Abstract of Presentations

Analysis of pathogenicity factor from three novel Streptomyces spp. causing potato scab in Korea. Duck Hwan Park1, Joon Soon Kim2, Jun Mo Chon3, Jung Hyun Hur3 and Chun Keun Lim1. 1Division of Biological Environment, College of Agriculture and Life Sciences, Kangwon National University, Chuncheon 200-701, Korea; 2Alpine Agricultural Experiment Station, Pyungchang, Kangwon 232-950, Korea.

Every one of the strains which were pathogenic on potato also gave a positive reaction in thaxtomin A assay. In this study, chloroform extracts of oatmeal broth from Streptomyces scabies, S. luridiscabiei, S. acidiscabiei and three novel Streptomyces spp. Korean strains produced a positive yellow band, which comigrated with the thaxtomin A standard. Inconsistent with our expectation, thaxtomin A was not detected from culture media of strains S. luridiscabiei (S63) and S. puniceiscabiei (S77) by HPLC analysis, however. The result clearly displays HPLC analysis, not TLC test alone, should be conducted to make sure positive identification of thaxtomin A. In a hypothesis that production of thaxtomin A by scab pathogens would require component(s) or signal(s) from potato tissues, all six pathogenic Korean strains were inoculated onto tuber slices. When chloroform extracts from tuber slices were analyzed by HPLC, again, thaxtomin A was not detected from strains S63 and S77, which clearly indicated that Korean strains S63 and S77 did not produce thaxtomin A either in the media or potato tissues. This is the first extensive demonstration by HPLC analysis that streptomycetes that are pathogenic to potato, do not produce thaxtomin A to our knowledge.

Description of three new species, Streptomyces luridiscabiei sp. nov., Streptomyces puniceiscabiei sp. nov., and Streptomyces niveiscabiei sp. nov., causing potato scab. Duck Hwan Park1, Soon Wo Kwon2 and Chun Keun Lim1. 1Division of Biological Environment, College of Agriculture and Life Sciences, Kangwon National University, Chuncheon 200-701, Korea; 2Division of Molecular Genetics, National Institute of Agricultural Science and Technology, Suwon 441-701, Korea.

Three new bacterial species were defined for which the name Streptomyces luridiscabiei, Streptomyces puniceiscabiei, and Streptomyces niveiscabiei were proposed. These phylogenetic analysis of 16S rRNA gene sequences showed that S. luridiscabiei was similar to the S. setonii (99.3%) and S. griseus subsp. griseus (99.9%), whereas, S. puniceiscabiei and S. niveiscabiei differed extensively from the other strains of Streptomyces tested (less than 96.5%). The levels of DNA-DNA hybridization among these three new species, pathogenic strains, and non-pathogenic strains analyzed, were low.

Identification of soybean sprout rot pathogens, H.N. Bae, C.K. Lim and J.H. Hur. Division of Biological Environment, College of Agriculture and Life Sciences, Kangwon National University, Chuncheon 200-701, Korea.

Soybean sprout, one of the traditional foods in Korea, has a problem of rot disease under humid and closed cultivation condition. There is lack of identification of pathogens of soybean sprouts, however. In this study, soybean sprout pathogens were isolated and identified. Thirty-three and sixteen strains of bacteria and fungi, respectively, were isolated from the rotten tissues of soybean sprouts. Pectobacterium carotovorum and Pseudomonas spp. were identified as bacterial pathogens, while Fusarium oxysporum, Alternaria sp., Rhizoctonia sp. and Macor sp. were identified as fungal pathogens. Alternaria sp. and Macor sp. have not been reported till now as pathogens of soybean sprouts.


Phylogenetic analysis of 11 Erwinia amylovora strains from different hosts and geographic origins was conducted by using the nucleotide sequences of the genes 16S rRNA, which have been determined by direct sequencing of PCR-amplified fragments. The sequence homology of 16S rRNA genes among the strains of E. amylovora was highly conserved. However, these strains were differentiated into three subclusters on the basis of the sequence analysis using UPGMA: subcluster I including the strains of E. amylovora from pomaceous plants; subcluster II including blackberry plant; and subcluster III raspberry plants. Base substitutions and base insertions in helix 6 and helix 18 in hypervariable regions could be identified, and differentiated into putative secondary structures among pomaceous, blackberry and raspberry strains of E. amylovora. The sequence diversity within different strains of E. amylovora could be assessed. The reliable identification of the bacterial strains by the 16S rRNA gene sequences will greatly facilitate to designate the strains of E. amylovora.

Identification and characterization of Coronatine-producing Pseudomonas syringae pv. actinidiae isolated from Korea. H.S. Han1, Y.J. Koh1 and J.S. Jung1. 1Dept. Biology, Sunchon National University, Sunchon 540-742, Korea; 2Dept. of Applied Biology, Sunchon National University, Sunchon 540-742, Korea.

Pseudomonas syringae pv. actinidiae strains, which causes canker or leaf spot on kiwifruits, were collected from the kiwifruit orchards in Korea. The 16S rDNA and 16S-23S internally transcribed spacer sequences were analyzed to clarify the pathovar of the isolates. The nucleotide sequences of two regions of the isolates were identical to
those of pathotype strain of P. syringae pv. actinidiae. A PCR test with primers designed to amplify DNA fragments from the phyto-
toxin related genes was performed to determine the phyto-
xic metabolites produced by the isolates. Although PCR with the patho-
type strain of P. syringae pv. actinidiae amplified DNA fragments
within the genes for phaseolotoxin biosynthesis and phaseolotoxin-
resistant ornithine carbamoyltransferase, these fragments were not
amplified with the isolates from Korea. On the contrary, PCR with
three primer sets derived from coronatine biosynthetic gene cluster
and genomic DNA from Korean strains of P. syringae pv. actinidiae
produced expected size of DNA fragments. The sequence analysis of
PCR products revealed that while P. syringae pv. glycinea and the
Korean strains of pv. actinidiae contained the, cork genes which
showed 91% of sequence similarity, they had identical cf. sequence.
The results suggested that these two genes in coronatine gene cluster
might have evolved differently from each other. The production of
coronatine, instead of phaseolotoxin, by the Korean strains of P.
syringae pv. actinidiae was confirmed by bioassay with reference
pathovars for coronatine and phaseolotoxin.

An inoculation method for screening resistance to bacterial soft
rot in Chinese cabbage. S.-H. Lee and J.-S. Cha. Department of
Agricultural Biology, Chungbuk National University, Cheongju,
Chungbuk 361-763, Korea.

Bacterial soft rot caused by Erwinia carotovora subsp. carotovora
(Ecc) is considered one of the most destructive diseases of Chinese
cabbage [Brassica rapa (syn. campestris) subsp. perkinensis] in China,
Japan, and Korea where the crop has been widely cultivated.
Control of bacterial soft rot is difficult due to a wide range of hosts,
ability of the bacteria to survive in plant debris in soil, and host sus-
ceptibility. Genetic resistance would be an ideal alternative approach.
Screening of resistance to bacterial soft rot is difficult because uni-
form induction of the disease by inoculation is difficult. Recently we
developed a novel inoculation method, which induces bacterial soft
rot with easy and uniformly. Incubation mixture of Ecc (10^7 cells/mL)
and mineral oil, 4:1 ratio, induced every inoculated plant of cultivat-
ing variety of Chinese cabbage. It induced, however, different level of
soft rot on selected breeding lines that had been reported as resistance
source of bacterial soft rot. The inoculation method has advantages
over the screening techniques reported previously because it is very
simple, applicable on anywhere, greenhouse or field, do not need any
special facility and, more importantly, any woundng of host plants.

The characterization of the hrp gene cluster isolated from
Xanthomonas axonopodis pv. glycines and Xanthomonas oryzae
pv. oryzae. Sang-Wook Han, Chang-Shik Oh and Sung-Gi Heu. Plant
Pathology Division, National Institute of Agricultural Science and
Technology, Suwon 441-707, Korea.

Xanthomonas oryzae pv. oryzae causes the very destructive bacterial
leaf blight on the rice and Xanthomonas axonopodis pv. glycines
caused the bacterial pustule disease on soybean. Since the pathoge-
nicity island including hpc gene cluster is the key element for the bac-
terio to cause the disease on the host plant and hypersensitive
response on the nonhost plant, we have cloned a hpc gene cluster
from X. oryzae pv. oryzae, and X. axonopodis pv. glycines. Two
clones covering about 50 kb of bacterial chromosomesal DNA con-
tained full set of genes for the type III protein secretion system and
several new hpc-associated loci located at left and right sides of the
genes for the type III protein secretion system. Using transposon
mutagenesis, possible functions of hpc genes in pathogenesis were
characterized. In contrast to other publications, the mutation on sev-
eral hpc genes and hpcC genes did not change the ability to cause
HR on nonhost, but the mutants lost the pathogenicity on host plant.
However, mutants which have a mutation on hrfP and hpAcC lost the
ability to cause disease on host and HR on nonhost. HrfP is a mainly
hydrophilic protein with two C-terminal hydrophobic segments.
HpAcC carries a leucine rich repeat domain and deduced amino acid
sequence of the HpAcC shows a high homology with well known rice
resistance gene, Xa21, mostly in the leucine rich repeat region. The
possible function of the HrfP and HpAcC in the plant microbe interac-
tion will be discussed in relation to the plant defense against the
pathogen.

Characterization of phenazine-deficient a Tn5 mutant of
Pseudomonas chlororafis strain O6. Beom-Ryong Kang, Mi-
Sung Kim and Young-Choel Kim. Applied Plant Science Division
and Institute of Biotechnology, College of Agriculture, Chonnam
National University, Kwangju 500-757, Korea.

Pseudomonas chlororafis strain O6 is a biological control bacte-
rium that inhibits several fungal pathogens including Fusarium spp.
and produces phenazines, hydrogen cyanide (HCN), and protease. P.
chlororafis O6 is known to produce at least three different phenae-
zares which include phenazine-1-carboxylic acid, 2-hydroxyphene-
azine-1-carboxylic acid, and 2-hydroxyphenazine. To investigate
global regulators in production of phenazines, we isolated several
phenazine-negative Tn5 mutants. One of the Tn5 mutants, N7 did not
produce phenazines and lost their ability to inhibit the fungal patho-
gen. The N7 mutant was not affected in their ability to produce hydro-
gen cyanide. The flanking sequence of the N7 mutant identified a
homolog of lon, which encodes an ATP-dependent protease found in
diverse organisms, including bacteria, plants, and animals. The
deduced amino acid sequences of the Tn5-flanking region is 90%
identical to the ATP-dependent protease of P. aeruginosa PA01. Our
results showed that Lon protease may be one of the global regulators
in phenazine production of P. chlororafis O6.

Occurrence of bacterial wilt of potato in Korea and distribution
of races and biovars of the pathogen. Young-Kee Lee', Seung-Don
Lee', Soon-Young Hong', Weon-Dae Cho' and Jong-Hyoung Lee'.
1 Plant Pathology Division, National Institute of Agricultural Science
and Technology, Suwon 441-707, Korea; 2 Jeju Agricultural Technol-
ology Administration, Jeju 690-170, Korea.

In 1999 and 2000 bacterial wilt of potato (Solanum tuberosum L.)
was surveyed in eight provinces of Korea. The disease was observed
in 12.8% and 15.8% of potato fields in years. Among the provinces
surveyed, the disease occurred in Jeonnam, Jeju, Gyeongbuk and
Gyeongnam. The disease incidence ranged from 0.1 to 70% in the
infected fields. Bacterial isolates from infected potatoes and contam-
nated soil were identified as Ralstonia solanacearum by routine iden-
tification tests. The bacterial isolates were classified into two races
based on pathogenicity tests. The isolation frequency of race 1 and
race 3 was 92.3% and 7.7%, respectively. Race 1 was most widely
distributed in the surveyed areas, while race 3 only in a few areas.
Based on physiological and biochemical tests, the isolates were clas-
sified into biovars 2, 3, and 4. Biovar 4 was predominant in the iso-
lates followed by biovar 3 and biovar 2.
Evaluation of mycelium virulence of mycoherbicide agent, fungal isolate BWC-101 to Aeschynomene indica L. Yeon Kyu Hong, Seok Bo Song, Dong Bun Shin, Dong Choon Lee, Dong Chang Lee and Huhn Pal Moon. National Yeongnam Agricultural Experiment Station, 1085, Miryang 627-803, Korea.

A summer annual that grows in the edges of rice paddies, ditches, and moist upland through Korea. It produces by seed, grows from May to November and flowers in July to September. Brown stem blight of Indian joint-veitch (Aeschynomene indica L.) observed at naturally occurred in rice paddy field, is first reported in Korea. The fungal isolate BWC-101 was successfully isolated from the diseased stems. Symptoms first appeared on stems of A. indica in June and the lesions rapidly elongated, expanded around the stems, and blighted completely August. Typical symptoms on stem having water soaked brown lesions were formed and which severely affected the seed malformation and the whole plants blighted. The fungus BWC-101 was grew well at 25-28°C, produced abundant aerial mycelial and dark brown sclerotinia on PDA at 15 days. The fungus was grown well in liquid culture media (PD broth) at 28°C and fully grown within 4 days in 250 ml. of flask. In host plant test, highly specific to A. indica but some Leguminosae were slightly infected by the pathogen. In pathology test, mycelial mat was the most effective to control the plant of the several kinds of inoculums. Under paddy field condition, mycelial mat of the fungus at the size of 1 cm² gave around 99% control. It was the very interesting event that diseased plants were severely blight of the whole body caused by this host specific fungal organism. Therefore, we conclude that the fungus may have a potential as a biological control agent of A. indica in rice paddy field.

Efficacy of cow dung in controlling root rot and Fusarium wilt diseases of cucumber plants. A. B. Basak* and Min Woong Lee. Department of Biology, Dongguk University, Seoul 100-715, Korea. (*Permanent address: Department of Botany, University of Chittagong, Chittagong-4331, Bangladesh)

Efficacy of fresh cow dung at 1.2 concentrations was tested against the artificially inoculated cucumber (Cucumis sativus L.) plants with root rot and Fusarium wilt pathogens- Fusarium solani f.sp. cucurbitae Synder and Hansen and Fusarium oxysporum f.sp. cucumaram Owen respectively. Results showed that cow dung solution controlled (100%) effectively both the pathogens and no plant was rotten or wilted at collar part after application of this bio matter. Cow dung might have characteristics of defense mechanism against the infecting pathogens producing a barrier at collar part of the plants. But direct use of fresh cow dung to the plants caused the death of growing plants within two days. This was the follow up experiment on growing plants in pots that was carried out after confirming positive response of cow dung to the inhibition of conidial germination and mycelial growth of the above two mentioned fungal pathogens of cucumber under laboratory conditions. Moreover, application of cow dung solution observed very helpful for healthy growth of plants supplying some nutrients to the plants.

Comparative efficacy and in vitro activity of cow urine and cow dung for controlling Fusarium wilt of cucumber. A.B. Basak* and Min Woong Lee. Department of Applied Biology, Dongguk University, Seoul 100-715, Korea. (*Permanent Address: Department of Botany, University of Chittagong, Chittagong-4331, Bangladesh)

An attempt was made to study on efficacy and in vitro activities of cow urine and cow dung for controlling Fusarium wilt caused by Fusarium oxysporum f.sp. cucumerinum Owen of cucumber following slide germination and mycelial growth inhibition tests. Both germination of conidia and the percentage inhibition of mycelial growth were effectively decreased and varied greatly with respect to different hours and days of incubation and kinds of bio matters. Result shows that, out of 200 conidia of F. oxysporum f.sp. cucumerinum, 42 conidia in cow urine and 76 in cow dung were germinated after 7 hours of incubation, while in control a total germinated conidia was 162. Morphological structure of conidia was deformed and length of germ tube of each conidium observed short in bio matters in comparison to conidia tested in control. The highest percentage (100%) inhibition of conidial germination in cow urine was recorded after two hours of incubation followed by 3 hr (95.45%) and 5 hr (93.22%) of incubation. On the other hand, mycelial growth and the percentage inhibition of mycelial growth was found more effective in cow dung potato dextrose agar than that of cow urine potato dextrose agar. So cow urine and cow dung may be used for controlling Fusarium wilt disease under field conditions initiating some experiments.

Development of microbial herbicide which is effective to weeds using Streptomyces sp. A6497. Hyang-Bok Lee¹, Sung Uk Kim², Jae Seong Yang¹, Sung Won Choi¹ and Kee Hyun Choi¹. ¹Green Biotech Ltd., 45-70, Yadang-ri, Gyohya-myeon, Paju, Gyeonggi-do, Korea; ²Antibiotics Research Laboratory, KIRIBB.

To search and develop new herbicides of microbial origin, we have screened and selected a Streptomyces sp. A6497 having inhibitory activity for cellulose biosynthesis, which is considered as excellent targets for herbicide screening system using their mode or site of action. The cellulose biosynthesis inhibitor was isolated from culture broth of Streptomyces sp. A6497 and identified as sangivamycin on the basis of various spectroscopic analysis. Sangivamycin, C12H15NSO5, is a deazaadenine glycoside compound. The result of biological control of weeds by culture broth of Streptomyces sp. A6497 as microbial herbicide exhibited a strong herbical activity against several weeds in greenhouse condition. In addition, sangivamycin, microbial metabolite of Streptomyces sp. A6497 also showed potent herbical activity in vivo. This is the first report that sangivamycin show a potent herbical activity.


The filamentous fungus Trichoderma harzianum is a potent mycoparasite, which is applied as a biocontrol agent against several plant pathogenic fungi. In order to enhance the biocontrol ability, the chitinase gene (chi54) of Chromobacterium sp. strain C-61 was introduced and expressed in T. harzianum under the promoter and the signal sequence of N-acetyl-β-D-glucosaminidase gene nagl. The chitinase of Chromobacterium sp. strain C-61 in the transgenic T. harzianum was produced and secreted on media containing chitin as carbon sources. The transgenic T. harzianum also produced its own chitinase. In the media containing chitin, chitinase activity from the
transgenic *T. harzianum* was the highest in 5 days cultivation and its total chitinase activity increased about 1.5-fold as compared with that of parental *T. harzianum*.

**Biological control of cucumber seedling damping-off caused by *Pythium ultimum* by specific carbon source amendment.** Sechul Chun, Sabong Paik and Jongmoon Lee. Dept. Food Resource, Konkuk University, Seoul 143-701, Korea.

The pathogenicity of *Pythium ultimum* on cucumber seedlings were tested in a growth chamber. Three pieces of *Pythium* grown on potato dextrose agar were inoculated in autoclaved moisturized mixture of sand and oatmeal (sand:oatmeal = 10:1, w:w) and incubated for 3 days at 28°C. The incubated mixture was used as inoculum sources for pathogenicity test of *Pythium* on cucumber seedling. No seedling was emerged in soil mixed with more than 50% of the inoculum sources. *Bacillus megaterium* 91-51 previously shown to be effective biological control agent in water-seeded rice could increase seedling emergence significantly by 53% compared to 22% of untreated seeds when seeds were coated with formulation. In addition, amendment of 8% D-galactose into seed coating formulation increased seedling emergence significantly by 8% compared to biological control agent alone, indicating that specific carbon substrate beneficial to biological agents could increase the efficacy of biological control.

**Antagonistic effect of bacterial strains on gray mold disease of cucumber caused by *Botrytis cinerea*.** Kee-Don Han1, Bye-Soo Lee2, Jung-Wan Kim1 and Min-Woong Lee1. 1 Dept. of Applied Biology, Dongguk University, Seoul 100-715, Korea; 2 Dept. of Biology, University of Incheon, Incheon 402-749, Korea.

Antagonistic effect of 300 promising bacterial strains isolated from phylloplane of some vegetable crops was tested on gray mold disease of cucumber caused by *Botrytis cinerea* Pers. following spore germination and mycelial growth inhibition test in laboratory conditions. It was revealed from the data that among the selected strains only 8 strains of *Bacillus* sp. #188 produced cent percent inhibition of spore germination after 3 days incubation in water agar. After selecting these strains, application of spore suspension was performed/done to the leaves surface of cucumber that were inoculated before a day with 10^7/mL spore density of *B. cinerea*.

Results showed that *Bacillus* strain #188 decreased disease incidence by 43.3% whereas at the same time the disease incidence in control was 19.6%.

**Anti-sporo adhesion and induce systemic resistance in cucumber by extrapoly saccharide (EPS) of *Burkholderia cepacia* 923-87.** Kyungseok Park, Eun-Young Kim, Yeong-Seok Bae and Choong-Hoe Kim. Plant Pathology Division, National Institute of Agricultural Science and Technology, Suwon 441-707, Korea.

*Burkholderia cepacia* strain 923-87 isolated from the rhizosphere of Chinese cabbage induced systemic resistance to cucumber anthracnose caused by *Colletotrichum orbiculare* and showed anti-sporo adhesion activity. To purify the active extrapoly saccharide (EPS), bacterial cultures grown on King’s B agar were harvested with ten liters of sterile distilled water and centrifuged. The compound was purified through dialysis and DEAE cellulose column chromatography after methanol decantation. Finally, the EPS fraction was treated with 1.0mM proteinase K to remove peptide protein in the EPS. The purified EPS significantly reduced the development of cucumber anthracnose at 50ppm when sprayed on two-leaf stage of cucumber seedling, showing 6.5 lesions per plant while the untreated control had 99 lesions per plant. The active EPS also showed anti-sporo adhesion of anthracnose fungus caused by *C. orbiculare* on cucumber leaf.

The percentage of anti-sporo adhesion was recorded 97.6% at 50 ppm by run-off treatment of EPS. Results demonstrated that the EPS obtained from *B. cepacia* strain 923-87 may act as a major component related to the mechanism of induce systemic resistance as well as direct effect on anti-sporo adhesion of anthracnose fungus.

**Effects of seed treatment of PGPR isolates on the barley growth in *rice-barley double cropping system*.** B.J. Lee1, N.S. Jun1, J.R. Choe1 and C.S. Park1. 1 Department of Agronomy, Gyeongsang National University, Jinju 660-701, Korea; 2 Department of Agricultural Biology, Gyeongsang National University, Jinju 660-701, Korea.

This study was aimed to evaluate the effects of PGPR strains on the barley growth in rice-barley double cropping system. The study was carried out in experimental farm of Gyeongnam Provincial RDA from November, 2000 to end of May, 2001. The test variety was Jin-yang, for breeding and PGPR strains are *Paenibacillus polymyxa* E681 and *Pseudomonas fluorescens* B16. The tested barley seeds were soaked into bacterial suspension of E681 or B16 at 108 cells/mL for 5 hrs and dried in shade place for 12 hrs then sowed in the barely field at rate of 10kg seed/10a. The emergency rates of barley plants at 30 days after sowing were observed 211/m^2 in E681 treated plots, 233/m^2 in B16 treated plot and 191/m^2 in untreated control. The emergency rates of test plots after over-wintering were 288/m^2 in E681, 260/m^2 in B16 and 233/m^2 in untreated control. The plant height, average tillers and fresh weight of test plants were measured with time sequence. The dry weight of stems, leaves, and heads were also measured. The leaf dry weight of control plot was continuously reduced after heading, however, that one in bacteria treated plots were not reduced up to 7 days before harvest. Average head weight and total yield were significantly higher in bacteria treated plots.

**Effects of bio-priming with bacterial strains and solid matrix priming on the germination of pepper seeds and their seedling growth.** S.M. Lee, S.S. Shen and C.S. Park. Division of Plant Resources and Environment, Gyeongsang National University, Jinju 660-701, Korea.

