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Phytoremediation of Metolachlor by Transgenic Rice Plants Expressing Human *CYP2B6*

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We introduced the human cytochrome P450 gene *CYP2B6* into rice plants (*Oryza sativa* L. cv. Nipponbare), and the *CYP2B6*-expressing rice plants became more tolerant to various herbicides than nontransgenic Nipponbare rice plants. In particular, CYP2B6 rice plants grown in soil showed tolerance to the chloroacetanilide herbicides alachlor and metolachlor. We evaluated the degradation of metolachlor by CYP2B6 rice plants to confirm the metabolic activity of the introduced *CYP2B6*. Although both CYP2B6 and nontransgenic Nipponbare rice plants could decrease the amount of metolachlor in plant tissue and culture medium, CYP2B6 rice plants could remove much greater amounts. In a greenhouse, the ability of CYP2B6 rice plants to remove metolachlor was confirmed in large-scale experiments, in which these plants appeared able to decrease residual quantities of metolachlor in water and soil.

KEYWORDS: Cytochrome P450 (CYP); tolerance; alachlor; acetoanilide; environmental pollution; paddy field

INTRODUCTION

Environmental contaminants such as sediments, pesticides, and nutrients (e.g., nitrate) can be transported from agricultural fields by rainfall and cultivation. It is estimated that about 1−5% of field-applied herbicides are removed by surface runoff (*1*). Metolachlor is a chloroacetanilide herbicide that inhibits very long chain fatty acid (VLCFA) synthesis in plants (*2*). It is usually applied to crops before plants emerge from the soil and is used to control various broadleaf and annual grassy weeds in fields of corn (maize), soybeans, peanuts, grain sorghum, potatoes, sunflowers, various vegetables, and cotton; along highway rights-of-way; and amid woody ornamentals (*3*). Because of its widespread use, metolachlor and its metabolites have been detected in streams, rivers, ponds, and wells (*4*, *5*). Pesticides have played very important roles against food shortages and plant diseases, and our modern life would be vastly different without their use (*6*). However, the possibility of nontarget effects of pesticides caused by agricultural runoff remains.

Phytoremediation is the use of plants and plant growth as a technique for detoxifying sites contaminated with organic and inorganic pollutants and is a promising method for reducing the risks of exposure of people and the environment to pesticides. This method exploits the ability of plants to extract and mineralize xenobiotics in the surrounding environment as well as the tolerance of these plants to the contaminants. The main benefit of phytoremediation is economic: it costs much less than chemical remediation treatments.

Plants can remove organic pollutants by root uptake of contaminants and subsequent accumulation of nonphytotoxic metabolites in plant tissue, leaf uptake of volatile contaminants from the surrounding air, and release of exudates and enzymes that enhance biochemical degradation and mineralization. According to Schnoor et al. (*7*), this technology is suitable for sites with shallow contamination (<5 m depth), moderately hydrophobic pollutants (log K_{ow} (log octanol/water partition coefficient) = 0.5−3), short-chain aliphatic chemicals, and nutrients. The log K_{ow} for metolachlor is 2.9 at 25 °C, and its water solubility is 488 mg/L (*3*). Therefore, metolachlor is a candidate for removal from the environment by phytoremediation.

Several studies on the metabolism of metolachlor in soil microorganisms, plants, and animals have been reported. O-Demethylation and hydrolytic dechlorination of metolachlor are the major pathways of metabolism in rat liver (*8*), bluegill sunfish (*9*), the soil fungus *Chaetomium* (*10*), and soil actinomycetes (*11*). In sorghum microsomes, metolachlor was catalyzed by O-demethylation to a single demethylated metabolite (*12*). The enzymes responsible for O-demethylation have not been well studied in plants, but this demethylation is thought to be catalyzed by cytochrome P450 monooxygenases (CYP).

