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Control of Seed Germination Rate by the Cysteine Proteinase Inhibitor in Transgenic Tobacco Plant

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Objectives

Phytocystatins (PhyCys) are widely distributed and have been found in several different species of higher plants. Although the metabolic role of this enzyme has not been fully defined, it has been reported that it is associated with multiple physiological processes, including seed ripening, germination and programed cell death. However, almost PhyCys information are *in vitro* data. To study the real *in vivo* roles of PhyCys in plant cell, we isolated a cDNA clone for PhyCys from Chinese cabbage flower bud cDNA library, introduced it in tobacco, and characterized phenotypic characteristics and its seed germination control effects.

Materials and Methods

1. Materials

- Plants: Chinese cabbage (*Brassica campestris* L. ssp. *pekinensis*)

Tobacco (Nicotiana tabacum cv. Xanthi-NN)

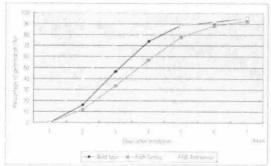


Figure 1. The comparison of germination rate between BCPI-1 transgenic and wild type tobacco plants.

- Vector: pBI 121-Linker
- Insert: BCPI-1 cDNA
- Host: Agrobacterium strain: LBA 4404

2. Methods

Vector construction, Tobacco transformation, Gene expression analysis (Western blotting, Cysteine protease inhibitory activity, PCR, RT-PCR), Seed germination rate

Results and Discussion

The morphologies of transformed *N. tabacum plants* expressing *BCPI-1* sense and antisense were compared to those of untransformed control plants. Transgenic plants did not exhibit any significant morphological abnormalities under normal growth conditions. However, a number of plants expressing BCPI-1 sense or antisense (as determined by protein immunoblot analysis) exhibited abnormalities, including flower number, seed pod size, growth and seed germination rate (Figure 1, 2). Our result suggest that the BCPI-1 functions are related with the regulation of seed germination and plant growth.

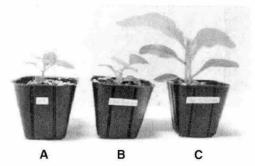


Figure 2. Growth of transgenic plants at soil condition. A, Wild type; B, BCPI-1 sense; C, BCPI-1 antisense plants.