

NURSERY VEGETATIVE GROWTH OF 'AROMAT DE IAȘI' AND 'GOLIA' GRAPE VARIETIES, BY ANALYSIS OF FOLIARY PHOTOSYNTHETIC PIGMENTS

STUDIU PRIVIND CREȘTERILE VEGETATIVE ÎN ȘCOALA DE VIȚE LA SOIURILE PENTRU STRUGURI DE VIN 'AROMAT DE IAȘI' ȘI 'GOLIA', PRIN ANALIZA PIGMENȚILOR FOTOSINTETICI FOLIARI

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Abstract. *The physiological role of the assimilating pigments is complex, being involved in the processes of oxidation-reduction, in the photosynthesis and protection processes against ultraviolet radiation. The study aims to track vegetative growth during the vegetation period by spectrophotometrically determining the content of chlorophylls (a and b) and carotenoids from the leaves of vine varieties (Vitis vinifera L.) for wine grapes Aromat de Iași and Golia, new varieties created within the Research and Development Station for Viticulture and Winemaking Iasi. The two varieties were grafted on three rootstocks (Riparia Gloire, Berlandieri x Riparia Selection Oppenheim 4-clone 4 and Berlandieri x Riparia Selection Crăciunel 2), thus creating six working variants. Following the completion of the observations, Golia/SO₄₋₄ variant recorded the best results in terms of vegetative growth in the vegetation period measuring 41.83 cm (before wood maturation) followed very close by Aromat de Iasi/Riparia Gloire, which recorded 41.71 cm at the last measurement. Regarding the determination of the content of photosynthetic pigments (chlorophyll a and b, respectively carotenoids), the best results were obtained in the Aromat de Iași variety on almost all variants. Golia grape variety registered smaller values.*

Key words: carotenoids, chlorophyll a and b, photosynthetic pigments, vegetative growths.

Rezumat. *Rolul fiziologic al pigmenților asimilatori este complex, fiind implicați în procesele de oxido-reducere, în procesele de fotosinteză, fructificare și de protecție față de radiațiile ultraviolete. Studiul are ca scop urmărirea creșterilor vegetative în perioada de vegetație prin determinarea spectrofotometrică a conținutului în clorofile (a și b) și carotenoizi din frunzele soiurilor de viță de vie (Vitis vinifera L.) pentru struguri de vin Aromat de Iași și Golia, soiuri nou create în cadrul Stațiunii de Cercetare și Dezvoltare pentru viticultură și vinificație Iași. Cele două soiuri au fost altoite pe trei portaltoi (Riparia Gloire, Berlandieri x Riparia Selectia Oppenheim 4-clona 4 și*

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Berlandieri x Riparia Selecția Crăciunel 2), creindu-se astfel șase variante de lucru. În urma finalizării observațiilor efectuate s-a putut constata că varianta Golia/SO_{4.4} a înregistrat cel mai bun rezultat în ceea ce privește creșterile vegetative din perioada de vegetație, la ultima măsurătoare, făcută înainte de maturarea lemnului, având 41,81 cm, iar cea mai bună variantă pentru al doilea soi luat în studiu a fost Aromat de Iași/Riparia Gloire care a înregistrat la ultima măsurătoare 41,71 cm. În privința determinării conținutului pigmenților fotosintetici (clorofila a și b, respectiv carotenoizi) cele mai bune rezultate au fost obținute la soiul Aromat de Iași pentru aproape toate variantele pe care a fost altoit, comparativ cu soiul Golia care a avut rezultate mai slabe.

Cuvinte cheie: pigmenți fotosintetici, clorofila a și b, carotenoizi, creșteri vegetative

INTRODUCTION

Chlorophylls (Chl) are probably the most important organic compounds on earth, as they are required for photosynthesis (Davies, 2004; Willows, 2004).

