

## 주제발표 초록

### 1. Induced Systemic Resistance by Selected Plant Growth Promoting Rhizobacteria.

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Induced systemic resistance (ISR), the activation of plant defense mechanisms in response to an extrinsic stimulus (including biological and chemical agent), is considered to be a plant disease control method in which the plant is the target, not the pathogens. Susceptible plants can be altered in their resistance against pathogens without genetic manipulation. The protection is based on the stimulation of defense mechanisms by metabolic changes that enable the plants to defend themselves more efficiently. The first review of ISR and the associated problems was published by Chester (1933), and as early as 1961, Ross did the first controlled laboratory study of induced resistance. ISR was observed in a wide variety of crop-pathogen systems and can be induced by prior inoculation with pathogens, microbial metabolites, plant derived materials, or chemicals. Many experimental evidence to support induced systemic resistance by selected rhizobacteria may come from several kinds of studies.

*Pseudomonas fluorescens* strain CHAO was found to provide biological protection against *Thielaviopsis basicola* on tobacco (Ahl et al., 1986). CHAO produces HCN which is shown to be associated with biological control by mutational analyses, and Voisard et al. (1989) suggested that HCN may induce resistance to *Thielaviopsis*.

Elevated levels of lignin were associated with plant defense mechanisms after inoculation of plant rhizobacteria. Treatment of potato tissue culture plants with a growth-promoting strain of *Pseudomonas* resulted in enhanced total plant lignification (Frommel et al., 1991) compared to non inoculated controls. These studies suggest that some PGPR may increase host defense.

Albert and Anderson (1987) demonstrated that bean roots colonized by a *P. putida* strain had increased and altered production of peroxidases, which have been implicated in induced resistance. In the systemic protection of carnation by *P. fluorescens* WC417 against Fusarium wilt, the purified bacterial outer membrane lipopolysaccharides (LPS) were as effective in inducing resistance as were live bacteria. This observation indicated that the bacterial LPS acts as a determinant of resistance induction. In more study, Cell wall extracts of *P. fluorescens* WC417, or purified LPS consisting of lipid A-inner core-O-antigenic side chain (OA), were as effective as live bacteria when applied to radish roots.

Experiments with *P. aeruginosa* TNSK2 as the including rhizobacterium and tobacco mosaic virus as the challenging leaf pathogen suggest that exogenous salicylic acid (SA) production contributes to the induced systemic resistance (De Mayer.,1997). Pieters (1996) reported that rhizobacteria producing SA and inducing pathogenesis related proteins (PRs) in the plant can be considered to trigger the SAR pathway rather than the ISR pathway. But many selected rhizobacteria-mediated ISR can induce ISR without SA production (unpublished data Park, 1998).

Siderophore, such as pseudobactin may involve in ISR. Leeman et al. (1996) reported that the purified pseudobactin of *P. fluorescens* strain WCS374 induced systemic resistance to *F. oxysporum* f.sp. *raphani* in radish and a pseudobactin-deficient mutant lacked the ISR activity (Duijff et al., 1994). Press (1998) reported that an increased the ferric iron level on *Serratia marcescens* 90-166 had led to induced systemic resistance to *Colletotrichum orbiculare*.

Induction of the gene encoding the pathogenesis-related protein, PR-1a, in tobacco was investigated to determine how plants respond biochemically to PGPR strain which induced systemic resistance. PR-1a is a protein of unknown function that is strongly induced during the onset of systemic acquired resistance (SAR) in tobacco (Uknes et al 1993). Recently, transgenic tobacco plants expressing high levels of PR-1a have shown significant resistance to downy mildew (Alexander et al., 1993). PR-1a protein have antifungal activity *in vitro*, and their expression correlated with the induction of SAR protein (Ryal et al.,1994). Park (1997) suggested that PGPR strain activates the PR-1a promoter on tobacco at different levels and times after treatment.

Anyway, PGPR can protect the plants from disease under commercial cropping system and, in certain cases, disease suppression is attributed to ISR. Leeman et al(1995) reported that in four consecutive years, seed treatment with the ISR inducing *P. fluorescens* strain WC374 reduced fusarium wilt of radish in a naturally infested greenhouse by up to 50%. Four PGPR strains applied as seed treatments followed by a soil drench at transplanting (Wei, 1991) suppressed angular leaf spot and anthracnose of cucumber in three field trails over a two-year period. In all three trials, most PGPR strains promoted plant growth in early season and enhanced yields. These observations indicate that treatments with PGPR-mediated ISR can both promote growth and reduce disease incidence under field condition.

