

CHLOROPHYLL AND CAROTENOID CONTENT IN WOLFBERRY (*LYCIUM BARBARUM* L.) LEAVES

Ion ROȘCA¹, Aliona GLIJIN¹, Nina CIORCHINĂ¹, Maria TABĂRA¹,
Alina CUTCOVSCHI-MUȘTUC¹, Tudor RALEA², Nina ZDIORUC²

1 – “Alexandru Ciubotaru” National Botanical Garden (Institute), Republic of Moldova;

2 – Institute of Genetics, Physiology and Plant Protection, Republic of Moldova

Abstract. The aim of the study included the spectrophotometric quantification of chlorophyll and carotenoid pigments and the estimation of the ratios of the respective pigments in the species *Lycium barbarum* L. from the spontaneous flora and in four cultivars of this species ('Licurici', 'New big', 'Amber Sweet' and 'Ning Xia N1'), obtained by tissue culture in the Laboratory of Embryology and Biotechnology of the "Alexandru Ciubotaru" National Botanical Garden (Institute).

The total content of chlorophyll pigments was between 6.934 and 11.604 mg/g plant mass. The minimum values, similarly to the separate fractions of chlorophyll *a* and *b*, were recorded in the leaves collected from wolfberry plants in the spontaneous flora. The maximum total chlorophyll content is characteristic of the 'Ning Xia N1' cultivar, which also demonstrated the highest chlorophyll *a* content, quantified separately. This is also true for the content of carotenoids. The ratio of chlorophyll *a* to *b* is about 3:1 in the leaves collected from the cultivated varieties and exceeds 4:1 in the plants found in the spontaneous flora. However, the ratio of total chlorophyll to carotenoids in the leaves of specimens from the spontaneous flora indicated a value equal to the average of this ratio calculated for the four cultivated varieties. The cluster analysis confirmed the differences of the contents of chlorophyll *a*, chlorophyll *b* and carotenoids between the cultivated goji varieties and the species *Lycium barbarum* L. from the spontaneous flora, and the similarities between the goji plants from the spontaneous flora and the cultivated varieties, particularly 'Licurici' and 'Ning Xia N1', in terms of the ratio of the total chlorophyll content to the carotenoids.

The generalization of the results obtained in the study on chlorophyll and carotenoid pigments and the estimation of the values established between their ratios allow us to assume that all four cultivars of *Lycium barbarum* L. ('Licurici', 'New big', 'Amber Sweet' and 'Ning Xia N1') have acclimatized very well to the pedoclimatic conditions of the Republic of Moldova, and the determined optimal values indicate a good development of the vital processes of the plants.

Key words: *Lycium barbarum* L., 'Licurici', 'New big', 'Amber Sweet', 'Ning Xia N1', goji, chlorophyll *a*, chlorophyll *b*, carotenoids.

INTRODUCTION

In the Chinese folk medicine, the leaves of *Lycium barbarum* L. (species known as goji or wolfberry) are called "Tianjingcao" (vitality and vigour of nature) because of the high ethno-botanical value of this species in China. They are considered a significant source of bioactive compounds, including mineral-rich polysaccharides, alkaloids, polyphenols, terpenoids, sterols, minerals (mainly calcium, iron and zinc), vitamins etc. (Yao et al., 2011; Dong et al., 2011; Liu et al., 2012; Ren et al., 2017; Lei et al., 2021). Flavonoids have been reported as the main functional components in *L. barbarum* leaves (Wu et al., 2015) and rutin – as the predominant flavonoid (Dong et al., 2009). The studies on the importance of *Lycium barbarum* have shown that its photosynthetic organs can exert diverse biological activities – antioxidant, anti-inflammatory, antidiabetic, antimicrobial (Duan, Chen and Chen, 2010; Mocan et al., 2014; Mocan et al., 2017) and they have a beneficial role in maintaining the functionality of the body, especially of the liver, eyesight (Dong et al., 2011),

in alleviating mineral deficiency, combating heat stress, toning etc. (Kim et al., 1997; Chen, Shao and Chen, 2015; Xiao et al., 2019).

The results obtained by Bae et al. (2019) suggest that *Lycium* fruits and leaves are potential anti-inflammatory therapeutic agents, and LBP (*Lycium barbarum* polysaccharides) found in leaves have a beneficial effect on immunostimulatory activity (Liu et al., 2012). Because they contain rutin, a compound known for its ability to protect cells from UV-induced damage, goji leaves could be good sources for anti-radiation foods or sun protection skincare products (Conidi, Drioli and Cassano, 2020).

Goji berries have also been used in food, for the preparation of functional teas, soups, sauces, salads or as food supplements (Yeh et al. 2008; Potterat, 2010; Gong et al., 2016; Mocan et al., 2017).

Due to the polyphenols contained in goji berries, they can be integrated into nutraceuticals, functional foods and cosmetics (Anunciato and da Rocha Filho, 2012). Mocan and his colleagues (2017) indicated that *L. barbarum* leaves have the potential to be marketed as ingredients for functional drinks and as a valuable source of bioactive compounds in other healthy foods. A recent study (Lei et al., 2021) led to the conclusion that LBP found in leaves have appreciable structural activity, and the proteins in goji leaves are worth studying. Goji berries are potential ingredients for the production of natural food additives, and for people who prefer to eat less sugar and more dietary fibre, goji leaves can be a good choice as an alternative to goji berries (Lei et al., 2021).

The yield of plant biomass depends on the photosynthetic process and, therefore, on the efficiency of the photosynthetic apparatus, and the assimilating pigments, including chlorophylls and carotenoids, are considered to be some of the most important chemical compounds that make this process possible.

Sims and Gamon (2002) mentioned that in most species, the content of photosynthetic pigments provides a valuable insight into the physiological performance of plants. Determining the chlorophyll content can be an important indicator of the vital processes of the plant, which can affect the biomass yield (Zielewicz, Wróbel and Niedbała, 2020).

The chlorophyll present in the leaves can act as an antioxidant or pro-oxidant depending on the presence of light (Bae et al., 2019). Carotenoids provide protection against excess light, detoxifying free radicals and limiting membrane damage (Cuttriss and Pogson, 2004). Because carotenoids are pigments with a chemically different structure, they also have a wider range of functionality. They can act as light-absorbing pigments or as protectors of chlorophyll pigments against distinctive photo-oxidation reactions (Valladares et al., 2000; Lichtenthaler et al., 2007). β -carotene is a source of vitamin A (Falkowski and Kukułka, 1977a). Another function of carotenoids is to mediate their responses to biotic and abiotic signals and to control plant architecture (Wurtzel, 2019). Carotenoids are lipophilic compounds that range in color from yellow to orange or even red. However, carotenoids do not determine the leaf color because they are masked by chlorophyll (Stahl and Sies, 2005).

