



THE CONTENT OF PHOTOSYNTHETIC PIGMENTS IN THE LEAVES OF *GALEGA OFFICINALIS* L. AND *GALEGA ORIENTALIS* LAM. CULTIVARS

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The present study aims to investigate the differences in leaf pigment content of plants of cultivars of *Galega* L. species. Plant raw material was collected in the spring vegetation stage, budding stage, flowering stage, and fruitage. Biochemical analyze of determination of pigments content was carried out with fresh mass of leaves (100 mg) of cultivars of *Galega officinalis* L. and *Galega orientalis* Lam. It was conducted the extraction of pigments in acetone. Obtained acetone extracts of leaves were prepared according to Musienko et al. (2001) and measured at wavelengths 662 nm (chlorophyll *a*), 644 nm (chlorophyll *b*) and 440 nm (carotenoids). To calculate concentration (mg% of fresh mass) of pigments was applied the equation of Holm-Wettstein. It was found the maximal sign of chlorophyll *a* in the acetone extracts of *Galega orientalis* cv. NBS-75 (2.38 mg%) at the flowering stage and minimal – in the leaves of *Galega officinalis* cv. Harant (1.20 mg%) at the fruitage. Highest level of chlorophyll *b* concentration was identified for *Galega orientalis* cv. NBS-75 (0.58 m%) at the flowering stage and lowest – for *Galega orientalis* cv. Kavkazskiy branets (0.19 mg%) at the spring vegetation period. As result, maximal accumulation of carotenoid concentration observed in the leaves of the plants of *Galega orientalis* cv. NBS-75 (1.05 mg%) at the flowering stage and minimal – in the leaves of the plants of *Galega orientalis* cv. Kavkazskiy branets (0.31 mg%) at the spring vegetation period. The results showed the ratio of chlorophylls content (*a/b*) was in range from 3.12 (*Galega orientalis* cv. Kavkazskiy branets at the fruitage) to 10.10 (*Galega orientalis* cv. Saliut at the spring vegetation). Maximal and minimal ratio between chlorophylls and carotenoids content identified for *Galega orientalis* cv. NBS-75 and was in range from 2.40 (budding stage) to 6.79 (spring vegetation stage).

Keywords: *Galega*; chlorophyll *a*; chlorophyll *b*; carotenoids; pigment ratio

Introduction

Physiological indexes quite often use for the characteristic of plant resistance to different factors of environment. Amount of chlorophyll in leaf tissue is influenced by nutrient availability and environmental stresses such as drought, salinity, cold and heat etc. (Palta, 1990).

Galega officinalis L. is widely used in folk medicine as an antidiabetic or for increasing lactation (Shojaee, 2015). This plant has been used for treatment of the plague, malignant fevers, and parasitic infection and was also widely cultivated as cattle feed in ancient times. The ethanolic extracts of *Galega officinalis* were tested against both gram-positive and gram-negative bacteria as the plant was suggested to hasten skin healing after surgery, and the antibacterial effect was shown (Karakas, 2012). Antimicrobial activity of extracts of *Galega officinalis* against bacteria was more

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effective than against fungus (Ozbuca et al., 2005). Plant raw material of these plants is rich source or biologically active compounds such as ascorbic acid, lipids, protein, carotene, amino acids etc. (Champavier, 1999; Balezentiene and Mikulioniene, 2006; Vergun, 2012). As reported Peiretti and Gai (2006) dry matter, organic matter, neutral detergent fibre, acid detergent fibre, lignin and gross energy increased during maturation, while the crude protein, ash of *Galega orientalis* decreased with increasing stage.

Moreover, as resulted Balezentiene and Spruogis (2011), biomass of *Galega orientalis* Lam. has better chemical content and metabolizable energy (9.6 MJ/kg) than traditional feed grasses and improved feed value and selective consumption of animal feeds.

