

THE INFLUENCE OF CERIUM DIOXIDE NANOPARTICLES ON SEED GERMINATION AND ACCUMULATION OF PLASTID PIGMENTS AND PHENOLIC COMPOUNDS OF SCOTS PINE SEEDLINGS (*Pinus sylvestris* L.)

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The aim of this work was to study the effect of cerium dioxide nanoparticles on the germination of Scots pine (*Pinus sylvestris*) seeds and the subsequent physiological changes in plastid pigments and phenolic compounds accumulation in the seedlings tissues. Brief soaking of pine seeds in solutions of cerium dioxide nanoparticles (2–4 nm) increases the germinating power and seed germination by 1.3 times. In pine seedlings, germinated from six month old seeds, reduction in chlorophyll *b*, phenols and phenylalanine content is observed in 14 days after treatment. Simultaneously, concentration of carotenoids and flavonoids increased. This indicates the overall decrease in phenylpropanoid synthesis. Hence, it is reasonable to assume that cerium dioxide nanoparticles, having entered the cells of Scots pine seeds, are engaged in the regulation of phenol and terpenoid synthesis in seedlings, which greatly affects the intensity of their growth and development.

Key words: Scots pine (*Pinus sylvestris* L.), cerium dioxide nanoparticles, germination of seeds, phenols, flavonoids, carotenoids.

One of the ways to improve the efficiency of production of planting material of valuable tree species is the use of biologically active substances (BAS) at the stage of presowing treatment of seeds [1, 2]. Nanoparticles of cerium dioxide that have strong antioxidant properties, deserve special attention among the BAS of new generation. Preparations based on cerium dioxide nanoparticles (CDN) have the potential to increase the resistance of plants to different adverse factors; however, their biocompatibility, environmental safety and effectiveness in low concentration are the subject of special studies. Physiological availability, pro- and antioxidant properties, the nature of the impact of nanoparticles on plant organism

as a whole are largely determined by the method of their preparation, their size, zeta potential and other physical and chemical characteristics. Detailed studies on various plant objects are required to determine the optimal concentrations of nanoparticles, their stress-protective action, translocation in plant tissues and localization in cells. It is known that the toxic properties of metals are strongly influenced by their degree of oxidation. It is shown that water-soluble compounds of cerium (III) are less toxic than its salts (IV). CDN have antibacterial effect *in vitro* against opportunistic microorganisms of different groups — clinical strains of *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans* [3].

CDN have a significant effect on important regulatory mechanisms, which control cell proliferation and survival in human astrocytes. There is an opinion that marked decrease of the expression of different transcription factors as well as growth regulatory factors by CDN represents its toxic effect. Specifically, the relation among endoplasmic reticulum stress-responsible genes expression, ERN1 signaling system function and cell proliferation processes in response to CDN action were studied [4].

In case of accumulation in the cells, the cytotoxicity of ceria appears in its ability to induce synthesis of reactive oxygen species (ROS). However, in tissues of rice discovered antioxidant activity of cerium dioxide in low concentrations [5]. The biological activity of ceria is largely determined by the proximity of ionic radii of Ce^{3+} and Ca^{2+} . This allows the cerium to partially substitute the calcium ions in the composition of biomolecules. However, the biological effect of ceria can deviate from normal dependence "dose — response". It is shown that CDN significantly inhibit the germination of seeds of *Lactuca sativa* L., *Cucumis sativus* L., *Solanum lycopersicum* L., *Spinacia oleracea* L. [6]. It is also established that CDN particles slowed the germination of *Raphanus sativus* L. seeds, but did not affect the germination rates. In field conditions the plants after the treatment showed accumulation of CDN in the roots, indicating its rather high mobility in plant tissues [7]. Intense movement and accumulation of cerium ions from the roots to the fruits discovered in experiments with tomatoes [8]. According to Zhang et al. [9], the intensity of lateral transfer is primarily dependent on particles size. In the experiment with *Cucumis sativus* seedlings 7 nm particles after joining to the conductive area of the root were transferred to all vegetative parts of the shoots. However, the question of the nanoparticles transport mechanism through tissue barriers of the roots remains open. The permeability of the cellular structures depends on the structural features of differentiated cells, the composition of primary and secondary cell walls, spatial orientation and the ratio of components of biopolymers. It is well known that impregnated with lignin cell walls have high adsorptive capacity for various metals. For this reason, the sawdust of wood is an effective adsorbent of cerium ions from aqueous solutions.

The findings have practical significance

in terms of environmental assessment of the potential risks associated with the migration of physiologically active elements in trophic chains of soil sphere. Literature data indicate the ambiguity of opinions about the effectiveness and appropriateness of the use of cerium nanoparticles in crop production practice.