It has been determined that the environment influences the expression of genes, inducing changes not only in the nutrients in the leaves (Sardans et al., 2006), but also in the content of leaf pigments (Valladares et al., 2000; Damesin, 2003; Larcher, 2003; Zúniga et al., 2006; Lichtenthaler et al., 2007; Soler et al., 2011). Thus, the content of chlorophyll pigments is considered an indicator of the response of plants to habitat, climate and anthropogenic conditions (Falkowski and Kukułka, 1977b; Kozłowski, Goliński and Golińska, 2001; Goliński, 2001; Selzer and Busso, 2016). Golińska (2007) mentioned that

the amount of chlorophyll can be considered an appropriate indicator of the vitality of plants and their resistance to thermal conditions and humidity stress.

Chlorophyll loss is associated with environmental stress, and any variation in the overall chlorophyll / carotenoid ratio may be a good indicator that the plant is affected by stress (Netto et al., 2005). Very low or high temperatures in the growth medium can be detrimental to various metabolic processes, including chlorophyll biosynthesis (Markwell et al., 1986). Lisar et al. (2012) found that water stress inhibits chlorophyll synthesis, while carotenoids are less susceptible to it. The capacity of accumulating photosynthetic pigments differs among varieties of a species, due to the morphological and anatomical features of the leaves: the surface, the perimeter and the thickness of the mesophyll (Salem-Fnayou et al., 2011). Drought reduces the content of chlorophyll and carotenoid pigments, and also changes the ratio of chlorophyll *a* to *b* (Ashraf et al., 1994; Farooq et al., 2009; Sourour et al., 2017). Besides, drought reduces the intensity of photosynthesis (Kalaji and Zebrowski, 2004). The level of soil moisture is another factor that influences the chlorophyll content of the leaves (Olszewska et al., 2010).

Taking into account the therapeutic, nutritional and cosmetic value of wolfberry leaves, but also the importance of the photosynthetic apparatus in obtaining the yield of plant biomass and as an indicator of plant vitality and adaptability, we aimed at evaluating the content of chlorophyll and carotenoid pigments so that we could also estimate the level of adaptation of *Lycium barbarum* cultivars, obtained *in vitro* by tissue culture and subsequently cultivated in open ground.

MATERIALS AND METHODS

The dried leaves of four cultivars of *Lycium barbarum* L. ('Licurici'; 'New big'; 'Amber Sweet' and 'Ning Xia N1') which had been obtained by tissue culture in the Embryology and Biotechnology Laboratory of the "Alexandru Ciubotaru" National Botanical Garden (Institute) (NBGI), served as biological material. The plants were grown in the experimental field of NBGI. We also studied leaves collected from wolfberry plants growing in the spontaneous flora.

The cultivar '**Licurici**' has been bred by the researchers of the Embryology and Biotechnology Laboratory of the NBGI. The fruits are red-orange, oblong or cylindrical, 1.8-2.0 cm long and 7-9 mm in diameter. They are juicy and sweet. The growing season lasts from April to November. Flowering begins in the middle of May. It prefers sunny places, sandy or clayey, well-drained soils. On rich soils, the plants are more vigorous and productive. This cultivar is resistant to pests, pathogens and adverse environmental conditions. The fruits of this cultivar are very suitable to be eaten fresh (Tabăra, 2020).

The cultivar '**New big**' was obtained in Poland in 2013. The fruits are large, elongated, deep red, 1 cm in diameter and up to 2 cm long. They are sweeter than the fruits of other cultivars. The plants bear fruit in July-November. The plant needs to be fixed to a support. The height reaches from 2.5 m to 3 m. It grows best in full sun and is resistant to frost. The fruits are suitable for drying. (<https://www.kraeuter-und-duftpflanzen.de/pflanzen-saatgut/gagelstrauch-guduchi/goji-beere/goji-beere-new-big-pflanze>).

'**Amber Sweet**' is a relatively new cultivar, obtained in 2016 by the researchers of the Research Centre Źródło Dobrych Pnaczy in Poland. The fruits are amber-yellow, 1.5 cm in diameter and 2-2.5 cm long. The plant begins to bear fruit 2-3 years after planting. It blooms in June-August. Fruit ripening takes place from August to November. It can keep its

shape without being fixed to a support. It has an annual growth of 0.5-1 m and can reach a height of 2-2.5 m. It prefers sunny places. This cultivar is tolerant to frost. (https://www.clematis.com.pl/ru/encyklopedia/?tx_plant_pi1%5Bplant%5D=947; <https://www.kraeuter-und-duftpflanzen.de/pflanzen-saatgut/gagelstrauch-guduchi/goji-beere/goji-beere-new-big-pflanze>).

'Ning Xia N1' is considered the most productive cultivar, obtained in 2007 by Chinese researchers. It is a thornless shrub with fast / vigorous growth. It has long shoots that can reach 3-4 m. It bears fruit from the first year when it is planted, unlike the older varieties, which begin to be productive after 3-5 years. The shrubs of this cultivar are more resistant to diseases and pests and are better adapted to harsh environmental conditions (very alkaline soils with a pH > 9, episodes of drought or excessive watering). The productivity of 3-year-old shrubs is 11.8 t/ha of fresh fruit with an average weight of a fruit of 1.29 g (Hummer et al., 2012).

***Lycium barbarum* L. from the spontaneous flora** is a species that was naturalized many years ago on the territory of the Republic of Moldova. It is a shrub with branches that can reach a height of 2.5 m, and the diameter of the shrub can vary between 1 and 3 m, depending on the environmental conditions. It blooms from June to September. The fruits are oblong-ovate, sometimes elliptical, with a sharp or obtuse tip and a glabrous surface. The colour can vary from bright red to yellow-orange, and the taste is sweet-faded.

The biochemical investigations were performed in the Plant Biochemistry Laboratory of the Institute of Genetics, Physiology and Plant Protection.

The determination of photosynthetic pigments (chlorophyll *a*, chlorophyll *b* and carotenoids). The method (according to Šlič) is based on the spectrophotometric quantification of the fraction strongly bound to pigments, which are extracted with acetone. The crushed plant sample (leaves) of 100 mg was transferred to a mortar, adding CaCO₃, and homogenized in 100% acetone to an even consistency, and then was filtered through a Schott filter with a vacuum pump into a Bunsen flask. The filtrate was adjusted with acetone to a volume of 25 ml. The extinction coefficients were determined for chlorophyll *a*, *b* and carotenoids in the red region of the spectrum and at the absorption maximum wavelength (chlorophyll *a* at 662 nm, chlorophyll *b* at 644 nm and carotenoids at 440.5 nm).

