G-72. A pepper CALRR1 gene encoding leucine-rich repeat (LRR) protein is only induced in pepper by microbial perception. Ho Won Jung[1], Eui hwan Jung[1], Sung Chul Lee[1], Sang Wook Han[1], Sunggi Heu[2], and Byung Kook Hwang[1]. [1]Laboratory of Molecular Plant Pathology, College of Life and Environmental Sciences, Korea University, Seoul 136-701, Korea, [2]Division of Plant Pathology, National Institute of Agricultural Science and Technology, Rural Development Administration, Suwon 441-707, Korea.

A novel *CALRR1* gene encoding three leucine-rich repeats (LRRs) was isolated from pepper plants. The *CALRR1*-encoded protein is rich in leucine at its N-terminus, and contains leucine-rich repeat domains at C-terminus. *CALRR1* includes three tandem repeats of a 24-amino acid leucine-rich repeat (LRR) sequence that can mediate molecular recognition and interaction processes. The *CALRR1* gene and its LRR consensus sequences share significant homology to the extracellular binding domains of the receptor-like protein kinase RLK5 and RMK1 of Arabidopsis, Cf-9 of tomato and Xa21 of rice, PGIP of tomato, pear and bean, SLRR of sorghum LRR proteins, and LRP from tomato LRR proteins. *CALRR1* proteins also are related to small leucine-rich repeat proteoglycans (SLRP) in animals. Northern blot analyses indicate that the *CALRR1* gene is strongly induced by inoculation of *Xanthomonas campestris* pv. *vesicatoria, Phytophthora capsici, Colletotrichum coccodes* and *C. gloeosporioides*. The CALRR1 gene was neither systemically induced by bacterial infection, nor transcriptionally activated in a host-pathogen-specific manner. Treatment with abiotic elicitors, including salicylic acid, ethylene, methyl jasmonate, DL--amino-n-butyric acid and benzothiadiazole did not activate the transcription of the *CALRR1* gene, indicating no involvement of the *CALRR1* gene in the signal transduction pathway regulated by the abiotic elicitors. The An in situ hybridization study showed that *CALRR1* mRNA was localized in phloem tissues of leaves, stems, and green fruits of pepper plants during the pathogen infection and ABA exposure. The structural characteristics and the spatio-temporal expression pattern of the *CALRR1* gene suggest that the *CALRR1* gene may mediate recognition and interaction in the pepper extracellular matrix as a component of a signal transduction pathway during the pathogen infection or some environmental stresses.

G-73. A thaumatin-like gene in nonclimacteric pepper fruits used as a molecular marker in probing ripening, sugar accumulation, and disease resistance. Young Soon Kim[1], Jung Yoon Park[1], Kwang Sang Kim[2], Moon Kyung Ko[1], Soo Jin Cheong[2], and Boung-Jun Oh[3]. [1]Kumho Life and Environmental Science Laboratory, Korea Kumho Petrochemical Co., Ltd., 1 Oryong-dong, Buk-gu, Gwangju 500-712, Korea, [2]PhytoCareTech Co., LTD., Chonnam National University Business Incubator, 300 Yongbong-dong, Buk-gu, Gwangju 500-757, Korea, [3]Plant & Microbe Co., LTD., Biotechnology Industrialization Center, 880-4 Ansan-Ri, Naju-Si, Jeonnam 520-811, Korea.

During pepper (*Capsicum annuum*) fruit ripening, the ripe fruit interaction with

the anthracnose fungus, *Colletotrichum gloeosporioides* is generally incompatible. However, the unripe fruit can interact compatibly with the fungus. A gene, designated *PepTLP* (for pepper thaumatin-like protein), was isolated and characterized using mRNA differential display. The *PepTLP* gene encodes a protein homologous to other thaumatin-like proteins and contains 16 conserved cysteine residues and the consensus pattern of thaumatin. The *PepTLP* gene expression is developmentally regulated during ripening. The expression of the *PepTLP* gene in the incompatible interaction was higher than that in the compatible one. Furthermore, the *PepTLP* gene expression was stimulated by both jasmonic acid treatment and wounding during ripening, but by wounding only in the unripe fruit. Immunolocalization studies showed that PepTLP accumulated during ripening, but highly only during incompatible interactions, and that it is localized in the intercellular spaces between cortical cells and vascular bundles. The development of anthracnose became reduced during fruit ripening, and the sum total of sugar accumulation increased. The results suggest that the *PepTLP* gene can be used as a molecular maker in probing for ripening, sugar accumulation, and disease resistance in the nonclimacteric pepper fruits

G-74. Regulation of H_2O_2 accumulation and proxidase acitivity by pepper ascorbate peroxidase, thioredoxin peroxidase, and peroxidase genes during the hypersensitive response to pathogen infection. J. K. HONG[1], H. M. Do[1], H. W. Jung[1], S. H. Kim[1], J. H. Ham[2], and B. K. Hwang[1]. [1]Laboratory of Molecular Plant Pathology, College of Life and Environmental Sciences, Korea University, Seoul 136-701, Korea, [2]Department of Plant Pathology, University of Wisconsin-Madison, Madison, WI 53706, USA.

