RESEARCH ARTICLE

SABRAO Journal of Breeding and Genetics
54 (2) 426-436, 2022
http://doi.org/10.54910/sabrao2022.54.2.18
http://sabraojournal.org/
pISSN 1029-7073; eISSN 2224-8978

DROUGHT EFFECTS ON MINERAL COMPOSITION OF THE LEAVES AND SEEDS OF *AMARANTHUS TRICOLOR* AND *AMARANTHUS CRUENTUS*

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SUMMARY

In global climate change, drought stress is one of the environmental restraining factors that can significantly influence the growth and development of crop plants. Drought stress conditions can also cause undesirable changes in plant physiological and metabolic processes. The influence of soil drought on the mineral composition of leaves and seeds of two species of amaranth (*Amaranthus tricolor* L. and *Amaranthus cruentus* L.) with C_4-type of photosynthesis was studied through energy dispersive spectrometry (ESD). The recent investigations were carried out during the years 2020–2022 at the Department of Genofonde and Bioresources of Plants, Federal Scientific Center for Horticulture, Moscow, Russia. The research results showed the leaves of both amaranth with major elements, i.e., K (11.23–15.33), Ca (5.15–7.61), P (3.91–3.92), Mg (2.81–3.36), and Cl (1.86–2.29), whereas, relatively lower values were recorded for Fe (0.05–0.48), and Na (0.07–0.11) mass% respectively. Regarding amaranth plants seed composition, the major elements were K (13.86–13.97), P (7.02–9.76), Mg (3.78–5.64), Ca (3.31–4.78), Cl (2.81–5.30), and Mo (2.80–2.86) mass% respectively. In the species, *A. tricolor*, a strong correlation was observed between the elements, i.e., S-Cu, Mg-Si, Na-Cu, Na-S, Na-Ca, Na-Si, and Si-S in leaves, while in seeds, these were between Ca-Cu, Mg-Cl, Si-Mn, Ca-Mo, and Cl-Mn. In the other species of amaranth, *A. cruentus*, the elements viz., Mg-S, Mg-Mo, S-Mo, Mg-Cl, S-Cl, Cl-Mo, Cl-P, P-S, Si-Cl, Ca-Mo, S-Ca, Mg-Ca, Mg-P, P-Mo, and Mg-Si in leaves, while Ni-Cu, Mg-P, Si-P, and Si-Cl in seeds also showed strong relationship. Effects of drought led to a weakening of these ties and the formation of new ones. The accumulation of mineral elements in the leaves of amaranth plants varies from species to species under drought conditions, and *A. tricolor* cv. Valentina was found most resistant to drought conditions.

Keywords: Mineral composition, leaves, seeds, drought stress, EDS analysis, *Amaranthus tricolor* L., *Amaranthus cruentus* L.

Key findings: For the first time, the drought stress effects on the mineral composition of the leaves and seeds of two amaranth species grown in Moscow (Russia) have been reported. The recent investigations also revealed that there were significant differences among the ratios of macro- and trace elements accumulation in the leaves and seeds of the two *Amaranthus* species (*Amaranthus tricolor* L. and *Amaranthus cruentus* L.) under controlled and drought conditions.

To cite this manuscript: Tetyannikov NV, Motyleva SM, Gins MS, Kozak NV, Panischeva DV, Mertvischeva ME, Kabashnikova LF, Domanskaya IN, Pilipovich TS (2022). Drought effects on mineral composition of the leaves and seeds of *Amaranthus tricolor* and *Amaranthus cruentus*. *SABRAO J. Breed. Genet.* 54(2): 426-436. http://doi.org/10.54910/sabrao2022.54.2.18

Communicating Editor: Dr. Kamile Ulukapi
Manuscript received: May 2, 2022; Accepted: June 2, 2022.

INTRODUCTION

Among abiotic stresses, the drought intensifies every year spreading widely all over the world. The most important objective for present breeding research is to determine the crops that are stable and perform better under drought conditions (Reddy *et al*., 2004; Agati and Tattini, 2010; Hasanuzzaman and Tanveer, 2020). Molecular indicators of water stress speeded accumulation of active forms of oxygen that leads to the development of oxidative stress, the change of chlorophyll structure, decrease in photosynthetic pigments and metabolites, and the plant cells damage (Hernandez *et al*., 2001; Munne-Bosch *et al*., 2013; Getko *et al*., 2019). Mineral elements are not only structural components, but also play an important role in the enzyme activity, osmotic pressure control for cell turgor and growth, and acid-base and water-salt metabolism (White and Brown, 2010; Nemtinov *et al*., 2020). Increased stability to drought stress mostly depends on the plant's mineral accumulation (Waraich *et al*., 2011; Khan *et al*., 2012).

