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Calcium and magnesium contents of Hungarian wines due to *Botrytis cinerea*, drought, and hardness of groundwater

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ABSTRACT

Drought and parasitic infections induce oxidative stress reactions in grape berries through the production of reactive oxygen species, which may involve alterations in calcium (Ca^{2+}) and magnesium (Mg^{2+}) levels. In the present study, 43 Hungarian wines were analysed for their Ca^{2+} and Mg^{2+} concentrations using atomic absorption spectrometry. Three groups of wines were investigated: (a) nine wines from the Tokaj region affected by *Botrytis cinerea*, (b) seventeen white wines free of *Botrytis*, and (c) seventeen red wines. The stress-inducing effects of drought and *Botrytis* infection were associated with statistically significant elevations in Ca^{2+} and Mg^{2+} concentrations. Wine samples were collected from different regions of Hungary representing varying hardness of groundwater and different climatic conditions. Differences in ion concentrations reflected the strength and type of stress factors affecting grape development. The highest parallel calcium and magnesium concentrations were observed in *Botrytis*-affected Tokaj wines, suggesting additive effects of multiple stress factors, including increased drought severity in regions associated with

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soft groundwater and enhanced defence responses against *Botrytis* infection. The results also indicate a previously underexplored role of groundwater hardness in environments influencing drought stress tolerance in grapevines.

KEYWORDS

calcium, magnesium, wines, drought, *Botrytis cinerea*

1. INTRODUCTION

Grapevine is one of the most important agricultural crops worldwide; however, a large proportion of vineyards are located in regions where environmental conditions impose substantial constraints on cultivation. Several abiotic stress factors, including drought (Porro et al., 2010; Bevacqua et al., 2024), heat waves and soil salinity increasingly threaten grapevine production, affecting vine health, grape yield and wine quality (Muhammad et al., 2024).

In addition to abiotic stress, biotic stress caused by bacteria, fungi, viruses, nematodes, and insects has become increasingly relevant, partly due to climate change (Santos and Figuiredo, 2023; Váczy et al., 2024). These stress factors induce significant alterations in plant metabolism while simultaneously activating defence mechanisms and stress tolerance pathways (Szepesi, 2020).

At the cellular level, plant stress responses frequently involve calcium signalling pathways. Increased intracellular Ca^{2+} concentrations activate stress-responsive genes (Gupta et al., 2023; Naz et al., 2024). Magnesium also plays an important role in plant adaptation to environmental stress conditions (Sarraf et al., 2026).

Since soil salinity has been shown to influence drought tolerance (Muhammad et al., 2024), the hardness of groundwater in vineyards may represent an additional environmental factor affecting grapevine physiology. Water hardness refers to the combined concentration of calcium and magnesium salts dissolved in water (Ingin et al., 2024). It is typically expressed in degrees and calculated relative to the concentrations of $CaCO_3$ or CaO (mg L^{-1}). According to WHO guidelines, five categories are distinguished: very soft (<40), soft (40–80), medium (80–180), hard (180–300), and very hard (>300°) (WHO, 2011; Ingin et al., 2024). Otherwise "groundwater" and "drinking water" can be used as synonyms (Grönwall and Danert, 2020).

The present study aimed to address the following questions: 1.) Are there differences in Ca^{2+} and Mg^{2+} concentrations among the three groups of Hungarian wines studied? 2.) Are there differences in Ca^{2+} and Mg^{2+} concentrations between red and white wines? 3.) Do drought conditions and *Botrytis cinerea* infection influence Ca^{2+} and Mg^{2+} concentrations in wines? 4.) Is there a relationship between Ca^{2+} and Mg^{2+} concentrations in wines and the hardness of groundwater where the grapes were grown?

To answer these questions, we analysed 17 commercial white wines without *Botrytis*, 17 red wines from various Hungarian regions representing different groundwater hardness, and 9 *Botrytis*-affected white wines from the Tokaj region.

