구두발표 초록

O-01. The Rapid and Accurate Species-specific Detection of *Phytophthora infestans* Through Analysis of ITSII Region in rDNA. K. S. Kim[1], S. J. Wool, H. J. Kim[1], H. S. Shinl. Y. I. Ham[2], J. W. Lee[2], B. S. Kim[3], Y. S. Leel, [1]Div. of Applied Plant Sciences, Kangwon National University, Chuncheon 200-701, [2]National Alpine Agricultural Experiment Station, RDA, Korea, [3]Kangnung National University, Kangnung

The late blight caused by *Phytophthora infestans* is a devastating disease of potato and tomato worldwide. We designed PISP-1 primer for species-specific detection of *P. infestans* through analyzing the internal transcribed spacer region II(ITS II) in ribosomal DNA. In the analysis of ITSII region sequence in rDNA, we conducted manual and automatic sequencing. Using *DNASTAR* program, we designed PISP-1 primer, and the pair primer, PISP-1 and ITS3, produced 453bp of DNA fragment in *P. infestans* only. However, in the screen test for the usefulness of PISP-1 as a species-specific marker using other species of *Phytophthora* and several fungal and bacterial pathogens, we were able to differentiate *P. infestans* isolates early from other fungal and baterial species.

O-02. Survey on Chestnut Blight Caused by *Cryphonectria parasitica* in South Korea. Young Jik Ju[1], Kyung Hoon Park[1], Dae-Hyuk Kim[2], and Byeongjin Cha[1]. [1]Dept. of Agricultural Biology, Chungbuk National University, Cheongju 361-763, Korea. [2]Div. of Biological Science, Chonbuk National University, Chonju 561-756, Korea

Stem and twig blights caused by *C. parasitica* were surveyed from four to five different localities of Kyungnam-, Kyungbuk-, Chonnam-, Chonbuk-Do in south Korea. The chestnut stands were away at least 20 km from each other and consisting of more than 200 trees. Bark disks were taken with cork borer (ϕ 10 mm) from above and below the canker, and stored separately in a plastic film bag. The disks were surface-sterilized with flame before isolation. Total 289 *C. parasitica* were isolated by morphological characteristics of the colony on PDA and PDA-MB. The isolation frequency of *C. parasitica* was 40.1% in Kyungnam-Do, 32.7% in Chonnam-Do, 20.1% in Kyungbuk-Do, and 13.9% in Chonbuk-Do. Pathogenicity was examined by inoculating 5 mm mycelial plugs of each isolates on excised stems (3.5-5.5 cm thick) and incubating 5 days at room temperature. Brown-discolored areas were measured for disease severity. The areas varied from 0.5 to 4.5 cm2, whereas standard virulent- (EP) and hypovirulent-isolate (UEP) from Chonbuk University discolored 1.3 and 0.6 cm2, respectively. After 2-3 weeks of incubation, yellow perithecium-like structures broke through the bark surface of inoculated stems. Curled conidial tendrils were the only structures to recognize the presence of pycnidia on the bark surface among perithecia. Asci and ascospores were examined from perithecia on naturally infected stems. In the stands, *C. parasitica* was responsible for swollen or sunken cankers on stems. Water sprouts grew below the canker, and some of the twigs showed flag symptom. Abundant pycnidia and perithecia or the trace of them were found around some cankers.

O-03. Immunolocalization of PR-protein P14 in Tomato Leaves Exhibiting Systemic Acquired Resistance Against *Phytophthora infestans* by Tobacco Necrosis Virus. Yong Chull Jeun and Heinrich Buchenauer. Institut f? Phytomedizin, Universit? Hohenheim, 70593 Stuttgart, Germany