Pepper ascorbate peroxidase-like (*CAPOA1*), thioredoxin peroxidase-like (*CAPOT1*), and peroxidase-like (*CAPO1*) clones were isolated from pepper leaves inoculated with avirulent strain Bv5-4a of *Xanthomonas campestris* pv. *vesicatoria*. *CAPOA1*, *CAPOT1* and *CAPO1* mRNA disappeared 18-30 h after the bacterial infection when the hypersensitive responses (HR) were visible. In contrast, peroxidase activity reached a peak at 18 h after infection and then declined at 24 and 30 h, when H_2O_2 accumulation level was maximized. These results suggest that the striking accumulation of H_2O_2 and strong decrease in peroxidase activity during the programmed cell death may be due to the strong suppression of *CAPOA1*, *CAPOT1* and *CAPO1* gene expression. Infection by *Phytophthora capsici* or *Colletotricum gloeosporioides* also induced the expression of the three putative peroxidase genes in pepper tissues. *CAPOA1* mRNAs were *in situ* localized in phloem areas of vascular bundles in pepper tissues infected by *Colletotrichum coccodes*, *P. capsici* or *C. gloeosporioides*. Exogenous treatment with H_2O_2 strongly induced the *CAPOA1* and *CAPOT1* transcription 1 h after treatment, while the *CAPO1* transcripts accumulated 12 h after H_2O_2 treatment. We suggest that pepper ascorbate peroxidase and thioredoxin peroxidase genes may function as regulators of H_2O_2 level and total peroxidase activity in the oxidative burst during the HR to incompatible pathogen interaction in pepper plant.

G-75. Isolation and characterization of pathogen-inducible putative zinc finger protein from hot pepper *Capsicum annuum* L. Sang-Keun Oh[1], Jeong-Mee

Park[1], Young-Hee Joung[1], Sanghyeob Lee[1], Soo-Yong Kim[1], Seung-Hun Yu[2], and Doil Choi[1]. [1]Lab. of Plant Genomics, KRIBB, P.O.Box 115, Yusung,Taejeon, 305-600, Korea, [2]Department of Agricultural Biology, CNU, Taejeon, 305-764, Korea

We have isolated a full-length cDNA, *Ca1244*, encoding a putative C2H2 zinc finger DNA-binding protein from hot pepper EST DB (http://plant.pdrc.re.kr). Ca1244 contains a putative DNA-binding domain that shares significant amino acid identity with DNA binding domain of members of the C2H2 zinc finger DNA-binding protein family and has a nuclear localization signal. Transcripts of the *Ca1244* gene were preferentially induced in hot pepper during an incompatible interaction with *Pseudomonas syringae* pv. *syringae* 61, *Xanthomonas axonopodis* cv. *vesicatoria* race 3, and *Pepper mild mottle virus*. Expression of *Ca1244* gene was also induced by treatment of abiotics such as wound and ethephone. Over-expression of Ca1244 in tobacco under control of CaMV 35S promoter resulted in resistance against fire blight pathogen (*Pseudomonas syringae* pv. *tabaci*). Furthermore, Northern blot analyses revealed that Ca1244 over-expressed transgenic tobacco plants showed increased expression of PR protein genes including *PR-1*, *PR-5*, and *SAR8.2*. Our data is presented the first evidence that a C2H2 type zinc finger transcription factor is preferentially induced by pathogen attack and suggesting that *Ca1244* gene may play an important role in the expression of plant defense-related genes.

G-76. Cloning and characterization of pathogen-inducible EREBP-like transcription factor(*CaNR19*) from hot pepper (*Capsicum annuum* L.). So-Young Yi[1], Jee-Hyub Kim[2], Seung-Hun Yu[3], and Doil Choi[1],. [1]Plant genomics Lab. KRIBB, P.O. Box 115, 305-600. [2]National Center for Genome Information. [3]Department of Agricultural Biology, CNU, Taejeon, 305-764, Korea.

