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ESTIMATION OF PHOTOSYNTHETIC PIGMENTS IN THE LEAVES OF *CRAMBE* SPP. DURING VEGETATION

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This study investigated the content of chlorophyll *a* (Chl *a*), b (Chl *b*), carotenoids (car) and their ratio in four species of *Crambe* L. during vegetation: *C. cordifolia* Steven, *C. koktebelica* (Junge) N.Busch, *C. maritima* L., *C. steveniana* Rupr. collected in the M.M. Gryshko National Botanical Garden of the NAS of Ukraine (Kyjiv). The middle height increment of investigated plants between stages of growth is measured. The concentration of chlorophylls *a*, *b* and *car* measured spectrophotometrically on UNICO 2800 at the wavelength 662, 644 and 440, respectively. Extraction procedure conducted in acetone. Obtained results are given in mg/g of fresh mass. The height increment in a period of spring vegetation-budding was 26.3–80.2 cm, budding-flowering 12.3–136.0 cm, flowering-fruitage 1.3–16.6 cm depending on species. *C. cordifolia* had the most value of this parameter at the budding-flowering period and *C. maritima* had the least value at the flowering-fruitage period. Content of Chl *a* at the spring vegetation period was 0.321–0.490 mg/g, at the budding period 0.237–0.680 mg/g, at the flowering period 0.349–0.680 mg/g and at the fruitage 0.478–0.962 mg/g depending on species. Content of Chl *b* was 0.133–0.203 mg/g at the spring vegetation stage, 0.158–0.275 mg/g at the budding stage, 0.158–0.275 mg/g at the flowering stage and 0.227–0.439 mg/g at the fruitage depending on species. The concentration of *car* was 0.038–0.078 mg/g at the spring vegetation period, 0.043–0.104 mg/g at the budding period, 0.070–0.201 mg/g at the flowering period and 0.117–0.435 mg/g at the fruitage depending on species. Correlation between the content of Chl *a* and increment of plants was strong at the spring vegetation (r = 0.78), moderate at the budding period (r = 0.52) and strong at the flowering (r = 0.76). At the flowering stage found a very strong correlation (r = 0.90) between Chl *b* content and increment of plants. Thus, obtained data showed that between the accumulation of photosynthetic pigments of investigated plants and increment of plants exists a positive correlation, which depends on the period of growth. Further, it's can be used for the study of total productivity and some grow parameters of these plants.

Keywords: *Crambe*, chlorophylls, carotenoids

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Introduction

Plants of the *Crambe* L. genus have studied in the Department of Cultural Flora of M.M. Gryshko National Botanical Garden. Investigation of the biochemical composition of underground parts of these plants showed high content of ascorbic acid, dry matter, sugars, etc. (Vergun et al., 2018). These plants also a good source of nutrients in the above-ground parts (Vergun et al., 2019). Some of these crops have industrial and pharmaceutical importance due to the content of oil in seeds and biologically active compounds, among which different groups of phenolic compounds (Bukhari et al., 2013). Also, these plants tested as forage and medicinal crops, on the other hand, selected species of this genus listed in the Red Book of Ukraine (Kalista, 2017).

The study of raw of these plants demonstrated different biological activities such as antioxidant, antimicrobial, cytotoxic, allelopathic, etc. (Razavi et al., 2009; Bukhari et al., 2013; Rashid et al., 2018; Kalista et al., 2019). One is the most important species of this genus is *C. abyssinica* that can produce oil with rich content of fatty acids (Bondioli et al., 1998). Oil from this species used for industrial products, such as industrial lubricants, corrosion inhibitors, etc. (Jasper et al., 2010). They also prospective candidates for use in biodiesel production (Silva et al., 2012).

Ecological aspects of *C. abyssinica* study showed results of the effect of different levels of soil salinity on growth parameters. As a result, this plant can be recommended as an alternative source of green biomass even under some levels of salinity (Ionov et al., 2013; Vasconcelos et al., 2015).