Induction of ISR might be dependent on colonization of the root system by the PGPR-mediated ISR in sufficient numbers to trigger the plant defense mechanisms. Some root-colonizing bacteria may enter plant roots and live as endophytes inside the plant. It may be speculated that endophytic behavior aids the induction of resistance, because more plant cells are being contacted by the bacteria than by isolates confined to the rhizosphere (Benhamou et al., 1996).

In conclusion, ISR might not be a solution to all plant disease problems, but the fact that induced resistance can be triggered in otherwise susceptible plants by various environmentally friendly treatments, like seed treatment with PGPR-mediated ISR, also this type of biological control can add to sustainable agriculture by widening the choice of control agents and by filling niches for which chemicals are not available.

## **2. Isolation and Expression of Defense-Related Genes During Disease Resistance Responses of Tobacco Against Pathogens.**

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Induced disease resistance is an intrinsic plant defense mechanism which restrict pathogen invasion without changing the genetic constitution of plants. Local resistance is induced after contact with pathogen which cause necrotic hypersensitive response (HR) and is followed by systemic acquired resistance. For better understanding the molecular mechanism disease resistance, we isolated TMV-induced HR-associated genes from tobacco using modified differential screening technique. Plasmid DNA was prepared by *in vivo* excision of cDNA library constructed with mRNA of *Nicotiana glutinosa* infected with TMV. The DNA was analyzed by the combination of slot blot and reverse Northern blot analysis. The probes used in slot blot were prepared from mRNA isolated from TMV-infected and mock-treated tobacco plant. From screening of 900 independent cDNA, 84 clones that showed increased or decreased expression after TMV

infection were selected and sequenced at their 5' end. DNA sequences of each gene were analyzed using an NCBI BLAST program and the genes were identified based on amino acid sequence homology. Sixty-eight clones (89%) show significant homology to the registered genes in the database. These include 25 clones known as pathogen- or wound-inducible, which indicate that the modified differential screening is an efficient approach to identify previously unknown defense-related genes. Expression of defense-related genes including novel genes during disease resistance of tobacco against pathogens will be presented.

### 3. Wound-inducible genes available for enhancing disease resistance in transgenic plants.

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Differential screening of an *Arabidopsis* cDNA library constructed from the plant tissues harvested 1 h after wounding resulted in the isolation of 13 wound-inducible cDNA clones. The corresponding genes were rapidly induced upon wounding. Cross hybridization and nucleotide sequence analysis revealed that 4 cDNA clones among the 13 are the same gene transcripts. Therefore, we have isolated 10 different wound-inducible cDNA clones in this experiment and designated AWI (*Arabidopsis* wound inducible) 13, 16, 20, 23, 24, 27, 31, 32, 34 and 39, respectively. The cDNA clones may be broadly classified into two groups according to the expression time of their transcripts. Nine different clones among the 10 were rapidly induced, reaching a maximum level in about 1h and then were progressively reduced after wounding. However, the cDNA clone AWI31 showed steady accumulation of the transcripts and reached the maximum value at 2-4h and then started to decline. Cloning and nucleotide sequence analysis of the *AWI31* gene revealed that the gene product predicts a protein of 386 amino acids and does not show any significant homology to other known proteins in the database. The *AWI31* gene transcription was not induced by other environmental stresses such as drought, high salt and low temperature. Nucleotide sequence analysis of the 9 different cDNA clones revealed that AWI 16, 23 and 24 cDNA clones encode a S-adenosyl-methionine synthetase, a chloroplast -3 fatty acid desaturase and a glutathione S-transferase, respectively, and others have no known function.

