V-01 Completion of Nucleotide Sequencing and Production of Biologically Active *In Vitro* RNA Transcripts of Cucurbits-Infecting Zucchini Green Mottle Mosaic Tobamovirus. Ju Yeon Yoon[1], Byung Eun Min[1], Jang Kyung Choi[2], Won Mok Park[3], and Ki Hyun Ryu[1]. [1]Plant Virus GenBank, Department of Horticultural Science, Seoul Women's University, Seoul, Korea 139-774. [2]Department of Agricultural Biology, Kangwon National University, Chun Cheon, Korea 200-701. 3Graduate School of Biotechnology, Korea University, Seoul, Korea 136-701.

The determination of total nucleotide sequence of the genomic RNA of zucchini green mottle mosaic tobamovirus (ZGMMV) was completed. Full-length cDNA copies of ZGMMV was amplified by RT-PCR with a set of 5'-end virus-specific upstream and 3'-end downstream primers. We could obtained the high quality full-length cDNA product of 6.5 kb long with high yield over 26.5 μg amounts PCR product from 0.1 μg of native viral genomic RNA. The full-length cDNA constructed under the control of the T7 RNA promoter could be successfully transcribed to produce *in vitro* transcipt RNA. The synthesized RNA transcripts of ZGMMV with cap analog (m^7GpppG) are infectious on its host plants. Infectivity was verified by RT-PCR with ZGMMV CP-specific primers and northern and western blots analyses. The first systemic symptom appeared on the 2nd true leaf with mottle 7 to 10 days post-inoculation (dpi) and was developed green mottle mosaic symptoms 12 dpi on the upper leaves on zucchini squash cv. Black Beauty. Progeny virus derived from infectious *in vitro* transcripts was efficiently transmitted by sap inoculation on its host plants, and its properties were the same with wild type virus. This is the first report on generating fully biologically active infectious transcript of cucurbits-infecting tobamovirus.

V-02 The Complete Nucleotide Sequence of Kyuri Green Mottle Mosaic Tobamovirus (KGMMV-C1 Strain). Ju Yeon Yoon[1], Byung Eun Min[1], Sang Hyeon Kim[1], Won Mok Park[2], and Ki Hyun Ryu[1]. [1]Plant Virus GenBank, Department of Horticultural Science, Seoul Women's University, Seoul, Korea 139-774. [2]Graduate School of Biotechnology, Korea University, Seoul, Korea 136-701.

The complete nucleotide sequence of the genomic RNA of kyuri green mottle mosaic tobamovirus (KGMMV-C1) was determined. The RNA genome of KGMMV-C1 is 6,514 nucleotides long and contains five open reading frames (ORFs 1 to 5) coding for proteins of M(r) 131 kDa (1,166 aa), 189 kDa (1,669 aa), 28 kDa (262 aa), 18 kDa (161 aa) and 58 kDa (503 aa), respectively. The 5' and 3' nontranslated regions consisted of 57 and 163 residues, respectively, and they were 42.1% to 33.3% and 45.4% to 35.0% identical to those of the other tobamoviruses, respectively. The sequences of the KGMMV RNA encoded proteins exhibit high homology to the proteins of the members of the Tobamovirus genus. The genomic organization and sequence analysis showed that KGMMV is more closely related to cucumber green mottle mosaic virus (CGMMV) than to other tobamoviruses. The total genomic sequence of the KGMMV-C1 was 1 nucleotide shorter than that of the KGMMV-Y. The amino acid sequences of five proteins were 95.5% (131 kDa gene) to 87.6% (18 kDa CP gene) identical between the KGMMV-C1 and KGMMV-Y.

V-03 RNA-dependent RNA Polymerase of Cucumber Mosaic Virus Is a Phosphoprotein. Sang Hyon Kim[1,2] , Peter Palukaitis[3], Ki Hyun Ryu[4], and Young In Park[1]. [1]Graduate School of Biotechnology, Korea University, Anam-dong 5ga, Sungbuk-ku, 136-701, Seoul, Korea. [2]Natural Science Institute, Seoul Women's University, Seoul 139-774, Korea. [3]Division of Pathology, Scottish Crop Research Institute, Dundee, Scotland, U.K., [4]Plant Virus GenBank, Department of Horticultural Science, Seoul Women's University, Seoul 139-774, Korea.

The phosphorylated form of 2a polymerase of cucumber mosaic virus (CMV) has been observed *in vivo*. CMV RNA was transfected into tobacco protoplasts by electroporation. Then, the protoplasts were cultured in the presence of ^{32}P orthophosphate for 60 hours, before 2a polymerase was immunoprecipitated by anti-2a antibody. We identified 2a polymerase is phosphorylated 48 hour after inoculation. Also, the protein kinases phosphorylating 2a polymerase were isolated from membrane fraction of tobacco (cv. Xanthi-nc) plant and designated them as tobacco protein 2a kinases (t2aks). The t2aks were chromatographically separated into three different proteins by in-gel kinase assay. When observed from heparin-Sepharose affinity chromatography, it has been identified that the affinity for heparin of 60 kDa t2ak was different from that of 55 kDa counterpart, in that the former has been eluted with 0.6 M NaCl whereas the latter 1.5 M. Assay for protein 2a kinase activity using truncation mutants of 2a polymerase showed that 2a polymerase has at least three different phosphorylation sites within full-length of 858 amino acids. One phosphorylation site is located in amino acids 605 to 679 containing GDD motif, and the others within the region of N-terminal 335 amino acids.

