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SALICYLIC ACID AS GROWTH REGULATOR FOR CADMIUM-STRESSED PLANTS

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Salicylic acid (SA) is known as a participant of stress reactions in plants. Effect of different SA treatments (pre-sowing soaking of grains and spraying 7-days-old seedlings with 100 and 500 μM SA) on Cd-stressed wheat and maize plants was investigated. We observed an adverse effect of Cd on leaf area, plant weight, biomass accumulation and pigment content. SA treatments alleviated the toxic effect of Cd on growth and photosynthetic pigments of investigated plants. Changes of protein level in plant tissues under stressful conditions and SA impact were observed. Pre-treatment with 500 μM SA manifested the most beneficial effect for both investigated plants.

Keywords: Triticum aestivum L., *Zea mays* L., salicylic acid, cadmium, growth.

Large areas in the world and particularly in Eastern Europe are polluted with heavy metals and radionuclides in natural and industrial regions. Heavy metal toxicity causes negative changes in growth and development of plants, animals and microorganisms. Also, heavy metals are seriously damaging human health, disrupting the normal function of central nervous system and internal organs. Some heavy metals were included into the list of the 20 most hazardous substances created by the Agency for Toxic Substances and Disease Registry (ATSDR) and Environmental Protection Agency (EPA, USA). The heavy elements such as arsenic, lead, mercury and cadmium, which pose serious ecological problems occupy the first, second, third and seventh positions respectively in a global toxicity ranking according to the ATSDR and the EPA in 2003 [22, 25].

Cadmium (Cd) is an extremely ecotoxic heavy metal that exhibits highly adverse effects on soil biological activity, plant metabolism and health of animals and humans. Metallic Cd and it compounds are frequently used in different industrial areas (production of accumulators, electric cables, motor radiators, fertilizers), as pigments in poligraphy, for coloring glass, enamels and ceramics. The most important anthropogenic source of Cd emission into the environment is steel production and burning of fuels [31]. Cd can be effectively absorbed by both the root and leaf systems and highly accumulated in plant organisms. When Cd is taken up in excess by plants, it induces various visible symptoms of phytotoxicity (e.g., leaf roll, chlorosis, growth reduction and, eventually, death). Physiological plant response to Cd toxicity are also alterations in various biochemical characteristics, such as activation of dark respiration and antioxidant defense system, disruptions in plant water relations, negative changes in the photosynthetic rate, which are associated with stomatal limitation, degradation of photosynthetic pigments, alterations in photochemical processes and biochemical reactions of Calvin's cycle [18, 37].

Salicylic acid (SA) is considered as an endogenous plant growth regulator which has been found to generate a wide range of physiological and metabolic responses in plants. SA is involved in eliciting specific responses to biotic and abiotic stresses as a potent signaling molecule [16, 27]. It has been shown that SA plays a key role in plant disease resistance [29, 33]. SA has been reported to provide protection against low-temperature stress [35], to induce thermotolerance [11, 38], and to modulate plant response to UV-radiation [24], salt [34] and osmotic [7] stresses, effect

of ozone [20], water deficit [14], impact of herbicides [3] and heavy metal stress [26].

Various investigations showed that SA might be able to protect photosynthesis against a toxic impact of heavy metals, such as copper [13], nickel [39], and cadmium [12, 21, 28]. It is shown that SA can produce stress solely under certain conditions [9].

In the present study, we made an attempt to explore whether exogenous application of SA could mitigate the adverse effect of Cd toxicity on maize and wheat plants.

Matherials and methods

Seeds of wheat (*Triticum aestivum* L. cv. Podolianka) and maize (*Zea mays* L. cv. Zakarpatska zhovta zubovydna) were sterilized and divided into three groups. Two groups of seeds were soaked in 100 and 500 μM SA solutions respectively for 5 h, another group was soaked in distilled water (control). Then both groups were allowed to germinate on moist filter paper in the dark. Two-days-old seedlings were transported in pots filled with 1.5 kg washed and inciderated sand, artificially contaminated with Cd as $CdCl_2 \cdot 2.5H_20$ at levels of 0 and 25 mg Cd kg^{-1} sand. Pot cultivation was carried out in greenhouse under controlled conditions. The pots were watered to 60% water holding capacity of the sand and fertilized twice a week with 25 ml modified Hoagland's nutrient solution. Part of seven-days-old seedlings were sprayed with 100 or 500 μM SA solutions, another seedlings were sprayed with distilled water. The 28-days-old plants were harvested for determination of their morphological and physiological parameters.

