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# Inducible cross-tolerance to herbicides in transgenic potato plants with the rat *CYP1A1* gene

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**Abstract** A gene of the enzyme involved in xenobiotic metabolism in mammalian liver was introduced into potato to confer inducible herbicide tolerance. A rat cytochrome P450 monooxygenase, CYP1A1 cDNA, was kept under the control of the tobacco PR1a promoter in order to apply the system of chemical inducible expression using the plant activator Benzothiadiazole (BTH). Transgenic plants were obtained based on the kanamycin resistance test and PCR analysis. Northern-blot analysis revealed the accumulation of mRNA corresponding to rat CYP1A1 in the transgenic plants treated with BTH (3.0 µmol/pot), whereas no accumulation of the corresponding mRNA occurred without BTH treatment. These transgenic plants also produced a protein corresponding to CYP1A1 in the leaves by BTH treatment. The transgenic plants with BTH application showed a much-higher tolerance to the phenylurea herbicides chlortoluron and methabenzthiazuron than non-transgenic plants. These findings indicated that the ability of metabolizing the two herbicides to less-toxic derivatives was displayed in the transgenic plants after BTH treatment. Transgenic plants harboring the CYP1A1 cDNA fused with the yeast P450 reductase (YR) gene under the control of PR1a were also produced. Although the plants showed a lower expression level of the fused gene than transgenic plants with CYP1A1 cDNA alone, they were tolerant to herbicides. These facts suggested that the CYP1A1 enzyme fused with YR showed a higher specific activity than CYP1A1 alone. This study demonstrated that the mammalian cDNA for the de-toxification enzyme of herbicides under the control of the PR1a promoter conferred chemical-inducible herbicide tolerance on potato.

**Keywords** Transgenic potato · Cytochrome P450 · PR1a promoter · BTH · Herbicide tolerance

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## Introduction

Recently, cultivation systems of a variety of crops have tended to rely increasingly on the use of a number of agrochemicals for the maintenance of regular agricultural production. However, their residues sometimes affect the ecosystems and resulted in the pollution of crops. Therefore, it is important to develop a system of rapid degradation of chemicals in the agricultural environment after use. Cytochrome P450 monooxygenases in higher plants play an important role in the oxidative metabolism of xenobiotics as well as endogenous substrates (Durst 1991; Sandermann 1992). It has been reported that some of these enzymes enhance the metabolism of agrochemicals, such as several herbicides, in maize, soybean, sorghum, rice and wheat plants (McFadden et al. 1990; Mougin et al. 1990; Leah et al. 1991; Moreland and Corbin 1991; Haack and Balke 1994). On the other hand, more information is available about the P450-dependent de-toxification of drugs in the mammalian liver than in plants. These xenobiotic metabolizing P450 cDNAs derived from mammals confer on transgenic tobacco and potato plants a high level of tolerance to many herbicides and metabolize them to

detoxified compounds (Shiota et al. 1994, 1996; Inui et al. 1998, 2000).

Although a number of transgenes under the control of a strong promoter such as 35S of cauliflower mosaic virus (CaMV) have been described in previous reports, a more-flexible expression system of the transgene is required in transgenic plant technology. It has been reported that endogenous plant genes respond to chemical treatment with 2-chlorobenzenesulfonamide, abscisic acid, methyl jasmonate and high-salt (Claes et al. 1991; Hershey and Stoner 1991; Guevara-Garcia et al. 1998; Koizumi et al. 1993). It is well-known that the pathogenesis-related 1a (PR1a) protein in tobacco is also induced upon pathogen attacks and chemical treatments with salicylic acid (SA) and 2,6-dichloroisonicotinic acid (INA) (White 1979; Ohashi and Matsuoka 1985; Métraux et al. 1991). Recently, Friedrich et al. (1996) have demonstrated that Benzothiadiazole (BTH) induced a high level of mRNA accumulation in PR1a protein in tobacco. BTH has been recently used as a non-phytotoxic plant-protection agent (Friedrich et al. 1996; Görlach et al. 1996; Benhamou and Bélanger 1998). It was reported that the application of BTH efficiently activates a number of systemic acquired resistance (SAR) genes containing PR1a in tobacco, *Arabidopsis* and wheat plants (Friedrich et al. 1996; Görlach et al. 1996; Lawton et al. 1996). Although chemical compounds such as SA and INA activated these genes, BTH showed a higher induction of SAR genes than the other chemicals (Görlach et al. 1996). Therefore, the PR1a promoter element which responds to these chemicals may be a suitable inducible promoter of transgenes in plants (Gatz and Lenk 1998).