Experiments were conducted to compare the germinability of pepper seeds primed by bacterial strain and solid matrix priming (SMP). Pepper seeds were soaked in the cell suspension of the bacterial strains for 1 hr and incubated at 28 for certain period of time then dried in shade and stored. Seed priming with *Bacillus* strains showed even higher germination rate than SMP or chemical osmotic controllers. In pots experiments, pepper seeds primed by *Bacillus* sp. B2-13 showed more than 80% seedling emergency within 7 days, while SMP treatment was 11 days and untreated control was 13 days. When the bio-primed seeds were planted in pots, significant increase of shoot weight and length as well as root weight and length were measured compare to other treatments. Bio-primed seedling revealed twofold more root biomass than untreated control.

**Identification of amino acids in the catalytic domain of *Chromobacterium* sp. strain C-61 as essential residues for chitinase activity.** C.W. Kim, S.C. Yin and S.K. Park. Dept. Environmental Agri-
cultural Science, Sunchon National University, Sunchon 540-742, Korea.

The chitinase (Chi54) of Chromobacterium sp. strain C-61 was composed of a chitin-binding domain and a family 18 catalytic domain (residues 212 to 536) containing four active sites. In this study, the amino acids essential or not essential for chitinase activity were identified by site-directed mutagenesis. In the active site I (residues 212 to 223; ISLGWTVWSKNF), five amino acids of S, GGW, S were highly conserved with other chitinases, and Gly216 and Trp217 were essential for chitinase activity. In the active site II (residues 265 to 273; FDGIDIDW), five amino acids of DG, D, D, E were highly conserved and Asp269, Asp271 and Glu273 were essential for chitinase activity. In the active site III (residues 399 to 405; KLNVGIP), Gly403 was highly conserved and essential for chitinase activity. In the active site IV (residues 490 to 501; LGGVFSWSDLGD), Gly492, Thr496 and Gly501 were highly conserved and essential for chitinase activity. On the other hand, Thr218 in the active site I, Ile268 and Ile270 in the active site II, Asn401 in the active site III, and Val493 and Ser497 in the active site IV were not conserved with other chitinase and not essential for chitinase activity. The Asp349, which is not present in the active sites, showed the reduced chitinase activity by replacement with another amino acid.


The signal sequence and the chitin-binding domain (ChBD) in the chitinase gene (chi54) of Chromobacterium sp. strain C-61 played an important role in secretion of chitinase in E. coli and hydrolysis of insoluble chitin, respectively. On the other hand, chitinase (Then-42) of Trichoderma harzianum was not secreted in E. coli and did not contain the ChBD. Thus, the catalytic domain of the then-42 was ligated in downstream of the signal sequence and the ChBD of the chi54, and then transformed in E. coli. The hybrid chitinase gene was expressed and secreted in E. coli. The chitinase activity of the fusion chitinase against insoluble chitin was enhanced in comparison with that of Then-42. The physicochemical characteristics of the fusion chitinase are investigating.


We already reported about several Tn5 insertional mutants of Chromobacterium sp. strain C-61 that differed in the quality and quantity of chitinolytic enzymes. The maximum chitinase activity was high by order of the parental strain C-61, and the mutants C61-A, -B, and -C, but the β-N-acetylhexosaminidase (Nahase) activity was higher in the mutants than in the strain C-61. Another mutant, C61-D, produced no detectable extracellular chitinase, Nahase and almost no extracellular protein. The in vitro and in vivo biocontrol activity on Rhizoctonia solani was high by order of strain C-61, C61-A and C61-B, but was not observed by C61-C and C61-D. This results indicate that biocontrol activity of chitinolytic bacteria is depend on productive ability of their chitinase, but is not appeared when the chitinase production is low. In order to identify role of chitinase on the biocontrol ability, a chitinase gene (chi54) of Chromobacterium sp. strain C-61 was introduced into the mutants by triparental mating. All of the mutants recovered the chitinase activity and the biocontrol ability like the strain C-61. In addition, E. coli harboring the chi54 suppressed growth of Rhizoctonia solani. This study provides clear evidence that the chitinase of Chromobacterium sp. strain C-61 are almost totally responsible for the biocontrol ability of this strain and that Nahase lacks antifungal activity.

In vitro interactions between Serratia plymuthica A21-4 and Pythium spp. in the suppression of damping-off in cucumber seedlings. S.S. Shen, S.M. Lee and C.S. Park. Division of Plant Resources and Environment, Gyeongsang National University, Jinju 660-701, Korea.

A bacterial strain, A21-4 was originally selected for biological control of Phytophthora blight of pepper. A21-4 inhibited the mycelial growth, germination of zoosporangia and cystospores, and strongly inhibited the formation of zoospore and zoosporangia of P. capsici. A21-4 inhibits exclusively for the fungal species belong to genus Pythium and Phytophthora. The biocontrol agent, A21-4 was identified as Serratia plymuthica. In this experiment, the effect of A21-4 metabolites on the mycelial growth of Pythium spp. was examined. We also examined the inhibitory effects of A21-4 on the formation of zoosporangia, germination of zoosporangia and germination of encysted zoospore. The biological control of Pythium seed rot and damping-off of cucumber by A21-4 was evaluated in corn meal agar and pot experiments. In pot experiment, cucumber seeds inoculated with A21-4 suppressed the development of seedling disease caused by P. ultimum and P. anphanidermatum. The A21-4 inoculated cucumber seeds showed significantly less damping-off, less root colonization by Pythium hence increase the fresh weight of cucumber seedlings.

Influence of soil biomass on growth and biocontrol efficacy of Trichoderma harzianum. Yeoung-Seuk Bae1, Guy Richard Knudsen2 and Louise-Marie Dandurand1. Plant Pathology Division, National Institute of Agricultural Science and Technology, Suwon 441-707, Korea; 2Department of Plant, Soil, and Entomological Sciences, University of Idaho, Moscow, Idaho 83844-2339, USA.

Isolates of Trichoderma spp. have been known as potential biocontrol agents for many plant diseases caused by soilborne fungal pathogens. For effective biocontrol of soilborne plant pathogens, the agent must explore through soil to the target propagules of soilborne pathogens during a short period of favorable environmental condition by means of hyphae of the agent. However, natural soils are resistant to introduced biocontrol agents due to abiotic or biotic factors. We examined whether soil biomass affects on the growth and biocontrol efficacy of the green fluorescent protein transformant T. harzianum strain Thz1D1-M3 using different levels of soil biomass. The hyphal radial growth of T. harzianum was significantly inhibited in the soil containing higher soil biomass compared with the soil containing lower soil biomass. When Thz1D1-M3 was added to the soils as an alginate pellet formulation, the recoverable population of Thz1D1-M3 varied, showing the highest populations in the soil containing higher soil biomass. When sclerotia of Sclerotinia sclerotiorum were added to soils with Thz1D1-M3, colonization of sclerotia by Thz1D1-M3 was sig-

Many plant growth-promoting rhizobacteria (PGPRs) have a beneficial effect on plants including biological control of soilborne pathogens, induced systemic resistance to plant pathogens, phytohormone production, and improvement of nutrient and water uptake of plants. Here we developed a simple method for screening of potential PGPRs using cucumber cuttings. Cucumber seeds were sown in a plastic pot containing vermiculite. After 7 days, the upper parts (approx. 2 cm in length) of cucumber seedlings were cut and used as cuttings. The lower part of the cutting was dipped in a micro-centrifuge tube containing 1.5 mL of a bacterial suspension and incubated at 23°C with a light. After 7 days, number, length, and weight of adventitious roots developed were examined. The cuttings showed various responses to the isolates tested. Some of the isolates tested, including Escherichia coli and Ralstonia solanacearum, showed wilt symptoms after 2 or 3 days of incubation, resulting in withering at the day of examination or in reduced number of roots developed in case of treatment of R. solanacearum. Several isolates stimulated initial development of adventitious roots giving more number of the adventitious roots than those of untreated cuttings, but not length of the adventitious roots developed. Furthermore, an isolate had more number and length of the adventitious roots developed than those of untreated control, while an isolate inhibited the root development of cucumber cuttings. Further experiments, relating to whether bacteria have the same effects in soil system, and settle down inside stem or roots, are examined. Our results indicate that this system may provide a useful method for differentiating PGPRs' functions involved in the development of root system.

Biorecontrol effects of formulated antagonistic rhizobacteria for root rot disease (Cylindrocarpon destructans) in natural infested soil. Sung Joon Yoo', Jae Beom Ra', Sun Ik Kim' and Hong Gi Kim'. 1Institute of Agricultural Science, Chungnam National University, Taejon 305-764, Korea; 2Department of Agricultural Biology, Chungnam National University, Taejon 305-764, Korea; 3Agriculture Technology and Extension Center, Kumsan Gun 312-835, Korea.

Antagonistic rhizobacteria Erwinia sp. (S21) and Bacillus sp. (16PS), which have biocide control effect against soil-borne gumos fungal disease, was applied to soil amendment in natural infested field soil which is located in Kumsan to study disease control effect. To see the control effect of antagonistic rhizobacteria Erwinia sp. (S21) and Bacillus sp. (16PS) for the root rot disease in a ginseng field, seven types of formulations (A-G) that combined two antagonistic rhizobacteria (S21 and 16PS) were tested. Within three months after application, high germination rate (90%) was showed with formulation C. The average isolation rate of S21 and 16PS obtained from the formulations which were 0.9x10^2 cfug soil and 7.1x10^5 soil, respectively. The rate of healthy plant after 6 months treatment of each formulation was 6.7%(A), 26.7%(B), 55%(C), 15%(D), 25%(E), 3%(F), 28%(G) and 6.7%(Control), respectively. Therefore, formulation C which was mixed antagonistic bacteria (S21, 16PS), 20 kg of saw dust, 10 kg of rice husk, 1kg of rice bran, 30g of MgO and 30g of Na2CO3, have high effect for the root rot disease of ginseng.

Sensitivity of Monilia fructicola isolates obtained from peach fruit in Korea to ergosterol biosynthesis inhibitor. Tae Heon Lim and Byeongjin Cha. Department of Agricultural Biology, Chungbuk National University, Cheongji 361-763, Korea.

The sensitivity to ergosterol biosynthesis inhibitors (EBIs) of Monilia fructicola causing brown rot and blossom blight on peach tree in Korea was investigated every year from 1998 to 2000. Against difenoconazole, the isolates isolated Kyongsan and Changdo showed wider range of EC50 values than those of isolates from Chochiyouon and Youngduk. However, the mean EC50 values of isolates were not statistically different between orchards treated with EBIs (difenoconazole, etc.) and poorly managed. The relative mycelial growth of most isolates of poorly-managed orchards on fungicide-medium (0.03 µg a.i./mL) was less than 50% of the growth on a fungicide-free medium during the same period. But, several isolates isolated from orchards treated with EBIs such as difenoconazole, bitertanol, and hexaconazole grew more than 60% on fungicide-medium (0.03 µg a.i./mL) compared to the growth on fungicide-free medium. In 2000, especially, the isolates of relative growth higher than 90% were detected. The isolate high EC50 values to one fungicide also showed a high value against other fungicides. The results suggested that the slight shift of sensitivity to EBIs of M. fructicola could be a potential risk in control of brown rot and blossom blight on peach trees with the fungicides in Korea.

Occurrence of pepper anthracnose fungus resistant to fungicides. Geum-jeong Kim', Yoon-Hee Kim', Ji-hee Jeong', Jae-choon Yi', Heung Tae Kim', and Byongjin Chal. 1Dept. of Agricultural Biology, Chungbuk National University, Cheongju 361-763, Korea; 2Screening Division, Korea Research Institute of Chemical Technology, Taejon 305-600, Korea.

More than 300 isolates of anthracnose fungi Colletotrichum spp. were isolated from typical anthracnose lesions on pepper fruit collected from pepper fields all over Chungbuk-do and their resistance several fungicides was investigated. To check the resistance, the media were amended with 100 µg a.i./mL of each of 9 fungicides: benomyl (BEN), thiophanate-methyl (THI), carbendazim (CAR), hexaconazol (HEX), nuiramol (NUA), fenamidol (FEN), iprodione (IBP), chlorothalonil (CHL), and folpet (FOL). As results, more than 90% of the isolates were thought to be resistant to at least one of these 9 fungicides. Among the fungicides, the rate of resistant isolates was high for benomyl and zero for DMI. Rate of resistant isolates to benomyl was 16.8% for BEN, 29.2% for THI, and 20.2% for CAR. CHL and FOL also showed high resistance and the rates were 59.8 and 75.5%, respectively. On the other hand, no resistant isolate had been detected to HEX, FEN, and NUA. Most of resistant isolates showed multi-resistance to more than one fungicide. Occurrence of resistant isolates to CHL or FOL was not much different among the locality, but the geographical distributions of the isolates resistant to
other fungicides showed difference according to the location.


Effectiveness of inorganic salts in controlling gray blight and anthracnose of tea (Camellia sinensis) were investigated. Six inorganic salts, ammonium bicarbonate, ammonium carbonate, sodium bicarbonate, sodium carbonate, potassium carbonate, and potassium bicarbonate, were tested in vitro at concentrations of 10, 20, 30, 40, 50, 60, 80, and 100 mM for inhibition of mycelial growth and spores germination of gray blight, Pestalotiopsis longiseta and anthracnose of tea, Colletotrichum theae-sinensis. Ammonium carbonate and ammonium bicarbonate, completely inhibited colony formation of P. longiseta at 10 mM and 30 mM within 5 days after colonies were transferred. Potassium carbonate and sodium carbonate inhibited mycelial growth completely at 60 mM and 80 mM. Effect of spores germination on salts was similar to inhibition of mycelial growth. Ammonium carbonate and sodium carbonate completely inhibited colony formation of C. theae-sinensis at 10 mM within 7 days after colonies were transferred. Ammonium bicarbonate, sodium bicarbonate and potassium carbonate inhibited mycelial growth completely at 20 mM. Ammonium carbonate at 20 mM and ammonium bicarbonate at 40 mM reduced disease incidence of gray blight to below 7.3% and 8.7% in the field, respectively. Ammonium bicarbonate and ammonium carbonate at 30 mM reduced disease incidence of anthracnose of tea to below 9.5% and 10.3% in the field, respectively.

Isolation of resistant isolates of Botrytis cinerea to benzimidazoles and development of mepanipirim for management of gray mold. S.J. An¹, H.C. Shin² and H.M. Koo³. ¹Kongju National Univ.; ²Fungicide Research, Central Research Institute, Kyungnong Co., Ltd.

Botrytis cinerea was isolated at greenhouse in Kyungju, Kyungsangbukdo, Korea for research about effective management of gray mold and development of mepanipirim. Existence of fungicide resistance and cross-resistance of Botrytis cinerea among benzimidazoles was examined. Thirty five % and 30% of isolated Botrytis cinerea were RSS and RSR type, respectively, and cross-resistance among same fungicides of benzimidazole class existed. This result showed that cross application of fungicides with different mode of action or of different class fungicides was important for effective management of gray mold. Treatment of mepanipirim in vitro was not effective in inhibition of spore germination but was rather effective in inhibition of mycelium growth of Botrytis cinerea. In vivo, application of mepanipirim on 1st leaf of cucumber inhibited penetration and lesion formation of Botrytis cinerea spores, which were absorbed in paper disk, with various phenotypes to benzimidazole. Thus, mepanipirim effect against gray mold was admitted in the tests and will be possible to manage gray mold in field. When formulation of mepanipirim such as wettable powder and soluble concentration was applied in real fields, control of gray mold was effective.

Distribution of benzimidazole-resistant isolates of Monilinia fructicola causing brown rot and blossom blight on peach in Korea. Tae Heon Lim and Byeongin Cha. Department of Agricultural Biology, Chungbuk National University, Cheongju 361-763, Korea.

The population shift of resistant isolates of Monilinia fructicola to benzimidazole fungicides in peach orchards around Korea was investigated every year from 1998 to 2000. In Chungdo (CD) and Kyongsan (KY), isolation rate of benzimidazole-resistant isolate (BRI) was relatively high compared to Chochiowon (CH) and Yungduk (YU) in this period. In CD and KY, BRI isolation rates were 20.6%, 33.4%, and 29.0% in 1998, 1999, 2000, respectively. However, the rate was less than 4.0% in CH and YU during the same period. BRI also showed cross-resistance against other benzimidazole fungicides. There were not isolates which showing negative cross-resistance to N-phenylcarbamate. Regardless of the year, the isolates having high EC50 more than 500 ug a.i./ml were isolated much frequently in mid season than the rest of year. In poorly-managed orchard of KY, the population of BRI had persisted without much difference for three years. The results suggested that benzimidazole resistance of M. fructicola, as other fungal pathogen, was must be a problem in control of brown rot and blossom blight on peach trees in the region like CD and KY.

Fungicide resistance of Colletotrichum spp. isolated from anthracnose lesions of pepper. Ji Hoe Jeong, Tae Heon Lim, and Byeongin Cha. Dept. of Agricultural Biology, Chungbuk National University, Cheongju 361-763, Korea.

About 100 isolates of Colletotrichum spp. were isolated from typical anthracnose lesions on pepper fruit collected from pepper plantations all over Chungbuk-do and their resistance to 3 fungicides, carbendazim (CAR), fenarimol (FEN), and dithianone (DIT), was investigated in vitro on the media amended with each fungicide. Mean EC50 values of isolates to CAR, DIT, and FEN were 44.03, 4.33, and 0.48 ppm, respectively. The lowest EC50 for each fungicide did not show much difference, but the highest EC50 of CAR was higher than 100 times of FEN. More than 90% of isolates had EC50 of lower than 1 ppm. On the other hand, more than 70% of isolates revealed EC50 of higher than 10 ppm. The standard isolates were selected from each fungicide according to their EC50 and Resistance Factor (RF) was calculated for each isolate by divide the EC50 of the isolate with EC50 of the standard isolate. Almost all isolates had the RF value of higher than 10 to CAR but the values to FEN were quite low in most isolates. When the EC50 values of each isolates to each fungicide were compared together, there was a positive correlation between DIT and FEN (Y=0.097X+0.17, R²=0.7813) and the isolate resistant to DIT had high EC50 to FEN. However, there was no correlation between CAR and DIT, and CAR and FEN.


Internal fruit rot of cucumber was observed in several locations in Korea. Incidence of the disease reached up to 21.5% and averaged 4.2% in the fields surveyed. The disease started at blossom ends of cucumber fruits. Internal tissues of infected fruit tips showed brown discoloration over 2 cm in length and 2 mm in diameter. Subsequently, the brown discoloration was extended into the carpels, and the surface of the infected fruit tips was rugged. Fungal isolates from
the internal tissues of diseased fruits were identified as *Didymella bryoniae* based on mycological characteristics. Aescul were cylindrical to subclavate with 8 spores, and ranged 60.5-90x10.6-15.5 μm in size. Ascospores were hyaline, uniseptate, ellipsoid with shape of mostly rounded ends and slightly constricted at the septum. The size of ascospore ranged 13.2-17.8x4-7.0 μm. Pycnidia were subspherical with dark brown color and sized 50-312x42-247 μm with 127.8x96.0 μm in average. The pycnidiospores were hyaline, cylindrical with rounded ends and averaged 8.2x4.0 μm with a range of 2.5-15x2.5-7.5 μm in size on diseased cucumber fruit. Temperature for mycelial growth of isolates ranged 5-32°C with optimal temperature between 26-28°C. Similar symptoms were developed in the internal part of the cucumber fruit when conidial suspensions of the isolates were inoculated to the flower of cucumber. Furthermore, *Didymella bryoniae* isolates from other plant parts of cucumber, watermelon, oriental melon, melon and pumpkin also showed the similar symptoms in the internal part of cucumber fruits by inoculation tests. Temperature range for occurrence of internal fruit rot of cucumber was 10-32°C with optimal temperature of 25-28°C. This is first report that *Didymella bryoniae* causes internal fruit rot of cucumber in Korea.

The nucleotide sequence of the coat protein genes of stem groove

*Development of the integrated disease forecasting model for control of apple blotch (Diplloecarpum mali)*. Jung Han Park, Kyoung Suk Han and Young Mun Choi. Div. of Horticultural Environment, National Horticultural Research Institute, RDA, Suwon 441-440, Korea.

Apple blotch outbreaks severely when increase of the number of rain-

*Phytophthora capsici* species and other fungi. Also, these PCR primers
detected *P. capsici* from infected pepper leaves artificially, and
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the resistance capacity of potential and current rice cultivars in Vietnam. Future research aims to identify a suitable diversification pattern of rice growing to minimize disease injury, achieve partial resistance and to meet IPM practices.


The effect of ozonated water on *Phomopsis* sp. was investigated with germination of the fungal conidia. The germination was inhibited by the water containing dissolved ozone concentration (DOC) of 0.1, 0.5, 1.0 and 2.0 ppm for 1- to 5-min exposure times. Spore inhibition was directly related with DOC and exposure times. Inhibition values over 90% for conidial germination was found at 0.1 ppm DOC for 5-min exposure and 0.5 ppm DOC for 1-min exposure, respectively. In particular, no spore was germinated in the water containing over 1 ppm DOC during the exposure times. When kiwifruits naturally infected in the field were treated with the ozonated water of 0.1 ppm DOC for 1 hr and then ripened at 25°C incubator for 2 weeks, total number of lesions and average lesion size of the treated kiwifruits were significantly decreased as much as 60% and 23%, respectively, compared with those treated with distilled water alone. This result suggests that ozonated water treatment can be implemented to IPM for soft rot disease of kiwifruits caused by *Phomopsis* sp. during post-harvest storage.

Occurrence and control of powdery scab caused by *Spongospora subterranea*. Joon-Soon Kim, Jong-Tae Kim and Young-II Hahn. Crop Division, National Alpine Agricultural Experiment Station, Pyungchang 232-955, Korea.

Several plants with root galls were found in greenhouse in Alpine area. A large amount of spore balls were observed in a root gall by the electron microscope. Spore balls were 30-80 µm in diam. and had irregular channels. Ten cultivars of disease free tubers were planted in greenhouse and field for resistance test. Jowon and Atlantic had no observed symptoms on the tubers. Slightly resistant cultivars were Superior, Jopung and Jashim. Gawon and other cultivars were susceptible. An experiment was performed to find chemicals to control tuber infection in Alpine area in 2001. Soil treatment of Fluazinam WP was most effective and Fluazinam D. and Flusulfimate D. were not significantly different to non-treatment.


Cultivation of sweet pepper (paprika) began with 10 ha in 1990 and increased rapidly to 135 ha in 2000 in Korea. The plant that is considered as a promising export crop is growing mainly in the aggregated rock-wool or perlite hydroponic system in the country. However, its cultivation is endangered by *Phytophthora capsici* in many farms. Incidence of the diseased plant was averaged 8.8% among surveyed farms and the disease was relatively severe in mono-cropping farms with increasing cultivation years. Primary inoculums were found to be sourced either from the nursery soil or flooding water. Plants growing in the system were highly susceptible to the pathogen showing 100% infection rate at 13, 11, 9 days after inoculation with the fungal sporangia at 10^7, 10^5, and 10^5/mL, respectively. Totally 17 chemicals registered for control of the pepper blight were evaluated for their control effect in the greenhouse. Among the chemicals, 15 showed control effect with different degrees, while, 6 showed severe chemical injury at double-strength concentration. Ethabinox WP, ethaboxam +dimethomorph SC, ethaboxam+ triflumizol WP, and dimethomorph+ copper oxychloride were found to be the most effective to control the disease either by pre- or post-infection trenching into the rock-wool system. In addition, those chemicals did not induce any chemical damage symptom. Further ecological studies on the causal pathogen and field applications of the chemicals are progressing.


