Characterization of some *Vitis vinifera* L. Indigenous Varieties by Analysis of Leaf Photosynthetic Pigments

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Abstract

Chlorophyll content in leaves of 11 *Vitis vinifera* L. indigenous varieties (Iasi vineyard, Romania) was determined by two independent methods: spectrophotometry (extraction) and with a Opti-Sciences CCM-200 plus chlorophyll content meter (non-destructive method) with the aim of comparing the results. Also, this study was conducted to establish the values of chlorophyll *a*/chlorophyll *b* and chlorophyll/carotenoid ratio in middle leaves of vine stocks, in the phenophase of progressive growing of shoots. Area and perimeter of leaves in this phenophase were determined. Total chlorophyll concentration in leaf extract varied from 0.67 ± 0.01 mg/g f.w. to 1.00 ± 0.03 mg/g f.w., being highly correlated with the chlorophyll content index values (CCI) recorded by CCM-200 ($R^2=0.9839$; $p<0.001$). Similar situation was identified in the case of the chlorophyll *b* and CCI correlation ($R^2=0.9638$; $p<0.001$). Chlorophyll *a/b* ratio in leaf extract varied significantly depending on variety, ranging from 1.06 ± 0.01 to 2.43 ± 0.01 , with a chlorophyll/carotenoid ratio reaching up to 3.19 ± 0.08 . Compared to spectrophotometry, CCM-200 plus affords fast, reliable, economical, non-destructive chlorophyll content measurement, however, providing no details regarding chlorophyll components and ratios.

Keywords: chlorophyll content meter, chlorophyll *a/b* ratio, chlorophyll/carotenoid ratio, spectrophotometry, *Vitis vinifera* L.

INTRODUCTION

First isolated by Caventou and Pelletier in 1817, chlorophyll (gr. *chloros* – green; *phyllon* – leaf) is a biomolecule critical in photosynthesis (gr. *photos* – light; *synthesis* – building a whole), which allow plants to absorb energy from light (Davies, 2004). Chlorophyll *a* and chlorophyll *b* are the major types of chlorophylls found in plants (Wilows, 2004). They have a characteristic green colour due to strong absorbance of blue and red light. The increased proportion of chlorophyll *b* is due to its absorption in the 450–480 nm range, capturing effectively light at low intensity (Lichtenthaler and Wellburn, 1983). The chlorophyll *a/b* ratio varies from 2.0–3.2 for shade adapted plants to 3.5–4.9 for plants

adapted to sunny conditions (Lichtenthaler *et al.*, 1982). In *V. vinifera* L. leaves chlorophyll *a/b* ratio is maximum at the beginning of the vegetation period, reaching 3/1 and decreases during grape maturation, while the chlorophyll/carotenoids ratio may reach up to 4/1 (Keller, 2010; Toma and Jităreanu, 2007). 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. Modification of chlorophyll *a*/chlorophyll *b* ratio is determined mainly by the decrease of chlorophyll *a* concentration during leaf vegetation (Burzo *et al.*, 2005).

Carotenoids are a large group of deeply red or yellow fat-soluble pigments (Pfander, 1992). Carotenoids are found in all photosynthetic organisms, being involved in photosystem assembly and contribute to light harvesting by absorbing light energy in a region of the visible spectrum where chlorophyll absorption is lower and by transferring the energy to chlorophyll. Also, carotenoids provide protection from excess light, free radical detoxification and limiting damage to membranes (Cuttriss and Pogson, 2004).

Determinations carried out on *V. vinifera* L. varieties showed that carotenoid concentration in leaves varied from 7.72 mg/100 g to 17.26 mg/100 g f.w., with a gradual accumulation during leaf maturation (Burzo *et al.*, 2005; Mendes-Pinto *et al.*, 2005).

