

## 진균 병학 (생리, 생태)

**FE-01 A Novel Wound-Induced Chitin-Binding Protein cDNA Gene from Pepper: Its Isolation and Differential Expression in Pepper Tissues Treated with Pathogens, Ethephon or Methyl Jasmonate.** Sung Chul Lee<sup>1</sup>, Young Jin Kim<sup>2</sup> and Byung Kook Hwang.<sup>1</sup> <sup>1</sup>Korea University, Anam Dong 5 ga 1, Seoul, Korea 136-701. <sup>2</sup>Boyce Thomson institute, Cornell University, Tower Road, Ithaca, NY14850, USA.

A chitin-binding protein (CBP) cDNA (*CACBPI*) was isolated from a cDNA library of pepper (*Capsicum annuum* L.) leaves infected with *Xanthomonas campestris* pv. *vesicatoria*. The deduced amino acid sequence of the *CACBPI* gene which has chitin-binding domain and hinge region shares a high level of identity with CBP sequences from tomato, potato and tobacco. The *CACBPI* gene was organ-specifically regulated in pepper plants, and differentially induced during the compatible and incompatible interactions of pepper with *X. campestris* pv. *vesicatoria* or *Phytophthora capsici*. Expression of the *CACBPI* gene was rapidly induced in the incompatible interactions upon pathogen infection. Transcripts of the *CACBPI* gene was highly inducible in the leaves of matured pepper plants by *Colletotrichum coccodes* infection. *In situ* hybridization results showed that *CACBPI* mRNA was expressed in the phloem area of vascular bundles in *C. coccodes*-infected leaf tissues, especially strongly in the incompatible interaction. The pathogen-inducible *CACBPI* gene was also strongly induced and accumulated in pepper leaves by ethephon, methyl jasmonate or wounding. These results suggest that ethylene and jasmonate may act as signal transduction pathways of the CBP gene induction during the pepper defense- or pathogenesis-related plant responses.

**FE-02 Application of a Computer Program on Evaluation of Cucumber Anthracnose by *Colletotrichum orbiculare*.** Ki Deok Kim, Min Sun Kwack, and Yun Sung Kim. Department of Agricultural Biology, Korea University, Seoul 136-701, Korea.

Cucumber anthracnose by *Colletotrichum orbiculare* was evaluated by a computer program, Matrox inspector 2.2 for its application of disease assessment. About 23-day-old cucumber plants (cv. Baeknokdadagi) were inoculated with various concentrations of conidia ( $10^3$ ,  $10^4$ ,  $10^5$ ,  $5 \times 10^5$ ,  $10^6$ , and  $10^7$ /ml) of *C. orbiculare*. Second and third leaves were scanned and evaluated for disease severity and number of lesions per leaf area ( $\text{cm}^2$ ) by the computer program 5 and 7 days after inoculation. Manual evaluation was also conducted for the comparison of the disease. Increased diseases were found as increased concentrations of conidia and  $10^6$  conidia/ml were determined as the optimum inoculum density for disease induction. More diseases in third leaves were found 7 than 5 days after inoculation, but disease assessment for second leaves by the program 7 days after inoculation was not feasible due to severe disease. Disease severity ( $r=0.764$  and  $0.800$ ) and number of lesions per leaf area ( $r=0.894$  and  $0.908$ ) of second and third leaves evaluated by the program 5 days after inoculation were correlated at  $P=0.0001$  with manual evaluation. This computer program can use stored image files and determine total and diseased leaf areas expressed as pixels that could not be done with manual disease assessment. Therefore, assessment of anthracnose by the computer program could be an effective and accurate method to evaluate cucumber anthracnose by *C. orbiculare*.

**FE-03 Comparison of detection specificity among *P. infestans* isolates with different mating type and metalaxyl sensitivity.** Youn Su Lee<sup>1</sup>, K. S. Kim<sup>1</sup>, and B. S. Kim<sup>2</sup>. <sup>1</sup>Div. of Applied Plant Sciences, College of Agriculture and Life Sciences, Kangwon National University, Chuncheon, Korea 200-092. <sup>2</sup>College of Life Sciences, Kangnung National University, Kangnung, Korea

Seventy-three *P. infestans* isolates were isolated from infected tubers, stems, and leaves from Kangwon-do in 1999 (10 isolates) and 2000(63 isolates). Their mating type and metalaxyl sensitivity were determined. Among the tested isolates, four isolates were found to be mating type A2, and the rest were A1 type. In metalaxyl sensitivity tests, 61 isolates were sensitive, 8 isolates were mild resistant, and four isolates were resistant. Metalaxyl sensitivity was not related with mating type, at all. Some A1s showed sensitivity and other A1s showed mild resistance or resistance. A2 types showed the same results. With these basic results, we tested the detection specificity among *P. infestans* isolates with different mating type and metalaxyl sensitivity.

**FE-04 Genetic relationships among the different mating types of *F. moniliforme* isolates and other *Fusarium* spp.** Youn Su Lee and H. J. Kim. Div. of Applied Plant Sciences, College of Agriculture and Life Sciences, Kangwon National University, Chuncheon, Korea 200-092.