The pigment concentration (mg/ml) was calculated according to the formula:

$$\begin{aligned} C_a &= (11,7 * D_{662}) - (2,09 * D_{644}) \\ C_b &= (21,19 * D_{644}) - (4,56 * D_{662}) \\ C_{a+b} &= (7,14 * D_{662}) + (19,1 * D_{644}) \\ C_{carotenoids} &= (4,695 * D_{440,5}) - (0,268 * C_{a+b}) \end{aligned}$$

To calculate the chlorophyll content in units of mass (mg/g plant mass), the following formula was applied:

$$C_{Chlorophyll\ a+b} = \frac{(C_{a+b} * V)}{(1000 * a)}$$

where, $C_{chlorophyll\ a+b}$ – chlorophyll *a* and *b* content (mg/g plant mass); *V* – volume of the extract (ml); *a* – initial weight of the sample (g).

The content of chlorophyll *a*, *b* and carotenoids was similarly calculated.

Statistical processing. The research results were analysed using the Microsoft Excel program. The data were expressed as the mean of replicates.

RESULTS AND DISCUSSIONS

The spectrophotometric estimation of chlorophyll pigment content showed values between 6.934 and 11.604 mg/g plant mass (Fig. 1). The maximum value of these pigments was recorded in the leaves collected from the 'Ning Xia N1' cultivar, and the minimum – in the photosynthetic organs of the specimens with the habitat in the spontaneous flora. If the difference in the chlorophyll content of leaves among the cultivated varieties is a maximum of 23%, then the value of this parameter is by 35.4% lower in the leaves of wild goji plants as compared with the average value determined in the cultivated varieties. The separate chlorophyll *a* and *b* contents also have the lowest values in the plants grown in the spontaneous flora. In contrast to the total chlorophyll content, the maximum value of chlorophyll *b* (2.645 mg/g plant mass) was quantified in the leaves of the 'Amber Sweet' cultivar, while the maximum level of chlorophyll *a* (9.065 mg/g plant mass) was determined in the 'Ning Xia N1' cultivar. However, the differences between the chlorophyll *b* content of the cultivated varieties are smaller as compared with those of the chlorophyll *a* and their overall content, respectively.

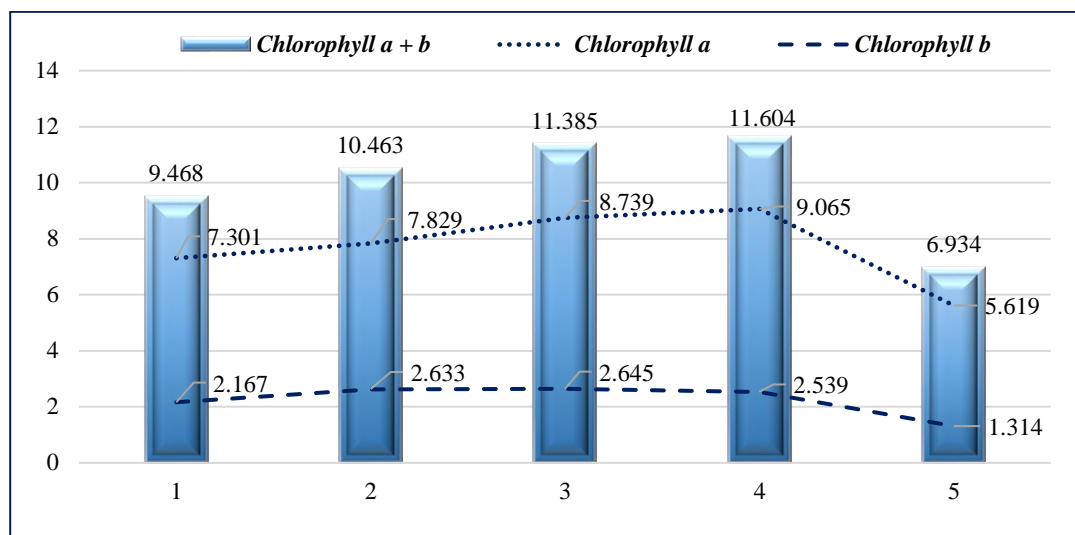


Figure 1. The quantity of chlorophyll *a* and *b* (mg/g plant mass) in the leaves of *Lycium barbarum* L.

(1 – 'Licurici'; 2 – 'New big'; 3 – 'Amber Sweet'; 4 – 'Ning Xia N1'; 5 – spontaneous flora)

The values of the chlorophyll pigment content recorded in our research are similar to those obtained by Chen et al. in a study on *Lycium chinense* leaves. The value of this parameter, according to the data included in the respective paper, was about 10000 µg/g of dried leaves (Chen et al., 2020). The congruence of our data with those of the above-mentioned paper is also determined by the fact that acetone was used as a solvent in both researches. However, in another investigation (Zhao Jian-hua et al., 2018) focused on studying the assimilating pigments of four species of goji (*Lycium chinense* Mill. var. *potaninii* (Pojark.) A. M. Lu, *Lycium chinense* Mill., *Lycium barbarum* L. and *Lycium yunnanense* Kuang & A. M. Lu), using water as solvent, the value of assimilating pigment

content was only 1.96-2.09 mg/g⁻¹ dry matter, thus demonstrating significantly lower values, indicating the importance of the nature of the solvent used in the pigment extraction process.

The elaboration of the distance matrix based on the values of chlorophyll *a* and chlorophyll *b* content (fig. 2) shows that the cultivars 'Amber Sweet' and 'Ning Xia N1' are the closest ($P_{3,4} = 0.34$) in terms of the capacity of biosynthesis and accumulation of chlorophyll pigments, followed by the cultivars 'Licurici' and 'New big' ($P_{1,2} = 0.70$). Respectively, the cluster analysis (fig. 2.) has made it possible to highlight two major clusters: $S_{(1,2,3,4)}$ and $S_{(5)}$. In the obtained dendrogram, it is obviously observed the distance between the values of the chlorophyll *a* and *b* content of the leaves collected from the *Lycium barbarum* plants grown in the spontaneous flora from the four cultivated varieties ($P = 1.89$).