The Opti-Sciences CCM-200 plus chlorophyll content meter is an instrument which provides fast and accurate chlorophyll content index (CCI) readings on the intact leaves of plants, without the need for destructive chlorophyll assays. The measurement is rapid and simple to obtain compared to chlorophyll extraction and spectrophotometric reading (Biber, 2007). Previous research conducted on different species showed significantly linear correlation of total chlorophyll (by spectrophotometry) and CCI values (by chlorophyll content meters), however with large differences in terms of data correlation (Callejas *et al.*, 2013; Ghasemi *et al.*, 2011; Khaleghi *et al.*, 2012; Richardson *et al.*, 2002; Schaper and Chacko, 1991; van den Berg and Perkins, 2004). Currently, the lack of a more consistent relationship between chlorophyll concentrations estimated by the extraction method and by the chlorophyll meter at *V. vinifera* L. for different genotypes and at different growth stages, limits the potential use of CCM-200 plus for this species.

MATERIALS AND METHODS

The research has been carried out on the young leaves of 11 *Vitis vinifera* L. indigenous varieties (Gelu, Milcov, Cetățuia, Napoca, Someșan, Splendid, Transilvania, Coarnă neagră, Coarnă neagră selecționată, Purpuriu and Radames), growing in the Ampelographic Collection of the University of Agricultural Sciences and Veterinary Medicine Iasi, Romania, in the phenophase of progressive growing of shoots (20 days after bud burst). Grapevines varieties were 25 years old

grafted on Kober 5 BB (Berlandieri × Riparia). Planting distances were 2.2 m (between rows)/1.2 m (between plants), half-high training system, bilateral cordon, with pruning in fructification rings providing an average load of 40–45 buds/vine. Soil maintenance was “black field” and technological operations were specific to industrial vineyard ecosystem.

Designed to non-destructively measure of chlorophyll concentration in leaf tissue without the need to detach and grind a sample, Opti-Sciences CCM-200 plus exploits that total chlorophyll has several distinct optical absorbance characteristics, in order to determine relative chlorophyll concentration. Chlorophyll content index (CCI) represents the ratio between transmittance at 931 nm and transmittance at 653 nm. One wavelength falls within the chlorophyll absorbance range while the other serves to compensate for mechanical differences such as tissue thickness. Calibration is required every time is started a new experiment (Gandia *et al.*, 2004). The Opti-Sciences CCM-200 chlorophyll content index (CCI) values were registered simultaneously, on the same leaves considered for extraction.

Leaf samples (5) for extraction were harvested manually, in the morning, from the middle third of vine shoots, rapidly frozen (in 10 minutes) and analyzed in same day. Frozen leaf samples (0.5 g) were ground in a mortar using sieved inert sand as a grinding aid. Solvent of 99.98% acetone (10 mL) was used to extract the photosynthetic pigments from the leaf tissue. The extract was placed in the refrigerator overnight to minimize phototransformation of chlorophyll and to complete extraction (Biber, 2007). Small amount of MgCO₃ (0.5 mg) was added during extraction to neutralize plant acids responsible for the formation of pheophytin *a* from chlorophyll *a* (Lee and Schwartz, 2005). Supernatant was transferred into 1 cm path length quartz cuvettes. 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 (xanthophylls and carotenes) as part of a full scan of the sample (400–800 nm). Turbidity (haze) can be checked by measuring absorbance (A) at 710 nm. For a fully transparent leaf pigment extract, A_{710–750} nm should equal zero, since chlorophylls *a* (Chl

a) and *b* (Chl *b*) and carotenoids do not absorb in this region (Lichtenthaler and Buschmann, 2001; Spectranomics Protocol, 2011). Photosynthetic pigment content was calculated in mg/g fresh weight (f.w.) to allow comparison among species, using the three “trichromatic” equations suggested by Lichtenthaler (1987) and Lichtenthaler and Buschmann (2001) and improved by Carnegie Institution for Science through Spectranomics Protocol, 2011:

