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THE METABOLITES OF AUTOTROPHIC AND HETEROTROPHIC LEAVES OF *Amaranthus tricolor* L. EARLY SPLENDOR VARIETY

M.S. GINS[1, 3] ✉, V.K. GINS[1], S.M. MOTYLEVA[2], I.M. KULIKOV[2], S.M. MEDVEDEV[2], V.F. PIVOVAROV[1]

[1]*Federal Research Center for Vegetable Growing*, 14, ul. Selektsionnaya, pos. VNIISSOK, Odintsovskii Region, Moscow Province, 143080 Russia, e-mail anirr@bk.ru (corresponding author ✉), anirr67@yandex.ru, pivovarov@vniissok.ru;
[2]*All-Russian Horticultural Institute for Breeding, Agrotechnology and Nursery*, 4, ul. Zagor'evskaya, Moscow, 115598 Russia e-mail motyleva_svetlana@mail.ru, vstisp@vstisp.org, mos_vstisp@mail.ru;
[3]*People's Friendship University of Russia (RUDN University),* 6, ul. Miklukho-Maklaya, Moscow, 117198 Russia
ORCID:
Gins M.S. orcid.org/0000-0001-5995-2696
Gins V.K. orcid.org/0000-0002-7053-4345
Motyleva S.M. orcid.org/0000-0003-3399-1958
Kulikov I.M. orcid.org/0000-0001-8071-0931
Medvedev S.M. orcid.org/0000-0002-4747-9835
Pivovarov V.F. orcid.org/0000-0003-1350-5852
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Abstract

An important area of systemic biology (metabolomics) is the study of the composition and properties of low-molecular metabolites of agricultural plants with different modes of nutrition. The use of metabolic technologies expands the possibilities of analyzing biochemical changes in the composition and structural modifications of metabolites occurring during the transition from autotrophic to heterotrophic nutrition. Most photosynthetic plants are capable of autotrophic nutrition, but in their lifetime, there are periods of appearance of the achlorophyllic organs which receive nutritients from the organic substances stored earlier. Thus, among *Amaranthus tricolor* L. plants there are varieties with leaves which differ from each other in the way of nutrition. For example, Early Splendor variety plants form brightly colored red heterotrophic leaves along with green photosynthesis leaf blades at the end of the vegetative phase. The comparative study of the low-molecular metabolites composition in these leaves is important for understanding the relationship between heterotrophic and autotrophic nutrition in the whole plant. In this paper, significant qualitative differences in metabolites composition between autotrophic and heterotrophic leaves were stated for the first time during the metabolome analysis of water and alcohol extracts of heterotrophic and autotrophic amaranth leaves of Early Splendor variety using the method of gas chromato-mass spectrometry. It was found that the low-molecular metabolites of autotrophic and heterotrophic leaves contained both non-specific metabolites common for both type of nutrition and specific metabolites characteristic for each of the ways separately. On the one hand, it indicates the close interaction between two ways of nutrition and, on the other hand, the ability to synthesize and modify the metabolites which demonstrates partial autonomy of heterotrophic leaves. The purpose of the work is to study the composition of low-molecular metabolites and to identify new biologically active metabolites antioxidants in heterotrophic and autotrophic amaranth leaves of Early Splendor variety. Experiments were carried out in 2017-2019 with amaranth plants of the Early Splendor variety at the end of flowering—the beginning of seed formation phase. The plants were grown in a film greenhouse (the Federal Research Center for Vegetable Growing). The fresh red-colored heterotrophic leaves formed at the top of the main stem and the underlying photosynthetic leaves with a fully formed leaf blade were collected for analysis. The leaves were homogenized (T18 homogenizer, IKA, Germany) and extracted for 30 min at 24 °C with either 96 % ethanol or distilled water (leaves weighing batch: extragent 1:10). The metabolites were profiled by gas chromato-mass spectrometry method (GH-MC) with a chromograph GH-MC JMS-Q1050GC (JEOL Ltd., Japan). According to the mass spectra library of the NIST-5 National Institute of Standards and Technology (USA), a total of 87 metabolites were totally identified. Heterotrophic leaves contained 19 substances in water extracts and 38 metabolites in alcohol extracts, while photosynthetic leaves contained 21 substances in the water extract and 57 metabolites in alcohol extracts. Twenty-nine identical metabolites were found in water and alcohol extracts. In heterotrophic and autotrophic amaranth leaves of Early Splendor variety squalene ($C_{30}H_{50}$), a biologically active compound with antioxidant properties was identified for the first time. Also, in heterotrophic leaves monopelargonine (mononononanoin) ($C_{15}H_{11}O_7$) was identified. Mon-

