

## 바이러스병학

- E-01. Molecular Cloning and Detection of Apple Mosaic Ilarvirus (ApMV) by Reverse Transcription and Polymerase Chain Reaction.** Ki Hyun Ryu, Chung Sun Kim<sup>1</sup>, Gung Pyo Lee, Sun Hee Choi, Seung Kook Choi<sup>2</sup> and Chang Hoo Lee<sup>1</sup>. Dept. of Horticultural Science, Seoul Women's University, Seoul 139-774, Korea. <sup>1</sup>Dept. of Horticultural Science, Korea University, Seoul 136-701, Korea. <sup>2</sup>Graduate School of Biotechnology, Korea University, Seoul 136-701, Korea

The coat protein (CP) gene of apple mosaic virus (ApMV), a member of the genus Ilarvirus, was selected for the design of the virus-specific primers for amplification and molecular detection of the virus in cultivated apple. A combined assay of reverse transcription and the polymerase chain reaction (RT-PCR) was performed with a single pair of ApMV-specific primers (PAPMCP5 and PAPMCP3-1) and crude nucleic acid extracts from virus-infected apple for rapid detection of the virus. The PCR product was verified by restriction mapping analysis and by sequence determination. The lowest concentration of template viral RNA required for detection was 100 fg. The specificity of the primers was verified using other unrelated viral RNAs. No PCR product was observed when cucumber mosaic virus (cucumovirus) or a crude extract of healthy apple was used as a template in RT-PCR with the same primers. The PCR product (669 bp) of the CP gene of the virus was cloned into the plasmid vector and resultant recombinant (pAPCP1) was selected for molecule of apple transformation to breed virus-resistant transgenic apple plants as the next step. The CP consists of 223 amino acid residues and shared 92.7 to 85.5 % amino acid identity with that of the known ApMV isolates.

- E-02. Identification and Detection of Lily Symptomless Carlavirus from *Lilium* spp. in Korea.** Jeong Uk Cheon, Kyung Gu Ahn, Jin Woo Park and Hong Su Choi. Department of Plant Pathology, National Institute of Agricultural Science and Technology, Suwon 441-707, Korea

Lily symptomless carlavirus (LSV) was purified and identified from lilies (*Lilium* spp.) showing mosaic symptoms with chlorotic stripes. Virions of lily symptomless carlavirus was purified from leaves of field-grown lilies. Virions were composed of a single polypeptide with Mr of about 32 KDa. LSV is a slightly flexuous filamentous particles of 600~700 nm long and occurs wherever lilies are grown in Korea. An A260/280 ratio of LSV was about 1.26~1.32.

Purified LSV was used as immunogen for immunizing BALB/c mouse. Hybridoma clones secreting specific monoclonal antibodies (MAbs) against the lily symptomless carlavirus were obtained by fusing murine myeloma cells (NS-1) with splenic cells from BALB/c mouse with the purified virus antigen. Four hundred and twenty-three cell lines secreting specific MAbs were screened from the obtained 480 hybridoma cell lines. Five hybridoma lines secreting specific MAbs against LSV were selected. The concentration of MAbs against LSV produced by the selected monoclonal was about 120~300 µg per 1ml culture supernatant. The isotype of MAbs secreted by 6 monoclonal, including 1G6-B1 and 1F2-B3 lines, was IgG 2a. But monoclonal, 1F2-B3 line, has two kinds of immunoglobulins such as IgG 2a and M. Specific MAbs against LSV were purified from culture supernatant and labelled with alkaline phosphatase marker for a detection of virus using dot immunobinding assay(DIBA) on nitrocellulose paper. Purified LSV and crude extract from leaves of lilies was detected by DIBA using monoclonal antibodies.

**E-03. Isolation and Purification of Carmovirus from *Lilium* spp. in Korea.** Jeong Uk Cheon, Hong Su Choi, Kyung Gu Ahn and Hae Im Jeong. Department of Plant Pathology, National Institute of Agricultural Science and Technology, Suwon 441-707, Korea

Isometric virus isolated from *Lilium* spp. collected from the middle part of Korea was identified as carmovirus based on the symptom expression on indicator plants, host range, morphological characteristics and the virus coat protein analysis. Carmovirus from lilies was propagated on *Chenopodium quinoa* and purified. The size of purified carmovirus from lilies was about 30 nm in diameter stained with 2% phosphotungstic acid. The concentration of virus was about 4.5 mg per 100 g leaves of *C. quinoa* inoculated mechanically with carmovirus isolated from lilies. An A260/280 ratio of purified virus was 1.59. Molecular weight of viral coat protein was about 37~40 KDa in electrophoretic analysis with 12% SDS-polyacrylamide gel.

**E-04. The Occurrence of Apple Scar Skin Viroid-Korean Strain (ASSVd-K) from Apples Cultivated in Korea.** Ju Hee Lee, Jean Kyung Park, Dong Hyuk Lee<sup>1</sup>, Jae Youl Uhm<sup>1</sup> and Jai Youl Lee. Department of Microbiology, Kyungpook National University, Taegu, 702-701, Korea, <sup>1</sup>Department of Agricultural Biology, Kyungpook National University, Taegu 702-701, Korea

Viroids are the smallest plant pathogens of single stranded, covalently closed circular RNA molecules. They may cause significant damage to the crop plants and fruit trees. Dapple apple and apple scar skin disease have been reported in the United States in 1950s, and have been reported in Canada, Japan, the United Kingdom and Italy subsequently. Another graft-transmissible disease of apple scar skin has been described from the United States, China and Japan. Apple scar skin viroid(ASSVd), dapple apple viroid(DAVd) and pear rust skin viroid(PRSVd) are economically important in apples and pears in China and Japan. Apple is the most economically important fruit in our country.

The low molecular weight RNA containing viroid RNA molecules were extracted from the peels of the apples (*Malus pumila* var. *dulcissima koidzumi*) var. Miegie Life bearing dapple symptoms. The Japanese variety of Miegie Life was improved as a hybrid of Senshu and Tsugaru in 1990. The RNA molecules extracted from the apples using Qiagen column chromatography. The purified RNAs were used for the synthesis of cDNA with RT-PCR. The PCR products were then ligated into a pGEM-T Easy vector, cloned and sequenced. The sequence of the viroid RNA molecule shows 331 nucleotides with one base difference ("G" insertion between the position of 133 and 134) compared to the apple scar skin viroid (ASSVd) reported in 1986 in Japan. It is the first report on the occurrence of the ASSVd in the apple trees cultivated in Korea as well as a new Korean strain of the ASSVd.

**E-05. The Occurrence of Peach Latent Mosaic Viroid(PLMVd) from Nectarine Cultivated in Korea.** Jin-Kyung Park, Ju-Hee Lee and Jai-Youl Lee. Department of Microbiology, Kyungpook National University, Taegu 702-701, Korea

Viroids are small, covalently closed, single-stranded circular RNA molecules that contain 246-371 nucleotides and possess no protein coat or mRNA activity. Some viroids cause significant damage to the infected host, usually economical crop plants and fruit trees. The casual agent of peach latent mosaic disease was reported first in France about 20 years ago and it was confirmed as peach latent mosaic viroid (PLMVd) in 1990. The PLMVd RNA molecule has been sequenced and found to have  $337 \pm 2$  nucleotides. The viroid adopts a branched conformation at the lowest free energy and is belong to the family *Avsunviroidae*.

