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Original Article

EFFECT OF MOLYBDENUM AND TUNGSTEN ON THE ACTIVITY OF MO-HYDROXYLASES AND THE ANTIOXIDANT SYSTEM IN PLANTS

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ABSTRACT

Modern studies in the field of plant molecular biology pay considerable attention to the mechanisms of regulation of the antioxidant system. In this work we study the effect of heavy metals molybdenum and tungsten on the modulation of Mo-hydroxylases and the functioning of the antioxidant system of plants using the model organism *Nicotiana benthamiana*.

The aim of the study is to analyse the effects of molybdenum and tungsten on the activity of antioxidant enzymes (SOD, CAT) as well as the Mo-hydroxylases aldehyde oxidase (AO) and xanthine dehydrogenase (XDH). The methods used include native gel electrophoresis and spectrophotometry.

The main results show that molybdenum application promotes the activation of plant antioxidant system by increasing the production of reactive oxygen species (ROS) and superoxide dismutase (SOD) enzyme activities, while catalase activity changed insignificantly. Also, AO activity increased, whereas XDH activity was almost unchanged. On the contrary, tungsten application reduced the activity of these antioxidant defence mechanisms and inhibited Mo-hydroxylases.

The practical significance of the work lies in the possibility of developing biotechnological approaches to increase plant resistance to abiotic stresses by regulating the activity of Mo-hydroxylases. The obtained data can be used in crop production and agriculture to improve stress tolerance of agricultural crops.

Key words: aldehyde oxidase, xanthine dehydrogenase, *Nicotiana benthamiana*, ROS, molybdenum, tungsten, heavy metals.

INTRODUCTION

During Kazakhstan's early years of independence, persistent environmental and management issues such as drought, water scarcity, outmoded facilities, habitat loss, and soil degradation posed significant constraints to agricultural productivity. The implementation of various restorative methods has made Kazakhstan's grain production variable throughout the last few decades. However, water scarcity and soil alkalization remain important limiting factors for wheat output, whereas soil alkalization is the case for barley [1]. Such abiotic stresses, as well as drought, salinity and soil contamination with heavy metals, have a negative impact on plant growth and development. These conditions reduce crop productivity, disrupt physiological processes and inhibit metabolism, which in turn leads to longer growing seasons and lower total yields.

A wide range of strategies are required to address this problem. Currently, considerable attention is being paid to the search for novel and affordable materials used to improve crop resistance and productivity.

Mo-hydroxylases play a key role in many biochemical reactions of plant organism. These enzymes are involved in various processes of adaptation to biotic and abiotic stress factors. Modulation of Mo-hydroxylase activity by exogenous addition of metals can serve as a useful methodological approach to develop new strategies for enhancing plant productivity and stress adaptation.

Molybdenum (Mo) is a vital component for most living things. Mo is found as a pterin-cofactor in the active core of plant enzymes that catalyze important processes in nitrogen, carbon, and sulfur metabolism, making them necessary for optimal growth under a variety of environmental conditions [2]. After being absorbed into the cells as molybdate, it is integrated into the molybdenum cofactor, which serves as the active site for various molybdenum-requiring enzymes and so plays critical roles in a variety of biological processes [3]. Molybdenum, used as a catalytic center in enzymes, is chemically adaptable and redox-active in physiological systems. Plants have around 50 Mo-dependent enzymes, including sulfite oxidase (SO), mitochondrial amidoxime reductase (mARC), xanthine dehydrogenase (XDH), nitrate reductase (NR), and aldehyde oxidase (AO) [4]. Mo is not physiologically active, but it is an essential component of an organic pterine complex known as the molybdenum cofactor (MoCo). MoCo is composed of a tricyclic pterin that coordinates Mo via a dithiol group in the third pyranocircle. All Mo-dependent enzymes have a pterin-type cofactor, with the exception of bacterial nitrogenase, which has a distinct iron-molybdenum cofactor that provides catalytic activity. MoCo exists in two forms in eukaryotic cells that differ from Mo. In the first form, Mo is covalently linked to a conserved cysteine residue in the MoCo connective domain. In the second form, the third terminal sulfur ligand is coupled to Mo, however in this version, MoCo remains non-covalently bound to the protein [5].

Tungsten (W) is a transition element of group VI, a refractory metal, with many similarities to its close element Mo, with which W frequently co-occurs. In soil, tungstate is the element's most stable form. This oxyanion is chemically identical to molybdate; however, unlike molybdenum, tungsten is not an essential micronutrient, and it has generally been considered inert in the soil environment and nontoxic to human health [6]. The consequences of W contamination on the biosphere have not been thoroughly assessed, however it is

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predicted that W may have negative impacts on species and may become more significant when it accumulates in the food chain or environment. The lack of mobility of W in acidic soil conditions has been shown to inhibit bioaccumulation in diverse plant species, but bioaccumulation was stronger in similarly contaminated alkaline soils [7].