V-04 Nucleotide Sequence Analysis of Ly2-CMV RNA 3. Hye Jin Jung[1], Ki Hyun Ryu[2], and Jang Kyung Choi[1]. [1]Division of Biological Environment, Kangwon National University, Chunchon, Korea 200-701. [2]Department of Horticultural Science, Seoul Women's University, Seoul, Korea 139-774.

A new strain of cucumber mosaic cucumovirus (CMV) isolated from *Lilium longflorum*, Ly2-CMV, show some peculiar characteristics; its host range is limited only to a small number of plant species (Jung et al., 2000) in contrast with common strains such as Fny-CMV, Y-CMV and Mf-CMV that can infect as many as several hundreds of plant species. The nucleotide sequence of RNA 3 of Ly2-CMV was determined and compared at both the nucleic acid and protein level with the previously reported, corresponding sequences of RNA 3 of nine other CMVs: Kin-CMV, Q-CMV, As-CMV, Nt9-CMV, Ix-CMV, Fny-CMV, Mf-CMV, O-CMV, and Y-CMV. Ly2-CMV RNA 3 is composed of 2226 nucleotides and contained two open reading frames, the 3a gene and coat protein gene. In comparison among the full-length RNA 3 of the CMV strains, the Ly2-CMV show similar to the nucleotide sequence from the subgroup IA strains. However, clustal multiple alignment of each region composed of the RNA 3 indicates the differences in each region-specific manner. In alignment of the 5' non-translated region (5'NTR) sequences, Ly2- CMV seems to be classified in subgroup IB. Phylogenetic analysis of the 3a gene of this RNA, however, forms a slightly distinct cluster from the subgroup IB, and is characterized with an intermediate relatedness of subgroups IA and IB. The nucleotide sequences of the intergenic region (IGR), coat protein gene and 3' non-translated region (3'NTR) are very similar to those of subgroup IA CMVs.

V-05 Identification and characterization of plant viruses from weeds. Sun-Jung Kwon, Jung-Heon Han, Hong-Soo Choi[1], and Kook-Hyung Kim
School of Agricultural Biotechnology, Seoul National University, Suwon 441-744, Korea. [1]Plant Pathology Division, National Institute of Agricultural Science and Technology, Suwon 441-707, Korea.

Weeds are widely grown in the field and are infected by many viruses. A survey was conducted to identify viruses infecting weeds in Korea. Virus-infected weed samples including *Rorippa indica* (L.) Hiern, *R. islandica* (Oed.) Bord, *Crepidiastrum denticulatum* (Houtt.) Pak&Kawanno, *Achyranthes japonica*(Miq.) Nakai, *Chrysanthemum boreale* (Makino) Makino were collected in Kyonggi Province. These weeds were grown in the greenhouse and were inoculated on 10 test plants. Several virus strains were isolated from infected tissues and were further studies by host range assay, serological test, electron microscopy (EM), reverse transcription-polymerase chain reaction (RT-PCR) and sequencing. Each isolated virus strain was mechanically transmitted to weeds and various hosts including *Nicotiana* spp., *Brassica* spp., *Vigna sinensis*, *Capsicum annume*, and *Cucumis sativus* and showed systemic mosaic, vein clearing, necrosis, mottle, malformation, chlorosis, and death of host plants in some cases. Each virus strain was then purified using infected leaves and observed by EM. From these results three viruses were isolated and identified as turnip mosaic virus (TuMV), broad bean wilt virus (BBWV), and cucumber mosaic virus (CMV). RT-PCR using specific oligonucleotide primers and the cloning were conducted to determine the nucleotide sequence of TuMV, BBWV and CMV coat protein as well as its deduced amino acid sequence. Results showed that the nucleotide sequence homologies were about 88.3 to 99.2%, 83.7 to 90.0%, and 93.8 to 98.9% to other reported TuMV, BBWV, and CMV strains, respectively. These results suggest that the management of viral diseases caused by TuMV, BBWV, and CMV can be achieved through weed control.

