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COMPARATIVE ANALYSIS OF CARBOHYDRATE METABOLITES IN AMARANTH LEAVES OF DIFFERENT AGE

E.M. GINS[1*], S.M. MOTYLEVA[2], V.K. GINS[3], I.M. KULIKOV[2], and M.S. GINS[3]

[1]Laboratory of Cell and Genomic Technologies, Russian Potato Research Center, Lyubertsy, Russia
[2]Federal Horticultural Center for Breeding, Agrotechnology, and Nursery, Russia
[3]Federal State Budgetary Scientific Institution, Federal Scientific Center for Vegetable Growing, Russia
*Corresponding author's email: katya.888888@yandex.ru
Email addresses of co-authors: motyleva_svetlana@mail.ru, gins1967@outlook.com, fncsad@fncsad.ru, anirr@bk.ru

SUMMARY

The recent study aimed to investigate the composition of monosaccharides and their derivatives in the leaves of vegetable species *Amaranthus tricolor* L. cv. 'Valentina' of different ages, as well as, their vital role in enhancing the adaptive potential of the plant. Forty-eight monosaccharides and 28 of their derivatives have been identified in the composition of amaranth leaves of different ages. The maximum number of hydrophilic carbohydrate metabolites clustered in the young leaf. However, in older leaves, the number of water soluble metabolites showed similarities to that of the soluble in ethyl alcohol. Along with the general carbohydrate metabolites, the old leaves also contained specific monosaccharides, such as, lixopyranose, glucose, sorbose, mannobiose, cellobiose, and monosaccharide derivatives, i.e., methyl galactoside, glucopyranosiduronic and glucuronic acids, and alcohol erythritol. However, in *Amaranthus tricolor* L., the young leaf is characterized by arabinofuranose and carbohydrate derivatives, i.e., alpha-ketogluconic, arabinohexane, glucaric, galactaric, xylonic-D, lactone acids, and alcohol pentatriol. The leaves of different ages' composition showed polyhydric alcohols (glycerin, ribitol, and myo-inositol). It also showed osmoprotective and antioxidant properties.

Keywords: *Amaranthus tricolor* L., cv. 'Valentina,' mono- and disaccharides and their derivatives, gas chromatography-mass spectrometry (GC-MS), antioxidants

Key findings: The gathered results show that the metabolism of *Amaranthus tricolor* L. leaves in different ages included general and specific monosaccharides. Their derivatives also exhibit antioxidant and osmoprotective properties.

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INTRODUCTION

The successful introduction and selection of the sparsely-distributed amaranth crop in the Non-Chernozem zone of Russia mainly resulted in studies of the plant's resistance due to its antioxidant potential and the possibilities for phytotherapeutic application on the human body (Pivovarov *et al*., 2019). The gluten-free proteins represent the nutritional and pharmacological values of amaranth leaf biomass with a complete set of essential amino

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acids: an increased content of ascorbic acid, phenolic compounds, betacyanin, and carotenoid pigments (Gins *et al*., 2017; Tetyannikov *et al*., 2022).

Earlier studies, using biochemical analysis, also revealed the accumulation of essential metabolites, i.e., ascorbic acid, carotenoids, amaranthine, and phenolic compounds with high antioxidant activity in the leaves and inflorescences of amaranth species *Amaranthus tricolor* L. cv. 'Valentina' (Gins *et al*., 2017). In addition to the above components, carbohydrates, in particular monosaccharides and their derivatives, play critical roles in the human and plant life. The metabolic substrates are involved in the modulation of various processes in the plant, from germination to seed maturity. Photosynthesis formed monosaccharides and most of them are in the form of polysaccharides, such as, starch, consisting of monomers of D-glucose, the most common hexose on earth. The usual sugars found in plants are quite fully detailed in literatures and include the four hexoses widely distributed in nature, i.e., D-glucose, D-fructose, D-mannose, and D-galactose (Zhang *et al*., 2017; Mochizuki *et al*., 2020).

Glucose, as a signaling molecule, can transform various metabolic processes occurring in plants at different stages of growth and development (Gibson, 2005). Glucose, together with fructose, accumulates in plants and affects their growth and development (Smeekens, 2000). These monosaccharides have been found to influence many physiological processes in plants (Dekkers *et al*., 2004). Glucose regulates the synthesis of various metabolites, such as, phenolic compounds and glucosinolates (Wei *et al*., 2011) and slows down seed germination and plant growth in a dose-dependent manner (Zhao *et al*., 2009). Exogenous fructose and glucose can enhance the level of soluble sugars (Bogdanović *et al*., 2008; Siddiqui *et al*., 2020). Mannose and galactose are also involved as intermediates in the synthesis of ascorbic acid (Sodeyama *et al*., 2021).