An EREBP/AP2-type transcription factor (*CaNR19*) was isolated by DDRT-PCR following inoculation of soybean pustule pathogen *Xanthomonas axonopodis* pv. *glycines* 8ra which induces HR on pepper leaves. Genomic Southern blot analysis revealed that the *CaNR19* gene is present as a single copy within the hot pepper genome. The deduced amino acid sequence of *CaNR19* has two potential nuclear localization signals, a possible acidic activation domain, and an EREBP/AP2 motif that could bind to a conserved *cis*- element present in promoter region of many stress-induced genes. The mRNA level of *CaNR19* was induced by both biotic and abiotic stresses. We observed higher-level transcripts in resistance-induced pepper tissues than diseased tissues. Expression of *CaNR19* is also induced upon various abiotic stresses including ethephon, MeJA, cold stress, drought stress and salt stress treatments. To study the role of *CaNR19* in plant, transgenic *Arabidopsis* and tobacco plants which express higher level of pepper *CaNR19* were generated. Global gene expression analysis of transgenic Arabidopsis by cDNA microarray indicated that expression of *CaNR19* in transgenic plants affect the expression of quite a few GCC box and DRE/CRT box-containing genes. Furthermore, the transgenic *Arabidopsis* and tobacco plant, expressing *CaNR19* showed tolerance against freezing temperature and enhanced resistance to *Pseudomonas syrnigae* pv. *tabaci*. Taken together, these results indicated that *CaNR19* is a novel EREBP/AP2 transcription factor in hot pepper plant

and it may has a significant role(s) in regulation of biotic and abiotic stresses in plant.

G-77. Characterization of a novel stress response of *Glycine max* cultivar PI96188. S. W. HAN[1], M. Choi[1], B. Hwang[2], and S. Heu[1]. [1]Plant Pathology Division, National Institute of Agricultural Science and Technology, Rural Development Administration, Suwon, Korea, [2]College of Life and Environmental Sciences, Korea University

The bacterial pustule disease of soybean (*Glycine max* (L.) Merr.), caused by *Xanthomonas axonopodis* pv. *glycines* (Nakano) Dye, is characterized by small yellow haloes to brown lesions with a raised pustule in the center. Seventy-five soybean cultivars had been tested for the response to several different *Xanthomonas axonopodis* pv. *glycines* races collected worldwide. Among the tested cultivars, one cultivar, *G. max* cultivar PI96188 showed very different response to all *Xanthomonas axonopodis* pv. *glycines* races which showed necrosis without chrolotic haloes but with pustules on the back of the necrosis symptoms. For testing the response of cultivar PI96188 with chemical reagents, cultivar PI96188 was treated with the herbicide, BastaR. Since BastaR is a systemic herbicide, control cultivar Jinjool were dried out finally. However, in the cultivar PI96188, dried regions did not extend to whole plant. Ultrastructural study showed the chloroplast degradation in control cultivar after pathogen treatment, but cultivar PI96188 did not. The contents of chlorophyll were measured to study the chrolophyll degradation. In the control leaves, chlorophyll levels declined steadily at 6 days after inoculation, so retained only about 35% of its chlorophyll at day 15. By contrast, chlorophyll levels of cultivar PI96188 started to decrease at 9 days after inoculation and retained 70% of their untreated levels. The bacterial multiplication in the cultivar PI96188 was 10-1~10-2-fold lower than that in susceptible soybean cultivars that produce typical disease symptoms., but it was 10-fold higher than that in resistant cultivar. For elucidating the response of PI96188 to stress in genetic level, northern blot analyses were performed using three group genes; ethylene biosynthesis pathway, photosynthesis system, and defense-related genes. These data will help to explain the mechanism of the chlorosis of the plant to respond the stress or the pathogen.

G-78. Identification and characterization of genes expressed during rice-*Magnaporthe grisea* interactions. Soonok Kim and Yong-Hwan Lee. School of Agricultural Biotechnology, Seoul National University, Suwon 441-744, Korea

