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PHOTOSYSTEMS FUNCTIONING, THE CONTENT OF CHEMICAL ELEMENTS, FLAVONOIDS AND BRASSINOSTEROIDS IN LEAVES OF CHIMERA *CORNUS CONTROVERSA*

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The formation of chimera is a well-known phenomenon in variegated ornamental and wild plants, but the biochemical peculiarities of this phenomenon are not well studied. The purpose of this work was to conduct a comparative study of the photosynthetic apparatus functioning, the content of macro-, microelements, flavonoids and brassinosteroids in the variegated and green leaves of the periclinal chimera Cornus controversa plant grown in the collection of M. M. Gryshko National Botanical Garden of the NAS of Ukraine. Sampling was made during the period from June to October 2022. The content of photosynthetic pigments and brassinosteroids was measured spectrophotometrically, autofluorescence of leaf tissues was studied using an inverted microscope with a multi-channel fluorescence imaging system, Normalized difference vegetation index (NDVI) – using GreenSeeker devices and fluorimeter, the level of macro- and microelements – using plasma emission spectrometer. Phenolic secondary metabolites were separated by HPTLC. To assess the protective capacity of flavonoids, a bioassay on the germination of radish seeds Raphanus sativus var. radicula in the presence of $AlCl_3$ *was used. It was shown that green leaves of C. controversa chimera, compared to variegated ones, had a twice as high NDVI index, maintained photosynthetic function longer in autumn, and had higher content of macronutrients (Mg, Ca and S) but lower concentration of toxic metals (Al, Cu, Fe, Zn, Ti). Instead, variegated leaves accumulated more brassinosteroids and phenolic metabolites, which were found not only to reduce the toxic effect of aluminum but also to have a growth-stimulating effect on the roots of radish seedlings. Such specialization of green and variegated leaves may contribute to better plant chimeras adaptation to environmental pollution.*

K e y w o r d s: periclinal chimera, Cornus controversa, photosynthetic pigments, NDVI index, tissue autofluorescence, macroelements, microelements, phenolic metabolites, brassinosteroids.

Variegation is the appearance of differently colored zones on the surface of leaves, stems and fruits of plants. It is a common phenomenon in the plant world, described for more than 1,700 plant species [1, 2]. In nature, species with variegated individuals are usually found in low-light undergrowth of deciduous forests or in other habitats beyond their ecological optimum, suggesting the possible adaptive nature of this trait. Variegation may be both genetically determined and a phenotypic response to external conditions [1].

Because of their aesthetic appeal, variegated forms are widely used in modern horticulture. In this context, the origin of variegation and the molecular mechanisms responsible for the inheritance of this trait are being actively investigated [1]. To date, two types of variegation have been established: (1) structural and (2) pigmentary, which is associated with the formation of abnormal plastids [3].

Despite a considerable number of studies on the physiological manifestations of variegation, there are still more questions than answers [2, 4, 5]. Although variegation slows plant growth due to reduced photosynthetic productivity, it has a number of advantages under certain conditions. In particular, Abadie et al. [6] studied the growth and metabolism of the variegated form of *Pelargonium×hortorum* L. H. Bailey, showed that under conditions of nitrogen deficiency, such plants accumulate leaf biomass more intensively and age more slowly than green plants. Differences in carbohydrate and amino acid metabolism of green and variegated leaves of *Coleus blumei* Benth. were found in the studies of Madore [7]. In particular, raffinose sugars were identified in green tissues, whereas in variegated tissues, sugars were hydrolyzed and converted into amino acids and organic acids. A comparative analysis of the metabolism in the mesophyll of non-chlorophyll, variegated and green leaves of *Camellia sinensis* showed that the content of amino acids and tannin was higher in the tissues of non-chlorophyll leaves than in variegated and green leaves. Intensification of lipid metabolism, retardation of sugar synthesis and increased content of amino acids in non-chlorophyll leaves of *Hydrangea macrophylla* “Maculata” compared to green leaves were shown by Li et al. [8].

Some differences in the functioning of enzymatic and non-enzymatic antioxidant protection systems were also noted. So, it was shown that the redox regulatory system in green leaves of *Pelargonium zonale* was based on ascorbate and activity associated with thylakoids, ascorbate peroxidase, and catalase. At the same time, ascorbate was localized mainly in the nucleus, peroxisomes, plastids, and mitochondria. On the other hand, chlorophyll-free leaves contained a higher amount of glutathione, which was mainly localized in mitochondria. The enzymatic antioxidant system in these tissues consisted of the ascorbate-glutathione cycle and Mn and Cu/Zn superoxide dismutase [9].

There is no consensus regarding tolerance to environmental stresses in variegated and green forms. On the one hand, due to the lower photosynthetic function and growth, variegated forms are supposed to be inferior to green ones in this respect [10]. However, there is evidence that variegation helps protect plants from ultraviolet radiation and low temperatures [11, 12].

The aim of our work was to study the peculiarities of photosynthetic function, non-enzymatic antioxidant accumulation and distribution of macro- and microelements in variegated and green leaves of the periclinal chimera *Cornus controversa* Hemsl.

Chimera refers to the fusion of two different forms in one individual. It usually arises from a bud, either apical or lateral. Depending on the location and nature of the arrangement of heterogeneous cells in the meristem layers, such plants are divided into periclinal, mericlinal, sectorial chimeras, and those with heterogeneous areas of cells without a pattern [13]. The periclinal chimera is the only stable type, while the other three are unstable. Periclinal chimeras are attractive to breeders because they can be used to transfer disease resistance and other useful traits [14].

The phenomenon of heterogenomicity in the plant kingdom has served as a tool for investigating fundamental questions of internal communications between dissimilar parts of a chimera organism. Until now, little understanding has been reached concerning the biological basis for the observed heterogenomicity. Today, there is very little knowledge about the biological sense of heterogenomic compatibility and the beneficial versus detrimental consequences of the observed genomic combinations.

