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NURSERY VEGETATIVE GROWTH OF 'GELU' AND 'PAULA' GRAPE VARIETIES, BY ANALYSIS OF FOLIARY PHOTOSYNTHETIC PIGMENTS

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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 table grapes 'Gelu' and 'Paula', 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, 'Gelu'/Riparia gloire variant recorded the best results in terms of vegetative growth in the vegetation period measuring 45.39 cm (before wood maturation) followed by 'Paula'/SO4-4, which recorded 37.32 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 'Gelu' variety on all rootstocks. 'Paula' grape variety registered smaller values.

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

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 inplants (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).

Photosynthetic pigments are represented by green chlorophyll pigments (chlorophylls a and b) and yellow carotenoid pigments (carotenes and xanthophylls), being essential compounds in light energy conversion (Toma and Jităreanu, 2007). 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 contributing 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).

Rootstock influences vegetative growth thereby increasing the photosynthesis of vine (Somkuwar et al., 2015; Köse and Çelik, 2017). The differences in vegetative growth patterns affect gas exchange by altering source/sink relations (Ezzahouani, 1995). The effect of rootstocks on photosynthetic activity is scion specific (During, 1994).

The study aims to track vegetative growth during the vegetation period depending on rootstock, measuring the content of chlorophylls (a and b) and carotenoids from the leaves of new vine varieties (*Vitis vinifera* L.) for table grapes 'Gelu' and 'Paula', new

created within the Research and Development Station for Viticulture and Winemaking Iaşi.

MATERIALS AND METHODS

In order to carry out this study, research was conducted within the Research and Development Station for Viticulture and Winemaking in Iaşi, in 2019. Two varieties of table grapes, 'Gelu' and 'Paula', obtained at SCDVV Iaşi (Table 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.

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.

Table 1. Studied biological material

Grape variety	Genitors	Author	Year of homologation
 Gelu	Free fecundation of local grape variety Coarnă neagră and irradiation with X rays of its seeds	Calistru Gheorghe Damian Doina	1999
 Paula	Intraspecific sexual hybridation of Bicane x Aromat de Iaşi	Calistru Gheorghe Damian Doina	1997

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.

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

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

Carotenoids (μg mL^{-1}) = (1000 × (A470 – A710) – 1.90× Chl *a* – 63.14 × 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 of 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 (Table 2).

From the table above 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. In June, the highest values were recorded for the variants 'Paula'/Crăciunel 2 (78.12%) and 'Gelu'/Crăciunel 2 (75.59%),

Table 2. Leaves moisture (%)

Scion	Rootstock	Humidity (%)		
		June	July	August
Gelu	Riparia Gloire	74.19	73.85	71.28
	Selection Oppenheim 4-clone 4 (SO_{4-4})	75.13	74.12	70.00
	Berlandieri x Riparia Selection Crăciunel 2	75.59	72.68	70.42
Paula	Riparia Gloire	74.84	73.81	72.87
	Selection Oppenheim 4-clone 4 (SO_{4-4})	76.56	75.16	71.67
	Berlandieri x Riparia Selection Crăciunel 2	78.12	76.79	73.91

after which they decreased slightly and the best results were in July registered in the 'Paula'/Crăciunel 2 (76.79%) and 'Gelu'/SO_{4-4} (74.12%) variants.

In August the results were even lower compared to the previous months, the best values being obtained at 'Paula'/Crăciunel 2 (73.91%) and 'Gelu'/Riparia gloire (71.28%).

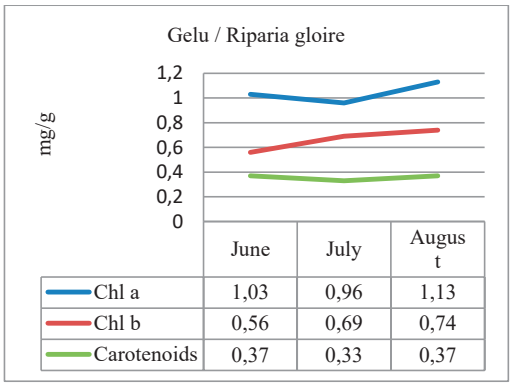
After determining the humidity of the harvested leaves, the analysis of the foliar photosynthetic pigments was studied (Figure 1).

