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Aluminum nanoscales as hormetic response effectors in *Fagopyrum esculentum* seedlings

Aluminum (Al) nanoscales have been applied in many areas of production industries to produce cosmetic fillers, packaging materials, cutting tools, glass products, metal products, semiconductor materials, plastics, etc. Several studies have demonstrated the contradictory data for positive and negative effects of Al nanoscales on plants. The total length of seedlings grown for 21 days and the relative water content are used to determine the stimulating effects. In addition, the enhancement effect of Al nanoscales on photosynthetic pigments and the total phenolic and anthocyanin contents are determined. The growth stimulation and increase of the content of photosynthetic pigments are observed at the addition of 50 and 250 mg/L of Al nanoscales. Plant growth stimuli and the fixed beneficial action of Al nanoscales on morphofunctional traits at physiological and biochemical levels are interpreted as the hormesis phenomenon.

Keywords: *Al nanoscales, colloidal solution, hormesis, buckwheat seedlings.*

Metal- and metal oxide-based nanoscales of titanium dioxide, silver, zinc oxide, cerium dioxide, copper, copper oxide, aluminum, aluminum oxide, nickel, and iron are most commonly used in industries and, therefore, are mostly studied for their influence on different biological objects including plants [1]. Al nanoscales characterized by a high wear-resistance, have good thermal conductivity, resist to strong acids and alkali-containing materials, are easily shaped, and have high strength and stiffness. This makes them a prime material to use in making products that include high-temperature electrical insulators, high-voltage insulators, thermometry sensors, wear pads, ballistic armor, and grinding media [2]. There is no single opinion on the influence of Al nanoscales on physiological and biochemical processes in plants in the literature available: positive and negative effects are noted. The direct exposure of Al nanoscales leads to phytotoxic and enhancement alterations in different plants at morphological, cellular, biochemical, and molecular levels [3].

The aim of this study was to investigate the influence of Al nanoscales colloidal solutions on buckwheat seedlings and to determine their dose-dependent effects on Al-resistant plant species.

Materials and methods. A colloidal solution of Al nanoscales was obtained by the electric-sparkle dispersing of an electric-conductive layer in water [4]. Submicron metal particles in water suspensions were obtained by the method of volumetric electric-spark destruction of metal granules. The pulse power source (thyristor pulse generators with a storage capacitor) was used to initiate the discharge and simultaneous formation of spark channels in contacts between the metal granules dipped into deionized water. The transformation of a liquid to vapor and its condensation with the following crystallization results in the creation of a fraction with size from 10 to 100 nm.

Seeds of common buckwheat (*Fagopyrum esculentum* Moench. cv. Rubra) were germinated in dark at 25 °C in Petri dishes with deionized water. After 2 days, seedlings were transferred in pots with sterilized sand and half-diluted Knop solution (pH 5.5) in trays. Experiments were conducted under controlled conditions: temperature — 25 °C, photoperiod of 16 h at a photosynthetic photon flux density of ≈ 200 μmol photons $m^{-2} \cdot s^{-1}$. For the investigation, 21-day controlled and treated plants were used. The treatment with a colloidal solution of Al nanoscales was performed according to the following scheme: 1 — control, half-diluted Knop medium, 2 — addition of 50 mg/L Al nanoscales; 3 — addition of 250 mg/L Al nanoscales; 4 — addition of 500 mg/L Al nanoscales; 5 — addition of 750 mg/L Al nanoscales; 6 — addition of 1000 mg/L Al nanoscales.

The investigated morphological parameters included the plant total length (TL), fresh biomass (FB), dry biomass (DB), and relative water content of leaves (RWC) [5]. The contents of photosynthetic pigments were determined by measuring the alcohol extract optical density at 662 nm for chlorophyll *a*, 644 nm for chlorophyll *b*, and 440.5 nm for carotenoids [6]. Folin—Ciocalteu reagent was used to determine the total phenolic content [7]. Rutin as the dominant phenolic substance in buckwheat plants was used to standard the curve construction; absorbance was measured at 765 nm [8]. Total anthocyanin was extracted and estimated by the method of Beggs and Wellmann with some minor modifications [9]. Absorbance was measured at 530 nm. Quantity of anthocyanin was calculated with using cyanidin-3-glucoside coefficients — the major anthocyanin in buckwheat (molar extinction coefficient of 26 900 $L\ cm^{-1} \cdot mol^{-1}$ and molecular weight of 449.2 $g \cdot mol^{-1}$). All spectrophotometric assays was measured using a spectrophotometer UV-1800 “Shimadzu” (Japan).

