Potential use of *MuS1* transgenic tobacco for phytoremediation of the soils contaminated with Cd

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Abstract
This study was carried out to identify the potential for phytoremediation of soils contaminated with Cd using *MuS1* (line 6) transgenic tobacco. The experiment was composed of two parts: i) Effect of cadmium treatment on germination rate; and ii) Effect of cadmium treatment on growth with hydroponic system. *MuS1* tobacco seeds geminated and grew better than wild-type tobacco seeds. The tolerance of *MuS1* transgenic tobacco to Cd stress was better than that of wild-type tobacco at all Cd treatment levels. Especially, wild-type tobacco showed chlorosis and withering with 200μM Cd treatment, but *MuS1* tobacco gradually recovered from Cd damage. Results of this study showed potential for phytoremediation of soils contaminated with Cd using *MuS1* transgenic tobacco. They also indicated potential for biofuel production from the area where soils are contaminated with Cd using *MuS1* transgenic tobacco.

Key Words
*MuS*1 gene, cadmium, phytoremediation, chlorosis.

Introduction
Soil contamination by human being goes back as far as the Bronze Age (2500 B.C.) (Kabata-Pendias and Mukherjee 2007). Industrial activities such as mining and smelting of metalliferous ores, brick and pipe manufacture as well as power generation and agricultural practices significantly contributed to soil contamination. Remediation of the contaminated soil and water is very popular not only scientifically but also for business aspects. Phytoremediation is a promising soil remediation technique among many remediation techniques including chemical, physical, biological and thermal ones. One of the disadvantages of the phytoremediation is that the amount of the biomass of most of the hyperaccumulators is not enough. If a plant has greater biomass as well as deep root system it would be perfect for phytoremediation. Since it is not easy to find such a plant, scientists try to prepare such plant with the help of genetic engineering. The *MuS1* is known to a multiple stress related gene with several lines extracted from sweet-potato (*Ipomoea batatas* L. cv. Yulmi). The previous study using RT-PCR showed that the expression of *MuS1* gene in tobacco plant induced the tolerance to cadmium stress. There the objective of this study was to identify potential of *MuS1* (line 6) transgenic tobacco for phytoremediation of the soils contaminated with Cd.

Materials and methods
Materials
MuS1 (line 6) transgenic tobacco and wild-type tobacco were used in this study. The previous study using RT-PCR showed that the expression of *MuS1* gene in tobacco plant induced the tolerance to cadmium stress (Yang, 2009). The total genomic DNA of *MuS1* gene was extracted from sweet-potato (*Ipomoea batatas* L. cv. Yulmi) using the modified CTAB method (Kim and Hamada 2005).

Methods
MS medium with 0, 50, 100 and 200μM Cd was used to compare germination rate between *MuS1* (line 6) transgenic tobacco seeds and wild-type tobacco (control) seeds. At each Cd concentration, fifteen seeds of each tobacco were placed in petri dish. Germination rate was determined after two weeks.
To find out effect of Cd stress on the growth of tobacco plants, *MuS1* (line 6) transgenic tobacco and wild-type tobacco seedlings were transplanted 2 weeks after germination and were cultivated in a hydroponic system with 0, 50, 100 and 200μM Cd treatment for 3 weeks (Kawashima *et al.* 2004). Three weeks later tobacco were harvested and fresh weight, dry weight, shoot length and relative damage content (RDC) of the leaves were measured. RDC is defined as number of damaged leaves relative to number of total leaves. After measuring fresh weight, dry weight, length of shoot and Cd damage of leaves, both *MuS1* (line 6) transgenic tobacco and wild-type tobacco (control) were separated into leaf, shoot and root, and then dried at 65℃ for 3 days. The concentration of Cd in each part of the plant was determined by AAS (Cresser and Parsons 1979) after digestion with mixed acid (conc. H_2SO_4:60% $HClO_4$= 1:10, v/v).

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Results and discussion

Germination rate

MuS1 (line 6) tobacco seeds showed better germination rate and growth than wild-type tobacco seeds. Especially, 100% of *MuS1* (line 6) tobacco seeds geminated with 100 µM Cd treatment at 14 days (Figure 1). There was visual difference of growth between *MuS1* (line 6) transgenic tobacco and wild-type tobacco. The cotyledon of *MuS1* (line 6) transgenic tobacco seedling was greener and bigger than that of the wild-type tobacco. The elongation of the roots of *MuS1* (line 6) transgenic tobacco the seedlings was better than that of wild-type tobacco.