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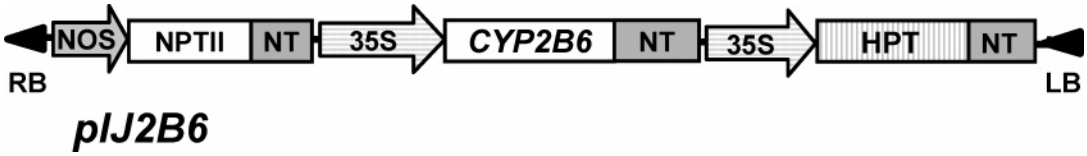

Figure 1. Schematic structure of T-DNA regions of the expression plasmid pIJ2B6. RB, right border; LB, left border; NOS, nopaline synthase promoter; NT, nopaline synthase terminator; NPTII, neomycin phosphotransferase II; 35S, cauliflower mosaic virus (CaMV) 35S promoter; HPT, hygromycin B phosphotransferase. Transformation of rice plants with this plasmid leads to constitutive expression of the *CYP2B6* gene.

P450 monooxygenases insert one atom of oxygen into hydrophobic molecules, making them more reactive and water-soluble (*13*). In plants, intermediate metabolites are converted to secondary conjugates or insoluble bound residues. These metabolites are deposited in vacuoles or into cell walls of plants. More than 1000 plant P450 sequences have been reported in the P450 database (*14*, *15*). These P450s are involved in secondary metabolism and the metabolism of xenobiotics (including herbicides), but molecular level information on plant P450s related to xenobiotic metabolism is limited. In contrast, in mammals, several microsomal P450s are involved in xenobiotic metabolism and have been well studied, and individual P450s show overlapping, broad substrate specificity. These animal P450s have abilities to metabolize a variety of hydrophobic compounds, including herbicides (*16*). Using an in vitro yeast microsome system, Inui et al. found that human CYP2B6 metabolized more than 10 herbicides (including chloroacetanilides, oxyacetamides, and 2,6-dinitroanilines), three insecticides, and two industrial chemicals (*17*). In particular, metolachlor was metabolized to its O-demethylated metabolite, which is the same as that reported in plants (*12*).

Phytoremediation has been shown to be useful in the dissipation of atrazine (*18*), chlorpyrifos (*19*), and other chlorinated compounds (*7*, *20*). Moor et al. (*21*) reported the removal of metolachlor by wetlands constructed to treat agricultural runoff. Rice is a good candidate for phytoremediation because it grows in paddy fields and can remove contaminants from streamwater. To enhance phytoremediation properties, the overexpression of endogenous plant genes or transgenic expression of bacterial or animal genes is required in many cases (*22*).

Use of mammalian P450s is one of the possibilities for phytoremediation. The introduction of these P450s into plants is considered to be a useful technique for producing crops with cross-tolerance to various herbicides (*23*). We have already produced transgenic rice plants that express the human *CYP2B6* gene (*24*). CYP2B6 rice plants detoxify several types of herbicides, including the chloroacetanilide metolachlor. The CYP2B6 rice plants show cross-tolerance to a number of herbicides that can be classified into different groups according to their site of action and chemical structure. In the present study, we evaluated the ability of CYP2B6 rice plants to decrease metolachlor concentrations under three different conditions: remediation of culture medium or nutrient water by young seedlings on a small scale, by mature plants on a large scale, and remediation of soil in stainless steel pools. We discuss the potential use of CYP2B6 rice plants for phytoremediation of metolachlor.

MATERIALS AND METHODS

Plant Materials. Transgenic rice plants expressing human *CYP2B6* were produced as reported previously (*24*). Briefly, a human *CYP2B6* cDNA was inserted into the expression vector pIG121-Hm to construct the expression plasmid pIJ2B6 (**Figure 1**), which then was used for *Agrobacterium*-mediated transformation of *Oryza sativa* cv. Nipponbare (*25*). Regenerated plants were screened for hygromycin resistance by a marker gene (*HPT*) and by PCR amplification with human *CYP2B6*-specific primers. The homozygous T_5 progeny of the selected line A11 were used in this study. Rice seeds were surface-sterilized and embedded in MS solid medium (*26*) containing 50 mg/L hygromycin and incubated at 27 °C with 16 h of light daily for 7−12 days. Seedlings then were used in experiments.