The periclinal chimeras with shoots that differ in the content of photosynthetic pigments are an interesting object for in-depth physiological studies of the relationship between photosynthesis and related physiological processes. Our analysis of the literature revealed that no such research had been conducted using *Cornus controversa* chimera as a target plant. Besides, as the object of our study was cultivated under stressful conditions due to technogenic pollution, it was interesting to check whether heterogenomicity gives any advantage to chimera to survive in an urbanized environment.

Material and Methods

Plant material. Five plants of the periclinal chimera of *Cornus controversa* ‘Variegata’, which has both natural type shoots with green leaves and variegated ones and grown in the collection of the fruit plant acclimatization department of the M. M. Gryshko National Botanical Garden of the National Academy of Sciences of Ukraine (Kyiv), were used for the study.

Cornus controversa is a deciduous tree of the *Cornacea*e family (Fig. 1), which is widely used as an ornamental and medicinal plant. The natural habitat of this species is East and Southeast Asia (China, Bhutan, North India, Japan, Myanmar, Korea, Nepal) [15]. The pharmacological properties of *C. controvers*a are associated with a rich content of ursolic acid, tannin [15-17], flavonoids, terpenoids, glycosides, including the iridoid loganin [17]. The content of the latter in the leaves of *C. controversa* in the spring period can reach 3.2% [16].

Sampling was made during the period of active vegetative growth (02.06.2022), fruiting (02.06.2022) and the end of the growing season (04.10.2022). Green and variegated leaves (10 green and 10 variegated from each of the 5 chimeras) were collected together with the rhizosphere soil (depth of sampling 20-30 cm). Photosynthetic pigments, flavonoids and brassinosteroids were determined in fresh foliar tissues. While air dry plant material was used for macro- and microelement analysis. Soil samples were air dried and sieved through a 2 mm sieve before analytic procedures.

Measurement of foliar tissue autofluorescence. The autofluorescence of leaf tissues was examined by inverted microscopy using a multichannel fluorescence imaging system (EVOS FL System, ThermoFisher Scientific, USA) with the filters DAPI (λ_{exc} = 357/44 nm; λ_{em} = 447/60 nm), GFP (λ_{exc} = 482/25 nm; λ_{em} = 524/24 nm) and Texas Red (λ_{exc} = 585/29 nm; λem = 628/32 nm). Photographic documentation and digital image processing were performed with the specialized program Image-Pro Premier 9.0 (Media Cybernetics, USA).

Measurement of chlorophyll content and effective quantum yield of photochemical energy conversion in PSII. For the analysis, 30 variegated and green leaves, located in the middle part of the crown of the periclinal chimera of *C. controversa*, were randomly selected. Chlorophyll content in leaves was determined using a field Chlorophyll Meter SPAD 502 (Konica Minolta, Japan) [18]. Photosynthetic pigments were extracted from the leaves with dimethyl sulfoxide [19]. The optical density was measured with a SPECORD 200 (Analytik Jena, Germany, 2003) at 665 nm for chlorophyll *a*, 649 nm for chlorophyll *b*, and 480 nm for carotenoids [20].

The quantum yield of electron transport (Y(II)), which determines the effective quantum yield of photochemical energy conversion to PSII, was determined using a field fluorimeter Y(II) meter&PlantStress Kit (Opti-SciencesInc., USA) and calculated using the formula (F'm –- Fs)/F'm. The maximum quantum yield of photosystem II was estimated after 20 min of dark adaptation, according to the formula Fv/Fm = (Fm – Fo)/Fm [21].

*Fig. 1. Periclinal chimera Cornus controversa (**a**), adaxial (**b**) and abaxial (**c**) surfaces of green and variegated leaves*

The NDVI index, which reflects the relative amount of photosynthetically active biomass, was determined using GreenSeeker (Trimble, Sunnyvale, California, CA, USA) [22, 23].

Chromatography procedures. The separation of phenolics was carried out by the method of high-performance thin-layer chromatography (HPTLC) on Silicagel G 60 plates (Merck, Germany) in the solvent system: ethyl acetate-formic acid-acetic acid-water (v/v/v/v – 100:11:11:25). The resulting chromatograms were treated with 0.5% NP-reagent in ethyl acetate followed by treatment with 1.0% PEG and heating at 90°C for 1 min. Flavonoids and other polyphenols were detected in the chromatogram under ultraviolet light (λ = 365 nm). The Rf indicator (retention index) of substances was determined photodensitometrically using the Sorbfil TLC Videodensitometer program). Purified solutions containing these flavonoids were obtained by eluting with a sorbent on a chromatographic plate.

Quantitative measurements of chemical elements. The preparation of the soil samples for the analysis of the content of macro- and microelements was carried out according to the Rinkis & Nolendorf method [24]. Acid-soluble forms of metal cations and other chemical elements were extracted using a 1.0 N solution of nitric acid (HNO_3). Preparation of plant samples for analysis was done by wet ashing with a solution of high purity nitric acid using Speedwave Xpert DAP-60X (Berghof, GmbH, Germany), a special system for microwave decomposition of samples. The concentrations of chemical elements in the solution were measured using an ICAP 6300 DUO plasma emission spectrometer (Thermo Fisher Scientific, USA). The relative uncertainty ranged from 7 to 12% for different chemical elements.

Quantitative assessment of brassinosteroids. For the analysis of brassinosteroids, samples (leaves) were transferred to a dark climate chamber at –8°C. The extraction was carried out in two steps: the first step with ethyl acetate (three times, 5 ml each) from the aqueous tissue extract (1 g tissue + 5 ml extraction solution). The ethyl acetate fraction was evaporated under vacuum, and the residue was extracted with cyclohexane (5 ml). The second step of extraction was performed with a mixture of ethanol and water (4: 1) (three times, 5 ml each) from the phase containing cyclohexane. The ethanol extract was evaporated under vacuum, and the residue was dissolved in a small amount of ethyl acetate. The content of brassinosteroids was measured spectrophotometrically at a wavelength of 450 nm [25] using a spectrophotometer SPECORD 200 (Analytik Jena, Germany, 2003).

