II. 진균병학(생리, 저항성)

B-01. Fungicide Resistance and Pathogenic Aggressiveness within *Colletotrichum gloeosporioides* Causing Anthracnose of the *Capsicum annuum*. Myoung Yong Shim[1], Sun-Sub Hwang[2], Chang Won Choi[2] and Sang Ho Park[3]. [1]Chungnam Agricultural Research & Extension Services, Taejon, Korea 305-313, [2]Department of Biology, Pai Chai University, Taejon, Korea 302-735, [3]Korea Research Institute of Bioscience and Biotechnology, Taejon, Korea 305-600.

Thirty seven isolates of *Colletotrichum gloeosporioides* were collected from pepper fields mostly in Chungnam in 1998. Mycelial growth response of each isolate on PDA amended with fungicides, mancozeb, fenarimol, and chlorothalonil was investigated. Also spore germination of each isolate was examined on water agar amended with the fungicides. EC_{50} value of each isolate of mancozeb, fenarimol, and chlorothalonil was calculated and compared. Pathogenic aggressiveness of these isolates were investigated by inoculating PDA agar plug cut from growing margin of the isolates onto green and red pepper.

B-02. Occurrence of Metalaxyl-Resistant Isolates of *Pythium* spp. Isolated from Turfgrasses at Golf Courses in Korea. Jin Won Kim and Eun Woo Park. Division of Applied Biology and Chemistry, College of Agriculture and Life Sciences, Seoul National University, Suwon 441-744, Korea.

Of 125 isolates collected from 35 golf courses in Korea, sensitivity of 44 isolates of *Pythium* species to metalaxyl was determined on Difco corn meal agar with various concentrations of metalaxyl. The isolates were able to be categorized into the sensitive and resistant groups based on hyphal growth measured in terms of colony diameters on the medium with 1.0 and 10.0 ㎍ a. i. /㎖. When compared with hyphal growth on the medium without metalaxyl, hyphal growth of the sensitive group which included 31 isolates was inhibited by 66~98% on the medium with 1.0 ㎍ a. i. /㎖, whereas the resistant group which included 13 isolates grew well and the hyphal growth was inhibited only by 6~26%. When 10.0 ㎍ a. i. /㎖ of metalaxyl was included in the medium, hyphal growth of the sensitive and resistant groups were inhibited by 82~99% and 27~47%, respectively. Control effects of metalaxyl were determined by applying metalaxyl to creeping bentgrass in pots, which had been inoculated with 4 and 3 isolates of sensitive and resistant isolates of *P. graminicola*, respectively. The minimum concentration of metalaxyl to control metalaxyl-sensitive isolates was 6.25 ㎍ a. i. /㎖, whereas the metalaxyl-resistant isolates could not be controlled with 12.50 ㎍ a. i. /㎖ of metalaxyl.

B-03. Field Evaluation of Adlay Cultivars or Lines for Resistance to Leaf Blight Caused by *Bipolaris coicis*. Seog Won Chang[1,2] and Byung Kook Hwang[2]. [1]North Agricultural Research Station, Kyonggi-do ARES, Yonchon, Korea. [2]Department of Agricultural Biology, Korea University, Seoul, 136-701, Korea.

Leaf blight caused by *Bipolaris coicis* is one of the destructive diseases of adlay in Korea. To screen adlay cultivars or lines resistant to leaf blight, thirty adlay cultivars or lines which have been mainly used as breeding materials in Korea were tested in 1997 and 1998 in field plots of Yonchon. Leaf blight that

usually initiated on mid-June in the field first appeared typically on lower leaves and spread to leaves in the middle and upper portion of the canopy. The response of adlay cultivars or lines tested to leaf blight was quantitative rather than qualitative and varied greatly among cultivars or lines. Zukuba group showed lower disease severity, whereas Huksuk group had higher disease severity. We selected some adlay cultivars or lines resistant to leaf blight for use in our breeding program in field plots. The reactions of six adlay cultivars or ines to leaf blight at various growth stages of plants and yield loss associated with leaf blight were evaluated in the field. Leaf blight infection gradually increased with aging of plants in six cultivars or lines tested, although the level of resistance varied among cultivars or lines. Based on differences in percent diseased leaf area of whole plants, the relative ranks of adlay cultivars or lines in levels of resistance were Zukuba B15> Moozu> Yulmu 1> Yonchon> Limgae> Huksuk 2. Reduction of yield by leaf blight was less in resistant cultivars or lines than in susceptible cultivars or lines. Kernel weight and percent of ripend grains were the yield components greatly affected by *Bipolaris coicis*. The line Zukuba B15 which was highly resistant to leaf blight showed a stable yield potential than the other tested cultivar or lines.