V-06 Occurrence and Inhibition Effect of Soil-Borne *Cucumber Green Mottle Mosaic Virus* by Skin Milk Treatment. Gug-Seoun Choi, Jae-Hyun Kim, Jong-Tae Kim, and Yong-Mun Choi. National Horticultual Research Institute, R.D.A., Suwon, Korea 441-440

Cucumber green mottle mosaic virus is readily transmissible by seeds, soil, and sap. The virus disease naturally occurred in 463ha, 17.3ha, and 26.8ha during cucurbits growing seasons of 1998, 1999, and 2000, respectively. In the virus-infected fields of previous year, the percent of soil transmission was 0~3.5% and numbers of the virus re-occurring fields were more in paddy fields than in dry fields for crop rotation. Biological activity of the virus was more than 20 months in the wetted soil but lost after 18 months in the dry soils.
To prevent the virus infection from soil, the roots of watermelon seedlings were soaked in 10% skin milk solution prior to transplanting. In the pot experiments mixed the infected plants and soil, infection rate of control seedlings and seedlings treated with skin milk solution was 11.6% and 1.4%, respectively. In the soil artificially infected with the virus of previous year, the seedlings treated with skin milk solution were not infected, while 6.4% of control seedlings were infected.

V-07 A Novel Strain of *Cucumber Mosaic Virus* Isolated from Paprika(*Capsicum annum* L.) in Korea. Jae-Hyun Kim[1], Gug-Seoun Choi[1], Yong-Mun Choi[1] and Jang Kyung Choi[2] [1] National Horticultural Research Institute, R.D.A., Suwon, Korea 441-440 [2] Department of Agricultural Biology, Kangwon National University, Chunchon 200-701,Koera

A virus was isolated from the paprika fruit which was showed swelling round shape symptoms at a plastic house in Puyngchang, Korea in 1999. The isolate from paprika, PaMF1-CMV, was identified and compared to the well characterized Mf-CMV(subgroup I) and Ls-CMV (subgroup II) by biological reactions in several hosts, serological property, electron microscopy, ds-RNA analysis, RT-PCR analysis, restriction enzyme mapping of the PCR product, and nucleotide sequence of coat protein gene. Mf-CMV and Ls-CMV revealed four major ds-RNA species with estimated molecular size of 3.4, 3.2, 2.1 and 1.0kbp. however, isolate PaMF1-CMV showed a slightly smaller RNA1 and larger RNA2 compared to Mf and Ls-CMV. Remarkable differences in symptoms of PaMF1-CMV were found between Mf and Ls-CMV in *Cucumis satives* and *Datura stramonium*. Mf and Ls-CMV caused systemic symptoms to upper leaves of *C. satives*. PaMF1-CMV was not infected in cucumber. Ls-CMV caused severe mosaic symptoms to upper leaves of *D. stramonium*. however, PaMF1-CMV caused mild mosaic symptoms. In experiments of RT-PCR of coat protein gene and restriction enzyme mapping analysis of the RT-PCR products produced that PaMF1-CMV was come within subgroup II CMV. In immunodiffusion tests, Ls-CMV as a virus of serotypeII formed additional spur against serotypeI antiserum. while, PaMF1-CMV did not formed any other spur. Now we are constructing the cDNA library to detect sequence variation between Ls-CMV and PaMF1-CMV.

V-08 Molecular Biological Characteristics of Grapevine Leafroll-associated 3 *Closterovirus*. Hyun-Ran Kim[1], Yong-Moon Choi[1], Bong-Choon Lee[2], Myoung-Soon Yiem[3], Jin- Woo Park[4], Young-Tae Kim[4]. [1]National Horticultural Research Institute, Rural Development Administration, 540-41 Tap-dong, Suwon, 440-310, Korea. [2]Geonnam National University. [3]National Alpin Agricultural Experiment Station, [4]National Institute of Agricultural Science and Technology, RDA.

This study was conducted to identify virus diseases in grapevine orchard in Korea, and to analyze the biological and molecular biological characteristics.
Leaf reddening and leafroll symptoms were observed in "Rubi" and some other species of red-fruited cultivars, which showed positive reactions to GLRaV-3 antiserum by ELISA method. In white-fruited cultivars, chlorosis and leafroll symptoms were observed. Mosaic, Rosette, or Bark necrosis were observed in different cultivars. Materials used in the virus purification were : symptomatic mature leaf, petiole and midrib, extracted from Kyoho vines reacting positively to GLRaV-3 antiserum and *in vitro* plantlet (node cultured of virus infected kyoho vines). The amount of purified virus was highest in *in vitro* plantlet, where long filamentous virus particles of 1800nm x 12nm were observed. In observation on the cytopathological characteristics of GLRaV-3, compact virus- like particles were observed in parenchyma cells in phloem and sieve tube. Virus particles surrounding vacuole membrane and in reddened leaf, inclusions assumed to be associated with anthocyanin were scattered in sieve tube. Western blot using GLRaV-3 antiserum resulted in the appearance of specific band in 43kda. Nucleotide sequence of Korean isolate GLRaV-3 coat protein gene was determined by cyclic sequencing with ABI Prism 377 Genetic Analyzer.

V-09 Identification and Cytopathological Characteristics of GFLV in Grapevine. Hyun Ran Kim[1], Yong Moon Choi[1], Myoung Rae Cho[1], Jeong Soo Kim[1], Moo Ung Chang[2]. [1]National Horticultural Research Institute, Rural Development Administration, 540-41 Tap-dong, Suwon, 440-310, Korea. [2]Youngnam University, 214-1 Daedong, Kyongsan, Kyongbuk 712-749, Korea.

