Response of plants and nitrogen-fixing symbiosis to the toxicity of cadmium and mercury using the pea mutant SGECD¹

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Key message. The combined effect of Hg and Cd on the growth, elemental composition, root exudation and interactions with rhizobia of pea SGE and its mutant SGECD¹ was studied in hydroponics and sand. The tolerance mechanisms of legume-rhizobia symbiosis to heavy metals are discussed.

Keywords: heavy metals, nitrogen fixation, nodulation, *Pisum sativum*, symbiosis

Heavy metals (HM) cadmium (Cd) and mercury (Hg) are among the most widespread and toxic soil pollutants, that inhibits plant growth and microbial activity. Legume plants are relatively sensitive to heavy metals. Although plant mutants with altered metal tolerance and accumulation have been used to study physiological mechanisms regulating these traits, using these plants to study the role of microorganisms in plant-HMs interactions and phytoremediation of polluted soils is limited. In this respect, the pea (*Pisum sativum* L.) mutant SGECD¹ with increased Cd tolerance and decreased Hg tolerance (Belimov et al., 2016) is of particular interest.

The previously developed gnotobiotic method of cultivating plants in open plastic vessels with forced aeration by sparging was used to study the combined effect of Hg and Cd on the growth, elemental composition and root exudation. The content of Cd, Hg and nutrients in the obtained plant samples was carried out using an ICPE-9000 emission spectrometer. The total nitrogen content in plants was determined using a Kjeltac 8200 automatic analyzer. The effect of HMs on the root exudation was studied using the UPLC Waters ACQUITY H-class system. Pot experiment was conducted in which plants were inoculated with nodule bacterium and grown in vessels containing washed silica sand enriched with Cd and Hg.

Inhibition of SGE root growth by Cd was approximately 2 times stronger than that of the SGECD¹ mutant. Mercury treatment did not affect the biomass of SGE roots, but reduced the biomass of SGECD¹ roots by 2 times. Combined treatment with Cd and Hg of plants did not have an additive or synergistic effect on root biomass of both genotypes. The additive effect of Cd and Hg appeared only on the SGECD¹ shoots. The Cd content in the roots and shoots of the mutant was higher than that of SGE. The Hg content was also higher in the roots of the mutant. Mercury prevented Cd from entering the roots and transporting this metal into the shoot of the mutant. Cadmium treatment of plants led to a decrease in the content of most nutrients in the roots and shoots, and this effect was manifested to a greater extent for SGE compared to SGECD¹. The negative effect of Hg on the consumption of nutrients by roots and their transport to the shoot was found in mutant plants. Important and original observations seem to be the opposite in the action of Cd and Hg on genotypic differences in the transport of elements from the root to the shoot and the leveling of these differences under combined stress.

The treatment with Cd significantly increased the exudation of several amino acids, sugars and organic acids. This effect was manifested to a greater extent in the mutant SGECD¹. Stimulation of the exudation of amino acids was also found in Hg treatment to a greater extent in wild-type SGE. The combined action of Cd and Hg did not lead to a further increase in exudation. Cluster dendrograms were constructed showing the similarity and differences of the exudation profiles by roots SGE and SGECD¹. The obtained results indicate various mechanisms of exudation of the studied fractions, while the most pronounced differences are observed between the fractions of amino acids or sugars with organic acids.

Enrichment of sand with Cd reduced the biomass of roots and shoots in control and inoculated plants of only wild-type SGE, and Hg reduced only the biomass of shoots of inoculated plants SGECD¹. Cadmium reduced the nodule biomass in the wild-type SGE, and mercury reduced the nodule biomass in the mutant SGECD¹. The combined effect of metals on the biomass of nodules was more negative than the effect of individual metals. The nitrogen fixation activity of plants also decreased. The results suggest the ability of the SGECD¹ to actively transport nitrogen from the nodule to the root and further to the shoot under stressful conditions. It was established that SGECD¹ has mechanisms to maintain the effective functioning of the nodule under stress. The reaction of the SGECD¹ mutant and its symbiotic apparatus to stress caused by the toxicity of Hg and the combination of Hg and Cd was characterized, and valuable information was obtained on the mechanisms of resistance of legume-rhizobial symbiosis to heavy metals.

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