Mo-hydroxylases aldehyde oxidase and xanthine dehydrogenase were studied in this paper. Aldehyde oxidase (AO) is a cytosolic enzyme with a molecular mass of 300 kDa that contains FAD, iron, and molybdenum cofactor (MoCo) as prosthetic groups and belongs to the molybdenum hydroxylase family [8]. Plant AOs accelerate the oxidation of several aromatic and aliphatic aldehydes. The conversion of abscisic aldehyde to ABA and indole acetaldehyde to indoleacetic acid by AOs, respectively, may be key regulatory elements; nevertheless, their regulatory role is still under question. Plant AOs have been examined from a variety of sources, including oat coleoptiles, potato tubers, cucumber seedlings, pea seedlings, and maize coleoptiles. Both animal and plant AOs have rather broad substrate specificity and can oxidize a number of different aldehydes, indicating that AOs play various biological roles [9]. The AO enzyme catalyzes the final steps of carotenoid catabolism and is an important enzyme in the abscisic acid (ABA) production. AO isoforms are found in the cytosolic compartment of tissues in many plants, where they promote the oxidation of aldehydes into carboxylic acid and catalyze the hydroxylation of certain heterocycles [10].

Xanthine dehydrogenase (XDH), a molybdenum enzyme, participates in purine metabolism by catalyzing the formation of ureides from xanthine and hypoxanthine [11]. It controls both normal development and non-biological stress-induced aging processes in plants. The mechanisms of XDH participation in ageing and stress tolerance have been investigated in Arabidopsis, pea, corn, and grapes. XDH involvement in premature leaf senescence in plants includes several mechanisms, such as nitrogen metabolism, reactive oxygen species (ROS), and hormonal regulation [12]. Plant xanthine dehydrogenases appear to be capable of employing both O_2 and NAD^+ as electron acceptors while also creating large quantities of ROS. It has previously been established that xanthine dehydrogenase plays an important role in *Arabidopsis thaliana* defense responses, most likely via producing ROS in epidermal cells and scavenging ROS in mesophyll cells [13].

The aim of the study was to investigate the effect heavy metals (molybdenum, tungsten) on the activity of Mo-hydroxylases, antioxidant enzymes (SOD, CAT) and the level of ROS accumulation (H_2O_2, O_2^-) in *N. Benthamiana*.

MATERIALS AND METHODS

Plant cultivation. *N. benthamiana* plants were grown in a specially equipped growth chamber, where optimal conditions for growth and development of these plants were created: long photoperiod 16 hours of simulated daylight and 8 hours of darkness. Spiral heating lamps (Klaus) with 6400 K spectrum at 24-28 °C were used for illumination. Plants were grown on enriched soil (Terra Vita) mixed with vermiculite until the third week. 30-day-old plants with similar initial morphological characters were selected for the experiment.

In this study, solutions of water-soluble salts Na2MoO4·2H2O and Na2WO4·2H2O of concentrations 2,5 and 5 mM were applied to the soil separately. As a control, the plant was watered with distilled water. 30-day-old plants were watered for 7 days with 30 ml of solutions at different concentrations, respectively.

Analysis of enzyme activity. To determine the enzyme activity, native gel electrophoresis was carried out, where leaves of *N. Benthamiana* were homogenized. The ratio of plant material to extraction buffer was taken as 1:2 (weight to volume). The extraction buffer consisted of 250 mM Tris-HCl (pH 7.5), 250 mM sucrose, 0.05 mM sodium molybdate ($Na_2MoO_4*2H_2O$), 1 mM ethylenediaminetetetraacetic acid (EDTA), 4 mM 1,4- dithiothreitol (DTT), 5 mM L-cysteine, 0.001 mM aprotinin, 0.1 mM phenylmethylsulfonyl fluoride (PMSF) and 0.001 mM pepstatin. After homogenisation, the samples were centrifuged at 10000 rpm and 4ºC for 30 minutes. Samples prepared for native PAGE were mixed with the Laemmli sample buffer.

Analysis of AO activity in gel. After native gel electrophoresis, a solution containing 0.1M Tris-HCl, pH 7.5, 0.1 mM phenazine methosulfate, 1 mM MTT (3 [4,5- dimethylthiazol-2-yl-2,5-diphenyltetrazolium-bromide), and 1 mM substrate (indole-3-carboxo-aldehyde) was used to determine the activity of AO, at room temperature for 30 minutes. AO activity was evaluated on the basis of MTT reduction, as a result of the appearance of specific formazan bands [8].

Analysis of XDH activity in gel. After native gel electrophoresis, the gel was washed in distilled water and stained with substrate solution containing 0.05 M Tris-HCl, pH 7.4, 1mM hypoxanthine, 0.5mM thiazolyl blue tetrazolium bromide (MTT) and electron carrier, 0.5 mM phenazine methosulfate (PMS) at 37°C for 40 min. The resulting bends on the gel were analysed [14].

Analysis of CAT activity in gel. After separation in native PAGE, a two-component substrate was prepared for catalase determination using 2% ferric chloride and 2% iron potassium cyanide. The prepared gel with samples was washed three times in distilled water for 5 minute each, incubated for 10 minutes in 0.03% hydrogen peroxide, then in a mixture of the above two substrate components until specific bands were manifested for 3 minutes [15].

Analysis of SOD activity in gel. Total SOD activity was estimated by nitroblue tetrazolium (NBT) photoreduction according to Giannopolitis and Ries, 1977 [16]. 10% vertical polyacrylamide gel was used for denaturing electrophoresis. The gel was placed in a 20 ml solution with 0.02 g NBT for 30 minutes. Then, the gel was placed in a 100 ml solution with 1.199 g of potassium dihydrogen phosphate, 0.01 g of riboflavin, and 2.8 µL of TEMED for 20 minutes. The gel was placed under UV. Visual and graphical data were derived from the obtained bands.

