

Chapter

Metabolite Profile of *Amaranthus tricolor* L. and *Amaranthus cruentus* L. in Adaptation to Drought

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Abstract

The Federal Research Center of Vegetable Growing has developed the cultivars Valentina (*Amaranthus tricolor* L.) and Krepysh (*Amaranthus cruentus* L.), which are successfully grown in several regions of Russia. The dry periods observed in recent years have a negative impact on the development of plants. The red-colored vegetable cultivar demonstrated a higher level of adaptability to drought than the green-colored grain cultivar. It was found that only in the leaves of cv. Valentina multiple spiked crystals consisting of four elements were formed, the predominant proportion belonged to Ca (38.59), then P (0.48), Mg (0.25), and K (0.16) followed, weight%, respectively. Under the conditions of moisture deficiency, the antioxidant activity of water and ethanol extracts in the leaves of both types of amaranth increased from 1.5 to 2.5 times. It was established that under drought conditions, the carbohydrate metabolism and the synthesis of secondary metabolites change. The leaves of the new cultivar of amaranth Valentina are a promising and reproducible source of antioxidants and can be used to create phytobiological preparations. The increased level of the main macro- and microelements—Ca, K, P, Mg, Mo, S and Cl in the seeds of cv. Valentina and Krepysh makes these cultivars promising for use in the food industry.

Keywords: amaranth, leaves, photosynthetic pigments, low-molecular-weight metabolites, ash composition of seeds

1. Introduction

Among abiotic stresses, drought is widely spread and strengthens from year to year all over the world. The stressful influence of drought conditions causes changes in morphological, physiological, and metabolic processes of plants that decrease the productivity and the quality of agricultural crops after all [1]. Molecular indicator of water stress is, first of all, speeded accumulation of active forms of oxygen that leads to the development of water stress, the change of chlorophylls structure, the decrease of photosynthetic pigments and metabolites, and the damage of plants cells [2–5].

Phenolic compounds and flavonoids are the most important and widely spread secondary products of plants. These metabolites enlarge enzymic antioxidant system and possess essential potential to decrease and prevent the cell damage [6]. Mineral elements are not only used as structural components, but also play an important role in the enzymes activity, osmotic pressure control for the cells' turgor and growth, take part in acid-base and water-salt metabolism [7–9]. Increased stability to drought mostly depends on the mineral composition of the plants [10, 11].

The most important and actual problem of genetic-breeding research studies is to determine the crops that are stable to drought. Metabolomic approach is a new direction of molecular-genetic research studies to identify the changes in plants under the influence of adverse environmental factors and to assess their nutritional value. Though, nowadays, the use of this approach remains a little used and poorly studied direction of breeding.

The fundamental knowledge about the characteristics of the leaves, seeds, and flour is crucial for the promotion of the crop for use in the food industry. The *Amaranthus tricolor* L. (cv. Valentina) leaf extracts do not only have beautiful crimson color, they also contain a large number of biologically active substances and can be used for tea drinks preparation. Amaranth gluten-free flour can be used for dietary bakery. This study is a first step toward a more efficient and successful application of amaranth as a commercially available pseudo-grain crop.

A. tricolor L. and *Amaranthus cruentus* L. species have been introduced and successfully grown in the Central region of Russia. However, during summer months, the dry period affects the productivity of these species negatively. Based on the foregoing, the present research work was also planned to evaluate and study the mechanisms of drought resistance of two species of amaranth under the conditions of artificial abiotic stress caused by drought.

2. Studies results

2.1 Studies place, objects, and methods

A vegetative experiment was conducted with amaranth species *A. tricolor* L. cv. Valentina (vegetable, red-colored cultivar, with the leaves and seeds having nutritional value for human organisms) and *A. cruentus* L. cv. Krepysh (grain, green-colored cultivar with the seeds having nutritional value for human organisms) in 2020–2021 in the department of gene pool and plant bioresources of the Federal Horticultural Research Center for Breeding, Agrotechnology and Nursery (FHRCBAN), Moscow. The plants of both species were placed outdoors with artificial protection from precipitation. The climate of the study site is moderately continental, the height above sea level is 168 m, the coordinates are 55° 7' 27" north latitude, 37° 56' 55". The amaranth plants were grown by seedlings and transplanted into plastic pots (250 and 175 mm in diameter and height, respectively) one plant per a pot. Totally 40 pots were planted, 20 pieces of each species: 10 control plants and 10 drought-affected ones.

