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ANALYSIS OF SOME BIOCHEMICAL COMPOUNDS INVOLVED IN ADAPTATION MECHANISMS OF VINE TO THE MINIMUM TEMPERATURES DURING THE DORMANT SEASON

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Abstract

The state of endodormancy in the vine as an adaptation reaction to unfavourable temperature conditions, has a complex character and is the result of multiple biochemical and physiological processes that take place at the level of the tissues of the vine shoots. In this study, the adaptation and response reactions of Merlot, Cabernet Sauvignon and Fetească neagră grapevine varieties to the temperatures during the dormant season in the Banu Mărăcine wine-growing centre are monitored, by following the evolution of some biochemical compounds involved in these mechanisms: evolution of free water (%), bound water (%), total water (%) and total dry matter (SUT %), as well as the evolution of carbohydrates (soluble sugar and starch) in annual and multiannual vine wood. A grouping of the analyzed varieties is made according to the storage potential of carbohydrates under the different conditions of minimum temperatures.

Key words: *dorm eat season, grapevine varieties, biochemical compounds.*

INTRODUCTION

Establishing the relationship between the climatic resources, soil and the vine variety as well as the biochemical processes that take place under their influence, confirms the viticulture vocation of the studied areas as well as the recommendation of some varieties that are required for the production of wines for current consumption and for higher quality wines (Călugăr et al., 2009; Ciupureanu et al., 2016; Khan et al., 2022; Chou et al., 2023). The conditions of climate changes that are related in particular to water and thermal stress, require, in horticulture, a thorough knowledge of the variety that must be cultivated in certain cultivation areas (Dejeu et al., 2005; Cristescu, 2010; Hall and Jones, 2010; Jones and Alves, 2012; Climaco et al., 2012; Nicu and Mandă, 2012; Van Leeuwen and Darriet, 2016; Alikadic et al., 2019; Cichi et al., 2021; Costea and Capruciu, 2022). The quality of the grapes is also directly influenced by variety, ecoclimatic conditions of the applied agrotechnical works and zoning (Makra et al., 2009; Bunea et al, 2013).

The temperature of the environment, the light and the humidity are essential features of the climate in a certain wine-growing area, with a decisive impact on the development of the biological, physiological and biochemical processes of the vine (according to Cristescu et al., 2010; Jones et al., 2010; Costea et al., 2015; Cichi et al, 2016; Costea et al., 2021). Temperature is an important environmental factor affecting almost all aspects of growth and development in plants. The grapevine (Vitis spp.) is quite sensitive to extreme temperatures (Venios et al., 2020). The free water and bound water have a special role in the life of plants due to the relationships that exist between the forms of water and the various biochemical components in connection with the frost resistance process (Chirilei et al., 1970; Amira et al., 2010; Kopali et al., 2021). The transition of water from the free form to the bound form occurs gradually and is influenced by environmental conditions and plant metabolism (Rossouw et al., 2017, Junges et al., 2020). The dynamics of total water during the dormant season in the grapevine show differences depending on the genetic factors (variety), the metabolic particularities of the varieties during the acclimatization period (accumulation of osmotically active substances), climatic factors (minimum temperatures during winter) as well as of the climatic characteristic of the previous vegetation period (Fraga et al., 2014; Bucur și Babeș, 2016; Bernardo et al., 2018). The defence mechanism of the vines against the action of low temperatures has two essential sides that have the same goal: increasing and maintaining the water bound in the cells at the limit of gelation and decreasing the freezing point of the cellular solution (Camps & Ramos, 2012; Trudi et al., 2015). Carbohydrates, a group of substances of particular importance for vines, have been studied by various researchers. Since 1926, Alexandrov and Makarevskaia, cited by Stoev, 1979, found that in winter the roots of the vine contain rich reserves of starch and sugar and the starch fills all the tissues of the bark and wood, representing one of the first works on the dynamics of carbohydrates in vines shoots. These researches were continued by Costea et al., 2010; Ferrara et al., 2022. The better the maturation of the wood, the greater the reserves of substances accumulated in the wood, the plant being more resistant to low temperatures in winter (Dejeu, 2005). The data recorded during the research show that the ecological factors, namely the water regime during the vegetation period, the drop in temperature in the autumn-winter interval and the protection by burial, significantly change the water balance (total, free, bound water) in woods of different ages (according to Bertamini et al, 2006; Bucur and Dejeu, 2020). All of these factors influence directly the solubility and transport of synthesized organic substances (carbohydrates, amino acids, lipids, etc.), ensuring a way of permanent regulation of the metabolic processes of synthesis and hydrolysis, with consequences on the frost resistance potential of the grapevines (Zapata et al., 2004; Stroe and Bucur, 2012; Bucur and Babes, 2016; Medici et al., 2014; Burzo, 2015; Căpruciu, 2022). The frost resistance of the grapevine is a complex physiological-biochemical property influenced by a series of genetic, ecological, technological factors etc., which condition each other (Cichi, 2006; Burzo, 2015, Ribeiro et al., 2018). Also, the dormant period can be seen as a genetically controlled process, the expression of its genes manifesting under the action of environmental factors and especially of photoperiod and temperature (Rotaru et al., 2008; Torregrosa et al., 2017).