Systemic acquired resistance (SAR) against late blight caused by *Phytophthora infestans* could be induced in tomato plants by pre-inoculation with tobacco necrosis virus (TNV). To investigate the possible role of pathogenesis-related proteins (PR-proteins) in defense mechanisms of tomato SAR, the accumulation and localization of tomato PR-protein P14 were studied by immunocytochemical methods. Immunofluorescence labeling revealed that pre-inoculation with TNV led to systemic accumulation of P14 in the untreated upper leaves of tomato plants before challenge inoculation with *P. infestans*. In contrast, this protein appeared in the leaves of control plants only after fungal inoculation. In the upper leaves of tomato plants pre-inoculated with TNV, P14 became apparent by immunogold labeling in cell wall thickenings of xylem vessels. In contrast to the leaves exhibiting SAR, P14 was rarely found in leaves of healthy control plants. After challenge inoculation with *P. infestans*, P14 was localized in fungal cell walls and in the space between fungal cell walls and invaginated plasma membrane. An accumulation of P14 was also observed in cell wall appositions (papillae) of the host cells. These results suggest that accumulation of P14 seems to be an important mechanism involved in tomato SAR against *P. infestans*.

O-04. Sporulation Pattern of *Pyricularia grisea* at Different Growth Stages in the Field. Chang Kyu Kim[1] and Reiichi Yoshino[2]. [1]Plant Pathology Division, NIAST, RDA, Suweon, Korea 441-707. [2]Research & Development Dept., ZENECA K. K. Agrochemicals, Akasaka Ohji Bldg., 8-1-22 Akasaka, Minato-ku, Tokyo, 107, Japan

Sporulation patterns of rice blast fungus were studied at relatively later stages of leaf blast and neck blast seasons at Icheon, Korea, by detaching lesion-bearing leaves and panicle bases. Number of conidia remained on the leaf blast lesions of different cultivars during Jul. 20 to Jul. 23 was variable ranging from 3,640 to 82,740 spores. More conidia were observed on the adaxial cells due to the release of conidia from abaxial cells. After heading, sporulation was observed from the lesions on the flag leaves, but the amount was less than in the late July. Detached panicle bases or uppermost internodes infected by *P. grisea* produced abundant amount of conidia. Among these panicle bases, 30.1 mm size lesion recorded the highest count, 224,560 spores. When we compared the sporulation amount using the KY-type spore trap, more conidia were observed from intact lesions than from the conidia and conidiophore removed ones. The ratio of conidia release against total sporulation ranged 20.5 - 25.0% for leaf blast and 8.2 - 25.3% in case of neck blast.

O-05. A Galactosyl Diacylglycerol from Cucumber Leaves Inhibits the Development of Cucumber Gray Mold Disease. Joong-Hyeop Park, Jin-Cheol Kim, Gyung Ja Choi, Heung Tae Kim, and Kwang Yun Cho. Screening Division, Korea Research Institute of Chemical Technology, Taejon 305-600, Korea

When the potted cucumber plants of the 4th leaf stage were artificially inoculated with spores of *Botrytis cinerea*, the disease severities were remarkably different among leaves; the first leaves showed most severe disease symptoms followed in order by 2nd, 3rd, and 4th leaves. Compared to 1st and 2nd leaves, 3rd and 4th leaves were highly resistant to *B. cinerea* infection. However, such a phenomenon did not occur in the cucumber leaves inoculated with mycelial discs of the fungus. Water extracts from individual leaves showed different inhibitory effects on the development of gray mold disease and the spore germination of *B. cinerea*. An active principle was purified from 4th leaves as white powders by MeOH extraction, chloroform partition, repeated silica gel column chromatography, preparative TLC, and *in vivo* bioassay. Its chemical structure was determined as 2,3-di-(9Z,12Z,15Z-octadecatrienoyl)-2,3-dihydroxypropylgalactopyranoside by IR spectroscopy, LC-ESI/MS, high resolution FAB/MS, and NMR spectrometry. Its structure was confirmed by TLC and GC-MS analyses after acid and alkali hydrolyses. The principle exhibited dose-dependent inhibitory effects on the disease development *in vivo* and the spore germination of *B. cinerea* without any effects on the mycelial growth of the fungus. This substance appears to be at least partially responsible for the disease resistance of young leaves of cucumber plants against *Botrytis cinerea* infection.