Determination of ROS accumulation. For the determination of O_2^- and H_2O_2, samples were extracted in 50 mM phosphate buffer (pH 7.5) at a ratio of 1:8 (wt./vol.) and centrifuged twice at 10000 rpm for 10 min. The reaction mixture for O_2^- determination consisted of 4 mM epinephrine as electron acceptor in 100 mM Tris-HCl buffer (pH 7.8) in the presence or absence of 2100 units/ml CuZn-SOD, as previously described by Yesbergenova et al. (2005). Absorbance was measured at 480 nm using a Biochrom Asys Expert 96

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microplate spectrophotometer [17].

The reaction mixture for H_2O_2 determination consisted of 0.85 mm 4-aminoantipyrin, 3.4 mm 3,5-dichloro-2-hydroxybenzene sulfonate, 4.5 units/ml HRP in 2 ml of 50 mm phosphate buffer (pH 7.5) as previously described by Yesbergenova et al. (2005). Absorbance was measured at 480 nm using a Biochrom Asys Expert 96 microplate spectrophotometer [17].

Statistical analysis. Data are expressed as arithmetic means ± standard deviations (SD) from three independent replicates. Statistical analysis was performed using one-way ANOVA, and Tukey's post hoc test was applied for multiple comparisons when differences were significant at $p \leq 0.05$. Student's t-test was conducted using GraphPad Prism (version 8.0). Enzymatic activity assays in gels were repeated at least three times. Data from three independent repeats were converted to numerical values (±SD) using ImageJ graphical editor.

RESULTS

In the present study, the impact of molybdenum (Mo) and tungsten (W) on the growth and development of plants was assessed based on morphometric indicators. The research aimed to evaluate not only the individual effects of Mo and W, but also their combined influence on the plant antioxidant defense system. A particular focus was placed on identifying potential antagonistic interactions between these two trace elements when applied simultaneously.

Given that both Mo and W are group 6 transition metals with chemical similarities, their interaction within biological systems can result in competitive or inhibitory effects, especially regarding molybdenum-dependent enzymes. Therefore, special attention was given to the modulation of Mo-containing hydroxylase activity under different treatment conditions. The obtained morphometric data (such as plant height, leaf number, and biomass accumulation) were analyzed to correlate physiological responses with enzymatic and biochemical changes induced by Mo, W, or their combination.

The effect of Mo and W on the growth of *N. benthamiana* is depicted in Figure 1.

As can be seen in the image, under the influence of 2.5 mM concentrations, the effect of heavy metals did not have a significant effect on plant growth. Under the influence of 5 mM concentrations, it can be observed that under the influence of Mo, the plant size is visually similar to the control plant. W, on the other hand, had a strongly negative effect on the plant. Under the combined effect, the negative effect of W was compensated by the effect of Mo.

Investigation of the effects of Mo and W on the level of ROS (H_2O_2, O_2^-) accumulation in *N. Benthamiana.* Hydrogen peroxide (H_2O_2) and superoxide (O_2^-) as previously described is one of the key components of ROS and plays an important role in the development of plant response to various stresses. To study the effect of Mo and W on ROS accumulation level, spectrophotometer experiments were performed (Figure 2).

The results showed that a significant increase in H_2O_2 and O_2^- levels was observed with W at concentrations of 2.5 and 5 mM compared to control plants. In addition, exposure to molybdenum (Mo) at concentrations of 2.5 and 5 mM and combined exposure to 2.5 and 5 mM Mo+W significantly increased the production of H_2O_2 and O_2^- compared to control plants.

Investigation of the effect of molybdenum and tungsten on aldehyde oxidase. To determine the activity of aldehyde oxidase (AO) and xanthine hydrogenase (XDH), native protein samples were separated on a 7.5% polyacrylamide gel

