PRELIMINARY FINDING ABOUT SUGAR COMPOSITION AND ORGANIC ACID STRUCTURE OF WOODLAND GRAPE (*VITIS SYLVESTRIS* GMEL.)

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Abstract

In this study, a total of 6 *Vitis sylvestris* GMEL. genotypes were compared to cultivated grapes of *Vitis vinifera* L. i.e. their sugar composition and acid structure were measured by

HPLC (High Performance Liquid Chromatography) and AAS (Atomic Absorption Spectroscopy) in two different years (2014 and 2015).

The predominant sugars in *Vitis sylvestris* GMEL. and *Vitis vinifera* L. (Welschriesling and Pinot noir) berries were glucose and fructose. Considering the total acid content of woodland grape berries, the amount of tartaric acid was lower than the amount of malic acid, in both years. Succinic acid was found in some *Vitis sylvestris* GMEL. genotypes (in 2014), which was absent from control European grapevine cultivars. Element analysis examinations by AAS indicated a higher concentration of potassium in the total mineral content of woodland grapes, than of other minerals.

The preliminary results of this study pointed out interesting differences in the constitution of organic acids, sugar and mineral element content of the grape-juice of *Vitis sylvestris* GMEL. genotypes and *Vitis vinifera* L. cultivars.

Keywords: minerals, sugars, organic acids, woodland grape

Összefoglalás

Kutatásunkban 6 ligeti szőlő genotípust és termesztett kerti szőlő fajtákat hasonlítottunk össze illetve ezek cukor-, valamint savtartalmát mértük HPLC-vel (High Performance Liquid Chromatography) és AAS-el (Atomic Absorption Spectroscopy) két különböző évben (2014-ben és 2015-ben).

A cukrok közül a glükóz és a fruktóz volt legnagyobb mértékben jelen a *Vitis sylvestris* GMEL.és a *Vitis vinifera* L. fajták (Olasz rizling, Pinot noir) mustjaiban. Mindkét évben a ligeti szőlő mustjának összes savtartalmát nézve a borkősav mennyisége alacsonyabb volt, mint az almasavé. A 2014-es évben a ligeti szőlő beltartalmi értékeit vizsgálva a

borostyánkősav is kimutatható volt, ami a kerti szőlő fajták mustjaiból hiányzott. Az AAS vizsgálatok kimutatták, hogy az összes ásványi anyagot tekintve a kálium mértéke volt a legmagasabb a többi elemhez képest.

Az elsődleges vizsgálatok alapján elmondhatjuk, hogy jelentős különbségek vannak a *Vitis sylvestris* GMEL és a *Vitis vinifera* L. fajták mustjának sav-, cukor- és ásványi elem összetétele között.

Introduction

The evolution of cultivated plants played an important role in the ascent of humanity. Research of their origin and evolution started at the beginning of the 20th century, but till now, a lot of questions have remained open. A large number of theories exist about the evolution of the European grapevine (*Vitis vinifer*a L.).

The *Vitis sylvestris* GMEL. is a protected species in Hungary (Farkas, 1999). The quest and reservation of its populations are significant in terms of nature conservation and reserve of biodiversity as well. Based on theoretical and practical researches, this species is supposed to be, either in itself, or in a crossing with other species the possible progenitor of the European grapevine (*Vitis vinifera* L.) (De Candolle, 1894, Kozma, 1991 and Terpó, 1986).

Vitis sylvestris GMEL. occurs in flood basins, with tendrils for climbing and lobed leaves. Its berries are blue with 1-5 seeds (usually 2-seeded), which are brownish, short-billed. The exocarpium is naturally thin with gelled berry flesh and a low amount of must can be gained from it. The colour is solely provided by anthocyanins containing monoglycoside (Bartha & Kevey, 2010).