*F. moniliforme* (*Gibberella fujikuroi*) is a primary pathogen of maize, but can also cause disease in many other crops. In this study, we evaluated *F. moniliforme* isolates from corn and *Allium thunbergii*, and other *Fusarium* species in Korea with AFLPs (Amplified Fragment Length Polymorphisms) to identify the relationships among different *F. moniliforme* mating types, and other *Fusarium* species. As a result, we selected six specifically amplified marker for the six different mating types in *F. moniliforme*, and isolated one common band specifically amplified for all the *Fusarium* species tested. In the AFLP analysis, different mating types of *F. moniliforme* showed similarities of 0.98-0.85 compared with other *Fusarium* species. *F. moniliforme* isolated from *Allium thunbergii* was shown to closely related to E+ mating type.

**FE-05 Ozone-Induced Suppression of Conidial Germination of the Rice Blast Pathogen *Magnaporthe grisea*.** Jae-Seoun Hur<sup>1</sup>, Ki Woo Kim<sup>2</sup> and Eun Woo Park<sup>2</sup>. <sup>1</sup>Sunchon National University, Suncheon, Korea 540-742. jshur1@sunchon.ac.kr <sup>2</sup>Seoul National University, Suwon, Korea 441-744.

Given the potential for concurrent impact of rice blast and ozone on rice plants during summer, investigation on the interaction between ozone and *M. grisea* is needed. Thus, we examined the direct effects of acute ozone on conidial germination of *M. grisea*. Acute exposure of 200 nl l<sup>-1</sup> ozone for 8 h significantly reduced conidial germination on water agar. The conidia produced under ozone exposure (200 nl l<sup>-1</sup> for 8 h per day for 3 days) showed enhanced formation of lipid bodies inside the cell and significant reduction in appressoria formation on a hydrophobic film. The ozone exposure also induced significant increase in polyamines content of the conidia. Recently, polyamines were suggested to inhibit the appressorium formation of *M. grisea* by regulating the level of intracellular cyclic AMP. Polyamines also play important roles in preventing cells from oxidative stress by eliminating active oxidants induced by ozone. Therefore, ozone-induced elevation of intracellular level of polyamines was implied to be involved in suppression of appressorium formation of the exposed conidia. This study suggested that the acute ozone would severely inhibit appressoria formation during conidial germination of the pathogen, resulting in decrease in rice blast development in the field during summer.

**FE-06 Method of Diagnosis on *Phytophthora* and *Pythium* Species by Potato Slice in Horticultural soil and crops.** Jung Sup Lee, Jong Han Park, Kyoung Suk Han, Hyeong Yong Jeon, Young Moon Choi Div. of Horticultural Environment, National Horticultural Research Institute, RDA, Suwon, Korea 441-440.

To development simple and precise diagnostic method to detect *Phytophthora* root rot fungus from tomato green house soil, potato slice were used as a baiting material. The standard selective medium for isolating Pythiaceae fungi, P<sub>5</sub>ARP(H)(cornmeal agar amended with 5mg/L Pimaricin, 250mg/L Ampicillin, 10mg/L rifampicin and 100mg/L PCNB), was compared with a modified method, potato slice(pimaricin added to 5mg/L and 250mg/L ampicillin+10mg/L rifampicin). On the tomato slice surface, grayish-white mycellia were abundantly observed 3 days after treatment. This new technique needed only 3~4 days for diagnosis of *P. capsici* with over 80% accuracy. Hymexazol(40mg/L) was added(i.e., P<sub>5</sub>ARPH and Potato slice) to suppress unwanted *Pythium* colonies for direct enumeration of *Phytophthora* from soil. Potato slice, when compared with P<sub>5</sub>ARP(H), 1) detected more germinable propagules of *P. capsici*, *P. cambivora* and *Pythium* species from naturally infested soils, 2) suppressed contaminating bacteria much better in the test soil, 3) supported mycelium growth of 2 isolates of *Phytophthora* and 2 isolates of *Pythium* equally well. PCNB was beneficial for reducing or eliminating background mycoflora on soil dilution plates. Hymexazol added to P<sub>5</sub>ARP and potato slice greatly inhibited *P. cambivora* and drastically limited the radial growth of one isolate of *P. capsici*. Potato slice is an simple and precise diagnostic method for isolating *Phytophthora* and *Pythium* species from plant tissue and soil.

**FE-07 Identification and distribution of new retrotransposon *Osr1* in rice.** Nam-soo Jwa, Sook-Yong Park, Chan-Ho Park, and Yong-Hwan Lee<sup>1</sup>. <sup>1</sup>School of Agricultural Biotechnology, Seoul National University, Suwon 441-744, Korea.