Photosynthesis in plants is dependent upon capturing light energy in the pigment chlorophyll, and in particular chlorophyll *a* (Blankenship, 2014). Photosynthetic activity is a very intense process (5 to 11 $\mu\text{mol CO}_2/\text{m}^2/\text{s}$) that provides all the organic material needed for the growth and life activity of the plant (Popescu and Popescu, 2014). This is why photosynthesising cells have to contain large amounts of assimilatory pigments, up to 5% of total dry solids (Rabinowitch and Govindjee, 1969). In most species, the photosynthetic pigment content of the leaves provides valuable insight into the physiological performance of plants (Sims and Gamon, 2002). Moreover, the assessment of photosynthetic pigments, and consequently their relationships, is an important indicator of leaf senescence (Netto *et al.*, 2005).

Chlorophyll is a bright green natural pigment found in all photosynthetic plants, allowing them to absorb energy from light (Hörtensteiner and Kräutler, 2011). Forms *a* and *b* are the major types of chlorophyll that predominate in the chloroplasts of all higher plants (Willows, 2004; Raven *et al.*, 2005). Most analytical studies have reported the total chlorophyll contents as the sum of Chl *a* and Chl *b* (Lanfer Marquez and Sinnecker, 2008).



On the other hand, carotenoids are a large group of deeply red or yellow lipophilic accessory 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 (Britton, 2008). Also, carotenoids provide protection from excess light, free-radical detoxification and limiting damage to membranes (Cuttriss and Pogson, 2004).

MATERIAL AND METHOD

In order to carry out this study, research was conducted within the Research and Development Station for Viticulture and Winemaking in Iași, in 2019. Two varieties of wine grapes, Aromat de Iași and Golia, obtained at SCDVV Iași (tab. 1), were grafted on three rootstocks (Riparia Gloire, Berlandieri x Riparia Selection Oppenheim 4-clone 4 and Berlandieri x Riparia Selection Crăciunel 2), thus creating six working variants.

Table 1

Studied biological material

Grape variety	Genitors	Author	Year of homologation
Aromat de Iași 	Free fecundation of local grape variety Coarnăneagră and irradiation with X rays of its seeds	Dănulescu Dumitru Sandu-Ville Gorun Popescu Gheorghe	1980
Golia 	Intraspecific hybridation of Sauvignon x Șarbă	Dănulescu Dumitru Calistru Gheorghe Damian Doina Crăcană Alexandru	1999

Using the two varieties taken into consideration, respectively the three rootstocks on which the varieties were grafted, two series of determinations were made:

Determinations regarding the content in photosynthetic pigments, chlorophyll (a and b) and leaf carotenoids for each created variant and determinations on vegetative growth during the vegetation period.

The determination of the content in photosynthetic pigments from leaves was performed by the extraction of chlorophyll (a and b) and carotenoids (xanthophylls and carotenoids). The harvested leaves were crushed and 0.5 g was weighed for each variant, after which this amount was infused with 10 mL acetone 99.98%. 0.5

mg of MgCO_3 was added during extraction to neutralize the acids responsible for the formation of pheophytin a in chlorophyll a. The samples thus obtained were stored overnight in a cold environment. The fractions obtained were subsequently centrifuged using a Nahita 2816 cooling centrifuge for 15 minutes, at 3000 rotations per minute, at a temperature of 10 °C. The analytical determinations were performed using a Shimadzu 1700 Pharmaspec UV-vis spectrophotometer at wavelengths 662, 645 and 710 nm for chlorophyll a and b, respectively 470 nm for carotenoids. The pigment content was calculated in mg/g fresh substance, using the equations proposed by Lichtenthaler and Buschmann and completed by the Carnegie Institute of Science by Spectranomis Protocol.