Due to the high pro- and antioxidant activity of nanodispersed CDN that are potentially capable to affect the stress resistance factors, the purpose of our study was to investigate the effect of CDN on germination indices of pine seed and plastids pigments and phenolic antioxidants accumulation in the tissues of sprouts.

Materials and Methods

Plant growth conditions. Seeds of Scots pine (*Pinus sylvestris* L.) were used in the experiment. 0.1 M solution of CDN was used for seeds processing. The size of CeO_2 nanoparticles is from 2 to 4 nm. The hydrodynamic diameter of particles is ~7 nm, ζ -potential ~ -20 mV. The 0.1, 0.5, 1.0 and 1.5 ml of the stock solution of CeO_2 nanoparticles were added per 1 l of distilled water to prepare solutions with different concentrations of CDN.

Seeds treatment was conducted by dipping into the solution with appropriate concentration of CeO_2 nanoparticles for a few seconds. The control group of seeds was soaked in distilled water. After treatment, the seeds were transferred to a specialized device (Jacobsen seed propagation machine) for germination. The temperature regime for sprouting seeds was 22 ± 2 °C.

The accounting of germination energy and seed similarity was carried out in accordance with State Standard of Ukraine 8558: 2015 "Seeds of trees and shrubs. Methods of determining the sowing qualities (similarity, viability, benignity)".

Determination of chlorophylls and carotenoids amount. Quantitative determination of chlorophylls and carotenoids amount in methanol extracts of 6-month old pine seedlings were performed spectrophotometrically according to the formula [10]:

$$Ca \text{ (mg/ml)} = 16.72A_{665.2} - 9.16A_{652.4},$$

$$Cb \text{ (mg/ml)} = 34.09A_{652.4} - 15.28A_{665.2},$$

$$c \text{ (x + c) (mg/ml)} = (1000A_{700} - 1.63Ca - 104.96Cb)/221.$$

Measurement of optical density of the extracts was performed on a spectrophotometer Optizen Pop (South Korea).

Determination of the total content of phenols and phenolic antioxidants. Quantitative determination of the total concentration of phenolic compounds in the seedlings (v/v 1/10) was conducted using Folin-Ciocalteu reagent [11]. The calibration curve was constructed for gallic acid. Quantitative content of flavonoids was measured spectrophotometrically at $\lambda = 419$ nm. To conduct this experiment, 200 μ l of 0.1 M solution of aluminium chloride (AlCl_3), and 300 μ l of 1M sodium acetate (CH_3COONa) were successively added to 300 μ l of methanol extracts. Quercetin (Sigma, Germany) was used as standard to construct the calibration curve. The concentration of phenolic antioxidants in the extracts was determined by the method based on the use of free stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. Water-soluble vitamin E (Trolox) was used as standard to construct the calibration curve. 6 mg of vitamin ($M_{\text{Trolox}} = 250.29$) was dissolved in 2.4 ml of 80% ethanol for preparation of stock solution. The reaction mixture contained 0.25 ml of plant extract, 1.75 ml of 80% ethanol, 2 ml of 0.2 mM solution of DPPH. 80% ethanol was added to the control vials instead of phenol extract. The reaction started after the addition of DPPH solution. The vials were intensively shaken and left for 30 min in the dark at room temperature. The optical density of the reaction mixture was determined at a wavelength of 517 nm. Antioxidant activity of the extracts was expressed in μM Trolox EQ [11].

Determination of the content of free amino acids. Chromatographic separation of free amino acids was carried out on HPTLC Cellulose plate (Merck, Germany) in the butanol-acetic acid-water solvent system (v/v/v — 3/1/1). 0.3% solution of ninhydrin in butanol was used as visualization reagent. Semi-quantitative analysis of the content of free amino acids was performed by densitometric method [12] with the use of Sorbfil TLC Vidiodensitometer program Ver. 2.3.0.

Statistical Analysis. Statistical data processing was performed using specialized software Statistica 7.0. Sigma Plot 12.0 program was used for regression analysis, mathematical modeling of dynamics of the content of plastid pigments, phenolic compounds and antioxidant activity.

Biochemical analyzes of plant tissues were

performed in four biological replicates ($n = 4$) with a meaningful difference compared to control at the threshold of significance level $P < 0.05$.

Results and Discussion

After short-time soaking of seeds in CDN solutions the natural process of hydration started. To obtain direct physiological effects, nanoparticles have to pass through the tissue barrier of the seed coat (perisperm), which consists of several layers of differentiated cells. The middle layer of pine seeds perisperm consists from cells with relatively thick lignified shells. Its secondary cell wall is mainly charged negatively. Nanoparticles of 2–4 nm with negative ζ -potential (-20 mV) are capable to easily overcome the tissue barrier and to penetrate into the living tissues of the embryo and a haploid endosperm. This is evidenced by the dependence of the main indicators of seed germination on the solution concentration. Cells of the embryo and female gametophyte have fairly thin cell walls with a high content of pectins. Uronic acids in their composition are capable to bind with Ca^{2+} ions, and as far as cerium ions may replace calcium in biomolecules [13], it is possible that CDN are actively immobilized in pectin components of cell walls of the primary meristem and endosperm.