The pots were filled with a mixture of peat and sand (5:1) with a drainage layer at the bottom. In the pots with the control samples, the humidity of the substrate for the plants was maintained at the level of 45–50%. Soil moisture was determined using soil moisture meter MC-7828 SOIL. All the plants were grown for 2 months in well-watered conditions in natural light (**Figure 1**). The average day/night temperature,



Figure 1.
 General view of control plants *A. tricolor* L. cv. Valentina and *A. cruentus* L. cv. Krepysh and drought-prone plants.

relative humidity, and the day length during the experimental period were 17.2°C/11.7°C, 64%, and 17 h, respectively. After 2 months of growth, the degree of stress from drought was determined according to the moisture content in the soil. The watering of the experimental plants was stopped until the signs of wilting. The duration of the soil drought period was 7 days. The plants were examined when the soil moisture dropped till 20–25%.

The biochemical research studies were held in the Laboratory of Physiology and Biochemistry of FHRCBAN.

The understudied parameters included the laboratory studies of the leaves (microscopy of cross sections of the leaf blade, photosynthetic pigments content, antioxidant activity, phenolic compounds sum, ash composition seeds, and quality content of the leaves' main metabolites). The leaves' microscopy and ash composition were determined on analytical REM JEOL JSM-6010 LA (JEOL Ltd., Japan). Photosynthetic pigments Chl a and b and total carotenoids (Car) were studied on spectrophotometer Helios Y UV-vis (USA) in accordance with the method [12]. Total phenolic amount was determined with Folin–Ciocalteu reagent in accordance with the method [13] and total antioxidant capacity, the scavenging activity for the 2, 2-diphenyl 1-picrylhydrazyl (DPPH) radical was determined in accordance with the method [14].

Metabolites quality composition contained in leaf extracts was analyzed on JEOL JMS-Q1050GC (JEOL Ltd., Japan) via the method of gas chromatate-mass-spectrometry in accordance with the method [15].

2.2 Biomineralization of amaranth leaves

An important morphological feature of *A. tricolor* L. cv. Valentina is biomineralization—the presence of multiple crystals in the leaf tissue. In the leaves of *A. cruentus* L. cv. Krepys, the crystals were not found. The round spiked crystals (metabolic products) are often located on the transverse sections of the leaf of *A. tricolor* L. cv. Valentina between the adaxial and abaxial sides (**Figure 2**).

The local energy dispersive spectrometry (EDS) analysis showed that the inclusions contained four elements. The main element was Ca (5.9–8.3 mass %); K (0.34–0.38 mass%); Mg and P—0.03–0.07 mass %. The combination of scanning electron microscopy (SEM) and EDS was a convenient method for determining the microstructure in the cross section of the leaves of *Amarantus* L. X-rays showing the location of all the elements are presented in **Figure 3**. K, P, and Mg are evenly distributed around the mineral inclusions. Ca is concentrated in the crystal. The SEM/EDX results allowed determining the concentration and distribution of elements in the mineral inclusions in the leaves of *A. tricolor* L. cv. Valentina. Calcium oxalate (Ca) crystals are found in many plant species and in most organs and tissues of photosynthetic organisms [16–19]. The modern research studies say that Ca^{2+} is a key element of signaling pathways and is mobilized during the adaptation process to biotic and abiotic stresses [20–22].

Calcium is involved in regulating metabolic processes, plant growth and development [23]. Under drought stress, Ca is an integral part of the recovery process after stress exposure, regulating the plasma membrane enzyme adenosinetriphosphatase, which is required to pump back nutrients lost during cell damage [24].

2.3 Effects of drought on influence on photosynthetic pigments synthesis

The content analysis of chlorophylls and carotenoids in the amaranth leaves showed that some changes were associated with drought (**Figure 4**). An increase of Chl a, b and Car was observed in the leaves of drought-affected amaranth species. In the leaves of *A. tricolor* L. cv. Valentina, the content of Chl a doubled; on the other hand, a slight decrease in the content of Chl b was noted. In the leaves of *A. cruentus* L. cv. Krepys, the content of Chl b increased slightly in comparison with the content of Chl a. The content of carotenoids in the amaranth leaves increased in the conditions of

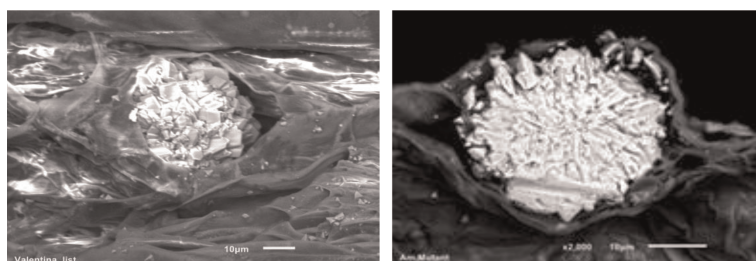


Figure 2.
The protrusions of crystals (a) and Mineral inclusions in the cross-section leaves *Amaranthus tricolor* cv. Valentina.