The aim of this study was to determine the accumulation of photosynthetic pigments in *Crambe* spp. leaves during vegetation. As reported by Dey et al. (2016), an important parameter of crop health is the leaf chlorophylls measurements. Plant pigments are necessary for photosynthesis and content of them can provide information about the physiological status of plants (Zhou et al., 2019).

Materials and methodology

Biological material

In this study leaves of four species of *Crambe* were evaluated: *C. cordifolia* Steven, *C. koktebelica* (Junge) N.Busch, *C. maritima* L., *C. steveniana* Rupr. Leaves collected from the experimental collection of the M.M. Gryshko National Botanical Garden of the NAS of Ukraine (Kyiv) during vegetation. Every two weeks was measured height of plants and during vegetation conducted phenological properties. Increment of plants measured at the stage of spring vegetation, budding, flowering and fruitage.

Determination of photosynthetic pigments

0.1 g of fresh leaves mass homogenized with 10–20 ml of 100% acetone and 0.5 mg $MgCO_3$ powder was added. The extract was then centrifuged at 2,500 rpm for 5 min. The measurement of pigments was conducted spectrophotometrically: chlorophyll *a* (Chl *a*) at the wavelength 662 nm, chlorophyll *b* (Chl *b*) at the wavelength 640 nm and carotenoids (*Car*) at

the wavelength 440 nm. For the measuring used 2,800 Uv/VIS Spectrophotometer (Unico). The results expressed in mg/g of fresh weight. The concentration of chlorophyll *a*, chlorophyll *b* and carotenoids were calculated using the equation proposed by Holm-Wettstein (Musienko et al., 2001).

Statistical analysis

Basic statistical analyses were performed using Excel 2016. Average and standard deviation given in Figure 1–5. Correlation coefficients were calculated by CORR analysis.

Results and discussion

Ecological aspects of plant study basically related to the conditions of growth. One of the most important ecological factors in plant life is light (Lichtenthaler, 1987).

Plant height in crops is generally considered to be a quantitative trait controlled by multiple loci and significantly affected by environmental factors (Chai et al., 2019). In our study, we used to measure the height of plants, increment and content of chlorophylls in leaves dynamically. The increment of plants during vegetation was measured and results represented in Figure 1. This parameter of investigated plants in the period of spring vegetation was from 16.3 to 26.6 cm, in the budding stage from 26.3 to 80.2 cm, in the flowering stage from 12.3 to 136.0 cm, the fruiting stage from 1.3 to 16.6 cm depending on species.

