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DROUGHT EFFECTS ON THE PHYSIOLOGICAL AND BIOCHEMICAL PARAMETERS OF AMARANTH (C-3) AND ACTINIDIA (C-4) PLANTS

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SUMMARY

Drought is one of the limiting factors affecting the environment worldwide. It adversely affects crop plant growth and development. The stressful effects of arid conditions cause changes in the morphological traits and physiological processes of plants. This study, photosynthetic pigment contents, photosynthetic system 2 (PS2) activity, antioxidant activity, and total phenolic compound (TPC) amounts were determined in the leaf extracts of two species of amaranth (with C4-type photosynthesis), namely, *Amaranthus tricolor* L. cultivar 'Valentina' and *Amaranthus cruentus* L. cultivar 'Krepysh'. The same parameters were determined in two species of actinidia (with C3-type photosynthesis), specifically, *Actinidia arguta* cultivar 'Taezhny Dar' and *Actinidia kolomikta* cultivar 'Narodnaya'. All parameters were measured before and after drought stress. The seedlings were initially grown in the laboratory, then planted in separate pots and kept in the greenhouse under a canopy to protect them from the rain. Chlorophyll (Chl) a and b, carotenoids, antioxidant activity, and phenolic compound contents were determined spectrophotometrically. PS2 activity was determined by using pulse amplitude modulation fluorometry. The functional state of PS2 was more resistant to water deficiency and drought in amaranth than in actinidia species. Certain differences were established in the resistance of PS2 to dehydration between the two species of amaranth under drought conditions, and the highest Chl fluorescence indexes were characterized in the tricolor amaranth cultivar 'Valentina'. Car content was 6.5 times higher in the leaves of *A. arguta* and 2.5 times higher in the leaves of *A. kolomikta* than in the leaves of the control plants. The highest correlation was found between Chl a and Car (r = 0.985) and Chl b and Car (r = 0.977) in the leaves of both species of amaranth. Under moisture deficit conditions, the antioxidant activity of water and alcohol extracts in the leaves of both species of amaranth increased from 1.5 time to 2.5 times. A high correlation (r = 0.77, r = 0.91) was found between the antioxidant activity of the water and alcoholic extracts and the TPC in the leaves of both amaranth species, respectively.

Keywords: Drought, leaves, photosynthesis, antioxidant activity, phenolic compounds, *Amaranthus tricolor* L., *Amaranthus cruentus* L., *Actinidia. arguta* L., *Actinidia kolomikta* L.

Key findings: As a manifestation of the reaction of photosynthetic apparatus, the total antioxidant capacity and phenolic compounds of amaranth and actinidia species varied under drought conditions depending on the species and their cultivars. The structural and functional state of PS2 were more resistant to water deficit conditions in both species of amaranth than in actinidia.

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INTRODUCTION

Under the current climatic change, drought is one of the environmental limiting factors that greatly influence the growth and development of crop plants. In agricultural plants the stressful influence of drought conditions causes changes in morphological, physiological, and metabolic processes that eventually decrease productivity (Hasanuzzaman *et al.*, 2013). The molecular indicators of water stress involve the accelerated accumulation of active oxygen forms that lead to the development of oxidative stress, change chlorophyll (Chl) structure, decrease the amount of photosynthetic pigments and metabolites, and damage plant cells (Reddy *et al.*, 2004; Munne-Bosch *et al.*, 2016: Getko *et al.*, 2019). Antioxidant systems act as protective mechanisms and provide protection to cell membranes and organelles under stressful conditions (Jaleel *et al.*, 2009).

Photosynthesis is the most fundamental and complex physiological process in green plants (Ashraf and Harris, 2013; Fang and Xiong, 2015), which are greatly stressed by different factors, including drought conditions, as a result of changes in the ultrastructure of Chl bodies and the content of photosynthetic pigments, metabolites, and enzymes (Hernandez *et al.*, 2001).

Although the general adverse effects of drought on plant growth are well known (Yordanov *et al.*, 2000; Zhu 2002; Chaitanya *et al.*, 2003; Chaves *et al.*, 2003), the main effects of water scarcity on physiological processes in actinidia species are not fully understood. Actinidia plants have a narrow range of soil water absorption and require very frequent watering to maintain adequate plant water status due to their poor stomatal regulation. The water requirement of actinidia species depends on climate and soil characteristics, as well as on plant morphology and physiology (Kulczewski, 1988). Amaranth is known as a drought-tolerant plant, and several previous studies have compared the responses of different types of amaranth to drought stresses.

The major part (>90%) of the Chl fluorescence in the leaves emanates from the Chl of photosystem 2 (PS2). Various Chl fluorescence parameters (Fv, Fm, Fo, Fm', Fv', NF, and ΔF), their ratios (Fv/Fm, Fv/Fo, and ΔF/Fm'), and photochemical quenching coefficients (qP), as well as nonphotochemical quenching coefficients (qN, qCN, and NPQ) measured via pulse amplitude modulation fluorometry (PAM) provide information about the functionality of PS2 and the photosynthetic apparatus (Lichtenthaler *et al.*, 2005; Claire *et al.*, 2006).

Phenolic compounds and flavonoids are the most important and widespread secondary products in plants. These metabolites complement the enzymatic antioxidant system and have considerable potential to reduce and prevent cell damage (Agati and Tattini, 2010). Their biosynthesis and accumulation are usually induced in response to biotic and abiotic stimuli, such as drought stress, in plant

tissues (Naczk and Shahidi, 2004). Under drought conditions, flavonoids can maintain the integrity of the chloroplast membrane by lipid remodeling to prevent oxidative damage (Inoue and Kinoshita, 2017).

