

PREPARATION OF TRANSGENIC FLAX WITH YEAST GENE FOR INCREASING HEAVY METAL ACCUMULATION

PŘÍPRAVA TRANSGENNÍCH LNŮ OBSAHUJÍCÍCH GEN PRO ZVÝŠENOU AKUMULACI TĚŽKÝCH KOVŮ

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Abstract:

In nature exist plants able to accumulate heavy metals in high concentration. These plants have very often slow growth, produce small amount biomass and therefore they are not suitable for phytoremediation. This problem could be solved by preparation of transgenic plants with appropriate properties.

The yeast CUP gene encoding yeast protein metallothionein has high affinity to heavy metals and therefore it was chosen for cloning into plant genome of flax (*Linum usitatissimum*). Plasmid pNOV2819 (Syngenta) containing gene for enzyme phosphomannose isomerase (for selection of transgenic plants) was chosen as plant cloning vector. Cassette RbcS with promoter (P-RbcS) and terminator (T-RbcS) of the RUBISCO enzyme was first cleaved out of plasmid pIV and then inserted into plasmid pNOV2819. HisCUP gene (CUP with histidine tail) was amplified by PCR from plasmid pTrcHisCUP, formerly used for preparation of transgenic tobacco plants. HisCUP gene was then cloned into prepared plasmid pNOV2819/RbcS. Afterwards, the engineered plasmid pNOV2819/RbcS/HisCUP was transferred to bacteria *Agrobacterium tumefaciens* C58-C1 (pCH32). The possible expression of HisCUP gene in plant of *Nicotiana tabacum* was studied by transient expression using *A. tumefaciens* C58-C1 (pCH32) harboring pNOV2819/RbcS/HisCUP. Metallothionein with histidine tail was isolated from plant tissue by affinity chromatography and then it was detected immunochemically by commercial antibody against histidine tail. Finally the transformation of flax was performed via agrobacterial infection. Regenerated plants are nowadays growing on selective medium with mannose as a selective agent.

Keywords:

phytoremediation, metallothionein, transgenic plant, gene HisCUP, phosphomannose isomerase

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