Effect of drought stress on metabolite synthesis in Actinidia Arguta Leaves

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Abstract. In the context of global climatic changes, water stress, which causes drought, is one of the limiting factors affecting the environment and negatively affects the growth and development of cultivated plants. The stressful impact of dry conditions causes changes in the biochemical processes of plants. Herein, we studied the change in antioxidant activity, the amount of phenolic compounds and the peculiarities of the synthesis of some metabolites in Actinidia. arguta leaves, Taezhny Dar variety, under drought stress. All parameters were measured in leaves of control plants and after drought stress. Biennial plants were grown in separate pots and kept in an open area under a canopy to keep out the rain. Antioxidant activity and the amount of phenolic compounds were determined spectrophotometrically. Under conditions of moisture deficiency, the antioxidant activity and the amount of phenolic compounds in the leaves are higher than in the control. The composition of metabolites in the leaf extract was determined by gas chromatography-mass spectrometry. Under drought stress, changes in the synthesis of primary and secondary metabolites occur. In the leaves of control plants, 14 substances were identified, of which 6 are organic acids and 8 are carbohydrate substances. In the leaves of plants under drought stress, 37 compounds were recorded, that is, more than 2 times more than in the leaves of control plants, 23 substances of a carbohydrate nature were identified, including Myo-Inositol, which has antioxidant properties. The main carbohydrates in the leaves of the control plants of actinidia were turanose and mannobiose; under drought conditions, sucrose; its content increased 15 times in comparison with the control plants. The phenolic compounds Quininic acid and Caffeic acid are synthesized in the leaves of Actinidia arguta plants subjected to drought.

1 Introduction

In recent years, due to the problems associated with climate change, drought is one of the main abiotic stresses that affects the biochemical processes of plants [1]. Molecular indicators of water stress accelerate the accumulation of reactive oxygen species, which leads to the development of oxidative stress, changes in the structure of chlorophyll, a decrease in the content of photosynthetic pigments, metabolites, and damage to plant cells

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[2-4]. Antioxidant systems protect the cell membrane and organelles under stressful conditions [5. 6]. Although the general negative effect of drought on plant growth is well known, the main effects of water deficit on physiological processes in actinidia species are not fully understood [7–9]. The metabolomic approach makes it possible to identify substances involved in various biochemical processes. In response to abiotic stress, plants can regulate their metabolic pathways and synthesize a number of metabolites that can help them repair damage [10]. The actinidia plant has a small range of soil water uptake and requires very frequent watering to maintain adequate plant water status due to low stomatal regulation. The water requirement of actinidia species depends on the climate and soil characteristics, as well as on the morphology and physiology of plants [11]. Phenolic compounds and flavonoids are the most important and widespread by-products in plants. These metabolites complement the enzymatic antioxidant system and have significant potential to reduce and prevent cell damage [12]. Their biosynthesis and accumulation are usually induced in response to biotic and abiotic stimuli, such as drought stress in plant tissues [13]. Representatives of the genus Actinidia Lindl. are hygrophilous and belong to C-3 plants. The species Actinidia arguta is characterized by weakly expressed xeromorphic characters; these are plants with large, smooth leaves, demanding of water [14]. Actinidia has been introduced and successfully grown in the Central region of Russia. However, during the summer months, the dry period negatively affects the growth and fruiting of plants. The effect of drought on the biochemical parameters of leaves, such as antioxidant activity, the amount of phenolic compounds, and the composition of metabolites in Actinidia arguta leaf extracts have not been previously studied. It is very important for breeders to understand the molecular responses of actinidia to drought stress and to develop new approaches to increase the drought tolerance of this crop. Therefore, the purpose of this study was to study the effect of soil drought on the biochemical composition of leaves of Actinidia arguta plants (for example, the Taezhny Dar variety).

2 Methods

A vegetation experiment with actinidia plants was carried out in 2020 - 2021 on an experimental site under a canopy from the rain in the department of the gene pool and biological resources of plants of the Federal Scientific Center for Horticulture, Moscow. The climate of the study site is temperate continental, altitude — 168 m, coordinates — 55° 7′27″, north latitude, 37° 56′55″ east longitude. Biennial plants Actinidia arguta, Taezhniy Dar variety, were individually planted in plastic pots 300 and 230 mm in diameter and height, respectively. The soil in the pots was filled with a mixture of peat and sand (5:1), with a drainage layer at the bottom of the pot. In pots with control samples, the substrate moisture content was maintained at a level of 54-60 % throughout the experiment. Soil moisture (SH) was determined using an MC-7828 SOIL soil moisture meter. All plants were grown for two months under good watering conditions under natural light. Average day/night temperature, relative humidity and day length during the experimental period were 17.2/11.7 °C, 64 % and 17 hours, respectively. After two months of growth, the degree of drought stress was determined by the moisture content of the soil. Watering of experimental plants was stopped until signs of wilting appeared (Figure 1)

The duration of the soil drought period of the test plants was seven days. Plants were examined with a decrease in soil moisture content up to 28-30 %. For all analyzes, the leaves of the middle layer were used.