The ability of pectin to form ionic bonds with calcium is determined by many factors including methylation of uronic acids [14]. In young tissues the degree of methylation is usually higher. During maturation and gradual differentiation of cells that occurs in the tissues of pine seedlings with high activity of pectinmethylesterase, the content of methyl groups decreases. This creates conditions for binding of calcium ions and, consequently, cerium dioxide.

In accordance with the theory of water migration in plant tissues, the rate of translocation of unbound cerium dioxide nanoparticles in apoplast can ten times exceed their movement through the symplast. This effect is explained by the significantly smaller throughput of plasmodesma channels. Moving through the intercellular spaces at a relatively high speed, the nanoparticles can lose ζ -potential charge. This greatly reduces their chemical reactivity, however, increases the penetrating capacity. When the surface charge is close to neutral, nanoparticles easily overcome cellular barriers and integrate

into the phospholipid layer of membranes [13]. Particles of extremely small size (2–4 nm) relatively freely penetrate into the periplasmic space, then to the cytoplasm and compartments of cells. The fact that smaller particles have greater total reaction surface creates preconditions for their active interaction with organic acids and other chelating agents. In our experiments, physiological indicators of pine seeds monotonously increased after swelling of seeds in aqueous solutions of cerium dioxide in the range of concentration ranging from 1.0 to 10.0 μM . Regression analysis revealed correlation between the concentration of CDN, energy of germination ($R^2 = 0.99$) and germination rate ($R^2 = 0.97$) of seeds, which can be described by the formula:

$$f = y_0 + a \cdot x^b.$$

When the concentration of nanoparticles increased to 150.0 μM , the physiological indicators of seed's germination activity decreased (Table 1).

At low concentrations of CDN (10.0 μM) the content of chlorophyll *b* in seedlings reduced (Fig. 1).

Instead, the content of chlorophyll *a* in the tissues of pine seedlings did not significantly change. The established effect of the relative stability of the main chlorophyll content is consistent with the data obtained when processing beans [15] and tomatoes [16] with CDN solutions. At the same time, for the rice culture Rico et al. [17] showed the ability

of CDN to decrease chlorophyll *b* content in leaves. A similar trend has been identified for pine seedlings.

For supplementary plastid pigments positive biological effect was observed in a narrow interval of CDN concentrations ranging from 10.0 to 50.0 μM . The dependence of indicators of chlorophylls and carotenoids in the tissues of pine seedlings from the CDN concentrations were determined by the lognormal function, which is described by the equation:

$$y = y_0 + \frac{a}{x} \exp \left[-0.5 \left(\frac{\ln(x/x_0)}{b} \right)^2 \right].$$

The accuracy of empirical data approximation ($R^2 \sim 0.97$ and 0.99) by this formula is sufficient to use it as a mathematical model of evaluation the effects of nanoparticles on physiological processes in seeds and seedlings of pine. The coefficients in the equation can have the following biological meanings: y_0 — the concentration of pigments in the tissues of the seedlings in the initial stages of growing; a, b — empirical constants, the first of which determines the saturation of plastid pigments, and the second characterizes the dynamics of their content; x_0 ($x_0 \neq 0$) is a coefficient of pigment complex inertia, which determines its response to external and internal stimulation; x is the variable, that depends on the concentration of active agent.

Carotenoids and phenolic compounds are important components of antioxidant

Table 1. Physiological and biochemical indicators of seeds and seedlings of pine (*Pinus sylvestris* L.) after treatment with CDN ($n = 4$)

Conc.	Fl	Ph	AO	Chl <i>a</i>	Chl <i>b</i>	K	E	Grm
0 (control)	2.8 \pm 0.14	32.2 \pm 1.45	33.2 \pm 1.99	1.34 \pm 0.07	0.69 \pm 0.06	0.04 \pm 0.002	48 \pm 2.4	60 \pm 2.4
10	3.0 \pm 0.12	26.1 \pm 1.57*	31.7 \pm 1.27	1.28 \pm 0.08	0.46 \pm 0.02*	0.16 \pm 0.008*	62 \pm 3.1*	74 \pm 3.7*
50	3.4 \pm 0.15*	19.9 \pm 0.80*	18.2 \pm 0.82*	1.32 \pm 0.06	0.51 \pm 0.03*	0.13 \pm 0.006*	64 \pm 2.9*	76 \pm 3.4*
100	4.7 \pm 0.28*	27.8 \pm 1.39	26.4 \pm 1.45*	1.33 \pm 0.06	0.54 \pm 0.02*	0.10 \pm 0.005*	68 \pm 2.7*	82 \pm 4.9*
150	5.4 \pm 0.27*	24.5 \pm 1.35*	29.4 \pm 1.47*	1.34 \pm 0.06	0.62 \pm 0.03*	0.07 \pm 0.003*	60 \pm 3.6*	68 \pm 3.4*