In this study, we attempted to generate transgenic potato plants harboring rat P4501A1 (CYP1A1) cDNA with a tobacco PR1a promoter and to control the expression of transgenes artificially by BTH treatment if necessary. The CYP1A1 cDNA fused with the yeast P450 reductase gene (YR) was also introduced into the potato plant, because Shiota et al. (1994) demonstrated that the activity of the fused enzyme genes was higher than that of CYP1A1 alone in transgenic tobacco plants. Furthermore, we examined whether the ability to de-toxify several herbicides is incorporated into transgenic potato plants.

## Materials and methods

### Plasmid construction

The plant expression plasmid pPR1A1 (Fig. 1A) was constructed by insertion of the tobacco PR1a promoter (Ohshima et at. 1987), rat CYP1A1 cDNA (Yabusaki et al. 1984) and Nos terminator into the *Cla*I site of the vector plasmid pBI121 (Clonetech Co.). The expression plasmid pPRT1A1 was constructed by replacing a PRT promoter (containing the region of +29 to +77 in PR1a, Yamakawa et al. 1998) with a PR1a promoter in pPR1A1 (Fig. 1B). The plasmid pPRTYR (Fig. 1C) was prepared by replacing CYP1A1 cDNA in pPRT1A1 with the fused enzyme unit between rat CYP1A1 and yeast P450 reductase (YR) from the previously reported plasmid pGFC2 (Shiota et al. 1994). The plasmid vector pPRTG consisted of the chimerical β-glucuronidase gene fused under the control of the PRT promoter (Fig. 1D).

### Plant materials and transformation

In vitro micro-tubers of *Solanum tuberosum* cv May Queen were used for the transformation experiment. The formation of micro-tubers and tuber-disk transformation with *Agrobacterium tumefaciens* strains LBA4404 were previously described by Ishige et al. (1991). The regenerated plants were selected for their ability to form roots in MS medium (Murashige and Skoog 1962) containing 100 mg/l of kanamycin and 200 mg/l of cefotaxime. The integration of the CYP1A1 cDNA in the kanamycin-resistant plants was verified by PCR with rat CYP1A1 specific primers.

### In vitro selection of highly tolerant plants to the herbicide chlortoluron

PCR-positive plants were cultured in liquid MS (0.5% sucrose) medium containing 20 µM of BTH and 80 µM of chlortoluron. The original BTH compound (CGA245704, Bion) and 5% wettable powder were kindly provided from Novartis Agro Co., Ltd. Based on the phenotypic changes of the transgenic plants cultured for 3 weeks, highly tolerant plants were selected. These plants were used for further analyses.

### Southern blotting

Total DNA was isolated from fresh leaves (2–3 g) by the method of Draper et al. (1988). After treatment with RNase A, polysaccharides were removed by ethanol-precipitation (Michaels et al. 1994). Approximately 10 µg of DNA was digested with *Eco*RI and subjected to electrophoresis on a 0.8% agarose gel. The DNA was then transferred to a nylon membrane (Hybond-$N^+$; Amersham, U.K). The fragment of rat CYP1A1 cDNA amplified by PCR (Fig. 1) was used as the probe in Southern-blot analysis. Probe-labeling, hybridization and detection were carried out using the ECL (enhanced chemiluminescence) direct nucleic acid labeling and detection system (Amersham, U.K).

### Inducible condition of the PRT promoter with BTH in vivo

After inoculation with *A. tumefaciens* harboring pPRTG, many transgenic plants were obtained. Based on the GUS activity of the leaf tissues treated with 2 mM of salicylic acid, one representative plant Ag2551 was selected and used for the determination of inducible conditions by BTH treatment. Ag2551 potted in the growth chamber was treated with BTH (3.0 µmol/pot) sprayed as a fine mist onto leaves with 0.02% Tween 20 and 0.015% sticker (Takeda, Japan). The GUS activity of the fresh leaves from Ag2551 treated with BTH was measured by the fluorescence method (Jefferson 1987). The specific activity was measured for each sample and expressed in pmols of 4-methylumbelliferone (4-MU) synthesis/min/mg of protein.

### Western blotting

The microsomal fraction from fresh potato leaves treated with BTH (3.0 µmol/pot) was prepared by the methods of Shiota et al. (1994). Ten µg and 20 µg of microsomal proteins were applied to SDS-PAGE on 12.5% and 7.5% running gels, respectively. Separated proteins were electrotransferred onto a nitrocellulose membrane (Amersham, U.K). Blotted proteins were immunodetected by using a polyclonal antibody against rat CYP1A1 (Daiichi Pure Chemicals Co., Japan) and a donkey anti-goat IgG horseradish peroxidase conjugate as a secondary antibody. Detection was carried out using the ECL Western-blotting detection system (Amersham, U.K).

