

## CARBOHYDRATE DYNAMICS IN SOME GRAPEVINE (*VITIS VINIFERA* L.) CULTIVARS DURING DORMANCY

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### Abstract

*The adaptation and resistance of the vine to low temperatures during the dormancy involves the realization of processes of accumulation and dynamics of some biochemical compounds in the tissues. In this study, between November and March (2022-2023), the dynamics of carbohydrates (soluble sugars and starch) was monitored at 10 days in the annual and multiannual vine wood in the 'Cabernet Sauvignon', 'Fetească neagră' and 'Merlot' from the Banu Mărăcine wine-growing center (plateau-type area, slightly leached reddish-brown soil) and Șimnicu de Sus wine-growing area (slope-type area, medium eroded reddish-brown soil). Regarding the carbohydrate content during the dormancy, significant differences between the two viticultural areas were highlighted only concerning the content of soluble sugars (%) in multiannual wood for the 'Merlot' and 'Cabernet Sauvignon'. As for the starch content (%) in annual woody shoots and in multiannual wood, no statistically significant differences were observed either between the studied cultivars or between the two areas.*

**Key words:** carbohydrate dynamics, freezing tolerance, dormancy.

### INTRODUCTION

Carbohydrates, a group of substances of particular importance for vines, are produced by photosynthesis and represent some of the most important elements that form the biochemical composition of the vine. They are the building blocks of organic compounds, store energy, and form support structures, such as cellulose, hemicellulose, and gluco-protein (Zufferey et al., 2012; De Rosa et al., 2022).

In the context of climate change, the observed increase in average surface temperatures causes an acceleration of plant phenology progression, exposing vulnerable green bud structures to a higher risk of late frost damage (Alikadic et al., 2019; Masson-Delmotte et al., 2021). In the context of some extreme weather and climate events have increased in frequency and intensity due to global warming, adaptive techniques in viticulture have become necessary to mitigate the resulting negative impacts (Ferrara et al., 2022).

The adaptation and resistance of the grapevine to low temperatures during the dormancy period involve the realization of processes of accumulation and dynamics of biochemical

compounds in tissues, with direct implications for protecting them against the destructive action of intracellular freezing (Holzapfel et al., 2010; Mohamed et al., 2012; Babajamali et al., 2022; Monteiro et al., 2022). The carbohydrate content of grapevine buds undergoes significant changes in an annual cycle, and there is a continuous interdependence between the content of soluble sugars and starch (Rogiers et al., 2011; Tixier et al., 2019). An important role is played by starch, which, through the hydrolysis process starting in January, leads to the formation of soluble sugars with a cellular protective function due to the promotion of water retention through osmotic means, thereby lowering the cryoscopic point of cellular sap (Zapata et al., 2004; Lebon et al., 2008). Several studies indicate that during winter, grapevine roots contain rich reserves of starch and sugars, with starch being present in all tissues of the bark and wood (annual or multiannual), actively contributing to the dynamics of carbohydrates in grapevine shoots (Caprara & Pezzi, 2013; Liu and Sherif, 2019; Călugăr et al., 2022; Ferrara et al., 2022; Costea et al., 2023). The larger the reserves of carbohydrates accumulated in the wood, the better the wood maturation, and the plant

becomes more resistant to low temperatures during the dormancy period (Cichi et al., 2016; Călugăr et al., 2019; Horiuchi et al., 2021). Essential climate characteristics in a vineyard area have a decisive impact on the evolution of the biochemical, biological, and physiological processes of grapevines during the dormancy period. These characteristics include the average minimum temperature, absolute minimum temperature, and others (Grant et al., 2013; Onache et al., 2020; Costea et al., 2021; Hernandez et al., 2021; Căpruciu et al., 2023). Among these, temperature is a crucial factor that affects nearly all aspects of plant growth and development, with grapevines (*Vitis* spp.) being quite sensitive to extreme temperatures (Bernardo et al., 2018; Venios et al., 2020; Cichi et al., 2021).

Pedo-climatic factors, as well as orographic characteristics and the grapevine variety itself, directly influence the solubility and transport of synthesized organic substances (carbohydrates, amino acids, etc.), ensuring a continuous regulation of metabolic processes involving synthesis and hydrolysis. This regulation has an impact on enhancing resistance to freezing in grapevine wood (Field et al., 2009; Bucur and Dejeu, 2020; Costea and Căpruciu 2022; Cichi et al., 2023; Costea et al., 2023).

The present study is focussed on the assessment of the content and dynamics of the starch and soluble sugars during the dormancy season of some wine grape cultivars grown in two wine-growing area from Oltenia and Muntenia Hills Region.