Notes: hereinafter* — Significant differences in comparison with the control values, $P \leq 0.05$, Conc. — CDN preparation concentration, μM ; FL — flavonoids, mg/g; Ph — phenols, mg/g; AO — antioxidants, mg/g; Chl *a* — chlorophyll *a*, mg/g; Chl *b* — chlorophyll *b*, mg/g; K — carotenoids, mg/g; E — the energy of germination, %; Grm — germination rate, %.

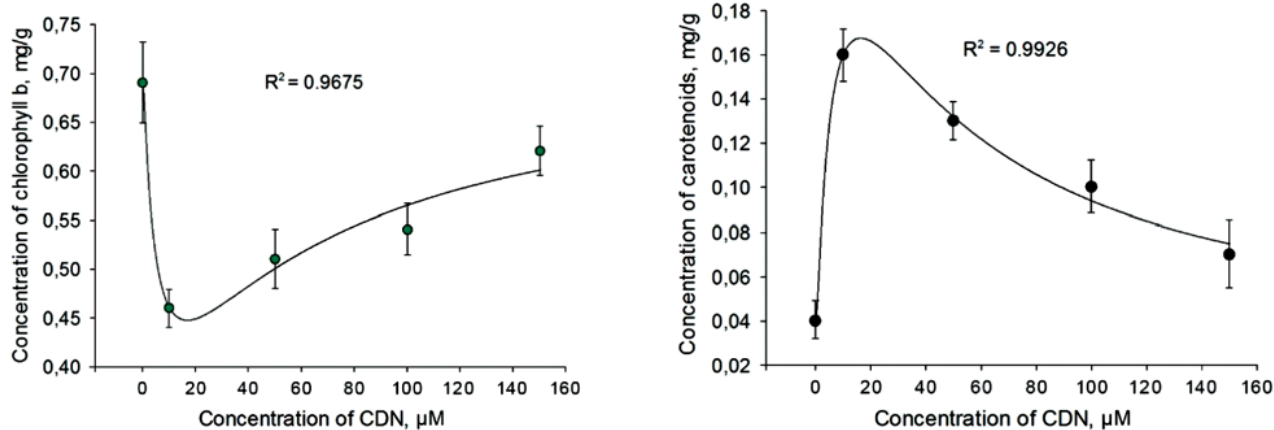


Fig. 1. The influence of CDN used in different concentrations on the content of chlorophyll b and carotenoids in the tissues of pine seedlings: hereinafter: $n = 4$

system that supports the stability of the pigment complex of photosynthetic apparatus in the cells. A significant part of simple phenols in higher plants is synthesized in the chloroplasts. They are optically active and able to perform photoprotective and antioxidant functions. Plant phenols are mainly generated by shikimate pathway. In angiosperm and gymnosperm species shikimate pathway genes are localized in the nuclear DNA and contain N-terminal signal polypeptide sequences that required for their transport into plastids. In the chloroplasts, where the formation of simple phenols takes place, the transport peptides are chipping off by special proteases, and basic polypeptides obtain typical for proteins appropriate spatial structure. This implies that the influence of the nanoparticles on the synthesis of phenols may be mediated in the cytoplasm through binding with the predecessors of enzymes at the stage of their transfer between compartments.

Key stage in phenylpropanoid synthesis in higher plants depends on the activity of phenylalanine-ammonia-lyase. This enzyme participates in deamination of L-phenylalanine with the formation of trans-cinnamic acid [18]. Ions of bivalent metals including Mg^{2+} , Fe^{2+} , Cu^{2+} , Zn^{2+} , Ca^{2+} are required as cofactors for proper functioning of the enzyme system of shikimate pathway. The latter may be partially replaced with cerium ions and, as a consequence, have a significant impact on the synthesis of phenolic compounds, and secondary metabolism in general. Suppression of phenylpropanoid synthesis was experimentally confirmed.

Thus, on the background of general suppression of the phenols accumulation, we observed a nonlinear increase in total antioxidant activity of plant tissues with increasing the concentration of CDN from 50.0 to 150.0 μM (Fig. 2).

This effect can be directly linked to the antioxidant properties of the particles themselves, as well as to their individual elements chelated by organic acids. The lack of monotony of a response function in increasing concentration gradient of CDN reflects the complexity of the effects of nanoparticles on cells and tissues of the seedlings.

