Genetics and Molecular Biology, 34, 4, 634-639 (2011)

www.sbg.org.br

Research Article

Transgenic plants of *Petunia hybrida* harboring the *CYP2E1* gene efficiently remove benzene and toluene pollutants and improve resistance to formaldehyde

Daoxiang Zhang, Taihe Xiang, Peihan Li and Lumin Bao

College of Life and Environmental Sciences, Hangzhou Normal University, Hangzhou, China.

Abstract

The CYP2E1 protein belongs to the P450 enzymes family and plays an important role in the metabolism of small molecular and organic pollutants. In this study we generated *CYP2E1* transgenic plants of *Petunia* using *Agrobacterium rhizogenes* K599. PCR analysis confirmed that the regenerated plants contained the *CYP2E1* transgene and the *rol*B gene of the Ri plasmid. Southern blotting revealed the presence of multiple copies of *CYP2E1* in the genome of transgenic plants. Fluorescent quantitative PCR revealed exogenous *CYP2E1* gene expression in *CYP2E1* transgenic plants at various levels, whereas no like expression was detected in either *GUS* transgenic plants or wild-types. The absorption of benzene and toluene by transgenic plants was analyzed through quantitative gas chromatography. Transgenic plants with high *CYP2E1* expression showed a significant increase in absorption capacity of environmental benzene and toluene, compared to control *GUS* transgenic and wild type plants. Furthermore, these plants also presented obvious improved resistance to formaldehyde. This study, besides being the first to reveal that the *CYP2E1* gene enhances plant resistance to formaldehyde, also furnishes a new method for reducing pollutants, such as benzene, toluene and formaldehyde, by using transgenic flowering horticultural plants.

Key words: Petunia hybrida, *CYP2E1* gene, benzene, toluene, formaldehyde.

Received: May 1, 2011; Accepted: June 2, 2011.

Introduction

Benzene and toluene are common chemical components in paints, dyes, adhesives, wallpapers, carpets, synthetic fibers and detergents. Furthermore, formaldehyde is a main pollutant in the process of room decoration. These three chemicals are considered as the major hazardousgases in indoor environments. In addition, besides being highly carcinogenic, benzene and toluene can be produced by facsimile machines, computer terminals and printers. Furthermore, tobacco smoke and gasoline exhaust also contain high levels of benzene and toluene (Shen *et al.*, 2001; Abhilash *et al.*, 2009). Although increased ventilation by opening windows may reduce the risk of pollution, the release process itself can be extremely slow (Isbell *et al.*, 2005), thereby placing in great risk those living in a polluted indoor environment.

Using plants to clean chemical air pollution is economic, effective and non-destructive. There are two steps in the purification of chemical air-pollution, viz., the withholding process, which involves plant interception, absorption and retention, and the removal process, through plant absorption, degradation, transformation, assimilation, and ultra-assimilation (Liao *et al.*, 2007; Kavamura and Esposito, 2010). Although some natural plants have the capacity to decompose and purify, this is usually extremely inefficient. Nevertheless, efficiency can be effectively improved by way of the transgenic technique (Hu *et al.*, 2005; Van Aken, 2008).

Cytochrome P450 2E1 is a decomposition enzyme in mammalian liver, with the strong and specific capacity of decomposing organic pollutants in animal bodies (Lee *et al.*, 1996; Gonzalez, 2007). The Doty group at the University of Washington successfully transferred the cytochrome P450 2E1 gene (*CYP2E1*) into the model plants tobacco and poplar. Transgenic tobacco efficiently decomposed benzene, toluene, trichloroethylene, chloroform, carbon tetrachloride, and bi-vinyl chloride, whereas the transgenic poplar exhibited increased removal rates of trichloroethylene, vinyl chloride, carbon tetrachloride, benzene, and chloroform. The latter also demonstrated superior removal of air pollutants, when exposed to gaseous trichloroethylene, chloroform, and benzene (Doty *et al.*, 2000, 2007; Banerjee *et al.*, 2002; James *et al.*, 2008). These studies opened the door to a new method of reducing environmental threats by air pollution.

Send correspondence to Taihe Xiang. College of Life and Environmental Sciences, Hangzhou Normal University no. 16, Xuelin Street, Hangzhou City 310036, Zhejiang Province, China. E-mail: xthcn@163.com, xthcn@hznu.edu.cn.