Fig. 1. Plants Actinidia arguta, Taezhny Dar variety. Control plant on the left, after a dry period on the right.

2.1 Analysis of antioxidant defense systems (Non-enzymatic Antioxidants).

2.1.1 Total phenolic compounds analysis

The total amount of phenolic compounds was determined using the Folin-Chocalteu reagent in accordance with the method described [15]. A gallic acid calibration curve was used. Various concentrations of gallic acid were prepared in distilled water and the absorbance was recorded at 750 nm. 100 µL of the diluted sample (1:10) was dissolved in 500 µL of the Folin - Chocalteu 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 % sodium carbonate solution (Na_2CO_3) was added. The prepared mixture was shaken and then incubated for 2 h in the dark at room temperature. The absorbance was measured at 750 nm using a Helios Y UV-vis spectrophotometer, and the results were expressed in mg of gallic acid calculated on the wet weight of the leaves.

2.1.2 Total antioxidant capacity 2, 2-diphenyl-1-picrylhydrazyl-radical-scavenging-activity

The absorption activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was determined spectrophotometrically as described in [16]. The principle of the analysis is based on the change in the color of the DPPH solution from purple to yellow as the radical is quenched by antioxidants. The homogenized leaves were mixed with distilled water and methanol. The samples were placed on a Lab-PU-01 shaker (Russia) for 6 hours, then filtered and the antioxidant activity was measured 10 minutes after the interaction of the extract with the reagent. The absorbance was recorded at 517 nm to determine the concentration of the remaining DPPH. All measurements were performed in triplicate. The radical absorption activity was calculated as a percentage as follows:

$$\text{DPPH radical (\%)} = [(AC - AAt) / AC] - 100 \quad (1)$$

AC – absorption by DPPH solution;

AAt – absorption in the presence of an antioxidant.

A lower absorption of the reaction mixture indicates a higher level of free radical scavenging activity.

2.1.3 Metabolic analysis by gas chromatography-mass spectrometry

Metabolites were analyzed on a JMS-Q1050GC chromatograph by gas chromatography-mass spectrometry (CMS). A capillary column DB-5HT (Agilent, USA) was used; length of 30 m, inner diameter of 0.25 mm, film thickness of 0.52 μm, carrier gas — helium). Identification of substances was carried out according to the values and mass spectra of the library of the National Institute of Standards and Technology NIST-5, USA. The scanning range was from 33 to 900 m/z. The probability of identifying identified substances ranged from 75 to 98 %. The temperature gradient during the analysis was from 40 to 280 °C; the furnace temperature varied from 40 to 130 °C at a rate of 1 °C min-1, from 130 to 200 °C at a rate of 2 °C min-1, from 200 to 280 °C at a rate of 4 °C min-1 and holding at 280 °C for 40 min; ion source temperature — 200 °C. The gas flow (helium) in the column was 2.0 ml/min, the split-flow injection mode was used, the sample was injected in a volume of 2 μL.

3 Results

The ability of actinidia leaf extracts to scavenge DPPH free radicals, which is used as a measure of total antioxidant activity, and total phenol content (TPC) are shown in Table 1. The antioxidant activity of aqueous and alcoholic extracts of actinidia leaves did not differ significantly. The influence of drought did not lead to a sharp increase in the content of antioxidant substances in the leaves of actinidia. The total content of phenols with a lack of moisture in the leaves of actinidia increased. The coefficient of variation of antioxidant activity and the total content of phenolic compounds was low, which indicates the relative homogeneity of the data obtained.

Table 1. The effect of drought on the antioxidant activity of aqueous (AAA) and pure methanol (AAM) extracts, expressed in %, and the total content of polyphenols (TPC), expressed in mg-equivalent of gallic acid (mg/g) in the leaves of Actinidia argute at Taezhny Dar.

