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Transgenic Note

Cadmium-tolerance of transgenic *Ipomoea aquatica* expressing serine acetyltransferase and cysteine synthase

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Abstract *Ipomoea aquatica* (water spinach) is a common aquatic plant growing in lakes and wetlands in Southeast Asia. Due to its vigorous growth, they were considered to be potentially useful for remediation of polluted water with, for example, high sulfate and heavy metals. In previous studies, we successfully constructed transgenic *I. aquatica* plants, which simultaneously expressed two genes encoding serine acetyltransferase and cysteine synthase involved in sulfate assimilation pathways. Resulting transgenic plants were shown to rapidly grow and to accumulate sulfate at a high level. In the present study, we tested the effect of cadmium on their physiological and biochemical features. Upon hydroponical cultivation in the presence of 200 μM cadmium for 7 days, two transgenic lines (SR1 and SR2) accumulated 2- to 4-fold higher levels of cysteine and glutathione than the wild type control plants. When plantlets were exposed to 100 μM cadmium for 30 days, wild type and transgenic SR2 plantlets died, or growth was greatly retarded with reduced biomass, whereas transgenic SR1 exhibited a 1.7-fold increase in total biomass in comparison with the initial weight at day-0 of cadmium treatment. These results suggested that some transgenic plants expressing serine acetyltransferase and cysteine synthase could mitigate detrimental effects of cadmium toxicity, perhaps by efficiently producing and accumulating sulfuric compounds.

Key words: Cadmium, cysteine synthase, heavy metal tolerance, *Ipomoea aquatica*, serine acetyltransferase.

Contamination of fresh water with chemicals and heavy metals has become a serious question for global environment. In order to cope with such problems, remediation using higher plants has been proposed as one of powerful approaches. In this context, genetic engineering of aquatic plants to confer enhanced ability to assimilate and detoxify pollutants was thought to be promising. Prototypes of such transgenic plants have indeed been constructed, although none of them have so far been practically applied in field (Zhu et al. 1999; Harada et al. 2001; Sakulkoo et al. 2005).

Our long-range aim is to clean up detrimental levels of sulfur and heavy metals including cadmium and copper from aquatic environment in Thailand. To this end, we selected *Ipomoea aquatica*, or water spinach, as a model plant due to its high ability in metal assimilation and rapid growth in rather polluted environment. In higher plants, sulfur and heavy metals are metabolized or detoxified through compounds such as glutathiones involving sulfur-containing amino acid, cysteine. Consequently we attempted to strengthen cysteine production in *I. aquatica* by introducing genes involved in the sulfur assimilation pathway, which is constituted from five enzymes (Saito 2000; Meerak et al. 2006). Our experimental results have so far indicated that such a strategy was essentially effective, as transgenic *I. aquatica* expressing adenosine phosphosulfate reductase became tolerant against sulfide and cadmium stresses (Sakulkoo et al. 2005). Another transgenic *I. aquatica*, simultaneously expressing two genes encoding serine acetyltransferase and cysteine synthase, also showed enhanced sulfate uptake with increased biomass (Meerak et al. 2006), suggesting that strengthening of sulfur assimilation pathways results in conferring plants broad spectra of stress tolerance. In this report, we further confirmed this idea by showing that above-mentioned transgenic lines expressing both serine acetyltransferase and cysteine synthase (Meerak et al. 2006) exhibited a clear tolerance against cadmium stress.

Transgenic *I. aquatica* was prepared as previously described (Meerak et al. 2006) by introducing a multi-gene transformation vector, pBIH1-IG(SX)-*SAT1-rcs1*, that contained genes encoding Arabidopsis serine acetyltransferase and rice cysteine synthase. Two lines were selected for this study, SR1 and SR2, of which more than five plantlets were cultured in a growth cabinet at

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25°C under a 16-h light/8-h dark photoperiod at a light intensity of 3,000 lux (white fluorescent tube). For biochemical analysis, plantlets were cultured in MS medium (Murashige and Skoog 1962) containing 200 μM cadmium chloride for 7 days and amounts of cysteine and glutathione were measured. For physiological analysis, plantlets were similarly cultured in the presence of 100 μM cadmium chloride for 30 days and dry weight was measured. For cysteine and glutathione content analysis, plantlets were cultured in Hoagland nutrient solution in a controlled-environment greenhouse for 30 days. Enzymatic activities for cysteine synthase and serine acetyltransferase were determined by the established methods (ref in Meerak et al. 2006). Protein concentration was quantified by the Bradford method (Bradford 1996). Cysteine and glutathione were quantified by HPLC as described (Noctor and Foyer 1998) using a 100-mg leaf tissue from transgenic and wild type plantlets as previously described (Sakulkoo et al. 2005).

