ASPECTS CONCERNING THE RELATIONSHIP BETWEEN PHOTOSYNTHETIC PIGMENTS AND SOLUBLE SUGARS AMOUNT OF SOME *PRUNUS AVIUM* CULTIVARS

ASPECTE PRIVIND RELAŢIA DINTRE CONŢINUTUL DE PIGMENŢI ASIMILATORI ŞI CANTITATEA DE GLUCIDE SOLUBILE LA UNELE SOIURI DE *PRUNUS AVIUM*

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Abstract. *The life essence of a photosynthetic organism is its assimilating pigments. Without them the light can`t be absorbed and thus no energy can be stored. This is why chlorophylls have been the subject of many years of research. Since more than two thirds of woody plant dry matter consists of processed sugars, their growth and development depends fundamentally by the synthesis of carbohydrates, their transport to the 'sinks' from 'sources' and systematic uptake by new tissues. Analyzing the results obtained we found differences in assimilating pigments contents of the cherry varieties taken in the study. The values of assimilating pigments quantities ranged from 5.222 mg·g*$^{-1}$ *(DW) in the 'Germersdorf' variety to 10.358 mg·g*$^{-1}$ *(DW) in the 'Van' variety. There were also significant differences in the levels of soluble carbohydrate content present in the leaves and cherry fruits of the same cherry varieties.*

Key words: chlorophyll a, chlorophyll b, carotenoids, soluble sugars.

Rezumat. *Esenţa vieţii unui organism fotosintetic sunt pigmenţii asimilatori ai acestuia. Fară ei lumina nu poate fi absorbită şi, astfel, nici energia nu poate fi stocată. De aceea, clorofilele sunt de mulţi ani subiectul numeroaselor cercetări. Deoarece mai mult de două treimi din substanţa uscată a plantelor lemnoase constă din zaharuri transformate, creşterea şi dezvoltarea lor depinde fundamental de sinteza carbohidraţilor, transportul acestora de la 'surse' la 'utilizatori' şi asimilarea sistematică în ţesuturile noi. Analizând rezultatele obţinute, am constatat diferenţe ale conţinutului de pigmenţi asimilatori la soiurile de cires luate în studiu. Valorile cantităţilor de pigmenţi asimilatori au fost cuprinse între 5.222 mg·g*$^{-1}$ *(S.U.) la soiul Germersdorf şi 10.358 mg·g*$^{-1}$ *(S.U.) la soiul Van. De asemenea, au fost înregistrate diferenţe semnificative ale valorilor conţinutului de glucide solubile prezente in fruzelele şi fructele soirilor de cireş luaţi în studiu.*

Cuvinte cheie: clorofila a, clorofila b, carotenoizi, glucide solubile.

INTRODUCTION

The lifeblood of a photosynthetic organism is its pigments. Without them light cannot be absorbed, and therefore energy cannot be stored. The biological process whereby the Sun's energy is captured and stored by a series of events that convert the pure energy of light into the biochemical energy needed to power life was called *photosynthesis*. Photosynthesis means literally "synthesis with light" as such it might be construed to include any process that involved synthesis of a new species of chemical compounds under the action of light. The most common form of photosynthesis involves chlorophyll-type pigments, and operates using light-driven electron transfer processes. The organisms will be considered to carry out what we will term “chlorophyll-based photosynthesis”.

Photosynthesis uses light from the Sun to drive a series of chemical reactions. The Sun, like all stars, produces a broad spectrum of light output that ranges from gamma rays to radio waves. Only some of the emitted solar light is visible to our eyes, consisting of light with wavelengths from 400 to 700 nanometers (nm). The entire visible range of light, and some wavelengths in the near infrared (700 to 1000 nm), are highly active in driving photosynthesis in certain organisms, although the most familiar chlorophyll *a*-containing organisms cannot use light longer than 700 nm. The spectral region from 400 to 700 nm is often called *photosynthetically active radiation*, although this is only strictly true for chlorophyll *a*-containing organisms.

Thus, knowing the importance of assimilating pigments and their role in the life of plants, we aimed to determine their content in 14 cherry varieties and hybrids, the ratio of the chlorophyll pigments and carotenoids, and correlate the results with pedo-climatic zone of culture. Also, we intended to pursue the relationship between the content of assimilating pigments and soluble carbohydrates content to highlight a possible correlation between these two parameters of studied cherry varieties and hybrids.