Samples	Determined indicators		
	AAA	AAM	TPS
Actinidia arguta, control	83.13±1.93	88.71±0.51	6.31±0.28
V %	2.33	0.57	4.51
Actinidia arguta, drought	85.85±3.19	87.81±0.61	7.39±0.08
V %	3.73	1.19	1.09

Using the gas chromatography-mass spectrometry (GCMS), the component composition of the main substances contained in the extract with pure methanol prepared from the leaves of actinidia was determined. The largest number of peaks of substances was recorded from 15 to 40 minutes of analysis (Figure 1). The figures show that the release time of substances and their amount in the extracts of control plants and after drought stress differ significantly. A total of 40 compounds have been identified. In the leaves of control plants, 14 substances were identified, where 6 are organic acids and 8 are carbohydrate substances (Table 2). The main carbohydrates in the leaves of control actinidia plants are mannobiose, turanose, and sucrose. In the leaves of plants under stress conditions, 37 compounds were recorded, that is, more than 2 times more than in the leaves of control plants. Organic acids 11, in addition to those found in the leaves of control plants, Phytol and Stearic acid are synthesized. Among substances of carbohydrate nature 23 were identified, where 16 were not found in the leaves of control plants, including Myo-Inositol, which has antioxidant properties. Under drought conditions, phenolic compounds Quininic

acid and Caffeic acid are synthesized. The sucrose content increases 15 times, the synthesis of turanose decreases 128 times, and mannobiose is not found.

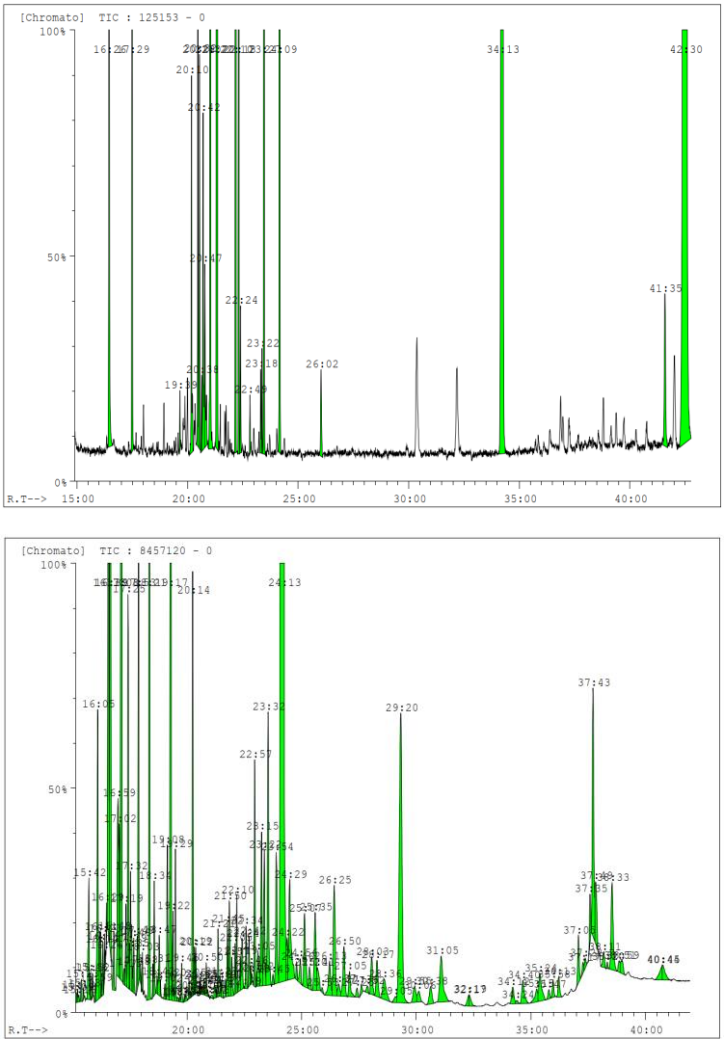

Fig. 1. Chromatographic profiles of Actinidia arguta leaf extracts at Taezhny Dar. Control (top) and drought conditions (bottom).

Table 2. Compounds found in the alcoholic extract (pure methanol) of actinidia leaves.

Ser. No.	Time	Name	Peak area (%)	
			control	drought
1	15.43	L-Arabitol	-	0.45
2	16.04	Levoglucosan	-	1.13
3	16.12	Azelaic acid	-	0.28
4	16.18	Ribonic acid	-	0.05
5	16.25	Protocatechoic acid	-	0.10
6	16.26	Malic acid	3.35	0.02
7	16.34	D-(-) - Fructofuranose	-	3.52