$\text{Chl } a \text{ (}\mu\text{g mL}^{-1}\text{)} = 11.24 \times (\text{A662} - \text{A710}) - 2.04 \times (\text{A645} - \text{A710});$

$\text{Chl } b \text{ (}\mu\text{g mL}^{-1}\text{)} = 20.13 \times (\text{A645} - \text{A710}) - 4.19 \times (\text{A662} - \text{A710});$

$\text{Carotenoids (}\mu\text{g mL}^{-1}\text{)} = (1000 \times (\text{A470} - \text{A710}) - 1.90 \times \text{Chl } a - 63.14 \times \text{Chl } b)/214.$

Simultaneously with the determination of the content in photosynthetic pigments from the leaves, the humidity of the harvested leaves was determined, by drying for four hours in the oven, at a temperature of 105 °C.

The determinations on the vegetative growths during the vegetation period were made by measuring the shoots every 15 days starting with 1st June 2019, the last measurement being on 15th August 2019.

RESULTS AND DISCUSSIONS

In order to determine the content of chlorophyll pigments, the leaves moisture content was also determined (tab. 2).

From the table below it can be observed that the leaf moisture for all six variants taken into account registered higher values in June, gradually decreasing in July and August respectively.

Table 2

Leaves moisture (%)

Scion	Rootstock	Humidity (%)		
		June	July	August
Aromat de Iași	Riparia Gloire	73.31	73.21	69.23
	Selection Oppenheim 4-clone 4 (SO ₄₋₄)	73.73	72.47	70.61
	Berlandieri x Riparia Selection Crăciunel 2	76.45	75.59	72.55
Golias	Riparia Gloire	73.32	70.51	68.58
	Selection Oppenheim 4-clone 4 (SO ₄₋₄)	73.00	72.28	70.79
	Berlandieri x Riparia Selection Crăciunel 2	74.25	72.37	69.71

In June, the highest values were recorded for the variants Aromat de Iași/Crăciunel 2 (76.45%) and Golia/Crăciunel 2 (74.25%), after which they decreased slightly and the best results were in July registered in the Aromat de Iași/Crăciunel 2 (75.59%) and Golia/Crăciunel 2 (72.37%) variants. In August the results were even lower compared to the previous months, the best values being obtained at Aromatde Iași/Crăciunel 2 (72.55 %) and Golia/SO_{4.4} (70.79%).

After determining the humidity of the harvested leaves, the analysis of the foliar photosynthetic pigments was studied (fig. 1).