The accumulation of large quantities of phenolic antioxidants associated with the synthesis of phenylalanine. In our experiments, chromatographic separation of amino acids contained in alcohol extracts of pine seedlings found lognormal dependence between the concentration of CDN and the content of phenylalanine (Fig. 3). A positive correlation ($r = 0.96$) between the content of proteinogenic amino acid and phenol substances in plant tissues confirmed the ability of CDN to influence the shikimate and phenylpropanoid pathways of synthesis in seedlings. Established pattern is also evident in the CDN concentrations ranging from 50.0 to 100.0 μM .

The narrow relationship between the amount of secondary metabolism products and concentration of CDN preparation was determined for the flavonoids, while the multiple correlation analysis revealed no significant relationship between the content of

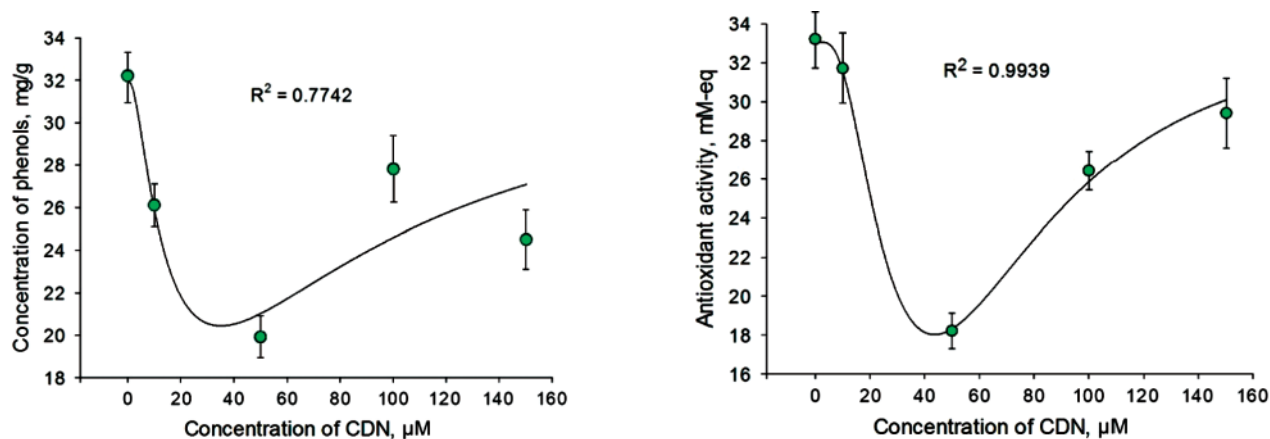


Fig. 2. The influence of CDN on the content of total phenols and phenolic antioxidants in the tissues of seedlings of pine (*Pinus sylvestris* L.)

Table 2. Correlation matrix of physiological and biochemical indicators of seeds and seedlings of pine (*Pinus sylvestris* L.) after treatment with CDN

	Concentration	Fl	Ph	AO	Chl a	Chl b	K	E	Grm
Concentration	1.00	0.98	-0.50	-0.60	0.21	0.00	0.00	0.40	0.40
Fl	1.00	1.00	-0.50	-0.60	0.21	0.00	0.00	0.40	0.40
Ph	-0.50	-0.50	1.00	0.70	0.36	0.50	-0.50	-0.30	-0.30
AO	-0.60	-0.60	0.70	1.00	0.31	0.40	-0.40	-0.80	-0.80
Chl a	0.21	0.21	0.36	0.31	1.00	0.97	-0.97	-0.56	-0.56
Chl b	0.00	0.00	0.50	0.40	0.97	1.00	-1.00	-0.60	-0.60
K	0.00	0.00	-0.50	-0.40	-0.97	-1.00	1.00	0.60	0.60
E	0.40	0.40	-0.30	-0.80	-0.56	-0.60	0.60	1.00	1.00
Grm	0.40	0.40	-0.30	-0.80	-0.56	-0.60	0.60	1.00	1.00

flavonoids and other physiological indicators of pine seedlings (Table 2). This fact confirms that the formation of these highly active substances from chalcone, which synthesized from p-cumaril-CoA and 3 molecules of malonyl-CoA under the action of chalcone synthase, retains some regulatory autonomy. The content of flavonoids, primarily in the mesophyll of cotyledons is determined by peculiarities of their synthesis and functionality.

Most of the known enzymes of flavonoid level are localized in the cytosol and endoplasmic reticulum [18], where, probably, large part of CDN is accumulated. On the basis of obtained data, CDN contribute to the synthesis of flavonoids in seedlings of pine (Fig. 4).

It is known that CDN after prolonged contact with water gradually change the value of ζ -potential due to the replacement

of protons absorbing on their surface by OH-groups [19]. The process of deproteinization of catechuic flavonol hydroxyls and, consequently, the formation of its complexes with metal facilitates under the condition of high content of hydroxyl groups on the surface of CDN.

