VARIATION OF SOME BIOCHEMICAL CHARACTERISTICS OF *VITIS VINIFERA* L.GREEN PARTS IN RELATION TO GROWING HEIGHT

VARIAȚIA UNOR CARACTERISTICI BIOCHIMICE LA UNELE ORGANE VERZI ALE VIȚEI DE VIE (*VITIS VINIFERA* L.) ÎN FUNCȚIE DE ÎNĂLȚIMEA DE CREȘTERE

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Abstract. *The purpose of the study was to evaluate and establish therelationships between some biochemical featrures of leaves collected from the top, middle and bottom of the vine stock in the phenophase of grapes tehnological mathurity. The determinations included analysis of the moisture and total dry matter, total polyphenolic compounds and peroxydase activity. The concentration of photosynthetic pigments and the ratios between them was also determinated. The varietys ana lysed were Fetească albă, Fetească Regală, Grasă de Cotnari, Tămâioasă românească and Frâncușă from Iași vineyard. The highest total polyphenolic content, was registrated in the top leaves while the middle collected leaves recorded a increased level of photosynthetic pigmentsdepending on variety and growing height.*
Key words: *leaves, phenolic compounds, assimilating pigments, growing height, Vitis vinifera L.*

Rezumat.*Scopul acestei lucrări a fost evaluarea si relaționarea unor caracteristici biochimice ale frunzelor prelevate din partea de superioară, partea mediană și partea inferioară a butucului de viță de vie, în fenofaza de maturare a strugurilor. Analizele efectuate au inclus determinarea conținutului total de umiditate și substanță uscată, a concentrației de compuși fenolici totali și a activității peroxidazei. A fost analizată, de asemenea, concentrația în pigmenți asimilatori, precum și raporturile dintre aceștia. Soiurile selectate pentru efectuarea determinărilor experimentale au fost Fetească albă, Fetească Regală, Grasă de Cotnari, Tămâioasă românească și Frâncușă, cultivate în arealul podgoriei Iași. Cel mai important conținut de compuși fenolici a fost identificat în cazul frunzelor din partea superioară a butucului, în timp ce concentrația de clorofile şi carotenoizi a variat semnificativ în funcție de soi şi înălțimea de creştere, frunzele din partea mediană înregistrând cele mai ridicate valori ale acestor parametri.*
Cuvinte cheie: *frunze, compuşi fenolici, pigmenți asimilatori, înălțime de creştere, Vitis vinifera L.*

INTRODUCTION

Climate change projections into the future suggest an increased variability of temperature and precipitation. Extreme climate conditions, such as dry spells,

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sustained drought, and heat waves can have large effects on vine. The timing of extreme events relative tosensitive phenological stages could affect growth and productivity of the vine.

The research conducted have shown only external effects of water and temperature stress, without going into the depths of physiological and biochemical changes that they induce in plants, and without considering the importance of the vine stock in ensuring resistance to grape vine.

Fewer studies were conducted on some variations of biochemical characteristics of vine leaves in grapes ripening phenophase and therefore this research produce a certain interest. During this stage the grapes are the main substances receptor and the vegetative organs reduce their growth, and also some of the some physiological and biochemical processes reduce their activity or even cease it (Mustea, 2004).

MATERIAL AND METHOD

The research has been carried out on the mature leaves of five*Vitis vinifera* L. indigenous grapes varieties for quality white wines (Fetească albă, Fetească Regală, Grasă de Cotnari, Tămâioasă românească and Frâncușă), growing in the Ampelographic Collection of the University of Agricultural Sciences and Veterinary Medicine Iasi, Romania, in the phenophase ofgrapes ripening.

Leaf samples were harvested manually, in the morning, from the top, middle, and bottomshoots of vine stock, rapidly frozen and analyzed in same day.

Peroxidase assay procedure was based on that of Bergmeyer (1974), in which the rate of decomposition of hydrogen peroxide by peroxidase, with guaiacol as hydrogen donor, is determined by measuring the rate of colour development spectrophotometrically at 436 nm and at 25°C (UV-vis Spectrostar Nano microplates spectrophotometer).