2. MATERIALS AND METHODS

2.1. Regions, soils, and wines

The hardness of groundwater was inferred from regional drinking water data. Data on the hardness of drinking water were obtained from surveys conducted by the National Institute of Health of Hungary (Budapest). A hydrological map (Fig. 1) shows the hardness of drinking water across Hungary (Sipka et al., 2023). The main wine-producing regions studied with their drinking water and *geological* characteristics were as follows: Tokaj (soft water, *volcanic soils*), Eger (hard water, *limestone soils)*, Balaton (hard water, *volcanic and clay soils*), Szekszárd–Villány (hard water, *volcanic and clay soils*), Etyek (medium hardness, *clay soils*), Neszmély (medium hardness, *alluvial soils*), Sopron (hard water, *volcanic soils*), and the Great Hungarian Plain (mixed soils: *clay or sandy*).

The geological characteristics of soils can influence moisture evaporation dynamics (Muhammed et al., 2024). Figure 2 illustrates the spatial distribution and intensity of drought conditions across Hungary during the summer of 2022. The drought intensity was markedly higher in the eastern and south-eastern parts of the country, regions frequently associated with softer drinking water types.

The following commercial bottled wines were analysed: 9 white wines from the Tokaj region affected by *B. cinerea,* 17 white wines without *Botrytis*, and 17 red wines. A small number of

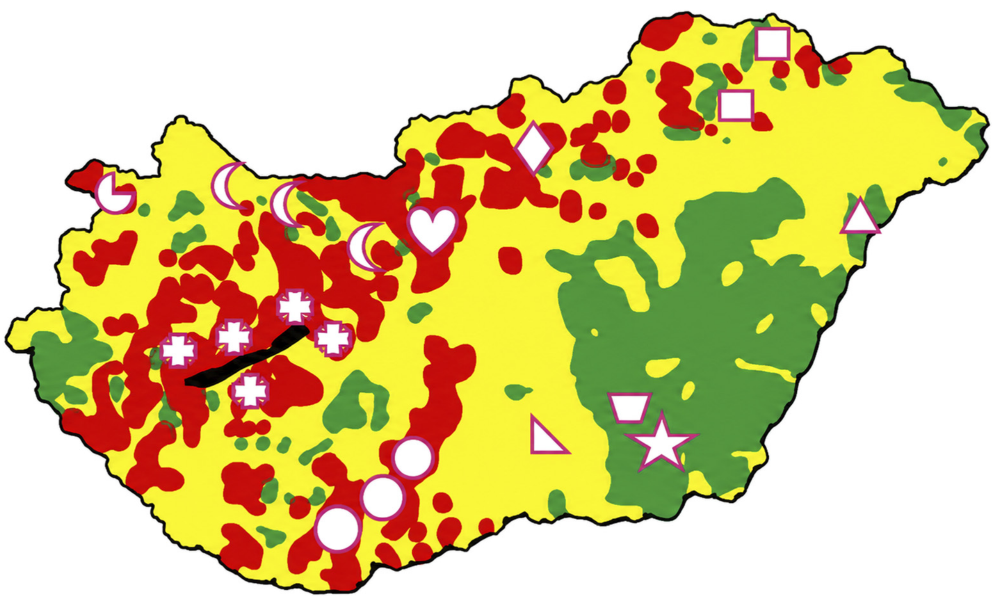

Fig. 1. Hydrologic map of Hungary showing regions with hard, medium, and soft groundwater and the locations of places from which the wines were derived
Colours of groundwater types: red = hard; yellow = medium; green = soft (Sipka et al., 2023)
♡ Budapest, □ Tokaj region, ☆ Hódmezővásárhely, △ Nagyléta, ▽ Csongrád, ◺ Kiskőrös, ◇ Eger, ○ Szekszárd-Villány, ✚ Balaton region, ◔ Sopron, ☾ Etyek-Neszmély