Notably, of the 34 hexoses existing in nature, 30 are rare monosaccharides (Jayamuthunagai *et al*., 2017). These were identified as monosaccharides and their derivatives, which are rarely found in plants (Granström *et al*., 2004). The most famous rare sugar is D-allulose (formerly called D-psicose). This monosaccharide is an epimer in the carbon position of 3D-fructose and has antihyperlipidemic (Matsuo *et al*., 2001), antihyperglycemic (Hayashi *et al*., 2010), and antioxidant (Suna *et al*., 2007) effects. In plants, D-allulose can induce disease resistance, as well as, inhibit the growth of *Oryza sativa* and *Lactuca sativa* (Kato-Noguchi *et al*., 2005; Kano *et al*., 2011; Hossain *et al*., 2015; Iwasaki *et al*., 2018). This monosaccharide inhibits growth induced by a regulatory mechanism associated with the plant growth hormone, gibberellin (Fukumoto *et al*., 2013).

The rare sugar tagatose controls a wide range of plant diseases. In particular, tagatose inhibits the growth of *Phytophthora infestans* and negatively affects mitochondrial processes (Perazzolli *et al*., 2020). In addition, tagatose can act as a human probiotic product, enhancing the growth of beneficial microorganisms and inhibiting the growth of bacterial pathogens (Bertelsen *et al*., 2001; Vastenavond *et al*., 2012). Similarly, the prebiotic effect of tagatose in plants showed an increased relative abundance of several beneficial microorganisms (Perazzolli *et al*., 2020). It also inhibited the growth of many phytopathogens, including the late blight of tomatoes and potatoes (Ohara *et al*., 2008).

Other known rare sugars sorbose, arabinose, and psicose can inhibit the activity of certain enzymes involved in the general metabolism of sugar in mammalian cells (Matsuo *et al*., 2001; Oku *et al*., 2014). Therefore, it is imperative to investigate their properties. Plants are often exposed to environmental stresses (Taiz and Zeiger, 2002; Motyleva *et al*., 2021). When establishing a balance between the formation of reactive oxygen species (ROS) and antioxidants, changing the time of day, the day and night temperature, and processing seeds with biologically active substances, various antioxidant protection mechanisms come into effect. ROS are extremely reactive molecules that can oxidize any cell component, modify protein, and cause DNA denaturation (Das and Roychoudhury, 2014).

Glucose and fructose play an important function in reducing cell damage caused by ROS (Bogdanović *et al*., 2008; Siddiqui *et al*., 2020). Glucose significantly increases the content of antioxidants in cabbage seedlings (Wei *et al*., 2011). Exogenous application of glucose increases the activity of antioxidant enzymes (Huang *et al*., 2013). It is also involved in the synthesis of non-enzymatic antioxidants, such as, ascorbate, glutathione, and phenolic compounds (Bolouri-Moghaddam *et al*., 2010).

Monosaccharides are known to have a protective effect on the protein-lipid

components of the cell, participating in the detoxification of free radicals (Siddiqui *et al*., 2020). Studies reported fructose has a higher antioxidant capacity than glucose (Streb *et al*., 2003; Bogdanović *et al*., 2008). The present study examines the metabolic rearrangements of carbohydrates in amaranth leaves of different ages during the flowering phase.

Although the antioxidant system is vital, it is far from the only stress-protective system of crop plants. About 10-11 different monosaccharides are involved in synthesizing all carbohydrate polymers that form the main structural backbone of the cell wall. One function of the cell wall is protection, which determines the strategy of the existence of the cell itself and the plant. The antioxidant properties of monosaccharides in the cell wall may contribute to the protective function. The study aimed to determine the composition of low-molecular-weight carbohydrate metabolites of aqueous and alcoholic extracts from different-aged leaves of the *A. tricolor* L. (cv. Valentine) and to identify metabolites exhibiting antioxidant and osmoprotective activities.

MATERIALS AND METHODS

Study material and procedure

Amaranth plants were grown in the experimental fields of the Federal State Budgetary Scientific Institution, Federal Scientific Vegetable Center (FSBSI FSVC), without pesticide and herbicide use. The soil of the experimental plot was sod-podzolic heavy loam, and the soil analysis showed diverse content, i.e., alkaline-hydrolyzable nitrogen (105 mg kg^{-1}), labile phosphorus (460 mg kg^{-1}), exchangeable potassium (210 mg kg^{-1}), pH (6.5), and humus content (1.80%).