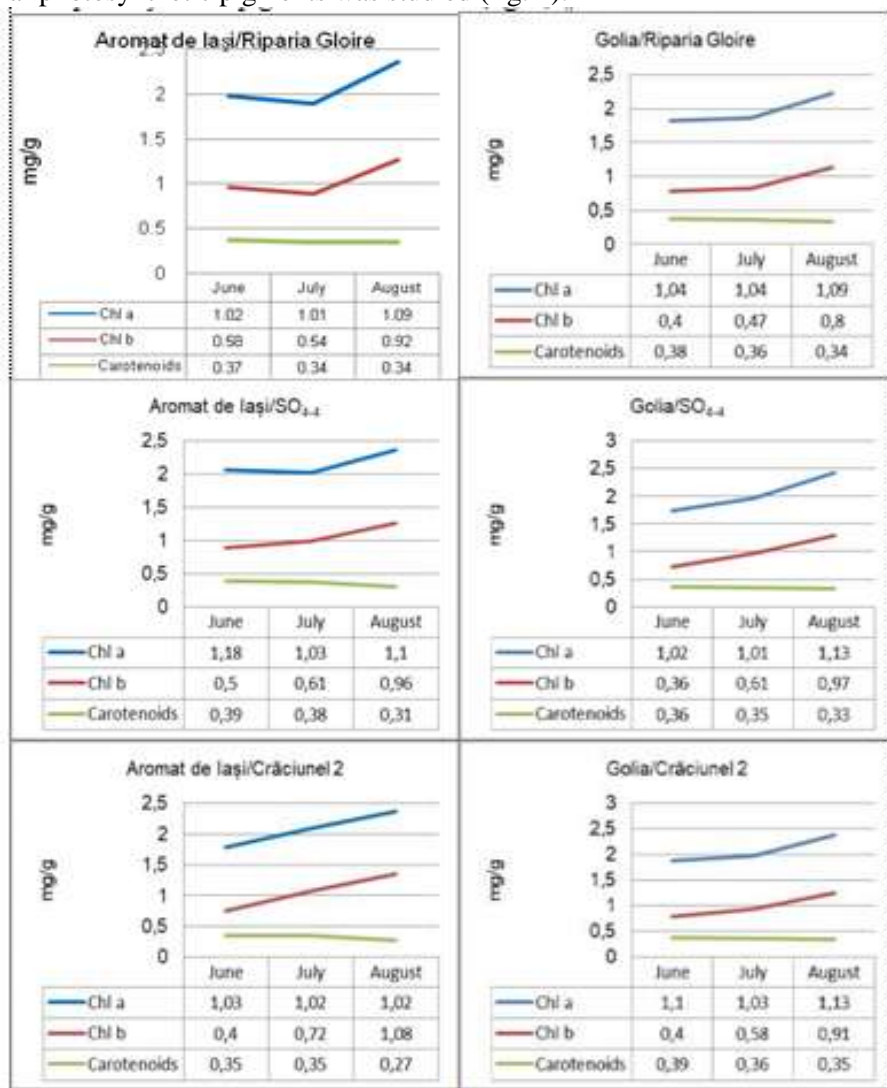


Fig.1 Dynamics of the content of photosynthetic pigments

Chlorophyll *a*, in June, in the variant Aromat de Iași/SO_{4.4} had the highest values of 1.18 mg/g fresh substance, and in the Golia variety the best result was recorded at the grafting with Crăciunel 2 having 1.10 mg/g fresh substance. For chlorophyll *b*, the best results were recorded for the variants Aromat de Iași/Ripariagloire (0.58 mg/g) and Golia grafted on Ripariagloire and Crăciunel 2 (0.40 mg/g), and for the carotenoids the best results were recorded at Aromat de Iași/SO_{4.4} (0.39 mg/g) and Golia/Crăciunel (0.39 mg/g).

The determination of the content of photosynthetic pigments from the leaves was carried out in July, where for the chlorophyll the best results were obtained by the variants Golia/Riparia gloire (1.04 mg/g), respectively Aromat de Iași/SO_{4.4} (1.03 mg / g). Chlorophyll *b*, recorded higher values compared to the previous month for almost all variants taken into consideration, and the best results were obtained at Aromat de Iași/Crăciunel 2 (0.72 mg/g) and Golia/SO_{4.4} (0.61 mg/g). For the last pigment analyzed, the carotenoids, the variants that were highlighted were Aromat de Iași/SO_{4.4} (0.38 mg/g) and Golia grafted on Riparia gloire and Crăciunel 2 (0.36 mg/g).

The last determination of the content of the photosynthetic pigments from the leaves was made in August, before the process of maturation of the shoots and of the appearance of the phellogen, the results obtained being higher in comparison with the other two months in which they were analyzed for Golia grape variety and smaller for Aromat de Iași. For chlorophyll *a*, the best results were recorded for the variants Golia/SO_{4.4} respectively Golia/Crăciunel 2 (1.13 mg/g) and Aromat de Iași/SO_{4.4} (1.10 mg/g). Chlorophyll *b* was found in the leaves of the Golia/SO_{4.4} variant (0.97 mg/g) and Aromat de Iași/SO_{4.4} (1.08 mg/g), and the last pigment analyzed, the carotenoids, had the highest results in the Golia/Crăciunel 2 variant (0.35 mg/g) and Aromat de Iași/Riparia gloire (0.34 mg/g).

The vine is a plant adapted to the conditions of insolation or semi-shade (Warren, 2013). Mittal *et al.* (2011), states that the ratio of chlorophyll *a* and *b* varies between 2.0 and 3.2 for plants adapted to shade conditions and 3.5 to 4.9 for plants adapted to insulation conditions.