opelargonine is an intermediate product of flavonoid o-glycosylation, is referred to phenolic compounds and possesses high antioxidant activity. Metabolites have been identified that are present in both autotrophic and heterotrophic amaranth leaves, which suggests a close interaction of the two types of nutrition during the appearance, growth and development of heterotrophic leaves. At the same time, photosynthesizing leaves serve as donors of key metabolites for heterotrophic leaves, while the latter are not only acceptors, but also can synthesize and modify metabolites necessary for cell formation. Due to revealed rich composition of carbohydrates, essential amino acids, lipids and organic acids, the photosynthesizing leaf biomass is a source of antioxidants and biologically active substances. It should be stressed that not all metabolites were identified. Nevertheless, the set of metabolites that we identified in the photosynthetic leaves allows us to suggest these substances to be key and sufficient compounds for the construction and functioning of cells and tissues in heterotrophic leaves.

Keywords: *Amaranthus tricolor*, low-molecular antioxidants, autotrophic leaf, heterotrophic leaf, gas chromatography, mass spectrometry

Photosynthetic plants are mostly autotrophs, however, their chlorophyll-less organs (flowers, leaves, bulbs, etc.) should utilize organic nutrients accumulated earlier. As all non-green organs and plant cells in the dark are heterotrophic, disclosing the mechanisms of heterotrophic nutrition is of fundamental importance.

Amaranth plants (*Amarantus tricolor* L.) are widespread throughout the world and recognized as a source of essential nutrients [1-3]. Healthy nutrition is an important element of human life. Deficiency of essential nutrients, bioactive compounds and minerals poses a serious threat to health [4]. Gaining more knowledge about the nutritional and pharmacological metabolites derived from amaranth leaves allows for a better development of safe functional foodstuff. Amaranth leaves contain vitamins, minerals [5-7], red betacyanins and yellow betaxanthins, flavonoids and other physiologically active compounds with antioxidant properties [8-11].

The amaranth (*A. tricolor*) Early Splendor variety is convenient for profiling organic substances in photo-synthesizing and chlorophyll-less leaves. The variety considered as leafy vegetable is so decorative that also decorate flower beds. During their life cycle, plants pass through stages characteristic only for the Early Splendor variety, i.e., the emergence of bright red heterotrophic leaves at shoot tops after growth completed, the formation of a green spot at the tip of each red leaf, and a gradual spread of the green zone throughout the leaf masking the red color.

Our previous findings have shown that the total content of antioxidants, photo-synthetic pigments and betacyanins in photosynthetic and heterotrophic leaves of Early Splendor plants differs. Moreover, the heterotrophic tissue of red leaves contains trace amounts of chlorophyll and is incapable of photosynthesis [12]. A number of studies have been reported on chlorophyll accumulation in red leaves [13, 14]. When assessing the expression of nine genes encoding eight enzymes that are involved in chlorophyll biosynthesis, a decrease was observed in the expression of the NADP-H-protochlorophyllide oxidoreductase gene. It catalyzes one of the stages of chlorophyll biosynthesis, therefore, a decrease in the NADP-H-protochlorophyllide oxidoreductase gene expression causes a loss of the ability to synthesize this photosynthetic pigment which plays a key role in the formation of red leaves in *A. tricolor* and its nutrition. Overproduction of the red-colored pigment amarantine can also be regulated by altering gene expression [15, 16].

The autotrophic leaves use inorganic substrates to photosynthesize while heterotrophic leaves of the same plant utilize pre-synthetized organic compounds [17]. However, scant data are available on the patterns of the main low-molecular-weight metabolites in auto- and heterotrophic leaves.