Herbicide Tolerance of CYP2B6 Rice Plants in Soil. Ten 7-day-old plants were transplanted to a glass pot (diameter, 9 cm; height, 19 cm) with 500 mL of water and 500 g of Kumiai-Ryujyou-Baido K soil (Kureha Chemical, Tokyo, Japan). Transgenic plants and nontransgenic Nipponbare plants were grown in a greenhouse at 28 °C during the day and 25 °C at night under 13 h of light daily for 2 weeks. Alachlor and metolachlor were added to each glass pot in two different concentrations, 16.2 and 32.3 μM (2.18 or 4.36 mg) or 12.4 and 24.8 μM (1.76 or 3.52 mg), respectively. Growth was noted after 3 weeks.

Small-Scale Analysis of Residual Herbicide in Plants and Culture Medium. Ten 12-day-old plants were transferred to a plastic plant box (length, 6.5 cm; width, 6.5 cm; height, 9.5 cm) (Magenta Corp., Chicago, IL) with 80 mL of MS culture medium (*26*) containing 30 μM metolachlor and incubated at 27 °C under 16 h of light daily for 1 or 6 days. The aerial part of the rice plants (mean ± standard deviation, 1.12 ± 0.21 g) was homogenized and then extracted with 50 mL of acetone, and the extract was concentrated to 18 mL in a 40 °C water bath. Each extract was applied to a ChemElute 1020 column (Varian Associates, Inc., Harbor City, CA) and Carbograph SEP cartridge (GL Sciences, Inc., Tokyo, Japan), eluted with 100 mL hexane, and evaporated to dryness. Each extract was dissolved using three 5-mL aliquots of hexane:diethyl ether (95:5 v/v) and then applied to a Sep-Pak Plus Florisil cartridge (Waters, Milford, MA). Metolachlor was eluted with 25 mL of hexane:acetone (95:5, v/v), evaporated to dryness, dissolved in acetone, and analyzed by GC−MS model numbers HP5890II (GC) and HP5971 (MS), Hewlett-Packard, Palo Alto, CA). The capillary column was an HP-5ms (30 m × 0.25 mm × 0.25 μm, Hewlett-Packard), and the temperature was programmed as follows: 70 °C (2 min) to 280 °C at 10 °C/min, 280 °C (hold). The sample was applied using the nonsplit method. The carrier gas (He) pressure was 72 kPa (1.5 min), increasing to 140 kPa at 3 kPa/min. The mass spectrometer was operated in EI ionization mode at 70 eV. The temperature of the source was kept at 175 °C. The retention time of metolachlor was 17.2 min, and metolachlor was determined at *m*/*z* 238 by selected ion monitoring (SIM).

In addition, a 20-mL aliquot of the culture medium of each plant was applied to a Chem Elute 1020 column (Varian), eluted with 100 mL of hexane, and evaporated to dryness in a 40 °C water bath. The extracts were dissolved in acetone, and 2-μL aliquots were analyzed by GC (HP5890, NPD, Hewlett-Packard) with a 15 m × 0.53 mm × 1.0 μm Rtx-5 column (Hewlett-Packard). The carrier gases were He at 10 mL/min, H_2 at 3 mL/min, and air at 100 mL/min; the injection temperature was 250 °C; column oven temperature was 250 °C; and detection temperature was 280 °C.

Large-Scale Analysis of Residual Herbicide in Hydroponic Medium. Residual herbicide in the culture medium of 1- and 3-month-old rice plants was analyzed. Hygromycin-resistant 10-day-old plants were transplanted into Kumiai-Ryujyou-Baido K soil in 1/10 000 Wagner pots and were grown for 3 or 12 weeks in a greenhouse. The roots of the plants were washed carefully, and fifteen 1-month-old plants (ca. 50 g fresh weight) or four 3-month-old plants (ca. 100 g fresh weight) were set into the hole of a polystyrene foam board that covered an enamel pot (diameter, 24 cm; height, 24 cm) holding 9 L of culture medium (10 mM NH_4NO_3, 9.4 mM KNO_3, 1.5 mM $CaCl_2$, 0.75 mM

$MgSO_4$, 0.63 mM KH_2PO_4) containing 6.2 μM metolachlor. The plants were incubated in a growth chamber at 28 °C during the day and 25 °C at night under 13 h of light daily. A 14-mL sample of the hydroponic medium was sampled after 14 days of incubation and loaded onto a Bond Elute LRC-C18 column (Varian). The bound herbicide was eluted with 2 mL of methanol and analyzed by high-performance liquid chromatography (HPLC; model LC 10AS, Shimadzu, Kyoto, Japan; column, Cosmosil 5C18-AR−II, 4.6 × 150 mm, Nakalai Tesque, Kyoto, Japan). The solvent system was acetonitrile:water (25:75, v/v), and metolachlor was detected at 230 nm. In addition, the plants were dried for 96 h at 60 °C and then the dry weight was measured.