Materials and methodology

Investigation was conducted in the laboratory of Cultural flora department of M.M. Gryshko National Botanical Garden of the NAS of Ukraine. Plant raw material of two cultivars of *Galega officinalis* and four cultivars of *Galega orientalis* collected during the vegetation period: at spring vegetation, budding stage, flowering stage, and fruitage. It was determined the content of leaf pigments (chlorophyll *a*, *b* and carotenoids) by spectrophotometric method according to Musienko et al. (2001) in solution of acetone extracts. Work stages: extraction with acetone (100 mg of fresh mass per 20 mL), spectrophotometric measuring at 662 nm (chlorophyll *a*), at 644 nm (chlorophyll *b*) and at 440 nm (carotenoids). Powder of $MgCO_3$ was added to avoid destruction of pigments. To determine the density of pigment solvent was used a spectrophotometer UNICO 2800. The concentrations of pigments were calculated according to the equation proposed by Holm-Wettstein (Musienko et al., 2001). The results are given in mg per 100 g of fresh mass. Mean values and standard deviations are given in Tables 1–3, Figure 1, 2.

Results and discussion

Chlorophyll content is one of the indices of photosynthetic activity (Larcher, 1995). The chlorophyll is also plays important role in plant physiology and it can be act as nutrition in decline blood sugar conditions, detoxification, digestion and decreasing allergens. Leaf chlorophyll concentration is an important parameter that is regularly measured as an indicator of chloroplast content, photosynthetic mechanism and of plant metabolism. Chlorophylls are an oxidant compounds which are present and stored in the chloroplast of green leaf, stems, and flowers (Kamble et al., 2015)

Leaves of investigated plants accumulated chlorophyll *a* in range from 1.20 to 2.38 mg% during vegetation (Table 1). The experimental results showed in the spring vegetation period, when shoots of plants have started to grow, the concentration of this pigment was in range 1.43–2.04 mg%, in budding period – in range 1.27–2.09 mg%, in flowering stage – in range 1.51–2.38 mg%, in fruitage – in range 1.20–1.88 mg%.

In Table 2 showed values of concentration of chlorophyll *b* in the leaves of investigated cultivars of *Galega officinalis* and *Galega orientalis*. In the start of spring vegetation its sign was in range 0.19–0.50 mg%, in the period of budding – in range 0.31–0.52 mg%, in the flowering stage – in range 0.38–0.58 mg%, in fruitage period – in range 0.24–0.54 mg%. Variation in leaf chlorophyll content can provide information about the physiological condition of a leaf or plant (Larcher, 1995).

Carotenoids play a very important role in the process of photosynthesis. Biosynthesis of carotenoids in plants is a genetic characteristic, but environmental conditions also have an essential role (Bojovic

and Stojanovic, 2005). Also, carotenoid pigments are important for animal organisms and decrease a risk of illness such as cancer, prostate, illness of the cardiovascular system.

Table 1 The content of chlorophyll *a* in the leaves of plants of genus of *Galega* L., mg% per fresh mass

Sample	Spring vegetation	Budding	Flowering stage	Fruitage
<i>G. officinalis</i> cv. Harant	2.04 ±0.07	2.09 ±0.06	1.51 ±0.04	1.20 ±0.02
<i>G. officinalis</i> cv. Flamingo	1.82 ±0.06	2.05 ±0.01	1.87 ±0.08	1.88 ±0.01
<i>G. orientalis</i> cv. Kavkazskiy branets	1.56 ±0.01	1.61 ±0.06	2.00 ±0.19	1.25 ±0.03
<i>G. orientalis</i> cv. Saliut	2.02 ±0.03	1.49 ±0.06	1.88 ±0.06	1.87 ±0.04
<i>G. orientalis</i> cv. NBS-75	1.43 ±0.01	1.52 ±0.07	2.38 ±0.19	1.27 ±0.10
<i>G. orientalis</i> cv. Riabchik	1.92 ±0.04	1.27 ±0.01	1.86 ±0.07	1.66 ±0.07

Table 2 The content of chlorophyll *b* in the leaves of plants of genus of *Galega* L., mg% per fresh mass