A new rice retrotransposon (*Osr1*) was detected on susceptible Pi-b open reading frame of rice cultivar Nipponbare. *Osr1* has total 6392bp-nucleotide sequence including 975bp LTRs on both ends. *Osr1* has 81% nucleotide identity with wheat *Tar1* retrotransposon on its reverse transcriptase sequences. They were up-regulated by inoculation of *Magnaporthe grisea*. Nucleotide divergence was noted among the individual LTR footprint clones by point mutations or small deletion on their sequences. Genomic Southern hybridization with the LTR clearly differentiates between Japonica, Indica and wild type rice cultivars by RFLP. The population density of the *Osr1* is significantly low in genome of wild type rice cultivars. The divergence on RFLP pattern was more obvious among Indica rice cultivars than that of Japonica cultivars from Korea, China and Japan. These data suggest that Japonica cultivars which have been cultivated in temperate zones including Korea, China and Japan have high homogeneity on their genetic background. This *Osr1* might play an important role in the evolution of rice and can be used as a marker for linkage analysis.

**FE-08 Population structure and genetic variability of the rice blast fungus in Korea.** <sup>1</sup>Sook-Young Park, <sup>1</sup>Nam-Soo Jwa, <sup>2</sup>Seokchan Kang, <sup>1</sup>Yong-Hwan Lee. <sup>1</sup>School of Agricultural Biotechnology, Seoul National University, Suwon 441-744, Korea; <sup>2</sup> Dept. Plant Pathology, Penn State University, University Park, PA 16802

Two hundred eighty three isolates of *Magnaporthe grisea*, sampled from four provinces of Korea, were analyzed using MGR586 as a probe and grouped based on their genetic relatedness. Nine DNA fingerprint groups were identified at the 75% similarity level (MG1-MG9). MG1 was a predominant group, consisting 80% of the population, and distributed in all regions sampled. To test the hypothesis that genetically distinct groups of strains preferentially infect either the leaf, neck or panicle of rice plants, respectively, additional isolates, collected from lesions on leaves, neck, and panicle in one field, were analyzed. Genetic lineages of the isolates from neck and panicle were distinct from those isolated from leaf tissues. We also compared the genetic variability of the fungus during successive rounds of asexual reproduction on artificial medium up to 10th generation. No clear difference was observed among asexual progenies.

**FE-09 Isolation and characterization of a cDNA encoding class III chitinase in rice.** Chanho Park, Nam-Soo Jwa, Sook-Young Park, Soonok Kim and Yong-Hwan Lee<sup>1</sup>. <sup>1</sup>School of Agricultural Biotechnology and Research Center for New Biomaterials in Agriculture, Seoul National University, Suwon 441-744, Korea.

A cDNA encoding *Oschib1* (AF296279) was isolated from cDNA library of *Oryza sativa* cv. Nipponbare. The 1,040bp of full length *Oschib1* clone contains an open reading frame of 861 nucleotides encoding 286 amino acid residue, of which pI is 5.06. GeneBank search showed that *Oschib1* exists on chromosome 10 and another Class III chitinase with 89% identity (designated to *Oschib2*) separated by about 10kbp. The deduced amino acid sequence of *Oschib1* has a high level of similarity with Class IIIb chitinase of *Gladiolus gandavensis* (46%) and *Tulipa bakeri* (49%). Southern blot analysis of genomic DNA indicates that two copy of *Oschib1* exists in the rice genome. In RFLP with *Oschib1*, there was polymorphism between japonica and indica cultivar. The expression of *Oschib1* was induced by 50mM H<sub>2</sub>O<sub>2</sub>, 50uM CuSO<sub>4</sub>, senescence and *M. grisea*. The High level of expression of *Oschib1* was detected at 72 hours after inoculation in compatible interaction and at 48 hour at incompatible. Thus, these data support that *Oschib1* might be involved in defense against *M. grisea* and be classified into PR-8 group.

**FE-10 Molecular characterization of the cDNA encoding an acidic isoform of PR-1 protein in rice.** Soonok Kim, Il-Pyung Ahn, Chanho Park, Sang-Geun Park, Sook-Young Park, Nam-Soo Jwa, and Yong-Hwan Lee<sup>1</sup> <sup>1</sup>School of Agricultural Biotechnology and Research Center for New Bio-Materials in Agriculture, Seoul National University, Suwon 441-744, Korea.

Rice cDNAs encoding an acidic type of pathogenesis-related protein -1 (PR-1a) were cloned and characterized. The deduced PR-1a protein was consisted of 168 amino acid residues including 24 hydrophobic signal sequences at the N-terminus. Predicted molecular weight of PR-1a was 15,728 Da with theoretical pI of 4.5, indicating an acidic protein. PR-1a showed high homology to an acidic PR-1 of *Zea mays* (74%), and to a previously identified basic type PR-1 of rice (64%). Both rice PR-1 and PR-1a genes were found to exist as small multigene families by Southern blot hybridization analyses. The PR-1 mRNA was accumulated only in leaves, while that of PR-1a was accumulated through entire plant at low level. Expression of both PR-1 genes was induced by infection of the rice blast fungus, *Magnaporthe grisea*, or the bacterial leaf blight pathogen, *Xanthomonas oryzae* pv. *oryzae*, and the treatment of benzo (1,2,3) thiadiazole-7-carbothioic acid S-ethyl ester, H<sub>2</sub>O<sub>2</sub>, or CuSO<sub>4</sub>. Expression of both PR-1 genes was more highly and rapidly induced in an incompatible than in a compatible interaction in rice *M. grisea* interactions.