According to Toma and Jitareanu (2007), the ratio of chlorophyll *a* / *b*, to the species *Vitis vinifera* L. is maximum at the beginning of the vegetation period, reaching up to a ratio of 3/1 and decreases during the maturation period of the grapes, while the ratio chlorophyll / carotenoids can record ratios of 4/1.

After determining the content of photosynthetic pigments, two reports were made between the analyzed pigments, the first being between chlorophyll *a* and *b*, and the second one was between the sum of the two chlorophyll pigments and carotenoids (tab. 3).

The chlorophyll *a* / *b* ratio for the Golia variety in June registered the highest value (2.83) at grafting on the SO_{4.4} rootstock while the same ratio for the Aromat de Iași variety, obtained the best result (2.57), at the grafting on the rootstock Crăciunel 2. In July both varieties had the best values of the ratio of chlorophyll *a* / *b* to the variants Golia/Riparia gloire (2.12) and Aromat de

Iași/Ripariagloire (1.87), and in August the variants with the highest values were Aromat de Iași/Ripariagloire (1.18) and Golia/Riparia gloire (1.36).

For the second report, between chlorophyll ($a + b$) / carotenoids, the variant that was highlighted in June, Aromat de Iași/Riparia gloire (4.32), was followed by Golia/Crăciunel 2 (3.84). In July the variants grafted on the Crăciunel 2 and SO₄₋₄ rootstock recorded the highest values, Aromat de Iași (4.97) and Golia (4.62). In the last analysis in August the variant Aromat de Iași/Crăciunel 2 (7.77), had the best result, while in the other variety taken into account the variant Golia/SO₄₋₄ (6.36) was highlighted.