### Northern blotting

Total RNA from fresh leaves treated with BTH was isolated by the method of phenol-chloroform extraction and lithium chloride precipitation (Lagrimini et al. 1987). Ten micrograms of the RNA were subjected to electrophoresis through a formaldehyde-agarose gel and transferred to a nylon membrane (Boehringer Mannheim, Germany). Labeling of riboprobes with digoxigenin-dUTP, hybridization and immunological detection were carried out according to the manufacturer's instruction (Boehringer Mannheim, Germany).

### In vivo herbicide tolerance test

Selected transgenic plants were transferred to a growth chamber and cultivated for 1 week. After BTH was applied to them, 7 µmol of chlortoluron, 6 µmol of methabenzthiazuron or 0.3 µmol of norflurazon each per pot were sprayed onto the plants as a fine mist with 0.02% of Tween 20 and 0.015% sticker, respectively. The level of tolerance to each herbicide was evaluated based on the degree of damage caused to the transgenic plants 2 weeks after spraying. This test was carried out with three independent replications.

## Results

### Transformation, regeneration and selection of transgenic plants

After the potato tuber-disks were inoculated with *A. tumefaciens* LBA4404 harboring each of the expression plasmids pPR1A1, pPRT1A1 and pPRTYR, 113, 156 and 137 regenerated plants were obtained, respectively. The integration of the corresponding rat CYP1A1 cDNA was confirmed by PCR in the kanamycin-resistant plants. Fifty seven PR1A1 plants, transformed with pPR1A1, 88 PRT1A1 plants transformed with pPRT1A1, and 85 transgenic PRTYR plants with pPRTYR, were selected through PCR analysis.

### In vitro selection of tolerant plants to the herbicide chlortoluron

The selected transgenic plants were cultured in liquid MS (0.5% sucrose) medium containing 20 µM of BTH and 80 µM of chlortoluron. Although non-transformed May Queen withered completely after 3 weeks, several plants displayed a normal phenotype. In total, four of each highly tolerant plants were selected by PR1A1, PRT1A1 and PRTYR, respectively, on the basis of the damage level caused by chlortoluron exposure (Table 1).

### Southern-blotting analysis

The genomic DNA of four PR1A1 plants (P2562, P2576, P2577 and P2586), four PRT1A1 plants (T2432, T2434, T2437 and T2500) and four PRTYR plants (F2355, F2365, F2371 and F2384) digested with *Eco*RI was examined in order to confirm the integration of the transgenes. Seven plants (P2577, T2432, T2437, F2355, F2365, F2371 and F2384) showed a single banding pattern corresponding to the fragment sizes 8.0 kbp or 4.4 kbp based on the *Eco*RI restriction site of inserted DNA in the expression plasmids (Fig. 1A–C, Fig. 2). However, the other plants (P2562, P2576, P2586, T2434 and T2500) exhibited different banding patterns, suggesting that two or more copies of the CYP1A1 cDNA were integrated (Fig. 2).

