Plant Pathol. J. 24(2) : 183-190 (2008)

The Plant Pathology Journal
©The Korean Society of Plant Pathology

Disease-resistant Transgenic *Arabidopsis* Carrying the *expI* Gene from *Pectobacterium carotovorum* subsp. *carotovorum* SL940

Joo-Hee Lee[1], Ja Bin Hong[2], Sang Bin Hong[2], Min-Seon Choi[1], Ki Yong Jeong[1], Hyoung-Joon Park[1], Duk-Ju Hwang[3], Seungdon Lee[1], Dongsoo Ra[1] and Sunggi Heu[1]*

[1]*Division of Plant Pathology, National Institute of Agricultural Science and Technology, Rural Development Administration, Suwon 441-707, Korea*

[2]*Korean Minjok Leadership Academy, Hoengseong 225-823, Korea*

[3]*Department of Molecular Physiology and Biochemistry, National Institute of Agricultural Biotechnology, Rural Development Administration, Suwon 441-707, Korea*

(Received on September 13, 2007; Accepted on February 28, 2008)

Plant-cell-wall-degrading enzymes (PCWDEs) of *Pectobacterium carotovorum* subsp. *carotovorum* are the key virulence factor in pathogenesis of soft rot disease of vegetables. The production of PCWDEs is controlled in a cell density dependent manner to avoid the premature production of PCWDEs and subsequent activation of plant defense. N-oxoacyl-homoserine lactone (OHL) is essential for quorum sensing in the soft rot pathogen and the *expI* gene is responsible for OHL production. The ExpI homolog isolated from *P. carotovorum* subsp. *carotovorum* SL940 had 94% identity with ExpI of *E. carotovora* subsp. *carotovora* scc3193 and 74% identity with CarI of *E. carotovora* subsp. *atroseptica*. The transgenic plants that express *expI* uner the control of CaMV35S promoter were able to produce diffusible OHL. Transgenic plants producing OHL were very resistant to the infection of *P. carotovorum* subsp. *carotovorum*. Since the *PR1* gene was strongly induced and *NPR1* and *NPR4* were induced weakly in transgenic plants compared to the wild type, salicylic acid-dependent pathways is likely involved in the resistance to the soft rot pathogen *P. carotovorum* subsp. *carotovorum* in ExpI transgenic plants.

***Keywords*:** disease resistance, *expI* gene, *Pectobacterium carotovorum* subsp. *carotovorum*

Many bacterial pathogens have developed the ability to multiply and survive on their hosts. Only well-adapted microbe species are able to infect the target host and cause a disease. *Pectobacterium carotovorum* subsp. *carotovorum* (=*Erwinia carotovara* subsp. *carotovora*) causes the soft rotting or tissue macerating disease in plants or plant organs of a number of economically important crops (Barras et al., 1994). When this pathogen attacks the plant tissue, it uses plant cell wall-degrading enzymes (PCWDEs) such as extracellular pectate lyase (Pel), cellulase (Cel), polygalacturonase (Peh), and protease (Prt) (Collmer and Keen, 1986). However, insufficient amounts of these enzymes are not able to affect plant tissues and instead turn on plant defense mechanism. In this reason, *E. carotovora* uses the quorum sensing system to cause a disease on their hosts (Pirhonen et al., 1993).

The quorum sensing system (QS) is a population density monitoring system in bacteria. The QS is used in physiological responses in bacteria such as bioluminescence, conjugation, production of secondary metabolites, and pathogenicity (Fuqua et al., 1996; Salmond et al., 1995; Swift et al., 1996). Many gram-negative bacteria synthesize diffusible signaling molecules called autoinducers (pheromones) and can regulate their behavior through their concentration. In other words, when bacteria propagate in sufficient numbers and concentration of the autoinducer reaches a threshold level, bacteria begin to respond to these pheromones and can activate PCWDEs or physiological response genes (Chatterjee et al., 1990; Fuqua et al., 1994; Jones et al., 1993; Kaplan and Greenberg 1985; Pierson et al., 1998; Pirhonen et al., 1993; Teresa and Barbara, 2000; Von Boldman and Farrand, 1995).

The QS is well known by the *lux* gene in *Vibrio fischeri*. Since discovery of *lux* genes in *V. fischeri*, similar molecules had been discovered in diverse bacteria (Bainton et al., 1992). *N*-(3-oxohexanoyl)-L-homoserine lactone (OHL) is required for a quorum sensing signaling as autoinducer. The signaling molecule OHL is synthesized by the action of the LuxI protein in *V. fischeri* (Salmond et al., 1995) and ExpI in *E. carotovora* (Pirhonen et al., 1993).

It had been reported that the *expI* gene encodes a 26 kDa protein that is similar to the *luxI* gene product in *V. fischeri* as OHL (Pirhonen et al., 1993) structurally and functionally. It was suggested that *E. carotovora* uses QS to avoid the premature production of PCWDEs controlled by OHL

*Corresponding author.
Phone) +82-31-290-0420, FAX) +82-31-290-0406
E-mail) heu@rda.go.kr

and subsequent activation of plant defense responses (Mäe et al., 2001). The *exp*I transgenic tobacco lines, producing OHL, showed enhanced resistance to *E. carotovora* and exogenous addition of OHL to wild-type plants resulted in increased resistance to *E. carotovora* (Mäe et al., 2001).

When plants are attacked by pathogen and show enhanced-resistance, a number of genes involved in the production of ethylene (ET), jasmonates (JA), and salicylic acid (SA) participate in this response (Fey and Parker, 2000; Kunkel and Brooks, 2002; Thomma et al., 2001). Among many factors that are related to plant defense mechanism, *PR* genes had been identified as plant defense marker genes (Ibeas et al., 2000; Niderman et al., 1995) and *NPR1* was required for expression of *PR1* (Kinkema et al., 2000) on the SA-dependent signaling pathway (Cao et al., 1994; Delaney et al., 1995; Shah et al., 1997). Recently, it was reported that NPR4 shared 36% identity with NPR1 and was required for basal defense. *NPR4* transcription levels were changed by SA or methyl jasmonic acid (MeJA) treatment. It was suggested that the NPR4 might be implicated in the cross-talk between the SA- and JA-dependent signaling pathways (Liu et al., 2005).