Grapevine fanleaf virus(GFLV) is one of the widespread and damaging viruses in grapevine. However, this virus has not been recorded in korea. Recently, many vines including rootstocks were introduced from other countries. Therefore, close inspection for the introduced vines and rootstocks is needed since the plants may carry the GFLV. Among the introduced grapevine germplasm maintained in NHRI, GFLV was detected for several vines. Fanleaf and mosaic symptoms in leaf and corky bark in stem were observed in different grapevine cultivars and artificially inoculated woody indicators. The purified virus has isometric particles of 30nm. In observation on the cytopathological characteristics in *Chenopodium quinoa* leaf infected with GFLV, virus-like particles were arranged consistently in form of several layers in cytoplasm. Several vesicles in cytoplasms and virus particles in tubular membrane associated with plasmodesmata were observed.

V-10 Purification and Detection of Contaminated Soil by Kyuri Green Mottle Mosaic Virus(KGMMV) in the zucchini green house. Young Gyu Lee[1], Joo Young Lee[1], Ji Eun Lim[1], Sang Mok Kim[1] and Key Woon Lee[1] [1]Department of Agricultral Biology, Kyungpook National University, Taegu, Korea, 702-701.

A viral disease of zucchini, caused by kyuri green mottle mosaic virus(KGMMV), occurred severely in Chunju province in 1999. KGMMV, a member of the tobamovirus group, is reported to be transmitted by seed, water, soil and mechanical transmission. So the soil transmission was worried over in KGMMV infected field because of repeated cultivation. To confirm the presence of the virus particles in the soil, the soil in and out of the green house and around roots of zucchini from the severely occurring of KGMMV last year(1999) was collected and tested. In modified the normal method of purification of tobamovirus, a lot of particles were obtained when treated with detrergent. Purified solution from virus-infected soil was observed virus like particles with the electron microscope by DN methods. The particles were identified by ISEM. The particles were decorated with antiserum to KGMMV. KGMMV antisera reacted positively with purified solution collected from the soil in the green house and around the root , however, showed weak reaction with the solution collected from the soil out of the green house by DIBA and ELISA test. In the bioassay using indicator plant, local lesions were found in *Datura stramonium*. The virus is active and abundant in amount in the soil and thought to be soil-transmitted.

V-11 Indexing of Potato Leafroll Virus Using Monoclonal Antibodies. Jeong Uk Cheon, Kang Kwon Kim, Jin Woo Park, Hong Soo Choi, and Su Heon Lee. Plant Pathology Division, National Institute of Agricultural Science and Technology, Suwon, Korea 441-707.

Potato leafroll virus (PLRV) was purified from freeze infected potato(*Solanum tuberosum* L.) leaves and used as immunogen for immunizing BALB/c mice. Hybridoma clones secreting specific monoclonal antibodies (MAbs) against the PLRV were obtained by fusing murine myeloma cells (NS-1) with spleenic cells from two BALB/c mice immunized with the purified PLRV. 212 cell lines secreting specific MAbs were seleted from the obtained 240 hybridoma cell lines. The titer of the MAb against PLRV produced by the selected monoclones in culture supernatent (CS) was 1,024 times shown by dilution end point in indirect ELISA test with crude antigen from PLRV-infected potato leaves. The MAbs secreted by monoclones, 1C6-D1-B4 and -B5 in CS belonged to IgG 2a isotype. The midrib vein sections of the infected potato leaves showed specific enzyme precipitation in phloem tissues when the sections stained with monoclonal antibodies. The crude extract of meristem-cultured potato plants was readily indexed using direct ELISA test with monoclonal antibodies against potato leafroll virus.

V-12 Occurrence of Peanut Stripe Virus in peanut in Korea. Hong-Soo Choi[1], Jeong-Soo Kim[2], Jeong-Uk Cheon[1], Jang-Kyung Choi[3], and H. R. Pappu[4]. [1] Plant Pathology Division , National Institute of Agricultural Science and Technology, Suwon, Korea 441-707, [2]Department of Vegetable Breeding, National Horticultural Research Institute, Suwon, Korea 441-707. [3] Department of Agricultural Biology, Kangwon National University, Chuncheon 200-701, Korea. [4]Department of Plant Pathology, Coastal Plain Experiment Station, University of Georgia, Tifton, GA 31793, USA.

A virus causing vein banding, sometimes yellow mosaic, and rugose symptoms was prevalent on peanut plants in the middle parts of Korea, where infected plants ranged from 78.8 to 100% depending on cultivars. The causal virus was identified as peanut stripe virus (PStV) and characterized based on biological, cytopathological, and molecular properties. All five peanut cultivars were shown to transmit PStV by seeds and its transmission rates ranged from 1.5 to 15.7%. The nine PStV isolates could be classified based on differences in symptoms, peanut cultivars and seed transmissions but not by host range. In host range studies, the P303 isolate caused mosaic on *Nicotiana benthamiana, Phaseolus vulgaris*, and *Arachis hypogaea*. Virus inclusion patterns were similar to those produced by members of *Potyvirus* subdivision III with the scroll, pinwheel and long laminated inclusions. In order to ascertain their taxonomic identity, 3' terminal region consisting of a part of the coat protein gene and 3'-untranslated region (UTR) of this isolate was cloned and sequenced. Multiple alignment as well as cluster dendrograms showed that the P303 isolate belongs to the PStV subgroup.

