Development of transgenic disease-resistant root stock for the growth of watermelon

Song-Mi Cho[1,2*], Soo-Jin Chung[2], Sun-Jin Moon[2], Kwang-Sang Kim[2], Young-Cheol Kim[1], and Baik-Ho Cho[1]

[1]*Applied Plant Science Division, College of Agriculture and Life Sciences, Chonnam National University, Gwangju 500-757, Korea;* [2]*PhytoCare Tech Co., LTD.*

Objectives

To protect the watermelon against soil-borne pathogens, we are producing disease-resistant transgenic root stock for the growth of watermelon.

Materials and Methods

1. Plant materials: watermelon, Kongdae watermelon, and gourd
2. Methods: Genomic DNA PCR, RT-PCR and Northern blot analysis

Abstract

To protect the watermelon against soil-borne pathogens, we are currently producing disease-resistant transgenic root stock for the growth of watermelon. A defensin gene (*J1-1*) from *Capsicum annum*, a ACC deaminase gene from *Pseudomonas syringae*, a galactinol synthase (*CsGolS*) gene from *Cucumis sativus*, and a WRKY (*CvWRKY2*) gene from *Citullus vulgaris* were used as transgenes for disease resistance. The genes were transformed into a inbred line (6-2-2) of watermelon, Kong-dae watermelon and a inbred line (GO702S-2) of gourd, respectively, by *Agrobacterium*-mediated transformation. Putative transgenic plants were selected in medium containing 100mg/L kanamycin, and then integration of the genes into the genomic DNA were demonstrated by PCR analysis. Successful integration of the gene in regenerated plants was also confirmed by PCR (Fig.1), genomic Southern blot (Fig.2), RT-PCR (Fig.3), and Northern blot analysis(Fig.4). Several T1 lines having different transgene were produced, and disease resistance of the T1 lines are under estimation.

Introduction

Agricultural yield tends to be reduced constantly by various soil borne pathogens which are generally caused by phytopathogenic fungi and bacteria. Root stock cultures of *cucurbitaceae* plant such as *Citrullus vulgaris*, *Cucumis sativus*, and *Cucumis melo* were used to avoid damage by soil borne pathogens. It was recently reported that *Fusarium oxysporium* f. sp *lagenaria* specially infect gourd plants, a root stock for the growth of watermelon. Therefore, alternative control methods are urgently demanding to protect root stock against the new outbreaking pathogen. Production of transgenic disease resistant root stock could be a valuable approach to improve disease resistance of root stock.

In response to pathogen infection, plants induce the expression of a complex set of defense-related genes. Plant defensin is a family of small (5 kDa) basic cysteine-rich peptide, and have been characterized in numerous plant species. The J1-1 of pepper, a defensin-type protein inhibited the mycelial spread of *F. oxysporium* and *Botrytis cinerea*.

The plant growth-promoting rhizobacteria (PGPR) are found in association with the roots of many different plants. A number of PGPR contain the enzyme 1-amino- cyclopropane-1-carboxylate(ACC) deaminase. The enzyme cleaves the ethylene precursor ACC, and thereby lower the level of ethylene in stressed plant. PGPR-treated plants became to be more resistant to deleterious effects of the hormone ethylene that was synthesized as a consequence of stressful conditions such as flooding, heavy metals, the presence of phytopathogens, drought, and high salt.

Among the several classes of transcription factors associated with plant defense response are the recently identified DNA-binding proteins containing WRKY domains. Transcript of the *CvWRKY2* gene was strongly accumulated in watermelon after infection with *C. cucumerium* compared with control plants. The *CvWRKY2* gene transcript is specifically accumulated by salicylic acid (SA) treatment, but not by jasmonic acid (JA).

Colonization of nonpathogenic rhizobacteria on roots can induced a systemic resistance (ISR) in plants. Priming phenomenon of defense response genes has been demonstrated in rhizobacterium-mediated ISR. We recently isolated the *CsGolS2* gene that may be involved in the priming phenomenon by employing a subtractive hybridization method using mRNA extracted from *Corynespora cassiicola* (challenger)-inoculated cucumber leaves with and without the root colonization of the rhizobacterium *P. chloraphis* O6.

Results and discussion

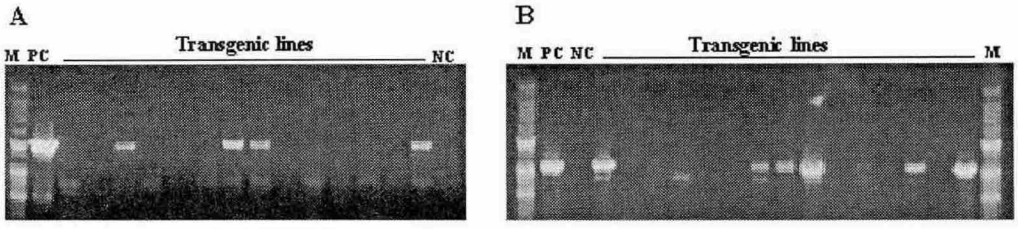


Fig. 1. Genomic DNA PCR in transformants.

The defensin (*J1-1*) (A) and *P. sryingae* ACC deaminase genes (B) of transgenic Kong-dye watermelon plants are amplified by PCR using the CaMV35S and the gene specific primers. M, size marker (1kb ladder, NEB); PC, positive control (plasmid); NC, negative control (non-transgenic plant). Putative transgenic plants obtained by *Agrobacterium*-mediated transformation were selected in medium containing 100mg/L kanamycin.