This study was conducted to determine the effects of crop rotation on the development of clubroot disease of Chinese cabbage and the population density of *Plasmiodiophora brassicae* in artificially infested experimental fields at Suwon and Icheon from 1998 through 2000. Cropping sequences were (i) continuous monoculture of Chinese cabbage for 2 yr (1998 through 1999); (ii) Chinese cabbage(0.5 yr)-shallow(0.5 yr)-astragalus(1 yr); (iii) Chinese cabbage(1 yr)-a resistant cv. of radish(1 yr); (iv) Chinese cabbage(0.5 yr)-shallow(1.5 yr); (v) Chinese cabbage(0.5 yr)-shallow(0.5 yr)-Welsh onion(1 yr). In 2000, Chinese cabbages were planted in all plots. The resistant radish and Welsh onion showed more than 50% of mean control value compared to the continuous monoculture of Chinese cabbage. Although astragalus showed 46.9% mean control value, the effect was unstable depending on the location and the cropping season, showing reduced yield of Chinese cabbage. The effect of resistant radish on the suppressiveness of the pathogen's population was prominent at both Suwon and Icheon. Welsh onion revealed to be suppressive at Suwon, but not at Icheon. These results suggested that the resistant radish and Welsh onion may be promising rotational crops for the control of clubroot disease.


We conducted to screen resistant varieties or lines of Chinese cabbage, radish, and cabbage to *Plasmiodiophora brassicae* for three years, from 1998 through 2000. The fields located at Suwon, Icheon, Pyeongtaek, Yeoncheon, and Pyongchang were repeatedly used in both spring and fall cropping seasons during experimental periods. Among fifty-five varieties of Chinese cabbage tested, there were two resistant and 20 moderate resistant varieties in the first year. In the
second year, only one resistant and 5 moderate resistant varieties among 17 which showed resistance or moderate resistance in the first year. In third year, there were only five moderate resistant varieties without any resistant varieties among selected ones in 1999. Among 58 radish varieties tested, 26 resistant and 9 moderate resistant varieties were selected in the first year. However, there were three resistant and 21 moderate resistant ones in the third year, showing similar response with Chinese cabbage. In case of cabbage, there were only one moderate resistant variety among 26 varieties tested for three years experiment. Our results suggested that the resistance crucifer crops to clubroot disease might decline to deteriorate in the field conditions over years.

**Biological control agent of post-harvest diseases in mandarin.**
Sung-Won Choi, Ja-Tae Kim, Ki-Suk Doh, Young-Bum Kim and Kee-Hyun Choi, Green Biotech Co., Ltd., 45-70 Tadjang-ri, Gyoja-myeon, Paju 413-830, Korea.

Post-harvest diseases of fruit cause 15 to 25% loses yearly in the fruit industry worldwide. Fungicides, the major weapon in controlling the disease, are often ineffective and pose hazards to human and the environment. Therefore, a critical need exists for new methods to control post-harvest diseases without posing such hazards to human or environment. The antifungal strain GB-0365 and GB-017 was showed prominently antagonism against mandarin pathogens, which in *Penicillium sp.*, *Fusarium sp.* and *Alterhenza sp.* The strain GB-0365 and GB-017 was identified as *Balbica subtilis* and *Balbica lentimobus*. As the result of the large scale field application, the rate of decay was considerably decreased than control.


A novel fungal strain of *Amelomycos quisquilis* 94013 was reported to be a hyperparasite of the causative fungi of powdery mildew in agricultural plants. *Amelomycos quisquilis* 94013 was liquid fermented large scale. Following fermentation, a suspension of conidia is subjected to a series of down stream processes. It was formulated as a wettable powder and termed Green-all AQ by Green Biotech Co., Ltd. Field trials were conducted to investigate the efficacy of Green-all AQ. Plants were sprayed with 0.1% Green-all AQ. In strawberry at Nonsan on 11 April 2001, 7 days after the third application, powdery mildew (Sphacotheca fuscata) incidence on fruits was 15.8% for untreated, 5.1% for Green-all AQ, and 5.5% for Sanco EC, respectively. In *Ligularia fischeri* (Ledebe) Turez at Icheon on 21 June 2001, 7 days after the third application, powdery mildew (Sphacotheca fuscata) severity on leaf surfaces was 96.7% for untreated, 13.3% for Green-all AQ, and 4.3% for Azoxystrobins SC, respectively. In grapes at Kunwi on 7 May 2001, 7 days after the third application, powdery mildew (Uncinula necator) incidence on fruits was 70% for untreated, 15% for Green-all AQ, respectively. In conclusion, Green-all AQ could be used effectively as a biofungicide for controlling powdery mildew on various crops.

**Trichoderma harzianum YC459 (TORY) for the control of grey mold of vegetable greenhouse crops.** S.K. Lee, H.B. Sohn and Y.R. Chung, Division of Applied Life Sciences, Kyungsoe National University, Chinchu 660-701, Korea.

**Trichoderma harzianum** YC459, isolated from saw dust compost, was found to be effective against *Botrytis cinerea* under commercial conditions on greenhouse crops. It was developed as a formulated product as TORY (20%, wettable powder) and is marketed in Korea for the control of grey mold on greenhouse tomatoes, strawberry, cucumber, and hot pepper. In greenhouse experiments, the efficacy of TORY was significantly better than that of the fungicide, procymidine. The formula with the conidial populations from 10^7 to 10^8 cfu/g dw had the mean control value of 70% in cucumber pot tests. Mixing of one to eight grams TORY (10^7 cfu/g dw) granules with one liter nursery container soil mix at seeding time produced synergistic effects in the control of grey mold with leaf spraying of wettable powder. Survival of conidia in the formula was good enough (10^6 cfu/g dw) until one year after preparation at room temperature.

**Distribution estimation of initial appearance of potato late blight based on meteorological information.** J.H. Ahn and Y.I. Hahm, National Alpine Agricultural Experiment Station, RDA, Pyongchang 232-950, Korea.

Potato late blight (*Phytophthora infestans*) has been known as a very serious disease in the alpine area located 800m above the sea level, one of the alpine region where most of seed potatoes are produced in Korea. To develop spatial analysis system of forecasting information, late blight data and weather information from Daegeum-yewong Weather Station were analysed and processed together. The conventional forecasting system, BLITECAST and the moving average method (NAAESCAS) developed by National Alpine Agricultural Experiment Station were applied in alpine area.

A computer system to estimate initial appearance distribution of late blight based on meteorological information was developed. Real time climatic data from the automated weather stations and daily climatic normals were analyzed and graphed in text and graphic file by program on 1km x 1km grid cell basis. Warning area distribution of initial appearance of late blight calculated from real time climatic data and two developed forecasting model were graphed on GIS and 1km x 1km mesh map.

**Selection and efficacy of soil bacteria for induced systemic resistance to Colletotrichum orbiculare on cucumber.** Min Sun Kwack and Ki Deok Kim, Department of Agricultural Biology, Korea University, Seoul 136-701, Korea; Division of Bioindustrial Science, Korea University, Seoul 136-701, Korea.

Soil bacteria inducing systemic resistance to *Colletotrichum orbiculare* on cucumber plants were selected for the purpose of biocontrol of the fungal pathogen. Sixty-four bacterial strains inhibiting the mycelial growth of eight fungal plant pathogens on the V8 juice agar were selected out of 1,400 bacteria isolated from various soils of Ansan, Chunan, Koyang, and Paju in Korea. Seeds (cv. Bae-knoodalagi) were sown in a potting mixture incorporated with the selected soil bacteria at a rate of 1 mL of cells adjusted to A600=0.5 per gram of the mixture. Two-week-old plants then transplanted into the steam-sterilized soil. Three-leaf-stage plants (about 24 days old) were inoculated with conidial suspension (5x10^7 conidia/mL) of *C. orbiculare*. Diseased leaf area (%) and number of lesions per cm² leaf were determined on third leaves of the cucumber 5 or 6 days after
inoculation. Nine soil bacterial strains, GC-B19, GC-B35, GK-B18, MM-B22, PK-B14, RC-B41, RC-B64, RC-B65, and RC-B77 with significant (P<0.05) disease reduction compared to the untreated control were selected for further experiments. After repeated experiments using the strains, strains MM-B22, RC-B65, and RC-B77 expressed significant (P<0.05) reduction of diseased leaf area (%) compared to the untreated control on third leaves of cucumber. Further research on expression of induced systemic resistance on cucumber using these strains against C. orbiculate is currently conducted.

**Biocontrol of Phytophthora capsici on pepper with rhizobacteria isolated from rhizosphere and rhizoplane of cucumber, pepper, and tomato grown in the field.** Hye Soon Kim¹ and Ki Deok Kim². ¹Department of Agricultural Biology, and ²Division of Biiodustrial Science, Korea University, Seoul 136-710, Korea.

Rhizobacteria as potential biocontrol agents against Phytophthora blight of pepper were isolated from soils of rhizosphere and rhizoplane of cucumber, pepper, and tomato grown in the field of 13 areas, Changnyong, Chiniu, Chungyang, Chunchon, Haman, Kimhae, Kurye, Kwangju, Namyangju, Puyo, Sacheon, Suchon, and Yangsan in Korea from 2000 to 2001. One-hundred-one bacterial strains were pre-screened from about 300 strains using in vitro seed rot test. The seed rot test was conducted with germinated pepper seeds (cv. Nokwang) dipped in bacterial suspension for 3 hours that were placed near the edges of mycelia of P. capsici grown on water agar amended with 0.02% glucose for 5 days. The selected strains were treated again in pepper seedlings grown in 9-cm² pots containing a potting mixture amended the seed cells (1 ml of cells adjusted to A₅₉₀ = 0.5 per gram of potting mixture). After 2 weeks, the seedlings were inoculated with P. capsici and 15 bacterial strains suppressed damping-off of the seedlings caused by the fungus. These 15 bacterial strains were subject to test with 5-week-old pepper plants that were inoculated with zoospores of P. capsici. From these tests, four bacterial strains, KJ1R5, KJ2C12, KJ9C8, and 11S16 showed significant (P<0.05) reduction in disease severity compared with the untreated, inoculated controls.

**Screening of effective fungicides and determination of their optimal application time for the control of the fruit rots diseases of kiwifruits.** J.G. Lee¹, D.M. Park², J.S. Hur² and Y.J. Koh³. ¹Dept. Applied Biology, Sunchon National University, 315 Maegok-dong, Sunchon 540-742, Korea; ²Namhae Sub-Institute, National Horticultural Research Institute, Namhae 668-812, Korea; ³Dept. Environmental Education, Sunchon National University, 315 Maegok-dong, Sunchon 540-742, Korea.

Fruit rots of kiwifruits are caused mainly by Botryosphaeria dothidea and Disporthea actinioides. Although the fruit rots diseases of kiwifruits usually occur after harvest, i.e., during storage, transportation, marketing and consumption, fruits were found to be infected with the causal organisms from June to August while in orchards. Therefore, it is important to prevent the infection on the fruits during the growing season of kiwifruits by spraying effective fungicides. To date, Benomyl WP and Thiophanate WP are the only registered fungicides in Korea for the control of the fruit diseases of kiwifruits. This study was performed to screen effective fungicides which can substitute for Benomyl WP and Thiophanate WP and to find their optimal application time. Among the several candidate fungicides tested, Vicloxolin WP, Iprodione WP, Tebuconazole WP, and Flusilazole WP effectively inhibited the mycelial growths of the causal organisms on laboratory media. Flusilazole WP, Iprodione WP, and Tebuconazole WP were more effective than Thiophanate WP and as effective as Benomyl WP when sprayed five times at 10-day-intervals from June 6. The control efficacies of the fungicides varied depending upon the spray schedule and applying the fungicides around June 26 was found to be most effective. Flusilazole WP, Iprodione WP, and Tebuconazole WP are recommended as potential fungicides which could substitute for Benomyl WP and Thiophanate WP in controlling the fruit rots diseases of kiwifruits.

**Biological control of Fusarium crown and root rot of tomato by use of endophytic bacteria.** Jong Tae Kim¹, In Hee Park¹, Young Ki Lee¹, Jeom Soon Kim¹ and Young Il Hahn¹. ¹Crop Division, National Alpine Agricultural Experiment Station, RDA, Pyungchang 232-950, Korea; ²Buyeo Tomato Experiment Station, ARES, Buyeo 414-810, Korea; ³National Institute of Agricultural Science and Technology, Suwon 441-707, Korea.

One hundred-ninety-eight bacterial strains isolated from internal tissues of tomato roots and stems were screened for the potential of biological control against crown and root rot of tomato caused by Fusarium oxysporum Schlecht. f. sp. radicis-lycopersici Jarvis & Shoemaker (FORL). Tomato seedlings were bacterized at 15 days after planting by root-dipping method with cell suspensions of each strain. Ten days later, the plants were inoculated by adding 30mL with microconidia of the pathogen. Thirty days after pathogen inoculation, symptom expression was evaluated by rating the disease severity with 0 to 4. Eight strains have shown potential as effective biological agents for control of FORL that reduced the disease severity below 1. These strains were DYT-03, PYT-40, PYT-86, PYT-93, PYT-143, HST-156, and GST-188, which were identified as Bacillus lentimorbus, Alcaligenes xylosoxydans, Stenotrophomonas maltophilia, B. lentimorbus, B. lentimorbus, B. subtilis, and Pseudomonas chlororaphis, respectively. In addition, the antagonistic effect of these strains were evaluated through in vitro on potato dextrose agar against F. oxysporum f. sp. lycopersici race 1, 2, F. oxysporum f. sp radicis-lycopersici, Verticillium dahliae, V. albo-atrum, Colletotrichum coccos, Pyrenochaeta lycopersici, Rhizoctonia solani, Sclerotinia sclerotiorum, Sclerotium rolfsii, Alternaria solanum, and Botrytis cinerea. Six bacterial strains (DYT-03, PYT-40, PYT-86, PYT-87, PYT-93, PYT-143) made strong inhibition for the mycelial growth of the above pathogens, but two strains (HST-156, GST-188) could not.

**Web based IPM system for Japanese pear diseases in Japan. I. Farming Diary System for farmers’ decision making.** K. Sugahara¹, K. Tanaka¹, M. Laurenerson¹, T. Ohtani¹, T. Watanabe¹ and S. Umemoz¹. ¹National Agricultural Research Center, Tsukuba, Ibaraki 305-8666, Japan; ²Chiba Prefectural Agriculture Research Center, Chiba 266-0006, Japan; ³Chiba Prefectural Agricultural Junior College, Togane, Chiba 283-0001, Japan.

Japanese pear (nashi) scab is one of the most serious diseases of nashi in Japan. To suppress the scab infections, fungicides are usually applied on a calendar based spray schedule, more than 10 times during a growing season. Increasing environmental concerns make such frequent spraying unacceptable, so accurate timing of fungicide applications has become more important. Combining alternative strategies such as cultural practices and optimally timed chemical sprays is a
practical way to control nashi scab. We propose a decision support system assisted by the Internet. It is designed to help farmers and extension officers make decisions about the effective timing of chemical sprays in their orchards. The system consists of several sub-systems, 1) a knowledge base regarding nashi scab control, 2) simulation models which can forecast the flowering period and the probability of scab infection associated with local weather data, 3) a model of effective fungicide residue, 4) weather data acquisition and retrieval system, and 5) a Web application, Farming Diary System. Farming Diary System enables users to input and retrieve the records of farm management and plant condition using Internet-enabled mobile phones, which are widely distributed and most convenient data-handling devices in Japan. Farmers can access the system in their orchards and immediately acquire information to help their decision making.

Web based IPM system for Japanese pear diseases in Japan. II. Forecasting models for scab infection associated with weather retrieval system. K. Tanaka1, M. Laurenson1, K. Sugahara1, T. Ohtani2, T. Watanebe1 and S. Umemoto1. 1National Agricultural Research Center, Tsukuba, Ibaraki 305-8666, Japan; 2Chiba Prefectural Agriculture Research Center, Chiba 266-0006, Japan; 3Chiba Prefectural Agricultural Junior College, Togane, Chiba 283-0001, Japan

To support farmers' and extension staffs' decision making related to fungicide spray against Japanese pear scab, two forecasting models have been developed and implemented for use on the Internet. The first model can estimate flowering date, which is used to time the first and most important fungicide application in the growing season. The flowering date is highly correlated with the maximum density of airborne ascospores. The second model can forecast scab infection probability on pear leaves, as a function of temperature and leaf wetness duration. Two methods are available for estimating the leaf wetness duration, measurement by wetness sensor and calculation from other weather variables, such as rainfall and humidity. The models are written in Java, and can run on most computers' Web browser (http://cse.naro.aaffrc.go.jp/ketanaka/model). Both weather sources, local weather data collected by users and database system maintained by some organizations, are available to the models through the MetBroker. MetBroker (http://www.agmonitor.net/MetBroker/) enables us to access the different kinds of weather database linked with this system. We can incorporate either kind of weather data to forecast site-specific scab infection risk and to optimize spray timing.

Web based IPM system for Japanese pear diseases in Japan. III. Weather data acquisition system to estimate leaf wetness duration and scab infection severity. T. Ohtani1, K. Sugahara2, K. Tanaka3, M. Laurenson4, T. Watanebe1 and S. Umemoto3. 1Chiba Prefectural Agriculture Research Center, Chiba 266-0006, Japan; 2National Agricultural Research Center, Tsukuba, Ibaraki 305-8666, Japan; 3Chiba Prefectural Agricultural Junior College, Togane, Chiba 283-0001, Japan

The duration of wetness on leaves is one of the most important factors associated with the nashi scab infection. We set weather sensors within and outside the canopy of an orchard to estimate leaf wetness duration and utilize those data for the scab infection model. Two sets of electronically wetness sensors, flat plate and cylindrical type, were mounted both inside and outside orchard. Temperature (dry and wet bulb), radiation, and wind speed, are also monitored within orchard. Those sensors are connected with a data logger, CR10X, and the weather data were logged at 15 minutes intervals. A laptop PC connected to the logger downloads data every morning automatically and sends it to an office PC, using e-mail over a 64Kbps Personal Handy phone system (PHS)-based dial-up Internet link. Weather data is in turn automatically transferred from the office PC to the weather server, and stored in a web-accessible database. A new leaf wetness estimation procedure was developed, that estimates "wetness" using rainfall and humidity data, from April to the half of May, which is the most important period to protect against the nashi scab. This procedure can be used to estimate leaf wetness where wetness sensor is not available. Mills' model and Dufhie's model were proposed to express nashi scab infection severity by wetness duration and temperature.


A prediction model (BLAST) simulating the disease cycles of rice leaf blast based on near real-time weather data was developed and evaluated for its accuracy in predicting leaf blast progression. Within the BLAST model, sub-models describing effects of hourly and daily weather conditions on sequential stages of the disease cycle were included along with cultivar-related components affecting disease development. Disease onset was simulated based on initial airborne spore density, which was calculated from favorable weather conditions for conidiphore formation. BLAST estimated sporulation, spore dispersal and deposition, penetration and colonization using hourly weather data. Latent period and lesion growth were estimated using daily weather data. Number of conidiphores and spores on individual leaves were estimated from the area of lesions of the previous infection cycles. Dispersal and deposition of spores were calculated for each 10 cm air layer within canopy based on the gradient transfer theory. Upon deposition, the spores on each leaf underwent penetration and colonization and then formed lesions after latent period. In addition, defoliation due to sheath blight, cultivar-specific susceptibility, and daily changes in adult plant resistance were also estimated and incorporated to improve accuracy in prediction of leaf blast severity. Disease severity was monitored at an experimental paddy in Hwasong, Korea at 3 to 10-day intervals during 1995-1999. Four rice cultivars were transplanted 3 or 4 times at 15-day intervals to evaluate cultivar specific susceptibility and adult plant resistance. Field data suggested that BLAST could be used for rice blast management although its accuracy in prediction of leaf blast severity still needs to be improved.

Determination of weather conditions favorable for development of Fusarium head blight in Korea. W. Kang1, E.W. Park1 and D.H. Shon1. 1Seoul National University, Suwon 441-744, Korea; 2Korea Food Research Institute, Seongnam 463-746, Korea.

Relationships between weather conditions and Fusarium head blight occurrence on 'Olbori' barley were analyzed using meteorological and disease records from 7 locations in Korea during the period from 1975 to 1995 except 1980. Among the 20 year data, only 12 data sets with more than 1% spikes being diseased were in this study. A total of 801 independent variables representing occurrence of certain daily weather conditions within various windows of time-frame during barley growing seasons were generated based on daily weather data like
air temperature, relative humidity, and rainfall. Considering subjective variations in disease ratings between years and locations, the amount of disease occurrence each year was standardized into 6 levels: 0 = no spikes diseased; 1 = 0-1%; 2 = 1-5%; 3 = 6-10%; 4 = 11-20%; and 5 = 21-100% of spikes diseased. Linear regression analyses between the independent variables and disease index suggested that frequent occurrence of daily average of RH > 85% and temperature > 16°C, and total rainfall > 5 mm in the early milk stage was critical for the disease to occur on upland barley. However, frequent occurrence of more than 8 mm rainfall during the late heading stage appeared important for the disease to occur on lowland barley, which were planted to drained paddy fields after rice harvest. In this study, a computer program for automatically replacing independent variables in regression models was developed in order to run regression analyses using 801 independent variables from varying windows of time frame during the entire barley growing season.

Evaluation of BC5F4 lines of crosses for incorporation of resistance to Phytophthora capsici into land races of pepper grown in Gyeongbuk province. D.W. Kim, Y.K. Kim, and B.S. Kim, Department of Horticulture, Kyungpook National University, Daegu 702-701, Korea.

BC5F4 lines of crosses between two land races of pepper, Chilsung and Subi, and resistance sources to Phytophthora capsici, AC2258 and SCM334, were tested for resistance to P. capsici at seedling stage. The selected plants were transplanted to the experimental net houses in Youngyang county and their horticultural characteristics were evaluated. BC5F4 lines of the cross Chilsung x AC2258 and Chilsung x SCM335 appeared to have recovered the horticultural characteristics of the recurrent parent. However, the BC5F4 lines of the cross Subi x AC2258 were relatively low in resistance to P. capsici and horticultural characteristics appeared not to be acceptable to farmers. Bacterial wilt interfered in the experimental field in Ilwol, Youngyang. Therefore, introduction of resistance to bacterial wilt would be also necessary.

Potential of a local-area agricultural weather simulation system (LAWS), for disease forecasting. R.D. Magarey1, R.C. Seem2, and J.W. Zack3, Cornell University, NYSAES, Geneva, NY 14456, USA; 3MESO, Inc, Troy, NY 12180, USA.

Weather data is important for disease forecasting. In this study, we examine a numerical atmospheric model that has potential to provide weather data at spatial scales of less than 1 km (<100 ha). The model is the Local-area Agricultural Weather Simulation System (LAWS). LAWS was developed from the MASS mesoscale model making simplifications to enable greater computational speed. The inputs to LAWS are upper air data, digital elevation, soil texture class, NDVI and landuse. In field validations, the LAWS model was compared to spatial interpolations made from hourly National Weather Service observations, (NWS-SIMOBS) and from the Finger Lakes district weather station network (FLV-SIMOBS). Comparisons were made to temperature records collected in 22 vineyards throughout the Finger Lakes, NY district (district scale) and to 12 loggers in a lake side transect (farm scale). The accuracy of the LAWS model was lower than spatial interpolations at the district scale. At the farm scale, LAWS had a comparable accuracy to the FLV-SIMOBS. However, at both scales the spatial interpolations tended to over-smooth the data, causing a loss of ability to determine temperature variation at the farm scale. The model can also be used to estimate other variables that are important for disease forecasting, such as surface wetness duration. At present the model requires approximately 8-12 hours to execute on a mid-priced workstation, but computer processing speeds are rapidly increasing.