MATERIALS AND METHODS

Sampling Sites - The Banu Mărăcine viticultural center has a heterogeneous landform of hilly areas, consisting of wide valleys with an asymmetric profile and a variation of the topoclimate conditions, with moderate to strong slopes (6-32%) that reduce the force of air flows, ensuring a calm circulation of the air favouring the development of the biochemical and physiological processes of the grapevine. Located at 176 m altitude with 44º19' north latitude and 23º48' east longitude, Banu Mărăcine belongs to the A_3 oenoclimatic zone which includes viticultural centers producing mainly high-class red and aromatic wines and, secondarily, quality table wines (Teodorescu et et al., 1987). The climate is temperate-continental with Mediterranean influence, characterized by mild winters, hot summers with a high number of tropical days, maximum temperatures being above 30˚C. The experiments were organized within the Banu Mărăcine viticultural area in parcels placed in identical orographic conditions (plateau conditions) on a slightly levigated reddish brown soil. The varieties included in the study are grafted on Berlandieri x Riparia SO4 rootstock, with 2 x 1.2 m planting distance, semi-high growth with multiple Guyot cutting system, with a crop load of 40 buds/vine.

Biological material - The observations and determinations were conducted for the Merlot, Cabernet Sauvignon and Fetească neagră wine grape varieties during the 2020-2022 period within Banu Mărăcine - the Didactic Research Station of the University of Craiova. The determinations were done on annual and multiannual wood (3 years) collected at an interval of 10 days during the dormant period (XI-III), observing the dynamics of the main biochemical compounds (free water, bound water, total dry matter, soluble sugar, starch). In this sense, 10 canes/variety/type of wood were collected from 10 stumps from each variant. The analyzes were carried out in the viticulture laboratory of the Faculty of Horticulture, University of Craiova.