**FE-11 Purple pigmentation of *Colletotrichum* sp. associated with dopa oxidase activity of a novel tyrosinase.** Soon Seop Hwang<sup>1</sup>, Sang Ho Park<sup>2,3</sup>, Myung Yong Shim<sup>4</sup>, Chang Won Choi<sup>1,2\*</sup>.  
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*Colletotrichum* sp. A1/6-3 developed purple pigmentation with whitish margin, showing strongly dopa oxidase-positive bands in the DOPA staining gel. In native gel electrophoresis, two isoforms were clearly time-dependent, designating as fungal tyrosinase 1 (FT1) and fungal tyrosinase 2 (FT2) and having same molecular masses about the 100 kDa. The intensity of enzyme activity and melanin contents depends on the composition of the growth medium, showing higher values in PDB than in minimal or medium of low nutrition media. The influence of amino acid supplemented into malt medium, on the production of dopa oxidase activity and melanin contents in *Colletotrichum* sp. A1/6-3, was various depending on the supplemented amino acid. Melanin production-inhibiting agents such as tricyclazole and kojic acid also inhibit the dopa oxidase activity, producing low melanin contents, but both of which were enhanced when cultures grown in PDB with added Cu<sup>++</sup>. A positive relationship was evident between dopa oxidase activity and melanin contents. Two isoforms of dopa oxidase have distinct patterns of binding metal ligands. 1,10-phenanthroline inhibits the dopa oxidase activity of FT1, but FT2 was resistant to phenanthroline. The localization of dopa oxidase activity in *Colletotrichum* sp. A1/6-3 is positive in the hyphae and spores. The medium pH also affected dopa oxidase activity, showing maximum peak activity at pH 5.6. When the medium pH was modified from pH 5.6 to a neutral pH 7.7, resulting in a lack of pigmentation. At basic pH 8.6, there was less pigmented with low enzyme activity. A1/6-3 became darker purple pigmented and had higher melanin contents when grown on more acidic media pH 3.5-5.6. In artificial inoculation, the pigmentation is partly associated with pathogenicity and melanin contents.

**FE-12 Effects of Silicon Application on Thickness of Epidermal Cell Wall of Rice Leaves.** Sang Gyu Kim, Ki Woo Kim, and Eun Woo Park. School of Agricultural Biotechnology, Seoul National University, Suwon 441-744, Korea.

Effects of silicon on thickness of epidermal cell wall of rice leaves were investigated using two rice cultivars, Hwasung and Jinmi, grown under a hydroponic culture system with Yoshida's nutrient solution containing 0, 50, 100, and 200 ppm of SiO<sub>2</sub>. Transmission electron micrographs of epidermal cell wall of leaves at the 4- and 8-leaf stages were obtained to measure cell wall thickness using an image analyzer. Cell wall of Hwasung at the 8-leaf stage became thicker with silicon application than without it. However, no significant difference was observed from Hwasung at the 4-leaf stage. In the case of Jinmi, silicon applications resulted in significant increase in cell wall thickness at both leaf stages as compared with the untreated control. It is speculated that adult plant resistance to leaf blast may be partially explained by increase in thickness of epidermal cell wall due to silicon uptake by rice plants. Increase in SiO<sub>2</sub> application resulted in decrease in blast development on rice plants grown under the hydroponic culture system.

**FE-13 Variations in Disease Resistance of Chestnut Varieties Against Chestnut Blight Fungus, *Cryphonectria parasitica*.** Sang-Hyun Lee<sup>1</sup>, Jong Kyu Lee<sup>2</sup>, Myoung-Soo Hwang<sup>3</sup>, and Byung-Ju Moon<sup>4</sup> <sup>1</sup>Department of Forest Biology and <sup>3</sup>Special Purpose Trees, Forest Research Institute, Seoul, 130-012, <sup>2</sup>Department of Forest Resources Protection, Kangwon National University, Chunchon, 200-701, <sup>4</sup>Faculty of Natural Resources and Life Sciences, Dong-A University, Pusan, 604-714, Korea.

Chestnut blight fungus, *Cryphonectria parasitica*, was isolated from the infected twigs, branches, stems, and trunks collected from 65 survey sites located in 9 provinces throughout south Korea. Ninety six virulent (V) strains and one hypovirulent (H) strains were identified and being kept in the deep freezer. For the selection and breeding of varieties resistant to *C. parasitica*, disease resistance of 25 chestnut varieties including Chuk-pa, Ok-kwang, Yi-pyung, Eun-gi, Eun-san, and so on were assayed by a rapid, simple, and reliable method. A typical, fast growing virulent strain (V-9) was cultured on PDA for 7 days, and then an agar plug with mycelium was inoculated on the center of inner surface of each bark tissue section. The variety Man-jeok, was shown to be the most resistant one among the tested varieties with the necrotic area of 0.45 cm<sup>2</sup>, while the varieties of Eun-san, Kwangju-joyul, and Eaton were ranged from 2.71 to 2.61 cm<sup>2</sup>, which indicate that these varieties are relatively susceptible to the fungus. Abundant pycnidial formation was observed on both inner and outer bark tissue sections 3 weeks after inoculation.