The low molecular weight RNA were extracted from the peels of nectarine var. Harko (*Prunus persica* Batsch var. *nucipersica*) fruits containing disordered color and mosaic symptoms. The RNAs were characterized with RT-PCR and 6% polyacrylamide gel electrophoresis. The synthesized PCR products were ligated to a pGEM-T Easy vector, cloned and sequenced. The sequence of the cloned RNA was a typical form of the circular viroid RNA molecule. The viroid RNA molecule consists of 338 nucleotides with 97% homology of the peach latent mosaic viroid(PLMVd) variant #21 among 94 PLMVd variants. It contains also the conserved sequences of the hammerheaded structure as all members of *Avsunviroidae* have. The secondary structure of the PLMVd RNA molecule shows the branched conformation with other typical viroid domains including the hammerheaded structure. It is the first report on the occurrence of peach latent mosaic viroid from the nectarine fruits cultivated in Korea.

**E-06. Detection of Phytoplasma in *Dendranthema grandiflorum* in Korea.** Bong Nam Chung, Gug Seoun Choi and Yong Mun Choi. National Horticultural Research Institute, RDA, Suwon 441-440, Korea

Typical phytoplasma symptoms of chlorosis, plant stunting and proliferation of axillary shoots were observed on chrysanthemum(*Dendranthema grandiflorum*) plants grown in Korea. Examination of ultrathin sections of leaf midribs by electron microscopy revealed that numerous phytoplasma bodies were localized in the phloem cells. The disease was transmissible to healthy chrysanthemum by grafting. DNA extracted from the infected leaves was amplified with a universal primer pair P1/P6 which was synthesized on the basis of phytoplasma 16S rDNA giving the expected PCR product of 1.5 kb. In nested PCR assays, the expected DNA fragments of 1.1 kb was amplified with the specific primer pair R16F1/R1 that was designed on the basis of AY phytoplasma 16S rDNA sequences. The phytoplasma was thought as a member of the aster yellows(AY) group by the result of digestion 1.1 kb PCR products with restriction endonuclease *Sau3A*, which produced three major DNA fragment.

**E-07. A Tobamovirus Infecting *Cucurbita pepo* in Korea.** Gug-Seoun Choi, Jae-Hyun Kim, Yong-Mun Choi, Soon-Bae Kwon, Myoung-Soon Yiem. National Horticultural Reserch institute, R. D. A., Suwon 441-440, Korea

A Tobamovirus causing chlorotic spots and mosaic on the leaf and malformation on the fruit of zucchini occurred at 17.5ha of the semi-forcing culture region of Chonju city in 1999. The virus, isolate ZT1, produced local necrotic spots on *Chenopodium amaranticolor* and *Datura stramonium* and systemic symptoms on *Nicotiana bentamiana* and *Cucurbita* species. However cucumber green mottle mosaic tobamovirus from watermelon, CGMMV-W did not infected in *D. stramonium*, *C. pepo* and *C. moschata*. ZT1 in electron microscopy revealed the presence of rigid shaped particles, 300nm long and 18nm wide, and these particles were arrayed in an infected zucchini cell. A titre of the antiserum produced using ZT1 was 1/512 in agar gel double diffusion test. The ZT1 antiserum was not serologically related to CGMMV-W. CGMMV-W was serologically closely related to ribgrass mosaic tobamovirus antiserum but not to ZT1. Reverse transcription and polymerase chain reaction(RT-PCR) techniques were used for differentiation of CGMMV and ZT1. RT-PCR used by two kinds of primers, one was a portion of coat protein gene of CGMMV and another was movement gene of the virus, amplified about 450bp and 810bp DNA fragments from CGMMV-W, respectively. But DNA fragments were not produced with these primers from ZT1. It is suggested that ZT1 be different from CGMMV.

**E-08. Cloning and Sequence Analysis of Coat Protein of Cucumber Mosaic Cucumovirus Isolated from Cultivated Lily Plants.** Seung Kook Choi, Moon Yeon Yoon, Won Mok Park, Jang Kyung Choi<sup>1</sup>, Jung Choul Lee<sup>2</sup> and Ki Hyun Ryu<sup>2</sup>. National Research Laboratory for GMO Plant Risk Assessment, Graduate School of Biotechnology, Korea University, Seoul 136-701, Korea. <sup>1</sup>Division of Biological Environment, Kangwon National University, Chunchon 200-701, Korea. <sup>2</sup>Department of Horticultural Science, Seoul Women's University, Seoul 139-774, Korea.

The coat protein (CP) gene of cucumber mosaic virus (CMV), a type species of the *Cucumovirus* genus, was selected for molecular analysis of the virus in cultivated lily plants (*Lilium lancitoium*, *L. leichtlini*, *L. × 'Casa Blanca'*) in Korea. The six viruses, designated as Li22, Li24, Li27, LiK2, LiK4 and Li02, were used for amplification of the target gene by the reverse transcription and polymerase chain reaction (RT-PCR) with the *Cucumovirus*-universal primer set (Choi et al., 1999. J. Virol. Methods, 82:In press) and amplified RT-PCR products of the viruses were cloned into the plasmid vector and their nucleotide sequences were determined. The CP cistrons of the viruses showed 92.0 % to 98.0 % and 97.7 % to 99.5 % identities at the nucleotide and amino acid sequences level, respectively. Phylogenetic tree analysis indicates that the viruses from lily plants are the subgroup IA. Multiple alignment of the CP gene of the viruses with those of other known CMV strains revealed that the viruses from lily plants have unique nucleotide and amino acid sequences boxes in the CP gene. Interestingly, restriction analysis showed that the CP gene of the viruses does not contain *EcoRI* and *HindIII* sites in which they are conserved in the corresponded region of other known CMV strains of subgroup I.

**E-09. An Icosahedral Virus, Isolated from Peach in Korea, Related to Strawberry Latent Ringspot Virus.** Elizabeth M. Martin<sup>2</sup>, Y. M Choi<sup>1</sup>, Jhon Q Xia<sup>3</sup> and Kyung-soo Kim<sup>2</sup>. <sup>1</sup>National Horticultural Research Institute, RDA, Suwon, Korea. <sup>2</sup>Dept of Plant Pathology, University of Arkansas, Fayetteville, AR 72701, <sup>3</sup>Agdia, Inc , 30380 Country Road 6, Elkhart, IN 46514

Mild, mosaic-like symptoms were noted on breeding line of nectarines ( *Prunus persica* Batsch ) at the National Horticultural Research Institute, KOREA. Four plant species from three families were found to be susceptible hosts of the viral-like agent. Those species were : *Chenopodium quinoa*, *C. amaranticolor*, *Gomphrena globosa* and *Nicotiana occidentalis*. Chip bud grafting of peach seedlings determined that the agent was transmissible to GF-305. Enzyme Linked Immunosorbent Assay (ELISA) determined that the agent was serologically related to strawberry latent ringspot nepovirus (SLRSV). Negative stain electron microscopy exhibited a great abundance of 28 nm spherical particles similar in morphology to known nepoviruses. However, thin section electron microscopy of seven day post-inoculated *C. quinoa* leaves revealed the consistent appearance of double membrane-bound viroplasmic inclusion bodies that are distinct from those characteristic of most nepoviruses, including strawberry latent ringspot virus. Therefore, the Korea peach mild mosaic virus, although serologically related to SLRSV, appears to be cytopathologically distinct from other nepoviruses.