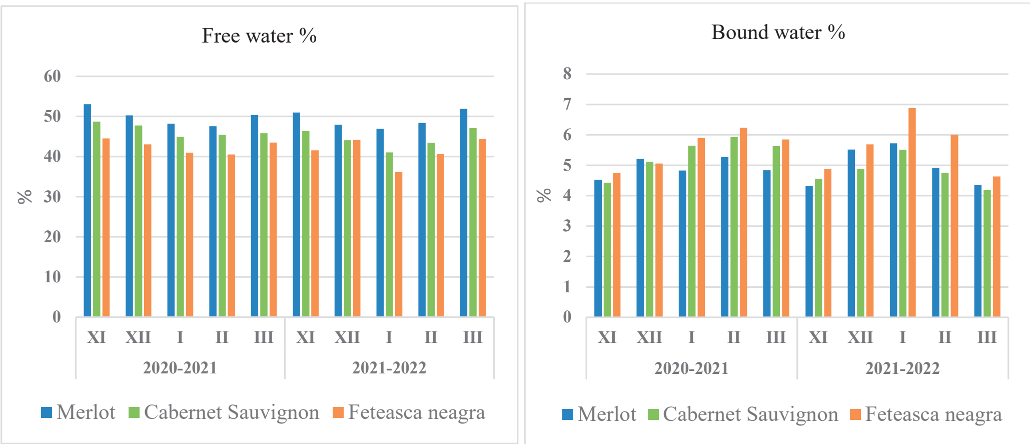
Methods - The monitoring of the minimum temperature during the dormant period was carried out at the Banu Mărăcine Meteorological Station. To determine the free water, consecutive weights were carried out on an analytical balance with a precision of 0.01 g until the difference between the weights did not exceed 0.03 g. In order to determine the bound water and the total dry matter, the oven - drying method was used (Căpruciu, 2016). The canes/woods were shredded and placed in an oven at a temperature of 105ºC. The evolution of the water was observed after 48h. The obtained data were calculated with the formula: $U = m_1 - m_2/m_1 \times 100$ (%), where, U = humidity, m_1 = sample mass before drying; m_2 = mass of the sample after drying. The total dry matter was calculated by the difference using the formula: SUT (%) = 100 - U (%). The carbohydrates were determined by spectrophotometric analysis. By this method, the extraction of soluble sugars is made with 80% volume alcohol solution, the starch with 52% volume perchloric acid solution and then the treatment is with 0.2% anthrone ($C_{14}H_{100}$) solution. The obtained colour intensity (with transparent blue-green colour shades) is measured colorimetrically, using UV-VIS Spectrophotometer at a wavelength of 620 nm (Comsa et al., 2013; Călugăr et al., 2010). The data resulting from the spectrophotometric reading were calculated as follows: Sugars = $E_c - E_a/E_b \times 50$; Starch = $E_d - E_a/E_b \times 50$; in which: E_a, E_b, E_c, E_d = extractions of solutions a, b, c, d; 50 = concentration of standard solution.

Statistical Analysis - In order to determine the content of biochemical compounds in the grapes of the Merlot, Cabernet Sauvignon and Fetească neagră varieties during the dormant season of 2020-2021 and 2021-2022 in the Banu Mărăcine viticultural center, the analysis of variance (ANOVA) was used. The differences between the means values of biochemical compounds were tested with the Duncan test (using the SPSS 16 program), the results being expressed as mean ± standard deviation (SD). Also, the coefficient of variation (CV %) was calculated.