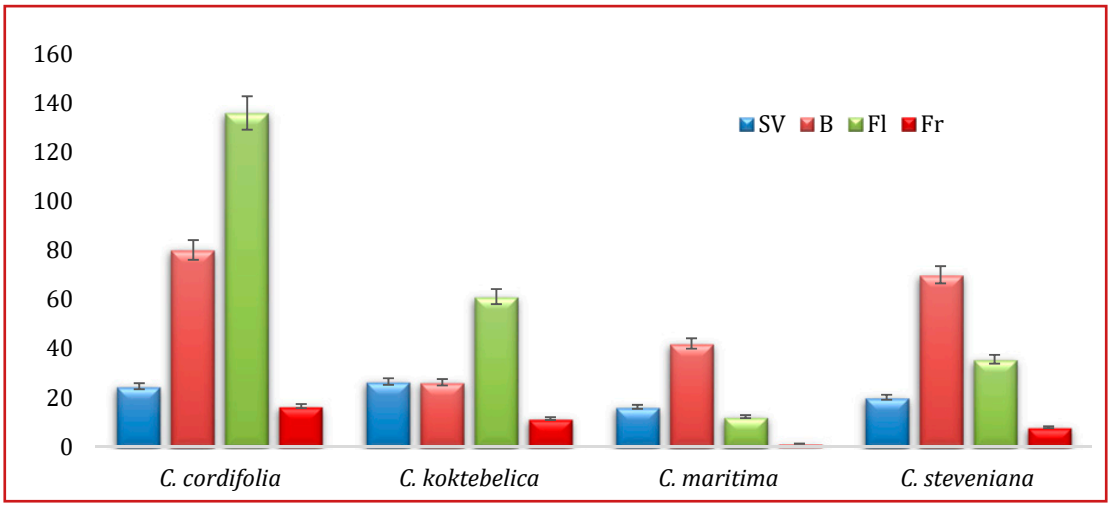

Figure 1 Increment of plants of *Crambe* L. species during vegetation(cm)
SV – spring vegetation; B – budding; Fl – flowering; Fr – fruitage

Investigation of leaf chlorophyll one of the most important vegetation parameter and is an indicator of photosynthetic activity (Lichtenthaler, 1987; Carmona et al., 2015). Chlorophyll responsible for green color and conversation of light into chemical energy and mainly determines the photosynthetic rate and primary productivity (Li et al., 2019). The most known method of investigation of chlorophylls and carotenoids content is conducted

spectrophotometrically (Lichenthaler, 1987). However, nowadays exist special equipment (portable chlorophyll meter) for measuring chlorophylls in field conditions is very simple and allows them to obtain results immediately (Dey et al., 2016; Kaspary et al., 2019).

Photosynthetic pigments are a group of nutrients that can demonstrate an antioxidant property (Zawiślak and Nurzyńska-Wierdak, 2014). Chlorophyll *a* and *b* associated with the light-harvesting process and are an indicator of plant health (Gogoi and Basumatary, 2018). Usually content of chlorophylls studies with the influence of different ecological factors (Li et al., 2019). The study of chlorophyll content can be used for vegetation health monitoring, forage quality assessment, biomass estimation, productivity measures, etc. (Hallik et al., 2017).

Content of Chl *a* in the stage of spring vegetation found from 0.32 to 0.49 mg/g FW, in the stage of budding from 0.24 to 0.68 mg/g FW, in the stage of flowering from 0.35 to 0.68 mg/g FW, and in stage of fruiting from 0.48 to 0.96 mg/g FW depending on species (Figure 2). It should be noted that in this period accumulation of Chl *a* in the leaves during vegetation for investigated species was uneven.

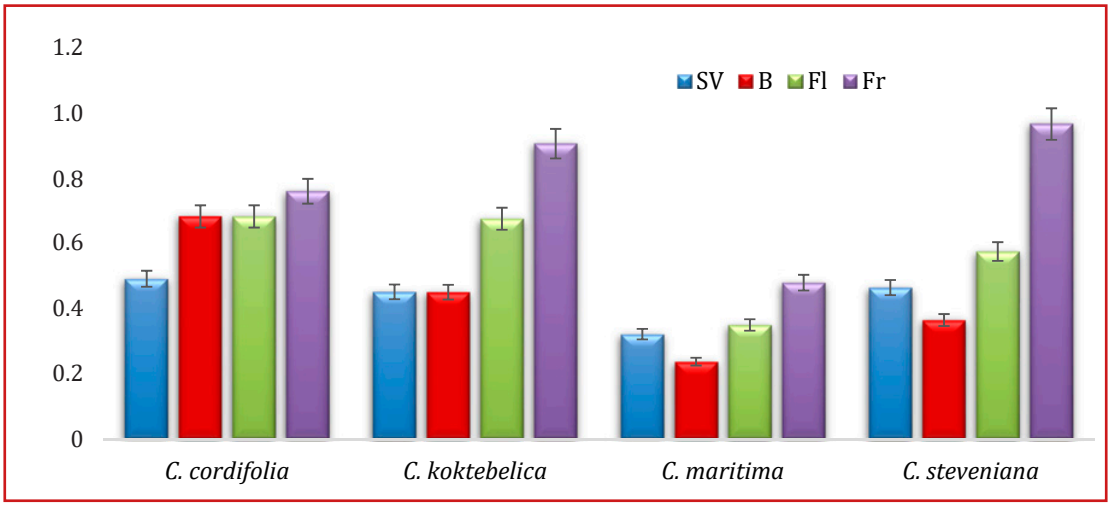

Figure 2 Content of chlorophyll *a* in the leaves of *Crambe* L. species during vegetation (mg/g) FW. SV – spring vegetation; B – budding; Fl – flowering; Fr – fruitage

