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ACCUMULATION OF HEAVY METALS IN SUNFLOWER SEEDLINGS UNDER THE INFLUENCE OF CADMIUM STRESS

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*Steady trend of recent decades is the rapid growth of soil pollution by heavy metals. Metals that do not play a significant role in plant life are particularly dangerous, but they can accumulate in plants and migrate along the food chain. Cadmium is one of such elements, its half-life from the human body is more than 50 years. For breeding programs aimed at creating varieties with low levels of cadmium accumulation, it is important to understand the role of genotype in the processes of metal intake to different parts of plants. An experiment was carried out to study the growth and absorption of trace metallic elements (TMEs) in Sunflower (Helianthus annuus L.) seedlings under Cd stress. The research was done on selection samples of sunflower selected by the method of vegetation evaluation in Sumy NAU in 2018. According to the evaluation results, line 62\3 was characterized by low, and line JB231AC – high level of resistance to cadmium accumulation. In 2020, the seeds of the samples were reseeded at the Henan Institute of Science and Technology. Sunflower seedlings (20-day-old) grown in plastic containers with Hogland's solution were investigated. From uniformly and well-developed plants were formed 4 samples which were for 7 days in a solution of $CdCl_2$ * 2.5 H_20 with a concentration of 0; 25; 50 and 100 μM. Ashing of the samples was performed in nitric acid. Determination of metal content was performed on an atomic adsorption spectrophotometer. In all samples there was a steady trend to decrease plant height and total seedling weight with increasing cadmium concentration. In the case of dried parts of plants, statistically significant, leveling the difference between the samples, the effect of cadmium was detected at a concentration of 50 μm or more for the dry matter of roots and at a concentration of 100 μm for the mass of the aboveground part. It was found that the threshold concentration that provided a significant difference between the cadmium content in the roots and stems was 100 μm. Compared to sample JB231AC, sample 64\2 had a higher cadmium content in the roots by 23 % and 12 % in the aboveground part. With respect to Li, Ni, and Sr, a significant difference between the samples was mostly observed at a concentration of 25 and 50 μM. The accumulation concentration of lithium (Li) and nickel (Ni) in high Cd variety 62\3 was lower than that in low Cd variety JB231AC. The Li and strontium (Sr) concentration in the root were similar to that in the aboveground part under Cd stress, while Ni accumulated in root of JB231AC in slightly higher concentrations than that in aboveground part. The presence of an inverse relationship between the concentration of cadmium and the concentrations of lithium and nickel may indicate both the genotypic features of their transport and the presence of antagonism between the accumulation of these metals. However, this statement requires further research.*

Key words: *sunflower, seedlings, Cd concentration, heavy metals, trace metallic elements, accumulation, growth.*

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Introduction. In recent years, with the rapid development of mining, industrial and agricultural production in the world, heavy metal soil pollution has become increasingly serious problem as a result of human activities, such as industrial wastewater, waste gas discharge, sewage irrigation and the abuse of chemical fertilizers and pesticides (Bashir et al., 2021; Singh & Prasad, 2014; Yan et al., 2021). Heavy metal cadmium (Cd) is a non-essential element for plants and animals, which inhibits the growth and development of plants (Li et al., 2019; Maria Celeste et al., 2013). Cd in plants interferes with normal metabolism, affecting photosynthesis and respiration, reducing root activity, slowing seedling growth, making leaves small and yellow, and eventually leading to plant death (Ahmad et al., 2015; Fan et al., 2011; Khadija et al., 2018). Consumption of Cd-contaminated plant material is one of the main sources of Cd intake for people, posing a threat to human health, even at low concentrations (Jaouani et al., 2018; Reyes-Hinojosa et al., 2019; Templeton and Liu, 2010). Cd repair and accumulation have been studied in many plant species, such as soybean (Li et al., 2012), wheat (Chen et al., 2017), barley (Chen et al., 2010), rice (Prerna et al., 2020), maize (Dakak & Hassan, 2020), rapeseed (Wu et al., 2015) and millet (Han et al., 2018), etc.

Sunflower (*Helianthus annuus* L.) is among the world's most important oilseed crops. As sunflower oil is one of the healthiest vegetable ones available for cooking, there is increasing demand due to a health-conscious diet (Bán et al., 2021). Studies have shown that sunflower has a high enrichment capacity to accumulate for heavy metals (Cornu et al., 2020; Zhou et al., 2020).