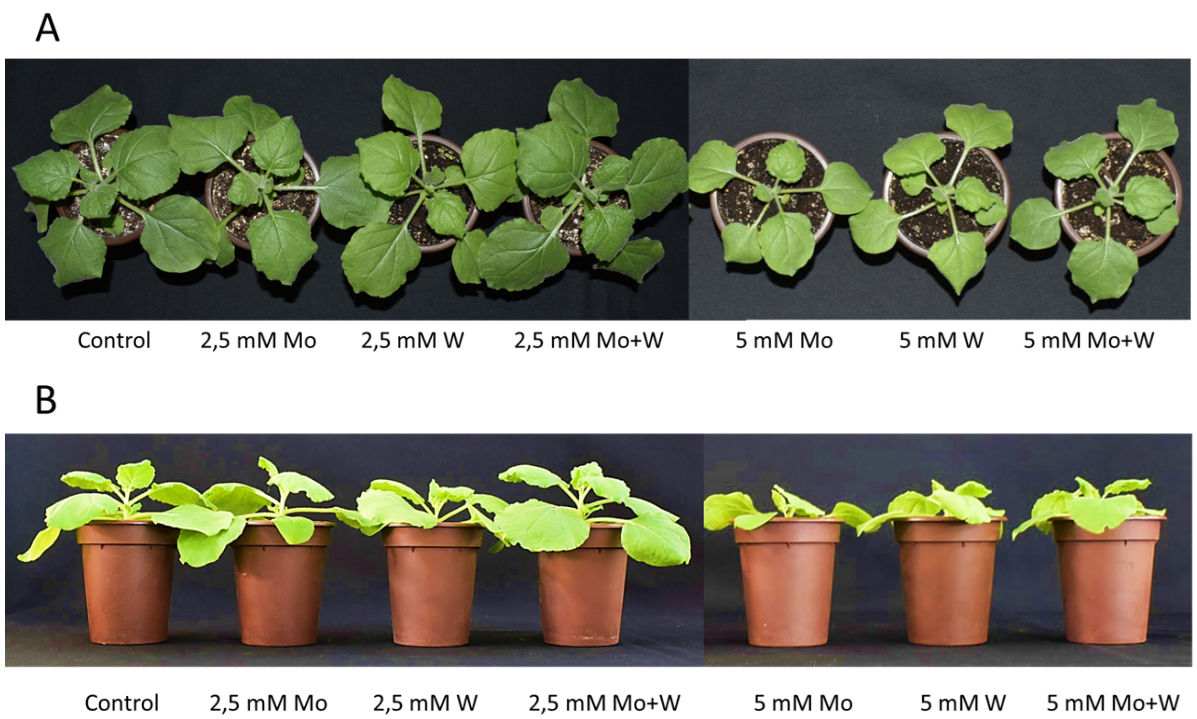

Figure 1 – Morphological effects of molybdenum (Mo), tungsten (W), and their combinations on plant growth. (A) Top-view photographs showing the growth of plants under control conditions and treatments with 2.5 mM Mo, 2.5 mM W, and 2.5 mM Mo + W, as well as 5 mM Mo, 5 mM W, and 5 mM Mo + W, (B) Side-view photographs of the same treatment groups illustrating differences in plant height and leaf development

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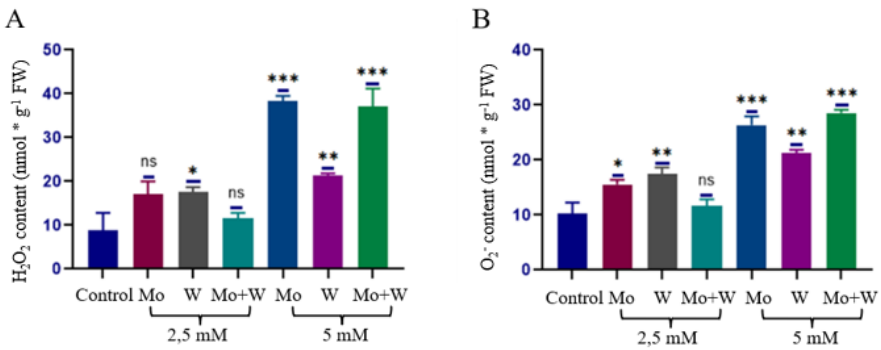

Figure 2 – Determination of ROS in *N. Benthamiana* under Mo and W exposure. (A) H_2O_2 content, (B) O_2^- content. Asterisks "*" - indicates significant ($P < 0.05$); "** and ****" - highly significant ($P < 0.01$ and 0.0001, respectively); "ns" - non-significant ($P > 0.05$) differences in the presented data. Data from three independent repeats were converted to numerical values (±SD) using statistical analyses (one-way Anova test, multiple comparisons) were performed using GraphPad Prism software (v.8.01)

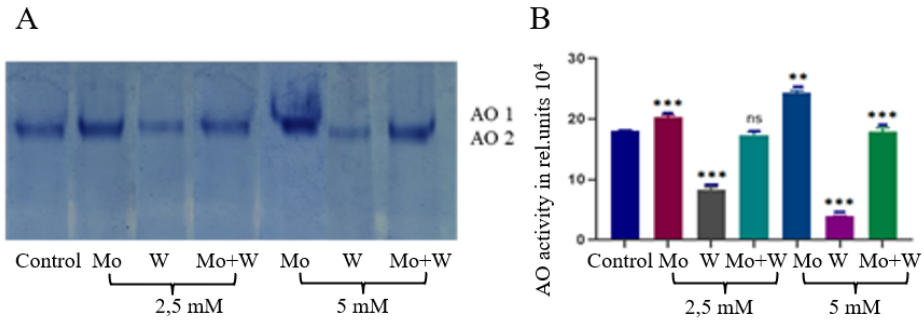

Figure 3 – Determination of AO activity by 7,5% native PAGE in *N. Benthamiana* upon exposure to Mo and W. (A) visualisation of AO activity, (B) graphical representation of AO activity. Asterisks "*" indicates significant ($P < 0.05$); "** and ****" - highly significant ($P < 0.01$ and 0.0001, respectively); ns - non-significant ($P > 0.05$) differences in the presented data. Data from three independent repeats were converted to numerical values (±SD) using ImageJ graphical editor, and statistical analyses (one-way Anova test, multiple comparisons) were performed using GraphPad Prism software (v.8.01)

and stained with substrate. The degree of activity was analysed using ImageJ software. Data were converted to percentage values.

Native electrophoresis on the resulting gels after incubation in the specific substrate revealed two isoforms of AO, AO1, AO2, respectively (Figure 3). When exposed to Mo at concentrations of 2.5 mM and 5 mM, significant AO activity appears compared to control plants. When exposed to W at concentrations of 2.5 mM and 5 mM, AO activity decreases markedly. When Mo+W was co-expressed at concentrations of 2.5 mM and 5 mM, an increase in AO activity was observed compared to the control plant. Moreover, under W, the AO1 isoform is activated to a small extent.

