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## TOTAL PHENOLIC, ANTHOCYANINS AND TBA-ACTIVE PRODUCTS IN BUCKWHEAT PLANTS UNDER NaCl IMPACT

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High salt concentration in soils cause osmotic and ionic stress for plants, along with oxidative stress and inhibition of growth and development, as a consequence, reducing the yield of many crops important for agriculture. Buckwheat (*Fagopyrum esculentum* Moench.) is classified as a salt-sensitive glycophyte plant, but salt tolerance of this culture is higher among many important food crops. The salt impact on the total levels of phenolics, anthocyanins and TBA-active products as the oxidative lipid peroxidation parameter, was determined in buckwheat plants (cv. Ukrainka) on 48, 72 h and 7<sup>th</sup> days of high NaCl exposure. Plants were grown on the ½ Hoagland–Arnon’s nutrient solution with/without addition of 100 mM NaCl. After 72 h of salt exposure, the TBA-active products increased in leaves and stems (130 and 204 %, respectively). Maximum of the TBA-active products was noticed at 72<sup>nd</sup> h, particularly in stems. On the 7<sup>th</sup> day of salt stress, the TBA-active products level decreased in leaves and roots, while in stems it remained at high level. Total amount of the phenolic compounds in the buckwheat roots treated with NaCl ranged from 35.55 to 64.95 µg/g DW in leaves – from 98.36 to 112.49 µg/g DW at different time points. In stems, the amount of total phenolic compounds was at the level in between 80.71 to 108.32 µg/g DW. After 48 h of NaCl impact, the total phenolic level decreased by 51 and 26 % in the roots and leaves, respectively. The same tendency was observed on the 3<sup>rd</sup> day of the experiment. Finally, on the 7<sup>th</sup> day the total phenolic content in roots approximated to the control level, however in leaves it was lower and in stems higher than in control. Content of the phenolic compounds was significantly higher in the above ground parts. An increase of the anthocyanin content during the long-term exposure to the stress was consistent with the statement regarding their secondary response to overcome an oxidative stress. Salt shock adversely affected physiological activity of the buckwheat

plants whereas a decrease of the TBA-active products, and a restoring of phenolics quantity in plant organs under prolonged salt stress points to an activated adaptive response.

**Keywords:** buckwheat, NaCl, phenolic compounds, anthocyanins, TBA-active products

## INTRODUCTION

High salt concentration in the produce osmotic and ionic stresses, oxidative stress and inhibition of plants growth and development, and, as consequence, reduce yield of many important crop cultures [4; 11; 12; 21]. Salinity with the increase of arid and semi-arid areas becomes one of the global problems for food security and agriculture. Studying and creating of salt-tolerant varieties are important for the future decades. The priority is getting a better understanding of salt responses at molecular, cellular, and whole plant levels [2; 4; 16; 21]. Buckwheat (*Fagopyrum esculentum* Moench.) is classified as a salt-sensitive glycophyte plant, but its' salt tolerance is higher if compare with many important crops [9; 26]. Nowadays, buckwheat becomes an important alternative source for human nutrition, because of its high nutrient value, possibility to use for health care, and high developmental potential [5; 12; 13; 19; 20; 25]. Usage of *F. esculentum* as a functional food largely benefits from the abundance of phenolic compounds with high antioxidant properties [6; 8; 12; 29]. On the other hand, high level of antioxidants, including anthocyanins, in plant tissues is one of the possible mechanisms of buckwheat ability to cope with salinity stress [20; 25]. A majority of current, published results is devoted to salt effects on phytochemicals in buckwheat sprouts or grains [13]. It was reported that the sprouts of common buckwheat contain an abundance of flavonoids, including C-glycosyl flavones (orientin, vitexin, and their isomers) and rutin, while tartary buckwheat contain only high concentration of rutin [25, 13; 14]. Recent studies demonstrated that 385 genes are differentially expressed under 100 mM NaCl stress [15]. There are also metabolic data on phenolic compounds under other stress conditions in *F. esculentum* and its relative *Fagopyrum tataricum* (L.) Gaertn. (tartary buckwheat) [6; 8; 9; 17; 23]. However, there are no data on phenolic compounds concentrations as antioxidant substances and salt response to the level of whole plant on early stages of growth, which are critical for further development and yield. Thus, the aim of present work was to determine levels of total phenolic compounds and anthocyanins in different organs of *F. esculentum* in connection with the lipid peroxidation processes on the 48<sup>th</sup>, 72<sup>nd</sup> h and 7 days of 100 mM NaCl influence.