Trace metallic elements (TMEs) are indispensable elements in nature, which are lost to land through mining and production (Robert and Stengel, 1999). Compared to other pollutants, TMEs are non-biodegradable. Certain heavy metals are necessary or even essential for living beings, and this at reasonable concentrations in certain environmental matrices. On the other hand, at a high threshold, they (Cd, Pd, Hg) exhibit more or less strong toxicity which harms most living organisms (Aristide et al., 2021). At present, some TMEs, such as Cd (Cornu et al., 2016; Silveira et al., 2021), As (Wang et al., 2020), Hg, Cu (Mahardika et al., 2018) and Pb (Alaboudi et al., 2018) have been studied in some crops, however there are little researches about TMEs in sunflower. Issam et al. (2015) studied the role of selenium (Se) in regulating oxidative stress of sunflower seed roots induced by Cd, and the results strongly suggested that exogenous selenium may improve the tolerance of plants to oxidative stress induced by Cd. Shoot nutrients uptake of plants decreased as nickel (Ni) levels increased, and high level of Ni decreased root colonization in sunflower (Jarrah et al., 2019). Studies have shown that lithium (Li) and strontium (Sr) can be absorbed by body and affect human health (Zaichick&Zaichick,2014; Zaichick et al., 2009), but little researches have been done about Li or Sr in crops.

In this paper, the Cd gradient experiment was carried out on high and low Cd accumulation sunflower sample varieties. The growth characteristics of the two varieties under Cd stress at seedling stage and the accumulation of TMEs Li, Ni and Sr in the two sunflower samples under Cd stress were studied. This paper provides a theoretical basis for the safe production of sunflower on metal contaminated soil.

Materials and methods. *Material treatment and acquisition*. Two genotypes accessions of sunflower 62\3 (high Cd accumulation) and JB231AC (low Cd accumulation), which were identified with different Cd accumulation capability in our previous study, were carried out in this study. The two varieties were obtained from Sumy National Agrarian University (Sumy, Ukraine) where they were reproducted in October 2020.

The healthy seeds of each accession were sterilized by 15 % H_2O_2 for half an hour, and rinsed by distilled water for at least three times, and soaked in deionized water at room temperature for four hours, then sown in germinated box (32 cm × 25.5 cm × 11 cm) containing vermiculite and moistened with deionized water. Seeds were incubated in the culture room with 16 h light (28°C, 5000 Lux) and 8 h dark (25°C) photoperiod for 6 days. Following germination, seedlings were transferred to plastic pots filled with 10 L of 1/4 strength modified Hoagland nutrient solutions for 7 days, then was increased to 1/2 strength for 7 days. After 20 days of Cd-free growth, seedlings with uniformed sizes were randomly assigned to four different Cd treatments: 0, 25, 50 and 100 μM $CdCl_2 \cdot 2.5H_2O$ for 7 days. Finally, the seedling were harvested, one part was used to measure morphological indexes and Cd content, and the other part was quickly isolated and frozen in liquid nitrogen and stored at – 80 ºC and was used to measure physiological indicators and transcriptome analysis. The 1/2 strength nutrient solution containing 2.5 mmol/L $Ca(NO_3)_2$, 1mmol/L $MgSO_4$, 0.5 mmol/L $NH_4H_2PO_4$, 2.5 μmol/L KCL, 2mmol/L NaCl, 0.2μmol/L $CuSO_4$, 1μmol/L $ZnSO_4$, 0.1mmol/L EDTA-FeNa, 0.02 mmol/LH_3BO_3, 5 nmol/L $(NH_4)_6Mo_7O_{24}$ and 1 μmol/L $MnSO_4$. All the treatments were replicated three times.

Identification of growth index and Cd content. For showing and evaluating of Cd treatment results, the plants were photographed by a digital camera, plant height and fresh weight were measured. The root and aboveground parts of seedlings were oven-dried at 80°C until constant weight and then weighed for dry weight.