The existent study investigated the vegetable species *A. tricolor* L., cv. 'Valentina' with the selection prepared at the FSBSI FSVC, Moscow region, Russia (Gins *et al*., 2017). The research material consisted of the fresh leaves and inflorescences of the amaranth plant at the beginning of the flowering phase. Preparation of water and alcohol extracts took place from leaves of different ages. The experiment used the first and second uniformly illuminated leaf from the base of the stem with a fully developed leaf plate and 35 leaves with a leaf volume of 0.5 of the fully developed underlying leaf. The gas chromatography-mass spectrometry (GC-MS) analyzed the composition of metabolites.

Chemicals

All the chemical substances chosen for the analysis were of an analytical grade and bought from Sigma Aldrich (USA) and Merck KgaA (Germany).

Total antioxidant content

Using the amperometric method determined total antioxidant content, adapted to determine the ethanol and water-soluble fractions (Mamedov *et al*., 2017). The quantity of a sample weighing 0.5-1.0 g was homogenized in the bidistillate and ethanol solution (50 mL), and these were used to produce extracts from the plant tissue. Then, the homogenate was centrifuged for 15 min at 5000 rpm at 4**°**C. The supernatant aliquot determined the content of total antioxidants dilution, as necessary. The measurements were made on the device 'Tsvet-Yauza-01-AA' in the constant current mode. The results were expressed as milligram gallic acid equivalents per gram fresh weight (mg GAE g^{-1} FW). The antioxidant activity of carbohydrates and their derived metabolites (pure substances) relative to glucose resulted in a quotient. It came from dividing the equivalent mass of glucose by the mass of pure substance, where the equivalent mass of glucose is the mass of glucose generating the same signal in the amperometric cell as a given mass of the pure substance.

Metabolic analysis by gas chromatography-mass spectrometry

The metabolites analysis was performed on chromatograph JMS-Q1050GC by the method of gas chromatography-mass-spectrometry (GC-MS). Capillary column DB-5HT (Agilent, USA, length 30 m, inner diameter - 0.25 mm, the film thickness - 0.52 μm, gas-carrier - helium) was used. The identification of substances was done following the holding values and mass-spectra of NIST-5 National Institute of Standards and Technology library, USA. The scanning range was 33–900 m/z. The probability of substance identification and determination is within 75%–98%. The temperature gradient during the analysis was between 40°C –280°C; oven temperature progressed from 40°C to 130°C at 1°C min^{-1}, from 130°C to 200°C at 2°C min^{-1}, from 200°C to 280°C at 4°C min^{-1}, and sustained at 280°C for 40 min; the temperature of the ion source = 200°C. Gas flow (helium) in the column was equal to 2.0 ml/min, and using a split-flow

injection mode, the sample was injected at a volume of 2 ml of the evaporated extract.

Processing of leaf probes for GC-MS analysis

The leaves of the fresh material *A. tricolor L*., cv. 'Valentina' were crushed using an IKA homogenizer (Germany), extracting 0.5 g leaf samples with 15 ml of pure methanol and water, then centrifuged on a Sigma 3-18KHS centrifuge (Germany). A 200 µl of centrifugate was evaporated to dryness in a helium stream using the BSTFA (N, O-Bistrifluoroacetamide trimethylsilyl) for trimethylsilylation according to the method described by Han *et al*. (2012) and Bergman *et al*. (2014). The BSTFA silylation was done for 30 min at 100°C.

RESULTS

Carbohydrate metabolites in amaranth leaves and inflorescence

Extensive information about the composition of carbohydrate metabolites in amaranth leaves of different ages can be obtained by analyzing their metabolic profiles. The chromatograms revealed the qualitative differences in the carbohydrate composition of aqueous and alcoholic extracts in their leaves of different ages (Figure 1). According to the library mass spectra, 27 individual substances belonging to mono- and disaccharides were identified in the old leaf and 21 in the young one (Table 1, Figure 2).