## MATERIALS AND METHODS

**Sampling Sites.** The Banu Mărăcine viticultural center is located at an altitude of 176 m, with 44°19' north latitude and 23°48' east longitude. Banu Mărăcine belongs to the A<sub>3</sub> oenoclimatic zone, which includes viticultural centers producing mainly high-class red and aromatic wines, and, secondarily, quality table wines (Teodorescu et al., 1987). Geographically, the Şimnicu de Sus area is located in the South-West of Romania, at 44°24'23 "N, 23°48'09" E, and is characterized by mild winters and long summers, with high sunshine duration and low rainfall (Table 1), climatic conditions required for the synthesis of carbohydrates. In the Banu

Mărăcine and Şimnicu de Sus wine areas, the climate is temperate-continental with Mediterranean influence, characterized by mild winters and hot summers with a high number of tropical days, where maximum temperatures often exceed 30°C. The experiments were conducted within the Banu Mărăcine viticultural area in parcels situated in identical orographic conditions (plateau-type area with slightly levigated reddish-brown soil) and within Şimnicu de Sus wine-growing area, characterized by slope-type area with medium-eroded reddish-brown soil.

Table 1. Description of the sampling sites for black grapes

Sampling sites	Şimnicu de Sus	Banu Mărăcine
Altitude (m)	175	176
Average January temperature (°C)	+1.12°C	+2.31°C
Radiation	High	High
Precipitation (mm)	565	540
Soil	slope-type area, medium eroded reddish-brown soil	plateau-type area, slightly leached reddish-brown soil

### Biological material

The cultivars included in the study are grafted on Berlandieri x Riparia SO4 rootstock, planted with a spacing of 2 x 1.2 m, exhibiting semi-high growth with a multiple Guyot cutting system, and a crop load of 40 buds/vine. These have been: 'Cabernet Sauvignon', 'Fetească neagră' and 'Merlot'.

The observations and determinations were conducted for the 'Cabernet Sauvignon', 'Fetească neagră', and 'Merlot' wine grape varieties during the 2022-2023 period within Banu Mărăcine - the Didactic Research Station of the University of Craiova and Şimnicu de Sus wine-growing area.

Parameters determined were done on both annual and multiannual wood (3 years), collected at a 10-day interval during the dormant period (XI-III). This involved observing the dynamics of the main biochemical compounds (soluble sugars % and starch %) on 10 canes/variety/type of wood harvested from 10 vines of each variety. The analyses were carried out at the Research Center for Life Sciences and Biotechnologies of the Faculty of Horticulture, University of Craiova.

**Methods.** The monitoring of the minimum temperature during the dormant period was conducted at the Banu Mărăciine Meteorological Station, and for Şimnicu de Sus, the AccuWeather application was utilized. Carbohydrates were determined through spectrophotometric analysis. In this method, soluble sugars were extracted using an 80% volume alcohol solution, the starch with a 52% volume perchloric acid solution, followed by treatment with a 0.2% anthrone (C<sub>14</sub>H<sub>10</sub>O) solution. The obtained colour intensity (with transparent blue-green colour shades) was measured colorimetrically using a UV-VIS Spectrophotometer at a wavelength of 620 nm (Călugăr et al., 2019). The data resulting from the spectrophotometric reading were calculated as follows: Sugars (%) =  $(E_c - E_a) / E_b \times 50$ ; Starch (%) =  $(E_d - E_a) / E_b \times 50$ ; where E<sub>a</sub>, E<sub>b</sub>, E<sub>c</sub>, E<sub>d</sub> = extractions of solutions a,b,c,d; 50 = concentration of the standard solution.

**Statistical Analysis.** Each variable was examined using analysis of variance (ANOVA). The differences between the mean values of biochemical compounds were tested with Tukey's HSD test (using the SPSS 16 program), and the results were expressed as mean ± standard deviation (SD). Additionally, the coefficient of variation (CV %) was calculated.

## RESULTS AND DISCUSSIONS

The adaptation and resistance of the vine to low temperatures (absolute minimum temperature) during the period of vegetative rest involves the realization of processes of accumulation and dynamics of some biochemical compounds in the tissues. In both studied viticultural areas, it has been determined that until the end of January, there was a constant decrease in starch content. During the month of February, the recorded decreases were noticeable, influenced by the recorded minimum temperatures, and starting from March, a slight increase in starch content became evident, particularly in the annual wood of all analysed cultivars (5.17% in annual wood compared to 4.28% in multiannual wood for the 'Cabernet Sauvignon' in the Banu Mărăciine viticultural area). The dynamics of starch (%) and soluble sugars (%) content in the annual vine wood during the dormant season in Banu Mărăciine and Şimnicu de Sus is presented in Figure 1 (a and b). Analysing the dynamic