Large-Scale Analysis of Residual Herbicide in Plants and Soil. For a large-scale experiment in a greenhouse, 24 7-day-old plants were transplanted to a stainless steel pool (length, 90 cm; width, 55 cm; height, 32 cm) with 50 L of water and 63 kg of Andosol (local soil) supplied with 19.8 g of chemical fertilizer (N−P−K, 14−14−14) and fused magnesium phosphate fertilizer on 30 April, 2004. The rice plants were planted 5 cm from the edge of the pool. A distance of 15 cm was left between rows running parallel to the 55 cm side, and 16 cm was left between rows running parallel to the 90 cm side. Both transgenic plants and nontransgenic Nipponbare plants were grown in the greenhouse, and 194 μL of Dual (Novartis Crop Protection Inc.), which contained 300 μmol of metolachlor (final concentration, 6.2 μM), was added to the water on 14 June, 2004. At 3 months after herbicide application, the aerial parts of the rice plants were harvested, and 12-cm soil samples were collected in a glass cylinder (diameter, 9 cm; height, 12 cm) from several points in the pool.

The residual herbicide in the aerial parts of the rice plants and in the soil was analyzed by supercritical fluid extraction and GC−MS spectrometry (Sumitomo Metal Technology Inc., Hyogo, Japan). (*27*). The soil and water were mixed well into a slurry and filtered over 2 mm mesh to remove plant roots. Plants were chopped and homogenized; 2-g samples were mixed with 3 g of WetSupport (ISCO Inc., Los Angeles, CA) and extracted using a supercritical fluid extraction system (SFX220, ISCO Inc.) at 13 790 kPa and 40 °C for 15 min in stable state and 15 min in dynamic state. The extracts were trapped in 20 mL of acetone, evaporated to dryness, and dissolved in 1 mL of acetone for analysis by GC−MS (GCMS-2010, Shimadzu) using a DB-5MS column (0.25 mm × 30 m, J&W Scientific, Folsom, CA). The temperature program was as follows: 80 °C (2 min) to 200 °C at 20 °C/min and then to 260 °C at 5 °C/min and 260 °C for 15 min. The injection temperature was 250 °C. A 2-μL aliquot was applied to the column by using the splitless method. The carrier He gas flow rate was 1.46 mL/min. The mass spectrometer was operated in EI ionization mode at 70 eV. The temperature of the source was kept at 250 °C, and the interface and detection temperatures were 250 and 200 °C, respectively. The retention time of metolachlor was 15.7 min, and metolachlor was determined at *m*/*z* 238 by selected ion monitoring (SIM).

RESULTS

Herbicide Tolerance of *CYP2B6*-Expressing Transgenic Rice Plants. We used T_5 seeds of the CYP2B6 and nontransgenic rice plants Nipponbare in previous germination tests for herbicide tolerance. In test tubes, CYP2B6 rice plants showed healthy growth in the presence of 2.5 μM alachlor or 5 μM metolachlor, whereas the control Nipponbare plants did not grow (*24*). Similarly, CYP2B6 rice plants showed healthy growth in soil containing alachlor (32.3 μM) or metolachlor (24.8 μM; **Figure 2A,B**). These concentrations are 4-fold higher doses than those in practical use in cornfields (*28*). In comparison, the growth of nontransgenic Nipponbare plants was inhibited by the presence of either herbicide at a concentration that is twice that typically used (**Figure 2**).

