Disease Resistance (F61-F71)

F-61. Structural defense mechanisms of pepper against *Colletotrichum gloeosporioides*. Kwang-Hyung Kim and Young Ho Kim. School of Agricultural Biotechnology, Seoul National University, Suwon, Korea

Fruit tissues of pepper plants, *Capsicum annuum* cv. Jejujaerae (susceptible) and *C. baccatum* cv. PBC80 (resistant) inoculated with anthracnose pathogen, *Colletotrichum gloeosporioides*, were examined by light and electron microscopy to compare cytological differences between compatible and incompatible interactions of peppers inoculated with the fungus. The susceptible pepper, *C. annuum* cv. Jejujaerae became extensively colonized by the fungus, leading to degeneration of cytoplasm, distortion of chloroplasts, and disruption of host cell walls. On the other hand, resistant fruit tissues were colonized little by the fungus. Resistant pepper fruits infected with *C. gloeosporioides* were characterized by apoptotic nuclear modifications showing heterochromatic nuclei, increased formation of electron-dense particles, and cuticle layer thickening into the epidermal cells. Programmed cell death (PCD) in the incompatible interaction was confirmed by using fluorescence microscopy, terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labelling (TUNEL), and annexin-V-FLUOS staining.

F-62. Different ultra-structures in the leaves of cucumber plants untreated or pre-treated with DL-3-aminobutyric acid after inoculation with *Colletotrichum orbiculare*. Y.C. JEUN[1] and E. W. PARK[2]. [1]Department of Plant Resource Science, Cheju National University, Jeju, 690-756, Korea, [2]National Instrumentation Center for Environmental Management (NICEM), Seoul National University, Suwon, 441-744, Korea.

DL-3-aminobutyric acid (BABA) has been well known as one of the effective chemical activators inducing resistance against various plant diseases. Our previous studies revealed that the pre-treatment with BABA in cucumber plants caused a remarkable reduction of lesion numbers after inoculation with *Colletotrichum orbiculare*. In this study ultra-structures of the cucumber leaves expressing systemic acquired resistance by BABA were compared with those in the untreated plants after inoculation with the anthracnose pathogen. In the leaves of untreated plants the fungal hyphae were broadly found e.g. in the epidermal, palisade and spongy parenchyma, bundle sheath cells and intercellular spaces at 5 days after inoculation with the pathogen. Most of the organs in the hyphae as well as in the plant cells were apparent and did not be degenerated, indicating a compatible interaction between the plant and the parasite. In contrasts, in the leaves of BABA pre-treated plants the growth of most hyphae was restricted to the epidermal cell layer at 5 days after the challenge inoculation. Most of the hyphae were electron dense or their organs were degenerated. The cell walls of some plant cells become thick at the site adjacent to the intercellular hyphae, indicating a direct defense reaction of the plant cells against the fungal attack. The organs of infected plant cells were rapidly destroyed and sometimes a number of vesicles were formed. Furthermore, hypersensitive reaction (HR) of the epidermal cells was often observed in which the intracellular hyphae were also degenerated. Based on the

observations, it is suggested that BABA causes the enhancement of defense mechanisms in the cucumber plants such as cell wall apposition or HR against the invasion of *C. orbiculare*.

F-63. Cytological and biochemical analyses of systemic resistance induced by soil bacteria against cucumber anthracnose. Eui Nam Kim[1], Min Sun Kwack[1], Yong-Chull Jeun[2], and Ki Deok Kim[1]. [1]Division of Bioscience and Technology, Korea University, Seoul 136-701, Korea, [2]Department of Plant Resource Science, Cheju National University, Cheju 690-756, Korea