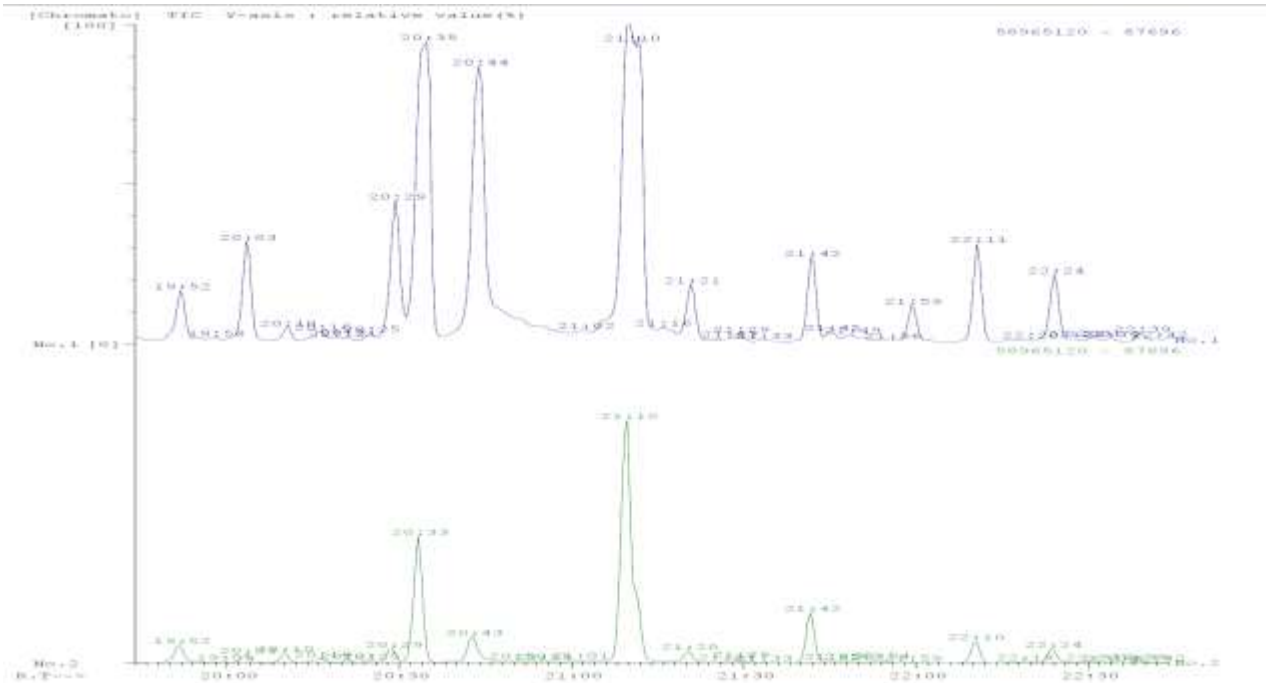

Figure 1. Plots of chromatograms of methanol extracts of the leaves of *Amaranthus tricolor* L., cv. 'Valentina'. Blue color - old leaf, green color - young leaf.

In the amaranth old leaves (the first and second), the study registered 2.5 to 4 times the total number of monosaccharides soluble in water and alcohol, compared with the young leaf. However, the aqueous extract of the young leaf showed a higher number of hydrophilic metabolites. Analysis of monosaccharide derivatives in the young amaranth leaf revealed 11 metabolites soluble in water and only one metabolite identified in the alcohol extract, gluconic acid, present in the water extract in the first leaf of the lower tier. Thus, in the amaranth plant, a pool of water-soluble carbohydrate metabolites and their derivatives gathered mainly in the young leaf and alcohol-soluble ones - in the oldest leaves of the lower tier.

Table 1. The number of metabolites of carbohydrate nature present in the inflorescence and leaves of different age of amaranth in the composition of aqueous and alcohol extracts.

Solvent leaf	The number of mono- and disaccharides in leaves of different ages				The number of monosaccharide derivatives in leaves of different ages			
	1st old	2nd old	Young	Inflorescences	1st old	2nd old	Young	Inflorescences
Water	5	6	9	1	6	3	11	1
Ethanol	10	10	9	3	6	8	1	-
Metabolites soluble in both ethanol and water	12	7	3	5	3	4	3	5