Assessment of protective effects of C. controversa secondary metabolites. The possible protective effect of phenolic secondary metabolites of *C. controversa* against toxic aluminum concentrations was assessed using bioassay on growth reactions of *Raphanus sativus* var. *radicula*. The choice of radish as an object is explained by the rapid growth of its seedlings, high germination rate and fairly high sensitivity to various toxicants. The radish seeds were surface sterilized with 1% sodium hypochlorite, washed with sterilized tap water and allowed to germinate in thermostat (28°C) in the dark for 24 h on two layers of filter paper saturated with sterilized tap water. After germination, uniform seedlings with 1-2 mm roots were transferred to Petri dishes with filter paper disks moistened with $AlCl_3$ (20 mg/ml) solution alone (control) or in the presence of purified *C. controversa* flavonoids obtained by elution with a sorbent on a chromatographic plate. Radish test plants were grown in a controlled environment chamber at +24–26°C, 14/10 h light/dark cycle and light at 80 μmol photons for 3 days. At the end of the bioassay the shoot and root lengths of radish seedlings were measured.

Statistical analysis. The results are presented as mean ± standard error (m ± SE). The reliability of the difference ($P < 0.05$) between the obtained data was determined by the method of variance analysis (one-factor variance analysis) using Tukey's a posteriori test in the XLSTAT program (Addinsoft Inc., USA, 2010).

Results and Discussion

Comparative micromorphological analysis of green and variegated leaves. Analysis of the micromorphological characteristics of green and variegated leaves of *C. controversa* chimera revealed some differences. In particular, cuticular folds up to 18-20 microns high are formed on the lower (abaxial) surface of green leaves. They surround the stomata with a dense ring and create a barrier to horizontal air currents, reducing transpiration moisture loss and generally improving the microclimatic conditions on the lower leaf surface. In addition to optimizing the water regime, cuticular folds may have another adaptive value, such as reducing insects' adhesion to plant surfaces [26]. Interestingly, the described micromorphological structures are rather rare in deciduous trees and shrubs of the temperate zone [27].

In variegated leaves, these folds were less pronounced. Ridge height reduced to 10-12 mm. The diameter of the folds also was reduced. The intensity of autofluorescence of variegated leaves in the green spectrum (λ_{exc} = 482/25 nm; λ_{em} = 524/24 nm), which reflects the presence of secondary metabolites such as cuticular waxes and terpenoids was also lower compared to green leaves. The cuticle of the upper epidermis in variegated leaves was also less developed and had less intense fluorescence.

Secondary metabolites, including phenolic acids and other products of phenylpropanoid synthesis, are normally formed in plastids and accumulate in the protoplasts of leaf cells, with the exception of polyphenolic compounds, which are components of the cell wall [28]. Phenolic secondary metabolites are usually optically active and their distribution is well visualized in cross-sections. Microscopic studies have shown that phenolic compounds in green leaves of *C. controversa* chimera were localized mainly in the cell walls of the upper epidermis and in the cuticular folds (Fig. 2).

A different trend was observed in variegated leaves. In the blue spectrum, the cell walls of the upper epidermis, as well as individual cells of the mesophyll, were intensely fluorescent. The distribution of metabolites with intense fluorescence in variegated leaves was uneven. The intensity of the autofluorescence of the folds on the lower (abaxial) surface of the variegated leaves was significantly lower than that of green leaves.

After processing the leaf samples with a natural (NP) reagent capable of enhancing the fluorescence of phenolic compounds (including flavonoids), an increase in the fluorescence of the folds of the lower epidermis was observed in green leaves (Fig. 2, *c*). At the same time, in variegated leaves, the intensity of the autofluorescence of the cell walls of the upper epidermis and the underlying layer of mesophyll cells increased in the blue spectrum (Fig. 2, *f*).

In the red spectrum, the autofluorescence intensity of the leaf tissues is mainly determined by the ability of chlorophyll and carotenoids to absorb electromagnetic energy and emit it in the orange and