To test whether this *exp*I gene isolated from *P. carotovorum* subsp. *carotovorum* exhibited enhanced-resistance in *Arabidopsis*, we created transgenic *Arabidopsis* plants harboring *exp*I under the control of the *CaMV35S* promoter and demonstrated the enhanced resistance against *P. carotovorum* subsp. *carotovorum*. To elucidate which genes are involved in the enhanced resistance of the transgenic plant, we performed RT (reverse transcription)-PCR.

Materials and Methods

Bacterial strains and growth conditions. *E. coli* strain, DH5α was used for plasmid maintenance and DNA manipulation (Hanahan 1983) and *P. carotovorum* subsp. *carotovorum* (=*E. carotovora* subsp. *carotovora*) strain L940 was used for infection of plants. *E. coli* cells were cultivated at 37 in LB medium (Sambrook et al., 1989) and *P. carotovorum* subsp. *carotovorum* strain L940 was propagated in L-medium (Miller, 1972) at 28°C. Ampicillin was added to a final concentration of 100 or 150 μg/ml and kanamycin to 50 μg/ml, when required.

Plant materials and treatments. Seeds of *A. thaliana* ecotype Columbia (Col-0) and transgenics were kept at 4°C at least 2 days. The surface of seeds were sterilized before placement on sterile MS medium (Murashige and Skoog, 1962) and replanted in new petridish after germination. Plants were grown for 2-3 weeks in growth chamber at 22°C with 16 hour light regime.

Cloning of *exp*I gene. A *exp*I gene sequence from *E. carotovora* subsp. *carotovora* had been previously described (GenBank accession no. X72891). Based on this information, gene-specific primers 5'-<u>GGATCC</u>ATGTTAGAAATATTCG-3' and 5'-<u>GGATCC</u>TTATCCGACCGGTTC-3' carrying *Bam*HI recognition sites (underlined) were designed. These primer pairs used to amplify a 654-bp fragment from genomic DNA of *P. carotovorum* subsp. *carotovorum* L940 by PCR. The full-length PCR fragment was cloned into pCR2.1 vector (Invitrogen, USA) and sequenced to verify the sequence. The plasmid harboring the 654-bp full-length fragment for *exp*I was digested with *Bam*HI and then subcloned into the corresponding sites of binary vector pCam-35S, which is derived from pCAMBIA 2301-1 containing the CaMV35S promoter. The sense orientation of the full-length *exp*I was conformed by restriction enzyme sites.

Automated sequencing was performed with ABI 310 sequencer (Genetic Analyzer ABI 310, PE Applied Biosystems). Accession Number for this gene is EU148355.

***Agrobacterium*-mediated transformation of *Arabidopsis*.** The *A. tumefaciens* strain GV3010 containing binary vector construct was grown 24 hr at 28°C in LB medium supplemented with 50 μg/ml kanamycin. Precultured cell (100 μl) was used to inoculate fresh LB media (100 ml) with 50 μg/ml kanamycin. Cells grown for 2 days were collected by centrifugation, and resuspended with transformation media (5% sucrose and 0.05% Silwer L-77) (Clough and Bent, 1998), and the concentration was adjusted to $O.D_{600}=1$. Approximately 50 ml of aerosol containing *Agrobacterium* suspension cells were sprayed onto 5-6 plants in a pot. After transformation, the plants were placed into a plastic flat and covered with a plastic dome to maintain humidity. The next day, dome was removed. The putative transgenic *Arabidopsis* plants (T1 plants) were selected by planting the seeds on a half strength MS (half MS salts and vitamins, B5 vitamin, 2 g Phytoagar) medium containing 100 mg/L kanamycin. After selection for 3 weeks on the medium, the kanamycin-resistant plants were transferred to the soil media and raised under a greenhouse condition.

Bioassay for AHL production. *E. coli* MG4 carrying pKDT17 which has lasB::lacZ tlanslational fusion was grown in LB broth overnight on a shaker at 37°C (Matthew et al., 1997). Five hundreds μl of the cultures containing approximately 2×10^9 CFU/ml bacteria, was spun down and resuspended in 5 ml of warm LB containing 0.5% agar. The bioassay agar suspensions were poured on a surface of the regular LB agar carrying X-gal, and leaves from aseptically grown seedlings were placed on a thin layer of soft agar containing the bacteria.

Total RNA isolation and RT PCR. Total RNA was isolated using the TRIzol Reagent (Invitrogen, Carlsbad, CA, U.S.A.) according to the manufacturer's protocol. RT-PCR analysis using AMV reverse transcriptase (Roche, USA) was performed with 1 μg of total RNA according to the manufacturer's instructions. Subsequent PCR was performed for 25-35cycles using specific primers. Uniform loading of RNA was checked by *EF1α*.

Sequence data from this article have been deposited with the GenBank data libraries under accession numbers U76707 (*NPR1*), AF141203 (*EIN2*), M90508 (*PR1*), AB023463 (*PR3b*), U01880 (*Hel*), and X97131 (*EF1α*). The primer pairs of these genes are described in Dong et al, 2004. NM118086 (*NPR4*) is described in Liu et al. (2005) and *PDF1.2* in Zimmerli et al. (2004).