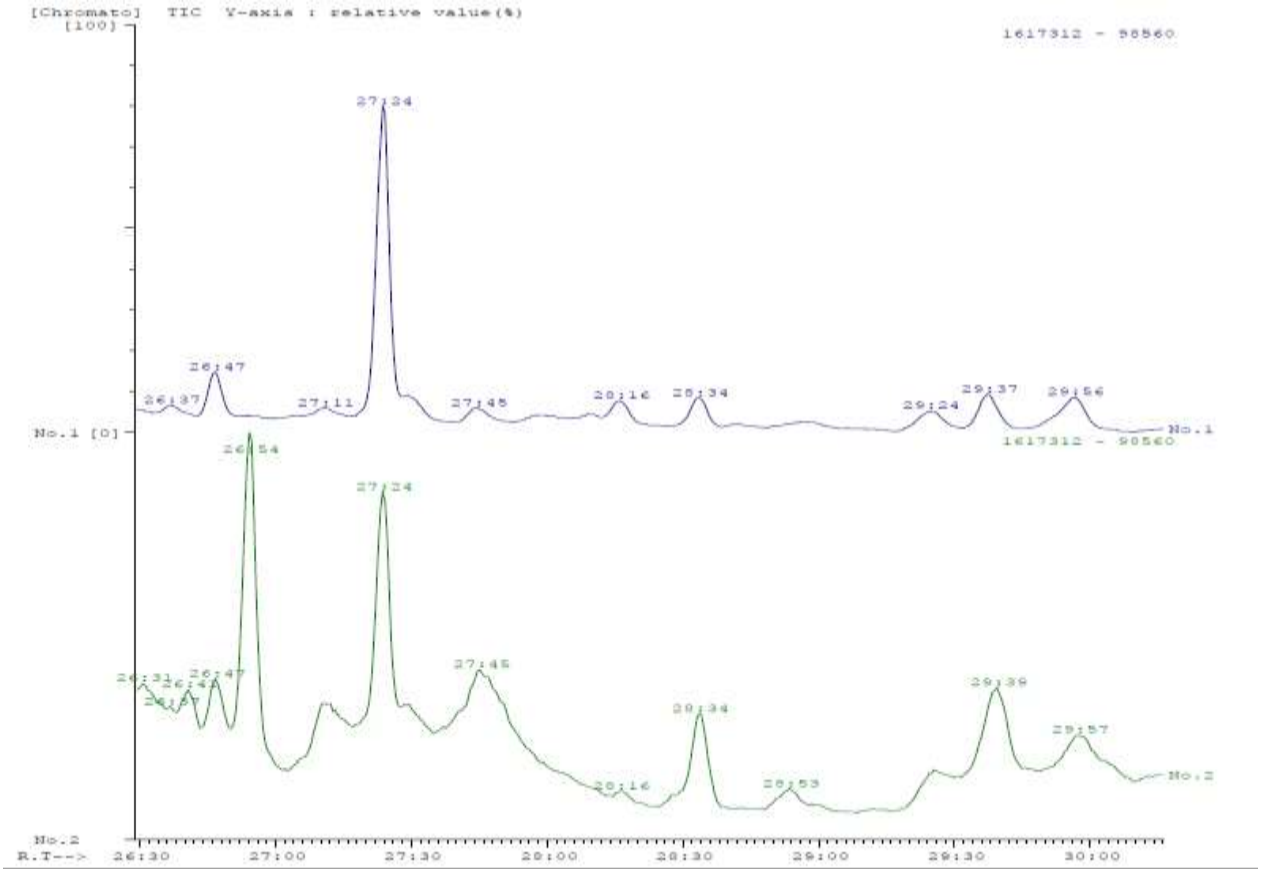

Figure 2. Plots of chromatograms of aqueous leaf extracts. Blue color - old leaf, green color - young leaf.

Similarly, the old leaves' lower tier displayed a large number of monosaccharides that were both soluble in alcohol and water. The ability of the same monosaccharide to pass only into aqueous or alcoholic extracts, or both, may indicate their different properties and structure in the cell: free or bound. Further, glucuronic and galacturonic acids are elements of polysaccharides extracted by alcohol, while in their free state may dissolve in water. Only nine monosaccharides and six of their derivatives were found in the inflorescences, while five metabolites dissolved in water and alcohol (Table 1).

Monosaccharides - pentoses in amaranth leaves

In the composition of amaranth leaves of different ages the study identified pentoses in a cyclic form. Simultaneously, xylose (old and juvenile leaves) occurred in the pyranose form,

Table 2. The composition of monosaccharides in the leaves of different age of *A. tricolor* L. (cv. 'Valentina'), soluble in polar (water) and nonpolar (96% ethyl ethanol) solvents (FSBSI FSVC, 2019).

The first old		The second old		The third young	
erythrofuranose	E	threose	E	erythrofuranose	E
-	-	D-erythrosis	E	-	-
-	-	erythritol-1,4-lactone	E	-	-
ribofuranose	E/W	ribofuranose	E/W	ribofuranose	E
xylopyranose	E	xylopyranose	W	xylopyranose	W
arabinopyranose	E	-	-	arabinopyranose	E/W
lyxopyranose	E	lyxopyranose	E	-	-
-		-	-	arabinofuranose	W
glucose	W	-	-	-	-
glucopyranose	E/W	glucopyranose	E/W	glucopyranose	W
galactose	E/W	galactose	E/W	galactose	E/W
galactopyranose	E	galactopyranose	E	galactopyranose	W
mannose	E/W	mannose	E/W	mannose	W
-		mannopyranose	E	-	-
fructose	E/W	-	-	-	-
fructopyranose	E	fructopyranose	W	fructopyranose	E
fructofuranose	E/W	fructofuranose	E/W	fructofuranose	E
hexopyranose	E	hexopyranose	W	-	-
sorbose	W	-	-	-	-
sorbofuranose	W	sorbofuranose	W	sorbofuranose	E
psicopyranose	W	psicopyranose	W	psicopyranose	W
allopyranose	E	-	-	allopyranose	W
talofuranose	E/W	talofuranose	E	talofuranose	E
tagatofuranose	E/W	tagatofuranose	E	tagatofuranose	E