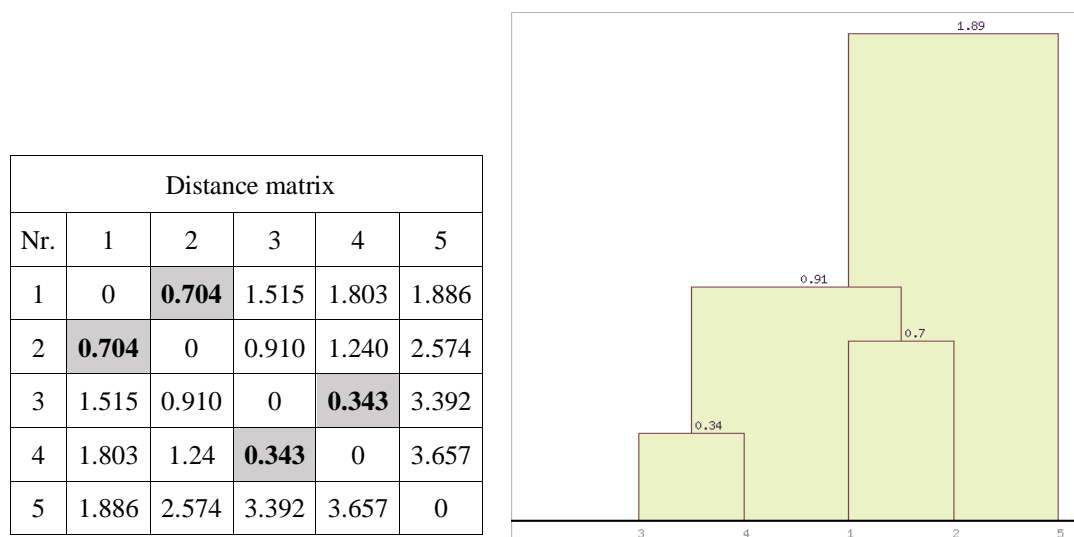


Figure 2. The distance matrix and the dendrogram obtained on the basis of the values of chlorophyll pigment content (1 – 'Licurici'; 2 – 'New big'; 3 – 'Amber Sweet'; 4 – 'Ning Xia N1'; 5 – spontaneous flora)

The quantitative study on carotenoids showed that the wolfberry leaves collected from plants in the spontaneous flora contain about 43% less carotenoids than the leaves of the 'Ning Xia N1' cultivar, for which the maximum value of this group of assimilating pigments is characteristic (2.665 mg/g plant mass). Unlike chlorophyll pigments, the amount of carotenoids quantified in the leaves of the 'Licurici' cultivar is about 12% higher than in the 'New big' cultivar, which has an almost 11% higher content of chlorophyll pigments. The quantitative differences of the carotenoids in the leaves of the cultivated varieties are smaller and constitute at most 28%, unlike the difference between the average value of this parameter in the cultivated varieties and in the goji plants from the spontaneous flora, which constitutes 35%. The difference between the content of chlorophyll pigments in the cultivated varieties is maximum 22.6%, and – between these plants and those grown in the spontaneous flora – much more, namely 35.4%, similar to the content of carotenoids (Fig. 3.).

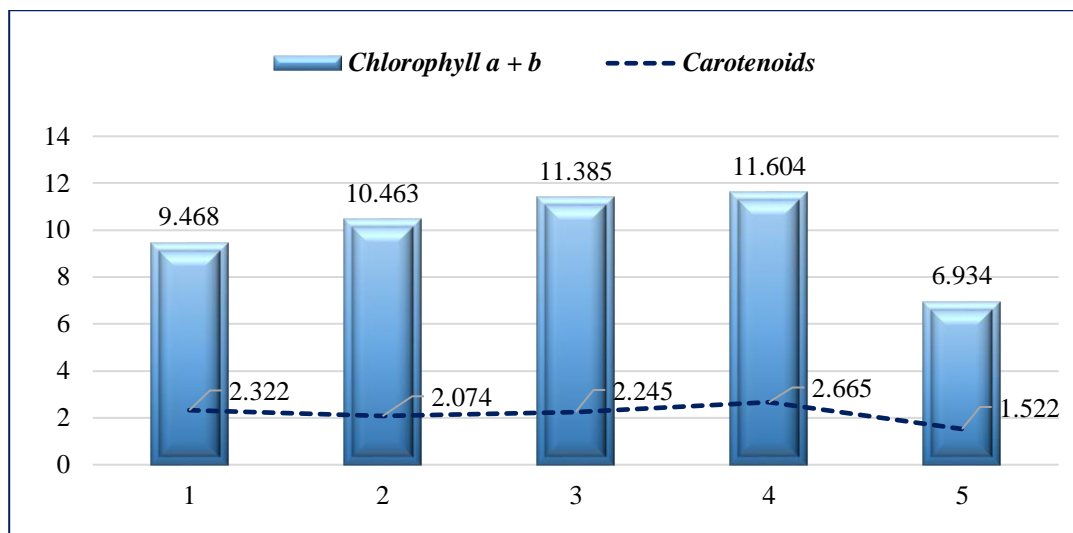


Figure 3. The total amount of chlorophylls and carotenoids (mg/g plant mass) in *Lycium barbarum* L. leaves (1 – 'Licurici'; 2 – 'New big'; 3 – 'Amber Sweet'; 4 – 'Ning Xia N1'; 5 – spontaneous flora)

On the distance matrix, elaborated based on the values of the total content of chlorophyll ($a + b$) and carotenoids (Fig. 4.), the same picture is seen, as described in the case of the fractions of chlorophyll a and chlorophyll b . Thus, the cultivars 'Amber Sweet' and 'Ning Xia N1' are the closest in terms of the content of chlorophyll and carotenoid pigments ($P_{3,4} = 0.47$), followed by the cultivars 'Licurici' and 'New big' ($P_{1,2} = 1.025$). The cluster analysis (Fig. 4.) also revealed two major clusters: $S_{(1,2,3,4)}$ and $S_{(5)}$, and the distance between the values of the plants grown in the spontaneous flora and the cultivated plants, according to the content of assimilating pigments, is much higher ($P = 2.66$).