The appearance of the AO1 isoform in plants under Mo+W exposure confirms that molybdenum replaced tungsten in the active centre of AO, since it was previously reported in the literature that the addition of tungsten (W) inhibited AO activity.

Investigation of the effect of molybdenum and tungsten on xanthine dehydrogenase. The effect of Mo and W on xanthine dehydrogenase activity was studied using the same samples that were used to analyse AO activity.

XDH is an important molybdoenzyme involved in purine catabolism and ureide synthesis. Our studies showed that, when exposed to Mo at concentrations of 2.5 mM and 5 mM, XDH activity was slightly increased compared to the control plant. When W at concentrations of 2.5 mM and 5 mM was applied, XDH activity was significantly decreased. Interestingly, the activity of XDH increases when Mo+W is co-expressed at concentrations of 2.5 mM and 5 mM compared to W-treated plants (Figure 4).

Our studies confirmed that tungsten (W) in non-toxic concentrations has no inhibitory effect on XDH activity in plants. The detected XDH activity in plants can be explained by two main factors. Firstly, it is the use of harmless low concentrations of tungsten for plant treatment. Second, it is the process of post-translational activation of XDH. In fact, most of the XDH enzyme in plants is in an inactive form that can be activated post-translationally in response to specific stimuli.

Investigation of the effects of Mo and W on superoxide dismutase activity in *N. benthamiana*. Superoxide dismutase (SOD) is an enzyme with the ability to convert superoxide anions into hydrogen peroxide and molecular oxygen (O_2) molecules. The estimation of SOD activity by the in-gel method in experimental *N. benthamiana* plants was performed using the same samples used to analyse AO and XDH activities. Four isoforms of SOD were detected by native electrophoresis on the resulting gels after incubation in the specific substrate. When Mo at concentrations of 2.5 and 5 mM was affected, little SOD activity appears in the plants, whereas when Mo+W was co-expressed at concentrations of 2.5 and 5 mM, SOD ac-

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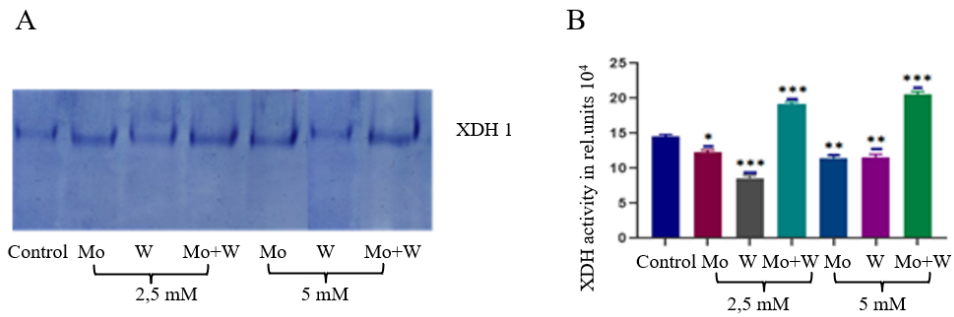

Figure 4 – Determination of XDH activity by 7,5% native PAGE in *N. benthamiana* upon exposure to Mo and W. (A) XDH isoforms are indicated by arrows to the right of the panels, (B) Graphical representation of XDH isoform activity. Asterisks "*" - indicates significant ($P < 0.05$); "** and ****" - highly significant ($P < 0.01$ and 0.0001, respectively); "ns" - non-significant ($P > 0.05$) differences in the presented data. Data from three independent repeats were converted to numerical values (±SD) using ImageJ graphical editor, and statistical analyses (one-way Anova test, multiple comparisons) were performed using GraphPad Prism software (v.8.01)

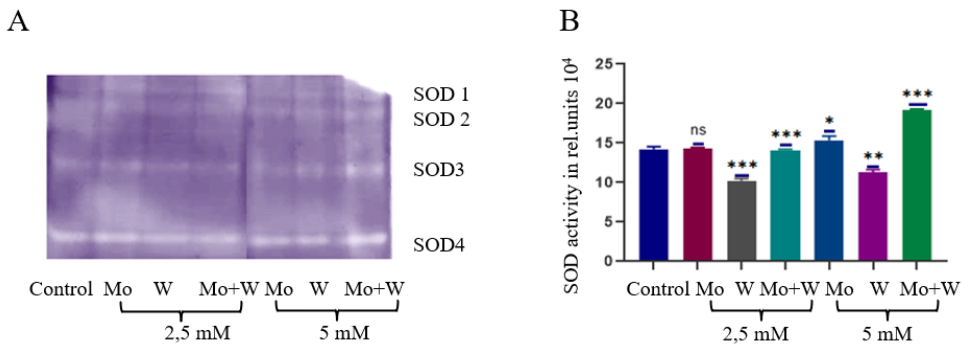

Figure 5 – Determination of SOD activity by 10% native PAGE in *N. benthamiana* upon exposure to Mo and W. (A) XDH isoforms are indicated by arrows to the right of the panels, (B) graphical representation of XDH isoform activity. Asterisks "*" - indicates significant ($P < 0.05$); "** and ****" - highly significant ($P < 0.01$ and 0.0001, respectively); "ns" - non-significant ($P > 0.05$) differences in the presented data. Data from three independent repeats were converted to numerical values (±SD) using ImageJ graphical editor, and statistical analyses (one-way Anova test, multiple comparisons) were performed using GraphPad Prism software (v.8.01)