and water-soluble ribose and arabinose (juvenile) appeared in the furanose form (Table 2). The study noted that ribose in the furanose form is part of ribonucleic acids. In the old leaves of the lower tier, a specific metabolite of lyxopyranose existed, and in the young— arabinofuranose. In addition, hydrophilic monosaccharides, xylopyranose, and arabinopyranose also appeared in the second and young leaves. Assumingly, they might be precursors of high molecular weight polysaccharides of pentosans. In the old (first) leaf, these monosaccharides occurred in the alcohol extract, indicating their formation during the hydrolysis of hemicellulose polysaccharides. In the young amaranth leaf, the above monosaccharides found in the aqueous extract suggest their participation in the synthesis of pentosans.

Monosaccharides – hexoses in amaranth leaves

The maximum number of monosaccharides (hexoses) ensued in the alcohol extracts of amaranth old leaves, and the young leaf's number of hexoses got evenly distributed between the water and alcohol extracts. It is also known that monosaccharides provide the metabolic activity of the leaves, including glucose. In amaranth young leaves, glucose, as a more reactive compound, was quickly metabolized and undetected in a non-cyclic form (Table 2).

The aqueous extract exhibited hydrophilic monosaccharides. The glucose fraction contained its non-cyclic (aldehyde) and all its cyclic forms, i.e., furanous and pyranous, but the glucose amount was only about 1%. In the first old leaf, the specific monosaccharides identified included water-soluble glucose and sorbose in non-cyclic form and alcohol-soluble hexapyranose, and in the second old leaf, manopyranose, threose, D-erythrose, erythro-1, 4-lactone, and water-soluble hexopyranose showed in the alcohol extract.

In addition to glucose, galactose, fructose, sorbose, and psicose came out in amaranth leaves in the cyclic form of pyranose, while thalose, fructose, and tagatose were displayed the furanose form. The maximum number of monosaccharides in the old leaf was soluble in both aqueous and alcoholic extracts. This indicates that most of the monosaccharides have osmoprotective properties in the amaranth old leaves. Young leaves were also found more sensitive to dehydration. A comparison of the antioxidant activity of old and young leaves of amaranth (cv. Valentina) revealed large content of low–molecular antioxidants - amaranthine and ascorbic acid in young leaves (Gins *et al*., 2017).

Relative antioxidant activity of soluble carbohydrates

The data presented in Table 4 on the relative antioxidant activity of widespread hexoses—common, as well as, rare sugars—sorbose and xylose, indicate their antioxidant properties and the ability to scavenge superoxide radicals. In the model system, mannose, galactose, and fructose have a higher antioxidant capacity compared with glucose. This shows that fructose, mannose, galactose, and rare sugars can perform protective functions in the plant during the accumulation of ROS.

Identification of disaccharides

In the studied amaranth leaves, lactulose disaccharide got detected in the form of ketose, and lactose in the form of aldose (Table 3). Notably, fewer disaccharides were identified in the composition of the young leaf compared with the older leaves of the lower tier. Interestingly, the study emphasizes that lactulose (milk sugar) is a rare disaccharide for the plant, which includes β-D-galactose and α-D-glucose. It is the second most important sugar from a nutritional point of view, which provides the nutritional value of milk and other products (Sitanggang *et al*., 2016).

Disaccharide (cellobiose) is the chief building block of fiber (cellulose) and represents the β-glucosidoglucose. It naturally exists in the sap of some trees. Cellobiose occurred in old amaranth leaves – its full name is β-D-glucopyranosyl-(1-6)-D-glucopyranose. Interestingly, the cellobiose appears in the plant only as a result of enzymatic hydrolysis of cellulose since it is not synthesized in the plant. The accumulation of cellobiose in the oldest amaranth leaves can be associated with the catabolism of cellulose structures in the cell caused by aging. The absence of cellobiose in young amaranth leaves may be an indication of its possible accumulation only in old leaves as a biomarker of aging.

Table 3. The composition of disaccharides in the leaves of different age of *A. tricolor* L. (cv. 'Valentina') (FSBSI FSVC, 2019).

The first old		The second old		The third young	
lactose	E/W	lactose	E	lactose	W
lactulose	W	lactulose	W	lactulose	W
mannobiose	E/W	mannobiose	E/W	-	-
cellobiose	E	-	-	-	-
-	-	cellulose	E	-	-
-	-	sucrose	E	-	-
turanose	E	-	-	turanose	E

Notice: W - water extract, E - alcohol extract, E/W - water-alcohol extract

Table 4. Relative antioxidant activity of carbohydrates in the aqueous extract.