Pathogen inoculation. For evaluation of resistance response of wild type and *exp*I transgenic *Arabidopsis*, 3-weeks old plants were infected with *P. carotovorum* subsp. *carotovorum*. *P. carotovorum* subsp. *carotovorum* were grown on PSA for 2 days at 28°C, resuspended in 10 mM $MgCl_2$, adjust $O.D_{600}$=0.5 and then sprayed on plants. The

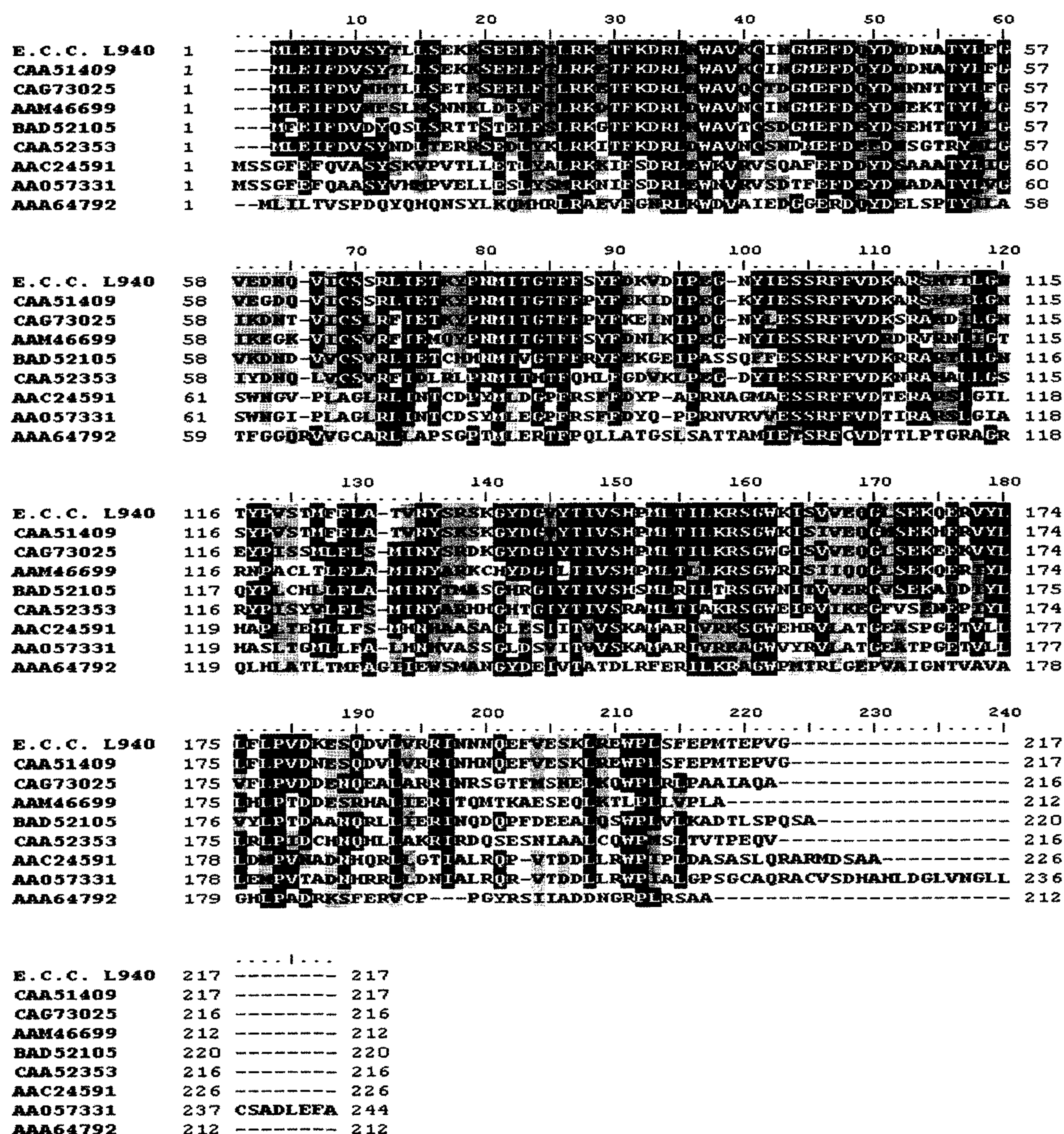

Fig. 1. Alignment of ExpI amino acid sequence of *P. carotovorum* subsp. *carotovorum* strain L940 and homologous sequences from different bacterial species. E.C.C. L940 represents ExpI sequence from *P. carotovorum* subsp. *carotovorum* L940 and another letters on the left indicate GenBank accession numbers. CAA51409, *E. carotovora* subsp. *carotovora* SCC3193 ExpI; CAG73025, *E. carotovora* subsp. *atroseptica* SCRI1043 ExpI; AAM46699, *Pectobacterium chrysanthemi* (=*Erwinina chrysanthemi*) EC16 AhlI; BAD52105, *Edwardsiella tarda* EdwI; CAA52353, *Pantoea agglomerans* (=*Erwinia herbicola*) EagI; AAC24591, *Pseudomonas syringae* pv. *syringae* B3A AhlI; AAO57331, *Pseudomonas syringae* pv. *tomato* DC3000 PsyI; AAA64792, *Agrobacterium tumefaciens* A348 TraI. Black-shaded boxes indicate identical amino acids and gray-shaded boxes mark conservative amino acid substitutions.

plants inoculated with *Erwinia* were incubated at 25°C chamber and then observed development of soft rotting disease.

