



Biochemical markers of the functional state of the *Stevia rebaudiana* and *Crambe cordifolia* plant-soil ecosystem

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A traditional approach to the study of plant-soil ecosystems is based on assessing the main physiological functions of plants (photosynthesis, respiration, mineral nutrition, regulation, protection) and main agronomic characteristics of soil. Such an approach is not very informative, and at the same time it is also time-consuming and expensive, which limits the number of objects that can be studied. Therefore, it is important to identify biochemical markers that can reflect the functional state of phytocoenosis in the most informative way. We analyzed the parameters of basic physiological processes, as well as agronomic and biochemical characteristics of soil in terms of their informative values for understanding the functional state of plant-soil ecosystem using the example of the medicinal plants candyleaf (*Stevia rebaudiana* (Bart.) Bertoni) and heartleaf crambe (*Crambe cordifolia* Stev.) from the collection of the M. M. Hryshko National Botanical Garden. In particular, we measured the contents of photosynthetic pigments, sugars, nitrogen (protein), amino acids, and brassinosteroids (BRs) in the plants. Also, we studied the contents of biogenic elements in the plant tissues and soil. The soil was subjected to allelopathic analysis. The study also considered the course of oxidation-reduction processes and the contents of phenolic compounds in the soil. The results of the analyses were processed using statistical methods of correlation, factor, discriminant, and cluster analyses. The correlation and factor analysis of the research results showed that the highest factor loadings (≥ 0.8) were observed for the characteristics of concentration of protective biomolecules (chlorophyll *b*, BRs). For the soil, the highest factor loadings were recorded for the characteristics of the content of free low-molecular-weight phenolic compounds, allelopathic activity, and redox potential, which are related to the root excretory function. The comparison of the metabolic profiles of *Crambe cordifolia* and *Stevia rebaudiana* revealed the species-specific features related to the synthesis of sugars and free amino acids (arginine, cysteine, lysine, and methionine). The allelopathic activity of *C. cordifolia* and *S. rebaudiana* is mainly due to low-molecular-weight phenolic compounds. Brassinosteroids do not seem to be involved in the allelopathic interactions of the studied species. On the other hand, BRs could be used as a marker for the intensity of assimilation processes in the studied plants.

Keywords: *Stevia rebaudiana*; *Crambe cordifolia*; mineral nutrition; brassinosteroids; aminoacids; allelopathy.

Introduction

Diagnosing the functional state of a "plant-soil" ecosystem is an important part of agronomic and ecological research. This objective is usually achieved by detecting the fluorescence emission from the photosynthetic apparatus, determining the concentrations of chlorophyll, protective biomolecules, phytohormones, and other markers of stress state in plants. At the same time, agronomic, physical, and chemical indicators of soil are considered, which can reflect a stressful effect on the plants. However, this approach is costly in terms of equipment, consumables, and time. In addition, plant and soil are considered independent components of this system. Moreover, detailed research is needed on the allelopathic, chemical, and physical effects of plant products on soil (Dmitriev et al., 2015; Pan et al., 2023).

By analogy to the organismic level, where regulatory and signaling biomolecules are considered markers of stress status, we hypothesize that in a plant-soil ecosystem, such markers may be biologically active exometabolites from plant root secretions that play regulatory and signaling roles at the ecosystem level. To verify this assumption, the basic physiological indicators (photosynthesis, mineral nutrition, primary metabolism) were analyzed in complex with the allelopathic activity, the content of low-molecular-weight phenolic exometabolites in the soil, as well as the indicator of soil redox status.

Low-molecular-weight phenolic exometabolites are the most abundant group of allelochemicals. Their content in the plant tissues reflects both the adaptive capacity (due to the regulatory and protective properties of allelochemicals) and the invasive potential of a studied plant (Dmitriev et al., 2015; Pan et al., 2023).

Another group of allelochemicals with regulatory activity are brassinosteroids (BRs). These plant hormones induce the plant resistance to abiotic stresses by modulating enzymatic and non-enzymatic antioxidant activities (Bartwal & Arora, 2020; Rattan et al., 2020). They also play important role in the crop yield and its quality. According to Divi & Krishna (2009), breeding cultivars with enhanced biosynthesis of brassinosteroids (BRs) is projected to achieve an increase in agricultural crop yields by 20–60%, owing to the diverse range of bioactive manifestations associated with BRs. In plant cells, BRs are present in a free state (active) or are bound (inactive) with fatty acids or sugars (Fujioka & Yokota, 2003). Both low-molecular-weight phenolics and BRs are recognized as stress biomarkers at the organismic level (Paes de Melo et al., 2022). In this study, we also addressed the question of the legitimacy of using these compounds as biomarkers of the functional state of the whole plant-soil ecosystem.

The objective of this study was analyzing the biochemical, allelopathic, and agronomic characteristics of the analyzed plants and the rhizosphere soil in relation to their informative value about the func-

tional state of a plant-soil ecosystem on the example of two species of medicinal plants: candyleaf (*Stevia rebaudiana* (Bart.) Bertoni) and heatleaf crambe (*Crambe cordifolia* Stev.) from the collection of the M. M. Hryshko National Botanical Garden of the National Academy of Sciences of Ukraine (Kyiv, Ukraine).

Stevia rebaudiana is a perennial herbaceous plant belonging to the Compositales order, Asteracea family, native to South and Central America (Gulko, 2007). The diterpene glycosides of *S. rebaudiana* exerted hypoglycemic effect, anti-inflammatory, reparative, and bactericidal properties, and were observed to help lower blood pressure, normalize the functions of the immune system, and prevent the de-

velopment of many diseases. The main sweet components of *S. rebaudiana* are the diterpene glycosides stevioside and rebaudioside. They were first isolated in 1931, and their chemical structure was determined in the 1950s. More than two dozen of these compounds are known to be derivatives of the aglycone steviol, a compound related to gibberellins. Our observations of the *S. rebaudiana* plants grown in the collection of M. M. Hryshko National Botanical Garden showed that the content of steviol glycosides in the leaves was within 3–9%, but this indicator can vary depending on the growing conditions and the morphotype (Fig. 1).

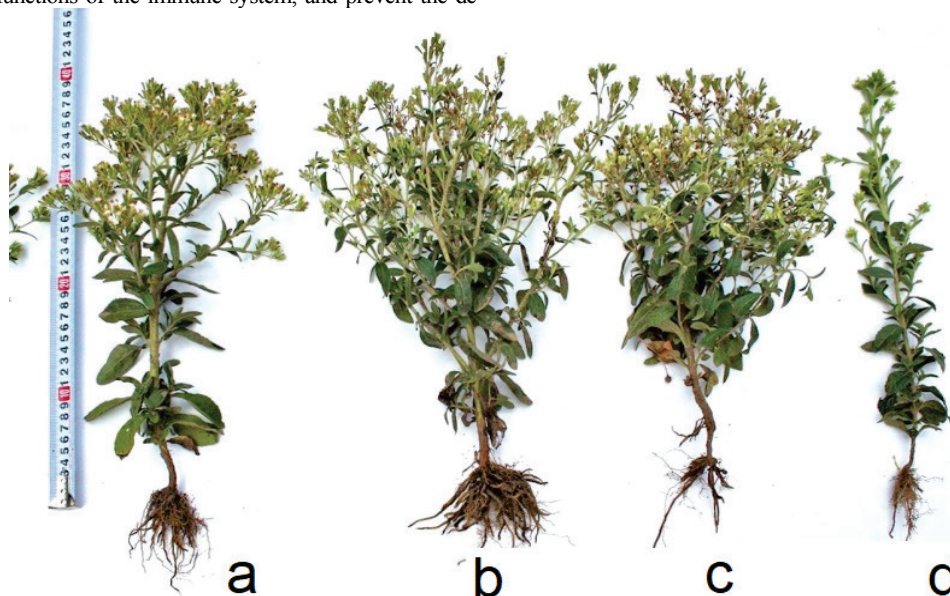


Fig. 1. *Stevia rebaudiana* morphotypes (a-d)