Chlorophyll content changing with the change of the external environment and definite as an important diagnostic indicator for plant growth study (Li et al., 2019). Content of chlorophyll *b* in the leaves of *Crambe* in a period of spring vegetation was from 0.13 to 0.20 mg/g FW, in the period of budding from 0.16 to 0.28 mg/g FW, in the period of flowering from 0.15 to 0.27 mg/g FW, and fruitage from 0.28 to 0.44 mg/g FW (Figure 3). Content of chlorophylls decrease more rapidly than carotenoids if plants in the stress conditions or during leaf senescence (Gitelson and Merzlyak, 1994; Peñuelas et al., 1995; Filella et al., 2009).

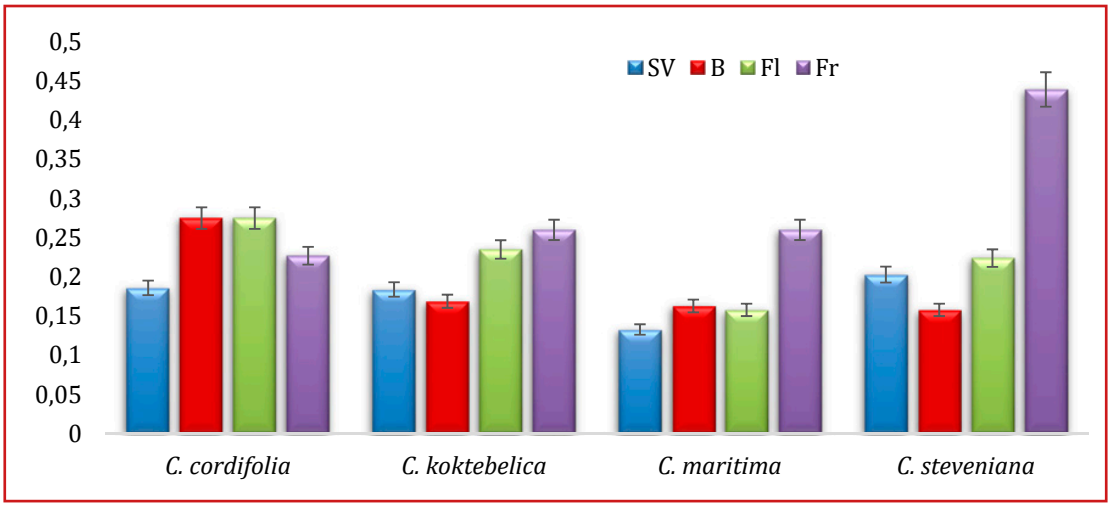

Figure 3 Content of chlorophyll *b* in the leaves of *Crambe* L. species during vegetation (mg/g)
FW. SV – spring vegetation; B – budding; Fl – flowering; Fr – fruitage

Carotenoids are involved in various defense mechanisms. They can inhibit the formation of free radicals preventing their negative effect on the organism. Carotenoids characterized as photoprotective agents with the ability to reduce sunburns, photo-allergy and some kinds of cancer (Veeru, 2009). This is mainly related to the yellow color during chlorophyll degradation (Li et al., 2019). In the study of Ivanišova et al. (2017), total carotenoid content and antioxidant activity of plant extracts by the phosphomolybdenum method had a strong correlation. Content of carotenoids in the stage of spring vegetation was from 0.04 to 0.10 mg/g FW, budding stage from 0.04 to 0.10 mg/g FW, flowering stage from 0.07 to 0.20 mg/g FW, fruitage stage 0.12 to 0.44 mg/g FW (Figure 4).