The opposite effects of the influence of CDN on the content of carotenoid seedlings and phenolic compounds in the tissues are interesting. These substances belong to the two most important groups of secondary metabolites. They are synthesized in various ways from the products of primary synthesis. Terpenes, which include carotenoids, are derivatives of isoprenes. They are formed from mevalonic acid, pyruvate and D-glyceraldehyde-3-phosphate. Phenols are synthesized mainly along the shikimate biosynthetic pathway from phenylalanine [18].

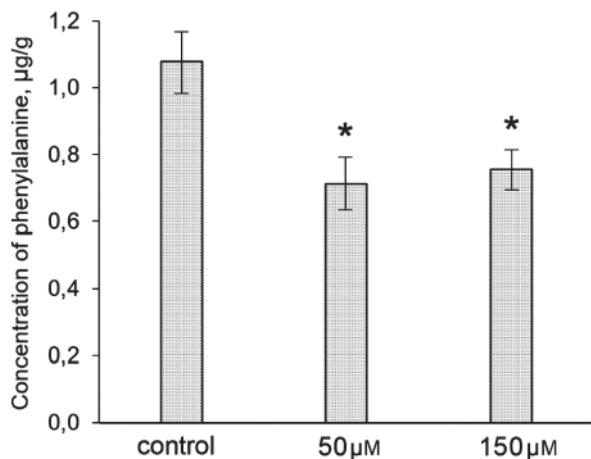


Fig. 3. The effect of CDN on the content of phenylalanine in the tissues of seedlings of pine (*Pinus sylvestris* L.)

Reduction in the content of phenylalanine and phenol in the tissues may indicate a reduction of the concentration of shikimic acid and its precursors in the cells: phosphoenolpyruvate and erythrose-4-phosphate.

Phosphoenolpyruvate is formed in the final stages of glycolysis. pyruvate — a precursor in the synthesis of terpenoids and flavonoids — is formed from it by substrate phosphorylation. CDN is able to catalyze dephosphorylation of substrates [13] and, possibly, to reduce the content of phosphoenolpyruvate in plant cells. For this reason CDN can have a significant effect on plants with C4 type of photosynthesis, in which phosphoenolpyruvate participates in carbon binding during photosynthesis. It is known, for example, that even a slight increase in CDN concentration leads to a slowing of the growth of roots and stems of corn [20]. Another factor in the supposed increase in glycolysis intensity is the ability of CDN to reversibly bind to phosphate groups [21] and, possibly, partially block the formation of ATP. The activity of phosphofructokinase-1, involving glucose-6-phosphate in the oxidative cycle, depends from the level of its content in cells. The increase in germination and seed germination rates observed by us can also be explained by the active glucose oxidation.

With glycolysis a linear increase in the content of flavonoids in pine seedlings is evidently associated with seed treatment with an increasing concentration of the CDN solution. Unlike most plant phenols, the synthesis of flavonoids is associated with an

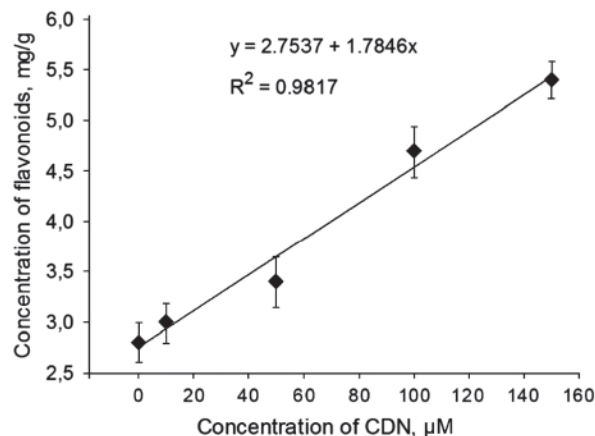


Fig. 4. The effect of CDN on the content of flavonoids in the tissues of seedlings of pine (*Pinus sylvestris* L.)

acetate-malonate route, the starting product of which is acetyl-CoA. This compound is formed in the mitochondria during the decarboxylation of pyruvate and is also associated with glycolysis.

So the rate of germination and sprouting of pine seeds increased in 1.3-1.4 times after short-time soaking of seeds in solutions of 2–4 nM CDN with negative z-potential used in concentrations ranging from 10.0 to 100.0 µM. When the concentration of CDN increased to 150.0 µM, these indicators decreased by 10–20%.

A single treatment of pine seeds with increased concentration of CDN solution resulted in non-monotonic change of content of chlorophylls, phenolic compounds and antioxidants in seedlings, which is described by lognormal function.