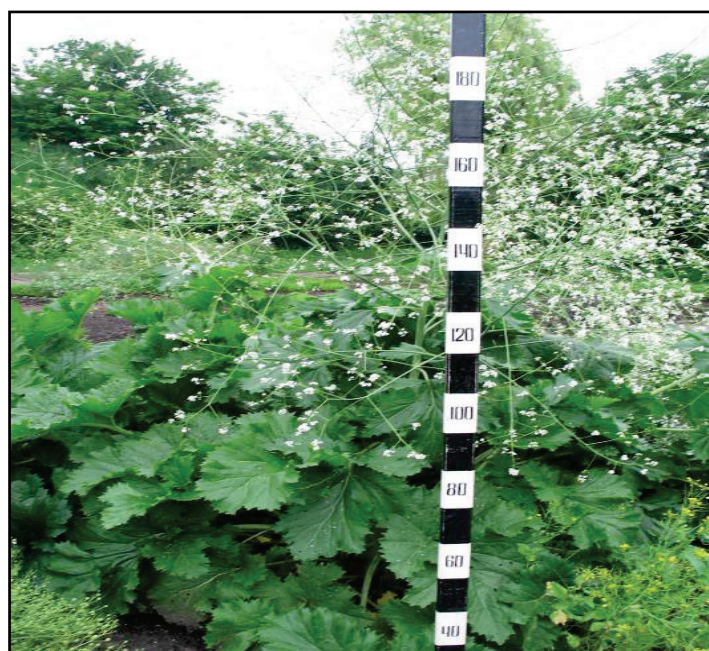


Fig. 2. *Crambe cordifolia* Stev.

Crambe cordifolia (Fig. 2) belongs to the Brassicaceae family and is native to the North Caucasus. In traditional medicine, both the aboveground and underground parts of the plant have long been used for antibacterial, antiviral, and adaptogenic applications (Gulko, 2007). Representatives of the genus *Crambe* L. are known for their high contents of biologically active compounds, in particular: amino acids, ascorbic acid, flavonoids, and vitamins (Bukhari et al., 2013; Pushkarova et al., 2016; Rashid et al., 2018; Vergun et al., 2019). The total content of sugars in the aerial part of plants of the genus *Crambe* ranges 6.54% to 33.18%.

Our preliminary studies have shown that *S. rebaudiana* and *C. cordifolia* successfully completed all the stages of acclimatization in the conditions of the Ukrainian Forest-Steppe and represent promising crops for mass cultivation in our region (Rakhmetov et al., 2016; Vergun et al., 2019). Therefore, identifying the key biochemical traits that reflect the functional state of their plant-soil ecosystem would help in the implementation of this species in agricultural production in our country. The biochemical indicators of the functional state of plant-soil ecosystems can be the key to understanding the mechanisms of ecocoenosis formation, including agrophytocoenoses.

Materials and methods

The plants of *S. rebaudiana* and *C. cordifolia* were grown in experimental plots of the Department of Horticultural Flora of the M. M. Hryshko National Botanical Garden of the National Academy of Sciences of Ukraine (Kyiv, Ukraine), in gray podzolized soil in compliance with appropriate agricultural techniques (regular watering, mechanical weeding, etc.). Sampling was carried out from May to October, 2022, during the stages of flowering induction, full flowering, and seed ripening using the random sampling technique. The replication was 20 plants of each species.

Photosynthetic pigments (chlorophyll *a*, *b*, and carotenoids) were extracted from the freshly collected crushed leaves using dimethyl sulfoxide (DMSO). The quantitative content was determined spectrophotometrically according to Wellburn (1994) using a Specord 2000 spectrophotometer (Analytic Jena, 2003).

The total sugar content in the plants was determined with the Bertrand's method. The content of nitrogen (protein) in the dry plant material was measured according to the Kjeldal method (using a Kjeldal Digestion Unit KDN-04D and a Kjeldahl Nitrogen Analyzer ATN-100).

The qualitative and quantitative composition of the amino acid was determined using a Hitachi L8900 analyzer (Tokyo, Japan).

The inorganic biogenic elements were extracted from the leaf tissue and soil using an ICAP 6300 DUO inductively coupled plasma spectrophotometer. The redox potential of the soil was determined using a pH/ORP Meter HI 2211 (Hanna Instruments, 2005).

The allelopathic analysis of the rhizosphere soil was carried out according to the method of direct bioassay using watercress (*Lepidium sativum* L.) seedlings as a test object (Pavliuchenko & Yang, 2021). The phenolic compounds were isolated from the soil with the method of ion exchange (desorption), using an ion exchanger KU-2-8 (H⁺) as a model of the root system with dissolving and absorbing capacity in relation to mobile organic compounds (Pavliuchenko et al., 2021).

For the analysis of endogenous BRs under optimal and stressful conditions, the plant samples were transferred to a dark climatic chamber with a temperature of -8°C and after various time intervals

extraction from the tissues was performed. The extraction was carried out in two stages: first, ethyl acetate was used to prepare an aqueous extract of the plant tissues (1 g of tissue + 5 mL of extraction solution). The ethyl acetate fraction was evaporated *in vacuo* and the dry residue was extracted using cyclohexane. The second extraction step, from the cyclohexane phase, was performed with a mixture of ethanol and water (4:1). The ethanol extract was evaporated under vacuum and the dry residue was dissolved in a small amount of ethyl acetate. The BR content was measured on a spectrophotometer at a wavelength of 450 nm (Kravets et al., 2011).

The research results were processed by the analysis of variance (ANOVA). The obtained data were expressed as the mean \pm standard error ($x \pm \text{SE}$), and the differences between the means were tested using the Tukey's HSD test. The differences were considered statistically significant at $P < 0.05$. ANOVA, and the correlation and factor analyses of the data were carried out by Statistica 10.0 (StatSoft). The orthogonal partial least squares discriminant analysis (OPLS-DA) and hierarchical cluster analysis were performed using StatPlus, AnalystSoft Inc., USA, 2023–2025 (www.analystsoft.com).

Results

A comparative analysis of the accumulation of macro- and microelements in the leaf tissues of *S. rebaudiana* and *C. cordifolia* revealed significant differences between these two species (Table 1). In particular, the *C. cordifolia* leaves contained a 10.1–14.6 times higher sulfur concentration and a 1.2–3.2 fold higher phosphorus concentration compared with *S. rebaudiana*, the leaves of which, on the other hand, accumulated a 1.5–5.4 times higher silicon concentration and a 2.1–4.3 fold higher magnesium content (Table 2). This tendency was observed throughout the growing season. The pattern of seasonal dynamics of the contents of studied macro- and microelements in the leaves of *S. rebaudiana* and *C. cordifolia* was similar for the vast majority of chemical elements, and was characterized, as a rule, by a gradual increase from the beginning to the end of the growing season. Such a tendency was particularly pronounced for Ba, Ca, Fe, Si, and Ti.