For assimilatory pigment extraction, frozenleaf samples (0.5 g) were grinded and washed with 10 mL of 99,98% acetone in order to extract the compounds from the leaf tissue. The extract was centrifugated (refrigerated laboratory centrifuge Nahita 2816) 15 min, 3000 rpm (10 °C).The analytical determination was conducted using a UV-vis Shimadzu 1700 Pharmaspec Spectrophotometer at the following wavelengths: 662 and 645 nm, for chlorophyll a and b and 470 nm for carotenoids. Photosynthetic pigment content was calculated in mg/g fresh weight (f.w.) according to the protocol presented by Lichtenthaler and Buschman, 2001.

Total phenolic content was determined by Folin-Ciocâlteu method, measuring the absorbance at 750 nm (Singleton and Rossi, 1965). A calibration curve using different concentrations of gallic acid solutions was used for expressing the results as gallic acid equivalent (GAE).

The method used to discriminate among the means was Fischer's least significant difference procedure at 95% confidence level. Simple regression analysis was performed to look for relationships between data registered by two independent methods. P values lower than 0.05 ($p<0.05$) were considered to be significant.

RESULTS AND DISCUSSION

According to Rotaru and Țârdea (2002), for leaves from the middle of the vinestock, the variability of ampelographic characters is the most reduced. Generally,leaves from the base and the top of vine stock have a lower content of assimilatory pigments and a lower photosynthetic activity, leaves between nodes 5–10 of shootsbeing the most photosynthetically active (Mustea, 2004).

Rate of photosynthesis, during grape ripeningis positively correlated with earliness or lateness character of grape maturation. Photosynthetic activity is favored by high temperatures in late August especially to middle maturing varieties (Ionescu and Condei, 1971).

Table 1

The content of chlorophyll a, chlorophyll b and carotenoids (mg/g f.w.) and the ratios between them in leaves of indigenous *V. vinifera* L. varieties

Variety	Position	Carot.	St. Dev.	Chl a	St. Dev.	Chl b	St. Dev.	Total chl	St. Dev.	Chl a/ Chl b	St. Dev.	Chl/ Carot.	St. Dev.
Fetească albă	T	0.53^{NS}	0.05	1.19^{00}	0.04	0.43^{0}	0.03	1.89^{NS}	0.06	2.78^{000}	0.02	3.56*	0.04
	M	0.59***	0.05	1.39**	0.06	0.60*	0.02	1.99*	0.08	2.32***	0.02	3.37^{NS}	0.07
	B	0.51^{000}	0.03	1.34^{NS}	0.03	0.55^{NS}	0.04	1.61^{0}	0.07	2.43^{000}	0.03	3.15^{00}	0.07
Fetească regală	T	0.67***	0.03	1.54***	0.02	0.53^{000}	0.07	2.07^{NS}	0.03	2.91***	0.07	3.08^{0}	0.06
	M	0.78***	0.02	1.92**	0.03	0.91***	0.05	2.83***	0.05	2.10^{000}	0.07	3.62*	0.01
	B	0.30^{000}	0.02	$0,69^{000}$	0.05	$0,30^{000}$	0.07	1.00^{000}	0.04	2.30^{0}	0.02	3.33^{NS}	0.08
Frâncușă	T	0.51^{NS}	0.03	1.27^{NS}	0.02	0.46^{0}	0.02	1.73^{00}	0.03	2.78***	0.02	3.39^{00}	0.07
	M	0.83***	0.02	2.03^{***}	0.07	0.93***	0.03	2.96***	0.05	2.19^{000}	0.03	3.56^{NS}	0.07
	B	0.35^{000}	0.07	$0,93^{000}$	0.04	$0,38^{00}$	0.02	1.31^{000}	0.03	2.42^{00}	0.06	3.74*	0.02
Grasă de Cotnari	T	0.35^{00}	0.05	1.26***	0.02	0.36^{00}	0.03	1.81***	0.02	2.66**	0.02	5.17***	0.02
	M	0.56**	0.03	1,37***	0.03	0.56**	0.07	1.89***	0.07	2.26^{00}	0.05	3.37^{00}	0.03
	B	0.50^{NS}	0.07	$0,89^{000}$	0.02	0.52^{NS}	0.06	1.24^{000}	0.05	2.46^{NS}	0.01	2.48^{000}	0.04
Tămâioasă românească	T	0.57^{NS}	0.03	1.20^{00}	0.05	0.45^{000}	0.03	2.04^{NS}	0.06	2.68***	0.02	3.57*	0.07
	M	0.59^{NS}	0.02	1.55**	0.03	0.64^{NS}	0.04	2.19***	0.02	2.41^{000}	0.03	3.71**	0.08
	B	0.55^{NS}	0.04	1.45*	0.01	0.59^{NS}	0.02	1.64^{000}	0.04	2.43^{000}	0.03	2.98^{00}	0.01
Mean		0.55	0.02	1.33	0.35	0.55	0.02	1.88	0,21	2.48	0.01	3.44	0.05
CV %		3.18	-	1.11	-	3.03	-	11,17	-	0.50	-	1.43	-

