COMPARATIVE STUDY OF BEHAVIOUR OF SOME BIOCHEMICAL PARAMETERS IN DIFFERENT PHENOPHASES OF SEABUCKTHORN CULTIVARS

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Abstract: Seabuckthorn is a shrub possible to be entirely capitalized because of the presence of biologically active principles in all plant organs. The seabuckthorn offshoots can be added as supplements in animal feed. For this reason, the study of chemical profile by quantification of total lipids, soluble proteins and assimilatory pigments is important. Our results established that the values of investigated biochemical parameters depend on phenophase when the biological material was harvested and also on analyzed cultivar. The biosyntheses are certainly more intensely in fruit maturation phenophase.

INTRODUCTION

Seabuckthorn (*Hippophae rhamnoides* L.) is, due to the high and diverse content in bioactive principles, a valuable biological material from medicinal and nutritive points of views as well as by its capability to influence the microclimate. The content and proportion of these components are variable and dependent on a variety of factors. Seabuckthorn is also a species of great interest because, unlike other plants, it can be entirely used (fruits, leaves, bark, and roots). In Romanian spontaneous flora, the species accommodates on the poor, dry and even brackish soils, without special preferences for lithologic substratum (Ciobanu, 2003; Bereşiu et al., 1983).

In this paper are presented the results of research carried out on offshoots from six seabuckthorn cultivars harvested from SC FRUCTEX SA Bacău with the aim to realize the chemical profile of plants – total lipids, soluble proteins, assimilatory pigments – in view of offshoots use as source of biologically active principles for animal feed supplement. In anterior research we carried studies about the biochemical profile for the fruits of these six seabuckthorn cultivars Olteanu et al., Zamfirache et al., 2007; Oprică et al., 2007).

MATERIAL AND METHOD

Biological material was represented by individuals belonging to six seabuckthorn cultivars from SC FRUCTEX SA Bacau, as follows: Şerpeni – variant **1,** Şerbăneşti I – variant **2,** Şerbăneşti 4 – variant **3,** Ciumaş – variant **4,** Delta 60M – variant **5,** Sfântu Gheorghe – variant **6**. The biochemical determinations were performed in two plant phenophases: offshoots maximum growth and fruit maturation. The leaves have been harvested from one year old offshoots as well as from two years old offshoots (in this case, leave from superior and from basal region of offshoot). The leaves have been entirely harvested and average samples were realized, from which biological material was used to estimate the biochemical parameters.

To determine the quantity of total lipids, the Soxhlet gravimetric method was used. The principle of this method consists in extraction of total lipids from analysed material at warm, with specific organic solvents, especially with petroleum ether (Artenie and Tanase, 1981; Cenkowski et al., 2006).

Bradford method, used to establish the content of soluble proteins, is based on colour reaction of proteins with Coomassie Brilliant Blue G250 (Bradford, 1976).

The assimilatory pigments have been analysed by spectrophotometer method based on multistep pigments extractions with acetone followed by their determination at wavelengths specific to each category of pigments.

RESULTS AND DISCUSSIONS

Hippophae rhamnoides L. have a high lipid level both in fruits and in seeds. Seabuckthorn oil is known as having important curative effects. Our results evidence a higher level of total lipids in leaves from offshoots basal region, comparatively with those detached from superior region (Fig. 1), excepting Delta 60M and Sfântu Gheorghe cultivars (variants noted 5, respectively 6) where the situation is inverse concerning the foliar lipid content. As general tendency, o higher intensity of lipid biosynthesis in inferior - more mature - leaves was observed.

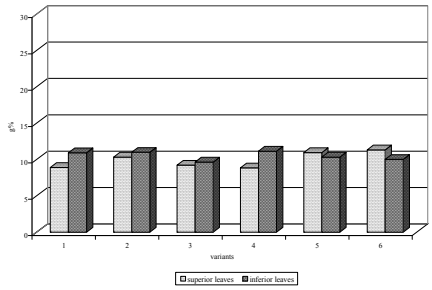

Figure 1. Variation of leaf total lipid content in different seabuckthorn cultivars, in maximum growth phenophase of offshoots

In a graphical representation of the average determinations, it is visible the existence of small variations of total lipid content in leaves harvested from one year offshoots (Fig. 2). Comparable values have on one hand Serbăneşti I, Delta 60M, and Sfântu Gheorghe cultivars and on other hand Şerpeni, Serbăneşti 4, and Ciumaş cultivars.