Results

Cloning of *expI* from *P. carotovorum* subsp. *carotovarum*. To produce *expI* transgenic plants, *expI* gene was amplified using DNA from *P. carotovorum* subsp. *carotovorum* SL940 isolated from Chinese cabbage in Korea as a template. The *expI* gene consisted with 654 nucleotides encoding a polypeptide of 217 amino acids was isolated and DNA sequence was confirmed (Fig. 1). Computer data base search with the deduced amino acid sequence of *expI* indicated that the *expI* gene product had sequence similarity to the *expI* gene product of *E. carotovora* subsp. of *carotovora* scc3193. The overall sequence contains 94% identical amino acids with ExpI of *Erwinia carotovora* subsp. *carotovora* scc3193, 74% identical amino acids with CarI of *E. carotovora* subsp. *atroseptica*, 61% identical amino acids with AhlI of *Erwinia chrysanthemi* EC16, 59% identical amino acids with EdwI of *Edwardsiella tarda*, 52% identical amino acids with EagIb of *Enterobacter agglomerans*. This result suggested that this protein may be a homoserine lactone synthase which synthesizes the quorum sensing signal molecule N-(3-oxohexanoyl)-L-homoserine lactone (OHL) (Fig. 2).

Generation of *expI* transgenic *Arabidopsis*. In order to produce the *expI* transgenic *Arabidopsis*, the isolated *expI* gene was cloned under the control of CaMV35S promoter (Fig. 3A), which has been shown to mediate high levels of expression in plants. This chimeric *expI* gene was introduced into *Arabidopsis* by *Agrobacterium* mediated floral

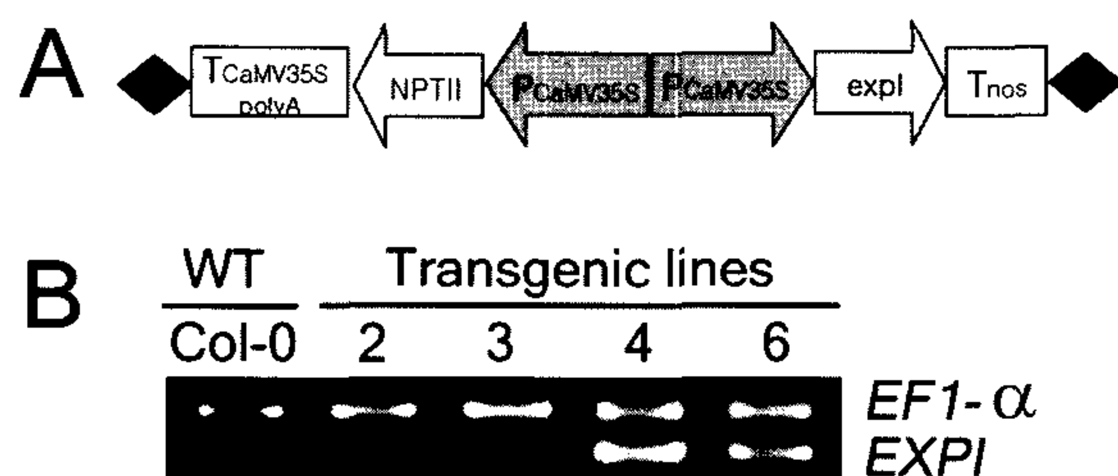

Fig. 2. Construction of chimeric *expI* gene and its expression in transgenic plants. (A) The binary vector containing the coding region of *expI* from *P. carotovorum* subsp. *carotovorum* L940 used in transformation. LB and RB, left-border and right-border; PCaMV35S, CaMV35S promoter; TCaMV35S polyA, CaMV terminator; Tnos, terminator of the nopaline synthase gene; NPTII, kanamycin resistance gene; expI, *expI* gene from *P. carotovorum* subsp. *carotovorum* L940. (B) RT-PCR analysis of *expI* expression in transgenic *Arabidopsis*. Col-0 is wild type. Numbers 2, 3, 4 and 6 indicate *expI* over-expression transgenic lines. *EF1-a* was used as a control.

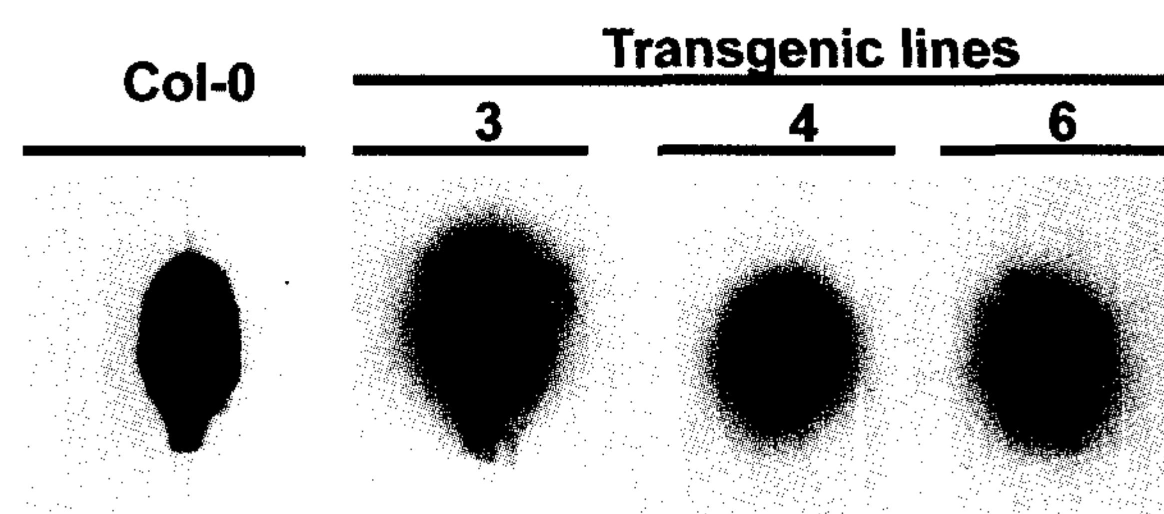

Fig. 3. Identification of N-acyl-homoserine lactones (acyl-HSL) signals in transformant and nontansformant arabidopsis leaves. Left leaves of all panels are wild type Arabidopsis cultivar Columbia and right leaves of all panels are transgenic Arabidopsis leaves. Stimulation of B-galactosidase activity in biosensor *E. coli* MG4 (pKDT17). Wild type leaves show lack of stimulation in *E. coli* MG4 (pKDT17) biosensor. Leaves of two weeks old Arabidopsis germinated and grown in media with antibiotic selection were placed on the surface of agar medium seeded with *E. coli* MG4 (pKDT17) biosensor strain supplemented with X-gal. A blue halo surrounding leaves indicate exudation of plant produced acyl-HSL.