Sample	Spring vegetation	Budding	Flowering stage	Fruitage
<i>G. officinalis</i> cv. Harant	0.50 ±0.02	0.50 ±0.09	0.38 ±0.09	0.24 ±0.04
<i>G. officinalis</i> cv. Flamingo	0.42 ±0.02	0.52 ±0.01	0.53 ±0.10	0.44 ±0.07
<i>G. orientalis</i> cv. Kavkazskiy branets	0.19 ±0.01	0.31 ±0.02	0.55 ±0.01	0.40 ±0.03
<i>G. orientalis</i> cv. Saliut	0.20 ±0.02	0.37 ±0.04	0.56 ±0.01	0.53 ±0.08
<i>G. orientalis</i> cv. NBS-75	0.28 ±0.03	0.36 ±0.01	0.58 ±0.12	0.35 ±0.01
<i>G. orientalis</i> cv. Riabchik	0.25 ±0.03	0.31 ±0.02	0.52 ±0.02	0.43 ±0.02

The possible justification could be that chlorophyll *a* is the primary pigment while other pigments including chlorophyll *b* are accessory pigments (Srichaikul et al., 2011; Kamble et al., 2015).

In Table 3 represented data of carotenoid concentration in the leaves of species of genus of *Galega*. Investigated plants accumulated carotenoids in the spring vegetation from 0.31 to 0.88 mg%, in the budding period – from 0.66 to 0.87 mg%, in the flowering stage – from 0.51 to 1.05 mg%, in fruitage period – from 0.57 to 0.85 mg%. The greatest values of carotenoid content were recorded in the flowering stage for plants of *G. orientalis* cv. NBS-75.

Table 3 The content of carotenoids in the leaves of plants of genus of *Galega* L., mg% per fresh mass

Sample	Spring vegetation	Budding	Flowering stage	Fruitage
<i>G. officinalis</i> cv. Harant	0.88 ±0.02	0.85 ±0.02	0.51 ±0.01	0.57 ±0.04
<i>G. officinalis</i> cv. Flamingo	0.80 ±0.02	0.87 ±0.02	0.76 ±0.01	0.81 ±0.01
<i>G. orientalis</i> cv. Kavkazskiy branets	0.31 ±0.03	0.76 ±0.07	0.88 ±0.06	0.60 ±0.03
<i>G. orientalis</i> cv. Saliut	0.34 ±0.07	0.73 ±0.01	0.85 ±0.02	0.85 ±0.03
<i>G. orientalis</i> cv. NBS-75	0.39 ±0.05	0.76 ±0.01	1.05 ±0.04	0.57 ±0.03
<i>G. orientalis</i> cv. Riabchik	0.32 ±0.03	0.66 ±0.01	0.83 ±0.02	0.78 ±0.02

According to Paliy et al. (2011) the content of carotenoids in *Galega officinalis* plants was 6.6 mg% that is 7.5 times more comparing with our maximal sign.

Analyzing data from Figure 1 it should be noted the ratio of chlorophylls concentration was in spring vegetation in range from 4.08 to 10.10, in the budding period – from 3.94 to 5.19, in flowering stage – from 3.36 to 4.11, in fruitage period – from 3.12 to 5.00. According to the majority of investigators, the ratio between chlorophylls *a* and *b* is 3 : 1. The weight ratio of chlorophyll *a* and chlorophyll *b* is an indicator of the functional pigment equipment and light adaptation of the photosynthetic apparatus (Bojovic and Stojanovic, 2005).