HPLC is used to analyse the content and composition of organic acids and sugars. Nowadays, several methods have been developed for identifying and quantifying these organic acids in grape juices and wines (so much) individually, like non-enzymatic spectrophotometric and enzymatic methods or as a group of them simultaneously, like chromatographic and electrophoretic methods (Mato, Suarez-Luque & Huidobro, 2005, Saavedra & Barbas, 2003 and Vereda, Garcia de Torres, Rivero & Cano, 1998).

Most methods for the analysis of sugars and organic acids in grapevine berries and wines that rely on high performance liquid chromatography (HPLC) have commonly used only the grape musts and/or juices with different sample extraction protocols (Castellari, Versari, Spinabelli, Galassi & Amati, 2000, Crippen & Morrison, 1986, Frayne. 1986, Liu, Wu, Fan, Li & Li, 2006, McCord, Trousdale & Ryu, 1984 and Sabir, Kafkas, & Tangolar, 2010). In analytical chemistry, the atomic absorption spectroscopy (AAS) technique is used for determining the concentration of a particular element (the analyte) in a sample to be analyzed.

Sugars and organic acids are important primary metabolites, which contribute to grapevine growth and berry development. These compounds are also considered key factors in grape and wine quality. The hexoses, glucose and fructose, as well as the organic acids, like malic and tartaric acid are the most abundant compounds contributing to the grape juices’ sweetness and acidity, respectively. Their concentrations and/or ratios vary during the berry development and maturation stages. Organic acids are produced in both the grape leaves and berries and start to accumulate in the grapevine berry at early stages of berry development (Conde et al. 2007).

It is very important to know the change in the acidity of the grapes since it affects the composition of the future wine (Kállay, 2010). It is important to determine the organic acids in grape juices and wines, because they have influence on the organoleptic properties (flavour, colour, and aroma) and on the stability and microbiological control of the products. Tartaric and malic acids are the predominant organic acids in grape juices and succinic, as well as citric acids are present in minor proportion. In the case of wines, a common differentiation is made between the acids which come directly from the grape (tartaric, malic and citric acids) and those that are originated, fundamentally, in the fermentation process (succinic, lactic and acetic acids) (Belitz & Grosch, 1992 and Peynaud, 1999).

Wineries need to monitor the concentrations of organic acids during the wine making process in order to ensure the quality of the products.

The low molecular organic acids can dramatically affect the pH values and can also have implications on biological stability, sensory properties (Caccamo, Carfagnini, Di Corcia & Samperi, 1986 and Tusseau & Benoit, 1987), and the colour of the wine (García-Romero, Sánchez-Munoz, Martín-Álvarez & Cabezudo-Ibánez, 1993).

The succinic acid is a two-phase acid with four carbon atoms. Formula: COOH-CH2-CH2-COOH. It has a slightly sour, unpleasant taste and is the secondary product of the alcoholic fermentation. In 1852, Pasteur (1859, 1969) showed that during the alcoholic fermentation, succinic acid always arises, in a concentration of approx. 1 g in 100 g of alcohol. In fact, the amount of generated succinate varies between 0.5 and 1.5 quantities g L^{-1} depending on the conditions of fermentation. This succinate content remains in the wine. With its typical complex salt and bitter - sour taste, it plays a great role in the typical wine taste, as the most flavourful acid in wine. It has an important physiological role, as the anion form of succinate is involved in the citric acid cycle (Kállay, 2010 and Krebs & Lowenstein, 1960).

The French paradox suggests that consuming red wine daily not only helps the cardiovascular system, but it also increases lifespan due to the resveratrol (found in the skins and tannins of red grapes) content in red wine (Catalgol, Batirel, Taga & Ozer, 2012). Resveratrol has been linked to preventing decline in cardiovascular function caused by age (Das, Mukherjee & Ray, 2011). France surpasses many countries in average life expectancy partly due to the common practice of drinking red wine with meals (Brownlee, 2006). However, not all wine is "created equal," with red wine containing eight times as many flavonoids as white wine (Catanese, 2013).