This paper is the first to report significant differences in the metabolite profiles between auto- and heterotrophic leaves of the Early Splendor amaranth plants. Gas chromatography-mass spectrometry (GC-MS)-based metabolomic analysis of water and ethanol leaf extracts has identified both nonspecific (common for different trophic types) and specific (characteristic of each type) low-molecular-weight metabolites. These findings broaden current knowledge of the adaptive changes in cells during the transition from phototrophic to heterotrophic nutrition type through modulation of the set and properties of low-molecular-weight metabolites.

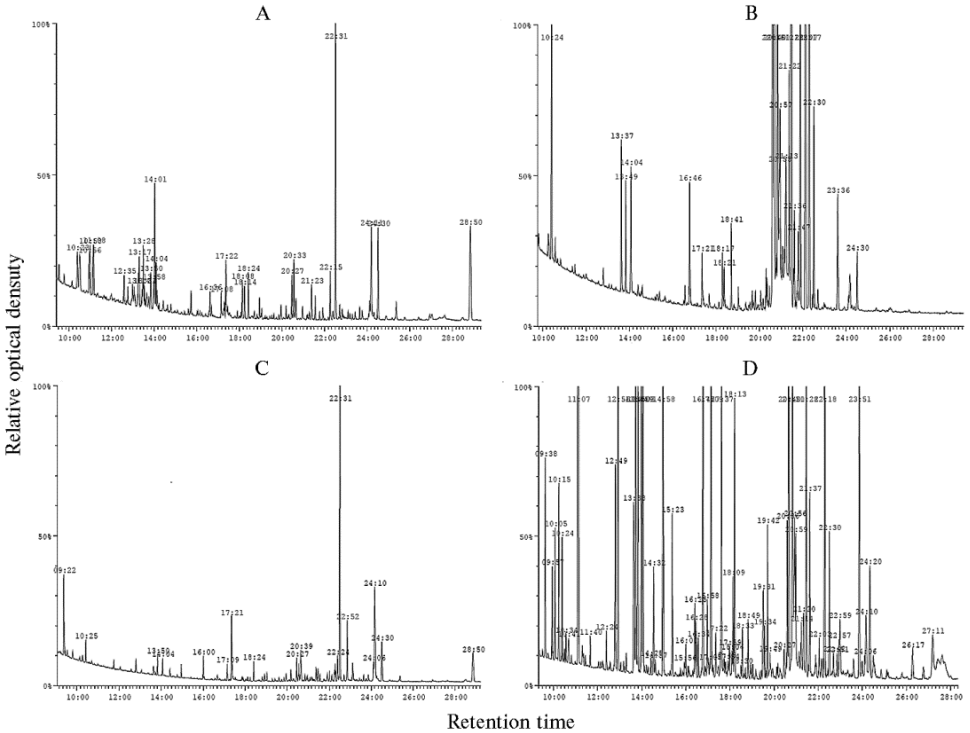
This work aimed to comparatively profile the low-molecular-weight metabolites from heterotrophic and autotrophic leaves of the amaranth plant and to identify new bioactive antioxidant metabolites.

Material and methods. The amaranth (*Amarantus tricolor* L.) Early Splendor cv. plants were grown in a film greenhouse (the Federal Scientific Center for Vegetable Growing, 2017-2019). During the period from the end of flowering to the beginning of seed formation, fully formed red-colored heterotrophic leaves from the top of the main shoot and the underlying photosynthetic leaves were collected. The fresh leaves were crushed (an A11 basic homogenizer, IKA, Germany) and extracted with 96% ethanol or distilled water at a ratio of 1:10 (leaves:extractant) at 24 °C for 30 min. Metabolites were analyzed by gas chromatography-mass spectrometry (GC-MS) using a JMS-Q1050GC chromatograph (JEOL Ltd, Japan) equipped with a DB-5HT capillary column (Agilent, USA; 30 m×0.25 mm, film thickness 0.52 μm). The temperature gradient ranged from 40 to 280 °C (250 °C for the injector and interface, 200 °C for the ion source). The helium carrier gas flow rate in the column was 2.0 ml/min, the analysis time was 45 min, the injection mode was split-flow, the volume of the injected sample was 1-2 μl of the evaporated extract. For derivatization, the silylation reagent N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) was used as described by Robbins [18]. The scanning range was 33-900 m/z. Substances were identified by retention parameters and mass spectra according to the of the library NIST-5 (National Institute of Standards and Technology, https://www.nist.gov, USA). The reliable probability of identification ranged within 75-98%.