For measuring content of Cd and the TMEs, the root of seedlings were rinsed with deionized water for at least three times to remove surface ions, and then, root and aboveground parts were harvested separately. The samples were dried at 105°C for 30 min, and then at 80°C in an oven until they were completely dried, then the dry sample were ground to powders. Dry powder of each sample was digested in 5 ml HNO_3 overnight (at least for three 3 h) at room temperature, then adding 2 ml H_2O_2 and continued digesting for approximately 3 h at 180°C. (Marguí et al., 2009) The digested solution volumetized to 25 mL, and then investigated by an atomic absorption spectrophotometer (ICP-OES, Optima 2100DV, Perkin Elmer).

Statistical analysis. All data were statistically analyzed using GraphPad Prism 8. Analysis of variance was performed on data sets, with the mean and SD of each treatment calculated. Multiple comparisons with Bonferroni t-test were mainly used to compare the mean values between treatments ($p < 0.05$).

Results. *1. Growth of two sunflower varieties under Cd stress.* In this experiment, the seedling height, fresh weight, aboveground and root dry weight of the two sunflower varieties decreased with the increase of Cd concentration, and the high Cd variety 62\3 grew weaker than the low Cd variety JB231AC under Cd stress (Fig. 1 A–B), and growth indexes of variety 62\3 were lower than variety JB231AC (Fig. 1 C–F).

Among them, the seedling height and fresh weight decreased with the increase of Cd concentration, but the change was not significant. The root dry weights of variety 62\3 and JB231AC were significantly difference when Cd concentration was 25μM, and the aboveground dry weight was significantly difference when Cd concentration was 25μM and 50μM.

3. *Accumulation of trace metallic elements in two sunflower varieties under Cd stress.* Fig. 2 (A–B) verifies that variety 62\3 is a high Cd variety and JB231AC is a low Cd variety, and the Cd concentration in the root is higher than that in the aboveground part. With the increase of Cd concentration, the Cd accumulation in root and aboveground part of the two cultivars increased, and higher Cd concentrations were accumulated in variety 62\3 under