An *Arabidopsis* glutathione S-transferase (GST) gene, designated *ATGST1*, was cloned using the AWI24 cDNA probe, and the gene product predicted a GST protein of 208 amino acids. The gene expression has known to be induced by pathogen attack and dehydration in the literature. Northern blot analysis using the gene specific DNA probe in 3' untranslated region revealed that expression of the gene was not only rapidly induced by wounding but also by other environmental stresses such as drought, low temperature and high salt. The promoter region of the gene contained the sequence motif ATTTCAA that has been known to be present in ethylene-responsive elements and other motifs that are highly conserved amongst stress-inducible gene promoters. A novel glutathione S-transferase gene, designated *ATGST11*, is tandemly arranged with the *ATGST1* gene. The *ATGST11* gene structure is very similar to the *ATGST1* gene with complete conservation of intron position and with a highly conserved nucleotide sequence, suggesting that they likely arose by duplication of a single primordial gene. The *ATGST 11* gene product predicts a GST of 209 amino acids. The gene was transcribed at very lower level and its transcripts may have very short half-life. Cis elements of the gene promoter possibly responsible for the transcriptional regulation are destroyed, and thereby the gene transcription is neither tissue-specific nor regulated by environmental stimuli.

About one third of the wound-inducible cDNAs that we have isolated (4 among 13) are the same gene transcript that had been known to encode a protein showing high similarity to blue copper-binding proteins. The corresponding gene of a representative cDNA clone AWI32 showed much stronger inducible expression than other 9 different wound-inducible cDNAs, showing about 30-fold increase in the amount of steady-state mRNA level at 1h after wounding. The gene transcription was also induced by other environmental stresses such as dehydration, high salt and low-temperature conditions. About 1.5 kb transcriptional regulatory region (from -1294 to +247) of the *AWI32* gene was cloned and fused to the -glucuronidase (*GUS*) gene in a plant transformation vector pBI101. Stable integration of the single chimeric gene into the tobacco genome led to strongly inducible *GUS* activity upon wounding in the homozygous T2 generations. The chimeric *GUS* gene expression driven by the *AWI32* gene promoter in the T2 plants was delayed and decreased compare to the *AWI32* gene expression in its native host, reaching the maximum level around 12h and showing about one third level of the wound-inducible activity. Nevertheless, the *AWI32* gene promoter may be potentially useful for the expression of chimeric gene in at least transgenic tobacco plants since the promoter activity reached to about half-fold level of the constitutive CaMV 35S promoter. The effect of progressive 5 deletions within the promoter region of the *AWI32* gene on wound-inducible expression of the *GUS* gene in the transgenic tobacco plants was analyzed. By deleting the promoter from -856 to -534 and further to -345, stepwise reductions in the promoter activity was observed, suggesting that at least two positive cis-elements were located in this region.

#### 4. CGA245704 - A Plant Activator for Disease Protection.

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CGA245704 (Benzo[1,2,3]thiadiazole-7-carbothioic acid-s-methylester) is the first compound of a new generation of plant protection products which activates the plant's own defence mechanisms, leading to an increased plant resistance against diseases. CGA245704 has been discovered and developed by the Ciba-Geigy (now Novartis).

CGA245704 and its metabolites have been tested in *vitro* 19 phytopathogenic fungi and bacteria and showed no significant toxic effect at 57 ppm a.i. However, by induction of the plant's own defence mechanisms, the compound provides a string protection of plants against pathogens. It stimulates the plant to become more resistant to diseases, imitating a natural phenomenon, called Systemic Activated Resistance (SAR).

Biochemical studies on tobacco, cucumber and other dicots showed that SAR reponses correlate with the accumulation of certain "pathogenicity related" (PR) proteins. Some of the PR proteins have been characterized as  $\beta$ -1,3-glucanases and chitinases which very likely play a certain role in SAR. Salicylic acid is involved in the transduction of the systemic signal and plays a central role in the biological induction of SAR. Treatment of plant with salicylic acid leads to the accumulation of the same PR proteins as after an application of CGA245704 and also achieves a certain disease protein. CGA245704 is also taken up by the plant more effectively than salicylic acid. In cereals, CGA245704 at 30ga.i/ha provides a long lasting protection against *Erysiphe graminis* with a single application at GS 25-32. CGA245704 at 1ga.i/box provides a unique long-lasting protection in rice against leaf blast (*Magnaporthe*

*grisea*) Partial protection against *Septoria* spp. and *Puccinia* spp. can be achieved. Good protection of tobacco against *Peronospora hyoscyami* f. sp. *tabacina* is obtained with 12ga.i/ha application.

