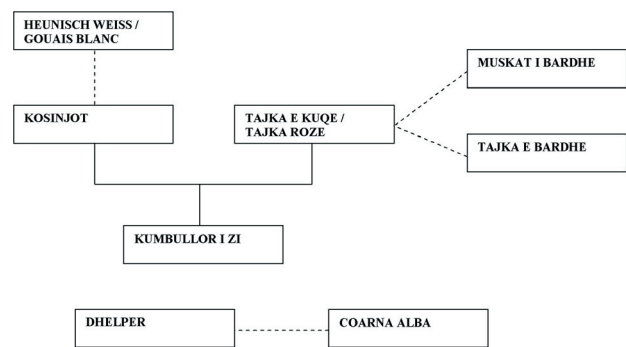


Figure: Illustration of the four parent-offspring relationships (---) and one full parentage (—) discovered within the study of thirteen Albanian old grapevine accessions by 21 SSR loci.

Acknowledgements

This work was carried out in the framework of COST Action FA 1003, "East-West Collaboration for Grapevine Diversity Exploration and Mobilization of Adaptive Traits for Breeding"

References

- BACILIERI, R.; THIS, P.; 2010: GrapeGen06, an European project for the management and conservation of grapevine genetic resources (<http://www1.montpellier.inra.fr/grapegen06/>).
- CARKA, F.; 2006: Ampelografia e disa kultivareve autoktone te hardhise. ADA, Tirane.
- CARKA, F.; CICI, I.; NAKO, R.; VORPSI, V.; PAKAKRONI, H.; 2010: Albanian Grapevine Germplasm. ISHS Acta Hort. **827**, 123-124
- CONSTANTINESCU, G.; 1966: Ampelografia Republicii Populare Romine, Vol. 7. Academiei Republicii Populare Romine.
- DEL ZAN, F.; FAILLA, O.; SCIENZA, A.; 2004: La Vite e l'Uomo, dal Rompicapo delle Origini al Salvataggio delle Reliquie. Editoriale Lloyd, San Dorligo della Valle, Trieste.
- EUROPEAN *VITIS* DATABASE (www.eu-vitis.de). ©JKI 2007-2011.
- FROLOV-BAGREEV, A. M.; NEGRUL A. M.; BLAGONRAVOV, P. P.; 1956: Ampelography of USSR, Vol. 6. Staatlich wissenschaftlich-technischer Verlag des Ministeriums für Lebensmittelproduktion.
- KARTAVCENKO, P. K.; FROLOV-BAGREEV, A. M.; BLAGONRAVOV, P. P.; 1966: Ampelografija SSSR, Vol. 3. Verlagshaus Lebensmittelindustrie, Moskau.
- LADOUAKIS, E. D.; LEFORT, F.; SOTIRI, P.; BACU, A.; KONGJIKA, E.; ROUBELAKIS-ANGELAKIS, K. A.; 2005: Genetic characterization of Albanian grapevine cultivars by microsatellite markers. J. Int. Sci. Vigne Vin **39**, 109-119.
- MAUL, E.; SUDHARMA, K. N.; KECKE, S.; MARX, G.; MÜLLER, C.; AUDEGUIN, L.; BOSELLI, M.; BOURSICQUOT, J. M.; BUCCHETTI, B.; CABELLO, F.; CARRARO, R.; CRESPIAN, M.; DE ANDRÉS, M. T.; EIRAS DIAS, J.; EKHVIAIA, J.; GAFORIO, L.; GARDIMAN, M.; GRANDO, S.; AGYROPOULOS, D.; JANDUROVA, O.; KISS, E.; KONTIC, J.; KOZMA, P.; LACOMBE, T.; LAUCOU, V.; LEGRAND, D.; MAGHRADZE, D.; MARINONI, D.; MALETIC, E.; MOREIRA, F.; MUÑOZ-ORGANERO, G.; NAKHUTSRISHVILI, G.; PEJIC, I.; PETERLUNGER, E.; PITSOLI, D.; POSPISILOVA, D.; PREINER, D.; RAIMONDI, S.; REGNER, F.; SAVIN, G.; SAVVIDES, S.; SCHNEIDER, A.; SERENO, C.; SIMON, S.; STARAZ, M.; ZULINI, L.; BACILIERI, R.; THIS, P.; 2012: The European *Vitis* Database (www.eu-vitis.de) - a technical innovation through an on-line uploading and interactive modification system. *Vitis* **51**, 79-86.
- MAUL, E.; EIBACH, R.; ZYPRIAN, E.; TÖPFER, R.; 2015: The prolific grape variety (*Vitis vinifera* L.) 'Heunisch Weiss' B (= 'Gouais blanc'): bud mutants, "colored" homonyms and further offsprings. *Vitis* **54**, 79-86.

- SOTIRI, P.; GJERMANI, T.; NINI, T.; 1973: Vitikultura. Tekst mesimor per Fakultetin e Agronomise. Instituti Larte Shteteror i Bujqesise, Tirana.
- THIS, P.; JUNG, A.; BOCCACCI, P.; BORREGO, J.; BOTTA, R.; COSTANTINI, L.; CRESPIAN, M.; DANGL, G. S.; EISENHELD, C.; FERREIRA-MONTEIRO, F.; GRANDO, S.; IBÁÑEZ, J.; LACOMBE, T.; LAUCOU, V.; MAGALHÃES, R.; MEREDITH, C. P.; MILANI, N.; PETERLUNGER, E.; REGNER, F.; ZULINI, L.; MAUL, E.; 2004: Development of a standard set of microsatellite references alleles for identification of grape cultivars. *Theor. Appl. Genet.* **109**, 1048-1058.
- ZULJ MIHALJEVIC, M.; SIMON, S.; PEJIC, I.; CARKA, F.; SEVO, R.; KOLS, A.; GASI, F.; TOMIC, L.; JOVANOVOG CVETKOVIC, T.; MALETIC, E.; PREINER, D.; BOZINOVIC, Z.; SAVIN, G.; CORNEA, V.; MARAS, V.; TOMIC MUGOSA, M.; BOTU, M.; POPA, A.; BELESKIO, K.; 2013: Molecular characterization of old local grapevine varieties from South East European countries. *Vitis* **52**, 69-76.