V-13 Detection of rice dwarf virus in individual green leaf hopper, *Nephotettix cincticeps*, by reverse transcription and polymerase chain reaction. Dong-Bum Shin, Mi-kyeong Lee, Soon-Do Bae, Yeon-Kyu Hong, and Do-Yeon Kwak, National Yeongnam Agricultural Experiment Station, 1085 Naidong, Milyang, Korea 627-130.

Rice dwarf virus is transmitted in a persistent manner by Nephotettix cincticeps in Korea. Disease incidence depends upon the population of the early generation, which synchronize with the attractive and infective stage of the rice plants. Detection of the virus in N. cincticeps have been achieved by a bioassay on seedling plant, and enzyme-linked immunosorbent assay(ELISA). Polymerase chain reaction(PCR) is a powerful tool to amplify small amounts of DNA(or RNA by using reverse transcriptase), for diagnosis of genetic as well as infectious diseases. In this study, RT-PCR techniques was applied to detection of RDV in individual green leaf hopper and rice plants. RT-PCR used by two set of primers, one was 5' 27-mer primer and another was 3' 25-mer primer, amplified 1427bp DNA fragments from individual vector and infected plant, and RDV was highly detected. The detection rate of viruliferous insect was appeared high as about two times in aritifically infected insect, and as eight times in naturally infected insect relation to bioassay on seedling plant used by susceptible variety, respectively. These results show that RT-PCR for RDV will extend the capability for diagnosing viral infections of vector or plants.

V-14 Characteristics of Chlorella Viruses Isolated from Fresh Water in Korea. Hyun-Hwa Cho, Hyoun-Hyang, Park, Jong-Oh Kim and Tae-Jin Choi. Pukyong National University, 599-1, Daeyeon 3 Dong, Nam-Gu, Pusan, , Korea 608-737.

Microalgae including chlorella species are the primary producer in fresh and marine water food chain. However, recent blooming of harmful green and red algae in aquatic systems awaked more attention in algae viruses as one possible control measure of these harmful microorganisms. We have isolated five chlorella virus strains from fresh water collected from streams and ponds near Pusan. Water samples were first filtered through bacterial proof filter and the viruses were amplified in chlorella strain NC64A, the most popular host for chlorella viruses. Pure virus strains were isolated by repeated plaque isolation. In plaque assay performed with NC64A, four strains formed bigger plaques than PBCV-1, the prototype chlorella virus and one virus strain formed smaller plaques. Spherical virus particles of about 190nm were observed in artificially inoculated chlorella. In SDS-PAGE analysis of purified virus, one of the virus strain F00ADJ-1 showed similar but distinct protein pattern to PBCV-1. In addition, restriction enzyme digestion mapping showed different fragment arrays. These results suggest that this new virus is a PBCV-1 related but distinct virus.

V-15 The expression of plastid ω-3 fatty acid desaturase gene involves in the HR lesion formation by TMV infection. Yang-Ju Im, Bong-Choon Lee and Baik-Ho Cho Applied Plant Science Division and Institue of Biotechnology, College of Agriculture, Chonnam National University, Kwangju 500-757, Korea

A plastid ω-3 fatty acid desaturase (FAD7) cDNA clone was obtained by differential screening of an *Arabidopsis* cDNA library. An antisense DNA, amplified from the FAD7 cDNA, transformed into tobacco plants under the control of a CaMV 35S promoter. Integrations of the T-DNA into the genome of five kanamycin-resistant T_1 lines were confirmed by PCR for NPT II gene detection and by northern blot analysis. Among the five T_1 lines, strong transcriptional activities of the antisense gene were observed in the AsFAD714 and 716 plants. The two T_1 lines contained reduced amounts of linolenic acid (18:3) in the total lipids. The FAD7 gene catalyze the formation of linolenic acid, the jasmonic acid (JA) precusor. Therefore, we inoculated TMV on the transgenic tobacco to investigate the role of FAD7 gene in plant-pathogen interaction. Compared with wild-type plants showing typical HR-like response, the antisense transgenic tobacco developed larger necrotic lesions by TMV infection. The lesion formation incidence also increased in the transgenic plants.

V-16 Detection of Cucumber Green Mottle Mosaic Virus in Bottle Gourd Seeds by RT-PCR. Sook-Kyung Lee, Wan-Yeob Song and Hyung-Moo Kim. Faculty of Biological Resources Science, Chonbuk National University, Chonju, Chonbuk 561-756, Korea.

