J-162. Characterization of double-stranded (ds) RNA mycovirus from *Fusarium graminearum* in Korea. Sang Jin Yea, Yeon Mee Chu, Yin-Won Lee, and Kook-Hyung Kim. School of Agricultural Biotechnology, Seoul National University

Mycoviruses have been detected in numerous species of yeasts and filamentous fungi. Most of them contain dsRNA genomes that are cryptic in phenotypic expression of the host cell. Exception of this are dsRNAs of chestnut blight fungus, *Cryphonectria parasitica*, satellite viruses encoding killer toxin found in *Saccaromyces cerevisiae* and *Ustilago maydis*. A genus of fungi, *Fusarium*, includes a number of plant pathogens. We found seventeen isolates (98-8-60, 98-56-12, 98-71-14, 23, 98-83-28, DK-21, JB-1, 33, 53, JNKY-19, YDP-16, YWD-3, 5, 6, 7, 9 and 10) of *Fusarium* containing 1 to 4 dsRNA molecules by screening 961 *Fusarium* isolates harvested from diseased barely, corn and wheat in Korea. These dsRNAs approximately 2.3 to 10 kb in length and they were inherited through the asexual cycle (conidia) and sexual cycle (ascospore) with percentage of 30~100%. DsRNA-containing strain, especially in case of isolate DK and YWD, had pronounced morphological changes, including reduction in mycelial growth rate, increased pigmentation, decreased (60-fold) production level of mycotoxin, and reduced virulence on wheat. DsRNAs in strain DK-21 could be transferred to dsRNA-free strain following anastomosis (hyphal fusion), and the recipient strain acquired the virus-associated phenotype of the donor strain. To characterize these dsRNAs, cDNA cloning and sequence analyses were performed. Partial nucleotide sequences of dsRNA in isolate DK, YWD and 98-8-60 revealed that it have some homology with polyprotein sequence and ATP-dependent RNA helicase of several viruses including C. *parasitica mycovirus*.

J-163. Characteristics of ds-RNA in Korean isolates of hypovirulent *Cryphonectria parasitica*. Jinyoung Lim[1], Michael G. Milgroom[2], Dae-Hyuk Kim[3], and Byeongjin Cha[1] [1]Dept. of Agricultural Biology, Chungbuk National University, Cheongju 361-763, Korea, [2]Dept. of Plant Pathology, Cornell University, Ithaca, NY 14853, [3]Institute for Molecular Biology and Genetics, Chonbuk National University, Chonju 561-756, Korea

From 670 isolates (585 virulent-like and 85 hypovirulent-like isolates in Bavendamm tests) of *Cryphonectria parasitica*, 161 virulent-like isolates were selected randomly for ds-RNA detection from mycelia of each isolates. In the tests, 51 (37 hypovirulent-like and 12 virulent-like) of total 225 isolates produced ds-RNA positive band(s) and the size of ds-RNA was 12 kb. In ITS-RFLP tests of ds-RNA positive isolates, two isolates revealed different band pattern from other isolates. In RT-PCR of 13 ds-RNA positive isolates that collected from different localities, RT-PCR products for two different regions of viral genome (3′ non-coding region and ORF B) were obtained using primer sequences of ds-RNA CHV-1 which was isolated from EP713, the hypovirulent type-species. In the comparison of RT-PCR product sequences with those of other countries including France, Italy, China, and Japan, one isolate of Japan was related to some Korean isolates in phylogenetic analysis of 3′ non-coding region. On the other hand, only one isolate of Korea, HE522, was closely related to EP713 and some

Chinese isolates in phylogenetic analysis of ORF B. These results might be an evidence that *C. parasitica* of Korea, Japan, and China have close relationships with each other.

J-164. *Hop Stunt Viroid* variant isolated from wild potatoes cultivated in Korea. Soon Jung Lee[1], Sung Joon Lee[1], Young-Il Hahm[2], and Jai Youl Lee[1]. [1]Department of Microbiology, Kyung Pook National University, Daegu, Korea, [2]National Alpine Agricultural Experiment Station, Gangwon, Korea

Low molecular weight RNA containing viroid molecules were extracted from the wild potatoes showing spindle tuber symptom using Qiagen column chromatography. The existence of viroid molecule was assayed using two-dimensional electrophoresis under denaturing conditions of 8M urea at 4 É. The viroid RNA molecules purified by the electroelution method were applicated for the synthesis of cDNA by the RT-PCR method. The synthesized PCR products were then ligated to a pGEM-T Easy vector, cloned, and sequenced. The sequence analysis of the cloned viroid RNA demonstrated a variation of four bases compared with the Korean strain of HSVd. The viroid RNA molecules of 295 nucleotides isolated from the wild potatoes could be a hop-type of the HSVd species.