Petunia hybrida is a perennial plant, known as the "king of parterre flowers". It can be planted not only on the ground to arrange parterres, but also in hanging pots to beautify balconies. It is much-loved and widely grown worldwide (Dai and Bao, 2004). In this study we successfully transferred a *CYP2E1* gene into *Petunia hybrida*, thus obtaining transgenic *Petunia* plants that could efficiently remove benzene and toluene, besides improving resistance to formaldehyde.

Material and Methods

Material

The QL01 variety of *Petunia hybrida* was used in this study. Sterilized seedlings were obtained by leaf *in vitro* tissue culture. The wild-type *Agrobacterium rhizogenes* K599 was raised in our laboratory, and the pSLD50-6 plasmid containing a *CYP2E1,* as well as the pKH200 plasmid containing the control *GUS* gene, were kindly provided by Dr. Doty at the University of Washington.

Transformation of pSLD50-6 and pKH200 plasmids into *Agrobacterium rhizogenes* K599

The freeze-thaw method was used to transform the pSLD50-6 and pKH200 plasmids into *Agrobacterium rhizogenes* K599. 0.1 μg of purified plasmid DNA was evenly mixed within 100 μL of K599 competent cells, and the mixture was put on ice for 10 min, followed by 5 min of quick freezing in liquid nitrogen and 5 min of heat shock in a water bath at 28 °C. Then, 500 μL of LB medium was added to the mixture, which was cultured for 2 h with slow shaking at 28 °C. Finally, 100 μL of bacteria solution was poured onto LB plates containing 50 mg/L of kanamycin (Km) and 50 mg/L of streptomycin (Str), and cultured for 48 h at 28 °C.

Induction of transgenic hairy roots and regeneration of *Petunia* plants

The modified leaf-disk method, previously described by Wang and Xiang (2009), was used to genetically transform leaf explants of *Petunia*. Specimens of *Agrobacterium rhizogenes* K599 bearing either pSLD50-6 or pKH200, were activated on LB plates with 50 mg/L of Km and 50 mg/L of Str. Single colonies were then selected for overnight culture in liquid medium containing 50 mg/L of Km and 50 mg/L of Str, with constant shaking at 200 r/min. Subsequently, 1 mL of bacteria solution was transferred to 50 mL of an MS medium containing 50 mg/L of Km and 50 mg/L of Str, for culture at 28 °C with 200 r/min shaking until the OD_{600} reached around 0.5. All the bacteria were then spun down and washed 3 times with MS medium. Under aseptic conditions, wounded leaves, which had been pre-cultured on MS medium containing 20 mg/L of As (acetosyrinone), were infected by bacteria for 8 min. After infection, the leaves were transferred to solid MS medium containing 20 mg/L of As and cultured for 3 days. Afterwards, they were washed 3 times with MS medium containing 500 mg/L of Cef (cefotaxime sodium), before transferred to the selection MS medium with 1.0 mg/L of 6-BA, 50 mg/L of Km, and 500 mg/L of Cef. After hairy roots had been induced and grown to about 2 cm in length, they were transferred to a fresh MS medium containing 500 mg/L of Cef and 50 mg/L of Km, to eliminate bacteria and reproduce.

Based on the sequences of the *CYP2E1* gene (GenBank accession number: M15061), *GUS* gene (GenBank accession number: AF354045) and *rol*B gene (GenBank accession number: X64255), the following primers were designed: CYP2E1-P1: 5'- TGA AGG GTG TGCA GCC GAT GAC AA -3', and CYP2E1-P2: 5'- CAT CGG GAA TCT TCT CCA GTT GG -3'; GUS-P1: 5'- CTG CGA CGC TCA CAC CGA T -3', and GUS-P2: 5'- TCA CCG AAG TTC ATG CCA GTC CAG -3'; rolB-P1: 5'- GCC AGC ATT TTT GGT GAA CT -3' and rolB-P2: 5'- CTG GCC CAT CGT TCT AAA AAA -3'. PCR analysis following the protocol described by Wang and Xiang (2009) were performed to identify transgenic hairy roots.

After sterilization and reproduction, hairy roots were cultured on MS medium containing 1.0 mg/L of 6-BA to induce calluses, which were used to generate multiple shoot-clumps on MS medium with 1.0 mg/L of 6-BA and 0.4 mg/L of NAA. These clumps were cultured on MS medium supplemented with 0.1 mg/L of NAA, to thus obtain entire plants. In addition, PCR amplification was employed to identify the regenerated plants, as previously done with hairy roots.