spray transformation. This transformation yielded six viable transformed (T1) lines. Among six *expI* transplant lines, five lines except line 1 were fertile, but seeds from T1 transplant line 5 were not able to germinate well during second kanamycin screening. More than 60% of seeds of line 2 and 3 and about 30% of seeds of line 4 and 6 were germinate and survive well in kanamycin media (Table 1). All four *expI* transgenic plant lines were fertile, and southern blot analysis indicated that kanamycin resistant progeny maintained the target gene without obvious rearrangement (data not shown). Expression of the chimeric *expI* gene in the transgenic lines was characterized by reverse-transcription (RT)-PCR with the control of *EF1a* gene expression at the same time (Fig. 3B). All four transgenic plant lines appeared to accumulate a specific *expI* related transcript. Whether the *expI* transgenic plants are able to produce OHL, the ability to induce X-gal production in the AHL biosensor *E. coli* MG4 carrying pKDT17 of

Table 1. Inheritance of the Kanamycin-resistant trait of *expI* transgenic seeds and survival rate of plants inoculated with *P. carotovorum* subsp. *carotovorum*

Transgenic Line	Kan^R seedling /plated seeds (no.)	Kan^R seedlings (%)	Survived plants[a] /plated seeds (no.)	Survived plants (%)
2	49/86	57	12/24	50
3	51/66	77	28/46	61
4	23/70	33	9/22	41
5	2/61	3	NT	NT
6	17/65	26	9/17	53

[a]plants were inoculated with *P. carotovorum* subsp. *carotovorum*, then checked for surviving plants 10 days after inoculation. NT: not tested

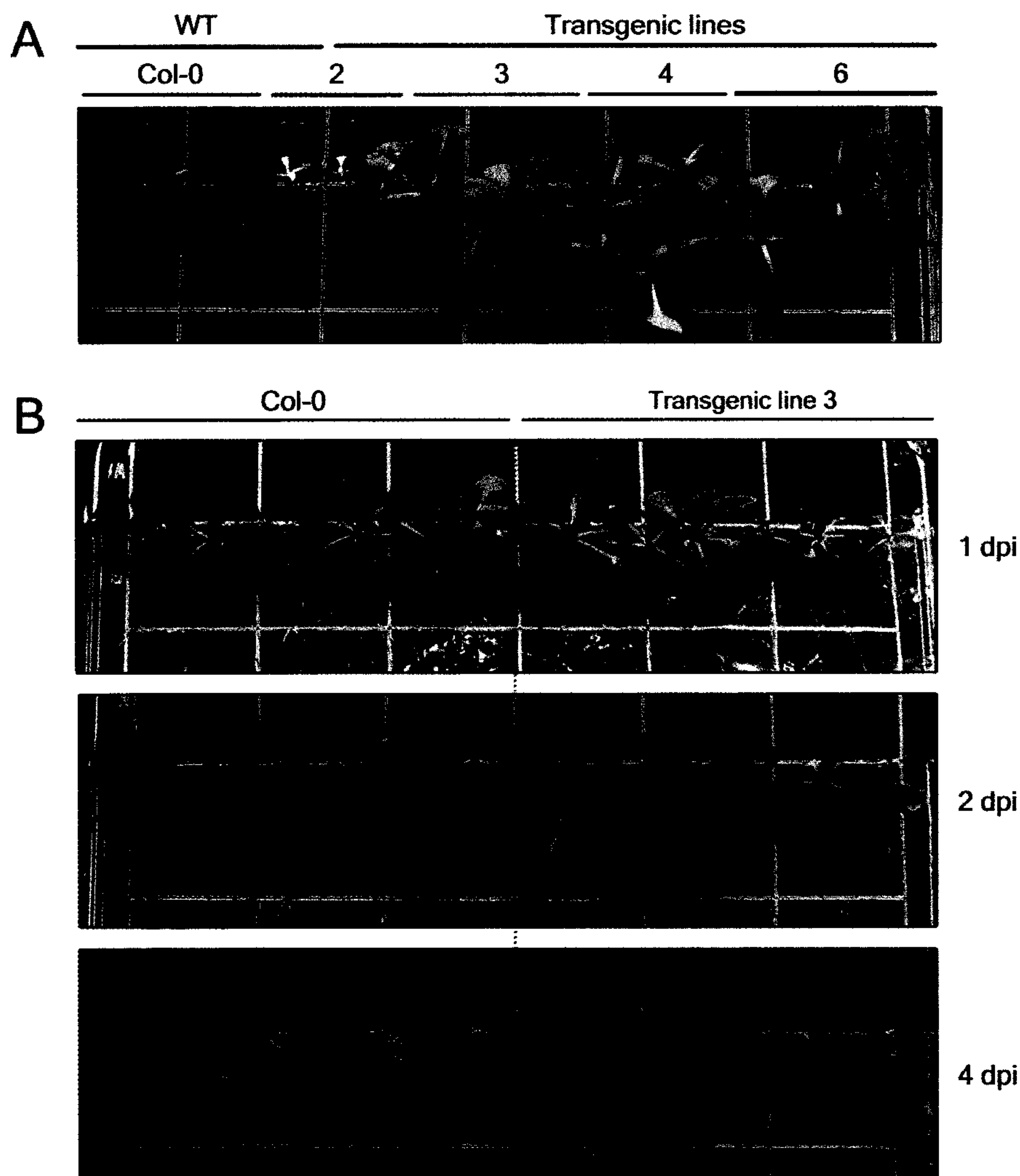

Fig. 4. Reaction of *expI* transgenic plants to *P. carotovorum* subsp. *carotovorum*. (A) in Col-0 and 4 different transgenic lines. (B) Observation of soft rotting development in Col-0 and transgenic line 3. Representative plants were photographed 1, 2, and 4 days after inoculation (dpi). All photographed plants are same plants.

transgenic plants were tested. When the leaves of transgenic plants were on the top of the biosensor bacteria, the plant-generated OHL of transgenic plants was diffused out and induced the X-gal production of the biosensor bacteria *E. coli* MG4 (Fig. 4).