**E-10. Identification of Satsuma Dwarf Virus(SDV) and Citrus Tatter Leaf Virus (CTLV) from Citrus in Korea.** Yong Mun Choi, Hyun Ran Kim and Myoung Soon Yiem. Division of Horticultural Environment, National Horticultural Research Institute, RDA, Korea

There has been reports on the occurrence of SDV and CTLV in Cheju-do, however, there was no report on their identification from citrus. In a 1992 survey on Cheju-do citrus farms, 61.9% of the trees showed the characteristic symptoms of SDV with boat-shaped leaves. This symptom could be induced by physiological disorder and closer observation was conducted. Citrus leaves showing the characteristic SDV symptoms were collected and sap transmission test was done.

In host range test, local lesion on inoculated leaves was observed in *C. quinoa*, *Datura stramonium*, *N. glatinosa*, and *Cucumis sativas* L. CV. Baikrok, and local symptom on inoculated leaves and mosaic symptoms on upper leaves were observed on *N. occidentalis* and *N. xthanti*. NC and *Physalis floridana*, mottling symptom on inoculated leaves and abnormal leaves and mosaic symptom on upper leaves were observed. In TEM observation of the infected *N. occidentalis* leaves, inclusion bodies formed by masses of spherical particles were found which were assumed to be induced by SDV.

Leaves were collected from the citrus trees showing symptoms of swollen graft union and necrosis on graft lines with dwarfing symptom. The leaves were tested for sap transmission on indicator plants for 3 weeks at 20°C. Inoculated *C. quinoa* showed abnormal leaves and dwarfing with mottling symptoms on upper leaves. Filamentous particles were observed under TEM after purification from diseased on *C. quinoa* with bentonite method. Antiserum was produced by injection of the purified particles. The Filamentous virus was identified as CTLV.

- E-11. A Single Amino Acid Change in Cucumber Mosaic Cucumovirus Coat Protein Induces the Severe Symptoms in Tobacco.** Hye Ja Kim<sup>1</sup>, Seung Kook Choi<sup>2</sup>, Ki Hyun Ryu<sup>3</sup>, Chikara Masuta<sup>4</sup>, Ichiro Uyeda<sup>4</sup> and Jang Kyung Choi<sup>1</sup>. <sup>1</sup>Kangwon National University, Chunchon 200-701, Korea. <sup>2</sup>Korea University, Seoul 136-701, Korea. <sup>3</sup>Seoul Women's University, Seoul 139-774, Korea. <sup>4</sup>Hokkaido University, Sapporo 060-8589, Japan

Two strains of cucumber mosaic cucumovirus (CMV), Mf-CMV and Fny-CMV, were used to genetically map phenotypic differences in severity of symptoms in tobacco (*Nicotiana tabacum* cv. Burley 21). Whereas Mf-CMV induces severe symptoms on which it causes chlorotic mosaic and stunting on Burley 21, Fny-CMV induces generally green mosaic symptoms. Pseudorecombinants were constructed between the two strains. Assessment of symptoms induced by the pseudorecombinant virus strains indicated that RNA 3 is responsible for the severity of symptoms elicited by Mf-CMV in Burley 21. Using full-length cDNA clones of all three genomic RNAs of Fny-CMV and RNA 3 of Mf-CMV, chimeric RNA 3s were constructed and infectious RNAs were generated from these clones. Chimeras formed between Fny- and Mf-CMV RNA 3 were indicated that the severe symptoms on Burley 21 induced at region of coat protein gene. The coat proteins of Fny- and Mf-CMV were shown to differ at two amino acid positions and a single amino acid change at position 137 (Ala to Thr) was demonstrated as essential for the severity of symptoms on Burley 21.

- E-12. The Complete Nucleotide Sequence and Infectious Transcripts Derived from cDNA Clones of Genomic RNAs of Cucumber Mosaic Cucumovirus Mf Strain.** Hye Ja Kim<sup>1</sup>, Hye Jin Jung<sup>1</sup>, Ki Hyun Ryu<sup>2</sup>, Sang Yong Lee<sup>1</sup> and Jang Kyung Choi<sup>1</sup>. <sup>1</sup>Kangwon National University, Chunchon 200-701, Korea. <sup>2</sup>Seoul Women's University, Seoul 139-774, Korea.

The complete nucleotide sequences of all three genomic RNAs of cucumber mosaic cucumovirus Mf strain (Mf-CMV) were determined using cloned cDNAs. The three genomic RNAs, RNA 1, RNA 2 and RNA 3, are 3,356, 3052 and 2,214 nucleotides long, respectively. RNA 1 and RNA 2 contain a single open reading frame (ORF) of 2,979 and 2,577 bases encoding proteins 1a and 2a, respectively, and the RNA 2 encodes an additional ORF, 2b, that overlaps the 3' end of ORF 2a. RNA 3 is involved two ORFs of 840 and 657 bases encoding proteins 3a and coat protein, respectively. There was almost complete sequence homology between Mf-CMV and Fny-CMV genomic RNAs (95.0% sequence homology at the nucleotide) and lower degree of homology between Mf-CMV and Q-CMV genomic RNAs (71.1% sequence homology at the nucleotide). The full-length cDNA clones of all three genomic RNAs of Mf-CMV have been cloned downstream from a bacteriophage T7 RNA polymerase promoter. The RNAs synthesized by *in vitro* run-off transcription in the presence of the 5' cap analog m7GpppG were infectious in *Nicotiana benthamiana*. Inoculations of the local lesion host *Chenopodium amaranticolor* indicated that the infectivity of the synthetic transcripts was about 1% of that of the native viral RNAs.

**E-13. Comparison of Nucleotide Sequences and Biological Properties of Three Satellite RNAs Associated with Cucumber Mosaic Cucumoviruses.** Jin Sung Hong, Jae Chan Park, Jung Eun Kim and Jang Kyung Choi. Kangwon National University, Chunchon 200-701, Korea

Three satellite RNA isolates of cucumber mosaic cucumovirus (CMV), Paf-, Ap- and Rs-CMV satellite RNAs (sat-RNAs) were obtained from CMV-infected *Physalis alkekengi*, *Artemisia princeps* and *Raphanus sativus* collected in Korea, respectively. Rs-CMV induced severe mosaic symptoms on tobacco, whereas Paf- and Ap-CMV caused a mild symptoms. The nucleotide sequences of the three sat-RNAs were determined. The sat-RNAs, Paf-, Ap- and Rs-sat-RNA, were 386, 347 and 335 bases long, respectively. The sequences were compared with each other and with other sat-RNAs, revealing high degree of nucleotide homology between Ap-sat- RNA and Rs-sat-RNA (92.5%), whereas Paf-sat-RNA revealed lower homology to other sat-RNAs (64.6 to 70.7%). The full-length cDNA clones of the three sat-RNAs have been cloned downstream from a bacteriophage T7 RNA polymerase promoter. The RNAs synthesized by *in vitro* run-off transcription in the presence of the 5' cap analog m7GpppG were infectious in *Nicotiana tabacum* cv. Xanthi nc which inoculated with purified M-CMV as a helper virus. The inoculations indicated that the symptoms on tobacco were differed from each sat-RNA.