The study of leaf and seed characteristics is very important for the promotion of the amaranth in the food industry usage. The amaranth plant leaves are used as part of salads, soups, and sauces, and both leaves and seed as a raw material for the production of food-grade natural dye. Leaf mass and seeds of *Amaranthus tricolor* L. have a high nutritional value. Leaf extracts contain a large number of biologically active substances, and can be used for tea drinks preparation. The amaranth gluten-free flour can be used for dietary bakery (Motyleva *et al*., 2022). Two different species of amaranth, i.e., *Amaranthus tricolor* L. and *Amaranthus cruentus* L., have been introduced and successfully grown in the Central region of Russia, as proven by studies on the economic efficiency of the cultivation of this crop (Pivovarov *et al*., 2019). According to different estimates, the area under amaranth is about 3,500 ha in Russia, with a tendency to increase. However, the average yield of green mass of amaranth was at 50.0 t/ha (Kononkov *et al*., 2013). Depending upon the cultivation technology, amaranth seed yields can be 1.7 to 2.9 t/ha (Saratovskiy *et al*., 2018). Nonetheless, although amaranth is drought tolerant, the problem of drought stress is still a concern for this crop. During the summer months, the dry spells affect the species' productivity.

In plant life, the mineral elements are very important and play a vital role in their resistance to biotic and abiotic stresses, growth, and development (Popov and Dementyev, 2014). The most important macronutrients include nitrogen-N, phosphorus-P, sulfur-S, potassium-K, calcium-Ca, and magnesium-Mg, while the micro-nutrients are iron-Fe, copper-Cu, chlorine-Cl, nickel-Ni, molybdenum-Mo, zinc-Zn, manganese-Mn, and boron-B (White and Brown, 2010; Nemtinov *et al*., 2020). Micronutrients are also involved in physiological, biochemical, and metabolic processes that occur under different abiotic stresses. Past findings reported that enhanced drought tolerance depends largely on the mineral composition of the plants (Waraich *et al*., 2011; Tetyannikov *et al*., 2021). Despite the quite important role of macro- and micronutrients in plants, the mechanisms of their accumulation and remobilization are still not fully clarified in crop plants under drought conditions (Etienne *et al*., 2018).

Drought effects on the physiological and biochemical status of the leaves include such parameters as relative water content (RWC), water deficit (WD), dry matter content (DMC), PS2 activity, photosynthetic pigment content, and antioxidant activity. The composition of phenolic compounds was also studied in amaranth and actinidia plants (Motyleva *et al*., 2021). Based on the foregoing discussion, the recent research aimed to study and evaluate the mechanisms of drought resistance in two different species of amaranth under the artificial abiotic stress conditions caused by drought.

MATERIALS AND METHODS

A vegetation experiment with amaranth species was carried out during the years 2020–2022 at the Department of the Genofonde Pool and Bioresources of Plants, Federal Scientific Center for Horticulture, Moscow, Russia. The plants of both amaranth species were placed in a greenhouse with artificial protection from rainfall. The study location has a temperate continental climate at 168 m above sea level,

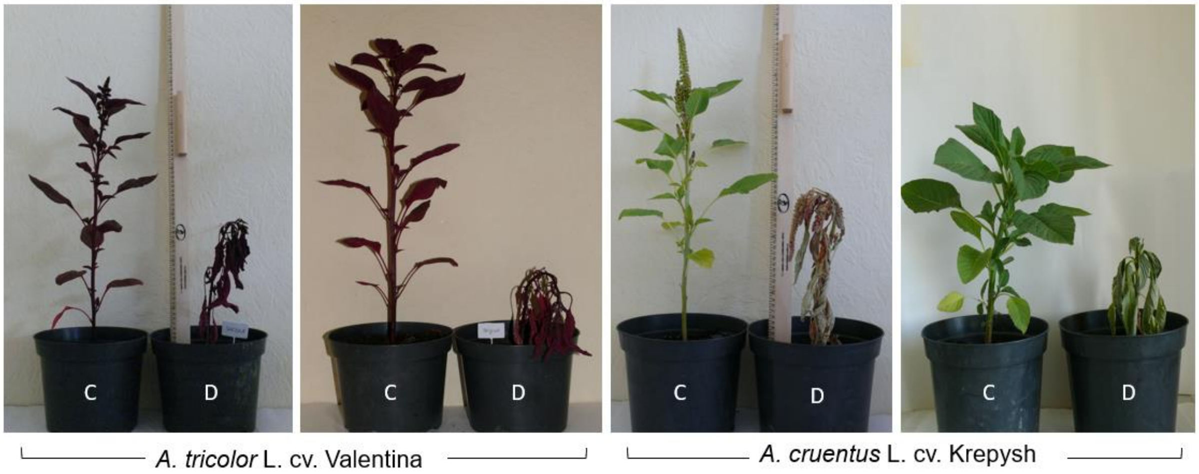

Figure 1. General view of control plants *A. tricolor* L. cv. Valentina and *A. cruentus* L. cv. Krepysh and drought-prone plants (c: plants in control, d: plants in drought stress).