Small-Scale Analysis of Residual Herbicide in Culture Medium. When both CYP2B6 plants and the control plants were transferred to a plant box and incubated for 1 or 6 days, 30 μM metolachlor had no effect on their growth (**Table 1**). The mean amount of metolachlor in the CYPB6 plants was 25% that of the Nipponbare plants after both 1 day and 6 days of incubation. While the control Nipponbare plants were able to reduce the amount of metolachlor in the medium by 31% at day 6, the CYP2B6 rice plants reduced metolachlor in the medium by 51%, a 1.65-fold increase. Therefore, the CYP2B6 rice plants removed metolachlor 1.65 times more effectively from the culture medium than Nipponbare plants after 6 days of incubation.

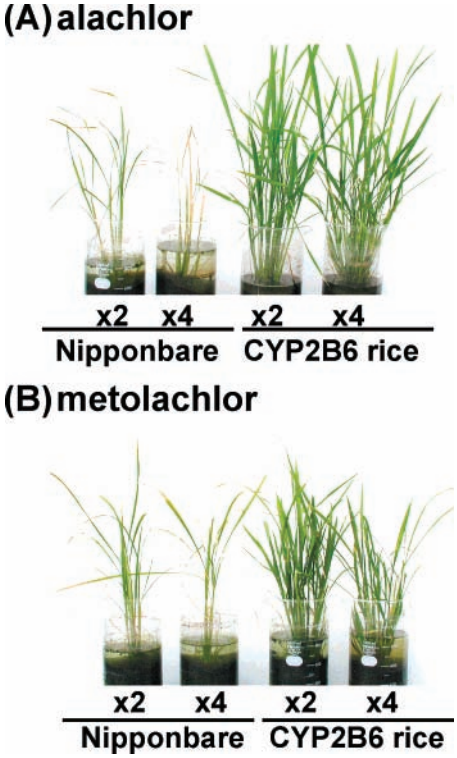

Figure 2. Herbicide tolerance of CYP2B6 rice plants to alachlor and metolachlor. (**A**) Tolerance to alachlor: ×2, 2.18 mg alachlor (twice what is used in cornfields); ×4, 4.36 mg alachlor (4 times more than typically used in cornfields). (**B**) Tolerance to metolachlor: ×2, 1.76 mg metolachlor (twice what is used in cornfields); ×4, 3.52 mg metolachlor (4 times more than used in cornfields).

Table 1. Small-Scale Residual Metolachlor Analysis[a]

	day	plants, μg/g (fresh weight)	culture medium, mg/L	%
Nipponbare	1	0.549 ± 0.10[b]	8.081 ± 0.25	95
	6	0.556 ± 0.24[c]	5.839 ± 0.39[b]	69
CYP2B6 rice	1	0.138 ± 0.05[b]	7.840 ± 0.44	92
	6	0.139 ± 0.09[c]	4.144 ± 0.26[b]	49
control medium	0	not determined	8.598 ± 0.10	100
control plants	6	not detected	not determined	

[a] We transferred 10 12-day-old plants to a Magenta box with 80 mL of MS culture medium containing 30 mM metolachlor and incubated them at 27 °C under 16 h of light daily. Values presented are the mean ± SD of three independent experiments. [b] $P < 0.01$ (*t*-test) between values for days 1 and 6 for the same type of sample. [c] $P < 0.05$ (*t*-test) between values for days 1 and 6 for the same type of sample.

Large-Scale Analysis of Residual Herbicide in Hydroponic Medium. We performed a large-scale residual herbicide analysis of the hydroponic medium of 1- and 3-month-old rice plants grown in a greenhouse (**Figure 3**). Rice plants were grown for 2 weeks in the hydroponic medium containing 6.2 μM metolachlor. One-month-old CYP2B6 rice plants removed 1.73 times as much metolachlor and 3-month-old CYP2B6 rice plants removed 1.41 times as much as nontransgenic control plants.

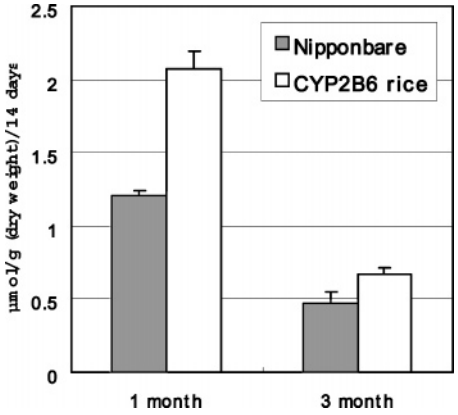

Figure 3. Reduction in the amount of metolachlor in the culture medium. The decrease in the metolachlor level in the culture medium of 1- and 3-month-old rice plants was analyzed by HPLC. CYP2B6 rice plants removed more metolachlor than did nontransgenic Nipponbare rice plants.