Note: Data expressed as mean values with standard deviation (n = 3). NS, *, **, *** - indicate nonsignificant and positive significant at $p \leq 0.05$, 0.01, 0.001, respectively; $^{0,\ 00,000}$ - negative significant at $p \leq 0.05$, 0.01, 0.001; T – top leaves; M – midlle leaves; B – bottom leaves.

Concentration of chlorophyll *a* in extracts presented wide variation between varieties, with a very positive statistical significance ($p<0.001$) in the case of variety Frâncușă (2.03±0.07 mg/g f.w.) middle leaves and a very negative statistical significance ($p<0.05$) in Fetească regală (0.69±0.05 mg/g f.w.) bottom leaves.

Chlorophyll *b* content of mature*V. vinifera* L. leaves varied also widely compared with the others varieties analysed, with a very positive statistical

significance ($p<0.001$) in Frâncușă and Fetească regală (0.91±0.05 mg/g f.w., respectively 0.93±0.05 mg/g f.w.) midlle leaves. Chlorophyll *a*/*b* ratio was specific to the phenophase of grape ripening, and varied within small limits between 2.10±0.07 (Fetească regală, middle leaves) and 2.78±0.02 (Frâncușă, Fetească albă, top leaves), with a mean of 2.48±0.01.

Research has shown that the leaves on the main shoots have a higher content of assimilating pigments than those of secondaryshoots and also the leaves that have a lower content of assimilating pigments are from the top and the base of the vine stock (Țâra, 1975).

Knowing that the main physiological processes, including photosynthesis that varies according to leaves age, it is necessary to determine at any time during the grapes ripining season,the concentration of photosynthetic pigments.In fact, each phenological phase corresponds to a particular floor of the photosynthetic activity when leaves reach the fully extended.

According to Gross (1991) and Wilows (2004), in mature leaves chlorophyll *a* is the major pigment and chlorophyll *b* is accessory pigment which exist in a ratio of approximately 3 to 1.Variation of chlorophyll a/b and chlorophyll/carotenoids ratio can be an indicator of senescence, stress, and damage to the photosynthetic apparatus, but can also provide distinctive informations on plant phenophase (Burzo et al., 2005).

Studies on the *V. vinifera* L. varieties growing in ecological conditions of Iași vineyard, established that at Fetească albă, Grasă de Cotnari and Tămâioasă românească genotypes, the maximum content of assimilating pigments are recorded in a more advanced stages of vegetation (50-70 days after leaf occurrence) and atthe Fetească regală and Frâncușă more later (after 90 days), fact that can be correlated with a longer vegetation period, noticing a superior production compared to the first three varieties.

Guaiacol peroxidase (E.C. 1.11.1.7) is widely distributed in plants where they catalyze the reduction of hydrogen peroxide (H_2O_2) to water, rendering it harmless (Bania and Mahanta, 2012). Peroxydase activity presented a wide variation between varieties, with a very significant ($p<0.001$) positive difference compared to the mean, in the case ofvine bottom leaves of all analysed varieties, with a maximumin Fetească regală leaves of 1.22±0.07 mg/g f.w.

Total polyphenol content of mature *V. vinifera* L. leaves harvested from the top of the plants was significantly higher compared to the midlle and bottom leaves, with a positive significance ($p<0.001$) in the case of Fetească albă (3.77±0.03 mg/g f.w.), Fetească regală (3.41±0.01 mg/g f.w.) and Frâncușă (2.84±0.01 mg/g f.w.) varieties.