Rice bacterial grain rot disease distribution in the field and the relationship between the weather conditions during the rice heading period and the disease occurrence were investigated to make the predict model of disease development in the field. Disease development with inoculation concentration and wetting periods were investigated. When the wetting periods were over 24 hours, rice panicles were diseased in 10^4 cfu/mL. The higher inoculated, the severer disease developed. In the non-wetting, disease was not developed except 12.6% of 10^4 cfu/mL. The ripened grains and 1000 grains weight were remarkably decreased with increasing the diseased panicles. The meteorological variables were processed in a time during 7 days from 3 days before heading date. Average of minimum temperature (MIN) and rainfall days (RAIN) was the variables that showed the association with disease incidence (RBG). Equation of RBG% = -12.99+0.65MIN+0.10RAIN (R^2=0.7626) was expressed using the data in 1998. Using this equation in 2000, the predicted values were higher than the actual disease incidence.


A computer program named EgyBlight was designed and tested using computer laboratory conditions to forecast late blight (Phytophthora infestans) occurrence and timing fungicide applications. EgyBlight is an integral linking and modification of late blight forecasting methods based on short-term observations over several potato growing seasons, analyzing the correlation between microclimatic data collected throughout real time automatic weather station (Adcon A733), and its effect on daily infection potential of late blight (DIP). In 1998 and 1999, EgyBlight correctly forecast potato late blight occurrence and totally reduced 75% of fungicides cost necessary for control under experimental conditions. The basic roles of system analysis for the model validation are discussed.

Ultastructural observations of vascular bundle in the leaves of cucumber plants untreated and treated with DL-3-amino butyric acid. Y.C. Jeon and E.W. Park, National Instrumentation Center for Environmental Management, Seoul National University, Suwon 441-744, Korea.

DL-3-amino butyric acid (BABA) is well known as one of the chemical activators inducing resistance against several plant pathogens. It has previously been shown that pre-treatment with BABA in the cucumber plants caused the decrease of disease severity after inoculation with Colletotrichum orbiculare. Also, the increase of salicylic acid (SA) level in the leaves of BABA-treated cucumber plants was reported. The accumulation of SA is necessary to trigger resistance in...
many host-pathogen interactions. However, the signal pathway of resistance mediated by BABA is not yet clearly illustrated. In this study, ultrastructures of the vascular bundle were observed in the leaves of untreated as well as BABA-treated cucumber plants using an electron microscope. The vascular bundle of the leaves was consistent of the tracheary elements of xylem, sieve elements and companion cells which contain the numerous large mitochondria and the abundant of small vacuoles. The plasmodesmata, through which the photosynthetic are transferred, was observed between the companion cells and the parenchyma cells. The ultrastructures of vascular bundle in the leaves of BABA-treated plants were similar with those of the untreated plants except the plasmodesmata. In the BABA-treated plants the plasmodesmata were numerous more formed compared to those of untreated plants. Based on these results it is suggested that the treatment with BABA may cause the activation of transfer system of vascular bundle, which may contribute to the translocation of the unknown signal compound triggering resistance.

Increase in resistance to leaf blast as accumulation of silicon in rice leaf tissues. Sang Gyu Kim1, Ki Woo Kim2 and Eun Woo Park1.

1School of Agricultural Biotechnology, Seoul National University, Suwon 441-744, Korea; 2Department of Plant Pathology, Washington State University, Pullman, Washington 99164-6430 USA.

Silicon is implicated as a factor influencing the degree of plant resistance to biotic or abiotic stresses. A cytological mechanism of silicon-induced resistance to blast was investigated in rice leaves by electron microscopy and X-ray microanalysis. A susceptible cv. Jimin and a partially-resistant cv. Hwaseong were grown under a hydroponic culture system with nutrient solution containing 0, 50, 100, and 200 ppm of silicon, respectively. Electron-dense silica layers were frequently found in epidermal cell walls of silicon-treated plants. Silicon was detected in outer regions of epidermal cell walls and the thickness of silica layers increased gradually with the increasing silicon concentrations. TEM/EDS revealed that silicon existed mainly in epidermal cell wall and middle lamella. SEM/EDS indicated that silicon was present in both trichomes and wart-like protuberances. Epidermal regions without wart-like protuberances, and stomatal guard cells also accumulated appreciable amounts of silicon. Quantitative analysis of silicon on leaf surface by WDS indicated that higher amounts of silicon exist in silicon-treated plants than in control plants. Amount of silicon deposition on rice leaves was affected by silicon applications, and silicon accumulation was greater in Jimin (4.05-5.54%) than Hwaseong (3.52-3.88%). Adult plant resistance to leaf blast may be partially explained by increase in silicon accumulation on epidermal cell wall due to silicon uptake by rice plants.

Evaluation of virulence on adlay of Korean isolates of Bipolaris coicis using an infection response rating scale. Seog Won Chang1 and Byung Kook Hwang2. 1Northern Agricultural Research Station, Gyeonggi Agricultural Research and Extension Services, Yondhon 486-833, Korea; 2Laboratory of Molecular Plant Pathology, College of Life and Environmental Sciences, Korea University, Seoul 136-701, Korea.

The virulence of 33 isolates of Bipolaris coicis from diverse geographic origins in Korea was evaluated on 6 adlay cultivars or lines under controlled environmental conditions. To evaluate infection levels of B. coicis isolates in adlay plants, a ten-class infection response (IR) rating scale was developed. The IR rating scale was based on the type and relative size of lesions on adlay leaves infected by B. coicis. Significant differences in virulence were found among isolates of B. coicis, although the differences were quantitative rather than qualitative. All isolates of B. coicis, except KG-9515, were pathogenic on adlay plants. Isolate BC-20136 was the most virulent capable of causing highly susceptible reactions on the adlay cultivars or lines. Significant differences in levels of resistance were found among 6 adlay cultivars or lines tested. A correlation was found between infection responses and percent diseased leaf areas on adlay plants. The polynomial regression model explained 78% of the variation in the percent diseased leaf area as a function of the infection response. The IR rating scale may be a reliable criterion to evaluate a large number of adlays or B. coicis isolates for resistance to leaf blast or virulence to adlays.

Molecular characterization of a calcineurin gene, MgCNA1, in Magnaporthe grisea. J.H. Choi1, Y. Kim1 and Y.H. Lee1. 1Dept. Agricultural Biology, Seoul National University, Suwon 441-744, Korea; 2Dept. Botany & Plant Pathology, Purdue University West Lafayette, Indiana 47907, USA.

Magnaporthe grisea, the causal agent of the rice blast, differentiates a specialized infection structure called an appressorium that is crucial for host plant penetration. Pharmacological data suggested that calcium/calcmodulin-dependent signaling involved in appressorium formation in this fungus. Calcineurin is a low molecular weight protein that participates in the Ca2+/calcmodulin signaling system in eukaryotes. To understand the molecular mechanisms in signaling system on appressorium formation, calcineurin gene (MgCNA1) of M. grisea was cloned from the strain 70-15 using PCR-based strategy. Sequence analysis showed that calcineurin gene contains a putative 1,683 bp open reading frame with calcmodulin-binding domain and auto-inhibitory domain. DNA blot analysis indicated that MgCNA1 is present as a single copy in the genome. Transformation of antisense construct of MgCNA1 caused pleiotropic effects on mycelial growth, sporulation, and colony morphology. The effect on infection-related morphogenesis will also be presented.

Complete sequencing of BAC clone containing 100 kb large DNA fragment flanking hrp gene cluster of Korean Xanthomonas oryzae pv. oryzae strain (KACC 11031). Dong-Suk Park, Hee-Wan Kang, Byoung-Moo Lee, Young-Jin Park, Gil-Bock Lee and Seung-Joo Go, Division of Molecular Genetics, National Institute of Agri. Science & Technology, Suwon 441-707, Korea

Korean Xanthomonas oryzae pv. oryzae (Xoo) strain which incites bacterial leaf blight (BLB) on rice, has unique feature that is virulent on rice resistant gene Xa 21. We have constructed BAC library of Xoo genome (Korean strain KACC 11031). BAC clone 4K15 harboring hrp gene cluster with approximately 110 kb insert size were isolated and used for this study. To sequence the insert DNA of the BAC, shotgun library was constructed using the sonicated BAC plasmid (4K15). Shotgun library containing 1000 clones (X10 coverage) with insert size of 1.5-2.5 kb was randomly sequenced and about 11.2 Mb sequences were assembled by Phred & Phrap programs. The sequencing gaps between contigs were filled by PCR techniques. Finally, nucleotide sequences of 100, 250 bp were determined. BLAST Search found hrp gene cluster, IS elements and divers genes flanking it. Furthermore, the sequences of the genes were compared to those from other strains from China and Japan.
Abstract of Presentations

Bacterial artificial chromosome (BAC) based-physical mapping of Xanthomomas oryzae pv. oryzae. Hee-Wan Kang, Dong-Suk Park, Byoung-Moo Lee, Young-Jin Park, Yong-Hwan Kim, Gil-Bok Lee and Seung-Joo Go, Division of Molecular Genetics, National Institute of Agri. Science & Technology, Suwon 441-707, Korea

Xanthomomas oryzae pv. oryzae, the causal agent of bacterial leaf blight (BLB), is the most destructive bacterial disease in rice (Oryza sativa L.) worldwide. Construction of bacterial artificial chromosome (BAC) library has widely been employed in building frameworks for genome-wide physical mappings. We here describe the physical mapping of X. oryzae pv. oryzae based on BACs. BAC library of X. oryzae pv. oryzae was constructed that provides a 36-fold genome coverage based on an estimated genome size of about 5.0 Mb. BAC library contains 1,536 clones with an average insert size of 112 kb. The BAC clones were arrayed on a membrane filters with high-density using Biomak 2000. To generate a physical map, the entire library was fingerprinted with EcoR I and the fingerprinted BACs were assembled into contigs using computer program. The ends of 75 BAC inserts selected from the respective contig assembly groups were sequenced to generate sequence-tagged connectors (STCs) framework. To join and orient the contigs, high-density BAC colony filters and primer generated from BAC end sequences were screened. Based on hybridization and PCR screening, physical map of X. oryzae pv. oryzae genome was constructed and additionally the known or unknown genes in the bacterium were positioned on the BACs.


Xanthomomas oryzae pv. oryzae (Xoo) which incites bacterial leaf blight (BLB) on rice, harbours complex gene clusters including avr, hrf and repetitive elements associated with pathogenicity. Understanding X. oryzae pv. oryzae genome would greatly advance our knowledge of how the bacteria defeat defense mechanisms and cause disease on different hosts. Especially, of diverse strains distributed in world, Korea strains of Xoo is uniquely virulent on resistant gene Xa21. Thus, we have performed X. oryzae pv. oryzae genome project that aim at its complete sequencing and functional analysis. We here describes whole genome sequencing of Korea X. oryzae pv. oryzae strain KACC 11031. To sequence whole genome, shotgun library was constructed using the sonicated genomic DNA fragments (1.6-2.5 kb and 9-15 kb). Shotgun library containing 50,000 clones was randomly sequenced and about 50 Mb sequences were assembled by Phred and Phrap programs. The sequencing information will be discussed.

Expression of three non-specific lipid transfer protein genes in Capsicum annuum are differentially regulated by pathogen attack and environmental stresses. Ho-Won Jung and Byung Kook Hwang. Laboratory of Molecular Plant Pathology, College of Life and Environmental Sciences, Korea University, Seoul 136-701, Korea

The three cDNA (CALP) clones corresponding to non-specific lipid transfer protein (nsLTP) genes were isolated from a pepper cDNA library from hypersensitive response (HR) lesions of leaves infected with an avirulent strain of Xanthomomas campestris pv. vesicatoria. The putative nsLTP proteins deduced from the three clones CALTP1, CALTP2 and CALTP3 have molecular masses of 11,291 Da, 11,563 Da, and 11,595 Da and basic isoelectric points of 8.83, 8.15, and 8.86, respectively. The CALP clones are homologous each other in the coding regions, whereas the S' untranslated regions greatly diverge between the clones. The expression of the three CALP genes were differentially regulated in leaf, stem and fruits following the infection by X. campestris pv. vesicatoria, Phytophthora capsici, and Colletotrichum gloeosporioides. The transcripts of the three CALP genes also were strongly induced in the systemic upper leaves after immunization on lower leaves by X. campestris pv. vesicatoria infection. In situ hybridization results showed that the CALP1 mRNA was localized in phloem cells of vascular tissues in pepper leaf, stem, and fruit tissues after pathogen infection. RNA gel blot analysis revealed that ethylene and methyl jasmonate were effective signal molecules for inducing the transcripts of CALP1 and CALP2 genes in pepper leaves. Expression of the CALP genes was strongly induced in pepper leaves in response to environmental stresses, such as drought, high salt, low temperature, abscisic acid, and wound treatments.

Rhizobacteria-induced resistance also perturbs viral disease progresses in tobacco and triggers defense-related gene expression. Il-Pyung Ahn1, Kyungsook Park2, Young-Seuk Ba3, Hyo-Won Choi4 and Choong-Hoe Kim5. 1Plant Pathology Division, National Institute of Agricultural Science and Technology, Suwon 441-707, Korea; 2Dept. Crop Science, Konkuk University, Seoul 134-701, Korea.

A selected strains of nonpathogenic rhizobacterium EXTN-1, Bacillus amyloliquefaciens, is capable of eliciting broad-spectrum induced systemic resistance (ISR crops that is phenotypically similar to pathogen-induced systemic acquired resistance (SAR)) in several. In tobacco (Nicotiana tabacum cv. Samsun nn), EXTN-1 treatment also perturbs the disease progress by Pepper mild mosaic virus (PMMoV), a member of Tobamovirus group. To investigate the defense mechanisms induced by this rhizobacterium, expression patterns of defense-related genes were analyzed. Pretreatment with EXTN-1 potentiated activation of genes encoding pathogenesis-related (PR) protein, 3-hydroxy-3-methylglutaryl CoA reductase (HMGR) and phenylalanine ammonia-lyase (PAL) genes at the earlier stage compared with those in water-treated mock. Induction of all these genes was subsequently detected in the uninoculated leaves; thus, their expression is associated with the development of both local and systemic resistance. In addition, coordinated reduction of viral genome accumulation was clearly detected in the tobacco leaves pretreated with EXTN-1. All these results suggested that timely recognition and rapid counterattack against the viral invasion, the key differences between incompatible interaction and compatible one, might be stimulated by ISR-inducing rhizobacterium.

Resistance induced by benzothiadiazole perturbs the biotroph-rice interaction, but not necrotroph-rice one. Il-Pyung Ahn1, Soonok Kim2 and Yong-Hwan Lee3. 1Plant Pathology Division, National Institute of Agricultural Science and Technology, Suwon 441-707, Korea; 2School of Agricultural Biotechnology, Seoul National University, Suwon 441-744, Korea.

To investigate the defense mechanisms in rice (Oryza sativa L.), com-
Molecular characterization of an extracellular matrix protein gene, EMP1 of Magnaporthe grisea. Namsook Ahn1, Woobong Choi1, Ralph Dean2, SooNok Kim1 and Yong-Hwan Lee1. 1School of Agricultural Biotechnology, Seoul National University, Suwon 441-744, Korea; 2Fungal Genomics Lab, NCSU, Raleigh, NC 27695-7251, USA.

Magnaporthe grisea, the causal fungus of rice Blast, differentiates a specialized infection structure called an appressorium that is crucial for host plant penetration. A cDNA clone (EMP1) of M. grisea showing a high sequence homology to FEM1 of Fusarium oxysporum encoding extracellular matrix protein was isolated from appressorium-forming stage cDNA library. Sequencing analysis of the corresponding genomic clone revealed that EMP1 contains an open reading frame of 691 nucleotides (intron 64 bps), which encode 208 amino acid residues. The estimated molecular weight was 20.5 kDa with pI of 7.84. Southern blot analysis revealed that the EMP1 exists as a single copy in the haploid genome of M. grisea. Northern blot analysis showed that the EMP1 transcripts are highly accumulated during differentiation of the appressorium. To evaluate the role of EMP1 in fungal pathogenicity and infection-related morphogenesis at the molecular level, experiment of EMP1 knock out is in the progress.

Agrobacterium tumefaciens-mediated transformation of the plant pathogenic fungus, Magnaporthe grisea. H.S. Roh1, S.C. Kang2 and Y.H. Lee1. 1School of Agricultural Biotechnology and Research Center for New Bio-Materials in Agriculture, Seoul National University, Suwon 441-744, Korea; 2Dept. Plant Pathology, The Pennsylvania State University, University Park, PA 16802, USA.

An effective way to study the infection mechanisms of fungal pathogens is to disrupt their genes via transformation, in both targeted and random manners, so as to isolate mutants exhibiting altered virulence. In this paper, we report the successful transformation of Magnaporthe grisea, the causal agent for rice blast, mediated by Agrobacterium tumefaciens, which has long been used to transform a wide variety of plants. Employing the binary vector pBHH2, carrying the bacterial hygromycin B phosphotransferase gene under the control of the Aspergillus nidulans trpC promoter as a selectable marker, led to the production of 500 to 800 hygromycin B-resistant transformants per 1 x 10^6 conidia of M. grisea. Transformation efficiency correlated with the number of A. tumefaciens cells used, pre-treating bacterial cells with acetosyringone prior to co-cultivation with fungal spores, and the duration of co-cultivation. All transformants tested remained mitotically stable, maintaining their hygromycin B resistance after several generations of growth in the absence of hygromycin B. Genomic Southern blot analysis showed that over 60% of the transformants contained a single T-DNA insert on their genome. Considering the efficiency and flexibility of A. tumefaciens-mediated transformation (ATMT), this technique offers a highly efficient means for characterizing those genes important for the pathogenicity of M. grisea.


Using the differential hybridization technique, a novel stellacyanin cDNA clone (CASLP1) was isolated from a pepper cDNA library from hypersensitive response (HR) lesions of leaves infected with an avirulent strain of Xanthomonas campestris pv. vesicatoria. The deduced amino acid sequences of CASLP1 are homologous to those of stellacyanins from cucumber, maize, pea and Arabidopsis. The CASLP1 stellacyanin mRNA was not constitutively expressed in all organs of pepper, but strongly induced and accumulated in pepper tissues infected with X. campestris pv. vesicatoria, Colletotrichum coccodes, Phytophthora capsici or C. gloeosporioides. In situ hybridization results revealed that CASLP1 transcripts were strongly localized in the phloem areas of vascular bundles in infected tissues of pepper stems and fruits. CASLP1 mRNA accumulation was found in lower pepper leaves infected by either virulent or avirulent strains of X. campestris pv. vesicatoria and non-pathogenic Pseudomonas fluorescens, whereas CASLP1 mRNA did not accumulate in uninfected upper leaves. Induction of this cDNA gene was only occurred in pepper leaves applied with methyl jasmonate (MeJA), but not with ethylene, salicylic acid, DL-β-aminono-butyric acid and benzethidiazole. Accumulation of CASLP1 transcripts was locally or systemically induced in pepper leaves upon mechanical wounding and was activated in a MeJA-dependent manner. The CASLP1 stellacyanin mRNA also was strongly induced in leaf and stem tissues after exposure of pepper plants to abscisic acid, salt, and drought.

Comparative assay of Magnaporthe grisea population between Korea and China. Seong-Sook Han1, Hong-Sik Shim2, Seong-Ho Choi3, Lei Cailin4, Jiulin Wang5 and ZhongZuan Ling6. 1Plant Pathology Division, National Institute of Agricultural Sciences & Technology, Suwon 441-707, Korea; 2Crop Environment Division, National Crop Experiment Station, Suwon 441-707, Korea; 3Rice Research Division, Institute of Crop Breeding and Cultivation, CAAS, Beijing 100081, China.

One hundred isolates of Magnaporthe grisea from Korea and China were characterized for pathogenicity using eight Korean differential varieties (KDV), six Chinese differential varieties (CDV), and six near isofenic lines (NILs) developed in China. The restriction length
polymorphism of *M. grisea* isolates from each country was also analyzed using MGRS86 as a probe. One hundred Korean isolates classified into 17 races on KDV were grouped into 29 pathotypes on Chinese near isogenic lines (NILs). Virulence of 46% of Korean isolates against all the six Chinese NILs indicated that the current six Chinese NILs alone was not enough to be used as differential varieties in Korea. Especially, susceptibility of the BL1 carrying resistance gene Pi-b to 70% of tested Korean isolates suggested that BL1 (Pi-b) may not be a useful resistance source to Korean blast. Based on the virulence assays of *M. grisea* populations from each country were divided into two groups. About 50% of Chinese isolates showed similarity to the 30% of the Korean isolates. Especially, the isolates from northern part of China, where Japonica rice cultivars were grown, showed high similarity to the Korean isolates, while isolates from southern part of China, where Indica rice were mainly grown, showed low similarity to Korean isolates. The genome RFLPs of Korean isolates were quite different from those of southern part of China using MGRS86 as a probe. These data indicated that the physiological and genetic characteristics of *M. grisea* population might be determined by strong interaction with cultivated rice.


Expressed sequence tag (EST) analysis was applied to identify rice genes involved in defense responses against infection by the blast fungus (*Magnaporthe grisea*) and fungal genes involved in growth within the host during a compatible interaction. A total of 511 clones were sequenced from a cDNA library constructed from rice leaves (cv. Nipponbare) infected with *M. grisea* strain 70-15, to generate 296 non-redundant ESTs. The sequences of 293 clones (57.3%) significantly matched NCBI database entries; 221 showed homologies with previously identified plant genes and 72 with fungal genes. Among the genes with assigned functions, 32.8% were involved in metabolism, 29.4% in cell/organism defense or pathogenicity, and 18.4% in gene/protein expression. cDNAs encoding a type I metallothionein (MT-I) of rice and a homolog of glucose-repressible gene 1 (GRG1) of Neurospora crassa were the most abundant representatives of plant and fungal genes, comprising 2.9 and 1.6% of the total clones, respectively. The expression patterns of ten ESTs, five each from rice and *M. grisea*, were analyzed. Five defense-related genes in rice, including four pathogenesis-related genes and MT-I, were highly expressed during *M. grisea* infection. Expression of five stress-inducible or pathogenesis-related genes of the fungus, including two hydrophobin genes, was also induced during growth within the host. Further characterization of the genes expressed in this study would be an aid in unraveling the mechanisms of pathogenicity of *M. grisea* and the defense responses of rice.


The *hrp* genes encode type III secretory pathways and are required by many phytopathogenic bacteria to elicit a hypersensitive response (HR) in nonhost or resistant host plants and for pathogenesis on susceptible hosts. Genes encoding effectors such as popA of *Ralstonia solanacearum* and harpins of *Pseudomonas syringae* secreted by the type III systems are commonly linked to the type III systems genes. Several elicitors had been shown to be present inside plants, however, the exact function of the bacterial elicitor inside plants are not understood yet. To investigate the function of the elicitor in the plant, two elicitor genes, *hmaA* isolated from *Pseudomonas syringae pv. syringae* Pss61 and popA isolated from *Xanthomonas oryzae* pv. *oryzae*, were chosen to make transgenic plants. Since the elicitor induces the programmed cell death of the plant, the specific promoter which can be expressed only by the pathogen attack is required. The promoter, hsr203J isolated from tobacco and reported to be expressed on the pathogen infection, was tested for the expression in the rice callus. The uidA gene (gus) under the hsr203J promoter was expressed in the rice callus only after the infection of the *Xanthomonas oryzae* pv. *oryzae* kko85. The chimeric constructions to deliver elicitors into the plants based on pSBOMAR were transferring into the embryogenic rice callus using Agrobacterium system.