Due to its particular modes of action, the development of resistance in pathogens seems to be very unlikely. CGA245704 offers a new, innovative way in plant in addition to fungicides. It is not only a new chemistry but also a new technology. The product has been introduced in Germany under the trade name Bion in 1996 and is under the registration trial in Korea.

## 5. Incidence of New Diseases of Major Crops in Korea.

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Since 1995 chronic but minor diseases have occurred severely along with the change of climate and cultivation methods. As an example, bacterial blight of rice (*Xanthomonas oryzae* pv. *oryzae*), which had been observed in a limited region with low incidence until mid 1990s, occurred severely in whole country from southern provinces to northern province like Kyunggi-do in 1998. Bacterial grain rot of rice was identified from 78 fields of 357 investigated (21.8%) and the average percentage of diseased panicle was 0.1% in 1998. Besides, false smut of rice, caused by *Ustilaginoidea virens*, also occurred in whole country. Severe incidence of the diseases assumed to be from high temperature and heavy rain-fall as well as from susceptibility of commercial cultivars. The three diseases tended to become increased and should be considered as major diseases to be controlled. In barley, scab disease caused by *Gibberella zeae* occurred severely in whole country in 1998.

In vegetables, Phytophthora blight and anthracnose of pepper occurred very severely in 1998 especially because of the heavy rain-fall. Because of increase of pathogen population and spatial distribution, the diseases have a potential to become even severer than before. Geographical distribution and disease severity of club root (*Plasmodiophora brassica*) in cabbage and radish has been rapidly expanded. In 1998, severe incidence of bacterial wilt of potato was investigated in Jeju-do and southern part of country. Therefore, bacterial wilt become a major disease in potato along with Phytophthora blight and anthracnose disease. In fruits, Phytophthora blight disease has been gradually expanded in apple, pear, peach and citron. Incidence of the disease in pear was observed from 272 ha in 18 cities and counties in 1998.

Diseases originated from contaminated seed were especially severe in pepper (bacterial canker caused by *Clavibacter michiganensis*) and watermelon (caused by cucumber green mottle mosaic virus, CGMMV). The watermelon CGMMV disease was first identified in 1989 in southern district but became very severe in 1998 mainly from the contamination of imported seed of root stock such as FR King II and Kajjidokki. The disease is still remained as a problem because field soil might be contaminated with the virus. Occurrence of bacterial canker of pepper was limited in southern district in 1997. However, the disease occurred severely in plug seedling farm in Seosan, caused by contaminated seed imported, and the infected seedlings distributed to farmers. In 1998, the disease was identified in whole country.

In the cultivation system under structure (vinyl, glass, hydroponic culture), tomato root rot and bacterial wilt become a major disease. Wilt of lettuce, severely occurred in hydroponic culture, was identified as a new disease caused by *Phytophthora* sp.. Fortunately, it could be controlled successfully by phosphoric acid ( $H_3PO_4$ ).

As a conclusion, incidence of some diseases, previously not a major problem, and new diseases have been gradually expanded. Main factors for the problems have been regarded as a recent meteorological disorder and change of cultivation methods, which suggest that recent disease pattern would be continued if environmental condition and cultivation method were not changed significantly. More consideration should also be needed to increase health of seed produced domestically and imported from abroad.

## 6. Characteristics and Genome Organization of Barley Mild Mosaic Virus in Korea.

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In this study, the barley mild mosaic virus (BaMMV-Kor) isolated from the southern part of Korea was characterized by mechanical inoculation onto barley cultivars, purification, production of antibody, and sequence analysis.

Particles of BaMMV-Kor purified from the infected barley plants were filamentous with a diameter of 13nm, and two model lengths of 250-300nm and 500-600nm. Antibody was produced in a rabbit by injecting the purified virus particles. The titer of the antibody was 1/680 in ring tests. In immuno-electromicroscopy, the purified virus particles reacted specifically with the antibody. In gel-diffusion tests with this antibody, BaMMV-Kor made no spur with a Japanese strain, BaMMV-Na1, but it formed spur with another Japanese strain BaMMV-Ka1 and a German strain BaMMV-M. However, BaMMV-Kor was distinguished from three other strains by ELISA. After mechanical inoculation with BaMMV-Kor, infected barley plants showed mosaic and yellowing symptoms. Among Japanese barley cultivars which had been used to discriminate BaMMV strains, BaMMV-Kor infected New Golden, Ishukushirazu (*Ym3*), Joshushirohadaka and Misato Golden (*Ym*), but it did not infect Shiromugi 6 or Tosangawa 73. Among barley cultivars recommended by Korean authority, it infected most of naked barley cultivars, but it did not infect Milyangketbori, Saealbori, Alchanbori, Yeongnambori and Chalbori. It infected all naked barley cultivars except Chalssalbori and Hinssalbori, and all malting barley cultivars tested.