8	16.38	b-Arabinofuranose	-	5.30
9	16.39	D-Fructose	-	0.21
10	16.59	D-Allofuranose	-	0.88
11	17.08	Quininic acid	-	7.40
12	17.25	a-D - (+) Talopyranose	-	1.17
13	17.29	Glyceric acid	1.51	0.10
14	17.53	Etyl-a-D-glucopyranose	-	1.37
15	18.03	D-Xylopyranose	-	0.09
16	18.21	b-D- Glucopyranose	-	2.30
17	18.34	Ribonic acid	-	0.31
18	18.47	D-(+)-Galactouronic acid	-	0.24
19	19.17	Myo-Inositol	-	5.20
20	19.29	Caffeic acid	-	0.42
21	19.39	Ribitol	0.12	0.05
22	19.45	Phytol	-	0.12
23	20.14	D-(+)-Cellobiose	0.91	1.33
24	20.18	b-Hydroxypyruvic acid	-	0.15
25	20.19	Stearic acid	-	0.15
26	20.22	Lactose	-	0.16
27	20.28	Mannonic acid	1.03	-
28	20.32	Gluonic acid	0.89	0.03
29	20.38	Malonic acid	0.17	0.04
30	20.47	Erythro-Pentonic acid	0.61	-
31	21.20	h-Ramnose	-	0.19
32	21.21	Glyceryl-glycoside	6.22	0.19
33	21.27	Methyl galactoctoside	6.22	0.06
35	22.10	D-Xylopyranose	-	0.32
36	22.24	D-(+)- Cellobiose	0.33	0.23
37	24.12	Sucrose	2.78	42.13
38	29.20	Galactitol	-	3.06
39	34.13	D-(+) Turanose	19.20	0.15
40	42.30	Mannobiose	39.81	-

4 Discussion

Herein, under the conditions of a vegetation experiment, biochemical changes were observed in the leaves of Actinidia arguta at Taezhny Dar under the influence of abiotic stress caused by drought. Biotic and abiotic stresses negatively affect the growth and development of plants due to a deficiency of nutrients, cause hormonal imbalance, osmotic and oxidative disorders. The most efficient mechanism is the biosynthesis of primary and secondary metabolites at the cellular level, which include organic compounds that help plants cope with stressful conditions. We have found that under conditions of water deficiency, the metabolic balance in actinidia plants is rearranged. There is an increased synthesis of primary metabolites — soluble sugars, which is also noted in papers [17-20]. The results showed that there are 3.8 times more carbohydrate substances in the leaves of actinidia plants subjected to drought than in the leaves of control plants. Enhanced sucrose synthesis has also been found in the leaves of drought-affected plants. As carbohydrate conjugates, maltotriose with glucose, sucrose, galactose, fructose, and trehalose were involved in plant response to abiotic stress [21, 22]. Sugars are not only a source of energy, but also precursors of carbon, substrates for polymers, compounds for storage and transportation, and signaling molecules [23]. Antioxidant activity plays a decisive role in maintaining a balance between the synthesis of free radicals and their uptake [24, 25]. According to our data, a positive correlation was observed between antioxidant activity and

the total content of phenolic compounds. It is possible that the synthesis of these metabolites is interrelated. The synthesis of Quininic acid and Caffeic acid noted by us in the leaves of actinidia plants subjected to drought suggests that these substances are closely related to the response of plants to drought. This result is consistent with the data obtained in the study of peach plants [24]. Quininic acid is involved in the resistance of plant cells to oxidation [26]. Quinic and caffeic acids are actively involved in the mechanisms of stress resistance, and are primarily used by plants for the synthesis of lignin, which ultimately thickens the cell walls and the plant becomes more resistant [27]. In most studies, organic acids and intermediate products of the tricarboxylic acid cycle (TCA) increase in response to drought or temperature stress [28], which we have also shown. Therefore, our results are comparable with the available reference phenotypic changes that we observed in Actinidia arguta at Taezhny Dar - leaf wilting (Fig. 1), may be associated with significant differences in the biochemical composition of leaves, which is formed under the influence of drought stress.

5 Conclusion

We analyzed the general changes in the metabolic profiles of leaves of Actinidia arguta at Taezhny Dar in control plants and those subjected to drought stress. Adaptive changes in actinidia leaves as a result of drought stress have been observed at the biochemical level. The mechanism of drought resistance caused a change in the synthesis of metabolites the synthesis of carbohydrates and secondary metabolites; the content of phenolic compounds and the general antioxidant activity increased and allowed the plants to survive in unfavorable environmental conditions. Actinidia adapts to water deficiency by synthesizing high levels of some protective metabolites such as sucrose, Etyl-a-D-glucopyranose, D-Xylopyranose, bD- Glucopyranose, Ribonic acid, D - (+) - Galactouronic acid, Myo-Inositol and others carbohydrates, Quininic acid and Caffeic acid. The metabolic responses to drought stress identified in this study help to reveal the complexity of drought tolerance at the molecular level and will be useful for breeding drought tolerant varieties. The genotype Taezhny Dar, Actinidia arguta, can be used in the future as a promising material for breeding for drought resistance of actinidia. In addition, the results are fundamental for studying the adaptation of Actinidia arguta to drier environments in the context of climate change.

The reported study was funded by RFBR and BRFBR, project number 20-51-00001. The reported study was also funded by BRFFR - project number B20R-298.

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