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Transgenic 'Seoulbaechu' plants with enhanced tolerance to multiple bacrerial pathogens by cysteine proteinase of *Ananas comosus*

Young-Jin Koh[1], Da-Yeung Seo[2], Hyo-Jin Jung[2], Bo-Ra-Mi Park[2], Yu-Jin Jung [3], Kwon-Kyu Kang[3], and Ill-Sup Nou[2*]

[1]Department of Plant Medicine, Sunchon National University, 315, Maegok-Dong, Sunchon 540-742, Korea
[2]Faculty of Plant Science, Sunchon National University, 315, Maegok-Dong, Sunchon 540-742, Korea
[3]Department of Horticulture, Hankyong National University, Anseong 456-749, Korea

Objectives

We have tried to make transformation of *Brassica rapa* L.('Seoulbaechu') through *Agrobacterium tumefaciens* and breeding of enhanced tolerance strains to multiple bacterial pathogens by cysteine proteinase.

Materials and Methods

1. Material

Plant - *Brassica rapa*('Seoulbaechu'), *Ananas comosus*('Pineapple')

Bacterial pathogens *Pectobacterium carotovorum* subsp. *Carotovorum*, *Pseudomonas margilnalis* pv. *Marginalis*, *Pseudomonas viridiflava*, *Xanthomonas campestris* pv. *campestris*

2. Methods: *Agrobacterium* mediation, Vector construction, PCR, RT-PCR, Real-time PCR, Bacterium inoculation by pinning method

Results and Discussion

Brassica rapa('Seoulbaechu') were transformed via *Agrobacterium tumefaciens* that EHA4404/pBI121 harbored genes for β-glucuronidase(GUS), hygromycin phophotransferase (HPT) and neomycin phosphotransferase gene (NPT II). A co-cultivation medium at pH5.2 with tobacco feeder cells was effective to enhance infection frequency evaluated by the number of hypocotyl sections. Transgenic plants in cv. Seoulbaechu were obtained by inoculating the hypocotyl sections in the bacterial inoculum for 30 min, and co-cultivation at 25℃ for 3 days. We finally obtained two T0 transgenic plants. After self-pollination, PCR analysis was carried out with *NPT*II, 35S promoter and cysteine proteinase specific primers in T1 generation. Expression of T1 generation plants were analyzed by RT-PCR and Real-time PCR. From these results, we know that the transgenic plants were over expressed mRNA for cysteine proteinase, and selected 5 homo-evented lines by the antibiotics tolerant test in T2 generation. The reactions of 5 homo-evented lines to 4 bacterial pathogens are under investigation.

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* *Corresponding author* : Ill-Sup Nou, TEL: 061-750-3249, E-mail: nis@sunchon.ac.kr