In agarose gel electrophoresis, RNA from the purified particles of BaMMV-Kor were separated into two bands of 7.5Kb (RNA 1) and 3.5Kb (RNA 2). In SDS-PAGE, the capsid protein from the BaMMV-Kor particles gave a major band of 33KDa. From the RNA extracted from the purified BaMMV-Kor particles strain, cDNA was synthesized by oligo dT-priming, and 14 clones greater than 2.0Kb were obtained. Among them, two large clones pKorKS 105 (2.5Kb) and pKorKS 68 (2.7Kb) were selected for sequence analysis. Northern hybridization showed that a pKorKS 105 reacted to RNA 1 and pKorKS 68 to RNA 2. Subclones were made from them and sequenced. Clone pKorKS 105 gave the sequence of 2,500 nucleotides excluding the 3'-terminal poly(A) and clone pKorKS 68 did 2,700 nucleotides excluding the 3'-terminal poly(A). The 5'-terminal portion of RNA 2 was cloned by the RACE method and sequenced.

The sequence of 3'-terminal region of RNA 1, determined by pKorKS 105, contained an open reading frame (ORF), with a UAG stop codon at position 2,159, encoding a polypeptide of 720 amino acids. A possible cleavage site (Q/S) between the capsid and Nib proteins was found at amino acid position 469. The partial Nib protein consisted of 469 amino acids. It contained the two conserved stretches SGXXXTXXXNT and GDD, which are thought to form the core of RNA-dependent RNA polymerase, at positions 273-282 and 315-317. The capsid protein was comprised of 251 amino acids. The two conserved blocks NGTS and AFDF, which had been found in the capsid proteins of bymoviruses and other

rod-shaped viruses, were also found in the capsid protein of BaMMV-Kor at positions 584-587 and 658-661 with a replacement of A by V in the second block. The 3'non-coding region(NCR) of BaMMV-Kor RNA 1 consisted of 342 nucleotides and contained the putative polyadenylation signal of UAUGU at position 2415-2419.

The RNA 2 of BaMMV-Kor consisted of 3,520 nucleotides excluding the 3'-terminal poly(A) and contained a large ORF starting with triplicated AUG codons at positions 142-150 and a UAA stop codon at position 2,823-2,825. This ORF encodes a putative polyprotein of 894 amino acids (98KDa). The amino acid motif domain GFCY was located at 117-120 and a putative cleavage GA site located at 228-229. The polyprotein of BaMMV-Kor RNA 2 was thought to consist of a N-terminal protein (P1; 25KDa) and a C-terminal protein (P2; 73KDa). The 5' NCR consisted of 141 nucleotides. The 3' NCR comprised 697 nucleotides, and contained a putative polyadenylation signal UAUGU at position 3,501-3,505.

The capsid protein of BaMMV-Kor had 97.2% homology to BaMMV-Na1, 92% to BaMMV-Ka1, 93.2% to BaMMV-G, 92.8% to BaMMV-UK. The partial NIB protein of BaMMV-Kor showed slightly higher sequence identity with BaMMV-Na1 (98.3%) than with BaMMV-Ka1 (97.4%). The 3' NCR of BaMMV-Kor also showed higher nucleotide sequence identity with BaMMV-Na1 (96.5%) than with BaMMV-Ka1 (92.1%), BaMMV-G (91.8%), or BaMMV-UK (92.9%).