**Variation analysis based on vegetative compatibility groups and genetic diversity of Fusarium oxysporum f. sp. radicus-lycopersici in Korea.** Sung Ho Kim1, Jong Tae Kim2, Sung Joon Yoo3, Jeong Young Song4 and Hong Gi Kim1. 1College of Agriculture, Chungnam National University, Taejon 305-764, Korea; 2National Alpine Agricultural Experiment Station; 3Institute of Agricultural Science, Chungnam National University, Taejon 305-764, Korea.

Vegetative compatibility groups (VCGs) of *Fusarium oxysporum* f. sp. *radicus-lycopersici* isolates collected from Korea were analyzed to determine the genetic characteristics and compared to those of foreign isolates. As a result, 28 of 31 isolates belong to VCG 0094 dominantly. In comparison of VCG specificity between foreign VCG subgroup testers and Korean VCG 0094, Korean isolates of VCG 0094 were appeared to be similar to those of Israel and Florida, USA having an “Universal” property, but differed from those of European. Genetic variation within identical VCG was investigated through restriction fragment length polymorphism (RFLP). Furthermore, correlation between genetic diversity of isolates from different regions and cultivation period of tomatoes was also studied. RFLP analysis with repetitive copy clone pT9F and pTF53 revealed DNA polymorphism between isolates. This tendency, especially, was stronger in the isolates collected from the region of longer tomato cultivation history. These results will contribute for the effective control of disease through precise estimation of fungal damage, the prediction of new pathogenic fungus appearance and the movement of foreign pathogens.

**Defense related gene expression in rice plants following silicon treatment and Magnaporthe grisea infection.** M.S. Jeon1, S. Yi1, D. Choi1 and E.W. Park. 1National Instrumentation Center for Environmental Management, Seoul National University, Suwon 441-744, Korea; 2School of Agricultural Biotechnology, Seoul National University, Suwon 441-744, Korea; 3Korea Research Institute of Bioscience and Biotechnology, Daejon 305-600, Korea.

Gene expression of chalcone synthase (CHS), phenylalanine ammonia lyase (PAL), and PR-1 was investigated in rice cv. Jimmi and Hwasung grown under a hydroponic culture system with Yoshida’s nutrient solutions containing 0, 50, 100, and 200 ppm of Na2SiO3. Silicon contents in rice leaves were increased by silicon application in the nutrient solution, reaching 6-10% of dry weight. Rice plants were
inoculated at five-leaf stage with three races of *M. grisea*: KI409, KI197, and KJ301, which are highly, moderately, and weakly virulent, respectively, to both cultivars. The number of lesions and leaf blast severity on both cultivars were decreased significantly by silicon treatment when inoculated with KI409 and KI197. Leaf samples were harvested everyday after inoculation and gene expression was analyzed by Northern hybridization. PAL and PR-1 transcripts were detected at low levels in healthy leaves regardless of silicon treatment. Expression levels of PAL and PR-1 genes increased after inoculation with each of KI409 and KI197. However, increase in silicon concentration in the nutrient solution resulted in decrease in expression levels of PAL and PR-1 genes. When KI409 was inoculated to both cultivars, strong expression of PR-1 gene was induced from 48 hours after inoculation. CHS gene was also strongly expressed at 48 hours after inoculation with KI409, KI197, and KJ301.

**Inhibition of mycelial growth, conidia germination and appressorium formation of *M. grisea* and *C. orbiculare* by phytase.** Y.J. Park 1, S. Yoon 1, S. Kim 2, E.W. Park 2 and Y.H. Lee 2. 1Research and Development Center, TS Corporation, Inchon 400-201, Korea; 2Dept. Plant Pathology, Seoul National University, Suwon 441-744, Korea.

Phytase is an enzyme that catalyzes the hydrolysis of phytic acid (Phytin) into myo-inositol and inorganic phosphate. To investigate the antifungal effects of phytase, mycelial growth, conidial germination, and appressorium formation were examined in *M. grisea* and *C. orbiculare*. Mycelial growth of *M. grisea* and *C. orbiculare* was decreased significantly on the complete and sporulation media treated with 10 U/ml phytase. The fungi on sporulation media with phytase were also significantly different from those without phytase in sporulation rate. Conidial germination of *C. orbiculare* was strongly inhibited when treated with phytase of greater than 10 U/ml. In the case of *M. grisea*, it was decreased to 41.3% with phytase of 100 U/ml. The addition of 10 U/ml phytase blocked appressorium formation completely in *M. grisea* and decreased appressorium formation rate to 5.8% in *C. orbiculare*. The inhibition activity was in a dose-dependent manner. Appressorium formation of *M. grisea* was restored by treatment with 10 mM cAMP and 10 M 1,16-hexadecanediol. These results suggest that phytase has antifungal activity and it may regulate appressorium formation by modulating the cAMP-dependent signal pathway in the fungi.

**Characterization of a Corynespora sp. causing leaf spot of cucumber in green house cultivation in Korea.** Mi Kyung Kwon, Beom Ryong Kang, Ki Chung Kim and Young Cheol Kim. Applied Plant Science Division and Biotechnology Research Institute, College of Agriculture, Chonnam National University, Kwangju 500-757, Korea.

In green house cultivation of cucumber (*Cucumis sativus* L.) fields in Chonnam province in 2000 revealed the presence of a severe leaf spot affecting old plant during winter season. Early leaf symptoms in disease development were brown spots with yellow halos. The lesions became irregular with larger diameter and eventually defoliation occurred. The fungus isolated from the infected leaves formed conidia singly or in chains on conidiophores. Conidia were transparent brown to pale brown, and were obclavate or cylindrical, straight or curved shaped with 7-11 pseudoascus. A PCR product, generated using primers to amplify ITS regions, was homologous (99% in identity) to the ITS sequence of *Corynespora cassicola*. Currently we are sequencing the ITS sequence of authentic strains of *C. melonis* and *C. cassicola* as references to determine the species of the *Corynespora* sp. from the Korean cucumbers. The fungus grew best at 30°C on Czapek solution agar medium. We are also investigating other factors that resulted in the epidemic.

**Isolation frequency of the fungus from anthracnose lesion and influence of inoculum density and wetness duration on occurrence of sweet persimmon anthracnose.** Tae Hoon Lim, Tae Hyun Chang, Chang Ho Sung and Bong Koo Chung. Dept. of Agricultural Biology, Chungbuk National University, Cheongju 361-763, Korea.

Two different *Colletotrichum* species were isolated from diseased fruits. The isolate with fusiform conidia and light-gray mycelium (FLM) similar to *Gloeosporium kaki* reported in Japan was the major, while the other isolate with cylindrical conidia and black-gray mycelium (CBM) similar to *Colletotrichum gloeosporioides* identified by previous study in Korea was the minor one. The FLM isolate caused lesion on a sweet persimmon fruit but not on apple by artificial inoculation with a mycelium plug. However, The CBM isolate caused symptoms on these fruits. The results suggested that a major pathogen of anthracnose on sweet persimmon was different from the one previously reported or had host-specificity. Effects of wetness duration and inoculum density on the development of sweet persimmon anthracnose were evaluated under controlled environment. There was a significant correlation (r=0.82) between severity of symptoms and wetness duration. Disease severity increased with increasing duration of wetness from 0 to 60 h. Wetness duration longer than 48 h caused severe symptoms on the foliage. As inoculum density of FLM increased from 10^3 to 10^5 conidia/mL, symptoms of anthracnose appeared on the leaf (Y=1.23+0.72X, R^2=0.82). Inoculum at 10^5 conidia/mL and 36 h of continuous wetness duration were enough to develop the disease on the tree. Inoculated with 10^5 conidia/mL, symptoms on the leaf initially appeared 8 days after inoculation. After 20 days, severity of the disease reached peak. The results suggested that occurrence of the disease in the field could increase after a few days of rain and fungicides must be sprayed as soon as stopping rain or after prolonged wetness duration to control the disease and reduce loss.

**First report of pink rot of palms (Palmae sp.) caused by *Gliocladium verrmoseni* (Biourge) Thom in Korea.** Young Suk Han, Jong Han Park and Young Mun Choi. Div. of Horticultural Environment, National Horticultural Research Institute, RDA, Suwon 441-440, Korea.

Pink rot of palm was occurred at Yeju area in 2000 and 2001. Infected plants showed rotting at the leaf-stock bases and killing of the terminal bud. The first symptoms are dark brown necrotic areas on the stem. Bases of infected frond may be covered with pink spores and the spots produced oozy gum pockets. Oozing lesions occur on the stems, and leaves turn brown and droop. The causal agents were isolated from salmon-pink spores sporulating on the leaf sheaths and necrotic stem tissues. Pathogen were isolated from freshly infected tissues were identified as *Gliocladium verrmoseni* based on mycological characteristics. Fungus were grown plenty on PDA culture. Temperature for mycelial growth was tested at 5 to 40 and optimal temperature was 25 and was not nearly grew at temperature above 35. Artificial pathogenicity were tested on 8 species of palm family in the wound inoculation and symptoms showed similar to those observed.
in the field. This is the first report on pink rot of palm in Korea.

**RAPD-PCR analysis and morphological segregation of small-spored Alternaria species.** B. R. Kim¹, H. S. Cho² and S. H. Yu³. ¹Dept. Agricultural Biology, Graduate School, Chungnam National University, Daejeon 305-764, Korea; ²Division of Applied Biology, Chemistry and Food Science, College of Agriculture, Chungnam National University, Daejeon 305-764, Korea.

The importance and diversity of the genus Alternaria highlights the need for accurate identification of species. However, many small-spored Alternaria isolates have been misidentified due to the use of spore size as the only identifying character. In this study ninety seven isolates of small-spored Alternaria were segregated into morphological groups or species and then subjected to RAPD-PCR analysis using total genomic DNA and ten different URP primers. Cluster analysis of RAPD fragment patterns showed that the ninety seven isolates segregated into the same distinct groups that are morphologically similar but identifiable as A. gaius, A. mali, A. tenuissima, A. longipes, A. citri, A. arborescens, A. infectoria, A. gossypina, A. nelumbii, A. alternata or Alternaria sp. from common pokeweed. The results showed that A. gaius, A. mali, A. tenuissima, A. longipes, A. citri, A. arborescens, A. infectoria, A. gossypina, A. nelumbii, A. alternata, and Alternaria sp. from common pokeweed are recognizable as genetically distinct taxa.

**Inhibit effect of bicarbonates to colony growth of Botrytis cinerea** from perilla plant cultivated under controlled cultivation conditions in drained paddies fields. Dong-Bum Shin, Yeon Kyu Hong and Bong-Choon Lee, National Yeongnam Agricultural Experiment Station, 1085 Naidong, Milyang 627-130, Korea.

Gray mold, caused by *Botrytis cinerea* in among the most important diseases on sheltered crops. Perilla as an vegetables cultivated at green houses on drained paddies fields in Korea. Gray mold is particularly damaging on perilla grown in greenhouse, as micro-climatic conditions are often highly conducive to disease development. Chemical control of gray mold has become increasingly difficult due to resistance to fungicides. Since bicarbonates have been demonstrated to control powdery mildew diseases and was found detrimental effects on disease incidence and *in vitro* growth of several cucurbit foliar pathogen, the purpose of this research was to determine the effectiveness of bicarbonates against *B. cinerea* from perilla plant cultivated under controlled cultivation conditions in drained paddies fields. Assessments were made of *in vitro* fungal colony growth in response to ammonium, potassium, and sodium bicarbonates. Bicarbonates inhibited colony growth at concentration as low as 20 mM. As pH increased from 7.0 to 8.5, colony growth decreased with ammonium bicarbonates. And it was not observed chemical injury on perilla plant until 200 mM below.

**A new species of Phytophthora associated with root rot of potted chrysanthemum.** Hyeong-Jin Je¹, Seung-Beum Hong² and Wen-Hsiung Ko³. ¹Plant Pathology Division, National Institute of Agricultural Science and Technology, Suwon 441-707, Korea; ²Molecular Genetics Division, National Institute of Agricultural Science and Technology, Suwon 441-707, Korea; ³Dept. of Plant Pathology, University of Hawaii at Manoa, Hilo, Hawaii 96720, USA.

Phytophthora root rot on hydroponically-grown potted chrysanthemum occurred severely at Goyang, Gyeonggi province in 2000. The causal pathogen showed strong pathogenicity to the plant was identified as a new species in the genus *Phytophthora* based on morphological characterization and the sequence analysis of ITS rDNA. The fungus belonged to *Phytophthora* group V according to non-papillate sporangium and homothallic sexuality. However, it readily distinguished from other species in the genus by unusual three types of antheridium; paragynous, amphiangynous, and devoid. In addition, the isolates showed only 70% nucleotide sequence homology with *P. insititia* in the ITS rDNA, which clustered in a group in the phylogenetic analysis with 53 *Phytophthora* spp. (GenBank). The oogonium and oospore without antheridium formed intercalary, unusually smaller than those of with antheridium, and sized from 20.0 to 32 (avr. 24.0±3.9) μm and 16.0 to 24.0 (avr. 20.4±3.0) μm, respectively. While, those with antheridium sized from 20.0 to 44.0 (avr. 38.0±6.0) μm and 22.0 to 40.0 (avr. 32.7±3.6) μm, respectively. The isolates produced abundant hyphal swellings in water and chlamydospores sized 20-48 (avr. 33.7±6.3) μm with cultural age. Non-papillate, internal or external sporangium formed in water was measured as (30-51)±2±1.2±1.2 (μm)(5±15-31±3.2±7.6-40) μm. The fungus grew relatively slow from 10 to 36°C and best at 30°C (4.0 mm/24 h).

**Leaf blight of welsh onion caused by Phytophthora nicotianae.** Hyeong-Jin Je¹, Jin-Hyuek Kweon², Won-Hae Yeh¹ and Choong-Hoe Kim¹. ¹Plant Pathology Division, National Institute of Agricultural Science and Technology, Suwon 441-707, Korea; ²Yungnam Agricultural Research and Extension Service, Jinju 660-360, Korea.

A severe Phytophthora leaf blight of Welsh onion is reported from Jinju, Goyang, and Guri in 2000 and 2001. Among 133 fields surveyed in the areas, 95 fields representing over 70% were infected by the disease and 23 fields showed over 50% infection rate. Population densities of the causal pathogen in the field soils were surprisingly high ranging from 15.5 to 53.5 cfu/g soil. The disease mainly occurred on leaves, however, basal stems and underground parts of the plant were also infected. The leaf lesions showed water soaking or a faint discoloring of the green color at the beginning and above parts of the lesion were wilted, twisted, and dried at the later stage. However, the whole plant rarely died unless basal portions were heavily infected. Totally 55 isolates of *Phytophthora* were isolated from infected leaves, basal stems or roots. Among the isolates, 52 were identified as *P. nicotianae* based on its following mycological characteristics: markedly papillate, ovoid to globose, and low-caducous sporangia; heterothallic sexuality and small oospores with amphiangynous antheridium; abundant chlamydospores; good growth at 35°C; arachnoid growth pattern on cultural media. However, the rest three isolates have not been identified yet. The leaf blight of Welsh onion caused by *P. nicotianae* was recorded as early as 1910 in northeast countries, however, no report has been found in the country, previously.

**Isolation and identification of an anthracnose fungus Colletotrichum musae from imported bananas.** Jin Young Yim, Tae Heon Lim and Byeongjin Cha. Dept. of Agricultural Biology, Chungbuk National University, Cheongju 361-763, Korea.

*Colletotrichum musae* that causes anthracnose was isolated from dark brown necrosis lesions on banana sold in the market. The fungus grew fast on PDA. Colony was loose and produced white aeral mycelium. Black masses of acervuli were developed abundantly on
the colony about 10 days after incubation at 25°C and acervuli produced dark orange masses of conidia on them. Conidial masses usually coalesced together and became larger. Conidia were aseptate, hyaline, straight, and ellipsoid to round shape, and measured 14.5 x 6.9 μm in size. Appressoria were readily formed from vegetative hyphae and the color and shape was black, clavate, round, or irregular. The size was 8.8 x 6.8 μm. Setae-like structure was not found from the colony on either the lesion or the medium. Sclerotium was absent, too. Among the media, PDA media was the best culture medium for the mycelial growth of C. musae. The optimum pH for mycelial growth ranged from 5.5 to 6.5. The optimum temperature for mycelial growth were 28°C. When the isolated fungus was inoculated on the banana fruit through needle-wound, black necrotic lesions appeared on the site and orange-colored spore masses were produced on the lesions. Spore suspension inoculation was more efficient than mycelial disc inoculation.

Vegetative compatibility grouping of **Cryphonectria parasitica** in Korea. Jin Young Yim1, Young Jik Ju2, Dae-Hyuk Kim1 and Byeongjin Cha1. 1Dept. of Agricultural Biology, Chungbuk National University, Cheongju 361-763, Korea; 2Heuksalim Research Institute, Taejon 305-333, Korea; 3Institute for Molecular Biology and Genetics, Chonbuk National University, Chonju 561-756, Korea.

The chestnut blight fungus **Cryphonectria parasitica** was isolated from the bark of blighted twigs collected from all over Korea. To decide the vegetative compatibility (VC), every isolates were cultured side by side with the rest isolates in the same petri plate which contains potato dextrose agar amended with methionine and biotin and the VC was checked with the dark brown line between the two isolates. In the grouping, total 668 isolates were divided into 188 VC groups (VCGs). Among the VCGs, the biggest VCG contained 164 of 668 isolates. The second biggest VCG had 30 isolates. The number of VCG in which more than 10 C. parasitica isolates belonged to was 11. On the other hand, 132 VCGs were consisted of sole member and only one isolate belonged to each VCG. Geographical distributions of VCGs consisted of many isolates were usually much wider than VCGs of small group size. Among the localities, the principal chestnut plantation area Kyungnam-do had 69 VCGs, and Chungbuk-do was the second as contained 51 VCGs. Chonbuk-do, the other chestnut plantation area, had 44 VCGs. On the other hand, Kyungbuk-do and Chonbuk-do had 19 and 17 VCGs respectively, and showed the simplest VCG distribution. More than 66% isolates of the biggest VCG was found out from three provinces, Kyungnam-, Kangwon-, and Chungbuk-do. These three provinces shared many of VCGs but it was very rare among other provinces.

Ectomycorrhizal formation on containerized pine seedlings and their resistance to **Rhizina root rot**. Jong Kyu Lee1, Sung Jae Lee1, Sang Hyun Lee1, Seung Kyu Lee1 and Kyung Hee Kim1. 1Division of Forest Resources, Kangwon National University; 2Kangwon Forest Development Research Institute, Chuncheon; 3Department of Forest Biology, Korea Forest Research Institute, Seoul, Korea.

**Rhizina root rot** caused by **Rhizina undulata** usually occurs after burning of pine forests in Korea. The disease is known to cause high mortality of coniferous seedlings in reforested sites after fire. Poor seedling establishment is usually attributed to Rhizina root rot, too. For the reforestation of fire-damaged pine forests, containerized pine seedlings were plentifully grown in well-equipped facilities. These seedlings usually formed ectomycorrhizae on roots in natural conditions, and showed relatively resistance to **Rhizina root rot**. The objective of this study was to investigate if ectomycorrhizal formation can protect pine seedlings from **Rhizina root rot**. Presence or absence of mycorrhizal formation on seedling roots was investigated through the process of seedling production. Seedling mortality and root growth of containerized and mycorrhizae-free pine seedlings were compared after inoculating **Rhizina undulata**. Seedling mortality of containerized pine seedlings was also investigated after transplanting to the fire-damaged forest sites, where abundant fruiting bodies of **Rhizina undulata** was observed last year.

Relationship between meteorological factors and potato late blight epidemics. M. Kato and T. Shimanuki. Natl. Agric. Res. Center for Hokkaido Region, Toyohira-ku, Sapporo 062-8555, Japan. Relationship between meteorological factors and the progress of potato late blight was studied in Hokkaido, Japan. Diseased leaf area was observed on low field resistant cultivar Irish Cobbler without fungicide applications from 1996 to 2000. Hourly temperature and hourly relative humidity were monitored within the canopy in 1999 and 2000. Daily maximum temperature, rainfall and global solar radiation were recorded at the weather station located within 300 m from potato fields in all years. Late blight progressed and defoliated whole leaves within 32 days after occurrence of lesions in 1996, 1997, and 1998, whereas diseased leaf area reached only 5% and 36.7% in 1999 and 2000, respectively at the harvest. In 1999 and 2000, consecutive hours of high relative humidity (90% or above) was more than 9 hours in most of days, mean temperatures during the highly humid periods ranged from 15 to 24 degree centigrade, and rain fell occasionally, indicating that days favorable for sporulation and infection by **Phytophthora infestans** frequently occurred. In 1999 and 2000 there were a number of days with high daily maximum temperatures, compared with in 1996, 1997, and 1998. Days with strong global solar radiation occurred more frequently in 1999 and 2000 than in the previous three years. These results suggest that even though days favorable for infection occurred frequently, an epidemic does not always occur, and that daily maximum temperatures and/or solar radiation affect the disease progress.

**Effect of Monosporascus root rot infection on photosynthetic activity and plant growth of oriental melon.** Noh-Youl Heo1 and Yong-Bum Lee1. 1National Plant Quarantine Service, Anyang 430-016, Korea; 2The University of Seoul, Seoul 130-743, Korea. **Monosporascus cannonellus** is recently described soilborne ascomycetes in Korea that cause root rot/vine decline of cucurbits. The effect of **Monosporascus root rot** disease on photosynthetic activity and growth was studied on infected oriental melon plants. At harvest stage, photosynthetic activity of diseased oriental melon plants was lower, stomatal resistance was higher than healthy plants, while xylem exudates was not observed in diseased plants. There was no difference in mineral contents of the leaves and stems between diseased and healthy plants. Leaf area, fresh weight and dry weight of plants, and fruit weight were severely decreased in diseased plants compared to healthy plants.

**Occurrence of Sclerotinia root rot caused by Sclerotinia sclerotiorum on some vegetable crops in Korea.** Seog Won Chang, Sung Kee Kim and Eun Sup Lee. Northern Agricultural Research Station,
Gyeonggi Agricultural Research and Extension Services, Yonchon 486-833, Korea.

Sclerotium rot occurred severely on some vegetable crops grown in Namyangju, Yangpyung, and Yonchon provinces of Korea in 2000 and 2001. The crops infected by Sclerotinia sp. were Solidago virgaurea var. asiatica, Allium victorialis var. platyphyllum, Adenophora remotiflora, Chichorium intybus, Armoracia lapathifolia, and Petroselinum crispum. A fungus associated with the disease was identified as Sclerotinia sclerotiorum (Sacc.) Shoem., based on the morphological characteristics of sclerotia and asci. The symptoms that often develop on lower leaves or young stems are water-soaked spots that may enlarge and become a watery soft rot. Infection parts become yellow and then turn brown followed by death of the whole plant. White mycelia may develop on higher stems, leaves, and on soil where these plant parts lay, and black sclerotia of variable size and shape form in the mycelial mass. Infection of plant parts before harvest often results in postharvest disease from spread of the fungus from diseased to healthy tissue in storage or shipping containers. Detailed epidemiological data are essential for the development of effective and economical control programs for disease caused by S. sclerotiorum.