Results. The Figure (A, B) shows typical GC-MS profiles of water and ethanol extracts from heterotrophic amaranth leaves. The analysis identified 87 metabolites of which 33 were found in water extracts and 74 in ethanol extracts (Table).

A total of 22 compounds were organic acids, including aliphatic, cyclic and high molecular weight acids which contain one or more carboxyl groups (see Table). In cells, organic acids can be free or bound. The physiological role of organic acids lies in their protective antibacterial activity, antioxidant properties, and the ability to buffer cell sap and participate in the Krebs cycle. Five organic acids were identified in the water extracts from heterotrophic amaranth leaves and nine in the ethanol extracts. Bromsebacic, monoamidoethylmalonic and succinic acids were found in both extracts of the heterogeneous leaves. Phenolic acids (i.e., benzoic, phenylacetic, and phthalic) were identified only in autotrophic leaves.

An important metabolite found in the ethanol extract was pantothenic acid (vitamin B_5), an amide of the amino acid β-alanine and pantothenic acid. Pantothenic acid as a coenzyme in CoA is involved in more than 130 metabolic reactions, participating in the synthesis of fatty acids, sterols, glycerides, citric acid, etc. [19].



Chromatographic profiles of water (left) **and ethanol** (right) **extracts from heterotrophic** (A, B) **and photosynthesizing** (C, D) **leaves of amaranth (*Amaranthus tricolor* L.) cv. Early Splendor.** Gas chromatography-mass spectrometer JMC-Q1050GC (JEOL Ltd, Japan).

Metabolites identified in extracts from heterotrophic and photosynthesizing tissues of amaranth (*Amarantus tricolor* L.) cv. Early Splendor leaves by gas chromatography-mass spectrometry

Rt, min	Compounds	Extract
	Organic acids	
20:02	D-arabinonic acid, $C_5H_{10}O_5$	EH, EP
19:57	Azelainic acid, $C_9H_{16}O_4$	WH, WP
22:42	2-Bromosebacic acid, $C_{10}H_{17}O_4Br$	WH, EH
10:23	Butanoic acid, $C_4H_8O_2$	EP
27;25	Dodecanedioic acid, $C_{12}H_{22}O_2$	EP
17:59	Glutaric acid, $C_5H_8O_4$	EH, EP
22:23	2-Hydroxyoctanoic acid, $C_4H_8O_3$	WP
12:09	Monoamidoethylmalonic acid, $C_{14}H_{33}NO_3$	WH, EH, EP
16:33	Malic acid, $C_4H_6O_5$	EH, EP
14:59	Malonic acid, $C_3H_4O_4$	EH, EP
10:24	Lactic acid, $C_3H_6O_3$	EH, EP
20:47	Pentonic acid, $C_5H_{10}O_2$	EH, EP
20:11	10-Undecynoic acid, $C_{11}H_{22}O_2$	WP
14:58	Erythronic acid, $C_{18}H_{30}O_5$	EP
22:59	Erythro-pentonic acid, $C_6H_{12}O_2$	EP
13:09	DL-malic (Butanedioic) acid, $C_4H_4O_5$	EP
14:04	Succinic acid, $C_4H_4O_4$	WH, EH
17:47	2-Isopropylmalic acid, $C_4H_4O_4$	EH
22:04	Pantothenic acid, $C_9H_{17}O_5$	EH
	Phenolic acids	
19:37	Benzoic acid, $C_7H_6O_2$	WP
21:09	Phenylacetic acid, $C_8H_8O_2$	EP
22:51	Isoferulic acid, $C_{10}H_{13}O_4$	WP
21:23	Phthalic acid, $C_8H_{12}O_4$	WP
	Fatty acids	
18:25	Dodecanoic acid, $C_{10}H_{20}O_2$	EH
19:07	Myristic acid, $C_{14}H_{28}O_2$	EH
21:15	Hexadecanoic acid, $C_{16}H_{32}O_2$	WH, EH, WP, EP
17:51	Octadecanoic (Stearic) acid, $C_{17}H_{35}COOH$	WH, EH, WP, EP
21:10	Linoleic acid (essential), $C_{12}H_{14}O_2$	WH, EH, WP, EP