Genomic DNA of transgenic plants, first digested with *Eco*RI at 37 °C overnight, was separated in 0.8% agarose gel and transferred to a nylon membrane. DNA fragments, amplified from genomic DNA with primers of CYP2E1-P1 and CYP2E1-P2, were used for preparing the probe. Probe labeling and hybridization were with the DIG DNA Labeling and Detection Kit (Roche Co.), according to manufacturer's instructions. Finally, hybridization results were scanned for the record.

Fluorescent quantitative RT-PCR analysis of *CYP2E1* genes in transgenic *Petunia* plants

Plant total RNA was extracted and cDNA produced by means of the RevertAid First Strand cDNA Synthesis Kit from Fermentas Co. The amplification reagent for fluorescent quantitative PCR was the SYBR Green Realtime PCR Master Mix from TaKaRa Inc. The following primers were designed for amplifying *Petunia GAPDH* as reference gene (GenBank accession number: GQ122207): GAPDH-F: 5'- AGC AAG GCA GTT AGT GGT GCA -3'; and GAPDH-R: 5'- TTG TGA TCT CCG CTC CTA GCA -3'. Based on the *CYP2E1* gene sequence, the following primers were designed for specific quantitative analysis: CYP2E1-F: 5'- ATT CCC AAG TCC TTT GGC AGG -3';

and CYP2E1-R: 5'- TGT GGT TCA ACA GCA TCT CCC -3'. Real-time PCR reactions were carried out in a Roche LightCycler 480 PCR machine, under the following conditions: 95 °C for 2 min, followed by 40 cycles of 95 °C for 30 s, 59 °C for 30 s and 72 °C for 20 s, whereupon the reactions were then maintained at 4 °C. Relative expression levels = $2^{-(\text{CT of target gene} - \text{CT of reference gene})}$ were calculated (Schmittgen and Livak, 2008).

Gas chromatography analysis of *Petunia* transgenic-plant benzene and toluene absorption capacity

A 5 mL aliquot of MS liquid-basal medium was placed into a 20 mL vial for volatile organic-compound analysis (VOA) and autoclaved. In an aseptic hood, tissue aliquots of 0.75 g of either transgenic or control aseptic plants were added to VOA vials, with a blank control containing only medium but no plant material. The vials were sealed with plastic film and placed into an incubator at 22-24 °C with 100 r/min agitation and a photoperiod of 16 h. One day later, 42.5 μg of benzene and 15 μg of toluene were added to each VOA vial with a micro-plus injector, whereupon the vials were sealed with caps with release-valves, and cultured under the same conditions for 2 h. The first samples were then obtained by using gas-tight gas samplers, and tested with a gas chromatographer of Aglient model 6890N with a column model of DB-624 (30 m x 0.53 mm x 3.0 μm). Testing conditions were: 200 °C of inlet temperature, 250 °C of test group, 5:1 of splitting ratio, 4.0 mL/min of constant elution with N2, and 60 °C of column temperature for 5 min, whereupon the temperature was increased to 200 °C at a rate of 20 °C/min, and kept there for 5 min, 80 °C of headspace for 30 min. Samples for gas chromatography analysis were collected at 48, 96 and 146 h.

Testing *Petunia*-plant ability for resistance to formaldehyde

Aliquots of 5 mL MS basal medium were poured into 20 mL VOA vials. In the hood, the medium was adjusted to contain 50 mg/L of formaldehyde. 0.4 g of *CYP2E1* gene transgenic plant, *GUS* gene transgenic plant and wild-type plant were cultured on this medium at 24-26 °C, 14/10 h light/ dark photoperiod and 1500-2000 Lux light.

Results

Hairy-root formation and regeneration in transgenic *Petunia* plants

About 10 days after infection by *Agrobacterium rhizogenes*, leaf-edges sprouted small white protrusions. Simultaneously, 15 days after infection, white hairy-roots appeared on the edges of cuts (Figure 1A). However, there was no root-formation on uninfected detached leaves. The hairy-roots grew well, with the formation of numerous branched roots after only 15-20 days. After a further 15 days, green calluses were induced from these roots onto MS medium containing 1.0 mg/L of 6-BA (Figure 1B). After 45 days, multiple shoot-clusters were differentiated from the calluses on MS medium containing 1.0 mg/L of 6-BA and 0.4 mg/L of NAA. These proceeded to produce roots on MS medium containing 0.1 mg/L of NAA, later forming complete plants (Figure 1C). They were finally survival-transplanted into soil (Figure 1D), where they developed a dwarf-like morphology with abundant roots. Four plants transplanted into soil flowered normally, although without setting seeds.