So, CDN significantly affects the processes of secondary metabolism of Scots pine sprouts. CDN stimulates the accumulation of carotenoids and flavonoids, the synthesis of which is closely related to the content of pyruvate and acetyl-CoA in cells. This significantly reduces the content of phenylalanine and phenols, which are formed by shikimat biosynthetic pathway by condensation of phosphoenolpyruvate and erythrose-4-phosphate.

REFERENCES

- Melnichuk M. D., Pinchuk A. P., Lihanov A. F., Maurer V. M., Klyuvadenko A. A. Influence of preparation of planting material on adaptive potential of pine seedlings (*Pinus sylvestris* L.). *Bioresursy i pryrodokorystuvannia*. 2013, 5(5–6), 92–98. (In Ukrainian).
- Savchenko Yu. M., Grigoryuk I. P., Lihanov A. F., Pinchuk A. P. The protective effect of the derivative N-oxide-2-methylpyridine on temperature stress in germination of pine seeds *Zbalansovane pryrodokorystuvannia*. 2016, 2, 83–88. (In Ukrainian).
- Babenko L. P., Zholobak N. M., Shcherbakov A. B., Voychuk S. I., Lazarenko L. M., Spivak M. Ya. Antibacterial activity of cerium colloids against opportunistic microorganisms *in vitro*. *Microbiol. J.* 2012, 74(3), 54–63.
- Minchenko D. O., Spivak M. Y., Herasymenko R. M., Ivanov V. K., Tretyakov Y. D., Minchenko O. H. Effect of cerium dioxide nanoparticles on the expression of selected growth and transcription factors in human astrocytes. *Mat.-wiss. u. Werkstofftech.* 2013, 44(2–3), 156–160. doi: 10.1002/mawe.201300091.
- Liu D. The effects of cerium on the growth and some antioxidant metabolisms in rice seedlings. *Environ. Sci. Pollut. Res.* 2012, 19(8), 3282–3291.
- Garcia A., Espinosa R., Delgado L., Casals E., Gonzalez E., Puntès V., Barata C., Font X., Sanchez A. Acute toxicity of cerium oxide, titanium oxide and iron oxide nanoparticles using standardized tests. *Desalination*. 2011, 269, 136–141.
- Corral-Diaz B., Peralta-Videa J. R., Alvarez-Parrilla E., Rodrigo-García J., Isabel Morales M., Osuna-Avila P., Niu G., Hernandez-Viezcás J. A., Gardea-Torresdey J. L. Cerium oxide nanoparticles alter the antioxidant capacity but do not impact tuber ionome in *Raphanus sativus* (L.). *Plant Physiol. Biochem.* 2014, V. 84, P. 277–285.
- Wang Q., Ma X., Zhang W., Peia H., Chen Y. The impact of cerium oxide nanoparticles on tomato (*Solanum lycopersicum* L.) and its implications for food safety. *Metallomics*. 2012, V. 4, P. 1105–1112. doi: 10.1039/C2MT20149F.
- Zhang Z., He X., Zhang H., Ma Y., Zhang P., Ding Y., Zhao Y. Uptake and distribution of ceria nanoparticles in cucumber plants. *Metallomics*. 2011, 3(8), 816–822. doi: 10.1039/C1MT00049G.
- Handbook of Food Analytical Chemistry: Pigments, Colorants, Flavors, Texture, and Bioactive Food Components. Ed. by E. Wrolstad. *Pigments and Colorants*. 2005, V. 4, P. 175–176.
- Sibgatullina G. V., Haertdinova L. R., Gumerova E. A. Methods for determining the redox status of cultured plant cells. Kazan: Kazanskiy (Privolzhskiy) Federalnyy universitet. 2011, 61. (In Russian).
- Heathcote J. G., Haworth C. The direct determination of amino acids on thin-layer chromatograms by densitometry. *Biochem. J.* 1969, 114, 667–668.
- Scherbakov A. B., Ivanova O. S., Spivak N. Ya., Kozik V. V., Ivanov V. K. Synthesis and biomedical applications of nanodispersed cerium dioxide. *Tomsk: Izd. Dom Tomskogo gos. un-ta*. 2016, 476 p. (In Russian).
- Gorshkova T. A. Plant cell wall as a dynamic system. *Moskva: Nauka*. 2007, 426 p. (In Russian).
- Majumdar S., Peralta-Videa J. R., Bandyopadhyay S., Castillo-Michel H., Hernandez-Viezcás J. A., Sahi S., Gardea-Torresdey J. L. Exposure of cerium oxide nanoparticles to kidney bean shows disturbance in the plant defense mechanisms. *J. Hazard. Mater.* 2014, V. 278, P. 279–287.
- Wang Q., Ebbs S. D., Chenc Y., Ma X. Trans-generational impact of cerium oxide nanoparticles on tomato plants. *Metallomics*. 2013, V. 5, P. 753–759. doi: 10.1039/C3MT00033H
- Rico C. M., Hong J., Morales M. I., Zhao L., Barrios A. C., Zhang J. Y., Peralta-Videa J. R., Gardea-Torresdey J. L. Effect of cerium oxide nanoparticles on rice: a study involving the antioxidant defense system and *in vivo* fluorescence imaging. *Environ. Sci. Technol.* 2013, V. 47, P. 5635–5642. doi: 10.1039/C3MT00033H
- Zaprometov M. N. Phenolic compounds. Distribution, metabolism and function in plants. *Moskva: Nauka*, 1993, 272 p. (In Russian).
- Vincent A., Inerbaev T. M., Babu S. Tuning Hydrated Nanocerium Surfaces: Experimental/Theoretical Investigations of Ion Exchange and Implications in Organic and Inorganic Interactions. *Langmuir*. 2010, V. 26, P. 7188–7198. doi: 10.1021/la904285g
- Diatloff E., Smith F. W., Asher C. J. Effects of lanthanum and cerium on the growth and mineral nutrition of corn and mungbean. *Ann. Botan.* 2008, V. 101, P. 971–982.
- Kuchma M. H., Komanski C. B., Colon J., Teblum A., Masunov A., Alvarado B., Babu S., Seal S., Summy J., Baker C. H. Phosphate ester hydrolysis of biologically relevant molecules by cerium oxide nanoparticles. *Nanomedicine*. 2010, V. 6, P. 738–744.