Transgenic lines SR1 and SR2 were originated from the same transformation SR series, which we previously characterized (Meerak et al. 2006). However, since their stock, age and culturing condition differed from previous experiments using SR3 and SR10, we carried out confirmation experiments to see whether or not introduced genes were stably expressed. Assays were performed using crude extracts prepared from mature leaves from three each of transgenic and wild type plantlets grown under non-stressed conditions in a growth cabinet. Cysteine synthase activity was found to be 1.6- and 1.8-fold higher in SR1 and SR2 lines than in the control, respectively (Figure 1A). Serine acetyltransferase activity was also 2.7- and 2.9-fold higher in transgenic SR1 and SR2 lines than in the wild type control, respectively (Figure 1B). These results were consistent with our previous observation, and indicated that transgenic lines simultaneously and stably expressed functional enzymes from the transgenes over prolonged cultivation period.

The biochemical effect of cadmium was then examined as a representative of heavy metals. Plantlets were hydroponically cultivated in the presence of 200 μM $CdCl_2$ for 7 days, and cysteine and glutathione contents in young expanding leaves were determined. The amount of cysteine in wild type control was 3.1 nmol g^{-1} fresh weight, while those in transgenic SR1 and SR2 were 5.3 and 12.3 nmol g^{-1} fresh weight, respectively (Figure 2A). The amounts of glutathione were 50.4 nmol g^{-1} fresh weight in wild type control, 127.9 nmol g^{-1} fresh weight in SR1, and 133.7 nmol g^{-1} fresh weight in SR2 (Figure 2B). Thus transgenic plantlets were able to assimilate cysteine at 1.7-fold and glutathione at 4.0-fold higher rates than wild type controls in the presence of cadmium.

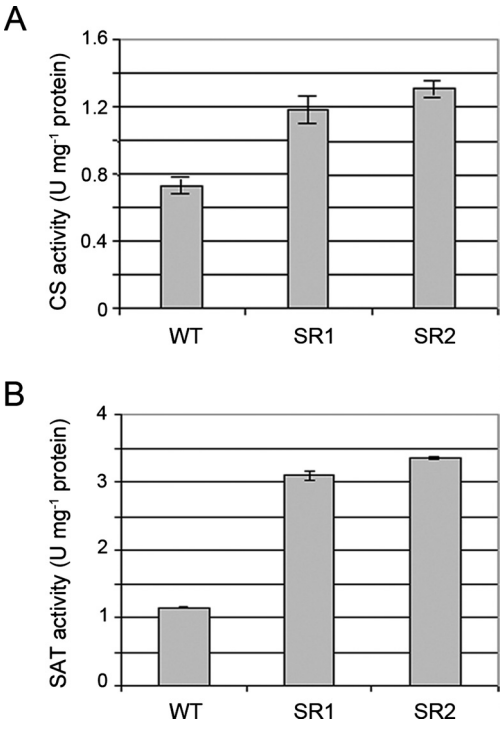

Figure 1. Enzymatic activities. Crude extracts were prepared from untreated healthy leaves of wild type and transgenic SR1 and SR2 plantlets as indicated, and activities of cysteine synthase (CS) (A) and serine acetyltransferase (SAT) (B) were measured as described in text. Standard deviations were calculated from measurements from three independent plantlets of each line.