Among polyphenols, trans-resveratrol can be emphasized, about which (some publications mention) is said to have beside its antioxidant quality, a lot of other positive effects, e.g. regulating the cholesterol level, preventing renal insufficiency, inhibiting prostate tumour, as well as having a liver defending function, HIV disablement, retardation of the cerebral aging processes, prevention of gastric ulcer and the diseases of the lungs etc. The presence of minerals is always ambivalent, as some of the physiologically important ions make the wines instable. These are for example the K^{+}, $Fe^{2+/3+}$ and the Ca^{2+}, which showed noticeable differences in these technological experiences (Kállay, Májer, Jahnke & Veress, 2007).

This research is interesting because to our knowledge, different *Vitis sylvestris* GMEL. genotypes have not previously been studied by HPLC and AAS.

Based on elementary studies, the positive physiology effects of woodland grapes can be used in our future breeding program.

Materials and methods

The *Vitis sylvestris* GMEL. genotypes came from the area of Szigetköz and Fertő- Hanság National Park and were ex-situ conserved in the NARIC Research Institute for Viticulture and Enology, Badacsony. Berry samples were collected from the different *Vitis sylvestris* GMEL. genotypes and *Vitis vinifera* L. cultivars at harvest time in 2014 and 2015. Grape juice was pressed out of the berries, and samples for analyses were prepared as described later.

The organic acids and sugars were determined by HPLC (High Performance Liquid Chromatography) and the minerals by AAS (Atomic Absorption Spectroscopy).

For the HPLC analyses, samples were cleaned by active carbon and 0,45 NYL syringe filter. Shimadzu (Japan) system was used with Rezex ROA-Organic Acid H^+ (8%) column. The analysis was processed at a constant temperature of 41°C. 70 µl/sample was injected. For data recording and processing, LC Solutions software provided by the producer was used. (Shimadzu, Japan).

For AAS analyses, samples were prepared as follows: the must was centrifuged, and the clear supernatant was filtered. 1 ml of ionising buffer (36 g L^{-1} strontium-chloride) was added to 1 ml cleared must. For the determination of calcium and magnesium, an additional 8 ml (10x dilution), as for potassium determination 98 ml (100x dilution) of distilled water was added. For the determination of sodium, only 1 ml of ionising buffer was added to 10 ml of original cleared must (1,1x dilution), because of the low Na concentration of the must.

For the analyses, GBC 932 plus system and for the data processing, GBC Avanta Ver 1.33 software (GBC, Australia) were used. For the determination of potassium and sodium content of the samples, the emission at 766,5 nm and at 589 nm wavelength with Curent Lamp were measured respectively. For the determination of calcium and magnesium content of the samples, the absorption at 422,7 nm or 285,2 nm wavelength with Cathode or Hollow Cathode Lamp were measured respectively.

For the statistical analysis, two way ANOVA (Excel 2013 Analysis Tool Pak) was used.

Results

Table 1. shows the meteorological data in both investigated years (2014 and 2015).

Table 1: Meteorological data in the year 2014 and 2015, in Badacsony (Hungary)

Month	Sum of sunny hours			Temperature (°C)			Rainfall (mm)		
	Average of many years	2014	Difference	Average of many years	2014	Difference	Average of many years	2014	Difference
January	63,2	51,3	-11,9	-0,4	2,7	3,1	36,2	24,2	-12,0
February	95,2	72,5	-22,7	1,6	4,1	2,5	36,3	114,4	78,1
March	149,5	174,3	24,8	6,3	10,0	3,7	38,5	18,2	-20,3
April	189,5	207,0	17,5	11,8	13,1	1,3	45,4	34,2	-11,2
May	243,1	259,6	16,5	16,9	15,4	-1,5	57,5	77,3	19,8
June	253,3	298,1	44,8	20,1	20,9	0,8	75,4	58,2	-17,2
July	272,9	257,7	-15,2	22,0	22,6	0,6	71,8	103,8	32,0
August	249,5	252,5	3,0	21,5	20,3	-1,2	71,4	236,9	165,5
September	187,8	153,0	-34,8	17,2	16,5	-0,7	52,3	137,8	85,5
October	145,5	139,0	-6,5	11,9	13,0	1,1	45,6	91,9	46,3
November	67,1	88,0	20,9	5,9	7,8	1,9	62,0	37,3	-24,7
December	45,0	95,0	50,0	1,3	2,5	1,2	47,9	47,7	-0,2
Total:	1961,6	2048,0	86,4	_	_	_	640,3	981,9	341,6
Average:	_	_	_	11,3	12,4	1,1	_	_	_