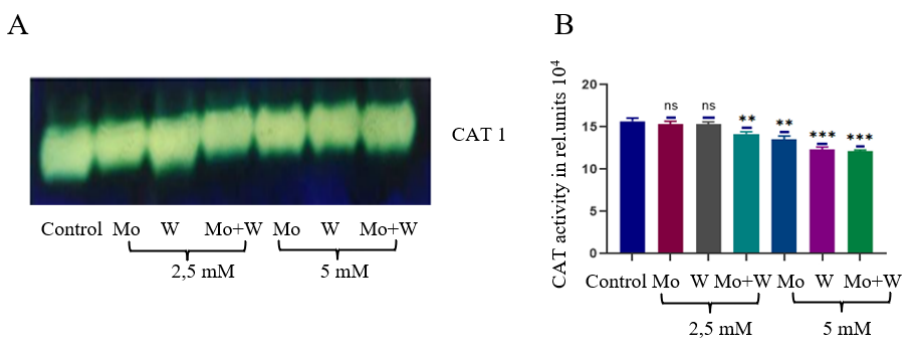

Figure 6 – Determination of CAT activity by 7,5% native PAGE in *N. benthamiana* upon exposure to Mo and W. (A) CAT isoform is indicated by arrow to the right of the panel, (B) graphical representation of CAT isoform activity. Asterisks "*" - indicates significant ($P < 0.05$); "** and ****" - highly significant ($P < 0.01$ and 0.0001, respectively); "ns" - non-significant ($P > 0.05$) differences in the presented data. Data from three independent repeats were converted to numerical values (±SD) using ImageJ graphical editor, and statistical analyses (one-way Anova test, multiple comparisons) were performed using GraphPad Prism software (v.8.01)

tivity was markedly increased. In addition, when exposed to W at concentrations of 2.5 and 5 mM in plants, SOD activity decreases in plants compared to the control plant (Figure 5).

Thus, it can be concluded that the joint exposure to Mo+W at concentrations of 5 mM negatively affects the activation of superoxide dismutase isoforms in *N. benthamiana* plants. Presumably, the reduced activation of superoxide dismutase isoforms in plants upon exposure to W can be interpreted as a strategy of this metal to reduce the level of hydrogen peroxide accumulation in plants by overcoming their defence mechanisms.

Investigation of the effects of molybdenum and tungsten on catalase activity in *N. benthamiana* plant. Catalase is one of the key antioxidant enzymes and is actively involved in the regulation of hydrogen peroxide content in plant tissues. Based on this, the present work also investigated the

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changes in catalase (CAT) activity upon exposure to heavy metals. To determine catalase activity, vertical electrophoresis under non-denaturing conditions was carried out using the same samples as in previous experiments for SOD and AO, XDH. The gel with samples was incubated alternately first in a weak hydrogen peroxide solution for 10 minutes, then in a two-component substrate consisting of potassium ferricyanide and ferric chloride solutions until the appearance of well-defined colourless bands characterizing catalase activity (Figure 6).

Under the influence of Mo at concentrations of 2.5 and 5 mM, CAT activity did not change compared to control plants. Under the influence of W at concentrations of 2.5 and 5 mM in plants, CAT activity increased slightly in comparison with control plants. Moreover, upon co-exposure to Mo+W at concentrations of 2.5 and 5 mM, CAT activity slightly decreased.

DISCUSSION

Effect of Molybdenum (Mo) and Tungsten (W) on morphometric parameters of plants. One of the experiments investigated the effect of Mo nanoparticles on *Nicotiana tabacum*. Two approaches were used: foliar sprays and root irrigation. Tobacco plants were treated to Mo NPs at varying concentrations (0-100 µg/mL) through root irrigation and foliar spray treatments on the 25th day of growth. The physiology and morphology of tobacco seedlings were then assessed. Mo NPs significantly increased the development of tobacco seedlings after 25 days of root irrigation treatment, whereas foliar spraying with Mo NPs had no significant effect on seedling growth [18]. Another study found that increasing molybdenum concentrations (0.1 mg kg^{-1} Mo, 0.2 mg kg^{-1} Mo, 0.4 mg kg^{-1} Mo, and 4 mg kg^{-1} Mo) resulted in a gradual increase in plant size. This dose-dependent growth enhancement suggests that molybdenum plays a positive role in promoting plant development, likely through its involvement in key physiological and metabolic processes [19]. Another study showed that molybdenum treatments (0.01-1 µM $(NH_4)_6Mo_7O_{24}$) in nutritional solutions caused only a minor decrease in shoot and root dry weights compared to the control trial findind no harmful consequences from molybdenum, even at its greatest dose [20].

As for W, based on previous studies, the first noticeable symptom of W poisoning was a general decrease in root and shoot development [21]. In our case, *N. benthamiana* also showed a decrease in plant size under the influence of tungsten. W dissolution is associated with a decrease in oxygen availability and hydrogen ion concentration in water and/or soil, which could explain why tungstate at plant toxicity levels causes delayed seedling growth. Other investigations have found that W, like most heavy metals, influences cell formation and elongation by breaking cortical microtubules, which inhibits root growth [22]. Interestingly, it has previously been documented that low soluble W concentrations can improve plant biomass production; however, this was only detected for shoot biomass in the acidic W 500 mg kg^{-1} clay, the soil with the lowest soluble W concentrations. That treatment resulted in slightly larger soybean nodule biomass and nodule fixation activity than the control soil [23].