Continued Table

	Carbohydrates	
19:43	L-(-)-Arabitol, $C_5H_{12}O_5$	EP
21:22	β-DL-Arabinopyranose, $C_5H_{12}O_5$	WH, EH, EP
19:03	β-L-Galactopyranose, $C_6H_{13}O_8$	EH, WP, EP
19:26	D-Galactose, $C_6H_{16}O_6$	EH, WP, EP
20:01	β-D-Xylopyranose, $C_6H_{10}O_5$	EH
22:07	D-Mannitol, $C_6H_{12}O_6$	EP
19:43	Ribitol, $C_5H_{12}O_5$	WP, EP
17:57	β-Erythrotetrofuranose, $C_5H_{14}O_5$	EP
22:41	Ribonic acid, pentakis, $C_5H_{12}O_5$	EP
22:17	β-D-Glucopyranose, $C_6H_6O_6$	EP
20:46	β-(DL)-Lyxopyranose, $C_5H_{10}O_5$	EP
20:00	Levoglucosan, $C_6H_{10}O_5$	WP, EP
36:53	3-α-Mannobiose, $C_{12}H_{22}O_{11}$	EP
20:11	D-(-)-Tagatofuranose, $C_6H_{12}O_6$	EH, EP
20:15	L-(-)-Sorbose, $C_6H_{12}O_6$	EP
20:36	α-l-(-)Sorbofuranose, $C_6H_{12}O_6$	EH, WP, EP
18:36	D-(-)-Ribofuranose, $C_5H_{10}O_5$	WH, EH, EP
20:36	D-(-)-Fructofuranose, $C_5H_8O_6$	EP
20:51	D-(-)-Fructopyranose, $C_5H_8O_6$	WP, EP
30:48	D-(+)-Turanose, $C_6H_{12}O_6$	EP
20:55	D-(+)-Talofuranose, $C_5H_{12}O_6$	EH
19:43	Ribitol, $C_5H_{12}O_5$	EH
21:17	Glyceryl glycoside	EP
20:18	Glucofuranoside, $C_6H_{12}O_6$	EP
19:43	Gluconic acid, γ-lacton, $C_6H_{12}O_6$	EP
21:51	Inositol, $C_6H_{12}O_6$	EP
19:30	D-(+)-Ribono-1,4-lactone, $C_5H_{12}O_5$	EH, EP
19:43	Gluconic acid, γ-lacton, $C_6H_{10}O_6$	WH, EH
13:50	Clycerol, $C_3H_8O_3$	WH, EH, WP, EP
14:57	2(3H)-Furanone, $C_4H_4O_2$	EH
	Amino acids and their derivates	
11:07	L-Alanin, $C_3H_7NO_2$	EH, WP, EP
12:06	L-Leucine, $C_6H_{13}NO_2$	EH, EP
14:03	L-Isoleucine, $C_6H_{13}NO_2$	EP
16:58	L-Aspartic acid, $C_4H_7NO_4$	EP
18:40	L-Asparagin, $C_4H_8N_2O_3$	EP
16:48	L-Proline, $C_3H_9NO_2$	EH, EP
13:47	L-Homoserine, $C_4H_9NO_3$	EH
13:20	L-Serin, $C_3H_7N_1O_3$	EP
12:55	L-Valin, $C_5H_{11}NO_2$	EH, EP
15:23	L-Treonin, $C_4H_9NO_3$	EH, EP
21:37	L-Tyrosine, $C_9H_{17}NO_2$	EP
18:12	L-Phenylalanin, $C_9H_{18}NO_2$	EP
22:38	L-Cistatione, $C_7H_{14}N_2O_2$	EH
	Terpenes	
36:06	Squalen, $C_{30}H_{50}$	WH, EH, WP, EP
23:01	Borneol, $C_{10}H_{18}O$	EH
20:10	Izoborneol, $C_{10}H_{18}O$	EH
22:42	Dehydroabietic acid, $C_{20}H_{29}O$	EH
	Others	
40:20	(+)-α-Tocopherol, $C_{29}H_{50}O_2$	EP
16:00	Niacinamide, $C_6H_5NO_2$	WP, EP
22:25	Stigmasterol, $C_{30}H_{18}O$	EH
20:29	Mononononanoin, $C_{15}H_{18}O_7$	EH
11:45	Carbamothioic acid, CH_3NO_2	EH, WP
20:08	Adenine, $C_5H_5N_5$	EH, WP
14:25	Uracil, $C_4H_6N_2O_2$	EP
11:18	Mono-ethylmalonate, $C_4H_4O_5$	EH
16:25	Trigonelline, $C_7H_7NO_2$	EP
16:08	2-Pyrrolidone-5-carboxylic acid, $C_9H_{27}N_2O_4$	EH
16:28	2,4(1H,3H)-Pyromidinedione, $C_4H_4N_2O_3$	EP