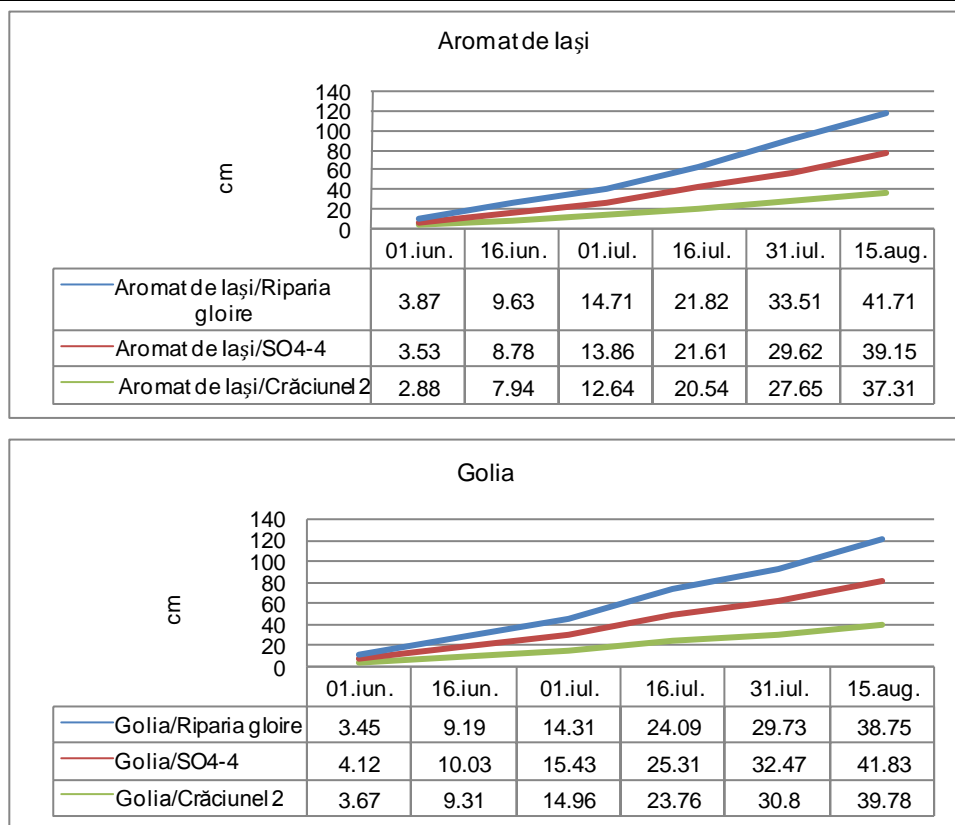
Table 3

Photosynthetic pigment reports

Scion	Rootstock	Chlorophyll a / b ratio			Chlorophyll ratio ($a + b$) / carotenoids		
		June	July	August	June	July	August
Aromat de Iași	Riparia Gloire	1.75	1.87	1.18	4.32	4.55	5.91
	Selection Oppenheim 4-clone 4 (SO ₄₋₄)	2.36	1.68	1.14	4.30	4.31	6.64
	Berlandieri x Riparia Selection Crăciunel 2	2.57	1.41	0.94	4.08	4.97	7.77
Golia	Riparia Gloire	2.60	2.12	1.36	3.78	4.19	5.55
	Selection Oppenheim 4-clone 4 (SO ₄₋₄)	2.83	1.65	1.16	3.83	4.62	6.36
	Berlandieri x Riparia Selection Crăciunel 2	2.75	1.77	1.24	3.84	4.47	5.82

In parallel with determining the content of foliar photosynthetic pigments, measurements were also made on the vegetative growths of the vines nursery. Six measurements were made, the first being on 01/06/2019. The measurements were made at an interval of 15 days from each other, the last being on 15/08/2019 (fig. 2).

From the above figure it can be observed that in the Aromat de Iași variety, the best results were recorded by the Aromat de Iași/Riparia gloire, on the other hand, in the Paula variety, the best results were recorded by the Golia/SO₄₋₄ variant for all the measurements made.

**Fig. 2** Dynamics of vegetative growth

At the first measurement performed on 01/06 the best results were recorded by the Golia/SO₄ variant which was 4.12 cm, and Aromat de Iași/Riparia gloire was 3.87 cm. The second measurement was made after 15 days, more precisely on 16/06, and the variants that had the largest increases were Golia/SO₄ (10.03 cm) and Aromat de Iași/Riparia gloire (9.63 cm). After another 15 days, the third measurement was made on 01/07, where the highest results were recorded in the Golia/SO₄ variant (15.43 cm), respectively Aromat de Iași/Riparia gloire (14.71 cm). The fourth measurement on 16/07 highlighted the variants Golia/SO₄ (25.31 cm) and Aromat de Iași/Riparia gloire (21.82). The fifth measurement on 31/07, highlighted the variants Golia/SO₄ (32.47 cm) and Aromat de Iași/Riparia gloire (33.51 cm), and on the last measurement on 15/08, the highest vegetative growths were in the Golia/SO₄ (41.83 cm) and Aromat de Iași/Riparia gloire (41.71 cm) variants.

Following the correlation between the vegetative growths and the amount of chlorophyll ($a + b$), a direct and linear relation is found, in the sense that the higher the content in the chlorophyll the higher the vegetative growths. This behavior was observed also in other situations, probably because of the mineral uptake stimulation by rootstocks (Fekete *et al.*, 2013). From the analysis of figure 3, it can be seen that the

values of the correlation coefficient (R^2) for the Aromat de Iași variety were 0.7826 for the grafted variant on the Riparia gloire rootstock, 0.7525 on the $SO_{4.4}$ rootstock and 0.9985 on the Crăciunel rootstock 2. For the other variety taken into consideration, Golia, the values of the correlation coefficient had values of 0.8602 in the variant grafted on the rootstock Riparia gloire, 0.9721 on the rootstock $SO_{4.4}$ and 0.9127 on the rootstock Crăciunel 2. In both cases the value of the coefficient correlation was over 75%, indicating that there is a direct linear correlation between the two factors analyzed.