Microsoft Excel 2010 was used for the data statistical analysis. Duncan’s multiple range test was used to evaluate the data. The results are expressed as the mean ± standard deviation, unless noted otherwise. Values of $P \leqslant 0.05$ were considered significant.

Results and discussion. Nowadays, the Buckwheat genus (*Fagopyrum* Mill.) is one of the most studied Al-resistant crops due to its Al-hyperaccumulating capability [10]. Analysis of the results of morphological traits showed that the total plant length, fresh and dry biomasses per plant were increased in variants with 50 and 250 mg/L of Al nanoscales. Addition of higher concentrations of nanoparticles (500, 750, and 1000 mg/L) led to a decrease of the plant growth parameters. Fluctuation of leaf RWC in variants with 50 and 250 mg/L of Al nanoscales was not detected. Level of RWC in leaves in variants with 500, 750, and 1000 mg/L of Al nanoscales showed a significant increase of the water deficit (Table).

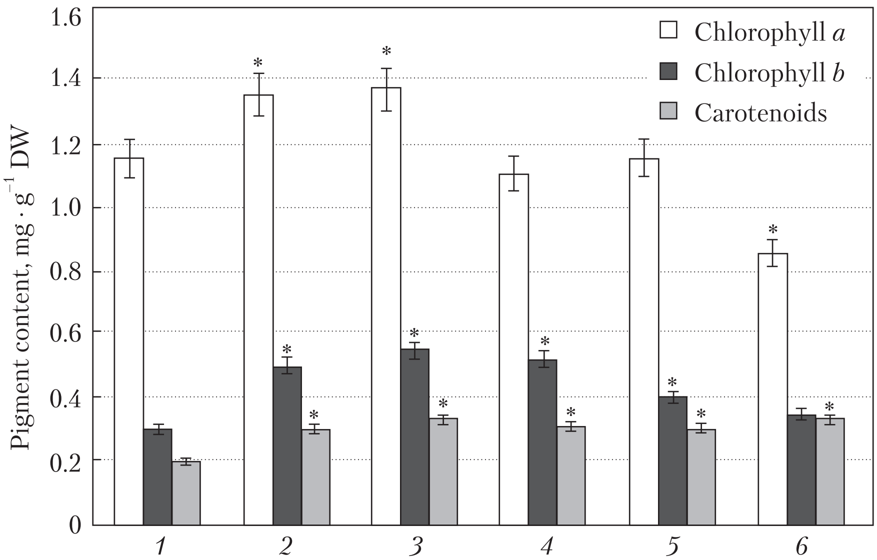

Fig. 1. Photosynthetic pigments content in buckwheat leaves under the treatment with colloidal solutions of Al nanoscales: *1* — control, half-diluted Knop medium, *2* — 50 mg/L Al nanoscales; *3* — 250 mg/L Al nanoscales; *4* — 500 mg/L Al nanoscales; *5* — 750 mg/L Al nanoscales; *6* — 1000 mg/L Al nanoscales; * — difference significant at $P \leqslant 0.05$ ($M \pm SD$; $n = 10$)

Morphological traits of buckwheat seedlings under the treatment with colloidal solutions of Al nanoscales

Variant of treatment	Plant length, cm	Fresh biomass, mg per plant	Dry biomass, mg per plant	Leaf relative water content, %
1	23.05 ± 0.31	197.43 ± 3.7	20.35 ± 0.78	87.7
2	24.78 ± 0.44*	209.27 ± 3.1*	21.07 ± 0.65*	86.9
3	24.67 ± 0.37*	211.58 ± 4.5*	21.82 ± 0.67*	88.7
4	22.21 ± 0.23*	188.73 ± 4.3*	19.57 ± 0.74*	70.6
5	20.35 ± 0.48*	156.05 ± 5.0*	14.72 ± 0.56*	66.7
6	18.93 ± 0.36*	153.91 ± 4.2*	14.39 ± 0.81*	54.3

* Difference significant at $P \leqslant 0.05$ ($M \pm SD$; $n = 20$).