Cytological and biochemical responses of cucumber to *Colletotrichum orbiculare* were examined in relation with induced systemic resistance (ISR) by soil bacteria. Cucumber anthracnose was significantly (P=0.05) reduced regardless of plant stages when 100ìg/ml (1ml per g of potting mixtures) of DL-â-amino-n-butyric acid (BABA), as an inducer for systemic resistance, were soil-drenched in pots. This BABA used as a positive control throughout these experiments. ISR-inducing strains, GC-B19, MM-B22, and RC-B65 significantly(P=0.05) reduced disease leaf area (%), but not number of lesions, at least two of three experiments compared with buffer-treated controls. BABA treatment also reduced both disease leaf area and number of lesions while a negative control strain PK-B09 did not. In addition, ISR-inducing strains and BABA treatment could produce hypersensitive responses and reductions of conidial germination and appressorium formation while caused increase of peroxidase activity 5 days after inoculation in cucumber plants. Therefore, these results may indicate that quantitative reductions of infection structures of the fungus and peroxidase accumulations by the ISR-inducing strains occurred in significant levels on the treated cucumber leaves that led to reduction of disease symptoms.

F-64. Mechanism associated with extracellular polysaccharides-mediated systemic resistance in tobacco against cucumber mosaic virus. Y.-S. Bae[1], K.-S. Park[1], C.-M, Ryu[2], and J. W. Kloepper[2]. [1]Plant Pathology Division, National Institute of Agricultural Science and Technology, Suwon, 441-707, Korea, [2]Department of Plant Pathology, Auburn University, Auburn AL, USA.

Extracellular polysaccharides (EPS) partially purified from the culture of *Burkholderia gladioli* strain IN26 have been reported to induce systemic resistance in cucumber and tobacco against fungal and bacterial plant pathogens. To determine the mechanism associated with EPS-mediated systemic resistance, cell suspension of IN26 or partially purified EPS was pre-infiltrated on one-month-old seedlings of wild-types (cv Xanthi-nc and Samsun-nn) or transgenic tobacco plants, which were genetically modified with NahG gene, encoding salicylate hydroxylase, or Tetr18 gene, encoding a defective ethylene receptor. Then, cucumber mosaic virus was challenged on those tobacco plants after 7 days of infiltration. Development of mosaic symptom was observed for 4 weeks. All treatments showed mosaic symptom after 7 days of challenge. However, the pre-infiltration of IN26 or EPS resulted in the reduction of mosaic symptoms compared to that of untreated control in wild-type and transgenic tobacco plants. This result indicates that mechanism associated with EPS-mediated ISR may

involve in different signal pathways other than salicylic acid or ethylene signaling pathway.

F-65. Rapid differential regulation of novel rice (*Oryza sativa* L.) MAP kinase kinase kinase gene, *OsEDR1* in response to signaling components and environmental stresses. Jung-A Kim[1], Keon-Seon Han[1], Joo-Hee Lee[1], Choong-Hyo Yun[2], Sunggi Heu[2], Yong-Hwan Lee[3], and Nam-Soo Jwa[1]. [1]Department of Molecular Biology, College of Natural Science, Sejong University, Seoul 143-747, Korea, [2]National Institute of Agricultural Science and Technology, Rural Development Administration, Suwon 441-707, Korea, [3]School of Agricultural Biotechnology, Seoul National University, Suwon 441-744, Korea

In search for major components on upstream of signal transduction pathway, a MAPKKK (mitogen-activated protein kinase kinase kinase) of rice (*Oryza sativa*L. cv. Nipponbare), is identified and designated as *OsEDR1*. The gene is cloned from jasmonic acid (JA) treated rice seedling leaves cDNA library and is a single copy. This gene has a 35 amino acid Arginine rich region at N-terminal region and 13 amino acids with a predicted serine/threonine protein kinase active-site signature with molecular mass of 113046.13 and a pI of 9.03. *OsEDR1* did show constitutive expression in leaves and was slightly induced within 15 min in response to wounding by cut. Using in vitro system, we show that the expression of *OsEDR1* mRNA was potently enhanced within 15 min by signaling molecules, protein phosphatase inhibitors, fungal elicitor, heavy metals, high salt and sucrose, and drought. *OsEDR1* expression was further modulated by co-application of JA, salicylic acid (SA) and ethylene, and required *de novo* synthesized protein factor(s) in its transient regulation. Moreover, high (37°C) and low temperatures (12°C) and environmental pollutants, ozone and sulfur dioxide differentially regulate the *OsEDR1* mRNA accumulation in leaves of intact plants. Present results demonstrating dramatic transcriptional and transient regulation of the *OsEDR1* expression by diverse biotic/abiotic stresses, a first report for any rice MAPKKK to date, suggest a role for *OsEDR1* in rice defense/stress response pathways.