ВПЛИВ НАНОЧАСТИНОК ДІОКСИДУ ЦЕРІЮ НА ПРОРОСТАННЯ НАСІННЯ ТА НАКОПИЧЕННЯ ПЛАСТИДНИХ ПІГМЕНТІВ І ФЕНОЛЬНИХ СПОЛУК У ПРОРОСТКАХ СОСНИ ЗВИЧАЙНОЇ (*Pinus sylvestris* L.)

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Метою роботи було дослідження впливу наночастинок діоксиду церію на проростання насіння з подальшими фізіологічними змінами в накопиченні пластидних пігментів та фенольних сполук у тканинах проростків сосни звичайної (*Pinus sylvestris*). Встановлено, що короткочасне замочування насіння сосни у розчинах наночастинок діоксиду церію (2–4 нм) збільшувало його схожість та енергію проростання в 1,3 раза. У проростках, що отримали з шестимісячного насіння, через 14 діб після їх оброблення спостерігалось зниження вмісту хлорофілу *b*, фенолів та фенілаланіну. Одночасно збільшувалася концентрація каротиноїдів і флавоноїдів. Це вказує на загальне зниження активності фенілпропаноїдного синтезу. На цій підставі зроблено припущення, що наночастинки діоксиду церію, які входять у клітини насіння сосни звичайної, у подальшому беруть участь у регуляції процесів синтезу фенолів і терпеноїдів у проростках, що відповідно впливає на інтенсивність їхнього росту та розвитку.

Ключові слова: сосна звичайна (*Pinus sylvestris* L.), наночастинки діоксиду церію, проростання насіння, феноли, флавоноїди, каротиноїди.

ВЛИЯНИЕ НАНОЧАСТИЦ ДИОКСИДА ЦЕРИЯ НА ПРОРАСТАНИЕ СЕМЯН И НАКОПЛЕНИЕ ПЛАСТИДНЫХ ПИГМЕНТОВ И ФЕНОЛЬНЫХ СОЕДИНЕНИЙ В ПРОРОСТКАХ СОСНЫ ОБЫКНОВЕННОЙ (*Pinus sylvestris* L.)

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Целью работы было исследование влияния наночастиц диоксида церия на прорастание семян с последующими физиологическими изменениями в накоплении пластидных пигментов и фенольных соединений в тканях проростков сосны обыкновенной (*Pinus sylvestris*). Установлено, что кратковременное замачивание семян сосны в растворах наночастиц диоксида церия (2–4 нм) увеличивало их всхожесть и энергию прорастания в 1,3 раза. В проростках, полученных из шестимесячных семян, через 14 дней после их обработки наблюдалось снижение содержания хлорофилла *b*, фенолов и фенилаланина. Одновременно увеличивалась концентрация каротиноидов и флавоноидов. Это указывает на общее снижение активности фенилпропаноидного синтеза. На этом основании сделано предположение, что наночастицы диоксида церия, которые входят в клетки семян сосны обыкновенной, в дальнейшем принимают участие в регуляции процессов синтеза фенолов и терпеноидов в проростках, что соответственно влияет на интенсивность их роста и развития.

Ключевые слова: сосна обыкновенная (*Pinus sylvestris* L.), наночастицы диоксида церия, прорастание семян, фенолы, флавоноиды, каротиноиды.