B-04. Genetic Diversity of *Fusarium graminearum* in Production of 8-Ketotrichothecene Chemotypes.

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Trichothecenes, produced by *Fusarium graminearum*, are sesquiterpene epoxides that inhibit eukaryotic protein synthesis and thereby impair human and animal health. A genetic variability of *F. graminearum* within chemotypes of 8-ketotrichothecene was assessed by random amplified polymorphic DNA (RAPD) analysis. Fifty-three isolates of *F. graminearum* were obtained from corn and barley samples from 7 provinces in Korea during 1991-1994. Eight out of 120 oligonucleotide primers tested were finally selected after screening by random selection and bulked segregant analysis (BSA). A total of 50 band positions were scored (1/0) for the 8 primers. Genetic distances between each of the isolates were calculated, and cluster analysis was used to generate dendrogram by NTSYS-pc using the UPGMA method. More genetic variability was observed in DON-type than NIV-type isolates. Primer OPE16 generated 0.25kbp DNA fragment from DON-type isolates and 0.36kbp from NIV-type isolates. These fragments were cloned and sequenced. No homology was shown between two fragments. Two chemotypes could be separated by cluster analysis and showed polymorphism by genomic southern blot. These data indicate that RAPD with selected 8 primers could be used to identify chemotypes of trichothecenes.

B-05. Analysis of the Genetic Diversity Among *Colletotrichum gloeosporioides* Isolates Causing Pepper Anthracnose Using RAPD Markers.

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Randomly amplified polymorphic DNA was used to generate efficiently identifying DNA profiles for close related thirty seven isolates of *Colletotrichum gloeosporioides* causing anthrancnose of hot peppers.

The RAPD assays were carried out using random of 10 nucleotides in length with high G+C contents (70-80%). The genetically diverse RAPD profiles of genomic DNA among isolates, analyzed correlation with the degree of fungicide resistance. The markers produced for each isolate were applied to a phylogenetic analysis to infer genetic relatedness. Accurate identification of isolate will be useful in population genetics including identifying individuals and in future pathological studies.

B-06. Parasitic Characteristics of *Ampelomyces quisqualis* to *P*owdery Mildew Fungus of Cucumber.

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An isolate of the prospective hyperparasite, *Ampelomyces quisqualis* was selected for biological control of cucumber powdery mildew caused by *Sphaerotheca fuliginea.* Examination for process of the parasitism by scanning electron microscopy and light microscopy showed that conidia of AQ94013 germinated on conidia, conidiophores and hyphae of *Sphaerotheca fuliginea* at 4hr after inoculation. Appressorium-like structures developed and attached to hyphae of *S. fuliginea* at 17hr after inoculation. Hyphae of AQ94013 penetrated into hyphae of *S. fuliginea* at 24hr after inoculation. Pycnidia of AQ94013 were produced in the hyphae and the basal part of conidiophores of *S. fuliginea* at 44hr after inoculation. The pycnidia of AQ94013 matured at 48hr after inoculation, and conidia were discharged from the ostioles of the pycnidia at 52hr after inoculation. At the same time, hyphae and conidiophores of *S. fuliginea* were distorted and died.

B-07. Coexpression of Two Thionin Genes via Different Signal Transduction Pathways and *Colletotrichum gloeosporioides* Interactions.

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The anthracnose fungus, *Colletotrichum gloeosporioides*, interacts incompatibly with the ripe fruit of pepper (*Capsicum annuum*). It interacts compatibly with the unripe-mature fruit. We isolated two thionin genes, *PepThi* and *j1-1*, expressed in the incompatible interaction by using mRNA differential display method. Both thionin genes were developmentally regulated during fruit ripening, organ-specifically regulated, and differentially induced during the compatible and incompatible interactions. The expression of *PepThi* gene was rapidly and strongly induced in the incompatible-ripe fruits upon fungal infection. The fungal-inducible *PepThi* gene is highly inducible only in the unripe fruit by salicylic acid. In both unripe and ripe fruits, it was induced by wounding, but not by jasmonic acid. The expression of *j1-1* gene is enhanced in the unripe fruit by jasmonic acid, while suppressed in the ripe fruit. These results suggest that both thionin genes are induced via different signal transduction pathways during fruit ripening to protect the reproductive organs against biotic and abiotic stresses.

