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Armenian grapevines: cytoembryological, morphological and chemical analysis

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Summary

The objectives of our study was to phenotype Armenian grapevines on the base of cytoembryological, morphometric and phenolic content analysis of 10 wine and 10 table cultivars *(Vitis vinifera* L.). The presented results suggest that Armenian table grape cultivars have higher level of abnormalities in the process of seed formation than wine cultivars. However, during ovule development, the observed differences between table and wine varieties were not significant. The berry morphometric analysis demonstrated that table cultivars formed significantly larger and heavier berries than wine cultivars. The obtained results show that wine grapes contained significantly higher phenol concentrations than table grapes, in both colored and white grapevine cultivars. The study of phenol composition, an important chemical descriptor in grapevine phenotyping, provides oenological information useful to improve the wine quality. Cultivar characterization could be used as marker for the selection of table and wine grape breeding programs. These results will be upgraded in the database for Armenian grapevine varieties. In future Armenian germplasm will be compared with datasets of neighboring countries, to determine the varietal origins relationships.

K e y w o r d s : Caucasus; cytoembryology; morphometric traits; polyphenols.

Introduction

Grapevines are an indigenous part of Armenia's flora. Archaeological excavations in the Areni-1 cave complex (southeastern Armenia) revealed installations and artifacts which suggest wine production around 4000 BC (Barnard *et al.* 2011).

Armenian grape cultivars were traditionally selected for thousands of years. Moreover, their variability was enlarged by crossing. Currently the viticulture is one of the most important branches of the Armenian agriculture and the production of brandy and wine provide an important contribution to the countrie's economy. Unfortunately, in the early 1990s, the main collection of Armenian grapes (850 cultivars) was eliminated. Now, in three existing ampelographic collections 140 cultivars are preserved, among them 125 are local accessions and 15 are varieties of foreign origin. Among the local ones, 70 are old autochthonous cultivars (Melyan and Gasparyan 2012). The wild grapevine (*Vitis vinifera* ssp. *sylvestris*) distribution areas in Armenia have recently sharply reduced. However, many wild populations are still growing in southern Armenia. Ten wild accessions were also planted in our ampelographic collection.

The grape characterization methods are based on morphological, cytoembryological, agronomical, physiological and genetic studies. A crucial stage in grapevine development is flowering because it affects the potential yield and some berry characteristics. This is particularly evident in table grapes, where stenospermocarpy or parthenocarpy generates seedless cultivars. Moreover, the new selected cultivars, are characterized by different qualities, yields and berry sizes, to satisfy the increasing interest in table seedless grapes (Longbottom 2007).

Armenia has a long tradition in grape growing and high quality wine making. Polyphenols are a class of compounds present in grapes and wine. They contribute to sensory attributes such as color, bitterness and astringency. Many polyphenols have positive effects on human health, including anti-inflammatory and antioxidant activity. Although external factors such as viticultural practices and weather conditions may affect phenol accumulation, genetic differences between cultivars could be significant.

Phenotypic and genotypic characterization of grape genetic resources in germplasm banks is a basic point for breeding programs. Phenotyping can be defined as precise and comprehensive analysis of traits, in which single components of the phenotype need to be observed and described. Phenotyping is required for a range of research applications, including genetic association studies and cultivar evaluation (Rustioni *et al.* 2014).

The objectives of our study were i) to characterize reproductive and carpological traits of Armenian wine and table grape cultivars and ii) to determine the content of polyphenols to assess the range of variation in these compounds among the studied accessions.

Material and Methods

G r a p e S a m p l e s : In 2012, 10 Armenian wine grape cultivars 'Sev Aldara', 'Karmrahyut', 'Tozot', 'Sev Khardji', 'Muscat tuyl', 'V1', 'Hadisi', 'Burmunq', 'Areni clone', 'Kakhet' and 10 Armenian table grape cultivars

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'Muscat Yerevanyan', 'Ayvazyani muscateni', '99/1 Derei', 'Podarok Rossii', 'Hayreniq', 'Erebuni', 'Ararati', 'Karmir itsaptuk', 'Hayastan', 'Shahumyani' were analyzed. Some of them survived only in low production vineyards or in germplasm collections.

Grape samples were harvested in their technological ripening stage. The sampling was randomly made by picking berries from the top, central, and bottom parts of the clusters. The samples were kept frozen until analysis. All the assays were performed in triplicate.

Total phenols assay: The total phenol content was determined using the Folin-Ciocalteu colorimetric method as suggested by Rustioni *et al.* (2014). The grape diluted extracts were oxidized by the Folin-Ciocalteu reagent and the reaction was neutralized with sodium carbonate. After 90 min at room temperature the absorbance was measured at 700 nm. The polyphenols were expressed as catechin (mg·L^{-1}). After that, data were converted in catechin (mg·kg^{-1} of grapes) based on the berry weights.