For the normal functioning of the photosynthetic apparatus, not only the absolute values of the pigment content are important, but also their ratios, which indicate the interaction of the reaction centres of the photosystems (Тужилкина, 2009). The variations in the ratios of chlorophyll a/b and chlorophyll/carotenoids are often used as indicators of senescence, stress or damage to the photosynthetic apparatus (Merzlyak et al., 1999; Gitelson et al., 2009; Fassnacht et al., 2015). The values of these ratios depend on both the species and the conditions of growth and development, especially on light intensity. One of the informative indicators that characterize the activity of the photosynthetic apparatus is the ratio between chlorophyll a and chlorophyll b . In higher plants, this ratio is about 3:1 (Rajalakshmi and Banu, 2015). The data obtained by us indicate that this ratio is more or less characteristic of the leaves collected from the four cultivated varieties. In the plants from the spontaneous flora, the value of the given ratio is higher, and constitutes 4.276 (Fig. 5.). It has been shown that the plants can adjust the ratio of chlorophyll a to b to respond to changes in environmental or internal conditions. For example, the chlorophyll a/b ratio may increase when the nitrogen content in the leaves decreases (Kitajima K., Hogan, 2003).

Distance matrix					
Nr.	1	2	3	4	5
1	0	1.025	1.919	2.163	2.657
2	1.025	0	0.938	1.285	3.572
3	1.919	0.938	0	0.474	4.509
4	2.163	1.285	0.474	0	4.808
5	2.657	3.572	4.509	4.808	0

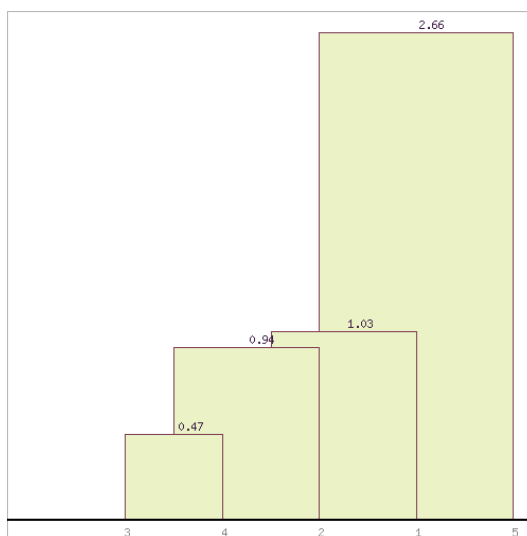


Figure 4. The distance matrix and the dendrogram elaborated on the basis of the content of chlorophyll and carotenoid pigments (1 - 'Licurici'; 2 - 'New big'; 3 - 'Amber Sweet'; 4 - 'Ning Xia N1'; 5 - spontaneous flora)

Among the cultivated varieties, the minimum value of this ratio (2.973) was found in the leaves of the 'New big' cultivar, and the maximum (3.57) – in the 'Ning Xia N1' cultivar (Fig. 5.). The ratio of chlorophyll *a* to *b* has been shown to increase sharply at low light intensity and gradually – at higher light intensities (Leong and Anderson, 1984). Probably the significantly higher value of the respective ratio in the plants grown in the spontaneous flora as compared with the cultivated varieties is due to the light intensity.

The variations in the total chlorophyll/carotenoid ratio (Hendry and Price, 1993) or the chlorophyll *a*/carotenoid ratio (Féret et al., 2008) can be used to assess the condition of plants in response to environmental stress. As photosynthetic pigments, both chlorophylls and carotenoids support the functionality of the photosynthetic apparatus, and the deviations of the ratio of these two groups of pigments indicate the mechanisms of light energy assimilation and finally the ability of plants to synthesize and accumulate biomass.

The estimation, in our research, of the ratio of total chlorophyll to carotenoids revealed values between 4.077 in the 'Licurici' cultivar and 5.071 – in the 'Amber Sweet' cultivar.

Unlike the ratio of chlorophyll *a* to *b*, which recorded the minimum value in the plants from the spontaneous flora, the ratio of chlorophylls to carotenoids in these plants has an average value, calculated for the cultivated varieties, which allows us to assume that even if the amount of chlorophyll and that of carotenoids is significantly lower than in the cultivated plants, the ratio between these two groups of assimilating pigments is similar to that determined in the cultivated varieties, and the photosynthetic capacity of goji plants in the spontaneous flora should not be affected.

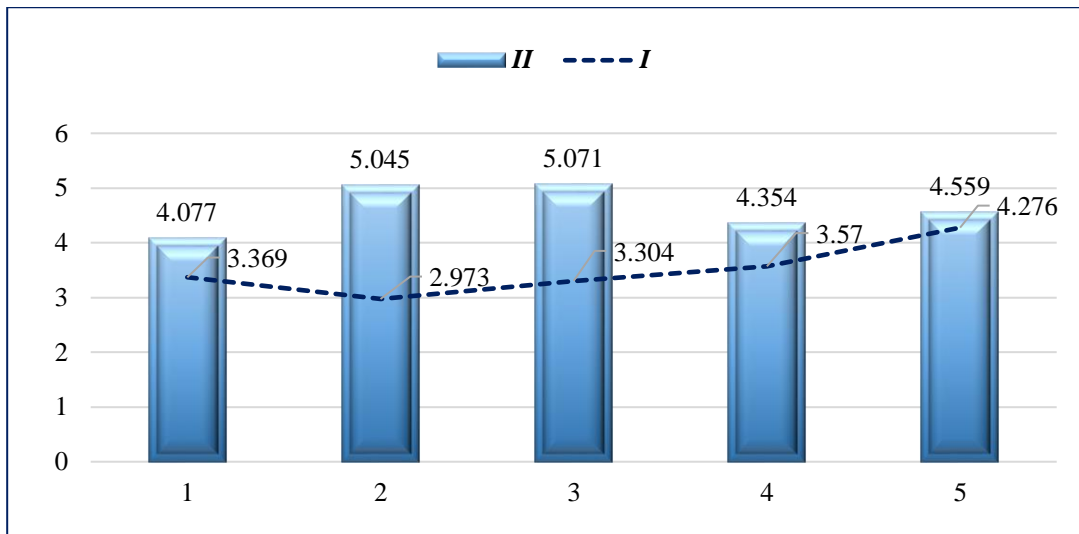


Figure 5. The ratio of the amount of chlorophyll *a* to *b* (I) and the ratio of the amount of chlorophyll to carotenoid pigments (II) in *Lycium barbarum* L. leaves. (1 – 'Licurici'; 2 – 'New big'; 3 – 'Amber Sweet'; 4 – 'Ning Xia N1'; 5 – spontaneous flora)

The elaboration of the distance matrix based on the ratios of chlorophylls to assimilating pigments (fig. 6.), showed that the closest cultivars, according to these parameters, are 'New big' and 'Amber Sweet' ($P_{3,4} = 0.33$), followed by 'Licurici' and 'Ning Xia N1' ($P_{1,2} = 0.34$).