F-66. Identification and characterization of novel ankyrin repeat gene from rice (*Oryza sativa* L.). Keon-Seon Han[1], Jung-A Kim[1], Yong-Hwan Lee[2], and Nam-Soo Jwa[1]. [1]Department of Molecular Biology, Sejong University, 98 Gunja-dong, Gwangjin-gu, Seoul 143-747, Korea, [2]School of Agricultural Biotechnology, Seoul National University, Suwon 441-744, Korea

Ankyrin repeats are randomly repeated modules of about 33 amino acids. They occur in a large number of functionally diverse proteins mainly from eukaryotes. Many ankyrin repeat regions are known to function as protein-protein interaction domains. The rice ankyrin repeat clone *OsAnk* was cloned and identified as consisting of 198 amino acids having 20kDa molecular weight with 4 ankyrin repeats. It was homologous to arabidopsis putative ankyrin repeat gene. This gene was transcriptionally regulated after inoculation of rice blast fungus *Magnaporthe grisea*. The *OsAnk* was not induced in the normal condition, but slightly induced after inoculation of blast fungus. The transgenic rices of overexpression of *OsAnk* showed enhanced resistance to rice blast disease,

while antisense transgenic rices were all susceptible to infection with virulent rice blast pathogen. The mechanism of the *OsAnk* gene through overexpression and antisense constructs is underway by selected transgenic lines. The function of the *OsAnk* in defense mechanism of rice to blast pathogen *Magnaporthe grisea* needs to be elucidated.

F-67. A pepper (*Capsicum annuum*) gene encoding esterase that expressed highly in a resistance response has a role for enhanced disease resistance in transgenic *Arabidopsis* plants. Moon Kyung Ko[1], Woong Bae Jeon[2], Young Soon Kim[1], Kwan Sang Kim[3], Hyun Hwa Lee[2], Hyo Hyoun Seo[1], Kyung Hoan Im[4], and Boung-Jun Oh[2]. [1]Kumho Life and Environmental Science Laboratory, Korea Kumho Petrochemical Co., Ltd., 1 Oryong-Dong, Buk-Gu, Gwangju 500-712, Korea, [2]Plant & Microbe Co., LTD., Biotechnology Industrialization Center, 880-4 Ansan-Ri, Naju-Si, Jeonnam 520-811, Korea, PhytoCareTech Co., LTD., Chonnam National University Business Incubator, 300 Yongbong-Dong, Buk-Gu, Gwangju 500-757, Korea, [4]Dept. of Biology, University of Incheon, 177 Dohwa-Dong, Nam-Gu, Incheon 402-749, Korea.