B-08. Molecular Cloning and Characterization of a Pathogen-induced Gene Encoding a Basic Class II Chitinase from *Capsicum annuum*. Jeum Kyu Hong[1] and Byung Kook Hwang[1]. [1]Department of Agricultural Biology, Korea University, 1 Anam-dong, Sungbuk-ku, Seoul 136-701, Korea.

A chitinase cDNA clone (designated *CAChi2*) was isolated from the cDNA library of pepper leaves infected with *Xanthomonas campestris* pv. *vesicatoria*. The 1004-bp full-length *CAChi2* cDNA encodes a basic chitinase with an N-terminal 24 amino acid signal peptide followed by a catalytic region. The mature protein has 253 amino acid residues with a predicted molecular mass of 28,768 Da and pI value of 9.39. An analysis of its sequence indicates that CAChi2 is class II chitinase, because it does not have chitin-binding domain and C-terminal extension sequence. The deduced amino acid sequence of CAChi2 has a high level of identity with class II chitinases from potato, tomato, tobacco and petunia. Southern analysis demonstrated that the CAChi2 chitinase is encoded by a single or two copy genes in pepper genome. Following *X. campestris* pv. *vesicatoria* or *Phytophthora capsici* infection, expressions of the *CAChi2* chitinase mRNA were markedly induced in the incompatible interaction, compared to those in the compatible interaction. *Colletotrichum coccodes* infection also induced *CAChi2* mRNA in pepper leaves during the lesion development. Treatment with ethephon resulted in a strong accumulation of the transcripts in the leaves, but benzothiadiazole induced a moderate level of the trascripts. In contrast, DL-β-amino-n-butyric acid, salicylic acid, methyl jasmonate were not effective in inducing *CAChi2* trascripts. The *CAChi2* mRNA was highly expressed in roots and flowers, but the expression was not detected in leaves, stems, and fruits, suggesting that constitutive expression of *CAChi2* gene is organ-specific in pepper plants.

B-09. A Viral Infection Upregulates Fungal Virulence in *Nectria radicicola*. Il-Pyung Ahn and Yong-Hwan Lee. Department of Agricultural Biology and RCNBMA, Seoul National University, Suwon, Korea 441-744.

Four distinct sizes of double-stranded RNA (dsRNA) molecules, 6.0, 5.0, 2.5, and 1.5 kbp, were detected from 24 out of 81 isolates of *Nectria radicicola* (anamorph: *Cylindrocarpon destructans*), the causal fungus of ginseng (*Panax ginseng*) root rot. They are present singly or in combinations. Curing tests of each dsRNA molecule suggested that the presence of 6.0 kbp dsRNA is responsible for increased virulence, sporulation ability, laccase activity, and pigmentation in this fungus. To understand the role of this dsRNA molecule further, 6.0 kbp dsRNA was reintroduced to cured isolates (dsRNA free) by hyphal anastomosis. Acquisition of this dsRNA molecule by dsRNA free isolates recovered all virulence-related phenotypes. Ultrastructural observation of mycelia by transmission electron microscope also supported the physiological changes by curing and reintroduction of this dsRNA molecule. These results are clear evidences that 6.0 kbp dsRNA upregulates fungal virulence in *N. radicicola* by demonstrating the cause and effect relationship. To characterize this 6.0 kbp dsRNA at molecular level, cDNA library was constructed. Sequencing of several cDNA clones revealed that this molecule harbors RNA-dependent RNA polymerase (RDRP) gene. Phylogenetic analysis of this gene to other RDRP genes indicated that this gene

is closely related to those of plant cryptic viruses. Although this dsRNA molecule is believed to be the genome of fungal virus, all efforts to detect typical virus particles were failed. We are in progress to elucidate the biochemical mechanisms how 6.0 kbp dsRNA upregulates fungal virulence in *N. radicicola*. Preliminary data suggested that this dsRNA molecule is involved in the regulation of cAMP-dependent protein kinase.

B-10. Molecular Cloning and Characterization of Calmodulin Gene in the Rice Blast Fungus. Yangseon Kim and Yong-Hwan Lee. Department of Agricultural Biology and RCNBMA, Seoul National university, Suwon, Korea 441-744.