J-165. A new variant of *Hop stunt viroid* (HSVd) detected dapple fruit disease from Plum trees cultivated in Korea. Seung-Lark Hwang[1], Sung-Joon Lee[1], Mi-Jo Kwon[1], Tae-Young Kwon[2], and Jai-Youl Lee[1]. [1]Department of Microbiology, Kyung Pook National University, Daegu, Korea, [2]Cheongdo Peach Experiment Station, Kyongbuk Provincial Agricultural Technology Administration, Gyeongbuk, Korea

Viroids are the smallest known pathogens and cause several economically significant crop plants, fruit trees ornamental plants. Hop stunt viroid(HSVd) is able to infect a number of herbaceous and woody hosts, such as grapevine, *Citrus* or *Prunus* plants. Previous phylogenetic analyses have suggested the existence of three major groups of HSVd isolates(plum-type, hop-type and citrus-type). The purified RNAs from plum were applicated 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 297 nucleotides with one base transition(G at position 205 instead of a U) compared to the Hop stunt viroid (HSVd) reported in 1989 in Japan. It is the first report on the occurrence of the HSVd in the plum trees cultivated in Korea as well as a new variant of the HSVd.

J-166. The detection and distribution of *Apple scar skin viroid*-Korean strain (ASSVd-K) from Apples Cultivated in Korea Mi Jo Kwon[1], Seung Lark Hwang[1], Sung Joon Lee[1], Dong Hyuk Lee[2], and Jai Youl Lee[1]. [1]Department of Microbiology, Kyungpook National University, Daegu 701-702 , Korea, [2]Daegu Apple Research Institute, Gyeongbuk 716-810 , Korea

Apple scar skin viroid has been developed on an extremely severe disease in

Korean apple farms, Sansa, Busa, Chukwang, Miegie Life, Honglo and Songbonkeum in Korea. The suspicious viroid disease of scar skin was detected from variable cultivars of apple cultivated at Kunwi, Andong, Youngduk and Muju in southern part of Korea.
The RNA molecules were extracted from the apples bearing virod symptoms with the application of CF-11 method. The purified RNAs were used for synthesis of cDNA with RT-PCR. The PCR products were cloned and sequenced. The viroid RNA molecule showed the same nucleotide sequence compared with the *Apple scar skin viroid*-Korean strain(ASSVd-K) reported before. Viroid RNA molecules extracted from 6 different cultivars bearing the apple symptoms showed all the same sequence.
ASSVd-K was detected in Kunwi, Andong and Youngduk of Gyeongbuk province in 2001 and in Muju of Jeonbug province in this year.
As the viroid disease could be propagated vegetatively, the apple viroid would be transmitted gradually wide in Korea.

J-167. Detection of *Apple scar skin viroid* from the bark of apple tree. K. H. Lee[1], N. Y. Heo[1], N. B. Lee[1], B. K. Kim[1], and T. Sano[2]. [1]Dept. Research & Technology Division, National Plant Quarantine Service, Ministry of Agriculture and Forestry, Anyang 430-016, Korea, [2]Faculty of Agriculture & Life Science, Hirodsaki University, Hirosaki 036-8561, Japan.

Apple scar skin disease caused by apple scar skin viroid(ASSVd) is economically important disease of apples(->*Malus domestica*) with symptoms of color dappling, cracking, and distortion of the fruit. Apple trees are propagated by cuttings of one year growth branches from mother stocks. A lot of seedlings and buds of apple are being imported from foreign countries every year. Detecting the viroid in a early stage of apple trees such as seedlings and buds is needed not only to archive quarantine purposes but also to minimize the economic losses. It is important to establish the best conditions of viroid detection for accurate and rapid detection of the disease in quarantine. Various parts of apple tree are tested by RT-PCR to find the efficient detecting condition of ASSVd. The viroid tends to be better detected at the basal and middle parts of apple tree branches than at the upper parts. The midribs were the most desirable parts for detecting the ASSVd and consequently leaves and barks as a results of test with various parts. Although the detection degree at the bark was the lowest among three parts of apple tree, midrib, leaf laminae and bark, ASSVd was efficiently detected from the bark. The concentration of RNA from bark was 1.15mg per ml. The detection limit of ASSVd in a serial dilution of RNA extracts from bark was ->10^5 in RT-PCR. ASSVd was also detected at the symptomless branches of ASSVd infected apple tree, as well as at branches showing symptom.