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Heavy metal resistance in transgenic plants

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Abstract. because of increasing in population and urban development, Heavy metals like lead, mercury, iron, cadmium, aluminum and magnesium are present in different sources There are hundreds of sources of heavy metal pollution, including the coal, natural gas, paper, and chlor-alkali industries. Toxic heavy metals in soil and water are global problems that are a growing threat to the environment. To overcome this problem, plants possess a variety of potential cellular mechanisms that are involved in the detoxification of heavy metals and tolerance to metal stress. In the pants exposed to excessive levels or heavy metals there is an increased production of metal binding proteins. In this study we investigated several researches which help us to suggest the best scientific way such as gene transfer to solve the one of the most important environmental problems that we are dealing with it.

Keywords: component: Heavy metals, detoxification, metal binding proteins, gene transfer.

1. Introduction

Heavy metal pollution such as Cd, Hg, Pb, As and Se is an increasing environment problem worldwide influencing both the condition of environment and human health. These metals and metalloids have toxic effect on both plants and animals, which are strongly poisonous to metal-sensitive enzymes, resulting in growth inhibition and death of the organism [20]. These metals also accumulating in plants reduce root growth rates, heighten permeability of membranes, change processes of cytoplasm vacuolization, inhibit DNA synthesis and photosynthesis, disturb transfer of assimilates and mineral nutrition, change water and hormone status of the organism, etc. [14, 16]. The ability of heavy metals to conjugate and form stable complexes with sulfur containing substances underlies their toxic action. However, in presence of toxic concentrations of heavy metals in soil, plants can induce different detoxication processes thus elevating (ensuring) resistance to toxic influence.

Contamination of soils with heavy metals, either by natural causes or due to pollution, often has pronounced effects on the vegetation, resulting in the appearance of metallophytes, and heavy-metal tolerant plants. A range of tolerance mechanisms have also been proposed, including phytochelatin-based sequestration, compartmentalization processes, as well as additional defense mechanisms, which are based on cell wall immobilization, plasma membrane exclusion, stress proteins, stress ethylene, peroxidase and metallothioneins (MT)[11, 19].

In plants exposed to excessive levels of heavy metals there is an increased production of metal-binding proteins such as metallothioneins (MTs) and phytochelatins [4].

Phytochelatin (PC) and heavy-metal transporters play important roles in heavy metal accumulation, transport and detoxification in plant [10, 19]. Several metal resistance-related genes, when overexpressed in

bacteria, yeasts and plants, can increase the metal tolerance of transformants [3, 6]. PvSR2 (*Phaseolus vulgaris* stressed-related) gene isolated from the bean cDNA library by differential screening is expressed especially under the heavy metal stress and encodes a new heavy-metal stress responsive protein. The putative amino acid sequences of PvSR2 have no any similarity with the metal transporter, PC and metallothionein registered in data base [9]. The transformed bacteria of PvSR2 displayed obvious resistance to CdCl2 [20].

A number of heavy metals such as Cu and Ni are essential micronutrients in plants, required for a variety of physiological mechanisms. However, in elevated concentrations in the soil, they cause to serious worldwide environmental and human health problems [8, 12]. To overcome this problem, plants possess a variety of potential cellular mechanisms that are involved in the detoxification of heavy metals and tolerance to metal stress [8].

Thlaspi caerulescens L. (*T. caerulescens*) is well known as a Zn, Cd, and Ni hyperaccumulator species, especially for Zn/Cd hyperaccumulation. Its ability to accumulate large amounts of heavy metals in its shoots (30,000 lg Zn g-1 d. wt, 14,000 lg Cd g-1 d. wt, and 4,700 lg Ni g-1 d. wt) without toxic symptoms makes this plant an excellent model species for plant heavy metal hyperaccumulation [21]. Using the remarkable ability of hyperaccumulators to extract and remove the metal pollutants from environment, termed ‘‘phytoremediation,’’ has been one of the most promising techniques for the clean-up of contaminated soils.

It is known that proline content increases in the presence of heavy metal ions [7, 15]. The protective action of proline is thought to be connected with its ability to interact with macromolecules keeping their spatial structure anbiological activity [17]. It was shown that treatment of 5-day-old seedlings of wheat Triticum aestivum and kidney bean Phaseolus aureus with solutions containing ions of cadmium and mercury led to higher proline content [2]. Free proline seems to have an antioxidant effect [13]. The affinity of heavy metals with substances binding them can differ depending on physical and chemical characteristics of ion and determining toxic influence on growth and development of plants.

However, in this research we study a several articles about Heavy metal resistance in transgenic plants and then we conclude the article for using gene transfer technique into useful plants that cultured in pollutions' soils.