The whole 3,520 nucleotides of BaMMV-Kor RNA 2 had 92.4% homology to BaMMV-Na1, 85.1% to BaMMV-UK-F, 72.2% to BaMMV-UK-M, 83.7% to BaMMV-ASL1 and 72.6% to BaMMV-M. The P1 protein of BaMMV-Kor had 94.0% homology to BaMMV-Na1, 55.2% to BaMMV-UK-F, 85.0% to BaMMV-UK-M, 85.0% to BaMMV-ASL1 and 85.3% to BaMMV-M. The P2 protein of BaMMV-Kor had 91.7% homology to BaMMV-Na1, 85.1% to BaMMV-UK-F, 84.9% to BaMMV-UK-M, 83.7% to BaMMV-ASL1 and 85.1% to BaMMV-M. The 5' NCR of BaMMV-Kor RNA 2 had 92.1% homology to BaMMV-Na1, 74.4% to BaMMV-UK-F, 71.2% to BaMMV-UK-M, 75.2% to BaMMV-ASL1 and 72.1% to BaMMV-M. Thus this region shows a relatively low homology. The 3' NCR of BaMMV-Kor RNA 2 had 96.8% homology to BaMMV-Na1, 93.7% to BaMMV-UK-F, 93.8% to BaMMV-UK-M, 93.8% to BaMMV-ASL1 and 94.4% to BaMMV-M.

The results suggest that BaMMV-Kor is most closely related to BaMMV-Na1. Although other mechanically-transmitted BaMMV isolates had deletions in their P2 proteins, no such deletion was found in that of BaMMV-Kor.

## 7. Bacterial Diseases of Major Crops in Korea.

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Occurrence of bacterial diseases in major crops had been surveyed in Korea from 1996 to 1998. Plant pathogenic bacteria belonged to 7 different genus, 16 different species and 25 different pathovars were identified from the survey. Among them ten diseases were found new in Korea. They are; bacterial leaf spot of angelica, bacterial blight of brown mustard, bacterial leaf spot of kale, soft rot of parsley, canker of pepper, bacterial wilt of perilla, soft rot of phalaenopsis, bacterial wilt of potato, bacterial leaf spot of rehmannia, and pith necrosis of tomato. Bacterial wilt by *Ralstonia solanacearum*, rice grain rot by *Burkholderia glumae*, soft rot by *Erwinia carotovora* subsp. *carotovora*, pepper bacterial leaf spot by *Xanthomonas campestris* pv. *vesicatoria*, potato scab by *Streptomyces* spp., and kiwifruit canker by

*Pseudomonas syringae* pv. *actinidiae* were the most severe diseases during 1996 and 1998. About 78% of total 89 fields were infected with the potato scab in Cheju, Kangwon province and southern part of Korea. Especially those fields in southern Cheju province and Mooankoon of Chunnam province, which had been cultivated with same crops for 10 years, were heavily infected with the diseases. Total 88% of 127 surveyed fields were infected by *Xanthomonas campestris* pv. *glycines* in soybean and crucifer black rot were found in 36% of 64 surveyed fields. Sixty two percent of surveyed vegetable fields were infected with soft rot disease, especially potato, radish, and chinese cabbage had serious infection. It was known that the bacterial leaf spot of pepper by *Xanthomonas campestris* pv. *vesicatoria* had been causing serious problems for a long time in Korea. However, most symptoms were mixed with two different symptoms which one was leaf spot and the other was canker. Isolated bacteria from the leaf spot was a *X. campestris* pv. *vesicatoria* but bacteria from canker was *Clavibacter michiganensis* subsp. *michiganensis*. The canker of pepper caused by *C. michiganensis* subsp. *michiganensis* was the first report in Korea, and new name "gueyangbyung" was given to that disease. Recently, hypotonic cultivation system had been extensively employed for vegetable cultivation in Korea and bacterial wilt caused by *Ralstonia solanacearum* became one of the most serious diseases in hypotonic cultivation of tomato. In the case of potato, the tissue culture creates seed potato for the potato growers in whole Cheju island and southern part of Korea. However, *R. solanacearum* was isolated even from the seed potato in Cheju island. The race 3 of *R. solanacearum*, which could infect only potato and tomato, was newly identified in Korea.

#### 8. Status of CGMMV occurrence and control measures in Korea.

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Among the seed production in Korea, seed production in foreign countries has been increased annually and the percentage was 65.7% in 1998. Therefore, there has been some possibilities in introduction of seed-borne diseases from the foreign countries. In the 1998 survey on watermelon showing abnormal growth, the causal agent was found to be the CGMMV in 408 ha from 949 farms of 25 cities and counties of 5 provinces. The source of infection of the virus was introduction of CGMMV infected in cucurbit rootstock seeds imported from foreign countries. The occurrence of CGMMV damage was due to a lack of concerns in seed companies and scientists. This paper was focused on the status of CGMMV occurrence in Korea, characteristics, ecology, and control of the virus.

