© B. KH. MEZHUNTS, M. A. NAVASARDYAN
bagratm@yahoo.com

UDC 581.132: 633.2: 577.4

PHOTOSYNTHETIC PIGMENT PARAMETERS OF GRASSES IN ARMENIA'S MOUNTAIN ECOSYSTEMS

SUMMARY. The content of a-chlorophyl and b-chlorophyl as well as the total amount of carotenoids in grasses growing in dry-steppe, meadow-steppe, and alpine zones of mountain ecosystems of Armenia (1250-3000 m AMSL) is studied. The purpose of the paper is to estimate the extent of photosynthetic pigment variation in plant leaves during a day, a vegetation period, and according to vertical zones. The target of the study is the following wild species: sagebrush (Artemizia fragrans), euphorbia (Euphorbia Marschalliana), geranium (Geranium tuberosum), dandelion (Taraxacum officinale, T. Stevenii), clover (Trifolium ambiguum, T. pratense), vetch (Vicia variabilis), sainfoin (Onobrychis transcaucassica), cocksfoot (Dactylis glomerata) and bluebell (Campanula tridentata). Pigments of plastids are extracted from fresh samples of leaves using the organic solvent of dimethylsulphoxide, according to the methodology developed by the authors of this paper for field conditions. The examination of the daily dynamics of pigment accumulation demonstrates that the maximum pigment content in plant leaves of the meadow-steppe zone is observed in the morning hours, and in the alpine zone it occurs at noon. In contrast to the daily dynamics, no clear pattern in pigment content variation is observed during the vegetation period of plants. It was also stated that the total amount of all studied pigments in plants of the dry-steppe zone varies within the range of 194-304 mg/100 g of wet sample, in the meadow steppe zone it is 236-280 mg/100 g, and in the alpine zone it is 134-225 mg/100 g.

KEY WORDS. Mountain ecosystems, grasses, chlorophylls, carotenoids.

During the photosynthesis process, solar energy is absorbed and transformed by photosynthetic pigments of plants, in particular by chlorophylls and carotenoids. Chlorophylls are characterized by a complex chemical structure, with porphyrin being its basic component. Porphyrin is a natural stable and inert organic compound, and its analogues can be met in crude oil, coal, and bituminous rocks. With the specific structure of a molecule of chlorophyll [1], even a minute amount of energy (about 1 quantum), allows graduating to excitation, i.e. increasing its energy level and reactionability. The absorption of energy by chlorophylls occurs in red and blue parts of the solar spectrum, maximum absorption is observed at the wavelength of 430 and 663 μm for *a*-chlorophyll, and at 450 and 645 μm for *b*-chlorophyll. In photosynthesis process, carotenoids, especially carotenes and xanthophylls, which absorb solar energy and carry it to the photochemical reaction center through *a*-chlorophyll, play an important role.

Many researchers prove the important role of photosynthetic pigments concentration in harvest forming and bioenergy accumulation in agrarian ecosystems

[4-6]. It evidently means even more for mountain ecosystems where plants are constantly exposed to unfavorable soil and climate condition and anthropogenic impact.

The works on photosynthetic pigments of wild plants in Armenia are not numerous, and they mainly deal with plant communities of the alpine zone [7-9]. Thus, the experimental data published earlier are not sufficient for representing the character of environmental factors impact on the accumulation and spatiotemporal variations of plant pigments in mountain ecosystems. We should note that this problem was insufficiently studied due to the absence of methods to determine pigments in field conditions. We developed such a method basing on our long-term experience [10]. The purpose of the paper is to estimate the average content of *a* and *b*-chlorophyls and carotenoid total in the leaves of wild plants and to determine the variation limit during a day, a vegetation period, and according to vertical zones.