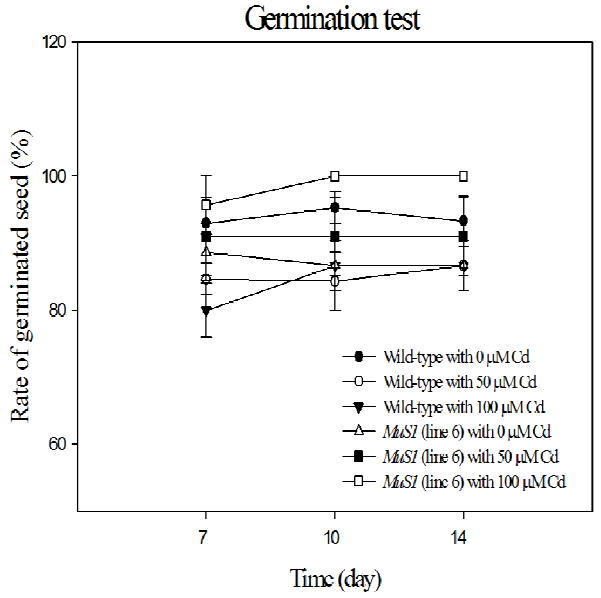

Figure 1. Comparison of the germination rate between *MuS1* (line 6) tobacco and wild-type tobacco with 0, 50, 100µM Cd treatment for 2 weeks.

Growth experiment

Figure 2 shows fresh weight and dry weight of wild-type tobacco and MuS1 transgenic tobacco with different Cd treatment. The growth of both plants decreased with increasing Cd concentration. The fresh and dry weight of *MuS1* transgenic tobacco was more than that of wild-type tobacco with 0-100 µM Cd treatment except for 200 µM Cd. Starting from the second day of the experiment, all of tobaccos with Cd treatment showed damage of leaves to Cd stress. Damaged leaves gradually got chlorosis and withered from undermost. With 200µM Cd treatment, the leaf damage of the wild-type tobacco became worse showing chlorosis and withering, whereas *MuS1* (line 6) transgenic tobacco gradually recovered from Cd damage from the tenth day of the experiment. *MuS1* transgenic tobacco expressed tolerance to cadmium stress at all Cd concentration, showing withered leaves at only some of the lower parts.

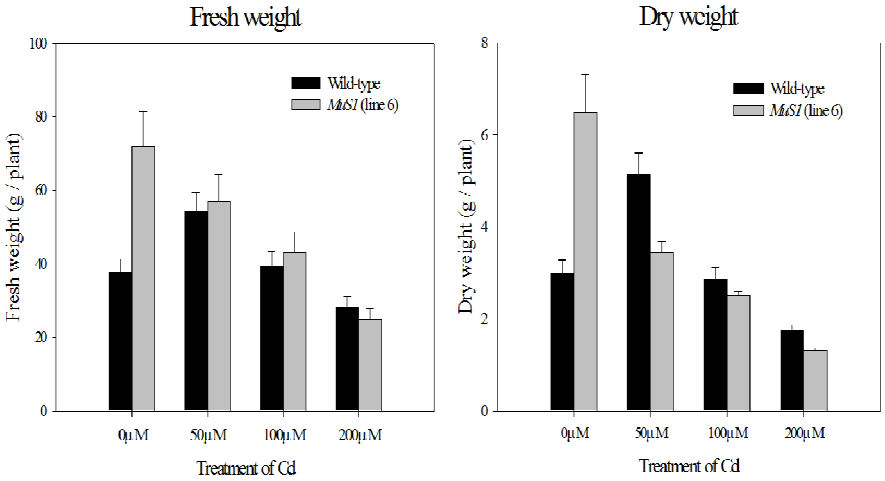

Figure 2. Comparison of the fresh and dry weight between *Mus1* (line 6) transgenic and wild-type tobacco treated with 0, 50, 100, 200µM Cd.

Figure 3 (a) shows RDC at harvest. RDC difference between wild-type tobacco and *MuS*1 transgenic tobacco increased with increasing Cd concentration, indicating Cd tolerance of *MuS*1 transgenic tobacco. Figure 3 (b) shows effect of 200 µM Cd treatment on growth of wild-type tobacco and *MuS*1 transgenic tobacco.