Amaranth and actinidia species were introduced and successfully grown in the central region of Russia. The leaves of amaranth plants are used as a raw material for the production of food-grade natural dye for coloring confectionery products and beverages and also can be used as part of salads, soups, and sauces, enriching them with biologically active substances (in particular, ascorbic acid and phenolic compounds), giving them an original taste and color. Pivovarov *et al.* (2019) also showed that growing three-colored amaranth is commercially and economically justifiable under open-air and protected conditions in the Moscow region of Russia. Many other countries also grow actinidia commercially (Williams *et al.*, 2003). Small actinidia farm plantations grow fruits for the domestic market, and the complex interaction of biologically active organic and inorganic components and phytochemical components, such as ascorbic acid, phenol compounds, amino acids, carbohydrates, fiber, and mineral substances, determines the high dietary value of the culture (Motyleva *et al.*, 2018). However, the dry period during the summer months negatively affects the performance of these species. Based on above discussion, the present research work was planned to evaluate the drought tolerance of the two species of amaranth and actinidia under abiotic stress caused through drought conditions.

MATERIALS AND METHODS

A vegetation experiment with amaranth and actinidia species was carried out during 2020 in a greenhouse under a canopy for protection from the rain at the Department of the Genofonde Pool and Bioresources of Plants of the Federal Scientific Center for Horticulture, Moscow. The plants of both species were placed in a greenhouse with artificial protection from rainfall. The study location has a temperate continental climate and was located at 168 m above sea level height and the 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. Biennial actinidia species (*Actinidia arguta* cultivar 'Taezhny Dar' and *Actinidia kolomikta* cultivar 'Narodnaya') were also individually planted in plastic pots (300 and 230 mm in diameter and height, respectively). A total of 20 pots of amaranth and actinidia plants were planted.

The pots were filled with a mixture of peat and sand (5:1) and had a drainage layer at the bottom. In the pots with the control samples, the substrate moisture content was maintained at 45%–50% for amaranth and 54%–60% for actinidia. Soil humidity was determined by using the soil moisture meter MC-7828 SOIL. All the plants were grown for 2 months under good watering conditions in natural light. The average day/night temperatures, relative humidity, and 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 drought stress was determined in accordance with soil moisture content. The watering of the experimental plants was stopped until signs of wilting appeared. The duration of the soil drought period was 5 days. The amaranth plants were studied when the soil moisture in the pots had decreased by 60%–70%, and actinidia plants were studied when soil moisture had decreased by 45%–50%. The plants involved in the experiments are shown in Figure 1 (amaranth) and Figure 2 (actinidia). The middle layer leaves were used for all analyses.

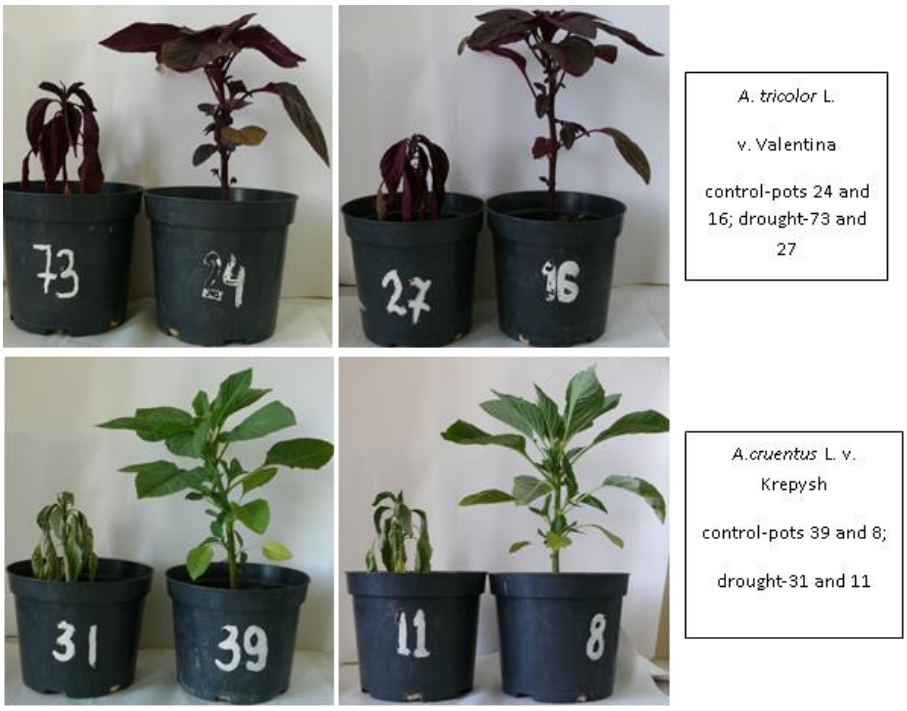

Figure 1. General view of the control plants *A. tricolor* L. cv. 'Valentina' and *A. cruentus* L. cv. 'Krepysh' and drought-prone plants.