Note. Rt — retention time, WH — water extract from heterotrophic tissues, EH — ethanol extract from heterotrophic tissues, WP — water extract from photosyntesizing tissues, EP — ethanol extract from photosyntesizing tissues.

The next group of identified compounds refers to fatty acids that contain an acidic group —COOH (carboxyl) in their molecule. Fatty acids are the lipid components and have protective functions. In addition to fatty acids, lipids include alcohol glycerol which has been identified in water and ethanol extracts of

heterotrophic leaves. In the course of plant adaptation to changing ambient conditions, an important role is assigned to an increase in the content of saturated and unsaturated fatty acids and their ratio, which determines the stability of the membrane lipids [20, 21].

In heterotrophic and autotrophic leaves, two saturated fatty acids (palmitic and stearic) and one unsaturated fatty acid (linoleic) were identified. In addition, in the aqueous extract of heterotrophic leaves, we found two saturated fatty acids, the myristic ($C_{14}H_{28}O_2$) and lauric ($C_{10}H_{20}O_2$), which indicates a high adaptability of photosynthetic leaves.

Low-molecular-weight sugars and polyhydric alcohols perform protective functions in the plant, exhibiting osmoprotective and antioxidant properties, and can also be part of signaling systems [22]. Monosaccharides serve as sources of energy and nutrients and are used for the synthesis of polysaccharides. The group of carbohydrate derivates is the largest and represented by 30 compounds, including simple carbohydrates, sugar alcohols, and lactones. Analysis of these compounds from the heterotrophic leaves revealed 12 substances, including two polyhydric alcohols, the glycerol and ribitol. The water extracts contained only four carbohydrate metabolites, the gluconic acid lactone, arabinopyranose, galactopyranose, and ribose

Amino acids are bioactive compounds with physiological activity. They are structural elements of synthesized protein molecules and participate in various metabolic events, in formation of plant resistance to stresses of various natures, and in detoxification of xenobiotics [23]. In the ethanol extract from heterotrophic leaves, we identified six amino acids of which three, the valine, threonine, and leucine are essential. It should be noted that cystathione serves as an intermediate in the biosynthesis of methionine.

Terpene metabolites, the borneol, isoborneol, dehydroabietic acid, and squalene were identified in the water extracts, while phytosterols and stigmasterol involved in the synthesis of cholesterol were identified in the ethanol extracts. The unique phytosterol squalene ($C_{30}H_{50}$) found in the heterotrophic leaves exhibits anticarcinogenic activity and wound-healing effects in humans and, being a powerful antioxidant, promotes intensive metabolism in cells [24]. As a food ingredient or as a special dietary supplement, squalene lowers cholesterol. Earlier, we first identified squalene in an aqueous extract from leaves of amaranth *Amaranthus tricolor* L. cv. Valentina [25]. Squalene is the compound from which steroids are formed. Currently, a number of steroid compounds have been found in plants that were previously considered typical for animal organisms, for example, cholesterol pregnenolone and progesterol [26].