MATERIAL AND METHOD

Our analysis was performed on 10 varieties and 4 hybrids (hybrids` author is Ph.D. Petre Ludovic) from Cherry National Germplasm Collection of Research and Development Department of Pomology, Iasi, Romania.

Assimilating pigments extraction has been realized in 85 % acetone. The readings were done using T70 UV-VIS Spectrophotometer.

Soluble sugar measurement has been made as follows: from cherry trees leaves fine grinded samples soluble sugars were extracted by hot ethanol (3 replicates). Five mL of 70% (v/v) ethanol were mixed by shaking it with 300 mg of grinded stem tissues in a test tube. The sample was then incubated at 80 ˚C for 20 min. by shaking and then centrifuged for 5 min. at 1500×g. Freshly prepared anthrone reagent (0.2% anthrone in concentrated H_2SO_4) was pipetted into a test tube and chilled in ice water. The extract was thoroughly mixed with the anthrone reagent, the tube heated in boiling water for 15 min. and then rapidly cooled. The absorbance was read at 630 nm. Total soluble sugar content was calculated as *g* per 100*g* biomass unit.

REZULTS AND DISCUSSIONS

The molecular chemical formula for chlorophyll *a* is $C_{55}H_{72}N_4O_5Mg$. This simple representation is entirely inadequate to convey the essential properties of this extraordinary molecule. It is a squarish planar molecule, about 10 Å on a side. The Mg atom in the center of the planar portion is coordinated to four nitrogen atoms. The nitrogen atoms are each part of a substructural element of the molecule that is derived from pyrrole, a cyclic organic compound with a nitrogen atom in a five-numbered ring with four carbons. For this reason, chlorophylls and related compounds are often referred to as tetrapyrroles. A fifth ring is formed in the lower right corner, and a long hydrocarbon tail is attached to the lower left (in the standard representation). Chemically, the chlorophylls are related to the porphyrins, which are also tetrapyrroles, but the porphyrins are generally more symmetric molecules.

Chlorophyll *b* is identical to chlorophyll *a* except at the C-7 position, where a formyl group replaces the methyl group. This change shifts the maximum absorption to shorter wavelengths.

Carotenoids are found in all known native photosynthetic organisms, as well as in many nonphotosynthetic organisms (Frank *et al.*, 2000). There are many hundreds of chemically distinct carotenoids. However, there are some consistent structural features that are common to most photosynthetic carotenoids. They are extended molecules with a delocalized *p* electrons system.

Experimental results from *table 1* show significant differences of the assimilating pigments content to the varieties and hybrids of cherry note study. The lowest values of chlorophyll amounts were recorded in variety Germersdorf and were around 3.18 $mg \cdot g^{-1}$ (DW) for chlorophyll *a*, 0.90 $mg \cdot g^{-1}$ (DW) for chlorophyll *b* and 1.14 $mg \cdot g^{-1}$ (DW) carotenoid pigments. Thus, registering a total amount of about 5.22 $mg \cdot g^{-1}$ (DW) pigments assimilated into the leaves of this variety of cherry. Maximum values of chlorophyll pigments and carotenoids was registered on Van cultivar and were about 5.47 $mg \cdot g^{-1}$ (DW) for chlorophyll *a*, 2.85 $mg \cdot g^{-1}$ (DW) for chlorophyll *b* and 2.04 $mg \cdot g^{-1}$ (DW) for carotenoids. Total assimilating pigments reached 10.358 $mg \cdot g^{-1}$ (DW).

Although the chlorophyll *b* is the major accessory light-absorbing pigment in the majority of eukaryotic photosynthetic organisms, with the exception of the red and brown algae, we obtained some higher values of carotenoids (a quantosome contains 160 chlorophyll *a* molecules + 70 chlorophyll *b* molecules + 48 molecules of carotenoids etc. (Park R.B. and Biggins J. 1964) in all varieties and hybrids. It is known that carotenoids have several well-documented essential functions in photosynthetic systems. First, they are accessory pigments in the collection of light, absorbing light and transferring energy to a chlorophyll-type pigment. Most antenna complexes contain carotenoids. Second, carotenoids function in a process called *photoprotection*. Carotenoids rapidly quench triplet excited states of chlorophylls before they can react with oxygen to form the highly reactive and damaging excited singlet state of oxygen. They also quench the singlet oxygen if it is somehow formed.

Table 1

Assimilating pigments content of some cherry varieties and hybrids (*Prunus avium* L.)