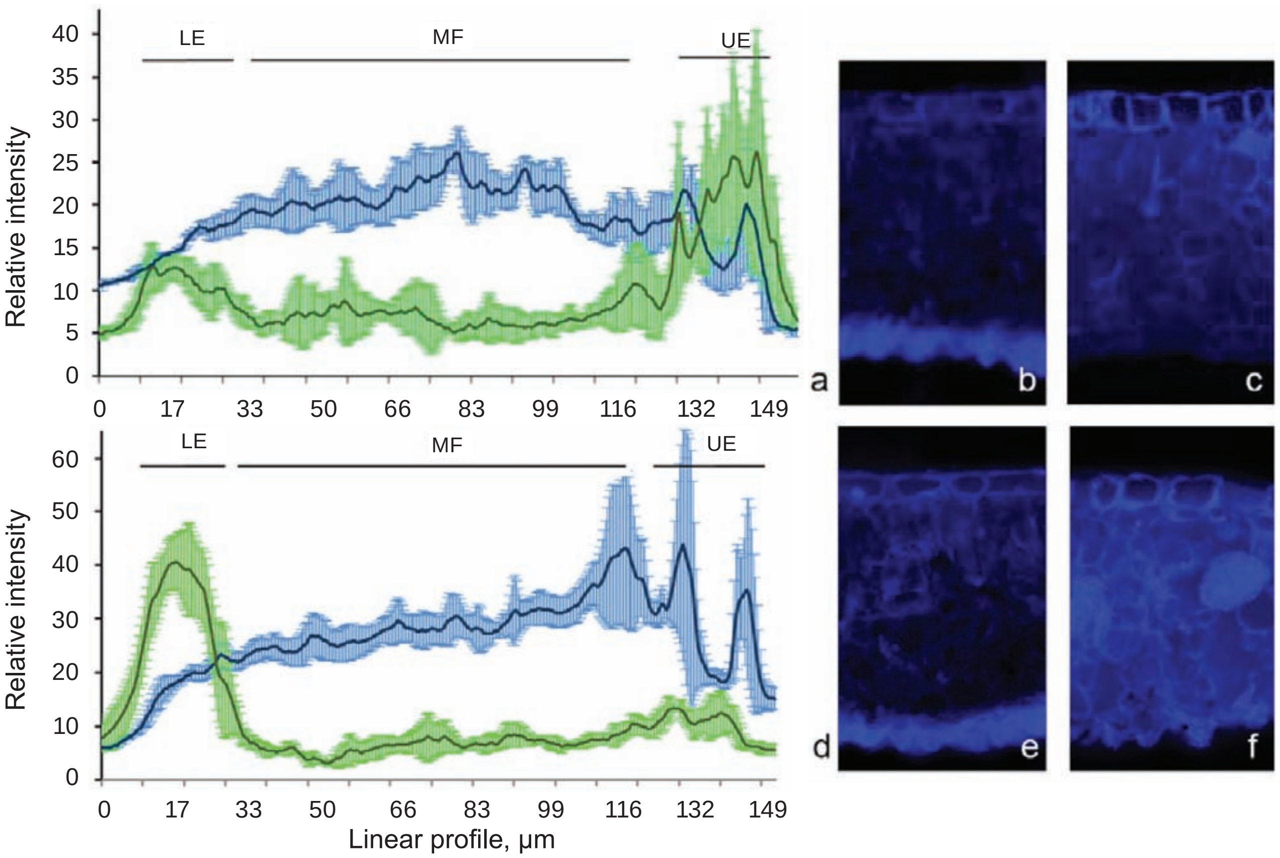


*Fig. 2. Profiles of autofluorescence of green (**b**, **c**) (green color) and variegated (**e**, **f**) (blue color) leaves of Cornus controversa chimera in the blue spectrum (λ_{em} = 447/60 nm); LE – lower epidermis, MF – mesophyll, UE – upper epidermis: before (**a**-**c**) and after (**d**-**f**) treatment with NP-reagent*

red spectra. In addition, lignin also exhibits optical activity in this part of the spectrum. In green leaves, the most intense fluorescence was observed in the mesophyll of the upper part of the leaf, as well as in the cuticular folds of the lower epidermis. At the same time, less fluorescence was observed in variegated leaves. After tissue treatment with the NP reagent, the fluorescence of the protoplasts of the upper epidermis and mesophyll in the upper part of the leaf blade increased in the red part of the spectrum (Fig. 3).

In variegated leaves, groups of cells containing optically active products were found. The intensity of their fluorescence almost did not change along the foliar blade linear profile. Such groups of cells were absent in green leaves. Fluorescence profiles in the green part of the spectrum were similar to the trend shown in Fig. 3.

To conclude, green leaves have more pronounced ultrastructural formations providing mechanical protection against drying out, phytophages, etc., such as better-developed cuticles of the upper epidermis with more pronounced folds, which accumulate higher amounts of protective secondary metabolites. While variegated leaves have less developed cuticles, their protective secondary metabolites are evenly distributed along the foliar blade linear profile.

Functional state of photosynthetic systems. The contents of chlorophylls and carotenoids in the green segments of variegated and in green leaves (Table 1) were not significantly different in June and August.

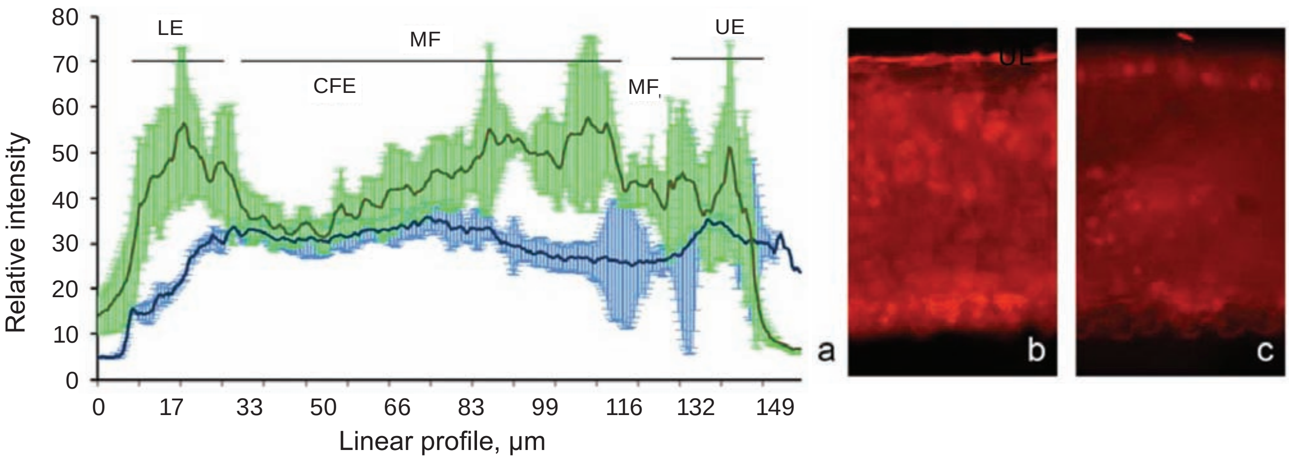


Fig. 3. Profiles of autofluorescence of green (green curve) and variegated (blue curve) leaves of Cornus controversa chimera after treatment with NP-reagent red spectrum (λ_{em} = 628/32 nm); LE – lower epidermis, MF – mesophyll, UE – upper epidermis: ***a*** *– linear graph,* ***b*** *– dissected green leaf;* ***c*** *– dissected variegated leaf*