$$\text{Chl } a \text{ (}\mu\text{g mL}^{-1}\text{)} = 11.24 \times (A_{662} - A_{710}) - 2.04 \times (A_{645} - A_{710});$$

$$\text{Chl } b \text{ (}\mu\text{g mL}^{-1}\text{)} = 20.13 \times (A_{645} - A_{710}) - 4.19 \times (A_{662} - A_{710});$$

$$\text{Carotenoids (}\mu\text{g mL}^{-1}\text{)} = (1000 \times (A_{470} - A_{710}) - 1.90 \times \text{Chl } a - 63.14 \times \text{Chl } b) / 214.$$

Area, length, width and perimeter of the leaf was measured (by scanning) with a portable ADC BioScientific AM 300 Area meter (non-destructive method).

A one-way Analysis of variance (ANOVA) test was initiated to investigate significant differences between methods. 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.

Statistical measure of the dispersion of data points around the mean was conducted using the coefficient of variation (CV), which represents the ratio of the standard deviation (St. dev.) to the mean (%).

RESULTS AND DISCUSSIONS

Determination of moisture is an essential step in vegetal tissue analysis, high proportion of water causing a poor stability of samples, favoring microbiological and enzymatic activity, and hydrolysis reactions (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 studied romanian varieties leaves moisture content was high, specific to the phenophase (shoot growing), and varying from 76.32% to 81.43%, with a mean of 78.61% (Tab. 1).

Total dry matter of leaves (%), represented by all their constituents excluding water, is naturally correlated with the moisture content of samples.

According to the absorbance spectra in the 400–800 nm range (Fig. 1), was calculated the amount of chlorophyll (*a* and *b*) and total carotenoids in leaf extracts.

Tab. 1. Moisture and dry matter content (%) in leaves of indigenous *V. vinifera* varieties

Variety	Moisture (%)	St. dev.	Total dry matter (%)	St. dev.
Cetățuia	76.32 ⁰⁰⁰	1.50	23.68 ^{***}	1.50
Someșan	76.45 ⁰⁰	1.21	23.55 ^{**}	1.21
Coarnă neagră	77.75 ^{NS}	0.89	22.25 ^{NS}	0.89
Coarnă neagră selecționată	77.88 ^{NS}	1.54	22.12 ^{NS}	1.54
Radames	78.03 ^{NS}	0.90	21.97 ^{NS}	0.90
Napoca	78.05 ^{NS}	1.02	21.95 ^{NS}	1.02
Milcov	78.98 ^{NS}	1.10	21.02 ^{NS}	1.10
Purpuriu	79.59 ^{NS}	1.80	20.41 ^{NS}	1.80
Splendid	80.03 ^{NS}	1.65	19.97 ^{NS}	1.65
Transilvania	80.18 ^{NS}	0.94	19.82 ^{NS}	0.94
Gelu	81.43 ^{***}	1.12	18.57 ⁰⁰⁰	1.12
Mean	78.61	1.59	21.39	1.59
CV%	2.02	–	7.4	–

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.

Previous research reported that new leaves usually have a low photosynthetic activity, concentration of assimilatory pigments reaching maximum values in leaves at the age of 20–30 days, furthermore, with a continuous accumulation until grape veraison (Burzo *et al.*, 2005; Keller, 2010; Lovisolo *et al.*, 1996). Chlorophyll *a*, chlorophyll *b* and carotenoid concentration in fresh young

leaves of romanian *V. vinifera* L. varieties studied is shown in Tab. 2, as mean values with standard deviation (significant differences to the mean are noted).

Concentration of chlorophyll *a* in extracts presented small variation between varieties, with a negative statistical significance ($p < 0.05$) in the case of Purpuriu variety (0.47 ± 0.01 mg/g f.w.).

