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Research Note

Preliminary study of Armenian grapevines phenolic contents

K. Margaryan[1), 2), 3)], R. Aroutiounian[1), 2)], G. Melyan[1)] and O. Failla[4)]

[1)] Armenian Academy of Viticulture and Wine-Making, Yerevan, Armenia
[2)] Department of Genetics and Cytology, Yerevan State University, Yerevan, Armenia
[3)] Research Group of Plant Genetics and Immunology, Institute of Molecular Biology of National Academy of Sciences, Yerevan, Armenia
[4)] Department of Agricultural and Environmental Sciences, University of Milan, Milan, Italy

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Introduction: According to archaeological data, viticulture and winemaking appeared in Armenia around the 7th millennium BC (Harutyunyan *et al*. 2005). Armenia has a very large wild grapevine population and grape cultivars offering a valuable gene pool to grapevine breeders.

Grapes contain large amounts of phytochemicals which account for most of the sensory characteristics of the respective wines, such as color, aroma and astringency (Waterhouse 2002). The most studied group of grape phytochemicals are polyphenols: secondary metabolites with diverse chemical structures and functions (Naczk and Shahidi 2004). Their biological activities have been extensively studied during the last decades, providing strong evidence of their potential health benefits.

Grape skins and seeds are known to be rich sources of phenolic compounds, both flavonoids and non-flavonoids (Arnous and Meyer 2008, Poudel *et al.* 2008). The concentration of phenolic compounds in grapes depends on the variety of grapevine and it is influenced by viticultural and environmental factors. Knowledge of polyphenolic content of grape skins is relevant for their future use.

Many Armenian grapevine cultivars have been already described and their genotypes determined. However, many local grapevine accessions remain unidentified and their phenotypic characteristics overlooked. At the same time an accurate phenotypic description of varieties needs to be done with combined methodologies which involve the determination of polyphenolic content as an important chemical descriptor. The aim of this study was characterization of Armenian local grapevine resources by determining the total polyphenol content in skins and seeds of grape cultivars for phenotyping the diversity of Armenian grapevines.

Correspondence to: Dr. K. Margaryan, Department of Genetics and Cytology, Yerevan State University, 0025, 1 Alex Manoogian Yerevan, Armenia. E-mail: kristina-margaryan@rambler.ru

Material and Methods: Grape samples: Ten colored and ten white Armenian grapes were analyzed to determine total phenols in skins and seeds extracts. Samples from the cultivars and germplasm accessions were harvested in their technological ripening stage.

Preparation of the grape skins and seeds for analyses: The pedicels were removed and the berries were manually skinned. The seeds were separated from the pulp, washed with distilled water and then blotted on paper to remove any residual pulp. The skins and seeds were then extracted in 20 mL of an ethanol: water: hydrochloric acid (70:29:1) solution for 24 h. The extracts were filtered before spectrophotometric the total phenols determination.

Analysis of total polyphenols: The Folin-Ciocalteu method was used for the determination of the total phenols as suggested by Rustioni *et al.* (2014). In brief, an aliquot (0.5 mL) of the appropriate diluted extracts was added to a 10 mL volumetric flask, containing 2.5 mL of distilled water. Then, 0.5 mL of Folin-Ciocalteu reagent was added and the contents mixed. After 3-5 min, 2 mL of 10 % Na_2CO_3 solution was added and made up to a total volume of 10 mL distilled water. After keeping the samples for 90 min at room temperature, their absorbance was read at 700 nm against distilled water as the blank. The total polyphenols were expressed as catechin ($mg \cdot L^{-1}$) concentration and calculated applying the formula "catechin ($mg \cdot L^{-1}$) = 186.5 x E_{700} x d" (E_{700} = absorbance at 700 nm; d = dilution). Then data were converted in $mg \cdot kg^{-1}$ of grape, based on the berry weight. All samples were prepared in triplicate.

Statistical methods: All data were expressed as mean ± standard deviation (SD) of three replications for each grape skin and seed extract tested. The data obtained were analyzed statistically by the one-way analysis of variance (ANOVA) and Multiple Range Test (STATGRAPHICS Plus).

Results and Discussion: Skin and seed extracts from twenty different Armenian grape cultivars were analyzed for determination their total phenol concentration. The content of total phenols expressed as catechin equivalents found in coloured grape cultivars traditionally grown in Armenia are presented in Tab. 1. According to the obtained data in skin extract of 'Avagi 2' cultivar presented significantly higher total phenolic content (1,355.8 $mg \cdot kg^{-1}$of grape) when compared to the other accessions, followed by 'Movsesi clone' and 'Movsesi Aghavnadzori' cultivars. However, significant differences in skin total phenolic content were not found among 'Avagi 3' and 'Lyustra', or among 'Areni sev' and 'Nalbandyan' cultivars ($p < 0.05$). The highest total phenolic content in seed extract was recorded in 'Armenia' cultivar (842.04 $mg \cdot kg^{-1}$of grape), followed by 'Vardabuyr' and 'Sev Sateni' cultivars ($p < 0.05$). Significant differences in seed total phenolic content were not found among 'Avagi 2' and 'Movsesi clone', or among 'Arevar' and 'Itsaptuk' cultivars ($p > 0.05$).