Compound	Relative antioxidant activity of the compound (GAE g $^{-1}$ FW)
D-(+)-Glucose	1,00±0,05
L-(-)-Sorbose	9,4±0,5
D-Galactose	4,9±0,5
D-(+)-Mannose	4,3±0,2
D-(-)- Fructose	4,1±0,2
D-Xylose	1,9±0,1

Monosaccharide derivatives in amaranth leaves

In the amaranth leaves of different ages, 33 derivatives of monosaccharides, mostly glucose and galactose got recognized (Table 5). Modified monosaccharides exhibit the properties of biologically active substances, including protective ones. Polyvalent alcohols were also found in all studied leaves, i.e., glycerin, ribitol, and myo-inositol, showing osmoprotective and antioxidant properties. Myo-inositol is a powerful antioxidant, and ribitol and glycerin also exhibit protective functions. The above polyols got detected in the amaranth leaves of different ages, and in addition, the specific alcohol erythritol was noted in the old leaf, and pentatriol in the young one (Table 5).

In uneven-aged leaves, the general and specific derivatives of aldonic acids came out. In the first old leaf, derivatives of a carbohydrate nature got noticed, i.e., ribonic

acid lactone, glucuronic and erythro-pentanoic acids, and glucopyranosiduronic acid in old leaves, and α-ketogluconic, glyceric, xylonic, and arabino-hexanoic in young leaves. Specific aric acids, i.e., glucaric and galactaric also revealed in the young leaf, however, their biological role has not been established yet. Methyl derivatives of galactose – methyl galactosides, which dissolve in water and alcohol, have been found in old amaranth leaves.

Gluconic, gulonic, and ribonic acids got recorded in the composition of aldonic acids in all studied amaranth leaves. Likewise, the erythro pentanic acid also occurred in the first and second old leaves (Table 5). The xylonic acid got observed in the second old and young leaves. From the composition of uronic acids, galacturonic acid accumulates in all leaves, and glucopyranosiduronic acid accumulates in old leaves only.

Table 5. Composition of monosaccharide derivatives in the leaves of different age of *A. tricolor* L. (cv. 'Valentina') (FSBSI FSVC, 2019).

The first old		The second old		The third young	
Methylated glycosides					
methylglycoside	E/W	-	-	-	-
methylgalactoside	E/W	Methylgalactoside	E/W	-	-
Glycosides					
glyceryl-glycoside	W	glyceryl-glycoside	E	glyceryl-glycoside	W
Aldonic acids					
glucofuranoside	E	-		glucofuranoside	E
-		gluconic acid lactone	W	-	-
gluconic	W	gluconic	E	gluconic	E
gulonic	E/W	gulonic	E	gulonic	W
-	-	-	-	α-ketogluconic	W
-	-	-	-	glyceric	W
ribonic acid	W	ribonic acid	E/W	ribonic acid	W
lactone of ribonic acid	EW	-	-	ribono lacton	W
-		-	-	arabino-hexonic acid	W
erythro-pentanoic acid		erythro-pentanoic acid	E	-	-
-		xylonic	E	xynolic acid D	W
Uronic acids					
glucopyranosiduronic	W	glucopyranosiduronic	E	-	-
galacturonic	E/W	galacturonic	-	galacturonic	E/W
glucuronic	E	-	E/W	-	-
Aric acids					
galactaric	E/W	galactaric	W	-	-
-	-	-	-	glucaric	W
-	-	-	-	galactoric	W
levoglucosan	E/W	Levoglucosan	E/W	levoglucosan	E/W
Sugar alcohols					
glycerol	E/W	glycerol	E/W	glycerol	E/W
ribitol	E	ribitol	E	ribitol	E/W
myo-inositol	E	myo-inositol	E/W	myo-inositol	W
-	-	-	-	pentatriol	W
erythritol	E	-	-	-	-
butantriol	W	-	-	-	-
-	-	isotridecyl alcohol	E	-	-
-	-	inositol	E	-	-

Notice: W - water extract, E - alcohol extract, E/W - water-alcohol extract

DISCUSSION

The presented results indicated that metabolomic profiling using GC-MS made it possible to identify carbohydrate metabolites in amaranth leaves of different ages, evaluate the dynamics of the composition of monosaccharides, and isolate common and specific metabolites from them, characteristic of an individual amaranth leaf. The study has shown low molecular weight monosaccharides in leaves of different ages form an individual phenotype (morphological and biochemical parameters) characteristic of each leaf.