Fig. 1. Absorption spectra of leaf extracts of *V. vinifera* L. indigenous varieties

Tab. 2. The content of chlorophyll *a*, chlorophyll *b* and carotenoids (mg/g f.w.) and ratios between them in leaves of indigenous *V. vinifera* L. varieties

Variety	Chl <i>a</i>	St. dev.	Chl <i>b</i>	St. dev.	Carote- noids	St. dev.	Total Chl	St. dev.	Chl <i>a</i> / Chl <i>b</i>	St. dev.	Chl/ Caroten.	St. dev.
Cetățuia	0.52 ^{NS}	0.02	0.37 ^{NS}	0.02	0.31 ^{NS}	0.01	0.89 ^{NS}	0.96	1.43 ^{NS}	0.01	2.87 ^{NS}	0.04
Coarnă neagră	0.52 ^{NS}	0.01	0.41 ^{NS}	0.01	0.33 ^{NS}	0.02	0.93 ^{NS}	1.00	1.27 ⁰⁰⁰	0.02	2.87 ^{NS}	0.07
Coarnă neagră selecționată	0.51 ^{NS}	0.03	0.40 ^{NS}	0.01	0.32 ^{NS}	0.01	0.91 ^{NS}	0.89	1.29 ⁰⁰⁰	0.01	2.87 ^{NS}	0.07
Gelu	0.52 ^{NS}	0.02	0.43 ^{NS}	0.01	0.31 ^{NS}	0.01	0.95 ^{***}	0.96	1.21 ⁰⁰⁰	0.02	3.04 ^{***}	0.01
Milcov	0.52 ^{NS}	0.01	0.49 ^{***}	0.03	0.32 ^{NS}	0.01	1.01 ^{***}	0.67	1.06 ⁰⁰⁰	0.01	3.19 ^{***}	0.08
Napoca	0.52 ^{NS}	0.02	0.44 ^{NS}	0.02	0.32 ^{NS}	0.02	0.96 ^{***}	0.88	1.18 ⁰⁰⁰	0.01	2.97 ^{**}	0.07
Purpuriu	0.47 ⁰	0.01	0.20 ⁰⁰⁰	0.01	0.28 ^{NS}	0.01	0.67 ⁰⁰⁰	0.92	2.43 ^{***}	0.03	2.42 ⁰⁰⁰	0.02
Radames	0.51 ^{NS}	0.03	0.37 ^{NS}	0.01	0.29 ^{NS}	0.01	0.88 ^{NS}	0.87	1.39 ^{NS}	0.02	3.02 ^{***}	0.03
Someșan	0.52 ^{NS}	0.01	0.40 ^{NS}	0.01	0.32 ^{NS}	0.01	0.92 ^{NS}	0.94	1.33 ⁰⁰⁰	0.01	2.84 ⁰⁰	0.07
Splendid	0.53 ^{NS}	0.01	0.35 ^{NS}	0.02	0.31 ^{NS}	0.02	0.88 ^{NS}	0.91	1.52 ^{***}	0.03	2.79 ⁰⁰⁰	0.04
Transilvania	0.52 ^{NS}	0.02	0.40 ^{NS}	0.01	0.32 ^{NS}	0.01	0.92 ^{NS}	0.92	1.29 ⁰⁰⁰	0.01	2.91 ^{NS}	0.07
Mean	0.52	0.02	0.39	0.07	0.31	0.01	0.90	0.09	1.40	0.36	2.89	0.19
CV%	3.92	–	17.95	–	3.22	–	10.00	–	25.71	–	6.57	–

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.

Chlorophyll *b* content of young *V. vinifera* L. leaves varied widely compared to chlorophyll *a*, with a high value of coefficient of variability between data (CV=17.95%), and reaching a maximum of 0.49 ± 0.03 mg/g f.w. at Milcov variety.

Chlorophyll *a/b* ratio was specific to each variety, and varied within the range of 1.06 ± 0.01 (Milcov variety) to 2.43 ± 0.03 (Purpuriu variety), with a mean of 1.40 ± 0.36 .