Table 1

The content of inorganic chemical elements in the leaves of *Crambe cordifolia* (mg/kg, $x \pm \text{SE}$, $n = 6$)

Element	Phenological phase			Seasonal mean
	flowering induction	full flowering	seed maturation	
B	27.3 \pm 2.2 ^a	81.2 \pm 5.1 ^b	31.0 \pm 1.8 ^a	46.5 \pm 3.3
Ba	14.4 \pm 0.9 ^a	25.0 \pm 3.3 ^b	29.6 \pm 1.6 ^b	23.0 \pm 1.2
Ca	17149.1 \pm 812.1 ^a	25011.1 \pm 1222.2 ^b	29890.2 \pm 1493.4 ^c	24016.7 \pm 1054.1
Fe	166.8 \pm 7.9 ^a	192.5 \pm 8.5 ^b	257.3 \pm 12.1 ^c	205.53 \pm 9.8
K	10811.0 \pm 522.2 ^a	7540.2 \pm 339.3 ^b	11271.2 \pm 513.1 ^a	9874.0 \pm 467.5
Mg	1483.2 \pm 68.1 ^a	2766.1 \pm 123.4 ^b	2159.0 \pm 104.2 ^c	2136.0 \pm 94.2
Mn	27.9 \pm 1.4 ^a	25.4 \pm 1.3 ^a	39.6 \pm 2.1 ^b	30.9 \pm 1.9
P	3375.2 \pm 158.5 ^a	2583.3 \pm 127.5 ^b	4447.1 \pm 218.3 ^c	3468.3 \pm 176.2
S	34141.3 \pm 1359.2 ^a	53131.1 \pm 2462.6 ^a	27790.2 \pm 1280.3 ^a	38354.0 \pm 1674.3
Si	384.5 \pm 17.6 ^a	933.9 \pm 29.1 ^b	853.4 \pm 98.7 ^b	723.93 \pm 57.6
Ti	8.6 \pm 0.5 ^a	18.3 \pm 1.9 ^b	21.6 \pm 1.1 ^c	16.17 \pm 0.8

Note: different letters in the line indicate significant differences ($P < 0.05$) for different sample sites according to the Tukey's test.

Table 2

The content of inorganic chemical elements in the leaves of *Stevia rebaudiana* (mg/kg, $x \pm \text{SE}$, $n = 6$)

Element	Phenological phase			Seasonal mean
	flowering induction	full flowering	seed maturation	
B	37.9 \pm 1.9 ^a	56.8 \pm 2.7 ^b	34.1 \pm 1.6 ^a	42.9 \pm 2.1
Ba	8.1 \pm 0.4 ^a	23.7 \pm 1.2 ^b	32.8 \pm 1.5 ^c	21.5 \pm 1.1
Ca	18135.5 \pm 883.2 ^a	21561.2 \pm 977.3 ^b	39192.2 \pm 1857.3 ^c	26296.0 \pm 1436.2
Fe	259.4 \pm 11.4 ^a	437.2 \pm 19.5 ^b	511.7 \pm 23.9 ^c	402.7 \pm 17.8
K	12288.2 \pm 589.3 ^a	9378.0 \pm 452.5 ^b	19875.0 \pm 952.2 ^c	13847.0 \pm 766.2
Mg	6415.2 \pm 318.1 ^a	9115.2 \pm 447.5 ^b	4519.5 \pm 220.3 ^c	6683.0 \pm 342.9
Mn	29.2 \pm 1.5 ^a	23.3 \pm 1.2 ^b	42.9 \pm 2.1 ^c	31.80 \pm 1.8
P	1359.2 \pm 62.1 ^a	2163.1 \pm 98.5 ^b	1391.3 \pm 64.3 ^a	1637.7 \pm 78.9
S	3392.4 \pm 152.5 ^a	3781.3 \pm 173.6 ^a	1897.3 \pm 89.4 ^b	3023.3 \pm 123.8
Si	1452.2 \pm 69.4 ^a	2099.2 \pm 86.1 ^b	1278.9 \pm 60.4 ^c	1609.7 \pm 74.6
Ti	12.5 \pm 0.5 ^a	29.4 \pm 1.3 ^b	35.3 \pm 1.7 ^c	25.7 \pm 1.1

Note: different letters in the line indicate significant differences ($P < 0.05$) for different sample sites according to the Tukey's test.

A comparative assessment of the qualitative and quantitative composition of free amino acids in the leaves of the studied species demonstrated a significant difference in their distribution (Table 3). In particular, *C. cordifolia* was characterized by a higher concentration of cysteine and methionine and low contents of arginine, serine, and aspartic acid, which was associated with a significantly higher level of sulfur supply to the plants.

Stevia rebaudiana accumulated 1.3–1.5 times higher amounts of sugars and BRs in the leaves as compared with *C. cordifolia* (Table 4). The seasonal dynamics of these two endometabolites was parabolic, with a maximum during the full flowering phase. A significant positive correlation was found between the total sugars and BRs in the leaves of *C. cordifolia* and *S. rebaudiana* ($R = 0.81$ and $R = 0.77$, respectively).

The seasonal dynamics of the content of photosynthetic pigments in the leaves of *C. cordifolia* differed from such of *S. rebaudiana* (Table 5). The *C. cordifolia* plants demonstrated maximum photosynthetic activity (content of chlorophyll *a* and *b*) at the beginning of flowering, slight decrease during the full flowering, and sharp reduction during the seed maturation. The *a/b* ratio rose steadily from the beginning to the end of the growing season. At the same time, the seasonal dynamics of the chlorophyll *a* content in *S. rebaudiana* was characterized by a maximum during the full flowering and a gradual

decrease during the seed maturation, accompanied by increases in the chlorophyll *b* and carotenoids contents. The *a/b* ratio in the *S. rebaudiana* leaves declined steadily from the beginning to the end of the growing season.

Table 3
Amino acid composition of the leaves of the *Stevia rebaudiana* and *Crambe cordifolia* plants ($\mu\text{g}/100$ mg of fresh weight, $x \pm \text{SE}$, $n = 5$)

Amino acid	<i>Stevia rebaudiana</i>	<i>Crambe cordifolia</i>
Aspartic acid	10.47 ± 0.50 ^a	3.74 ± 0.17 ^b
Threonine	3.68 ± 0.19 ^a	0.83 ± 0.04 ^b
Serine	5.25 ± 0.25 ^a	1.12 ± 0.06 ^b
Glutamic acid	13.71 ± 0.65 ^a	1.86 ± 0.09 ^b
Alanine	5.61 ± 0.27 ^a	2.16 ± 0.11 ^b
Valine	3.88 ± 0.19 ^a	1.28 ± 0.06 ^b
Histidine	1.55 ± 0.07 ^a	12.19 ± 0.63 ^b
Lysine	4.31 ± 0.21 ^a	1.13 ± 0.06 ^b
Arginine	9.27 ± 0.45 ^a	1.97 ± 0.08 ^b
Tyrosine	2.45 ± 0.12 ^a	0.83 ± 0.04 ^b
Phenylalanine	3.87 ± 0.17 ^a	1.08 ± 0.05 ^b
Glycine	4.50 ± 0.20 ^a	0.79 ± 0.04 ^b
Proline	3.39 ± 0.15 ^a	0.93 ± 0.05 ^b
Cysteine	0.37 ± 0.02 ^a	4.96 ± 0.22 ^b
Methionine	2.69 ± 0.13 ^a	5.11 ± 0.24 ^b

Note. * – significant differences ($P < 0.05$, ANOVA).

Table 4
Content of sugars (%) and brassinosteroids (BRs) ($\mu\text{g}/\text{g}$ of dry weight) in the leaves of *C. cordifolia* and *S. rebaudiana* ($x \pm \text{SE}$, $n = 5$)

Index	<i>Crambe cordifolia</i>			<i>Stevia rebaudiana</i>		
	phenological phase					
	flowering induction	full flowering	seed maturation	flowering induction	full flowering	seed maturation
Total sugar	2.97 ± 0.04 ^a	7.32 ± 0.06 ^b	5.68 ± 0.05 ^c	4.35 ± 0.07 ^e	10.32 ± 0.07 ^d	7.82 ± 0.06 ^b
BRs	1.62 ± 0.03 ^a	1.78 ± 0.05 ^b	1.70 ± 0.04 ^b	2.11 ± 0.04 ^d	2.53 ± 0.02 ^e	2.38 ± 0.03 ^f

Note: different letters in the line indicate significant differences ($P < 0.05$) for different sample sites according to the Tukey's test.