In the research, metabolic profiling of individual organs of the amaranth revealed

that the inflorescences and the leaves of different ages are characterized by an individual composition, including both common metabolites characteristic of all studied leaves and specific ones inherent only in a single leaf (Tables 1, 2, 3, and 5). During the growing season, the plant constantly experienced the effects of stress factors, the duration and strength of which did not negatively affect its vital activity, but can cause significant metabolic rearrangements in its organs and leaves, slowing down or enhancing the synthesis of metabolites that exhibit protective properties.

The study of the ability of monosaccharides and their derivatives to dissolve in polar and non-polar solvents revealed their different composition depending on the age of the leaf (Table 1). In the old leaf, most of the monosaccharides have osmoprotective properties. According to past data, water-soluble monosaccharides, i.e., glucose, mannose, galactose, and fructose can neutralize reactive oxygen species and free radicals (Bogdanović *et al*., 2008; Siddiqui *et al*., 2020;). The presented results have shown that the hexoses in the model system exhibit the ability to neutralize free radicals. Moreover, fructose, galactose, and mannose have a higher antioxidant capacity compared with glucose (Table 4). It indicates that the above sugars may play an important role in regulating the balance of ROS in the cell as one of the non-enzymatic defense mechanisms. Considering the previous discussion, the old amaranth leaves can be assumed to have the greater resistance, for example, to the effect of temperature differences as determined by the composition of alcohol-soluble monosaccharides with antioxidant properties, the number of which is higher in the old leaf compared with the youngest one. With a constant difference in night and day temperatures, for example, during spring or autumn, part of the pool of carbohydrate metabolites can participate in the formation of cold resistance of cell components. Hydrophobic monosaccharides exhibit antioxidant properties as part of the composition of membranes and other structures which need protection from oxidation and destruction.

Water-soluble hexoses - monosaccharides of the old leaves, i.e., glucose, sorbose, sorbofuranose, and psycopyranose, as well as, of the young leaves, i.e., glucopyranose, galactopyranose, mannose, psycopyranose, and allopyranose, are part of the antioxidant defense system of the cell. As a result, three pools of monosaccharides were identified in the composition of carbohydrate metabolites in leaves of different ages, differing in their solubility in aqueous and alcoholic solvents, which may be related to their protective functions, to name a few. In addition, the known monosaccharides from amaranth leaves, i.e., glucose, mannose, galactose, xylose, and arabinose, as well as, carbohydrate derivatives – glucuronic and galacturonic acids, were involved in the construction of all carbohydrate polymers that form the cell wall. These metabolites gave protection from pathogenic organisms as part of the cell wall defense. Also, the above monosaccharides determine the stability of the cell and the strategy of the existence of the cell itself and the plant (Verbančič *et al*., 2018).

The presence of hydroxyl, aldehyde, and ketone groups in monosaccharides allows them to enter reactions characteristic of alcohols, aldehydes, and ketones. Modification of these groups in the monosaccharide molecule leads to the formation of monosaccharide derivatives. In plants, during the biological oxidation of the terminal groups of aldoses to carboxylic, three different aldose derivatives are formed, i.e., aldonic, uronic, and aronic acids. Some of them take part in the synthesis of polysaccharides. For example, the starting material for the biosynthesis of hemicelluloses and pectin substances are D-glucuronic and D-galacturonic acids, the formation of which occurs by oxidation of UDP-glucose and UDP-galactose.

The formation of alcohol-soluble monosaccharides may indicate that the accumulation of monosaccharides in old leaves is not only due to sugars formed during photosynthesis but also due to soluble carbohydrates formed from fiber and hemicelluloses contained in cell walls. Noteworthy to state that myo-inositol formed D-glucuronic and D-galacturonic acids and, along with glucose, can serve as starting compounds for ascorbic acid synthesis. The L-gulonic acid occurred in all studied leaves and participates as an intermediate in the synthesis of ascorbic acid, where the starting material is glucuronic acid (Akram *et al*., 2017). Other monosaccharide derivatives are intermediates of various biochemical reactions of the cell metabolism.

The presented results suggested that common and rare monosaccharides in the leaves of different ages are involved in the plasticity formation of the amaranth cultivar, Valentina; hence, the plant could adapt to the

ever-changing growing conditions. For the first time, a study showed that common and rare sugars neutralize superoxide radicals, which can over-accumulate in the cell when environmental conditions change. The osmoprotectant sugars can play a significant role in stabilizing cell homeostasis as part of the non-enzymatic defense mechanisms of plants.

CONCLUSIONS

Common and rare monosaccharides are involved in the formation of resistance in the plants of amaranth cultivar, Valentine, to constantly changing growing conditions. For the first time, the study presented that common sugars, i.e., galactose, fructose, mannose and glucose, and rare sugars, i.e., sorbose and xylose and their derivatives can scavenge superoxide radicals, exhibiting antioxidant and osmoprotective properties.

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