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The Physiological Mechanism of Improved Formaldehyde Resistance in *Petunia hybrida* Harboring a Mammalian *cyp2e1* Gene

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Abstract

Cytochrome P450 CYP2E1 is mainly present in hepatocytes in the livers of mammals, where it plays an important role in the metabolism of xenobiotic organic substances. Previous studies showed that transgenic petunia (*Petunia hybrid*) plants harboring a mammalian *cyp2e1* gene (designated *cyp2e1*-transgenic petunia) exhibited increased resistance to formaldehyde stress. In this study, we used *cyp2e1*-transgenic petunia plants to analyze physiological indexes related to formaldehyde stress responses. The results indicated that under formaldehyde stress, the malondialdehyde content in *cyp2e1*-transgenic petunia plants was lower than in *β*-glucuronidase gene (*gus*)-transgenic and wild-type petunia plants. The activities of both superoxide dismutase and peroxidase in the *cyp2e1*-transgenic plants were higher than in *gus*-transgenic and wild-type plants. The alcohol dehydrogenase activity was slightly increased and more glutathione was consumed. Additionally, under formaldehyde stress, the levels of plant hormones including indole-3-acetic acid, zeatin and abscisic acid in *cyp2e1*-transgenic petunia plants displayed decreasing trends, whereas the level of gibberellic acid displayed an increasing trend. In contrast, the indole-3-acetic acid, zeatin and abscisic acid levels in *gus*-transgenic and wild-type petunia plants displayed increasing trends, whereas the gibberellic acid level displayed a decreasing trend. At 72 h after incubation of 0.5 g of *cyp2e1*-transgenic petunia plants in 40 mL of treatment solution containing formaldehyde at 50 mg · L^{-1}, the formaldehyde content remaining in the treatment solution was close to zero while approximately half of original formaldehyde remained in the treatment solutions containing *gus*-transgenic and wild-type petunia plants.

Keywords: *Petunia hybrida*; cytochrome P450 CYP2E1; formaldehyde stress; transgenic plant

1. Introduction

Cytochrome P450s are a class of heme-containing enzymes that can interact with heavy metals and play important roles in the metabolism of xenobiotic organic substances. Cytochrome P450 2E1 (CYP2E1), which is encoded by the *cyp2e1* gene, is a member of the cytochrome P450 super family and is mainly distributed in the hepatocytes of mammalian livers. In mammalian livers, CYP2E1 catalyzes alcohol oxidation and p-hydroxylation of molecules such as phenylamine, and thus has a very important detoxifying capability (Lieber, 1997; Leng and Qiu, 2001; Gonzalez, 2007).

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While some plants have the capability to detoxify formaldehyde, the efficiency is generally quite low. Transgenic technology can be used to generate and cultivate transgenic plants that have the capability to remove organic pollutants in the environment with high efficiency (Abhilash et al., 2009).

Doty's research group at the University of Washington in the United States was the first to generate transgenic plants with a mammalian *cyp2e1* gene (Doty et al., 2000). With the *Agrobacterium rhizogenes*-mediated transgenic technology, Zhang et al. (2011) introduced the rabbit hepatic *cyp2e1* gene into petunia and found that *cyp2e1*-transgenic petunia plants exhibited significantly increased resistance to formaldehyde. A study by Li et al. (2012) indicated that in *cyp2e1*-transgenic tobacco plants, the endogenous NADPH P450 oxidoreductase and cytochrome b5 played roles similar to those of their mammalian counterparts and were involved in the electron transfer chain during the catalysis process by CYP2E1, which was related to CYP2E1-mediated detoxification in the transgenic plants. However, the cellular and physiological mechanisms by which the transgenic *cyp2e1* gene enhances the capability of transgenic petunia to resist formaldehyde stress are currently unclear. In the present study, we treated the previously generated *cyp2e1*-transgenic petunia plants with formaldehyde and conducted cellular and physiological analysis on *cyp2e1*-transgenic, *gus*-transgenic, and wild-type petunia plants to explore the physiological responses of *cyp2e1*-transgenic plants to formaldehyde stress.

2. Materials and methods

2.1. Experimental materials and pre-treatments

The *cyp2e1*-transgenic, *gus*-transgenic, and wild-type petunia plants (without transgenes) were generated in our previous study (Zhang et al., 2011). These plants were sub-cultivated on 1/2 MS culture medium for about 15 days. Uniform healthy seedlings of petunia were used as experimental materials. The experiments were conducted in the Key Laboratory of Plant Science of Hangzhou Normal University during 2013–2014 (Hangzhou, Zhejiang, China).

Approximately 0.5 g of the upper branches of the sub-cultivated petunia seedlings was taken and put into an ampoule containing 40 mL of formaldehyde treatment solution (MS culture medium plus 50 mg · L^{-1} formaldehyde) or 40 mL of MS liquid culture medium (Fig. 1) and incubated at (20 ± 2) ℃ with continuing illumination at an intensity of 100 mol · m^{-2} · s^{-1} and shaking at 100 r · min^{-1} for 24 h.

The samples were taken out and washed with water. The excess water residue was absorbed with a paper towel. The samples were put into a pre-cooled homogenizer and 2 mL of pre-cooled 0.05 mol · L^{-1} phosphate buffer (pH 7.8) was added. The samples were homogenized on an ice bath to generate 20% homogenates, which were then centrifuged at 4 000 r · min^{-1} and 4 ℃ for 20 min. The supernatants were saved and used as crude enzyme extracts for subsequent assays of physiological indexes.

2.2. Measurement of malondialdehyde (MDA) content

The MDA content in the crude enzyme extract was measured with a plant MDA ELISA kit (Shanghai Xinran Bio-Tech Co., Ltd, Shanghai, China). A micro-well plate was coated with purified anti-plant MDA antibody to generate a solid antibody matrix. MDA solution was loaded into the wells pre-coated with antibody and then bound to horseradish peroxidase (HRP)-labeled anti-MDA antibody to form a complex of antibody-antigen-enzyme labeled with antibody. After thorough washing, the substrate tetramethylbenzidine (TMB) was added to develop the color. Under HRP catalysis, TMB was converted to a blue substance, which was further converted to a yellow substance under the action of an acid. The depth of the yellow color was positively proportional to the MDA content present in the samples. The absorbance at 450 nm (OD_{450}) was read with a microplate reader and a standard curve was drawn. The MDA content in the samples was calculated according to the standard curve.

2.3. Assays of superoxide dismutase (SOD) activity

Total SOD activity in the crude enzyme extract was assayed with a total SOD assay kit (Nanjing Jiancheng Bioengineering Research Institute, Nanjing, Jiangsu, China). By measuring the superoxide radical (O_2^-) generated in the xanthine-xanthine oxidase system, which is oxidized to form nitrite and a substance with an amaranth color, the absorbance can be measured with a visible spectrophotometer.

When SOD is present in the samples, it causes specific reduction of the superoxide radical and reduces the formation of nitrite; thus, the absorbance of SOD-containing samples is lower than that of controls without SOD and the SOD activity in the samples can be calculated. One unit (U) of SOD activity was defined as the amount of SOD required for an inhibitory rate greater than 50% for 1 g of tissue in 1 mL of reaction solution.

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