In heterotrophic amaranth leaves, monopelargonin (mononanoin) ($C_{15}H_{11}O_7$) was identified for the first time. Mononanoin is a phenolic compound, an intermediate of o-glycosylation of flavonoids, and has high antioxidant activity [27, 28].

The Figure (C, D) shows typical profiles of water and ethanol extracts of autotrophic leaves of amaranth cv. Early Splendor. GC-MS analysis revealed 67 low-molecular-weight compounds in photosynthesizing green-violet leaves, of which 59 metabolites from autotrophic tissues were present in the ethanol extracts, and only 21 metabolites were identified in the water extracts (see Table 1).

It should be noted that more organic acids were present in autotrophic leaves than in heterotrophic. We found five organic acids in the water extracts from autotrophic leaves. It is known that natural antimicrobial substances with antioxidant activity synthesized in the leaves of amaranth *A. tricolor* (malate, oxalacetate, ferulic, benzoic, gallic and other organic and phenolic acids) con-

tribute to food preservation [29-32]. Among natural metabolites of amaranth, these organic and phenolic acids involved in cell metabolism attract special attention [33-35].

Organic acids of different chemical nature (i.e., polyoxycarboxylic and phenylcarboxylic acids) were found in the ethanol extracts of green amaranth leaves. For example, the bioactive phenylacetic acid ($C_8H_8O_2$) serves as a plant hormone (auxin).

Of the fatty acids, we found palmitic, stearic, and linolenic acids both in water and ethanol extracts. In addition, lauric and myristic saturated fatty acids were detected in the water extracts.

In green leaves, five monosaccharides and a polyhydric alcohol ribit were identified in the water extracts, erythrofuranose and ribonic acid were additionally found in the ethanol extracts (see Table). The ethanol extracts of autotrophic leaves contained the largest number of monosaccharides and their derivatives, a total of 24, of which five, the mannitol, ribitol, glycerin, arabitol, and inositol (a vitamin-like substance cyclohexane-1,2,3,4,5,6-hexol, the vitamin B_8) belong to polyhydric alcohols.

In water and ethanol extracts of autotrophic amaranth leaves, we found essential amino acids valine, leucine, isoleucine, threonine, and phenylalanine, as well as stress-protective amino acids serine, proline, aspartic acid, asparagine, alanine, and tyrosine. Six amino acids were detected in heterotrophic leaves.

Ore earlier studies have shown that the leaves of the vegetable amaranth *A. tricolor* cv. Valentina contain a full set of free and protein-bound essential amino acids, as well as a large number of bioactive metabolites with antioxidant activity which determine the pharmacological property of a bioactive supplement (herbal tea) Amarantil [7, 36].

Trigonelline (betaine) which is formed by methylation of nicotinic acid and plays a significant role in nitrogen metabolism [31, 37] was also found in the ethanol extracts from photosynthesizing amaranth leaves.

The E group vitamins identified in the ethanol extracts of photosynthesizing leaves, being strong antioxidants, regulate free radical activity in the cell and thus protect unsaturated fatty acids of membrane lipids from oxidation. Tocopherols reduce the risk of chronic free radical pathologies and suppress the enzyme responsible for cholesterol synthesis [38]. The profiles of bioactive metabolites in water and ethanol extracts from amaranth leaves identified in this work and obtained earlier [24] indicate that amaranth can be used to develop safe functional products and drugs exhibiting antioxidant [39], hepatoprotective [40, 41], and antidiabetic [42, 43] effects.

