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# PREPARATION OF TRANSGENIC PLANTS WITH ENHANCED HEAVY METAL ACCUMULATION

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## Abstract

Phytoremediation has among bioremediation techniques the highest potential for decontamination of heavy metals. This method uses plants for accumulation, transformation or degradation of organic and inorganic pollutants. Plants exploitable to decontaminate inorganic pollutants have to take up, transfer and accumulate high concentrations of metals from the contaminated soil into harvestable shoots. To make the process effective, these plants should form also a large amount of biomass. To find suitable plant species fitting this requirement can be solved by preparation of transgenic plants overexpressing proper binding domains.

The aim of this work is to prepare genetically modified plants of *Linum usitatissimum* with increased ability to bind heavy metals and plants of *Nicotiana tabacum* containing genetic elements allowing remediation of environment contaminated by both inorganic and organic pollutants together. *CUP1* gene encodes a yeast protein metallothionein known for high affinity to heavy metals. In order to increase metal accumulation ability, the gene *CUP1* was fused with gene for an additional metal binding domain. In this case a polyhistidine tail was the peptide of choice yielding the construct *HisCUP*. With the aim to prepare transgenic plants containing *HisCUP* gene two plant vectors were prepared: (1) using plasmid pNOV2819 (Syngenta) containing a gene for phosphomannose isomerase enabling selection of transgenic plants on medium with mannose, in this vector *HisCUP* gene was cloned under the control of RUBISCO promoter; (2) using plasmid pGreen0019 enabling selection of transgenic plants on medium with antibiotic kanamycin. In this case *HisCUP* gene was cloned under the control of constitutive CaMV 35S promoter. First the transient expression in tobacco plants was accomplished and the presence of mRNA of *HisCUP* gene in plant tissue was confirmed. Further plants of *Linum usitatissimum* AGT-952 were transformed by prepared vectors with *HisCUP* gene using agrobacterial infection. The presence of *HisCUP* gene was confirmed by PCR in eight plant regenerants grown in medium with kanamycin.

With the aim to prepare plants able to remove simultaneously organic and inorganic pollutants, *HisCUP* gene was inserted into the genome of the transgenic tobacco plants containing *bphC* gene. *BphC* gene encodes bacterial enzyme 2,3-dihydroxybiphenyl-1,2-dioxygenase involved in bacterial degradation pathway of PCBs (polychlorinated biphenyls). Transgenic plants expressing *bphC* gene together with *HisCUP* gene thus could solve the problem of mixed soil contamination.

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