2. Several researches

2.1. Tobacco Transformants Expressing Antisense Sequence of Proline Dehydrogenase Gene Possess Toleranceto Heavy Metals

In the earlier works, using cultivar SR1, tobacco (Nicotiana tabaccum) plant transformants expressing fragment of Arabidopsis proline dehydrogenase gene in antisense orientation were developed. Analysis of the transformants showed that such plants are characterized by a higher proline content, elevated osmotic pressure, and salinity resistance. This work was aimed at investigation of resistance to high concentrations of some heavy metals in the progeny of the third-generation transformants obtained by self-pollination. To estimate resistance of plants to higher content of heavy metal ions, the experiments on growing seeds of transgenic line 8 and cultivar SR1 (control) on Murashige and Skoog medium (MS) and on MS medium supplemented with cadmium chloride (0.1, 0.2 mM), lead nitrate (0.1, 0.3 mM), and nickel sulfate (0.1, 0.2,0.4 mM) were carried out. Such concentrations were chosen in accordance with the results of the experimental data presented by Kawahima. On these media in plant growth chamber conditions (21 C, 16 h photoperiod) the plants were cultivated for 2 months. The experiments were repeated twice with 50 to 70 plants per sample. For statistical estimation of the effect of heavy metals on the growth of the experimental and control plants, the weight estimation using reliability criteria of sample difference was performed. The content of chlorophyll a and b was determined according to the method described by Shlyk. The homogenate obtained by grinding 200 mg of plant material with 3 ml of 96% ethanol was centrifuged for 3 min at 3000 rpm (Eppendorf MiniSpin centrifuge). The concentration was determined using spectrophotometer SF-46 at wave length 649 and 665 nm and then calculated using formulas where D is the optical density of the solutions examined. In comparing the germinating capacity of seeds from experimental and control lines on the MS medium and on the MS with addition of cadmium, nickel or lead, no significant differences were found. The counts were performed on day 14–15 after sowing. After growing plants on these media for 6 to 8 weeks, some visible changes were observed.

It was found that growth of plants of cultivar SR1 on MS medium with addition of 0.1 mM cadmium chloride was slower than that of the transformed plants; some features of chlorosis (loss of pigmentation in plants) were also noted. After elevation of the cadmium chloride concentration to 0.2 mM, the complete chlorosis of leaves of nontransgenic plants was observed, while in transformants leaf chlorosis was less expressed. Estimation of weight of plants cultivated on media with different cadmium chloride concentrations showed significant differences between control plants of cultivar SR1 and transformants.

$$C\text{Chl a} = 13.7D665 - 5.76D649,$$
$$C\text{Chl b} = 25.8D665 - 7.6D649,$$

The growth of plants on the MS medium containing 0.1 mM of lead nitrate was slowed and features of chlorosis were also observed. However, growth of tobacco transformants bearing antisense suppressor of proline dehydrogenase was decelerated to a lesser degree. In case of seeds sowed on the MS media with 0.3 mM lead nitrate, the death of either experimental or control plants was observed after 8 weeks but transformed plants were visually of larger size, characterized by higher weight, and features of chlorosis were less expressed in them.

Presence of 0.1 mM nickel sulfate in the MS medium significantly reduced the growth rate of control plants, but had virtually no effect on the growth of transformants and features of chlorosis were not visually detected in both cases. Concentration of 0.2 mM inhibited growth of both control and experimental plants in the same degree, while biomass measurements showed no significant differences. Being planted on the medium with addition of 0.4 mM nickel sulfate, seedlings died immediately after germination.

Analysis of all experiments performed and positioning metals in order of decrease of their toxic action produced the following series: Cd > Ni > Pb. In literature, other series have been described, e.g., Cd > Pb > Ni, which is probably connected to unequal resistance of different plant species. But in all cases cadmium manifests the highest degree of toxicity. It should be also mentioned that all experiments were performed cultivating tobacco plants on the MS medium containing a standard spectrum of micro- and macroelements. The reaction of plants on the presence of heavy metal ions may depend on the type of medium, and in case of performing similar experiments with plants grown in soil or on other nutritive media, the quantitative estimation of stress tolerance of transformants compared with control plants can have other values. However, our results showed higher resistance of tobacco plants bearing antisense suppressor of PDG to ions of nickel, cadmium and lead. Transformants do not differ phenotypically from plants of the original cultivar SR1. This method can probably be used in plants modification aimed at obtaining forms able to grow in conditions of elevated concentrations of heavy metals [18].