Plant height, leaf area and fresh weight (FW) were determined immediately after organ separation. Dry weight (DW) was determined after drying in the oven at 80°C until reaching a constant mass.

Variability of biomass production sensitivity index (SI) as the difference between dry matter production of treated plants and the control, expressed in percent of the latter, was calculated according to the following formula: $SI_{treatment} = (100 \text{ x } (DW_{treatment} - DW_{control})/DW_{control})$ [5].

Relative water content (RWC) was also measured and expressed as a percentage according to the following equation: $RWC\ (\%) = (FW - DW)/FW^x 100$.

For determination of photosynthetic pigments content 100 mg of plant leafs was homogenized with 25 ml 100% acetone, with the addition of the small amount of $CaCO_3$. Chlorophyll and carotenoids content was measured spectrophotometrically at 662, 644 and 440.5 nm according to Holm and Wettstein [1].

For protein extraction, 50 mg of tissue samples from shoots and roots were homogenized with sodium phosphate buffer (pH=6.8). Homogenate was centrifuged at 9600*g* for 15 min and supernatant was used for determination of protein according to Bradford [8], using bovine albumin serum as a standard.

Student's t-test was used to determine the significance of the results in different treatments.

Results and discussion

Changes of plant height and leaf area are represented in Table 1. Cd treatment did not affect plant height in wheat, but caused significant decrease of it in maize plants. 500 μM SA treatment by soaking grains led to the increase of plant height both under stressful and non-stressful conditions. Alterations in plant growth caused by SA and Cd treatments were more important if expressed through the plant leaf area (Table 1). Cd ions led to noticeable reduction of the leaf area in wheat and maize plants. Different variants of SA treatment had different effects on this parameter. Soaking grains with 500 μM SA increased leaf area of plants, other treatments did not have stable and visible effect. All SA treatments had powerful impact on Cd-stressed plants.

Analyzing fresh and dry mass of plants under the influence of SA and Cd, we can see essential changes in both plants. The presence of Cd reduced FW and DW in wheat and FW in

maize, and water balance was not disturbed (Table 1, 2). SA treatments recovered FW in Cd-affected plants and initiated accumulation of dry mass. Treatment with 500 μM SA (soaking) led to 200% increase of DW in maize plants, accompanied with RWC decline. The data of biomass production, expressed as SI, are represented in Table 2. 500 μM SA (soaking) had most pronounced effect on biomass accumulation of plants. Wheat plants were more affected than maize plants; this can be related to hyperaccumulating ability of maize plants [32]. Soaking grains with 500 μM SA had the greatest impact on biomass production of maize plants; all SA treatments had positive effect on the growth of Cd-stressed wheat plants.

Table 1

Effect of Cd and salicylic acid treatments on plant height (sm), leaf area (sm^2) and fresh weight (g) of 28-days-old wheat and maize plants (M±m; n=25)

Cd, mg kg^{-1}	SA, μM	Plant height		Leaf area		Fresh weight	
		T. aestivum	*Z. mays*	*T. aestivum*	*Z. mays*	*T. aestivum*	*Z. mays*
0	0 (control)	21.9 ± 1.2	38.7 ± 2.3	12.40 ± 0.79	78.64 ± 5.17	0.19 ± 0.01	1.61 ± 0.11
	100 soak	23.1 ± 2.0	42.3 ± 3.2	14.81 ± 0.83 [a]	71.68 ± 5.99	0.23 ± 0.02	1.64 ± 0.12
	100 spr	20.2 ± 1.7	42.0 ± 3.2	11.93 ± 0.85	75.49 ± 7.86	0.20 ± 0.01	1.56 ± 0.11
	500 soak	26.7 ± 1.8 [a]	47.6 ± 2.2 [a]	18.36 ± 0.62 [a]	92.99 ± 3.63 [a]	0.31 ± 0.02 [a]	1.88 ± 0.09
	500 spr	19.4 ± 2.0	46.7 ± 2.2 [a]	10.53 ± 0.72	80.37 ± 3.54	0.17 ± 0.01	1.86 ± 0.06 [a]
25	0	21.2 ± 1.7	32.8 ± 2.4	10.88 ± 0.57	55.12 ± 5.85 [a]	0.15 ± 0.01 [a]	1.36 ± 0.10
	100 soak	19.6 ± 1.9	36.2 ± 2.0	12.33 ± 0.44 [b]	64.93 ± 4.04 [a]	0.19 ± 0.01 [b]	1.36 ± 0.05 [a]
	100 spr	21.8 ± 2.3	36.5 ± 2.3	14.40 ± 0.61 [a,b]	74.44 ± 4.22 [b]	0.21 ± 0.01 [b]	1.51 ± 0.12
	500 soak	23.3 ± 1.6	35.9 ± 2.2	14.94 ± 0.33 [a,b]	67.20 ± 3.27	0.21 ± 0.01 [b]	1.46 ± 0.08
	500 spr	23.2 ± 1.8	27.7 ± 1.6 [a]	14.29 ± 0.57 [a,b]	69.89 ± 2.64 [b]	0.23 ± 0.01 [a,b]	1.54 ± 0.09