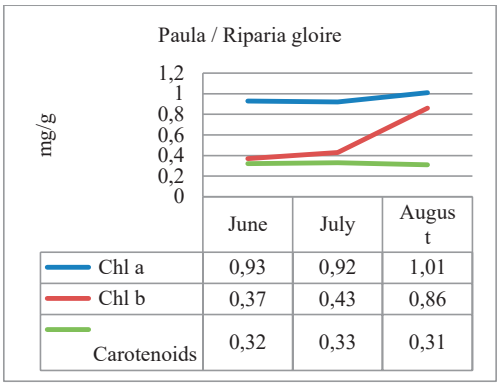
Regarding the determination of the content of photosynthetic pigments (chlorophyll a and b, respectively carotenoids), the best results were obtained in the 'Gelu' variety for all the variants on which it was grafted, compared to the 'Paula' variety which had weaker results.

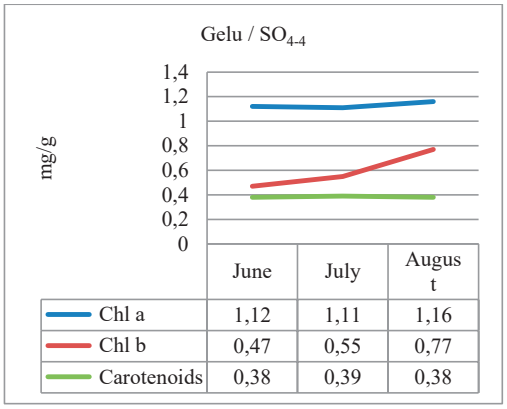
Chlorophyll a, in June, in the variant Gelu/SO_{4-4} had the highest values of 1.12 mg/g fresh substance, and in the Paula variety the best result was recorded at the grafting with Riparia gloire having 0, 93 mg/g fresh substance. For chlorophyll b, the best results were recorded for the variants 'Gelu'/Riparia gloire (0.56 mg/g) and 'Paula'/SO_{4-4} (0.39 mg/g), and for the carotenoids the best results were recorded at 'Gelu'/SO_{4-4} (0.38 mg/g) and 'Paula'/Riparia gloire (0.32 mg/g).

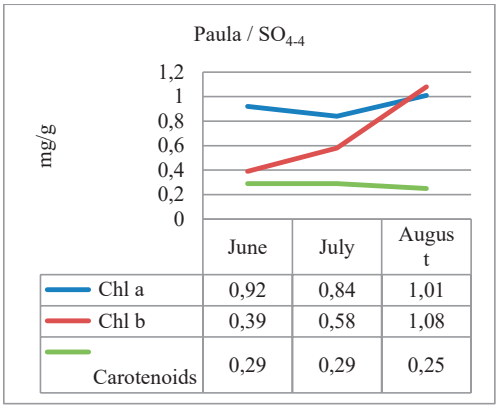
The determination of the content of photosynthetic pigments from the leaves was carried out in July, where for the chlorophyll the best results were obtained by the variants 'Gelu'/SO_{4-4} (1.11 mg/g), respectively 'Paula'/Riparia gloire (0.92 mg/g). Chlorophyll b, recorded higher values compared to the previous month for all variants taken into consideration, and the best results were obtained at 'Gelu'/Riparia gloire (0.69 mg/g) and 'Paula'/SO_{4-4} (0.58 mg/g). For the last pigment analyzed, the carotenoids, the variants that were highlighted were 'Gelu'/SO_{4-4} (0.39 mg/g) and 'Paula'/Riparia gloire (0.33 mg/g).