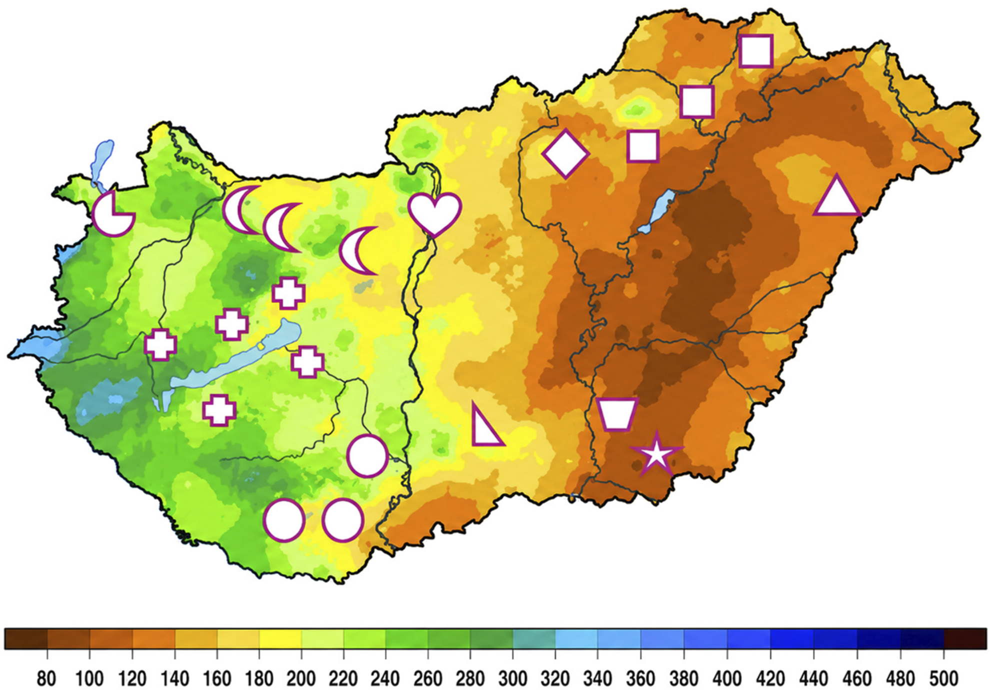

Fig. 2. Distribution and severity of drought in Hungary in June, 2022.
The scale of colours shows the amount of precipitation (mm) recorded in Hungary between January and June, 2022. The data were obtained from HungaroMet (15, July, 2022)
♡ Budapest, □ Tokaj region, ☆ Hódmezővásárhely, △ Nagyléta, ▽ Csongrád, ◺ Kiskőrös,

French and Italian wines were also measured for comparison but were not included in the statistical analysis.

2.2. Determination of calcium and magnesium concentrations

Calcium and magnesium concentrations were determined using atomic absorption spectrometry (Analyst 800 Atomic Absorption Spectrometer). For these measurements, 10 mL of sample was mixed with 0.6 mL of lanthanum solution (concentration 5%). The burning head of equipment was set at 45°. The analyses were performed at the Controlling Laboratory of Water Quality for the Tisza Region in Debrecen, an accredited reference laboratory specialising in water quality measurements (NAH-1-1294/2019). All results were certified and reported as official laboratory measurements.

2.3. Statistical analysis

Data distribution and normality were evaluated using the Shapiro–Wilk test. Normally distributed variables were analysed using analysis of variance (ANOVA), whereas non-normally distributed data were analysed using the Kruskal–Wallis test. Bartlett's test was used to assess the homogeneity of variances. When significant differences among variances were detected, Brown–Forsythe and Welch ANOVA tests were applied, followed by Dunnett's post hoc test. The group sizes varied between 9 and 21 samples. Statistical analyses were performed using GraphPad Prism version 9.1.2 (GraphPad Software Inc., San Diego, USA). Results are presented as mean ± SD, and P values <0.05 were considered statistically significant.

