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Short Communication

Transgenic Tobacco-Bearing *p-cV-ChMTIIGFP* Gene Accumulated More Lead Compared to Wild Type

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Abstract

Heavy metal pollution, including lead (Pb), is a common global pollution problem. Transgenic (*p-cV-ChMTIIGFP*) and non-transgenic (*Nicotiana tabacum* L. cv Petit Havana SR-1) tobacco plants grown for 6 weeks in pot experiments containing 0, 25, 50, 100, 200, and 400 mg Pb kg·soil^{-1} were investigated. Lead toxicity symptoms, chlorophyll content, dry weight, and Pb concentration of plants were measured in order to evaluate phytoremediative potential of the plants. Neither plant showed any toxicity symptoms during the growth period. The *p-cV-ChMTIIGFP* tobacco plant accumulated more Pb (5.6 mg·kg^{-1}) than the wild type tobacco (3.6 mg·kg^{-1}). Therefore, the transgenic *p-cV-ChMTIIGFP* tobacco has promising potential for reclaiming slightly lead-contaminated soils.

Keywords: transgenic tobacco, lead, phytoremediation, soil pollution, metallothionein

Introduction

Lead is commonly used in many branches of industry such as batteries, paints, and leathers, etc. Especially, human activities like burning of fossil fuels, mining, and manufacturing cause lead pollution in the environment [1-3]. Despite its common use Pb is one of the most toxic metals for living organism and environment by eventually accumulating in the human body and causing a series of health problems [1, 2, 4, 5].

There are various methods to reclaim heavy metal contaminated areas. Phytoremediation is defined as cleaning of the contaminated areas using plants. It is a cheaper, more efficient, and a more environmentally friendly method than the physical and chemical methods. High metal accumulation in the shoot part, rapid growth, deep rooting, harvest ease, and high biomass production are required properties for ideal planting for phytoremediation [1, 2, 6, 7]. There are hyperaccumulator plants in nature with a capacity for accumulating high amounts of various heavy metals such as 10,000 mg Zn·kg^{-1} dry matter (DM), 1,000 mg Pb·kg^{-1} DM, and 100 mg Cd·kg^{-1} DM [7], but their slow growth and little biomass limit their usage for phytoremediation. However, this limitation by developments in genetic engineering can be compensated for by genetically modifying transgenic plants having high biomass production, high tolerance, and high accumulation ability for various heavy metals [2, 6]. Tobacco is mostly the preferred model plant in genetic studies because of its high shoot production ability, deep root system, and non-selective property to different soils and climates. Thus, tobacco was chosen for this study.

Metallothionein genes from different sources were used to develop transgenic plants for remediation of heavy metal-contaminated soils. Metallothionein is a protein of metal binding, rich in cysteine, and low molecular weight (6-7 kDa) [6]. The objective of this study was to compare phtoremediative potentials of the transgenic tobacco plants,

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Table 1. The effect of Pb treatments on chlorophyll (on old and young leaves), dry weights, and Pb concentrations of transgenic (*p-cV-ChMTIIGFP*) and non-transgenic tobacco (SR-1).

Tobacco cultivars	Chlorophyll (SPAD)		Dry Weight ($g·plant^{-1}$)	Pb Concentration ($mg·kg^{-1}$)
	Old leaves	Young leaves		
p-cV-ChMTIIGFP	37.86a	48.30a	10.64b	2.34a
SR-1	37.18b	48.02a	11.95a	1.64b
LSD ($p<0.05$)	0.25*	n.s.	0,13*	0.21*

*significant, n.s. – not significant

p-cV-ChMTIIGFP, expressing MT gene isolated from Chinese hamster and wild type SR-1 tobacco as the control.

Experimental Procedures

Non-transgenic tobacco variety (*Nicotiana tobacum* L., cv. Petite Havana SR-1) and genetically modified line of the same variety (bearing the ChMTII gene under a constitutive promoter) were used. Details of *p-cV-ChMTIIGFP* gene transfer were given elsewhere [6].

Mahmutlu soil series from a depth of 0 to 30 cm was used in this study. The properties of soil were: clay loam texture [8], pH: 7.60 [9], $CaCO_3$ 11.4% [10], organic matter 2.16% [11], total Pb 3.92 $mg·kg^{-1}$ [12], and DTPA-extractable-Pb 0.29 $mg·kg^{-1}$ [13].

The soil (2 kg) was placed in 2.5 L plastic pots and then the following fertilizer applications were done: 200 mg/kg N as $(NH_4)_2SO_4$ form, 100 mg/kg P and 125 mg/kg K as KH_2PO_4 form and 2.5 mg/kg Fe as FeEDTA. Serial Pb concentrations 0, 25, 50, 100, 200, and 400 mg/kg as $Pb(NO_3)_2$ were applied to the soil in solution. Non-transgenic tobacco seeds were germinated in a mixture of peat and sand (2:1, V/V), whereas transgenic tobacco seeds were germinated in Murashige and Skoog minimal organics medium (Sigma, Germany).

Nicotiana tobacum Petit Havana SR-1 and transgenic tobacco plants were grown under controlled environmental conditions with a 16 h light period (light intensity of 10 klux), a 25/20ºC light/dark temperature cycle, and 60% relative humidity. Leaves' chlorophyll content was determined using a Chlorophyll meter (Konica-Minolta SPAD-502) before harvest. Plants were harvested after 6 weeks of growth. Plant samples were rinsed briefly in deionized water and dried with tissue paper, then processed for Pb quantitation by drying at 70ºC. After dry biomasses were measured, particle size was reduced with an agat mill (Retsch RM200), and then samples were digested by wet combustion procedure with HNO_3 and H_2O_2 in microwave oven (MarsXpress CEM). Total Pb concentration of digested samples were measured using an ICP-AES (Inductively Coupled Plasma-Atomic Emmission Spectrometry; Varian Series-II).