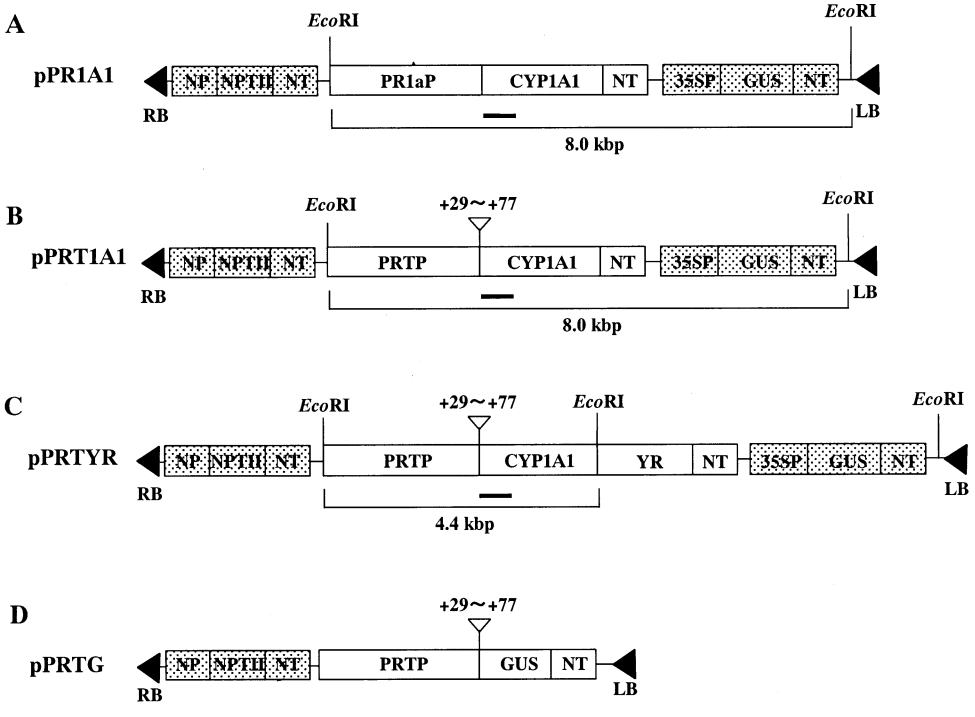


**Fig. 1A–D** Structure of the expression plasmids constructed. **A** pPR1A1. **B** pPRT1A1. **C** pPRTYR. **D** pPRTG. RB, LB, NP, NPTII, NT, PR1aP, PRTP, CYP1A1, YR, 35SP and GUS denote right border, left border, Nos promoter, neomycin phosphotransferase-II gene, Nos terminator, PR1a promoter, PRT promoter, rat CYP1A1 cDNA, yeast P450 reductase gene, CaMV 35S promoter and β-glucuronidase gene, respectively. PRT promoter contains the +27 to +77 region of the PR1a gene. *Eco*RI denotes the restriction site of *Eco*RI enzyme in the expression plasmids. The DNA fragment used for probes in Southern-blot analysis is indicated under the expression plasmids by *bold lines*

**Table 1** Selected transgenic plants with the in vitro chlortoluron tolerance test

| P450 species | Promoter | Expression plasmid | Selected plants |
|---|---|---|---|
| Rat CYP1A1 | PR1a | pPR1A1 | P2562, P2576, P2577, P2586 |
| Rat CYP1A1 | PRT | pPRT1A1 | T2432, T2434, T2437, T2500 |
| Rat CYP1A1/YR | PRT | pPRTYR | F2355, F2365, F2371, F2384 |

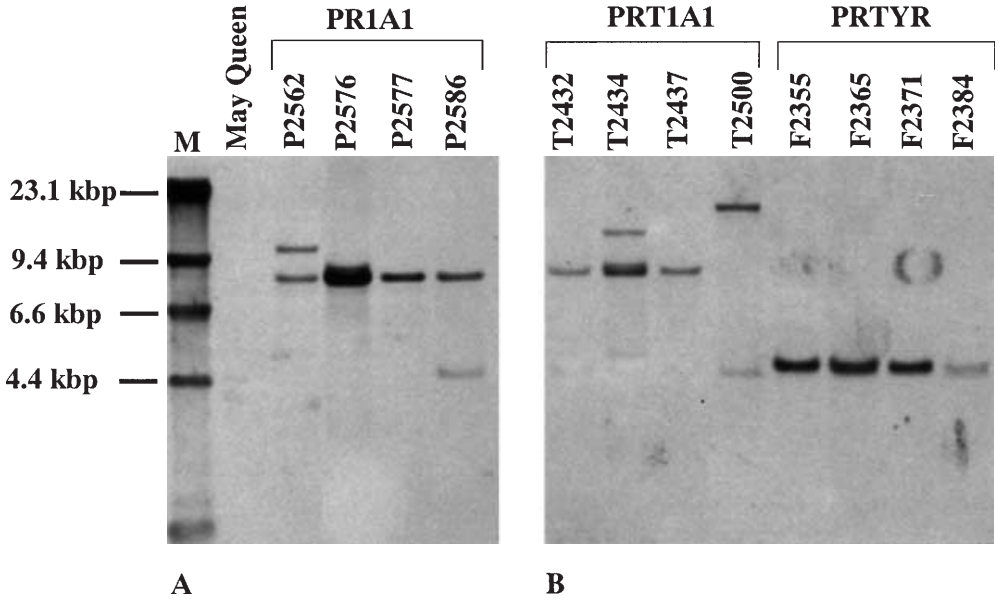


**Fig. 2A, B** Southern-blot analysis of the representative transgenic plants. Genomic DNA was digested with *Eco*RI and probed with a labeled DNA fragment of the CYP1A1 cDNA (see Fig. 1). *M*, molecular-weight marker (lambda DNA digested with *Hin*dIII). **A** PR1A1 plants. **B** PRT1A1 and PRTYR plants

## Western-blotting and Northern-blotting analyses in transgenic plants treated with BTH

The GUS activity of Ag2551 had increased 3 days after the BTH treatment (3.0 µmol/pot) and continuously reached the maximum level at 10 days after treatment (Fig. 3). Based on the above-mentioned results, we assumed that treatment with 3.0 µmol/pot of BTH treatment was sufficient to induce the expression of CYP1A1 cDNA and its fused enzyme gene with YR. Therefore, microsomal fractions were isolated from the leaves at 7 days after the BTH treatment. Although no protein band was detected in most of the transgenic plants without BTH treatment, bands corresponding to rat CYP1A1 or its fused enzyme were observed in all the transgenic plants treated with BTH. A high accumulation of CYP1A1 protein was observed in the leaves from P2577 (PR1A1 plant), T2432 and T2434 (PRT1A1 plants) treated with BTH. Two PRT1A1 plants, T2434 and T2500, were found to contain the protein corresponding to CYP1A1 even in non-treated leaves. On the other hand, the accumulation level of CYP1A1 fused with YR protein in the PRTYR plants was much lower than that in the PR1A1 and PRT1A1 plants even after treatment with BTH. In the three plants (P2577, T2432 and F2355) treated with BTH, mRNA bands that hybridized with the CYP1A1 riboprobes were detected (Fig. 4A–C). Although a slight expression of CYP1A1 was observed in P2577 and T2432 without BTH treatment, Northern-blot patterns showed that the amount of mRNA of rat