The phenotype of line 3, 4, and 6 were the same as wild type but the phenotype of T1 transgenic plant line 2 was different from that of wild type. All T1 transgenic plant line 2 showed severe stunting (Fig. 5A).

Resistance to pathogens. It was previously suggested that *P. carotovorum* subsp. *carotovorum* employs the quorum sensing system to avoid premature detection by the plant defense response (Pirhonen et al., 1993) and was shown that the OHL producing tobacco lines exhibited enhanced resistance to infection by wild type *P. carotovorum* subsp.

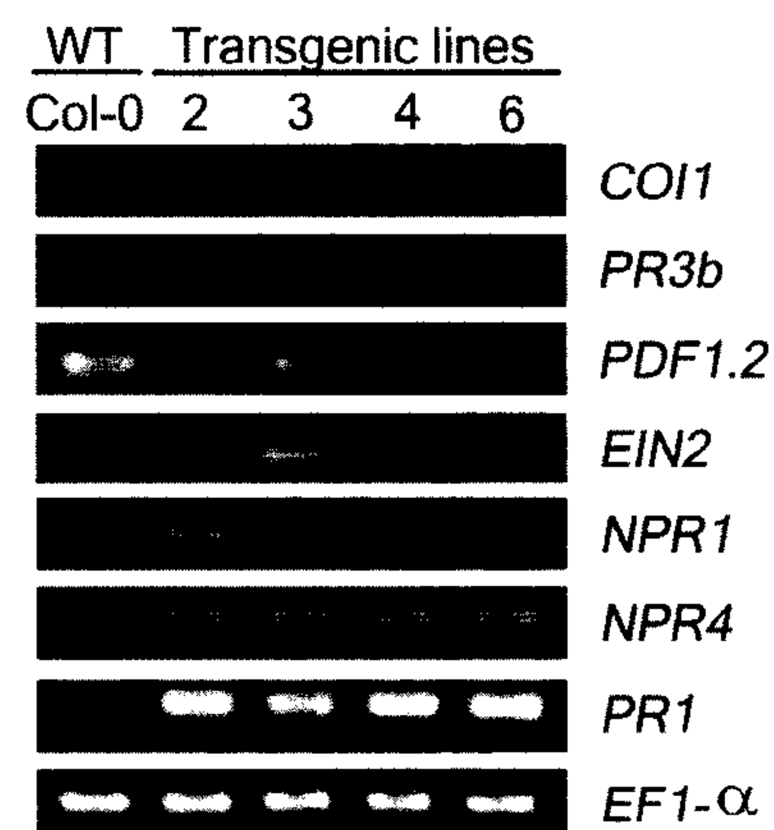

Fig. 5. Differential expression of *PR1* and SA-responsive genes in Col-0 and 4 different transgenic lines. Col-0 is wild type. *EF1-a* was used as a standard.

carotovorum (Mae et al., 2001). Since *P. carotovorum* subsp. *carotovorum* is one of the most destructive pathogen to crucifer plants, it was tested whether *exp*I transgenic *Arabidopsis* showed resistance to pathogen or not. In order to get rid of other environmental factors that may influence the interaction, *P. carotovorum* subsp. *carotovorum* was applied in axenic culture of transgenic *Arabidopsis*., The wild type *Arabidopsis* started to show the water-soaking symptoms at just one day after application of *P. carotovorum* subsp. *carotovorum* whereas transgenic plants did not show water-soaking symptom at all. Soft rot symptoms were shown from two days after pathogen application in wild type and all wild type *Arabidopsis* were completely killed at four days after pathogen application (Fig. 5A & B). All transgenic plants were healthy until 4 days after pathogen application and some individual transgenic plants exhibited to get soft. However, in most individual transgenic plants, the rate of disease development was much slower than wild type or soft rotting did not progress and almost half of transgenic plants were survived at 10 days after pathogen application (Fig. 5A & Table 1).

Expression of defense related genes. To analyze whether *exp*I had a role in the regulation of the defense mechanism, we selected several SA-, JA- and ET- dependent marker genes and RT-PCR with these defense related genes were performed. SA-dependent marker genes, *NPR1*, *NPR4* and *PR1* expression were characterized in wild type plants and *exp*I transgenic plants. JA- and ET- dependent marker gene, *PDF1.2* and *PR3b* were employed. *EIN2* was chosen for the ethylene responsive gene in this experiment. In RT-PCR analysis, *PR1* gene expression was strongly induced in all *exp*I transgenic lines than wild type plant and *NPR1* and *NPR4* gene expressions were weakly increased in transgenic lines. Whereas no significant changes of other defense related genes were not observed (Fig. 6).