Expressed sequence tag (EST) analysis was conducted to identify rice genes involved in defense responses against infection by the blast fungus *Magnaporthe grisea* and fungal genes involved in infectious growth within the host during a compatible interaction. Two different cDNA libraries were constructed. A cDNA library was constructed with RNA from rice leaves (*Oryza sativa* cv. Hwacheong) infected with *M. grisea* strain KJ201 into lZAP XR vector, of which 1,980 clones were sequenced to generate 1,543 non-redundant ESTs. In the second approach, the polymerase chain reaction based suppression subtractive hybridization technology was adopted to enrich the fungal genes expressed during infectious growth in the host plant. The subtraction library was constructed with RNA from infected rice leaves as a tester and that from

uninfected rice leaves as a driver. A total of 1,996 clones from this library were sequenced to generate 972 non-redundant ESTs. Of the 2,315 non-redundant ESTs generated from two cDNA libraries, 1,594 ESTs (68.9%) significantly matched with NCBI database entries. About 63% (1,477) of ESTs showed homology to genomic sequences of Indica type rice generated by the Beijing Genome Institute. This strongly implies that about 36% (838) of ESTs were from the blast fungus. cDNAs encoding senescence-associated protein and translation elongation fator eEF-1 were the most abundant representatives of plant and fungal genes, respectively. Transcriptional profiling of fungal genes expressed *in planta* is in progress.

G-79. Large-scale insertional mutagenesis of *Magnaporthe grisea* using *Agrobacterium tumefaciens*-mediated transformation. Myung-Hwan Ji, Sook-Young Park, Soonok Kim, and Yong-Hwan Lee. School of Agricultural Biotechnology, Seoul National University, Suwon 441-744, Korea

Rice blast, caused by *Magnaporthe grisea*, is considered as a model system for studying fungal pathogen-plant interactions not only due to the great economic importance involved, but also due to the genetic and molecular genetic tractability of both the fungus and the host. To understand pathogenicity factors of this fungal pathogen at genome-wide level, we developed large-scale insertional mutagenesis technique using *Agrobacterium tumefaciens*-mediated transformation (ATMT) and high-throughput phenotype assay system. Fifteen thousands of transformants were generated thus far, and their phenotypes including fungal growth rate, pigmentation, ability of conidiation, conidial germination, appressorium formation and pathogenicity are being evaluated. Among the first screened 5,040 transformants, 528 pathogenicity-defective mutants that contain one or more developmental defects were obtained. The tagged sequences from pathogenicity-defective mutants are being identified by TAIL-PCR technique. This approach would offer highly efficient means for characterizing fungal genes that are important for pathogenicity of *M. grisea*.

G-80. Development of Arabidopsis-*Cochliobolus miyabeanus* pathosystem. Ju-Young Park, Soonok Kim, and Yong-Hwan Lee. School of Agricultural Biotechnology, Seoul National University, Suwon 441-744, Korea

Cochliobolus miyabeanus is a necrotrophic fungal pathogen that causes brown leaf spot in rice. We established an experimental system for analysis of the interaction of *C. miyabeanus* with *Arabidopsis thaliana*, the most representative dicotyledonous model plant. Small brownish necrotic lesions with marginal yellowing appeared on infected Col-O leaves at 3 days after inoculation, expanded throughout the entire leaf as time increases, and the infected leaves eventually withered and died at 9 days after inoculation. The fungus recovered from the necrotic lesions can induce the same lesions on healthy Arabidopsis plant. Expression of both *PR-1* and *PDF1.2* was induced by the *C. miyabeanus* infection. Pretreatment of signaling molecules had diverse effects on disease development: ethylene increased susceptibility, methyl jasmonate had little effect, and surprisingly, salicylic acid increased resistance. These results suggest that the establishment of resistance against *C. miyabeanus* might be based on SA-dependent

pathway, and JA/ET-dependent signaling pathway underlying the susceptibility could be dissected more finely.

G-81. Characterization of *cppk1* null mutant and identification of CpPK1 target protein of *Cryphonectria parasitica*. Moung-Ju Kim, Seung-Moon Park, Young-Ho Kim[1], Moon-Sik Yang, and Dae-Hyuk Kim. Institute for Molecular Biology and Genetics, Chonbuk National University, Dukjindong 664-14, Chonju, Chonbuk 561-756, Korea, [1]School of Agricultural Biotechnology, Seoul National Univerisity, Suwon 441-744, Korea

Infection of double stranded RNA (dsRNA) forms of Cryphonectria Hypovirus 1(CHV1) into Chestnut blight fungus *Cryphonectria parasitica* reveals the characteristic symptoms of hypovirulence and aberrant expression of specific fungal genes. The *cppk1* is a gene encoding the ser/thr protein kinase of *C. parasitica* and is transcriptionally up-regulated by infection of CHV1. To characterize the CpPK1, we constructed a *cppk1* null-mutant by transforming a vector that containing hygromycin resistant gene (*hph*) as a selectable marker. The *cppk1* null mutant was initially isolated as a heterokaryotic form, which contains wild type and *cppk1* deleted nucleus. Southern blot analysis and genomic PCR confirmed their different type of nucleus. The real *cppk1* null mutant was further isolated after single sporing of heterokaryotic form, and showed characteristic morphological symptoms: restricted growth, swollen and shortened mycelia. We introduced antisense *cppk1* into wild type strain, and the resulting transformants also showed slow growth symptoms than that of wild-type strain. We previously identified two potential intracellular protein substrates by the kinase assay using *E.coli* derived CpPK1. To further isolate the CpPK1 dependent substrates, we conducted the two dimensional gel electrophoresis, MALDI TOF analysis and amino acid sequencing of phosphorylated substrate proteins, and the enolase was deduced as a putative substrate of CpPK1.