These authors contributed equally to this work. Ripe fruits of pepper (*Capsicum annuum*) showed disease resistance to the anthracnose fungus, *Colletotrichum gloeosporioides*. However, unripe-mature fruits showed susceptible response. Deduced amino acid sequence of *PepEST* cDNA showed homologous to both esterases and lipases, and contained -HGGGF- and -GXSXG- motifs and a catalytic triad. Expression of the *PepEST* gene was fruit-specific in response to *C. gloeosporioides*, and upregulated by wounding or jasmonic acid treatment during ripening. Accumulation of the *PepEST* mRNA and protein was higher in the resistant interaction than in the susceptible interaction. Immunolocalization showed that the PepEST accumulation was localized in epidermal and cortical cell layers in the resistant interaction. However, in the susceptible interaction, the PepEST was rarely localized even in the epidermal cell layers. Inhibition of PepEST activity by a specific inhibitor of serine hydrolase revealed that a serine residue was essential for the esterase enzyme activity. These results suggest that the esterase activity of the PepEST is involved in the defense mechanism of the ripe fruit of pepper against *C. gloeosporioides*. Transgenic *Arabidopsis* plants with over-expression of the *PepEST* showed enhanced disease resistance to *Alternaria brassicicola* and constitutive expression of *PR-2* and *PR-4* genes, suggesting that the PepEST play a role for defense mechanisms in *Arabidopsis* by mediating *PR* gene expression.

F-68. An *Arabidodpsis* ubiquitin-conjugating enzyme gene that negatively controls jasmonate/ethylene regulated defense. Hyun Hwa Lee[1], Woong Bae Jeon[1], Young Soon Kim[2], Kwang Sang Kim[3], Nam Ju Hong[1], and Boung-Jun Oh[1]. [1]Plant & Microbe Co., LTD., Biotechnology Industrialization Center, 880-4 Ansan-Ri, Naju-Si, Jeonnam 520-811, Korea, [2]Kumho Life and Environmental Science Laboratory, Korea Kumho Petrochemical Co., Ltd., 1 Oryong-dong, Buk-gu, Gwangju 500-712, Korea, [3]PhytoCareTech Co., LTD., Chonnam National University Business Incubator, 300 Yongbong-Dong, Buk-Gu, Gwangju 500-757, Korea.

Defense signaling pathway is initiated through recognition of stress signal or pathogen-derived molecules called elicitors, which trigger the biosynthesis of one or a combination of hormone-like compounds. The defense related hormones are salicylic acid (SA), jasmonic acid (JA)/ethylene (ET). To understand the mechanisms of disease resistance, *Arabidopsis* mutants with altered defense response have been identified. SA-dependent pathway is well characterized by defense related mutants. However, mutants related with JA/ET signaling pathway in plant defense were not well characterized. Both plant hormones JA and ET are concomitantly required for disease resistance to a necrotrophic fungus *Alternaria brassicicola* that demonstrated activation of plant defensin gene *PDF1.2* in *Arabidopsis*. A T-DNA tagged *Arabidopsis* mutant *eda1* that en! hanced di sease resistance against *A. brassicicola* and had constitutive expression of *PDF1.2* was isolated. *EDA1* gene encodes a protein with homol ogy to ubiquitin-conjugating enzyme (E2). A recombinant EDA1 protein formed a thioester linkage with ubiquitin. Gene expression and mutant complementation demonstrated that *EDA1* negatively regulated disease resistance, suggesting that ubiquitin-mediated proteolysis is involved in JA/ET regulated defense mechanism.

F-69. Characterization of a rice deletion mutant gained resistance to rice blast.

S.-W. LEE[1], C. H. Lee[2], K. S. Jang[1], Y. H. Choi[1], H. K. Lim[1], J.-C. Kim[1], G. J. Choi[1], I. C. Shin[4], J. E. Choi[2], S.-.N Ahn[2], H. T. Kim[3], and K. Y. Cho[1]
[1]Agrochemical Screening Team, Korea Research Institute of Chemical Technology, Taejon 305-600, Korea, [2]Department of Agriculture, Chung-Nam National University, Taejon 305-764, [3]Department of Agricultural Biology, Chungbuk National University, Cheongju 361-763, Korea, [4]Plant Breeding by Using Radiation Mutation, Korea