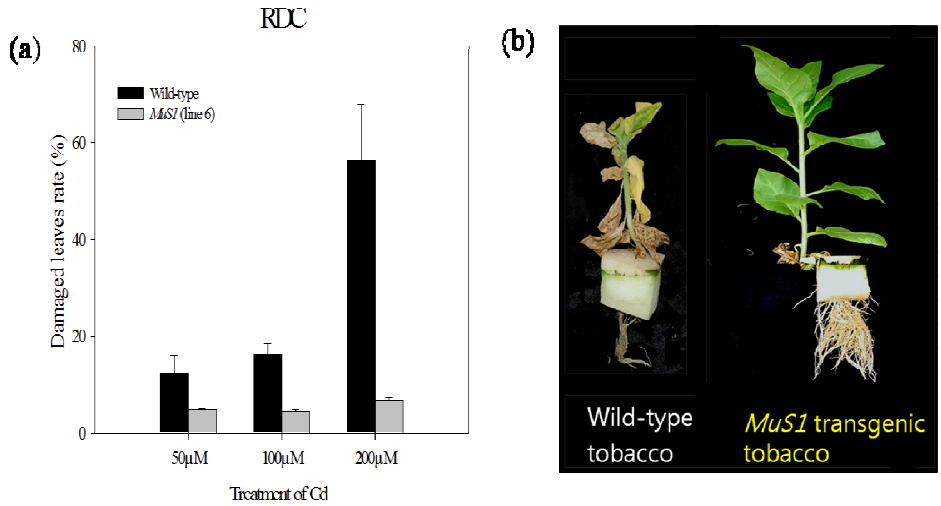

Figure 3. Comparison of the growth of *MuS1* transgenic tobacco and wild-type tobacco with Cd treatment: (a) Relative damage contents (RDC) of leaves of both tobacco with 50, 100, 200µM Cd; (b) Symptoms of both tobacco to Cd stress with 200µM Cd at harvest.

Concentration and accumulation of Cd

Expression of the Cd resistant *MuS1* gene resulted in less Cd uptake by *MuS1* (line 6) transgenic tobacco than by wild-type tobacco with 100 and 200 µM Cd treatment. Wild-type tobacco accumulated more Cd (2.28 mg per plant) with 200 µM Cd treatment than *MuS1* (line 6) transgenic tobacco (Table 1). Rate of Cd translocation from root to leaves was 81.8 % for wild-type tobacco, while 37.1 % for *MuS1* transgenic tobacco. *MuS1* gene may reduce the translocation of Cd from roots to shoot, leading to an overall decrease of Cd in leaves, which was similar to the results by Maiti *et al.* (1989). According to these results, the mechanism of the recovery of the *MuS1* (line 6) transgenic tobacco plant is not by high level of Cd uptake and accumulation in the plant but by revealing resistance to Cd through inducing less Cd uptake and/or more Cd immobilization around roots, resulting in less translocation to shoot.

Table 1. Cd concentration and accumulated amount for *Mus1* (line 6) transgenic tobacco and wild-type tobacco in root, shoot and leaf with 50, 100, 200µM Cd treatment at harvested.

Treatment of Cd	Root		Shoot		Leaf		Accumulated amount	
	Wild-type	*MuS1*	Wild-type	*MuS1*	Wild-type	*MuS1*	Wild-type	*MuS1*
	(---------- mg/kg ----------)						(----------mg----------)	
50 µM	1165.8	1206.9	105.4	109.5	662.8	595.8	3.24	2.11
100 µM	3645.4	2366.7	160.2	104.2	1264.6	1121.8	4.08	2.88
200 µM	7796.8	5262.4	1355.6	622.2	1746.8	865.3	4.65	2.37

Conclusion

This study showed the potential for phytoremediation of the soils contaminated with Cd using *MuS1* (line 6) transgenic tobacco. They also indicated potential for biofuel production from the area where soils are contaminated with Cd using *MuS1* (line 6) transgenic tobacco in the future.

References

Cresser MS, Parsons JW (1979) Sulfuric-perchloric acid digestion of plant material for the determination of nitrogen, phosphorus, potassium, calcium and magnesium. *Analytica Chimica Acta* **109**, 431-436.

Kabata-Pendias A, Mukherjee AB (2007) 'Trace Elements from Soil to Human'. (Springer).

Kawashima CG, Noji M, Nakamura M, Ogra Y, Suzuki KT, Saito K (2004) Heavy metal tolerance of transgenic tobacco plants over-expressing cysteine synthase. *Biotechnology Letters* **26**, 153-157.

Kim SH, Hamada T (2005) Rapid and reliable method of extracting DNA and RNA from sweet potato (*Ipomoea batatas* (L.) Lam. *Biotechnology Letters* **27**, 1841-1845.

Maiti IB, Wagner GJ, Yeargan R, Hunt AG (1989) Inheritance and Expression of the Mouse Metallothionein

Gene in Tobacco. *Plant Physiology* **91**, 1020-1024.

Yang YS (2009) Characterization and Expression Pattern of *MuS1*, a Novel Stress Related Gene from Sweet potato. MS. thesis, The University of Seoul, Seoul, Korea.