Investigation of the effect of molybdenum and tungsten on aldehyde oxidase.A similar investigation on the effect of molybdenum in tobacco found that molybdenum treatment altered the expression of the AO gene. The control group had the lowest AO gene expression levels. Gene expression increases dramatically with molybdenum content, peaking at 0.2 mg kg^{-1} Mo, which has the highest relative expression level. In the 0.4 mg kg^{-1} Mo group, expression reduces slightly but remains high. At the maximum concentration, 4 mg kg^{-1} Mo group, expression reduces further, but remains above the control level [19].

In a previous study, it was observed that Mo application (1 µM $Na_2MoO_4*2H_2O$) raised AO activities in leaf tissues by 36.10%, 46.93%, and 29.34% in Mo-efficient cultivars, and by 64.70%, 88.56%, and 46.87% in Mo-inefficient cultivars under NO3⁻, NH4NO3, and NH4+ sources, respectively [24].

According to another study, using indole-3-carboxaldehyde as an AO substrate in barley resulted in the identification of one isoform in leaves. Increasing molybdenum concentrations (0.1, 0.5, 1 mM) increased enzyme activity significantly. The combination of both metals considerably increased leaf AO activity, outperforming molybdenum alone, which does not agree with our results, since the AO activity is slightly lower when Mo and W are co-exposed than when Mo is exposed separately [25].

In addition, according to the literature, the mechanism by which W exerted negative impact has not been thoroughly investigated, although widely evident flaws were linked to decreasing activity of the Mo-enzymes in response to increasing levels of tungstate. Within the cell, W effectively antagonizes and may replace Mo in the molybdenum cofactor (MoCo) of the Mo-enzymes [21]. In our case, we can observe the same thing: W strongly decreased the activity of AO.

Investigation of the effect of molybdenum and tungsten on xantidehydrogenase. In one of the previously mentioned studies, the effect of molybdenum on XDH gene expression in *Nicotiana tabacum* was investigated. XDH activity increased gradually with increasing Mo concentrations (0.1 mg kg-¹ Mo, 0.2 mg kg-¹ Mo, 0.4 mg kg-¹ Mo, and 4 mg kg-¹ Mo) [19].

Considering W, as previously stated, the activity of Mo enzymes is decreased according to the literature. As soon as W reaches the plant cell, it may have an unfavorable effect on certain targets. The first targets identified were Mo-enzymes, primarily due to W-Mo antagonism. Because of their similar physical and chemical properties, W and Mo have been integrated into the active sites of important enzymes throughout evolution, greatly increasing their catalytic diversity in biological systems [21].

Investigation of the effects of Mo and W on superoxide dismutase (SOD) activity in *N. benthamiana.* As noted in the earlier research about the effect of molybdenum nanoparticles on *Nicotiana tabacum*, SOD activity was also studied. At 0 µg/mL, SOD activity is the lowest for both treatment strategies (root irrigation and foliar spraying), acting as a control. SOD activity increases considerably as concentrations rise, particularly in the foliar spray group. SOD activity increases significantly at 25 µg/mL and 100 µg/mL, especially for foliar application [18]. Compared to our results, the SOD activity also increased with increasing Mo concentration. Considering the effect of W on SOD activity, the effect of different concentrations (0 to 60 mg/litre) in celery and pepper plants was examined. In both cases, it was possible to observe the

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activation of SOD with increasing concentration [22].

Investigation of the effects of molybdenum and tungsten on catalase (CAT) activity in *N. benthamiana* plant. Comparing the data obtained with the study on the effect of molybdenum particles on *Nicotiana tabacum*, different trends can be observed. In the above-mentioned study at the control concentration (0 µg/mL), both treatments exhibit moderate CAT activity, with the root-irrigation group showing slightly higher levels. At a concentration of 25 µg/mL, root irrigation considerably boosts CAT activity in plants, but foliar application has no noticeable effect. At 100 µg/mL, root irrigation leads to the maximum CAT activity, while foliar treatment shows just a small increase [18]. In the aforementioned study on the effect of W on antioxidant enzyme activity in pepper and celery plants, the effect of tungsten on CAT was also mentioned. When the concentration was increased from 0 to 60 mg/L, a direct relationship could be observed: increasing tungsten concentration increased catalase activity [22]. However, in another study, where broccoli was studied at concentrations of 0-100 mg/kg soil, a slight decrease in catalase activity could be observed with increasing concentration. CAT activity increased gradually with tungstate exposure up to 50 mg/kg soil, but decreased at 100 mg/kg soil relative to the control, indicating little involvement of CAT during W exposure [26]. In our case, the activity of catalase under Mo and W changed insignificantly and decreased with increasing concentration (2,5 mM and 5 mM) which is possibly due to the fact that the concentrations chosen in this study are too low and had a negligible effect on catalase activity.

CONCLUSION

The following main conclusions were drawn from the results of the research conducted within the framework of the project effect of molybdenum and tungsten on the activity of Mo-hydroxylases and the antioxidant system in plants.

Exogenous Mo application promotes accumulation of reactive oxygen species (ROS) such as H_2O_2 and O_2^-, and leads to increased activity of the antioxidant enzyme, superoxide dismutase (SOD) while catalase (CAT) activity changed insignificantly. In addition, Mo causes activation of the Mo- hydroxylases enzyme aldehyde oxidase (AO) whereas xanthine dehydrogenase (XDH) activity remained almost unchanged, indicating enhanced defence and metabolic processes in plants.