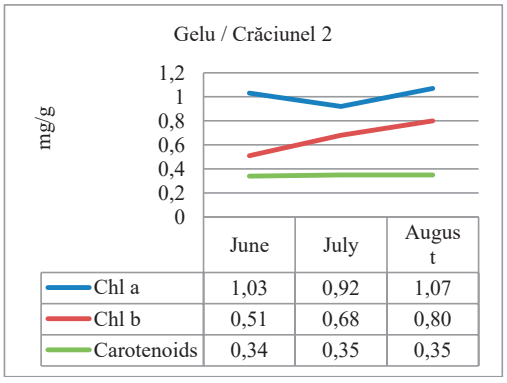
The last determination of the content of the photosynthetic pigments from the leaves was made in August, before the process of maturation of the shoots and of the appearance of the phellogen, the results obtained being higher in comparison with the other two months in which they were analyzed. For chlorophyll a, the best results were recorded for the variants 'Gelu'/SO_{4-4} (1.16 mg/g) and 'Paula/Riparia gloire respectively 'Paula'/SO_{4-4} at which the same values were obtained (1.01 mg/g). Chlorophyll b was found in the leaves of the Paula/SO_{4-4} variant (1.08 mg/g) and Gelu/Crăciunel 2 (0.80 mg/g), and the last pigment analyzed, the carotenoids, had the highest results in the 'Gelu' variant./SO_{4-4} (0.38 mg/g) and 'Paula'/Riparia glory (0.31 mg/g).











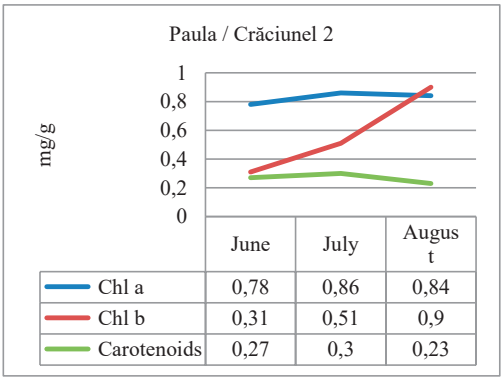


Figure 1. Dynamics of the content of photosynthetic pigments

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 ratiosof 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 (Table 3).

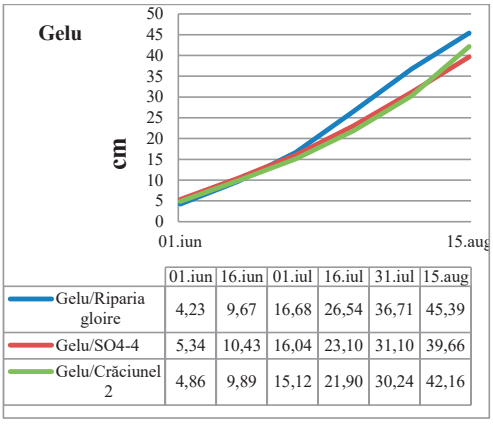
Table 3. Photosynthetic pigment reports

Scion	Rootstock	Chlorophyll a/b ratio			Chlorophyll ratio (a + b) / carotenoids		
		June	July	August	June	July	August
Gelu	Riparia Gloire	1.83	1.39	1.52	4.29	5.00	5.05
	Selection Oppenheim 4-clone 4 (SO_{4-4})	2.38	2.01	1.50	4.18	4.25	5.07
	Berlandieri x Riparia Selection Crăciunel 2	2.01	1.35	1.33	4.52	4.57	5.34
Paula	Riparia Gloire	2.51	2.13	1.17	4.06	4.09	6.03
	Selection Oppenheim 4-clone 4 (SO_{4-4})	2.30	1.44	0.93	4.51	4.89	8.36
	Berlandieri x Riparia Selection Crăciunel 2	2.51	1.68	0.93	4.03	4.56	7.56

The chlorophyll a/b ratio for the Gelu variety in June registered the highest value (2.38) at grafting on the SO_{4-4} rootstock while the same ratio for the Paula variety, obtained the best result (2.51), both at the grafting on the rootstock Riparia Gloire and on the rootstock Crăciunel 2. In July both varieties had the best values of the ratio of chlorophyll a / b to the variants Gelu/SO_{4-4} (2.01) and Paula/Riparia gloire (2.13), and in August the variants with the highest values were Gelu/Riparia gloire (1.52) and Paula / Riparia gloire (1.17).

For the second report, between chlorophyll (a + b) / carotenoids, the variant that was highlighted in June, Gelu / Craciunel 2 (4.52), was closely followed by Paula/SO_{4-4} (4.51). In July the variants grafted on the SO_{4-4} rootstock recorded the highest values, Paula (4.89) and Gelu (4.25). In the last analysis in August the variant Paula/SO_{4-4} (8.36), had the best result, while in the other variety taken into account the variant Gelu/Crăciunel 2 (5.34) was highlighted.