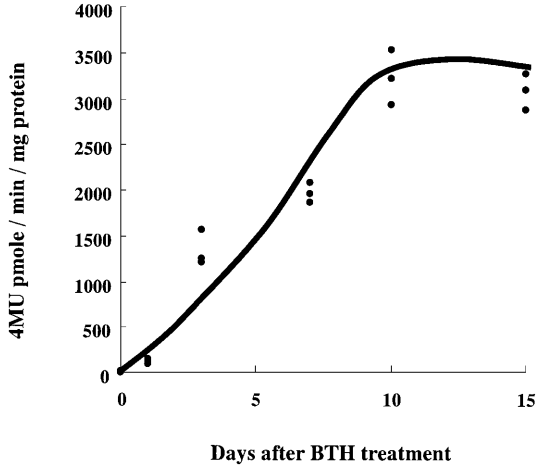


**Fig. 3** GUS activity in the transgenic plant Ag2551 harboring the PRT-GUS gene. The level of GUS activity was assayed at 0, 1, 3, 7, 10 and 15 days after the treatment with BTH (3.0 µmol/pot). The activity data expressed the results of three independent experiments

CYP1A1 markedly increased in these transgenic plants 15 days after the BTH treatment (Fig. 4A, B).

## In vivo herbicide tolerance test

For the tolerance test to the herbicides chlortoluron, methabenzthiazuron and norflurazon, P2577, T2432 and F2355 were used and each herbicide was sprayed onto

**Table 2** Tolerance test to chlortoluron, methabenzthiazuron and norflurazon

| Plant lines | Chlortoluron | | Methabenzthiazuron | | Norflurazon | |
|---|---|---|---|---|---|---|
| | Non-BTH | BTH | Non-BTH | BTH | Non-BTH | BTH |
| P2577 | Plants died | No damage | Slightly damage | No damage | Chlorosis | Chlorosis |
| T2432 | Plants died | No damage | Plants died | No damage | Chlorosis | Chlorosis |
| F2355 | Plants died | No damage | Plants died | No damage | Chlorosis | Chlorosis |

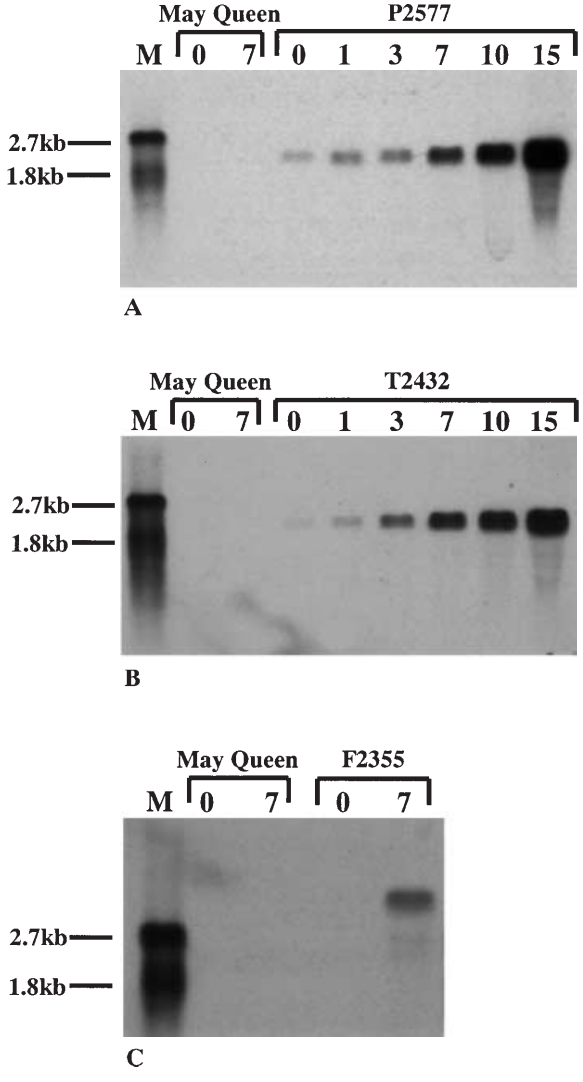


**Fig. 4A–C** Northern-blot analysis of the representative transgenic plants. Total RNA was extracted from the leaves in May Queen and transgenic plants. 0, 1, 3, 7, 10 and 15 indicate the number of days after BTH treatment (3.0 µmol/pot). *M*, RNA molecular-weight marker. **A, B** 10 µg of total RNA hybridized with rat CYP1A1 riboprobes. **C** 20 µg of total RNA hybridized with rat CYP1A1 riboprobes