**E-14. Characterization of Two Alfalfa Mosaic Alfamoviruses Isolated from Adzuki Bean and Potato Plants.** Hye Ja Kim<sup>1</sup>, Hye Jin Jung<sup>1</sup>, Hyo Won Jung<sup>2</sup>, Young Il Hahm<sup>2</sup> and Jang Kyung Choi<sup>1</sup>  
<sup>1</sup>Kangwon National University, Chunchon 200-701, Korea. <sup>2</sup>National Alpine Agricultural Experiment Station, Pyungchang 232-950, Korea.

Two alfalfa mosaic alfamoviruses (AIMV), Az-AIMV and Po-AIMV, were isolated from adzuki bean and potato plants showing yellow mosaic and calico symptoms on each plant, and were identified using electron micrograph, dsRNA analysis, serological assay, reverse transcription-polymerase chain reaction (RT-PCR), SDS-PAGE of the coat protein and genomic RNAs, physical properties and host range tests. Electron micrograph of Az- and Po-AIMV exhibited bacilliform particles of about 18 to 60 x 18 nm. Both dsRNA preparations from two AIMV-infected tobacco leaves were appeared four bands of 3.6, 2.6, 2.0 and 0.9 kbp. The viruses could be detected with RT-PCR using two specific primers designed to amplify about 900 bp of the coat protein gene. SDS-PAGE of the Az- and Po-AIMV coat proteins showed one major protein band which have a same migration site as well as that of common strain of AIMV (ATCC PV-92). In immunodiffusion test, the isolates were positively reacted with antiserum of common AIMV. Thermal inactivation points of the viruses were 70°C and retained their infectivities at the dilution of 10<sup>-4</sup>. Longevity *in vitro* of the crude sap of tobacco leaves infected with the viruses were 3 days. All isolates caused systemic symptoms on upper leaves of *Chenopodium amarnaticolar*, *Nicotiana tabacum* and *Gomphrena grobosa*. Local lesions were produced on the inoculated leaves of *Vigna sinensis*, *Tetragonia expansa*. However, Po-AIMV induced yellow mosaic and necrosis on *C. quinoa* and *Capsicum annum* instead of a mild mosaic induced by other strains.

**E-15. Symptom Expression and Virus Accumulation by Cucumber Mosaic Cucumovirus Infection in Transgenic Tobacco Plants Expressing the Satellite RNA.** Jin Sung Hong, Sang Yong Lee and Jang Kyung Choi. Kangwon National University, Chunchon 200-701, Korea

The objective of this work was to confirm steps in virus infection which were inhibited in transgenic tobacco plants (*Nicotiana tabacum* cv. Xanthi nc) that express the satellite RNA of the As strain of cucumber mosaic cucumovirus (As-CMV). These plants were shown to be protected against disease development after inoculation with Co-CMV (Lee et al., 1995). In this experiment, we used to M-CMV as a challenge virus, which is expressed a distinct chlorosis symptoms on tobacco. The chlorotic symptoms were considerably attenuated in the M-CMV-infected transgenic tobacco plants. The numbers of local lesions produced on inoculated leaves of *Chenopodium amaranticolor* and *C. quinoa* infected with M-CMV derived from the transgenic tobacco plants were 30% or less of those from the non-transgenic tobacco plants. The accumulation degree of the viral RNA and coat protein in the transgenic tobacco was investigated using the dsRNA, RT-PCR and western blot analyses.

**E-16. Identification of a Virus Inducing Pepper Yellowing Disease.** Bong Choon Lee, Yong Mun Choi and Myoung Soon Yiem. National Horticultural Research Institute, Suwon 441-440, Korea

Recently, an apparent yellowing symptom in pepper was newly observed in experimental plots of the Horticultural Research Institute in Suwon. The affected plants had a bright yellowing on their leaves and immature fruits. Cucumber mosaic virus(CMV) and satellite RNA(satRNA) were always recovered by dsRNA analysis and RT-PCR in the pepper plants. It is reported that large amount of satRNA were found in RNAs of naturally occurring CMV isolates and induced a unique brilliant yellow symptom on tobacco(Takanami, 1980). Choi reported pepper yellowing disease by aphid and graft transmissions(1997). In this study, pepper plants were transmitted by mechanical sap, aphid and grafting, which produced the same yellowing symptom. We could also detect CMV and satRNA. It is supposed that CMV and satRNA are related to pepper yellowing symptoms. The cDNAs of CMV-CP and satRNA were cloned and sequenced.



- E-17. **Potyriviruses Isolated from Peanut in Korea.** Bong Jin Koo, Hyun A Jung, O Eok Kwon, Moo Ung Chang<sup>1</sup>, Eui Kyoo Cho<sup>2</sup>. <sup>1</sup>Yeungnam University, 214-1 DaeDong, Kyungsan, Korea 712-749. <sup>2</sup>Department of Agricultural Biology, Colley of Natural Sciences, Andong National University, Andong 760-749, Korea

Peanut(*Arachis hypogaea*) plants showing dark green stripes along the lateral leaf veins, mild mottle and green mosaic symptoms were collected from their growing areas in Kyongbuk province. Electron microscopic examination of negatively stained preparations showed filamentous particles of ca. 780 nm in length as well as inclusion bodies. By the immunosorbent electron microscopy(ISEM) and electronmicroscope(EM), filamentous particles were strongly decorated with antisera of peanut mottle virus(PMV, ATCC-413), peanut stripe virus(PStV, from Ohki), but these particles were partially decorated with antiserum of peanut green-mosaic virus(PgMV, ATCC-607). In an ultrathin sections of the tissues of naturally infected peanuts, virus particles and inclusion bodies(pinwheel-, scrolls-type) were observed in the cytoplasm of mesophyll cells. By direct immunostaining assay(DISA), PMV and PStV were detected from seeds of naturally infected peanut.

- E-18. **Two Viruses Isolated from Yam in Korea.** Bong Jin Koo, Dong Soo Jung, Moo Ung Chang<sup>1</sup>, Dong Kyoon Kang<sup>2</sup>, Yong Moon Choi<sup>3</sup>. <sup>1</sup>Yeungnam University, 214-1 Dae Dong, Kyungsan, Korea 712-749. <sup>2</sup>Kyongbuk provincial ATA Taegu, Korea 702-320, <sup>3</sup>National Horticultural Research Institute, Suwon 441-440, Korea

Yam plants, *Dioscorea batatas* showing necrotic mosaic and *Dioscorea bulbifera* showing mosaic or malformation were collected from their growing areas in Taegu, Kyongbuk province. One virus isolated from *D. batatas* was identified as chine yam necrotic mosaic maculavirus(ChYNMV) and another virus isolated from *D. bulbifera* was identified as yam mosaic potyvirus(YMV) by ISEM and EM. ChYNMV has previously been considered to be a member of the carlavirus. But, Kondo's studies(1999) reported the ChYNMV belongs to the new genus Macluravirus. Electron microscopic examination of negatively stained preparations showed that ChYNMV and YMV were filamentous particles of ca. 660 nm and 780 nm in length as well as inclusion bodies. By ISEM, the particles of 660 nm were decorated with antiserum of ChYNMV(from Kondo), and the particles of 780 nm were decorated with antiserum of YMV(ATCC-471). In ultrathin sections of the tissues of naturally infected *D. bulbifera*, virus particles and inclusion bodies(pinwheel-, scroll- and laminated aggregate-type) were observed in the cytoplasm.