Occurrence of Streptobotrys blight on columbine caused by Streptobotrys caulliphylli. Sung-Kee Hong1, Wan-Gyu Kim1, Weon-Dae Cho1 and Hong-Gi Kim1. 1Plant Pathology Division, National Institute of Agricultural Science and Technology, Suwon 441-707, Korea; 2Department of Agricultural Biology, Chungnam National University, Daegu 305-764, Korea.

Blight symptoms on leaves and flowers of columbine (Aquilegia caerulea Sieb. et Zucc.) were frequently observed during a disease survey in 2000 and 2001 in Gangwon and Chungnam provinces, Korea. Lesions on leaves appeared as pale purple to brown spots with dark purple margins. Sometimes, gray molds were produced on the leaves. The spotted lesions enlarged later and the severely infected leaves were blighted with sporulation. Infected flowers were also rot- ted and blighted with gray molds. All fungal isolates obtained from the infected leaves and flowers of columbine were identified as Streptobotrys caulliphylli Hennetert based on their morphological and cultural characteristics. Conidiophores were brown, erect, septate, cylindrical, repetitively branched, with twisted branches, and up to 2 mm long. Conidiogenous cells were produced at ends of branches. Conidia were globose to subglobose, pale brown, unicellular, warty and measured 6.5-12.5 μm (usually 8-10 μm) in diameter. Blight symptoms were induced on leaves and flowers of columbine by artificial inoculation with the isolates. This is the first report that S. caulliphylli causes blight of columbine.


Anthracnose symptoms severely occurred on leaves of May lily (Convallaria keiskei Miquel) grown in fields of Gapyeong and Yangpyong areas in Korea during July 2001. Incidence of the disease reached up to 100% in the fields of May lily. Symptoms appeared as circular to irregular, small spots with brown to dark brown discoloration on leaves of the plant at the early stage. The spotted lesions enlarged and coalesced at the late stage. Severely infected leaves blighted later. Colletotrichum sp. was consistently isolated from lesions on the diseased leaves. All isolates of Colletotrichum sp. were identified as Colletotrichum liliacearum Ferr. based on their morphological and cultural characteristics. Conidia were unicellular, falcate, fusiform, tapered gradually to each end and measured 14-24x3-4 μm. Appressoria were dark brown to black, circular or ovate to lobed and measured 6-14x5-8 μm. Setae were dark brown to black, needle-shaped, 1-3 septate and measured 52-154x4-6 μm. Leaf spots similar to the original anthracnose symptoms were induced on the host leaves by artificial inoculation with the isolates of the fungus. This is the first report on anthracnose of May lily caused by C. liliacearum in Korea.

Relative efficiency of two rotary inertial impaction spore samplers for collection of airborne conidia of Pyricularia grisea. Y.K. Han1, E.W. Park1, S.S. Hong1, K.K. Kim1 and K.Y. Park1. 1Gyeonggi Province Agricultural Research and Extension Services, Hwasung 445-972, Korea; 2Dept. Agricultural Biology, Seoul National University, Suwon 441-744, Korea.

Rotor-type spore collectors have been used in Korea during the last three decades to monitor airborne spores of P. grisea in order to forecast blast development. The rotor-type spore collector has two collector rods of each 26.0 mm wide, which is too wide to collect small particles like P. grisea conidia whose size is approximately 23.2±8.7 μm. This study was conducted to evaluate efficiency of airborne spore collection by the rotor-type sampler as compared with Rotorod11 sampler and Burkard volumetric sampler. The rotor-type sampler was installed next to Rotorod and Burkard samplers in a rice paddy field during the period from July to September in 2000 and 2001. In addition, four Rotorod samplers were installed at 0.5, 0.8, 1.1, and 1.4 m above the ground level in the paddy field to determine vertical variations in the airborne spore density. Hourly weather conditions during the experiment period were monitored using an automated weather station installed in the paddy field. The relative collection efficiency of the rotor-type sampler to Rotorod was 0.005-0.287 (average 0.036) and 0.002-0.293 (average 0.031) in 2000 and 2001, respectively. Rotorod was most efficient in spore collection in 2000, whereas Burkard was better than Rotorod in 2001. Spore collections at 0.8 m above the ground appeared to be most appropriate to describe leaf blast development in the field. Daily spore release occurred mainly during the period from 22:00 until 12:00 next day.

Occurrence of stem rot on potato caused by Sclerotium rolfsii. Jong Tae Kim, Jeom Soon Kim and Young Il Hahn. Crop Division, National Alpine Agricultural Experiment Station, RDA, Pyungchang, Gangwon 232-950, Korea.

Stem rot of potato (Solanum tuberosum) caused by Sclerotium rolfsii occurred in many potato growing areas in Gangwon province of Korea in 2001. The first symptoms of affected plants are a general wilting. The wilting progresses without a change in foliage color until the plants finally die. An appressed, white, fainlike mycelial growth radiates over the soil surface, and numerous round, tan sclerotia form in the older mycelia at the stem base and soil surface. The mycelium is white when young, becoming tan as it gets older. Sclerotia are numerous, round 0.35-2.5 mm in diameter, white when young, then tan, and dark brown when old. Optimum temperatures for mycelial growth of this fungus were 30-35°C, and tuber surfaces were also highest virulence at these temperatures. Base on these cultural and
morphological characteristics, the fungus was identified as *Sclerotium rolfsii* Sacc.. This is the first report on stem rot of potato in Korea.

**The corky root of tomato caused by Pyrenochaeta lycopersici in Korea.** Jong Tae Kim¹, Hyo Park⁰, Jeom Soon Kim¹ and Young Il Hahn¹. Crop Division, National Alpine Agricultural Experiment Station, RDA, Pyungchang, Gangwon 232-950, Korea; ²Buyeo Tomato Experiment Station, AREDS, Buyeo 414-810, Korea.

In 1999, *Fusarium oxysporum*, *Verticillium dahliae*, *Colletotrichum coccodes*, and *Pyrenochaeta lycopersici* were isolated from roots and stems of tomato plants which showing wilt symptoms in greenhouses of Dalseong area, Daegu in Korea. Among them, except *P. lycopersici*, the other 3 fungi were reported already as *Fusarium* wilt or *Fusarium* crown and root rot, *Verticillium* wilt and black dot root rot, respectively. Symptoms of corky root caused by *Pyrenochaeta lycopersici* were appearing stunted and generally lacking vigor. After fruits set, plants may die back from the foliage tips. Brown lesions appearing with bands around the roots are characteristic symptoms of the disease. The lesions become swollen and cracked along the length of the root with corky appearance. The top roots and stem base may eventually turn brown and rot. Pycnidia were solitary, globose to sub-globose, brown to black, darker around the neck region, and measured as 45-370 µm diameter with septate setae up to 135×4.6 µm. Conidia are hyaline, unicellular, and 5-7×1.5 µm long. Optimum temperatures for mycelial growth were 20-25°C. Based on these cultural and morphological characteristics, the fungus was identified as *Pyrenochaeta lycopersici* Schneider & Gerlach. This is the first report of corky root on tomato caused by *Pyrenochaeta lycopersici* in Korea.

**Occurrence of Verticillium wilt on potato caused by Verticillium albo-atrum.** Jong Tae Kim, Jeom Soon Kim and Young Il Hahn. Crop Division, National Alpine Agricultural Experiment Station, RDA, Pyungchang, Gangwon 232-950, Korea.

In 2001, Verticillium wilt of potato (*Solanum tuberosum*) was occurred at Milyang area which is one of major potato producing area of Korea. The wilted potato plants showed the typical symptoms including gradual yellowing and interveinal necrosis. The vascular tissues of the infected stems were discolored with light brown. Fungal isolates from discolored vascular tissues were whitish to creamy color with folding on potato dextrose agar medium, where they produced resting dark mycelium but not microsclerotia. Conidiophores are septate with side branches, swollen at the base, and arranged in a whorl. First-formed conidia are 5-13.5×23.5 µm. They were borne in small clusters at the tips of phialides. Optimum temperatures for mycelial growth were 20-25°C. Base on these cultural and morphological characteristics, the fungus was identified as *Verticillium albo-atrum* Reink & Berth. Pathogenicity tests by root dipping method revealed that the fungus caused the same symptoms as observed in the naturally infected potato plants. This is the first report of Verticillium wilt on potato caused by *Verticillium albo-atrum* in Korea.

**Effect of pesticides and microorganisms on the russet of Whangkeumbae.** Young-Soob Park, Kyung-Hyong Hong and Hung-Su Seo. Naju Pear Research Institute, NHRI, RDA, Korea.

This study was conducted to examine the effect of pesticides and microorganisms to find out the cause of russet occurring in Whangkeumbae pear during 1999-2000. Out of 12 isolates obtained from the Whangkeumbae pear russet, six isolates and skin russet were used for pathogenicity tests to pear by inoculation using spores and mycelia. They didn't induce russet on pear fruit. Accordingly, it is considered that the russet is not caused by microorganism. In the effect of the pesticides application, pesticides-application bags were begged fruit in 45 days (2000) and 110 days (1999) after full bloom. No difference in russet was found between the treatments until postharvest. When fruits were sprayed formulations, emulsions gave higher rusting percentage compared with wettable powders.

**Diseases recently occurred on citrus fruits (cv. Shiranuhi) in Jeju Island.** J.W. Hyun, D.W. Kim and K.S. Kim. Citrus Experiment Station, National Jeju Agricultural Experiment Station, Rural Development Administration, Jeju-Do, 699-803, Korea.

'Siranuhi' is a hybrid of 'Kiyomi' (*Citrus unshiu* Marc. x *C. sinensis* (L.) Osh.) and Ponkan (*C. reticulata* Blanco), generally called as 'Hanfla Bong' in Korea, and the cultivated area in plastic film house are rapidly increasing. At recent, some diseases took place on 'Shiranui' fruit, and were to be important in some orchards. They were *Phytophthora citrophthora*, *Alternaria* sp., *Diplodia natalensis*, *Penicillium digitatum*, and *Botrytis cinerea*, which were generally minor diseases on citrus, especially satsuma mandarin. *P. citrophthora* took place on everywhere of surface of fruit at early to middle growth stage of fruit. *Alternaria* sp. and *D. natalensis* appeared firstly on stolar-end at early to middle stage of fruit and advanced in the rind and core. The symptom was initially yellowish on stolar-end around, and gradually proceeded through the rind and eventually the fruit was dropped. Surface mycelium appears only advanced stages of infection on stolar-end in case of *D. natalensis*. *B. cinerea* and *P. digitatum* infected the tissues of well around which were to be softened and wounded by stagnated water in well formed on arround of stem-end and typical characteristics of 'Shiranui' at late stage of fruit.

**Changes in distribution of mating type and sensitivity to metalaxyl of Phytophthora infestans in Gangwon area in Korea.** Byung-Sup Kim¹, Xuan-Zhe Zhang¹, Eun-Kyoung Chung², Kyoung-Yul Ryu², Young-Il Hahn² and Youn-Su Lee³. ¹Department of Horticulture, Kangnung National University, Jibyang Dong 123, Gangnung 210-702 Korea; ²Crop Division, National Alpine Agricultural Experiment Station, RDA, Korea; ³Division of Applied Plant Science, Gangwon National University, Chuncheon, Korea.

Isolates of *Phytophthora infestans* obtained from several locations of Gangwon area in 1998-2000 were examined for their mating types and sensitivities to metalaxyl. Both A1 and A2 mating type isolates were isolated in 1998, 1999, and 2000. The majority of the *P. infestans* isolates were A1 mating type. About 64.3% of the isolates collected in 1998, 99.1% in 1999, and 81.4% in 2000 were determined as A1 mating type. Sensitivity of the *P. infestans* to metalaxyl was examined by mycelial growth on V8 juice agar amended with metalaxyl. About 44.6% of the isolates examined in 1988 were resistant to metalaxyl, 55.4% of the isolates were intermediate resistant, but none of the isolates tested were sensitive. In 1999 and 2000, 10.5 and 41.9% of the isolates examined were sensitive, 88.6 and 39.5% of the isolates were intermediate resistant, and 0.9 and 18.6% of the isolates were resistant to metalaxyl, respectively. In addition, metalaxyl effectively controlled potato late blight in two field tests in Gangwon area in 2001. Therefore, it is possible to assume that A1 mating type
is displacing A2 mating type, and metalaxyl sensitivity of the *P. infestans* isolates of Gangwon area is increasing. These results are quite different from those of early 1990s.

**Occurrence of blossom blight of *Chrysanthemum boreale* by *Didymella chrysanthemi*, Dong Kil Kim1, Chang Ki Shim2, Dong Won Bae2, Sun Chul Lee1 and Hee Kyu Kim1. 1Department Agricultural Biology, Research Institute of Life Science, Gyeongsang National University, Chiju 660-701, Korea; 2Department Agricultural Biology, Central Laboratory, Gyeongsang National University, Chiju 660-701, Korea.

The blossom and flower buds of a wild chrysanthemum, *Chrysanthemum boreale*, were blighted to black in the experiment field in Hanamyang in 1998. The infection rate of the disease on the plant was ranged from 40 to 91.8%. The pathogen isolated from infected flower buds produced numerous conidia in pycnidia. The pycnidia immersed on petals emerged through the epidermis by short ostiolate neck. Conidia have 0-3 septate (mostly uniseptate), sized 10-27.5 x 5-7.5 μm. The fungus produced pseudeocchia on potato dextrose agar (PDA), uniseptate ascospores were produced in ascii, sized 10 x 2.7 μm. The pathogen also produced pycnidia and pycnidiospores on PDA media in 4 weeks in the dark condition. The conidia produced on PDA were smaller than those from infected plants. Based on the examined mycological characteristics, the fungus was identified as *Didymella chrysanthemi*.

**Antifungal compound extracted from Cocklebur, *Xanthium strumarium* against *Phytophthora drechsleri*, Kim Dong Kil1, Chang-Ki Shim2, Min-Suk Yang2 and Hee Kyu Kim1. 1Department of Agricultural Biology, Research Institute of Life Science, Gyeongsang National University, Chiju 660-701, Korea; 2Department of Agricultural Chemistry, Gyeongsang National University, Chiju 660-701, Korea.

Crude extracts of *Xanthium strumarium* inhibited mycelial growth and zoospore germination of *Phytophthora drechsleri* in vitro. Flesh sap of 50 fold dilution from *X. strumarium* was recognized as highly effective for controlling the disease incidence in pot and field trials. Purified extracts from cocklebur inhibited mycelial growth at 12.5 μg concentration and zoospore germination at 15.6 μg/mL in vitro. Hypothial affects by the compound was malformed as corals. The antifungal compound purified from *X. strumarium* was identified as sesquiterpene lactone of xanthanolide type, 4-oxo-1(5),2,11,(13)-xanthatriene-12,8-olide having the molecular formula C_{15}H_{16}O, and MW 246.

**Genetic diversity of *Gibberella zeae* isolates from corn by AFLP**, J. J. Jeon1, H. Kim1, T. Lee1, S. H. Yun1 and Y. W. Lee1. 1School of Agricultural biotechnology, Seoul National University, Suwon 441-744, Korea; 2Division of Life Sciences, Soonchunhyang University, Asan 336-745, Korea.

A total of 496 isolates of *Gibberella zeae* were obtained from corn in Kangwon province of Korea during 1999-2000. Among them, 456 were deoxynivalenol (DON) producers and 40 were nivalenol (NIV) producers. A genetic diversity of *G. zeae* isolates differing in trichothecene production was assessed by amplified fragment length polymorphism (AFLP) method. AFLP was performed by using the three primer-pair combinations and the bands were scored. Cluster analysis was used to generate a dendrogram by NTSYS-pc using UPGMA method. The genetic diversity was significant between DON- and NIV producers; the band similarity between the two chemotypes was about 65% and that within each chemotype was 86-98%. This result indicates that similarity values within each chemotype are higher than those between the chemotypes. Therefore, AFLP analysis could differentiate DON- and NIV-chemotype of *G. zeae*.

**Polymorphism at trichothecene biosynthesis genes among deoxynivalenol- and nivalenol-producing *Gibberella zeae* strains**, T. Lee1, H.-S. Kim1, S.-H. Yun1, N.J. Alexander2, R.L. Bowden3, J.F. Leslie4 and Y.-W. Lee1. 1Seoul National University, Suwon 441-744, Korea; 2Sunchunhyang University, Asan 336-745, Korea; 3USDA/ARS, Peoria, IL 61604-3902, U.S.A.; 4Kansas State University, Manhattan, KS 66506-5502, U.S.A.

A total of 174 strains of *Gibberella zeae* were analyzed for investigating genetic diversity in trichothecene production. Korean strains from barley, corn, or wheat produced either deoxynivalenol (DON) or nivalenol (NIV) whereas all US strains from corn tested produced only DON. Genomic DNAs from these strains were digested with *MseI* and probed with a 0.6 kb fragment of *Tri5*. Hybridization pattern revealed a single band polymorphism among these strains; a 1.7 kb and a 2.2 kb band were seen from DON and NIV producers, respectively. The same set of strains was subjected to a PCR assay developed previously for differentiating DON and NIV chemotypes using *Tri7*. The PCR assay also resulted in a single band polymorphism between DON and NIV chemotypes, which was consistent with the hybridization result with the *Tri5*. The polymorphism at *Tri5* and *Tri7* was not associated with geographical origins or hosts of the strains and thus both of the methods could serve to differentiate DON and NIV producers.

**How many root-knot nematodes develop in root gall?**, D.G. Kim. Seongju Fruit Vegetable Experiment Station, Seongju, Kyungbuk 719-861, Korea.

To estimate total number of root-knot nematodes in the root galls, a fruit melon, *Cucumis melo* L. cv. Geumussaragi-euncheon, grazed on Shintoozoa (*Cucurbit maxima* x *Cu. moschata*) was planted on 4th February in a greenhouse infested with *Meloidogyne arenaria* and root gall was examined six months after planting. A grain of root gall volume 10 cm³ contained in an average of 270 females, 1,600 juveniles (J2), and 9,800 eggs. In a conservative estimation, an oriental melon plant could accommodate ca. 2 million eggs and J2 per root system which is covering 0.8 m² area. There were 5,625 J2 per 100 cm³ soil around the infested plant. These eggs and J2 in root gall are important inoculum source to the next crop and the fate is well worth of further investigation.

**Relationship in biological and genetic diversity of *Bipolaris cassivela* causing stem rot of cactus**, Jung Ho Kim and Young Ho Kim. School of Agricultural Biotechnology, Seoul National University, Suwon 441-744, Korea.

In 1999-2000, Cactus stem diseases were surveyed in the areas of Suwon, Anseong, Eumseong, Cheonan, Daeug, Yeou, Kimcheon, and Goyang, from which 62 isolates of *Bipolaris cassivela* were isolated. Colony morphology of the isolates on potato-dextrose agar could be differentiated to 4 types designated as A, B, C, and D. The
morbidity of conidia was distinguished by I and II types which included A, C, and D colony types and B type, respectively. We conducted RAPD (random amplified polymorphic DNA) in order to classify I and II types. Twenty-eight amplified fragments were produced by polymerase chain reaction (PCR) with a set of 2 random primers, and the sizes of amplified DNA fragments ranged approximately from 0.1 to 2.3 kb. The 62 isolates of B. cinerea were classified into 2 major genomic DNA RAPD groups at the genomic similarity of 97.7% and 95.1%, respectively. Cluster analysis of genetic similarity among the isolates generated the dendrogram that clearly separated all isolates into I or II conidium type.

**Rice leaf blast forecasting based on blast fungus population dynamics and weather condition in Icheon, Hong-Sik Shim, Chang-Kyu Kim, Jae-Dang Ryu, Seong-Sook Han and Hong-Sik Min, Plant Pathology Div, National Institute of Agricultural Science and Technology, RDA, Suwon 441-707 Korea.**

Rice disease forecasting technology based on pathogen activities and weather data is very important to establish disease control strategy. This experiment was conducted at Icheon experimental paddy field to analyze the rice blast occurrence together with weather data. Blast fungus was collected daily from June 11 to September 10 since 1974 and the weather data were collected by CR10 since 1993. The number of collected spores was closely related with weather condition. It was more in the cloudy day and/or day after rainfall. The peak of leaf blast occurrence was July 19 until 1970s but it became July 12 after 1980s. The speed of leaf blast increase was slow at the standard nitrogen fertilizer level on Jinheung variety but was very fast at the double amount nitrogen fertilizer plot. The most favorite condition for leaf blast development was 20°C-21°C night temperature and 90%-99% relative humidity. Especially, it was most rapidly developed under 98% RH at night.

**Effect of extraction methods of gourd seeds on detection of Cucumber green mottle mosaic virus by enzyme-linked immunosorbent assay.** Sechul Chun, Kurniawati, Y.S. Song, K.I. Ko and B.K. Kim, 1 Dept. Food Resource, Konkuk University, Seoul 143-701, Korea; 2 National Plant Quarantine Service, Anyang 430-016, Korea.

Seeds were soaked in sample extraction buffer (Agdia, Elkhart, IN, U.S.A.) for 30 min i 48 hrs to exude cucumber green mottle mosaic virus. In addition, 2 hrs soaking in sample extraction buffer and 2 hrs of freezing followed by 10 hrs thawing extract (2S-2F-10T) was used for the detection of the virus by enzyme-linked immunosorbent assay (ELISA). Whole seed and seed coat only were also extracted for the detection of Cucumber green mottle mosaic virus (CGMMV) by ELISA. In addition, the effect of amount of seeds on the rate of detection of the virus by ELISA was studied. Soaking seeds for 14hr and 2S-2F-10T methods were similar to seed and seed coat alone crushing methods on the efficiency of virus detection, indicating that seed soaking methods for the extraction could be used instead of mechanical breaking seeds with similar detection rate of the virus from gourd seeds. The results suggested that the chance of detection for the virus by increasing amount of seeds up to 20 seeds per well could be similar to one seed per well, although total amount of seeds for detection of the virus would be same for one seed and 20 seeds per well, respectively.

**Development of biofungicides for tomato gray mold rot by Botrytis cinerea L VF 12.** Choul Soung Kim, Hyun Ju Kim, Jae Pil Lee, Eun Kyung Lim, Ju Hee Song, Soon Je Jung and Byung Ju Moon. Faculty of Natural Resources and Life Sciences, Dong-A University, Busan 604-714, Korea.

This study was carried out to develop biofungicides formulated by 7 different formulation categorized into 3 different groups such as wettable, liquid and emulsified types. Also, the suppressive effect of each formulation against tomato gray mold rot by Botrytis cinerea L VF 12 was respectively investigated in a growth chamber and in a green house. To see the control effects using 7 different formulations, each formulation was respectively treated on tomato leaves, petals and fruits in a growth chamber and in a green house. According to the results, the control values of NIE and N1W3 formulations showed the highest values as 92.1% and 85.7%, respectively. And the aforementioned results were similar with that of a chemical fungicide, Diethofencarb+Carbazim (WP), showed 89.7% in the growth chamber experiment. Also, the control values of NIE and N1W3 were more effective than that of other formulations and that of chemical fungicide in the green house experiment. For the control of petals and fruits, control value of NIE was also higher than N1W3 and chemical fungicide. In addition, the taking number of fruits according to the treatment of NIE, N1W3 and the chemical fungicide were respectively 3.2, 0.9 and 0.9 in average. Furthermore, when NIE was treated, fruit numbers, fruit weight, fruit length and fruit width increased into 18, 49.8g, 6.0cm and 8.0 cm in average, respectively. For N1W3 treatment, those values were increased into 13, 45.6g, 6.0 cm and 8.0 cm, respectively. Those results of NIE and N1W3 treatment were significantly higher than those of the chemical fungicide in the green house experiments. According to the all aforementioned results, N1E and N1W3 formulation was selected as biofungicides for tomato gray mold rot.