Table 5
Content of photosynthetic pigments in the leaves of *Crambe cordifolia* and *Stevia rebaudiana* (mg/g of dry weight, $x \pm \text{SE}$, $n = 6$)

Phenological phase	<i>Crambe cordifolia</i>				<i>Stevia rebaudiana</i>			
	chlorophyll			carotenoids	chlorophyll			carotenoids
	<i>a</i>	<i>b</i>	<i>a/b</i>		<i>a</i>	<i>b</i>	<i>a/b</i>	
Flowering induction	1.20 ± 0.02 ^a	0.82 ± 0.03 ^a	0.46	0.19 ± 0.01 ^a	1.18 ± 0.02 ^a	0.44 ± 0.02 ^a	22.68	0.29 ± 0.01 ^a
Full flowering	1.16 ± 0.02 ^a	0.54 ± 0.01 ^b	22.14	0.37 ± 0.03 ^b	1.26 ± 0.03 ^b	0.52 ± 0.03 ^b	22.42	0.31 ± 0.03 ^a
Seed maturation	0.81 ± 0.03 ^b	0.35 ± 0.10 ^c	22.31	0.39 ± 0.03 ^b	1.06 ± 0.02 ^c	0.54 ± 0.01 ^b	11.96	0.35 ± 0.01 ^b

Note: see Table 4.

Table 6
The content of free nitrogen (% to dry weight) and protein (% to dry weight) in the leaves of *Crambe cordifolia* and *Stevia rebaudiana* (flowering phase) ($x \pm \text{SE}$, $n = 6$)

Index	<i>Crambe cordifolia</i>	<i>Stevia rebaudiana</i>
Free nitrogen	6.34 ± 0.53 ^a	39.64 ± 0.44 ^b
Protein	2.06 ± 0.24 ^a	12.88 ± 0.28 ^b

Note: different letters in the line indicate significant differences ($p < 0.05$) for different sample sites according to the Tukey's test.

Regarding the contents of free nitrogen and total protein, *S. rebaudiana* was significantly superior to *C. cordifolia* (Table 6). These two indices were positively correlated with the contents of total sugars and BRs ($R = 0.63$ – 0.85). The obtained results suggest the regulatory function of sulfur ions in the processes of protein and sugar synthesis and nitrogen metabolism.

The agrochemical analysis of the rhizosphere soils associated with *C. cordifolia* and *S. rebaudiana* (Tables 7 and 8) revealed significant decreases in the Ca, Mg, Fe, Ba, and Ti contents from the beginning to the end of the growing season for both the species. This tendency was consistent with the intensive uptake and accumulation of nutrients in the foliar tissues of the studied plants (Tables 1 and 2). A 3.3-fold decline in the potassium concentration in the rhizosphere soil of *C. cordifolia* was not accompanied by increase in the accumulation of the macronutrient in the plant's foliar tissues. During the flowering period, the potassium concentration in the leaves of

C. cordifolia decreased by 43%, and afterwards increased by 45%, reaching the level insignificantly higher than those observed during the beginning of the observation period (Tables 1 and 7).

Table 7
The content of inorganic chemical elements in the rhizosphere soil of *Crambe cordifolia* (mg/kg , $x \pm \text{SE}$, $n = 6$)

Element	Phenological phase			Seasonal mean
	flowering induction	full flowering	seed maturation	
B	5.1 ± 0.3 ^a	2.7 ± 0.1 ^b	7.9 ± 0.4 ^c	5.2 ± 0.3
Ba	45.6 ± 2.1 ^a	33.8 ± 1.5 ^b	13.5 ± 0.6 ^c	30.9 ± 1.4
Ca	2754 ± 328 ^a	4853 ± 236 ^b	889 ± 43 ^c	2832 ± 240
Fe	6376 ± 316 ^a	4651 ± 224 ^b	2182 ± 109 ^c	4403 ± 268
K	1671 ± 80 ^a	618 ± 31 ^b	507 ± 25 ^c	932 ± 51
Mg	961 ± 47 ^a	480 ± 23 ^b	289 ± 15 ^c	576 ± 34
Mn	171.6 ± 8.3 ^a	159.2 ± 7.8 ^a	258.2 ± 12.7 ^c	196.3 ± 10.7
P	391 ± 19 ^a	149 ± 7 ^b	436 ± 22 ^a	325 ± 20
S	438 ± 21 ^a	311 ± 16 ^b	512 ± 25 ^c	374 ± 20
Si	1907 ± 95 ^a	116 ± 6 ^b	798 ± 39 ^c	940 ± 71
Ti	271.8 ± 13.1 ^a	242.5 ± 11.8 ^b	101.2 ± 4.9 ^c	205.17 ± 10.7

Note: different letters in the line indicate significant differences ($P < 0.05$) for different sample sites according to the Tukey's test.

A different tendency was recorded for the seasonal dynamics of potassium concentration in the rhizosphere soil of *S. rebaudiana* (Tables 2 and 8). This parameter declined by 15% during the flowering period, with a subsequent 22% increase until the seed maturation.

This increase in the potassium concentration in the rhizosphere soil was accompanied by an almost 2-fold increase in the potassium concentration in the *S. rebaudiana* foliar tissues.

Table 8

The content of inorganic chemical elements in the rhizosphere soil of *Stevia rebaudiana* (mg/kg)

Element	Phenological phase			Seasonal mean
	flowering induction	full flowering	seed maturation	
B	6.9 ± 0.3 ^a	4.7 ± 0.2 ^b	9.2 ± 0.5 ^c	6.9 ± 0.4
Ba	41.8 ± 1.9 ^a	42.5 ± 2.1 ^a	21.7 ± 1.1 ^b	35.3 ± 1.5
Ca	7902 ± 384 ^a	7542 ± 369 ^a	3046 ± 149 ^b	6163 ± 324
Fe	7031 ± 351 ^a	6103 ± 301 ^b	5306 ± 256 ^c	6146 ± 310
K	1364 ± 68 ^a	1183 ± 59 ^b	1506 ± 70 ^c	1351 ± 61
Mg	10404 ± 52 ^a	938 ± 45 ^a	496 ± 38 ^b	824 ± 860

Table 9

Allelopathic and biochemical characteristics of the rhizosphere soils of *Stevia rebaudiana* and *Crambe cordifolia*

Index	<i>Crambe cordifolia</i>			<i>Stevia rebaudiana</i>		
	phenological phase					
	flowering induction	full flowering	seed maturation	flowering induction	full flowering	seed maturation
Allelopathic activity, % control	72.3 ± 2.1 ^a	68.9 ± 2.1 ^a	45.0 ± 1.3 ^b	88.1 ± 2.6 ^c	70.0 ± 2.0 ^d	54.0 ± 1.6 ^c
Redox potential, mV	265.1 ± 5.3 ^a	255.0 ± 5.1 ^a	106.2 ± 2.1 ^b	264.1 ± 5.2 ^a	209.4 ± 4.2 ^c	101.4 ± 2.4 ^b
Phenolic allelochemicals, mg/kg	88.2 ± 2.6 ^a	96.0 ± 2.9 ^b	120.0 ± 3.6 ^c	75.0 ± 2.2 ^d	83.2 ± 2.5 ^a	94.1 ± 2.8 ^b

Note: see Table 7.

Moderate redox processes were observed in the rhizospheres of *S. rebaudiana* and *C. cordifolia* at the beginning and in the middle of the growing season, while intensive redox processes were observed at the end of the growing season. The ORP values of the soil decreased by 2.6 times at the end of the growing season compared with the beginning (Fig. 4). The observed trend suggests the accumulation of allelochemicals with antioxidant potential.

Gradual increases in the contents of low-molecular-weight phenolics in the rhizosphere soils associated with *S. rebaudiana* and *C. cordifolia* were observed throughout the growing season, peaking towards the end (Fig. 5).