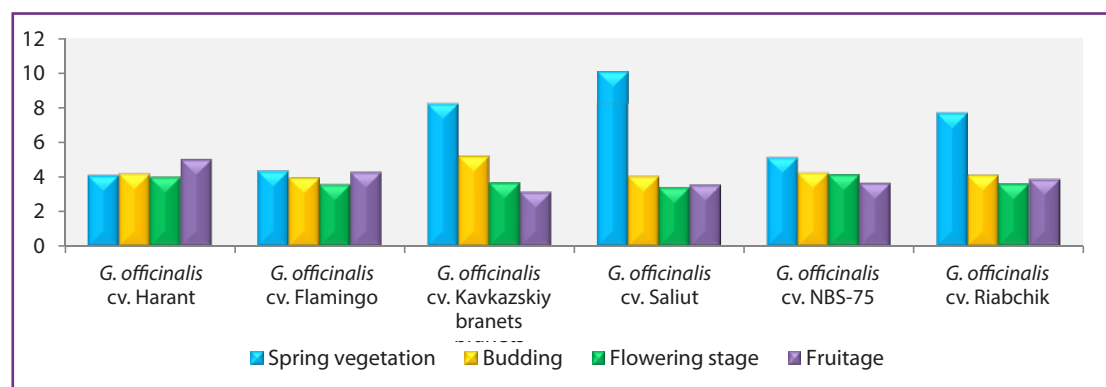


Figure 1 The chlorophyll content ratio *a/b* in the leaves of plants of genus of *Galega* L.

In Figure 2 demonstrated ratio of sum of chlorophylls to carotenoid content during vegetation. At spring vegetation ratio of chlorophylls content to carotenoid content was from 2.80 to 6.79, in budding stage – from 2.40 to 3.05, in flowering stage – from 2.82 to 3.71, in fruitage – from 2.53 to 2.86.

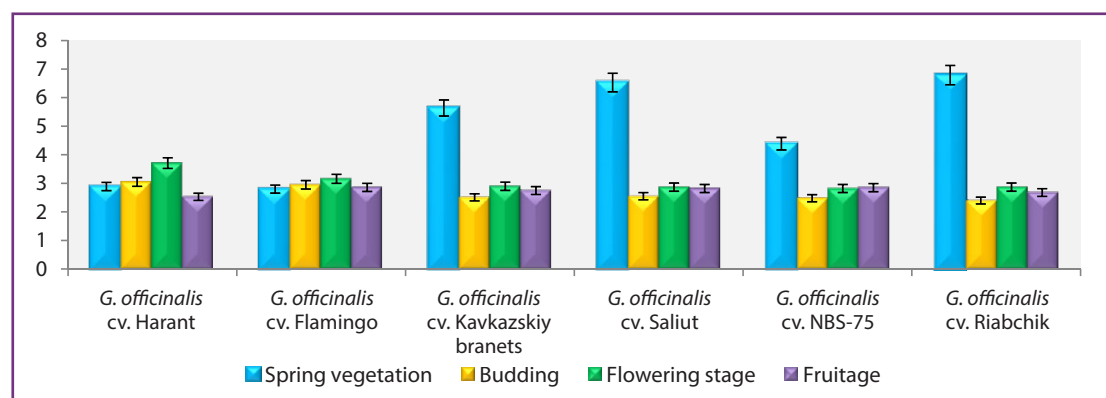


Figure 2 The ratio of the content of the sum of chlorophylls to carotenoids in the leaves of plants of genus of *Galega* L.

According to Penuelas et al. (1995) increasing in the relative concentration of carotenoids are often observed when plants are subjected to stress. The weight ratio of chlorophylls to carotenoids is an indicator of the greenness of plants. It is normally from 4.2 to 5.0 in sun leaves and sun-exposed

plants, and between 5.5 and 7.0 in shade leaves and shade-exposed plants. Lower values of this ratio are an indicator of senescence, stress, and damage to the plant and the photosynthetic apparatus (Brix, 1962).

Conclusions

Thus, in conditions of M.M. Gryshko National Botanical Garden of the NAS of Ukraine the plants of cultivars of *Galega officinalis* and *Galega orientalis* accumulated chlorophyll a in the leaves more than chlorophyll b. Obtained data have resulted that for all cultivars of *Galega officinalis* and *Galega orientalis* the chlorophyll a concentration was higher than that of chlorophyll b. In addition, different ratio between a sum of chlorophylls and carotenoid content says about various functional pigment equipment and light adaptation (3.12–10.10 for a/b and 2.40–6.79 for $(a + b)/car$). The most accumulation of chlorophyll *a*, *b* and carotenoid concentration was fixed for *G. orientalis* cv. NBS-75 (2.38, 0.58 and 1.05 mg% respectively).

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