O-06. The Responses of *Magnaporthe grisea* to High Ozone Concentration. Sung-Chul Yun[1], Jae-Seoun Hur[2], Eun Woo Park[1]. [1]Seoul National University, 103 Seodun Dong, Suwon, Korea 441-744. [2]Sunchon University, 315 Maegok Dong, Sunchon, 540-742, Korea

Rice blast fungus, *Magnaporthe grisea* (race KI-197), was exposed to ozone at 300ppb/hr for 0, 8, 16, and 24 hours. Ozone exposure was started from 3 days after inoculation on oatmeal agar plates. The four ozone treatments had no detrimental effects on the hyphal growth for 4 days ($p=0.407$ - 0.717) after finishing the ozone exposure. Conidia in each plate were collected after the last measurement of the hyphal growth. The numbers of spores were 2.9 105-4.2 105 spores/㎖ in each treatment, but not changed by the ozone treatments ($p=0.849$). The size of spore was 5.1-5.2 ㎛ wide and 18.1-18.6 ㎛ long, but ozone did not change either width ($p=0.846$) or length ($p=0.734$) of the spores. The spore suspensions were incubated for 16 hours at 20℃, then the percent of germination and appresorium formation were measured as 82-93% and 17-22%, respectively. These percent data were transformed to arc sine for statistical analysis. Ozone did not change either germination ($p=0.618$) or appresorium formation ($p=0.902$). Because 300ppb/hr is 3 times higher than the ozone standard and has been rarely reached in Korea, our results show rice blast fungus would be highly resistant to ozone. In addition, the fungal resistance to ozone can help interpreting the interactions among ozone, rice and the blast fungus in the field.

O-07. Biochemical Aspects of GlycinecinA Produced by *Xanthomonas campestris* pv. *glycines* 8ra. Jonghee Oh[1], Moonjae Cho[2], Sangryeol Ryu[3], and Yongsup Cho[1]. [1]School of Applied Biology and Chemistry, [3]Department of Food Science and Technology, Seoul National University, Suwon, Korea 441-744, and [2]Medical school, Che-ju National University, Che-ju, 690-756, Korea

Xanthomonas campestris pv. *glycines* 8ra produces proteinaceous bacteriocin, glycinecinA against the related pathogen, however biochemical characterization of glycinecinA has not been reported. Biochemical aspects of glycinecinA are an essential goal to study its mode of action on sensitive cells, with a view at evaluating its possible utilization as a biological control agent. Recently, about 1.7 kb DNA fragment responsible for production of glycinecinA has been cloned, and sequenced. Possible open reading frames were expressed in *Escherichia coli* DH5α, and expressed proteins were was purified with usual protocols of bacteriocin purification, consisting of ammonium sulfate precipitation and column chromatography. The molecular mass of glycinecinA was estimated to be approximately 54 kDa polypeptide, consisting of two subunits as analyzed by SDS-PAGE. The purified active glycinecinA was heterodimer of these two subunits deduced from cross-linking reaction and gel-filtration chromatography. The result of N-terminal protein sequencing indicated that these two subunits have signal peptides necessary to pass through the cytoplasmic membrane.

O-08. Role of Domains of a Chitinase from *Chromobacterium* sp. Strain C-61 in Chitin Degradation. Seur Kee Park, Ki Beum Park, and Young Jun Kim. Department of Applied Biology and Horticulture, Sunchon National University, Sunchon, Chonnam, 540-745, Korea

The chitinase from *Chromobacterium* sp. C-61 consisted of a signal peptide of 26 amino acids and a mature protein of 510 amino acids. The deduced mature protein contained three functional domains such as a chitin-binding domain (CBD) in N-terminal, a bacterial extracellular solute-binding protein (BBP) in the middle region and a catalytic domain in C-terminal. The catalytic domain had three chitinase active sites (CAS I, II and III) that were belong to family 18 of glycosyl hydrolases. To define the roles of these domains in chitin degradation, domains of the chitinase gene (*ChiCV*) was deleted by deletion- and site-directed mutagenesis. *Eschericha coli* harboring the intact *ChiCV* gene formed clear zone on the chitin agar plates and secreted the chitinase into culture supernatant, which had molecular weight of 54- and 53-kDa on SDS-PAGE. However, deletions of signal sequence from *ChiCV* did not secrete the chitinase into culture supernatant, although chitinase of 54- and 53-kDa was produced in the cytoplasm. Deletions of BBP, and CAS I, II and III from *ChiCV* did not have a chitinase activity. Deletion of CBD were lower in ability to degrade colloidal chitin on the agar plate, in chitinase activity toward native or colloidal chitin, although not differed in chitinase activity toward *p*-nitrophenyl-β-D-*N,N*-diacetylchitobiose.