The biochemical composition of low-molecular-weight bioactive substances, including those with anti-stress and pharmacological effects, is being actively studied in various organs of amaranth, but especially in the leaves [44, 45]. With the advent of metabolomic technologies, the list of identified low-molecular-weight metabolites in various amaranth species has increased [46]. In leaves of green- and red-colored vegetable and grain amaranth species, compounds were found that are valuable not only for the food, but also for the pharmaceutical industry (squalene, inositol, glycerin, stigmasterol, linoleic acid, glucopyranose, mannose, etc.), which is similar to the data obtained in this work.

The relationship between hetero- and phototrophic leaves is of particular interest. Metabolic profiling showed that the heterotrophic leaves of cv. Early Splendor contain 13 carbohydrate derivates out of 29 those present in autotrophic leaves (see Table). It should be noted that carbohydrates in plants play a key role as energy sources and carbohydrate skeletons for organic compounds,

storage substances, signaling molecules, they also participate in plant defense response to abiotic stresses.

Of the monosaccharides, eight compounds were detected in the heterotrophic leaves, which was significantly less compared to autotrophic leaves containing 13 compounds. It should be noted that among the latter, xylose and talose which are involved in the synthesis of complex carbohydrates were not identified. The revealed ability of monosaccharides to be both water- and ethanol-extracted from heterotrophic (arabinopyranose and ribose) and autotrophic (galactopyranose, galactose, sorbofuranose and fructofuranose) leaves indicates the amphiphilic (osmoprotective) properties of their molecules.

Metabolic profiling revealed five polycyclic alcohols in the autotrophic leaves and glycerol and ribitol in heterotrophic leaves.

Monosaccharides, polycyclic alcohols and proline in amaranth leaves are compatible osmolytes that help maintain the osmotic balance of the cell, stabilize proteins and cellular structures, exhibiting a protective function. However, heterotrophic leaves contained significantly less compatible osmolytes compared to autotrophic ones. Probably, in heterotrophic leaves, carbohydrates perform the function of signaling molecules and are necessary to construct cell membranes.

The obtained results suggest the formation of three pools of monosaccharides and their derivates in hetero- and autotrophic amaranth leaves which perform different, possibly interchangeable functions. The water-soluble pool of carbohydrate metabolites plays a decisive role in cell metabolism, providing optimal metabolic activity in the cytoplasm and antioxidant protection. The ethanol-soluble pool of monosaccharides and their derivates ensures the preservation and activity of cell structures. The pool of amphiphilic monosaccharides and their derivates maintains the osmotic balance, participates in the stabilization of proteins and cell structures, and provides antioxidant protection.

In cv. Early Splendor amaranth plants, after the growth of the main shoot is completed and the formation of heterotrophic tissues are initiated at its apex, complex metabolic links arise between red and photosynthesizing leaves. We assume that they are mediated by the transport of assimilates from photosynthetic leaves (donor) to newly formed growing heterotrophic leaves (acceptor). This study indicates that the heterotrophic tissues of red leaves contain 37 low-molecular-weight metabolites found in photosynthesizing leaves. These are carbohydrate substances, organic acids, amino acids, and essential fatty acids. Identical metabolites found in heterotrophic and autotrophic leaves seem to be key for the formation of heterotrophic tissue.

Thus, gas chromatography-mass spectrometry profiling of water and ethanol extracts from photosynthesizing and heterotrophic leaves of amaranth cv. Early Splendor revealed 87 low-molecular-weight metabolites, including organic acids, monosaccharides and their derivates, fatty and amino acids, and secondary metabolites (phenolic compounds, terpenes, and glycosides). Heterotrophic leaves contain almost 1.5 times less metabolites compared to autotrophic leaves. The appearance of nonspecific and specific metabolites in both photosynthesizing and heterotrophic leaves, on the one hand, indicates a close interaction of these trophic ways, and on the other, the partial autonomy of heterotrophic leaves due to their ability to synthesize and modify metabolites. Most of the identified compounds have nutritional and pharmacological value. Therefore, leaves of the amaranth cv. Early Splendor are rich in bioactive metabolites and can be used as a raw material for production of dietary and prophylactic bioactive food additives and herbal medicines. The detection of identical metabolites

in photosynthesizing and heterotrophic tissues indicates that in-deep study of their interaction is of fundamental and practical matter.

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