The experiment. The research was conducted in the conditions of the dry-steppe, meadow-steppe, and alpine zones of the Geghama mountain chain in 2006-2008. The wild species under study included sagebrush (*Artemizia fragrans Willd.*), euphorbia (*Euphorbia Marschalliana Boiss.*), geranium (*Geranium tuberosum L.*), dandelion (*Taraxacum officinale Wigg., T. Stevenii D.C.*), clover (*Trifolium ambiguum L, T. pratense L.*), vetch (*Vicia variabilis Fr. et Sint.*), sainfoin (*Onobrychis transcaucassica Grossh.*), cocksfoot (*Dactylis glomerata L.*), and bluebell (*Campanula tridentata Schreb.*). The main part of the research was timed to the anthesis and beginning of seed forming phases of the studied plants that occur in the dry-steppe zone in May, in the meadow-steppe zone in July, and in the alpine zone in August (7-23-fold replication of measurement). The pigment content was measured at 8-9 a.m., 1-2 p.m., and 6-7 p.m; and according to vegetation it was measured on the June, 25; July, 11; and July, 26 (in the phase of mass anthesis, in the beginning and in the end of seed forming, respectively). Pigments of plastids were extracted from fresh samples of leaves using the organic solvent of dimethylsulphoxide. The extraction method, storage conditions for samples, and the procedure of determining chlorophyls and carotenoids were described in the Patent for Invention [10]. The pigments were measured with the SF-16 spectrophotometer; and their concentration was calculated according to conventional equations [11]:

a-chlorophyl = $12.7*E663 — 2.69*E645$,
b-chlorophyl = $22.9*E645 — 4.68*E663$,
a-chlorophyl and *b*-chlorophyl total= $8.02*E663+20.2*E645$,
carotenoid total = $4.695*E440 — 0.268$ * (*a*-chlorophyl and *b*-chlorophyl total),

where E is the spectrophotometer indication, and 663, 645 и 440 are spectrum wave lengths, corresponding to *a*-chlorophyl, *b*-chlorophyl, and carotenoid total.

Results and discussion. It should be noted that the photosynthetic pigments (*a* and *b*-chlorophyls and carotenoids) were measured in the same hitch. However, in the discussion of the results on the daily and seasonal dynamics, the data on *a*-chlorophyl only is presented (Fig.1, 2); it is taken into account that the variation of content of other pigments was generally identical to the variation of this pigment.

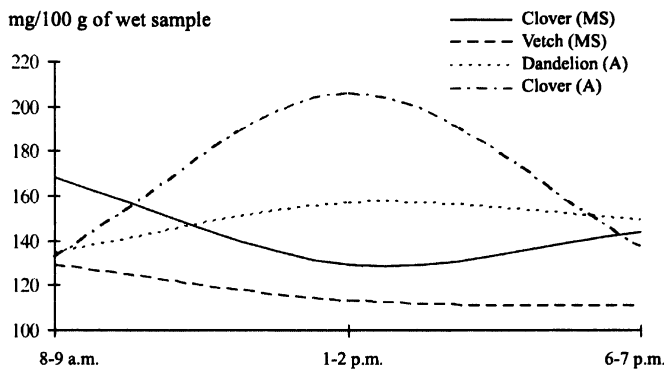

Fig. 1. Daily dynamics of a-chlorophyl accumulation in the plants of the meadow-steppe (MS) and alpine (A) zones

The daily dynamics of variations in photosynthetic pigments of plants was first studied by R. Willstätter and A. Stoll [1], who did not register appreciable variations during this period. However, in later research [12] conducted on the plants of tomato, potato, lilac, etc. some variation was registered at 6 a.m., 12 a.m., 6 p.m., and 12 p.m. Subtle variations of pigment concentration in the plants of oats, barley, soybeans, millet, and spring wheat were registered by E. Komaritskaya [13].

In our experiment (Fig.1) conducted in the conditions of the meadow-steppe zone, the maximum content of a-chlorophyl in the leaves of clover and vetch was registered in the morning (168 and 128 mg/100 g of wet sample, respectively), In the alpine zone, the maximum content of a-chlorophyl was registered in the leaves of clover and dandelion at noon (206 and 157 mg/100 g of wet sample, respectively). Obviously, the main reason for the difference in the trend of curves is the difference in lighting intensity, air temperature, and soil moisture, which was also registered by the above-mentioned scientists. Basing on three measurements of pigments conducted during a day, we calculated the average daily value (for clover and vetch in the meadow-steppe zone, it was 147 and 117 mg/100 g of wet sample; for clover and dandelion in the alpine zone, it was 158 and 147 mg/100 g, respectively). Comparing this value with the curves in Fig.1, we determined the most appropriate terms of probe extraction for each species.

