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Cloning and characterization of a cold inducible Pal promoter from *Fagopyrum tataricum*

Research Article

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Abstract: Phenylalanine ammonia-lyase (PAL) catalyzes the first reaction in biosynthesis pathway of flavonoids and plays an important role in plant stress resistance. In this study, the 5' flanking region of phenylalanine ammonia-lyase gene was isolated from *Fagopyrum tataricum* by thermal asymmetric interlaced PCR method, named P*FtPal* (GenBank: KF463139). To investigate the functional properties of P*FtPal*, we constructed a series of plant expression vectors that contained different promoter fragments resulting from nest deletions and had successfully transformed them into tobacco leaves by *Agrobacterium tumefaciens*. Histochemical assay of GUS suggested that P*FtPal* could drive GUS gene expression in leaves and roots, while GUS activity was not detected in the stem. In addition, the region of -274 bp to -1 bp was enough to drive normal expression of GUS gene. Low temperature treatment of transgenic tobacco plants demonstrated that P*FtPal* conferred cold-induced expression. Taken together, our study will help to better understand the *Pal* promoter, and provides a candidate promoter for molecular breeding in Fagopyrum plants.

Keywords: *Phenylalanine ammonia-lyase • Tissue-specific expression • Cold-induced expression*

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1. Introduction

Fagopyrum tataricum is well known as a crop with abundant flavonoids, has nutrition and health value for human beings. Flavonoids are secondary metabolites related to plant development and stress resistance [1]. Phenylalanine ammonia-lyase (PAL), which catalyzes the deamination of L-phenylalanine to produce trans-cinnamic acid, is the first key enzyme of flavonoid metabolic pathway. Expression of PAL could be induced by various stress-related stimuli including low temperature, bending stress, and light in many plant species. The expression level of PAL effectively influences the biosynthesis of flavonoids [2,3]. As an important component of eukaryote gene expression regulation, the promoter plays an important role in spatial and temporal regulation of gene expression *via* controlling the efficiency of start and start frequency. In order to adapt to different environment, various *cis*-elements in the promoter respond to elicitors outside to control the expression of genes. A number of *cis*-elements have been identified from varied species that have been shown to involving responding to stimuli of light, signal molecule and invading bacteria [4-6].

Previous findings indicate that *Pal* promoters play important roles during the various developmental stages and stress conditions in plants. The results of transgenic experiments have showed that the GUS gene is expressed in a spatial and temporal pattern under the control of *Pal* promoters [7-9]. In addition, *Pal* promoter of sugar beet is involved in recognition of the repression signal from fungal pathogens. As the expression and enzyme activity level of PAL reduce, plant defense reactions are repressed resulting in plant diseases [10]. *Pal* promoter in *Pinus taeda* significantly increases the activity of genes for lignin biosynthesis

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