RESULTS AND DISCUSSIONS

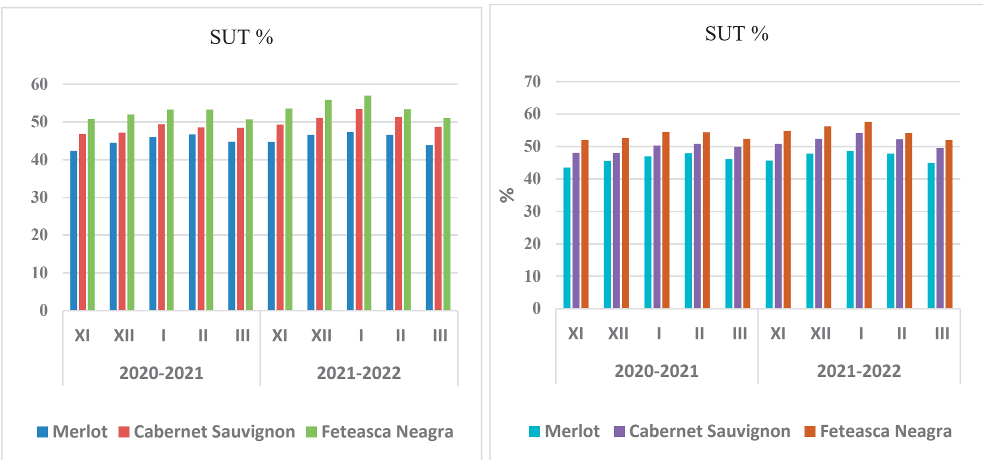
The biochemistry of plants can be directed towards the increasing resistance to adverse climatic conditions, the assessment of resistance can be defined with the help of biochemical and physiological indices such as water or carbohydrate content. The ratio between free and bound water is an essential indicator in determining the behaviour of vines at low temperatures during the dormant period (Gezici-Koç et al., 2017). In the woods of the Merlot variety, in the 2020-2021 period, the highest values were recorded in free water (53.03% in annual wood and 52.06% in multiannual wood in December 2021) with minimum values recorded in the 2021-2022 period of 46.91% in annual wood and 45.81% in multiannual wood within low temperatures in January (absolute minimum temperature of -10.5ºC). In December, the lowest free water content was recorded in the Fetească neagra variety among the analysed varieties (44.5% in annual wood and 43.4% in multiannual wood in 2020). Chapman et al. recorded similar results regarding the water content of the canes/woods of the Cabernet Sauvignon variety in 2005 and Tomoiagă et al. for the Fetească neagră variety in 2020. The behaviour of the Cabernet Sauvignon variety at low temperatures was also studied by Adams in 2017. The water content of the Merlot variety was maximum in January with similar values for both the studied intervals as well as for the type of wood (5.8% in January 2021 in annual wood respectively 5.7% in January 2022 in annual wood and 5.5% bound water was recorded in multiannual wood) within absolute minimum temperatures of -10.5^{0}C. And Junges et al., 2020 analyse the content in biochemical compounds of the Merlot variety with similar results. Regarding the dynamics of the accumulation of bound water, it is found that in all the analysed varieties at the beginning of the dormant period the values are low, increasing with the decrease in temperatures, and decreasing again at the beginning of March. It is found in all the studied varieties that in multiannual vine wood both free and bound water have lower values compared to annual wood (Figure 1). Similar studies were conducted by Jităreanu et al. in 2011 in