**E-19. Classification of Soybean Mosaic Virus Strains Based on RT PCR-RFLP Analysis.** Yul-Ho Kim<sup>1</sup>, Ok-Sun Kim<sup>1,3</sup>, Bong-Choon Lee<sup>2</sup>, Jae-Hwan Roh<sup>1</sup>, Myoung-Ki Kim<sup>1</sup>, Dae-Joon Im<sup>1</sup> and Il-Bong Hur<sup>1</sup>. <sup>1</sup>National Crop Experiment Station, RDA, Suwon, Korea 441-100. <sup>2</sup>National Horticultural Research Institute, RDA, Suwon, Korea 441-440. <sup>3</sup>Kangwon National University, Chuncheon 200-701, Korea

Strains of soybean mosaic virus(SMV) were classified by reverse transcription polymerase chain reaction-restriction fragment length polymorphism(RT PCR-RFLP) analysis and virulence tests. A primer pair that amplified a 1385 bp fragment of the cylindrical inclusion(CI) coding region was used. The RFLP profiles of the RT PCR products were determined with the restriction enzymes, *EcoRI* and *RsaI*, which were derived from the nucleotide sequences of G2, G5, G5H, G7 and G7H. The RFLP profiles of G2, G4 and G7H strains when digested with *EcoRI* had their specific band patterns. The *RsaI* enzyme distinguished each of G4, G5, G6 and G7 strains from the others. Seventeen isolates of SMV used in this study were from seedborne local varieties. They were inoculated to more five plants of each differential cultivar, Jangbeakong and Hwanggeumkong and classified into nine groups.

**E-20. Detection of Barley Yellow Dwarf Virus(BYDV) by RT-PCR and Distribution of BYDV Strains in Korea.** Yul-Ho Kim<sup>1</sup>, Mi-Ok Woo<sup>3</sup>, Ok-Sun Kim<sup>1</sup>, Bong-Choon Lee<sup>2</sup>, Jung-Hyun Nam<sup>1</sup>, Dae-Joon Im<sup>1</sup> and Nam-Chun Pack<sup>3</sup>. <sup>1</sup>National Crop Experiment Station, RDA, Suwon, Korea 441-100. <sup>2</sup>National Horticultural Research Institute, RDA, Suwon, Korea 441-440. <sup>3</sup>Seoul National University, Suwon 441-744, Korea

Barley Yellow Dwarf Virus(BYDV) is a group of closely related viruses which cause economic losses in a wide range of graminaceous species throughout the world. BYDV has not yet reported in Korea. Therefore we report the detection method by RT-PCR and distribution of BYDV strains in Korea.

A diagnostic assay for BYDV was developed using the reverse transcription and polymerase chain reaction(RT-PCR). Three primer pairs were derived from the previously published RNA sequences of four BYDV strains (MAV, PAV, RMV, and RPV). A single primer pair(Primer A) designed by comparing each of the RNA sequences of the conserved regions of the coat protein could detect all strains used. A major RT-PCR product of 388bp amplified by Primer A was present in samples from four strains provided by CIMMYT.

In Korea, BYDV was distributed at 11 regions among the investigated 19 regions. Of 101 samples collected, 67 were detected as BYDV and BYDV-PAV was most common (60%), followed by MAV (4%) and RMV (2%).

**E-21. Incidence of Three Major Citrus Viruses in Cheju Island.** Dae-Hyun Kim, Duck-Chul Oh<sup>1</sup>, Hyeog-Mo Kwon<sup>2</sup>, Chae-Wook Hyun, Dong-Hwan Kim and Seong-Chan Lee. Citrus Experiment Station, National Cheju Agricultural Experiment Station, Rural Development Administration, Cheju 699-800, <sup>1</sup>Department of Biology, College of Science, Cheju National University, <sup>2</sup>Extension Planning Division, Rural Development Administration

The virus infection rates and disease symptoms of three major citrus viruses, citrus tristeza virus (CTV), satsuma dwarf virus (SDV), and citrus tatter leaf virus (CTLV) were investigated at 35 citrus orchards in Cheju Island from 1995 to 1997.

The infection rates of CTV, SDV, and CTLV were 69.8%, 8.6%, and 9.3%, respectively. However, according to cultivars, there were significant difference in the infection rates. The infection rates of CTV were highest in early satsuma mandarin (*Citrus unshiu*) with 80.9% and lowest in very early satsuma mandarin with 51.9%. In SDV, the highest was in very early satsuma mandarin with 23.1% and the lowest was in early satsuma mandarin with 6.3%. And the highest infection rate in very early satsuma mandarin with 17.9% and the lowest in tangors with 7.3% in CTLV.

The symptoms of virus-infected citrus were very diverse ; small and abnormal shape of fruits, abnormal leaves such as narrow boat and small spoon shapes of leaves, stem-pitting on the twig, bud-union crease and swelling of the craft part, reduction of the plant vigor and poor yields.

**E-22. Ecology and Pathogenicity of Cucumber Green Mottle Mosaic Tobamovirus (CGMMV).** Jin Ho Yeo and Eui Kyoo Cho. School of bioresource science, Andong national University, Andong 760-749, Korea

In some northern parts of Kyungbook Prov. where Cucumber Green Mottle Mosaic Tobamovirus (CGMMV) was observed severely in 1998, results of soil-borne transmission of both rotational cropping cucumber plants and after rice growing the previous year were no difference in incidences. Cucumber was very useful for source of virus because mosaic symptom produced 15 days by being inoculated with infected leaves of the plants above. And in test plants *Chenopodium amaranticolor* produced local lesions and no symptom on *Nicotiana glutinosa*, *Petunia hybrida*, and *Datura stramonium*. Investigation on the disease infection and movement in both water melon and grafting plant used in Korea indicated that some of cultivars of the water melon and all cultivars of the grafting plants produced mosaic symptom 20 days after the sap inoculation. From 200g of the grafting plants the CGMMV was purified 4.16mg/ml, and proved through the electric microscopy and the agar gel double diffusion test.

- E-23. Tobacco Mosaic Virus(TMV) Isolated from Buckwheat Plant (*Fagopyrum esculentum* Moench) in Korea.** Hahm Young Il, Jung Hyo Won and Shin Gwan Yong<sup>1</sup>. <sup>1</sup>National Alpine Agricultural Experiment Station, Pyungchang 232-950, Korea

Buckwheat(*Fagopyrum esculentum* Moench) plants showing severe mosaic symptoms on leaves was collected from Bongpyung area, Kangwon, in 1999. Isolate of virus was identified as tobacco mosaic tobamovirus(TMV) by some experiments of host range, serology, electron microscopy and sodium dodecyl sulfate-polyacrylamide gel electrophoresis(SDS-PAGE). The virus was produced local lesions on the inoculated leaves of *Gomphrena globosa*, *Datura stramonium*, *Nicotiana glutinosa*, *N. rustica*, *N. tabaccum* cv. Xanthi-nc and *Tetragonia expansa*, and systemically infected on *Chenopodium amaranticolor*, *C. quinoa*, *N. debneyi*, *N. tabaccum* cv. Samsun NN and *Physalis floridana*. However, the virus did not infected on *Phaseolus vulgaris*, *Pisium sativum*, and *Vicia faba*. The virus was clearly reacted with antiserum to TMV-P3 strain, but not with antisera to TMV-N strain, cucumber green mottle virus(CGMMV), and pepper mild mottle virus(PeMV). The shape of virus particle was rod type about 280~300nm in length by dipping method of electron microscopy. Molecular weight of the viral coat protein was 18,000~21,000 dalton by mean of SDS-PAGE. This is the first report that TMV is a pathogen of buckwheat in Korea.

