separated into subgroup I and II by some molecular properties. Nucleotide sequence analyses of the coat protein genes of the cucumoviruses were determined to confirm the subgrouping. RT-PCR products amplified by the CP gene-specific primers of *Cucumovirus* were cloned in pGEM T-easy vector (Promega Co.). The nucleotide sequences were analysed and compared with published cucumoviruses data using programs of the DNA sequence analysis computer package for PC (DNASTAR, Madison, Wis., USA). The percent nucleotide sequence similarity between CMV-Y CP gene and the CP gene sequences from CMV-Fk and CMV-Hm were 93.5 and 91.9%, respectively. The percent nucleotide sequence similarity between PSV-J and PSV-Rp were calculated to be 71.8%. Phylogeny analysis of the CP genes further classified CMV-Hm, -Fk and PSV -Rp into CMV subgroup IA, IB and PSV subgroup I, respectively. Restriction enzyme analysis of RT-PCR products from the CP genes showed the same characteristics as the phylogeny analysis.

Early warning of rice ragged stunt disease based on viruliferous brown planthopper population and their implementation in Thailand. Dara Chettanachit. Division of Plant Pathology and Microbiology, Department of Agriculture, Chatuchak, Bangkok 10900, Thailand.

Rice ragged stunt disease has caused severe damage on rice production in Thailand since 1977. The *Rice ragged stunt virus* (RRSV), causal agent is transmitted by the brown planthopper *Nilaporvata lugens* Stal. in persistent manner. Light trap was set up in six locations to catch and monitor the migrating brown planthopper, which is presumably a short distance migration. The ELISA technique was used in detecting the virus from insect vector. Enzyme-linked immunobinding technique or tissue blotting was introduced to determine the RRSV infected plant in the farmer field. One hundred rice plants per plot were selected randomly. The stem was cut and pressed gently on nitrocellulose membrane (NCM). Blocked the free binding site on NCM with 5% skimmed milk in PBS-T. The NCM was then treated with RSV-antiserum. After added anti-rabbit alkaline phosphatase conjugates, followed by the BCIP/NBT substrate mixture. The presence of virus was shown by the corresponding reaction. Percentage of RRSV viruliferous insect were both highest at Pisanulok Rice Research Center and Chainat Rice Experiment Station in March and October which is the harvesting period of crop. The symptom of RSV appeared one month after the huge mass migration BPH population caught by light trap or after the highest peak of BPH population in each location.

Destruction and inactivation of *Cucumber green mottle mosaic virus* by seed heat treatment. Sang-Min Kim[1], Sang-Hyun Nam[2], Jung-Myung Lee[3] and Kook-Hyung Kim[1]. [1]School of Agricultural Biotechnology, Seoul National University, Suwon 441-744, Korea; [2]Seminis Korea, Inc., Cheongwon 363-955, Korea; [3]Deptartment of Horticulture, Kyung Hee University, Yongin 449-701, Korea.

Heat treatment is commonly and widely used to control virus contamination on seeds in commercial scale. But the mechanism of virus inactivation is not clearly known. To get the clue for virus inactivation, we treated *Cucumber green mottle mosaic virus* (CGMMV) contaminated seeds using various heat treatment conditions. Virus was purified and observed using electron microscope. CGMMV paticles were physically destructed as increasing temperature and duration of treatment. Viral RNAs were extracted and assayed through RT-PCR using specific primers that are designed to amplify about 1 kb fragments spanning CGMMV genome. Expected size of amplified fragments were observed when oligonucleotide primers for 5' and 3' terminus. In contrast, no amplification was observed at regions 2-3 and 3-4 kb position from 5 terminus of the genome. These results suggest that terminus of CGMMV genome is strongly protected while central regions are not and provide clue for understanding the mechanisms of virus inactivation by heat treatment.

Cellular protein binds to sequences near the 5′ terminus of *Potato virus X* RNA that are important for virus replication. Sun-Jung Kwon[1], Cynthia Hemenway[2] and Kook-Hyung Kim[1]. [1]School of Agricultural Biotechnology, Seoul National University, Suwon 441-744, Korea; [2]Department of Biochemistry, Box 7622, North Carolina State University, Raleigh 27695-7622, North Carolina.

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

Isolation and characterization of dsRNA mycovirus isolated from *Fusarium graminearum*. Yeon-Mee Chu, Jae-Jin Jeon, Yin-Won Lee and Kook-Hyung Kim. School of Agricultural Biotechnology, Seoul National University, Suwon 441-744, Korea.

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

Variation of *Potato virus Y* isolated from potatoes, tobaccoes and weeds in Korea on C-terminal region of coat protein gene and 3' non-translated region. W.S. Yun[1], H.W. Jung[1], M.H. Oh[2], Y.I. Hahm[3] and K.-H. Kim[1]. [1]School of Agricultural Biotechnology, Seoul