Varieties	Chl. *a* $mg \cdot g^{-1}$ DW	Chl. *b* $mg \cdot g^{-1}$ DW	Carotenoids $mg \cdot g^{-1}$ DW	Σ
Van	5.47±0.52	2.85±0.18	2.04±0.17	10.36
Stella	4.75±0.42	1.35±0.12	1.48±0.12	7.58
Maria	3.78±0.36	1.20±0.10	1.34±0.11	6.32
Bucium	4.36±0.38	1.20±0.11	1.47±0.12	7.03
Rivan	4.58±0.41	1.27±0.14	1.46±0.12	7.30
George	3.90±0.33	1.08±0.08	1.30±0.11	6.28
Golia	3.63±0.31	1.10±0.09	1.22±0.10	5.95
New Star	4.74±0.44	1.42±0.12	1.45±0.12	7.61
Boambe de Cotnari	3.78±0.35	1.15±0.11	1.25±0.11	6.17
Germersdorf	3.18±0.29	0.90±0.06	1.14±0.10	5.22
H.C. 840808	3.59±0.31	1.02±0.08	1.29±0.13	5.91
H.C. 840933	4.23±0.39	1.20±0.10	1.44±0.12	6.87
H.C. 871616	4.60±0.42	1.34±0.11	1.51±0.13	7.44
H.C. 893705	4.80±0.45	1.42±0.12	1.63±0.14	7.85

Each value is shown as the mean ± S.D. of 12 samples.
DW – dry weight.

Finally, carotenoids have recently been shown to be involved in the regulation of energy transfer in antennas. So, as a preliminary conclusion, we can say that both cherry varieties and hybrids had a high photosynthetic activity and efficiency and also, due to high concentration of carotenoids, an effective photoprotection mechanism.

All chlorophyll-based photosynthetic organisms contain light-gathering antenna systems (Green and Parson, 2001). These systems function to absorb light and transfer the light's energy to a trap, which quenches or deactivates the excited state. Light-induced electron transport generates ATP and NADPH, which are high-energy compounds of intermediate stability. But they are not suitable for long term storage of energy, such as building plant biomass, or for storage in seeds, tubers or fruits. For these, it is necessary to convert the energy into a more stable form. Most plants produce sugars or more complex carbohydrates such as starch for long-term energy storage, although some plants produce large quantities of proteins or oils. Some of these conversions are carried out within the chloroplast, while in other cases they take place in the cell cytoplasm from building blocks that are exported from the chloroplast.

Carbon fixed by the Calvin cycle is processed for longer-term storage in two distinct forms. One of these forms is starch, which is made and stored, during the day, in the chloroplast. The other form is sucrose, which is made in the cytoplasm. Both processes take place at significant rates and the interplay between them is highly regulated. In both cases, the storage product is a nonreducing oligosaccharide that is not phosphorylated. The monosaccharide glucose is rather easily oxidized and it's not suitable as a storage product.

Soluble sugars (fructose, glucose, raffinose, sorbitol, stachyose, sucrose) content synthesized in leaf cells (*table 2*) ranged out 3.78% (DW) on cherry hybrid HC 893705 and 7.89% (DW) on 'Golia' variety. A higher amount of foliar soluble sugars (over 7% DW) showed on varieties 'Stella' (7.85%), 'Boambe de Cotnari' (7.39%), 'Georgia' (7.25%), cherry hybrids HC 840808 (7.17%) and H.C. 871616 (7.04%). That's why we tend to believe that a higher amount of sugars is produced by trees which have a bigger content of assimilating pigments in their leaves. But, analyzing *figure 1* we can see another picture from which we expect. So, for example, variety 'Van' had the biggest content of assimilating pigments ($10.36g\cdot100g^{-1}$ DW) but soluble sugars amount is far to be the biggest one in leaves ($5.86g\cdot100g^{-1}$ DW) and fruit flesh ($12g\cdot100g^{-1}$ DW). On the other hand, variety 'Golia' showed almost the lowest content of assimilating pigments ($5.95g\cdot100g^{-1}$ DW) and the biggest amount of soluble sugars in leaves ($7.89g\cdot100g^{-1}$ DW) and, also, a bigger one in fruit flesh ($14g\cdot100g^{-1}$ DW).

Table 2

Soluble carbohydrate content of leaves and fruits of some sweet cherry varieties and hybrids (*Prunus avium* L.)