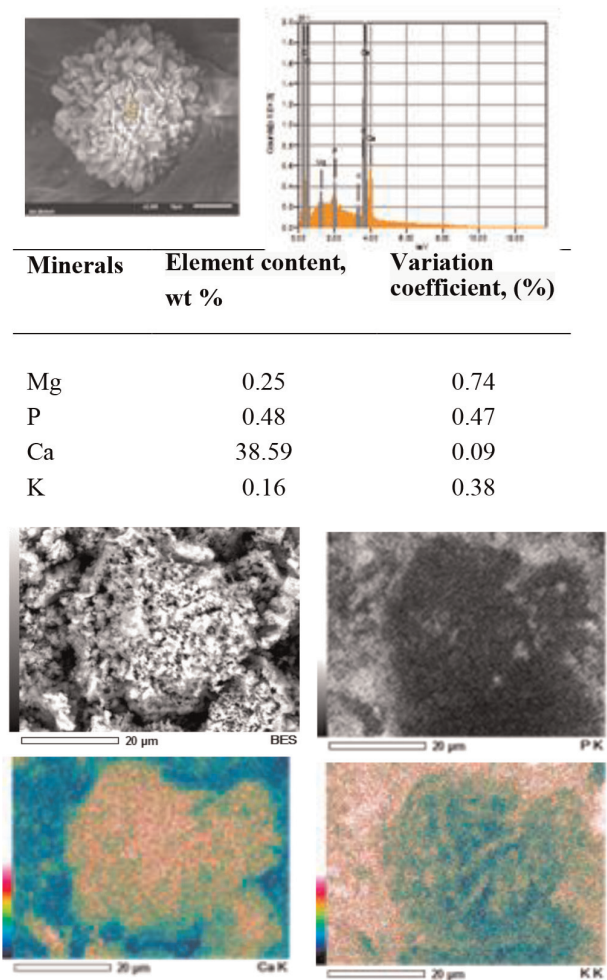


Figure 3. Mineral inclusions in the cross section of amaranth leaves and EDS crystal analysis. SEM micrographs and corresponding EDX spectra of elements in the cross-section leaves *Amaranthus tricolor* cv. *Valentina*., sample 2000 X. The energy spectrum of the X-rays character emitted from the element of K, P and Ca.

moisture deficiency: in the leaves of *A. tricolor* L., it increased 2.5 times, and in the leaves of *A. cruentus* L.—1.5–2.0 times compared with the control. These results are consistent with the data received from the testing on Choy sum in the dry season [25], which reported an increase in total carotenoid content under drought stress.

A high correlation was found between Chl a and Car ($r = 0.985$) and Chl b and Car ($r = 0.977$) in the leaves of *A. tricolor* L. and *A. cruentus* L., respectively. The observed changes in photosynthetic pigments of the leaves in drought are probably associated with free-radical-induced oxidation of Chl pigment [26], the destruction of some chloroplasts, and an increase in the activity of Chl catabolizing enzyme of chlorophyllase [27]. The increase in chlorophyll concentration under drought stress can be determined as an indicator of the plant tissues' resistance to abiotic stress under the drought conditions, which is fully consistent with the data of Jain et al., [28], who reported similar observations. Carotenoids are involved in drought stress resistance due to their ability to capture singlet oxygen. They can also inhibit lipid peroxidation