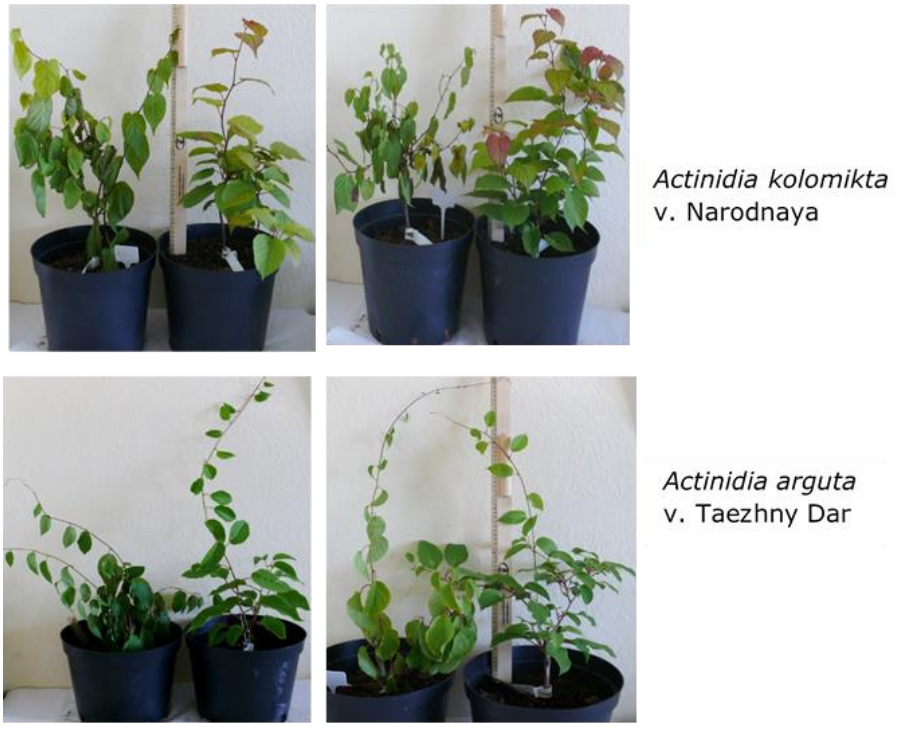

Figure 2. General view of the control plants of *A. arguta* cv. 'Taezhny Dar' and *A. kolomikta* cv. 'Narodnaya'. Each pair of pots represents drought (left side) and control (right side).

Data recorded

The parameters determined included relative water content (RWC), water deficit (WD), dry matter content (DMC), PS2 activity, photosynthetic pigment content, antioxidant activity, and the sum of phenolic compounds in the leaf extracts of actinidia (*A. arguta* and *A. kolomikta*) with C3-type photosynthesis and amaranth (*A. tricolor* and *A. cruentus* L.) with C4-type photosynthesis.

Chemicals

The chemical substances chosen for all analysis were of analytical grade and were bought from Sigma Aldrich, USA.

Leaf RWC

Plant water exchange was investigated by determining the DMC and RWC of the plants (Schonfeld *et al.*, 1988; Talbi *et al.*, 2015). Mean leaf samples in triplicate were weighed on HT 224RCE analytical weighing scales (Japan) then dried to the constant weight at 105 °C to determine DMC. DMC was determined by using the formula:

$$DMC = DW/ FW \times 100\%,$$

where, DMC – dry matter content,
DW – dry leaf biomass,
FW – crude leaf biomass.

RWC was determined by applying the formula:

$$RWC = (FW - DW) / (TW - DW) \times 100\%$$

where, RWC – relative water content,
TW – the biomass of a leaf when its cells reach the state of full turgor after 24 h of incubation in distilled water.

WD was determined with the formula:

$$WD = (TW - FW)/ TW \times 100\%$$

Determination of PS2 photochemical activity

The activity of PS2 was determined via PAM, which allows the real-time registration of the kinetic curve of the induction of Chl a fluorescence. The measurements were carried out on a "Dual-PAM-100" fluorimeter (Heinz Walz, Germany) by using the method described in a previous paper (Krause, 1991; Kramer, 2004). Actinidia and amaranth leaves were previously adapted to darkness for 20 min. Then, the background fluorescence was excited with a low-intensity measuring light (0.04 μquant m^{-2} s^{-1}, 460 nm) modulated at 20 Hz frequency. When the actinic light was switched on (125 micromol of m^{-2} s^{-1} quanta, 635 nm), the fluorescence intensity reached the maximum value of Fm then decreased due to decontamination via the photochemical and dissipation pathway. The use of a flash of saturating light (10 000 micromol of m^2 s^{-1}, 635 nm quanta) against the background of actinic light led to an increase in the fluorescence intensity from F_0 to Fm. After the flash of the saturating light, the actinic light was turned off, and the high-beam red light source, which excited only PS1, was turned on. In this case, the pool of electron carriers was rapidly and completely oxidized. The value of fluorescence reached the value of F_0. The obtained values of F_0, Fm, and F were used to calculate the maximum (potential) quantum yield of the photochemical reactions of PS2 – (Fv/Fm); the effective quantum yield of PS2 – Y(PS2); the quantum yields of unregulated – Y(NO) and regulated – Y(NPQ), the nonphotochemical quenching of Chl fluorescence; the coefficient of the nonphotochemical quenching of Chl fluorescence – qN and NPQ; and the coefficients of the photochemical quenching of Chl fluorescence – qP, an indicator of the proportion of open reaction centers – qL (Kramer, 2004; Makarenko, 2016):

$$Fv/Fm = (Fm - F_0)/Fm$$

$$Fv = Fm - F_0$$

$$qP = (Fm - F)/(Fm - F_0)$$

The coefficient of the nonphotochemical quenching of Chl a fluorescence (NPQ) was estimated by using the formula given by Bilger and Schreiber (1986).

$$NPQ = SV_N = F_m/F_{m'} - 1$$

The Chl fluorescence decrease ratio R_{Fd} measured at saturation irradiance in the red band near 690 nm is directly correlated with the net CO_2 assimilation rate (PN) of leaves (Lichtenthaler *et al.*, 2005) as follows:

$$R_{Fd} = F_d/F_s$$

where Fp, F_0, and Fs – maximum, initial, and steady Chl fluorescence,
F_d – Chl fluorescence,
Fp to Fs; Fp - maximum Chl fluorescence under nonsaturating light conditions.