Discussion

This paper proved that the production of bacterial pheromone in transgenic plants made soft rot resistant. The phenotype of *exp*I transgenic lines was not different from that of the wild type. Since the isolated ExpI-homolog from *P. carotovorum* subsp. *carotovorum* SL940 showed 94% identity with ExpI from *E. carotovora* subsp. *carotovora* scc3193 and 74% identity with CarI of *E. carotovorasub* sp. *atroseptica*, it may have the similar function with those genes. *Exp*I transgenic lines 3, 4, and 6 showed strong expression of *exp*I gene and also production of OHL and secreted out this OHL without any secretion signal. All tested transgenic plants producing OHL were resistant to bacterial soft rot disease. This results support the previous hypothesis by Mae et al. (2001) that the transgenic plant derived production of QS will result in PCWDE production at low bacterial population densities, eliciting a plant defense when the bacterial population is not sufficient to suppress this responses.

Previously ExpI transgenic tobacco was reported to produce OHL and showed resistant to *E. carotovora* subsp. *carotovora*. Our data also proved that ExpI transgenic *Arabidopsis* was able to produce and diffuse OHL out and became a soft rot resistant. In addition to the construction of disease resistant OHL producing transgenic plants, we supported the defense gene involvement in this disease resistant caused by *exp*I gene by the increase of several defense related gene expressions. Especially the PR1 expression was increased dramatically in transgenic plants than wild type *Arabidopsis*. In addition, the expression of NPR1 and NPR4 were slightly increased in comparison with the wild type line. PR1 is the best-known SA induced gene. NPR1 and NPR4 all are part of the SA signaling pathway but play different roles in controlling the expression of SA-related genes. Therefore the expression of all genes representing SA signal pathway were increased. The enhancement of NPR1 gene expression may explain the secretion of OHL from transgenic plants without secretion signals. Dong et al. (2005) reported that NPR1 not only induce the PR genes but also prepare the cell for secretion of PR proteins by first making more secretary machinery components. More NPR1 expression induced more secretary machinery components and small OHL molecule was diffused out from the transgenic plants.

The RT-PCR results show no dissimilarity between wild type and transgenic expression of the JA pathway related genes COI1, PR3, andPDF1.2 and ethylene related gene EIN2. These results suggested that the acquired resistance of *exp*I transgenic plants to soft rot pathogen *P. carotovorum* subsp. *carotovorum* is related with SA signaling pathway not JA or ethylene related pathways. However, these gene expression results are somewhat contrary to the previous results that the *E. carotovora* culture filtrates induced resistance in *Arabidopsis* by SA independent pathways (Norman-Setterbald et al., 2000; Vidal et al., 1997; Vidal et al., 1998). Since the hypothesis of the disease resistance of *exp*I transgenic plants was that the transgenic plant derived production of QS will result in PCWDE production at low bacterial population densities, eliciting a plant defense when the bacterial population is not sufficient to suppress this responses, the expression of defense related gene in *exp*I transgenic plant would be the same as the expression of defense related gene by culture filtrate treatment of *E. carotovora*. However, the induced resistance of *exp*I transgenic plants was SA dependent but the induced resistance of culture filtrate of *E. carotovora* was SA

independent. Since QS controls many gene expressions in addition to PCDWE production, other products besides PCDWE may be induced by the high expression of *exp*I gene in transgenic plants. Also the high expression of *exp*I gene in transgenic plants may directly affect the defense signaling pathway in *Arabidopsis*. All these possibilities should be investigated in further.

References

Bainton, N., Bycroft, B., Chhabra, S., Stead, P., Gledhill, L., Hill, P., Rees, C., Winson, M., Salmond, G., Stewart, G. and Williams, P. 1992. A general role for the *lux* autoinducer in bacterial cell signaling: control of antibiotic biosynthesis in *Erwinia*. *Gene*. 116:87-91.

Barras, F., van Gijegem, F. and Chatterjee, A. 1994. Extracellular enzymes and pathogenesis of soft-rot *Erwinia*. *Annu. Rev. Phytopathol.* 32:201-234.

Cao, H., Bowling, S., Gordon, A. and Dong, X. 1994. Characterization of an Arabidopsis mutant that is nonresponsive to inducers of systemic acquired resistance. *Plant Cell* 6:1583-1592.

Chatterjee, A., Chun, W. and Chatterjee, A. 1990. Isolation and characterization of an *rcs*A-like gene of *Erwinia amylovora* that activates extracellular polysaccharide production in *Erwinia* species, *Escherichia coli*, and *Salmonella typhimurium*. *Mol. Plant-Microbe Interact.* 3:144-148.

Clough, S. and Bent, A. 1998. Floral dip: a simplified method for *Agrobacterium*-mediated transformation in *Arabidopsis thaliana*. *Plant J.* 16:735-743.

Collmer, A. and Keen, N. 1986. The role of pectic enzymes in plant pathogenesis. *Annu. Rev. Phytopathol.* 24:383-409.

Delaney, T., Friedrich, L. and Ryals, J. 1995. Arabidopsis signal transduction mutant defective in chemically and biologically induced disease resistance. *Proc. Natl, Acad. Sci. USA* 95: 6602-6606.

Feys, B. and Parker, J. 2000. Interplay of signaling pathways in plant disease resistance. *Trends Genet.* 16:449-455.

Fuqua, W., Winans, S. and Greenberg, E. 1994. Quorum sensing in bacteria: The LuxR-LuxI family of cell density-responsive transcriptional regulators. *J. Bacteriol.* 176:269-275.

Fuqua, W., Winans, S. and Greenberg, E. 1996. Census and consensus in bacterial ecosystems: The LuxR-LuxI family of quorum-sensing transcriptional regulators. *Annu. Rev. Microbiol.* 50:727-751.

Hanahan, D. 1983. Studies on transformation of *Escherichia coli* with plasmids. *J. Mol. Biol.* 166:557-580.

Ibeas, J., Lee, H., Damsz, B., Prasad, D., Pardo, J., Hasegawa, P., Bressan, R. and Narasimhan, M. 2000. Fungal cell wall phosphomannans facilitate the toxic activity of a plant PR-5 protein. *Plant J.* 23:375-383.