**Biological control of strawberry gray mold rot using NIE powder formulation by Bacillus licheniformis N1.** Choul Soong Kim, Hyun Ju Kim, Jae Pil Lee, Eun Kyung Lim, Ju Hee Song, Soon Je Jung and Byung Ju Moon. Faculty of Natural Resources and Life Sciences, Dong-A University, Busan 604-714, Korea.

Occurrence of strawberry gray mold rot, which is mainly caused by high moisture condition in the green houses, have been causing great damages in the quantity and quality of strawberry fruits. Several chemical fungicides have been used to control the disease, although showing no satisfied results and causing more problems. Nowadays, more safe and promising methods of disease control are strongly requesting for the safe strawberry production. Thus, this study was conducted to develop new biofungicide using Bacillus licheniformis N1 for a new biological control method for the disease, and the suppressive effect by the biofungicide for strawberry gray mold rot was investigated in a growth chamber and in a green house. The biofungicide was formulated by 7 different formulation categorized into 3 different groups such as wettable powder (WP), liquid concentrate (LC), and emulsified concentrate (EC). Also, the suppressive effect of each formulation against tomato grey mold rot by Botrytis cinerea L VF 12 was respectively investigated in a growth chamber and in a green house. For the pathogenicity test of B. cinerea L VF 12 on strawberry leaves, various concentration (10^3, 10^4, 10^5 conidia/mL) of the conidial suspensions were prepared in 30% of tomato juice and 0.1M
of KH₂PO₄. According to the results, the most effective inoculum concentration was found to be 10⁷ conidia/mL. For the selection of remarkable formulation to control strawberry gray mold rot, the suppressive effect of each formulation was examined on strawberry leaves in a growth chamber. According to the results, NIE and NIW3 among 7 different formulation were more effective than others, although those effects were not significantly different with others. However, control values of NIE and NIW3 were significantly higher than that of a chemical fungicide, Iprodione (WP). In the green house experiment, the control value of effective two formulations showed same result as the growth chamber experiment. For control of petals, the control effect of NIE showed the highest value as 79.5% which was more effective than that of NIW3 as 17.8% and that of chemical fungicide as 42.5%. Also, In comparison of weights and number of fruits between NIE and NIW3, control value of NIE was slightly higher than that of NIW3. According to the all aforementioned results, NIE formulation, wettable powder type, was selected as a suitable biofungicide for strawberry gray mold rot amongst the 7 different formulation.

Control effect of NIK formulation using Bacillus licheniformis N1 on lettuce gray mold rot. Eun Kyung Lim, Hyun Ju Kim, Choul Soung Kim, Ju Hee Song, Soon Je Jung and Byung Ju Moon. Dept. of Agricultural Biology, Graduated School, Dong-A University, Busan 604-714, Korea.

For the biological control of lettuce gray mold rot, seven different types of formulations using Bacillus licheniformis N1 were developed as biofungicides, and those control effect was also investigated. For the biological control, The most suitable inoculation density of pathogene was tested prior to the biological control test. For the selection of the suitable inoculum, the conidial suspension of Botrytis cinerea LVF 12 made by 30% of tomato juice and 0.1M of KH₂PO₄, which was prepared as various concentrations (10⁴, 10⁵, 10⁶, 10⁷ conidia/mL), and each suspension was then inoculated on lettuce leaves cultivated in the greenhouse. Disease incidence two days after inoculation according to the inoculation density were respectively 27.5%, 45.5%, 83.5%, 86.0% and 88.0%, and the most suitable inoculation density of pathogene was determined as 10⁶ conidia/mL. To select the most suitable biological control agent among 7 formulations for lettuce gray mold rot, each formulation was treated on the lettuce leaves after inoculation of conidial suspension of pathogene, and the control effect of each formulation was examined on 7th day. According to the results, NIK formulated by the type of wettable powder showed the highest control value as 79.6%, and followed by NIW3 and NIE, liquid type, as 70.3% and 68.6%, respectively. Those control values of the aforementioned biofungicides were significantly higher than that of a chemical fungicide, Procymidione, showed as 48.3%. To find the change of control effect with time, the changes of control effects of formulations with time showed similar tendency, although the control effect of NIK was maintained for a longer time. The control effect of NIK was maintained as 77.5% for 2 weeks, and it showed 10% of decrement after 4 weeks. Therefore, NIK formulation was found to be the most suitable biofungicide for lettuce gray mold rot by Botrytis cinerea LVF 12.


Living modified organisms (LMOs), especially genetically modified bacteria(GMB) could have the potential risks to the environment. But GMB may not reveal their full potential risks until they are released to the environment. This is the reason that they must be released into the environment in safe way. Most of scientists have been interested in just developing LMOs by using biotechnology even though we lack knowledge on the possible impacts and the handling methods of GMB. It is time that we have to be paid more attention to the risk assessment and management of LMOs. Therefore, we are going to introduce one of good guidances on environmental risk evaluation of GMB, tracking the fate of GMB and monitoring their changes in microbial ecosystems after their introduction into the environment.


Hydroponic culture is being practiced as a strategy to avoid root diseases. However, various soilborne pathogens, especially semi-aquatic species as Ralstonia solanacearum, Phytophthora spp., or Pythium spp. still commonly occur and often cause greater losses than that in soil once introduced into the system. A number of chemical, physical, or mechanical measures to suppress the diseases have been developed and being practically used. Among the control systems, effects of UV/Ozone advance oxidation process (AOP) system (UV: 254.7 nm, Ozone: 184.9 nm) on control of tomato bacterial wilt caused by R. solanacearum was evaluated in this study. The original bacterial density in a nutrient tank (500 liter) was adjusted to 7.3×10⁷ cfu/mL and the population changes were checked by hourly. The bacterial population increased to 4.9×10⁷ cfu/mL in the non-treated control tank at 24 h after, while the bacterium was not detectable in the plot treated with UV/Ozone AOP System. In the other trial, the bacterial density of 2.3×10⁷ cfu/mL increased to 1.2×10⁸ cfu/mL in the control plot at 50 min after treatment. However, the bacterial population was not discovered from 10 min after treatment in the control system. Results indicated that the UV/Ozone AOP System is highly effective to sterilize the contaminated nutrient solution by R. solanacearum in hydroponics. Further assessment of the system on control of Phytophthora and Pythium is under task.

The occurrence pattern on Barley yellow mosaic virus according to the sowing time. Jong Nae Hyun, Bong Choon Lee, Dong Soo Park, Sang Ik Han, So Hee Kweon, Soo dong Kim and Huhn Pal Moon. National Yeongnam Agricultural Experimental Station, 1085, Milyang, 627-803 Korea.

Barley yellow mosaic virus (BaYMV) are responsible for one of the most important disease of malting barley in Korea, Japan, China, and Europe. Its symptom was similar to Barley mild mosaic virus (BaMMV), Barley yellow dwarf virus (BaYDv), and Soil-borne wheat mosaic virus (SBWYM), Due to transmitted by soil-borne fur- gus, Polymyxa graminis, the chemical control against the disease was very difficult. To estimate the occurrence pattern of BaYMV according to the sowing time, the sixteen varieties were sown at Oct. 10,
Oct. 20, and Oct. 30 in infected field with BaYMV in 2000. The external symptoms were investigated by the standard evaluation method of RDA on Dec. 16, Jan. 12, Feb. 19, and Mar. 3, respectively, also it was tested the leaves and roots with RT-PCR method, the primer sequence was based on the coat protein specific site of BaYMV (Gene bank accession No. Z24677). The upstream primer 5’ AAAGCGTTGCTAATTAGGTGTGTT 3’ corresponds to bases 503 to 523 of BaYMV. The downstream primer 5’ AGTGGGAGGGTGGTGATTATG 3’ which is corresponds to bases 1088 to 1109 of BaYMV. The disease occurrence was more severe degree when the seeds were earlier sown and was recorded the highest rate of occurrence sowing on Mar. 3. Some varieties such as Ea52, Otml, Geurumil, and Dahongmil didn’t infected by BaYMV and, however, Misatogolden and Moksokko 3 were no symptom in field but they were responded by RT-PCR. The Sacheon 6 revealed that the root bagon to infected at Dec. 16, it is about 8 week after the sowing.

Seralogy and molecular characteristics of Chlorella virus strains isolated in Korea. Hyun-Hwa Cho, Hyoun-Hyang Park, Jong-Oh Kim and Tae-Jin Choi. Department of Microbiology, Pukyong National University, Pusan 608-737, Korea.

We have isolated 23 Chlorella virus isolates from 10 cities in Korea. The viruses were first amplified in Chlorella strain NC64A, and pure virus isolates were obtained by repeated plaque isolation. Digestion of purified genomic DNA with 10 restriction enzymes revealed different DNA fragment patterns among these strains, and one strain, SS-1, was resistant to HindII and Alul digestion. The RNA coding regions of 8 selected strains were cloned by PCR, which contain 14-16 3RNA genes in 1.2-2 kb region except the SS-1 strain that has a 1005bp spacer in the middle. The spacer of SS-1 contains an open reading frame (ORF) of 304 amino acids that is similar to PBCV-1 ORF A478L. The major capsid protein (MCP) of all the isolated strains did not react to antiserum against Chlorella virus EPA-1 but 5 strains reacted to antiserum against PBCV-1 and 3 strains reacted to antiserum against VY-2A. Nucleotide sequences of the MCP genes of 5 strains were determined by cut-and-walk method, which showed over 90% homology among the strains.

Characterization of a Korean hypovirus of chestnut blight fungus Cryphonectria parasitica. M.J. Kim1, E.J. Hong, B.J. Cha2, S.M. Park1, M.S. Yang1 and D.H. Kim1. 1Div. Biological Sciences, Chonbuk National University, Chonju 561-756, Korea; 2Dep. Agricultural Biology, Chungbuk National University, Cheongju 361-763, Korea.

A total of 688 isolates was collected from 8 subpopulations of Cryphonectria parasitica in Korea and the presence of Cryphonectria hypovirus (CHV) was examined among 63 Korean isolates of C. parasitica which show the viral symptoms such as reduced pigmentation and conidiation. The overall incidence of virus infection in Korean C. parasitica population was 19%, 12 out of 63 isolates. Five different types of dsRNA were identified based on their molecular sizes on the agarose gel. Most of them consisted of multiple dsRNA segments consisting of approximately 5 kb to 0.9 kb in size. Among them, the hypovirus containing a 3.3 kb segment was most common in that it was observed in six isolates. The other two types were in two isolates for each and two were observed in a single isolate. In addition to the reduced pigmentation and conidiation, the strains of C. parasitica containing a 3.3 kb segment showed reduced laccase activity and hypovirulence. Moreover, the most common pattern of dsRNA was detected in two major subpopulations and also observed in the most common Korean VC group comprising 25% of a whole population.

Identification of Japanese yam mosaic potyvirus from Dipsaco opposita Thunb. cv. Dung-Gun-Ma (Tsukuneimo) in Korea. Dong Kyoon Kang1, Bong Jin Koo2, Hyeon A Jung2, Hye Yeung Shin3 and Moo Ung Chang2, 1Experiment Station of Bieresources, Kyungbuk Provincial ATA, Andong 760-891, Korea; 2Dept. Biology, Yeungnam University, 214-1 Daedong, Kyungsan 702-320, Korea.

Japanese yam mosaic potyvirus (JYMV) was identified from Dipsaco opposita Thunb. cv. Dung-Gun-Ma (Tsukuneimo) showing mosaic and malformation on leaves. Electron microscopic examination of negatively stained preparation revealed that JYMV were filamentous particles of ca. 780 nm in length as well as inclusion body. Also the filamentous particles purified from the infected leaves were decorated with antiserum of JYMV (from Fuji). In ultrathin section of the tissues of infected D. opposita, virus particles and inclusion bodies (pinwheel-, scroll, and laminated aggregate-type) were observed in the cytoplasm. The coat protein (CP) gene of JYMV was amplified by RT-PCR using a pair of specific primers. The amplified RT-PCR products were cloned into the plasmid vector and their nucleotide sequences were determined. The cloned CP genes were 773 bases long and showed 96% homology to the JYMV CP gene at the nucleotide sequences level.


Toobacco rattle virus (TRV) was detected Gladiolus hybridus, Crocus spp. and Narcissus spp. leaves showing notched or stripe on the leaf and malformation symptoms collected from Daegu and Kyungbuk province by electron microscopy, immunoblor electron microscopy (ISEM) and host range. TRV isolated from Gladiolus hybridus propagated with Nicotiana tabacum, which was good source of virus. Purification method of TRV was modified Lister (1967)'s method. TRV antiserum was made by the purified virus. The RNA molecule extracted from purified viruses by phenol: chloroform extraction method followed by ethanol precipitation. PCR primers for TRV were designed based on the sequence data in GenBank accession number AF034621. They were a forward 20-mer primer, 5'-CATTGACCGAAAGTCTAA-3' and a reverse 20-mer primer, 5'-CCTAAATCCGAACAGAAGAA-3'. The primer are corresponding to intergenic region of coat protein (CP) gene on RNA-2. RT-PCR was done. PCR products were ligated into pGEM-T Easy vector, cloned and sequenced.

Identification and characterization of Prunus necrotic ringspot virus, the family Ilarviridae, from peach. Hyeon Ran Kim, Bong Nam Chung, Gui Seoung Choi, Yong Mun Choi and Jeong Seo Kim. Division of Horticultural Environment, National Horticultural Research Institute, Rural Development Administration, Suwon 441-440, Korea.

Prunus necrotic ringspot virus (PNRSV) was isolated from peach showing mild mosaic on leaves in Korea. Virus infection rate ranged from 0 to 16.4% by ELISA in main cultivation area. The virus isolate produced ringspot in the inoculated cotyledons and systemic mosaic
and malformation in the upper leaves of *Cucumis sativus* cv. Back-bong. Systemic mottles were appeared in *Chenopodium quinoa*. The virus could not infected in *C. amaranthicolor* and *Nicotiana tabacum*. In woody indicator indexing, when the buds of virus-infected stem were grafted to the healthy GF305 young seedlings, the line pattern with mosaic was appeared in 4-6 weeks. Virus-like particles, shaped isometric having diameter of about 25 nm were found in parenchyma cells and plasmodesmata of *C. sativus* leaves inoculated mechanically. For RT-PCR, specific primers were designed based on the nucleotide sequences of *PNSV* coat protein and total RNA was extracted from virus-infected leaves of *C. sativus* by Qiagen RNeasy Plant Mini kit. The cDNA fragments of *PNSV* CP region, approximately 674 bp, were synthesized from genomic RNA extracted from virus-infected leaves by RT-PCR using specific primer pairs. And the nucleotide sequences of this *PNSV* isolates was determined and analyzed with the known *PNSV*.

**Seed and soil transmission of *Cucumber green mottle mosaic virus* (CGMMV) on cucurbits.** Jin-Woo Park1, Ta-Ho Jiang2, Jeong-Uk Cheon2 and Sook-Joo Ko1. 1Plant Pathology Division, National Institute of Agricultural Science and Technology, Surwon 441-707, Korea; 2National Plant Quarantine Service, Incheon 400-210, Korea; 1Jinam Agricultural Research and Extension Service, Naju 520-715, Korea.

Seed and soil transmission of *Cucumber green mottle mosaic virus* (CGMMV) on cucurbits were investigated in this study. The bioactivity of CGMMV on contaminated bottle guard seeds remained up to 30 months and 64% of the contaminated seeds showed positive reaction in the bioassay on *Nicotiana benthamiana*. However, less than 5% of the seedlings showed symptoms indicating seed transmission. Total amounts of CGMMV in seed coat of watermelon and bottle gourd were 25.0 and 1.4 times higher than those in the inner membrane and embryo in ELISA test. In the artificial inoculation test, infection rate of CGMMV to wounded or non-wounded bottle gourd seed coat was not different. However, injection of CGMMV solution (10 ng/mL) into the embryo increased 15-20% of infection rate compared to the non-injected control. The bioactivity of CGMMV in contaminated soil either flooded or non-flooded remained up to 17 months. However, only 1.0-3.6% of watermelon seedlings were infected in the soils. When watermelon roots were wounded and planted into the soils, infection increased 2-18 times compared to non-wounded plants. Over 50% of heavily contaminated watermelon residues in flooded and non-flooded soils showed bioactivities after one year. Results indicated that epidemics of CGMMV may be caused by not only the primary inoculums of seeds, soils, and plant residues but also by the secondary mechanical transmission.

**Garlic mite-borne mosaic viruses were surveyed their regional distribution in Korea and identified by differential RT-PCR.** Bong Jin Koo, Sang Gu Kang and Moo Ung Chang. Dept. of Biology at Yeungnam University, 214-1 Dae-dong, Kyungsan, Kyungbuk 712-749, Korea.

In the survey of viruses infected in garlic cultivated in Korea from 1997 to 2000, 3 viruses including garlic mite-borne mosaic allexivirus (GMBMV), leafy yellow stripe potyvirus-garlic strain (LYSV-G), garlic latent carlavirus (GLV) were detected from cultivated garlic plants and bulbs collected from growing 9 region 46 sites in Korea. These viruses were tested on immunosorbent electron microscopy (ISEM) with each antisera. In case of tissue cultured virus-free garlic, 1st, 2nd year of garlic were not detected of viruses, but 3rd year of garlic detected of viruses with ISEM. In most cases, virus-free garlic would be infected again with viruses when planted in field. In this survey, GMBMV were purified from infected garlic leaves and bulbs according to the CsCl-Sucrose density gradient method. Purified virus particles (0.5 mg) were emulsified in 0.5 mL of complete and incomplete Freund's Adjuvant (Gibco BRL) and injected intramuscularly into rabbit. Viral RNAs were extracted from purified viruses by phenol/chloroform extraction method followed by ethanol precipitation. After cDNA was produced, sequencing and amino acid analysis was done. cDNAs of complementary to the 3'-terminal regions of ORF V and ORF VI genomes were sequenced and characterized using of conserved region and 4 of differential primers. As a results, dIfA and dIfT of differential primers shows about 95%, 98% with GV-D and GV-A, respectively.

**Carlaviruses detected from plants in Korea.** Bong Jin Koo, Hyun A Jung, O Eok Kwon and Moo Ung Chang. Dept. of Biology at Yeungnam University, 214-1 Dae-dong, Kyungsan, Kyungbuk 712-749, Korea.

In the survey of carlaviruses of wild and cultivated plants in Korea from 1999 to 2000, 13 viruses including *Carnation latent virus* (CLV), *Lily symptomless virus* (LSV), *Chrysanthemum B virus* (CBV), *Chinese yam necrotic mosaic virus* (ChYMV), *Nerine latent virus* (NeLV), *Butterbar mosaic virus* (BtMV), *Daphne virus* S (DVS), *Kalanchoe latex virus* (KLV), *Lilac ringspot virus* (LaRSV), *Narcissus latent virus* (NLV), *Shallot latent virus* (SLV), *Potato virus M* (PVM), *Potato virus S* (PVS) were detected from the 250 plants in 47 species of 35 genera, based on shape and size of virus particles, host range, cytopathology, and serology. Under the electron microscope, negatively stained preparation of viruses appeared filamentous with 650 nm in CLV, SLM, PVM, and PVS, 640-650 nm in LSV, 650-660 nm in NLV, 685 nm in KLV, 680 nm in DVS, 700 nm in LaRSV, respectively. The same shape of virus particles were found in each infected plant cells, and infections consisting of aggregates of parallel virions in CLV, LSV, SLM, PVM, and LaRSV, amorphous bodies in PVM, were found in the infected cells, but infections in CBV were not found in the infected cells by ultra-thin sections. By sap inoculation, the viruses with CLV, CBV, SLM, PVM, PVS, NeLV, BtMV, KL, LaRSV were transmitted to several test plants, producing systemic and local symptoms. Each of the viruses with the LSV, CLV, CBV, SLM, ChYMV, PVM, PVS, NLV showed specific serological reaction to each antiserum in Immunosorbent Electron Microscopy (ISEM) or direct blotting immunoassay (DTBIA), ascertaining the identification of the viruses in this experiment.

**Characterization of a new potyvirus infecting Calla lily.** Soon Bae Kwon1, Su Jeong Heo2, Ju Yeon Yoon2, Ki Hyun Ryu1 and Jeong Ki Hong1. 1Regional Crop Experiment Station, Kangwondo Agricultural Research and Extension Services, Chuncheon 200-822, Korea; 2Plant Virus GenBank, Seoul Women's University, Seoul 139-774, Korea.

A novel virus causing mosaic and malformation symptoms was isolated from Calla lily (*Zantedeschia spp.* in Korea and its properties were determined. The size and shape of its virion and other properties suggest that the virus is a potyvirus. The virus was compared to the
well characterized several potyviruses by biological and serological properties, size of coat protein and RNA in PAGE and nucleotide sequences analysis of partial coat protein gene. The virus inducing mosaic symptom on only Calla lily plant, did not infect 15 species of indicator plants using this experiment. The average $A_{max}$ and $A_{max}$ ratios of purified virion were 1.06 and 1.25, respectively. Antiserum prepared to purified virus particles were virus specific in immunodiffusion test and had homologous titer of 1:512 in microprecipitin test. DAS-ELISA determined that the virus was not serologically related to *Dasheen mosaic virus*, which have been reported as causal virus of Calla lily. Molecular weight of the viral coat protein was about 34 kDa in electrophoretic analysis with SDS-PAGE. Electrophoretic pattern of viral RNA appeared as single band and was similar to those of other potyviruses (TuMV and LMV) RNAs. The virus could be detected with RT-PCR using several potyvirus detectable primer designed to amplify about 340 bp of the partial CP gene of potyvirus. The PCR product (340 bp) was cloned and its nucleotide sequences were determined. The nucleotide sequences showed 48.2 to 98.0% (with ZMV) identities when compared with other potyviruses. Our sequence data indicate that the virus is a strain of ZMV.

A new strain of *Kyuri green mottle mosaic virus* isolated from cucumber. Su Heon Lee1, Jeong Uk Cheon1, Hong Soo Choi1, So Hee Kwon1, Sook Joo Ko2 and Key Woon Lee3. 1Plant Pathology Division, National Institute of Agricultural Science and Technology, Suwon 441-701, Korea; 2Chenag Agricultural Research and Extension Services, Naju 520-715, Korea; 3Department of Agricultural Biology, Kyungpook National University, Taegu 702-701, Korea.

A new *Kyuri green mottle mosaic virus* (KGMV) strain (KGMV-YC1) was isolated from cucumber in Yeosu during a survey for the presence of KGMV in the major cucurbits growing area of Korea. In reaction to indicator plants, KGMV-YC1 strain was distinguished from *Cucumber green mottle mosaic virus* (CGYMV) in * Chenopodium amaranticolor*, *Spinacia oleracea*, *Cucumis melo* var. *makawa*, *Cucurbita pepo*, and *Datura stramonium*. Host reaction of KGMV-YC1 strain differed from that of the other KGMV strains in *Tetragonia expansa*, *Comphrena globosa*, *Zinnia elegans*, *Phaseolus vulgaris*, and *Petunia hybrida*. DNA fragments including coat protein region were amplified by reverse transcriptase-polymerase chain reaction (RT-PCR) using GYMV specific primers, and sequenced. The coat protein (CP) gene of KGMV-YC1 strain was 486 nucleotide residues. Comparison of the KGMV-YC1 CP with those of KGMV-Z, KGMV-KC, KGMV-C, KGMV-Y, *Cucumber fruit mottle mosaic virus* (CFMV) and KGMV-KW1 showed 96.3, 96.1, 79.6, 78.4, 70.2, and 44.0% nucleotide identity, and 99.8, 96.3, 78.3, 77.0, 78.3, and 46.0% amino acid identity, respectively. Results indicated that KGMV-YC1 is a new strain of KGMV and closely related to KGMV-KC and KGMV-Z isolated from cucumber and zucchini, respectively.