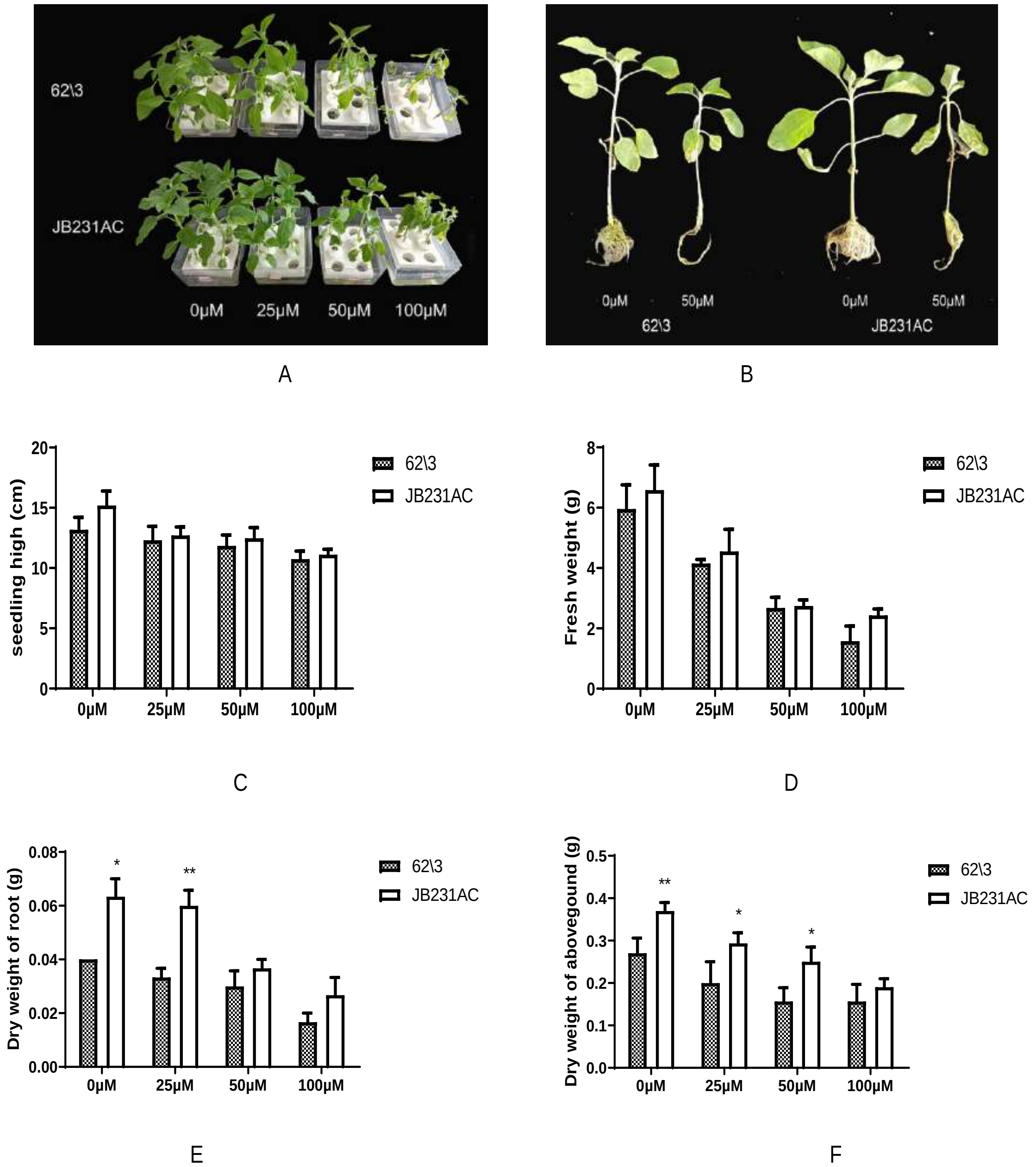

Fig. 1. Growth performance of variety 62\3 and JB231AC seedling.
A–B, Growth status under Cd conditions for 7 days. C-F, growth indices of the seedling. For C–F the statistical analyses were conducted using all the data of the two varieties under Cd conditions. Data presented are the means (n = 3), and error bars denote the standard deviations. The asterisk represents the significant difference between two varieties. *: P value < 0.05, **: P < 0.01

different Cd gradients. When the Cd concentration was 100 μM, the Cd accumulation in root and aboveground part of variety 62\3 showed extremely significant differences.

The Li concentration in the root and aboveground part of the two varieties was approximately the same under Cd stress, and the high Cd variety 62\3 accumulated less Li than the low Cd variety JB231AC (Fig. 2 C–D). In root, Li accumulation was significantly differences when the Cd concentration was 50 μM and 100 μM. With the increase of Cd concentration, the Li concentration in root increased, the change in the aboveground part was not regular, and there might be experimental error.

The Ni accumulation in sunflower increased firstly and then decreased with the increase of Cd concentration, and the maximum value appeared at 50 μM in the root and 25 μM in the aboveground part (Fig. 2, E–F). The Li concentration in root was higher than that in aboveground part. The Ni concentration in the high CD variety 62\3 was lower than that in the low Cd variety JB231AC, and there was a very significant difference in the Cd concentration at 25 μM and 50 μM in the root and aboveground part, respectively.

With the increase of Cd concentration, the Sr accumulation in root of the two sunflower varieties varied, but remained basically flat, which may be caused by experimental error,