T a b l e 1. Content of photosynthetic pigments (mg/g dry weight) in green segments of variegated and in green leaves of Cornus controversa

Sample	Date of sampling								
	May			August			October		
	Chlorophyll		Carotenoids	Chlorophyll		Carotenoids	Chlorophyll		Carotenoids
	a	*b*		*a*	*b*		*a*	*b*	
Green leaves	2.14 ± 0.08	1.17 ± 0.03	0.63 ± 0.05	2.21 ± 0.11	1.19 ± 0.06	0.64 ± 0.05	2.17 ± 0.09*	1.13 ± 0.04*	0.54 ± 0.03*
Variegated leaves	2.34 ± 0.09	1.23 ± 0.05	0.64 ± 0.04	2.31 ± 0.08	1.26 ± 0.05	0.69 ± 0.04	2.54 ± 0.08*	1.36 ± 0.04*	0.64 ± 0.02*

Note. Significant differences between green and variegated leaves are indicated with at *$P < 0.05$

However, in October, the content of photosynthetic pigments in the green segments of green leaves was significantly higher than that of variegated leaves, indicating earlier senescence of variegated leaves compared to green leaves.

Fractal measurement is a very sensitive method of obtaining spectral information about the geometric configuration, shape, reflectivity of leaves and allows analysis of the adaptive potential of plants [29-31].

The NDVI index, which reflects the proportion of photosynthetically active biomass in green leaves (0.80 ± 0.07), was 2 times higher compared with variegated leaves (0.39 ± 0.11). Also, the quantum yield of electron transport (Y(II)) was significantly higher in the green leaves of *Cornus controversa* compared to the green parts of variegated leaves. This may be a compensatory mechanism that increases the effective quantum yield of photochemical energy conversion in PSII, which is affected by the closure of reaction centers caused by the scattering of light and heat involved in non-photochemical quenching mechanisms, and is therefore sensitive to many types of plant stress. Y(II) allows the process of photosynthesis to be studied as it occurs. Dark adaptation is not necessary.

At high levels of air pollution, AQI = 80, in September 2022, a tendency to decrease the quantum yield of electron transport was observed in the green segments of variegated leaves (Table 2). In the white part of the leaves, however, the quantum yield of electron transport was not identified. With the restoration of air quality, a tendency to increase the levels of quantum yield of electronic transport was observed on the green parts of variegated leaves compared to green leaves. The quantum yield of electron transport (Y(II)) in leaves is also a direct estimate of the stress state of plants under given environmental conditions. In conclusion, green leaves maintained a more stable photosynthetic function than variegated leaves under air pollution conditions.

In the test, after 20 min of dark adaptation, Fv/Fm was determined, representing the maximum potential quantum efficiency of PSII when all reaction centers are open. 0.79-0.85 is an approximate optimal value for most plant species, and lower values indicate plant stress [32]. In the green part of the variegated leaves, the value of Fv/Fm did not differ significantly. The Fv/Fm index was not determined in the white part of the leaves (Table 3).

To sum up, though the NDVI index in green leaves was 2-fold as much as in variegated the intensity of photosynthetic processes in the green parts of variegated leaves was only slightly lower as compared to green leaves during the period from active vegetative growth till fruiting. At the end of the growing season the aging of photosynthetic apparatus in green parts of variegated leaves was slower as compared to green leaves.

Table 2. The quantum yield of electron transport (Y(II)) in leaves of Cornus controversa (date of analysis 09/05/2022 and 09/09/2022)

Sample		Y(II)	
		05.09.2022	09.09.2022
Mean air quality index (AQI)		80	10
Green leaves		0.77±0.04*	0.72±0.05*
Variegated leaves	Green area	0.71±0.04*	0.75±0.04*
	White area	Not detected	

Note. Significant differences between green and variegated leaves are indicated with at *$P < 0.05$. AQI (Air Quality Index) https://www.saveecobot.com/maps/kyiv.

Allocation of micro- and macroelements between the variegated and green leaves of Cornus controversa chimera. The photosynthetic processes, to a great extent, depend on mineral nutrition. Chlorosis or weak pigmentation may indicate a deficiency of certain mineral elements [33]. On the other hand, it was established that chlorotic areas of variegated leaves differ from green ones in their chemical composition. For example, chlorotic areas of citrus leaves were deficient in P and K, while concentrations of Fe, Mn and Zn were significantly higher as compared to green leaves [34]. Although such observations of differences in the composition of macro- and microelements in variegated and green leaves are not rare, this phenomenon does not have a sufficient physiological explanation today.

Table 3. Maximum quantum efficiency of electron transport (Fv/Fm) in the green and variegated leaves of Cornus controversa (date of analysis 09.09.2022)

Sample		Fv/Fm = (Fm - Fo)/Fm
Green leaves	0.800*	
Variegated leaves	Green area	0.798*
	White area	Not detected

Note. Significant differences between green and variegated leaves are indicated with at *$P < 0.05$

T a b l e 4. The content of chemical elements in the variegated and green leaves of Cornus controversa (mg/kg)