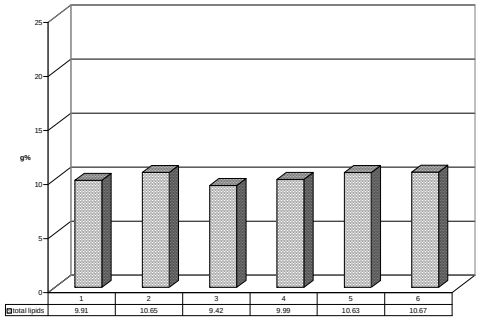

Figure 2. Variation of average leaf lipid content in different seabuckthorn cultivars, in maximum growth phenophase of offshoots

In fruit maturation phenophase, the lipid content variations depend on analyzed cultivar (Fig. 3). So, in Şerpeni, Şerbăneşti I and Sfântu Gheorghe cultivars, in leaves from one year offshoots and from superior half of two years offshoots a lower lipid biosynthesis takes place than in those with inferior placement. In Serbăneşti 4, Ciumaş, and Delta 60M seabuckthorn cultivars an inverse behaviour is evidenced. By comparing the results registered for the two phenophases, we see that the tendency noted for Şerpeni, Şerbăneşti I and Delta 60M cultivars is maintained.

Regarding the average values of lipid levels in fruit maturation phenophase, a considerable increase of value of investigated parameter appeared (Fig. 4). The increase amplitudes are different, depending on analyzed cultivar. The highest increase (2.38 fold) was registered for Ciumaş cultivar, the smallest one (1.71 fold) for Sfântu Gheorghe cultivar.

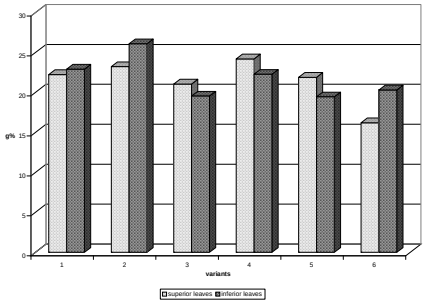

Figure 3. Variation of leaf total lipid content in different seabuckthorn cultivars, in fruit maturation phenophase

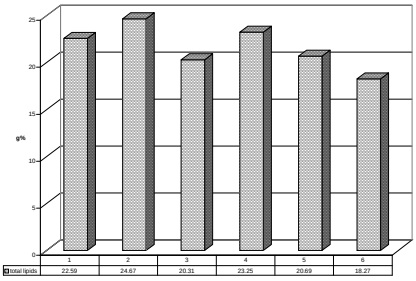

Figura 4. Variation of average leaf lipid content in different seabuckthorn cultivars, in fruit maturation phenophase

Leaf assimilatory pigments, with their specific fractions (chlorophyll *a*, chlorophyll *b*), represent the main antennae of higher plants to capture and transform light energy into chemical energy, incorporated in chemical bonds of the products newly synthesized during photosynthesis process. The average content of assimilatory pigments of investigated *Hippophae* cultivars (leaves harvested from one year old offshoots) presents quantitative variations typical for each of them (Fig. 5)

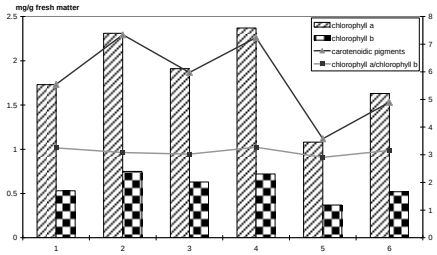

Figure 5. Variation of average content of chlorophyll and carotenoid pigments and of chlorophyll *a*/ chlorophyll *b* in seabuckthorn cultivars (2007)

The maximum quantity of chlorophyll *a* is registered in Şerbăneşti I (2) and Ciumaş (4) cultivars. The lowest content of chlorophyll *a* is recorded in the leaves of Delta 60 M (5) cultivar.

The other investigated varieties display a content of chlorophyll *a* comprised between 1.63 mg/g and 1.91 mg/g fresh matter.