with coordinates of 55°7'27" north latitude, 37°56'55" east longitude. Amaranth species (*Amaranthus tricolor* L. cultivar 'Valentina' and *Amaranthus cruentus* L. cultivar 'Krepysh') were grown as seedlings and transplanted into plastic pots (250 and 175 mm in diameter and height, respectively) with one plant per pot. A total of 20 pots were planted with amaranth plants. The pots were filled with a mixture of peat and sand (5:1) and had a drainage layer at the bottom. In pots with the control samples, the substrate moisture content was maintained at 45% to 50% of the full water holding capacity of soil for amaranth plants of both species. Humidity was determined by using the soil moisture meter MC-7828 SOIL. All the plants were grown for two months under good watering conditions under natural light. After two months of growth, the degree of drought stress was determined following soil moisture content. The watering of the experimental plants was stopped until signs of wilting appeared. The soil drought duration was for five days. The amaranth plants were studied when the soil moisture in the pots decreased by 60% to 70% from the initial control. Control and experimental plants at the time of the experiment were in the phase of flowering and seed formation. The plants in the experiment are shown in Figure 1. The middle layer leaves were used for analyses. After sampling in the drought variant, these plants were returned to irrigation until the soil moisture reached the control level. A recovery of leaf turgor in *A. tricolor* occurred within 3–5 h, while in *A. cruentus,* it was observed after 8–10 h. Until the seed maturation phase, the soil moisture content of the control and experimental plants was maintained at 45% to 50% of the full water holding capacity of soil. Seeds obtained from control plants and plants after drought stress were investigated for the content of ash elements.

Leaf and seed material

Fresh leaf material, with an average mass of 10 g, was air-dried in a drying cupboard at 80 °C. Ripe seeds weighing 15 g (from a representative sample not less than 100 g) were taken for mineralization. The dried samples of leaves and seeds were mineralized in a muffle furnace (Naberterm, Germany) at T = 400 °C. The obtained ash was dispersed by ultrasound at an 18 kHz frequency for 15 min. An even layer of the dispersant was applied to the object table covered with carbonic scotch.

Energy dispersion spectrometry (EDS) of mineral elements

The minerals (ash) composition was determined by an energy dispersion spectrometry (EDS) analyzer combined with the scanning electron microscope JEOL JSM 6090 LA (Japan) by following the methodology of Motyleva (2018) and Motyleva *et al*. (2021). Spectra and element distribution data were obtained together with images on a raster electron microscope. The EDS method was used for the qualitative and quantitative analysis of the existing mineral elements in the X-ray spectra acquired through the electronic beam scanning of the observed image. The X-

Table 1. Mineral (ash) composition of the amaranth species *A. tricolor* L. cv. Valentina and *A. cruentus* L. cv. Krepysh leaves.

Macro- & micro-elements	*Amaranthus tricolor* L.				*Amaranthus cruentus* L.			
	Control		Drought		Control		Drought	
	$\bar{x}\pm S_x$	CV (%)	$\bar{x}\pm S_x$	CV (%)	$\bar{x}\pm S_x$	CV (%)	$\bar{x}\pm S_x$	CV (%)
Na	0.07±0.02	51.10	0.09±0.01	30.16	0.11±0.02	40.48	1.36±0.24*	31.66
Mg	2.81±0.31	24.83	2.60±0.30	25.72	3.36±0.40	26.84	3.48±0.42	23.94
Si	0.64±0.13	43.92	0.38±0.06	33.43	0.40±0.05	28.00	0.44±0.02	10.19
P	3.91±0.27	15.69	2.35±0.29*	27.46	3.72±0.45	26.99	1.89±0.05*	5.86
S	0.38±0.09	56.10	0.69±0.20	65.64	0.26±0.05	44.98	0.32±0.06	36.98
Cl	1.86±0.31	37.23	4.34±0.50*	25.93	2.29±0.45	44.26	2.25±0.46	41.00
K	15.33±0.76	11.12	16.65±1.12	17.09	11.23±1.12	22.40	6.15±0.23*	7.63
Ca	5.15±0.64	28.11	5.91±0.85	0.85	7.61±0.57	16.76	8.42±0.61	14.39
Fe	0.48±0.12	55.69	0.51±0.25	108.5	0.05±0.02	89.10	0.05±0.01	18.24
Cu	0.37±0.15	91.17	0.38±0.12	68.47	0.43±0.09	48.18	0.28±0.11	82.12
Mo	4.28±0.49	25.89	3.78±0.49	29.21	2.89±0.18	13.67	3.85±0.29*	15.03
∑	35.28		37.68		32.35		28.49	

* = Significant at $P < 0.05$

ray microanalysis data were obtained by standard protocols and included the microstructure image of the sample under study, and the table with data on weight (mass%) and atomic correlations, spectra, and histograms. Ten measurements were taken for each ash sample. The local analytical area was 3 mm, and the scanning area was at least 12 µm. The average quadratic deviation did not exceed 1.2%–6.9%. All the analytical observations were performed in triplicate.

Statistical analyses

The recorded data for various parameters were presented as mean values (n = 10) in standard error (S_x), and coefficient of variation (CV). Statistical analyses and correlation analyses were carried out with Excel and Statistica (v. 7.0) packages. The treatments, which showed significant differences for drought-stress-dependent characteristics, were further determined by *t*-test.