## MATERIALS AND METHODS

**Plant material and growth conditions.** The object of investigations were 14 days buckwheat plants (*Fagopyrum esculentum* Moench) cv. Ukrainka. Seeds were pre-germinated for 3 days at 22±1°C in the darkness. Uniform seedlings were transferred into pots with perlite as substrate for further growth in the greenhouse under controlled conditions with 16 h photoperiod and 22±1 °C temperature. Seedlings were irrigated by a ½ Hoagland–Arnon's nutrient solution. After two weeks of growth (period when biomass accumulated), 100 mM NaCl was added to the part of plants as described [3]. The control set was grown with adding a ½ Hoagland–Arnon's nutrient solution. Samples of roots, stems and leaves were collected on 48<sup>th</sup> and 72<sup>nd</sup> h and on the 7<sup>th</sup> day of salt treatment.

**Lipid Peroxidation.** The level of lipid peroxidation in cells of buckwheat leaves, stems and roots was measured as the amount of the TBA-active products (malondial-

dehyde (MDA)) determined by the tiobarbituric acid reaction [16]. The absorbance of the supernatant was monitored at 532 nm, subtracting the value for non-specific absorption at 600 nm spectrophotometrically with SF-43. MDA content was calculated in nmol MDA per gram of dry weight (DW), and the level of lipid peroxidation was expressed in regarding to control (100 %).

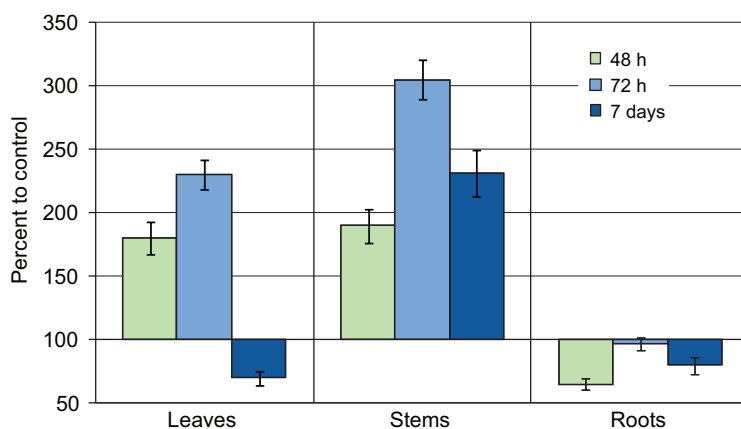
**Total phenolic compounds** were quantified by the reaction with Folin–Ciocalteu reagent, absorbance was measured at 725–730 nm. Phenolic compounds were calculated with a calibration curve using chlorogenic acid as standard and expressed in  $\mu\text{g}$  per g DW.

**Determination of anthocyanins content.** Anthocyanins were extracted by the method of Jaleel et al. [7]. The absorbance was measured at 525 nm with the spectrophotometer (SF 43) against HCl: methanol (1:100 v/v) as a blank. Anthocyanin content was calculated as cyanidin-3-glucoside using 29,600 as a molecular extinction coefficient and 445 as a molecular weight. Three or four independent replicates were analyzed, for roots, stems and leaves, separately. The results were expressed in micrograms per gram of dry weight (DW).