The whole plant growth fluctuation under nanoscales treatments is the most evident trait of the plant functional status and becomes a useful bioassay due to its normativity, simplicity, non-destructivity, and sensitivity [11]. In turn, RWC is probably the most appropriate trait of the plant water balance in terms of the physiological consequence of a plant water deficit, since water accounts for 80—90 % of the fresh weight of most herbaceous plant structures. Analysis of morphometric results considered that the addition of Al nanoscales in concentrations of 500, 750, and 1000 mg/L was perceived as a stress condition accompanied by the growth inhibition and water balance disruption. Low concentrations of Al nanoscales (50 and 250 mg/L) led to the fresh and dry biomass accumulation and did not cause changes in the water status of treated plants.

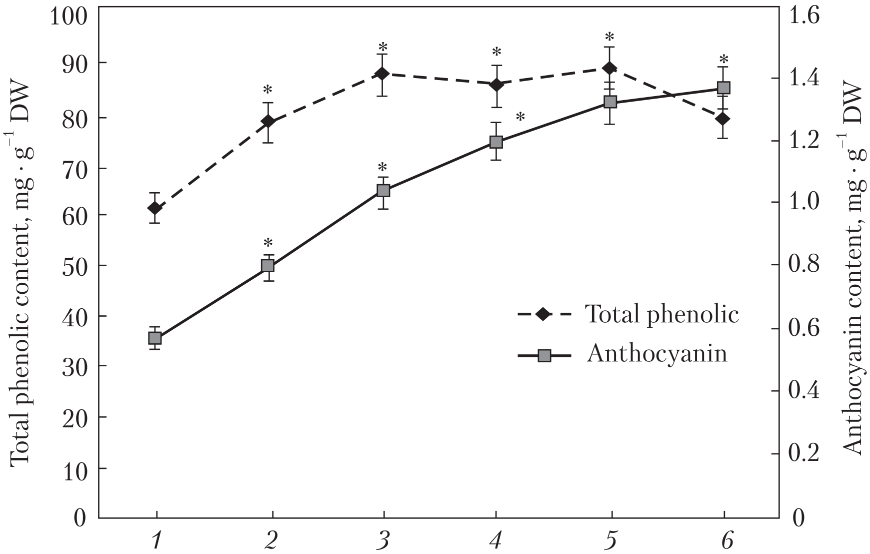

Fig. 2. Total phenolic and anthocyanin content in buckwheat leaves under the treatment with colloidals solution of Al nanoscales: *1* — control, half-diluted Knop medium; *2* — 50 mg/L Al nanoscales; *3* — 250 mg/L Al nanoscales; *4* — 500 mg/L Al nanoscales; *5* — 750 mg/L Al nanoscales; *6* — 1000 mg/L Al nanoscales; * — difference significant at $P \leqslant 0.05$ ($M \pm SD$; $n = 10$)

Our study showed a significant fluctuation of the photosynthetic pigment content under the Al nanoscales treatment (Fig. 1).

Enhancement effects of Al nanoscales on all pigments classes were noted in variants with 50 and 250 mg/L. The increases of chlorophyll *b* and carotenoids were observed in variants with 500 and 750 mg/L of Al nanoscales. Our results of the study of the influence of Al nanoscales on the photosynthetic pigments content showed significant increases of chlorophylls and carotenoids in variants with treatment by 50 and 250 mg/L of Al nanoscales. Ascending the nanoscales concentration to 1000 mg/L led to decreasing the chlorophyll *a* content, while the contents of chlorophyll *b* and carotenoids remained above the control level. These pigments are considered adaptogens due to adaptive capabilities of molecules, which consist in quenching an excited state of chlorophyll and preventing the formation of singlet oxygen and other reactive oxygen species [12].

The results of the study of the effects of Al nanoscales on the phenolic and anthocyanin contents showed that the addition of nanoscales induced the anthocyanins accumulation with a dose-dependent relation (Fig. 2).