The CYP2E1-P1 and CYP2E1-P2 primers amplified a 410 bp band from both *CYP2E1* transgenic plants and pSLD50-6 plasmids, while no PCR products were detected using these two primers for plants regenerated by tissue culture. The rolB-P1 and rolB-P2 primers amplified a band of around 450 bp from all the 7 randomly chosen *CYP2E1* transgenic plants, as well as from Ri plasmids borne by wild-type *Agrobacterium rhizogenes*, while no such PCR band was observed using these primers for plants regenerated by tissue culture (Figure 2A, B). A similar strategy for PCR-characterization was used to confirm *GUS* transgenic plants, in this case using rolB-P1, rolB-P2, GUS-P1, and GUS-P2 primers (data not shown).

Furthermore, Southern blot analysis revealed that the *CYP2E1* gene had multiple copies in the genome of four survival-plants transplanted into soil, which were denominated CYP2E1-1, CYP2E1-2, CYP2E1-3 and CYP2E1-4 (Figure 2C).

Fluorescent quantitative RT-PCR for *CYP2E1* transgenic *Petunia* plants

Fluorescent quantitative RT-PCR was undertaken for the following selected plants, with *GAPDH* as the reference gene: the four *CYP2E1* transgenic plants, CYP2E1-1, CYP2E1-2, CYP2E1-3 and CYP2E1-4; a *GUS* transgenic control plant; and a wild-type (non-transgenic) plant. The exogenous *CYP2E1* gene was expressed in all the examined *CYP2E1* transgenic plants, with significantly higher expression in CYP2E1-1 and CYP2E1-4, whereas no expression was detected in the *GUS* control and the wild type plant (Figure 3).

CYP2E1 transgenic *Petunia* plant benzene and toluene absorption and decomposition capacity

The absorption of benzene and toluene by transgenic plants was measured by quantitative gas chromatography. Six days later, the levels of benzene and toluene had already been significantly reduced by the *CYP2E1* transgenic plant tissues of CYP2E1-1 and CYP2E1-4. *GUS* transgenic and wild type plants revealed no significant changes in benzene and toluene absorption (Figure 4A, B). Furthermore, there was no significant difference between CYP2E1-2 and