The correlation and factorization of the research results clustered all the studied indices into three groups in the factor space (Fig. 6). The concentration of protective biomolecules (chlorophyll *b*, BRs) exhibited the highest factor loadings (≥0.80). For the soil, the highest factor loadings were observed for phenolic allelochemical content,

Mn	155.6 ± 7.5 ^a	169.2 ± 8.2 ^a	246.8 ± 11.5 ^b	190.5 ± 9.3
P	775 ± 37 ^a	737 ± 36 ^a	767 ± 38 ^a	760 ± 35
S	549 ± 27 ^a	532 ± 26 ^a	1218 ± 60 ^b	766 ± 43
Si	1550 ± 78 ^a	1413 ± 70 ^b	1692 ± 83 ^a	1552 ± 75
Ti	265 ± 13 ^a	238 ± 12 ^b	201 ± 10 ^c	235 ± 11

Note: see Table 7.

The allelopathic analysis of the rhizosphere soils revealed the presence of phytotoxic allelochemicals in the *S. rebaudiana* and *C. cordifolia* (Fig. 3). The inhibition of root growth in the examined plants of *Lepidium sativum* ranged 12% to 46% depending on the plant phenological phase, with the maximum inhibition observed in the soil samples collected at the end of vegetation. The soil phytotoxicity was further aggravated by an increase in the soil manganese content and declines in the calcium and iron concentrations (Table 7 and 8).

allelopathic activity, and redox potential. At the same time, certain correlations were observed among the studied biochemical characteristics. In particular, in both *S. rebaudiana* and *C. cordifolia*, the content of BRs was reliably positively correlated with the content of total sugar (correlation coefficients $R = 0.86$ and 0.89 , respectively), chlorophyll *b* ($R = 0.85$ and 0.78 , respectively), and carotenoids ($R = 0.76$ and 0.54 , respectively). A negative correlation was found between the sulfur ($R = -0.81$) and potassium ($R = -0.79$) contents of the rhizosphere soil and the accumulation of BRs in the foliar tissues of *S. rebaudiana* and *C. cordifolia*, while the contents of iron and titanium in the soil were positively correlated with the concentration of BRs ($R = 0.68$ and 0.73 , respectively). The allelopathic activity of the rhizosphere soils of *C. cordifolia* and *S. rebaudiana* was positively correlated with the redox potential of the soil solution ($R = 0.88$) and was negatively correlated with the content of low molecular weight phenolic compounds ($R = -0.89$).

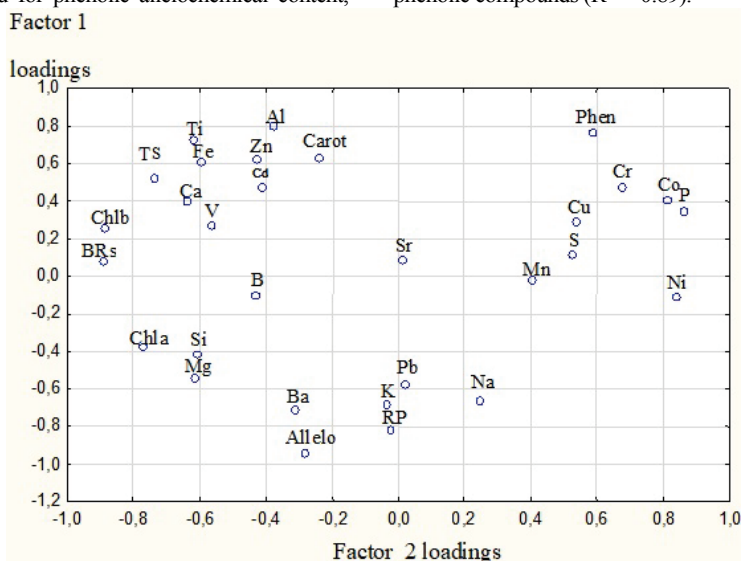


Fig. 6. Geometric interpretation of factor analysis of biochemical and agronomical characteristics: TS – total sugar, Chla – chlorophyll *a*, Chlb – chlorophyll *b*, Carot – carotenoids, BRs – brassinosteroids; Allelo – allelopathic activity; Phen – phenolics, RP – redox potential

To determine whether the observed patterns were due to the metabolic similarity between *S. rebaudiana* and *C. cordifolia*, we compared the specificity of the molecular phenotypes of *C. cordifolia* and *S. rebaudiana* according to the contents of free amino acids, proteins,

BRs, and sugars in the leaves. The principal component analysis (PCA) and orthogonal partial least squares (OPLS) were used to identify the metabolites in which these two species differ. The reliability of the OPLS-DA model was confirmed by the results of cross-

validation, which calculated the value of R^2 as 0.946 and Q^2 as 0.999 (Fig. 7a). The results indicate that the amino acid inventory enables biochemical differentiation between *C. cordifolia* and *S. rebaudiana*.

According to the evaluation of the variables important for the prediction (VIP) estimates, the amino acids arginine, cysteine, lysine,

and methionine made the greatest contribution to determining the level of differences between the two species, as well as parameters such as total protein content, free forms of nitrogen, BRs, and sugars (Fig. 7b).