Total phenolic compounds and anthocyanins are also able to function as potential antioxidants and are involved in the adaptive response under stressors influence [13]. The most significant increasing of anthocyanins was fixed in the variant with the maximal 1000 mg/L concentration of Al nanoscales — addition of 1000 mg/L of Al nanoscales led to the maximum stimulatory response — 241 % of the control level. The sharp increase of the total phenolic content fixed in all variants of Al nanoscales treatments. The total phenolic content showed no significant difference between variants with 250, 500, 750, and 1000 mg/L. After a sharp increase in the variant with 250 mg/L of Al nanoscales, the total phenolic content remained at 130—145 % of the control level.

While some results have been concerned with the toxicity of Al nanoscales to plants, others have focused on the possibility of using Al nanoscales as a fertilizer seeing enhancement effects. It was showed that, at the addition of certain concentrations of Al nanoscales, the plant growth, photosynthetic pigments, total phenolic and anthocyanin contents increase. Our experiments have provided evidence to characterize the morphofunctional responses of buckwheat plants as the hormesis — a dose-response phenomenon with the low-dose stimulation and high-dose inhibition [14].

Conclusions. Thus, the presence of aluminum nanoscales in low concentrations (50 and 250 mg/L) induced the plant growth, phenolic compounds, anthocyanin accumulation, and led to increasing the chlorophylls and carotenoids content. Shoot and root growth stimuli and fixed beneficial action of Al nanoscales on morphofunctional traits at physiological and biochemical levels were interpreted as the hormesis phenomenon.

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НАНОЧАСТИНКИ АЛЮМІНІЮ ЯК ГОРМЕТИЧНІ ЕФЕКТОРИ В ПРОРОСТКАХ *FAGOPYRUM ESCULENTUM*

Наночастинки алюмінію (Al) використовуються у багатьох сферах промислового виробництва для отримання косметичних наповнювачів, пакувальних матеріалів, різальних матеріалів, виробів зі скла, металевих виробів, матеріалів з напівпровідниковими властивостями, пластмас тощо. Існують суперечливі дані про позитивні та негативні ефекти наночастинок Al на рослини. Запропоновано для визначення стимулюючих ефектів наночастинок Al використовувати загальну довжину 21-добових проростків та відносну тургесцентність. Крім того, визначено позитивний вплив наночастинок Al на вміст фотосинтетичних пігментів, загальний вміст фенольних сполук та антоціанів. Додавання наночастинок Al у концентрації 50 та 250 мг/л спричиняє стимуляцію росту та збільшення вмісту фотосинтетичних пігментів. Стимуляція росту рослин і позитивний вплив наночастинок Al на морфофункціональні характеристики на фізіологічному та біохімічному рівнях інтерпретовані як феномен гормезису.

Ключові слова: *наночастинки алюмінію, колоїд, гормезис, проростки гречки.*

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НАНОЧАСТИЦЫ АЛЮМИНИЯ КАК ГОРМЕТИЧЕСКИЕ ЭФФЕКТОРЫ В ПРОРОСТКАХ *FAGOPYRUM ESCULENTUM*

Наночастицы алюминия (Al) используются во многих сферах промышленного производства для получения косметических наполнителей, упаковочных материалов, режущих материалов, изделий из стекла, металлических изделий, материалов с полупроводниковыми свойствами, пластмасс и т. п. Существуют противоречивые данные о положительных и отрицательных эффектах наночастиц Al на растения. Предложено для определения стимулирующих эффектов наночастиц Al использовать общую длину 21-суточных проростков и относительную тургесцентность. Кроме того, определено положительное влияние наночастиц Al на содержание фотосинтетических пигментов, общее содержание фенольных соединений и антоцианов. Добавление наночастиц Al в концентрации 50 и 250 мг/л приводит к стимуляции роста и увеличению содержания фотосинтетических пигментов. Стимуляция роста растений и положительное влияние наночастиц Al на морфофункциональные характеристики на физиологическом и биохимическом уровнях интерпретированы как феномен гормезиса.

Ключевые слова: *наночастицы алюминия, коллоид, гормезис, проростки гречихи.*