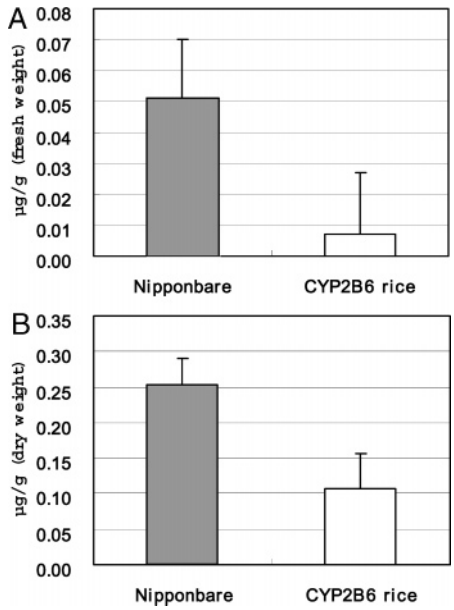

Figure 4. Large-scale analysis of residual herbicide in plants and soil. We added 300 μmol metolachlor (final concentration, 6.2 μM) to the soil of plants in stainless steel pools. Residual metolachlor in rice plants (**A**) and soil (**B**) was analyzed 3 months after application. Data are presented as mean ± SD ($n = 3$ for plants, $n = 4$ for soil, $P < 0.01$ by t-test).

Large-Scale Analysis of Residual Herbicide in Plants and Soil. We added 300 μmol metolachlor (final concentration, 6.2 μM) to the soil of plants grown in stainless steel pools and analyzed the amount of herbicide present 3 months after application (**Figure 4**). Whereas CYP2B6 rice plants showed healthy growth (fresh weight, 74.1 ± 19.1 g), nontransgenic Nipponbare plants showed poorer growth (fresh weight, 31.8 ± 6.1 g). The mean amount of metolachlor was 0.051 ± 0.019 μg/g (fresh weight of plants) in Nipponbare plants and 0.007 ± 0.02 μg/g in CYP2B6 rice plants, only 14% of that in Nipponbare (**Figure 4A**). The mean concentration of metolachlor in the soil was 0.254 ± 0.036 μg/g (dry weight of soil) for Nipponbare, compared with 0.108 ± 0.048 μg/g for CYP2B6 rice plants, which is 42.5% that of Nipponbare plants (**Figure 4B**).

DISCUSSION

Phytoremediation is a relatively new field of science and technology that uses plants to clean up polluted soil, water, or air (*29*). The main benefit of phytoremediation is economic: it costs much less than chemical remediation treatments. With the help of genetic engineering, plants can be used to extract and detoxify a wide variety of environmental contaminants (*22*, *30*). We introduced CYP2B6 into Nipponbare rice plants to enhance their metabolic activity and decrease the residual herbicide in the plants and their environments.

In germination tests, the CYP2B6-transgenic rice plants showed strong tolerance to several herbicides with different chemical structures and modes of action at dosages similar to those in practical use (*24*). Chloroacetanilide herbicides (e.g., alachlor, metolachlor) inhibit VLCFA synthesis in plants, and nontransgenic Nipponbare failed to grow in the presence of these herbicides in germination tests. Moreover, when alachlor or metolachlor was applied to rice plants in soil at doses 4 times those used in corn fields, CYP2B6 rice plants showed strong tolerance toward these herbicides during both germination and vegetative growth, whereas the growth of nontransgenic Nipponbare plants was retarded. The observed herbicide tolerance of the CYP2B6 rice plants was consistent with in vitro catalysis of these herbicides by recombinant yeast microsomes expressing human *CYP2B6* (*17*).