Continuing Table 1: Meteorological data in the year 2014 and 2015, in Badacsony (Hungary)

Month	Sum of sunny hours			Temperature (°C)			Rainfall (mm)		
	Average of many years	2015	Difference	Average of many years	2015	Difference	Average of many years	2015	Difference
January	62,9	87,0	24,1	-0,3	1,9	2,2	36,0	63,2	27,2
February	94,8	104,0	9,2	1,6	1,9	0,3	37,7	57,4	19,7
March	150,0	176,0	26,0	6,3	6,8	0,5	38,1	16,2	-21,9
April	189,8	266,0	76,2	11,9	11,7	-0,2	45,2	4,0	-41,2
May	243,4	242,0	-1,4	16,9	16,4	-0,5	57,9	104,7	46,8
June	254,2	315,0	60,8	20,1	21,2	1,1	75,1	12,9	-62,2
July	272,6	300,0	27,4	22,0	24,7	2,7	72,3	38,7	-33,6
August	249,6	303,0	53,4	21,4	24,8	3,4	74,3	56,6	-17,7
September	187,1			17,2			53,8		
October	145,4			11,9			46,4		
November	67,5			5,9			61,6		
December	46,0			1,3			47,9		
Total:	1963,3			_	_	_	646,3		
Average:	_	_	_	11,4			_	_	_

Results of the HPLC and AAS analyses are detailed in Table 2.

Table 2. Sugars, organic acid and Na, K, Ca, Mg contents in grape berries (Badacsony, 2014-2015).

Accessions	Year	Glucose (g/l)	Fructose (g/l)	Glucose+ Fructose (g/l)	G/F ratio	Citric acid (g/l)	Tartaric acid (g/l)	Malic acid (g/l)	Succinic acid (g/l)
S-4/1	2014	84,77	84,61	169,38	1,00	0,61	8,43	15,10	4,14
S-4/2	2014	79,62	73,15	152,77	1,09	0,62	8,03	21,17	4,23
S-6/1	2014	83,15	75,64	158,79	1,10	0,44	5,23	24,92	6,22
S-6/2	2014	64,46	65,70	130,16	0,99	1,00	7,94	25,10	6,69
S-B.1	2014	99,56	108,43	207,99	0,92	1,53	8,65	21,52	1,87
S-B.48	2014	90,5	101,60	192,10	0,89	0,48	5,23	27,96	5,04
Vinifera	2014	80,48	61,67	142,14	1,31	0,38	6,29	5,38	0,10
S-4/1	2015	69,89	86,46	156,36	0,81	0,02	5,19	6,705	0,06
S-4/2	2015	70,72	86,32	157,04	0,81	0,03	4,79	7,605	0,12
S-6/1	2015	66,97	81,11	148,08	0,82	0,04	5,45	7,73	0,11
S-6/2	2015	44,90	59,51	104,41	0,75	0,06	8,45	8,05	0,13
S-B.1	2015	53,08	69,26	122,34	0,77	0,12	3,70	9,62	0,08
S-B.48	2015	64,74	84,01	148,75	0,77	0,02	7,74	7,03	0,08
Vinifera	2015	81,81	91,55	173,36	0,89	0,03	4,04	6,15	0,04