RESULTS

Drought effects on amaranth leaf composition

Analysis of amaranth leaf ash composition revealed the presence of 11 macro- and micro-elements, i.e., sodium-Na, silicon-Si, Mg, P, S, Cl, K, Ca, Fe, Cu, and Mo. The potassium and calcium accounted for the largest proportion of the total composition, with a mass% of 15.33 and 5.15, and 11.23 and 7.61 for the amaranth species *A. tricolor* and *A. cruentus*, respectively. However, in both amaranth species, the sequence, and accumulation of macro- and micro-elements in leaf ash were as follows in the descending order:

A. tricolor =
K>Ca>Mo>P>Mg>Cl>Si>Fe>S>Cu>Na

A. cruentus =
K>Ca>P>Mg>Mo>Cl>Cu>Si>S>Na>Fe

In response to the drought, the leaves of amaranth species *A. tricolor* (cv. Valentina) showed a total increase in elements from 35.28 to 37.68 mass%, whereas, in the leaves of the other species *A. cruentus* (cv. Krepysh), the stress conditions contributed a decrease from 32.35 to 28.49 mass% (Table 1). Considering each element individually, differences in the content of the various macro- and micro-elements were also authenticated.

The K and Ca, being the major elements in amaranth leaves, showed an ambiguous response to stress conditions. The *A. tricolor* leaves showed a slight increase in elements from 15.33 and 5.15 to 16.65 and 5.91 mass%, respectively. On the contrary, the *A. cruentus* significantly decreased its K content twice (from 11.23 to 6.15 mass%), whereas Ca increased from 7.61 to 8.42 mass%. In both amaranth species, the P content has significantly decreased from 3.91 to 2.35 (*A. tricolor*) and from 3.72 to 1.89 (*A. cruentus*), mass%, respectively. In response to drought, chlorine has significantly increased twice its mass% from 1.86% to 4.34% in the leaf ash of *A. tricolor*. In the leaves of *A. cruentus*, however, the Cl has slightly decreased from 2.29 to 2.25 mass%.

In the leaves of *A. tricolor* plants, a relative decrease was recorded for the Mo and Mg content. Under irrigated conditions, the content of these elements was at 4.28 and 2.81 mass%; however, after exposure to drought stress, it showed a decrease to 3.78 and 2.60 mass%, respectively. In the experiments, the Mo content in *A. cruentus* increased from 2.89 to 3.85 mass% and the Mg content was observed from 3.36 to 3.48 mass%. In *A. tricolor*, the Si content in drought stress environment decreased almost twice to 0.38 mass%, whereas, in the leaf ash of *A. cruentus,* the mentioned element was insignificantly increased.

For elements, such as, Cu, Fe, S, and Na in leaves of *A. tricolor* there was an increase, depending on the element, where the increase ranged from 2.70 to 81.57 mass%. However, at the same time, the response of *A. cruentus* species to stress conditions was different, where a significant increase in Na by 12 times in comparison with the control (1.36 mass%) was observed, and the S content was least significant from 0.26 to 0.32 mass%.

The coefficient of variation (CV) values were also calculated between the mineral elements. The elements, i.e., Cu, Fe, Cl, S, Si, and Na had high values of the coefficient of variation, more than 30% versus the control for both amaranth species. However, the elements, Mo, Ca, K, P, and Mg, were characterized by medium variability. Stress conditions contributed to changes in the limits of variation in the leaf ash of *A. tricolor*. For elements, such as, Cu, Ca, K, Cl, Si, and Na, a decrease of 1-2 times was observed. For other elements, there was an increase in the coefficient of variation. In *A. cruentus* species, the most abundant elements were K and P, in which the CV decreased by more than 3-5 times, to the level of 5.89 to 7.63 mass%. The least decrease in the CV was also observed for the other elements. Similarly, only Cu and Mo were characterized by an increase in this index by 1-2 times.

Correlation analysis allows for determining the relationship between the mineral elements found in the leaf ash of the amaranth species, as well as, assessing the impact of stress conditions on the degree of their conjugation. In *A. tricolor* with control, there was a strong correlation between the following elements, i.e., S-Cu (*r* = 0.99), Mg-Si (*r* = 0.97), Na-Cu (*r* = 0.96), Na-S (*r* = 0.96), Na-Ca (*r* = 0.93), Na-Si (*r* = 0.92), and Si-S (*r* = 0.91) (Table 2). Under stress conditions, the identified correlation decreased and weakened, however, between Mg-Ca, Na-Fe, and P-Ca at *r* = 0.95, *r* = 0.94, and *r* = 0.94, respectively. The strong correlation between the elements Si-S is almost unchanged (*r* = 0.92) (Table 3).