**FE-14 Changes of Chemicals Response of *Phytophthora infestans* Causing Potato Late Blight in Korea.** Ryu Kyoung Yul, Cho Il Chan, Kim Jeom Soon, Hahm Young Il, and Kim Byoung Sup<sup>1</sup> Crop Division, National Alpine Agricultural Experiment Station, RDA, Pyungchang, Kangwon, Korea 232-950. <sup>2</sup>Dept. of Horticulture, Kangnung National University, Kangnung, Kangwon, Korea 210-702.

Isolates of *Phytophthora infestans* collected from the major potato cultivation areas in Korea were examined for the mating type distribution and chemicals response of pathogen. All of Kangwon isolates were A1 mating type and Cheju isolates were A2 mating type, respectively. The pattern of mating type distribution of isolates in 2000 was showed similar pattern to the one in 1999. Response to 10µg/ml metalaxyl concentration of Kangwon and Kyungnam isolates were sensitive reaction but Cheju isolates were resistance reaction. At 6 days after artificial inoculation with 10<sup>3</sup> zoospores/ml concentration, Cheju isolates were developed more severe water soaking lesion area on leaves than Kangwon isolates. Comparing with 8 promising potato breeding lines and 4 potato cultivars on late blight resistance, two lines, Daekwan 1-69 and Daekwan 1-70 were showed highly resistance against Kangwon and Cheju isolates. Several chemicals were tested for the field control of late blight, among them, azoxystrobin, dimethomorph, ethaboxam and metalaxyl were significantly effective to late blight in Daekwallryung area.

**FE-15 Measurement of Intracellular cAMP and Proteome analysis of Newly synthesized Proteins with 2-DE during Appressorium formation in *Magnaporthe grisea*.** Sun Tae Kim<sup>†</sup>, Yu Sin Jang<sup>†</sup>, Kyu Seong Cho, Seok Yu, Sang Gon Kim, Chul Hyung Chu, and Kyu Young Kang\* Department of Agricultural Chemistry, PMBBRC, Gyeongsang National University, Chinju660-701, Korea <sup>†</sup> Equal contribution, \*Corresponding author

Rice blast fungus, *Magnaporthe grisea*, differentiates into an infection structure called the appressorium in order to penetrate their host. During appressorium formation in germinating *Magnaporthe grisea* induced by the surface wax of its host, the molecular and signal transduction events such as accumulation of cAMP, activation of protein kinases are triggered in the fungus. To study molecular events triggered by the rice leaf surface wax contact, we monitored the changes in intracellular cAMP level and protein patterns during appressorium formation on non-wax coated condition and wax-coated condition. Levels of cAMP were measured on wax-coated plate and IBMX-treated plate. Basal concentration of cAMP were 758 fmol/8.3 × 10<sup>4</sup> conidia. Maximum concentration of cAMP was 1192, 1033 fmol/ 8.3 × 10<sup>4</sup> conidia on wax-coated plate and IBMX-treated plate, respectively, at 4hr after incubation. More than 700 polypeptides were resolved by 2-DE in both <sup>35</sup>S methionine labeled control and wax-treated samples. Total 30 induced or enhanced proteins were detected during appressorium formation. Newly induced and enhanced proteins were 21 and 9, respectively. These results suggest that appressorium formation was triggered by levels of intracellular cAMP and translation of these proteins triggered by rice leaf surface wax are associated with germination and appressorium formation during the germination processes.

**FE-16 Proteome Analysis by 2-DE of Rice Suspension Cell Treated with Rice Blast Fungus.** Kyu Seong Cho, Sun Tae Kim, Chul Hyung Chu, Sang Gon Kim, and Kyu Young Kang\* Department of Agricultural Chemistry, Gyeongsang National University, Chinju 660-701, Korea

We investigated response of rice suspension-cultured cell against a rice blast fungus, *M. grisea*, to study host regulated-response at the protein level. Proteins were extracted with PEG fractionation from suspension calli over time course of 24 and 48hr after rice blast fungus inoculation. In suspension cells co-inoculated with blast fungi, directly or indirectly by membrane separation, ten proteins were expressed at 24, 48hr after inoculation. Upon treatment of suspension-cultured cell with rice blast fungal elicitor from mycelium, nine out of twelve proteins from fungal pathogen induced proteins were identified. However, treatment of salicylic acid (SA, 5mM) or hydrogen peroxide(H<sub>2</sub>O<sub>2</sub>, 5mM) did not induced new proteins compared to those of control. Whereas, six proteins were induced by jasmonic acid (JA, 250 μM). Ten induced proteins were N-terminal sequenced from rice blast fungus spores-treated rice suspension cell. Five out of ten proteins were sequenced. Four proteins (No. 1, 2, 3 and 4) showed homology to probenazole-inducible protein (PBZ). Two of them (No. 6, 7) showed homology to salt stress related protein (Salt). The rest of three protein spots showed homology to pathogen-related protein (PR-10), isoflavone reductase (IFR) and novel protein, respectively. These results suggest that proteins expressed by fungal infection, elicitor, and JA treatment might be involved in defense mechanism in rice plant.