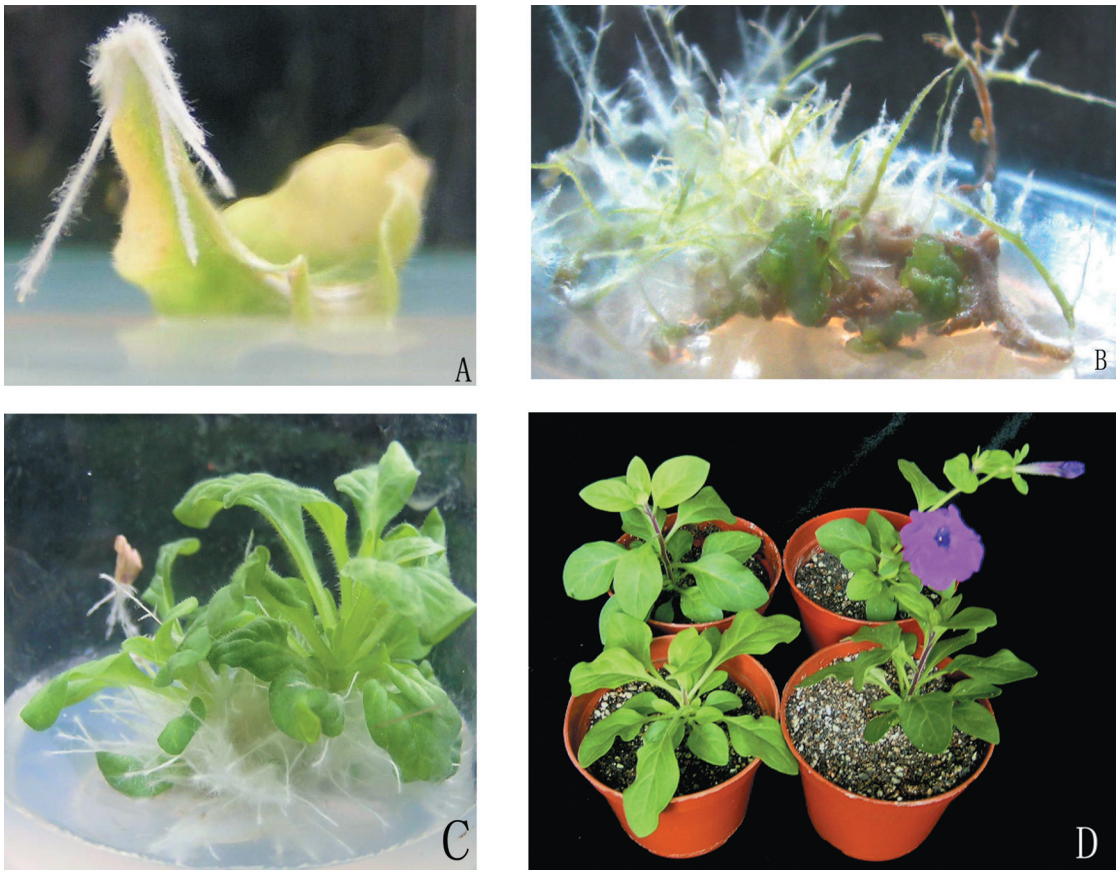

Figure 1 - Transgenic hairy roots and regenerated plants of *CYP2E1* transgenic *Petunia*. (A) transgenic hairy roots from cuts in the edges of *Petunia* leaves; (B) calluses induced from transgenic hairy roots; (C) plants regenerated from calluses; (D) four transgenic plants survival-transplanted into soil.