Chl and carotenoids

Leaf pigment content was determined in accordance with the method described by Lichtenthaler and Bushmann (2001). A total of 0.5 g of leaves was extracted with 25 mL of methanol (pure solvent) by using a mortar. The pigment extracts were centrifuged at 2500 rpm for 9 min. The resulting extracts were assayed spectrophotometrically. The concentrations of Chl a and b and total carotenoids were determined by using the following equations and estimated in mg/g FW as follows:

$$Chl_a = 16.72\ A_{665.2} - 9.16\ A_{652.4}$$

$$Chl_b = 34.09\ A_{652.4} - 15.28\ A_{665.2}$$

$$Car = (1000\ A_{470} - 1.63\ Chl_a - 104.96\ Chl_b)/221$$

Analysis of antioxidant defense systems (nonenzymatic antioxidants)

Total phenolic compound analysis

The total phenolic amount was determined with Folin–Ciocalteu reagent in accordance with the method described by Velioglu *et al.* (1998). A total of 10 g of leaves was crushed on an IKA homogenizer (Germany). A total of 0.5 g of the crushed fraction was taken and extracted with 25 mL of deionized distilled water for 5 h. After centrifugation at 4000 × *g* on Rotofix 32 A (Hettich, Germany) for 20 min, the supernatant was used for analysis. A standard curve with gallic acid was used. Different concentrations of gallic acid were prepared in distilled water, and absorbance was recorded at 750 nm. A total of 100 µL of diluted sample (1:10) was dissolved in 500 µL of Folin–Ciocalteu reagent and 1000 µL of distilled water. The solutions were mixed and incubated at room temperature for 1 min. After 1 min, 1500 µL of 20% Na_2CO_3 solution was added. The final mixture was shaken and then incubated for 2 h in the dark at room temperature. The absorbance was measured at 750 nm by using a Helios Y UV–vis spectrophotometer. The results were expressed in mg of gallic acid (GEA) calculated on the basis of the fresh weight of plants.

2, 2-diphenyl-1-picrylhydrazyl radical scavenging activity

Total antioxidant capacity

The scavenging activity for the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was determined spectrophotometrically in accordance with the method described by Brand-Williams *et al.* (1995). The principle of this analysis is based on the color change from purple to yellow exhibited by the DPPH solution as the radical is quenched by antioxidants. Homogenized leaves were mixed with distilled water and pure methanol. The samples were placed

on the shaker Lab-PU-01 (Russia) for 6 h and then filtered. The antioxidant activity was measured in 10 min after interaction between the extract and the reagent. The absorbance was recorded at 517 nm to determine the concentration of the remaining DPPH. The radical scavenging activity was calculated as a percentage as follows:

$$\text{DPPH radical-scavenging (\%)} = [(AC - AAt) / AC] \times 100$$

where, AC – DPPH solution absorption;
AAt – absorption in the presence of the antioxidant.

The low absorbance of the reaction mixture indicates a high level of free radical scavenging activity.

Statistical analysis

All the analyses were performed in triplicate. The results were expressed as mean values (n = 3) in standard error (SE). Statistical analyses were performed with Excel package (Microsoft Excel, v. 2016).

RESULTS

The results revealed significant ($P \leq 0.051$) differences for all the drought-stress-dependent characteristics.

Water exchange

Under the influence of the osmotic factor, significant changes were observed in the leaves of amaranth cv. 'Valentina' for DMC (Figure 3). However, the leaves of both actinidia species revealed small alterations for DMC (Figure 4). The WD value increased in amaranth leaves but decreased in actinidia leaves. Reparation systems were likely activated in actinidia plants, allowing the maintenance of a high level of water content in the leaves.

Photochemical processes

The results for the analysis of the water deficit effects on the parameters of Chl fluorescence in the leaves of amaranth plants are presented in Table 1 and Figure 5. The photochemical quenching coefficient of PS2 (qP) was stable in the leaves of cv. 'Valentina' and decreased by 2.45 times under the action of dehydration in the leaves of cv. 'Krepysh' (Table 1). Water deficit had a great inhibitory effect on the proportion of the open PS2 in the leaves of cv. 'Krepysh'. NPQ decreased in the dehydrated leaves of the both species of amaranth (cultivars 'Valentina' and 'Krepysh') by 4.58 and 1.69 times compared with that in the control (Table 1). The RFd parameter in the dehydrated leaves of amaranth was lower than that in the control with 12.4% for cv. 'Valentina' and 2.39 times for cv. 'Krepysh'. The obtained data illustrated the inhibition of photosynthesis processes in amaranth species under water deficit conditions. However, the drought resistance of the photosynthetic apparatus was higher in cv. 'Valentina' than in cv. 'Krepysh'.

The effects of water deficit conditions on actinidia species are illustrated in Table 2 and Figure 5. An increase in the Chl fluorescence parameter F_0 was observed in both species of actinidia (*A. arguta* and *A. kolomikta*) under stress conditions. Therefore, the F_0 parameter showed an increase of 1.42 times in the leaves of *A. arguta* and 1.57 times in the leaves of *A. kolomikta*. By contrast, the parameter Fm decreased (17.7%) in the leaves of species *A. kolomikta* and remained unchanged for the species *A. arguta*. The quantum yield of PS2 (Fv/Fm) was lower in dehydrated leaves than in the control (1.20 and 1.37 times) for *A. arguta* and *A. kolomikta*. The coefficient of the photochemical quenching of Chl fluorescence (qP) also decreased by 1.41 and 2.23 times as a result of