G-82. Cloning and characterization of a YERK1 homologue, CpMK2, from *Cryphonectria parasitica*. Eun-Sil Choi[1], Seung-Moon Park[1], Myoung-Ju Kim[1], Byeongjin Cha[2], Moon-Sik Yang[1], and Dae-Hyuk Kim[1]. [1]Institute for MolecularBiology and Genetics, Chonbuk National University, Dukjindong 664-14, Chonju, Chonbuk 561-756, Korea, [2]Dept. of Agricultural Biology, Chungbuk National University, Cheongju, Chungbuk 361-763, Korea

We cloned the gene, *cpmk2*, encoding a mitogen-activated protein kinse (MAPK) of *C. parasitica* and examined what the biological function of *cpmk2*. The sequence comparision of *cpmk2* shows the highest homology to pmk1 of *Magneporthe grisea* and reveals that it belongs to the yeast extracellular signal-regulated kinase (YERK1) subfamily. The deduced protein product had a predicted mass of 39 kDa and a pI of 5.6. The *E. coli*-derived CpMK2 showed the basal level of kinase activity indicating the gene product is a catalytically active protein kinase. The *cpmk2* disruption resulted in several, but not all, hypovirulence-associated phenotypic changes such as reduced pigmentation, conidiation and hypovirulence. No growth defect of the *cpmk2*-null mutant was observed on EP complete broth, but partial growth defect was appeared on PDAmb plate. A molecular symptom of the down-regulation of mating pheromone gene *Mf2/1* was also observed in the *cpmk2*-null mutant. Virulence assay indicated that the

disruption of the *cpmk2* resulted in a reduced canker area as more severe as that of hypovirulent strain. These results suggest that CpMK2 could be related to pathogenecity

G-83. Cloning and hypoviral regulation of HOG1 homologue, CpMK1, from *Cryphonectria parasitica*. Seung-Moon Park[1], Eun-Sil Choi[1], Myoung-Ju Kim[1], Byeongjin Cha[2], Moon-Sik Yang[1], and Dae-Hyuk Kim[1]. [1]Institute for Molecular Biology and Genetics, Chonbuk National University, Dukjindong 664-14, Chonju, Chonbuk 561-756, Korea, [2]Dept. of Agricultural Biology, Chungbuk National University, Cheongju, Chungbuk 361-763, Korea

We cloned the gene, *cpmk1*, encoding a mitogen-activated protein kinse (MAPK) of *Cryphonectria parasitica*. The sequence comparision of *cpmk1* shows the highest homology to *osm1*, a *hog*1-homologue from *Magneporthe grisea* and reveals that it belongs to the yeast stress-activated protein kinase (YSAPK) subfamily. The deduced protein product had a predicted mass of 40.9 kDa and a pI of 5.36. The *E. coli*-derived CpMK1 showed the basal level of kinase activity indicating the gene product is a catalytically active protein kinase. Kinase assay using cell-free extract and heterologously expressed CpMK1 suggested that the CpMK1 pathway was specifically affected in a hyperosmotic condition by the presence of hypovirus CHV1. No growth defect of the *cpmk1*-null mutant was observed on standard culture conditions whereas the *cpmk1*-null mutant was not able to grow on the high osmotic condition indicating that the *cpmk1* functionally belonged to the subfamily of YSAPK. Moreover, the virus-infected hypovirulent UEP1 strain, compared with the growth of virus-free isogenic strain EP155/2, also exhibited the growth inhibition on a high osmotic condition, which is an additional indicative of viral regulation of *cpmk1*. In addition to the osmosensitivity, the *cpmk1* disruption resulted in several, but not all, hypovirulence-associated phenotypic changes such as reduced pigmentation, conidiation, and laccase production on a tannic acid plate. A molecular symptom of the down-regulation of hydrophobin, cryparin, gene was also observed in the *cpmk1*-null mutant. However, comparing with a hypovirulent strain, the *cpmk1*-null mutant displayed an opposite expression pattern of mating pheromone gene *Mf2/1*. Virulence assay indicated that the disruption of the *cpmk1* resulted in a reduced canker area, but not as severe as that of hypovirulent strain.