Chemical element	June		August	
	Variegated	Green	Variegated	Green
Al	403 ± 19**	219 ± 10**	182 ± 9**	97.0 ± 4.5**
B	18.6 ± 0.8	19.1 ± 0.8	43.05 ± 2.01**	28.13 ± 1.24**
Ba	12.8 ± 0.1**	17.1 ± 0.8**	22.35 ± 1.02**	29.74 ± 1.33**
Ca	11825 ± 52**	16790 ± 792**	17500 ± 811	17540 ± 802
Cu	9.21 ± 0.41	8.57 ± 0.37	13.34 ± 0.76*	10.45 ± 0.49*
Fe	303 ± 15**	167 ± 8**	115 ± 6**	94 ± 5**
K	22595 ± 1033**	13530 ± 642**	12337 ± 592**	8720 ± 415**
Mg	2702 ± 13**	3310 ± 154**	3103 ± 147**	2892 ± 135**
Mn	29.1 ± 1.2**	34.5 ± 1.5**	32.3 ± 1.48**	36.78 ± 1.72**
Na	86.4 ± 4.3	85.9 ± 3.8	18.1 ± 0.9**	11.5 ± 0.5**
P	3802 ± 182**	2075 ± 99**	2905 ± 144**	1103 ± 51**
S	8971 ± 432	9476 ± 465	12320 ± 608	13360 ± 632
Si	1281 ± 59**	1009 ± 51**	1828 ± 88**	1142 ± 51**
Sr	35.04 ± 1.56**	50.50 ± 2.41**	39.26 ± 1.75**	54.0 ± 2.34**
Ti	109.6 ± 5.1**	11.80 ± 0.47**	11.5 ± 0.5**	8.07 ± 0.37**
Zn	16.0 ± 0.7**	11.0 ± 0.5**	22 ± 1**	14 ± 1**

Note. Significant differences between green and variegated leaves are indicated with at $*P < 0.05$ and with at $**P < 0.01$

The results of our study demonstrated that the allocation of micro- and macroelements differed significantly in the variegated and green leaves of *C. controversa* chimera. In particular, concentrations of such macronutrients as Mg, Ca, S were significantly higher in the green leaves, while content of K, P as well as micronutrients such as Al, Cu, Fe, Si, Zn was higher in variegated leaves (Table 4).

At the beginning of vegetation, the content of rather toxic aluminum in depigmented parts of variegated leaves was 1.8 times higher than in the green leaves. At the same time, by the end of the growing season, the content of this element, regardless of pigmentation, decreased 2.2 times. This proves the ability of *C. controversa* to effectively remove the excess of this toxic metal from the assimilating organs. However, it is interesting to note that the aluminum content in the rhizosphere soil under the studied plant also decreased 1.2 times by the end of the growing season.

It is known that aluminum prevents the entry of phosphorus, calcium, and magnesium into plant tissues, although we have revealed a rather interesting feature regarding the accumulation of phosphorus and silicon, the content of which was 1.8 and 1.6 times higher, respectively, in the foliar tissues rich in aluminum. A higher content of Mg and Ca in green leaves reflected their higher photosynthetic activity compared to the variegated ones. The lower content of toxic metals such as Al, Cu, Fe, Zn, Ti observed in green leaves compared to variegated leaves suggests that the latter may extract toxic metals from green leaves, thus protecting the photosynthetic apparatus of the latter.

The analysis of the rhizosphere soil under *C. controversa* shows a decrease in the concentration of most macro- and micronutrients towards the end of the vegetation period, except for iron, sodium and sulphur, whose content increased 1.2, 5.9 and 2.3 times, respectively (Table 6).

The higher accumulation of silicon in the variegated leaves of *C. controversa* indicates their higher tolerance to toxic metals due to the activating effect of this microelement on enzymatic and non-enzymatic protective antioxidant systems, which was demonstrated in our previous studies on wheat, rice, corn, soybean and sugar beet, apple, peach, Chinese magnolia vine and cornelian cherry [35].

Table 5. Content of chemical elements in the rhizosphere of Cornus controversa chimera (mg/kg)

Chemical element	June	August
Al	9695 ± 452**	7811 ± 379**
B	17.69 ± 0.81**	9.9 ± 0.4**
Ba	88.9 ± 4.1**	484.2 ± 22.7**
Ca	8065 ± 375**	6232 ± 296**
Cu	23.9 ± 1.0**	29.3 ± 1.3**
Fe	9304 ± 421**	11040 ± 542**
K	2732 ± 127	2781 ± 119
Mg	1602 ± 73**	1166 ± 54**
Mn	247 ± 12	257 ± 12
Na	387 ± 18**	2277 ± 106**
P	589 ± 28**	504 ± 23**
S	453 ± 21**	1039 ± 50**
Si	1791 ± 83	1651 ± 78
Sr	38.96 ± 1.54	40.8 ± 1.9
Ti	374.5 ± 16.2**	416 ± 19**
Zn	97.0 ± 4.5**	187 ± 9**
pH	6.58	6.55
C %	10.9 ± 0.2	10.1 ± 0.2

Note. The significant differences between soil samples collected in June and August are indicated with *$P < 0.05$ and with **$P < 0.01$

Table 6. Content of brassinosteroids in the leaves of Cornus controversa (mg/kg)

Samples	Sample collection time		
	June	August	October
Green leaves	1.50 ± 0.06*	0.60 ± 0.01**	0.40 ± 0.01**
Variegated leaves	1.30 ± 0.05*	2.30 ± 0.08**	2.50 ± 0.11**

Note. Significant differences between green and variegated leaves are indicated with at *$P < 0.05$ and with at **$P < 0.01$

Thus, significant differences in the allocation of micro- and macroelements between green and variegated leaves have been found. Green leaves concentrated higher amounts of Mg and Ca, which reflects their more intense photosynthetic activity, but lower content of Al, Cu, Fe, Zn, Ti as compared to variegated ones.

Accumulation of protective antioxidants. The influx of toxicants adversely affects the membranes of plant cells through lipid peroxidation and protein oxidation, and brassinosteroids (Table 6), which stimulate protective enzymes, mitigate the effects of toxicity and contribute to the restoration of normal osmoregulation [36].

The accumulation of elevated concentrations of toxic metals in plant tissues can trigger antioxidant defense mechanisms, in which brassinosteroids and phenolic secondary metabolites play an important role [36-38]. Therefore, we carried out a comparative analysis of the concentration of these biomolecules in variegated and green leaves of *C. controversa*.