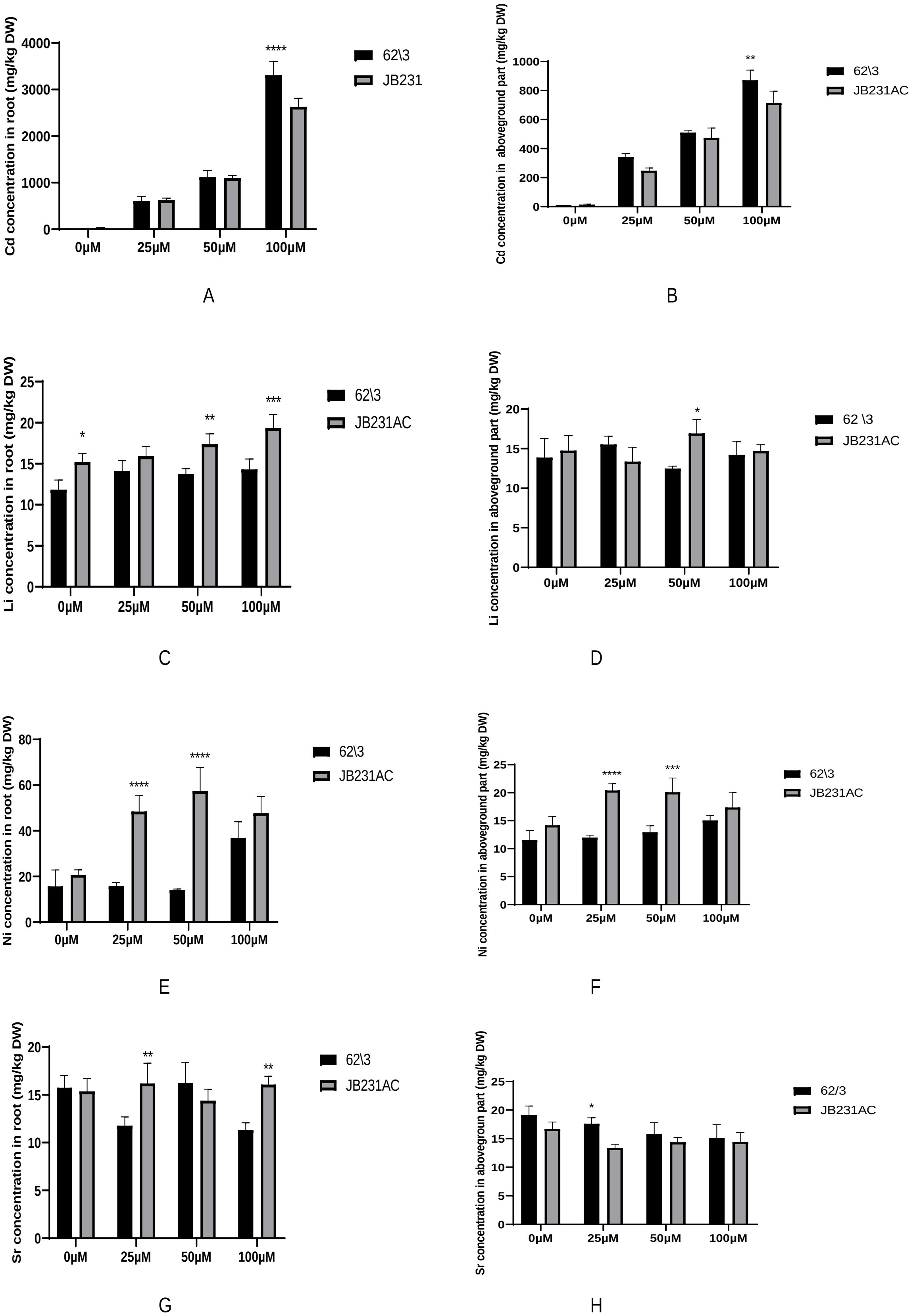

Fig. 2. Accumulation of trace metallic elements in the root and aboveground part of variety 62\3 and JB231AC seedling.

A–B, Cd accumulation. C–D, Li accumulation. E–F, Ni accumulation. G–H, Sr accumulation. For A–H the statistical analyses were conducted using all the data of the two varieties under Cd conditions. Data presented are the means (n = 3), and error bars denote the standard deviations. The asterisk represents the significant difference between two varieties *: P value < 0.05, **: P < 0.01,***: P < 0.001,****: P < 0.0001 and basically showed a downward trend in aboveground part (Fig. 2G–H). Sr accumulates in roughly equal concentration in root and aboveground part. In aboveground part, the high Cd variety 62\3 accumulated relatively higher Sr concentration than the low Cd variety JB231AC.

Discussion. In hydroponics under controlled conditions, Cd stress (5 μM Cd for 14 days) induced strong phytotoxic effects, as indicated by a decrease in all growth parameters (Hawrylak-Nowak et al., 2015). At Cd concentration of 50 μM the seedling growth of sunflowers was affected significantly in a short period (< 7 days) in the experiment, and the growth indexes such as plant height, dry and fresh weight decreased obviously. Cd accumulation of both varieties was more in roots than in aboveground parts. Cd accumulation in sunflower was different in different genotypes. Varieties 62\3 with high Cd accumulation might accumulate more Cd, causing greater toxicity to themselves, so their plant height, dry and fresh weight were lower than varieties JB231AC with low Cd accumulation under different Cd gradient. The accumulation characteristics of different TMEs in the two sunflower species under Cd stress were different, and the accumulation of TMEs in the root and aboveground part of the same variety might be different. The accumulation concentration of Li and Ni in high Cd variety 62\3 was low, so it is speculated whether they have antagonistic effect with Cd absorption. Accumulation was more in roots than shoots for most of the heavy metals (Belhaj et al., 2016). In the paper, the Li and Sr concentration in the root were similar to that in the aboveground part under Cd stress, while Ni accumulated in root of JB231AC in slightly higher concentrations than that in aboveground part.