Atomic Energy Research Institute, Taejon 305-600 Cultivation of disease resistant rice cultivar has been an effective way for the safe production of rice. To identify rice genes involved in disease resistance and defense response, a blast-susceptible rice cultivar, Sanghaehyanghyella, was subject to mutagenesis by gamma-ray irradiation. Eight mutant lines were selected at the M3 stage and some of them showed different disease and insect response. One of the mutant lines, SHM-11, was identified as a gain-of-function mutant to rice blast and rice brown plant hopper, but susceptible to bacterial leaf blight. The mutant line SHM-11 was resistant to the tested four *Magnaporthe grisea* races, such as KI-1113a, KI-313, KI-409, and KJ-201, while the wild type was susceptible to all tested races. In order to dissect the mechanism of the gain of function in the mutant line, we take a PCR-based subtractive hybridization approach to identify the deleted chromosomal loci. The genomic subtraction and subsequent Southern hybridization analysis identified two loci disrupted in the mutant lines among the tested 7 subtracted clones. Both mutations were located in rice chromosome 10. Deleted portion and length in the mutant SHM-11 will be determined by comparing the loci between wild type and the SHM-11. Further identification of deleted loci in the mutant line SHM-11 is in progress.

F-70. Candidate defense response and resistance genes from rice, barley and

maize and their association with quantitative blast resistance loci in rice. S. W. LEE[1], S. S. Han[2], S. H. Choi[2], D. S. Ra[1], and J. E. Leach[3]. [1]Plant Pathology Div., NIAST, Suwon, 441-707, Korea, [2]NCES, Suwon, 441-100, Korea, [3]Dept. Plant Pathology, Kansas State University, Manhattan, KS 66506 USA.

A candidate gene approach was applied to a population of 164 recombinant inbred (RI) lines derived from a cross between a japonica/indica hybrid derivative (Milyang 23) and a japonica variety (Gihobyeo) to determine association between defense response genes and resistance gene analogs (RGA) with quantitative trait loci (QTL) for rice blast resistance. The parents (Milyang 23, Gihobyeo) were screened for polymorphism with candidate gene probes. Of 157 genes tested with five restriction enzyme digests, 96 were polymorphic. Of 96 polymorphic markers, RGA (29) and defense response genes (2) from rice, barley and maize were mapped on the rice chromosomes and analyzed for their association with blast QTL. All markers produced single loci and were well distributed among all the chromosomes except on 12, where no markers were observed. Based on diseased leaf area, and lesion density, size and number using Korean and Philippine blast fungal isolates, a total of nine putative QTL were identified on chromosomes 1, 2, 4, 6, 7, 10 and 12. Five RGA markers, rNBS84, rNBS81, rNBS5, rNBS72 and r8, were associated with three different QTL. These preliminary results demonstrate that the candidate gene approach can be used to establish the association between candidate genes and functionality.

F-71. Ozone-induced resistance to rice blast fungus, *Magnaporthe grisea* in rice plant. M. Kim[1], Y. J. Koh[2], and J.-S. Hur[3]. [1]Dept. Biology, [2]Dept. Applied Biology, [3]Dept. Environmental Education, Sunchon National University, Sunchon, 540-742, Korea.

The phytotoxic air pollutant ozone spontaneously generates reactive oxygen species in the leaf apoplast and induces defense reactions that significantly overlap with pathogen and other oxidative stress responses. Increasingly, concerns have been raised regarding the interactions between ozone and pathogens and plant competition. This study was conducted to improve our understanding of rice plant responsese to ozone in the presense of ricr blast pathogen. Ozone exposure (80 nl l-1, 6h day -1, 6 days) to healthy plants clearly induced expression of pathogensis-related (PR) gene of *PR1* and *PR8*. Whereas rice blast disease was severely devloped on unfunigated rice leaves after inoculation of conidia at the termination of ozone exposure, the leaves predisposed by ozone-induced PR gene expression retarded disease developement and alleviated disease indicence and severity. This result implies that ozone can suppress rice blast developement by induction of defense reactions in rice plants. In addition to direct damage to the fungal pathogen, ozone-induced modification of rice plant responses to the pathogen would weaken the rice blast potential for disease development in the field.