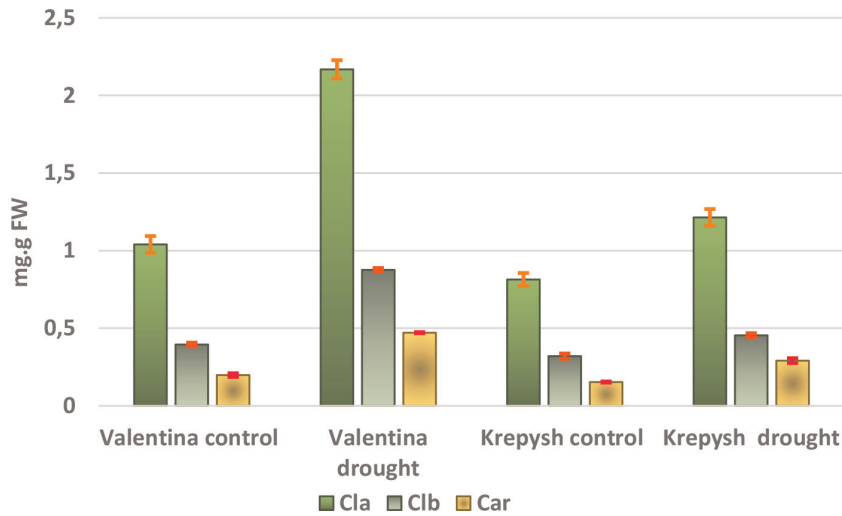


Figure 4.
The content of chlorophyll a (Cla), chlorophyll b (Clb) and carotenoids (Car) in the leaves of amaranth (C4) under stress conditions of drought. Data are the mean $\pm$ SE of three replicates.

and superoxide formation by dehydrating factors. Carotenoids and beta-carotene may play the main protective role in photosynthetic tissue as they directly help plants resist drought [29].

2.4 Effects of drought on influence on antioxidant activity and phenol compounds sum accumulation

The ability of amaranth leaf extracts to absorb DPPH + free radicals, which is used as a measure of total antioxidant activity (TAA), and total phenol content (TPC) are shown in **Table 1**. The antioxidant activity of the water extracts of *A. tricolor* L. leaves was significantly higher than that of *A. cruentus* L. leaves. The antioxidant activity of the alcohol solution differed slightly between the types of amaranth. In the conditions

Samples	Determined indicators		
	AAA	AAM	TPS
<i>A. tricolor</i> L., control V %	24.11 $\pm$ 1.87 7.75	16.26 $\pm$ 0.65 0.43	2.28 $\pm$ 0.37 16.06
<i>A. tricolor</i> L. drought V %	66.82 $\pm$ 1.36 2.03	27.08 $\pm$ 0.87 3.24	6.61 $\pm$ 0.56 8.59
<i>A. cruentus</i> L. control V %	1.35 $\pm$ 0.21 14.93	16.08 $\pm$ 0.24 1.53	1.15 $\pm$ 0.07 6.09
<i>A. cruentus</i> L. drought V %	7.71 $\pm$ 1.01 13.56	26.05 $\pm$ 0.56 2.15	3.19 $\pm$ 0.45 14.18

Table 1.
The effect of drought stress on the antioxidant activity of water (AAA) and methanol (AAM) extracts, expressed in %, and the total content of polyphenols (TPC), expressed in mg equivalent of gallic acid (mg/g TW) in the leaves of *Amaranthus* species.

of water deficiency in the leaves of both types of amaranth, the antioxidant activity of water and alcohol extracts increases by 1.5–2.5 times. Antioxidant activity plays a crucial role in maintaining the balance between free radical synthesis and capture [30–32]. With a lack of water, the total content of phenols in the leaves of both types of amaranth increases by three times. The variation coefficient of the antioxidant activity and the total amount of phenolic compounds was low, which indicates the relative homogeneity of the data obtained. A high correlation was established between the antioxidant activity of water and alcohol extracts and the TPC content in the leaves of both amaranth species ($r = 0.77$, $r = 0.91$), respectively.

Hence, the leaf mass of *A. tricolor* L. can be considered as a source of plant antioxidants that can normalize the ability of the human body to counteract free radicals caused by stress.

2.5 The influence of drought on the contents of metabolites in the leaves of *A. tricolor* L. (cv. Valentina) и *A. cruentus* L. (cv. Krepysh)