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 (Figure 2).



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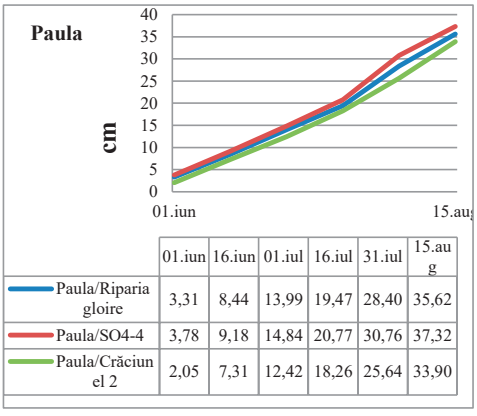
Gelu/Riparia gloire	4,23	9,67	16,68	26,54	36,71	45,39
Gelu/SO4-4	5,34	10,43	16,04	23,10	31,10	39,66
Gelu/Crăciunel 2	4,86	9,89	15,12	21,90	30,24	42,16



	01.iun	16.iun	01.iul	16.iul	31.iul	15.aug
Paula/Riparia gloire	3,31	8,44	13,99	19,47	28,40	35,62
Paula/SO4-4	3,78	9,18	14,84	20,77	30,76	37,32
Paula/Crăciunel 2	2,05	7,31	12,42	18,26	25,64	33,90

Figure 2. Dynamics of vegetative growth

From the above figure it can be observed that in the 'Gelu' variety, the first two measurements the largest vegetative growths were recorded at the graft on the SO_{4-4} rootstock, after which the 'Gelu'/Riparia gloire variant had the most significant increases. On the other hand, in the 'Paula' variety, the best results were recorded by the 'Paula'/SO_{4-4} variant for all the measurements made.

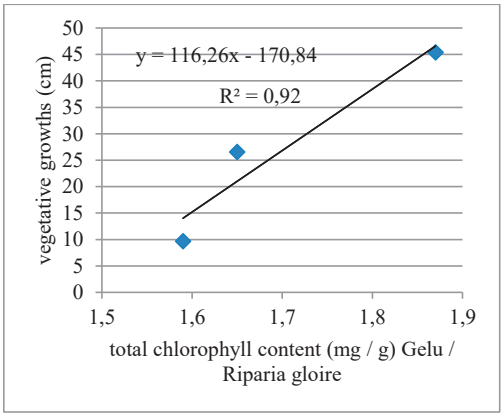
At the first measurement performed on 01/06 the best results were recorded by the 'Gelu'/SO_{4-4} variant which was 5.34 cm, and

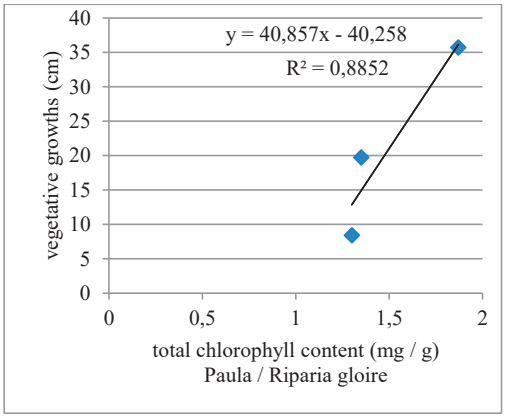
'Paula'/SO_{4-4} was 3.78 cm. The second measurement was made after 15 days, more precisely on 16/06, and the variants that had the largest increases were 'Gelu'/SO_{4-4} (10.43 cm) and 'Paula'/SO_{4-4} (9.18 cm).