- E-24. Ultrastructural Aspects of the Mixed Infections of Watermelon Mosaic Potyvirus and Cucumber Green Mottle Mosaic Tobamovirus V. Double Infections of CGMMV from Watermelon and Three Isolates of WMV from Pumpkin.** Jeom-Deog Cho<sup>1</sup>, Hong-Soo Choi<sup>2</sup>, Jeong-Soo Kim<sup>3</sup>, Kyung-Soo Kim<sup>4</sup> and Kook-Hyung Kim. <sup>1</sup>Seoul National University, Suwon 441-744, Korea, <sup>2</sup>National Institute of Agricultural Science and Technology, Suwon 441-707, Korea, <sup>3</sup>National Horticultural Research Institute, Suwon 441-440, Korea, <sup>4</sup>University of Arkansas, Fayetteville 72701, USA

Two unrelated viruses of watermelon mosaic potyvirus (WMV) and cucumber green mottle mosaic tobamovirus (CGMMV) were mechanically co-inoculated to investigate ultrastructural aspects of the mixed infections. Three WMV isolates collected from different pumpkin cultivated areas (WMV-PA from Andong, WMV-PS from Suwon and WMV-PE from Euiryung) were individually co-inoculated with CGMMV isolated from watermelon (CGMMV-W) on Cucurbits. Cucurbits were watermelon 'Dalgona', oriental melon 'Eunchon' and cucumber 'Jangilibchu'. Synergistic symptom expression was occurred in watermelon by the all sets of mixed infections, however, no synergistic symptoms were produced in oriental melon and cucumber. In the cells infected mixedly with WMV-PA and CGMMV-W, the specific ultrastructures of nonagon, a ring of nine tobamovirus particles having a potyvirus particle in center, and angled-layer aggregates made by tobamovirus particles were induced. The mixed virions of WMV-PS and CGMMV-W also induced nonagon. In the combination of WMV-PE and CGMMV-W, the two different viruses were presented independently in a same cell and therefore no specific ultrastructures were induced. There was no difference in the ultrasturctures of three Cucurbits infected with the mixed virions.

**E-25. Ultrastructural Aspects of the Mixed Infections of Watermelon Mosaic Potyvirus and Cucumber Green Mottle Mosaic Tobamovirus VI. Double Infections of CGMMV from Cucumber and Three Isolates of WMV from pumpkin.** Jeom-Deog Cho<sup>1</sup>, Hong-Soo Choi<sup>2</sup>, Jeong-Soo Kim<sup>3</sup>, Kyung-Soo Kim<sup>4</sup> and Kook-Hyung Kim. <sup>1</sup>Seoul National University, Suwon 441-744, Korea, <sup>2</sup>National Institute of Agricultural Science and Technology, Suwon 441-707, Korea, <sup>3</sup>National Horticultural Research Institute, Suwon 441-440, Korea, <sup>4</sup>University of Arkansas, Fayetteville 72701, USA

The combinations of the mixed infections for watermelon mosaic potyvirus (WMV) and cucumber green mottle mosaic tobamovirus (CGMMV) were CGMMV-C + WMV-PA, CGMMV-C + WMV-PS and CGMMV-C + WMV-PE. CGMMV-C was isolated from cucumber. The isolates of WMV-PA, WMV-PS and WMV-PE were identified from pumpkin at Andong, Suwon and Euiryung area, respectively. Three Cucurbits of watermelon 'Dalgona', oriental melon 'Eunchon' and cucumber 'Jangilibchu' were used for the mixed infections through mechanical inoculation. Synergism in symptom expression was occurred only in watermelon infected mixedly with WMV-PA and CGMMV-C. Nonagon, a potyvirus particle surrounded by evenly spaced nine tobamovirus particles, was induced in the cells infected mixedly with WMV-PA + CGMMV-C and WMV-PS + CGMMV-C. The other specific ultrastructure of angled-layer aggregate was observed in the mixedly infected cells with WMV-PA + CGMMV-C. The mixed virions of WMV-PE and CGMMV-C were observed in a same cell but did not produce nonagons or angled-layer aggregates.

**E-26. Identification of Peanut Stunt Virus Isolated from *Robinia pseudo-acacia* L. Using RFLP Techniques.** Ju Hee Bang<sup>1</sup>, Sun Jung Park<sup>1</sup>, Sang Yong Lee<sup>1</sup>, Jang Kyung Choi<sup>2</sup> and Ki Hyun Ryu<sup>3</sup>, <sup>1</sup>Department of Forest Resources Protection, Kangwon National University, Chuncheon 200-701, Korea. <sup>2</sup>Department of Agricultural Biology, Kangwon National University, Chuncheon 200-701, Korea. <sup>3</sup>Department of Horticultural Science, Seoul Women's University, Seoul 139-774, Korea

An isolate of peanut stunt virus (PSV) was isolated from *Robinia pseudo-acacia* L. showing severe mosaic symptom, designated as PSV-Rp. RT-PCR and RFLP techniques were used to identification and differentiation of PSV-Rp. A single pair of primer was designed for Cucumovirus-genus specific. Downstream primer, CPTALL3, is 18-mer nucleotide which located in completely conserved region of the 3' noncoding part. Upstream primer as degenerate primer, CPTALL5, is 20-mer nucleotide which located in the intercistronic region of MP and CP part. RT-PCR used by the primer amplified about 950bp DNA fragments from the crude sap of virus-infected acacia leaves. And, RFLP analysis of RT-PCR products using *EcoRV*, *XhoI* and *MboI* showed that PSV-Rp belonged to PSV-Japan strain with some variation. Nucleotide sequence of the RT-PCR product from PSV-Rp was analysed, and compared the sequence with other PSV strains.

**E-27. Nucleotide Sequence of Coat Protein Gene of Kyuri Green Mottle Mosaic Virus(KGMMV) Isolated from Zucchini(*Cucurbita pepo*) in Korea.** Su-Heon Lee<sup>1</sup>, Young-Gyu Lee<sup>2</sup>, Jin-Woo Park<sup>1</sup>, Jeong-Uk Cheon<sup>1</sup>, Key-Woon, Lee<sup>2</sup> and Yong-Chul Choi<sup>1</sup>. <sup>1</sup>Division of Plant Pathology, National Institute of Agricultural Science and Technology, Suwon, Korea 441-707. <sup>2</sup>Department of Agricultural Biology, Kyungpook National University, Taegu 702-701, Korea

The coat protein(CP) gene of kyuri green mottle mosaic virus(KGMMV) isolated from zucchini(*Cucurbita pepo*) in Chonju, Korea in 1999 was sequenced by the reverse transcription and polymerase chain reaction with degenerate and generate primers originated from tobamoviruses. The gene consisted of 486 nucleotides and had the same nucleotide length compared with those of cucurbit-infecting tobamoviruses. The sequence of the KGMMV CP gene compared with three CGMMV strains, CGMMV-W, CGMMV-SH, and CGMMV-KT1 showed 43.0-43.6% similarity, whereas, three CGMMV strains among themselves showed 98.6-99.8% similarity. The deduced amino acids of KGMMV CP gene were 160 amino acid residues with the molecular weight of 17,049 daltons. The first 15 codons of them corresponded to the sequences of the N-terminal amino acid of the viral capsid protein. The amino acid sequences of KGMMV CP had 43.5% similarity compared with those of three CGMMV strains. However, the similarities among three CGMMV strains were 100%. These results showed that two cucurbit-infecting tobamovirus members, KGMMV and CGMMV were genetically distantly related.