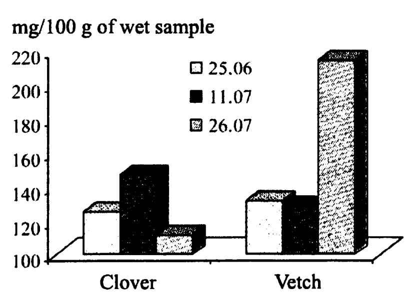

Fig. 2. Seasonal dynamics of a-chlorophyl accumulation in the leaves of wild plants in the meadow-steppe zone

In Fig.2 the results of measuring *a*-chlorophyl content in different vegetation periods of clover and vetch are presented. The research was conducted in natural hayfields of the meadow-steppe zone, the probes were extracted a day before, 15 days before, and 15 days after haying. As it can be seen, the *a*-chlorophyl content in the leaves of the plants under study varied essentially by the date of measurement: for clover it varied during a month within 112-147 mg/100 g of wet sample; for vetch it varied within 128-214 mg/100 g. In the experiments with clover, the maximum content was registered in the second measurement period, and in the experiments with vetch, it was registered in the third one. Great variations in the pigment concentration in seasonal dynamics were registered by other scientists [14-15]. We should note that red clover is much shorter than vetch; thus, after the anthesis phase, clover occupies the middle layer on natural hayfields, and vetch usually occupies the top one. The location of plants on different layers of sward greatly influences the water content of plant leaves: tall plants of the top layer, being under direct sunlight, evaporate more water, and its tissues lose more water, than short plants, occupying the middle and bottom layers. We suppose that this phenomenon may have a great influence on the pigment content, taking into account that the recalculation is done for the weight of wet leaves. This can apparently explain such great increase of *a*-chlorophyl content in the leaves of tall vetch and its decrease in the leaves of short clover registered in the third measurement period.

The results of numerous measurements of green and yellow pigment content in plants of three vertical zones are summarized in Table 1. According to the data from this table, the average *a*-chlorophyl content in sagebrush, euphorbia, geranium, and dandelion growing in the dry-steppe zone is 120, 116, and 155 mg/100 g of wet sample. The average *b*-chlorophyl content is 37, 46, 61, and 81 mg/100 g. The carotenoid total is 54, 61, 71, and 68 mg/100 g. The maximum content of *a* and *b*-chlorophyls is registered in the leaves of dandelion, the minimum one is registered in the leaves of sagebrush. The statistical treatment of experimental data [16] demonstrates essential variations of values from the average value of sampling (see data on regular errors and variations, Table 1). The maximum variation range of *a*-chlorophyl is registered for euphorbia. The maximum variation range of *b*-chlorophyl is registered for sagebrush. The maximum variation range of the carotenoid total is registered for dandelion. The studied species of wild plants growing in the dry-steppe zone demonstrate great variations in pigment proportion. Thus, e.g., *a*-chlorophyl-to-*b*-chlorophyl-to-carotenoid-total ratio is: 2.8, 1.9, and 0.7 (sagebrush); 2.6, 2.0, and 0.8 (euphorbia); 1.9, 1.6, and 0.9 (geranium); 1.9, 2.3, and 1.2 (dandelion). Within this zone, the *a*-chlorophyl fraction in the total pigment composition is 51%, the *b*-chlorophyl fraction is 22.5%, and the carotenoid total fraction is 26.5%.

