Transient expression of organophosphorus hydrolase to enhance the degrading activity of tomato fruit on coumaphos*

Jie-hong ZHAO1,2, De-gang ZHAO†‡1,2
(MOE Key Laboratory of Green Pesticide and Agricultural Bioengineering; Guizhou Key Laboratory of Agricultural Bioengineering, Guizhou University, Guiyang 550025, China)
†E-mail: dgzhao@gzu.edu.cn
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Abstract: We constructed an expression cassette of the organophosphorus pesticide degrading (opd) gene under the control of the E8 promoter. Then opd was transformed into tomato fruit using an agroinfiltration transient expression system. β-Glucuronidase (GUS) staining, reverse transcription-polymerase chain reaction (RT-PCR), wavelength scanning, and fluorescent reaction were performed to examine the expression of the opd gene and the hydrolysis activity on coumaphos of organophosphorus hydrolase (OPH) in tomato fruit. The results show that the agroinfiltrated tomato fruit-expressed OPH had the maximum hydrolysis activity of about 11.59 U/mg total soluble protein. These results will allow us to focus on breeding transgenic plants that could not only enhance the degrading capability of fruit but also hold no negative effects on pest control when spraying organophosphorus pesticides onto the seedlings in fields.

Key words: Bioremediation, E8 promoter, Organophosphorus hydrolase (OPH), Transient expression

INTRODUCTION

Organophosphorus pesticides are used worldwide to control major pests. Their residues not only exist on the surface of agricultural products, but also can be soaked into tissues based on contact absorption. In addition, plants absorb and enrich these residues through vascular bundles from surrounding soil and water, leading to a high risk of residue exceeding limits in food and vegetables. Organophosphorus pesticide residues have been detected above residue limits in several commercial grape juices (Picó and Kozmutza, 2007), fresh apples (Nie et al., 2005), and tomato juice or paste (Aysal et al., 2004). It seems that degradation was slower in fruit juices than in water due to natural antioxidants (Picó and Kozmutza, 2007).

Because organophosphorus pesticides are potent inhibitors of acetylcholinesterase (AchE) that is also present in all vertebrates, non-target organisms will be damaged by residues of this kind of agents (Zhang et al., 2006). As reported, organophosphorus pesticide poisoning has become a major global health problem, with hundreds of thousands of deaths each year, although the majority of deaths occurred in developing countries (Eddleston and Phillips, 2004). To solve this problem, there is intense interest for us to breed fruit that could rapidly detoxify pesticide residues spontaneously to minimize the potential damage to people, but, meanwhile, would not affect pest control when spraying organophosphorus pesticides onto seedlings.

Organophosphorus hydrolase (OPH), encoded by the organophosphorus pesticide degrading (opd) gene, can hydrolyze a wide range of organophosphorus compounds including pesticides, chemical warfare agents, and herbicides (Dumas et al., 1989;
Kolakowski et al., 1997; Di Sioudi et al., 1999). This enzyme has received considerable attention on its use as a bioremediation agent. The opd gene was first isolated from Pseudomonas diminuta and Flavobacterium spp. (Munnecke and Hsieh, 1976; Harper et al., 1988), and has been cloned and expressed in Escherichia coli (Lan et al., 2006; Shimazu et al., 2001), Moraxella spp. (Shimazu et al., 2001), Saccharomyces cerevisiae (Takayama et al., 2006) and other microbial strains.

The researches mentioned above focused on the production of stable and efficient hydrolase by genetically modified microorganisms for environmental remediation. Recently, opd has been also expressed in transgenic maize for establishing a new selection marker in transgenic research (Pinkerton et al., 2008). In the current study, we studied the feasibility of transient expression of OPH to enhance the degrading activity of tomato fruit on coumaphos.

MATERIALS AND METHODS

Promoter clone and plasmids construction

A 1.1-kb E8 promoter that is specific for fruit expression was polymerase chain reaction (PCR) amplified from tomato DNA using LA Taq DNA polymerase with the primer pairs designed based on the sequence (GeneBank accession No. X13437) described by Deikman et al. (1992). The 5′- and 3′-primer sequences were 5′-CCG GTG ACC AAG CTT AGG AAT TTC ACG AAA TCG-3′ and 5′-GCC GTC GAC GGA TCC TCT TTT GCA CTG TGA ATG-3′, respectively.

The amplified segment was cloned into pGEM-T (Promega, USA) for sequencing and other manipulation. The fragment from pGEM-T was then cut by HindIII/BamHI and subcloned into pSH (constructed by Prof. Zhao Degang and conserved in Guizhou University) to exchange the double 35S promoter to generate plasmid pSE8. The opd gene (GeneBank accession No. AX384799) of p9137 kindly gifted by Prof. John A. Howard, Cal Poly State University of USA, was subcloned into the EcoRI site of pSH and pSE8. Then the plant expression vectors pSOP and pSE8OP were obtained and transformed to Agrobacterium tumefaciens LBA4404. The cauliflower mosaic virus 35S (CaMV 35S) promoter or E8 promoter drives the expression of the opd gene preceded by a fragment of the ubiquitin intron. The transferred DNA (T-DNA) regions of the two expression cassettes are depicted as Fig.1.

![Fig.1 T-DNA regions of the two expression cassettes of pSOP (a) and pSE8OP (b)](https://example.com/fig1)

Agrobacterium-based transient transformation

Growth and induction of Agrobacterium were carried out mainly according to the methods of Spolaore et al. (2001) and Orzaez et al. (2006). Agrobacterium tumefaciens LBA4404 harboring one of the two vectors was grown separately overnight at 28°C in 5 ml of induction medium (5 g/L beef extract, 1 g/L yeast extract, 5 g/L peptone, 2 mmol/L MgSO₄, and 20 μmol/L acetylsyringone), and then buffered with 10 mmol/L 2-(N-morpholino) ethanesulphonic acid (MES) to pH 5.6, followed by the addition of rifampicin (20 mg/L) and kanamycin (100 mg/L). Then, the sample was transferred to 50-ml induction medium. When the culture reached an optical density at 600 nm (OD₆₀₀) of about 0.8, it was recovered by centrifugation, then resuspended in infiltration medium (10 mmol/L MgCl₂, 10 mmol/L MES (pH 5.6), 20 g/L sucrose, and 200 μmol/L acetylsyringone) up to a final OD₆₀₀ of 2.0, and incubated at room temperature with gentle agitation (20 r/min) for a minimum of 2 h. Agrobacterium cultures were collected with a syringe when required, and then fruit agroinjection was performed next.

Commercially ripe tomato fruit was rinsed thoroughly in distilled water before injection. The Agrobacterium suspensions harboring one of the two expression cassettes were evenly injected throughout the entire fruit using a 10-ml sterile syringe with a 4-cm needle. The needle was inserted 2~3 cm deep into the fruit tissue and injected the infiltration solutions gently into the fruit. A total of about 6 ml of the infiltration solutions was injected into commercially ripe tomatoes,