Comments: [a] significantly differing from 0 SA, [b] significantly differing from 0 Cd at $p<0.05$.

Table 2

Effect of Cd and salicylic acid treatments on dry weight (mg), RWC (%) and sensitivity index of 28-days-old wheat and maize plants (M±m; n=25)

Cd, mg kg^{-1}	SA, μM	Dry weight, mg		RWC, %		Sensitivity Index	
		T. aestivum	*Z. mays*	*T. aestivum*	*Z. mays*	*T. aestivum*	*Z. mays*
0	0 (control)	3.1 ± 0.2	14 ± 0.7	83.6 ± 0.4	91.1 ± 0.5		
	100 soak	3.6 ± 0.3	15 ± 1.1	84.3 ± 0.4	90.8 ± 0.5	+ 16.7	+ 7.8
	100 spr	3.6 ± 0.2	14 ± 0.7	82.4 ± 0.7	90.8 ± 0.6	+ 16.4	+ 2.5
	500 soak	4.8 ± 0.2 [a]	20 ± 0.8 [a]	84.7 ± 0.6	89.5 ± 0.4 [a]	+ 57.0	+ 41.0
	500 spr	2.9 ± 0.1	21 ± 1.1 [a]	83.3 ± 0.4	88.7 ± 0.4 [a]	− 4.6	+ 50.1
25	0	2.4 ± 0.1 [a]	13 ± 0.7	83.7 ± 0.5	90.2 ± 0.5	− 21.0	− 7.1
	100 soak	3.3 ± 0.2 [b]	13 ± 0.6	82.8 ± 0.4	90.5 ± 0.5	+ 8.5	− 4.3
	100 spr	4.1 ± 0.2 [a,b]	16 ± 1.0 [b]	80.8 ± 0.4 [a,b]	89.5 ± 0.4 [a]	+33.4	+ 13.3
	500 soak	3.5 ± 0.2 [b]	26 ± 1.3 [a,b]	83.7 ± 0.3	82.2 ± 0.3 [a,b]	+13.4	+ 85.6
	500 spr	4.6 ± 0.3 [a,b]	14 ± 0.7	80.2 ± 0.4 [a,b]	90.7 ± 0.5	+49.5	+ 2.3

Comments: [a] significantly differing from 0 SA, [b] significantly differing from 0 Cd at $p<0.05$.

Growing in Cd-polluted environment induced reduction of chlorophyll content by 28% in wheat and 10% in maize plants (Table 3). Considerable changes of chlorophyll *b* (decline by 43%) were observed in wheat, but no changes were found in maize. Chlorophyll *a/b* ratio increased in wheat, but decreased in maize (Table 4). All variants of SA treatment recovered chlorophyll content in both plants. The pre-treatment of maize with 500 μM SA led to increase of chlorophyll content by 28%, which exceeds control meanings by 18%. This treatment had a positive effect for non-stressed maize plants, but decreased chlorophyll *b* content in wheat. Soaking wheat seeds with 100 μM SA and spraying plants with 500 μM SA gave a more pronounced effect – 13% and 16% increase respectively.

Cd revealed an adverse effect on carotenoids content in investigated plants (Table 4). SA treatment recovered carotenoids in most variants. Also, SA provided increase of carotenoids in non-stressed plants.