3. RESULTS AND DISCUSSION

3.1. Presentation of calcium and magnesium concentrations in the various types of Hungarian wines ($n = 43$)

Tables 1a–c contain data on calcium and magnesium concentrations in all tested wine samples, together with the corresponding types of groundwater.

3.2. Statistical analysis and comparison of calcium and magnesium concentrations in Hungarian wines

Statistically significant differences in Ca^{2+} and Mg^{2+} concentrations were observed among the three groups of 43 Hungarian wines analysed. The highest calcium concentrations were found in the 9 white wines affected by *B. cinerea* from the Tokaj region. Magnesium concentrations were also the highest in this group. However, magnesium concentrations were significantly higher in the 17 red wines compared with the 17 white wines that were not affected by *Botrytis*.

The 9 *Botrytis*-affected wines originated from vintages between 2014 and 2023 and were produced exclusively from vineyards located in regions with soft groundwater. The white wines

Table 1a. Calcium and magnesium concentrations of wines affected by *Botrytis cinerea* in relation to groundwater hardness in Tokaj region

White wines with *Botrytis* infection	Year	Calcium (Ca^{2+}) (mg L^{-1})	Magnesium (Mg^{2+}) (mg L^{-1})	Type of groundwater
Late-harvested sweet Tokaj	2014	209	264	Soft
Late-harvested dry Tokaj	2016	146	135	Soft
Late-harvested sweet Tokaj	2016	165	115	Soft
Late-harvested sweet Tokaj	2018	170	109	Soft
Furmint Aszú 5 baskets (puttony) Tokaj	2001	146	127	Soft
Furmint Aszú 6 baskets (puttony) Tokaj	2014	135	156	Soft
Muscatel Aszú 6 baskets (puttony) Tokaj	2014	205	147	Soft
Muscatel Aszú 6 baskets (puttony) Tokaj	2000	253	164	Soft
Essencia Tokaj	2005	107	156	Soft
Mean		**170.67**	**152.55**	
± SD		**44.72**	**45.93**	

Table 1b. Calcium and magnesium of *Botrytis*–free white wines in relation to groundwater hardness in Hungarian wine-growing regions

White wines	Year	Calcium (Ca^{2+}) (mg L^{-1})	Magnesium (Mg^{2+}) (mg L^{-1})	Type of groundwater
Linden-leaved semi-sweet Tokaj	2017	159	107	soft
Furmint dry Tokaj	2019	96	100	Soft
Furmint dry Tokaj	2020	132	91	Soft
Furmint dry Tokaj	2020	108	78	Soft
Muscatel semi-dry Tokaj	2020	132	83	Soft
Furmint dry Tokaj	2021	94	91	Soft
Cuvée dry Nagyléta	2023	71	81	Soft
Cuvée dry H.Vásárhely	2023	141	80	Soft
Kövidinka dry H.Vásárhely	2023	121	111	Soft
Irsai Olivér dry Kiskőrös	2023	99	90	Medium
Chardonnay dry Etyek	2022	122	85	Medium
Cuvée dry Eger	2023	110	73	**Hard**
Furmint dry Balaton	2019	87	73	**Hard**
Riesling dry Balaton	2022	86	86	**Hard**
Riesling dry Balaton	2023	127	84	**Hard**
Sauvignon Blanc dry Villány	2023	68	107	**Hard**
Riesling dry Villány	2023	98	74	**Hard**
Touraine dry France	2021	117	67	**Hard**
Chardonnay dry Italy	2022	117	68	**Hard**
Mean		**108.50**	**83.37**	
± SD		**23.72**	**15.84**	

without *Botrytis* infection and the red wines were produced between 2017 and 2023 in regions characterised by soft, medium, or hard groundwater types. The summarised analytical data are presented in Table 2.