Our previous study showed that CYP2B6 rice plants metabolize metolachlor into its nonphytotoxic or less phytotoxic O-demethylated metabolite (*24*). In plant box experiments, when young rice seedlings were cultured in 30 μM metolachlor, concentrations of this herbicide were 4 times higher in nontransgenic rice plants than in CYP2B6 rice plants after 6 days of incubation (**Table 1**), and the residual metolachlor in the culture medium of CYP2B6 rice plants was decreased 1.65 times more than that in the culture medium of Nipponbare (**Table 1**). Thus, CYP2B6 rice plants clearly showed remediation ability on a small scale in laboratory experiments.

In greenhouse experiments using enamel pots, both 1- and 3-month-old rice plants were able to decrease the metolachlor concentration in the hydroponic medium. In both cases, CYP2B6 rice plants showed higher remediation ability than nontransgenic Nipponbare plants. The remediation ability of 1-month-old rice plants was higher than that of 3-month-old rice plants; the difference was a factor of 2.5 for nontransgenic Nipponbare rice plants and 3.1 for CYP2B6 rice plants. Furthermore, some researchers have found that pesticide absorption decreases as age increases, and tolerance increases with age (*31*, *32*). It is not unexpected that plant tolerance to herbicides may change depending on plant size and age. Although we did not check for differences in herbicide tolerance between 1- and 3-month-old CYP2B6 plants, our results show that the absorption of metolachlor per weight decreased as age increased. Therefore, the detoxification ability of metolachlor seems to be correlated with the growth rate of the plants.

P450 oxidation and glutathione conjugation are two important mechanisms of detoxification of herbicides (*13*), and a change in the P450 or glutathione (GSH) content may change the tolerance of a plant. Hatton et al. (*33*) reported that glutathione-S-transferease and GSH levels in corn decreased during 30 days of growth and that herbicide tolerance might decrease with increasing age. Transgenic poplar plants expressing γ-glutamylcysteine synthetase showed increased levels of GSH, which led to increased tolerance to chloroacetanilide herbicides (*34*). This scenario is similar to that of our CYP2B6 rice plants, which showed enhanced detoxification of and increased herbicide tolerance to chloroacetanilide herbicides.

We also checked the remediation ability of CYP2B6 rice plants grown in a stainless steel pool 3 months after herbicide application. The residual amount of metolachlor in the soil of

CYP2B6 rice plants was 42.5% of that in the soil of Nipponbare. The total residual amounts of metolachlor in the soil of Nipponbare and CYP2B6 rice plants were 11.9 and 5.1 mg, respectively, which were 14% and 6% of the amount of metolachlor applied. Therefore, the difference in remediation ability between Nipponbare and CYP2B6 rice plants is 13.5 g/1000 m^2 in 3 months. Our combined results (small- and large-scale evaluations of remediation of culture medium and soil) show that CYP2B6 rice plants can decrease the amount of metolachlor in contaminated water and soil.

Water quality and agriculture are closely linked, because non-point-source pollution of lakes, rivers, and streams is caused by agricultural runoff. Metolachlor is a commonly used agricultural pesticide in the United States and often is detected in both surface and groundwater samples (*35*). The long-term effects from exposure to low levels of herbicides are difficult to detect. In addition, extensive use of pesticides can lead to considerable risk to nontarget organisms within adjacent aquatic and terrestrial ecosystems. As Cooper mentioned (*6*), some herbicides may decrease primary productivity because of their toxicity to phytoplankton, and changes in habitat can lead to profound alterations in aquatic macrophyte communities.

For successful phytoremediation, plants need to have tolerance to the chemicals or pollutants to be removed, the ability to metabolize and immobilize them, and a large biomass to remedy chemicals dispersed widely in the field. In East Asian countries, rice plants have a large biomass and are thought to remediate pollutants both from soil and from the water passing through paddy fields. Our results show that *CYP2B6*-transgenic rice plants make it possible to remove metolachlor faster from the culture medium and soil than can nontransgenic Nipponbare. However, further investigations, including a safety assessment of these transgenic plants, are needed. In the future, CYP2B6 rice plants may become useful in degrading metolachlor and thus decreasing the environmental loads of this herbicide in paddy fields and connected water streams. In addition, presumably other pesticides, industrial chemicals, and endocrine-disrupting pollutants that can be metabolized by CYP2B6 will be remediated by our transgenic rice plants.

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