The experimental design was completely randomized with three replications. Analysis of variance was performed using a general linear model procedure [14]. Comparisons between means were carried out using the least significant difference (LSD) test at $p < 0.05$.

Results and Discussion

Transgenic and nontransgenic plants at the highest Pb treatment did not exhibit any Pb toxicity symptoms. Accordingly, the absence of toxicity symptoms indicates that the plant can tolerate higher Pb doses. While the effects of Pb treatments on the chlorophyll levels of old leaves of *p-cV-ChMTIIGFP* tobacco plants and SR-1 were significant at $p \leq 0.05$, it was not significant for the young leaves' chlorophyll levels (Table 1).

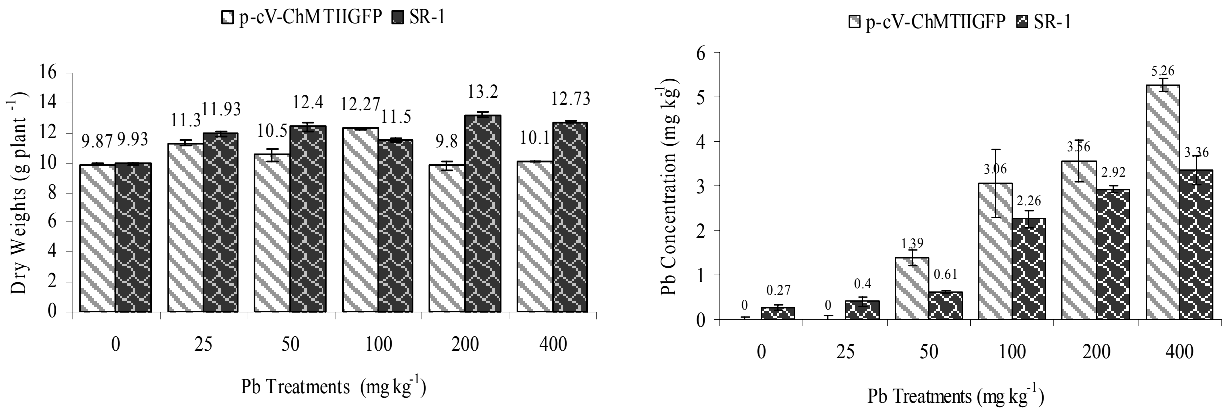

Fig. 1. The interreaction between variety and dose at different Pb treatments on dry weights and Pb concentrations of transgenic (*p-cV-ChMTIIGFP*) and non-transgenic tobacco (SR- 1) plants.

The effect of Pb treatments on the dry shoot biomass of both plants were statistically significant at $p \leq 0.05$. The dry weight range was 11.95 and 10.64 g·plant^{-1}, but SR-1 showed slightly higher growth performance. Beside, SR-1 tobacco plants compared to *p-cV-ChMTIIGFP* have higher dry weight as shown in Fig. 1, whose dry weight difference was found higher at doses above 200 mg Pb·kg^{-1}. Similar results were reported by some researchers [2, 15, 16].

The effects of Pb treatments on shoot Pb concentrations of *p-cV-ChMTIIGFP* and SR-1 tobacco plants were significant at $p \leq 0.05$ (Table 1).

p-cV-ChMTIIGFP tobacco plants accumulated higher Pb than SR-1 tobacco with increasing Pb doses (Fig. 1). This indicates that the Pb uptake and tolerance ability of transgenic (*p-cV-ChMTIIGFP*) tobacco plants are much higher than the wild type. In that respect the highest Pb concentration was determined for the *p-cV-ChMTIIGFP* tobacco plants at treatment of 400 mg Pb·kg^{-1} as 5.6 mg Pb·kg^{-1}.

Gisbert et al. [17] reported that transgenic *TAPCS1* (wheat gene encoding phytochelatin synthase) tobacco accumulated double the concentration of Pb of its wild type in mining soils containing high levels of Pb (1,572 ppm). Similarly, *Arobidopsis thaliana* bearing the YCF1 gene was found to be very tolerant to high concentrations of Zn and Pb [18]. These results were in agreement with the findings in this study. Such an increase in tolerance to heavy metals indicate that these plants can be used for phytoremediation.

In conclusion, higher depletion of Pb amounts by the transgenic tobacco from soil compared to the wild one is an expected result. No exhibition of any toxicity symptom of the *p-cV-ChMTIIGFP* tobacco plant improved for the phytoremediation method until the dose of 400 mg Pb·kg^{-1} indicates that the plant in question can have the tolerance to higher Pb doses, too. The obtained results showed that the transgenic (*p-cV-ChMTIIGFP*) tobacco plant can be used for phytoremediation of the slightly Pb-contaminated soils. The overexpression of MTs can increase plant tolerance to specific metals, for example Zn, Cd, or Cu. However, these findings should be confirmed under field conditions. Only in a few reports were MT overexpression results in slightly increased accumulation of metals in shoots. Thus, the use of MTs in phytoremediation still shows limitations but offers great potential for improvement.

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