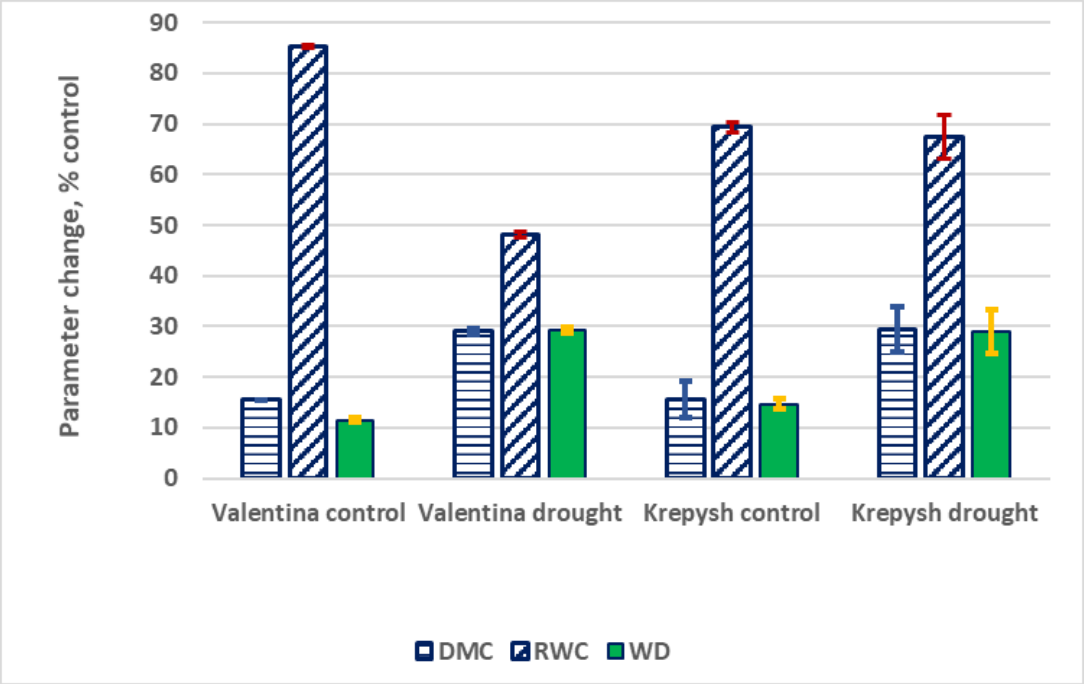

Figure 3. Change in dry matter content (DMC), relative water content (RWC), and water deficit (WD) in the leaves of *A. tricolor* L. cv. 'Valentina' and *A. cruentus* L. cv. 'Krepysh'.

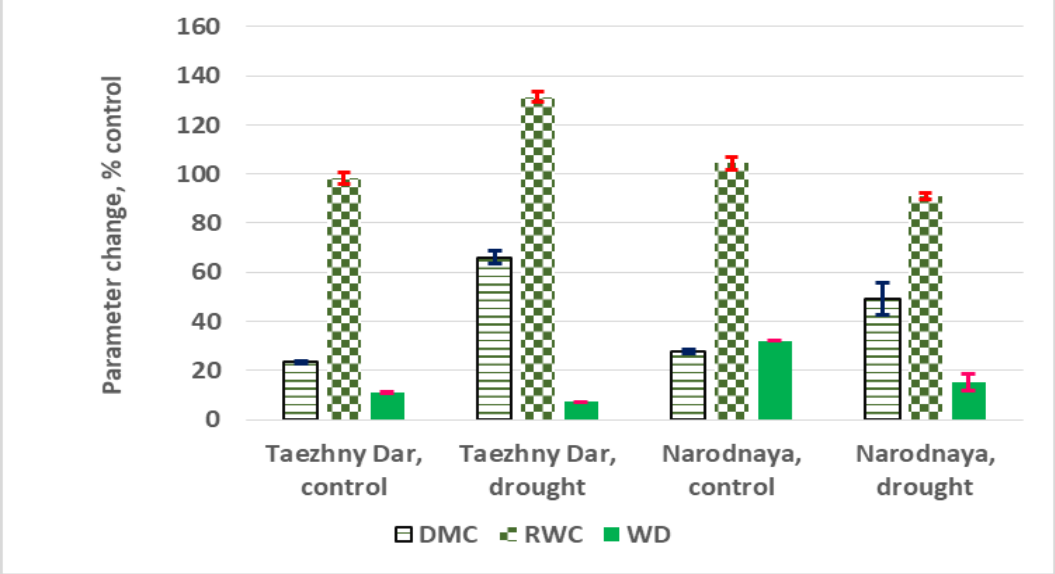

Figure 4. Change in dry matter content (DMC), relative water content (RWC), and water deficit (WD) in the leaves of *A. arguta* cv. 'Taezhny Dar' and *A. kolomikta* cv. 'Narodnaya'.

Table 1. Influence of water deficit conditions on the parameters of fluorescence in the leaves of amaranth species. Values represent the mean of three replicates ± SE.

Determinable indicators	*A. cruentus* cv. 'Krepysh': drought	*A. cruentus* cv. 'Krepysh': control	*A. tricolor* cv. 'Valentina': drought	*A. tricolor* cv. 'Valentina': control
Fo	0.207 ± 0.005	0.200 ± 0.010	0.271 ± 0.021	0.252 ± 0.019
Fm	1.033 ± 0.042	1.017 ± 0.068	1.148 ± 0.060	1.231 ± 0.083
Fp	0.985 ± 0.044	0.940 ± 0.066	1.121 ± 0.064	1.166 ± 0.092
Fv/Fm	0.799 ± 0.003	0.803 ± 0.006	0.765 ± 0.009	0.792 ± 0.006
Rfd	0.772 ± 0.061	1.258 ± 0.130	1.513 ± 0.047	1.702 ± 0.176
Qp Lss	0.334 ± 0.011	0.462 ± 0.021	0.227 ± 0.059	0.463 ± 0.016
NPQ Lss	0.600 ± 0.046	1.225 ± 0.135	1.338 ± 0.059	1.410 ± 0.180

Table 2. Influence of water deficit conditions on the parameters of fluorescence in the leaves of actinidia species. Values represent the mean of three replicate ± SE.