leaves in the transgenic and control (May Queen) plants 7 days after the BTH treatment (3.0 µmol/pot). Although the control and three transgenic plants without BTH treatment had withered completely within 2 weeks after spraying with chlortoluron, three transgenic plants treated with BTH did not show any phytotoxic changes (Table 2). Among the transgenic plants not treated with BTH, only P2577 (PR1A1 plant) was slightly damaged by methabenzthiazuron whereas the other plants almost withered (Table 2). Three transgenic plants treated with BTH also showed a normal phenotype after methabenzthiazuron spraying (Table 2). On the other hand, all the plants exhibited chlorosis around the leaf vein after norflurazon application (Table 2).

## Discussion

BTH has been developed under the trade name ‘Bion’ in the agrochemical market and used as a non-phytotoxic plant-protection agent (Kunz et al. 1997). It was reported that BTH activated a number of systemic acquired resistance (SAR) genes including PR1a in tobacco, *Arabidopsis* and wheat (Friedrich et al. 1996; Görlach et al. 1996; Lawton et al. 1996). Because the PR1a (2.4 kbp) and PRT (containing the region of +29 to +77 in PR1a) promoters used in this study contained also the promoter region described by Friedrich et al. (1996), transgenes under the control of these promoters were easily induced by BTH treatment. The GUS activity had increased after 3 days of BTH application in the Ag2551 transgenic plant harboring the PRT-GUS fusion gene. This concentration of BTH was considered to be sufficient to activate the PR1a and PRT promoters. Western-blot analysis in the PR1A1, PRT1A1 and PRTYR plants 7 days after BTH treatment showed that rat CYP1A1 and its fused proteins with YR accumulated in the transgenic plants treated with BTH. These facts indicated that BTH application efficiently led to the expression of *CYP1A1* and its fused genes, as well as to the expression of the PRT-GUS gene in Ag2551. In the T2434 and T2500 transgenic plants, a large amount of CYP1A1 protein was detected in the leaves from plants not treated with BTH, indicating that the basal expression of the CYP1A1 cDNA was much higher than that in the other plants. Several copies of transgenes were estimated to be integrated into genomic DNA in T2434 and T2500, because the Southern-blotting pattern showed several signal bands hybridized with the CYP1A1-specific probe. Each two bands in

T2434 and T2500 showed unexpected fragment sizes, namely the fragment sizes were larger than 8.0 kbp based on the *Eco*RI restriction site in the expression plasmid (Figs. 1B and 2). The large copy number of the transgene, whether or not defective in construction, might be associated with the high basal expression of the CYP1A1 cDNA.

Northern-blot analysis showed that the mRNAs of CYP1A1, or its fused gene with YR, were induced in the transgenic plants P2577 (PR1A1), T2432 (PRT1A1) and F2355 (PRTYR) treated with BTH, respectively. The mRNA of CYP1A1 in P2577 and T2432 began to accumulate 1 day after BTH application and did not reach a maximum level even at 15 days after treatment (Fig. 4A, B). The mRNA accumulation in the endogenous PR1a gene reached the maximum level at 3 days and 1 day after BTH application in the tobacco and Arabidopsis plants, respectively (Friedrich et al. 1996; Lawton et al. 1996). The extended accumulation might depend on the absence of a feedback mechanism for suppressing the exogenous genes. Although the amount of mRNA of CYP1A1 in P2577 and T2432 had increased even 15 days after BTH treatment (3.0 µmol/pot), these plants showed that the expression level of CYP1A1 at 7 days after BTH treatment was the same as that of the transgenic plants harboring CYP1A1 cDNA under the control of the CaMV 35S promoter in both Western- and Northern-blot analyses (data not shown). The transgenic plants harboring CYP1A1 cDNA under the control of the CaMV 35S promoter efficiently metabolized the herbicides chlortoluron and DCMU to de-toxified compounds (Inui et al. 1998). These facts indicated that the expression level of CYP1A1 in P2577 and T2432 at 7 days after the BTH pre-treatment might be sufficient to metabolize these herbicides to de-toxified compounds. F2355 showed a high tolerance to chlortoluron and methabenzthiazuron in the same way as P2577 and T2432. However, the accumulation level of fused protein was lower than that of CYP1A1 alone. These findings suggested that the CYP1A1 fused enzyme with YR shows a higher specific activity than the CYP1A1 alone and confers herbicide tolerance, although the levels of enzyme protein was low.