Cotnari. Following the dynamics of SUT (%) in Figure 2, a maximum of accumulated dry matter can be observed in January 2022 in the Fetească neagră variety (57.03% in annual wood and 57.60% in multiannual wood) which coincides with a minimum of total water (42.9% in annual wood respectively 42.3% total water in multiannual wood). It is observed that the SUT (%) from the multiannual wood is higher than that recorded in the annual wood for all the analyzed varieties. The content in SUT (%) recorded during the dormant period of 2021-2022 in wood recorded higher amounts compared to the previous year (Figure 2). Analyzing the dynamics of carbohydrates in the Merlot variety, lower contents of soluble sugar can be noted in annual vine wood, with a maximum of 10.76% and in multiannual wood with 9.85% at the end of January (abs. min. temp. of -10.5^{0}C) corresponding to a minimum starch of 3.01% in annual vine wood and 3.25% in multiannual vine wood (January 23, 2021). The maximum average values were recorded in the Cabernet Sauvignon variety in both years, with higher values in January 2021 in annual wood (11.13% soluble sugar compared to 10.15% in January 2022). In multiannual wood, the soluble sugar content is lower (9.33%) with a minimum starch of 4.03% (January 2021), respectively 9.23% soluble sugar and 4.14% starch in January 2022 (Figure 3). The maximum values of soluble sugar were recorded in the annual wood of the Fetească neagră variety with 13.33% in January 2021 and 12.56% in January 2022. The values recorded in the multiannual wood are lower (10.12% and 11.33%). Regarding the starch content in the annual vine wood, the differences are significant with a maximum at the end of the dormant period of 7.21% in the annual wood of the Cabernet Sauvignon variety. Both in the annual and in the multiannual vine wood, a constant increase in starch can be found at the end of February and the beginning of March, in all the analyzed varieties (Figure 3). The quality of Fetească neagră and Merlot varieties depending on the cultivation area and climatic conditions was also studied by Onache et al in 2020, Trejo-Martínez together with the research team in 2009 as well as Bucur and Dejeu in 2020. In terms of the starch hydrolysis potential in the Banu Mărăcine viticultural center, the Fetească neagră variety stands out, followed by Cabernet Sauvignon and Merlot (Figure 3). Observing the dynamics of the evolution of carbohydrates in the annual and multiannual vine wood of the studied varieties during the dormant period, a quantitative increase in soluble sugar and a decrease in starch can be observed in all varieties, the differences being given by the low temperatures and the metabolic particularities of the varieties. In the cells of plant tissues, two types of water are highlighted: free and bound water. Furthermore, Khan et al. (2016) mention in plant-based food materials three types of water: intercellular, intracellular water and cell wall water. Free water, or capillary water, comes mainly from the intercellular space, while intracellular water and cell wall water is known as bound water, being osmotically or physically (Côme, 1992; Khan et al., 2016) linked to various substances with a protective role (proteins, soluble sugars, etc.). A series of researches have demonstrated that in freezing tolerant plants, ice formation is extracellular and results in the dehydration of living cells as intracellular water is drawn to extracellular ice masses (Levitt, 1980; Côme, 1992; Dereuddre et al., 1992). Based on the recorded results regarding the average content of total water (%), the free water and bound water during the two dormant seasons, it is observed that there are differences between the three varieties. Among the three varieties, Merlot has the highest content in total water and free water, but also the lowest content in bound water in both annual woody shoot and in multiannual wood (Table 1). Although there are differences between Fetească neagră and Cabernet Sauvignon regarding the types of water, they are statistically significant only for the total water content in both annual and multiannual wood ($p \leq .01$). Some studies show that the varieties that stood out through a higher tolerance to negative thermal stress during the winter have the ability to fix a greater amount of bound water in the cells of the canes/wood tissues (Cichi, 2005; Adams, 2017; Bernardo et al., 2018). Among the analyzed varieties, the highest content in bound water was recorded in the Fetească neagră variety, the differences being significant compared to the Merlot variety ($p \leq .05$). During the dormant season, the differences were highlighted between the three varieties regarding the content of some biochemical compounds with a protective role at the negative critical temperatures during winter (Table 2). The Feteasca neagra variety recorded the highest SUT content (%) both in annual woody shoot and in multiannual wood, the differences being significantly positive compared to Cabernet Sauvignon (Tukey HSD, $p \leq .05$) and Merlot ($p \leq .01$). Although there are differences between the three varieties regarding the content of soluble sugars, these are not statistically significant (Table 2). The differences are also observed in terms of starch content, the Merlot variety recording the lowest values both in the annual woody shoot and in the multiannual wood, the differences being significantly negative compared to the Feteasca neagra variety ($p \leq .05$).



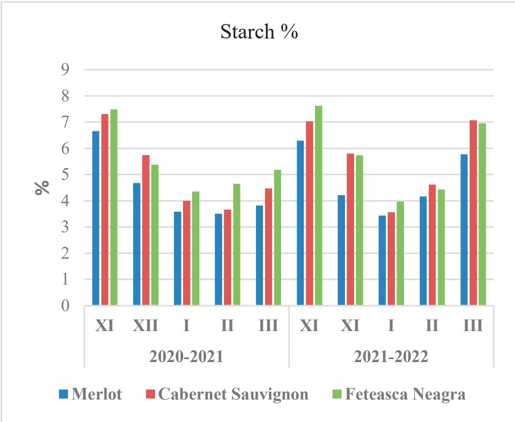
(a) (b)

Figure 1. The dynamics of free water (a) and bound water (b) content in annual vine wood during the dormant season