Nr.	1	2	3	4	5
1	0	1.046	0.996	0.342	1.027
2	1.046	0	0.332	0.913	1.391
3	0.996	0.332	0	0.765	1.099
4	0.342	0.913	0.765	0	0.735
5	1.027	1.391	1.099	0.735	0

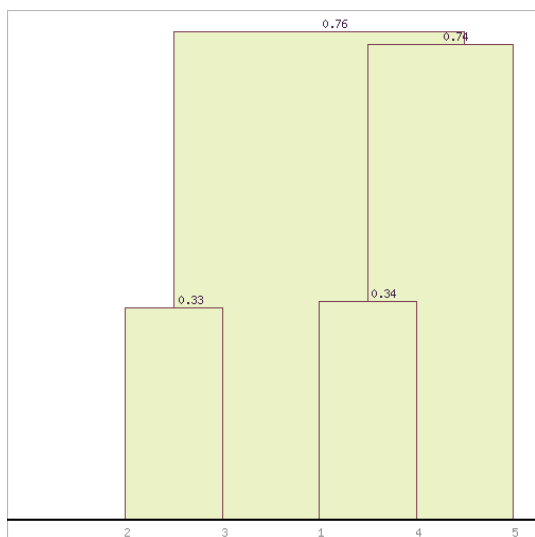


Figure 6. The distance matrix and the dendrogram elaborated on the basis of the ratios of the amount of chlorophyll *a* to *b* and the ratio of the amount of chlorophyll to carotenoid pigments (1 – 'Licurici'; 2 – 'New big'; 3 – 'Amber Sweet'; 4 – 'Ning Xia N1'; 5 – spontaneous flora)

Unlike the other two dendrograms, the one elaborated as a result of the cluster analysis of the values of the chlorophyll *a/b* ratio and the chlorophyll/carotenoid ratio indicates another clustering picture, also highlighting two clusters, but different from those described above. They include: S_(1,4,5) and S_(2,3), and the distance between them is 0.76. This result shows that according to the separate content of chlorophyll *a*, chlorophyll *b* and carotenoids, the leaves collected from goji plants in the spontaneous flora of the Republic of Moldova differ significantly from the cultivated varieties in the same parameters of the photosynthetic organs, but in the ratios of chlorophyll *a* to *b* and chlorophylls to carotenoids, this difference is not so pronounced. Moreover, in terms of the above-mentioned ratios, the leaves of the plants from the spontaneous flora are located in the same cluster as those of the 'Licurici' and 'Ning Xia N1' cultivars (S_(1,4,5), P = 0.74).

CONCLUSIONS

The spectrophotometric estimation of the content of chlorophyll pigments showed values of chlorophyll *a* between 5.619 and 9.065 mg/g plant mass and chlorophyll *b* – between 1.314 and 2.645 mg/g plant mass. Both fractions of chlorophyll recorded minimal values in the plants grown in the spontaneous flora of the Republic of Moldova. The carotenoid content also showed significantly higher values in the cultivated varieties of *Lycium barbarum*.

The evaluation of the ratio of chlorophyll *a* to *b* is about 3:1 in the leaves collected from the cultivated varieties and exceeds 4:1 in the plants grown in the spontaneous flora. However, the ratio of total chlorophyll to carotenoids in the leaves of specimens from the spontaneous flora indicated a value equal to the average of this ratio calculated for the four cultivated varieties.

The cluster analysis confirmed the distancing of the cultivated varieties from the *Lycium barbarum* L. plants grown in the spontaneous flora in terms of chlorophyll *a*, chlorophyll *b* and carotenoid content, but also the similarity between the original species and the cultivated varieties, especially 'Licurici' and 'Ning Xia N1' according to the ratio of total chlorophyll to carotenoids.

The general results of the study on chlorophyll and carotenoid pigments and the estimation of the values established between their ratios allow us to assume that all four cultivars of *Lycium barbarum* L. ('Licurici', 'New big', 'Amber Sweet' and 'Ning Xia N1') have acclimatized very well to the pedoclimatic conditions of the Republic of Moldova, and the determined optimal values indicate a good development of the vital processes of the plants.

The research was carried out within the project 20.80009.7007.19 (2020-2023) "*The introduction and development of technologies for propagation and cultivation of new species of woody plants by conventional techniques and tissue culture*".

BIBLIOGRAPHY

1. Anunciato, T. P., da Rocha Filho, P. A. (2012). Carotenoids and polyphenols in nutricosmetics, nutraceuticals, and cosmeceuticals. *J. Cosmet. Dermatol.*, 11, 51-54.
2. Ashraf, M. Y., Azmi, A. R., Khan, A. H., Ala, S. A. (1994). Effect of water stress on total phenols, peroxidase activity and chlorophyll content in wheat. *Acta Physiol. Plant.*, 16, 185-191.