Varieties	Year	Na (mg/l)	K (mg/l)	Ca (mg/l)	Mg (mg/l)
S-4/1	2014	0,05	1159,12	187,99	106,00
S-4/2	2014	0,49	1510,95	94,70	57,00
S-6/1	2014	1,04	1943,95	228,85	89,30
S-6/2	2014	4,94	1803,06	280,46	211,80
S-B.1	2014	3,57	1001,81	131,79	136,20
S-B.48	2014	0,87	998,27	125,19	60,16
Vinifera	2014	3,02	1250,67	33,80	60,00
S-4/1	2015	0,585	1237,43	165,53	83,98
S-4/2	2015	0,90	1275,42	119,94	72,59
S-6/1	2015	1,54	1431,715	166,56	94,43
S-6/2	2015	0,85	1135,12	158,15	125,13
S-B.1	2015	1,77	1648,42	94,98	75,75
S-B.48	2015	0,90	1013,26	138,59	63,86
Vinifera	2015	1,36	1548,77	41,59	34,59

Figure 1. shows the average organic acid content of berries of *Vitis sylvestris* GMEL. accessions and *Vitis vinifera* L. cultivars in 2014 and in 2015.

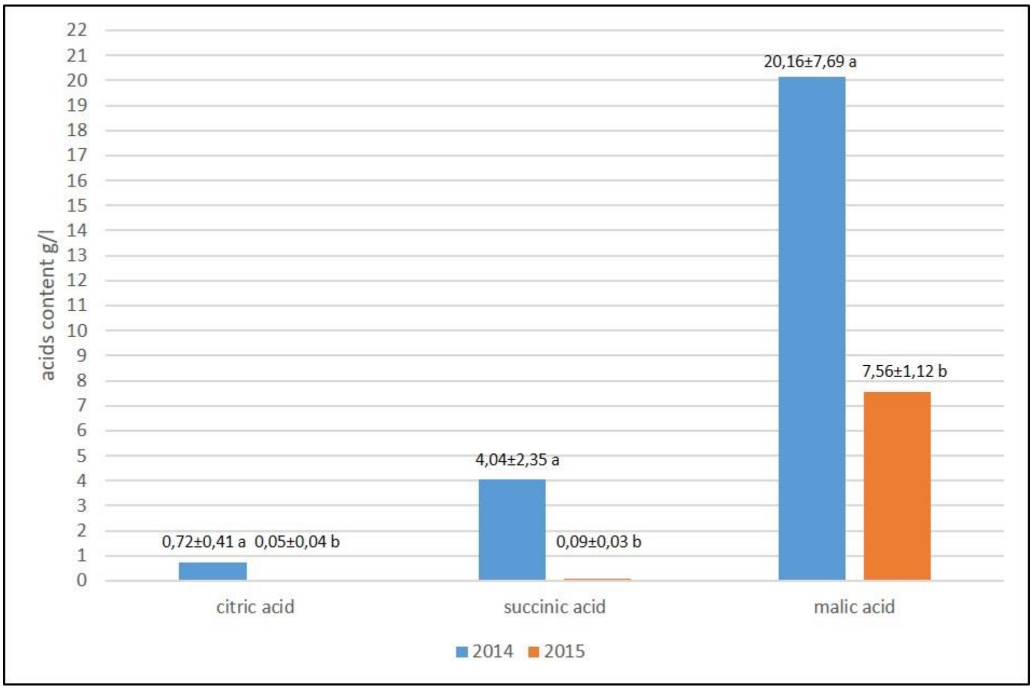

Figure 1.: Average organic acid content of berries of Vitis sylvestris GMEL: accessions and Vitis vinifera L. cultivars in 2014 and in 2015. Different letters mean significant differences between the investigated years of each organic acid (p< 0.05)

Figure 2. shows glucose and fructose contents of musts by years (2014 and 2015).