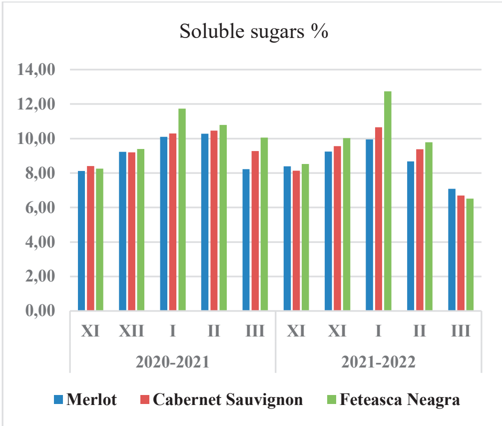


(a) (b)

Figure 2. The dynamics of SUT content (%) in annual (a) and multiannual vine wood (b) during the dormant season



(a)



(b)

Figure 3. The dynamics of the content of starch (a) and soluble sugars (b) in annual vine wood during the dormant season

Table 1. The content of types of the water during the dormant season*

Variety	Total water %		Bound water %		Free water %	
	Annual woody shoot	Multiannual wood	Annual woody shoot	Multiannual wood	Annual woody shoot	Multiannual wood
Merlot	54.65 ± 1.54^{a}	53.44 ± 1.61^{a}	4.79 ± 0.34^{b}	4.77 ± 0.29^{b}	49.55 ± 0.47^{b}	48.51 ± 0.46^{a}
Cabernet Sauvignon	50.52 ± 2.00^{b}	49.36 ± 1.93^{b}	5.06 ± 0.29^{ab}	4.91 ± 0.26^{ab}	45.47 ± 1.50^{ab}	44.49 ± 1.29^{bc}
Feteasca neagra	46.91 ± 2.11^{c}	45.91 ± 1.83^{c}	5.58 ± 0.13^{a}	5.44 ± 0.20^{a}	41.95 ± 0.82^{a}	41.07 ± 0.48^{c}

*Average of seasons 2020/2021 and 2021/2022/ Note: Means separation by HSD Tukey`s test at p≤0.05. Means with the same superscript are not statistically significant

Table 2. The content of the main biochemical compounds during the dormant season*

Variety	SUT %		Soluble sugars %		Starch %	
	Annual woody shoot	Multiannual wood	Annual woody shoot	Multiannual wood	Annual woody shoot	Multiannual wood
Merlot	45.35 ± 1.54^{c}	46.54 ± 2.60^{c}	9.14 ± 1.12^{a}	8.60 ± 0.99^{a}	4.61 ± 1.26^{b}	6.49 ± 1.20^{b}
Cabernet Sauvignon	49.44 ± 2.01^{b}	49.65 ± 3.91^{b}	9.33 ± 1.54^{a}	8.77 ± 1.08^{a}	5.49 ± 1.78^{ab}	7.45 ± 1.46^{ab}
Feteasca neagra	53.09 ± 2.11^{a}	54.07 ± 2.86^{a}	10.12 ± 1.69^{a}	8.96 ± 1.47^{a}	5.76 ± 1.73^{a}	7.93 ± 1.25^{a}

*Average of seasons 2020/2021 and 2021/2022 / Note: Means separation by HSD Tukey`s test at p≤0.05. Means with the same superscript are not statistically significant

CONCLUSIONS

It is observed in all the studied varieties, that both free and bound water are found in larger quantities in the annual wood compared to the multiannual wood. It is also found that the free form of water decreases progressively with the entry of the vine into the dormant period until February, after which it starts to grow again, the dynamics being different from one variety to another. Parallel to the decrease in the content of free water in all the studied varieties, an increase in the content of bound water is observed, with variable intensity depending on the variety and thermal conditions. During the dormant period in the studied varieties, it is observed that along with the decrease in the water content of the annual and multiannual wood, there is an increase in the content of total dry matter (SUT (%).

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

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