**Engineering expression of polyphosphate confers cadmium resistance in tobacco**

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**ABSTRACT** — The feasibility of transgenic tobacco, genetically engineered to express bacterial polyphosphate (polyP) for phytoremediation of cadmium pollution was examined. The transgenic tobacco showed more resistance to Cd\(^{2+}\) and accumulated more Cd\(^{2+}\) than its wild-type progenitors. These results suggest that polyP has abilities to reduce Cd\(^{2+}\) toxicity, probably via a chelation mechanism, and to accumulate cadmium in the transgenic tobacco. Based on the results obtained in this study, polyP-mediated Cd\(^{2+}\) accumulation may serve as a useful strategy for Cd\(^{2+}\) phytoremediation.

**Key words:** Transgenic tobacco, Polyphosphate, Cadmium, Resistance, Accumulation

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**INTRODUCTION**

Cadmium is a toxic and carcinogenic non-essential metal (Vido et al., 2001) that has caused serious adverse effects on human health and the environment. The cadmium level in the environment has risen with advances in industrialization. Chronic exposure to cadmium results in preferential renal cadmium accumulation, thereby leading to nephrotoxicity in human (Goering et al., 1995). There is thus an urgent need to remove cadmium from the environment. Recent research in the sphere of heavy metal removal from wastewater, sediments and soils has focused on development of materials with increased affinity, capacity and selectivity for target metals (Gadd and White, 1993; Sandstede et al., 1993; Wasay et al., 1995; Totura, 1996; Pan-Hou et al., 2001, 2002).

Inorganic polyphosphate (polyP), a linear polymer of many tens or hundreds of orthophosphate residues in anhydrous linkage, is widely distributed in bacteria, amoebae and mammals (Kulaev, 1979). The conservation and ubiquitous distribution of this polymer suggests that it may play important roles in living cells. Although the physiological functions of polyP are not fully elucidated at present, polyP has been implicated as a strong chelator of essential divalent metals including Ca\(^{2+}\), Mg\(^{2+}\) and Mn\(^{2+}\) in vivo (Archibald and Fridovich, 1982; Kornberg, 1995). In previous papers, we reported that tobacco plant genetically engineered to express bacterial \(ppk\)-specified polyphosphate was more resistant to Hg\(^{2+}\) and accumulated significantly more mercury than its wild-type progenitors from Hg\(^{2+}\)-containing soils (Nagata et al., 2006a, 2006b). These results suggested that the polyP-mediated accumulation of mercury from an Hg\(^{2+}\)-containing medium could serve as a useful strategy for phytoremediation of Hg\(^{2+}\) in the environment. In view of these findings, it is of interest to determine whether polyP could serve as a chelator for cadmium and reduce cadmium toxicity when cadmium is taken up into \(ppk\)-transgenic tobacco.

The aim of this study is to examine the impact of expression of polyP on cadmium tolerance and accumulation in tobacco. Here, we describe that over-expression of polyP contributes to tobacco plant resistance to cadmium, probably via chelation formation with polyP, and accumulates more cadmium in tobacco.

**MATERIALS AND METHODS**

Plants and growth conditions. The wild-type tobacco (Nicotiana tabacum cv. Samsun NN), kindly supplied by Japan Tobacco and the \(T_1\) progeny of \(ppk\)-transgenic tobacco, engineered to express bacterial \(ppk\)-specified polyP (Nagata et al., 2006a) were photoautotrophically cultured in Murashige-Skoog (MS) medium according to the method of Takeda et al. (1990).

Evaluation of heavy metal resistance. The sterilized seeds from the wild-type and \(ppk\)-transgenic tobacco
were sterilized, germinated and grown in MS agar medium containing various concentrations of Hg\(^{2+}\) or Cd\(^{2+}\). After culture for 2 weeks at 25°C, the tobacco was collected, then washed with distilled water. The sensitivity of tobacco plants to heavy metals was evaluated by monitoring the dry weight of the tobacco shoot.

**Accumulation of cadmium.** The sterilized seeds from the wild-type and *ppk*-transgenic tobacco were aligned in a horizontal array, and germinated and grown in MS agar medium. After 3 weeks culture at 25°C, the seedlings were transferred to MS liquid medium and cultured for 1 week at 25°C. The 4 weeks-old seedlings of tobacco were hydroponically grown in MS liquid medium containing 0.1 or 0.5 μM \(^{109}\)Cd\(^{2+}\) (specific activity 17.55GBq/mmol, GE Healthcare UK Ltd., Buckinghamshire, England). After culture for 1 week at 25°C, the tobacco shoot radioactivity was measured by auto well gamma system ARC-380CL (ALOKA Co., Ltd., Tokyo, Japan).

**RESULTS AND DISCUSSION**

To develop the potential of a plant to remove and accumulate mercury from contaminated sites, we have engineered a tobacco to express a bacterial *ppk* gene, which encoded polyphosphate kinase (PPK), a key enzyme for polyP synthesis under a plant promoter (Nagata *et al.*, 2006a). Expression of polyP in transgenic tobacco has been reported to confer resistance to Hg\(^{2+}\) (Nagata *et al.*, 2006b). Our interest has turned to examine the effect of polyP expression on cadmium resistance in *ppk*-transgenic tobacco. As shown in Fig. 1, the seedling growth of wild-type tobacco was largely inhibited not only by Hg\(^{2+}\) but also by Cd\(^{2+}\) in a dose-dependent manner. By contrast, the transgenic tobacco was substantially more resistant to Hg\(^{2+}\) and Cd\(^{2+}\) than its wild-type progenic over a range of metal concentrations employed. The cadmium tolerance could also be seen when plants were grown hydroponically in the range of cadmium concentration employed (data not shown). These results suggest that the transported Cd\(^{2+}\) was converted to a less toxic molecule, probably via chelation formation with polyP. Due to chelation with polyP, the transported Cd\(^{2+}\) is never free in the plant tissues, and the transgenic tobacco, therefore, expresses a resistant phenotype to Cd\(^{2+}\).

Next, to ascertain whether polyP expression altered total cadmium accumulation, four weeks-old transgenic tobacco was hydroponically grown in MS liquid medium containing various concentrations of CdCl\(_2\). After 1-week culture at 25°C, cadmium accumulations in tobacco leaves were measured. As shown in Fig. 2, polyP-expressing tobaccos contained almost two times the total cadmium in leaf tissues as the wild-type control tobacco. Accumulation of Cd\(^{2+}\) during the experimental period did not cause any considerable morphological abnormalities in the transgenic tobacco. These results clearly demonstrate
that ppk-specified polyP is able to confer tobacco-accumulating Cd\(^2^+\) from the medium containing Cd\(^2^+\).

From the results obtained here, we communicate that expression of polyP in transgenic tobacco may provide an ecologically compatible approach for environmental phytoremediation of cadmium pollution in soils.

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