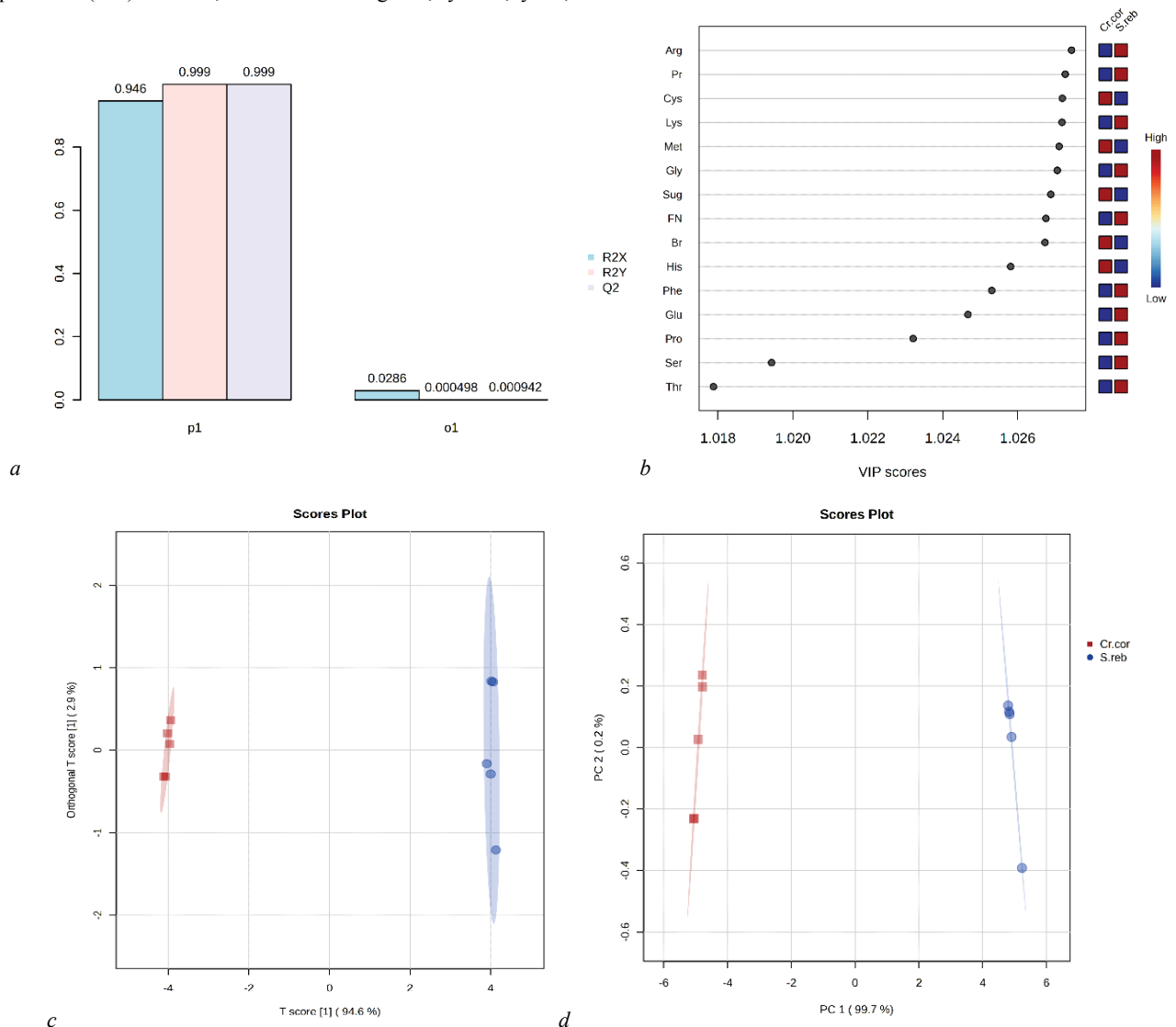


Fig. 7. Graphs of orthogonal discriminant analysis by least squares (OPLS-DA) scores: *b* – indicators that are important for prognostic assessment (VIP) of 19 main metabolites, allowing to separate the metabolomic profiles of *Crambe cordifolia* and *Stevia rebaudiana* with respect to components 1 and the results of the analysis principal components (PCA)

The analysis showed that based on the complex of metabolites, these two species are distributed along the T-score axis, which describes 94.6% of the variance (Fig. 7c). At the same time, the variability in the composition of metabolites among samples within a class, which is determined by the orthogonal axis (orthogonal T), was insignificant and amounted to only 2.4%. According to the principal component analysis, the first principal component describes 99.7% of the total variance, confirming the significant difference in plant metabolism that, for the most part, according to our study, is related to the synthesis and accumulation of free amino acids (Fig. 7d).

To visualize the differences in metabolomics between *S. rebaudiana* and *C. cordifolia* based on selected compounds and their total contents, the normalized data were analyzed using a heat map and hierarchical cluster analysis (Fig. 8). The analysis of the isolated compounds confirmed significant differences between the two species, which were clearly divided into two separate classes.

The comparison of the metabolic profiles of *C. cordifolia* and *S. rebaudiana* revealed species-specific features related to the synthesis of sugars. In the *C. cordifolia* plants, the levels of BRs and sugars were positively related to the levels of methionine, cysteine, and histidine, which were also closely correlated with each other. No such

trend was observed in *S. rebaudiana*. The volcano plot demonstrates exactly how the synthesis and content of individual metabolites and their general indicators change in the studied plant species (Fig. 9).

Figure 9 shows metabolites on the left (blue), including 5 amino acids and indices of total nitrogen and protein content, that are characterized by lower regulatory activity. Amino acids bearing no reliable significant information are shown in gray on the graph, metabolites with high regulatory activity are shown in red on the right, corresponding to statistical thresholds for fold differences (FC) and P-value <0.05. Note that (FD) > 2 was found for only 3 amino acids (methionine, cysteine, and histidine).

Discussion

The availability of mineral nutrients in soil rhizosphere plays an important role in plant growth and health. Therefore, physiological indices characterizing plant nutrition are a primary focus in any research related to the functioning of plant-soil ecosystem. The results of the agronomic analysis of the rhizosphere soils associated with *C. cordifolia* and *S. rebaudiana* showed that at the beginning of the growing season, the content of macro- and micronutrients did not

differ significantly between these two species. However, after reaching the full flowering/seed maturation phase, the concentration of certain mineral nutrients in the rhizosphere soil associated with these two species was drastically different. In particular, *C. cordifolia* accumulated sulfur and phosphorus much more intensively in its leaf tissues compared with *S. rebaudiana*. The prominent ability of *C. cordifolia* to accumulate sulfur could be related to the specificity of its secondary metabolism, i.e. synthesis of glucosinolates and their deriv-

atives (nitriles, isothiocyanates, thiocyanates, etc.), which are known to protect plants from negative effects caused by stress conditions (Sokólski et al., 2020). The higher concentration of sulfur in the *C. cordifolia* leaves also indicates a higher intensity of carbohydrate assimilation and synthesis (Narayan et al., 2023). Sulfur is involved in redox reactions and protein metabolism, is a component of some amino acids, and plays an important role in secondary metabolism (Künstler et al., 2020; Fatma et al., 2023).

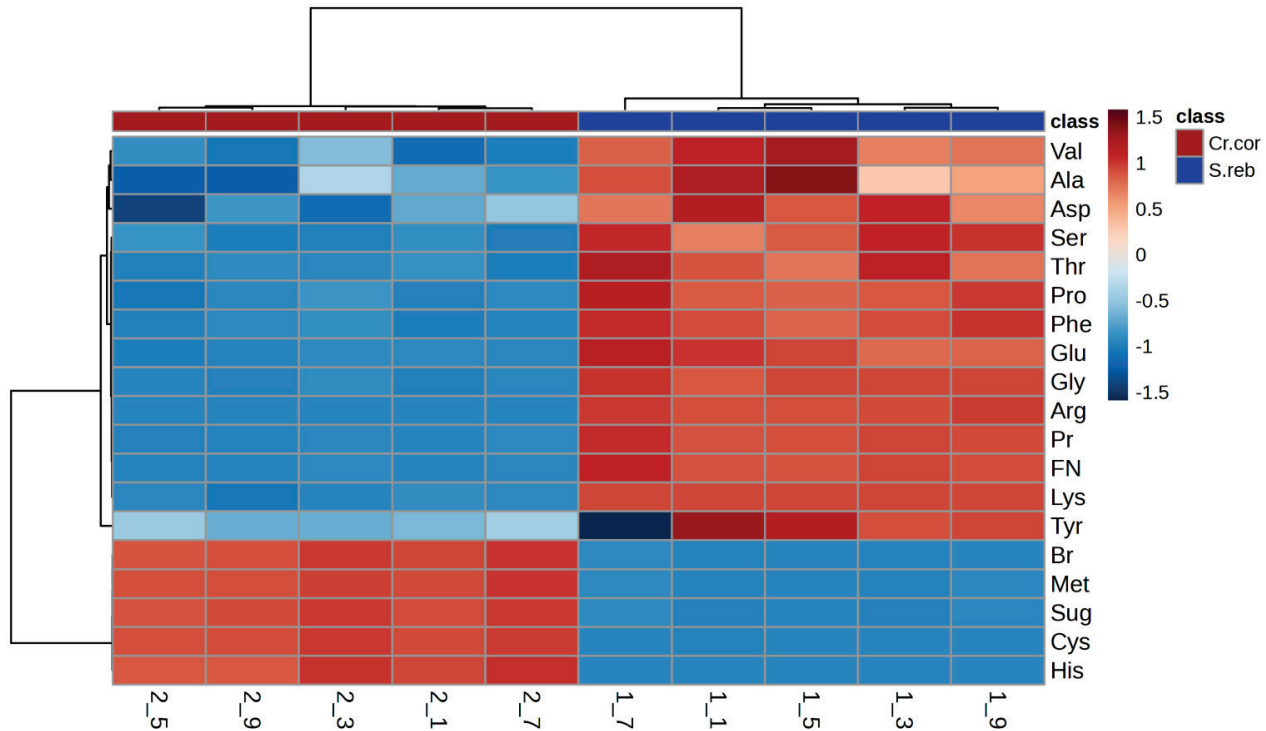


Fig. 8. Heat map and hierarchical cluster analysis for 15 proteinogenic amino acids and 4 general biochemical indices of *Crambe cordifolia* and *Stevia rebaudiana* leaves