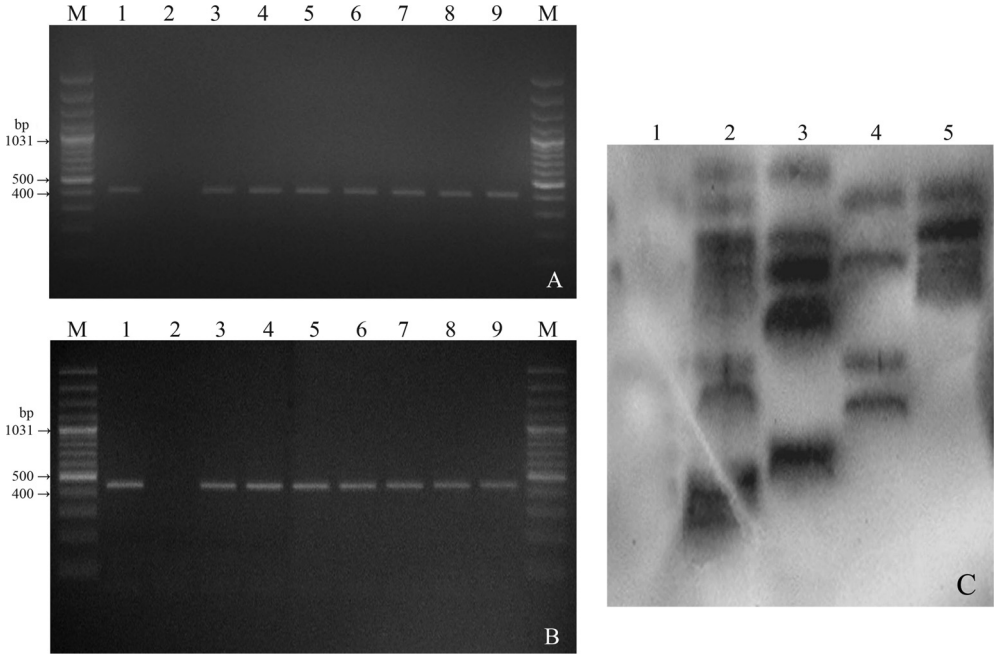

Figure 2 - Molecular identification of regenerated plants of *CYP2E1* transgenic *Petunia*. (A) PCR amplification of *CYP2E1* gene; M: 100 bp ladder plus DNA molecular marker (MBI Co.); 1: 410 bp band amplified from Ri plasmid carried by wild-type *Agrobacterium rhizogenes* K599; 2: no PCR bands were observed in plants regenerated by tissue culture; 3-9: 410 bp bands amplified from each of the 7 *CYP2E1* transgenic plants. (B) PCR amplification of the *rol*B gene; M: 100 bp ladder plus DNA molecular marker (MBI Co.); 1: amplification of an approx. 450 bp band from Ri plasmids carried by wild-type *Agrobacterium rhizogenes* K599; 2: no PCR bands were observed in plants regenerated by tissue culture; 3-9: 450 bp bands amplified from each of the 7 *CYP2E1* transgenic plants. (C) Southern blot analysis of *CYP2E1* transgenic plants; 1: no bands in wild-type (non-transgenic) plants; 2-5: respective bands in CYP2E1-1, CYP2E1-2, CYP2E1-3 and CYP2E1-4 transgenic plants.

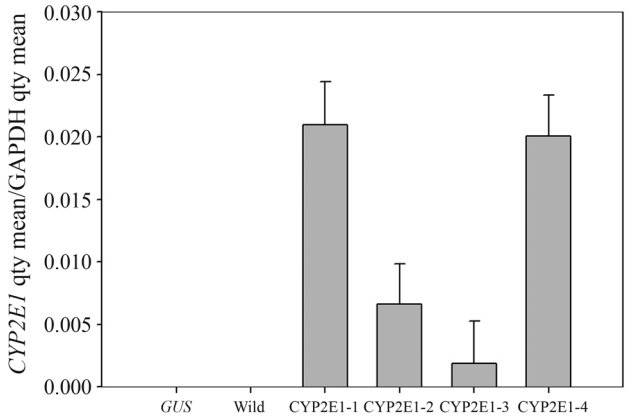

Figure 3 - Fluorescent quantitative RT-PCR analysis of *CYP2E1* gene expression in *CYP2E1* transgenic, *GUS* transgenic and wild type (non-transgenic) plants. The exogenous *CYP2E1* gene, detected in all the examined *CYP2E1* transgenic plants, CYP2E1-1, CYP2E1-2, CYP2E1-3 and CYP2E1-4, were especially highly expressed in two, CYP2E1-1 and CYP2E1-4, while *GUS* control and wild type plants did not express *CYP2E1*.

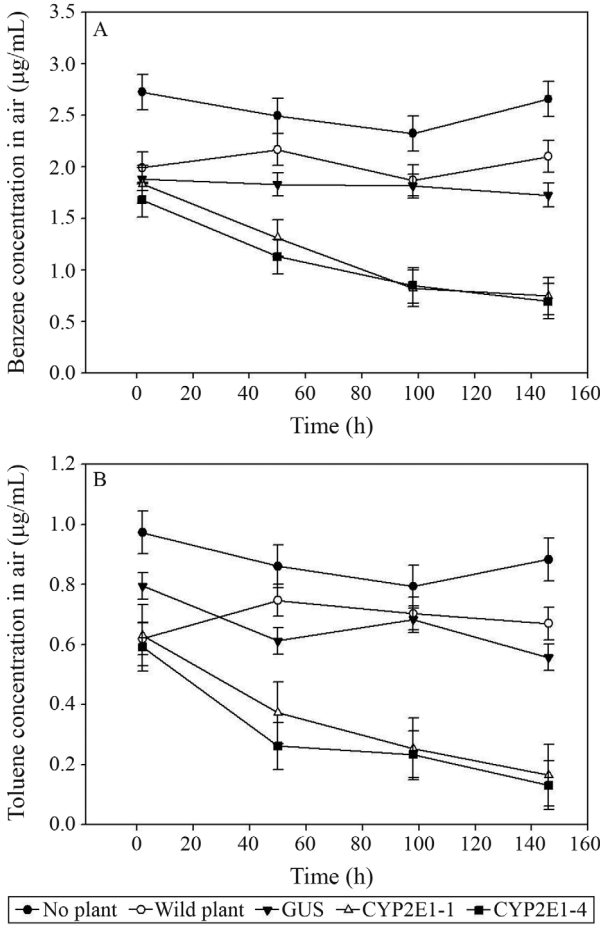

Figure 4 - Gas chromatographic analysis of benzene (A) and toluene (B) decomposition and absorption by *CYP2E1* transgenic, *GUS* transgenic and wild-type (non-transgenic) *Petunia* plants. Benzene and toluene levels were significantly reduced by *CYP2E1* transgenic plant tissue of CYP2E1-1 and CYP2E1-4, whereas while *GUS* transgenic plant and wild type plant did not show significant changes in absorption of benzene and toluene over time.