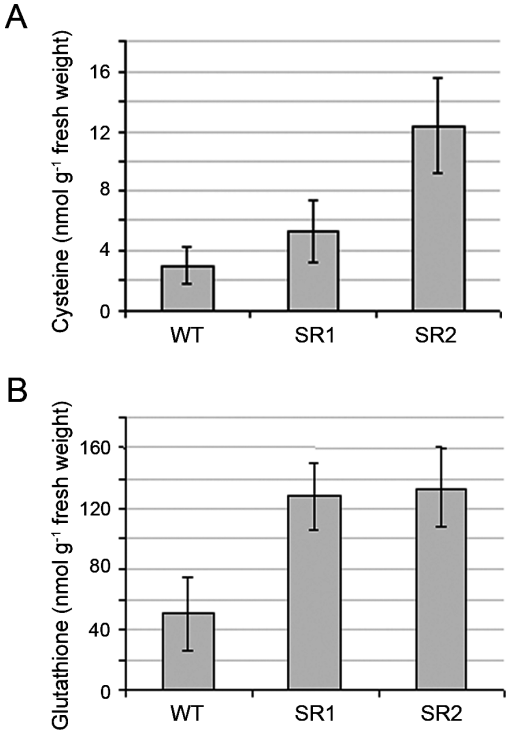

Figure 2. Effects of cadmium. Wild type and transgenic SR1 and SR2 plantlets were hydroponically cultivated in MS medium containing 200 μM cadmium chloride for 7 days. Young expanding leaves were sampled and amounts of cysteine (A) and glutathione (B) were measured by HPLC as described in text. Standard deviations were calculated from measurements from three independent plantlets of each line.

Table 1. Effects of cadmium on growth.

Plants	Dry weight (mg) Day-0	Day-30	Change (Δ) (mg)	Relative change[b]
WT-1	19.0	—[a]	—	—
WT-2	19.5	—	—	—
WT-3	14.7	11.0	−3.7	0.75
WT-4	19.0	13.9	−5.1	0.73
SR1-1	6.6	10.8	+4.2	1.64
SR1-2	9.6	14.2	+4.6	1.48
SR1-3	13.6	26.5	+12.9	1.95
SR1-4	11.6	16.3	+4.7	1.41
SR1-5	9.1	18.0	+8.9	1.98
SR2-1	13.0	—	—	—
SR2-2	25.6	—	—	—
SR2-3	20.8	20.8	0	1.0
SR2-4	20.2	—	—	—
SR2-5	17.9	13.4	−4.5	0.75

Plantlets were hydroponically cultivated in the presence of 100 μM cadmium chloride, harvested 30 days later, dried and weighed.

[a] Plant died during treatments.

[b] Relative values to those at Day-0 taken as 1.

To determine whether or not increased levels of cysteine and glutathione contributed to plant survival under cadmium stress, wild type and transgenic plantlets were hydroponically cultivated in MS medium containing 100 μM $CdCl_2$ for 30 days, and their weight was measured (Table 1). When wild type plants were exposed to cadmium, their growth immediately ceased, and died during the treatment. Some survived but their total biomass decreased to 75% of the initial weight, and ultimately died as well. In contrast, transgenic SR1 continued to grow under cadmium stress, and total biomass increased 1.7-fold that of the initial weight after 30 days culture. In the case of transgenic SR2, growth was retarded, dying during the treatment, or showing reduced biomass. This feature was similar to the wild type control (Table 1).

The present observation revealed two features in cadmium tolerance: First, the increased level of cysteine and glutathione does mitigate cadmium toxicity. Second, however, this alone is not sufficient, but additional mechanism such as efficient utilization of these compounds *in planta* might be necessary. It is established that glutathione plays a central role in protecting plants from environmental stresses, including reactive oxygen species, xenobiotics and heavy metals (May et al. 1998). In the latter case, γ-glutamylcysteine peptides, or phytochelatins, which are formed by polymerization of glutathiones, were proposed to be responsible (Grill et al. 1985, 1987; Chen et al. 1997). Indeed, overexpression of γ-glutamylcysteine synthetase was shown to enhanced cadmium tolerance (Zhu et al. 1999). In this context, our present observation suggested that conversion of glutathione into phytochelatins *in planta* is important to confer cadmium tolerance. Perhaps transgenic SR1 line could efficiently synthesized this compound using overproduced glutathione, while SR2 failed to do so. Further analyses including phytochelatin measurements will determine detailed molecular mechanism of cadmium tolerance in *I. aquatica*.

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