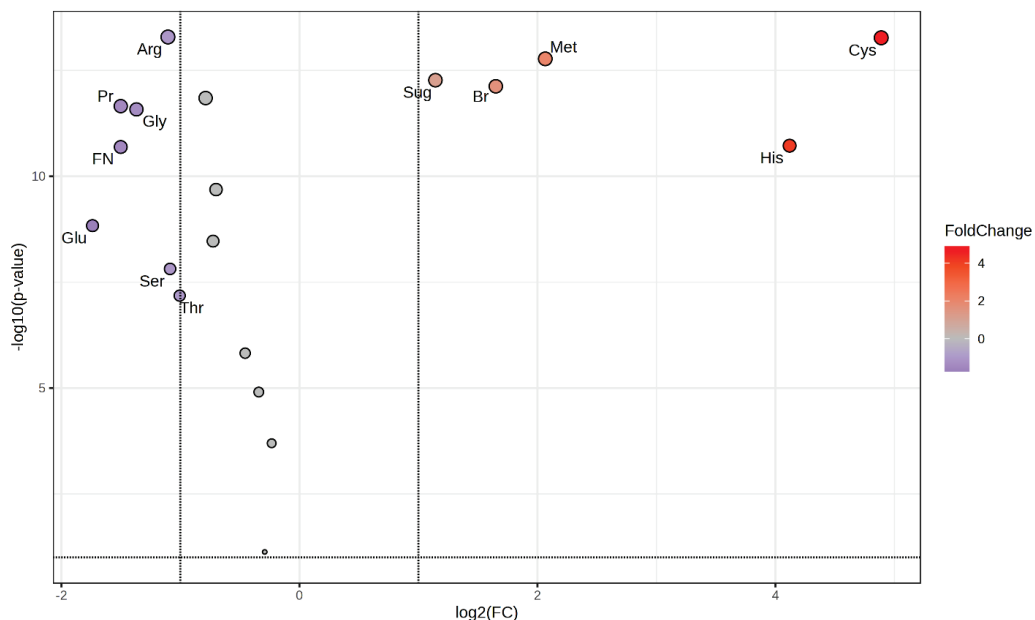


Fig. 9. Volcano plot of the differential distribution of metabolites in *Crambe cordifolia* and *Stevia rebaudiana* foliar tissues with threshold values for multiple differences (FC) > 2 and P < 0.05

Phosphorus is an essential nutrient participating in diverse physiological processes, including energy metabolism, cell division, DNA synthesis, and phospholipid biosynthesis (Isidra-Arellano et al., 2021). The observed notable capacity of *C. cordifolia* to accumulate high amounts of phosphorus in its leaves could be related to the prominent growth rate and the ability to accumulate high biomass (form-

ing up to 2.5 m tall and 1.5 m wide clumps) during short period between the mid spring to early summer when full flowering occurs. Our results confirmed the observations of other authors declaring *C. cordifolia* to be a rich source of mineral nutrients (including phosphorus, calcium, and other essential minerals) and vitamins (Vergun et al., 2019). Despite the ability of *C. cordifolia* to quickly consume

and utilize phosphorus and sulfur, the deficit of these macronutrients in the soil was observed only during a short period in the full flowering phase. At the same time, from this stage to the seed maturation, the concentrations of phosphorus and sulfur in the soil increased in parallel with the growth of concentrations of these macroelements in the leaves of *C. cordifolia*. Obviously, this species has developed certain adaptations by which it can regulate the solubility of sulfates and phosphates constituting soil minerals. *Crambe cordifolia* is known to produce and accumulate large amounts of various organic acids in its tissues, which could be exudated by the roots to the rhizosphere soil and increase the solubility of phosphate compounds that are insoluble in soil (Vergun et al., 2019). Similar adaptations in relation to sulfur and potassium availability in soil could be suggested for *S. rebaudiana*. The latter species demonstrated a notable ability to draw up silicon and magnesium from the soil and accumulate it in its leaves. The concentration of silicon in the *S. rebaudiana* leaves was 1.5–3.8 fold higher than such in *C. cordifolia*. Silicon is known to be involved in the resistance of higher plants to biotic and abiotic stress factors, and promote photosynthesis productivity and growth processes (Zaimenko et al., 2018). The increases in the foliar boron concentrations (2.6–3.0 fold in *C. cordifolia* and 1.5–1.7 fold in *S. rebaudiana*) could be related to flowering phase, indicating its important role in pollination and redistribution of assimilates in the direction of formation of the generative organs.

The sharp decrease in the potassium concentration in the soil of the *C. cordifolia* rhizosphere during the period of full flowering – seed maturation was not accompanied by increase in its concentration in the leaf tissue. Apparently, it was either immobilized or leached out of the soil solution during this period. Therefore, root exudation of *C. cordifolia* could play some role in this process. At the same time, the seasonal dynamics of potassium concentration in the rhizosphere soil and foliar tissues of *S. rebaudiana* were similar, with a minimum during the full flowering phase and a maximum during the seed maturation.

The observed seasonal changes of certain micronutrient concentrations in the leaves of *C. cordifolia* and *S. rebaudiana* could be related to meteorological factors. In particular, the 1.7–4.0 fold increase in barium concentration in the leaves of the studied species during the seed ripening period may be associated with activation of antioxidant enzymes, including catalase, which are involved in the plant's resistance to drought stress. It was found that increase in barium content in dicotyledonous plants intensified the activities of catalase, guaiacol peroxidase, and ascorbate peroxidase in different tissues, indicating oxidative damage due to barium and induction of an enzymatic system of antioxidant defense of plants (Sleimi et al., 2021). This assumption is supported by the fact of increase in the iron content, which suggests activation of redox processes. The increase in the titanium concentration proved the increased resistance to biotic stresses, as well as intensification of photosynthetic function and growth processes (Jajor et al., 2021).

The above-described peculiarities of mineral nutrition of *C. cordifolia* and *S. rebaudiana* were reflected in the amino acid composition and carbohydrate accumulation in the leaves. Sulfur assimilation processes are known to be coordinated with nitrogen due to the involvement of these elements in protein synthesis processes (Kopriva & Rennenberg, 2004). In turn, the coordination of sulfate assimilation with carbon metabolism is similar to that of nitrogen, regulated by low CO₂ levels and induced by glucose and sucrose (Kopriva et al., 2002). It is due to high sulfur content in the leaf tissue of *C. cordifolia*, especially during seed maturation, that sugars accumulate and plant resistance to stress increases (Hesse et al., 2003; Zhang et al., 2021). This is confirmed by the high levels of free histidine in the leaves, the synthesis of which is directly related to phosphorylated sugar compounds. The primary product of sulfate assimilation in plants is cysteine, a proteinogenic amino acid, as well as a source of renewed sulfur for metabolism. Cysteine synthesis is the point of convergence of the three main pathways of primary metabolism: carbon, nitrate, and sulfate assimilation. Currently, there is very little information regarding the coordination and regulation of these pathways in metabolic system (Jobe et al., 2019). However, it should be noted that it was found

that an increase in sulfur accumulation can limit the uptake and consequently the toxicity of barium in plants (de Souza Cardoso et al., 2023). At the same time, decreases in concentrations of arginine and lysine in the leaves indirectly indicates a sufficient supply of phosphate to the plants (Ljungdahl et al., 2012; Kawade et al., 2023). The *S. rebaudiana* leaves were characterized by a higher concentration of proline, which was consistent with the higher silicon content, and suggests activation of systemic resistance mechanisms to stress factors.