**FE-17 Genetic diversity of the small-spored *Alternaria* species based on the intergenic spacer region of the ribosomal DNA.** Byung Ryun Kim and Seung Hun Yu. Chungnam National University, 220 Gung-Dong, Taejon, Korea 305-764

Using PCR, we amplified the intergenic spacer (IGS) of the rDNA in four species of small-spored *Alternaria*; *A. gaisen*, *A. mali*, nonpathogenic *A. alternata* and *A. infectoria*. The primer pair amplified a DNA fragment from each of the isolates ranging in size from 2.0 to 2.5 Kb. Within a species, there were two or three types of polymorphism in the IGS region. Seven restriction endonucleases, *Dpn* I, *Hae*III, *Hha* I, *Msp* I, *Rsa* I, *Taq* I, and *Alu* I, generated polymorphic fragments and showed several IGS haplotypes. These haplotypes were not correlated with the species. Moreover, a IGS fragment generates different RFLP pattern. The results indicate that IGS variation, in terms of size or restriction pattern, was present within and among the species tested, and we interpret each combination of size and RFLP pattern as an IGS haplotype.

**FE-18 Comparison of nitrate nonutilizing mutants and vegetative compatibility groups of *Fusarium oxysporum* f. sp. *lycopersici* and *radicis-lycopersici* from Korea, Belgium, Israel and United Kingdom.** Sung Ho Kim, Sung Joon Yoo<sup>1</sup>, and Hong Gi Kim. Department of Agricultural Biology, Chungnam National University, Taejon, 305-764, Korea. <sup>1</sup>Institute of Agricultural Science, Chungnam National University, Taejon, 305-764, Korea.

Nitrate nonutilizing (*nit*) mutants and vegetative compatibility groups of *Fusarium oxysporum* ff. sp. *lycopersici* (FOL) and *radicis-lycopersici* (FORL) from Korea were compared to those of FORL from other countries. Twenty-four isolates of FOL from Korea, FORL from Korea and other countries were examined for analysis of vegetative compatibility group (VCG). Nitrate nonutilizing (*nit*) mutants were produced by cultures on the minimal medium (MM) containing chlorate (CLM). All of the 24 isolates irregularly produced chlorate-resistant sectors on CLM. The frequency at which chlorate-resistant sectors were produced depended upon the strains and the amount of chlorate in the MM. Isolates of FORL formed sporodochia during culture at 27°C, but isolates of FOL didn't form them. Isolates of FOL produced *nit*-mutants on 1.5% CLM, but isolates of FORL produced them over 1.5% CLM. Isolates of FOL produced *nit*-mutants on 1.5% CLM. Isolates of FORL, however, in dissimilar with FOL, could produce them over 1.5% CLM. It was found out with MM that about 60% and 35% among thin-growth mutant colonies, which isolates in 2.0% and 2.5% CLM were *nit*-mutants, respectively. In addition, the vegetative compatibility between Korean *nit*-mutants and foreign *nit*-mutants was analyzed.

**FE-19 Correlation among some factors affecting the genetic structure of *Phytophthora capsici* population.** Jeong Young Song, Moon Nam and Hong Gi Kim. Department of Agricultural Biology, Chungnam National University, Taejon, 305-764, Korea.

In order to analyze the genetic diversity and variation factors of the red-pepper pathogen, *Phytophthora capsici* population totally 973 isolates were collected from 75 fields throughout the country from 1995 to 1998. Mycological characteristics as mating type, pathogenicity, and metalaxyl sensitivity of the isolates were examined. Genetic variation of the pathogen population on DNA level and inheritance of some genetic characteristics through the sexual reproduction were investigated using molecular techniques. It was summarized that the major factors affecting the genetic diversity in *Phytophthora capsici* population in Korea were sexual reproduction among compatible isolates showing different degrees of pathogenicity, metalaxyl resistance, and mating types. Continuous adaptation and mutation of the pathogens to the chemicals could be the basis of the genetic diversity of this fungal population.

**FE-20 Transformation of *Scrophularia buergeriana* and selection of resistant lines against fungal infections.** J. D. Lim, C. Y. Yu, H. J. Kim, and Youn Su Lee. Div. of Applied Plant Sciences, College of Agriculture and Life Sciences, Kangwon National University, Chuncheon, Korea 200-092.