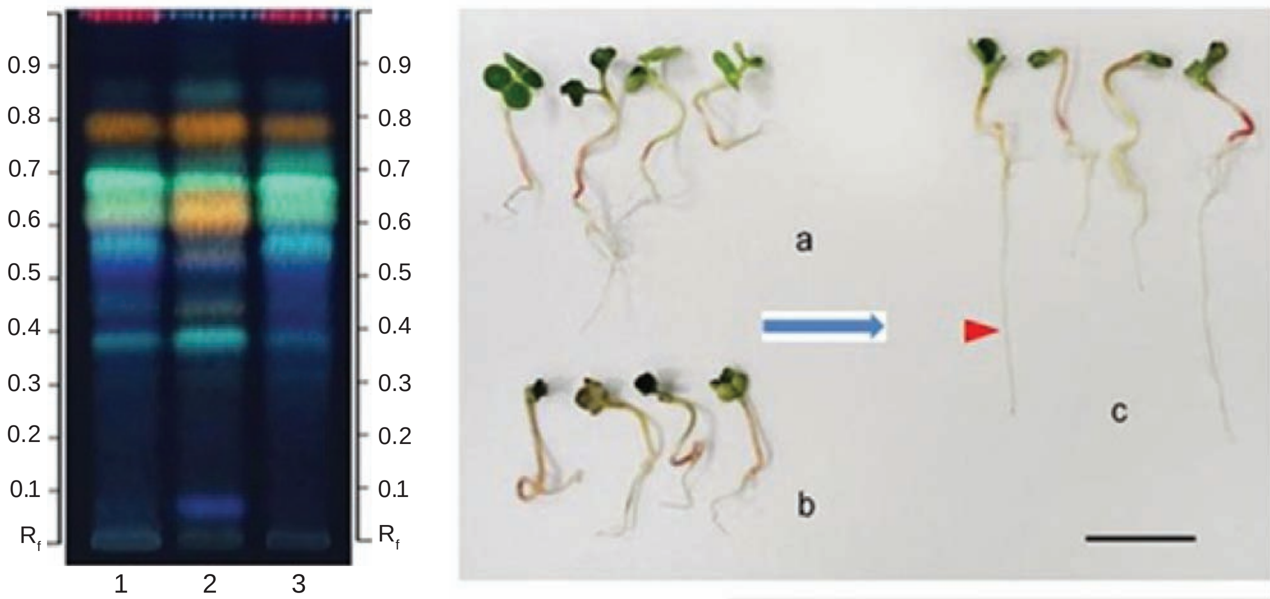
The analysis of the seasonal dynamics of brassinosteroids in green variegated leaves of *C. controversa* showed that at the beginning of the growing season, the levels of brassinosteroids in green variegated leaves did not differ significantly. However, in August and October, the levels of these protective metabolites were 3.8-fold and 6.3-fold higher in variegated leaves compared to green leaves. This indicates their higher resistance to abiotic and biotic stresses towards the end of the growing season.

In addition, one of the mechanisms for reducing aluminum phytotoxicity is to increase the concentration of sodium and potassium cations [37]. The potassium concentration in variegated leaves was 1.7 and 1.4 times higher at the beginning and the end of the growing season, respectively and the concentration of sodium was 1.6 times higher at the end of the growing season compared to green leaves.

The study of the metabolic profiles of phenolic compounds in the white area of variegated leaves revealed that one flavonoid (R_f ~ 0.62) was absent in the green leaves (Fig. 4). In addition, three more specific phenolic compounds were found in the tissues of variegated leaves. Considering the higher aluminum tolerance of the variegated form of *C. controversa* and the ability of flavonoids to chelate this metal, we hypothesized that the detected secondary metabolites might be associated with aluminum resistance. To test this hypothesis, purified extracts containing flavonoids with R_f ~ 0.62 and 0.64 were tested on the germination of radish seeds *Raphanus sativus* var. *radicula* in the presence of $AlCl_3$ (20 mg/ml). Purified solutions containing these flavonoids were obtained by elution with a sorbent on a chromatographic plate. The eluate obtained was then added to Petri dishes in which seeds of *Raphanus sativus* var. *radicula* were germinated in the presence of $AlCl_3$ solution (20 mg/ml).

As a result, flavonoids isolated from the green part of variegated leaves were found to not only reduce the toxic effect of aluminum, but also have a growth-stimulating effect on the roots of radish seedlings (Fig. 5).

In toxic concentrations, aluminum prevents cell division, root formation, contributes to the compaction of cell walls and the reduction of polysaccharide deposition, reduces the activity of enzymes, inhibits the transport of cytokines, changes the structure of plasma membranes, disrupts the absorption and transport of nutrients [38]. The mechanisms of chemical detoxification of Al are poorly understood, one of them is secretion and chelation by organic acids, mainly oxalic acid or citrate [39]. The important role of organic acids in the external and internal neutralization of phytotoxic concentrations of aluminum, especially malate in wheat plants, citrate in leguminous crops, citrate and malate in triticale, and oxalate in buckwheat, was shown [40]. The protective effect of salicylic acid mixed with the natural siliceous mineral analcite to ash (*Fraxinus excelsior* L.), birch (*Betula pendula* Roth.),



The left photo of illustrates the chromatogram of extracts of variegated leaves of Cornus controversa green part (1) and non-chlorophyll part (2) and green leaves (3); the right photo illustrates the effect of $AlCl_3$ solution on seedlings (Raphanus sativus var. radicula): ***a*** *– control,* ***b*** *– $AlCl_3$ solution (20 mg/ml),* ***c*** *– $AlCl_3$ solution with the addition of flavonoid (R_f ~ 0.62) (track 2) isolated from the green part of the variegated leaves*