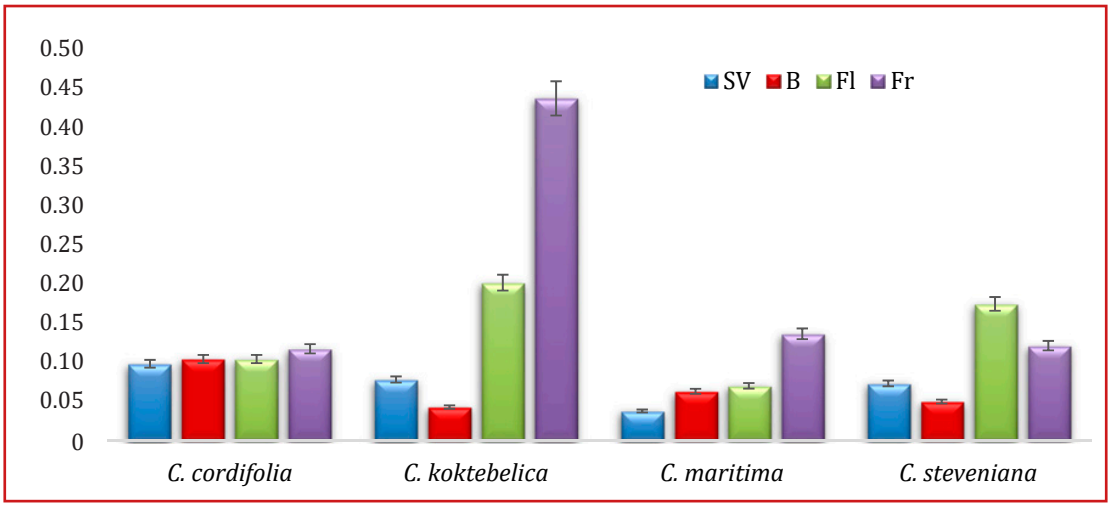

Figure 4 Content of carotenoids in the leaves of *Crambe* L. species during vegetation (mg/g)
FW. SV – spring vegetation; B – budding; Fl – flowering; Fr – fruitage

Any stress effects on regulation processes in plant organisms and photosynthetic properties. This can be noticed in the relation between carotenoids and chlorophylls. Carotenoids/chlorophylls ratio, in this case, increased (Filella, 2002). As reported before, the knowledge of the proportion between chlorophylls and carotenoids in leaves can provide more information for indicating plant physiological status (Zhou et al., 2019). According to Gogoi and Basumatary (2018), chlorophyll a/*b* (Chl *a*/*b*) ratio indicates the developmental state of the photosynthetic apparatus in plant organisms and connected with plant growth. The Chl a/b ratio in this study was from 2.28 to 2.64 in the period of spring vegetation, from 1.45 to 2.65 in the period of budding, from 2.28 to 2.87 in the period of flowering, from 1.84 to 3.50 (Figure 5). As reported by Anderson (1986), the typical Chl a/b ratio for shade plants is 1.6–2.2 and light plants 2.6–3.4.