A rapid and specific detection method of cucumber green mottle mosaic virus (CGMMV) from bottle gourd (*Lagenaria siceraria*) seeds was developed by using simplified virus concentration procedure from the seeds and RT-PCR. Some serological methods such as enzyme linked immuno sorbent assay and mulit-rapid immunofilter paper assay have been used for assaying infected plant parts but a more sensitive, reliable and labour-saving assay is needed to detect the virus from low level of infected seeds. Primers, Wmf1 and Wmr1, specific for CGMMV were designed from coat protein gene sequences of CGMMV-W and used for amplifying 420-bp product in RT-PCR. To simplify the virus extraction procedure and reduce an inhibitor from seed samples for the RT-PCR, some methods using filtration, polyethylene glycol precipitation, ethanol precipitation and phenol-chloroform procedure were compared and the phenol-chloroform procedure was selected by its enhanced sensitivity. This detection method using the extraction step and primers for RT-PCR could reliably detect an infection level of one CGMMV-infected seed in 1000. This rapid and sensitive RT-PCR assay provides a useful tool for the specific detection of CGMMV in bottle gourd seed samples containing high levels of background inhibitors so that it can be used for the sensitive routine testing of bottle gourd seeds.

V-17 A Subpopulation of Cucumber Mosaic Virus RNA1 Contains 3' Terminal Originating from RNA 2 or RNA3. Seung Kook Choi[1], Thomas Canto[2], Peter Palukaitis[2], Ki Hyun Ryu[3], and Won Mok Park[1] [1]Graduate School of Biotechnology, Korea University, Seoul, Korea 136-701. [2]Division of Pathology, Scottish Crop Research Institute, Dundee, Scotland, U.K. [3]Plant Virus GenBank, Department of Horticultural Science, Seoul Womens University, Seoul, Korea 139-774.

Transgenic tobacco plants expressing full-length RNA1 of cucumber mosaic virus (CMV) and inoculated with transcripts of RNA2 and RNA3, regenerated viral RNA1 from the transgenic mRNA and the plants became systemically infected by the reconstituted virus. The cDNA fragments corresponding to the 3' noncoding region (NCR) of viral RNA1 were amplified, cloned and sequenced. In some clones, the termini of 3' NCR corresponded to those of viral RNA2 or 3. This suggested that in some cases RNA1 had regenerated during replication by a template switching mechanism between the inoculated transcript RNAs and the mRNA. However, encapsidated, recombinant RNA1 with the 3' NCR ends originating from RNA 2 or 3 also was found in virus samples that had been passaged exclusively through non-transgenic plants. Therefore, these chimeras occur naturally due to recombination between wild-type viral RNAs, and they are present encapsidated in low level, but detectable amounts.

V-18 Initial Ultrastructural Changes in Tobacco During TMV-Induced Programmed Cell Death. Jun-Sung Shin and Young Ho Kim . School of Agricultural Biotechnology, Seoul National University, Suwon, Korea 441-744.

TMV-induced programmed cell death (PCD) has already been described as condensation and vacuolation of cytoplasm and cleavage of nuclear DNA, which was studied 1 and 2 days after PCD induction by temperature shift of TMV-infected tobacco plants. We studied some of microscopy of Xanthic-nc NN tobacco cells 0, 6 and 17 hours after PCD induction, when neither PCD symptoms nor pathogenesis-related (PR) protein gene were induced (0 hour after PCD induction), PR gene induced without definite PCD symptoms (6 hours after PCD induction), and both PR gene and PCD symptoms induced (17 hours after PCD induction), respectively. TMV particles and structures of X-body were observed and no aberrations of cytoplasm and chloroplasts and nucleus were noted 0 hour PCD induction. Six hours after PCD induction, cytoplasm appeared to be condensed and somewhat depleted to be compartmented, but nucleus and mitochondria were appeared to intact. At 17 hours after PCD induction, most mesophyll cells had completely depleted cytoplasm and degenerated nucleus with sparsely granulated appearance. Dense-looking cytoplasm was necrotized, containing TMV particles.

V-19 Characterization of a protein kinase from the chestnut blight fungus *Cryphonectria parasitica* and its transcriptional regulation by hypovirus. Jin-Won Choi, Seung-Moon Park, Moon-Sik Yang, and Dae-Hyuk Kim Institute of Molecular Biology and Genetics, Basic Science Research Institute, Chonbuk National University, Dukjindong 664-14, Chonju, Chonbuk 561-756, Korea