content of carbohydrates in the annual canes of the studied cultivars, it is observed that 'Fetească neagră' records the highest values of soluble sugars content (13.11% on 22.02.2023 in Banu Mărăciine and 12.93% on 22.02.2023 in Şimnicu de Sus), as shown in Figure 1b. Against the backdrop of absolute minimum temperatures in February (-12.6°C), it is noted that all varieties registered maximum quantities of soluble sugars (13.11% for 'Fetească neagră', 13.06% for 'Cabernet Sauvignon', and 12.87% for 'Merlot' in the Banu Mărăciine viticultural area). In the Şimnicu de Sus viticultural area, maximum values of soluble sugars content were also recorded in February, albeit lower compared to those obtained for the same cultivars cultivated in the Banu Mărăciine viticultural area (12.93% for 'Fetească neagră', 12.75% for 'Cabernet Sauvignon', and 12.55% for 'Merlot'), as illustrated in Figure 1b. Alongside the decrease in starch content in all studied cultivars, there is a slow and continuous increase in the content of soluble sugars. This increase became evident from the second decade of January until the end of February, amid absolute minimum temperatures of -12.6°C in Banu Mărăciine and -11.2°C in Şimnicu de Sus. Starting from the first decade of March, both annual and multiannual cane starch values began to rise, while the content of soluble sugars followed a decreasing dynamic with variations between different cultivars based on their metabolic characteristics (Figure 1a and 1b). Also, Călugăr et al. (2019) state that due to climatic conditions during dormancy, starch began to resynthesize already in January and February, with significantly high levels for all cultivars. Field et al. (2009) and Zufferey et al. (2012) note that the conversion of starch into soluble carbohydrates occurred during the winter, coinciding with lower temperatures. These results confirm earlier observations made on different grapevine cultivars and other woody species. Towards the end of the dormancy period (March 24, 2023), 'Fetească neagră' recorded the highest content of soluble sugars, both in annual wood (9.94% in Banu Mărăciine and 9.82% in Şimnicu de Sus) and in multiannual wood (9.43% in Banu Mărăciine and 8.71% in Şimnicu de Sus). The lowest content of soluble sugars at the end of the dormancy period was noted for the 'Merlot' (Figure 1b). The quality of 'Fetească neagră'

and ‘Merlot’, depending on the cultivation area and climatic conditions, was also studied by Călugăr et al. (2019), Onache et al. (2020), as well as Bucur and Dejeu, (2020). The impact of climate change on grapevine phenology, investigating the role of cultivars and microclimates in the areas, was studied by Alikadic et al. (2019). The average content of soluble sugars (%) during the dormancy period 2022-2023 in annual woody shoots ranged from 9.81% (‘Merlot’ in Şimnicu de Sus) to 11.02% (‘Fetească neagră’, Şimnicu de Sus). In the Şimnicu de Sus viticultural area, there were no statistically significant differences between cultivars regarding the content of soluble sugars in annual woody shoots (Table 2). Statistically significant differences between ‘Merlot’ and ‘Fetească neagră’ ( $p \leq .05$ ) and ‘Cabernet Sauvignon’ ( $p \leq .05$ ) were only observed in the Banu Mărăciine viticultural area. Furthermore, regarding the influence of the viticultural area on the average content of soluble sugars (%) in annual woody

shoots for each of the three studied cultivars, no statistically significant differences were established. The average content of soluble sugars (%) during the dormancy period in multiannual wood ranged from 8.76% (‘Merlot’, in Şimnicu de Sus) to 10.03% (‘Fetească neagră’, Banu Mărăciine). Statistically significant negative differences in the content of soluble sugars in multiannual wood were observed between ‘Merlot’ and ‘Fetească neagră’ ( $p \leq .05$ ) in the Şimnicu de Sus viticultural area. Additionally, statistically significant positive differences in the average content of soluble sugars (%) during the dormancy period in multiannual wood were noted for ‘Cabernet Sauvignon’ ( $p \leq .05$ ) and ‘Merlot’ ( $p \leq .01$ ) in the Banu Mărăciine viticultural area compared to the Şimnicu de Sus area. The starch content (%) during the dormancy period 2022-2023 in annual woody shoots ranged from 4.84% (‘Merlot’, in Şimnicu de Sus) to 6.07% (‘Fetească neagră’, Şimnicu de Sus).