**Statistical analysis.** All experiments were performed using four replicates. The results were analyzed statistically, the mean value (M) and the standard error ( $\pm m$ ) were calculated using Microsoft Excel. Significance between control and treatment was compared at 0.05 probability levels using the Student's test.

## RESULTS AND DISCUSSION

One of harmful effects of salinity is the overproduction of free radicals, which can cause protein denaturation, lipid peroxidation and cell membranes damage, etc [14; 21; 24; 28]. Lipid peroxidation is often used as a marker of oxidative stress in biosystems [2; 8; 13; 24]. Short-term impact of salt led to a significant increase of the TBA-active products content in leaves and stem of buckwheat plants (Fig. 1). After 72 h of salt exposure, the level of TBA-active products increased in leaves and stems by 130 and 204 %, respectively. On the contrary, in the roots of experimental plants the decrease in TBA-



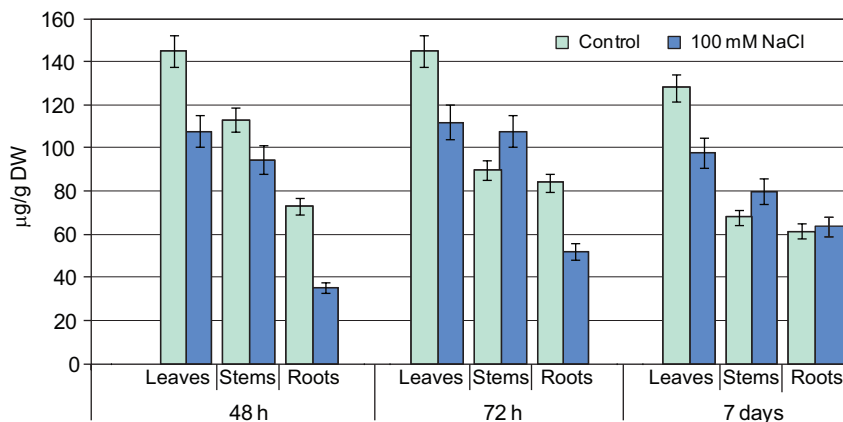
**Fig. 1.** Results of estimation of influence of 100 mM NaCl on the level of lipid peroxidation (TBA-active products formation) in *F. esculentum* (cv. Ukrainka) plants. Control –  $\frac{1}{2}$  Hoagland–Arnon's nutrient solution

**Рис. 1.** Вплив 100 мМ NaCl на рівень пероксидного окиснення ліпідів (за утворенням ТБК-активних продуктів) у рослин *F. esculentum* (с. Українка). Контроль –  $\frac{1}{2}$  поживне середовище Хогланда–Арнона

active products content was revealed. Generally, the maximum of TBA-active products were detected on the 72<sup>nd</sup> h of salt impact, especially in stems. On the 7<sup>th</sup> day of salt stress, the content of TBA-active products decreased in leaves and roots, while in stems it remained at high level.

Thus, under the short term salt impact the level of the TBA-active compounds increased, inducing defence responses, which led to a decrease of this parameter on the 7<sup>th</sup> day of salt stress. An oxidative stress activates an enzymatic and non-enzymatic antioxidant systems, including phenolics. Phenolic compounds and flavonoids play crucial roles in plants as growth regulators, cell wall components, and protective substances under the biotic and abiotic stress [2; 4; 22; 27]. To analyze a possible involvement in the salt responses, the level of the phenolic compounds and anthocyanins in buckwheat were determined.