3. Bae, S.-M., Kim J.-E., Bae, E.-Y., Kim, K.-A., Ly, S. Y. (2019). Anti-inflammatory effects of fruit and leaf extracts of *Lycium barbarum* in lipopolysaccharide-stimulated RAW264. 7 cells and animal model. *Journal of Nutrition and Health*, 52(2), 129-138.
4. Chen, C., Shao, Y., Li Y., Chen, T. (2015). Trace elements in *Lycium barbarum* L. Leaves by inductively coupled plasma mass spectrometry after microwave assisted digestion and multivariate analysis. *Spectrosc Lett.*, 48, 775-780.
5. Chen, P.-Y., Shih, T.-H., Chang, K.-C., Wang, J.-S., Yang, C.-M., Chang, Y.-S. (2020) Potential of galled leaves of Goji (*Lycium chinense*) as functional food. *BMC Nutrition*, 6(26), <https://doi.org/10.1186/s40795-020-00351-w>.
6. Conidi, C., Drioli, E., Cassano, A. (2020). Biologically Active Compounds from Goji (*Lycium Barbarum* L.) Leaves Aqueous Extracts: Purification and Concentration by Membrane Processes. *Biomolecules*, 10, 935, doi:10.3390/biom10060935.
7. Cuttriss, A., Pogson, B. (2004). Carotenoids. In Davies K. M. (Eds.). *Plant pigments and their manipulation*. CRC Press, Boca Raton. (pp. 57-92).
8. Damesin, C. (2003). Respiration and photosynthesis characteristics of current-year stems of *Fagus sylvatica*: from the seasonal pattern to an annual balance. *New Phytol.*, 158, 465-475.
9. Dong, J. Z., Gao, W. S., Lu, D. Y., Wang, Y. (2011). Simultaneous extraction and analysis of four polyphenols from leaves of *Lycium barbarum* L. *J. Food Biochem.*, 35, 914-931.
10. Dong, J. Z., Lu, D. Y., Wang, Y. (2009). Analysis of flavonoids from leaves of cultivated *Lycium barbarum* L. *Plant Food Hum. Nutr.*, 64, 199-204.
11. Duan, H., Chen, Y., Chen, G. (2010). Far infrared-assisted extraction followed by capillary electrophoresis for the determination of bioactive constituents in the leaves of *Lycium barbarum* Linn. *J. Chromatogr A.*, 1217(27), 4511-4516.
12. Falkowski, M., Kukułka, I. (1977a). Zawartość karotenu jako cecha charakterystyczna roślin łąkowych. *Roczn. Nauk. Roln. Seria F.*, 79, 105-112.
13. Falkowski, M., Kukułka, I. (1977b). Zawartość chlorofilu jako wskaźnika biologicznych właściwości roślin łąkowych. *Roczn. Nauk. Rol. Seria F.*, 79, 87-104.
14. Farooq, M., Wahid, A., Kobayashi, N., Fujita, D., Basra, S. M. A. (2009). Plant drought stress: Effects, mechanisms and management. *Agron. Sustain. Dev.*, 29, 185-212.
15. Fassnacht, F. E., Stenzel, S., Gitelson, A. A. (2015). Non-destructive estimation of foliar carotenoid content of tree species using merged vegetation indices. *J. Plant Physiol.*, 176, 210-217.
16. Féret, J.-B., Francois, C., Asner, G. P., Gitelson, A. A., Martin, R. E., Bidel, L. P. R., Ustin, S. L., leMaire, G., Jacquemoud, S. (2008). PROSPECT-4 and 5: Advances in the leaf optical properties model separating photosynthetic pigments. *Remote Sens. Environ.* 112, 3030-3043.
17. Gitelson, A. A., Chivkunova, B. O., Merzlyak, M. N. (2009). Nondestructive estimation of anthocyanins and chlorophylls in anthocyanic leaves. *Am. J. Bot.*, 96(10), 1861-1868.
18. Golińska, B. (2007). Chlorofil jako wskaźnik azotowej kondycji *Poa pratensis* (Poaceae) w warunkach wielokrotnej defoliacji jej runi. *Fragm. Flor. Geobot. Pol.*, 9, 137-145.
19. Goliński, P. (2001). Efektywność nawożenia azotem w produkcji nasion *Lolium perenne* L. *Rozpr. Nauk. Akad. Rol. Pozn.*, 321, 20-29.
20. Gong, G., Fan, J., Sun, Y., Wu, Y., Liu, Y., Sun, W., Zhang, Y., Wang, Z. (2016). Isolation, structural characterization, and antioxidativity of polysaccharide LBLP5-A from *Lycium barbarum* leaves. *Process Biochem (Elsevier Ltd)*, 51, 314-324.
21. Hendry, G. A. F., Price, A. H. (1993). Stress Indicators: Chlorophylls and Carotenoids. In Hendry, G.A.F. and Grime, J.P., Eds., *Methods in Comparative Plant Ecology*, Chapman Hall, London (pp. 148-152).
22. Hummer, K. E., Pomper, K. W., Postman, J., Graham, C. J., Stover, E., Mercure, E. (2012). Emerging fruit crops. In Badenes M, Byrne D, editors. *Fruit Breeding. Handbook of Plant Breeding*, New York: Springer, 8, 97-147.
23. Kalaji, H. M., Zebrowski, M. (2004). Intensywność fotosyntezy jedno- i dwuliściennych roślin C₃ i C₄ w różnych warunkach środowiska. *Zesz. Probl. Post. Nauk. Rol.*, 496, 133-142.

24. Kim, S. Y., Lee, K. H., Chang, K. S., Bock, J. Y., Jung, M. Y. (1997). Taste and flavor compounds in box thorn (*Lycium chinense* Miller) leaves. In Food Chem., 58(4), 297-303.
25. Kitajima, K., Hogan, K. P. (2003). Increases of chlorophyll a/b ratios during acclimation of tropical woody seedlings to nitrogen limitation and high light. Plant Cell Environ, 26(6), 857-865.
26. Kozłowski, S., Goliński, P., Golińska, B. (2001). Barwniki chlorofilowe jako wskaźniki wartości użytkowej gatunków i odmian traw. Zesz. Probl. Post. Nauk. Rol., 474, 215-223.
27. Larcher, W. (2003). Physiological Plant Ecology: Ecophysiology and Stress Physiology of Functional Groups. Springer, Berlin, -514 p.
28. Lei, Z., Chen, X., Cao, F., Guo, Q., Wang, J. (2021). Phytochemicals and bioactivities of Goji (*Lycium barbarum* L. and *Lycium chinense* Mill.) leaves and their potential applications in the food industry: a review. International Journal of Food Science and Technology, doi:10.1111/ijfs.15507.
29. Leong, T. Y., Anderson, J. M. (1984). Adaptation of the thylakoid membranes of pea chloroplasts to light intensities. I. Study on the distribution of chlorophyll-protein complexes. Photosynth. Res., 5, 105-115.
30. Lichtenthaler, H. K., Ac, A., Marek, M. V., Kalina, J., Urban, O. (2007). Differences in pigment composition, photosynthetic rates and chlorophyll fluorescence images of sun and shade leaves of four tree species. Plant Physiol. Biochem. (Paris), 45, 577-588.
31. Lisar, S. Y. S., Motafakkerazad, R., Hossain, M. M., Rahman, I. M. M. (2012). Water stress in plants: Causes, effects and responses. In Rahman I. M. M. & Hasegawa, H. (Eds). Water Stress. (pp. 1-14).
32. Liu, H., Fan, Y., Wang, W., Liu, N., Zhang, H., Zhu, Z., Liu, A. (2012). Polysaccharides from *Lycium barbarum* leaves: isolation, characterization and splenocyte proliferation activity. International Journal of Biological Macromolecules, 51(4), 417-422.
33. Merzlyak, M. N., Gitelson, A. A., Chivkunova, O. B., Rakitin, V. Y. (1999). Non-destructive optical detection of leaf senescence and fruit ripening. Physiol. Plant., 106(1), 135-141.
34. Mocan, A., Vlase, L., Vodnar, D. C., Bischin, C., Hanganu, D., Gheldiu, A. M., Oprean, R., Silaghi-Dumitrescu R., Crisan G. (2014). Polyphenolic content, antioxidant and antimicrobial activities of *Lycium barbarum* L. and *Lycium chinense* mill. Leaves. Molecules., 19(7), 10056-10073.
35. Mocan, A., Zengin, G., Simirgiotis, M., Schafberg, M., Mollica, A., Vodnar, D. C., Crisan, G., Rohn S. (2017). Functional constituents of wild and cultivated Goji (*L. barbarum* L.) leaves: phytochemical characterization, biological profile and computational studies. In J. Enzym. Inhib. Med. Chem., 32(1), 153-168.
36. Netto, A. T., Campostrini, E., De Oliveira, J. G., Bressan-Smith, R. E. (2005). Photosynthetic pigments, nitrogen, chlorophyll a fluorescence and SPAD-502 readings in coffee leaves. Sci. Hortic., 104, 199-209.
37. Olszewska, M., Grzegorzczak, S., Olszewski, J., Bałuch-Małecka, A. (2010). Porównanie reakcji wybranych gatunków traw na stres wodny. Grassld. Sci. Pol., 13, 127-136.
38. Potterat, O. (2010). Goji (*Lycium barbarum* and *Lycium chinense*): Phytochemistry, pharmacology and safety in the perspective of traditional uses and recent popularity. Planta Med., 76, 7-19.
39. Rajalakshmi, K., Banu, N. (2015). Extraction and estimation of chlorophyll from medicinal plants. Intern. J. Sci. Res., 4, 209-212.
40. Ren, L., Li J., Xiao, Y., Zhang, Y., Fan, J., Zhang, B., Wang, L., Shen, X. (2017). Polysaccharide from *Lycium barbarum* L. leaves enhances absorption of endogenous calcium, and elevates cecal calcium transport protein levels and serum cytokine levels in rats. J. Funct. Food., 33, 227-234.
41. Salem-Fnayou, B. A., Bouamama B., Ghorbel A., Mliki A. (2011). Investigations on the leaf anatomy and ultrastructure of grapevine (*Vitis vinifera*) under heat stress. Microsc. Res. Tech., 74(8), 756-762.