2.2. Gene Manipulation of a Heavy Metal Hyperaccumulator Species Thlaspi caerulescens L. via Agrobacterium-mediated Transformation

Thlaspi caerulescens L. is well known as a Zn/Cd hyperaccumulator. The genetic manipulation of T. caerulescens through transgenic technology can modify plant features for use in phytoremediation. Here, we describe the efficient transformation of *T. caerulescens* using *Agrobacterium tumefaciens* strain EHA105 harboring a binary vector pBI121 with the nptII gene as a selectable marker, the gus gene as a reporter and a foreign catalase gene. Based on the optimal concentration of growth regulators, the shoot cluster regeneration system via callus phase provided the basis of the genetic transformation in *T. caerulescens*. The key variables in transformation were examined, such as co-cultivation period and bacterial suspension density. Optimizing factors for T-DNA delivery resulted in kanamycin-resistant transgenic shoots with transformation efficiency more than 20%, proven by histochemical GUS assay and PCR analysis. Southern analysis of nptII and RT-PCR of catalase gene demonstrated that the foreign genes were integrated in the genome of transformed plantlets [21].

2.3. Expression of the PsMTA1 gene in white poplar engineered with the MAT system is associated with heavy metal tolerance and protection against 8-hydroxy-20 deoxyguanosine mediated-DNA damage

Marker-free transgenic white poplar (*Populus alba* L., cv 'Villafranca') plants, expressing the PsMTA1 gene from Pisum sativum for a metallothionein-like protein, were produced by *Agrobacterium* tumefaciens-mediated transformation. The 35SCaMV-PsMTA1-NosT cassette was inserted into the ipt-type vector pMAT22. The occurrence of the abnormal ipt-shooty phenotype allowed the visual selection of transformants,

while the yeast sitespecific recombination R/RS system was responsible for the excision of the undesired vector sequences with the consequent recovery of normal marker-free transgenic plants. Molecular analyses confirmed the presence of the 35SCaMV-PsMTA1-NosT cassette and transgene expression. Five selected lines were further characterized, revealing the ability to withstand heavy metal toxicity.

They survived 0.1 mM CuCl2, a concentration which strongly affected the nontransgenic plants. Moreover, root development was only slightly affected by the ectopic expression of the transgene. Reactive oxygen species were accumulated to a lower extent in leaf tissues of multi autotransformation (MAT)-PsMTA1 plants exposed to copper and zinc, compared to control plants. Tolerance to photooxidative stress induced by paraquat was another distinctive feature of the MAT-PsMTA1 lines [1].

2.4.Cadmium resistance in transgenic tobacco plants enhanced by expressing bean heavy metal-responsive gene PvSR2

PvSR2 (Phaseolus vulgaris stress-related gene) has been cloned from French bean and shown to be expressed specifically upon heavy metal treatment. In order to investigate the role of PvSR2 in plant, PvSR2 gene under the control of cauliflower mosaic virus 35S promoter was introduced into tobacco mediated with *Agrobacterium tumefaciens* LBA4404. The regenerated plantlets were selected on medium with 100 mg/L kanamycin. PCR and Southern blot analysis showed PvSR2 gene was integrated in tobacco genome. Gus and Northern blot analysis indicated PvSR2 gene was expressed in transgenic seedling. The heavy metal resistance assay showed that the transgenic tobacco seedlings with the PvSR2 coding sequence exhibited higher tolerance to Cd compared with wild-type (WT) under Cd exposure. The Cd content accumulated in root between transgenic and WT seedlings had no obvious difference at lower Cd external concentration (0.05—0.075 mmol/L CdCl2), whereas transgenic plant showed a lower root Cd content than the control at higher external Cd concentration (0.1 mmol/L CdCl2) [5].

3. CONCLUSION

Excessive metal concentration in soils pose significant hazard to human, animal and plant health, and to the environment in general. Contamination of soils with toxic metals has often resulted from human activities, especially those related to mining, industrial emissions, disposal or leakage of industrial wastes, application of sewage sludge to agricultural soils, manure, fertilizer and pesticide use. Due to the potential toxicity and high persistence of metals, soils polluted with these elements are an environmental problem that requires an effective and affordable solution.

However, the first article results showed higher resistance of tobacco plants bearing antisense suppressor of PDG to ions of nickel, cadmium and lead. Transformants do not differ phenotypically from plants of the original cultivar SR1. This method can probably be used in plants modification aimed at obtaining forms able to grow in conditions of elevated concentrations of heavy metals.

Moreover, the second article showed the activity of catalase enzyme in transgenic plants was obviously higher than in wild-type plants. This method offers new prospects for the genetic engineering of this important hyperaccumulator species.

The results of third article showed that the low levels of DNA damage were detected by quantifying the amounts of 8-hydroxy-20-deoxyguanosine in leaf tissues of the transgenic plants exposed to copper.

The results of forth article suggested that the expression of PvSR2 can enhance the Cd tolerance, and PvSR2 may be involved in Cd transportation and accumulation at the test concentration of 0.1 mmol/L Cd.