Different distribution of phenolic compounds in plants organs with the significant predominance of their contents in leaves and stems, less extent in roots was revealed (Fig. 2). Total amount of the phenolic compounds in roots treated with NaCl ranged from 35.55 to 64.95  $\mu\text{g/g}$  DW in leaves – from 98.36 to 112.49  $\mu\text{g/g}$  DW at different time points. In stems, the amount of total phenolic compounds was at the level ranged from 80.71 to 108.32  $\mu\text{g/g}$  DW. After 48 h of NaCl impact the total phenolic level decreased by 51 and 26 % in the roots and leaves, respectively. This trend remained to a 3<sup>rd</sup> day of the experiment. Finally, on the 7<sup>th</sup> day of the NaCl impact, the total phenolic content in roots became close to the control level, in leaves it was lower and in stems higher than in control. Thus, we assume that the depletion of total phenolics pool points to their involvement on early salt responses of buckwheat and probably altered phenolics biosynthesis or changed direction of metabolic pathways. Some phenolic compounds serve as precursors for anthocyanins, that belong to a non-enzymatic ROS scavenging system and also involved in the salt stress responses of other plant species [1; 4; 10; 14; 21; 22; 24].



\* – significant at  $p \geq 0.95$

**Fig. 2.** Content of total phenolic compounds ( $\mu\text{g/g}$  DW) in *F. esculentum* (cv. Ukrainka) leaves, stems and roots of 14-days old plants under the 48, 72 h and 7 days of NaCl impact; control –  $\frac{1}{2}$  Hoagland–Arnon's nutrient solution

**Рис. 2.** Загальний вміст фенолів (мкг/г сухої речовини) у листках, стеблах і коренях 14-добових рослин *F. esculentum* (с. Українка) за 48, 72 год і 7 днів впливу NaCl; контроль –  $\frac{1}{2}$  поживне середовище Хогланда–Арнона

Differences in the total anthocyanins content in the buckwheat organs under salt influence are presented in Table. There were no significant changes in anthocyanins during the first 48–72 h of NaCl impact. However, on the 7<sup>th</sup> day of salt stress, the anthocyanins content decreased by 59 and 55 % in leaves and roots, respectively. In contrast, in stems was increased by 75%. A decrease in the anthocyanins content in roots was detected on all time points of the salt influence.

An increase of TBA-active products concentration in leaves of *F. esculentum* caused by NaCl treatment was also reported by Ž. Jovanović et al. [9] with plants cultivated hydroponically on ½ Murashige–Skoog nutrient solution. Salinity increased TBA-active compounds on the 2<sup>nd</sup> and 7<sup>th</sup> days of treatment, and elevated the level of TBK-active compounds was related to an increasing of ROS in early responses to the salinity.

The results of H. J. Kim et al. (2008) on romaine lettuce demonstrated that 2 days salinity caused phenolic content decrement, whereas there were no significant differences under the long term (15 day) treatments [11]. Similar result with a reduction in total phenol and flavonoid in artichoke leaves under NaCl influence was obtained by A. Rezazadeh et al. [21].

**The results of measuring the impact of 100 mM NaCl on total anthocyanin content ( $\mu\text{g/g DW}$ ) in *F. esculentum* (cv. Ukrainka) plants.**

**Control – ½ Hoagland–Arnon's nutrient solution**

**Вплив 100 мМ NaCl на загальний вміст антоціанів (мкг/г сухої речовини в рослинах *F. esculentum* (с. Українка). Контроль – ½ поживне середовище Хогланда–Арнона**

Time of exposition	Control			NaCl		
	Leaves	Stems	Root	Leaves	Stems	Root
48 hours	899.1±35.4	871.0±134.6	60.2±3.7	887.2±48.0	647.7±82.7↓	40.8±7.3↓
72 hours	869.4±51.5	586.5±61.3	163.5±14.5	683.8±49.2	520.41±30.25↓	124.9±9.7↓
7 days	1285.7±27.5	449.9±22.2	225.8±11.2	1523.6±16.9*↑	786.5±29.7*↑	102.5±4.8*↓