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. It has been generally reported that chlorophyll *a/b* ratio of leaves is maximum at the beginning of vegetation period (Lichtenthaler *et al.*, 1981; Toma and Jitoreanu, 2007). Chlorophyll *a/b* ratio in leaves of romanian grapevine varieties studied in the phenophase of progressive growing of shoots had lower values than those presented in international literature, but comparable to data presented by researchers from Romania, from 1.3 to 1.4 (Acatrinei and Andor, 2006; Coțovanu *et al.*, 2012). Thereby, lower values of chlorophyll *a/b* ratio could be a peculiarity of romanian indigenous vine varieties, especially for those growing in N-E of the country, an areal with continental climate characterized by alternating hot and cold days, with frequent moisture deficit especially to the hills, that can frequently cause blockages of photosynthesis processes (Teodorescu *et al.*, 1987; Cotea *et al.*, 2000). On the other hand, according to Hopkins and Hüner (2009), typically

photosystem (PS) 1 has a chlorophyll *a/b* ratio of about 4/1, and PS 2 contains 50 to 60% of the total chlorophyll, with a chlorophyll *a/b* ratio of about 1.2, most of the chlorophyll *b* and carotenoids (xanthophyll). These data along with those reported by Jiang *et al.* (2005) and Jiang *et al.* (2006), which shows that the probability for energy transfer among PS 2 units in newly initiating vine leaves was distinctly higher than in mature ones and that only a few functional PS 1 were developed at the initial stages of leaf growth, could also explain the low values of the chlorophyll *a/b* ratio in young leaves of analysed varieties.

Burzo *et al.* (2005) reported that the carotenoid content of grapevine leaves has a specific accumulation, with lower values at the beginning of vegetation period. Carotenoid pigments (carotenes and xanthophylls) were present in lower concentration in grapevine young leaves compared to chlorophylls, without exceeding 0.33 ± 0.01 mg/g f.w., with a proper chlorophyll/carotenoid ratio of 2.42 ± 0.02 – 3.19 ± 0.08 (Tab. 2). These values are within the published range for *V. vinifera* L. leaves presented by Burzo *et al.* (2005), but are slightly lower than those reported by Young and Britton (1993) and Bertamini and Nedunchezian (2003), where chlorophyll/carotenoid ratio was in the range of 3 to 4.

Chlorophyll content index (CCI) in leaves of *V. vinifera* L. varieties, determined by means of hand held CCM-200 plus, ranged from 4.10 ± 0.19 to 6.26 ± 0.17 (Tab. 3).

Tab. 3. Chlorophyll content index (CCI) in leaves of indigenous *V. vinifera* L. varieties analysed

Variety	Minimum	Maximum	Mean	St. dev.
Purpuriu	3.90	4.40	4.10^{000}	0.19
Splendid	4.20	5.70	5.20^{NS}	0.59
Radames	5.20	5.80	5.54^{NS}	0.26
Cetățuia	5.40	5.90	5.60^{NS}	0.23
Coarnă neagră selecționată	5.20	6.40	5.64^{NS}	0.48
Transilvania	5.40	5.80	5.66^{NS}	0.24
Someșan	5.60	5.80	5.76^{NS}	0.21
Napoca	5.80	6.10	5.92^{NS}	0.13
Coarnă neagră	5.50	6.20	5.98^{NS}	0.29
Gelu	5.40	6.10	6.00^{NS}	0.60
Milcov	5.40	6.40	6.26^{**}	0.17
Mean	5.18	5.87	5.61	0.31
CV%	11.35	9.30	10.23	–

Note: Data expressed as mean values with standard deviation (n = 5). ^{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.