CGMMV was firstly reported by Answorth in England in 1935 and this virus is reported from many countries including Iran, Israel, Indonesia, Germany, The Netherlands, Russia, India, Poland, Spain, and Japan. The occurrence of CGMMV in Korea was found in southern provinces in 1989 and reported in 1990. The record of CGMMV in Japan was done in 1971. The survey of CGMMV has been conducted in Korea for the occurrence of the disease, however, there has been no report until 1989.

CGMMV belongs to Tobamovirus group with rod shape particles of  $300 \times 18\text{nm}$  and single stranded RNA. Transmission occurs by sap and contact. Host range is limited in Curcubitaceae, but local symptom is observed in *D. stramonium*, petunia, and *Chenopodium* sp, according to the different isolates. Differentiation of isolates were done by host responses, serological relationship, and amino acid composition and structure of coat protein. There are three isolates of CGMMV-C from cucumber, CGMMV-G from watermelon, and CGMMV-Y from cucumber. Among the isolates, they show different



response in relation to their relationship, therefore, attention should be paid in serological diagnosis. The virus is very stable as TMV.

Watermelon infected with CGMMV shows symptoms on fruits and leaves. Irregular discoloration and mosaic symptoms and dark green island appear on leave when the infection is severe. Growth of the whole plant is retarded and wilted. In cucumber, vein banding symptom and discoloring of inter-veinal tissues are characteristic. Patches or central necrosis are observed on the surface of infected fruit. Fruit stalk has brown necrotic stripes. Characteristic fruit inside symptoms are yellow soaking between the peel and flesh. When the infection progresses the flesh around the seeds become bloody-pink, soaking yellow fibers and cork symptom. The flesh is not crunch and tastes somewhat bitter, and sound is different from uninfected fruit when knock on the infected fruit.

CGMMV in watermelon is transmitted by seed (watermelon and rootstock), soil, contact, and sap. The sources of transmission are infected seed and soil, contaminated materials, and infected plants. Most transmission occurs by seeds in the rate of 3-4%. Seed transmission rate is relatively lower than the infection rate of in the field because the secondary transmission occurs by contact during cultivation activities such as grafting, transplanting and pinching. Virus density is high in pollen and pistil, but the virus is not transmitted by pollen. Insect damage is not related to the virus transmission, however, severe damage by insect having chewing type mouthpart promotes virus infection.

To control the CGMMV, it is desirable to collect seed from healthy plant to prevent primary transmission and dry heat treatment is recommended when the virus infection is suspected. Heat treatment is done for more than 3 days in 70°C and more than 2 days in 73°C, and then pretreatment in lower temperature can increase the germination rate. Steam and MB treatments are effective for contaminate soil sterilization. In consecutively cultivated soils, virus lose activity in 2-3 months under the condition of well decaying of remaining diseased root and stem, but the virus is active for over a year when the drainage is poor or submerged in water. Therefore, root and stem should be removed from infected field, and drainage improvement, promotion of decay, and application of lime are desirable.

Above descriptions are based on the research results from other countries. To prevent the damage by CGMMV and other possible virus disease imported from foreign countries, more attention should be paid by seed companies, farmers, researchers, and especially by quarantine officers. Seed companies have to select CGMMV-free area for seed production with cautious field management and inspection during seed collection from healthy plants. After seed inspection on virus, import have to be done through regular quarantine procedure. And attention should be paid on the occurrence of the disease in farms using the seeds.

In case of seed production in foreign country, suspected plant can be collected and tested in Korea since the CGMMV is relatively stable and easy to detect.

Due to soil transmission of CGMMV, it is desirable to avoid consecutive cultivation in contaminated field and should plant non-host crop. When the CGMMV infection is observed, the diseased plant should be removed with 3-4 more plants around the infected plant.

There is few research results on CGMMV in Korea since the research began in 1998. Diagnosis methods of RIPA and High density latex agglutination method were developed, and studies on the ecology and control are underway. In Japan, CGMMV was firstly reported in 1971, however, there's no report on the severe damage. Therefore, the virus problem can be solved when virus-free seed production is done and more attention is paid on field sanitation to prevent infection of the virus during cultivation.