The gene encoding protein kinase (*cppk*-1) was isolated from chestnut blight fungus, *Cryphonectria parasitica*. Degenerated primers for *cppk*-1 was designed based on conserved catalytic domain of many fungal PK. The expected size of 350-bp amplicon was obtained by using PCR and cloned into plasmid vector. Sequence comparison of the cloned fragment showed the highest similarity to Ser/Thr protein kinase from *Trichoderma reesei* and it was 82% identity. The *cppk*-1 consisted of three exons with two intervening sequences of 67 bp and 71 bp in size, and the deduced *cppk*-1 protein product, CPPK-1, had an estimated molecular mass of 70.5 kDa and a pI of 7.45. Primer extension experiment revealed the major transcription initiation site located at -49 bp from the translation start codon and the sequencing of cDNA clone indicated the poly(A) occurred at 569 bp down-stream of the stop codon. Northern blot analysis and RT-PCR were conducted to examine the expression pattern of *cppk*-1 from virus-free *C. parasitica* strain EP155/2 as well as its isogenic hypovirulent strain UEP1. The expression of *cppk*-1 was low at both 1 and 5 day after the liquid culture of EP155/2 while its expression increased considerably at 5 day after the liquid culture by the presence of the hypovirus CHV1-713. This indicates the implication of hypovirus in the signal transduction pathway of *C. parasitica* through *cppk-1* and it is one of the first evidences that hypovirus disturbs fungal signal transduction pathway at the transcriptional level. Correlation of an aberrant expression of *cppk*-1 to the specific viral symptom is under investigation.

V-20 Complete Nucleotide Sequence and Helper Virus Specificity of Satellite Tobacco Mosaic Virus Strain Korean. Su Heon Lee[1], Key Woon Lee[2], Sang Mok Kim[2], and Jeong Uk Cheon[1]. [1]Plant Pathology Division, National Institute of Agricultural Science and Technology, Suwon, Korea 441-707. [2]Department of Agricultural Biology, Kyungpook National University, Taegu, Korea 702-701.

The complete nucleotide sequence of satellite tobacco mosaic virus strain korean (STMV-K) was determined. The genome of STMV-K consisted of single stranded RNA molecules of 1,058 bases. Sequence analysis showed that STMV-K shared 99% nucleotide sequence identity with the genome of STMV type strain. Four bases of coat protein (CP) region were substituted, but the deduced amino acids were the same. The 6.8 kDa protein translated *in vitro* by STMV type strain did not exist in case of STMV-K due to frameshifts resulted from the deletion of guanine in base from 60 to 63. Helper virus specificity of STMV-K was examined using purified virions. Using RT-PCR, STMV-K was detected in the tissues inoculated with not only natural helper virus, tobacco mild green mottle virus (TMGMV-KT1), but also tomato mosaic virus (ToMV-KP1), pepper mild mottle virus (PMMoV-KP1), or ribgrass mosaic virus (RMV-KC1) as a helper virus. However, STMV-K was not detected in the tissues coinoculated with cucumber green mottle mosaic virus (CGMMV-KW1). Comparison of 3'-terminal sequence between STMV-K and nine tobamoviruses revealed that there were three highly homogeneous regions which are considered to play an important role in recognizing RNA-dependent RNA polymerase.

V-21 A New Strain of Kyuri Green Mottle Mosaic Virus Isolated from Cucumber : Biological Properties and Nucleotide Sequence of Coat Protein Gene. Jeong Uk Cheon[1], Su Heon Lee[1], Hong Soo Choi[1], So Hee Kwon[1], Sook Joo Ko[2], and Key Woon Lee.[3] [1]Plant Pathology Division, National Institute of Agricultural Science and Technology, Suwon, Korea 441-707. [2]Chonnam Agricultural Research and Extension Services, Naju, Korea 520-715, [3]Department of Agricultural Biology, Kyungpook National University, Taegu, Korea 702-701.

A survey for the presence of kyuri green mottle mosaic virus in the major cucurbits growing area of Korea was conducted, and a new strain (KGMMV-KC) was isolated from cucumber in Kangjin. In reaction of indicator plants, KGMMV-KC is distinguished from CGMMV in *Chenopodium amaranticolor*, *Datura stramonium*, and *Cucurbita pepo*, however KGMMV-KC differs little from KGMMV-C and KGMMV-Z isolated from cucumber and zucchini, respectively. DNA fragments including coat protein region were amplified by reverse transcriptase-polymerase chain reaction (RT-PCR) using KGMMV specific primers, and sequenced. The coat protein (CP) gene of KGMMV was 486 nucleotide residues which was the same nucleotide length with those of cucurbit-infecting tobamoviruses. Comparison of the KGMMV-KC CP with those of KGMMV-Z, KGMMV-C, KGMMV-Y, and CGMMV showed 95.7, 79.0, 78.8, and 43.2% nucleotide identity, and 96.3, 75.8, 75.2, and 44.1% amino acid identity, respectively. These results indicate that KGMMV-KC is closely related to KGMMV-Z isolated from zucchini rather than KGMMV-C and KGMMV-Y reported from cucumber. This is the first report of KGMMV on cucumber in Korea.

V-22 Occurrence and identify of mosaic viruses in cucumber in Chonnam Province. Sook-Joo Ko[1], Kwang-Hong Cha[1], Yong-Hwan Lee[1], Hyo-Jeong Kim[1], Jin-Woo Park[2], Hong-Soo Choi[2] and In-Jin Park[1]. [1]Chonnam Agricultural Research and Extension Service, 206-7 Sanjae-Ri, Sanpo-Myoun, Naju, Chonnam Province, Korea 520-715, [2]Plant Pathology Division, National Institute of Agricultural Science and Technology, Suwon Korea 441-707.