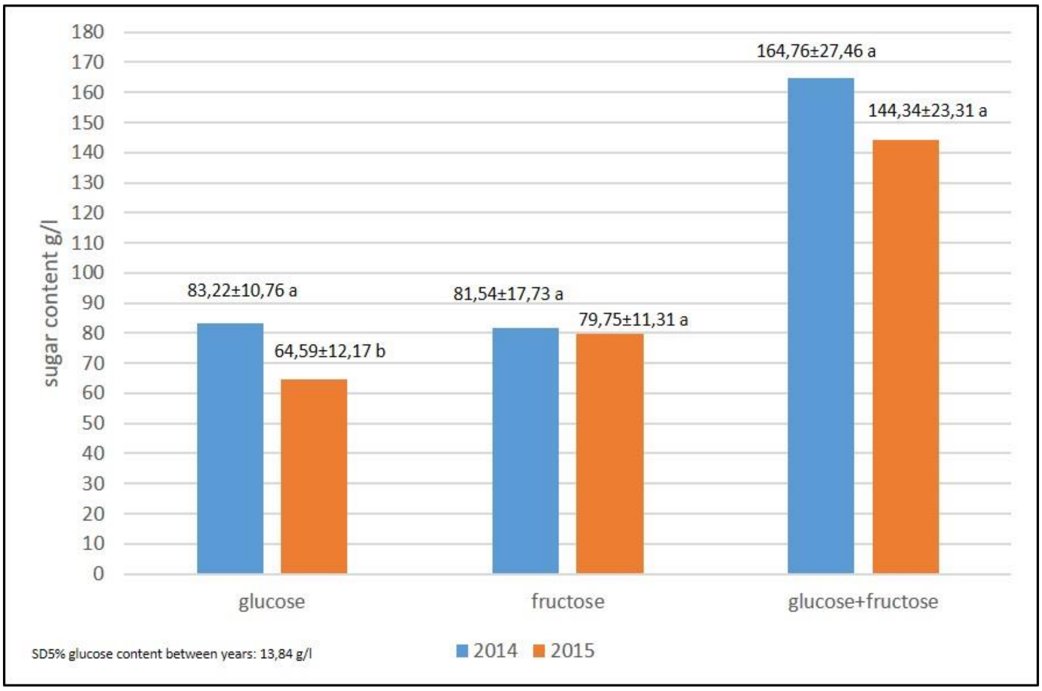

Figure 2.: Average glucose and fructose contents of musts by years (2014 and 2015) of Vitis sylvestris GMEL.accessions and Vitis vinifera L. cultivars. Different letters mean significant differences between the investigated years of each organic sugar (p< 0.05).

Figure 3. shows the glucose/fructose ratio (G/F) in grape berries in 2014 and 2015.