The transgenic plants harboring the rat CYP1A1 cDNA metabolized the herbicide chlortoluron through *N*-demethylation and ring-methyl hydroxylation (Shiota et al. 1994; Inui et al. 1998). In this study, three transgenic plants P2577, T2432 and F2355 treated with BTH showed a high tolerance to chlortoluron, indicating that these plants metabolized it to de-toxified compounds using CYP1A1 or its fused enzyme with YR induced by BTH treatment. Furthermore, these transgenic plants treated with BTH exhibited no damage with methabenzthiazuron treatment. On the other hand, no tolerance to chlortoluron and methabenzthiazuron was observed in T2432 and F2355 without BTH treatment. Only P2577 showed a tolerance to methabenzthiazuron without BTH treatment. In the Northern-blotting analysis, a slight level of mRNA for CYP1A1 was detected in both P2577 and T2432 in the absence of BTH. From these results, it was considered that the basal level of CYP1A1 expression in P2577 without the BTH treatment was sufficient to de-toxify methabenzthiazuron, but unlike chlortoluron the basal level in T2432 was not sufficient to de-toxify both herbicides. Inui et al. (2000) reported that transgenic potatoes harboring the human CYP1A1 cDNA showed a high tolerance to norflurazon. All the transgenic plants used in this study developed chlorosis around the leaf vein even on the application of 0.3 µmol/pot of norflurazon. The transgenic plants harboring rat CYP1A1 cDNA under the control of the CaMV 35S promoter also were not tolerant to norflurazon, as in the case of the other transgenic plants (data not shown). These facts indicate that the plants with rat CYP1A1 could not metabolize norflurazon to de-toxified compounds sufficiently. The sensitivity to norflurazon in these transgenic plants is not associated with the level and organ specificity of the promoter activity, but the rat CYP1A1 enzyme may have a much lower ability to metabolize norflurazon than human CYP1A1 and the other P450 species in rat liver.

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## References

Benhamou N, Bélanger RR (1998) Benzothiadiazole-mediated induced resistance to *Fusarium oxysporum* f. sp. *radicis-lycopersici* in tomato. Plant Physiol 118:1203–1212

Claes B, Smalle J, Dekeyser R, Van Montagu M, Caplan A (1991) Organ-dependent regulation of a plant promoter isolated from rice by promoter-trapping in tobacco. Plant J 1:15–26

Draper JJ, Scott R, Armitage P, Walden R (1988) Plant genetic transformation and gene expression. A laboratory manual. Blackwell Scientific Publication, London, pp 212–214

Durst F (1991) Biochemistry and physiology of plant cytochrome 450. In: Ruckpaul K, Rein H (eds) Frontiers in bioremediation, vol 4. Akademie Verlag, Berlin, pp 191–232

Friedrich L, Lawton K, Ruess W, Masner P, Specker N, Gut Rella M, Meier B, Dincher S, Staub T, Uknes S and Métraux J-P, Kessmann H, Ryals J (1996) A benzothiadiazole derivative induces systemic acquired resistance in tobacco. Plant J 10:61–70

Gatz C, Lenk I (1998) Promoters that respond to chemical inducers. Trends Plant Sci 3:352–358

Görlach J, Volrath S, Knauf-Beiter G, Hengy G, Beckhove U, Kogel K-H, Oostendorp M, Staub T, Ward E, Kessmann H, Ryals J (1996) Benzothiadiazole, a novel class of inducers of systemic acquired resistance, activates gene expression and disease resistance in wheat. Plant Cell 8:629–643

Guevara-Garcia A, Lopez-Ochoa L, Lopez-Bucio J, Simpson J, Herrera-Estrella L (1998) A 42-bp fragment of the pmas1′ promoter containing an ocs-like element confers a developmental wound- and chemically inducible-expression pattern. Plant Mol Biol 38:743–753

Hacck AE, Balke NE (1994) Aryl-hydroxylation of bentazon by bentazon 6-hydroxylase, a cytochrome P450 monooxygenase, from sorghum, corn, and rice. In: Abstract of the 8th IUPAC Congress of Pesticide Chemistry, vol. 2, pp 839

Hershey HP, Stoner TD (1991) Isolation and characterization of cDNA clones for RNA species induced by substituted benzenesulfonamides in corn. Plant Mol Biol 17:679–690

Inui H, Shiota N, Ishige T, Ohkawa Y, Ohkawa H (1998) Herbicide metabolism and resistance of transgenic potato plants expressing rat cytochrome P4501A1. Breeding Sci 48:135–143