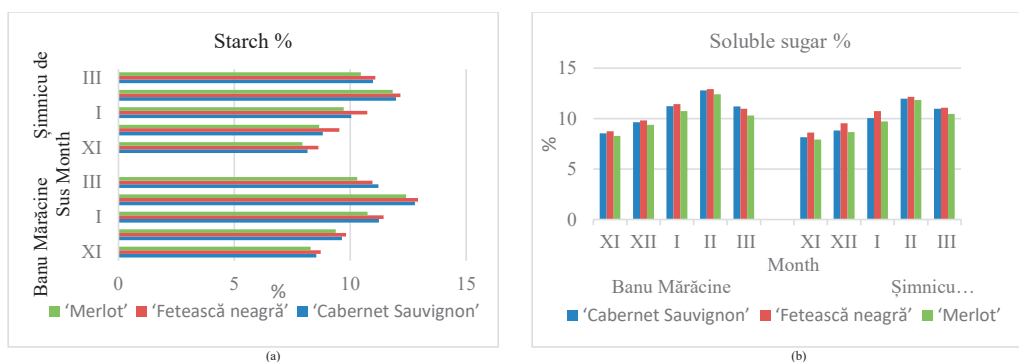


Figure 1. The dynamics of the content of starch (a) and soluble sugars (b) in annual vine wood during the dormancy period in Banu Mărăciine and Şimnicu de Sus (XI- November, XII-December; I-January, II- February; III-March)

Table 2. The content of the carbohydrate during the dormant season\*

Cultivar	Wine Area	Soluble sugars %				Starch %			
		Annual woody shoot	CV%	Multiannual wood	CV%	Annual woody shoot	CV%	Multiannual wood	CV%
‘Cabernet Sauvignon’	Banu Mărăciine	10.91±0.21 <sup>aA</sup>	1.92	9.85±0.23 <sup>aA</sup>	2.33	5.73±0.47 <sup>aA</sup>	8.20	5.55±1.01 <sup>aA</sup>	18.19
	Şimnicu de Sus	10.60±0.82 <sup>aA</sup>	7.73	9.06±0.40 <sup>abB</sup>	4.42	5.64±0.44 <sup>aA</sup>	7.80	5.37±0.73 <sup>aA</sup>	13.59
‘Feteasca neagra’	Banu Mărăciine	10.81±0.10 <sup>aA</sup>	0.92	10.03±0.24 <sup>aA</sup>	2.39	5.48±0.68 <sup>aA</sup>	12.41	5.88±0.67 <sup>aA</sup>	11.39
	Şimnicu de Sus	11.02±0.67 <sup>aA</sup>	6.08	9.82±0.40 <sup>aA</sup>	4.07	6.07±0.96 <sup>aA</sup>	15.82	5.66±0.77 <sup>aA</sup>	13.60
‘Merlot’	Banu Mărăciine	10.23±0.27 <sup>bA</sup>	2.64	9.55±0.13 <sup>aA</sup>	1.36	4.84±0.59 <sup>aA</sup>	12.19	4.62±0.71 <sup>aA</sup>	15.37
	Şimnicu de Sus	9.81±0.41 <sup>aA</sup>	4.18	8.76±0.25 <sup>bB</sup>	2.85	5.10±0.69 <sup>aA</sup>	13.53	4.77±0.68 <sup>aA</sup>	14.26

\* Note: Data are Means±SD; Means separation by HSD Tukey’s test at  $p \leq 0.05$ . Means with the same superscript are not statistically significant difference. In the column, lowercase letters indicate the significance of differences between cultivars for each studied viticultural area, while uppercase letters represent the significance of differences between the two areas for each cultivar.

In multiannual wood, the starch content (%) during the dormancy recorded values between 4.62% ('Merlot', in Banu Mărăcine) and 5.88% ('Fetească neagră', in Banu Mărăcine). Although there were some differences in starch content (%) in annual woody shoots and multiannual wood among the studied cultivars and between the two areas, these differences were not statistically significant (Table 2).

## CONCLUSIONS

The dynamics of the starch and soluble sugars content show variations depending on the cultivar and thermal conditions from November to March, with the most intense metabolic transformation of starch into soluble sugars being recorded in the months of January and February.

Regarding the carbohydrate content during the dormancy, significant differences between the two viticultural areas were highlighted only concerning the content of soluble sugars (%) in multiannual wood for the 'Merlot' and 'Cabernet Sauvignon'.

In the Banu Mărăcine viticultural area, the average content of soluble sugars (%) in multiannual wood for both cultivars was higher compared to the Șimnicu de Sus area.

Significantly negative differences between cultivars regarding the content of soluble sugars (%) in annual woody shoots were determined for the 'Merlot' compared to 'Cabernet Sauvignon' and 'Fetească neagră' in Banu Mărăcine. Significantly negative differences between varieties regarding the content of soluble sugars (%) in multiannual wood were noted between 'Merlot' and 'Fetească neagră' in the Șimnicu de Sus viticultural area.

As for the starch content (%) in annual woody shoots and in multiannual wood, no statistically significant differences were observed either between the studied cultivars or between the two areas.

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