Jones, S., Yu, B., Bainton, N. J., Birdsall, M., Bycroft, B. W., Chhabra, S., Cox, A. J. R., Colby, P., Reeves, P. J., Stephens, S., Winson, M. K., Salmond G. P. C., Stewart, S. A. B. and Williams, P. 1993. The *lux* autoinducer regulates the production of exoenzyme virulence determinants in *Erwinia carotovora* and *Pseudomonas aeruginosa*. *EMBO J.* 12:2477-2482.

Kaplan, H. and Greenberg, E. 1985. Diffusion of autoinducer is involved in regulation of the *Vibrio fischeri* luminescence system. *J. Bacteriol.* 163:1210-1214.

Kinkema M., Fan W. and Dong X. 2000. Nuclear localization of NPR1 is required for activation of PR gene expression. *Plant Cell* 12:2339-2350.

Kunkel, B. N. and Brooks, D. 2002. Cross talk between signaling pathways in pathogen defense. *Curr. Opin. Plant Biol.* 5:325-331.

Liu, G., Holub, E. B., Alonso, J. M., Ecker, J. R. and Fobert, P. R. 2005. An Arabidopsis *NPR1*-like gene, *NPR4*, is required for disease resistance. *Plant J.* 41:304-318.

Mäe, A., Montesano, M., Koiv, V. and Palva, E. T. 2001. Transgenic plants producing the bacterial pheromone *N*-acyl-homoserin lactone exhibit enhanced resistance to the bacterial phytopathogen *Erwinia carotovora*. *Mol. Plant-Microbe Interact.* 14:1035-1042.

Matthew, R. Parsek, Amy, L. Schaefer and Greenberg, E. P. 1997. Analysis of random and site-directed mutations in *rhl*I, a *Pseudomonas aeruginosa* gene encoding an acylhomoserine lactone synthase. *Mol. Microbiology* 26:301-310.

Miller, J. H. 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, U.S.A.

Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol. Plant.* 15:473-497.

Niderman, T., Genetet, I., Bruyere, T., Gees, R., Stintzi, A., Legrand, M., Fritig, B. and Mosinger, E. 1995. Pathogenesis-related PR-1 proteins are antifungal. Isolation and characterization of three 14-kilodalton proteins of tomato and of a basis PR-1 of tobacco with inhibitory activity against *Phytophthora infestans*. *Plant Physiol.* 108:17-27.

Norman, C., Vidal, S. and Palva, E. T. 1999. Oligogalacturonide-mediated induction of a gene involved in jasmonic acid synthesis in response to the cell-wall-degrading enzymes of the plant pathogen *Erwinia carotovora* . *Mol. Plant-Microbe Interact.* 12:640-644.

Norman-Setterbald, C., Vidal, S. and Palva, E. T. 2000. Interacting signal pathways control defense gene expression in Arabidopsis in response to cell wall-degrading enzymes from *E. carotovora* subsp. *carotovora*. *Mol. Plant-Microbe Interact* 13:430-438.

Pierson, L. S., III, Wood, D. W. and Pierson, E. A. 1998. Homoserine lactone-mediated gene regulation in plant-associated bacteria. *Annu. Rev. Phytopathol.* 36:207-235.

Pirhonen, M., Flego, K., Heikingeimo, R. and Palva, E. T. 1993. A small diffusible signal molecule is responsible for the global control of virulence and exoenzyme production in the plant pathogen *Erwinia carotovora*. *EMBO J.* 12:2467-2476.

Salmond G. P., Bycroft B.W., Stewart G. S. and Williams P. 1995. The bacterial 'enigma': cracking the code of cell-cell communication. *Mol. Microbiol.* 16:615-624.

Sambrook, J., Fritsch, E. F. and Maniatis, T. 1989. Molecular

cloning: A laboratory manual, 2nd ed. Cold spring harbor laboratory press, Cold spring harbor, NY, U.S.A.

Shah, J., Tsui, F. and Klessig, D. F. 1997. Characterization of a salicylic acid-insensitive mutant (*sai1*) of *Arabidopsis thaliana*, identified in a selective screen utilizing the SA-inducible expression of the *tms2* gene. *Mol. Plant-Microbe Interact.* 10: 69-78.

Swift, S., Throup, J. P., Williams, P., Salmond, G. P. C. and Stewart, G. S. A. B. 1996. Quorum sensing: A population-density component in the determination of bacterial phenotype. *Trends Biochem. Sci.* 21:214-219.

Teresa, R. and Barabara, H. 2000. Bacterial quorum sensing in pathogenic relationships. *Infect. Immun.* 68:4839-4849.

Thomma, B. P. H. J., Penninckx, I. A. M. A., Cammue, B. P. A. and Broekaert, W. F. 2001. The complexity of disease signaling in *Arabidopsis*. *Curr. Opin. Immunol.* 13:63-68.

Vidal, S., Ponce de Leon, L. and Palva, E. T. 1997. Salicylic acid and the plant pathogen *Erwinia carotovora* induce defense via antagonistic pathways. *Plant J.* 11:115-123.

Vidal, S., Eriksson, A. R. B., Montesano, M., Demecke, J. and Palva, E. T. 1998. Cell wall-degrading enzymes from *Erwinia carotovora* cooperate in the salicylic acid-independent induction of a plant defense response. . *Mol. Plant-Microbe Interact* 11:23-32.

Von Bodman, S. B. and Farrand, S. K. 1995. Capsular polysaccharide biosynthesis and pathogenicity in *Erwinia stewartii* require induction by *N*-acyl-homoserine lactone autoinducer. *J. Bacteriol.* 177:5000-5008.

Zimmerli, L., Stein, M., Lipka, V., Schulze-Lefert, P. and Somerville, S. 2004. Host and non-host pathogens elicit different jasmonate/ethylene responses in Arabidopsis. *Plant J.* 40:633-646.