Varieties	**Leaf soluble sugars** *$g\cdot100g^{-1}$ DW*	**Fruit flesh soluble sugars** *$g\cdot100g^{-1}$ FW*	**Leaf soluble sugars/fruit flesh soluble sugars ratio**
Van	5.86±0.47	12±0.87	2.0
Stella	7.85±0.68	14±0.95	1.8
Maria	6.23±0.56	14±0.93	2.2
Bucium	5.49±0.42	14±0.98	2.6
Rivan	4.64±0.33	16±1.02	3.4
George	7.25±0.61	15±1.07	2.1
Golia	7.89±0.65	14±0.99	1.8
New Star	6.84±0.54	13±0.98	1.9
Boambe de Cotnari	7.39±0.61	17±1.21	2.3
Germersdorf	6.95±0.56	14±1.03	2.0
H.C. 840808	7.17±0.60	14±0.89	2.0
H.C. 840933	6.65±0.53	15±1.12	2.3
H.C. 871616	7.04±0.59	15±1.17	2.1
H.C. 893705	5.86±0.47	16±1.18	4.2

Each value is shown as the mean ± S.D. of 12 samples for leaves and 3 for fruits.
DW – dry weight.

So, by performing this analysis we found that it is not necessary for a bigger content of assimilating pigments to have a higher amount of soluble sugars in tree`s leaves cells. We believe that it is rather due to cells osmotic pressure pattern and stress adaptation. Therefore, a certain average concentration of soluble sugars in leaves cells during vegetative period can be considered a marker for each species and their varieties. Plants resort many adaptive strategies in response to abiotic environmental stress such as dehydration and excessive osmotic pressure.

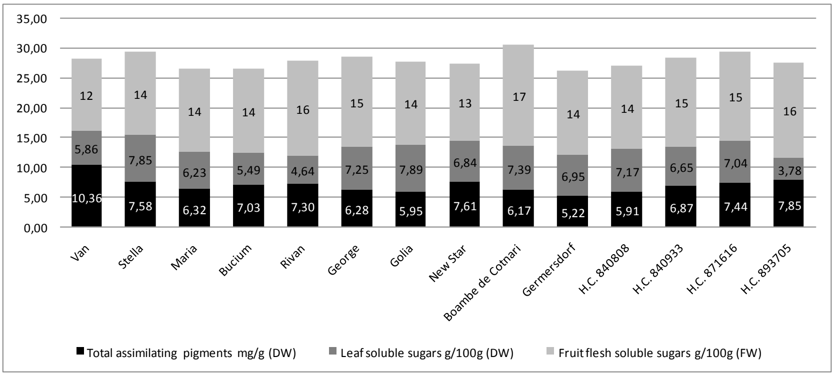

Fig. 1. Assimilating pigments, leaf and fruit soluble sugars amount of some *Prunus avium* L. varieties and hybrids

These adaptive mechanisms include changes in physiological and biochemical processes. Adaptation to stress is associated with metabolic adjustments that lead to the accumulation of several organic solutes like sugars, polyols, betaines and proline (Yancey et all, 1982).

CONCLUSIONS

Performing our analysis data on 10 cherry varieties and four hybrids we observed that the assimilating pigments average content during vegetative period are not correlated with leaves soluble sugars amount.

We believe that the content of soluble sugars in leaves is rather due to cells osmotic pressure pattern and stress adaptation of each studied varieties.

Acknowledgement:

This study has been financed by the National Department of Programs Management, Grant No. 52-151/2008, with title "*Genetical and biochemical researches concerning sweet cherry assortment breeding for a better agro productivity and quality*".

REFERENCES

1. **Blankenship E. R., 2002** – *Molecular mechanisms of photosynthesis*. Published by Blackwell Science Ltd.
2. **Frank H., Young A. J., Britton G. and Cogdell R. J., 2000** – *The Photochemistry of Carotenoids: Applications in Biology.* Kluwer Academic Publishers, Dordrecht.
3. **Green B. R. and Parson W. W., 2001** – *Light-Harvesting Antennas*. Kluwer Academic Press, Dordrecht.
4. **Park R.B. and Biggins J., 1964** – *Quantasome size and composition*. Science 144, pp. 1009–1011
5. **Yancey P.H., Clark M.E., Hand S.C., Bowlus R.D. and Somero G.N., 1982** – *Living with water stress: Evolution of osmolyte system*. Science, 217: 1214-122.