Disease incidence of virus were surveyed on cucumber cultivating under structural houses from Kangjin, Kwangyang, Kurye, Boseung and Suncheon in year 1999 and 2000. Disease incidences were varied among the regions in which cucumber of some regions were severely infected by virus, whereas no symptom of virus were found on cucumber of the other regions. Using the transmission electron microscope(TEM), enzyme-linked immunosorbent assay(ELISA) and polymerase chain reaction(PCR) the samples from each regions were analyzed to identify the virus causing disease. Most of the virus were cucumber green mottle mosaic virus(CGMMV) and watermelon mosaic virus(WMV) and some cucumber were also infected by cucumber mosaic virus(CMV) and zucchini yellow mosaic virus(ZYMV). CGMMV were mostly identified from the samples of Kurye, Sunchon, Kwanyang and Boseung in year and additionally samples from Kangjin in 2000. Disease severity by CGMMV were different by varieties of cucumber plants. Seeds of cucumber varieties were analyzed by ELISA test, in which 5.6% of one variety was infected by CGMMV but the other 5 varieties were virus free. From these detections it is suggested that the occurrence of disease by CGMMV on cucumber plants may be caused by seed infection or the virus.

V-23 Study on Soybeans and Soybean Mosaic Virus Strains Interactions. Yul-Ho Kim[1], Ok-Sun Kim[2], Hae-jin Lee[3], Bong-Choon Lee[3], Jae-Hwan Roh[1], Dae-Joon Im[1], Il-Bong Hur[1] and Jang-Kyung Choi[2]. [1]National Crop Experiment Station, RDA, Suwon, Korea 441-100. [2]Kangwon National University, Chunchon, Korea 200-701. [3]Kyungpook National University, Taegu, Korea 702-701.

Soybeans x soybean mosaic virus(SMV) strains interactions affected plant growth, strain survival and seed transmission. Strain virulence of SMV depended on host cultivars. Kwangankong and Tawonkong all were susceptible to G7H and G5, causing mosaic symptoms. In the first treatment, two primary leaves in a single plant were infected with both strains by means of one strain per leaf. The leaves of Kwangankong and Tawonkong at V2 and V4 stages were doubly infected with the two strains and the upper leaves than those had only G7H strain. Secondly, the two soybeans were inoculated with G7H, and 24 h after followed by the other strain inoculation. The leaves of V6 and V8 stages in all infected plants showed mosaic symptoms caused by G7H strain and there was no detection of G5. In contrast, the reverse treatment with G5 and G7H induced different results. Pre-inoculated G5 strain detected in every stage besides G7H strain. Host x SMV strain compatibility influenced seed coat mottling, yield, plant height, number of pod per plant. Among 205 seeds from G7H-infected Kwangankong, 202 seeds were mottled. G7H had a seed mottling rate of 98.5% in Kwangankong, while G5 had an incidence of seed mottling of 1.4% in the same cultivar. G5 was more virulent to Kwangankong and had a lower affinity for infecting soybean seed. Additional inoculation of G7H protected soybean yield and growth from G5-inducing loss in Kwangankong.

V-24 Pathogenicity of Alfalfa Mosaic Virus Strains Isolated from Soybeans. Yul-Ho Kim[1], Hae-Jin Lee[2], Ok-Sun Kim[3], Young-Gyu Lee[2], Bong-Choon Lee[2], Il-Bong Hur[1], and Key-Woon Lee[2]. [1]National Crop Experiment Station, RDA, Suwon, Korea 441-100. [2]Kyungpook National University, Taegu, Korea 702-701. [3]Kangwon National University, Chunchon, Korea 200-701.

AMV isolates showing specific yellow mosaic in soybean fields were collected. They induced various reactions like bud bending, yellow mosaic, mild mosaic, necrosis, and mottling, when inoculated to 26 soybean varieties. They were classified into two groups based on symptoms and pathogenicity, and designated as K1 and K2. Two AMV representitives were isolated from Eunhakong and Sinpaldalkong2, respectively, and maintained for this study. All 26 soybean varieties were susceptible or resistant when inoculated with the virus K1 and K2 , though not to the same degree. Duyoukong and Sodamkong were resistant to K1 but the other 24 cultivars generally showed yellow mosaic and mottling. Especially, 4 soybeans including Muhankong showed bud bending. K1 induced necrosis in the infected leaves of Muhankong, Saealkong, Samnamkong and Baegunkong, and occasionally caused necrosis of the veins and veinlets of the inoculated leaves of them. On the other hand, Sobaegnamulkong, Jagsukong, Danyeobkong, Pureunkong were resistance to K2, but the other 22 soybean cultivars produced systemic yellow and mild mosaics. There was no necrotic symptom by K2 inoculation. These results suggested that pathogenicity of AMV-K1 is higher than that of AMV-K2. To confirm the identities of virulence test, the nucleotide sequences of K1 and K2 in the coat protein coding regions of approximately 665bp were determined and compared.