CYP2E1-3 with low-level *CYP2E1* expression, and *GUS* transgenic and wild-type plants (data not shown).

CYP2E1 transgenic *Petunia* plant capacity for resistance to formaldehyde

CYP2E1-1 and CYP2E1-4 transgenic plants maintained normal growth when cultured on MS medium with 50 mg/L of formaldehyde, whereas CYP2E1-2, CYP2E1-3, *GUS* transgenic and wild-types all withered and died after 30 days (Figure 5). Transgenic *Petunia* plants with high *CYP2E1* expression levels presented significantly improved ability for resistance to formaldehyde.

Discussion

Plants play an important role in the removal of near-surface atmospheric pollutants. Their use is economic, effective, and non-destructive. Not only is integration with the environment easier, but plant-use can also be extended to greening and aesthetics. However, only a limited number of plants in nature possess the capacity for atmospheric-pollutant decomposition and purification, efficiency is usually very low. Transgenic technology has been shown to be an effective way for improving plant environmental-remediation capacity (Hu *et al.*, 2005; Van Aken, 2008).

CYP2E1 belongs to the P450 enzyme gene family and is specifically expressed in mammalian liver. The CYP2E1 protein plays an important role in the metabolism of small molecular and organic pollutants. The Doty group was the first to transfer the *CYP2E1* gene into the model plants tobacco and poplar, both of which showed highly effective decomposing capacity for benzene, toluene, trichloroethylene, chloroform, carbon tetrachloride and vinyl chloride (Doty *et al.*, 2000, 2007; Banerjee *et al.*, 2002; James *et al.*, 2008).

In this study we successfully transferred the cytochrome P450 2E1 gene into *Petunia* by using *Agrobacterium rhizogenes* transgenic technology and obtained transgenic

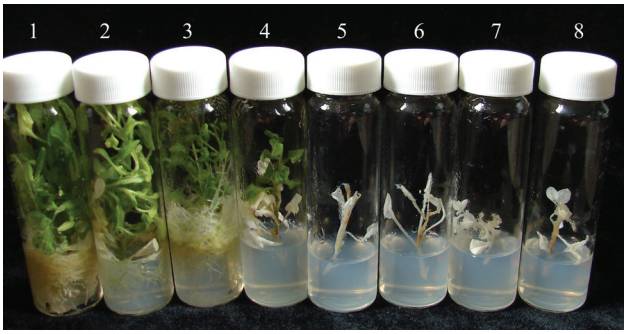

Figure 5 - *CYP2E1* transgenic, *GUS* transgenic and wild-type plants tolerance to formaldehyde. 1, 2: After 30 days, *CYP2E1* transgenic plants of CYP2E1-1 continued to maintain normal growth, when cultured on MS medium with 50 mg/L of formaldehyde; 3, 4: After 30 days, *CYP2E1* transgenic plants of CYP2E1-4 continued to maintain normal growth, when cultured on the same medium; 5-8: After 30 days, *CYP2E1* transgenic plants of CYP2E1-2, CYP2E1-3 and wild-type and *GUS* transgenic plants died on MS medium with 50 mg/L of formaldehyde.

plants with high *CYP2E1* expression. The transgenic plants with high levels of *CYP2E1* were efficient in removing benzene and toluene, and these plants had obviously improved ability of resistance to formaldehyde. Our study is the first one to report that the *CYP2E1* gene enhances the resistance ability of plants to formaldehyde.

CYP2E1 gene expression levels in the four transgenic lines, CYP2E1-1, CYP2E1-2, CYP2E1-3 and CYP2E1-4, were different, possibly caused by different sites of insertion that silenced *CYP2E1* expression in some transgenic plants. This variation is common in transgenic plants (Kumar and Fladung, 2001; Butaye *et al.*, 2005).

Although further investigation is required, in order to understand how the *CYP2E1* transgene improves plant-capacity for cleaning pollutants, it is proposed that certain specific electron transfer enzymes are produced in *Petunia*, so as to facilitate the CYP2E1 role in decomposing organic pollutants, as occurs with the mammalian CYP2E1 enzyme, which requires oxidoreductase NADPH-P450 and cytochrome b5 for electron transfer (Doty *et al.*, 2007).