Forty-three secondary metabolites were totally determined in ethanol extracts of amaranth leaves. Forty-two substances were identified in the leaves of *A. tricolor* L. (cv. Valentina) and 35 metabolites in the leaves of *A. cruentus* L. (cv. Krepysh) (Table 2). Among nine compounds that possess antimicrobial characteristics, five belong to organic acids—Lactic acid, Pyruvic acid, Glyoxylic acid, Acetamide, Malic acid, and Tartaric acid; one to sugar alcohol—Glycerol; one to amide—Acetamide; and one to phenolic compounds—Benzoic acid. The content of Lactic acid, Benzoic acid, Malic acid, and Mannonic acid is 40, 6, 2, and 1.5 times higher in the leaves of cv. Valentina, than in the leaves of cv. Krepysh, respectively. Glyoxylic acid is found only in the leaves of green-colored amaranth; Acetamide is only in the leaves of red amaranth. Other organic acids are represented by the following compounds: Butanoic acid, Glycolic acid, Oxalic acid, 2-Butanedioic acid, Monoethyl malonic acid, Succinic acid, Glyceric acid, 2-Oxopentanoic acid, Malonic acid, 2,3,4-Trihydroxybutiric acid, Arabinoic acid, Ketosuccinic acid, Fumaric acid, 2-Propenoic acid, and Citric acid. The phenolic compounds are presented by Caffeic acid, Vanillic acid, and Cinnamic acid. The following compounds were also detected: glycoside Apigenin, keto-acid—1,2-Ketoglutaric acid, sugar acid—Myo-inositol; 4 aminoacids—Lauric acid, Myristic acid, Palmitic acid, and Stearic acid (only in the leaves of cv. Valentina). Under drought conditions, the following compounds were synthesized in a significantly larger amount in the leaves of *A. tricolor* L. cv. Valentina: Mannonic acid—by 70 times; Myo-inositol—by 40 times; Caffeic acid—by 23 times; Tartaric acid—by 15 times; Glycerol—by 7 times; L-Proline and Serine—by 4 times; and Glycolic acid, Oxalic acid, and Lactic acid—by 2–3 times. The differences in the synthesis of these compounds were less obvious in the leaves of *A. cruentus* L. (cv. Krepysh). Our results are consistent with earlier findings that the accumulation of Proline and other amino acids increases with water potential decrease in the leaves [33, 34]. Myo-inositol is necessary for absolutely everyone for the synthesis of the substances involved in the transmission of intracellular signals from receptors. It is a vitamin-like substance that affects metabolism and normalizes the levels of sugar and insulin in the blood. It increases the sensitivity of body cells to hormones, supports hormonal balance, and stimulates the proper functioning of the hormone insulin and the stabilization of carbohydrate metabolism [35]. The present study confirmed that the leaves of cv. Valentina and Krepysh are the sources of biologically active compounds and have an enriched antioxidant profile.

N	Tmin	Metabolite	Peak height, % of scale cv. Valentina cv,Krepysh		Biological characteristic
1	10:20	Lactic acid	15–8	0.3–0.2	Antimicrobial 93
2	10:23	Butanoic acid	1.4–0.5	1.2–0.3	Organic acid
3	10:27	Glycolic acid	5–15	5–7	Organic acid
4	10:28	Oxalic acid	10–15	8–5	Organic acid
5	10:42	Pyruvic acid	0.2–0.2	0.3–1.2	Antimicrobial 118
6	10:49	2-Butanedioic acid	0.2–1.5	0.1–7	Organic acid
7	11:00	L-Alanine	1.5–4	1.2–1.8	Amino acid
8	11:29	Monoethyl malonic acid	8–10	5–10	Organic acid
9	12:16	Glyoxylic acid	—	2.5–3	Antimicrobial 78
10	13:23	Acetamide	0.8–0	—	Antimicrobial 40
11	13:43	Glycerol	8–60	8–70	Antimicrobial 77
12	14:04	Succinic acid	11–15	3–4	Organic acid
13	14:23	Glyceric acid	40–13	13–7	Organic acid
14	15:03	Glycine	0.4–3	0.2–1.5	Amino acid
15	15:24	2-Oxopentanoic acid	2–3.2	8–10	Organic acid
16	15:29	Malonic acid	6–7	2–3	Organic acid
17	16:27	Malic acid	14–27	8–19	Antimicrobial 96
18	16:40	L-5-Oxoproline	1.5–2	1.2–2	Amino acid derivative
19	16:48	L-Proline	5–20	4–11	Amino acid
20	17:30	2,3,4.-Trihydroxybutiric acid	22–43	—	Organic acid
21	17:54	1, 2-Ketoglutaric acid	0.2–0.4	—	Keto acid
22	18:14	Arabinoic acid	0.3–0.25	0.3–0.3	Organic acid
23	18:16	Ketosuccinic acid	11–8	—	Organic acid
24	18:24	Lauric acid	0.2–0.4	0.1	Saturated fatty acid
25	19:33	Vanillic acid	2–2.5	—	Phenolic acid
26	19:37	Benzoic acid	3–4.1	0.5–1.6	Antimicrobial 60
27	16:46	Fumaric acid	0.1–0.5	—	Organic acid
28	16:58	Serine	2.5–11	3–8	Amino acid
29	25:00	2-Propenoic acid	0.1–0.3	—	Organic acid
30	20:08	Adenine	1–4	1–2.5	Amino acid
31	20:21	Citric acid	15–40	8–15	Organic acid
32	21:48	Cinnamic acid	2.5–2.8	1.2–1.0	Phenolic acid
33	22:24	Myristic acid	4–13	4–10	Saturated fatty acid
34	22:26	Acrylic acid	8–10	6–10	Antimicrobial 44
35	22:30	Palmitic Acid	0.1	0.05–0.1	Saturated fatty acid
36	22:46	Tartaric acid	4–62	3–15	Antimicrobial 126
37	22:48	Caffeic acid	1.2–28	0.2–0.8	Phenolic acid