W, unlike Mo, has an inhibitory effect on the same enzymatic systems. Its application leads to a slight increase in the level of ROS, decrease in SOD and CAT activity, and reduces the activity of AO and XDH. This confirms its role as a functional Mo antagonist and Mo-hydroxylase inhibitor.

It was found that Mo is able to activate aldehyde oxidase isoforms (AO1 and AO2) and enhance the functioning of Mo-dependent redox enzymes. In contrast, tungsten suppresses these isoforms, indicating its negative effects on enzymatic activity and potential stress tolerance of plants.

Thus, the results demonstrate that Mo and W have opposite effects: molybdenum activates, and tungsten inhibits both the antioxidant system and Mo-hydroxylases. This emphasises the importance of the balance of trace elements in the regulation of metabolic and defence processes in plants.

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ВЛИЯНИЕ МОЛИБДЕНА И ВОЛЬФРАМА НА АКТИВНОСТЬ МО-ГИДРОКСИЛАЗ И АНТИОКСИДАНТНУЮ СИСТЕМУ РАСТЕНИЙ

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АБСТРАКТ

Современные исследования в области молекулярной биологии растений уделяют значительное внимание механизмам регуляции антиоксидантной системы. В данной работе мы изучили влияние тяжелых металлов молибдена и вольфрама на модуляцию Мо-гидроксилаз и функционирование антиоксидантной системы растений с использованием модельного организма *Nicotiana benthamiana.*

Целью исследования является анализ влияния молибдена и вольфрама на активность антиоксидантных ферментов (СОД, КАТ), а также Мо-гидроксилаз альдегидоксидазы (АО) и ксантиндегидрогеназы (КДГ). Используемые методы включают нативный гель-электрофорез и спектрофотометрию.

Основные результаты показывают, что применение молибдена способствует активации антиоксидантной системы растений за счет увеличения продукции активных форм кислорода (АФК) и активности фермента супероксиддисмутазы (СОД), в то время как активность каталазы изменилась незначительно. Также активность АО увеличилась, тогда как активность КДГ практически не изменилась. Напротив, внесение вольфрама снижало активность этих механизмов антиоксидантной защиты и ингибировало Мо-гидроксилазы.

Практическая значимость работы заключается в возможности разработки биотехнологических подходов к повышению устойчивости растений к абиотическим стрессам путем регуляции активности Мо-гидроксилаз. Полученные данные могут быть использованы в растениеводстве и сельском хозяйстве для повышения стрессоустойчивости сельскохозяйственных культур.

Ключевые слова: альдегидоксидаза, ксантиндегидрогеназа, *Nicotiana benthamiana*, АФК, молибден, вольфрам, тяжелые металлы

МОЛИБДЕН МЕН ВОЛЬФРАМНЫҢ ӨСІМДІКТЕРДЕГІ МО-ГИДРОКСИЛАЗАЛАРДЫҢ БЕЛСЕНДІЛІГІНЕ ЖӘНЕ АНТИОКСИДАНТТЫҚ ЖҮЙЕГЕ ӘСЕРІ

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АБСТРАКТ

Өсімдіктердің молекулалық биологиясы саласындағы қазіргі зерттеулер антиоксиданттық жүйенің реттелу механизмдеріне көп көңіл бөледі. Бұл жұмыста ауыр металдардың молибден мен вольфрамның Мо-гидроксилазалардың модуляциясына әсерін және *Nicotiana benthamiana* модельдік организмін пайдаланып өсімдіктердің антиоксиданттық жүйесінің қызметін зерттейміз.

Зерттеудің мақсаты молибден мен вольфрамның антиоксиданттық ферменттердің (СОД, КАТ), сондай-ақ альдегидоксидаза (АО) және ксантиндегидрогеназа (КДГ) Мо-ферменттерінің белсенділігіне әсерін талдау болып табылады. Қолданылатын әдістерге табиғи гельдік электрофорез және спектрофотометрия жатады.

Негізгі нәтижелер молибденді қолдану оттегінің белсенді түрлерінің (ОБТ) және супероксиддисмутазасының (СОД) ферменттерінің белсенділігін арттыру арқылы өсімдіктің антиоксиданттық жүйесінің белсендірілуіне ықпал ететінін көрсетеді, ал каталаза белсенділігі шамалы өзгерді. Сондай-ақ, АО белсенділігі өсті, ал КДГ белсенділігі дерлік өзгерген жоқ. Керісінше, вольфрамды қолдану осы антиоксиданттық қорғаныс механизмдерінің белсенділігін төмендетті және Мо-гидроксилазаларды тежеді.

Жұмыстың практикалық маңыздылығы Мо-гидроксилазалардың белсенділігін реттеу арқылы өсімдіктердің абиотикалық кернеулерге төзімділігін арттырудың биотехнологиялық тәсілдерін жасау мүмкіндігінде. Алынған мәліметтерді ауылшаруашылық дақылдарының стресске төзімділігін арттыру үшін өсімдік шаруашылығында және ауыл шаруашылығында пайдалануға болады.

Кілт сөздер: альдегидоксидаза, ксантиндегидрогеназа, *Nicotiana benthamiana*, ОБТ, молибден, вольфрам, ауыр металлдар