G-84. Isolation and characterization of *lac3* encoding inducible laccase from *Cryphonectria parasitica*. Hea-Jong Chung, Seung-Moon Park, Moon-Sik Yang, and Dae-Hyuk Kim. Institute for Molecular Biology and Genetics, Chonbuk National University, Dukjindong 664-14, Chonju, Chonbuk 561-756, Korea

The chestnut blight fungus, *Cryphonectria parasitica*, and its hypovirus are a useful model system to study the mechanisms of hypoviral infection. We cloned a novel gene, *lac3*, encoding inducible laccase from a genomic library of *C. parasitica* using a 800 bp of probe which isolated by PCR with degenerate primers based on the copper binding region I and II of fungal laccase. We also cloned the corresponding cDNA by RT-PCR. The *lac3* gene contained six introns, and the transcription initiation site was

207 bp upstream of the start codon. The deduced amino acid sequence consisted of a putative 18 amino acid leader peptide and a mature protein with an estimated molecular mass of 60.3 kDa containing four copper binding regions. A molecular symptom of the *lac3* gene was observed by northern blot analysis, and the results appeared that the lac3 was specifically induced by tannic acid and expressed maximally at 24 hours of induction time. Moreover, *lac3* was down-regulated by the hypovirulence-causing dsRNA virus, CHV1.

G-85. Transformation of the filamentous fungi *Cryphonectria parasitica* using *Agrobacterium tumefaciens*. Eun-Ju Hong, Seung-Moon Park, Moon-Sik Yang, and Dae-Hyuk Kim. Institute for Molecular Biology and Genetics, Chonbuk National University, Dukjindong 664-14, Chonju, Chonbuk 561-756, Korea

Since it is known that *Agrobacterium tumefaciens*, which has long been used to transform of plants, can transfer the T-DNA to yeast *Saccharomyces cerevisiae* during tumourigenesis, variety of fungi were subjected to transformation to improve their transformation frequency. We here report the successful *A. tumefaciens*-mediated transformation (ATMT) of filamentous fungus *Cryphonectria parasitica*, the causal agent for chestut blight. Transfer of the binary vector pBIN9-Hg, containing the bacterial hygromycin B phosphotransferase gene under the control of the *Aspergillus nidulans trpC* promoter and terminator as a selectable marker, led to the selection of 800-1000 hygromycin B-resistant transformants per 1 x 106 or 1 x 107 conidia of *C.parasitica*. All transformants tested remained mitotically stable maintaining their hygromycin B phosphotransferase gene. Genomic Southern blot and PCR analysis appeared that over 70% of the transformants contained a single T-DNA insert per fungal genome. Considering the efficiency and flexibility of ATMT, this technique offers a highly efficient means for chrarcterizing those genes important for the pathogenicity of *C. parasitica*.

G-86. *Pseudomonas chlororaphis* O6 induces plant disease resistance by activating ethylene-dependent signal pathway. A. J. Anderson[1], K. Y. Yang[1], C. M. Ryu[2], B. H. Cho[3], and Y. C. Kim[3]. [1]Dept. Biology, Utah State University, Logan, UT 84322-5305, USA, [2]Dept. Entomology and Plant Pathology, Auburn University, Auburn, Alabama 36849, USA, [3]Agricultral Plant Stress Research Center, Chonnam National University, Gwangju 500-757, Korea

An aggressive plant root colonizer, *P. chlororaphis* O6 produces several antimicrobial secondary metabolites, such as phenazines and protease, and induces disease resistance against foliar plant pathogens, such as *Erwinia carotovora* causing soft-rot disease and *Pseudomonas syringae* causing wild-fire disease. Two different signal pathways, salicylic acid (SA) dependent and jasmonic acid/ethylene (JA/ethylene) dependent pathways, are known for induced systemic resistance (ISR) by different rhizobacteria. In this work, the signal transduction pathway involved in ISR activity by *P. chlororaphis O6* was determined by northern hybridization with several molecular markers for SA or JA/ethylene pathway and by using several tobacco transgenic lines

defected SA or JA/ethylene pathway. Hybridization of RNA extracted from leaf tissues of tobacco that colonized by O6, displayed increasing accumulation of transcripts to probes for the tobacco defense genes PR-1g (JA/ethylene pathway indicator) in seedlings. No accumulations were observed with the PR-1a probe (SA pathway indicator). In suspension cultured cells, both O6 and sensor kinase GacS mutant induced increasing transcript accumulation of HMGR and PR-1g but not Lox nor PR-1a. ISR activity of *P. chlororaphis* O6 differed in tobacco transgenic lines deficient different signal transduction pathway in disease resistance. Loss of the SA pathway in the NahG tobacco had no effect on protection, but loss of the ethylene pathway eliminated the protection against *E. carotovora*. These results indicated that *P. chlororaphis* O6 may induce plant defense connected to the ethylene pathway.