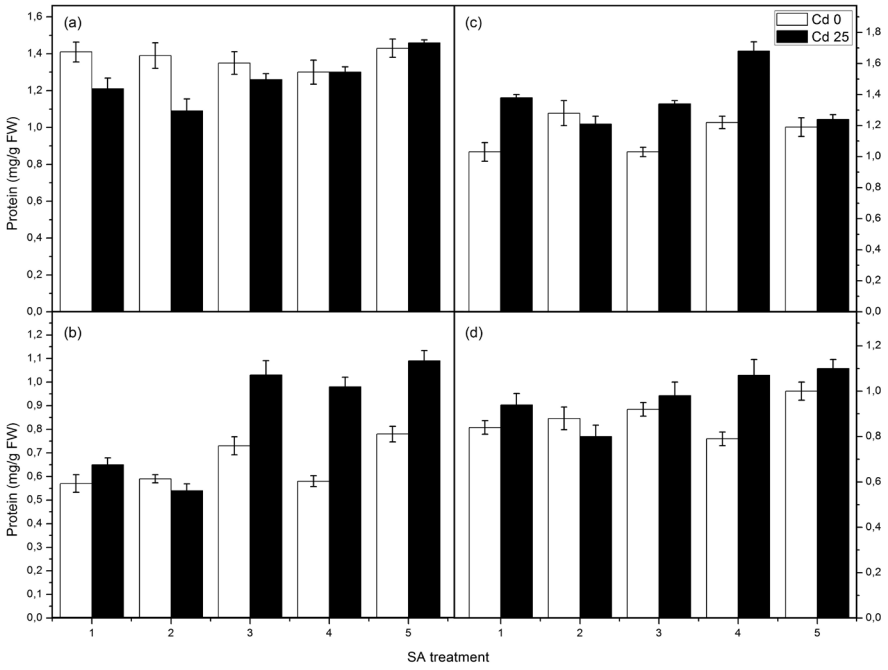
Table 3

Effect of Cd and salicylic acid treatments on chlorophyll content in 28-days-old wheat and maize plants (mg g^{-1} FW; M±m; n=3)

Cd, mg kg^{-1}	SA, μM	Chlorophyll *a*		Chlorophyll *b*		Chlorophyll *(a+b)*	
		T. aestivum	*Z. mays*	*T. aestivum*	*Z. mays*	*T. aestivum*	*Z. mays*
0	0 (control)	1.50 ± 0.07	1.33 ± 0.07	0.63 ± 0.03	0.41 ± 0.02	2.13 ± 0.10	1.74 ± 0.09
	100 soak	1.68 ± 0.09	1.39 ± 0.08	0.73 ± 0.05	0.44 ± 0.03	2.41 ± 0.14	1.83 ± 0.09
	100 spr	1.60 ± 0.06	1.29 ± 0.06	0.50 ± 0.03	0.42 ± 0.02	2.10 ± 0.09	1.71 ± 0.10
	500 soak	1.46 ± 0.08	1.31 ± 0.08	0.46 ± 0.01 [a]	0.61 ± 0.02 [a]	1.92 ± 0.10	1.92 ± 0.10
	500 spr	1.78 ± 0.07	1.33 ± 0.07	0.70 ± 0.05	0.50 ± 0.03	2.48 ± 0.12	1.83 ± 0.09
25	0	1.15 ± 0.06	1.14 ± 0.05	0.36 ± 0.02 [a]	0.42 ± 0.02	1.51 ± 0.07 [a]	1.56 ± 0.08
	100 soak	1.40 ± 0.06	1.30 ± 0.07	0.40 ± 0.03 [a]	0.34 ± 0.02	1.80 ± 0.09	1.64 ± 0.07
	100 spr	1.34 ± 0.05	1.19 ± 0.05	0.37 ± 0.02 [a]	0.42 ± 0.02	1.71 ± 0.08	1.61 ± 0.08
	500 soak	1.42 ± 0.08	1.53 ± 0.08 [b]	0.38 ± 0.01 [a]	0.53 ± 0.04	1.80 ± 0.09	2.06 ± 0.10
	500 spr	1.59 ± 0.08 [b]	1.22 ± 0.09	0.47 ± 0.02 [a]	0.36 ± 0.03	2.06 ± 0.10 [b]	1.58 ± 0.09

Comments: [a] significantly differing from 0 SA, [b] significantly differing from 0 Cd at $p<0.05$.