Conclusions. The accumulation and distribution of cadmium in plants have a great relationship with species, varieties, parts and growing environment, and eventually lead to different accumulation and distribution in different parts of plants. An inverse relationship between cadmium concentration and lithium and nickel concentrations has been established, which may be due to genotypic features, but the presence of antagonism between the accumulation of these metals is not excluded. In general, studies have confirmed the sufficient level of efficiency of vegetative evaluation of sunflower initial material for resistance to cadmium accumulation and stability of this trait in subsequent generations.

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Накопичення важких металів у проростках соняшнику під впливом кадмієвого стесу

Стійкою тенденцією останніх десятиліть є стрімке зростання забруднення грунту важкими металами. Особливу небезпеку становлять метали, які не відіграють суттєвої ролі у житті рослин, але можуть накопичуватися у рослинах та мігрувати харчовим ланцюгом. Одним із таких елементів є кадмій, період напіввиведення якого з організму людини складає більше 50 років.

Для селекційних програм, спрямованих на створення сортів із низьким рівнем накопичення кадмію, важливим є розуміння ролі генотипу в процесах надходження металу до різних частин рослин. Дослідження проводились на селекційних зразках соняшнику, виділених за методикою вегетаційного оцінювання в Сумському НАУ в 2018 році. За результатами оцінювання лінія 62\3 характеризувалася низьким, а лінія JB231AC – високим рівнем стійкості до накопичення кадмію. У 2020 році насіння зразків було пересіяно в умовах Науково-технологічного інституту м. Хенань. Досліджували 20-денні проростки соняшнику, що вегетували у пластикових контейнерах з розчином Хогланда. Із рівномірно розвинених рослин було сформовано 4 вибірки, які протягом 7 днів знаходились у розчині $CdCl_2 \cdot 2,5\ H_20$ з концентрацією 0; 25; 50 та 100 мкМ. Для аналізу зразки розчиняли у нітратній кислоті. Визначення вмісту металів проводили на атомно-адсорбційному спектрофотометрі (ICP-OES, Optima 2100DV, Perkin Elmer).

В обох зразків було відмічено стійкий тренд до зменшення висоти рослин та загальної маси проростків із збільшенням концентрації кадмію. Різниця між зразками за згаданими показниками нівелювалася при концентрації 50 і більше мкМ. У випадку із висушеними частинами рослин статистично суттєвий вплив кадмію виявлено при концентрації 50 і більше мкМ для показника маси сухої речовини коренів та при концентрації 100 мкМ для показника маси надземної частини.

Встановлено, що пороговою концентрацією, яка забезпечувала суттєву різниці між показниками вмісту кадмію в коренях та стеблах, було 100 мкМ. Порівняно із зразком JB231AC, зразок 64\2 мав вищий вміст кадмію у коренях на 23 % та на 12 % у надземній частині. Відносно Li, Ni, та Sr суттєва різниця між зразками здебільшого спостерігалася за концентрації 25 та 50 мкМ.

Накопичення і розподіл кадмію в рослинах мають значний зв'язок з видами, сортами, частинами та середовищем вирощування і в кінцевому підсумку призводять до різного накопичення та розподілу в різних частинах рослин. Встановлено зворотну залежність між концентрацією кадмію та концентраціями літію та нікелю, що може бути зумовлено генотипними особливостями, але не виключається наявність антагонізму між накопиченням цих металів. Однак таке твердження потребує додаткових досліджень.

Загалом дослідження підтвердили достатній рівень ефективності вегетативної оцінки вихідного матеріалу соняшнику на стійкість до накопичення кадмію та стабільність цієї ознаки в наступних поколіннях.

Ключові слова: *соняшник, проростки, концентрація Cd, важкі метали, мікроелементи, накопичення, ріст.*