Under optimal irrigation conditions, the strongest relationships in *A. cruentus* were noted between Mg-S (*r* = 1.00), Mg-Mo (*r* = 1.00), S-Mo (*r* = 1.00), Mg-Cl (*r* = 0.99), S-Cl (*r* = 0.99), Cl-Mo (*r* = 0.99), Cl-P (*r* = 0.94), P-S (*r* = 0.93), Si-Cl (*r* = 0.93), Ca-Mo (*r* = 0.93), S-Ca (*r* = 0.93), Mg-Ca (*r* = 0.93), Mg-P (*r* = 0.92), P-Mo (*r* = 0.92), and Mg-Si (*r* = 0.90) (Table 4). Under the drought conditions, these correlations became weak, with the conjugations between the elements were, i.e., Cl-Na (*r* = -1.00), Mg-P (*r* = -0.97), S-Na (*r* = -0.95), P-Na (*r* = 0.94), Cu-Ca (*r* = 0.94), Cl-P (*r* = -0.93), and Cl-Mg (*r* = 0.90) (Table 5).

Table 2. Correlation matrix of mineral (ash) composition of *A. tricolor* L. cv. Valentina control leaves.

Macro- & micro-elements	Na	Mg	Si	P	S	Cl	K	Ca	Fe	Cu
Mg	0.82*									
Si	0.92*	0.97*								
P	0.87*	0.72*	0.83*							
S	0.96*	0.85*	0.91*	0.80*						
Cl	0.06	0.01	-0.04	-0.40	0.22					
K	0.14	0.56	0.40	0.19	0.37	0.10				
Ca	0.93*	0.68*	0.80*	0.68*	0.83*	0.21	-0.14			
Fe	-0.31	0.11	0.00	0.05	-0.35	-0.76*	0.33	-0.47		
Cu	0.96*	0.79*	0.87*	0.74*	0.99*	0.31	0.25	0.89*	-0.48	
Mo	0.43	0.71*	0.66*	0.30	0.32	-0.14	0.15	0.48	0.38	0.31

* = significant at *P*<0.05

Table 3. Correlation matrix of mineral (ash) composition of *A. tricolor* L. cv. Valentina leaves under drought stress.

Macro- & micro-elements	Na	Mg	Si	P	S	Cl	K	Ca	Fe	Cu
Mg	0.80*									
Si	0.24	0.74*								
P	0.65*	0.82*	0.80*							
S	0.41	0.78*	0.92*	0.83*						
Cl	0.69*	0.57	0.31	0.74*	0.25					
K	0.53	0.56	0.13	0.04	0.30	-0.18				
Ca	0.76*	0.95*	0.81	0.94*	0.89*	0.60*	0.37			
Fe	0.94*	0.79*	0.20	0.48	0.41	0.42	0.78*	0.69*		
Cu	-0.09	-0.02	0.38	0.53	0.43	0.27	-0.64*	0.28	-0.30	
Mo	0.03	-0.51	-0.79*	-0.35	-0.78*	0.29	-0.45	-0.51	-0.14	-0.04

*significant at $P<0.05$

Table 4. Correlation matrix of mineral (ash) composition of *A. cruentus* L. cv. Krepysh control leaves.

Macro- & micro-elements	Na	Mg	Si	P	S	Cl	K	Ca	Fe	Cu
Mg	0.78*									
Si	0.79*	0.90*								
P	0.59	0.92*	0.84*							
S	0.74*	1.00*	0.86*	0.93*						
Cl	0.75*	0.99*	0.93*	0.94*	0.99*					
K	-0.38	-0.67*	-0.86*	-0.74*	-0.64	-0.75*				
Ca	0.84*	0.93*	0.76*	0.73*	0.93*	0.89*	-0.41			
Fe	-0.26	-0.45	-0.32	-0.18	-0.46	-0.43	0.25	-0.60		
Cu	-0.62*	-0.58	-0.25	-0.43	-0.62*	-0.50	-0.21	-0.77*	0.27	
Mo	0.75*	1.00*	0.86*	0.92*	1.00*	0.99*	-0.62*	0.93*	-0.46	-0.63*

* = significant at $P<0.05$

Table 5. . Correlation matrix of mineral (ash) composition of *A. cruentus* L. cv. Krepysh leaves under drought stress.

Macro- & micro-elements	Na	Mg	Si	P	S	Cl	K	Ca	Fe	Cu
Mg	-0.90*									
Si	-0.34	0.24								
P	0.94*	-0.97*	-0.10							
S	-0.95*	0.74*	0.50	-0.80*						
Cl	-1.00*	0.90*	0.38	-0.93*	0.96*					
K	-0.05	0.22	-0.89*	-0.33	-0.19	0.01				
Ca	-0.63*	0.69*	-0.50	-0.81*	0.42	0.60	0.81*			
Fe	0.86*	-0.62	-0.67*	0.65*	-0.97*	-0.88*	0.41	-0.19		
Cu	-0.68*	0.84*	-0.33	-0.88*	0.43	0.65*	0.71*	0.94*	-0.21	
Mo	0.50	-0.11	-0.01	0.32	-0.66*	-0.49	0.01	-0.32	0.63*	-0.07

* = significant at $P<0.05$

Drought effects on amaranth seed composition

The content of 14 main elements that make up the mineral part of amaranth seeds was studied (Figure 2). The features of the mineral elements accumulation in the seeds of two species of amaranth were noted. The main proportion of the seed ash elements in the species *A. tricolor* was K, P, and Mg, and in the seed ash of *A. cruentus,* the mainly observed elements were K, P, and Cl. The seed ash composition varies significantly, and the descending series of the accumulation of different elements were as follows:

A. tricolor =
K>P>Mg>Ca>Cl>Mo>Cu>Mn>Si>Ni>S>Fe>Zn>Co

A. cruentus =
K>P>Cl>Mg>Ca>Mo>Cu>Mn>Ni>S>Fe>Zn>Si>Co

Under drought stress conditions, there was a tendency to increase the accumulation of mineral elements, i.e., Mg, P, K, and Mo, in the seeds of both amaranth species (Figure 2). In the seeds of *A. tricolor,* an increase of these elements and Cl was observed 1-2 times. Under drought conditions, the other macro- and micronutrients remained at the level of the control values or even decreased by almost two times. The fractions of Mg, Si, P, and Mo in *A. cruentus* were significantly increased from 3.78, 0.09, 7.02, 2.86 to 5.03, 0.14, 10.08, and 3.77 mass%, respectively. A two times significant decrease was noted in S and Zn in *A. tricolor.*

Considering the relationships between the elements in amaranth seeds, a wide range of trait variability was found. The highest value of the CV in *A. tricolor* with control was characterized by cobalt-Co (117.94%) and Zn (78.79%), while *A. cruentus* was distinguished by Ni (74.29%) and Co (68.06%). Drought stress contributed to a decline in the variability of these elements, Co (82.39%) and Zn (61.29%). in *A. tricolor*. Similarly, the Cu increased (78.62%), whereas, for the other elements, there was a decrease in the CV. Drought stress conditions significantly increased the variation in *A. cruentus*— Co (96.02%), Fe (from 41.51% to 84.18%), and S (from 36.89% to 69.46%). For other elements, the increase was insignificant. The minimum variability of 11.82% was observed for Mg, but in the control the CV was 35.49%.

In *A. tricolor* species under normal irrigation regime, the strong correlation was recorded between Ca-Cu (*r* = 0.91), Mg-Cl (*r* = 0.90), Si-Mn (*r* = 0.87), Ca-Mo (*r* = 0.85), and Cl-Mn (*r* = 0.84). The effects of the drought stress conditions destroyed these bonds, and the formation of new interactions between the elements was observed, i.e., Mg-Si (*r* = 0.97) and Cu-Zn (*r* = 0.84). The relationship between Ca-Mo decreased to 0.81, whereas, in Cl-Mn the experimental variant increased to 0.92. In the control for *A. cruentus* species, the relationship of the elements was strong between Ni-Cu (*r* = 0.95), Mg-P (*r* = 0.92), Si-P (*r* = 0.86), and Si-Cl (*r* = 0.82). Under drought stress conditions, the relationship of Mg, Si, P, Ca, Cu, and Mn with other elements increased insignificantly. However, the strongest correlation was noted between S-Fe (*r* = 0.91) and Si-Ca (*r* = 0.84).

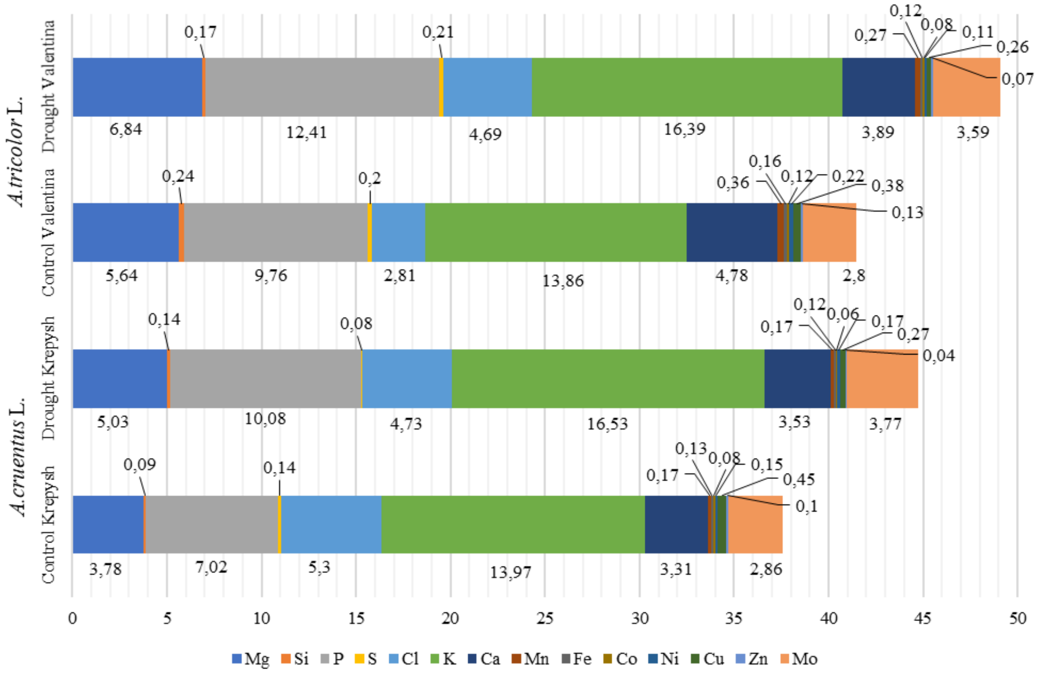

Figure 2. Effect of drought stress on the accumulation of mineral elements in *A. tricolor* L. cv. Valentina and *A. cruentus* L. cv. Krepysh seeds.