N	Tmin	Metabolite	Peak height, % of scale cv. Valentina cv,Krepysh		Biological characteristic
1	10:20	Lactic acid	15–8	0.3–0.2	Antimicrobial 93
38	23:17	Apigenin	0.4–1.2	—	Glycosides
39	23:31	Myo-inositol	10–40	11–15	Sucar acid
40	24:19	Stearic acid	1–1.4	—	Saturated fatty acid
41	34:14	Mannonic acid	10–70	8–30	Organic acid

Table 2.
Metabolites discovered in ethanol extracts of Amaranthus L. leaves.

2.6 The ash residue comparative composition of *A. tricolor* L. (Valentina cultivar) and *A. cruentus* L. (Krepysh cultivar) amaranth seeds

The content (in mass %) of 11 main elements that make up the mineral part of amaranth seeds was studied (**Table 3**). The ash composition of the seeds varies significantly. The descending series of the elements accumulation is the following:

A. tricolor L. – Ca > P > K > Mg > Mo > S > Si > Se > Fe > Zn > Mn.

A. cruentus L. – K > P > Ca > Mg > Mo > S > Se > Zn > Fe > Si > Mn.

At the same time, the main proportion of ash elements in the seeds of *A. tricolor* L. is Ca, and in the seeds of *A. cruentus* L.—K.

Ca is the main ash element in the seeds of *A. tricolor* L., and its portion is 17.83 mass %. The proportion of Ca in the seeds of *A. cruentus* L. is less, and its portion is 11.54 mass %. Ca is a part of coenzymes and cell nuclei, it is involved in the most important processes for human organisms, such as metabolism, immunity, regeneration, and others [36]. The proportion of K in the seeds of *A. cruentus* L. is 1.8, and the proportion

Mineral Elements	<i>Amaranthus tricolor</i> L. cv. Valentina			<i>Amaranthus cruentus</i> L. cv. Krepysh		
	$\bar{x} \pm Sx$	min-max	V,%	$\bar{x} \pm Sx$	min-max	V,%
K	8.94 ± 0.20	7.78–9.07	13.35	15.78 ± 0.19	11.71–13.32	17.75
P	9.67 ± 0.08	8.49–9.98	8.82	14.38 ± 0.15	13.29–14.87	27.78
Ca	17.83 ± 0.08	16.71–18.08	13.39	11.54 ± 0.12	9.76–14.37	29.85
Mo	2.54 ± 0.04	2.12–3.35	17.62	3.43 ± 0.04	3.21–4.86	45.16
Mg	7.33 ± 0.42	6.31–8.89	13.09	5.76 ± 0.22	4.06–6.06	38.21
S	1.84 ± 0.20	1.08–2.35	19.45	2.23 ± 1.04	1.49–2.41	38.60
Si	0.48 ± 0.07	0.41–0.64	20.68	0.21 ± 0.08	0.17–0.37	22.97
Mn	0.17 ± 0.11	0.12–0.21	27.81	0.19 ± 0.08	0.10–0.29	54.20
Fe	0.23 ± 0.04	0.18–0.36	36.38	0.23 ± 0.03	0.13–0.39	58.40
Zn	0.21 ± 0.06	0.17–0.34	30.35	0.26 ± 0.08	0.17–0.24	29.45
Se	0.41 ± 0.06	0.37–0.54	29.45	0.35 ± 0.06	0.27–0.44	31.18
Σ	49.65			54.36		

Notice: *significant at $P < 0.05$.