Magnaporthe grisea is a heterothallic ascomycete and the causal agent of rice blast. Rice blast is one of the most destructive factors of rice production and occurs in most rice-growing regions of the world. This fungus differentiates a specialized cell, an appressorium, a dome-shaped and melanized structure for infection of its host. Environmental cues and signal transduction pathways that induce appressorium formation have been elucidated extensively during last a few years. Calmodulin is a low molecular weight, calcium binding protein that modulates calcium-related signaling pathway in eukaryotes. The gene encoding calmodulin was cloned from *Magnaporthe grisea* using a nested polymerase chain reaction (PCR) based strategy. PCR primers designed after highly conserved regions in the same gene from several filamentous fungi were used to amplify genomic DNA fragments. The PCR product was used to identify genomic clones in Bacterial artificial chromosome (BAC) library. Southern blot hybridization analysis revealed that calmodulin gene is present as a single copy in the genome of *M. grisea*. Calmodulin gene consists of 987 bp, including five short introns sufficient to encode a 149 amino acids. Functional analysis of this gene is being proceeded.

B-11. Vitamin Regulation of Appressorium Formation in *Magnaporthe grisea*. Yun-Kyung Cho and Yong-Hwan Lee. Department of Agricultural Biology and RCNBMA, Seoul National University, Suwon, Korea 441-744.

Magnaporthe grisea, the causal agent of rice blast, differentiates a dome-shaped melanized infection structure, an appressorium, to penerate its hosts. Environmental cues and signal transduction systems required for appressorium formation of this fungus have been studied extensively during last a few years. Recently, α-factor pheromone of *Saccharomyces cerevisiae* was reported as an effective inhibitor of appressorium formation with mating-type specific manner. Yeast extract treatment also inhibited the appressorium formation, whereas those of glucose and sucrose did not exhibit significant differences on the inductive surface. In order to investigate the possible role of another components, effects of amino acid complexes and various kinds of vitamin were assessed in the respect of appressorium formation of *M. grisea*. Among them, vitamin B_1 (thiamine) and B_6 (pyridoxine) showed distinguishable inhibitory effects as dosage-dependent manner. These inhibitions were also recovered by the addition of cAMP. These evidences demonstrate that inhibition of appressorium formation by yeast extract was largely due to the vitamin and

their target site was more upstream of adenylate cyclase and/or phosphodiesterase. Vitamin treatment restored the inhibition of appressorium formation by the addition of EGTA, a calcium chelator, neomycin, a phospholipase C inhibitor, and W-7, a calmodulin antagonist. Vitamin is involved in the cAMP-dependent pathway as well as Ca^{2+}-dependent pathway.

B-12. A Novel and Target-site Specific Screening System for Antifungal Compounds on the Rice Blast Fungus. Hong-Sik Oh and Yong-Hwan Lee. Department of Agricultural Biology and RCNBMA, Seoul National University, Suwon, Korea 441-744.

Control of plant diseases has been accomplished by breeding of resistant cultivars and treatment of chemical fungicides. Developments of new fungicides have been dependent upon *in vivo* screening whereas target-oriented screening systems were available in pharmaceutical field. The assessment of target site (an appressorium formation) specific screening system was performed with antifungal compounds of *M. grisea*. Among 1,000 culture filtrates of actinomycetes and fungi, 5 methanol extracts (A5005, A5008, A5314, A5387, A5397) showed specific inhibitory effects on appressorium formation of *M. grisea* as dosage-dependent manner. Inhibition of appressorium formation by A5005, A5008, A5397 was recovered by addition of cAMP or 1,16-hexadecanediol, whereas inhibition by A5314, A5387 was not recovered by any effecter chemicals. Methanol extracts of A5314 also exhibited inhibitory effect on mycelial growth of several plant pathogenic fungi such as, *Colletotrichum gloeosporioides*, *Fusarium oxysporum* f.sp. *cucumerinum*. Methanol extracts of 3 culture filtrates (A5005, A5314, A5387) were further fractionated by ethyl acetate and acetone. Ethyl acetate fraction of A5005 specifically inhibited appressorium formation and the inhibition was effective on controlling of rice blast. This rapid, massive and target-oriented screening system is applicable to screening new fungicides. This screening system could be also applicable to screen antifungal compounds for appressorium-forming plant pathogenic fungi.