DISCUSSION

In amaranth seeds, the minerals play an important role in meeting human dietary needs and have a significant contribution to the recommended diets (Park *et al*., 2020). In both amaranth species, the mineral elements, K and Ca in the leaves, and K and P in the seeds were more pronounced. Potassium is the main ash element in the leaves (15.33 mass%) and seeds (9.76 mass%) of *A. tricolor*. The proportion of K in leaves and seeds of *A. cruentus* was less and its accumulation was 11.23 and 7.02 mass%, respectively. The macro element, K is responsible for regulating the majority of metabolic reactions occurring in living organisms. Potassium can also be used by the plants in photosynthesis, maintaining cell turgor, activating enzymes, and regulating excessive Na and Fe uptake. Potassium is also necessary for photosynthetic carbon dioxide fixation (Wang *et al*., 2013), and it also controls osmotic pressure, transmembrane potential, charge equilibrium, cathode-anion balance, pH—everything that makes up the homeostasis of cells and tissues (Meathnis *et al*., 1997). Drought stress conditions trigger a loss of potassium in chloroplasts, which suppresses photosynthesis in the leaves (Waraich *et al*., 2011).

Calcium is also an important substance in plant nutrition, and its deficiency can lead to stunted growth, and poor plant development (Ahanger *et al*., 2016). In *A. cruentus* leaves, the proportion of Ca was 1.5, which was higher than in the leaves of *A. tricolor*. On the contrary, in the seeds of *A. tricolor*, the Ca content was 1.4 times higher than that of *A. cruentus*. Calcium is a part of coenzymes and cell nuclei, and is also involved in the most important processes in humans, such as, metabolism, immunity, and regeneration (Gusev, 1998; Gins and Gins, 2011). A study related to the identification of calcium stress-induced genes in amaranth leaves revealed the Ca involvement in response to stress, which shows its important role in signal transduction pathways in response to biotic and abiotic stress conditions (Aguilar-Hernandez *et al*., 2011).

The P content in leaves and seeds of *A. tricolor* were 3.91 and 9.76 mass%, which was 1 to 1.4 times higher than the same in the *A. cruentus*. Phosphorus is important in amaranth plants vegetation, and for high productivity (Ojo *et al*., 2011). In the human body, P is a part of DNA and RNA, phospholipids, phosphate esters, and nucleoside phosphates - ATP, ADP, and NATP, where it performs structural and metabolic functions (Childers *et al*., 2011). Under drought stress conditions, phosphorus can have a positive effect on plant growth by participating in various processes, including higher cell membrane stability and water relation, leaf area, and photosynthesis (Kang *et al*., 2014).

The chlorine content in the leaves and seeds was highest by 1.2 to 1.9 times in *A. cruentus.* Chlorine is necessary for the activation of various enzymes, maintenance of water and electrical balance, and participation in water photolysis by photosystem II (Pessarakli *et al*., 2015; Wage *et al*., 2017). It is also noted that Cl ions, together with K and Na, contribute to osmotic adjustment in plants to tolerate drought stress (Heckman, 2007).

Magnesium and molybdenum in the leaves and seeds of both amaranth species did not differ significantly. In *A. cruentus* leaves, the Mg was 2.81 and Mo was 4.28, whereas, in *A. tricolor* the content of Mg was higher (3.36) and Mo, on the contrary, was least (2.89), mass%, respectively. In seeds, no differences were observed for Mo content between both species, while Mg content was 1.5 times higher in *A. tricolor* (5.64 mass%). In plants, the Mg takes the main role in chlorophyll molecules, involved in energy conservation and conversion, and protein synthesis (Amtmann and Blatt, 2009). 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 CO_2 and nitrogen. In the cytosol, Mg balances organic compounds (groups of sugars, nucleotides, organic, and amino acids). Magnesium is necessary to maintain cathodic-anionic balance and regulate pH (Sharifnabia *et al*., 2014). Molybdenum is necessary for plant nutrition in small quantities and is also a part of the enzymes that participate in essential redox reactions in the global C, N, and S cycle, and its deficiency can induce N deficiency (Silva, 2010). Molybdenum is an important element in the diet, catalyzes the reactions of oxygen transfer from or to substrates, using water as a donor or acceptor of oxygen, and is a part of enzymes (Schwarz and Belaidi, 2013).