4. References

[1] A. Balestrazzi, et al. Expression of the PsMTA1 gene in white popla engineered with the MAT system is associated with heavy metal tolerance and protection against 8-hydroxy-20 deoxyguanosine mediated-DNA damage. Plant Cell Rep: 28:1179–1192. 2009.

[2] B. P. Shaw, N. P. Rout. Hg and Cd Induced Changes in Proline Content and Activities of Proline Biosynthesizing Enzymes in Phaseolus aureus and Triticum aestivum. Biol. Plant: pp. 267–271. 2002.

[3] B. Van der Zaal, L. W. Neuteboom, J. E. Pinas. Overexpression of novel Arabidopsis gene related to putative zinc-transporter genes from animals can lead to enhanced zinc resistance and accumulation. Plant Physiol. 119 (3):1047-1055. 1999.

[4] C. Cobbett, P. Goldsbrough. Phytochelatins and metallothioneins: roles in heavy metal detoxification and homeostasis. Annu Rev Plant Biol. 53:159–182. 2002.

[5] C. Tuanyao, C. Qiong, Z. Yuxiu, D. Juan, A. Chengcai. Cadmium resistance in transgenic tobacco plants enhanced by expressing bean heavy metal-responsive gene PvSR2. Science in china. 2003.

[6] F. Odaware, M. Kauraski, M. Suzuki-Kurasaki. Expression of human metallothionein-2 in Escherichia coli: Cadmium tolerance of transformed cells. J. Biochem. 118 (6): 1131-1137. 1995.

[7] I. V. Seregin, A. D., Kozhevnikova. Physiologic Role of Nickel and Its Toxic Effect on Higher Plants. Fiziol. Rastenii: pp. 285–308. 2006.

[8] J. L. Hall Cellular mechanisms for heavy metal detoxification and tolerance. J. Exp. Bot. 366: 1–11. 2002.

[9] L. Huang, J. Gitschier. A novel gene involved in zinc transport is deficient in the lethal milk mouse. Nat. Genet. 17 (3): 292-297. 1997.

[10] M. G. Guerinot. The ZIP family of metal transporters. Biochimica Biophysica Acta. 1456 (1-2): 190-198. 2000.

[11] M. Mejáre, L. Bülow. Metal-binding proteins and peptides in bioremediation and phytoremediation of heavy metals. Trends in Biotech. 19 (2): 67-73. 2001.

[12] S. Clemens. Molecular mechanisms of plant metal tolerance and homeostasis. Planta. 212: 475–486. 2001.

[13] S. Siripornadulsil, S. Traina, D. P. S Verma, R. T. Sayre. Molecular Mechanisms of Proline-Mediated Tolerance to Toxic Heavy Metals in Transgenic Microalgae. Plant Cell: pp. 2837–2847. 2002.

[14] T. V. Chirkova, Fiziologicheskie osnovy ustoichivosti rastenii, (hysiological Bases of Plant Tolerance), St. Petersburg : Gos. Univ. 2002.

[15] V. P. Kholodova, K. S. Volkov, V. V. Kuznetsov. Ice Plant Adaptation to High Copper and Zinc Salt Concentration and Their Usage in Phytoremediation, Fiziol. Rastenii: pp. 848–858. 2005.

[16] V. V. Kuznetsov, G. A. Dmitrieva. Fiziologiya rastenii (Plant Physiology), Moscow: Vysshaya Shkola. 2005.

[17] V. V. Kuznetsov, N. I. Shevyakova. Proline during Stress: Biological Role, Metabolism, and Regulation. Fiziol. Rastenii: pp. 321–336. 1999.

[18] Y. S. Kolodyazhnaya, S. E. Titov, A. V. Kochetov, E. A. Trifonova, A. V. Romanova, M. L. Komarova, V. S. Koval, and V. K. Shumny. Tobacco Transformants Expressing Antisense Sequence of Proline Dehydrogenase Gene Possess Tolerance to Heavy Metals. Russian Journal of Genetics: pp. 825–828. 2007.

[19] Y. X. Zhang, T. Y. Chai, G. Burlard. Heavy metal tolerance mechanisms in plants. Acta Bot. Sin. 41 (5): 453-457. 1999.

[20] Y. X. Zhang, T. Y. Chai, W. M. Zhao. Cloning and expression analysis of the heavy-metal responsive gene PvSR2 from bean. Plant Science. 161 (4): 783-790. 2001.

[21] Z. Guan, T. Chai, Y. Zhang. J. Xu, W. Wei, L. Han, L. Cong. Gene Manipulation of a Heavy Metal Hyperaccumulator Species Thlaspi caerulescens L. via Agrobacterium-mediate Transformation. Humana Press. 2008.