Determinable indicators	*A. arguta* cv. 'Taezhny Dar': Drought	*A. arguta* cv. 'Taezhny Dar': Control	*A. kolomikta* cv. 'Narodnaya': drought	*A. kolomikta* cv. 'Narodnaya': control
Fo	0.588 ± 0.076	0.414 ± 0.022	0.642 ± 0.005	0.408 ± 0.007
Fm	2.260 ± 0.210	2.208 ± 0.240	1.760 ± 0.188	2.072 ± 0.053
Fp	2.241 ± 0.192	2.084 ± 0.240	1.753 ± 0.189	2.012 ± 0.049
Fv/Fm	0.676 ± 0.055	0.810 ± 0.011	0.587 ± 0.072	0.802 ± 0.001
Rfd	0.706 ± 0.178	1.057 ± 0.069	0.502 ± 0.002	1.145 ± 0.1
Qp_Lss	0.728 ± 0.182	1.025 ± 0.083	0.518 ± 0.012	1.156 ± 0.1
NPQ_Lss	0.056 ± 0.054	0.163 ± 0.043	0	0.066 ± 0.002

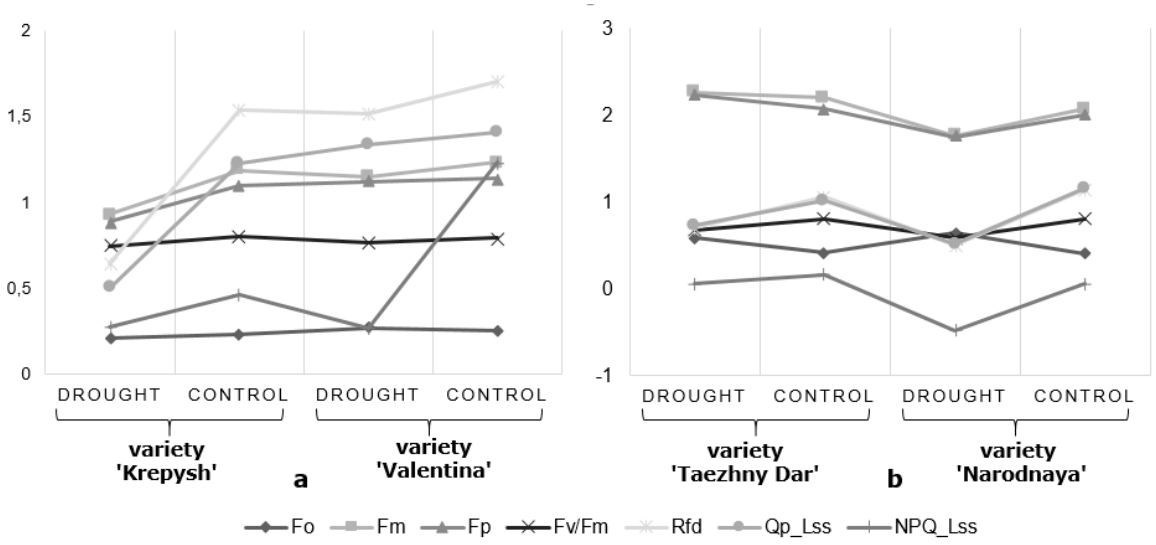

Figure 5. Influence of water deficit on the parameters of fluorescence in the leaves *A. tricolor* L. cv. 'Valentina' and *A. cruentus* L. cv. 'Krepysh' and in the leaves *A. arguta* cv. 'Taezhny Dar' and *A. kolomikta* cv. Nadeghda.

dehydration in the leaves of actinidia species *A. arguta* and *A. kolomikta*, respectively. The NPQ coefficient decreased in the dehydrated leaves of *A. arguta* (2.9 times) in comparison with that in the control and was not pronounced in the leaves of *A. kolomikta* under drought conditions.

Under water deficit conditions, the Chl fluorescence ratio (RFd) decreased in direct correlation with photosynthesis rate to 1.5 and 2.28 times in the leaves of actinidia species *A. arguta* and *A. kolomikta*, respectively. The effects on Chl fluorescence in the leaves of amaranth species under water deficit conditions are presented in Table 1 and Figure 5. No changes in the parameters of initial fluorescence (F_0) and maximal fluorescence (Fm) were observed under control and abiotic stress conditions in amaranth cv. 'Valentina' in contrast to cv. 'Krepysh', wherein the indicators decreased to 11.6 and 27.3% (Table 1) with dehydration. However, some differences were observed between the level of the maximum quantum yield of PS2 (Fv/Fm) in the leaves of amaranth species under control and stress conditions (Table 1). These data indicated the lack of damage to the photosynthetic membranes under water deficit in the leaves of both species of amaranth.