**Genome structure of a Korean isolate of *Potato virus X* and production of infectious full-length cDNA transcript.** S.H. Choi1, S.A. Choi1, W.M. Park1, J.K. Choi1 and K.H. Ryu2. 1Plant Virus GenBank, Department of Horticultural Science, Seoul Women's University, Seoul 139-774, Korea; 2Graduate School of Biotechnology, Korea University, Seoul 136-701, Korea; 3Division of Biological Environment, Kangwon National University, Chunchon 200-701, Korea.

The complete nucleotide sequence of a Korean isolate of *Potato virus X* (PVX-KR), a type species of the *Potexivirus* genus, has been determined. The RNA genome of PVX is 6,436 nucleotides long and contains five open reading frames coding for proteins of 165 kDa (viral replicase), 25 kDa (triple gene block; TGB-1), 12 kDa (TGB-2), 8 kDa (TGB-3) and 25 kDa (viral coat protein) from the 5 to 3 end, respectively. The sequences of the PVX-KR RNA-encoded proteins exhibit high similarity to the proteins of the known PVX strains; 68.5 to 98.6% and 70.4 to 99.6% at the nucleotide and amino acid levels, respectively. Strains of PVX could be divided into two subgroups (subgroup A and subgroup B) based on the sequence analysis. Phylogenetic tree analyses of the coding regions demonstrate that PVX-KR fell in subgroup B in contrast to PVX-RG-1, -Roth1, -X3, -RU1 and -RU2 strains. Full-length cDNA of PVX-KR was directly amplified by reverse transcription polymerase chain reaction (RT-PCR) using the 5'-end primer containing a T7 RNA promoter sequence and virus-specific 3'-end primer and cloned into the pUC18. Capped in *vitro* transcripts from the RT-PCR amplicon as well as full-length clone were infectious on tobacco, pepper and *Nicotiana benthamiana* plants. There was no difference between progeny virus from *in vitro* transcripts and native PVX when the progeny virus was passaged several times on its host plants. This highly infectious transcript system from full-length cDNA clone for PVX-KR can be useful for recombinant molecules for expression of foreign gene in *planta* as well as for reverse genetics for plant-PVX interaction study.

**Generation of pepper-infectious full-length cDNA transcript of *Tobacco mosaic virus*.** J.Y. Yoon1, J.K. Choi1 and K.H. Ryu2. 1Plant Virus GenBank, Department of Horticultural Science, Seoul Women's University, Seoul 139-774, Korea; 2Division of Biological Environment, Kangwon National University, Chunchon 200-701, Korea.

Full-length cDNA of pepper isolate of *Tobacco mosaic virus* (TMV-P1) was directly amplified by reverse transcription polymerase chain reaction (RT-PCR) using the 5'-end primer containing a T7 RNA promoter sequence and virus-specific 3'-end primer and cloned into the pUC18. Capped in *vitro* transcripts from the RT-PCR amplicons as well as from full-length clone were infectious on hot pepper and *Nicotiana benthamiana* plants. Progeny virus derived from transcripts was indistinguishable in the biological and biochemical properties of those wild-type virus. Progeny virus from the transcripts is stable after passaged successively several times on its host plants. Our data suggest that the infectious transcript system can be useful for recombinant molecules for expression of foreign gene in *planta* and for reverse genetics for pepper-virus interactions.

**Nucleotide sequence analyses of the coat protein genes of three cucumoviruses isolated from woody plants.** Ju Hee Bang1, Sun Jung Park1, Keum Hee Lee1, Jang Kyung Choi2 and Sang Yong Lee3. 1Department of Forest Resources Protection, Kangwon National University, Chunchon 200-701, Korea; 2Institute of National Plant Quarantine Service, Anyang 430-012, Korea; 3Department of Agricultural Biology, Kangwon National University, Chunchon 200-701, Korea.

Three cucumoviruses were isolated from *Forsythia koreana* (CMV-Fk), *Hydrangea macrophylla* for. *atoka* (CMV-Hm), and *Robinia pseudo-acacia* (PSV-Rp). Previously it was reported that CMV-Fk, CMV-Hm, and PSV-Rp belonged to *Cucumber mosaic virus* subgroup I and *Pea stunt virus* by host reaction, serological property and RT-PCR analysis. Recent analysis of the CMV CP genes can be further divided the subgroup I into IA and IB. PSV strains can be also
RT-PCR using specific primers that are designed to amplify about 1 kb fragments spanning CGMMV genome. Expected size of amplified fragments were observed when oligonucleotide primers for 5’ and 3’ terminus. In contrast, no amplification was observed at regions 2-3 and 3-4 kb position from 5 terminus of the genome. These results suggest that terminus of CGMMV genome is strongly protected while central regions are not and provide clue for understanding the mechanisms of virus inactivation by heat treatment.

Cellular protein binds to sequences near the 5' terminus of Potato virus X RNA that are important for virus replication. Sun-Jung Kwon¹, Cynthia Hemenway² and Kook-Hyung Kim¹. 'School of Agricultural Biotechnology, Seoul National University, Suwon 441-744, Korea; Department of Biochemistry, Box 7622, North Carolina State University, Raleigh 27695-7622, North Carolina.

The sequences in the 5' non-translated region (NTR) of Potato virus X (PVX) genomic RNA were previously reported to contain several regulatory elements that are required for genomic and subgenomic RNA accumulation. To investigate whether cellular proteins bind to these elements, we conducted electrophoretic mobility shift assays (EMSA) with protoplast protein extracts and RNA sequences within the PVX 5′ non-translated region. These analyses showed that the 5′ region of PVX positive-strand RNA formed complexes with cellular proteins. UV cross-linking studies of complexes formed with various deletions of the PVX RNA indicated that a 54 kDa cellular protein was bound to nt 1-46 at the 5′ terminus of PVX RNA. Site-directed mutations introduced within this 46 nt region further indicated that an ACCA sequence element located at nt 10-13 was important for optimal binding. In addition, mutations that decreased the affinity of the template RNA for the cellular factor decreased PVX plus-strand RNA accumulation in protoplasts. These studies suggest that the p54 plays an important role in PVX RNA replication by binding to the 5′ terminus of the viral genomic RNA.


One to four different segments of dsRNAs were found in several isolates of Fusarium graminearum obtained from diseased barley and corn in Korea. The dsRNAs were approximately 8 kb, 6-7 kb, and 3 kb in length, respectively, and were transmissible through spores with incidence of 30-100%. Altered culture morphology and reduced virulence were observed in one of dsRNA containing Fusarium isolate, DK-21. It was proved that dsRNA had certain effect on morphological expression of isolate DK-21, through anastomosis (hyphal fusion) with hygromycin-resistant Fusarium isolate. dsRNAs were extracted, gel purified, and used for cDNA cloning and sequence analysis. Partial nucleotide sequences of the 8 kb dsRNA genome revealed that it has some homology with polyprotein sequence and ATP-dependent helicase of several other viruses including Cryptonectria parasitica hypovirus, Barley yellow mosaic virus, and Wheat yellow mosaic virus.

Variation of Potato virus Y isolated from potatoes, tobaccoes and weeds in Korea on C-terminal region of coat protein gene and 3' non-translated region. W.S. Yun¹, H.W. Jung², M.H. Oh³, Y.I. Hahn² and K.-H. Kim¹. 'School of Agricultural Biotechnology, Seoul
National University, Suwon 441-744, Korea; 2 Suwon Experimental Station, Korea Ginseng & Tobacco Research Institute, Suwon 440-302, Korea; 3 Alpina Agricultural Experimental Station, Pyungchang, Kangwon 232-950, Korea.

Potato virus Y (PVY) is one of the most important viruses of infectious diseases in Korea. In this study, the thirty-one PVY isolates were isolated from the infected potatoes (Solanum tuberosum), tobaccos (Nicotiana tabacum) and weeds showing different mosaic symptoms in Chonbuk, Chungnam, Kangwon and Kyungbuk areas in Korea. The 640 nucleotide region containing C-terminal portion of coat protein (CP) gene and 3non-translated region (NTR) was amplified by reverse transcription-polymerase chain reaction (RT-PCR) using specific oligonucleotide primers. Sequence analyses of the amplified DNA fragments showed that the C-terminal portion of CP gene was not significantly different from those of previously reported PVY strains from potato (PVY-OK and -T) and tobacco (PVY-VN) in Korea. Homologies of the deduced CP amino acid sequences was 93.3% to 99.0% to corresponding regions of the other PVY strains including PVY-N, PVY-O, PVY-OK, PVY-T, and PVY-VN. In contrast, the sequences located at the 3'-NTR showed more diverse sequence homologies (76.4% to 99.7%). These results indicated that the C-terminal portion of the CP gene was relatively conserved while sequences at the 3'-NTR were more diverse and variable over the host species and the regions where they isolated.

Complete nucleotide sequences and infectious cDNA clones of a Korean strain of Tomato aspermy virus. S.K. Choi, I.K. Cho, K.H. Ryu and W.M. Park. 1 Graduate School of Biotechnology, Korea University, Seoul 136-701, Korea; 2 Division of Biological Environment, Kangwon National University, Chunchon 200-701, Korea; 3 Plant Virus GenBank, Department of Horticultural Science, Seoul Women’s University, Seoul 139-774, Korea.

Infectious full-length cDNA clones of a Korean strain of Tomato aspermy virus (KC-TAV) was constructed using long-template reverse transcription-polymerase chain reaction. The RNA transcripts in vitro produced using T7 RNA polymerase from full-length cDNAs could systemically infect Nicotiana tabacum cv. Xanti-nc plants and induce systemic symptoms on the upper leaves similar to the wild-type KC-TAV. The complete nucleotide sequences of genomic RNAs of KC-TAV were determined from the infectious full-length cDNA clones, respectively. RNA 1 and RNA2 of KC-TAV contain 3412 nucleotides and 3074 nucleotides, respectively. RNA 3 of KC-TAV, 2222 nucleotides long encodes 3a protein and coat protein separated by 295 nucleotides intergenic region. Overall sequence analysis of the whole genome of KC-TAV revealed strong homology (99%) to the genome of V-TAV strain, the only strain whose entire genomic nucleotide sequence had been available in the database, and an overall 60% homology to those of other cucumber mosaic virus strains and peanut stunt virus strains. The sequence comparison analysis of the deduced amino acid sequences of cDNAs of KC-TAV RNA 1, 2, and 3 indicates that there is no genetic diversity in TAV population, although the virus exists in different geographical distribution.

Necrotic spot disease of cineraria caused by Impatiens necrotic spot virus (INSV). Tanina, K., Inoue, K., Date, H., Okuda, M., Hanada, K., Nasu, H. and Kasayama, S. 1 Agricultural Experiment Station, Okayama Prefectural General Agriculture Center, Sanyocho, Okayama 709-0801, Japan; 2 Kyushu National Agricultural Experiment Station, 2421, Suya, Nishigoushi, Kikuchi, Kumamoto 861-1192, Japan.

In February 1999, cineraria (Senecio x hybrida) plants with necrotic ring spot were found in Okayama Prefecture, Japan. A spherical virus with a diameter of ca. 85-120 nm was isolated. The virus infected seven plant species of four families after mechanical inoculation. Original cineraria plants showed severe yellow mottling with necrotic spots, and were subsequently stunted. After flowering, petals in the flower showed malfomation and flower head color breaking. In serological tests, the virus was closely related to Impatiens necrotic spot virus (INSV). In RT-PCR with INSV specific primers, a fragment of about 900 bp was amplified. The fragment had a high degree of nucleotide sequence homology with the S RNA of INSV. Based on these results, the virus isolated from cineraria was identified as INSV. This is the first report of INSV on cineraria in Japan. Since diseases caused by INSV have been reported on many crops in other countries and these plants are already present in Japan, we must be aware that INSV would become an important virus in Japan in future.


Rice stripe virus (RSV) diseases transmitted in a persistent manner by the smaller brown planthopper Lachnophyax striatellus (Fallen) occurred widely this year (2001) in Gyeonggi province. The area infected with RSV was 22,005 ha in 1980, 470ha in 1981, 10,905ha in 1982, but there has been no occurrence since 1983. No incidence of RSV disease after 1983 was probably due to the decline in planted areas of winter wheat and barley. However, the area infected with RSV was found to be 3,064ha in 2001, which was 2.5% of total cultivated rice area. The major infected regions were, Hwasung, Siheung, Uiwang, Kimpo, and Kangwha. Severe occurrence of RSV was observed in Ilpumbyeo, Chuchungbyeo and Surabyeo but there was a little infection in some varieties such as Hwasungbyeo, Daenbyeo and Daejinbyeo. First occurrence of RSV was in the early of July and the peak of occurrence was in the end of July. RSV causes yellow and white stripe on leaves, leaf distorsion and the ear malfomation after heading. The percentage of overwintering population of RSV vector L. striatellus was 28.2% compared to the previous 10 years. Density of L. striatellus in light trap was low of 42.8% compared to the previous 10 years. Density of viruliferous vectors from rice fields at Hwasung area was 12.0% in Aug. 2001 by ELISA test.

Expression profile of putative transcription factor genes during the nonhost pathogens attack into hot pepper plants (Capsicum annum L.), Sang-Kum Oh1; Sanghyeob Lee, Soo-Yong Kim2, Young-Hee Chung3, Eunsook Chung4, Young-Chul Kim5, So-Young Yi5, Seoung-Hun Yu4 and Doil Choi. 1 Genome Research Center, KRRIBB, PO Box 115, Yusung, Taejon 305-600, Korea; 2 Chungnam National Univ., 220 Kang-Dong, Taejon 305-764, Korea.

Toward the understanding of regulatory mechanisms in defense response of plant-pathogen interactions, we used hot pepper plant (Capsicum annum cv. Bukang) and nonhost pathogens (Xanthomonas campestris pv. glycines; Xcg8ra) as a model system. We are generating random EST sequence database from hot pepper tissues showing nonhost hypersensitive response during Xcg8ra inoculation.
In this study, ninety putative transcription-factor genes were selected from the EST sequence database (http://plant.pdr.c.re.kr/gene) for study the expression profiles. To examine transcription profiles of these selected genes following inoculation with Xcc88a, dot blot analyses were carried out using reverse transcription and Northern hybridization methods. As results, either induced or repressed genes were detected with different time point after Xcc88a infection. More detailed expression patterns of the selected genes were investigated by Northern blot analysis. Four putative defense-response regulatory genes, which were induced by Xcc88a inoculation, were selected and expressions in relation to development of nonhost hypersensitive response in hot pepper were examined. More detailed study of mRNA expression of these genes during various pathogen and stress conditions will be presented.

Promoter analysis of HR cell death-induced gene from Nicotiana glutinosa. Young-Chul Kim1,2, Kyung-Hee Paek2 and Doil Choi1. 1Genome Research Center, KRIBB, P.O. Box 115, Yusung, Taejeon 305-600, Korea; 2Graduate School of Biotechnology, Korea University, Seoul 136-701, Korea.

In previous experiments, a HR cell death-associated gene induced by infection of TMV was isolated using modified differential screening and characterized. As results, this gene was expressed by infection of HR-inducing bacterium, Pss61 as well as TMV but not by treatment of SA, MeJA, and H2O2. Additionally, the expression of this gene was not associated with SAR. In this experiments, we isolated the promoter region of this gene using by I-PCR. Analyses of the promoter region using publically available cis-acting element analysis program revealed that several stress-related cis-acting elements were found. To further analyze transcriptional regulation of this gene and select cis-acting element respond to HR cell death, 6 different nested deletions of the promoter (approximately, 1, 0.7, 0.5, 0.3, 0.2, and 0.1 kb) were constructed into pBI101 binary vectors. Constructed recombinant binary vectors were transformed into Agrobacterium, strain LBA4404 and transferred into tobacco plant, N. tabacum cv. Xanthi in. The results of histochemical GUS staining and GUS enzyme assay were suggested that the promoter region from 0.3 kb to 0.5 kb was responded to HR cell death by infection of TMV and Pss61.

Cloning and characterization of pathogen-inducible EREBP-like transcription factor (CaNR19) from hot pepper (Capsicum annum L.) So-Young Yi1,2, Seung-Hun Yu1 and Doil Choi1. 1Genome Research Center, KRIBB, P.O. Box 115, Taejeon 305-600, Korea; 2Department of Agricultural Biology, CNU, Taejeon 305-764, Korea.

An EREBP-like transcription factor (CaNR19) was isolated by DDRT-PCR following inoculation of soybean pestle pathogen Xanthomonas campestris pv. glycines 8ra which induces HR cell death on pepper leaves. Southern blot hybridization revealed that the CaNR19 gene is present as a single copy within the hot pepper genome. The deduced amino acid sequence has two potential nuclear localization signals, a possible acidic activation domain, and an EREBP/AP2 motif that could bind to a conserved cis-element present in promoter region of many stress-induced genes. The mRNA level of CaNR19 was induced by both biotic and abiotic stresses. We observed higher level transcripts in resistance-induced pepper tissues than diseased tissues. In hot pepper plant, CaNR19 gene is also activated upon ethenon, MeJA, and SA treatment. Cold stress clearly induces expression of CaNR19 mRNA but drought, high salinity and high temperature treatment did not. For further characterization of the role of CaNR19 in stress resistance in plant, transgenic Arabidopsis plants, which overexpresses CaNR19, were produced. Analysis of the transgenic plants and biochemical experiments to confirm CaNR19, as a transcription factor is under way.

Random EST analysis and expression profiling in nonhost pathogen infected hot pepper plants. Soo-Yong Kim1,2, Cheol-Goo Hur1, Sanghyeob Lee1, Sang-Keu Oh1, Young-Chul Kim1, So-Young Yi1,2, Seung-Hun Yu1 and Doil Choi1. 1Genome Research Center, KRIBB, Yusung PO BOX 115, Taejeon 305-600, Korea; 2Chungnam National Univ., 220 Kung-Dong, Taejeon 305-764, Korea.

Identification of a complete set of genes involved in the defense process is an essential step toward understanding whole scheme of plant defense mechanism. To contribute to the goal a cDNA library was constructed from pepper leaves (Capsicum annum cv. Bukan) showing non-host hypersensitive response against bean rust pathogen Xanthomonas campestris pv. glycines and 6,599 ESTs were sequenced. To increase the genes diversity, flower bud and anther (Capsicum annum cv. Happy dry) libraries were constructed and sequenced about 2,000 ESTs, respectively. A total of 11,015 ESTs were generated and 8,525 ESTs had enough quality to be analyzed. About 50% of total ESTs (4,865) were shown to be unique genes. About 74% of EST transcripts showed sequence similarity to already known proteins. Hot pepper gene index DB (http://plant.pdr.c.re.kr/gene) have been created and detailed informations are available. Available the unique genes, 1,152 EST clones were arrayed on a slide glass and transcription profiles following non-host pathogen infected versus healthy leaves were analyzed. According to the analyzed data, subsets of genes induced or repressed in pathogen inoculated leaves were identified. Among them, several genes were previously known as being regulated by biotic stress. Some others are novel genes in non-host resistance response.

Generation of expressed sequence tags from hot pepper (Capsicum annum L.) and sequence analysis in relation to hypersensitive response against pathogen. Sanghyeob Lee, Soo-Yong Kim, Young-Hee Chung, Hyung-Joo Shin, Sung-Ho Goh, Cheol-Goo Hur, Hyun-Sook Pai and Doil Choi. Genome Research Center, KRIBB, P.O. Box 115, Yusung, Taejeon 305-600, Korea.

Large-scale single-pass sequencing of cDNA has proven to be a useful tool for discovery of new genes and understanding of biological mechanisms. As a first step to understand the complexity of plant defense mechanism, expressed sequence tags (EST) were generated from hot pepper leaf cDNA library constructed from combined leaves prepared at different time after inoculation with soybean rust pathogen Xanthomonas campestris pv. glycines. For further extension of gene diversity, ESTs were also generated from cDNA libraries constructed from anther, and flower buds using dye termination method. Among total of 10,061 generated ESTs, 9434 had good quality to be analyzed and clustering analysis revealed that 50% of total ESTs (4685) were unique. Here, we describe detailed sequence analysis data. Although we focused on the genes related to plant defense response, our data are the useful depository for further comparative studies or other purposes. In addition, this is the first work of the massive sequence information about hot pepper plant (Capsicum annum L.).
Detection of *Rice stripe virus* on rice and its insect vector small brown planthopper. Bong Choon Lee, Dong Bum Shin, Yeon Kyu Hong, Do Yeon Kwak, Sang Jong Lim and Dong Chang Lee. Plant Protection Division, National Yeongnam Agricultural Experiment Station, 1085, Milyang 627-803, Korea.

*Rice stripe virus* (RSV), which causes severe damage to rice in Korea, Japan, and China, is a type member of the tenuivirus group and is transmitted by the small brown planthopper, *Laodelphax striatellus*, in a persistent manner (Gingery, R.E. 1988, Toriyama, S. 1986). Until now, occurrence of RSV is limited in southern part of Korea. However recently the occurrence of RSV is increasing and spreading in central part of Korea including Chungcheongdo and Kyonggido province. It is very difficult to distinguish RSV symptoms on virus symptom from physiological damage of rice. The symptoms induced of infected plants includes general leaf stripping, yellowing, a distinct white coloring of the leaf stripes. We detected RSV viral RNA using reverse transcription (RT)-PCR. Gene expression of RNA is termed ambisense, therefore we used specific primer correspond to sense (RNA polymerase) and antisense (coat protein). Primer for specific amplification of nucleic acid sequence from each of the RNA polymerase (GenBank Accession No. D31879) and coat protein (GenBank Accession No. X35563) gene were designed as follows. Primers RNApol5 5’agt acg aca cca cct ctc gtt at 3’, 5’ctt aat cct tga ccc tta tag tgg t 3’ (upstream) and RNApol3 5’act aag tgt gga aca taa ct 3’ (downstream) correspond to nucleotide 58-1080, 476-1080, respectively of the RSV RNA polymerase gene and amplify a fragment of 1,023bp and 605bp. Antisense ORF3 Primers RNAcp5 5’agt ggt acc aac aag cca ggc act c 3’, 5’aca ccc tga tac aag ggt tta tat a 3’ (upstream) and RNAcp3 5’ctc gtc atc tgc act ttc tgc gtc ctc a 3’ (downstream) correspond to nucleotide 2,414-1,444, 2,412-1,913, respectively of the RSV RNA coat protein gene and amplify a fragment of 968bp and 499bp. Total RNA was extracted from infected rice plants using RNAgent Total RNA Isolation System (Promega). Total RNA of infected insect was extracted from using TRIzol Reagent ( Gibocto BRL). The result of RT-PCR, we observed specific band including RSV-polymerase (1,023, 605 bp) and CP (968, 499 bp) in both host of rice and insect vector. And we are processing cloning and partial sequencing of RSV-polymerase and CP gene.