\* –significant at  $p \geq 0.95$

However, these results are opposite to those reported by H. J. Lim et al. [13] for leaves of *F. esculentum* under the effect of 10, 50, 100, and 200 mM NaCl. Accumulation of low molecular weight antioxidants, such as anthocyanins and phenolic compounds, in salt sensitive plants, may have multiple functions under stress conditions [4]. The total phenolic contents usually decrease in salt sensitive plants and increase in salt tolerant plants. Differences between the results of various studies are caused by analyzed organ (fruit, leaves, root), determination methods, values expression in terms of dry or fresh weight. A decrease in the phenolic compounds and anthocyanin concentrations in roots and leaves of the buckwheat under the short term impact of 100 mM NaCl suggests that they are involved in salt shock responses, but their pool is gradually depleted. The increase in the level of anthocyanins on the 7<sup>th</sup> day of salt stress indicates that protective mechanisms consists of flavonoid pigments. Therefore, the increase in the anthocyanin content during the long-term stress exposure could be considered as a response to the secondary oxidative stress.

## CONCLUSIONS

Short term 48–72 h salt shock caused by 100 mM NaCl led to the increase in TBA-active products, total phenolics concentrations and the decrease in anthocyanins levels. An induction of adaptive responses was revealed by the increase in anthocyanins and restore in phenolic compounds quantity in plant organs under the prolonged (7-days) salt stress.

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## ПРОДУКТИ ПЕРЕКИСНОГО ОКИСНЕННЯ ЛІПІДІВ, ФЕНОЛЬНІ СПОЛУКИ Й АНТОЦΙΑНИ У РОСЛИНАХ ГРЕЧКИ ЗА ДІЇ NaCl

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Висока концентрація солі у ґрунтовому розчині спричиняє осмотичний та іонний стреси, вторинний окислювальний стрес, пригнічення росту й розвитку рослин і, як наслідок, зниження врожайності важливих сільськогосподарських культур. Гречка (*Fagopyrum esculentum* Moench.) належить до групи солечутливих рослин-глікофітів, але здатність витримувати засолення у цієї культури вища порівняно з багатьма іншими культурами. Вплив засолення на загальний вміст фенольних сполук, антоціанів і ТБК-активних продуктів як показника перекисного окиснення ліпідів у рослинах гречки (с. Українка) визначали на 48, 72 год (сольовий шок), через 7 діб (сольовий стрес) впливу NaCl. Рослини вирощували на ½ поживному середовищі Хогланда–Арнона з/без додавання 100 мМ NaCl. Максимальний рівень перекисного окислення ліпідів мембран виявляли через 72 год дії солі: вміст ТБК-активних продуктів зростав у надземній частині (відповідно 130 і 204 % на листках і стеблах). За 7-добового сольового стресу концентрація ТБК-активних продуктів знижувалася порівняно з контролем у листках і коренях, залишаючись на високому рівні у стеблах. Загальний вміст фенольних сполук був значно вищим у наземних частинах рослин. Концентрація фенолів у коренях за обробки NaCl на різних часових точках експерименту була в межах від 35,55 до 64,95 мкг/г сухої речовини, у листках – від 98,36 до 112,49 мкг/г сухої речовини. У стеблах сума фенольних сполук була на рівні 80,71–108,32 мкг/г сухої речовини. Через 48 год впливу NaCl загальний вміст фенолів знизився на 51 та 26 % у коренях і листках, відповідно. Ця тенденція зберігалася і на 3-тю добу експерименту. На 7-му добу дії солі загальний вміст фенольних сполук у коренях наближався до контрольних значень, у листках він залишався достовірно нижчим, ніж у контролі. Зростання вмісту антоціанів у листках і стеблах гречки під час довготривалого впливу стресу узгоджувалося з їхньою антиоксидантною здатністю. Загалом короточасний сольовий шок несприятливо позначився на фізіологічній активності рослин гречки. Поступове зниження вмісту ТБК-активних продуктів, відновлення до контрольного рівня загальної кількості фенольних сполук і зростання вмісту антоціанів за тривалішого сольового стресу свідчать про індукцію адаптивних реакцій у досліджуваних рослин.

**Ключові слова:** гречка, NaCl, фенольні сполуки, антоціани, ТБК-активні продукти

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