Fig. 2. Correlation of total chlorophyll and CCI values in leaves of *V. vinifera* L. indigenous varieties in the phenophase of progressive shoot growing

Fig. 3. Correlation of chlorophyll *a* and chlorophyll *b* with CCI values in leaves of *V. vinifera* L. indigenous varieties in the phenophase of progressive shoot growing

According to Van den Berg and Perkins (2004) and Yamamoto *et al.* (2002) small differences in the structures of the two main chlorophylls produce differences in the absorption maxima of chlorophyll *a* and chlorophyll *b*, which helps in their individual analysis. This fact can be exploited spectrophotometrically (by extraction), but not by a chlorophyll content meter.

The relationship between total extractable chlorophyll (*a* + *b*) and CCI values was significantly linear, with R^2 indicating that 98% ($p < 0.001$) of the variation was explained by a linear model, with conversion equation: $y = 6.4995x - 0.2475$ (Fig. 2).

Chlorophyll *a* and CCI values correlation in young leaves of *V. vinifera* L. romanian varieties resulting in a weak relationship ($R^2 = 0.6296$), while chlorophyll *b* and CCI values was significantly linear, similar to total chlorophyll content, with R^2 indicating that 96%

($p < 0.001$) of the variation was explained by the model obtained (Fig. 3).

However, for most CCI values, it appears that the chlorophyll meter CCM-200 plus is able to provide a relative estimate of total chlorophyll in grapevine leaves based on a linear model, at least for the phenological phase under study.

Variety influences photosynthesis process through both photosynthetic pigment content and leaf morphological particularities. The elements that compose the leaf structure (e.g. thickness of mesophyll), and leaf shape and area have an important role in the process of photosynthesis and are specific to each variety (Lebon *et al.*, 2005). Also, technological factors such as training system, type of pruning, number of buds, rootstock used, along with fertilization, irrigation, soil tillage, attack of diseases and pests, affect the accumulation of photosynthetic pigments and

certainly their determination (Keller, 2010; Rotaru *et al.*, 2011).

In order to determine whether the chlorophyll and carotenoid content of leaves is influenced by their biometric characteristics, leaf size and shape (area and perimeter) were determined by an AM-300 portable instrument (Tab. 4). Measurements are made optically using a simple scanning process. Both measurements and the scanned shape were stored and transferred to a personal computer.

Leaves selected for the measurements were collected from the middle of the vine stock (the middle third of shoots), where, according to Rotaru and Țârdea (2002), the variability of ampelo-

graphic characters is the most reduced. Generally, leaves from the base and the top of shoots have a lower content of assimilatory pigments and a lower photosynthetic activity, leaves between nodes 5–10 being the most photosynthetically active (Mustea, 2004).

Leaf area ranged from 2907±56 mm (Transilvania variety) to 6792±125 mm (Coarnă neagră selecționată variety), with a mean of 5210 mm and a standard deviation between varieties of 1157 mm. A high value of coefficient of variability between data was calculated in the analysis of leaf perimeter (CV=31.7%), as a result of an high variability between varieties in respect to this parameter.

Tab. 4. Area and shape of leaves of indigenous *V. vinifera* L. varieties in the phenophase of shoot growing