The treatment with Cd caused increase of protein content in wheat roots and maize (refer to figure). Slight reduction of protein accumulation was observed in wheat roots. Spraying plants with 100 and 500 μM SA led to enhancement of protein in roots. Pre-treatment with 500 μM SA also stimulated protein accumulation in maize shoots. SA treatment enhanced protein level in Cd-affected wheat plants; changes were more significant in roots. Pre-treatment maize plants with 500 μM SA led to increase of protein content – 14% in roots and 22% in shoots. Spraying with the same concentration of SA caused increase of protein level in maize roots (17%), but diminution in shoots was observed.



Effect of Cd and salicylic acid treatments on protein content in 28-days-old wheat (a,b) and maize (c,d) shoots (a,c) and roots (b,d), mg/g FW: 1 – SA 0; 2 – 100 μM SA (soak); 3 – 100 μM SA (spr); 4 – 500 μM SA (soak); 5 – 500 μM SA (spr).

Searching compounds with protective ability against environmental and anthropogenic stresses, including heavy metal pollution, and studying mechanisms of their impact on plant functioning may be important in its practical and theoretical aspects. The increase of own resistance of plants to different stresses with natural plant metabolites is important for current agronomy and horticulture. Plant hormones and their synthetical analogues are widely studied as growth regulators both in natural and stressful environments [23, 36]. SA and its derivatives can be considered in a role of a natural stress metabolite of plants, which can also result in non-stressful conditions.

In present study, the protective role of SA against Cd stress and separate SA influence have been presented; different SA concentrations and methods of treatment have been demonstrated. Pre-treatment with 500 μM SA revealed significant positive effect on growth parameters of both plants, but changes of chlorophyll content were not observed. Carotenoids content increased in both plants, but this effect was more visible in maize plants. Chlorophylls/carotenoids ratio was depressed, which is related to carotenoids accumulation. Such SA treatment did not affect protein content in plant tissues. Similar changes of photosynthetic apparatus were also observed in pea [28] and hemp [30] plants under 500 μM SA pretreatment.

Table 4

Effect of Cd and salicylic acid treatments on carotenoids content, Chl *a*/Chl *b* and Chl *(a+b)*/carotenoids ratio in 28-days-old wheat and maize plants (mg g^{-1} FW; M±m; n=3)

Cd, mg kg^{-1}	SA, μM	Carotenoids, mg g^{-1} FW		Chl *a*/Chl *b*		Chl *(a+b)*/carotenoids	
		T. aestivum	*Z. mays*	*T. aestivum*	*Z. mays*	*T. aestivum*	*Z. mays*
0	0 (control)	0.97 ± 0.05	1.24 ± 0.07	2.4	3.2	2.2	1.4
	100 soak	1.17 ± 0.04	1.26 ± 0.06	2.3	3.2	2.1	1.5
	100 spr	1.18 ± 0.07	1.40 ± 0.10	3.2	3.1	1.8	1.2
	500 soak	1.03 ± 0.06	1.73 ± 0.09 [a]	3.2	2.2	1.9	1.1
	500 spr	0.90 ± 0.05	1.52 ± 0.08	2.5	2.7	2.8	1.2
25	0	0.77 ± 0.05	0.89 ± 0.05 [a]	3.2	2.7	2.0	1.8
	100 soak	1.04 ± 0.06	1.26 ± 0.06 [b]	3.5	3.8	1.7	1.3
	100 spr	1.16 ± 0.07 [b]	1.19 ± 0.05 [b]	3.6	2.8	1.5	1.4
	500 soak	0.72 ± 0.03 [a]	1.17 ± 0.06	3.7	2.9	2.5	1.8
	500 spr	0.85 ± 0.06	1.22 ± 0.06 [b]	3.4	3.4	2.4	1.3

Comments: [a] significantly differing from 0 SA, [b] significantly differing from 0 Cd at $p<0.05$.

Other SA treatments have an ambiguous effect on plants. The slight increase of growth and pigment content was observed in plants pre-treated with 100 μM SA, the effect being more pronounced in wheat. Chlorophylls/carotenoids ratio was not changed. Some growth of protein content occurred in maize shoots. Spraying plants with SA did not give a pronounced effect, and there was no relationship with concentration.

SA treatments did not show a deteriorative effect on plant growth and development; treatment with 500 μM SA by soaking grains was the most effective for plants. Experiments with higher concentrations gave contradictory results [10, 19].