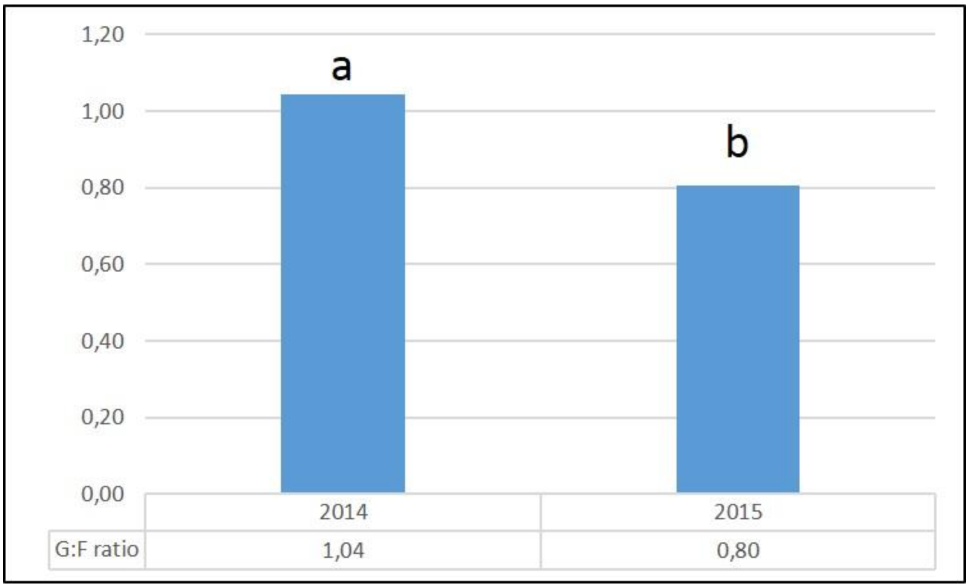

Figure 3. Glucose/fructose ratio (G/F) in grape berries in 2014 and 2015. Different letters mean significant differences (p< 0.05).

Figure 4. shows the Ca and Mg contents in grape berries in 2014-2015.

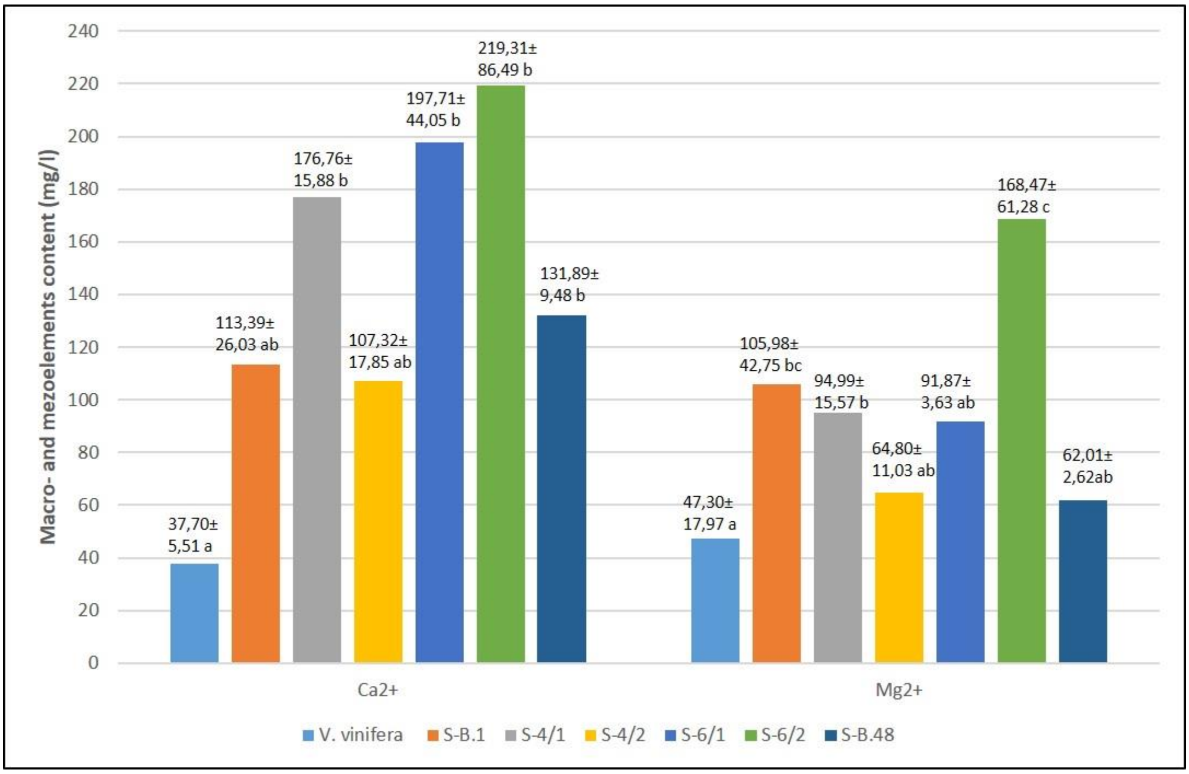

Figure 4. Ca and Mg contents in grape berries (Badacsony, 2014-2015). Different letters mean significant differences between the investigated genotypes and cultivars of each element ($p < 0.05$).