The content of total phenols found in white grape cultivars traditionally grown in Armenia are presented

Table 1

Content of total phenols in Armenian colored grape cultivars

Colored grape cultivars	Skin phenols (mg·kg^{-1} of grape)	Seed phenols (mg·kg^{-1} of grape)
Avagi 2	1355.8 ± 107.7^{f}	462.4 ± 99.7^{bc}
Avagi 3	652.2 ± 166.5^{ab}	327.8 ± 101.9^{ab}
Areni sev	529.9 ± 117.4^{a}	319.04 ± 83.3^{ab}
Armenia	710.3 ± 101.9^{abc}	842.04 ± 166.8^{d}
Lyustra	671.8 ± 117.6^{ab}	558.4 ± 63.3^{c}
Movsesi clone	1108.1 ± 141.1^{e}	404.1 ± 67.7^{bc}
Movsesi Aghavnadzori	1008.9 ± 207.4^{de}	226.2 ± 36.9^{a}
Nalbandyan	482.1 ± 91.0 ^{a}	145.3 ± 94.3^{c}
Sev Sateni	920.4 ± 106.7^{cde}	736.7 ± 55.01^{d}
Vardabuyr	869.9 ± 184.4^{bcd}	832.4 ± 107.7^{d}

Average value ± standard deviation (n = 3), number with no letters in common is significantly different ($p < 0.05$). Avagi 2 and Avagi 3 are temporary names, because cultivars were found only in 2012 in old vineyards, in East Armenia. Genetic and ampelographic characterization of these cultivars are still in progress.

in Tab. 2. Analysis of white grape cultivars revealed that in 'Tokun' (744.4 mg·kg^{-1} of grape) and 'Khachi khardji' (740.3 mg·kg^{-1} of grape) skin extracts presented significantly higher total phenolic content ($p < 0.05$) with respect to the other accessions. However, significant differences in skin total phenolic content were not found among 'Ararati' and 'Arevar', or among 'Khatun khardji' and 'Parvana' cultivars ($p > 0.05$). A significantly higher total phenolic content in seed extract was found in 'Mskhali' cultivars (523.2 mg·kg^{-1} of grape) ($p < 0.05$) with respect to the other white grape varieties. Significant differences in seed total phenolic content were not found among 'Ararati', 'Arevar' and 'Itsaptuk' cultivars ($p > 0.05$).

The obtained results revealed that 'Avagi 2', 'Armenia' and 'Movsesi clone' cultivars with black skin have the highest total phenolic content. Among white grapes high level of total phenolic content was determined for 'Mskhali', 'Tokun' and 'Khachi khardji' cultivars.

Genetic, agronomic or environmental factors play crucial roles in phenolic composition and concentration. It is well known that the composition of phenols in grapevines depends from variety, species, season and environmental and management factors such as soil conditions, climate and crop load. The total phenol content of red grape skins is higher than that of white grapes probably due to the loss of the ability to produce anthocyanins in the skins of white grapes. Our results indicate that the phenolic content of different grapes and distribution of these compounds in skins and seeds depend mainly on the grape skin color and variety.

Table 2

Content of total phenols in Armenian white grape cultivars

White grape cultivars	Skin phenols (mg·kg^{-1} of grape)	Seed phenols (mg·kg^{-1} of grape)
Ararati	209.2 ± 22.2^{b}	120.8 ± 7.2^{a}
Arevar	382.1 ± 90.5^{b}	121.9 ± 18.3^{a}
Itsaptuk	428.7 ± 26.4^{bc}	110.6 ± 3.9^{a}
Khachi khardji	740.3 ± 91.5^{e}	202.2 ± 7.2^{cd}
Khatun khardji	328.6 ± 76.6^{ab}	352.2 ± 6.6^{e}
Mskhali	527.5 ± 17.2^{cd}	523.2 ± 40.9^{f}
Parvana	304.8 ± 34.4^{ab}	136.9 ± 33.4^{ab}
Qrdi khaghogh	248.6 ± 41.6^{a}	174.1 ± 30.2^{bc}
Tokun	744.4 ± 49.2^{e}	199.2 ± 21.5^{c}
Voskehat	638.1 ± 34.9^{de}	240.3 ± 32.04^{d}

Average value ± standard deviation (n = 3), number with no letters in common is significantly different ($p < 0.05$).

Conclusions: The conservation of grapevine biodiversity in Armenia is particularly important because of the large number of traditional local varieties out of cultivation. These resources could be relevant for the development of new cultivars. The presented work is a first step towards identification and conservation of genetic resources of Armenian grapes, where phenotyping is one of the basic steps. Results reported here can be used for Armenian grapevine phenotyping as an important chemical descriptor. In the next future, these data could also find application for selection of improved grape varieties targeted to fresh consumption and wine production.

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