Chl and carotenoid contents

The analysis of the Chl and carotenoid contents in the leaves reveled that some changes were associated with drought (Figures 6 and 7). Chl a, b, and Car contents increased in the leaves of C3 and C4 species subjected to drought. The content of Chl in the leaves of *A. tricolor* cv. 'Valentina' and *Actinidia kolomikta* cv. 'Narodnaya' increased by two-fold. A slight decrease in Chl b was noted in the leaves cv. 'Valentina'. In the leaves of other species of amaranth and actinidia, the content of Chl b increased insignificantly in comparison with that of Chl a. In the leaves of C3 and C4 species, the content of carotenoids increased under moisture deficit conditions similar to the change observed in Chl b. Compared with that in the control, the content of Car increased by 6.5 times in the leaves of *Actinidia arguta* and by more than 2.5 times in the leaves of *Actinidia kolomikta*. In amaranth leaves, Car content increased by 1.5–2.0 times vs. control. 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* and *A. cruentus*, respectively.

Antioxidant activity and phenolic compounds

The capacity of amaranth and actinidia leaf extracts to scavenge DPPH+ free radicals, which has been used as a measure of total antioxidant capacity, and total phenolic content (TPC) are shown in Tables 3 and 4. Actinidia leaf extracts had greater antioxidant activity than amaranth leaf extracts. Interestingly, the antioxidant activity of the water and alcoholic extracts of actinidia leaves did not differ significantly. The effect of drought did not lead to a sharp increase in antioxidant substances in actinidia leaves. The antioxidant activity of water extracts of *A. tricolor* leaves was significantly higher than that in the leaves of *A. cruentus*.

The antioxidant activity of alcohol extracts differed slightly between amaranth species. Under moisture deficit conditions, the antioxidant activity of water and alcohol extracts increased from 1.5 time to 2.5 times in both species of amaranth. The total content of phenols was increased in the leaves of both species of amaranth and actinidia after subjecting the plants to drought. However, the greatest increase in TPS (3 times) was observed in the leaves of both species of amaranth. The low coefficient of variation of antioxidant activity and total phenolic compounds indicated the relative uniformity in the obtained data. A high correlation was established between the antioxidant activity of water and alcohol extracts and the TPC content in the leaves of both species of amaranth (r = 0.77, r = 0.91).

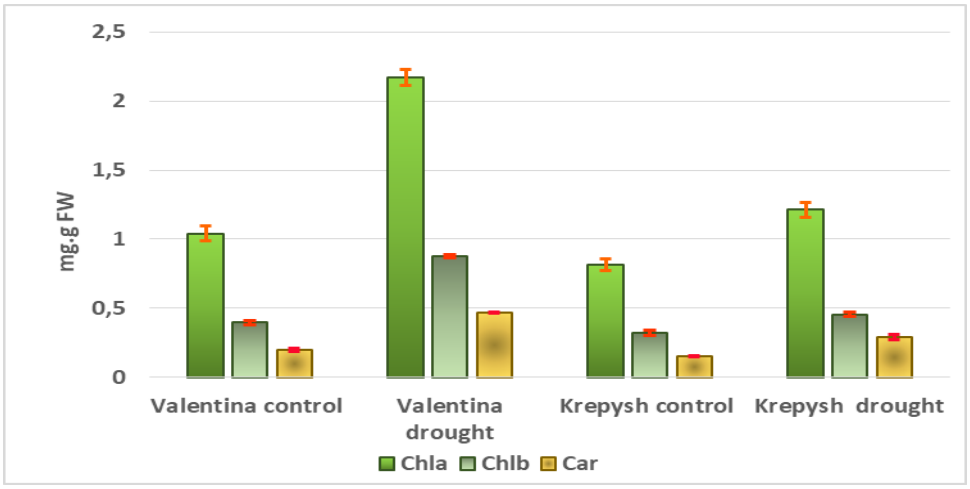

Figure 6. Chlorophyll (Chl) a, Chl b, and carotenoids (Car) in the leaves of *A. tricolor* L. cv. 'Valentina' and *A. cruentus* L. cv. 'Krepysh' under drought stress conditions.

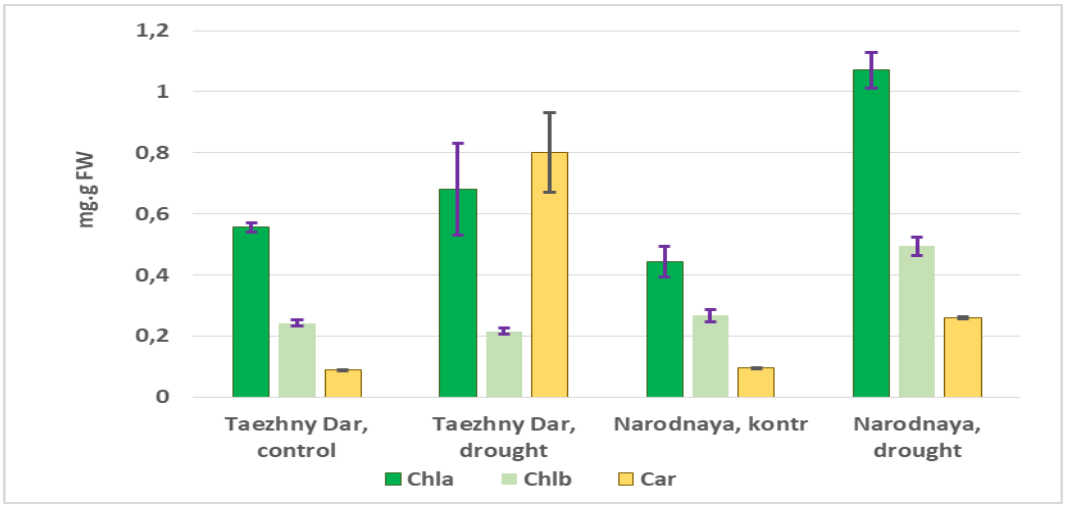

Figure 7. Chlorophyll (Chl) a, Chl b, and carotenoids (Car) in the leaves of *A. arguta* cv. 'Taezhny Dar' and *A. kolomikta* cv. 'Narodnaya' under drought stress conditions.

