

## Molecular Cloning and Characterization of A Novel Multiple Stress-inducible Peroxidase Promoter from Sweetpotato

Sun-Hwa Ryu, Soo-Young Park, Yun-Hee Kim, Suk-Yoon Kwon, Sang-Soo Kwak\*

Laboratory of Environmental Biotechnology, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Daejeon 305-806, Korea

### Objectives

We have cloned more than 20 peroxidase (POD) cDNAs from suspension cultures of sweetpotato (*Ipomoea batatas* (L.) Lam.). Among them, *swpa4* was most highly induced by both abiotic and biotic stress, suggesting that this gene was regulated by a multiple stress-inducible promoter (Park et al. 2003). In this study, a stress-inducible *SWPA4* promoter was isolated by chromosome walking and its 5'-deletion mutants were characterized in transgenic tobacco plants and transient assay using tobacco BY-2 protoplasts.

### Materials and Methods

1. Plant Materials : Sweetpotato (*Ipomoea batata* (L.) Lam. cv. White Star), Tobacco BY-2 suspension cells, Tobacco (*Nicotiana tabacum* cv. Xanthi)
2. Methods : - Transient assay by PEG transfection  
- *Agrobacterium tumefaciens*-mediated transformation

### Results and Discussion

A promoter of genomic clone (referred to *SWPA4*) with 2,433 bp of 5'-upstream sequence from the translation start site was obtained from sweetpotato by chromosome walking. Sequence analysis reveals that *SWPA4* promoter contains putative binding sites for several transcription factors including ELRE, MYBGAHV, ASF1, ABRE, GCN4, W-box and HSF. Employing a transient expression assay in tobacco BY-2 protoplasts, 2.4 kb promoter and five progressive deletion mutants, -1934, -1467, -1199, -818 and -433, were fused to the GUS gene and their GUS activity was analysed. The GUS activity of *SWPA4* was enhanced by hydrogen peroxide treatment. Each deletion mutants showed diverse GUS activity. The -1934 bp deletion mutant showed about 8 times higher GUS expression than the CaMV 35S promoter. The further characterization of *SWPA4* and deletion promoters is under study in tobacco transgenic plants.

### Reference

Park SY et al. (2003) Differential expression of six novel peroxidase cDNAs from cell cultures of sweetpotato in response to stress. *Molecular General and Genetics* 269: 542-552