Inui H, Kodama T, Ohkawa Y, Ohkawa H (2000) Herbicide metabolism and cross-tolerance in transgenic potato plants co-expressing human CYP1A1, CYP2B6 and CYP2C19. Pestic Biochem Physiol 66:116–129

Ishige T, Ohshima M, Ohashi Y (1991) Transformation of Japanese potato cultivars with the β-glucuronidase gene fused with the promoter of the pathogenesis-related 1a protein gene of tobacco. Plant Sci 73:167–174

Jefferson RA (1987) GUS fusions: assaying chimeric gene in plants: The GUS gene fusion system. Plant Mol Biol Rep 5:387–405

Koizumi M, Yamaguchi-Shinozaki K, Tsujii H, Shinozaki K (1993) Structure and expression of two genes that encode distinct drought-inducible cysteine protein *Arabidopsis thaliana*. Gene 129:175–182

Kunz W, Schurter R, Maetzke T (1997) The chemistry of Benzothiadiazole plant activators. Pestic Sci 50:275–282

Lagrimini LM, Burkhart W, Moyer M, Rothstein S (1987) Molecular cloning of complementary DNA encoding the lignin-forming peroxidase from tobacco: molecular analysis and tissue-specific expression. Proc Natl Acad Sci USA 84:7542–7546

Lawton KA, Friedrich L, Hunt M, Weymann K, Delaney T, Kessmann H, Staub T, Ryals J (1996) Benzothiadiazole induces disease resistance in *Arabidopsis* by activation of the systemic acquired resistance signal transduction pathway. Plant J 10:71–82

Leah JM, Worrall TL, Cobb AH (1991) A study of bentazon uptake and metabolism in the presence of cytochrome P-450 and acethyl-coenzyme A carboxylase inhibitors. Pestic Biochem Physiol 39:232–239

McFadden JJ, Gronwald JW, Eberlein CV (1990) In vitro hydroxylation of bentazon by microsomes from naphthalic anhydride-treated corn shoots. Biochem Biophys Res Commun 168:206–213

Métraux J-P, Ahl Goy P, Staub T, Speich J, Steinemann A, Ryals J, Ward E (1991) Induced resistance in cucumber in response to 2, 6-dichloroisonicotinic acid and pathogens. In: Hennecke H, Verma DPS (eds) Advances in molecular genetics of plant – microbe interactions, vol. 1. Kluwer Dordrecht, pp 432–439

Michaels SD, John MC, Amasino RM (1994) Removal of polysaccharides from plant DNA by ethanol precipitation. Bio Tech 17:274–276

Moreland DE, Corbin FT (1991) Influence of safeners on the in vivo and in vitro metabolism of bentazon and metolachlor by grain sorghum shoots – A preliminary report. Z Naturforschung 46:906–914

Mougin C, Cabanne F, Canivenc M-C, Scalla R (1990) Hydroxylation and *N*-demethylation of chlortoluron by wheat microsoma l enzymes. Plant Sci 66:195–203

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassay with tobacco tissue culture. Physiol Plant 15: 473–497

Ohashi Y, Matsuoka M (1985) Synthesis of stress protein in tobacco leaves. Plant Cell Physiol 26:473–480

Ohshima M, Matsuoka M, Yamamoto N, Tanaka Y, Kano-Murakami Y, Ozeki Y, Kato A, Harada N, Ohashi Y (1987) Nucleotide sequence of the PR-1 gene of *Nicotiana tabacum*. FEBS Lett 225:243–246

Sandermann HJ (1992) Plant metabolism of xenobiotics. Trends Biochem Sci 17:82–84

Shiota N, Nagasawa A, Sakaki T, Yabusaki Y, Ohkawa H (1994) Herbicide-resistant tobacco plants expressing the fused enzyme between rat cytochrome P4501A1 (CYP1A1) and yeast NADP-cytochrome P450 oxidoreductase. Plant Physiol 106:17–23

Shiota N, Inui H, Ohkawa H (1996) Metabolism of the herbicide chlortoluron in transgenic tobacco plants expressing the fused enzyme between rat cytochrome P4501A1 and yeast NADPH-cytochrome P450 oxidoreductase. Pestic Biochem Physiol 54:190–198

White RF (1979) Acetylsalicylic acid (aspirin) induces resistance to tobacco mosaic virus in tobacco. Virology 99:410–412

Yabusaki Y, Shimizu M, Murakami, Nakamura K, Oeda K, Ohkawa H (1984) Nucleotide sequence in a full-length cDNA coding for 3-methylcholanthrene-induced rat liver cytochrome P-450MC. Nucleic Acids Res 12:2929–2938

Yamakawa H, Kamada H, Satoh M, Ohashi Y (1998) Spermine is a salicylate-independent endogenous inducer for both tobacco acidic pathogenesis-related proteins and resistance against tobacco mosaic virus infection. Plant Physiol 118:1213–1222