Variety	Area (mm)	St. dev.	Length (mm)	St. dev.	Width (mm)	St. dev.	Perimeter (mm)	St. dev.	Scanned image
Cetățuia	3903 ⁰⁰⁰	64	71.6 ⁰⁰⁰	8.9	77.5 ^{NS}	6.8	528.7 ⁰⁰	28.1
Coarnă neagră	6401 ^{***}	101	95.5 [*]	12.5	95.0 ^{NS}	4.9	684.2 ^{***}	14.3
Coarnă neagră selecționată	6792 ^{***}	125	103.1 ^{***}	14.8	94.7 ^{NS}	10.4	527.3 ⁰⁰	15.1
Gelu	5885 ^{***}	124	94.2 [*]	9.4	88.1 ^{NS}	11.2	516.4 ⁰⁰	18.9
Milcov	5714 ^{***}	59	95.0 [*]	8.7	90.2 ^{NS}	8.6	597.1 ^{**}	10.4
Napoca	4864 ⁰⁰⁰	102	87.6 ^{NS}	11.4	83.8 ^{NS}	7.4	402.1 ⁰⁰⁰	19.2
Purpuriu	4196 ⁰⁰⁰	96	83.1 ⁰⁰	11.6	86.1 ^{NS}	10.8	594.3 ^{NS}	20.7
Radames	5237 ^{NS}	147	92.7 ^{NS}	10.4	87.1 ^{NS}	10.2	486.3 ⁰⁰⁰	16.1
Someșan	5465 ^{***}	88	91.7 ^{NS}	9.4	84.6 ^{NS}	6.5	1018.6 ^{***}	11.1
Splendid	5943 ^{***}	197	100.1 ^{***}	8.2	87.9 ^{NS}	9.3	670.5 ^{***}	12.4
Transilvania	2907 ⁰⁰⁰	56	72.9 ⁰⁰⁰	11.0	62.7 ⁰⁰⁰	7.4	310.8 ⁰⁰⁰	10.6
Mean	5210	1157	89.8	10.2	85.2	8.9	576.0	182.8	–
CV%	22.2	–	11.3	–	10.4	–	31.7	–	–

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.

Tab. 5. Correlation of photosynthetic pigments concentration, CCI values and biometric data of leaves of indigenous *V. vinifera* L. varieties

<i>V. vinifera</i> L.	Chl <i>a</i>	Chl <i>b</i>	Total chl (<i>a+b</i>)	Carotenoids	CCI	Leaf Area	Leaf perimeter
Chl <i>a</i>	1						
Chl <i>b</i>	0.7877	1					
Total chl (<i>a+b</i>)	0.8522	0.9936	1				
Carotenoids	0.7389	0.7786	0.7966	1			
CCI	0.7934	0.9817	0.9791	0.7838	1		
Leaf Area	0.2304	0.3177	0.3120	0.2974	0.3220	1	
Leaf perimeter	0.0493	-0.0753	-0.0549	0.1178	-0.0299	0.4331	1

Note: Bolded values suggests that correlation coefficients are statistically significant in ANOVA test ($p < 0.05$)

Pearson's correlation (Data analysis, Microsoft Excel), between area and perimeter of leaves, extractable photosynthetic pigment content and CCI values, reported that there was no positive or negative relationship between experimental data (Tab. 5).

However, a linear relationship was noticed between chlorophyll *b* and total chlorophyll with a correlation coefficient of 0.9936 ($p < 0.001$), and was also confirmed the correlation between total chlorophyll content and CCI values ($r = 0.9791$; $p < 0.001$).

CONCLUSION

In young leaves of *Vitis vinifera* L. varieties native to Romania, concentration of photosynthetic pigments and the relationship between them was specific to each variety. Low chlorophyll *a/b* ratio may be a peculiarity of indigenous grapevine varieties from Romania and can be attributed to the specific climatic conditions and/or to the phenophase considered (progressive growing of shoots). This relation shall be established in the further studies.

Carotenoid pigments were present in low concentration in grapevine leaves compared to chlorophylls, with a chlorophyll/carotenoids ratio that falls within the published range for this species, from 2.4 to 3.2.

Compared to spectrophotometry (that require extraction of assimilatory pigments), Opti-Sciences CCM-200 plus chlorophyll content meter provide fast, reliable, economical measurement

of chlorophyll concentration, but with no details regarding chlorophyll components and ratios.

The data in this study indicate that chlorophyll meter CCM-200 plus appears to be able to provide a relative estimation of extracted chlorophyll in *Vitis vinifera* L. young leaves based on a linear model (by an equation of conversion). Once this relationship is established, the CCM can become an effective tool in plant management (e.g. plant health status, assessment of physiological changes over time, or in treatments affecting plant growth and development).

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