AFP(4 lines) or PAP(3 lines) transformed *Scrophularia buergeriana* were inoculated with *Fusarium oxysporum* and *Collectotrichum* sp. in pot tests for the selection of fungal disease resistant lines. As a result, control plants showed high sensitivity against *Fusarium oxysporum*, whereas transformed lines AFP-S-10-2-1 and PAP-P-10-8 showed resistance against the pathogen *F. oxysporum* infection. Line AFP-P-10-8 produced new leaves after the inoculations. In pathogenicity tests with *Collectotrichum* sp., control plants showed mild sensitivity, whereas transformed plants showed resistance against the infection of *Collectotrichum* sp.

**FE-21 Induction of mutation of *Rehmannia glutinosa* with EMS and selection of *F. oxysporum* resistant lines.** H. J. Kim, J. D. Lim, C. Y. Yu, and Y. S. Lee. Div. of Applied Plant Sciences, College of Agriculture and Life Sciences, Kangwon National University, Chuncheon, Korea 200-092.

Mutation of *Rehmannia glutinosa* were induced with EMS, and *F. oxysporum* resistant mutant lines were selected through pathogenicity tests, both in pots and in the field. In pot tests, all the control plants were sensitive to the infection, whereas EMS treated plants showed resistance. The concentrations of EMS treated also showed differences in their reactions. Plants treated with higher concentrations of EMS showed higher sensitivity on the *F. oxysporum* infection. In the field tests, control plants also showed very low level of resistance, whereas, the mutagen treated plants showed higher level of resistance. Also, the fresh weights of the root systems were higher in the mutagen treated plants compared to those of control plants.

**FE-22 The Stimulation and Inhibitory Effects of Various Sugar Analogues on Spore Germination of *Botrytis cinerea*.** Jin Sook Ahn, Eugene Rha, Chang-Won Lee, Young Ryun Chung and Jae Won Kim. Division of Life Science, Institute of Natural Sciences, Gyeongsang National University, Chinju 660-701, Korea

*Botrytis cinerea* is a well known plant pathogenic fungus causing gray mould. This fungus invade plant cell walls to macerate plant tissues. However, the pathogenic factors of this fungus are not clear yet, although hydrogen peroxide and several enzymes such as polygalacturonase, laccase, and cutinase were mentioned as putative pathogenic factors. The infection of *B. cinerea* in laboratories requires the presence of sugars in the inoculum for the successive infection, which is thought to be an unique phenomena in this organism. In this study, we examined the effect of sugars on the spore germination of *B. cinerea* to know whether the stimulation effect of sugars on the infection of the fungus is due to germination efficiency of spore or not. The spore was not germinated in pure water and Czapeck medium in the absence of sugar upto 18 hours. The rate of spore germination was below 0.1%. Under the same circumstances, spore germination was observed within 7-24 hours in the presence of sugars such as glucose, xylose, galactose, mannose, fructose, cellobiose, trehalose, maltose, and lactose, sucrose, raffinose, sorbitol, inulin, pectin(1%) showing germination rate of above 90%. However, the germination rate was reduced in the presence of myo-inositol (3%), which could not stimulate infection of *B. cinerea*. Interestingly, the germination of spore requires stereospecific isomers, that is, spore was not germinated in the presence of D-arabinose, but germinated by L-arabinose. Furthermore, 2-deoxy-D-glucose and 2-deoxy-D-galactose did not stimulate spore germination. Specific inhibition of germination by 2-deoxy-D-glucose was observed even when glucose was present in incubation medium. These results suggest that there are specific machineries to detect sugar or environmental signals for the germination of spore in *B. cinerea*.

**FE-23 Transgenic *Arabidopsis* Plants Expressing a Pepper Esterase Show Enhanced Disease Resistance Against *Alternaria brassicicola*.** Moon Kyung Ko, Young Soon Kim, Kwang Sang Kim, Jeon Woong Bae, Hyun Hwa Lee and Boung-Jun Oh. Kumho Life and Environmental Science Laboratory, Korea Kumho Petrochemical Co., Ltd., 1 Oryong-Dong, Puk-Ku, Kwangju, Korea 500-712.

The ripe fruit of pepper (*Capsicum annuum*) showed an incompatible interaction with anthracnose fungus, *Colletotrichum gloeosporioides*. However, the unripe-mature fruit showed a compatible interaction with the fungus. We isolated and characterized a *PepEST* gene highly expressed in the incompatible interaction by using mRNA differential display. The amino acid sequence of the encoded protein showed limited but significant sequence homology to both lipases and esterases of mammals and prokaryotic organisms, and contains the -HGGGF- and -GX SXG- motifs and the catalytic triads. The accumulation of *PepEST* mRNA and PepEST protein was higher in the incompatible interaction than in compatible interaction from 48 h after inoculation (HAI). Immunolocalization showed that the accumulation of PepEST was localized in the epidermal and cortical cell layers in the incompatible interaction from 48 and 72 HAI. However, in the compatible interaction, the PepEST was rarely localized even in the epidermal cell layers. The recombinant protein expressed in *Escherichia coli* degraded *p*-nitrophenylbutyrate, as a general substrate for carboxylesterases. To reveal if the *PepEST* is involved in plant defense mechanism, we have made transgenic *Arabidopsis* containing 35S viral promoter and *PepEST*. Several lines among T3 generation transgenics tested showed disease resistance to *Alternaria brassicicola*. The transgenics expressed the *PepEST* as well as other defense-related genes.