The transgenic *Petunia* plants obtained in this study have potential industrial value, through providing a novel method for reducing indoor air-pollution by benzene and toluene. In addition, transgenic plants produced by *Agrobacterium rhizogenes* transgenic technology presented dwarf-morphology with abundant roots, which makes this technology fit for screening novel forms of breeding material for *Petunia*. Furthermore, four plants transplanted into the soil could flower normally, but were incapable of forming seeds. Whether this phenomenon is related to *CYP2E1* transgenis will have to be addressed in subsequent studies.

Acknowledgments

The project was supported by the Zhejiang Provincial Natural Science Foundation (No. Y3080184) and the Hangzhou Science and Technology Development Plan (No. 20091133B07).

References

Abhilash PC, Jamil S and Singh N (2009) Transgenic plants for enhanced biodegradation and phytoremediation of organic xenobiotics. Biotechnol Adv 7:474-488.

Banerjee S, Shang TQ, Wilson AM, Moore AL, Strand SE, Gordon MP and Doty SL (2002) Expression of functional mammalian P450 2E1 in hairy root cultures. Biotechnol Bioeng 77:462-466.

Butaye K, Cammue B, Delauré S and De Bolle M (2005) Approaches to minimize variation of transgene expression in plants. Mol Breed 16:79-91.

Dai SP and Bao MZ (2004) Advances in genetics and breeding of *Petunia hybrida Vilm*. Chin Bull Bot 21:385-391 (in Chinese, Abstract in English).

Doty SL, James CA, Moore AL, Vajzovic A, Singleton GL, Ma C, Khan Z, Xin G, Kang JW, Park JY, *et al.* (2007) Enhanced phytoremediation of volatile environmental pollutants with transgenic trees. Proc Natl Acad Sci USA 104:16816-16821.

Doty SL, Shang QT, Wilson AM, Westergreen AD, Newman LA, Strand SE and Gordon MP (2000) Enhanced metabolism of halogenated hydrocarbons in transgenic plants containing mammalian cytochrome P450 2E1. Proc Natl Acad Sci USA 97:6287-6291.

Gonzalez FJ (2007) The 2006 Bernard B. Brodie Award Lecture CYP2E1. Drug Metab Dispos 35:1-8.

Hu GZ, Wang YF and He YK (2005) Uptake, transformation and degradation of organic pollutants in transgenic plants. J Plant Physiol Mol Biol 31:340-346 (in Chinese, Abstract in English).

Isbell MA, Stolzberg RJ and Duffy LK (2005) Indoor climate in interior AK: Simultaneous measurement of ventilation, benzene and toluene in residential indoor air of two homes. Sci Total Environ 345:31-40.

James CA, Xin G, Doty SL and Strand SE (2008) Degradation of low molecular weight volatile organic compounds by plants genetically modified with mammalian cytochrome P450 2E1. Environ Sci Technol 42:289-293.

Kavamura VN and Esposito E (2010) Biotechnological strategies applied to the decontamination of soils polluted with heavy metals. Biotechnol Adv 28:61-69.

Kumar S and Fladung M (2001) Controlling transgene integration in plants. Trends Plant Sci 6:155-159.

Lee SS, Buters JT, Pineau T, Fernandez-Salguero P and Gonzalez FJ (1996) Role of CYP2E1 in the hepatotoxicity of acetaminophen. J Biol Chem 271:12063-12067.

Liao XY, Chen TB, Yan XL and Nie CJ (2007) Enhancement of heavy metal removal in phytoremediation of soils contaminated with heavy metals. Acta Scient Circum 27:881-892 (in Chinese, Abstract in English).

Schmittgen TD and Livak KJ (2008) Analyzing real-time PCR data by the comparative C(T) method. Nat Protoc 3:1101-1108.

Shen XY, Luo XL and Zhu LZ (2001) Progress in research on volatile organic compounds in ambient air. J Zhejiang Univ (Sci Edition) 28:547-556 (in Chinese, Abstract in English).

Van Aken B (2008) Transgenic plants for phytoremediation: Helping nature to clean up environmental pollution. Trends Biotechnol 26:225-227.

Wang L and Xiang TH (2009) Cloning of flavonoid-3', 5'-hydroxylase gene (F3',5'H) of *Petunia hybrida* and construction of a novel vector expression F3',5'H driven by flower-specific promoter. J Trop Subtrop Bot 17:358-364 (in Chinese, Abstract in English).

Associate Editor: Carlos F.M. Menck