The variability of chlorophyll *b* content is smaller than that of chlorophyll *a*. The values are generally 0.7 mg/g fresh matter. Delta 60 M (5) cultivar must be noted in this case by the absolute minimum value registered in connection with the biosynthesis and accumulation of this type of pigment (0.37 mg/g fresh matter).

Chlorophyll undergoes a continuous process of biosynthesis and biodegradation. As a result, the chlorophyll is permanently renewed in the leaves of higher plants, to a rate of 40% over 2-3 days (Burzo et al., 2004). In higher plants, quantitative preponderance of chlorophyll *a*, comparatively to chlorophyll *b*, imprint to the chlorophyll *a*/chlorophyll *b* ratio values of about 3:1 or even 3:2; in this manner, this ratio offers some indications on the optimum degree of lighting assuring an adequate plant growth and development (Burnea et al., 1977). In this context, it must be specified the fact that chlorophyll *a*/chlorophyll *b* and carotene/xanthophylls ratios reach higher values in the leaves of heliophylous plants compared with those of ombrophyilous plants, where chlorophyll *b* and xanthophylls are in larger quantities (Murariu, 2002).

In our experiments, the chlorophyll *a*/chlorophyll *b* ratio is generally slightly superior to 3 because to quantitative preponderance of chlorophyll *a* compared with chlorophyll *b*. Delta 60 M cultivar have also in this case the minimum registered amount, even if in terms of value, it remains close to the above mentioned amount. By the evolution of the chlorophyll *a*/chlorophyll *b* ratio, the investigated seabuckthorn cultivars are the behaviour of sun-loving plants.

The leaf carotenoid pigments accompanying the chlorophylls and being part of plant photosynthetic apparatus have superior values to chlorophylls in all investigated cultivars. The 2 and 4 conventionally noted varieties show the maximum values, while the variant 5 has a minimum value.

The high content of leaf carotenoid pigments, evidenced in all investigated seabuckthorn cultivars, could be motivated by their function to protect the chlorophylls against destructive action of ultraviolet radiation from solar spectrum (Burzo et al., 2005). During 2007 summer there were many days with strong insolation, so that the seabuckthorn plants were for large intervals subjected to intense lighting. This is also the explanation of specific variation of chlorophyll *a*, comparatively with chlorophyll *b*.

Analyses of chlorophyll pigments performed on superior and inferior segments of one year old offshoots (Fig. 6, Fig. 7) evidenced quantitative variations specific to each investigated cultivar.

Thus, for **chlorophyll *a***, in both segments of the offshoots, Şerbăneşti I and Ciumaş cultivars show the highest rates of biosynthesis and accumulation, followed by Şerbăneşti 4, Şerpeni and Sfântu Gheorghe varieties. The smaller value of chlorophyll *a* level was registered in Delta 60M (variant 5).

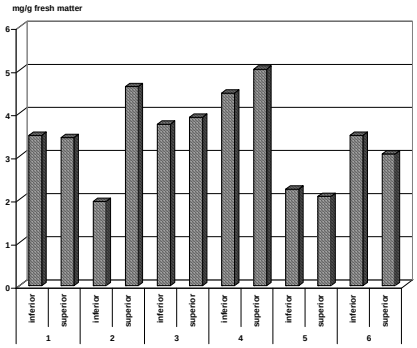

Figure 6. Variation of leaf chlorophyll *a* content in 2007 seabuckthorn cultivars

For **chlorophyll *b***, the hierarchy is similar to that for chlorophyll *a*, but at 3-4 times smaller levels of pigment.

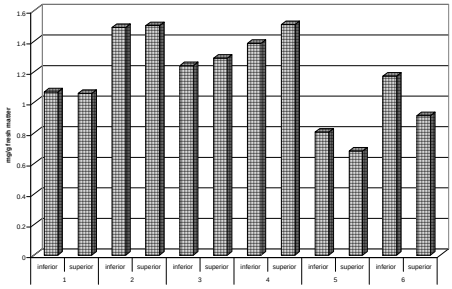

Figure 7. Variation of leaf chlorophyll *b* content in 2007 seabuckthorn cultivars

Carotenoid pigments (Fig. 8) generally maintain a rhythm of biosynthesis and accumulation similar to that of chlorophyll fractions, but the levels of this category of pigments are much smaller.