Table 1

Pigment content in the leaves of wild plants of different vertical zones, mg/100 g of wet sample

Plant	Content	*a*-chlorophyl	*b*-chlorophyl	Carotenoids	Total
Dry-steppe zone (1250-1400 m AMSL)					
Sagebrush	Average	102±4.1	37±3.1	54±4.6	194±5.2
	variation	89-117	28-52	35-70	178-215

The end of Table 1

Euphorbia	Average	120±11	46±2.7	61±3.2	226±12
	variation	79-150	39-58	50-72	187-267
Geranium	Average	116±4.9	61+4	71±5.4	248±14
	variation	98-135	43-76	56-88	205-297
Dandelion	Average	155±4.1	81±2.1	68±2	304±6.5
	variation	114-186	64-100	44-98	240-355
Pigment fraction, % from the total		51.0	22.5	26.5	-
Meadow-steppe zone (1900-2200 m AMSL)					
Clover	Average	132±7.7	43±1.9	69±3.1	244±11
	variation	86-196	32-59	49-104	166-350
Vetch	Average	147±11	46±4.1	60±2.9	253±17
	variation	92-244	22-92	41-94	160-425
Cocksfoot	Average	161±9.4	27±2.6	92±5.4	280±16
	variation	131-193	17-37	72-112	220-340
Dandelion	Average	137±4.7	36±1.2	63±2.6	236±8.3
	variation	119-152	31-41	53-73	207-262
Sainfoin	Average	155±5.4	40±1.4	65±3	260±10
	variation	136-170	35-45	55-75	231-286
Pigment fraction, % from the total		57.4	15.2	27.4	-
Alpine zone (2800-3000 m AMSL)					
Clover	Average	133±8.7	34±1.7	58±4.8	225±15
	variation	61-214	17-50	18-90	96-333
Dandelion	Average	108±8.7	32±2	51±5.4	191±16
	variation	32-174	7-48	7-87	46-285
Bluebell	Average	75±6.8	28±3.3	31±5.5	134±15
	variation	32-113	7-53	7-75	46-225
Pigment fraction, % from the total		57.3	17.3	25.4	-

The average *a*-chlorophyl content in the leaves of the plants in the meadow-steppe zone varies within the range of 132-161 mg/100 g of sample. The average *b*-chlorophyl content varies within the range of 27-46 mg/100 g. The average carotenoid total varies within the range of 60-92 mg/100 g. The concentration of *a*-chlorophyl and carotenoid total is something higher in the leaves of cocksfoot, and the concentration of *b*-chlorophyl is higher in the leaves of vetch (see Table 1). On the whole, for pigment total, cocksfoot proves to have more advantage, while the other plants do not demonstrate essential difference. In this zone, *a*-chlorophyl-to-*b*-chlorophyl ratio for studied plants varies within the range of 3.2-6.0; *a*-chlorophyl-to-carotenoid-total and *b*-chlorophyl-to-carotenoid-total ratio is 1.8-2.4 and 0.3-0.7, respectively. On the average, for studied plants growing in the meadow-steppe zone, the *a*-chlorophyl fraction is 57.4%, the *b*-chlorophyl fraction is 15.2%, and the carotenoid total fraction is 27.4%.

On the pastures of the alpine zone, the pigment concentration in the leaves of clover, bluebell, and dandelion varies within the range: 67-144 mg/100 g of wet sample for *a*-chlorophyl, 26-36 mg/100 g for *b*-chlorophyl, 26-65 mg/100 g for carotenoids. The maximum value is registered for clover; the minimum value is registered for bluebell (see Table 1). Besides, in the alpine zone, the variation of total pigment content to the sample average is expressed much stronger than in the dry-steppe and meadow-steppe zones. It is also seen from Table 1 that in the leaves of alpine plants the *a*-chlorophyl fraction is 57.3%, the *b*-chlorophyl fraction is 17.3%, and the carotenoid total fraction is 25.4%, i.e. we can state that in the plants of meadow-steppe and alpine zones mainly *a*-chlorophyl is accumulated, while for the pigment total, the alpine plants with the average of 183 mg/100 g of wet sample prove to have less advantage than the dry-steppe and meadow-steppe ones (243 and 253 mg/100 g of wet sample, respectively). The same phenomenon was registered by V. Voskanyan [9], who, in addition to alpine plants, studied the content of green and yellow pigments in the conditions of the Yerevan Botanic Gardens (dry steppe, 800-1000 m *AMSL*).