Our study shows that Cd has an adverse effect on leaf area, fresh and dry weight of plants and height of maize plants. Alterations in pigment content were observed. These data are similar with typical reaction of plant organism to heavy metal toxicity. Some mechanisms of Cd toxicity can be assumed: (a) destructive impact of free radicals caused by Cd-induced lipid peroxidation of chloroplast membranes; (b) influence of Cd on transport processes in plant organism; (c) degradation of enzymes which take part in chlorophyll biosynthesis; (d) modulation activity of chlorophyll-degradation enzymes; (e) substitution of Mg^{2+} of chlorophyll [6, 31]. We observed increase of protein level in maize roots and shoots and wheat roots, protein content in wheat

shoots being less than in control plants. Such protein accumulation can be related to the activation of synthesis protein compounds with metal-protective abilities (phytochelatins, metallothioneins, heat shock proteins, etc.) [17]. Differences in protein accumulation of wheat and maize plants may be associated with a genotypic contrast in reaction to heavy metal toxicity of our experimental plants. Maize is characterized by powerful root/shoot barrier which makes Cd penetration in shoot tissues less intensive and induces stronger protective mechanisms including hypersynthesis of phytochelatins and metallothioneins.

Salicylic acid is known as the compound which may play a protective role in stressful conditions [15, 16, 27]. In our study SA is studied as a potential protector for Cd-stressed plants. We observed increase of growth parameters in plants with SA+Cd treatment. Pre-soaking with 500 μM SA had more a beneficial effect on plant than the treatment with 100 μM SA. Spraying with SA showed results only for wheat plants. SA treatments (except soaking with 100 μM SA) revealed a stimulative effect on protein accumulation in wheat plants, especially in roots. Significant changes of protein content in maize were observed in variant with 500 μM SA treatment (soaking). We can assume that protein accumulation is related to the synthesis of stress-protective metal-binding proteins. SA is reported to be able to increase specific and non-specific protective mechanisms of stress tolerance in plants, such as enhancing efficiency of antioxidant system and inducing synthesis of metal-binding compounds, regulating different physiological and biochemical processes and participating in plant signaling networks [4]. We can summarize that SA is able to mitigate adverse effect of Cd on plants, and pre-treatment with 500 μM SA manifested beneficial effect for both investigated plants. Our results can be implemented in agronomy and horticulture of cereal plants. The results of investigations used in application of patent for a utility model [2].

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САЛІЦИЛОВА КИСЛОТА ЯК РЕГУЛЯТОР РОСТУ РОСЛИН ЗА УМОВ КАДМІЄВОГО СТРЕСУ

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Саліцилова кислота (СК) відома як учасник стресових реакцій рослин. Досліджувався вплив різних методів обробки СК (попереднє замочування насіння та обприскування 7-добових проростків 100 та 500 мкМ СК) на рослини пшениці та кукурудзи. Виявлено негативний вплив іонів Cd на площу листкової поверхні, масу рослин і вміст фотосинтетичних пігментів. Вплив СК знижує токсичний ефект іонів Cd на ріст і пігментну систему досліджуваних рослин. Виявлені зміни у вмісті білка в тканинах рослин за стресових умов та дії СК. Показано, що допосівне замочування насіння у 500 мкМ СК має найпомітніший ефект для обох досліджуваних рослин.

Ключові слова: Triticum aestivum L., *Zea mays* L., саліцилова кислота, кадмій, ріст.

САЛИЦИЛОВАЯ КИСЛОТА КАК РЕГУЛЯТОР РОСТА РАСТЕНИЙ В УСЛОВИЯХ КАДМИЕВОГО СТРЕССА

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Салициловая кислота (СК) известна как участник стрессовых реакций растений. Исследовали влияние различных способов обработки СК (предварительное замачивание семян и опрыскивание 7-суточных ростков 100 и 500 мкМ СК) на растения пшеницы и кукурузы. Обнаружено отрицательное влияние ионов Cd на площадь листовой поверхности, массу растений и содержание фотосинтетических пигментов. Влияние СК снижает токсический эффект ионов Cd на рост и пигментную систему исследованных растений. Обнаружены изменения содержания белка в тканях растений под влиянием стрессовых условий и воздействия СК. Показано, что предпосевное замачивание семян в 500 мкМ СК имеет наиболее заметный эффект для обоих исследованных растений.

Ключевые слова: Triticum aestivum L., *Zea mays* L., салициловая кислота, кадмий, рост.