Brassinosteroids are crucial plant hormones regulating various physiological processes, including uptake, distribution, and utilization of macro- and micronutrients (Usmani et al., 2025). They induce plant tolerance to a wide range of stresses such as salt, extreme temperatures, water deficit, heavy metals, and pathogens (Bartwal & Arora, 2020). Increase in endogenous BRs is considered a marker of stress responses in higher plants (Paes de Melo et al., 2022). The seasonal dynamics of BRs in the leaves of *C. cordifolia* showed low variation during the growing season, which indicated its good acclimatization in the conditions of Forest-Steppe climate. The content of BRs in the *S. rebaudiana* leaves exhibited pronounced increase during mid-summer – early autumn period, indicating the plant's response to drought. On the other hand, the observed increases in the BRs and sugar contents during the full flowering period were accompanied by increase in the chlorophyll *a* content, which suggests successful adaptation to environmental stress. The revealed significant positive correlation between contents of BRs and total sugar confirmed the involvement of the latter in carbohydrate metabolism, which was reported by other authors (Xu et al., 2015).

Content of chlorophylls in the leaves is an indicator of the intensity of photosynthetic processes, which are directly related to the synthesis of primary and secondary metabolites. The observed seasonal dynamics in the content of photosynthetic pigments reflect the differing strategies of these two species in adapting to the unfavorable high temperatures and drought conditions typical of the mid to late summer period in the study region. The intensive photosynthesis and biomass accumulation observed during the spring in *C. cordifolia* was followed by sharp decreases in the photosynthetic activity and vegetative growth during the seed maturation, when the accumulated resources are redistributed between the seeds and the root system. It is during this period that the excretory activity of the root system is at its maximum, as confirmed by the results of its allelopathic, biochemical, and chemical analyses. It is possible that the high content of sulfur and the lower levels of iron and magnesium in *C. cordifolia* inhibited the biosynthesis of chlorophyll *b*, especially at the end of the growing season, due to the demethylation process with a shift in the balance towards the auxiliary pigment, which was accompanied by the increase in the concentration of protective carotenoids with a high antioxidant potential. Unlike *C. cordifolia*, the vegetative growth of *S. rebaudiana* continued and its photosynthetic activity reduced slightly during the transition period from flowering to seed maturation. Its adaptation to summer drought mainly occurred at the biochemical level, which included sharp decrease in the chlorophyll *a/b* ratio and increase in the contents carotenoids, as well as in the accumulation of prolin, potassium, and calcium in the foliar tissues.

The agrochemical analysis of the rhizosphere of *C. cordifolia* and *S. rebaudiana* (Tables 7 and 8) revealed significant decreases in the calcium contents, which were consistent with the intensive uptake of this element by the studied plants at the end of the growing season (Tables 1 and 2). It is calcium that establishes a link between the redox state of the membranes and the regulation of the cation channel, through which proteins pass due to the presence of specific thiol groups in the channel. Thanks to notable accumulation of calcium by *S. rebaudiana* in the leaves during seed maturation, silicon accumulates in the plant tissues. It should be noted that the rhizosphere of *C. cordifolia* was characterized by more pronounced fluctuations in the concentrations of chemical elements, especially iron, potassium, aluminum, calcium, and silicon, compared with *S. rebaudiana*, which could be explained by a much larger biomass and therefore greater impact on its environment.

The allelopathic analysis of the rhizosphere soil under these two species revealed greater phytotoxicity of the *C. cordifolia* root exuda-

tions, with difference especially obvious during the flowering induction phase. The seasonal dynamics of allelopathic activity in the rhizosphere soil under these two species was similar and characterized by gradual increase from the flowering induction to seed maturation. This tendency coincided with the seasonal dynamics of phenolic allelochemicals in the rhizosphere soil, evidencing an important contribution of this group of organic compounds to allelopathic interaction of the studied species. Besides, the increase in soil phytotoxicity during seed maturation coincided with the increase in soil manganese content and the decreases in calcium and iron concentrations (Table 7 and 8), suggesting some contribution of the studied plant's root exudations to the regulation of solubility of metals cations, thus coordinating their availability to the plants. This phenomenon was also described by other authors (Liu et al., 2024). The sharp decrease in ORP index of the rhizosphere soil during the seed maturation suggested corresponding changes in the pool of allelochemicals and associated soil microflora.

The correlation and factor analysis of the available data resulted in clustering of all the analyzed indices into three groups in the factor space. The highest factor loadings (≥ 0.8) were observed for the indices of non-specific plant response to stress (chlorophyll *b*, BRs, and phenolic secondary metabolites). Soil phenolics, allelopathic activity, and redox potential exhibited the highest sensitivity to hypothetical environmental changes. Moreover, the BRs were well correlated with basic physiological processes such as photosynthesis, assimilation, antioxidant protection, and mineral supply. Therefore, this index could be proposed as a marker of the functional state of plant-soil ecosystem. The allelopathic activity, content of low-molecular-weight phenolics, and redox potential of the soil solution did not display reliable correlations with the concentration of BRs in the studied plant species. This indicates the insignificant (or even absent) contribution of the latter to the formation of the biochemical environment of the rhizosphere and the allelopathic activity of the soil. No reliable correlation was found between the content of chemical elements and the allelopathic properties of the rhizosphere soil. This conclusion was also confirmed by the results of the factor analysis: the indices Phen (the content of low molecular weight phenolic compounds) and Allelo (the allelopathic activity of the soil) were located in the factor space significantly distant from the other studied agrochemical characteristics.

The comparison of the specificity of the molecular phenotypes of *C. cordifolia* and *S. rebaudiana* indicated that the greatest contribution to determining the level of differences between the two species was made by amino acids, in particular, arginine, cysteine, lysine, and methionine. The orthogonal analysis of the complex of metabolites of these two species, as well as the principal component analysis, confirmed the significant differences, especially in the synthesis and accumulation of free amino acids and sugars. These two species also differed in relationships of BRs with sugars and amino acids. In particular, in the *C. cordifolia* plants, the levels of BRs and sugars were positively related to the levels of methionine, cysteine, and histidine, which were also closely correlated with each other. No such tendency was observed in *S. rebaudiana*. High regulatory activity was found for only three amino acids: methionine, cysteine, and histidine. The high regulatory activities of cysteine and histidine, as well as of the BRs, emphasize their close relationship with the synthesis and metabolism of sugars, known to be important in the adaptation strategy of *C. cordifolia*.

In conclusion, as markers of the functional state of soil-plant ecosystem for both the studied species, we propose using contents of BRs, chlorophyll *b* in the leaves, as well as concentration of low-molecular-weight phenolics and the allelopathic activity of rhizosphere soil. These characteristics were the most sensitive to the action of environmental factors, and at the same time were correlated with the basic physiological processes of the plant organism. The observed similarity of the patterns of interdependencies between the variables in these two taxonomically distant species suggests that the proposed markers of the functional state of plant-soil ecosystem might be common to higher plants.

Conclusions

We carried out the analysis of the biochemical characteristics of basic physiological processes in *C. cordifolia* and *S. rebaudiana*, as well as agronomic indices of the rhizosphere soil in terms of their informative value and the possibility of using them as markers of the functional state of a whole plant-soil system. The characteristics that were the most sensitive to changes in the environment and at the same time related to basic physiological processes were the contents of BRs and chlorophyll *b* in the leaves, as well as the accumulation of free low-molecular-weight phenolics, allelopathic activity, and redox potential of rhizosphere soil. The comparison of the metabolic profiles of *C. cordifolia* and *S. rebaudiana* revealed the species-specific features related to the synthesis of sugars and free amino acids (arginine, cysteine, lysine, and methionine). The allelopathic activities of *C. cordifolia* and *S. rebaudiana* were found to be mainly due to low molecular weight phenolics. Brassinosteroids do not seem to be involved in the allelopathic interactions of the studied species. On the other hand, BRs can serve as a marker of the intensity of assimilation processes in these plants and determine the level of competitive ability of the species in a community.

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The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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