3.3. Comparison of wines from drought-affected and non-affected areas

Table 3 shows a statistically significant difference in Ca^{2+} and Mg^{2+} concentrations between wines derived from drought-affected regions and those produced in regions without significant drought exposure (Ca^{2+} $P = 0.0039$; Mg^{2+} $P = 0.4332$ n.s.).

3.4. Relationship between ion concentrations and groundwater hardness

Table 4 demonstrates a significant association between Ca^{2+} concentrations and groundwater hardness in vineyards. Wines originating from regions associated with soft groundwater showed significantly higher calcium concentrations compared with wines derived from regions associated with hard groundwater (115.2 *vs* 87.8 mg L^{-1}; $P = 0.0015$). Magnesium concentrations did not differ significantly between these groups. (Geological soil types were not evaluated because of the insufficient number of samples within the groups.)

This study presents the first statistically supported evaluation of calcium and magnesium concentrations in Hungarian wines in relation to both abiotic (drought) and biotic (*B. cinerea*)

Table 1c. Calcium and magnesium concentrations of red wines in relation to groundwater hardness in Hungarian wine-growing regions

Red wines	Year	Calcium (Ca^{2+}) ($mg\ L^{-1}$)	Magnesium (Mg^{2+}) ($mg\ L^{-1}$)	Type of groundwater
Cuvée dry H.Vásárhely	2023	107	131	Soft
Kadarka dry Csongrád	2023	92	113	Soft
Cuvée dry Nagyléta	2023	129	95	Soft
Cuvée dry Kiskőrös	2023	96	191	Medium
Pinot noir dry Etyek	2020	73	105	Medium
Rose dry Etyek-Neszmély	2022	87	104	Medium
Blue Franconian dry Eger	2019	77	105	**Hard**
Cabernet sauvignon dry Eger	2020	83	104	**Hard**
Cuvée dry Eger	2021	82	99	**Hard**
Bull's blood dry Eger	2023	85	81	**Hard**
Blue Franconian dry Szekszárd	2014	105	108	**Hard**
Cuvée dry Szekszárd	2019	68	118	**Hard**
Kadarka dry Szekszárd	2023	70	120	**Hard**
Cabernet Sauvignon dry Villány	2018	65	121	**Hard**
Portuguese dry Villány	2023	111	93	**Hard**
Cabernet Franconian dry Balaton	2018	75	100	**Hard**
Pinot noir dry Sopron	2023	87	107	**Hard**
Cuvée dry Bordeaux France	2019	75	78	**Hard**
Chianti dry Italy	2022	97	106	**Hard**
Mean		**87.84**	**108.37**	
± SD		**16.46**	**23.57**	

Table 2. Calcium and magnesium concentrations in Hungarian wines ($n = 43$)

Parameters ($mg\ L^{-1}$)	*Botrytis*-infected white wines (1) ($n = 9$)	White wines (2) ($n = 17$)	Red wines (3) ($n = 17$)
Calcium (Ca^{2+})	170.67 ± 44.72	108.50 ± 23.72	87.84 ± 16.46
Magnesium (Mg^{2+})	152.55 ± 45.93	83.37 ± 15.84	108.37 ± 23.57
Significance			
Calcium (Ca^{2+})	1–2: $P < 0.0001$	1–3: $P < 0.0001$	2–3: $P = 0.1033$ n.s.
Magnesium (Mg^{2+})	1–2: $P < 0.0001$	1–3: $P = 0.1006$ n.s.	2–3: $P = 0.0144$

Mean ± SD; n.s. = not significant.

Table 3. Calcium and magnesium concentrations of wines from drought-affected vs. non-affected areas ($n = 34$)

Wines (red and white)	Calcium (Ca^{2+}) ($mg\ L^{-1}$)	Magnesium (Mg^{2+}) ($mg\ L^{-1}$)
From areas with drought ($n = 13$)	113.92 ± 24.23	96.23 ± 15.64
From areas without drought ($n = 21$)	88.67 ± 17.86	101.81 ± 25.34
Significance	$P = 0.0039$	$P = 0.4332$ n.s.