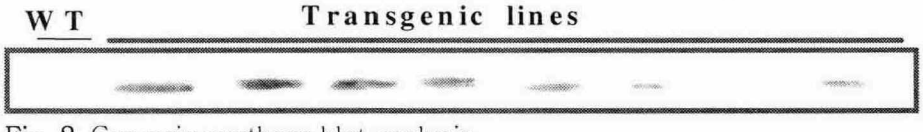


Fig. 2. Genomic southern blot analysis.

Genomic DNA of transgenic Kong-dye watermelon plants and that of non-transgenic plant (WT) were completely digested with *EcoR* I, and probed with a 32P-labeled *PepJ1-1* cDNA.

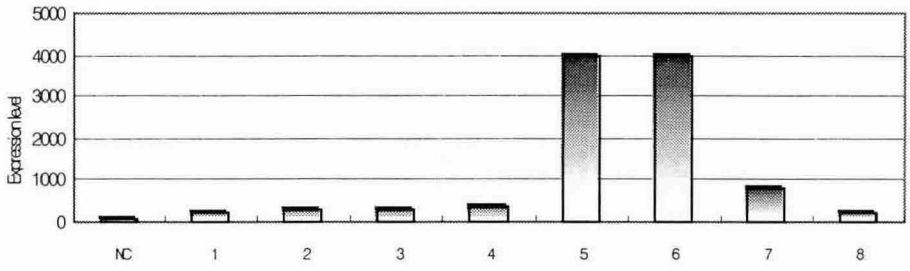


Fig. 3. A quantative real-time RT-PCR(Q-PCR) profile measuring the expression of *P. syringae* ACC deaminase gene in transgenic Kongdae watermelon. NC, non-transgenic plant control; lane1-8, putative transgenic plant. Samples were snap frozen in liquid nitrogen, and total RNA were extracted. Equal amount of RT reaction product were used as templates to amplify selected region of the ACC deaminase gene with specific primer pairs. Expression levels are represented by arbitrary units which were set at 100 for control.

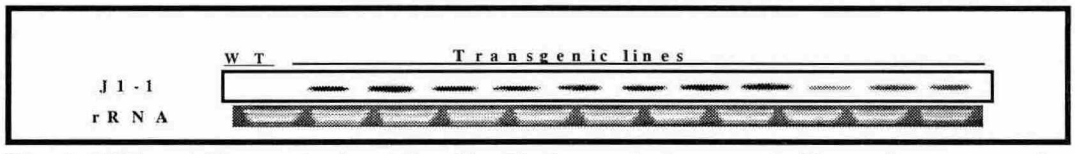
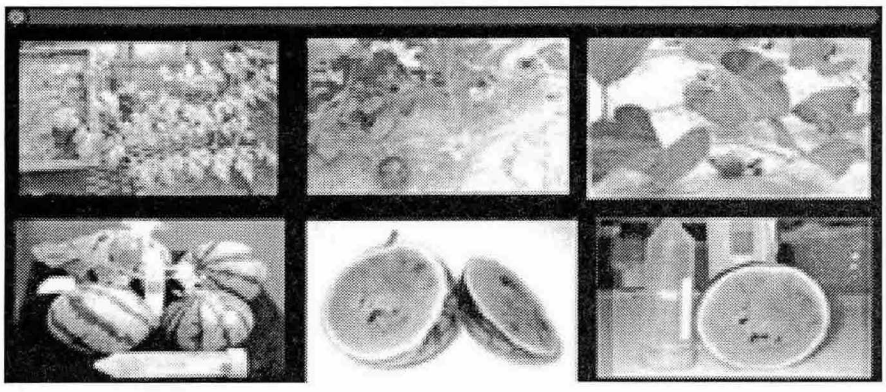


Fig. 4. RNA gel blot analysis of the *PepJ1-1* gene in transgenic Kong-dye watermelon. For each lane, 20μg of total RNA was fractionated on a formaldehyde containing agarose gel and blotted on nylon membrane. The membrane was hybridized with a 32P-labeled cDNA. WT, untransformed plant.

Trasgenic plants and the T1 seed



Conclusion

1. We have isolated several plant- and microbe-derived genes that could confer disease resistance in transgenic root stock.
2. Putative transgenic plants were selected in medium containing 100mg/L kanamycin and integration of the Pep J1 -1 and ACC deaminase into the genomic DNA were demonstrated by the PCR .
3. The successfully regenerated plants were further confirmed by southern blot, RT-PCR, and northern blot analysis, and T1 seeds were obtained from the regenerated plants.

References

1. R. Dirks and M. van Buggenum (1989). *In vitro* plant regeneration from leaf and cotyledon explants of *Cucumis melo* L. Plant Cell Reports 7: 626-627.
2. M. Kalde, M. Barth, I. E. Somssich, and B. Lippok (2003). Members of the *Arabidopsis* WRKY group III transcription factors are part of different plant defense signaling pathways. MPMI 16: 295-305.

Acknowledgement

This work was supported by a granted from BioGreen 21 program, Rural Development Administration, Republic of Korea.

Corresponding author: Baik Ho Cho , Tel: 062-530-2075 , E-mai: chobh@chonnam.ac.kr