Table 3.
Mineral (ash) composition of Amaranthus L. seeds, mass %, X (2020–2021).

of P is 1.5 times higher than that in the seeds of *A. tricolor* L. The macroelement K is responsible for regulating the majority of metabolic reactions occurring in living organisms. It controls osmotic pressure, transmembrane potential, charge equilibrium, cathode-anion balance, pH—everything that makes up the homeostasis of cells and tissues [37]. In the human body, P is a part of DNA and RNA, phospholipids, phosphate esters, nucleoside phosphates—ATP, ADP, NADP, where it performs a structural and metabolic function [38].

The content of Mg and Mo in the seeds of *A. tricolor* L. (7.33 and 2.54 mass %) differs slightly from the content in the seeds of *A. cruentus* L. (5.76 and 3.43 mass %). In the human body, Mg is necessary for the processes of regeneration and renewal of cells, tissues, and organs. It activates a large number of enzymes involved in the assimilation of CO₂ and nitrogen. In cytosol, Mg balances organic compounds (groups of sugars, nucleotides, organic and amino acids). Mg is necessary to maintain cathodic-anionic balance and regulate pH [39]. Mo is an important element in the diet, catalyzes the reactions of oxygen transfer from substrates or to substrates, using water as a donor or acceptor of oxygen, is a part of enzymes [40]. The content of trace elements S, Mn, Fe, and Zn in amaranth seeds of the studied cultivars differs slightly (Table 2).

S is a biogenic element in the composition of proteins and glutathione, has antioxidant activity, provides the process of energy transfer in the cell by transferring electrons, participates in the transfer and fixation of methyl groups, the formation of covalent, hydrogen, and mercaptide bonds, provides the transfer of genetic information. Mn is a cofactor and activator of many enzymes (pyruvate kinase, decarboxylase, superoxide dismutase), participates in the synthesis of glycoproteins and proteoglycans, has antioxidant activity.

In active centers (hemoproteins and iron-sulfur proteins), Fe determines the structure and activity of space and participates in redox reactions. Organic Fe is a necessary compound for the human body. This element is part of catalytic centers of many redox enzymes. Zn stabilizes the structure of molecules, plays an important role in the metabolism of DNA and RNA, in protein synthesis and cell division, in the processes of signaling within the cell [41–43].

Si is not only the basis of the framework element of tissues, but also controls a number of biological and chemical processes in a living organism, increases the resistance of a living organism to the effects of biogenic and abiogenic stressors, is a necessary trace element that is part of active centers in the form of selenocysteine aminoacystide [44]. The concentration of Si in *A. tricolor* L. seeds is two times more than that in the seeds of *A. cruentus* L.

The minerals found in amaranth seeds are important for meeting human dietary needs and can make a significant contribution to recommended diets.

2.7 Biologically active components of the studied cultivars of *Amaranthus* L.

The previous studies of the extracts from cv. Valentina fresh leaves detected the following physiologically active substances with antioxidant activity: Amarantin—1.5 mg/g, Ascorbic acid—150–170 mg/100 g, simple phenols and phenolcarboxylic acids, Chlorogenic, Ferulic, Gallic acids, and Arbutin—2.05, 0.01, 1.51, and 473 mg/g, respectively. All metabolites are biologically active substances [45]. Phenolic acids and Betacyanin (Amarantin) are characterized by antibacterial [46–48], antimycotic, anti-inflammatory, and wound-healing properties. Ferulic acid has radioprotective properties, glycosylated hydroquinone Arbutin exhibits antioxidant activity [48]. The

pigment Amarantin is a multifunctional pigment of red-colored amaranth leaves. Amarantin is a nitrogenous heterocyclic compound that has a strong physiological effect on living organisms. The study of the biochemical properties of Amarantin extracted from the leaves of the red-colored cv. Valentina revealed the following physiological activities: antibacterial, antimycotic, antioxidant, antitumor. The extracts from fresh and dried leaves of cv. Valentina stimulated the growing activity of vegetable seeds, which allows its extracts to be used in phytobiology for stimulation of seeds and sprouts (in the concentration of 10⁻⁴, 10⁻⁵ M) [49]. The mechanism of antioxidant activity of Amarantin is associated with its ability to neutralize the superoxide radical and inhibit lipid peroxidation. This allows the leaves to be used to obtain Amarantin extract as a dietary supplement and a phytopreparation.