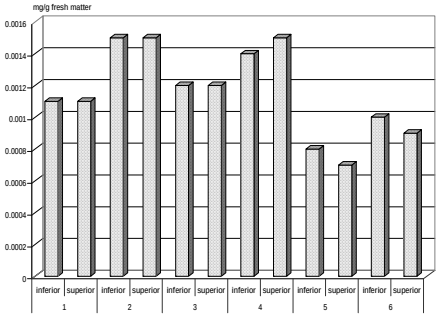

Figure 8. Variation of leaf carotenoid content in 2007 seabuckthorn cultivars

Concerning the two segments of one year old offshoots, for the most analyzed seabuckthorn cultivars, they were observed quantitative differences in favour of superior segment having young leaves, functionally active. Şerbăneşti 4 and Ciumaş cultivars represent an exception, because they have a preponderance of leaf chlorophylls and carotenoids in the inferior segments of offshoots. This situation can be explained by the exposition of inferior segments of respective analyzed offshoots to a supplementary lighting.

Leaf protein content. The seabuckthorn leaves represent an important protein source. The study of Li and Wardle (2003) regarding the effect of leaves harvesting moment in male and female seabuckthorn individuals on protein content, pointed out the existence of a small variation of this indicator between individuals but an important variation regarding the harvesting moments of biological material. The results obtained in phenophase of offshoot maximum growth evidence two tendencies. In Şerbăneşti I (variant 2) and Delta 60M (variant 5) cultivars a tendency to diminution of soluble protein content appears, from leaves belonging to superior segment to those harvested from the inferior segment. In the other four cultivars an inverse tendency was evidenced. The maximum protein content was registered in Ciumaş cultivar.

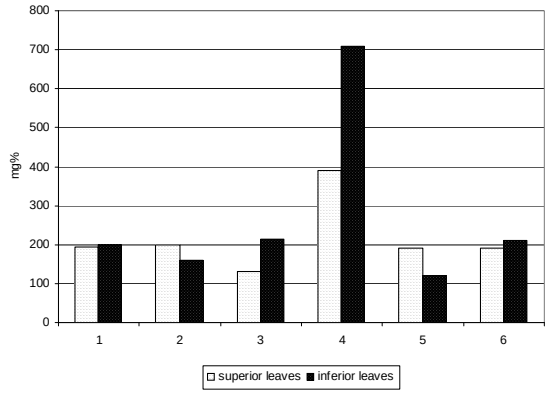

Figure 9. Variation of leaf protein content in seabuckthorn cultivars, in phenophase of offshoot maximum growth

The analysis of leaves harvested in phenophase of fruit maturation evidences a reversal of the tendency visible in previously described phenophase. At this stage, the soluble protein levels are increased in all cultivars. The values are comparable; some small differences are attributed to the cultivars characteristics.

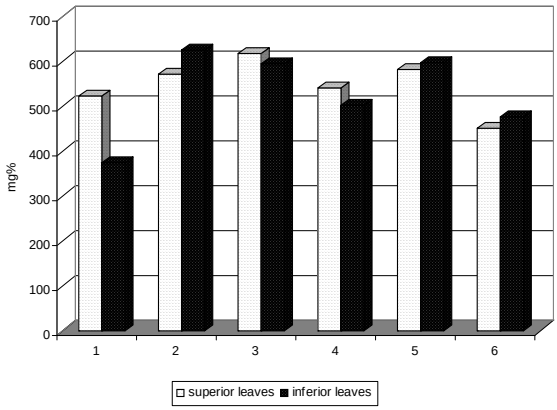

Figure 10. Variation of leaf soluble protein content in seabuckthorn cultivars, in fruit maturation phenophase

CONCLUSIONS

In phenophase of offshoot maximum growth, the results indicate a general tendency to the increase of lipids and proteins to the basal segments of seabuckthorn offshoots. In fruit maturation phenophase, the intensity of the two biosynthesis processes is amplified and depends on analyzed cultivar.

The leaf chlorophyll content varies depending on cultivar, assuring an adequate photosynthesis in fruit carrying one year old offshoots. The superior segments of seabuckthorn offshoots present a slight preponderance of these pigments because of the fact that they are a better light exposition, their leaves being more active from functional point of view.

The leaf carotenoid pigments assure a good protection to chlorophylls in the cases of strong lighting of analyzed individuals, allowing them an optimum functioning, with adequate photosynthesis efficiency.

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