Discussion

The citric-, malic- and succinic acid content showed higher values in 2015 than in 2014, which was possibly caused by the difference in the weather conditions in the growing period (see Table 1. for the detailed meteorological data). The malic acid content in berries decreases during the ripening, while tartaric/malic acid ratio is in close connection with the maturation process itself. During the growing of the berries and even at veraison, malic acid content of the berries is prevalent, while tartaric/malic acid ratio is lower than 1 (Kliewer 1965, Kliewer 1967b). In 2014, the succinate content of the grape berries were considerably higher than in

2015 (Fig. 1.), which shows that the berries were less mature at harvest in 2014 than in 2015, due to the different weather conditions.

Musts of *Vitis vinifera* L. cultivars regularly contain 0,1-0,5 g L^{-1} citric acid, but musts originating from Botrytis-infected grapes can contain 1g L^{-1} (Kállay, 2010). The increased citric acid concentration of musts in 2014 can be traced back to the Botrytis infection.

The tartaric acid concentrations didn't show any significant difference between years or genotypes. The malic acid concentrations were significantly higher in 2014, than in 2015, because of the different weather conditions as mentioned before. No significant differences were found between genotypes regarding the malate concentrations of the musts.

The mature berries of the European grapevines contain a very low amount of succinate, but it always evolves during alcoholic fermentation (Pasteur 1859, 1969). The remarkable succinate content of woodland grape berries is a surprise.

The amount of fructose was almost the same in both years, but the glucose content showed significant differences (Fig. 2.). Significant difference was observed in the glucose/fructose ratio (G/F) between vintage years, the value was lower (0.80) in 2015 than in 2014 (1.04) (Fig. 3). As this ratio corresponds to the ripeness of the berries, and remarkable declines in G/F ratios can be observed up to veraison, this difference can be traced back to the different weather conditions in the years as well. During veraison, when the greater infiltration of sugars begins, the fructose content increases and the glucose/fructose ratio decreases rapidly. At the end of veraison, the glucose/fructose ratio approaches to 1 (Kliewer 1965, Kliewer, 1967a; Kliewer 1967b, Sabir, Kafkas & Tangolar, 2010.).

The musts of European grapevine cultivars contain small amounts of sodium (10-20 mg L^{-1}), which stagnate during the maturation (Table 2.). Plants take up potassium in high quantity, because it plays an important role in the regulation of the transport processes. The K concentration in must decreases in colder years and in case of stronger drought (Kállay, 2010). The observed pH of musts is primarily a reflection of the extent to which protons from the total acidity have been exchanged for potassium and sodium ions. During maturity, the uptake of potassium and sodium at constant total acidity can lead to a rise in pH of the must (Boulton, 1980).

According to the results, there were no significant differences in the sodium or potassium content of musts concerning the *Vitis sylvestris* GMEL. accessions and *Vitis vinifera* L. cultivars, or between the different vintage years (Table 2)

The amount of calcium and magnesium decreases in grape berries during ripening. Musts contain about 40-160 mg L^{-1} or 50-160 mg L^{-1} respectively (Kállay, 2010).

Magnesium ions have a positive effect on the protection of yeast cells against heat shock during fermentation (Birch & Walker, 2000).

In the measured values of Ca and Mg content, there were significant differences between genotypes. The *Vitis sylvestris* GMEL. accessions showed significantly higher values (Fig. 4.), which can be traced back to the partly immature state of the berries.

Conclusions

Based on the results, the following conclusion can be drown: at harvest time, the woodland grape (*Vitis sylvestris* GMEL.) accessions showed the same sugar content (glucose + fructose) and acidity as the European grapevine (*Vitis vinifera* L.), but the G/F ratio, acid composition (remarkable succinate in berries), and the significantly higher calcium and magnesium contents account for the unripe phenomenon in woodland grapes. Considering the climate change (global warming) processes, this phenomenon can be advantageous.

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