Under the conditions of drought and high solar radiation, the content of Amarantin in the leaves of cv. Valentina decreases to 40%. The received data indicate that Amarantin performs an important protective function of the photosynthetic apparatus in the plant [50, 51]. The advantage of Amarantin as a water-soluble antioxidant is its rapid synthesis (within 4 hours) after the cessation of drought. The data obtained by us and investigated in the literature data indicate an important role of Amarantin in photosynthetic, metabolic, and protective reactions of an amaranth plant.

Consequently, the data found in literary sources and the results received by us prove that *A. tricolor* L. cv. Valentina is not only highly drought-tolerant, but is also a promising, reproducible source of antioxidants and can be used to create functional foods and phytobiological preparations. The presence of potential antinutrients may limit the use of amaranth in a human diet. To inactivate or reduce these antinutrients, various pretreatment methods are used, such as heat treatment, extrusion, etc. Therefore, further profiling of the metabolomic profile is necessary to improve the nutritional properties of the food product.

3. Conclusions

In the present study, the representatives of species C4 (amaranth) *A. tricolor* L. cv. Valentina and *A. cruentus* L. cv. Krepys have observed several adaptive responses to drought stress under the conditions of water deficit. The features of specific changes in photosynthetic pigments, antioxidant activity, the amount of phenolic compounds, and the composition of metabolites in the leaves were revealed. The increase in the content of phenolic compounds, the total antioxidant activity allowed the plants to survive in adverse environmental conditions. The greatest adaptive potential to drought stress, taking into account the complex of studied physiological and biochemical parameters, was demonstrated by amaranth of cv. Valentina. *A. tricolor* L. cv. Valentina used in the present study can be further investigated as a promising cultivar that can accelerate the breeding for drought tolerance in amaranth.

The leaves of *A. tricolor* L. cv. Valentina contain a sufficient amount of nutraceuticals, phytopigments, and phytochemicals, and the seeds contain a set of macro- and microelements. The leaves of amaranth cv. Valentina can also be used to produce juice as a source of potential nutritional value, phytopigments, antioxidants, flavonoids, phenols, and ascorbic acid in the diet. The present study showed that the cultivars are the sources of biologically active compounds and have an enriched antioxidant profile. The leaves of amaranth *A. tricolor* cv. Valentina contain a sufficient amount of nutraceuticals, phytopigments, phytochemicals, and the seeds

contain a set of macro- and microelements. Valentine's amaranth leaves can also be used to produce juice as a source of potential nutritional value, phytopigments, antioxidants, flavonoids, phenols, and ascorbic acid in the diet. The increased level of the essential macro- and microelements such as Ca, K, P, Mg, Mo, S stipulates the perspective of the functional products creation on the base of the seeds of the studied amaranth (cv. Valentina and Krepysh). The mineral elements concentration in different organs of the plant and their influence on the human life activity are an actual (global) problem, as the deficit of macro- and microelements in the industrial food stuff is extremely huge and dangerous for the human health, because the major part of food stuff is depleted in mineral substances.

The present study showed that the *A. tricolor* L. cv. Valentina and *A. cruentus* L. cv. Krepysh are sources of biologically active compounds and have an enriched antioxidant profile. Recently, the demand for healthy food has increased substantially due to the fact that the link between the health and the consumed products has been shown. In addition to high nutritional value, pseudocereal plants, which include amaranth, contain a large amount of biologically active substances necessary for health. The high protein content of amaranth seeds is characterized by a well-balanced amino acid profile. Seeds are a good source of unsaturated fatty acids, dietary fiber, and essential trace elements. In addition, they contain a wide variety of biologically active compounds. Due to the lack of gluten, these pseudocereals are also interesting ingredients for gluten-free products. Currently, the gluten-free food market is expanding rapidly due to the increasing prevalence of gluten-related diseases such as celiac disease, i.e., gluten intolerance. Amaranth seeds can be used to produce new products, as well as to be an additive to enrich traditional food. Red-colored amaranth leaves can be used to make herbal teas and natural food dyes. The detailed fundamental knowledge of the composition and properties of amaranth seeds is crucial for their introduction into industrial production.

Acknowledgements

The reported study was funded by RFBR and BRFFR, project number 20-516-00012. The reported study was also funded by BRFFR-project number B20R-298.

Conflict of interest

The authors declare no conflict of interest.

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