**E-28. Detection of Tobacco Rattle Tobravirus (TRV) Using RT-PCR from *Narcissus* spp. in Korea.** O Eok Kwon, Dong Soo Jung, Bong Jin Koo, Hyun A Jung, Moo Ung Chang<sup>1</sup> and Yong Mun Choi<sup>2</sup>. <sup>1</sup>Yeungnam University, Kyungsan 712-749, Korea. <sup>2</sup>National Horticultural Research Institute, Suwon 441-440, Korea

*Narcissus* spp. showing yellow-green stripes or mottling-mosaic on the leaf surface, chlorotic streaks especially in the leaf bases, leaf distortion, and sometimes flower color breaking symptoms were collected from its growing areas in Taegu and Kyungbuk province of Korea. An electron microscopic examination of negatively stained preparations showed rod-shaped particles of 180-210 nm, 46-114 nm length and 22 nm wide, filamentous particles of ca. 800 nm, and spherical particles of 28 nm in a diameter. The reverse transcription-polymerase chain reaction (RT-PCR) and an immunosorbent electron microscopy (ISEM) were carried out for detection of the TRV in the narcissus samples. The RT-PCR was used by a set of 18-mer primers (upstream 5'-ATGACGTGTGTACTCAAG-3', downstream 5'-AGAAACAGATAACAATAT-3'), designed in CP-gene of TRV. The fragments of amplified cDNA were about 450-500 bp size. The RT-PCR was suitable for the identification and differentiation of TRV as rapid, time-saving, and reliable detecting method. By the ISEM, rod-shaped virus particles were decorated antiserum of TRV (ATCC PVAS-820) and detected mainly in the narcissus showing yellow-green stripes and mottling-mosaic symptoms.

**E-29. RT-PCR Detection of *Closterovirus* GLRaV-3 and *Nepovirus* GFLV.** Hyun Ran Kim, Yong Mun Chio, Bong Choon Lee, Myoung Soon Yiem and Jae Dong Chung<sup>1</sup>. Horticultural Environment Division, National Horticultural Research Institute, RDA. <sup>1</sup>Kyungpook National University

Application of the PCR techniques in routine virus diagnosis is limited by difficulties in extracting good quality nucleic acid free of PCR inhibitors in woody plants such as grapes. To detect the phloem limited closterovirus GLRaV-3 and the nepovirus GFLV in grapes, the viral RNAs were extracted using RNA extraction kit(Qiagen, Inc.) and RT-PCR diagnostic method was applied. Petiole and midrib, infected plantlet cultured *in vitro* and young leaves were ground in liquid nitrogen. The total RNAs were extracted with phenol or using commercially RNeasy Plant kit. DNA amplification primers were designed based on the published nucleotide sequences of the coat protein coding regions of GLRaV-3 and GFLV. RT-PCR was conducted using a Access RT-PCR system(Promega Co.) and the products were electrophoresed on 1.2% Agarose gel and stained with EtBr.

In RNA extraction by phenol, PCR products could not be detected, while a specific amplication products could be detected by the RNA purification kit. *In vitro* cultured plantlet showed the best results. Specific bands of about 941bp in GLRaV-3 and about 1515bp in GFLV were detected.

**E-30. Molecular Immunological Studies About Host Cell Division Induced by BCTV Gene Product in Arabidopsis.** Hyunsik Hwang<sup>1</sup>, Jongbum Park<sup>2</sup> and Sukchan Lee<sup>1</sup>. <sup>1</sup>Sungkyunkwan Univ. Suwon, Korea, 440-746. <sup>2</sup>Silla Univ., Pusan 617-736, Korea

BCTV is a single-stranded DNA virus that is proving useful for basic studies of the interaction of Arabidopsis with a viral host and provides a system for studying both resistance and the molecular basis of symptom development. Arabidopsis Sei-O ecotype was found to be 'hypersusceptible' to the BCTV-Logan strain in that it developed very severe symptoms, including severely deformed inflorescences with callus-like structures, and accumulated high levels of viral DNA. Microscopic studies of the BCTV-induced cell division that was dramatically expressed in Sei-O demonstrated that the activation of cell division is preceded by the disruption of the phloem. We have further definded the factors important for symptom development caused by BCTV using a molecular genetic approach based on expressing BCTV- encoded proteins in transgenic plants. Results of these studies indicate that the BCTV ORF L4 is a primary symptom determinant. Expression of L4 from BCTV- Logan in transgenic plants results in a range of phenotypes that include typical BCTV inducible symptoms. We currently analyzed the BCTV L4 protein expression by western hybridization and by RT-PCR and have been utilizing the molecular immunocytological approaches to localize L4 protein in the cellular and subcellular levels with transformed Arabidopsis and BCTV infected plants.

**E-31. Identification of Loci in Arabidopsis that Confer Tolerance to Hypervirulent BCTV Infection.** Sunghee Lee<sup>1</sup>, Jongbum Park<sup>2</sup>, Keith Davis<sup>3</sup> and Sukchan Lee<sup>1</sup>. <sup>1</sup>Sungkyunkwan Univ. Suwon, Korea, 440-746. <sup>2</sup>Silla Univ. Pusan, Korea, 617-736. <sup>3</sup>Plant Biotechnology Center, Ohio State Univ., 43210, USA

The infection of susceptible plant hosts by single stranded DNA virus in the geminivirus group depends on the infection of host and viral factors for the replication of viral DNA, the expression of viral genes, and the movement of virus throughout the plant. This work reports that three strains of beet curly top virus (BCTV) differ in their ability to infect certain ecotypes of *Arabidopsis thaliana*. Symptoms appeared on susceptible plants approximately 2-3 weeks after inoculation with BCTV-Logan and BCTV-Worland and after 10-15 days with BCTV-CFH. Symptoms were more severe in BCTV-CFH infected plants and included leaf curling, the formation of stunted, deformed inflorescence structures and the accumulation of anthocyanin pigments in symptomatic tissues. Analysis of viral DNA accumulation indicated that symptom development and severity were correlated with the amount of viral DNA present in the plants. One ecotype did not produce any disease symptoms against BCTV-CFH but the other ecotypes all were died with BCTV-CFH infection. Studies of viral DNA replication and virus movement in three excised organs demonstrated that BCTV-CFH could replicate viral DNA and move systemically in this ecotype, suggesting that tolerance was due to a block in symptom development. Genetic analysis of this ecotype indicated that tolerance is due to a single, recessive locus.