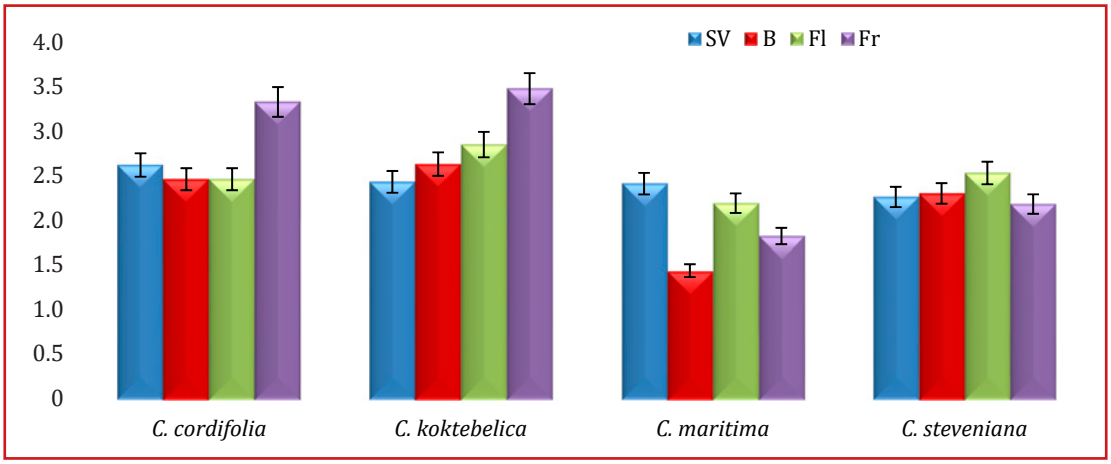

Figure 5 Relation between the content of chlorophyll *a* and chlorophyll *b* in the leaves of *Crambe* L. species
SV – spring vegetation; B – budding; Fl – flowering; Fr – fruitage

Correlation analysis of obtained results showed very strong, strong, moderate, etc., relations between investigated parameters (Table 1).

It should be noted that very strong correlation at the period of spring vegetation found between the content of *car* and increment of plants and between all investigated pigments. At the budding stage determined very strong correlation between Chl *a* and Chl *b* and between Chl *b* and car. At the flowering stage, very strong correlation found between chlorophylls content, increment of plants and Chl *b*, and between ratio of chlorophylls and car. At the fruitage period found strong correlation between increment of plants and chlorophylls ratio that had the highest value during vegetation.

Table 1 Coefficient of correlation between increment of plants and accumulation of pigments during vegetation

Characters	Increment	Chl *a*	Chl *b*	*Car*
Spring vegetation				
Chl *a*	0.782	1		
Chl *b*	0.620**	0.929**	1	
Car	0.836**	0.962	0.794**	1
Chl *a*/*b*	0.455	0.239**	-0.136*	0.492*
Budding				
Chl *a*	0.521*	1		
Chl *b*	0.625	0.898	1	
Car	0.694**	0.701**	0.940**	1
Chl *a*/*b*	0.130	0.719**	0.341**	0.014
Flowering				
Chl *a*	0.762*	1		
Chl *b*	0.901	0947**	1	
Car	-0.013*	0.636	0.389*	1
Chl *a*/*b*	0.260	0.797**	0.564	0.923
Fruitage				
Chl *a*	0.548	1		
Chl *b*	-0.259**	0.547	1	
Car	0.175**	0.353**	-0.261	1
Chl *a*/*b*	0.880	0.470	-0.478**	0.592

Note: Significant correlations are indicated by $^{**}p < 0.01$ and $^{*}p < 0.05$

Conclusions

Obtained data showed that maximal values of chlorophyll *a* found at the stage of fruitage for all investigated species. Minimal values of it found at the stage of spring vegetation for *C. cordifolia* and *C. koktebelica*, and at the stage of budding for *C. maritima*, *C. steveniana*. The least value of chlorophyll *a*/*b* ratio found for *C. maritima* at the stage of budding indicates some changes in the photosynthetic apparatus.

It is found a correlation between the content of chlorophyll *a* and the growth of the plant in the start of vegetation between the process of growth and accumulation of photosynthetic pigments exists the correlation. Very strong and strong correlation found between increment and *car* content at the spring vegetation and budding period, respectively. Very strong correlation identified between the increment of plants and the ratio of chlorophylls at the end of vegetation.

Thus, the obtained results showed that a positive correlation between the accumulation of photosynthetic pigments of investigated plants and the increment of plants depends on the

period of growth. Taking into account fewer reviews about pigment status of *Crambe* spp., obtained data can be used for the study of total productivity and some grow parameters of these plants.

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