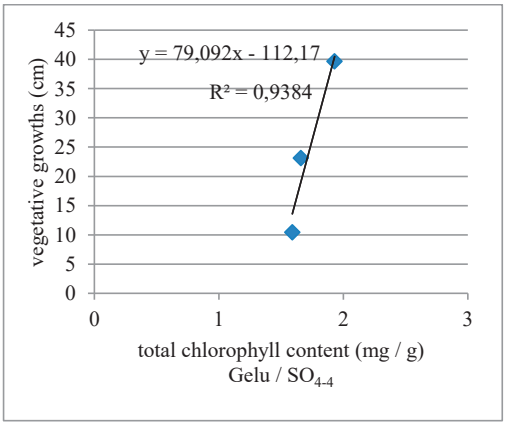
After another 15 days, the third measurement was made on 01/07, where the highest results were recorded in the 'Gelu'/Riparia gloire variant (16.68 cm), respectively 'Paula/SO_{4-4} (14.84 cm). The fourth measurement on 16/07 highlighted the variants Gelu/Riparia gloire (26.54 cm) and Paula / SO_{4-4} (14.84).

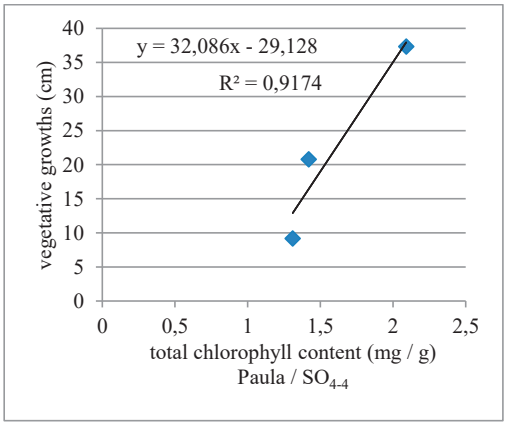
The fifth measurement on 31/07, highlighted the variants 'Gelu'/Riparia gloire (36.71 cm) and 'Paula'/SO_{4-4} (30.76 cm), and on the last measurement on 15/08, the highest vegetative growths were in the 'Gelu'/Riparia gloire (45.39 cm) and 'Paula'/SO_{4-4} (37.32 cm) variants.

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 (R2) for the Gelu variety were 0.9200 for the grafted variant on the Riparia gloire rootstock, 0.9384 on the SO_{4-4} rootstock and 0.9583 on the Crăciunel rootstock 2. For the other variety taken into consideration, Paula, the values of the correlation coefficient had values of 0.8852 in the variant grafted on the rootstock Riparia gloire, 0.9174 on the rootstock SO_{4-4} and 0.9995 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.









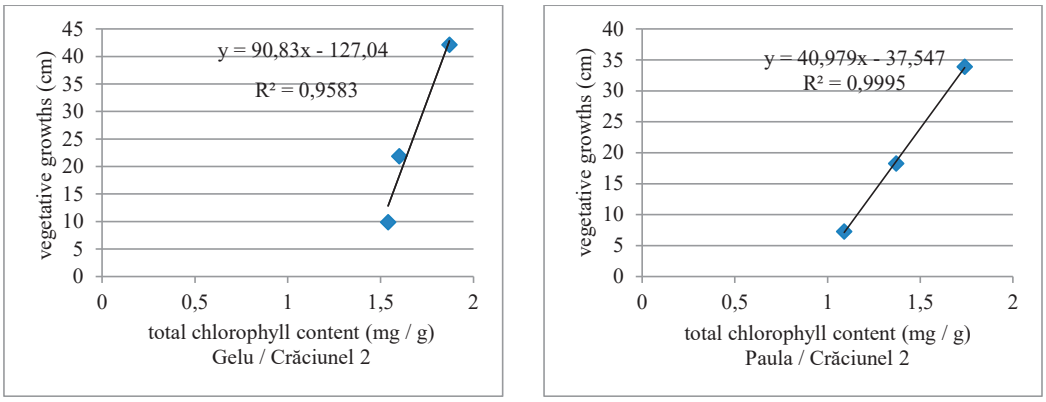


Figure 3. Correlation between total chlorophyll content and vegetative growth of vines

CONCLUSIONS

From the analysis of the moisture of studied vine leaves, it is ascertained that it decreases as they go towards the senescence phase; however, the values are kept within the physiological limits of the plant. The highest humidity was recorded in June on the 'Paula'/Crăciunel 2 version, and the lowest on the 'Gelu/'SO_{4-4} 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 1.83-2.38 in the variety 'Gelu' and 2.30-2.51 in the Paula 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 8.36 in the Paula/SO_{4-4} variant. The correlation coefficient R^2 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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