Statistical methods: All data were expressed as mean ± standard deviation (SD) of three replications for each grape skin and seed extracts. The obtained data were analyzed by the one-way analysis of variance (ANOVA) and Multiple Range Test for comparison of means (STATGRAPHICS Plus). The probability lower than 0.05 was accepted as significant.

Cytoembryological and morphometric analysis of Armenian grapevines: Grape flowers on growth stage 6 (from early flowering: 30 % of flower caps fallen to full flowering: 50 % of flower caps fallen) **(**Lorenz *et al.* 1994**)** for cytoembryological analysis and ripe berries for morphometric analysis were collected from the grapevine germplasm collection of the Scientific Center of Viticulture, Fruit-Growing and Wine-Making (Merdzavan, Armenia). Paraffin-embedded slides were prepared by common cyto-embryological techniques and stained by Mayer's hematoxylin and eosin (H&E) (Ruzin *et al.* 1999, Bancroft and Gamble 2008). Morphometric features were described according to the IPGRI, OIV and UPOV phenotypic descriptors (IPGRI 1997) using the ImageJ software.

Results and Discussion

The content of total phenols found in ten wine and ten table grape cultivars traditionally grown in Armenia are presented in the Figure.

Obtained results show that the wine grapes, in both colored and white grapevine cultivars, contain significantly higher concentrations of total phenols when compared to table grapes. Moreover, a grape skin color effect was evidenced by our data. Skin total phenols concentration of red grapes was higher than that one of white grapes, probably due to the loss of the ability to produce anthocyanins. In particular, the cultivars 'Sev Aldara' (3463.88 mg·kg^{-1} of grape), 'Karmrahyut' (3143.31 mg·kg^{-1} of grape) and 'Tozot' (1990.18 mg·kg^{-1} of grape) had the highest total phenol contents, and, traditionally, they are used as wine grapes. 'Shahumyani' and 'Hayastan' with yellow-green skins had the lowest total phenol content and they are used as table grapes.

Genetic, agronomic and environmental factors play a crucial role in grape phenol composition and concentration. The polyphenols profiles in grapevines depend on their species, cultivar, environmental and management factors (e.g.: soil and weather conditions, yield …). However, also genotype should play a key role in phenol concentration.

The results of cytoembryological and morphometric analysis in ten wine and ten table grape cultivars are presented in the Table. Cytoembryological analysis revealed, that all investigated grapes cultivars had abnormalities in macrogametogenesis. The development of an embryo sac was arrested in some ovules either before or after meiosis, in seeded or parthenocarpic cultivars. Some ovules in ovary were not developed or were degenerated. Grape ovaries could potentially develop 4 ovules. However, an average of 1.44-2.67 ovules in wine cultivars and of 1.51-2.82 in table cultivars were developed. Then, considering the embryogenesis, only 58-90 % of seeds were normally developed in table grapes, beside the 75-99 % seed development success in wine cultivars. However, no significant differences have been found between ovule and seed number between wine and table grapes.

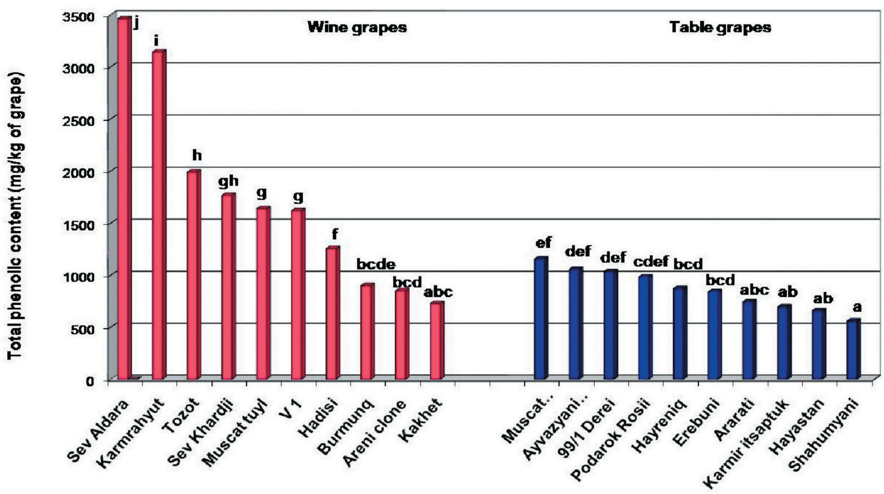

Figure: Total phenolic content in Armenian wine and table grapes. Bars which have no common letters significantly differ ($p < 0.05$) each other.