**FE-24 An Improved Method for Estimating Number of Resting Spores of *Plasmodiophora brassicae* in Soil.** Choong Sik Lee, Sang-Bum Lee, and Choong-Hoe Kim, Institute of Agricultural Science and Technology, RDA, Suwon 441-707 Korea.

A new procedure was developed to improve the detection limit of the method for estimating the number of resting spores of *Plasmodiophora brassicae* in soil. The procedure is as follows: the infested soil (10 g) was filtered with 2 mm diameter sieve to remove coarse soil particles. The soil was shaken with 0.05% (v/v) tween-80 solution for two hours and filtered through sieves of 150, 270, and 500 meshes by turns. Each sample of 2 ml of the filtrate was mixed with 40  $\mu$ l of 1.0 N NaOH. The suspension was layered on a 40%(w/v) sucrose solution for 10 min and the upper fraction (1.5 ml) was recovered. This process settled much of mineral particles leaving most resting spores in the upper fraction. However, without NaOH treatment, most of resting spores settled down with the mineral particles into the sucrose solution. The recovered fraction (1 ml) was centrifuged (3,000 rpm, 10 min) and the pellet was resuspended in 100  $\mu$ l of freshly prepared fluorescence staining solution [1:1 mixture of Calcofluor White M2R (100  $\mu$ g/ml) and ethidium bromide (100  $\mu$ g/ml) in 40% aqueous glycerol]. Resting spores in the suspension were counted under an epifluorescence microscope. Glycerol in staining solution prevented rapid drying of the specimen and the motion of spores under observation. The recovery efficiency of this method ranged from 88.8 to 99.6% in loam soil and clay loam soil containing  $10^4$ ,  $10^5$ , and  $10^6$  spores/g, respectively. This improved method could be applied to infested soils containing  $10^3$  spores/g soil or less.

**FE-25 Yield Loss Caused by Neck Blast at Different Infection Times.** Hong-Sik Shim, Seong-Sook Han. Plant Pathology Div., National Institute of Agricultural Science and Technology, RDA, Suwon 441-707 Korea.

This experiment was conducted at Icheon experimental paddy fields to analyze the yield loss of rice by neck blast when infected at different times after panicle emergence. Thirty-day-old seedlings of two rice cultivars(cv), Jinmiby eo(early maturing cv) and Chucheongbyeo(mid-late maturing cv) were transplanted on May 20. Insecticides were only applied when needed. Disease incidence was recorded at 3-day interval until 40 days after heading. More than 100 plants at each investigation were marked and later harvested for yield assay. The first neck blast symptom was developed six days after heading on Jinmiby eo, and 14 days after heading on Chucheongbyeo. Percentage of yield loss by neck blast was calculated by comparison with the grain weight of healthy panicles. Yield loss of Jinmiby eo amounted 83.9% and 44.3% when diseased at six and 30 days after heading, respectively. The regression equation for the yield loss by neck blast in Jinmiby eo was  $y = -1.9729X + 71.878$  ( $R^2=0.9764$ ), where X = days after heading and Y = yield loss. Yield loss of Chucheongbyeo was 64.9% and 29.1% when diseased at 13 and 39 days after heading, respectively. The regression equation for the yield loss estimation by neck blast in Chucheongbyeo was  $y = -1.6563X + 60.363$  ( $R^2=0.9441$ ). This indicates that late infection of neck blast resulted in a significant yield loss and therefore, disease control strategy for the late infection of neck blast should be considered significantly.

**FE-26 An analysis for Rapid Increase of Rice Blast Fungus Race KI-1117(a) in Korea.**

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This experiment was carried out to analyze the recent epidemic of rice blast in southern provinces in 1999. Incidence of leaf blast and panicle blast in 1999 was 1.5 and 2.9 times greater than that in 1998, respectively. During this time cultivation area of those rice cultivars, Daesan, Ilmi and Dongan bred from Milyang 95 as a recurrent parent increased over the country from 11% in 1998 to 29.4% in 1999. More than 67 % of total paddy fields in Cheonnam province was grown with those cultivars in 1999. Race population of *Pyricularia grisea* in fields had been changed from predominance of KJ-301 to KI-1117 in 1999. Distribution ratio of the KI-1117 race in 1998 in the southern provinces was 1.7%, but increased to 30% in 1999. Cultivars Daesan, Ilmi and Dongan possessive several resistance genes have shown wide spectrum of resistance to many races including KJ-301, but susceptible to race KI-1117. The KI-1117 race could be further classified into two sub-races, KI-1117 and KI-1117a based on virulence to Daesan; both races were virulent to Ilmi and Dongan, but only KI-1117a was virulent to Daesan. This indicates that rice blast epidemic in southern provinces in 1999 was resulted from the breakdown of the resistance of Daesan, Ilmi and Dongan by the rapid increase of virulent race KI-1117 and KI-1117a.