Determination of moisture is essential in vegetal tissue analysis, high proportion of humidity causing a poor stability of samples, favoring microbiological and enzymatic activity (Beceanu et al., 2011; Maltini et al., 2003). In a typical grapevine leaf water content depends on the physiological condition, age and intensity of plant metabolism, ranging from 70 to 85% (Boyer et al., 1997; Mustea, 2004). In mature leaves harvested in the grape ripening stage moisture content was low, specific to this phenophase and varying from 58.59% to 72.93%,

with a mean of 66,96%. Total dry matter of leaves (%) is represented by all their constituents excluding water and is correlated with the moisture content (Tab. 2).

Table 2

Moisture dry matter (%)total polyphenol contentand peroxidase activityin mature leaves of indigenous *V. vinifera* varieties

Variety	Position	Moisture (%)	St. Dev.	Totaldry metter(%)	St. Dev.	POD (U/min/g)	St. Dev.	TPC(gGAE/ 100g f.w.)	St. Dev.
Fetească albă	T	71.09^{NS}	1.21	35.27**	1.21	0.11^{00}	0.05	3.77***	0.03
	M	72.93**	0.59	27,07^{00}	0.59	0.33^{NS}	0.01	2.15**	0.02
	B	64,73^{00}	1.50	28,91^{NS}	1.50	0.39**	0.02	1.60^{000}	0.05
Fetească regală	T	70.34^{NS}	0.90	28.21^{000}	0.90	0.24^{000}	0.03	3.41***	0.01
	M	71.79***	1.59	29.66^{NS}	1.59	1.03^{NS}	0.02	1.74**	0.01
	B	61.65^{000}	1.19	38.35***	1.19	1.22***	0.07	1.62^{000}	0.02
Frâncușă	T	66.98^{NS}	1.80	37.72**	1.80	0.56^{NS}	0.01	2.84***	0.01
	M	68.68**	1.65	31.32^{00}	1.65	0.40^{000}	0.01	2.16**	0.03
	B	62.28^{00}	0.94	33.02^{NS}	0.94	0.72***	0.02	1.70^{00}	0.01
Grasă de Cotnari	T	63.11*	1.65	41.41***	1.65	0.79^{NS}	0.01	2.50*	0.02
	M	67.45***	1.10	32.55^{000}	1.10	0.79^{NS}	0.01	2.25^{NS}	0.02
	B	58.59^{000}	1.02	36.89*	1.02	0.88**	0.01	1.90^{0}	0.01
Tămâioasă românească	T	68.54^{NS}	1.46	34.59**	1.46	0.51^{NS}	0.02	2.85*	0.01
	M	70.88***	1.35	29.12^{00}	1.35	0.34^{000}	0.02	2.33^{NS}	0.01
	B	65.41^{000}	0.89	31.46^{NS}	0.89	1.20***	0.02	1.75^{0}	0.02
Mean		66.96	1.26	33.04	1.26	0.63	0.02	2.30	0.01
CV %		1.88	-	3.80	-	2.48	-	0.64	-

Note: Data expressed as mean values with standard deviation (n = 3). NS, *, **, *** - indicate nonsignificant and positive significant at $p \leq 0.05$, 0.01, 0.001, respectively; $^{0, 00, 000}$ - negative significant at $p \leq 0.05$, 0.01, 0.001.T – top leaves; M – midlle leaves; B – bottom leaves.

Itwas found a significant correlation between low levels of moisture and a high peroxidase activity esspecialy in the leaves situated on the base of vine stock which can be related with the drought resistance of varietiesas was previously reported bySenaratna, 1985.

CONCLUSIONS

Biochemical characteristics investigated showed significant variations indicating different metabolic rates, particularly leaf age-related, which responds unitary to the cumulative action of stress factors.

Variation of chlorophyll a/b and chlorophyll/carotenoids ratio provide distinctive informations on plant phenophase, data recorded being within the range of values presented in the literature for *Vitis vinifera* L. varieties.

Carotenoid pigments were present in low concentration in grapevine leaves compared to chlorophylls, with a proper chlorophyll/carotenoids ratio ranging from 2.4 to 5.2.

The analysis of total polyphenolic compounds reported a higher content in the top leaves of vine, in the middle leaves beeing reported a higher concentration

of photosynthetic pigments. Peroxidase activity was higer in the leaves collected from the base of the vine and correlated to the lower levels of moisture phenomenon that can be related with the drought resistance of varieties.

Experimental data obtained provide further evidence that growing height of vine green parts influence their biochemical composition, in relation to plant defence activity against temperature and water stress.

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