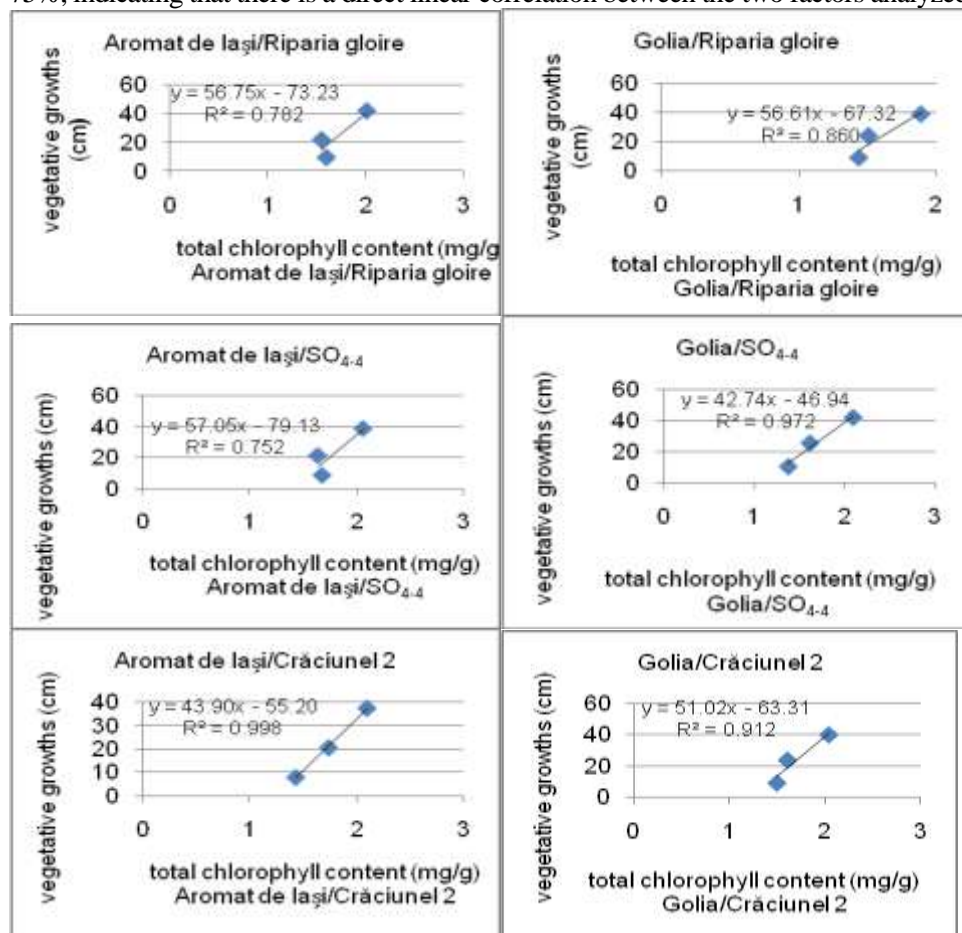


Fig. 3 Correlation between total chlorophyll content and vegetative growth of vines

CONCLUSION

From the analysis of the moisture of the vines, it is ascertained that it decreases as they go towards the senescence phase, however, they are kept within the physiological limits of the plant. The highest humidity was recorded in June on the Aromat de Iași/Crăciunel 2 version, and the lowest on the Golia/Riparia gloire version, in August.

Regarding the ratio of chlorophyll *a*/chlorophyll *b*, it is found that it is specific to a semi-shade plant, being the highest in June, between 1.75-2.57 in the variety Aromat de Iași and 2.60-2.80 in the Golia variety. As the plant grows older, it has lower values, due to the fact that here we are witnessing the development of a vine and an adult plant.

The ratio of chlorophyll (*a* + *b*)/carotenoids has been 4/1 higher since June, increasing as the age progresses, to 7.77 in the Aromat de Iași/Crăciunel 2 variant.

The correlation coefficient R² was calculated in order to establish the direct correlation between the vegetative growths and the total chlorophyll content. It had values indicating the existence of a direct and linear correlation between increasing the content of chlorophyll pigments and the vegetative growths of the vines, having values of over 0.75.

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