The content of trace elements, i.e., S, Mn, Fe, and Zn in the seeds of both amaranth species cultivars differs slightly. Sulfur 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, and hydrogen and mercaptide bonds; and provides the transfer of genetic information. Leaves and seeds of *A. tricolor* were characterized by a higher S content. Sulfur is the component of chlorophyll, vitamins, and some amino acids, which are essential for protein synthesis (Heckman, 2007). Past studies also showed an important role of sulfur in alleviating the effects of drought stress (Chan *et al*., 2013). Drought limits the availability of sulfate to shoot, thereby causing the downregulation of the sulfur assimilating pathway in the leaves (Ahanger *et al*., 2016).

Manganese was identified only in the amaranth seeds, and the highest content was observed in *A. tricolor* (0.36 mass%) under both optimal and stress conditions. Iron content in leaves and seeds, as well as, Mn content, was higher in *A. tricolor*. Manganese is a cofactor and activator of many enzymes (pyruvate kinase, decarboxylase, and superoxide dismutase), which participates in the synthesis of glycoproteins and proteoglycans, and has an antioxidant activity. In the active centers (hemoproteins and iron-sulfur proteins) Fe determines the structure and activity of space and participates in redox reactions. Organic Fe is an essential compound for humans. Iron, as a part of active centers—hemoproteins and iron-sulfur proteins—determines the space structure and activity and takes part in oxidation-reduction reactions. The alternative form of Fe is the molecule of protein ferritin that may accumulate up to 4500 atoms of Fe in soluble nontoxic form. In the amaranth seeds, the mineral elements, Fe and Cu, were localized in the corcule (Shmalko and Roslyakov, 2011).

The observed Zn content revealed its insignificant excess in the seeds of *A. cruentus*, while under stress conditions, its accumulation was decreased by 1.9 to 2.5 times in both amaranth species. Zinc stabilizes the structure of molecules and plays an important role in the metabolism of DNA and RNA, protein synthesis and cell division, and in the processes of signaling within the cell (Pedersen *et al*., 1987; Avtsyn *et al*., 1991; Nechaev *et al*., 2007). Zinc is also needed in carbohydrate metabolism, protein synthesis, and the metabolism of auxins (Reddy, 2006).

The concentration of Si in *A. tricolor* leaves was 1.6 times more than in the leaves of *A. cruentus,* and 4.7 times more in the seeds. Silicon is not only the basis of the framework element of tissues, but also controls several biological and chemical processes in a living organism, increases the resistance of a living organism to the effects of biogenic and abiogenic stressors, and is also a necessary trace element of the active centers in the form of selenocysteine animoacystide (Vikhreva *et al*., 2001). Under drought stress conditions, Si is involved in the plant water retention and stomata function, enhances photosynthesis, and maintains the integrity of chloroplasts and membranes (Waraich *et al*., 2011).

The content of Cu and Na was higher by more than 1.2 to 1.6 times in the leaves of *A. cruentus* compared with *A. tricolor*. In the seeds, the Cu content was higher in *A. cruentus* species, while Na was not detected. Copper is an important micronutrient, as a part of low molecular weight substances and proteins, and enzymes (Rehman *et al*., 2019). The presence of Cu in cells helps to mitigate the effects of drought, yellowing of leaves, and stunted growth, and improves CHO and nitrogen metabolism (Waraich *et al*., 2011; Kabata-Pendias and Szteke, 2015). Sodium can be involved in Na-K synergism for the cytokinin-dependent betacyanin synthesis (Elliot, 1979). Sarker *et al*. (2022) also reported that under drought stress conditions, the amount of nutrients in amaranth leaves increases, which is consistent with the recent findings.

CONCLUSIONS

In this study, the C4 (amaranth) species, *A. tricolor* L. cv. Valentina and *A. cruentus* L. cv. Krepysh, were observed for responses to drought stress conditions through minerals metabolism. The results confirmed that the accumulation of 11 mineral elements in the leaves under drought stress conditions and 14 elements in the seeds of two amaranth species varied depending upon the species. Coefficients of variation and correlation between various mineral elements were formulated. A strong correlation was observed between S-Cu, Mg-Si, Na-Cu, Na-S, Na-Ca, Na-Si, and Si-S in the leaves and Ca-Cu, Mg-Cl, Si-Mn, Ca-Mo, and Cl-Mn in the seeds of *A. tricolor*. In *A. cruentus,* a strong correlation was observed between the mineral elements, Mg-S, Mg-Mo, S-Mo, Mg-Cl, S-Cl, Cl-Mo, Cl-P, P-S, Si-Cl, Ca-Mo, S-Ca, Mg-Ca, Mg-P, P-Mo, and Mg-Si in the leaves, while Ni-Cu, Mg-P, Si-P, and Si-Cl in the seeds. However, the drought stress conditions weakened these ties. The role of ash elements in the adaptation processes occurring in *A. tricolor* L. cv. Valentina and *A. cruentus* L. cv. Krepysh plants under drought stress has been established. The greatest adaptive potential to drought stress was demonstrated by amaranth *A. tricolor* L. cv. Valentina. Therefore, this cultivar can be used as a base material in future breeding for development of drought tolerant genotypes in amaranth.

ACKNOWLEDGMENTS

The reported study was funded by RFBR and BRFBR - project number 20-516-00012\21, and by BRFFR - project number B20R-298.

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