Mean ± SD; n.s. = not significant.

Table 4. Calcium and magnesium contents of *Botrytis*-free red and white vines from regions with soft and hard groundwater (Means ± SD) ($n = 29$)

Wines from regions with *soft* groundwater ($n = 12$)		Wines from regions with *hard* groundwater ($n = 17$)	
1. Calcium (Ca^{2+}) (mg L^{-1})	2. Magnesium (Mg^{2+}) (mg L^{-1})	3. Calcium (Ca^{2+}) (mg L^{-1})	4. Magnesium (Mg^{2+}) (mg L^{-1})
159	107	110	73
96	100	87	73
132	91	86	86
108	78	127	84
132	83	68	107
94	91	98	74
71	81	77	105
141	80	83	104
121	111	82	99
107	131	85	81
92	113	105	108
129	95	68	118
		70	120
		65	121
		111	93
		75	100
		87	107
115.2 ± 24.8	96.75 ± 16.2	87.3 ± 17.6	97.2 ± 16.3

Significances:
1–3: $P = 0.0015$.
2–4: $P = 0.937$ n.s.
n.s. = not significant.

stress factors. A novel aspect of the study is the simultaneous comparison of three wine categories: *Botrytis*-affected Tokaj wines, white wines without *Botrytis* infection, and red wines.

Both drought and *Botrytis* infection were associated with significant increases in Ca^{2+} and Mg^{2+} concentrations. Furthermore, wines derived from regions associated with soft groundwater exhibited higher Ca^{2+} concentrations than those from regions associated with hard groundwater, regardless of whether the wines were red or white. This was not true for Mg^{2+}.

The majority of the *Botrytis*-free wines analysed were produced during the period 2020–2023, when Hungary experienced severe summer drought conditions (Bevacqua et al., 2024). As illustrated in Fig. 2, drought intensity varied considerably between western and eastern regions of the country.

In the *Botrytis*-affected wines of Tokaj, the combined influence of drought, soft groundwater conditions, and fungal infection (Temme and Tudzynsky, 2009; Cheung et al., 2020) likely contributed to the highest concentrations of both ions. However, it is noteworthy that the elevated magnesium levels observed in red wines may also have potential implications for cardiovascular health (Ye et al., 2023; Sipka et al., 2024a).

Stress factors affecting grape berries can trigger oxidative stress responses involving antioxidant enzyme activation (Hasanuzzaman et al., 2020; Kidwai et al., 2020; Sipka et al., 2024b) as well as alterations in Ca^{2+} and Mg^{2+} signalling pathways (Gupta et al., 2023; Naz et al., 2024; Sarraf et al., 2026).

Importantly, these biochemical changes occurring in grape berries are transmitted to grape must during wine production, ultimately influencing the chemical composition of the resulting wines. Concerning the quality of wines, it has also been observed that moderate drought may positively influence wine quality (Savoi et al., 2020).

4. CONCLUSIONS

This study showed the additive effects of multiple stress factors, including increased drought severity in soils associated with soft drinking water and enhanced defence responses against *B. cinerea* infection on the Ca^{2+} and M^{2+} levels in Aszú wines of Tokaj. The results also indicate a previously underexplored role of groundwater hardness influencing drought stress tolerance in grapevines. Further studies are required to determine whether calcium and magnesium measurements could serve as indicators of wine quality and could be used for practical purposes. Moreover, these results suggest that these ions may represent useful markers for assessing plant stress conditions in viticulture.

Conflict of interest: No conflict of interest exists among the authors.

Data are available upon request.

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Note: The typo in the third location name in the legend to Fig. 1 has been updated after online first publication, to correctly read ‘Hódmezővásárhely’ (the original version contained a code for the letter ‘ő’: ‘Hódmezővásárhely').
The update was implemented in the final paginated version of the paper, published in the issue.