G-87. A sensor kinase GacS of *Pseudomonas chlororaphis* O6 are involved in induced systemic resistance (ISR). A. J. Anderson[1], C. M. Ryu[2], B. Y. Kang[3], B. H. Cho[3], and Y. C. Kim[3]. [1]Dept. Biology, Utah State University, Logan, UT 84322-5305, USA, [2]Dept. Entomology and Plant Pathology, Auburn University, Auburn, Alabama 36849, USA, [3]Agricultral Plant Stress Research Center, Chonnam National University, Gwangju 500-757, Korea

Root colonization by *P. chlororaphis* O6 induces foliar resistance to *Pseudomonas syringae* pv. tabaci and *Erwinia carotovora* subsp. carotovora SCC1 in tobacco. GacS/GacA two component sensor kinase system is a key regulator in production of antifungal compounds and/or virulence factors in other plant associated bacteria. To examine the role of *gacS* gene in triggering plant defenses, we created GacS mutants and tested ISR activity against *P. syringae* pv. tabaci and *E. carotovora* subsp. carotovora. The GacS knock-out mutant was deficient in productions of phenazine, acyl-homoserine lactones and extracellular protease. Unlike wild-type O6, the responses to *Erwinia* and *Pseudomonas* pathogens differed for plants colonized by GacS mutant. GacS mutant protected tobacco at the same level as the wild-type against the wild-fire disease, but the mutant was ineffective against the soft rot disease. The ISR activity of GacS mutant against the soft rot disease was restored as wild type level in the tobacco expressing hexanoyl homoserine lactone. These results indicate that root colonization by *P. chlororaphis* O6 induces disease resistance against foliar pathogens. The global regulator protein kinase GacS regulates the expression of traits that differentially result in protection for two diseases, the soft-rot disease and the wild-fire disease. Induction of resistance appears to involve traits in the bacterium that are regulated by acyl-homoserine lactone.

G-88. A sensor kinase *gacS* gene of *Pseudomonas chlororaphis* O6 involved in induced systemic resistance against abiotic and biotic stresses, but not in root colonization and oxidative stress. Kang, B. R.[1], Yang, K. Y.[2], Cho, B. H.[1], and Kim, Y. C.[1] [1]Agricultural Plant Stress Research Center, Chonnam National University, Gwangju 500-757, Korea, [2]Department of Biology, Utah State University, Logan, Utah 84322-5305, USA

A root-associating bacterium, *Psedomonas chlororaphis* O6, has the ability to

inhibit fungal pathogens by producing secondary metabolites, to confer induced systemic resistance to cucumber against leaf spot disease causing by *Corynespora cassiicola*, and to induce drought resistance. To investigate the role of a sensor kinase *gacS* on biological properties of *P. chlororaphis* O6, we isolated *gacS* mutants by marker exchange mutagenesis. The *gacS*mutant did not produce phenazine, protease, and homoserin lactone. The ability of induced disease resistance and drought resistance of *P. chlororaphis* O6 was abolished in *gacS* mutant. However, sensor kinase *gacS* gene of *P. chlororaphis* O6 is not essential for colonization on cucumber root and on survival under oxidative stresses. In Gel Kinase(MAP-kinase) assay was conducted to compare kinase activity between WT and *gacS* mutant. WT and complemented mutant showed about kinase activity of 80kD band, while *gacS* mutant at 45 kD.

G-89. Identification of cucumber genes involved in induced systemic resistance by root colonization of a nonpathogenic rhizobacterium *Pseudomonas chlororaphis* O6. M. KIM, Y. C. Kim, and B. H. Cho. Agicultural Plant Stress Research Center and Applied Plant Science Division, Chonnam National University, Gwangju 500-757, Korea

Plants can acquire systemic resistance to a broad spectrum of pathogens after treatment with nonpathogenic root-colonizing rhizobacteria. Root colonization of *P. chlororaphis* O6 on cucumber induced disease resistance against a foliar disease, target leaf spot caused by *Corynespora cassiicola*. To identify the cucumber genes involved in induced systemic resistance (ISR) by O6, we employed a subtractive hybridization method using mRNAs extracted from *cassiicola*-inoculated cucumber leaves with and without O6 root colonization. We isolated several cDNA clones that were induced in cucumber leaves upon root colonization of O6. Northern hybridization study reveal that galactinol synthase and raffinose synthase gene expressions may be involved in ISR by O6 against foliar target leaf spot disease in cucumber.