Table

Cytoembryological and morphometric characteristics of Armenian grape cultivars

Cultivar	Number of ovules in a flower	Number of seeds in a berry	Berry weight (g)	Berry size	
				length (mm)	width (mm)
Wine cultivars					
Sev Aldara	1.57 ± 0.05	1.53 ± 0.08	2.35 ± 0.13	19.29 ± 2.08	17.90 ± 2.03
Karmrahyut	1.97 ± 0.15	1.64 ± 0.07	1.38 ± 0.07	12.74 ± 2.01	12.35 ± 1.96
Tozot	1.65 ± 0.12	1.64 ± 0.11	2.60 ± 0.11	19.52 ± 1.59	17.00 ± 1.53
Sev Khardji	2.67 ± 0.16	2.42 ± 0.11	2.20 ± 0.09	18.62 ± 1.53	16.13 ± 1.52
Muscat tuyl	2.06 ± 0.10	1.76 ± 0.25	2.85 ± 0.09	20.43 ± 1.90	18.19 ± 2.49
V1	2.14 ± 0.14	1.88 ± 0.12	1.86 ± 0.09	16.52 ± 1.65	14.51 ± 1.47
Hadisi	2.07 ± 0.16	1.89 ± 0.13	1.68 ± 0.13	14.95 ± 1.44	13.88 ± 1.25
Burmunq	1.54 ± 0.10	1.16 ± 0.12	1.29 ± 0.09	15.13 ± 1.13	13.94 ± 1.26
Areni clone	1.44 ± 0.09	1.26 ± 0.05	2.91 ± 0.15	22.20 ± 2.07	17.72 ± 1.91
Kakhet	2.12 ± 0.12	2.03 ± 0.14	1.78 ± 0.07	16.34 ± 1.32	15.50 ± 1.32
Table cultivars					
Muscat Yerevanyan	2.77 ± 0.13	1.65 ± 0.06	3.51 ± 0.13	16.56 ± 1.86	16.36 ± 1.69
Ayvazyani muscateni	2.05 ± 0.08	1.52 ± 0.12	3.80 ± 0.18	21.42 ± 2.87	16.84 ± 2.43
99/1 Derei	1.96 ± 0.08	1.71 ± 0.10	5.02 ± 0.14	31.08 ± 2.13	20.10 ± 1.70
Podarok Rosii	1.51 ± 0.09	1.17 ± 0.06	4.00 ± 0.15	22.94 ± 2.35	18.22 ± 1.84
Hayreniq	2.28 ± 0.10	1.33 ± 0.06	5.01 ± 0.13	25.03 ± 2.63	24.50 ± 2.52
Erebuni	2.82 ± 0.09	2.38 ± 0.09	5.63 ± 0.12	25.02 ± 3.56	23.98 ± 2.57
Ararati	1.83 ± 0.12	1.32 ± 0.08	4.87 ± 0.15	21.84 ± 3.20	20.40 ± 2.70
Karmir itsaptuk	2.05 ± 0.10	1.44 ± 0.07	3.58 ± 0.08	24.86 ± 3.24	16.84 ± 1.92
Hayastan	1.81 ± 0.14	1.65 ± 0.11	5.60 ± 0.10	25.03 ± 2.83	24.05 ± 2.71
Shahumyani	2.76 ± 0.14	2.10 ± 0.13	6.40 ± 0.12	31.18 ± 2.04	19.30 ± 1.53

Morphometric analysis revealed the formation of significantly larger and heavier berries in table cultivars with respect to wine cultivars. The average weight of berries varied from 1.29 to 6.40 g. Berry sizes ranged from 12.74 to 31.18 mm. The largest berries were registered in 'Shahumyani' with an average weight of 6.40 g, length of 31.18 mm and width of 19.30 mm. In wine cultivars the values of morphometric traits were generally lower than table grapes. Average weight of wine berries ranged between 1.29 and 2.91 g and sizes varied between 12.8 and 22.2 mm. The smallest berries were found in 'Karmrahyut': average weight 1.38 g, length 12.74 mm and width 12.35 mm. Generally, it appeared that table berry weights ($p < 0.001$), lengths ($p < 0.001$) and width ($p < 0.05$) were statistically higher than these one recorded in wine berries. Morphometric study of the investigated accessions demonstrated that table cultivars formed significantly larger and heavier berries than wine cultivars.

Conclusions

Recently, some Armenian grapevine varieties have been characterized. However, many local accessions remain unidentified and their ampelographic characteristics overlooked. Accurate descriptions have to be done by combined approaches, including phenotyping and genotyping.

A preliminary study of phenolic composition could support wine industries. As an example, berry color variation is also used to differentiate cultivars (Lijavetzky *et al.* 2006).

Our data could also be a useful basis for targeted selection to improve grape quality in cross-breeding programs.

The presented results suggest that Armenian table grape cultivars have a higher level of seed formation abnormalities than wine cultivars, although ovules development differences were not significant. Morphometric carpological characteristics of investigated cultivars could be used as markers for parental selection in table grape breeding programs since the production of large berries without or with small number of seeds is generally highly appreciated.

Our research is the first step towards true-to-type grape cultivars identification in Armenia. In this way, cultivars could be restarted, and important practical knowledge could be available for growers.

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