42. Sardans, J., Penuelas, J., Rodá, F. (2006). Plasticity of leaf morphological traits leaf nutrient content, and water capture in the Mediterranean evergreen oak *Quercus ilex* subsp. *ballota* in response to fertilization and changes in competitive conditions. *Ecos*, 13, 258-270.
43. Selzer, L. J., Busso, C. A. (2016). Pigments and photosynthesis of understory grasses: Light irradiance and soil moisture effects. *Russ. J. Plant Phys.*, 63, 224-234.
44. Sims, D. A., Gamon, J. A. (2002). Relationships between leaf pigment content and spectral reflectance across a wide range of species, leaf structures and developmental stages. *Remote Sens. Environ.*, 81(2-3), 337- 354.
45. Soler, R., Martínez, Pastur G., Lencinas, M. V., Moretto, A., Peri, P. (2011). Above- and below-ground nutrient tissue concentration and leaf pigment changes in Patagonian woody seedlings grown under light and soil moisture gradients. *J. Plant Nutr.*, 34, 2222-2226.
46. Sourour, A., Afef, O., Mounir, R., Mongi, B. Y. A. (2017). Morphological, physiological, biochemical and molecular plant responses to water deficit stress. *Intern. J. Eng. Sci.*, 6, 1-4.
47. Stahl, W., Sies H. (2005). Bioactivity and protective effects of natural carotenoids. *Bioch. Bioph. Acta*, 1740, 101-107.
48. Tabăra, M. (2020). Dezvoltarea și multiplicarea microclonală a speciei *Lycium barbarum* L. (goji). Teza de doctor în științe biologice, Chișinău, -156 p.
49. Valladares, F., Martínez-Ferri, E., Balaguer, L., Pérez-Corona, E., Manrique, E. (2000). Low leaf-level response to light and nutrients in Mediterranean evergreen oaks: a conservative resource-use strategy? *New Phytol.*, 148, 79-91.
50. Wu, S. H., Wang, Y. Y., Gong, G. L., Li, F., Ren, H. T., Liu, Y. (2015). Adsorption and desorption properties of macroporous resins for flavonoids from the extract of *Chinese wolfberry* (*Lycium barbarum* L.). *Food Bioprod. Process.*, 93, 148-155.
51. Wurtzel, E. Changing Form and Function through Carotenoids and Synthetic Biology. // *Plant Physiology*, March 2019, 179(3), p. 830-843.
52. Xiao, X., Ren, W., Zhang, N., Bing, T., Liu, X., Zhao, Z., Shangguan, D. (2019). Comparative study of the chemical constituents and bioactivities of the extracts from fruits, leaves and root barks of *Lycium barbarum*. *Molecules*, 24, 1585-1607.
53. Yao, X., Peng, Y., Xu, L. J., Li, L., Wu, Q. L., Xiao, P. G. (2011). Phytochemical and biological studies of *Lycium* medicinal plants. *Chemistry & Biodiversity*, 8, 976-1010.
54. Yeh, Y. C., Hahm, T. S., Sabliov, C. M., Lo, Y. M. (2008). Effects of Chinese wolfberry (*Lycium chinense* P. Mill.) leaf hydrolysates on the growth of *Pediococcus acidilactici*. *Bioresour. Technol.*, 99, 1383-1393.
55. Zhao, Jian-hua, Li, Hao-xia, Zhang, Cun-zhi, An, Wei, Yin, Yue, Wang, Ya-jun, Cao, You-long (2018). Physiological response of four wolfberry (*Lycium* Linn.) species under drought stress. *Journal of Integrative Agriculture*, 17(3), 603-612.
56. Zielewicz, W., Wróbel, B., Niedbała, G. (2020). Quantification of Chlorophyll and Carotene Pigments Content in Mountain Melick (*Melica nutans* L.) in Relation to Edaphic Variables. *Forests*, 11, 1197; doi:10.3390/f11111197.
57. Zúniga, R., Alberdi, M., Reyes-Díaz, M., Olivares, E., Hess, S., Bravo, L. A., Corcuera, L. J. (2006). Seasonal changes in the photosynthetic performance of two evergreen *Nothofagus* species in south central Chile. *Rev. Chil. Hist. Nat.*, 79, 489-504.
58. Практикум по физиологии растений под ред. Н. Н. Третьякова, 3-е издание, Москва, Агропромиздат, 1990, с. 86-94.
59. Тужилкина, В. В. (2009). Реакция пигментной системы хвойных на длительное аэротехногенное загрязнение. *Экология*. 4, 243-248.