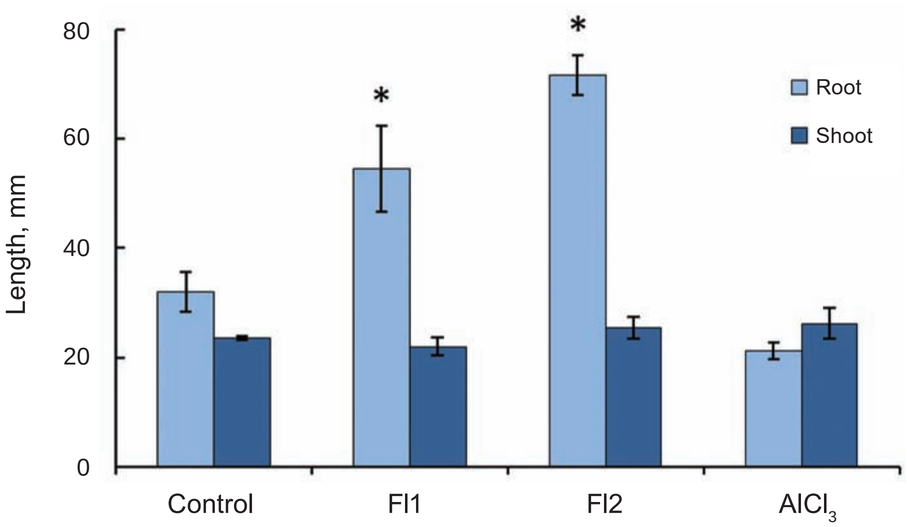


*Fig. 5. Protective effect of flavonoids isolated from the green part of variegated leaves of Cornus controversa on seedlings of Raphanus sativus var. radicula: control – seedlings germinated in water; $AlCl_3$ – seedlings germinated in $AlCl_3$ solution (20 mg/ml); Fl1 and Fl2 – seedlings germinated when flavonoids with R_f ~ 0.62 and R_f ~ 0.64 were added to the $AlCl_3$ solution, respectively; note: the reliability of the difference between the options was assessed using one-way analysis of variance (ANOVA); *the difference is significant compared to the control (H_2O) and $AlCl_3$ solution at $P < 0.05$ (Tukey's test)*

Japanese spirea (*Spiraea japonica* L.f.), Magnolia × soulangeana Soul.-Bod., maple (*Acer platanoides* L.) and privet (*Ligustrum vulgare* L.) grown in soil polluted with toxic metals (Al, Cd, Cr and Pb) was shown in our previous studies [41].

Thus, variegated leaves accumulated higher amounts of phenolic secondary metabolites and brassinosteroids, which are known for their protective effect against multiple environmental stress factors, including toxic metals and other technogenic pollutants.

Conclusions. The conducted studies showed certain differences in the functioning of photosynthetic systems, redistribution of macro- and microelements, and secondary metabolism between green and variegated leaves of the periclinal chimera of *Cornus controversa*. The NDVI index in green leaves was 2-fold as much as in variegated ones. However, comparing the intensity of photosynthetic processes in the green parts of variegated leaves and green leaves revealed a slight difference during the period from active vegetative growth to fruiting. While at the end of the growing season, the aging of photosynthetic apparatus in green parts of variegated leaves was slower as compared to green leaves. The latter have a significantly higher content of Mg and Ca and a lower content of metal cations (Al, Cu, Fe, Zn, Ti) compared to variegated leaves. Variegated leaves not only draw off toxic metals from green leaves, but also accumulate higher amount of phenolic secondary metabolites and brassinosteroids with protective effect against multiple environmental stress factors. Such specialization of green and variegated leaves may contribute to better adaptation of the chimeras to urban pollution.

Conflict of interest. The authors have completed the Unified Conflicts of Interest form at http://ukrbiochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

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ФУНКЦІОНУВАННЯ ФОТОСИСТЕМ, ВМІСТ ХІМІЧНИХ ЕЛЕМЕНТІВ, ФЛАВОНОЇДІВ І БРАСИНОСТЕРОЇДІВ У ЛИСТКАХ ХИМЕРИ *CORNUS CONTROVERSA*

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Утворення химери є добре відомим явищем у варієгатних декоративних та диких рослин, проте фізіологічні та біохімічні особливості цього явища вивчені недостатньо. Метою цієї роботи було провести порівняльний аналіз функціонування фотосинтетичного апарату, вмісту макро-, мікроелементів, флавоноїдів та брасиностероїдів у варієгатних та зелених листках переклинальної химери рослин *Cornus controversa* колекції Національного ботанічного саду імені М. М. Гришка НАН України. Відбір зразків проводили протягом періоду з червня по жовтень 2022 р. Вміст фотосинтетичних пігментів та брасиностероїдів визначали спектрофотометрично, аутофлуоресценцію тканин листків вивчали за допомогою інвертованого мікроскопу з багатоканальною системою флуоресцентної візуалізації, унормований індекс різниці рослинності індекс (NDVI) – за допомогою приладів GreenSeeker та флуорометра, кількість макро- та мікроелементів – за допомогою емісійного плазменного спектрометра. Фенольні вторинні метаболіти розділяли методом HPTLC. Для оцінки захисної здатності флавоноїдів використовували біотест на схожість насіння редиски *Raphanus sativus* var. *radicula* за присутності $AlCl_3$. Показано, що зелені листки химери *C. controversa* мали вдвічі вищий індекс NDVI, довше зберігали фотосинтетичну активність восени та мали вищий вміст макроелементів (Mg, Ca та S), але нижчу концентрацію токсичних металів (Al, Cu, Fe, Zn, Ti) ніж варієгатні. Тоді як, варієгатні листки акумулювали більше брасиностероїдів та фенольних метаболітів, які проявили здатність, не тільки знижувати токсичний вплив алюмінію, а також стимулювали ріст корінців проростків редиски. Така спеціалізація зелених та варієгатних листків може сприяти кращій адаптації рослинних химер до забруднення навколишнього середовища

К л ю ч о в і с л о в а: периклінальна химера, *Cornus controversa*, фотосинтетичні пігменти, індекс NDVI, аутофлуоресценції тканин, фенольні метаболіти, макроелементи, мікроелементи, брасиностероїди.

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