G-90. Analysis of expressed sequence tags during rice/*Xanthomonas oryzae* pv. *oryzae* interactions. Sang-Ryeol Park, Dool-Yi Kim, Hae-Sook Song, Duk-Ju Hwang, Tae-Ho Kim, Seung-Joo Go, and Myung-Ok Byun. Division of Molecular Physiology, National Institute of Agricultural Biotechnology, RDA, Suwon 441-707, Korea

Expressed sequence tag (EST) analysis was applied to identify rice genes involved in defense responses against infection by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), the causal agent of bacterial leaf blight (BLB), is the most destructive bacterial disease in rice (*Oryza sativa* L.). A cDNA library was constructed from rice leaves (cv. Dongjin) infected with *Xoo* strain 10331. A total of 963 clones were sequenced, 752 non-redundant ESTs were generated from these clones. The sequences of 533 (70%) ESTs showed significantly high homology in deduced amino acid sequences with other sequences deposited in databases. Among the genes with assigned functions, 37% were involved in metabolism, 12% in rescue and defence, and 11% in protein destination transport, 18% in cellular organization aqnd biogenesis, 12% in signal transduction and 10% in transcription. Further characterization of the genes represented in this study is

useful not only an aid in unraveling the mechanisms of pathogenicity of *Xoo* 10331 but also the defense responses of rice.

G-91. Mutational analysis of HpaG, a harpin-like protein elicitor, from *Xanthomonas axonopodis* pv. *glycines*. Eunkyung Jeon, Jung-Gun Kim, Chang-Hyuk Yoo, Jonghee Oh, and Ingyu Hwang. School of Agricultural Biotechnology, Seoul National University, Suwon 441-744, Korea.

HpaG is a harpin-like protein elicitor identified from *Xanthomonas axonopodis* pv. *glycines*. We have determined the critical amino acid residues important for hypersensitive response (HR) elicitation of the protein by deletion and site-directed mutagenesis. From the serial deletion analyses, we found that the amino-terminal 67 amino acids were sufficient for inducing HR in tobacco plants. We then mutagenized the plasmid clone carrying *hpaG* by error-prone PCR and site-directed mutagenesis. We obtained forty six mutants, including three nonsense mutants, thirty five single missense mutants, and eight double missense mutants. Three nonsense mutants and L39A, L43P, I47A, Q52A substitutions abolished the activity. On the other hand, seven single missense mutants and three double missense mutants showed reduced activity, and the other mutants had the same HR elicitation activity as the wild-type. These results indicate that residues between K37 and G67 have important roles in HR elicitation in tobacco plants.

G-92. Transient expression of two different bacterial elicitor genes, *hpa*1 and *hrm*A, in the plant cell. M. S. CHOI[1], N. C. Baek[2], D. S. Ra[1], and S. Heu[1]. [1]Plant Pathology Division, National Institute of Agricultural Science and Technology, Suwon, Korea, [2]Department of College of Agricultural and life science, Seoul National University, Suwon, Korea

The *hrp* genes encode type Ⅲ secretory pathways and are required by many phytopathogenic bacteria to elicit a hypersensitive response(HR) in nonhost or resistant host plants and for pathogenesis on susceptible hosts. Genes encoding type III secretion systems are present on pathogenicity island (Pai) of plant pathogenic bacteria. Genes encoding effectors such as Harpins of *Pseudomonas syringae* and PopA of *Ralstonia solanacearum* secreted by the type III systems are commonly linked to the type III system genes. We have been isolated Pai region from very destructive rice pathogen, *Xanthomonas oryzae* pv. *oryzae*. Up to now we been sequenced 53 kb DNA region carrying more than 30 open reading frames (ORFs) including genes for type III secretion system. Among those, a *pop*A-like gene, *hpa*1 gene encoded a 13 kDa glycine-rich protein with a composition similar to those of Harpins and PopA. Since we believe that the Hpa1 is a kind of a elicitor, we expressed transiently *hpa*1 gene in nonhost plants with *uid*A encoded beta-glucuronidase and *hrm*A isolated from *Pseudomonas syringae* pv. *syringae* Pss61 under the control of 35s promoter. Transient expression of *hpa*1 and *hrm*A in soybean and chinese cabbage induced HR that become visible beginning 3 days after particle bombardment. Control bombardments with *uid*A showed no HR.