**E-32. Induced Lignification During Symptom Development by Geminivirus Infection on Arabidopsis thaliana.** Hyekyung Shim<sup>1</sup> and Sukchan Lee<sup>1</sup>. <sup>1</sup>Sungkyunkwan Univ. Suwon 440-746, Korea

The responses of *Arabidopsis thaliana* ecotype to the phytopathogenic geminivirus were examined. Beet curly top virus (BCTV) induce typical disease symptoms including malstructure of shoots, leaf curling and stunting. One of Arabidopsis ecotype, Sei-O showed very severe curling of shoot tips compared to any other Arabidopsis ecotypes and we examined the symptom development by analyzing lignification induced by virus infection. LTGA assay and tissue printing analysis showed that lignin was accumulated on severely curled shoots compared to mock inoculated plants. The symptomatic shoots contained more phenolic compounds rather than other organs such as rosette leaves and roots. PAL and peroxidase genes, which were involved in lignification, were also induced during symptom development. PAL was expressed dramatically earlier stage on 1 to 2 week after inoculation than peroxidase on shoot tips. However, peroxidase transcripts were accumulated more and longer period up to 3 week after inoculation than PAL. Peroxidase enzyme activity were gradually up to 3 to 4 week after inoculation. The important observation from this study is that BCTV infection induce lignification on symptomatic tissues followed by increasing transcription and translation of PAL and peroxidase during symptom development in hypersusceptible Sei-O ecotype.



**E-33. Identification of Citrus Tristeza Virus Isolated from *Citrus unshiu* and *C. junos* in Cheju Island.** Daehyun Kim<sup>1</sup>, Jaewook Hyun<sup>1</sup>, Hyunsik Hwang<sup>2</sup> and Sukchan Lee<sup>2</sup>. <sup>1</sup>Citrus Experiment Station, Rural Development Administration, Cheju, Korea, 699-800, <sup>2</sup>Sungkyunkwan Univ., Suwon 440-746, Korea

Citrus tristeza virus (CTV) was identified from CTV infected satsuma mandarin (*Citrus unshiu*) and yuzu (*C. junos*) by RT-PCR. The total RNAs were isolated from citrus tissues infected with CTV and reverse transcription was followed with primers designed for amplifying CTV coat protein gene. 738 bp DNA fragments were amplified by RT-PCR and these products were subcloned for further analysis. Based on the sequence analysis, this gene product has 90% sequence homology to CTV CP gene isolated from USA. The sensitivity of CTV detection by RT-PCR was much more effective than ELISA assay which was done with anti-CTV CP antibody purchased from USA. This is the first report about CTV identification in Cheju island, Korea.

**E-34. Influences of Cucumber Green Mottle Mosaic Virus on the Growth and Quality of Watermelon at Different Growth Stages.** Sook-Joo Ko, Yong-Hwan Lee, Kwang-Hong Cha, Tae-Seon Lee, Jin-Woo Park<sup>1</sup>, Hong-Soo Choi<sup>1</sup> and In-Jin Park. Chonnam Agricultural Research and Extension Service, 206-7 Sanjae-Ri, Sanpo-Myoun, Naju, Chonnam Province, Korea 520-830, <sup>1</sup>Plant Pathology Div. Dept. of Crop Protection, National Institute of Agricultural Science and Technology, Suwon 441-707, Korea

Influence of cucumber green mottle mosaic virus (CGMMV) on the growth and quality of watermelon was investigated by inoculation at different growth stages. Leaves of watermelon was inoculated mechanically with juice inoculum at transplanting time, vegetative growth stage, fruiting time, and 20 days after fruiting time. Mosaic symptoms appeared on watermelon leaves after 16, 11 and 11 days when inoculated at transplanting time, vegetative growth stage, and fruiting time, respectively. Symptom severity became severer when infected at earlier stage. Incidence and severity of 'Juksaekgwa' in which inner pulp of fruit turned to dirty red with water soaking were severer and growth of plant also retarded more severely as infected at earlier stage. Vine and internode length and fruit weight of watermelon were 390 cm 9.9 cm and 5.3 kg, respectively, when inoculated at transplanting time, while those of non-inoculated plant were 517 cm, 11.0 cm and 7.3 kg, respectively. However there was no mosaic symptom appeared when inoculated at 20 days after fruiting time. Fruit stalk necrosis and mosaic-like dark green on the fruit skin were not developed at all the treatments

**E-35. Kyuri Green Mottle Mosaic Virus(KGMMV) Occurred on Zucchini(*Cucurbita pepo*) in Korea.** Key-Woon Lee<sup>1</sup>, Young-Gyu Lee<sup>1</sup>, Su-Heon Lee<sup>2</sup>, Joo-Young Lee<sup>1</sup>, Hae-Jin Lee<sup>1</sup>, Ok-Gyu Bok<sup>1</sup>. <sup>1</sup>Department of Agricultural Biology, College of Agriculture, Kyungpook National University, Taegu 702-701, Korea. <sup>2</sup>Division of Plant Pathology, National Institute of Agricultural Science and Technology, Suwon 441-707, Korea

Severe virus disease causing deformed fruit, chlorotic mosaic leaf, and stunt growth were occurred on zucchini in Chon-Ju, Chonbuk province and Ye-chun, Kyungpook province, over April and May in 1999.

The virus particles from the zucchini were rigid rod shaped tobamovirus of 300×18nm, which found also in seed coat. In cells infected with the tobamovirus, the virus particles arranged in cytoplasm.

The reactions of indicator plants showed local lesions in *Datura stramonium*, no symptom in *Chenopodium amaranticolor*, and mottle mosaic in watermelon, cucumber, oriental melon, melon, and zucchini.

SDS-PAGE of the capsid protein results showed a little difference between the virus isolated from the diseased zucchini and the CGMMV isolated from the diseased watermelon, cucumber, and oriental melon.

The agar-gel double diffusion test with the antiserum prepared from the causal virus showed a highly serologically homogenous reaction with the virus from the diseased zucchini, but no reaction with CGMMV from the diseased watermelon, cucumber, and oriental melon.

Based on these results, this tobamovirus isolated from zucchini was considered a KGMMV which has not been reported in Korea.

**E-36. Comparison of Infection Rate of Viruses between Domestic and Imported-Chinese Garlic Bulbs for Planting.** Keum-Hee Lee, Se-Chul Chun, Noh-Youl Heo and Kyoung-Il Ko. Pathogen Research Division, National Plant Quarantine Service, 433-1 Anyang 6-Dong, Manan-Gu, Kyungki-Do 430-016, Korea

Two hundred twelve tons of garlic bulbs for planting were imported from China between January and August, 1999. In order to investigate the infection rates of domestic and imported garlics from China, one hundred garlic bulbs were collected, respectively. The imported garlics were sampled from the port of Incheon and Pusan, and domestic garlics were collected from Yisung, Danyang, Sinan, Koheung, Yeosu, Muan, Seosan and Namhae. The infection rates of Onion yellow dwarf virus (OYDV) using ELISA (Agdia, Elkhart, IN. U.S.A) were 82% and 94% for domestic and importation, respectively. However, Shallot latent virus (SLV) were not detected from both of domestic and importation. The infection rates of OYDV in garlics depending on the port of entry were 90% and 98% for Incheon and Pusan, respectively. The infection rates were 40%, 50%, 70% for Danyang, Seosan, Yisung, respectively and all 100% from the garlics of Sinan, Koheung, Yeosu, Muan, and Namhae. The results indicated that the infection rates were significantly different from the garlic collections.