PLANT-MICROBE SYMBIOTIC SYSTEM BASED ON CADMIUM TOLERANT PEA MUTANT, MYCORRHIZAL FUNGUS, RHIZOBIA AND PGPR

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Introduction

Plants are inextricably linked with symbiotic microorganisms that enhance growth and nutrition of plants and increase their adaptation to adverse environmental conditions, including contamination of soils with heavy metals (HM). In particular, many symbiotic bacteria contain the enzyme ACC deaminase, which increases plant resistance to abiotic stresses by reducing biosynthesis of phytohormone ethylene (Glick et al., 2007). Legumes most actively form various symbioses with microorganisms and can play a special role to restore fertility of HM-contaminated soils (Safronova et al., 2011). However, application of legumes for phytoremediation constrained by low HM tolerance and accumulation. The aim of our research was to create symbiotic plant-microbial system, consisting of a legume plant and a complex of symbiotic microorganisms, with increased adaptive capacity to restore healthy ecosystems.

Methods

The subjects were: the Cd-tolerant/Cd-accumulating pea (*Pisum sativum* L.) mutant SGECd^t (Tsyganov et al., 2007), Cd-tolerant Indian mustard (*Brassica juncea* (L.) Czern.) genotype VIR263 (Belimov et al., 2007) and association of specially-selected Cd-tolerant symbiotic microorganisms: arbuscular mycorrhizal fungus *Glomus* sp. 1Fo, ACC deaminase containing nodule bacterium *Rhizobium leguminosarum* bv. *viciae* RCAM1066 and PGPR *Variovorax paradoxus* 5C-2. Pot experiments were carried out in a greenhouse with natural lighting and temperature in summer (June-August, St. Petersburg). Plants were grown in pots containing sod-podzolic light loamy soil fertilized with $^{15}NH_4{}^{15}NO_3$ and supplemented with 15 mg Cd/kg as $CdCl_2$. Plant biomass, mycorrhizal structures in roots, nodule number, root colonization by *V. paradoxus* 5C-2, N_2-fixation by acetylene-reduction assay), total N and ^{15}N content, total Cd and nutrient elements (P, K, Mg, S, Ca, Fe, Zn, Mn, Na, Cu, Ni, Cr, Co) contents were determined.

Results

In the presence of Cd a better formation and functioning of symbiotic structures (development of mycorrhizae, nitrogen-fixing nodules and colonization of roots by *V. paradoxus* 5C-2) was observed on SGECd^t as compared to wild type pea. Inoculation of both pea genotypes with the mixture of microorganisms increased biomass by 2-3 times and improved assimilation of nutrients along with removal of Cd from soil. Biomass and Cd accumulation by SGECd^t was about 60% more than the wild type pea. Growth response of Indian mustard (which does not form mycorrhizal and rhizobial symbioses) to inoculations was not significant and did not affect accumulation of Cd by plants. The proposed symbiotic system significantly increases the adaptation of legume plant to toxic Cd, making it comparable in growth and phyto-extracting capacity with a known Cd-accumulating plant species *Brassica juncea.* An additional advantage of this system was the enrichment the soil with beneficial microorganisms.

Conclusion

The observed effects on growth, mineral nutrition and Cd accumulation by the studied legume-microbe symbiotic model were due to a combination of genetic modification of the plant and selection of Cd-tolerant and efficient symbiotic microorganisms. The results can be used to develop approaches for creation of efficient environmentally friendly, resource- and energy-saving technologies of phytostabilization and restoration healthy ecosystems. The financial support by the RFBR (09-04-01614-a and 12-04-01501-a), MES RF (16.512.11.2162) and the RSF (14-16-00137) is greatly appreciated.

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