Conclusions:

1. The results of numerous measurements demonstrated that the photosynthetic pigment content in grass communities of mountain ecosystems greatly varies by the terms of probe extraction, botanical species, and vertical zones.
2. It is stated that the total concentration of pigments under study was essentially higher in the leaves of dry-steppe and meadow-steppe plants; *a*-chlorophyl concentration was higher in the leaves of alpine and meadow-steppe plants; *b*-chlorophyl concentration was higher in the leaves of dry-steppe plants; the carotenoid content did not essentially vary in the studied zones.
3. There are opposite trajectories of curves representing daily dynamics of *a*-chlorophyl variation in the leaves of plants; and in the conditions of meadow-steppe zone, the maximum value is registered in the morning, while in the alpine zone the maximum one is registered at noon, which can be apparently explained by daily temperature and lighting variations; in particular, in the alpine zone, these parameters reach their peak values at noon.

REFERENCES

1. Heath, O.V.S. The Physiological Aspects of Photosynthesis. California: Stanford University Press, 1969, 315 p.
2. Nichiporovich, A.A. Light and Carbonic Feed (Photosynthesis). Moscow: Publishing House of USSR Academy of Science, 1955, 287 p.
3. Cohen-Bazire, G. & Stanier, R.Y. Specific Inhibition of Carotenoid Synthesis in a Photosynthetic Bacterium and its Physiological Consequences. // Nature. 1958. Vol. 181. P. 250-252.
4. Ustenko, G.P. & Yagnova, S.N. The Experience of Programmed High Corn Harvest by Given Efficiency of Solar Radiation Energy. Photosynthetic Systems of High Productivity. Moscow: Nauka, 1966. P. 178-193.
5. The Physiology of Winter Wheat in the South-East of Kazakhstan / Ed. by V.P. Bedenko. Alma-Ata: Nauka, 1976. 187 p.
6. Tarchevsky, I.A. & Andrianova, Yu. Ye. Pigment Content as Power Factor for Photosynthetic Apparatus Development of Wheat // Plant Physiology. 1980. Vol. 27. No. 2. P. 341-347.

7. Narinyan, S.G., Della-Rossa, R.G., Voskanyan, V.Ye. On the Impact of Ultraviolet Radiation on the Growth of Plants and the Content of Plastid Pigments in the Conditions of Aragats Mountain // Proc. of Academy of Science, Armenian Soviet Socialist Republic. Biology. 1965. Vol. 18. No .2. P. 37-40.

8. Shaidurov, V.S. On Plastid Pigments and Photosynthesis of Plants in the Subniveal Zone of Aragats Mountain // Problems of Botany. 1967. Vol. 9. P. 398-406.

9. Voskanyan, V.Ye. Dynamics of Plastid Pigment Content in the Leaves of Plants in the Alpine Zone of Aragats Mountain // Problems of Botany. 1967, Vol. 9, P. 334-350.

10. Mezhunts, B.Kh, Navasardyan, M.A. The Method of Determining a and b-Chlorophyls and Carotenoids in Plant Leave Extracts // Patent for Invention. No 2439 A., Yerevan, 2010 (in Armenian).

11. Shlyk, A.A. Determining Chlorophyls and Carotenoids in Green Leave Extracts. Biochemical Methods in Plant Physiology. Moscow: Nauka, 1971. P. 154-157.

12. Godnev T.N., Shabelskaya, E.F. On the problem of Presense of Daily Variations of Chlorophyls and Carotenoids in the Leaves of Some Plants // Plant Physiology. 1964. Vol. 11. No. 3. P. 385-390.

13. Komaritskaya, E.N. On Daily Variation in the Content of Photosynthetic Pigments in the Leaves // Proc. of the Ukrainian Agricultural Academy. 1970. No. 31. P. 127-132.

14. Lukyanova, L.M. Ecological and Physiological Factors of Studying Pigment System of Plants. I. External Factor Influence, Seasonal and Daily Dynamics // Botanical Life. 1982. Vol. 67. No. 3. P. 265-271.

15. Sestak, Z. Photosynthetic Characteristics during Ontogenesis of Leave. I. Chlorophylls. // Photosynthetica. 1977. № 11 (4). P. 367-448.

16. Statistics for Windows. Release 6,0A. StatSoft Inc. USA, 1998.