Table 3. Effects 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 amaranth species.

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

Table 4. Effects of drought stress on the antioxidant activity of aqueous (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 actinidia species.

Samples	Determined indicators		
	AAA	AAM	TPS
A. arguta, control V%	83.13 ± 1.93 2.33	88.71 ± 0.51 0.57	6.31 ± 0.28 4.51
A. arguta, drought V%	85.85 ± 3.19 3.73	87.81 ± 0.61 1.19	7.39 ± 0.08 1.09
A. kolomikta, control V%	69.84 ± 2.24 3.24	88.05 ± 1.34 1.87	6.38 ± 0.89 13.98
A. kolomikta, drought V%	78,43 ± 2,38 3.05	87.59 ± 0.33 0.38	9.83 ± 1.92 17.51

DISCUSSION

In this study, several adaptive reactions were observed in representatives of C4 (amaranth) and C3 (actinidia) species under the conditions of water scarcity. The tested species and their cultivars showed physiological and biochemical changes carotenoids; and phenolic compounds. Important findings for the photochemical process were found. The level of the photochemical quenching coefficient of PS2 (qP) provides an indication of the proportion of the reaction centers of PS2 that were open. Plants have evolved several photoprotective mechanisms to eliminate damage from excessive light (Cisse *et al.*, 2020).

The mechanism to dissipate excessive excitation energy as heat in the PS2 antenna complex is known as the nonphotochemical quenching (NPQ) of Chl. Although ubiquitous, the role of NPQ in plant productivity remains uncertain because it momentarily reduces the quantum efficiency of photosynthesis. The obtained data were in line with most past findings, indicating an increase in the NPQ parameter under stress conditions. According to Lichtenthaler *et al.* (2005), the RFd parameter, when measured under saturation irradiance, is directly correlated to the net CO_2 assimilation rate (PN) of the leaves. This correlation suggests that even with a limited soil moisture supply, tricolor amaranth cultivars can grow and maintain water balance. The present results were consistent with previous under the influence of abiotic stress due to drought. The evaluated species of amaranth and actinidia and their cultivars revealed greater variability for the studied parameters, i.e., the parameters of water metabolism; the activity of PS2; the substances of the nonenzymatic antioxidant system, such as total findings. Although the lack of water retards growth, maintaining plants with low water availability in the soil will allow them to continue to grow, albeit at a slower rate than under complete irrigation conditions (Chaves and Oliveira, 2004; Jatoi *et al.*, 2011; Sarker and Oba, 2018). The observed changes in the photosynthetic pigments of the leaves under drought stress were probably associated with the free radical-induced oxidation of the Chl pigment (Kato and Shimizu, 1985); the destruction of some chloroplasts; and the increased activity of the Chl-catabolizing enzyme chlorophyllase (Parida *et al.*, 2002). Lutts *et al.* (1996) showed that the concentration of Chl in stressed tissues can be considered as an indicator of the resistance of tissues to abiotic stress under drought conditions. The present results were also in complete agreement with the findings of Jain *et al.* (2015), who reported similar observations.

Carotenoids participate in resistance to drought stress through their capability to capture singlet oxygen (Reddy *et al*., 2004). They can also inhibit the peroxidation of lipids and the formation of superoxide under

dehydrating forces. The main protective role of carotenoids and beta-carotene in photosynthetic tissue may be the one that directly extinguishes triplet Chl and helps plants resist drought (Farooq *et al.*, 2009). Thus, the role of antioxidants and beta-carotene pigments in the regulation of photosynthetic electron transport is assumed to be crucial (Bartwal *et al.*, 2013; Ma *et al.*, 2013).

In this study, carotenoid content increased under drought stress. These results were in parallel with the past findings on Choy sum trials during the dry season by Hanson *et al.* (2011), who reported an increase in total carotenoids under drought stress conditions. Antioxidant activity plays a crucial role in maintaining the balance between free radical synthesis and capture (Lin *et al.*, 2006; Bettaieb *et al.*, 2011; Espinoza *et al.*, 2013). A positive correlation was observed between antioxidant activity and the total content of phenolic compounds. Perhaps these metabolites were organized accordingly in relation to each other. On the basis of the obtained data, a wide range of adaptive processes in PS2 structure was observed in the leaves of *A. tricolor* cv. 'Valentina' than *A. cruentus* cv. 'Krepysh'. However, a detailed study is required to further understand the whole mechanism of NPQ reduction in various crop plants.

CONCLUSIONS

Adaptive changes at the physiological and biochemical levels due to drought stress were observed in amaranth and actinidia species. On the physiological level, the changes in water status parameters and photosynthesis processes were marked in the plant leaves. The structural and functional condition of PS2 was more stable under water deficit conditions in amaranth (C4) than in actinidia (C3). Under drought conditions, the amaranth cv. 'Valentina' was characterized by high indexes of Chl fluorescence. The other mechanism of drought resistance was the change in secondary metabolite synthesis. The increase in the content of phenol compositions and total antioxidant activities allowed the plants to survive under unfavorable environmental conditions. Therefore, the amaranth cv. 'Valentina' showed the highest adaptive potential to drought stress considering the complex of physiological and biochemical parameters studied. The present observations could help biologists in their future research on the interrelation between secondary plants metabolites and abiotic stresses, such as drought. The *A. argute* genotype 'Taezhny Dar' and the *A. tricolor* cv. 'Valentina' used in this study can be further studied as promising material that can accelerate selection for drought resistance in amaranth and actinidia species. The results also provided some valuable pieces of evidence that can potentially help and guide in the selection of promising parental genotypes in future breeding programs.

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