O-09. Occurrence of a Chrysanthemum Stunt Viroid in *Dendranthema grandiflorum* in Korea. Bong Nam Chung, Gug Seoun Choi and Yong Mun Choi. National Horticultural Research Institute, RDA, Suwon, 441-440, Korea

A viroid was detected in chrysanthemum cv. Chunkwang plants, exhibiting symptoms of stunting in commercial fields in Korea. It was transmissible to *Dendranthema grandiflorum, Gynura aurantiaca* and *Lycopersicon esculentum* cv. Rutgers. RNA were extracted from Chrysanthemum stunt viroid(CSVd) infected chrysanthemum and were amplified by reverse transcription and polymerase chain reaction (RT-PCR) using a pair of primers specific for CSVd sequence. The expected amplified cDNA products were detected by agarose gel electrophoresis. The cDNA were cloned and those sequence was determined. CSVd Korean isolate consists of 354 nucleotides. In comparing the nucleotide sequence, it showed a homology of 100%, 99.8% and 97.8% with CSVd isolate of Japan, America and Australia, respectively.

O-10. Detection of Citrus Tristeza Virus by RT-PCR and Identification of two CTV Strains in Cheju Island. Hyoun-Hyang Park[1], Dae-Hyun Kim1, Doo-Khil Moon[2], Young-Jin Koh[3] and Tae-Jin Choi[1]. [1]Pukyong National University, 599-1, Daeyeon Dong, Pusan, Korea, 608-737. [2]Cheju National University, Ara-1-Dong, Cheju, 690-756. [3]Sunchon National University, 315 Maekokdong, Sunchon, 540-742, Korea

Citrus tristeza virus (CTV) is the causal agent of one of the most important diseases of citrus. Recently, CTV has been detected from Cheju Island by ELISA. CTV was detected by RT-PCR and the nucleotide sequence of the cloned PCR products were analysed in this study. Citrus leaves were collected from trees showing decline symptoms from various region of Cheju Island in the summer of 1998 and 1999. Viral RNAs were extracted by common methods and with viral RNA purification kit designed for animal virus RNA purification, and used for RT-PCR. PCR products of 560 base pairs corresponding to the part of the coat protein open reading frame were obtained from all the tested samples. Sequence analyses of the cloned PCR products showed that two CTV strains present in Cheju Island. Viruses collected from Sogwipo area in 1998 and Cheju City area in 1999 showed 93-98% nucleotide sequence homology with CTV T36 strain. Viruses collected from Cheju City area in 1999 showed 91% nucleotide sequence homology with CTV SY568 stain. Purification of viral RNA from woody plants has been problematic. Animal virus RNA isolation kit used in this experiment was very effective for viral RNA purification from hard plant tissue and more effective than common viral RNA purification method. PCR product was detected from one tenth of RNA purified from as small as 45mg fresh or frozen tissue.

O-11. Development of Broad-Spectrum Virus Resistant Plants by Using Recombinant Antibody.

Eulyong Park[1], Youngju Jang[2], and Sukchan Lee[1]. [1]Sungkyunkwan Univ. Suwon, Korea, 440-746. [2]Research Institute for Medical Sciences Aju Univ Medical School. Suwon, 442-749, Korea

Virologists have been numerous attempts to produce virus resistant plants by using the host plant protein or virus protein. The purpose of this study was to produce broad-spectrum virus resistant plants using the ssDNA specific single-chain antibody fragments (scFv). Single-chain antibody fragments are recombinant proteins composed of variable domains of an immunoglobulin fused by a flexible linker DNA. Anti-ssDNA monoclonal antibodies were isolated and cloned from the MRL mouse which produce ssDNA specific antibodies. scFv gene was isolated and amplified by polymerase chain reaction (PCR) with mixed primers designed by conserved sequences in each variable fragments. Recombinant scFv gene was cloned into plant expression vector pBI121 to express it in plants. The cloned scFv gene (pBI-scFv) was transformed into tobacco and Arabidopsis by agrobacterium mediated transformation. The scFv transcription from putative transgenic plants was confirmed by reverse transcription polymerase chain reaction.